

# PROGRAM AND ABSTRACT BOOK

**SETAC Latin America 15<sup>th</sup> Biennial Meeting**

17–20 September 2023 | Montevideo, Uruguay

“Cutting-Edge Knowledge and Technologies for Environmental  
Health Management and Research”



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# Program and Abstract Book

## SETAC Latin America 15<sup>th</sup> Biennial Meeting

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This book comprises the abstracts of the presentations for the platform and poster sessions of the Society of Environmental Toxicology and Chemistry (SETAC) Latin America 15<sup>th</sup> Biennial Meeting, conducted from 17–20 September 2023 in Montevideo, Uruguay. The abstracts are reproduced as accepted by the Scientific Program Committee and appear in numerical order. In each abstract, the presenting author’s name is underlined. The author index cross-references the corresponding abstract numbers.

# DRAFT

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# About SETAC

The Society of Environmental Toxicology and Chemistry (SETAC), with offices in North America and Europe, is a not-for-profit, worldwide professional organization composed of more than 16,000 researchers, students, and expert practitioners from universities, institutions, governmental authorities, businesses, and nongovernmental organizations as well as 85 partner organizations in more than 90 countries dedicated to advancing environmental science and environmental management.

Specific goals of the society are:

- Promote research, education and training in the environmental sciences
- Promote the systematic application of all relevant scientific disciplines to the evaluation of chemical hazards
- Participate in the scientific interpretation of issues concerned with hazard assessment and risk analysis
- Support the development of ecologically acceptable practices and principles
- Provide a forum (meetings and publications) for communication among professionals in government, business, academia and other segments of society involved in the use, protection and management of our environment

These goals are pursued through the conduct of numerous activities, which include:

- Conduct meetings with study and workshop sessions, platform and poster presentations, and achievement and merit awards
- Publish peer-reviewed scientific journals, *Environmental Toxicology and Chemistry* (ET&C) and *Integrated Environmental Assessment and Management* (IEAM), as well as electronic newsletters and special technical publications
- Provide funds for education and training through the SETAC grants program
- Organize and sponsor chapters and branches to provide a forum for the presentation of scientific data and for the interchange and study of information about local and regional concerns
- Provide advice and counsel to technical and nontechnical persons through a number of standing and ad hoc committees

For further information, contact the Pensacola office if you are in Latin America, Asia-Pacific or North America, or the Brussels office if you are in Europe or Africa.

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**MEETING  
PROGRAM**

## Meeting Program | Sunday, 17 September

Time (UYT)	Program	Room
9:00–13:00	<b>Chemical Management Symposium (Registered participants only)</b>	Auditorium
	Training Course 1 - <b>Eco-innovation and the Transition to the Circular Economy</b>	1
	Training Course 2 - <b>Biomarkers as Tools for Ecotoxicological Studies in Sentinel Species in Latin America</b>	2
	Training Course 3 - <b>Development of Adverse Outcome Pathway</b>	3
	Training Course 4 - <b>Environmental Monitoring and Remediation: Study of Environmental Contaminants and Evaluation of Effects by Ecotoxicity Bioassays</b>	4
	Training Course 5 - <b>Modeling Tools in Assessing Pesticide Exposure to Ground and Surface Water</b>	5
	Training Course 6 - <b>Plastic Pollution, Chemicals and Their Impacts: From Macro to Microplastics Analysis</b>	6
13:00–14:00	<b>Break</b>	
14:00–18:00	<b>Chemical Management Symposium (Registered participants only)</b>	Auditorium
	Training Course 7 - <b>O uso do bioindicador <i>Allium cepa</i> em ensaios de citogenotoxicidade de xenobiontes e de amostras ambientais</b>	7
	Training Course 8 - <b>Using Fish for Monitoring Multiple Stressors in Aquatic Systems</b>	8
	Training Course 9 - <b>Ecological Risk Assessment of Contaminated Land</b>	9
	Training Course 10 - <b>Chemical, Analytical and Ecotoxicological Tools to Assure Sustainability of Agroecosystems</b>	10
	Training Course 11 - <b>Implementation of Alternative Methods to the Use of Laboratory Animals in Safety Studies of Products for Human Use and Agrochemicals</b>	11
18:00–19:00	<b>Break</b>	
19:00–20:00	<b>Opening Ceremony</b>	Auditorium
20:00–21:00	<b>Cocktail Party Reception and Show</b>	

## Meeting Program | Monday, 18 September

Time (UYT)	Program	Room
9:00–10:30	1A - <b>Environmental Risk Assessment</b>   Julie Brodeur, Ximena Patino, Carla Pozzi	Speaker Room
	6A - <b>Emerging Contaminants and Environmental Remediation: Monitoring of Effects by Ecotoxicological Bioassays</b>   Jairo Lisbo Rodrigues, Márcia Cristina da Silva Faria	Auditorium
	25A - <b>Toxic Cyanobacterial Blooms: Causes of a Growing Problem</b>   Carla Kruk, Claudia Piccini	Orquideas
	23A - <b>Planetary Health: Chemical Pollution as Driver for Loss of Ecosystem Services and Biodiversity</b>   Hennert Hollert, Francisco Sylvester	CoWork
	15A - <b>Toxic Metal and Metalloids Pollution in Surface and Groundwater Hydric Resources</b>   Paula Collazo, Valerie Buhl	Camelia
10:30–11:00	<b>Break</b>	Showroom
11:00–12:30	1B - <b>Environmental Risk Assessment</b>   Julie Brodeur, Ximena Patino, Carla Pozzi	Speaker Room
	6B - <b>Emerging Contaminants and Environmental Remediation: Monitoring of Effects by Ecotoxicological Bioassays</b>   Jairo Lisbo Rodrigues, Márcia Cristina da Silva Faria	Auditorium
	25B - <b>Toxic Cyanobacterial Blooms: Causes of a Growing Problem</b>   Carla Kruk, Claudia Piccini	Orquideas
	23B - <b>Planetary Health: Chemical Pollution as Driver for Loss of Ecosystem Services and Biodiversity</b>   Hennert Hollert, Francisco Sylvester	CoWork
	15B - <b>Toxic Metal and Metalloids Pollution in Surface and Groundwater Hydric Resources</b>   Paula Collazo, Valerie Buhl	Camelia
12:40–13:40	<b>Plenary: Santiago Mirazo, “One Health Paradigm for Pandemic Preparedness in the Anthropocene Era”</b>	Speaker Room
13:40–14:40	<b>Lunch</b>	Showroom
	<b>Bayer Sponsored Seminar</b>	Orquideas
	<b>Universidad Montevideo Sponsored Seminar</b>	Auditorium
	<b>General Student Assembly</b>	
14:45–16:15	16A - <b>Microplastics</b>   Nedia Ghisi, Lorena M. Rios Mendoza, Carlos M. Alonso Hernandez	Speaker Room
	14A - <b>Nanomaterials</b>   Marta Margarete Cestari, Roberto Martins, Jimena Cazenave	Auditorium
	26A - <b>Cutting-edge Monitoring Technologies for Health Protection</b>   Claudia Elizondo, Alejo Silvarrey, José Valles	Orquideas
	13 - <b>Detection, Toxicity and Environmental Risk of UV Filters and Cosmetic Ingredients in Aquatic Ecosystems</b>   Carys L. Mitchelmore, Charles A. Menzie	Camelia
	<b>SETAC CHEM PANEL Horizon Scanning</b>	CoWork
16:15–16:45	<b>Break</b>	Showroom
16:45–18:15	16B - <b>Microplastics</b>   Nedia Ghisi, Lorena M. Rios Mendoza, Carlos M. Alonso Hernandez	Speaker Room
	14B - <b>Nanomaterials</b>   Marta Margarete Cestari, Roberto Martins, Jimena Cazenave	Auditorium
	26B - <b>Cutting-edge Monitoring Technologies for Health Protection</b>   Claudia Elizondo, Alejo Silvarrey, José Valles	Orquideas
	18 - <b>Development and Acceptance of New Methodological Approaches (NAMs) in Latin America</b>   Juan Ignacio Pina, María Laura Gutiérrez, Ana Cione	Camelia
	<b>Risk Assessment Certification Program Presentation</b>	CoWork
18:15–19:45	<b>Poster Session</b>	Showroom

## Meeting Program | Tuesday, 19 September

Time (UYT)	Program	Room
9:00–10:30	1C - <b>Environmental Risk Assessment</b>   Julie Brodeur, Ximena Patino, Carla Pozzi	Speaker Room
	7A - <b>Tracking Primary Pollutants and Their Toxicologically Relevant Transformation Products</b>   George P. Cobb, Karina Miglioranza, John Giesy	Auditorium
	4A - <b>Wildlife Ecotoxicology</b>   John Jairo López Perea, Laura Addy Orduna, Maria Belen Poliserpi	Orquideas
	3 - <b>Advances in Genotoxicity Biomarkers Application in Latin America</b>   Maria Fernanda Simoniello, Sonia Soloneski	Camelia
	12A - <b>Tropical Ecosystem Health Assessment: Effects of Contaminants on Marine and Estuarine Ecosystems</b>   Paola Calle, Frank Von Hippel, Gustavo Dominguez	CoWork
10:30–11:00	Break	Showroom
11:00–12:30	1D - <b>Environmental Risk Assessment</b>   Julie Brodeur, Ximena Patino, Carla Pozzi	Speaker Room
	7B - <b>Tracking Primary Pollutants and Their Toxicologically Relevant Transformation Products</b>   George P. Cobb, Karina Miglioranza, John Giesy	Auditorium
	4B - <b>Wildlife Ecotoxicology</b>   John Jairo López Perea, Laura Addy Orduna, Maria Belen Poliserpi	Orquideas
	8 - <b>Environmental Mutagenesis in Exposed Populations</b>   Juliana Da Silva, Gisela Poletta	Camelia
	12B - <b>Tropical Ecosystem Health Assessment: Effects of Contaminants on Marine and Estuarine Ecosystems</b>   Paola Calle, Frank Von Hippel, Gustavo Dominguez	CoWork
12:40–13:40	<b>Plenary: Prakash Hande, “Environmental Toxicogenomics: Approaches to Analyzing Biological Consequences of Environmental Toxicant”</b>	Speaker Room
13:40–14:40	<b>Lunch, Lunch With Mentors</b>	Showroom
	<b>Syngenta Sponsored Seminar</b>	Orquideas
	<b>LATU Sponsored Seminar</b>	Auditorium
	<b>Group Photo During Lunch</b>	Showroom
14:45–16:15	16C - <b>Microplastics</b>   Nedia Ghisi, Lorena M. Rios Mendoza, Carlos M. Alonso Hernandez	Speaker Room
	2A - <b>Research and Management Approaches to Achieve a Balance Between Food Production and Nature Conservation - How to Conserve Biodiversity in an Agricultural Context?</b>   Breno Freitas, Jose Ricardo Cure, Maria Teresa Almanza	Auditorium
	21A - <b>Nature-based Solutions for Remediation and Restoration of Water Quality</b>   Helena Cristina Silva de Assis, Tatiana Heid Furley	Orquideas
	5 - <b>Challenges and Strategies for Linking Adverse Effects to Endocrine Activity to Identify Endocrine Disruptors</b>   Diana Miguez, Laurent Lagadic, Steven Levine	Camelia
16:15–16:30	<b>Break</b>	Showroom
16:30–18:00	16D - <b>Microplastics</b>   Nedia Ghisi, Lorena M. Rios Mendoza, Carlos M. Alonso Hernandez	Speaker Room
	2B - <b>Research and Management Approaches to Achieve a Balance Between Food Production and Nature Conservation - How to Conserve Biodiversity in an Agricultural Context?</b>   Breno Freitas, Jose Ricardo Cure, Maria Teresa Almanza	Auditorium
	21B - <b>Nature-based Solutions for Remediation and Restoration of Water Quality</b>   Helena Cristina Silva de Assis, Tatiana Heid Furley	Orquideas
	10 - <b>Understanding Contamination Threats to Amphibians and Reptiles of Latin America to Promote Their Conservation</b>   Julie Brodeur, Paola M. Peltzer	Camelia
	<b>Mate-Debate (students)</b>	CoWork
18:00–19:30	<b>Poster Session</b>	Showroom



## Meeting Program | Wednesday, 20 September

Time (UYT)	Program	Room
9:00–10:30	9A - <b>Aquatic Ecotoxicology: Test System &amp; New Approaches</b>   Juan Manuel Perez Iglesias, Leticia Peluso	Speaker Room
	20A - <b>Environmental Assessment of Pesticides for Soil Organisms in Latin America</b>   Cintia Carla Niva, Clara Wandenkolck Silva Aragão, Gregor Ernst	Auditorium
	24 - <b>Impacts of the Expansion of Anthropogenic Activities on Water Quality and Biota in the Amazon and Pantanal Biomes</b>   Bruno do Amaral Crispim, Lucilene Finoto Viana	Orquideas
	27 - <b>Advances and Challenges in the Analysis of Toxicity and Environmental Data: Statistics, Models, Databases, Index, and Risk Assessment</b>   Lidwina Bertrand, Fernando Gastón Iturburu	Camelia
10:30–11:00	<b>Break</b>	Showroom
11:00–12:30	9B - <b>Aquatic Ecotoxicology: Test System &amp; New Approaches</b>   Juan Manuel Perez Iglesias, Leticia Peluso	Speaker Room
	20B - <b>Environmental Assessment of Pesticides for Soil Organisms in Latin America</b>   Cintia Carla Niva, Clara Wandenkolck Silva Aragão, Gregor Ernst	Auditorium
	17 - <b>Biological Consequences of the Environmental Radiation Exposure</b>   Prakash Hande, Rosario Odino, Wilner Martínez-López	Orquideas
	11 - <b>Physiologically Based Pharmacokinetic Modeling: Applications in Biopharmaceutics, Precision Dosing and Toxicokinetic Predictions</b>   Pietro Fagiolino, Manuel Ibarra, Alan Talevi	Camelia
	<b>Women in Science</b>	CoWork
12:40–13:40	<b>Lunch</b>	Showroom
	<b>Agilent Sponsored Seminar</b>	
	<b>General Assembly</b>	
13:40–14:40	<b>Plenary: Karla Pozo, “Plastic Pollution, Chemicals and Their Impacts: From Macro to Microplastics”</b>	Speaker Room
14:45–16:15	<b>Poster Session</b>	Showroom
16:30–17:30	<b>Plenary: Henner Hollert, “Environmental Impacts of Chemicals – Beyond the Planetary Guardrails”</b>	Speaker Room
17:30–until	<b>Closing Ceremony and Awards</b>	Speaker Room

# Sessions

- 01 Environmental Risk Assessment
- 02 Research and Management Approaches to Achieve a Balance Between Food Production and Nature Conservation
- 03 Advances in Genotoxicity Biomarkers Application in Latin America
- 04 Wildlife Ecotoxicology
- 05 Challenges and Strategies for Linking Adverse Effects to Endocrine Activity to Identify Endocrine Disruptors
- 06 Emerging Contaminants and Environmental Remediation: Monitoring of Effects by Ecotoxicological Bioassays
- 07 Tracking Primary Pollutants and Their Toxicologically Relevant Transformation Products
- 08 Environmental Mutagenesis in Human Populations
- 09 Aquatic Ecotoxicology: Test System and New Approaches
- 10 Understanding Contamination Threats to Amphibians and Reptiles of Latin America to Promote Their Conservation
- 11 Physiologically Based Pharmacokinetic Modeling
- 12 Tropical Ecosystem Health Assessment: Effects of Contaminants on Marine and Estuarine Ecosystems
- 13 Detection, Toxicity and Environmental Risk of UV Filters and Cosmetic Ingredients in Aquatic Ecosystems
- 14 Nanomaterials
- 15 Toxic Metal and Metalloids Pollution in Surface and Groundwater Hydric Resources
- 16 Microplastics
- 17 Biological Consequences of the Environmental Radiation Exposure
- 18 Development and Acceptance of New Methodological Approaches (NAMs) in Latin America
- 20 Environmental Assessment of Pesticides for Soil Organisms in Latin America
- 21 Nature-Based Solutions for Remediation and Restoration of Water Quality
- 22 Eco-Economy: New Economies for Sustainability
- 23 Planetary Health: Chemical Pollution as Driver for Loss of Ecosystem Services and Biodiversity
- 24 Impacts of the Expansion of Anthropogenic Activities on Water Quality and Biota in the Amazon and Pantanal Biomes
- 25 Toxic Cyanobacterial Blooms: Causes of a Growing Problem
- 26 Cutting-Edge Monitoring Technologies for Health Protection
- 27 Advances and Challenges in the Analysis of Toxicity and Environmental Data

# Presentation IDs

The scientific program is organized by sessions. Within each session, presentations are organized by oral (O) or poster (P) presentation type and which day they were presented.

**1A.O-Mo-1**

Session: 1A    O for Oral    Mo for Monday

**DRAFT**

**PLATFORM  
PRESENTATIONS**

# Session 1: Environmental Risk Assessment

## 1A.O-Mo-1

### Waterborne Toxicity on Neotropical Invertebrates and Hazard of Cigarette Butt Leachates to Marine Environments

Lucas Buruaem Moreira<sup>1</sup>, Wanessa Gentil Mandelli<sup>1</sup>, Rodrigo Brasil Choueri<sup>1</sup>, Denis Moledo de Souza Abessa<sup>2</sup>, Ítalo Braga Castro<sup>1</sup>

<sup>1</sup>Instituto do Mar da Universidade Federal de São Paulo (Imar UNIFESP), Brazil. <sup>2</sup>Núcleo de Estudos em Poluição e Ecotoxicologia Aquática da Universidade Estadual Paulista (NEPEA, UNESP), Brazil

#### Abstract

Cigarette butts (CB) are the most common litter on beaches worldwide and are known to contain a complex mixture of chemicals in their composition. Once released into water, CBs decomposition begins, releasing contaminants generated during smoking and retained in filters, which may have an increased toxic potential. Given the recent interest in this emerging problem, it is essential to assess the toxicity of CBs leachates to a range of species from different regions and with different sensitivities and ecological traits. In this study, the waterborne toxicity of smoked CBs leachates to tropical invertebrates was evaluated in the context of Environmental Risk Assessment (ERA) in coastal and marine environments. Leachates of smoked cigarettes were prepared in the laboratory and characterized for trace elements (Mn, Fe, Co, Ni, Cu, Zn, As, Cd, and Pb), ammonia nitrogen, and polycyclic aromatic hydrocarbons (PAHs). Then, a set of toxicity tests with marine invertebrates was performed and both acute and chronic effects of samples were determined. In summary, the main toxicity metrics were: the brine shrimp *Artemia* sp. (nontoxic); the amphipod *Tiburonella viscana* (LC<sub>50</sub> of 0.038 CB.L<sup>-1</sup>); the tanaid *Monokalliapseudes schubarti* (LC<sub>50</sub> of 0.126 CB.L<sup>-1</sup>); the copepods *Tisbe biminiensis* (EC<sub>50</sub> of 0.038 CB.L<sup>-1</sup>) and *Nitokra* sp (EC<sub>50</sub> of 0.009 CB.L<sup>-1</sup>); pluteus stage larvae of the sea urchin *Echinometra lucunter* (EC<sub>50</sub> of 0.152 CB.L<sup>-1</sup>) and the sand dollar *Mellita quinquiesperforata* (EC<sub>50</sub> of 0.054 CB.L<sup>-1</sup>), and D-stage larvae of the mussel *Perna perna* (EC<sub>50</sub> of 0.005 CB.L<sup>-1</sup>). A hazard assessment was performed by means of Species Sensitivity Distributions (SSD), producing an HC5 (hazardous concentration that affects 5% of the species of a community) of 0.019 CB.L<sup>-1</sup>. This is proposed as a preliminary threshold for the toxic effects of CB leachates on marine biota for further evaluations. The results of this study contribute to the advancement of knowledge about the contamination, toxicity, and ecological risks of cigarette residues in coastal environments.

## 1A.O-Mo-2

# PAH Contamination in Sediments and Biota Following the 2019/2020 Brazilian Oil Spill: Implications for Human Health and the Environment

Mariana de Freitas, Tatiane Combi, Ana Cecília Albergaria-Barbosa  
Federal University of Bahia, Brazil

### Abstract

Petroleum is an essential element of modern society, serving as a primary energy source and used in numerous products, including plastics, tires, and asphalt. It consists of various hydrocarbon groups, including polycyclic aromatic hydrocarbons (PAHs), which can be hazardous to humans and the environment, potentially causing endocrine disorders, neurological problems, and cancer. Oil spills can be a source of PAHs in the environment, and when they occur, they can have environmental, social, and economic effects, as seen in previous incidents (e.g., Spain, South Korea, and USA). In late 2019, oil from an unknown source began appearing along several locations on Brazil's NE coast, with tons of oil continuing to appear on beaches, estuaries, and mangroves until the beginning of 2020. The goal of this study is to determine the bioaccumulation of PAHs from sediment to biota in a mangrove in the south of Bahia state, near the traditional fishing community of Belmonte. This study will also assess the health risks related to the consumption of the marine bivalves *Mytella guyanense*, *Crassostrea rhizophorae*, and *Anomalocardia flexuosa*. The samples were collected in February 2021 and freeze-dried before chemical analysis. The extraction was performed with ultrasound for sediments and with Accelerated Sample Extraction (ASE) for biota. The 16 priority PAHs were analyzed by gas chromatography coupled to a triple quadrupole mass spectrometer (GC-MS/MS). The average sediment PAH levels were  $174.74 \pm 8.70$  ng g<sup>-1</sup>, which is lower than those found in previous studies following oil spills in Spain after the Prestige in 2002 and South Korea after the Hebei Spirit in 2007. The  $\Sigma$  16 priority PAHs in biota were 515.24 ng g<sup>-1</sup> for the *M. guyanense*, 176.17 ng g<sup>-1</sup> for *C. rhizophorae*, and 229.29 ng g<sup>-1</sup> for the *A. flexuosa*. These organisms live directly associated with mangrove sediments or roots, presenting a low metabolism which can result in accumulation in their soft tissues. The bioaccumulation factor, carcinogenic potential, daily dietary intake, and incremental lifetime cancer risk were calculated to assess the potential risks of consuming these bivalves, which are commonly used in regional cuisine. All samples showed medium to high risk of cancer development over the years. To comprehend the potential long-term risks and impacts of the oil spill, as well as the susceptibility of the local population, continued monitoring of the sediments and bivalves in the region is necessary. This work was funded by the Programa de Recursos Humanos em Petróleo e Meio Ambiente da Universidade Federal da Bahia (PEMA/UFBA) - PRH36.

## 1A.O-Mo-3

### Comparing Toxicological Data of Three Stingless Bee Species With Honeybees for Pesticide Risk Assessments

Helen Thompson<sup>1</sup>, Gustavo Santos<sup>2</sup>, Ana Cione<sup>2</sup>

<sup>1</sup>Syngenta, United Kingdom. <sup>2</sup>Syngenta Proteção de Cultivos, Brazil

#### Abstract

The study conducted by Lourencetti et al. in 2023 published in Environmental Pollution, presents an analysis of the acute oral toxicity of thiamethoxam to stingless bees, comparing it with published data for honeybees (*Apis mellifera*). The authors concluded that *A. mellifera* is less sensitive to thiamethoxam than stingless bees and, therefore, not representative in bee risk assessment. However, the derivation of the acute oral toxicity of thiamethoxam to *A. mellifera* by Lourencetti et al. has significant errors that raise critical concerns about the conclusions of the paper. Lourencetti et al. calculated the acute oral toxicity of thiamethoxam to *A. mellifera* using a concentration from one study and a mean consumption value of untreated sucrose from a different study, rather than measuring consumption directly, which resulted in an incorrect value. Furthermore, the bodyweight of honeybees used by Lourencetti et al. was significantly lower than the widely accepted values. There are numerous publications on the acute oral toxicity of thiamethoxam to *A. mellifera* that follow the OECD guideline cited by Lourencetti et al. These studies identify the acute oral LD50 of thiamethoxam to *A. mellifera* as 5 ng/bee, which is 43-fold lower than the value used by Lourencetti et al. By replacing the values used by Lourencetti et al. with the correct values, a very different conclusion is drawn about the relative sensitivity of the species. The sensitivity of *A. mellifera* is similar to that of *Melipona scutellaris* and 6 to 8-fold greater than those of *Tetragonisca angustula* and *Scaptotrigona postica*. Risk assessment of the toxicity of thiamethoxam also depends on the exposure level of the bees. Using the daily consumption data to indicate relative exposure suggests nectar consumption is lower for the three species of stingless bees than for *A. mellifera*. Risk assessments in Brazil are based on far greater assumed consumption by honeybees, and, therefore, are likely to be protective of species of similar or lower sensitivity to thiamethoxam consuming lower amounts of nectar. In conclusion, the errors in the cited acute oral LD50 for *A. mellifera* in the study by Lourencetti et al. mean that the subsequent discussion and conclusions on both sensitivity and risk assessment are flawed.



## 1A.O-Mo-4

# Developing Regulatory Risk Assessment Schemes for Birds and Mammals for Agrochemicals in Latin America: Importance of Country Agronomic Practice

Juan Pascual<sup>1</sup>, Leticia Scopel Camargo Carniel<sup>2</sup>, Ximena Patino<sup>3</sup>, Joachim Nopper<sup>1</sup>

<sup>1</sup>BASF SE, Germany. <sup>2</sup>BASF S.A., Brazil. <sup>3</sup>Bayer S.A., Colombia

### Abstract

As described in the joint platform presentation submitted by Pascual et al., the EFSA approach as a whole is very much targeted on the conditions of the European Union (EU) territory. Many of such conditions are largely different in LATAM countries and therefore, any adoption of such scheme in LATAM should first evaluate and consider key differences. The agronomic conditions are not the same in all LATAM countries due to the large differences across this large continent. Our presentation will focus on Brazil, with a rich, diverse, and very important agriculture. There are major parameters that differ between the Brazilian and EU agricultural landscapes and conditions: field size and landscape structure (presence of natural habitats), crop diversity and crop cycles, pests and diseases pressure (agrochemical use) and agrochemical application methods. Brazil has a large surface area of truly natural ecosystems with very rich fauna diversity, some interspaced with agroecosystems. In the EU, such scenario does not exist as the agroecosystem landscapes are fully man-made and bird and mammal much less diverse. The size of the fields in Brazil are extremely large in some cases (thousands of hectares) while in the EU are much smaller. This has an important implication on the in-crop/off-crop scenarios for the risk assessment (RA). Brazil has tropical or sub-tropical conditions while the EFSA approach is developed for a temperate climate and agriculture, which leads to major differences with Brazil's agriculture. Climate conditions and shorter crop cycles imply that the pest and diseases pressure in Brazil require in some cases a more frequent and higher use of agrochemicals than in Europe. The particularity of Brazilian agriculture entails application methods, like aerial applications, that are practically ruled out in Europe. In our platform presentation, we would provide data underpinning the differences between the EU and Brazilian agricultural landscape and outline ideas that could be considered when developing a regulatory risk assessment scheme for birds and mammals in Brazil or other LATAM countries.

## 1A.O-Mo-5

### Ecological Risk Assessment to Soil Organisms – How to Advance in a Tiered Approach?

Leticia Scopel Carniel<sup>1</sup>, Tiago Natal-da-Luz<sup>2,3</sup>, Osmar Klauberg-Filho<sup>4</sup>, Julia Niemeyer<sup>5</sup>, Frank Staab<sup>6</sup>, Jose Paulo Sousa<sup>3</sup>

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#### Abstract

The Ecological Risk Assessment (ERA) must be appropriately protective, consistent, practical, and cost-effective. To fulfil an ERA procedure considering effects of Plant Protection Products (PPPs) to non-target organisms, the tiered approach is usually used where the realism is increased, and conservatism reduced in a stepwise approach. For in-soil organisms, the European Food Safety Authority (EFSA, 2017) highlighted some open points in this context: between the lower tier (based on lab studies and conservative exposure as a worse case scenario) and the higher tier (realistic, complex, expensive field studies with heterogenous data) there is a need for additional intermediate tier options or even some refinements, e.g., mitigations that could embrace different agricultural practices – to fill the gap. Among the methods already proposed, we can find the species sensitivity distribution (SSD) curve as an option for the intermediate tier. In fact, this approach is currently used to refine ERA for aquatic organisms- however, for in-soil fauna, there are still open questions. Even though SSD methodology has many scientific advantages (transparency to stakeholders; versatility to choose percentiles and confidence limits), it is not easily applied to the edaphic fauna. It requires some fundamental work and more data (as indicated by many authors), to achieve a standardized and agreed approach (including statistics). Community tests, another methodology which is often mentioned, are still under development with remaining challenges to solve. Nevertheless, the intermediate tiers can be helpful to complement the ERA in soil and the current options are presented and summarized. In addition, other methods already mentioned in the literature that could be useful for discussion could be advancing in tests with natural soils and the multigeneration tests, already performed with *Collembola*. It is also fundamental to discuss the already available good agricultural practices, especially on tropical countries under the consideration of the high-level performance in agriculture (e.g., Brazil) – they are good allies in keeping soil healthy, meeting the protection goals criteria, and contributing for a sustainable and economical food production.

## 1B.O-Mo-1

### **Exploring Relationships between Environmental Risk Factors linked to socioeconomical activities and Human Health in a Suburban agricultural Population of Cochabamba, Bolivia**

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#### **Abstract**

Human health is mainly influenced by social and economic factors, geographical location and physical environment. In suburban communities of Cochabamba, Bolivia, living conditions are considered unhealthy (e.g. lack of basic services, inefficient waste management), increasing health vulnerability. As for other developing countries, farming in Bolivia is mostly carried out in a rudimentary way, leading to an increased risk of exposure to harmful chemicals. Understanding key factors that influence health in suburban areas communities is a necessity for public policies aimed to improve health quality of these populations. The current study analysed environmental exposure related to social characteristics and economic activity as risk factor for human health in La Maica, Cochabamba, Bolivia, a suburban community mostly dedicated to farming and cattle. The main data were collected through familial and individual surveys to the members of 237 families. The results confirm that the main economic activity of La Maica is farming. Local fumigation is a recurring activity in which children and adolescents are also involved. It appeared that the level of knowledge about the use and handling of pesticides is low; thus the community only use basic protection to pesticide exposure. On the other hand, there are no patterns that could aware of a public health problem neither in adults nor in children. Indeed, environment and health problems are not considered as major preoccupation for the families. Parents are unaware of their children's vaccination status, as well as children are unaware of their parents' health history. Since chronic exposure to environmental pollutants have cognitive consequences, the study focused on a smaller group considering this aspect in particular. Cognitive development was evaluated in 58 children from 4 to 7 years old using two tests for Spanish speakers (Peabody and Cumanin). The results of these tests reveal an association between low level of cognitive development and nonverbal neuropsychological maturity with different environmental risk parameters linked to the socioeconomic characteristics of families. Therefore, although major public health concerns cannot be evidenced in the community, the troubles in cognitive development observed in the children of the area are worrying considering that these troubles are not adequately diagnosed by the Bolivian public health system and can have an environmental origin.

## 1B.O-Mo-2

### Developing Regulatory Risk Assessment Schemes for Birds and Mammals for Agrochemicals in Latin America: Learnings From a Well-Established Scheme (EU Approach)

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#### Abstract

Regulatory authorities in several Latin American (LATAM) countries have been or are active on the revision or development of risk assessment (RA) schemes for birds and mammals for agrochemicals. Examples include the countries of the Andean Pact, Costa Rica and Brazil. Of the two well established RA schemes worldwide [(United States EPA and European Union (EU) Food Safety Agency, EFSA)], the EFSA approach was taken by Costa Rica and it seems to be the one that might be preferred by Brazil's IBAMA. The EU RA schemes (EFSA Guidance Documents of 2009 and 2023) are based on a sustained long-term effort (started in 2006) by experts from authorities, academia, industry and contract laboratories and underpinned by abundant scientific information and data, which are specific to Europe. While their tiered-approach scheme is sound, its implementation is based on data hungry requirements and refinement approaches targeted for the local species and specific agricultural conditions in Europe. For this SETAC conference, two closely related are proposed. In this one, we would outline elements of the EFSA approach that could be suitable for any worldwide RA scheme and others which are primarily EU-driven and should be fully adapted to the fauna and agronomic characteristics of each country. The second one (see separate abstract) would cover some of the differences in the agronomic practice and use of agrochemicals between the EU and some LATAM countries (mainly Brazil).

A fundamental principle of the EFSA approach is the definition of feeding guilds and different types of species ('indicator', 'generic' and 'focal' species) which, in the case of the EU, is based on the actual bird and mammal species in EU agroecosystems. The number of farmland bird and mammal species in EU countries is largely inferior to the numbers in tropical and sub-tropical LATAM countries, like Brazil (>1100 birds & >300 mammals, data from a recent IBAMA workshop in February 2023). Thus, the relevance of different species in LATAM countries should be substantially re-evaluated. Another crucial factor for the correct implementation of an EFSA-approach scheme is having adequate information on which species, and with which frequency, they use a particular crop at different growing stages. This proved to be a very time and effort consuming exercise in Europe. Other biological parameters influencing the scientific validity of the EFSA approach for LATAM will also be presented.

## 1B.O-Mo-3

### A Framework for Species Extrapolation from Standard Ecotoxicological Datasets

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#### Abstract

Environmental risk assessment is a procedure which has been implemented by many Latin American countries as a means to achieve their goal of protecting the local occurring biodiversity with all the species present in the country. In contrast toxicity data is in most cases only available for a few standard species, which can be tested in the laboratory and for which global testing guidelines are available by OECD.

Therefore, one of the main challenges when performing an environmental risk assessment is to clarify how protective these standard species are for the local ones. Differences can occur due to intrinsic sensitivity (sensitivity at target site), differences in toxicokinetics (uptake, distribution and elimination) and exposure due to different behaviour (e.g. feeding patterns).

Different methods to extrapolate from the standard species to focal species were evaluated and put into context for an overall extrapolation framework. Those includes empirical approaches like species-sensitivity distributions (SSDs), interspecies correlation estimation, critical body burden as well as mechanistic approaches like toxicokinetic-toxicodynamic modelling.

If we base our extrapolation efforts on the traditional approach of animal toxicity studies like an acute daphnia or bee test it needs to be considered that a given endpoint from these studies will always merge the intrinsic sensitivity and the toxicokinetics. Therefore, a comparison of those endpoints is highly uncertain and often not a straightforward exercise. In addition, there remains the constraint that the empirical relationship cannot be extrapolated outside the calibration domain. These fundamental limitations result in the conclusion that SSDs are only valid for a given species-compound combination, and adding data for more species may change the outcome of an SSD.

In contrast, mechanistic approaches like the critical body burden concept or the more sophisticated toxicokinetics-toxicodynamics modelling have the advantage to separate the toxicokinetics from the intrinsic sensitivity and will allow a much better basis for extrapolation.

In this presentation we will present the framework and demonstrate the advantages of mechanistic modelling for species extrapolation for effects on pesticides for aquatic invertebrates, bees and birds.

## 1B.O-Mo-4

### Ecological Risk Assessment of a Contaminated Area due to a Pipeline Leak

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#### Abstract

The Ecological Risk Assessment (ERA) emerges as a tool to assist decision-making for the management of contaminated sites around the world. The triad approach uses three lines of evidence (chemistry, ecotoxicological and ecological) for estimate the integrated risk. This work aimed to perform an ERA in a site contaminated by petroleum-derived substances, occasioned by a pipeline leak, as a study case under tropical conditions. Twenty-two sampling points (including a Reference Site) were evaluated for the terrestrial compartment; ten sampling points for groundwater and five for surface water and sediment, upstream and downstream of the affected stream. Lethality and avoidance tests with earthworms *Eisenia andrei* and lettuce germination (*Lactuca sativa*) were performed in phase 1 (screening). The aquatic compartment was evaluated with an immobility test of *Daphnia similis*. In phase 2, evaluations included reproduction tests with soil invertebrates of the species *Folsomia candida* and *Enchytraeus crypticus*, growth and biomass of *L. sativa* and of the arboreal species *Mimosa scabrella* (bracatinga); reproduction of *Ceriodaphnia dubia* with groundwater and sediments; and growth and biomass of *Lemna minor* with elutriates. As ecological evaluations in situ, the bait lamina test in soil and ecological evaluation of the benthic community in sediments were performed. All data were analyzed using the ANOVA followed by Dunnett's test ( $p < 0.05$ ), when the criteria of normality and homogeneity of variances were met; or using non-parametric statistics with the Kruskal-Wallis test followed by Dunn's test ( $p < 0.05$ ). Chemical analysis detected hydrocarbons in soil deep layers and groundwater, but not at soil surface (0-20 cm). Ecotoxicity tests showed significant reduction of invertebrate's reproduction and plant's growth in points corresponding to the hotspot of the study area, related to some metals. Aquatic invertebrates indicated impairment in groundwater quality. In the stream, ecotoxicity tests and the benthic community indicated impairment of the water quality including upstream points, indicating anthropic influence not related with the pipeline leak. This work proved the effectiveness of using the triad approach for decision making in a petroleum contaminated site, disentangling a complex study case.



## 1B.O-Mo-5

### Trace Elements Trigger Values for Cu and Zn in Subtropical Brazilian Soils

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#### Abstract

Despite the reports on the negative effects of trace element pollution on soil bioindicators, differences in pedological characteristics observed between different soils motivate new evaluations. As a large number of studies in the field of ecotoxicology focus mainly on soils from the northern hemisphere, despite the fact that Brazil contains within its geographical limits the largest portion of important terrestrial biomes, evaluations of the implications of contamination of Brazilian soils are required in order to serve as a basis for decision making regarding the adoption of environmental preservation policies and management of contaminated areas. In this sense, contamination tests with copper ( $\text{CuCl}_2$ ; at 0; 25; 50; 75; 150; 300 and 600  $\text{mg kg}^{-1}$ ) and zinc ( $\text{ZnCl}_2$ ; at 0; 25; 50; 75; 150; 300 and 600  $\text{mg kg}^{-1}$ ) were conducted on a subtropical soil (Red-Yellow Ultisol; collected in Lauro Müller, Santa Catarina State) with three species of potworms (*Enchytraeus crypticus*, *E. dudichi* and *E. bigeminus*) according to the procedures listed in ISO 16387. The results indicate that the current legislation is not at all effective, since the effect concentrations ( $\text{EC}_{50}$ ) were sometimes lower than the critical limits established in the federal standard (60  $\text{mg kg}^{-1}$  for copper and 300  $\text{mg kg}^{-1}$  for zinc). From this perspective, there is an urgent need for the reevaluation of the legal provisions, especially the Resolution n. 420 of the National Council of the Environment (CONAMA). Such updates are possible by conducting ecotoxicological tests with bioindicators of soil quality and will be discussed considering the natural concentrations of chemical elements in the soil.

## 1B.O-Mo-6

### Run-off Mitigation in Exposure Modelling of Plant Protection Products

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#### Abstract

Surface runoff is an important transport pathway of eroded sediment and dissolved and particle-bound chemicals from agricultural fields. This results in the loss of fertile topsoil material, irrigation water, nutrients, and plant protection products (PPP) into adjacent surface water bodies, wetlands, or terrestrial habitats. Effective in-field and edge-of-field mitigation measures such as conservation tillage, micro-dams, cover crops, and vegetated filter strips can significantly reduce these off-site movement processes.

This presentation will showcase latest model developments to predict the effectiveness for the reduction of pesticides due to edge-of-field measures by the mechanistic vegetated filter strip model VFSSMOD and in-field measures by modifying USDA run-off curve numbers (RCN) and MUSS (version of Modified Universal Soil Loss Equation) erosion parameters in the run-off model PRZM.

The latest version of VFSSMOD contains three new features, i.e., the dynamic estimation of the median particle diameter to predict the retention of sediment, a mechanistic mass balance equation to quantify pesticide trapping, and a new remobilization and leaching algorithm for residues trapped in the filter strip.

To quantify the effect of in-field measures (conservation tillage, micro-dams, cover crops) on the reduction of pesticides in run-off and erosion, this work demonstrates the derivation of RCN and the MUSS C-factor based on results from own field trials and comprehensive literature data with arable crops such as maize and potatoes.

The implementation of these methods into the regulatory risk assessment for PPPs enables to operationalize and quantify the impact of run-off mitigation measures by determining the effect on EECs (Expected Environmental Concentration in surface water) calculated with the PWC (pesticide in water calculator) model suite.

Finally, a brief outlook will be provided into the future of precision risk management at the scale of the individual field.

## 1C.O-Tu-1

### **Environmental Risk Assessment of Chemicals used in Aquaculture in Chile: Progress and Challenges for the Future**

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#### **Abstract**

The Environmental Risk Assessment procedures for the use of chemicals in aquaculture were enacted in 2020 by the maritime authority (DIRECTEMAR) in Chile. Given the new requirements, based on a three tiered approach, a more rigorous process was established, starting with a hazardous properties step (i.e. mutagenic, carcinogenic, endocrine disruptors etc.), followed by a Persistence, Bioaccumulation and Toxicity (PBT) Assessment and finally an Environmental Risk assessment with an exposure and effects assessment part, the use of multimedia mass balance models adapted to the aquaculture use of different type of chemicals in the productive activity, such as pesticides, disinfectants, antibiotics, etc. for the predicted environmental concentrations (PEC) estimated after the normal use of chemicals under a worst case environmental scenario. The effects assessment is made considering local marine or freshwater species for the risk quotient calculations under deterministic and probabilistic methodologies. In these years of application, several problems arise, among them, the issue of the lack of flexibility for those chemicals that surpass the Risk Quotient of 1 established by the authority and the absence of risk mitigation procedures to reduce the risks in the current chemicals application scenarios. On the other side, the industry have now a more clear picture of what chemicals could be allowed to be used in aquacultural activities, reducing environmental risks for non target species. In response to the industry/authority concerns we have been working in assessing different chemicals, by elaborating different modeling scenarios and strategies for assessing mixtures assessment, since under the current evaluation the analysis is performed on active principles for the PEC and the commercial formulation for the toxicity assessment. Some of the issues raised during this period, is the slow process of bioassays development for local species in different labs across the country, since some of the requested species to be tested are not available all year around, making delaying the process of delivering results and look for alternatives for testing. The need of different validated modeling approaches as well as the development of better mixtures assessment procedures, since a large amount of chemicals used are in the form of mixtures. This research was funded by ANID/FONDAP 15130015 and ANID Millennium Science Initiative Program ICN 2019\_015.

## 1C.O-Tu-2

### Ecological Risk Assessment of PFOS Contamination in Surface Waters From Sulfluramid-Treated Watersheds in Brazil

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#### Abstract

The use of sulfluramid as an insecticide in Brazil poses environmental risks as it may convert into the persistent and toxic perfluorooctanesulfonic acid (PFOS), a chemical listed in Annex B of the Stockholm Convention that can accumulate in the environment and threaten human and wildlife populations. This study evaluated the ecological risk associated with the presence of PFOS in surface waters of watersheds with intensive use of sulfluramid ant bait. To assess the exposure levels, surface water samples were sampled monthly for a year (October 2020 to September 2021) from eight watersheds containing planted forests using ant baits. A stainless-steel collector was used for the sampling, and the samples were stored in polypropylene bottles, refrigerated, and sent to the laboratory within 48 hours. Water samples were concentrated by solid phase extraction (SPE) and subsequent analysis by UPLC-ESI-QqQ-MS/MS. To assess the impact of PFOS in surface waters, the risk quotient (RQ) approach was employed. RQs were calculated by dividing the measured environmental concentrations (MECs) by the Annual Average Environmental Quality Standard (AA-EQS) proposed by the European Union for PFOS (0.65 ng L<sup>-1</sup>). For samples with PFOS concentration above the limit of detection (LOD) but below the limit of quantification (LOQ), the concentration was estimated using the equation  $(LOQ + LOD)/2$ . PFOS was detected in 85.3% of surface water samples, with the highest concentration observed in October 2020 and the lowest concentrations found between March 2021 to July 2021. The PF1 sampling point had the highest average concentration of PFOS at 8.2 ng L<sup>-1</sup>. The PF6 point had the lowest mean concentration at 1.5 ng L<sup>-1</sup>. In all the samples where PFOS was detected, risks to the preservation of aquatic life were observed. The samples collected in PF6 had the same RQ value (2.3) because the concentrations of PFOS were between LOD and LOQ. The highest RQs obtained were 77.8 (PF1), 20.5 (PF8), 19.2 (PF4), 18.9 (PF5), 18.5 (PF3), and 7.7 (PF2 and PF7). All the highest RQs were found in the samples collected in October 2020, except for sites PF6 (sample not analyzed in this campaign) and PF8 (highest RQ obtained in the sample collected in February 2021). All the lowest RQs found had a value of 2.3 because the concentrations of PFOS were between the LOD and the LOQ, except for the sampling site PF1, which had the lowest RQ of 3.4.

## 1C.O-Tu-3

### Methylmercury in Freshwater Ecosystems along Chile and Mapping Risk of Exposure from Fish Consumption in a Latin America Context

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#### Abstract

Methylmercury is bioaccumulated and biomagnified in the aquatic food web reaching concerning levels in predator fish. Mining activity is the most important economic activity in Chile and one of the most relevant in Latin America, but also a source of global anthropogenic mercury emission. Rivers in central-northern Chile have been historically exposed to mercury pollution from a vast range of anthropogenic (mining) and natural (orogenic processes, volcanic activity) processes. We sampled rainbow and/or brown trout in 8 river ecosystems from northern (18°S) to southern (54°S) Chile, extending across > 4,000 km. When possible, primary consumer invertebrates and sediments were also obtained. Methylmercury (MeHg) was quantified following U.S. EPA Method 1631 (2001), Acadia University. In addition,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotope ratios were estimated via Elementar VisION EA-IRMS, Universidad de Antofagasta. MeHg in fish muscle ranged 0.001-0.492  $\mu\text{g g}^{-1}$  ww. Trout from the rivers associated with mining in the north of the country had significantly higher MeHg concentrations, following by rivers from areas where mining transitioned to agriculture. Across sites, trout had significantly higher MeHg concentration than macroinvertebrates, and the trophic biomagnification factor (TMF) ranged from 1.53 to 3.01. Later, the risk of mercury exposure and consequently health hazard due to fish consumption in Chile and Latin America and the Caribbean countries were estimated. The 7.38% of the 171 fish species studied were found to have concentrations above the recommended ingestion of THg  $\geq 0.95 \mu\text{g g}^{-1}$  ww, independently of the water habitat. Furthermore, high risk values (HQ  $\geq 1$ ) were estimated in 13 out of the 18 countries studied, and even higher-risk values (HQ  $\geq 10$ ) were estimated in some fish species inhabiting watersheds in Trinidad and Peru. Finally, only one site in the north of Chile has HQ  $\geq 1$ , however 3 out of the 8 sampled sites had THg concentration in trout's to be consumed with restricted meals. Funded by FONDECYT 11180914

## 1C.O-Tu-4

# Risk Communication for Mitigation Actions and Human Health Protection in Contaminated Residential Areas with Tension Increase

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### Abstract

Risk communication is part of the management of a contaminated area and is prescribed in Brazil and in the State of São Paulo by Resolution Conama 420/2009 and by the State Law 13.577/2009, respectively. It is a process involving representatives of all social groups and requires the stakeholders to actively participate in a constructive way.

However, there is no description of strategies and procedures on how risk communication should be held. In this context, this article presents two case studies where communication and tools among all stakeholders were considered a key component for the implementation of risk mitigation actions as the pressure from the residents and regulatory agencies raised.

Both case studies relate to residential areas contaminated with chlorinated solvent vapors that migrated from the tetrachloroethene and trichloroethene plume in groundwater whose source came from the upstream industries that had used those substances in their process. The concentrations in the indoor air were above standards and required mitigation actions to protect the resident's health.

A Communication Strategy was conceived, mapping all the stakeholders - district attorney, environmental agency, health surveillance, municipality, the responsible party for the environmental liabilities, residents, consultants and subcontractors – and establishing information channels and a risk communication matrix.

The risk communication was performed using several communication channels and approaches: a) individual and small groups meetings, with the participation of governmental and non-governmental agencies; b) preparation and distribution of informative notes; c) toxicologist technical support; d) providing a contact number or an e-mail for residents to contact at any time with their questions and schedule work; e) the technical team in the field answered resident's technical questions with accessible language; and f) above all transparency, constant feedbacks and empathy.

Therefore, the risk communication process promoted in both case studies the cooperation between the stakeholders during the implementation of the vapor intrusion investigation and mitigation of the potential risks (applied engineering actions and subslab depressurization system), which were supported by the environmental agencies.

## 1C.O-Tu-5

### The RISK21 Framework: Applications in Risk-Based Evaluation and Risk Communication

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#### Abstract

The HESI Risk Assessment in the 21st Century (RISK21) project developed an integrated risk assessment framework that enhances efficiency and risk management. The project was initiated to develop a scientific, transparent, and efficient approach to risk assessment, relying on a problem formulation-based, exposure driven, tiered data acquisition. This RISK21 framework allows informed decisions on safety to be made when sufficient evidence is available, and it maximizes the ability to inform decisions and optimize resource usage. The program also developed a web-based tool that allows users to easily graph their risk assessment data and effectively communicate risk-based decisions, whether for a screening and prioritization purpose or a definitive risk assessment. This tool is freely available at [www.risk21.org](http://www.risk21.org).

The utility and uptake of this approach and the web-tool has been demonstrated via several hands-on case study workshops, led by RISK21 team members and hosted or sponsored by various groups, including government agencies. These workshops have engaged participants in real-world case examples across the world, including the US, Canada, China, Taiwan, Thailand, Brazil, Argentina, Panama, Colombia and Costa Rica,. Moreover, several RISK21 case studies were developed to show the applicability of the framework Risk21 tool to study the impact of pharmaceutical, and agrochemical exposure assessments to the environment. The approach and the associated web-tool are broadly applicable across disciplines for risk assessment, prioritization, communication, and outreach. This presentation will provide a demonstration of the utility of the tool with a focus on applications for ecological risk assessment.

## 1C.O-Tu-6

### Legacy and Novel Brominated Flame Retardants in Indoor Environments and Cars in Major Cities of Colombia: Exposure and Risk Characterization

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#### Abstract

Legacy and novel brominated flame retardants (N-BFRs) are a growing environmental and public health concern due to their ubiquity and toxicity. In particular, dust is a major exposure pathway, which can inadvertently lead to ingestion via hand-to-mouth activity. Our study aimed to characterize the exposure and risk to legacy and novel N-BFRs in office, house, and car dust from three major Colombian cities: Bogota, Medellin, and Cartagena. We collected a total of 44 dust samples from cars, houses, and offices using a household vacuum cleaner, which were then placed in solvent-rinsed aluminum foil, sealed in plastic zip lock bags, and stored at  $-20\text{ }^{\circ}\text{C}$ . Analysis of PBDEs and N-BFRs was performed using a gas chromatography coupled to a mass spectrometer. On average, the most abundant concentrations of BFRs detected were decabromodiphenyl ethane (DBDPE) in cars at  $3531 \pm 5376\text{ ng/g}$  and decabromodiphenyl ether (BDE-209) in offices at  $6808 \pm 10576\text{ ng/g}$ . BDE-28 had the lowest value detected below of LOQ ( $\leq 0.2\text{ ng/g}$ ) in cars and houses. Spearman correlation coefficient and Principal Component Analysis (PCA) were used to evaluate the relationship among the concentrations of BFRs and to identify the major factors explaining the variability in the dataset. The PCA showed that each microenvironment aligned with specific areas, with the samples from offices having the largest concentrations of PBDEs compared to cars and houses. PBDEs had similar concentrations in samples collected from cars and houses. Furthermore, the compounds DP<sub>s</sub> showed a decrease in anti-DP proportion to syn-DP in the office samples, while these compounds in cars and houses maintained similar profiles. BDE-209 was detected in 92% of samples from office environments, but in cars and houses, it had greater than 98% and 99%, respectively. Overall, the PBDE compounds detected in all samples of different microenvironments were BDE-99, BDE-183, and BDE-47. Our study provides valuable insights into the levels and distribution of legacy and novel BFRs in the Colombian environment and their potential health risks to humans. Our findings could inform policymakers and stakeholders in developing and implementing more efficient e-waste management strategies and safer consumer products.



## 1D.O-Tu-1

### Disproportional Health Risk of Trace Metals in Soil in the Houston Metropolitan Area

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#### Abstract

Urban surface soils are contaminated by heavy metals from various anthropogenic sources such as traffic and industrial facilities. Socio-economically disadvantaged communities are generally located closer to these sources and likely exposed more to heavy metals. This study was to investigate if there is a negative correlation between surface soil contamination by trace metals and economic status (i.e., average annual household income) of surrounding communities. Surface soils were collected from community parks (n=75) and playgrounds of elementary schools (n=6) located in 33 zip codes split into four groups with different economic status and industrial emissions. Trace metals (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, and Pb) were analyzed using ICP-MS and portable XRF, and cancer and noncancer risks were calculated using an USEPA model. Lead is responsible for more than 80% of noncancer risk in most sites. Hazardous index was significantly higher in low income neighborhoods close to industrial facilities and exceeded 1 in about 10% of the sites. Cancer risk of arsenic ranged from  $1.2 \times 10^{-6}$  to  $2.3 \times 10^{-5}$  ( $4.6 \pm 3.1 \times 10^{-6}$ ) without significant differences between the all 4 groups, indicating that arsenic is unlikely originated from anthropogenic sources. But, arsenic concentrations exceeded soil screening level in all sites and needs additional investigation for arsenic speciation to predict risk from arsenic more accurately. Health risk associated with organic pollutants such as polycyclic aromatic hydrocarbons is also expected to be higher in the economically disadvantaged communities close to industrial facilities, and the overall health risk of toxic chemicals in residential surface soils needs more attention to minimize adverse health effects especially children in these communities.

## 1D.O-Tu-2

### Ecological Risk Assessment of Pesticides in an Urban Tropical Aquatic Ecosystem

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#### Abstract

The Ecological Risk Assessment (ERA) is associated with threats arising from activities with potential of causing continuous or accidental impacts to the environment. The ERA tool was originally developed to assess soil contamination scenarios and more recently, it has been conducted to assess ecological risks associated to aquatic environments. The Jacarepaguá Lagoon (JPAL), one of the four lagoons in the Jacarepaguá lagoon complex in Rio de Janeiro city suffers from the discharge of untreated or poorly treated urban sewage from residential and commercial condominiums and slums built up in the drainage basin. In the present investigation, the Dutch Triad methodology for ERA was adapted to perform an ERA for JPAL based not on three, but on four Lines of Evidence (LoE). Surface water samples were collected during six bimonthly campaigns at five sampling points in JPAL. The additional LoE (Water Quality LoE) was based on conventional physical-chemical and biological parameters to calculate the Water Quality Index and subsequently estimate the Water Quality Risk. For the Chemical LoE the presence of 107 pesticides was investigated with gas chromatography coupled to mass spectrometry, to estimate the Chemical Risk Index. The Ecotoxicological LoE was based on chronic ecotoxicity tests with the microalgae *Chlorella vulgaris* and with the microcrustacean *Ceriodaphnia dubia*, to estimate the Ecotoxicological Risk Index. For the Ecological LoE, the richness and abundance of microalgae species in the lagoon were analyzed to estimate the Ecological Risk Index. The Environmental Risk Index was then, obtained after the integration of these four LoE risks indexes. Meanwhile the Water Quality Risk ( $0.68 \pm 0.06$ ), the Chemical Risk ( $0.61 \pm 0.21$ ) and the Ecological Risk ( $0.72 \pm 0.08$ ) were classified as high, the Ecotoxicological Risk ( $0.78 \pm 0.22$ ) was classified as very high. Finally, the Integrated Environmental Risk based on all LoE was considered very high ( $0.77 \pm 0.13$ ). The result of this assessment, strongly indicate the lagoon ecosystem is in an advanced stage of contamination and degradation, requiring urgent actions to mitigate the risks, such as improvement in the sewage collection and treatment, with interruption of clandestine discharge into the lagoon. Additionally, a detailed investigation is needed to elucidate the main sources and routes of the pesticides found in JPAL to prevent them from reaching this sensitive water body.

## 1D.O-Tu-3

### **Arsenic Concentration in Groundwater and Raw Bovine Milk in Dairy Farms From Córdoba, Argentina. Is There Any Risk to Human Health?**

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#### **Abstract**

Arsenic (As) is mainly a naturally toxic metalloid that can cause adverse effects on human health. Drinking water contaminated with As is the most important route of exposure and accumulation in organisms. Dairy cattle often suffer subclinical As toxicity, as the metalloid is retained in blood and milk. This study aimed to determine As concentration in groundwater and raw bovine milk, as well as the bioaccumulation factor (BAF), and risks it is likely to pose for human health. Seventeen dairy farms in the Pampean plain of Córdoba were analyzed during November 2021. Total As concentration in groundwater and bovine milk was determined by inductively coupled plasma-mass spectrometry. Potential human health risks, associated with As consumption, have been assessed by estimating the hazard quotient (HQ), target hazard quotient (THQ) and carcinogenic risk (CR) in children and adults. The BAF was used to evaluate the degree to which As from water can bioaccumulate in milk. Total As was quantified in 100% of the groundwater (at values ranging from 4.5 to 498.7 µg/L), and in 71% of the bovine milk samples (from 7.4 to 470.2 µg/g). The As levels in 94% and 53% of the groundwater samples (n=17) exceeded the limits established for human consumption (10 µg/L) and animal drinking (50 µg/L), respectively. While 71% of the bovine milk samples exceeded the internationally suggested concentrations (10 µg/kg). The As concentration in groundwater samples was more prevalent in the south-east (discharge area) of the Córdoba province. Therefore, As is controlled by lithology (fine sediments) and distance of the boreholes from the piedmont recharge area in the western Pampean mountain. The HQ mean values of the metalloid classified as “adverse effect” on human health for children and adults in groundwater (child= 25.9, adult= 3.6). The THQ mean values were negligible in milk (child= 1.0E-2, adult= 2.7E-3). The CR values classified as “unacceptable” to “need further evaluation” (child= 4.5E-4 – 5.0E-2, adult= 2.1E-4 – 2.0E-2 in groundwater; child= 1.3E-3 – 3.0E-2, adult= 2.5E-4 – 1.0E-2 in milk). Nevertheless, BAF was higher than 1 in 35% of the dairy farms, suggesting that dairy cows can incorporate As from abiotic matrices. The results presented indicate that the status of As contamination in the study area should continue to be monitored in order to assess its long-term impact on public health.

## 1D.O-Tu-4

### Interdisciplinary Working Methodology and Contribution to the Knowledge of Toxicology and Risk Analysis

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#### Abstract

The Institute for Scientific Cooperation in Environment and Health (ICCAS) is an area of transdisciplinary interaction based on a tripartite working model: academia, industry and government. It brings together specialists to address science- and technology-based issues in an integrated manner. Its mission is to promote joint learning, connect, cooperate and bring together leading scientists to exchange knowledge, opening the doors to innovation. The pillars of the institution are: scientific integrity, collaborative work and professional ethics. ICCAS is supported by volunteer professional who devote their time and economical resources from donors and other institutions, which provide funding or in-kind contributions. Multiple thematic areas such as research integrity, food safety, food residues and contaminants, good agricultural practices, water quality, emerging contaminants, medicine and environment, and evidence-based nutrition are addressed. In the Working Group on Risk Analysis and Toxicology (WG-ARyT), contribution is focused on the training of professionals in risk assessment, toxicology and epidemiology, residues in food, regulatory toxicology, and the use of the Risk21 tool, developed by the Health and Environmental Sciences Institute (HESI), for problem formulation and risk communication, stands out. In addition, science communication and publications are produced on water quality, environmental pollution, toxicology and risk assessment. In relation to water, several articles have been published for non-specialists on arsenic, impact and safety of phytosanitary products, emerging pollutants, microplastics, among others.

We are currently working on a collaborative project on pharmacontamination, focused on providing tools for diagnosis and proposing mitigation measures. In this presentation we would like to share our broad and independent approach and methodology used to exchange ideas and knowledge based on a rigorous analysis of scientific evidence, based on principles of scientific integrity

## 1D.O-Tu-5

### Estimating the Aquatic Risk from Exposure to up to Twenty-two Pesticide Active Ingredients in Waterways Discharging to the Great Barrier Reef

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#### Abstract

Pesticides decrease the quality of water reaching the Great Barrier Reef (GBR), Australia. Up to 86 pesticide active ingredients (PAIs) were monitored between 2015 to 2018 at 28 sites in waterways that discharge to the GBR. Twenty-two frequently detected PAIs were selected to calculate the risk when they co-occur in water samples. Species sensitivity distributions (SSDs) for the 22 PAIs to fresh and marine species were developed. The SSDs, the multi-substance potentially affected fraction method, Independent Action model of joint toxicity and a Multiple Imputation method were combined in a Pesticide Risk Metric that converted PAI concentration data to estimates of the Total Pesticide Risk (i.e., for the 22 PAIs) expressed as the average percentage of species affected during the wet season (i.e., 165 days). The Total Pesticide Risk ranged from < 1% to 42% of aquatic species being affected. Approximately 84% of the Total Pesticide Risk estimates were greater than 1% — meaning they did not meet the Reef 2050 Water Quality Improvement Plan's pesticide target for waters entering the GBR. There were marked spatial differences in Total Pesticide Risk values — regions dominated by grazing had lower values while those where sugar cane was a large land use had higher values. Nine PAIs (diuron, imidacloprid, metolachlor, atrazine, MCPA, imazapic, metsulfuron, triclopyr and ametryn) were responsible for more than 99% of Total Pesticide Risk across all the monitored waterways.

The above method only allows the estimation of pesticide risk for waterways with pesticide monitoring data. Therefore, we developed relationships that use land use, hydrological and spatial variables to predict the Total Pesticide Risk at sites without pesticide monitoring data. Relationships were developed to predict the risk posed by all 22 PAIs, PSII herbicide AIs, Other Herbicide AIs and Insecticide AIs. The relationships were able to explain 60 to 80% of the variation in pesticide risk for the four pesticide groups. The relationships were used to predict the risk for basins, regions and the entire area of land that drains to the GBR. While the relationships are specific to the GBR catchments the approach of developing pesticide mixture toxicity – land use relationships can be applied to waterways internationally.

## 1D.O-Tu-6

# Bioavailability-Based Environmental Risk Assessment Approaches for Nickel: Providing Ecological Protection for This Participant in the Renewable Energy Transition

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### Abstract

Nickel is increasingly acknowledged as playing a role in the green energy transition given its role as a cathode in batteries used to fuel electronic vehicles, in addition to its more traditional role as a component of sustainable corrosion-resistant stainless steel. The role that nickel will play has led to increased global demand. Recently, mining operations have commenced in South America, and notably in Brazil. Ecosystem protection involves a wide range of tools, including the derivation of threshold concentrations that can be used as the basis for emission limits and in various stages of environmental risk assessment, e.g., the establishment of clean-up goals. This is challenging for naturally occurring substances like nickel, as threshold concentrations need to be at once protective of ecosystem structure and function while avoiding situations where standards are set at concentrations occurring below those that occur naturally. One methodology that has received global attention is *bioavailability-based approaches*. Nickel toxicity to organisms is influenced by the naturally-occurring constituents of different environmental matrices. For example, nickel toxicity to aquatic organisms is determined by pH, water hardness, and dissolved organic carbon concentrations, such that effects of nickel can vary considerably from one system to another. We have worked with regulatory authorities around the world to develop bioavailability-based approaches for nickel that have been demonstrated to be equally protective for temperate and tropical ecosystems. The basis for these approaches is an extremely diverse ecotoxicity database and a bioavailability normalization process that can yield local- and regional-level thresholds that are adaptable to the desired level of ecosystem protection. We will present examples showing the degree to which nickel toxicity can vary among different South American ecosystems, and suggest approaches that can be implemented to provide cost-effective ecological protection.

# Session 2: Research and Management Approaches to Achieve a Balance Between Food Production and Nature Conservation – How to Conserve Biodiversity in an Agricultural Context?

## 2A.O-Tu-1

### Environment Assessment of Mezcal Supply-Chain in Oaxaca

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#### Abstract

Agricultural production and its subsequent transformation into food and beverages have a significant impact on the environment. Mezcal, a Mexican distilled beverage, is predominantly produced in Oaxaca, southern Mexico, with an annual production of 6.5 million liters. Over the last 10 years, production has increased by 700% due to high demand, causing an expansion in the agricultural frontier for agave planting and extraction, promoting the monoculture of the espadin maguey (*Agave angustifolia* Haw), and increased use of resources such as water and firewood. Therefore, it is essential to assess the ecological impact of the production chain. The aim of this study was to analyze the environmental effects generated by the agave-mezcal production chain using the Life Cycle Assessment methodology to calculate potential environmental impact. The functional unit considered is 1 liter of young white mezcal made from agave espadin. Results were obtained for three environmental indicators: global warming potential (GWP). The results indicate that the Mezcal agro-industrial system in Oaxaca contributes to GWP, mainly due to CO<sub>2</sub> emissions caused by the transport of inputs such as agave, firewood, bottles, and the use of fuel in agricultural machinery. The net result is 1.48 kg CO<sub>2</sub> eq/liter of mezcal. The occupation of land for the cultivation of mezcal is 2 m<sup>2</sup>/liter. The expansion of the agricultural frontier for agave cultivation and extraction, along with increased use of resources. It was identified that large-scale monoculture cultivation of agave can result in the elimination of natural habitats and a reduction in biodiversity. Additionally, the intensive use of pesticides and herbicides can be toxic to wildlife and their habitats. Furthermore, monoculture farming can lead to soil depletion and degradation, resulting in reduced soil quality, erosion, and loss of nutrients. Unlike a diverse range of plants, a single crop species cannot effectively retain nutrients in the soil, further exacerbating the environmental impact. These findings underscore the importance of adopting sustainable production practices. The results of this study can inform efforts to develop alternative approaches that reduce the environmental impact of mezcal production.

## 2A.O-Tu-2

### **A Pesticide Decision Support Tool to Guide the Selection of Less Environmentally Harmful Pesticides for the Sugar Cane Industry**

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#### **Abstract**

Pesticides applied to agricultural land have been shown to decrease the quality of water entering the Great Barrier Reef lagoon, in eastern Australia. This issue is addressed by the Reef 2050 Water Quality Improvement Plan which includes a pesticide reduction target. As part of a wider educational strategy, one method that could help meet the target is to provide stakeholders with information that assists in the selection and use of pesticide active ingredients (PAIs) that pose a lower risk to aquatic environments compared to those currently used. This study developed a Pesticide Decision Support Tool (PDST) in collaboration with stakeholders for the sugar cane industry. The PDST covers the active ingredients of all PAIs registered and applied to sugar cane in Australia and four additional PAIs registered for use on crops grown in rotation with sugar cane. The PDST uses the toxicity, persistence and mobility of the PAIs to determine their potential aquatic risk and as such can be applied internationally. An interactive spreadsheet allows characteristics, including application rate and tank mixes, to be considered when assessing the potential risk.



## 2A.O-Tu-3

### Toxicity of Ametrine and Carbendazim on histopathological endpoints in the *Danio rerio* (Zebrafish) experimental model

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#### Abstract

Currently, thousands of different commercial products are being used as pesticides, among them the fungicide Carbendazim and the herbicide Ametrine stand out, due to their high environmental risk. The Zebrafish (*D. Rerio*), a fish native of South Asia, is one of the most important species as a vertebrate model in studies of genetics, developmental biology, neurophysiology, ecotoxicology and biomedicine. Histological endpoints are considered great biomarkers, as they allow the rapid identification of changes in different tissues and organs. Among the organs usually used for such analyzes, the gills and the liver can be highlighted, due to their direct contact with the environment, in the case of the gills, and the detoxification of the organism, in the case of the liver. This work aimed to evaluate the histopathological effects in these two organs after exposure to a range of environmentally relevant concentrations of the pesticides Ametrine and Fipronil in the Zebrafish experimental model. For this, acute exposure (96h) was performed with a partial change of water after 48h of exposure. For each substance, 5 concentrations were used, being 2; 20; 200; 1000; 2000  $\mu\text{g/L}$  for Ametrine and 5, 10, 50, 150 and 200  $\mu\text{g/L}$  for Carbendazim. After exposure, the organs were collected, processed in paraffin and submitted to Hematoxylin and Eosin reaction, for analysis aiming at obtaining the histopathological alteration index. This index was calculated taking into account the changes classified into five basic classes (circulatory disorders, regressive changes, progressive changes, inflammatory processes and neoplasms), both for the gills and for the liver. No neoplastic changes were observed for any of the pesticides studied. Ametrine showed a predominance of regressive alterations for both tissues with observation of foci of inflammatory infiltrates in the liver. Carbendazim showed a higher frequency of regressive and progressive alterations in the gills and regressive in the liver. These data will be used as a basis for calculating environmentally relevant mixtures, the focus of the next stages of this project. Grant: 2022/03094-5, São Paulo Research Foundation (FAPESP)

## 2A.O-Tu-4

### Phytotoxic and Tick-Killing Activity of Colombian Essential Oils

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#### Abstract

Pest control in agriculture is generally carried out through the application of synthetic insecticides that cause deterioration of health and the environment. Essential oils (EOs) extracted from plants are seen as alternatives to synthetic pesticides.

In this work, essential oils from plants of the Piperaceae, Apiaceae and Rutaceae families were isolated by hydrodistillation and their volatile chemical composition was determined by gas chromatography with a mass spectrometry detector (GC-MS). The phytotoxic activity was evaluated using Stock solutions, in ethanol, of A.E. from an initial concentration of 10  $\mu\text{g}/\mu\text{L}$ , and tested on seeds of *Lolium perenne* and *Lactuca sativa*, in which the germination inhibition capacity was determined in a period of 7 days. The insecticidal activity was evaluated using Stock solutions, in acetone, of the EOs from a curve with an initial concentration of 20  $\mu\text{g}/\mu\text{L}$  up to 1.25  $\mu\text{g}/\mu\text{L}$ , and tested on *Hyalomma lusitanicum* larvae.

From these results it is concluded that the EOs of *P. coruscans*, *P. ottoniaefolium*, *P. reticulatum*, *E. foetidum*, and *T. trifolia*, do not present phytotoxic activity to the tested species, since they do not exceed 50% in inhibition of the Seed germination, on the other hand, have a high insecticidal activity, since low concentrations are required for the mortality of 50% of the evaluated *H. lusitanicum* population.

In conclusion, the essential oils evaluated can be considered as potential biopesticides for future integrated pest management programs.

## 2A.O-Tu-5

### **Relative Impact of Extensive Agriculture and Horticulture to Sediment Quality: Ecotoxicological Bioassays with the Latin-American Amphipod *Hyaella curvispina***

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#### **Abstract**

Argentina's current agricultural production model is based on the use of agrochemicals, such as fertilizers and mainly pesticides. Extensive agriculture and horticulture are the two major productive activities that use up pesticide in large quantities. While extensive agriculture is characterized by areas of 50 to 100 ha of a single crop, horticulture is characterized by intense use of the land, with farms ranging from 1 to 3 ha, being cultivated with multiple crops. Despite pesticide relevance, data on the effects on the biota associated to nearby watercourses is scarce. Given this paucity, the objective is to assess the relative impact of extensive agriculture and horticulture to sediment quality on representative watercourses by conducting whole-sediment toxicity bioassays with the native amphipod *Hyaella curvispina*. The lower Gualeguay basin, in the Province of Entre Ríos, was selected as a representative watercourse impacted by extensive agriculture, and the Carnaval creek, in the periphery of La Plata, Province of Buenos Aires, as a horticulture-impacted watercourse. Survival in all the campaigns and sites of the Gualeguay basin was, on average, greater than 90%, in some instances reaching the survival of all test organisms. Growth inhibition for this system was around 10% to 20%, with higher percentages in the spring-summer campaigns, these being the ones that presented statistically significant differences with respect to the control. Mortality was largely observed in the horticulture-impacted Carnaval Creek, with no organisms surviving some of the sediments. In those sediments where survival was >50%, growth inhibition reached values of up to 40% and generally presented statistically significant differences. Concentrations of cypermethrin, chlorpyrifos and  $\lambda$ -cyhalothrin were above effects-based sediment benchmarks. Glyphosate and AMPA were the most frequently detected pesticides in both production systems, but no mortality was recorded in sediments where no other pesticide was detected. Observed effects were correlated with pesticide concentrations by multivariate analysis, revealing toxicity was mainly due to insecticides, principally to  $\lambda$ -cyhalothrin. According to our results, both productive systems negatively impacted the associated waterbodies, with the sediments from the horticulture system being the ones that produced a greater frequency of acute ecotoxicological effects.

## 2A.O-Tu-6

### Ecotoxicological Footprint of Rice Rotations With Different Agricultural Intensity

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#### Abstract

In the framework of Sustainable Development Goal 2 - creating a world free of hunger by 2030 - is one of the most significant technological demands. The development of sustainable agriculture intensification (SI) has emerged as a paradigm for improving agricultural sustainability and productivity to enhance food production with reduced environmental footprints on existing farmland.

In this context, rice accounting for 21% of global calorie intake while using 11% of global cropland. Uruguay is an important exporter, whose intensification strategies and production system have reported one of the best environmental performances at farms in using nutrients, carbon footprint, and energy balance. In this framework, the National Institute of Agriculture Research of Uruguay holds a 7-years long-term experiment (LTE) that represents different scenarios of rice cropping systems for the characterization of the agronomic and environmental performance of each one. Since a Life Cycle Assessment (LCA) view the environmental impact assessment requires a standardized way to allow the comparison between alternative technology of intensification.

According to mentioned above, this work determined the changes in the ecotoxicological footprint of six rice-based cropping systems - that belong to LTE - with different intensification levels that were estimated for rice in rotation with long-term pastures, with short-term pastures, with soybean crop, and continuous rice. The ecotoxicological footprint was estimated using USEtox database, and Toxicological Units, using the University of Hertfordshire's pesticide database, for the impacts on soil and freshwater ecosystems. The functional unit used was the energy equivalent embodied in the harvest crops.

The results allow for hierarchizing pesticides according to its respective impacts in each cropping system and comparing rotations and crops. This assessment will allow the prioritization of pesticides whose use must be replaced or eliminated to reduce the overall impact.

## 2B.O-Tu-1

### Reduction of Glyphosate Loads in Soybean Production Systems Using Service Crops and Roller Crimping

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#### Abstract

Currently, weed resistance to herbicides is a critical issue negatively affecting agricultural production worldwide. In general, management of herbicide-resistant (HR) weeds has meant an increase in the use of chemical products which, at the same time, has risen environmental concerns. In this context, service crops (SC) and roller crimping, as a desiccation method, are promising alternative tools for managing HR weeds that would allow to reduce the use of chemical herbicides and contribute to developing more sustainable agricultural systems.

One experiment replicated in three different agricultural locations in Uruguay conducted to evaluate: i) the weed control achieved with different planting densities (0; 6.25; 12.5; 25; 50, and 100 kg ha<sup>-1</sup>) of black oats as a SC, and ii) the desiccation of the SC using a roller crimper, and its effect on the weed infestation and yield of the subsequent soybean crop. The highest densities of the SC (50, and 100 kg ha<sup>-1</sup>) oats covered the soil more quickly although, this did not translate into a decrease in the initial weed germination infesting the SC. However, weeds' biomass was reduced by at least 85% at the time of the desiccation of the SC at sowing densities equal or above 25 kg ha<sup>-1</sup>. The suppression of weeds at these densities was maintained even during the soybean crop's initial development, decreasing weeds' germination of summer weeds. The roller crimping of the SC did not affect the population or the yield of the soybean crop. These results indicate that weed interference and commonly used amount of herbicides can be reduced by the effect of a competitive SC in combination with the roller crimper for its desiccation.

## 2B.O-Tu-2

### Dust Abraded From Thiamethoxam-treated Seed During Sowing: Refining the Risk Assessment for Native Bees in Brazil

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#### Abstract

During sowing using pneumatic machinery, dust may be abraded from pesticide-treated seed and contaminate adjacent bee-attractive off-crop areas. This study quantified the risk to native bees of dust released during sowing of Brazilian crop seeds treated with a thiamethoxam formulation (Cruiser 350FS). To address toxicity to native bees, adult acute contact LD<sub>50</sub> data for thiamethoxam were collated from the literature, a species sensitivity distribution generated, and the HD<sub>5</sub> calculated.

The LD<sub>50</sub> HD<sub>5</sub> was used to refine the default safety factor applied to the honeybee acute contact LD<sub>50</sub> from 10 to 5.45 for thiamethoxam. Crop-specific abraded dust data (Heubach dust and Heubach AI) were generated for seeds treated with Cruiser 350FS sourced from on-farm and industrial facilities. The mean Heubach dust levels was ranked as cotton = maize > sunflower = soybean > drybean. There was no correlation between the measured residues of thiamethoxam (Heubach AI) and those estimated in dust based on the thiamethoxam content of Cruiser 350FS. A hazard quotient (HQ) for each crop (based on application rate, the default dust deposition factor, and the honeybee contact LD<sub>50</sub>/10) identified risks during sowing for all crops. Refinement of the application rate with the measured 90th percentile Heubach dust (assuming 100% thiamethoxam) resulted in sowing of industrially treated soybean and on-farm treated cotton being identified as risks. Further refinement using either the measured 90th percentile Heubach AI or the acute contact LD<sub>50</sub> (HD<sub>5</sub>) resulted in sowing of all crops treated with Cruiser 350FS as being identified as low risk. Similar high quality seed treatment should be demonstrated for other formulations containing insecticides with high toxicity to bees. Data on dust drift from machinery and crops more representative of those in Brazil may allow further refinement of the default dust deposition value of 17% used in this study.

## 2B.O-Tu-3

### Alterations in Cartilage Growth in Swines (*Sus scrofa domesticus*) Exposed Orally to the Fungicide Captan

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#### Abstract

Brazil is one of the world's largest consumers of pesticides, which directly contributes to the increase in agricultural productivity, but which also leads to environmental contamination and the manifestation of intoxication in humans and production animals. In the state of Mato Grosso there are records of sick animals that were exposed to pesticides, including Captan, and that presented several clinical-pathological symptoms involving the digestive, neurological, and musculoskeletal systems, and whose causes are still not fully understood. Thus, both environmental damage and intoxication caused by pesticides demonstrate the urgent need to expand knowledge about the mechanisms of action and the harmful effects of these toxicants on different animal species. Pigs (*Sus scrofa domesticus*) are one of the most important species economically and as a source of food, which is also considered an excellent translational model for toxicological evaluation of chemicals. In this context, our objective was to evaluate the subchronic toxic effects of the fungicide Captan on the growth of the long bones (femur and tibia) of the pelvic limbs of orally exposed pigs. No statistical differences were observed in relation to the macroscopic damage between the control and groups exposed to Captan. However, at the same doses, several foci of fibrosis, chondrocyte necrosis and tissue disorganization in the cartilage growth plate were observed. The replacement of cartilaginous tissue by fibrous tissue produces catastrophic effects for the development of the growth plate. These events demonstrate the risks arising from exposure to this fungicide for the normal development of long bones in pigs, which serves as an alert for the need to control the indiscriminate use of this fungicide.

## 2B.O-Tu-4

### Influence of Systemic vs. Non-Systemic Pesticide Applications on Contamination of Nectar

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#### Abstract

Exposure to pesticides is one potential factor contributing to the loss of pollinators and pollinator diversity observed over the recent past. Pollinators may be exposed to pesticides by ingestion or contact when collecting nectar and/or pollen from contaminated flowers. Given that pesticides are necessary tools for economically viable ornamental plant production at large scales, pest management practices should be optimized to reduce exposures of pollinators as much as possible, while simultaneously providing adequate control of pests. Before practices can be optimized, an understanding of the influence of pesticide application and cultural practices on contamination of floral resources is needed. Few studies have specifically focused on the relationship between pesticide management during ornamental plant production and contamination of nectar. This project evaluated the influence of pesticide application with the systemic insecticide thiamethoxam (Flagship), and the non-systemic fungicides boscalid and pyraclostrobin (Pageant) on contamination of nectar in *Salvia* x 'Indigo Spires'. Applications were made at the highest labeled rates for the commercially available products, comparing the influence of application method (drench vs. spray), and timing (relative to flowering) within each pesticide. Nectar was sampled using 50  $\mu$ L microcapillary tubes and analyzed by LC-MS/MS. Results indicate that concentrations of the systemic insecticide thiamethoxam and its metabolite clothianidin in nectar exceeded published toxicity thresholds for native bees and/or honeybees, with the highest concentrations occurring with drench applications regardless of application timing. Although thiamethoxam/clothianidin concentrations from the spray applications were much lower, they also exceeded published toxicity thresholds in all cases. In contrast, concentrations of boscalid and pyraclostrobin (non-systemics) in nectar were far below published toxicity thresholds for honeybees. While much research is needed to develop a comprehensive pollinator-friendly best management practices guide, this study provides insight into how pesticide applications with systemic vs. non-systemic pesticides may influence contamination of nectar in ornamental plant species. It also provides insight into the potential risks for pollinator species associated with these uses.



## 2B.O-Tu-5

### **Sinergistic Effects on Zebrafish Gill Biomarkers After Pesticides Mixtures Exposition**

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#### **Abstract**

The occurrence of runoff contaminated with pesticides in tropical ecosystems after field applications leads to the toxicity of these compounds to non-target organisms. In addition, contamination from aerial spraying in agricultural fields close to water bodies is also significant and can affect the aquatic ecosystems. Regarded that, the objective of this study was to evaluate the effects of the Kraft 36EC® and Score 250EC® pesticides considering the runoff and the spray drift situation. Isolated and mixtures experiments were conducted using the adults of *Danio rerio* fish. After acute exposure (96h) in mesocosms (ecosystem models), biomarkers of aquatic contamination were analyzed on the fish gills. From the results obtained, a little alteration was verified in the enzymatic activities when evaluated separately, highlighting only the inhibition of the antioxidant enzymes catalase and glutathione reductase and the occurrence of oxidative stress (MDA levels) in organisms exposed to Kraft 36EC® spray drift. From the analysis of IBR (Integrated Biomarker Response), it was observed that the exposure to Kraft 36EC® spray drift in the field promotes the greatest deleterious effects on the metabolism of *Danio rerio*, followed by exposures to runoff contaminated with Kraft 36EC® and Score 250EC®. Differences in the pattern of biomarker responses between the experiments carried out in mesocosms and the laboratory are evident due to the influence of the concentrations used, the ecological interactions between the communities present in the environment, and the environmental variables in the experiments in mesocosms. Funding by FAPESP (Process 2014/14621-0).

## 2B.O-Tu-6

### **Pesticide Residues on Brazilian Agricultural Scenario: a Survey to Validate Environmental Risk Assessment of Birds and Mammals**

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#### **Abstract**

Environmental risk assessment (ERA) is the process that evaluates the probability that an adverse ecological effect may occur, or is occurring, as a result of exposure of non-target organisms to one or more stressors, such as pesticides. The presence of birds and mammals in agroecosystems and their surroundings makes them potentially exposed to the use of pesticides in the field, with the ingestion of residues in food items supplied by the crops being the main route of contamination. To estimate the amount of residues in avian and mammalian food items, focus of this study, current ERA approaches use databases of residue concentrations on agricultural crops normalized to a standard application rate to obtain the “residue unit dose” (RUD). In Brazil, the ERA of birds and mammals is conducted with the same RUD data as in the U.S. Environmental Protection Agency (US-EPA), based on Kenaga’s nomogram. However, these data were obtained under specific conditions and may not be appropriate to the environmental conditions in Brazil, considering the differences in substances and usage of pesticides in each country. Therefore, the aim of the present study was to carry out a survey of recommended use/doses and maximum residue limits (MRLs) of all chemical pesticides registered in Brazil, in order to analyze whether RUD values used by the US-EPA represent the local conditions of use. From the collected data of 270 active substances (a.s.), it was possible to calculate the RUD for 137 crops. Maximum RUD ranged from 0.05 mg/kg for ryegrass (in one a.s.) to 250 mg/kg for pasture (in 18 a.s.). Except for the “broadleaf forage” plant category, we found higher maximum RUDs in all other groups used by the US-EPA. Rice, in 79 active substances, presented a maximum RUD of 200 mg/kg, approximately two times higher than the nomogram predictions. In fruit category, crops such as grapes and cashew presented a maximum RUD of 33.33 mg/kg (in 77 and 25 active substances, respectively), exceeding about 2.5 times the US-EPA values. Even though the best-case scenario is to calculate RUD from specific studies for ERA in birds and mammals, our survey indicates that RUD values for several crops in Brazil are not covered by RUD data used in the US-EPA. Thus, more effort should be put in to define better criteria for risk assessment of non-target organisms in Brazil, taking into account the substances authorized for use, as well as the agricultural activity and biodiversity of our land.

# Session 3: Advances in Genotoxicity Biomarkers Application in Latin America

## 3.O-Tu-1

### Pesticides Exposure: How to Evaluate the DNA Damage?

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#### Abstract

The exposure to pesticides could cause several human health effects. While the acute exposure could lead to episodes of acute intoxication, the chronic exposure is associated to delayed health effects such as reproductive, metabolic, and respiratory disorders, neurotoxicity, and carcinogenicity. Many of these effects could be associated to DNA damage caused by pesticides exposure. Thus, the use of several DNA damage markers is an important tool to monitoring the pesticides exposure effects. Hence, the aim of this study is to evaluate genomic instability, biomarkers in rural workers living in the State of São Paulo, Brazil. The study population included 81 pesticide-exposed farm workers (69 males and 12 females) with a mean age of  $49.16 \pm 10.06$  years and a mean time job of  $=30.00 \pm 14.00$  years, and 81 non-exposed individuals (62 males and 15 females) with a mean age of  $47.87 \pm 10.66$  years. Results of buccal micronucleus cytome assay showed significantly higher levels of cell damage (micronuclei and binucleated cells) and cell death (karyorrhectic and condensed chromatin cells) in subjects exposed to pesticide when compared to those non-exposed ( $p < 0.05$ ). The exposed group demonstrated a reduction in mitochondrial genome copy number when compared to non-exposed individuals ( $p < 0.01$ ). On the other hand, telomere length and global DNA methylation did not show significant differences among exposed and non-exposed groups. Each biomarker also presented influences of different characteristics, such as age, specific pesticide exposure. Thus, our findings demonstrated different genomic instability results due to pesticides exposure, depending on the biomarkers used.

### 3.O-Tu-2

## Genotoxicity in Field Studies: What Do We Know Today About DNA Damage in Fish and Aquatic Macrophytes From Contaminated Sites?

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### Abstract

Aquatic ecosystems are affected by several anthropic activities. However, most of the knowledge about its effects in non-target organisms come from bioassays. Particularly the use of aquatic plants as sentinel species of pollution in field studies is scarce. Indeed, a recent review highlighted that most of the studies on effects on macrophytes were carried out under controlled laboratory conditions (93%), 5% in controlled field, and only 2% in natural field. The fragmentation of DNA through the “Comet” assay has been used in *Lemna minor* exposed to polluted surface water contaminated by a fertilizer factory effluent rich in fluorides, metals, and polycyclic aromatic hydrocarbons. Importantly, the authors found comparable responses in *L. minor* and fish regarding DNA damage and oxidative stress. The genotoxicity observed from in situ exposure was the result of numerous interactions between contaminants themselves and environmental factors, an area of research also scarcely addressed. Other sensitive techniques like the chromosomal abnormalities in roots of aquatic plants are common in bioassays. However, in *Elodea canadensis* this cytogenetic analysis has been used as a tool for testing genotoxicity of bottom sediments in the field, concluding that it can be used as a relevant and sensitive genotoxicity endpoint. On the other hand, in fish several field studies in Latin America have been carried out. The fish species commonly used are *Jenynsia lineata*, *Olygosarcus jenynsii*, *Prochilodus lacustris* and several species of the genera *Astyanax* (*A. lacustris*, *A. jacuhiensis*, *A. bifasciatus*) from freshwater ecosystems and *Plagioscion squamosissimus* from estuaries. The in situ biomonitoring of the water quality conducted with these species allowed to associate DNA damage to areas subjected to petrochemical influence, and exposure to metals and pesticides. Most of the results have shown the sensitivity of the techniques applied and the species used, making genotoxicity a suitable effect to be studied in pollution research.

### 3.O-Tu-3

## Evaluation of Genotoxicity and Biomarkers of Oxidative Stress in Fish (*Oreochromis niloticus*) from the Lagoon System of Rio de Janeiro (RJ), Brazil

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### Abstract

Due to anthropic actions derived from intense and accelerated urbanization process, constant discharge of sewage introduces contaminants in Jacarepaguá Lagoon - part of the lowland complex of the city of RJ - with a high potential to cause damage to health. The aim of this study was to evaluate the effects of environmental contamination using biomarkers of oxidative stress and genotoxicity. Samplings were carried out in 2019 (C1, C2, C3 and C4). Genotoxicity analyses were performed by the comet assay (CA) on blood samples and biomarkers of oxidative stress on liver and muscle. Previously, the chemical analysis in water samples comprised the detection and quantification of drugs, hormones and pesticides revealed presence of substances that prove contamination. The results were performed by evaluation of normality and confidence limit  $\geq 95\%$ . The drug ibuprofen (IBP) was observed in C4 and the hormone 17 $\alpha$ -ethinylestradiol (EE2) was found in all samplings. Pesticides were quantified in C1. The results of CA were represented in arbitrary unit (AU) and categorized by damage class (0,1,2 and 3). Higher genotoxic damage was observed in C1. The highest Liver SOD activity was in C4-  $4.66 \pm 3.02 \times 10^3$  U.g ptn-1 (median  $\pm$  standart error). In muscle, SOD activities were highest in C3- $4.54 \pm 0.55$  and C4- $4.98 \pm 0.50 \times 10^3$  U.g ptn-1. SOD constitutes one of the first stages of the antioxidant system - transform O<sub>2</sub>- into H<sub>2</sub>O<sub>2</sub>, preventing the formation of OH-. The highest SOD activities in both tissues were in C4 precisely in the collection where the IBP was found. The increase can be explained by the fact that oxidative metabolism of IBP favors the formation of O<sub>2</sub>- which is responsible for SOD activity. Accumulation and binding of IBP in membranes can cause structural damage and disintegration of cells which results in the release of the SOD into the bloodstream. The highest Liver LPO levels were C1- $6.39 \pm 3.26$  and C2- $16.11 \pm 2.12$   $\mu$ mol.g ptn-1 while in muscles was C1- $0.11 \pm 0.06$   $\mu$ mol.g ptn-1. In the liver, higher damage was in C1 where LPO can be related to exposure to pesticides. In muscles, the gradient followed C1>C4>C2>C3 where damage to cell membranes, in addition to being related to exposure to contaminants but can also be a result of deregulation of the antioxidant system of fish (stress condition). More analyzes must be carried out to verify the magnitude of the effects of environmental contamination on aquatic species but damage is already observed from exposure to contaminants.

### 3.O-Tu-4

## Metal and Metalloids Bioaccumulation and Genotoxicity of Aquatic Macrophytes From Lakes Under Metallurgical Influence

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### Abstract

Pollution is defined as any change in the composition and characteristics of the environment that causes disturbances in ecosystems and the biota. Aquatic plants are widely used as bioindicators of water quality, playing a very important role in this process. Due to these characteristics, macrophytes are used for monitoring aquatic environments. The objective of this work was to determine the metal and metalloids occurrence in water and sediment, bioaccumulation and genotoxic potential in leaves and roots of three species of aquatic macrophytes collected in three lakes (1) Carapebus (P1, P2 and P3), (2) Juara (P1, P2 and P3) (3) Maembá (P1, P2 and P3) and in (4) artificial lakes in Alegre (P1 and P2). Samples of leaves and roots of *Typha angustifolia* L. were collected in Carapebus and Alegre P2; *Eichhornia crassipes* in Juara and Alegre P1 and *Nymphaea alba* in Maembá. Generalized procrustes analysis (GPA) of studied parameters from each sampling site and the macrophyte analysed showed spatial differences in which the correlation with metals bioaccumulated and genotoxicity were observed. Spearman's rank correlation was applied for each specie. Considering the *Eichhornia crassipes* macrophyte, Alegre P1 showed the higher levels of metals and metalloids. In Alegre P1 the genotoxicity of leaves showed correlation with Mn bioaccumulated in leaves and Cu and Ni bioaccumulated in root. The root genotoxicity were correlated with Al, Ti, V, Cu, Y, Mo, Ba, La, Ce in leaves and Mn, As, Se, Mo, Cd, Ba, W in roots. For *Typha angustifolia* L., Alegre P2 showed the higher levels of metals and metalloids. The genotoxicity in leaves were correlated with Ti, Mn, Ni, Rb, Y, Ba, Ce in leaves and with Ti, Ni, Se, Rb, Nb, Ba, Cd, W in roots. Root genotoxicity were correlated with Al, Ti, V, Cu, Y, Mo, Ba, La, Ce in leaves and Mn, As, Se, Mo, Cd, Ba, W in roots. For *Nymphaea alba*, collected only in Maembá, the metal and metalloids bioaccumulation were higher in P2 followed by P1 and P3. The genotoxicity in leaves were correlated with Pb in root and the genotoxicity in roots were correlated with As in root. Thus, it is possible to confirm the presence of genotoxic alterations correlated with the metals presence in these ecosystems.

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### 3.O-Tu-5

## Environmental and Occupational Exposure Assessment in Miners of Itinga, Brazil, Using Oxidative Stress and Genotoxicity Biomarkers

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### Abstract

Mining activity has been related to the exposure to chemical, physical, and biological agents, in addition to the environmental degradation and contamination by toxic metals. Such factors can potentiate the imbalance that exists between oxidant and antioxidant compounds, causing a damage to biomolecules and leading to several diseases such as cancer. Gem miners from the district of Taquaral de Minas, in the city of Itinga, are exposed to contaminating agents. Thus, this study aimed to evaluate the occupational exposure in miners through biomonitoring techniques with biomarkers. A total of 22 miners and 17 workers not occupationally exposed were recruited, totaling 39 participants, as approved by the CEP/UFVJM in opinion 3.692.758. For the study, the following parameters were evaluated: Lipoperoxidation, Catalase activity and Microelements, an environmental analysis was performed using the Ames test. The results of lipid peroxidation showed a statistical significance ( $p < 0.05$ ), with increased frequencies in the exposed groups (20-130=0.787±0.0; 30-140=0.588±0.0; 40-150=1.849±0.047; 50-160=1.892±0.233; 60-170=2.051±0.101 and 70-180=2.030±0.173  $\mu\text{M}$ ) when compared to the control group (0.564±0.186  $\mu\text{M}$ ). The catalase enzyme activity values for the group (50-160=0.044±0.006  $\mu\text{mol}/\text{min}/\text{mg}$  of protein) showed a statically significant difference ( $p < 0.05$ ) compared to the control group (0.034±0.002  $\mu\text{mol}/\text{min}/\text{mg}$  of protein). For the exposed group the micronutrients Fe (50-160=549363.50; 70-180= 321043.61), Cu (30-140=972.83; 50-160= 937.96) Se (30-140= 49.63; 40-150=56.12; 50-160=49.61; 60-170=43.85 and 70-180= 28.49  $\mu\text{g}\cdot\text{L}^{-1}$ ) showed lower median blood concentrations when compared to unexposed Fe(580573.83  $\mu\text{g}\cdot\text{L}^{-1}$ ) and Se (72.82  $\mu\text{g}\cdot\text{L}^{-1}$ ) subjects. For the study of genotoxicity and mutagenicity in samples of soil (SS), Soil dust (P) and Stone dust (PP) the Ames test was applied and RM calculations were performed: TA100 without metabolization (BOD=3.21; PIR=3.45; PIN=3.37; LAJ =3.74; MAR=4.00), TA100 with metabolization (BOD=2.80; LAJ =2.36; MAR=2.13), TA98 without metabolization (BOD=3.83; PIR=2.11; PIN=2.22; LAJ =3.83; MAR=2.19) and TA98 with metabolization (BOD=2.51; LAJ =2.29; MAR=2.23). Thus, the present study indicates a possible environmental contamination and a potential health risk for miners, which suggests that further studies are important in the region.

### 3.O-Tu-6

## Mutagenicity of Polyethylene Microplastics and TiO<sub>2</sub> Nanoparticles in Bullfrog Tadpoles

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### Abstract

The Anthropocene has brought many new ecotoxicological concerns, so the contamination of aquatic ecosystems gained major attention among scientists. The increased consumption of plastic materials (especially polyethylene; hereafter PE MPs) was accompanied by the generation of residues, and its degradation into microplastic fragments is known to cause serious pollution in aquatic environments. Allied to that, nanotechnology has intensified the use of various nanoparticles, whose residues also end up reaching aquatic communities. However, the possible effects of environmental mixtures of these pollutants remain unknown, especially if considering the amphibians group, whose ecotoxicity studies are scarce. Thus, this study aimed to evaluate the chronic (15 days) mutagenicity of environmentally relevant concentrations of TiO<sub>2</sub> nanoparticles (TiO<sub>2</sub> NPs: 10 ug/ L) and PE MPs (60 mg /L) (isolated or combined) on bullfrog (*Aquara catesbeiana*) tadpoles, using micronucleus and erythrocytic abnormalities as biomarkers. Although none of the experimental groups presented alterations in micronuclei quantification, the isolated exposures to MP, and to TiO<sub>2</sub> NPs increased the number of moved nuclei. Also, the isolated MP exposure also increased the incidence of notched nuclei in bullfrog's erythrocytes, while other abnormalities were not statistically relevant (reniform, blebbed, multilobulated nuclei, binucleate or non-nucleated cells). Our results indicate that the interaction of MP and TiO<sub>2</sub> NPs may have reduced the uptake and/or bioavailability of the pollutants, which prevented a synergistic action in the mixture group. Additionally, depending on the recycling time of the animals' blood cells, such biomarkers may be more efficient in acute studies to assess earlier responses.



## Session 4: Wildlife Ecotoxicology

### 4A.O-Tu-1

#### Assessing Avian Toxicity Resulting from the La Pampilla Refinery Oil Spill in Peru

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#### Abstract

On 15 January 2022, large waves resulting from volcanic eruptions in Tonga reached the coast of Lima, Peru. Wave action destabilized the Italian tanker, Mare Doricum, transporting a shipment of crude oil to the Repsol-operated La Pampilla refinery. An estimated 12,000 barrels of oil were spilled into coastal waters, impacting 11,061 hectares of Peruvian coastline; more than 1,200 oil-covered birds were also identified post-spill. In order to assess the short- and long-term impacts of oil exposure on local seabirds, a suite of toxicity endpoints in blood collected from guanay cormorant (*Leucocarbo bougainvilliorum*) from the impacted island of Pescadores and the unimpacted island of Ballestas were measured. In September of 2022, blood was collected from 27 birds from Ballestas and 30 birds from Pescadores. Cytochrome P450 1A activity, measured as ethoxyresorufin O-deethylase (EROD) was measured in red blood cells; polycyclic aromatic hydrocarbons (PAHs), glutathione peroxidase (GPx), thyroxin (T4), antioxidants, and RNA/DNA damage were measured in blood plasma. Both the impacted and unimpacted plasma samples were extracted and analyzed for 42 parent and alkylated PAHs. Results showed low concentrations of all PAHs in both the exposed and control birds, with naphthalene (including the parent and homologues) accounting for the vast majority of detected PAHs in all samples. There was no difference in PAH pattern or distribution between the impacted and unimpacted birds. Similarly, there was no difference in EROD activity, an early biomarker of PAH exposure, between birds from the two islands. However, GPx activity, another common biomarker of PAH exposure, was significantly elevated in birds from the impacted island. T4 and antioxidant levels were depressed in birds from the impacted island, a biomarker previously shown in birds exposed to oil. There was no RNA or DNA damage, a longer-term biomarker of PAH exposure, measured in blood plasma from birds from either island. Overall, these results suggest birds from the impacted island may have been exposed to elevated concentrations of PAHs resulting from the oil spill, but long-term impacts of this exposure are still unclear. This study leveraged analytical chemistry techniques and biomarker analysis to better understand the ecological impacts to wildlife in the months following oil exposure.

## 4A.O-Tu-2

### **Atrazine Effects on the Biochemical Parameters (Health Status) of the Eared Dove (*Zenaida auriculata*)**

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#### **Abstract**

Pesticides impact on wildlife has been subject of research in recent decades, and its massive use has been proposed as one of the main causes of biodiversity loss. Atrazine herbicide (ATZ) is widely used in Argentina and the world associated with corn and sorghum crops. Environmental concentrations of the herbicide in waterbodies around the world and some harmful effects on biota are known, highlighting endocrine disruption in amphibians. In birds, a few reports show effects on reproduction, growth, hematological and biochemical parameters. But there is no information about ATZ effects in bird native species. The objective of this study was to elucidate how ATZ affects the health status of *Z. auriculata* males by biochemical parameters evaluation (hematocrit, alkaline phosphatase, creatinine, glucose, hepatic transaminases (AST and ALT), cholesterol, low density lipoprotein, total protein, triglycerides, uric acid, and ALAD activity). Additionally, body mass (BM) and water intake (WI) were evaluated. Three treatments, two ATZ concentrations (25 and 250 mg/kg) and a control group were evaluated. ATZ was diluted in corn oil and administered by gavage technique intermittently for 15 days. The control group underwent the same procedure as the exposed birds, with intermittent gavage administering corn oil, without herbicide. Each treatment had 6 replicates. At the beginning and the end of the bioassay, BM and WI was measured, and blood samples were taken from the brachial vein for hematocrit and ALAD evaluation. After exposure, blood extraction for biochemical parameters determination were performed during morning to avoid the effect of circadian rhythm. RM-ANOVA was performed to contrast ATZ effect at start and end of the experiment for BM, WI, hematocrit and ALAD. One-way ANOVA was performed to contrast biochemical parameters (Tukey post-hoc test,  $p < 0.05$ ) between groups. Data that failed to meet ANOVA assumptions were analyzed by Kruskal-Wallis test. No effects were observed for BM, WI, hematocrit and ALAD activity ( $p > 0.05$ ). For biochemical parameters, only ALT resulted significantly higher in doves exposed to 25 and 250 mg ATZ/Kg ( $p < 0.05$ ). To the best of our knowledge, this represents the first report of effects on ALT in ATZ exposed birds. An expected result, since high value of ALT indicates drug intoxication. However, further studies involving effects at the level of the hepatic response could help to understand how ATZ are affecting wild birds.

## 4A.O-Tu-3

### The Patagonian Penguin as a Bioindicator of Current Use and Contamination by Organochlorine Pesticides on the Argentine Shelf

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#### Abstract

The current agricultural model, based on the simplification of ecosystems, is sustained by the intensive use of pesticides. The environmental dynamics of pollutants lead them to easily reach surface water bodies and eventually coastal areas, affecting the biological integrity of ecosystems. The coastal areas of southeastern Buenos Aires represent an important area of transit and/or feeding for the Magellanic penguin (*Spheniscus magellanicus*) in Argentina. The aim of this work is to evaluate the levels of organochlorine pesticides and chlorpyrifos in body feathers of males and females with different body condition (starvation and non-starvation) in juvenile penguins, in order to relate contaminant levels with intrinsic factors such as sex and body condition.

The determination of these levels was carried out using gas chromatography with electron capture detection (GC-ECD). Chlorpyrifos showed the highest values among all pesticides analyzed in individuals of both sexes and body conditions, probably due to its recent intensive use. Male specimens showed higher concentrations than females (60.48 ng/g and 41.33 ng/g, respectively). Differences between sexes could be related to differences in exploited areas and/or diets or to the larger size and weight of male individuals, beyond their state of starvation or non-starvation. On the other hand, individuals in a state of starvation of both sexes presented higher concentrations than those in a state of non-starvation (54.40 ng/g and 47.42 ng/g, respectively). This fact may be related to the greater availability of contaminants from fatty tissues during periods of dietary stress. In the case of OCPs, a distribution pattern of contaminants was observed: DDTs > HCHs > Heptachlors +drins > endosulfans > chlordanes. The most commonly found compounds within each group were the p,p'-DDE metabolite, the g-HCH isomer, and the endosulfan Ssulfate. DDT concentrations were higher in females in good body condition and HCH concentrations in males and females in a state of starvation.

Long-term monitoring of agrochemical levels in seabirds is suggested in order to provide information on pesticide dynamics and exposure pathways in these ecosystems. These results may contribute to the development of a tool to promote other possible land uses, which consider the complexity of ecosystems, to be considered as a possible conservation strategy for the seabirds that inhabit the southwestern Atlantic.

## 4A.O-Tu-4

### **Metal Accumulation in the South America Sea Lion *Otaria flavescens*: A Study Addressing Environmental Monitoring**

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#### **Abstract**

The present study characterized concentrations of essential (Copper [Cu], Iron [Fe] and Zinc [Zn]) and non-essential (Cadmium [Cd]) metals in different tissues (liver, kidney, heart, muscle, skin, fur) of the South American sea lion *Otaria flavescens* stranded in the southern Brazil coast and evaluated whether these metal accumulations are related to biological parameters such as sex, total length and decomposition. Zn and Fe concentrations were measured by flame atomic absorption spectrometer, and Cu and Cd by graphite furnace absorption spectrometer. Among the analyzed metals, the highest concentration of Cd was detected in the kidney ( $7.09 \pm 1.15 \mu\text{g/g dw}$ ) and of Cu in the liver followed by fur ( $31.08 \pm 6.43 \mu\text{g/g dw}$  and  $24.04 \pm 7.25 \mu\text{g/g dw}$ , respectively). As for Cd and Cu, the total length of the animal seems to influence the accumulation of these metals, being directly related to Cd in the kidney and inversely related to Cu in the fur. Fe and Zn were found with similar values in all tissues ( $1186,85 \pm 160$  and  $66,70 \pm 6,34 \mu\text{g/g dw}$ , respectively). The present study provides new information on trace element concentrations in the tissues of South American sea lion in the western South Atlantic Ocean and highlights the value of strandings in detecting the pattern and factors affecting concentrations.

## 4A.O-Tu-5

### Behavioral Responses of Imidacloprid-Dosed Red-Legged Partridges (*Alectoris Rufa*, Phasianidae) to a Simulated Predation Risk

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#### Abstract

Neonicotinoids have the potential to produce alterations in the nervous system of birds, which could affect complex behaviors linked to survival such as antipredator behaviors. The objective of this study was to evaluate the effect of sublethal doses of imidacloprid (0, 1, and 6 mg/kg bw) on the behavioral responses of the red-legged partridge (*Alectoris rufa*) to a simulated predator threat. To do this, 66 partridges (33 males and 33 females), were exposed in groups or individually to the playback of intra and interspecific alarm calls (auditory stimuli), to a simulated raptor attack using a harrier model flying over the birds or to a terrestrial predator approach using a fox model (both as visual stimuli). The behavioral responses were divided into active antipredator responses (escape, vigilance with displacement) or passive responses (vigilance without displacement, crouching, and freezing). Latency (the time it takes for the bird to respond to the stimulus), percentage of individuals who responded and duration of each response, speed during the active responses, and, only in the individual experiment, vocalizations and neurological status, were also measured. We found a treatment effect on the duration of the crouching in response to visual stimuli during the group experiment ( $p = 0.040$ ) and on the vigilance behavior without displacement in response to the intraspecific alarm call ( $p = 0.007$ ). Imidacloprid caused an overexcitation of birds, as birds dosed with 6 mg a.i./kg bw showed shorter crouching and longer static vigilance than controls. Future studies with formulated imidacloprid would be convenient to continue elucidating the effect in the field of this neonicotinoid on the antipredator behaviors of birds.

## 4A.O-Tu-6

### Birds Living In Areas Influenced By Wastewater Suffer Changes In Biochemical And Physiological Biomarkers

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#### Abstract

The use of effluents of wastewater treatment plants (WWTP) for maintenance of the wetlands is becoming a strategy to alleviate the problem of drought in arid and semi-arid regions. However, this solution is becoming in a new problem, because this practice has led to the input of chemical pollutants coming from urban and industrial sources and also have a high prevalence of pathogenic bacteria. On the other hand, these wetlands have a high productivity, and this makes them very attractive for waterbirds feeding on aquatic invertebrates, with the consequent risk of chemical exposure for them. Here, we evaluated changes and effects on the sex hormones, immune response, oxidative balance and carotenoid-based ornaments of common moorhens from Navaseca Pond (Central Spain), which receives the effluent of a WWTP, and the more pristine Tablas de Daimiel National Park (TDNP). Previous studies have shown that birds from Navaseca Pond exhibited a high burden of toxic compounds with effects as endocrine disruptors. Plasma levels of sex hormones were lower in Navaseca Pond than in TDNP and gender-related differences were less marked than in TDNP. Moreover, moorhens from Navaseca Pond have shown a higher bactericidal activity and haptoglobin levels than in TDNP, which can be associated with a higher capacity to cope with pathogens from sewage water. Birds from Navaseca Pond also showed a depletion of circulating antioxidants and a greater activity of GPX, which could explain the lowest lipid peroxidation exhibited. In addition, these birds exhibited a darker red color of the bill, and metal levels had a negative effect on Hue or Chroma (red bill), which could mean an investment of carotenoids and other antioxidant in maintaining health status at the expense of ornamental coloration. The observed adverse effects on health status and fitness of waterbirds inhabiting WWTP wetlands show the risk of these polluted sites as ecological traps.

## 4B.O-Tu-1

### Long Term Consumption of Tiamethoxam Coated Seeds Causes Multilevel Effects to the Passerine *Agelaioides Badius*

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#### Abstract

The wide use of neonicotinoid insecticides seed treatment represents a hazard for farmland birds that feed on treated seeds. Tiamethoxam (TMX) is one of the main used neonicotinoids for row crops seed coating. The toxicity of TMX to birds is considered low, however, their sublethal effects are scarcely studied in birds. This study aimed to characterize the multilevel effects of TMX in the passerine grayish baywing (*Agelaioides badius*) exposed to TMX treated seeds. Adults were exposed to peeled millet treated with nominal concentrations of 0 (CON), 0.05 (TMX 1), 0.5 (TMX 2), and 5 (TMX 3) g TMX/kg seed for 21 days. These experimental concentrations represent the range of approved use for coating seeds in the Pampa Region of Argentina. Daily seed consumption and survival were estimate. Weight of birds was assessed weekly. At the end of exposure, the hematocrit and the enzymatic activities of catalase (CAT), cholinesterase (ChE), and glutathione-S-transferase (GST) in multiple tissues (i.e., plasma, brain, liver, kidney, and muscle) were measured. Results demonstrated that significant mortality was observed in the TMX3 group by 50%. No mortalities were registered for the other treatments. Mean consumption of seeds per group was (mean  $\pm$  S.E): 115.0 $\pm$  1.65, 111.0 $\pm$  1.30, 115.83 $\pm$  1.90, and 144.8 $\pm$  2.07 g seed/kg bw, for CON, TMX1, TMX2, and TMX3 respectively. Moreover, consumption by birds from TMX3 was higher than the rest of the groups, representing 126% of the CON consumption ( $p < 0.05$ ). Mean daily dose ingested were different between the experimental groups analyzed ( $p < 0.05$ ), with values of (mean  $\pm$  S.E): 2.94  $\pm$  0.047, 38.00  $\pm$  0.54, and 619.7  $\pm$  15.313 mg TMX/ kg bw for TMX1, TMX2, and TMX3 respectively. Birds from TMX3 experienced an early loss of body weight that remained decreased throughout the experiment. At the end of exposure, the hematocrit was reduced in both TMX2 and TM3 groups, in a dose-response way. Compared with CON group, the birds from TMX3 showed an increase in the activities of GST (i.e., in muscle and kidney), ChE (i.e., in muscle), and CAT (i.e., in kidney); while the birds from TMX1 had increased activities of ChE and GST in the liver ( $p < 0.05$ ). This study highlights that ingestion of environmentally relevant doses is sufficient to trigger multilevel effects and death. In the wild, these effects may have ecological consequences, representing a risk to farmland bird populations.

## 4B.O-Tu-2

### **Use of Freshly Drilled Dry Bean Fields by Birds and Mammals in Brazil: Insights from a Field Study and its Use in Pesticide Risk Assessment**

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#### **Abstract**

Research into the occurrence and diversity of wildlife in agricultural areas is essential to understand the impact of agriculture (including the use of pesticides) on wildlife populations. Dry bean production in Brazil is economically essential but simultaneously must prioritize the preservation of wildlife habitats. This study aims to describe the occurrence and diversity of birds and mammals on and around freshly drilled dry bean fields in different agricultural landscapes in the Brazilian Cerrado and Atlantic Forest. Two different scenarios were analyzed, with one set of fields within a mixed landscape and adjacent to permanent natural off-crop vegetation and the other set of fields within a landscape of other dry bean fields and no significant natural off-crop vegetation. Two types of bird observations were conducted. First, point counts were conducted to assess the in-crop and off-crop occurrence and abundance of individual species and overall avian diversity. Second, foraging observations were conducted from blinds to assess feeding habits of birds occurring on freshly drilled dry bean fields. Mammal occurrence was assessed by using wildlife cameras set up at field borders and adjacent non-crop habitat. Abundance and diversity of birds and mammals was significantly higher in non-crop vegetation compared to freshly drilled dry bean fields. Bird species occurring within drilled dry bean fields were mainly small insectivorous or granivorous birds. These species were generally not capable of consuming dry bean seeds due to seed size. During foraging observations, no evidence of birds feeding on dry bean seeds was observed. Also, potential feeding on dry bean seeds by mammals was not confirmed. Overall, it can be concluded that freshly drilled dry bean fields are unattractive habitats for birds and mammals occurring in agricultural landscapes in Brazil. Despite high densities and diversity in non-crop habitats, abundance and diversity within dry bean fields were low. The data presented here indicate that exposure to pesticides used in freshly drilled dry bean fields by birds and mammals is expected to be relatively low.



## 4B.O-Tu-3

### Amazon River Dolphins: Bioaccumulation of Mercury and Stable Isotopes of Carbon and Nitrogen as Ecological Tracers

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#### Abstract

The Amazon Basin hosts an immense diversity of aquatic environments. Several endemic species inhabit the floodplain, such as the Amazon river dolphin (*Inia geoffrensis*), an endangered species, with its population decreasing dramatically. Exposure to contaminants, especially mercury, is one of the threats to this species and information on feeding ecology and trophic level are valuable tools to assess this exposure. This work aimed to determine total mercury (THg) in the blood of *I. geoffrensis* and to understand its relationship with their habitat use, feeding ecology and trophic position through carbon and nitrogen stable isotopes analyses. Blood samples of 33 Amazon river dolphins were collected from wild and live animals in the Amazon basin in 2011. The carbon and nitrogen isotopic ratio determination was performed by an isotopic ratio mass spectrometer coupled to a C-N-S elemental analyzer. THg concentrations were determined by Cold Vapor/Atomic Absorption. The THg mean concentrations were  $184 \pm 133$  ng/g and  $167 \pm 76$  ng/g in females ( $n=15$ ) and males ( $n=18$ ), respectively. Significant differences were found for  $\delta^{13}\text{C}$  values between sexes, with males ( $-29.4 \pm 1.1$  ‰) presenting higher values than females ( $-30.3 \pm 1.3$  ‰) (Mann-Whitney U test,  $p < 0.05$ ). The THg concentrations and  $\delta^{13}\text{C}$  values were positively correlated with animals' total length and weight (Spearman test,  $p < 0.05$ ), whereas no correlations were found between  $\delta^{15}\text{N}$  and biological parameters. The THg concentrations were positively correlated to the  $\delta^{13}\text{C}$  values, which were significantly higher in males. Additionally, a general linear model considering sex, total length and the interaction between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values to explain the THg concentrations were obtained ( $p < 0.05$ ;  $R^2 = 0.60$ ), and the sex was pointed out as a significant variable for the model ( $p < 0.05$ ). These findings may indicate that males exhibit a foraging area distinct from females, exploring areas where  $\delta^{13}\text{C}$  values may be higher. The investigation of THg concentration and stable isotope values in this iconic and threatened species of the Amazon provides novel and important knowledge on its trophic ecology and tracking different sources of this toxic metal bioaccumulation. Studies are being conducted to increase the sample size and period and refine information.

## 4B.O-Tu-4

### The Chilean Marine Otter (*Lontra felina*) as a Bioindicator for Mercury Contamination in Chile's Continental Coast: Toxicokinetics and Latitudinal Distribution of Mercury

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#### Abstract

Mercury (Hg) is a trace metal with no known biological function, and with potential toxic effect in different organs. Its organic form, methylmercury (MeHg), is highly neurotoxic and of great biological concern, especially in top predators, as it is lipophilic and has high bioaccumulation potential in marine organisms and biomagnification through food webs. In Chile, anthropogenic mercury emissions are mainly associated to mining activity, particularly copper smelters (widely distributed in the central and northern regions of the country), as well as industrial activities such as coal-fired power plants. Marine mammals are considered sentinels of ocean health and bioindicators of environmental contamination. Additionally, there is a rising interest in ecotoxicological studies for the use of non-destructive samples, such as hair, blood and faeces. The Chilean marine otter, or chungungo (*Lontra felina*), is a top predator distributed through all of Chile's exposed coast, resident of relatively small sections of it, sharing a similar diet and living along with humans in areas with high levels of anthropogenic activities. Furthermore, it's a protected species classified as Endangered, with a decreasing population, and hypothetically recognizing heavy metal contamination as one of its threats. The present study evaluates the distribution of Hg between different biological samples (muscle, liver, kidney, brain, heart, lungs, fur, blood and faeces), as well as its latitudinal distribution in Chile's continental coast. We used a Direct Mercury Analyzer (DMA-80 evo) to measure the total mercury (THg) in fresh and freeze-dried samples (for wet-weight [ww] and dry-weight [dw] bases, respectively). As preliminary results we found mean THg concentrations (as ppm dw, and/or ww between brackets) of 5.53 in fur (N=22), 5.17 [1.22] in liver (N=10), 4.12 [0.84] in kidney (N=13), 1.19 [0.29] in muscle (N=13), 1.04 [0.22] in heart (N=6), 1.06 [0.17] in lung (N=1), 0.63 in brain (N=5), [0.07] in blood (N=18), and 0.10 in faeces (N=29). This liver, kidney and brain concentrations are higher than those detected for *Enhydra lutris* in Washington and Alaska. Further analyses are being undertaken that may reveal latitudinal differences in mercury contamination. This is the first ever assessment of Hg levels in *Lontra felina*. This study is possible thanks to Fondecyt 1221348, Fondecyt 1100139, Fondecyt 1171417 and Regular-Unab DI-07-20.

## 4B.O-Tu-5

### Occurrence and Bioaccumulation of Metals in Black Necked Swans (*Cygnus melancoryphus*) From Río Cruces in a Climate Change Context

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#### Abstract

The presence and effect of metals has been the subject of many investigations worldwide. In Chile, the Cruces River, a RAMSAR site and a protected area, have been the receptor of metal pollution with result of aquatic plants die-off and Black necked swans' emigration. Iron (Fe), Zinc (Zn) and Aluminum (Al) are among the most concentrated metal on this system. Although the most concentrated metals in the water column are Fe>Al>Zn, only Fe and Zn concentrations have been correlated with their concentrations in the (*Egeria densa*) - main food items for Black necked swans-. In 2004, severe changes in environmental conditions were determined and correlated with anthropogenic activities in the Cruces River wetland. One of the main conclusions of the 2004 environmental changes events, was the presence of metals, particularly iron, in concentration that exerted pollution type effects on the system. Decreased black necked swam (*Cygnus melancoryphus*) population numbers due migration and mortality were correlated with metal pollution in their main food item, the Brazilian Seaweed (*Egeria densa*). Currently, despite an increase of Black necked swans' abundance, there are yet substantial concentrations of metals in the system that may be correlated with climate change pattern in rain and water volume on the river. We investigate Fe, Zn and Al concentration in Brazilian Seaweed (*Egeria densa*) and in Black necked swan feathers during a 5-year period (2018-2022) using atomic absorption spectrophotometry. In general, concentrations of each metal were highly variable in feathers and aquatic plants. In the swans' feathers, Fe average and range were 46.4 (3 to 158) mg/kg; 88.4 (31 to 148) mg/kg for Zn; and 756.6 (1.8 to 4,430) mg/kg for Al. We observed idiosyncratic patterns of bioaccumulation of each of the three analyzed metals in Black necked swans when compared with concentration found in the main food item, the aquatic plant. Our results suggest caution in the interpretation of metal concentration data in matrices such as water and aquatic plants in a large wetland in southern Chile.

## 4B.O-Tu-6

### Total Mercury in Elephant Seals From the Antarctic Peninsula

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#### Abstract

Antarctica is the coldest and driest continent in the world and is of great importance for scientific research due to its uniqueness and biological diversity. Antarctica contains 90% of the Earth's ice, making it a vital reserve of fresh water. However, this ecosystem is not exempt from anthropogenic influence such as mercury contamination that threatens the fauna that inhabits this continent. Its fauna includes penguins, whales, seals, albatrosses, sea elephants among others. Southern elephant seals are one of the largest pinniped species and play an important role in the ecological balance of the region as indicators of the health status of the Antarctic ecosystem and are important to the ecosystem because they are top predators and control populations of their prey. Mercury is a toxic chemical element that can accumulate in the environment and infecting living organisms can severely affect the health of southern elephant seals. Mercury can accumulate in animals' tissues over time and cause damage to their nervous system and other vital organs. In this study, average values of total mercury in hair samples of  $=1.27 \text{ mg/kg} \pm 1.01$  are reported, which does not represent any risk, however, one organism exceeded the threshold of affectation to the neurological system ( $5.4 \text{ mg/Kg}$ ). The conservation of this species is important to maintain the biodiversity of Antarctica and protect the ecological balance of the region

DRAFT

# Session 5: Challenges and Strategies for Linking Adverse Effects to Endocrine Activity to Identify Endocrine Disruptors

## 5.O-Tu-1

### Demonstration of a Novel Integrated Risk Assessment Framework of Endocrine Disruptors at a Large River Scenario under “One Health”, Reduction in Experimentation Animals and Weight of Evidence Concepts

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#### Abstract

Most of the risk assessment frameworks are designed in tiers, but in general they are directed to test chemicals for endocrine disruption. For instance, the OECD has developed a framework including four tiers of testing, ranging from *in silico* methods and *in vitro* assays to *in vivo* studies in laboratory animals such as the Hershberger and uterotrophic assays. The US Environmental Protection Agency (EPA) recommends a battery of *in vitro* and *in vivo* assays such as the pubertal female assay, pubertal male assay, and two-generation reproductive toxicology assay. In turn, the EU prescribes the use of both *in silico* and *in vitro* methods and progressing to *in vivo* studies in laboratory animals such as the fish short-term reproduction assay and amphibian metamorphosis assay.

The question on how to assess the risks both to humans and to aquatic environmental receptors, through a holistic “One Health” approach was addressed in a case-study at a large river (River Uruguay) in a PhD investigation based at Cranfield University and the Technological Laboratory of Uruguay, from 2008-2013.

Mixture toxicity in large rivers is a phenomenon whereby multiple chemicals or pollutants in a large river system interact with each other to create a cocktail of substances that can have harmful effects on the aquatic ecosystem and inhabitants in the catchment. This is a problem of significant environmental concern due to a potential biodiversity loss, but also many large rivers are used to provide drinking water and support fisheries, and pollution can lead to health risks and economic damage.

Hazard identification was done considering the different sources (industrial, domestic, and agricultural). Bayesian statistics, artificial neural networks, and other machine learning techniques aided the understanding of the databases to determine the river health and the results of native fish biomonitoring. In this case scenario, a novel endocrine disruptors framework was developed including in the exposure assessment phase *in silico* models and *in vitro* assays, such as vitellogenin test (ELISA and PCR) and endocrine receptor binding tests, as well as invertebrate and model fish short term toxicity tests with *Pimephales promelas* in a quantity that would diminish the use of animal species. Risk characterization followed an innovative approach to establish the hot spots in certain reaches of the river and highlighted the top priority endocrine disruptors regarding risks to humans and wildlife.

## 5.O-Tu-2

### Today or Not Today? a Study to Optimize Seasonal Variation and Sample Size of Sex Steroid Production in Small Bodied Fish Used for Endocrine Disruption Monitoring

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#### Abstract

It is well known that aquatic environments are especially vulnerable to pollution. In vitro steroid production measurements it has been a useful tool in environmental monitoring program for the last have been used for >20 years at least, almost related to endocrine disruption. However, to be able to understand an effect of Endocrine Disrupting Compounds (EDCs) on hormone production in an individual, and to discriminate natural variability from an induced changed that could lead to a biological/ecological impact, it is necessary to know the natural hormone cycle. In this study, we analyzed the seasonal variation of Testosterone (T, in males and females), 17 $\beta$ -estradiol (E2, females) and 11-ketotestosterone (11-KT, males) in 4 species of small bodied fish used as sentinel organisms in Canada and Chile. Here we showed that all the species presented here spawn in spring so sampling near this period could enable to establish direct correlations between hormones variations and reproductive success. At the same time gonad maturation is preceded by a 11-KT and E2 peak (late fall and early winter), and may be determinant for gonad maturation and so, for reproductive success. Thus, it could be a key period to study. At the same time, using sex mature fish above certain size for each specie (total length) we could decrease the sample size for every sex steroid between 8-39 individuals. Sampling timing (month) and sample size are not only crucial to observe a proper relationship to the stressor (EDCs) but could help us to diminish the pressure over wild fish populations already vulnerable to multiple impacts in a given basin.

## 5.O-Tu-3

### Approaches for Differentiating Between Non-Endocrine and Endocrine Modes of Action Causing Adrenocortical Pathologies and Potential Adversity

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#### Abstract

The adrenal glands produce a diversity of hormones mainly from two discrete zones of the outer cortex. The innermost layer of the cortex, the zona reticularis, produces androgens that are converted to fully functional sex hormones in other organs, and the next layer, the zona fasciculata, produces the glucocorticoids cortisol and cortisone. The adrenal gland is one of the most common toxicological target organs within the endocrine system, with adrenal cortical vacuolization being a common observation following xenobiotic exposure. Vacuolization of the zona reticularis and zona fasciculata is often associated with vacuolization of neutral lipids and cholesterol caused by mitochondrial cytotoxicity and swelling to the smooth endoplasmic reticulum (SER) thus disrupting normal cellular function. Such vacuolization could be due to an inhibition of steroidogenesis as the rate limiting step in this pathway is the importing of cholesterol into the mitochondria followed by the conversion of cholesterol to pregnenolone and progesterone. Cytotoxicity may also be observed in the adrenal gland at relatively high exposure levels for some xenobiotics due to the unique anatomical architecture and composition (i.e., high perfusion rates and high lipid content) of the gland. The combination of high perfusion rates and high compound hydrophilicity provides a mechanism to achieve high tissue concentrations for some xenobiotics. Therefore, it may not be evident if cortical vacuolization of the zona reticularis and zona fasciculata is caused by a direct effect on steroidogenesis (i.e., inhibition) or an indirect effect on the steroidogenic pathway (i.e., cytotoxicity). Currently, there is a lack of guidance on the interpretation and significance of adrenal cortical vacuolization, nor is there a strategy for testing/performing a functional assessment of effects. This talk will present approaches to differentiate direct versus indirect effects to adrenal steroidogenesis to help differentiate between endocrine and non-endocrine cytotoxic effects. In addition, the presentation will discuss approaches to assess the potential for adversity related to adrenal cortical pathologies.

## 5.O-Tu-4

### Yeast estrogenicity screening (YES) bioassay in daily use products and environmental samples in Maldonado, Uruguay

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#### Abstract

On a daily basis, human are exposed to chemical formulations that finishing in the sewage network system and end in aquatic ecosystems. Some chemical has been associated with diseases related to endocrine systems, known as Endocrine Disrupting Pollutants (EDC's). Some of these substances can mimic estrogen signals, called xenoestrogens. The Yeast Estrogen Screening bioassay using *Saccharomyces cerevisiae* is a useful technique to quantify estrogenicity. The YES bioassay was set in the laboratory of CURE- Universidad de la República, Maldonado, Uruguay). We quantify estrogenicity in daily-use products, and in environmental samples (i. e. sediments and water samples of Maldonado basin, Uruguay). Fifty nine products were evaluated (including detergents, shampoos, resin, pesticides) and sediment and water samples of 19 sites of Maldonado basin were screening. Sediments were extracted by Quechers, and superficial water were extracted by solid phase extraction. For the color reaction of the bioassay, we used CPRG as a substrate. Several products presented estrogenic activity, including household cleaning, personal hygiene, biocides, resins and thermal paper. The EC50 of some products reached 332 ng/ L of EQ-E2. In Maldonado basin, most of sites showed values below the detection limit. Only in one sediment sample down-stream a sewage treatment plant of San Carlos city and two water samples, one countryside and an urban creek showed estrogenicity. Indicating the multisource of EDCs and the accumulation in sediments exposed to sewage discharge. The results show that YES is a valuable tool to determine estrogenic activity in different type of samples.



## 5.O-Tu-5

### **Weight of Evidence Versus Key characteristics for Assessing Endocrine Disruption: Case Study on Why Glyphosate Is Not an Endocrine Disruptor**

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#### **Abstract**

A weight of evidence (WoE) approach assesses all available information and weighs data by considering relevance, reliability, and quality. Using a WoE approach the US Environmental Protection Agency's Endocrine Disruptor Screening Program (EDSP) in 2015, the European Food Safety Authority in 2017, a peer reviewed article by Bayer Crop Science in 2020, and the Assessment Group on Glyphosate, the rapporteur Member States who led the European renewal of glyphosate in 2021, all evaluated glyphosate's potential to interact with the estrogen, androgen, thyroid and steroidogenic (EATS) pathways. These evaluations included results from in silico screening, the EDSP Tier 1 battery, guideline regulatory studies with endpoints that informed an endocrine assessment, and open literature studies. The conclusions from each of these reviews were that glyphosate does not have endocrine-disrupting properties through EATS modalities. In striking contrast to these conclusions, a recent paper evaluated glyphosate using the ten proposed key characteristics (KC) of endocrine-disrupting chemicals (EDCs), concluding that glyphosate satisfied at least eight KCs of an EDC. The KC approach for EDCs fails to apply the international consensus definition of EDC and it is not amenable to empirical testing or validation. The proposed KCs for EDCs ignore principles of hormone action and characteristics of dose-response in endocrine pharmacology and toxicology. The approach lacks a means to reach a negative conclusion about a chemical's EDC properties and appears incapable of distinguishing EDCs from non-EDCs. The most relevant and reliable documents assessing glyphosate's potential endocrine activity and toxicological profile were not discussed. Rather, this publication relied on a small subset of publications that used non-standard in vitro/in vivo methods that tested glyphosate and its formulations often at unrealistically high concentrations exceeding those recommended for endocrine testing by international guidelines. Additionally, the studies were not designed to differentiate between endocrine and non-endocrine activity, which confounded the conclusions of the study and cited studies were not evaluated for relevance, reliability, and quality, which is required for systematic reviews. In conclusion, application of KCs and not performing a WoE for evaluating glyphosate's potential to interact with the EATS modalities resulted in an incorrect classification.

## 5.O-Tu-6

### **A Bright New World! Making the Best Use of New Approach Methods in the Assessment of Endocrine Disruption**

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<sup>1</sup>ER2, USA. <sup>2</sup>Bayer CropScience, USA

#### **Abstract**

Over the last two decades, the implementation of legislative frameworks focused on evaluating substances for endocrine activity/disruption (EA/ED) have fostered the improvement of existing test methods and the development of new test systems and approaches (NAMs) that include in silico, in vitro, and in vivo lines of evidence. Integrating activity-based screens and apical testing into endocrine system specific adverse outcome pathways (AOP) may allow for more rapid prioritization, screening, and evaluation of a causal link between endocrine mechanisms and adverse effects, a key criterion for determining whether a particular chemical should be considered an endocrine disruptor. High throughput (HTP) assays and other NAMs have the potential to not only increase the amount of data available for assessments while reducing the cost and time of generating the data, but also to help develop better predictive models of responses to contaminants. However, these same novel endpoints and rapid screening tools could add uncertainty rather than clarity and result in unintended consequences if not considered in a transparent and systematic hypothesis-testing weight-of-evidence process. The misidentification of non-endocrine or indirect effects as truly ED has serious consequences in terms of triggering animal and resource intensive testing and potentially severe regulatory consequences. Thus, it is important to establish the reliability, relevance, fit for purpose, and consistency of the reported results from enhanced tests and new methodologies, to ensure that any additional animal- and resource-intensive testing and regulatory actions are truly warranted. Case studies will be discussed that make use of these NAMs in the context of both data rich and data poor substances to illustrate best practices and key watch-outs in the use of this new, mechanistically-focused data.

# Session 6: Emerging Contaminants and Environmental Remediation: Monitoring of Effects by Ecotoxicological Bioassays

## 6A.O-Mo-1

### Bioaccumulation and Depletion of the Antibiotic Sulfadiazine $^{14}\text{C}$ in Lambari (*Astyanax bimaculatus*)

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#### Abstract

Antibiotics are present in the environment primarily due to their release through wastewater treatment plants, agricultural practices, and improper disposal of unused medications. In the environment, these drugs can be bioaccumulated by organisms and transferred along the food chain. This is a problem for the consumption of fish meat. The legislation provides that the maximum residue limit for sulfadiazine (SDZ) should not exceed  $100 \mu\text{g Kg}^{-1}$ . The Lambari fish has potential economic importance in aquaculture, as they are relatively easy to breed and can be raised in small-scale operations. Finally, studying the biology and ecology of lambari can provide valuable information about freshwater ecosystems and their inhabitants. This work aimed to perform the bioaccumulation and depletion of the antibiotic SDZ  $^{14}\text{C}$  in Lambari (*Astyanax bimaculatus*). For this purpose, the tests were divided into two stages 07 days of exposure and 07 days of depletion, where one fish was randomly selected and sampled every day. In the exposure phase, the fish were fed the medicated feed three times a day at a concentration of  $2.5 \text{ mg g}^{-1}$ . The control was fed uncontaminated feed. For the depletion phase, the remaining Lambaris were transferred to clean tanks and fed uncontaminated feed three times a day. The fish samples were burned in the Oxidizer and the reading of radioactivity was in a liquid scintillation spectrometer. It is worth noting that on day 7 and day 14, the water in the aquariums was filtered through filter paper to collect the metabolic excrement. SDZ concentrations increased over days, and accumulation occurred in the fish, with day seven being the maximum accumulation value of  $91.7 \text{ ng g}^{-1}$  due to feeding uptake. After the depletion phase on day 13, the value found was  $0.83 \text{ ng g}^{-1}$ . The bioconcentration factor calculated was  $0,02 \text{ L g}^{-1}$ . After the bioaccumulation period, the concentration of SDZ in the water and excreta was  $4.5 \mu\text{g L}^{-1}$  and  $363.5 \text{ ng g}^{-1}$  respectively. In the depletion period, the concentration in the water and excreta was  $0,01 \mu\text{g L}^{-1}$  and  $5,96 \text{ ng g}^{-1}$  respectively. These results imply that SDZ bioaccumulated little in the fish, but distributed itself in larger amounts to water. This is due to the physicochemical properties of the molecule with the low log Kow value. Regarding the maximum residue limit, the value was below the established value. This study contributes to understanding SDZ dynamics in an aquatic species native to Brazil.

## 6A.O-Mo-2

### **Bioassays With *Ceriodaphnia silvestrii* Can Assess the Toxicity of Six Pharmaceuticals in Artificial Wastewater**

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#### **Abstract**

Pharmaceuticals are released into the environment and can cause toxic effects on several aquatic organisms even at low concentrations. These emerging contaminants are not completely removed in effluent treatment plants, although anaerobic reactors have already shown potential for their degradation. In this context, this research aims to evaluate the ecotoxicity of six pharmaceuticals to the Neotropical cladoceran *Ceriodaphnia silvestrii*, including atenolol, carbamazepine, diclofenac, ibuprofen, propranolol, and triclosan. In the first stage, the target compounds will be tested individually to assess the potential acute and chronic effect concentrations for the exposed organisms. Then, the mixture of pharmaceuticals and their interactions will determine the potential enhanced effects in an artificial wastewater matrix. We hypothesize that: i) the effect concentrations of pharmaceuticals are expected to be higher than reported concentrations in wastewater, according to the literature; ii) the presence of multiple contaminants in the wastewater may increase the toxicity of pharmaceuticals; iii) a reduction in ecotoxicity to bioindicators is expected with the removal of pharmaceuticals in an anaerobic system operated with phase separation (acidogenesis and methanogenesis). The results can reinforce the application of ecotoxicological tests to evaluate the treatment and removal of target compounds by anaerobic bioreactors. Choosing native species is relevant to assess water and wastewater quality and evaluating ecological risk in tropical environments.

## 6A.O-Mo-3

### Investigation of the Effects of Pharmaceutical Nimesulide on Seeds: A Study of Comparative Sensitivity

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#### Abstract

The excessive use of pharmaceuticals has been a global concern, especially with regard to their effects on the environment. Because they are bioavailable after human and animal disposal, these emerging pollutants are introduced into environmental matrices through wastewater disposal and the absence of drug-removing treatment. As a result, more and more of these pollutants and their metabolites are found in water bodies and soil, where they can affect the flora and fauna of various ecosystems.

The presence of pharmaceuticals in soil is a growing problem that negatively affects biodiversity and human health. Its origins can be in the disposal of sludge contaminated with pharmaceuticals in agricultural areas as well as irrigation water with these pollutants present. Among the recurring classes of drugs in environmental studies are antibiotics, endocrine disruptors, and anti-inflammatory drugs. Regarding the last mentioned class, nimesulide stands out, which is widely consumed around the world, with the exception of European countries that have a marketing ban, due to studies related to the risk of liver damage.

How methodologies, phytotoxicity test is widely used to assess the effects of drugs on the environment, and the choice of seed testing is a consolidated practice, because it is low cost, easy to use, and offers results in a short period of time. Therefore, the aim of this study was to evaluate the difference in sensitivity between two seeds, mustard and wheat, exposed to the anti-inflammatory drug nimesulide, determined through the results of root length and germination. The methodology of this study followed ISO 11269-2 and OECD Test No. 208. Statistical analysis was performed using ANOVA test, Dunnett's test and Tukey's test.

The results of the phytotoxicity test showed statistical difference between the sensitivity of the analyzed seeds, in both observed parameters. The elongation of the mustard roots, as well as the germination, suffered a reducing effect with  $p < 0.05$ . The EC50 values with commercial drug nimesulide for mustard seed was  $614.15 \text{ mg.L}^{-1}$ , and wheat seed suffered no negative effect at the concentrations evaluated. Finally, with the present study it was possible to conclude that the anti-inflammatory drug nimesulide presented different toxicological effects between mustard and arugula seeds.

## 6A.O-Mo-4

### Shining a Light on the Dark Side: The Toxicity and Environmental Impacts of Lightsticks

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#### Abstract

Lightsticks (LS) consists of artifacts that emit light up to 48h through a chemical reaction between an oxalate ester and hydrogen peroxide, catalyzed by a viscous solvent (phthalate); the color is often associated with polycyclic aromatic hydrocarbons (PAHs). LS are mainly used as party bracelets and for surface longline fishing. Longline fishing vessels use a multitude of LS each night, but countless LS are lost or discarded in the sea and carried to the beaches. LS also may leach their contents and contaminate the water and sediment. This study evaluated the toxicity of the liquid inside LS from the beaches of Costa dos Coqueiros (Bahia, Brazil), assessing the effects on several marine species: mussel *Perna Perna* (embryo, ammonia excretion rate, oxygen consumption, and behavior), macroalgae *Ulva lactuca* (chlorophyll-a and phaeopigment concentrations), sea urchin *Arbacia lixula* and sand dollar *Mellita quinquiesperforata* (embryo development), and the mysid *Promysis atlantica* (mortality, behavior). *P. atlantica* and *A. lixula* embryos were also used in a phase 1 of Toxicity Identification and Evaluation - TIE. The liquid inside LS caused deformations in mussel, sand dollar and sea urchin larvae at low concentrations (EC50 24 h 0.000086% [0.00005-0.00012], 48 h 0.00018% [0.00008-0.0004], and 24 h 0.00031% [0.00022-0.0004] respectively), altered the metabolic rate of mussels at lower concentration EC50 (20 min) 0.00024% (0.0000 -0.0007), caused the mussels to close their valves to avoid contamination EC50 (20 min) 0.0007% (0.00029-0.0017), and even made them try to climb the aquarium by using their byssus to scape (0.002%). A similar effect was observed to the mysids, which tried to escape the contaminants by swimming to the surface (0.005%). The macroalgae exhibited loss of Cl-a (EC50 96 h 0.00058% [0.0005-0.0007]) and increased levels of phaeopigments (EC50 96 h 0.003% [0.0002-0.024]), leading to their death. In TIE experiments, treatment with a C18 column significantly reduced toxicity, demonstrating that polar compounds like PAHs may be responsible for the toxicity. Based on the mysid test, volatile and oxidizable compounds also reduced toxicity, suggesting that compounds such as hydrogen peroxide and phthalates contribute to the toxicity. It can be concluded that LS pose an ecotoxicological risk to the marine environment from the concentration of 0.00008%, and their disposal must be regulated and monitored to prevent any harmful effects to marine biota.

## 6A.O-Mo-5

### Monensin Antibiotic Degradation in Agricultural Soil Amended with Poultry Litter

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#### Abstract

The application of organic amendments such as poultry litter (PL) is a common practice in horticulture, as it contributes nutrients, such as N, P and K, and it improves the physical, chemical and biological properties of the soil. PL is one of the three main residues from poultry farming and one of its functions is to receive the animal excreta. Ionophore polyether antibiotics (IPA) are one of the most used families in poultry farming, of which monensin (MON) is the most relevant one. The IPAs are incorporated into the balanced feed, with up to 90% of them being excreted without metabolizing. Once the PL is used to amend the soil, the IPAs in it can degrade, runoff into surface watercourses, percolate into the groundwater, or be incorporated and translocate within the plant. Therefore, the objective was to evaluate the degradation kinetics of MON in a horticultural soil with and without the addition of poultry litter. The soil was collected from the horticultural greenbelt surrounding La Plata, Buenos Aires, Argentina. The test was carried out in plastic trays and the application dose was 30 tn/ha. Three treatments were carried out: a control (C), soil spiked with MON (T1) and soil with PL previously spiked with MON (T2). The trial was conducted for 50 days. The extraction was carried out with methanol and sonication, with the IPA maduramicin as the internal standard. The analysis of IPA was carried out via HPLC Waters Alliance coupled to Quattro Premier XE Tandem Quadrupole Mass Spectrometer equipped with an ESI source set in the positive mode. Under the test conditions, for both treatments a first-order degradation fit was observed, with a half-life for MON of 50 h for T1, while for T2, the half-life value turned out to be more than three times higher than that for T1. After 7 d, the residual MON concentration in T1 was 2.8% with respect to the initial concentration, while for T2 it was 82.8%. It is then that the degradation of MON in soil slows down when it is introduced via poultry litter. Due to its continuous use as an organic amendment and the calculated half-life times, poultry litter with antibiotic residues could lead to the accumulation of these contaminants in the soil and generate various environmental consequences associated with their presence, such as the spread of microbial resistance, effects on terrestrial organisms or their mobilization into watercourses.

## 6A.O-Mo-6

### Use of High-resolution Respirometry for Ecotoxicological Evaluation of Benzoylecgonine at Environmental Concentrations

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#### Abstract

Cocaine and its metabolites are considered emerging contaminants, which are chemical substances exogenous to the environment with the potential to cause toxic effects to human and animal life. After consumption, cocaine is metabolized in the liver and excreted in the urine, mainly in the form of the metabolite benzoylecgonine (BE). Toxicological studies related to cocaine are dedicated to evaluating effects caused to health from the point of view of its consumption as a drug of abuse and, therefore, based on concentrations of the order of milligrams per liter. Thus, there is a need to evaluate the toxic effects of cocaine and its metabolites seen as environmental contaminants. Therefore, this work aims to evaluate the ecotoxicological effects of BE through high-resolution respirometry at the cellular level. The BE concentrations used in this study varied between 0.05 and 1000  $\mu\text{L}^{-1}$  according to values reported in the literature on the presence of this substance in the environment and in ecotoxicity tests on test organisms. Cells of the VERO lineage (from African green monkey kidney) were inoculated per mL of RPMI medium supplemented with fetal bovine serum, penicillin and L-glutamine, in 75  $\text{cm}^2$  flasks. After 24 h of incubation at 37°C, each flask had its culture medium replaced as follows: CT (supplemented RPMI medium only); ME (supplemented RPMI and addition of methanol); and BE (supplemented RPMI and addition of a concentration of BE). The flasks remained for another 24 h at a temperature of 37°C and were subsequently added to a high-performance oxygraph (Oroboros Co., model 2k, Austria) with a buffered breathing solution. When oxygen flow readings reached stable values, antimycin A was added to make sure that the measured oxygen consumption corresponded only to mitochondrial respiration. The first tests were conducted with concentrations of 0.05  $\mu\text{L}^{-1}$  (BE1) and 5  $\mu\text{L}^{-1}$  (BE2) of benzoylecgonine. The median values of oxygen consumption rate were 19, 18.55, 20.62 and 18.12  $\text{nmol O}_2 \text{ min}^{-1} 10^{-7}$  cells for CT, ME, BE1 and BE2, respectively. The Kruskal-Wallis statistical test indicated that these values do not differ statistically, suggesting that, at these concentrations, the profile of mitochondrial respiration remains unaltered. The next tests will evaluate the mitochondrial respiration of cells exposed to BE concentrations varying between 50 and 1000  $\mu\text{L}^{-1}$ .



## 6B.O-Mo-1

### Monitoring of the Cocaine and Benzoyllecgonine Seasonal Behavior in River Waters in Rio de Janeiro/Brazil

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#### Abstract

Cocaine (COC) and benzoyllecgonine (BE) have been detected in surface waters associated with domestic sewage. In this work, COC and BE were monitored in 5 urban rivers in Rio de Janeiro/Brazil in the dry and rainy seasons. Samples from Canal do Cunha (PCC), Canal do Mangue (PCM), Rio Carioca (PRC), Rio Irajá (PRI) and Rio Meriti (PRM), were acidified to pH 2, transported at  $T \approx 8$  °C, filtered through glass microfiber (1.2  $\mu\text{m}$ ) and cellulose acetate membrane (0.2  $\mu\text{m}$ ) and submitted to solid phase extraction (250 mL of sample) with Oasis HLB® cartridges (6 mL, 500 mg). COC and BE were analyzed in LC-EMAR (Dionex 3000 UHPLC, Thermo Scientific), with a C18 column (Thermo Scientific) at 40 °C, and aqueous and methanolic mobile phases. The Q-Exactive Plus mass spectrometer (Thermo Scientific) equipped with electrospray ionization was set to positive  $[M+H]^+$  mode and  $RT=4.44$  (COC) and 4.36 (BE). Analyte area/internal standard area (COC-d3) was applied for quantification of COC and BE in the samples and analytical curve ( $r^2 > 0.990$ ). The recovery was between 92-84% and 73-61%, DPR% between 7.0-7.1% and 4.7-13.8%,  $LD=8.94$  and 3.09 ng.L<sup>-1</sup> and  $LQ=29.80$  and 10.29 ng.L<sup>-1</sup> for COC and BE, respectively. T °C and pH were analyzed with a multiparameter probe and N-NH<sub>3</sub> and COD were analyzed in a spectrophotometric method. T °C, pH, N-NH<sub>3</sub> and COD resulted in mean values of 22.33 °C, 7.07, 18.96 mg.L<sup>-1</sup> and 180.15 mg.L<sup>-1</sup> during the dry season and 24.69 °C, 7.12, 12.47 mg.L<sup>-1</sup>, 177.86 mg.L<sup>-1</sup> in the rainy season, respectively. COC and BE were quantified ( $>LQ$ ) in 100% of the samples during the monitoring, with higher average levels of COC (383.68 ng.L<sup>-1</sup>) and BE (1022.16 ng.L<sup>-1</sup>) in the rainy season, highlighting COC in PCC (1089.96 ng L<sup>-1</sup>) and BE in PRM (2004.39 ng.L<sup>-1</sup>). COC and BE were detected on average levels at 192.27 and 504.30 ng.L<sup>-1</sup> in the dry season, respectively. The highest concentrations observed in the rainy season contrast with the lowest observed results for N-NH<sub>3</sub> and COD, suggesting a greater association with recreational COC consumption, commonly associated with spring and summer (rainy season). Higher levels of BE are expected in these environments, considering the hydrolysis of COC when pH close to 7 and room temperature. Our findings highlight to the social/sanitary problem of the presence of COC and BE in urban rivers, indicating the input of sewage into these sources and suggesting the drugs of abuse consumption profile of the related population.

## 6B.O-Mo-2

### Occurrence and Implications of Chemicals of Emerging Concern (CECs) in Wastewater Systems of Cartagena and Barranquilla, Colombia

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#### Abstract

Chemicals of Emerging Concern (CECs) are a diverse group of compounds, including pharmaceuticals, personal care products, illicit drugs, and estrogen-like endocrine disruptors, that are found in water systems and have significant biological activity, impacting both human and aquatic life. In this study, samples were collected from 25 different locations in Cartagena and Barranquilla, Colombia, including submarine discharge, marshes, and rivers associated with wastewater systems. A total of 28 CECs, including some metabolites, were detected and categorized into 10 groups, which included illicit substances (such as cocaine, benzoylecgonine, 3,4-Methylenedioxyamphetamine, MDA), stimulant substances (such as nicotine, cotinine, caffeine, dimethyl xanthine, fexofenadine), antihistamines (Fexofenadine), pain relievers (Tramadol, Odesmethyltramadol, Codeine, Didydrocodeine), hypertension medications (Irbesartan), lipid regulators (Atorvastatin, 2-Hydroxy-Atorvastatin), preservatives (Methylparaben), UV filters (Benzophenone-1, Benzophenone-2, Benzophenone-4), non-steroidal anti-inflammatories (Naproxen, O-Desmethylnaproxen, Acetaminophen, Ketoprofen, Ibuprofen, 2-Hydroxyibuprofen, Diclofenac, 4-Hydroxy-Diclofenac), and plasticizers (Bisphenol A). The samples were passed through GF/F filters and adjusted to a pH range of 6.5-8.0. The samples were spiked with 50 ng of all internal standards and loaded onto Oasis HLB cartridges. Elution was carried out using MeOH at a flow rate of 1 mL/min. The extracts were dried under a nitrogen evaporator and then adjusted to 500  $\mu$ L with MeOH. The vials were analyzed using a UPLC coupled with a Triple Quadrupole Mass Spectrometer. A mobile phase consisting of 80:20 H<sub>2</sub>O: MeOH with 1 mM NH<sub>4</sub>F was used to separate acidic compounds, while basic compounds were separated using 5 mM NH<sub>4</sub>OAc in 80:20 H<sub>2</sub>O: MeOH with 0.3% CH<sub>3</sub>COOH (mobile phase A) and MeOH (mobile phase B). Both methods utilized a reversed-phase BEH C18 column. The results of this study revealed that the concentrations of acetaminophen, naproxen, caffeine, ibuprofen, 2-Hydroxyibuprofen, cocaine, and benzoylecgonine were relatively higher, with mean  $\pm$  SD values of  $26 \pm 0.28$ ,  $39 \pm 19$ ,  $20 \pm 0.9$ ,  $5.9 \pm 5.2$ ,  $1.4 \pm 0.67$ ,  $0.34 \pm 0.053$ , and  $1.7 \pm 0.46$  ng/L, respectively. These findings were further normalized according to population size and mass loads (mg/day/1000 inhabitants) to enable meaningful comparison with other sites.

## 6B.O-Mo-3

### Contaminants of Emerging Concern (CECs): From Wastewater to Food Crops

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#### Abstract

Municipal treated wastewater and biosolids are historically under-utilized resources, but are finding increasing uses in agriculture. Due to urbanization and climate-change induced water scarcity, treated wastewater is viewed as a valuable water source for agricultural irrigation, especially in arid and semi-arid regions. Biosolids, the primary by-product of wastewater treatment, are widely used as soil amendments for their nutrient value. Irrigation with treated wastewater and application of biosolids or animal waste introduce numerous contaminants of emerging concern (CECs) into our food crop production systems. Transfer of CECs in these “waste” sources from soil to our food produce constitutes a potential food safety and human health risk. We have carried out systematic research to evaluate uptake of common CECs into vegetables and to mechanistically understand their plant metabolism pathways. A comprehensive risk assessment and management of CECs in agroecosystems is constrained by multiple factors, the utmost of which is the enormous number and diverse characteristics of CECs. Here we provide an overview of current knowledge, and then outline a bottom-up approach as the path forward to better address this concern. We further propose several strategies to minimize the potential risk of CECs during the beneficial reuse of treated wastewater and biosolids. This research contributes to environmental sustainability while ensuring safety in beneficial reuse.

DRAFT

## 6B.O-Mo-4

### Seasonal Variations of Human Pharmaceuticals in Rivers of the Metropolitan Region of Buenos Aires

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#### Abstract

Urban wastewaters represent a primary source of human pharmaceuticals (HPs) in aquatic environments. This work aimed to assess seasonal variation of HPs and identify those more relevant in the rivers of the metropolitan area of Buenos Aires (AMBA). Two campaigns were carried out, one in summer and the other in winter, along seven watercourses within the AMBA, sampling at 3 to 5 sites per stream. From north to south, selected watercourses were Lujan, Reconquista, and Matanza-Riachuelo rivers, and Del Gato, Maldonado, El Pescado, and Espinillo streams. Samples were filtered in situ and, in the laboratory, submitted to solid phase extraction (SPE) and analyzed by HPLC-MS. Thirteen HPs were analyzed: Acetaminophen (ACE), Alprazolam (ALP), Atenolol (ATE), Bromazepam (BRO), Carbamazepine (CBZ), Clonazepam (CLO), Diphenhydramine (DIP), Fluoxetine (FLX), Ibuprofen (IBU), Lorazepam (LOR), Metformin (MET), Salbutamol (SAL) and Sildenafil (SIL). HPs were classified according to their concentrations ( $\mu\text{g/L}$ ) and frequencies (%) in four groups: dominant ( $>1; >50$ ), frequent ( $<1; >50$ ), occasional ( $>1; <50$ ), and rare ( $<1; <50$ ). Results showed that IBU and ACE were dominant, while CRV, ALP, and DIP were rare and the others frequent. Both average frequencies and mean total HPs concentration were significantly different among seasons. However, while frequencies were higher in summer ( $85 \pm 11\%$ ) than in winter ( $51 \pm 26\%$ ), concentrations were higher in winter ( $8.8 \pm 10.1 \mu\text{g/l}$ ) than in summer ( $3.1 \pm 3.7 \mu\text{g/l}$ ). Only 6 out of 13 HPs showed significant differences in concentration among seasons, three were higher in winter (ACE, ATE, and SAL), and three were more elevated in summer (BRO, ENA, and SIL). In conclusion, the dominant HPs in the region were ACE, CBZ, and IBU. Additionally, despite seasonal variations being observed with higher frequencies in summer, the behaviors of the concentrations were particular to each HP.

## 6B.O-Mo-5

### **Ecotoxicity of Silver Nanoparticles in the Presence of Natural Organic Matter: Exposure Waterborne and Dietborne Simple Food Chain Algae-Microcrustacean**

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#### **Abstract**

The current expansion of nanotechnology and the massive use of nanoparticles (NP) in commercial products can provoke a contamination of fresh waters and aquatic organisms. Generally, nanomaterials (NMs) add ionic or polymeric coatings to improve their mobility and stabilization in terms of size. The present work aims evaluate the toxic effects of two manufactured coated (Ppolyethylene glycol and Citrate, Peg and Cit, respectively) silver nanoparticles (AgNP) in absence and presence of humic substances (HS) on the algae *Raphidocelis subcapitata* and microcrustacean *Daphnia similis* in order to explore the impact of natural molecules on their environmental risk of these NMs in the aquatic environment. Additionally, waterborne and dietborne exposition using a simple food chain Algae-Daphnia for the, neonatal and adult *Daphnia similis* test-organism. The organisms were exposed to AgNP with coatings manufactured in the absence and presence of HS and naturally EPS (since they will be excreted by algae simultaneously). In parallel to the exposures of NPs, the effects of equivalent concentrations of total and free Ag in solution were quantified. Growth inhibition of algal growth was determined by the biomass produced after the tests using the count of the initial and final cell numbers. Firstly, the toxic effect for silver ions observed did not changed with the presence of natural organic matter. The difference of capping agents as well as nanoparticle concentration shows different toxicological responses by observing a AgPeg toxicity compared to the same Ag concentration with Citrate which did not presented toxic effect. The toxicity of Peg can be related to the dissolution behavior assessed in previous chapter, also associated to the solution exposed and exposition time. For bioaccumulation, the presence of natural organic matter enhanced the silver amount in the algae probably due to surface bound of HS-Ag. Finally, the presence of NOM influences in the silver bioaccumulation, and for citrate capped nanoparticles, there is a decrease of silver accumulated with the increase of humic material. A greater toxic sensitivity of micro crustaceans compared to algae can also be observed. For algae-microcrustacean simple food chain experiment, it was possible to observe that the toxic effect provided by microalgae dietary can be noticed, evidencing the importance of the studies considering the fate of nanomaterials in the environment.

## 6B.O-Mo-6

### Evaluation of the Potential Chemical Risk of the Use of Disinfectants in Freshwater Environments. A Theoretical Study

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#### Abstract

The increase in the use of disinfectants from the year 2020, due to the pandemic with COVID-19, an absence of an adequate regulation, from the perspective of environmental protection, for the registration and authorization of the use of disinfectant/sanitizing products in Chile was identified. For this, a theoretical exercise of environmental risk evaluation was carried out for the active principles registered in the Institute of Public Health of Chile. To this end, a preliminary hazard analysis was carried out for a freshwater organism (*Daphnia magna*). The hazard assessment was carried out using a new regulation currently in force for the registration of chemicals products in aquaculture activities in Chile. This consists of evaluating certain hazard properties (eg, carcinogenicity, teratogenicity, mutagenicity, endocrine disruption, etc., as well as persistence properties, bioaccumulation potential and toxicity, as well as scenarios for estimating the level of exposure to disinfecting agents. After analyzing more than 25 active principles, the following active principles: sodium hypochlorite, alkyldimethylbenzyl ammonium chloride, didecyldimethyl ammonium chloride, 2-phenylphenol and copper particles and nanoparticles, sodium chlorite and sodium dichloroisocyanurate, were considered a potential hazard for freshwater organisms due to their properties of persistence, bioaccumulation and toxicity in the bioindicators *Raphidocelis subcapitata* (microalgae), *Daphnia magna* (invertebrates) and *Danio rerio* (fish), and in some cases, endocrine disrupting properties. In the same way, the active ingredients: alkyldimethylbenzyl ammonium chloride, didecyldimethyl ammonium chloride, 2-phenylphenol and copper particles and nanoparticles were considered a potential risk for freshwater organisms due to the expected risk coefficients greater than 1, considered a worst case exposure scenario for the invertebrate, *Daphnia magna*. It is discussed that there are conditions to improve registration procedures to ensure that the potential to affect freshwater ecosystems by this type of chemicals substances is reduced. Supported by FONDAP/ ANID 15130015.

## 6D.O-Tu-3

### North Pacific Garbage Patch: Where are Microplastics Finally Sinking?

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#### Abstract

The extent of microplastic pollution in deep-sea sediments remains poorly quantified, but this knowledge is imperative for predicting the distribution and potential impacts of global plastic pollution. The Clarion Clipperton Zone (CCZ) is a submarine area of 1,700,000 km<sup>2</sup>, located in the North Pacific Ocean with particular potential for submarine mining due to the presence of polymetallic nodules but also is nearby to the North Pacific Garbage Patch. In this work, the first 5 cm of the bottom sediments and nodules (depth ~4500 m) obtained with an octopus multi-core were used to evaluate the presence of microplastics (MPs). MPs were separated from dry sediments and nodules using a saturated NaCl solution and the supernatant was filtered using a glass filter previously muffled. Retained particles in the filters were visually inspected under a stereomicroscope, and Raman spectroscopy was performed to identify their polymer composition. A total of 23 sediments and nodules samples were analyzed from the CCZ. Results showed that 41,1% and 34,7% of sediment and nodules samples, respectively, showed at least 1 microplastic particle. The average concentration of MPs in sediments was 16.5 items/kg whereas in nodules was 13.6 items/kg with an average size of  $0.94 \pm 0.66$  mm. Fibers predominated all samples (82%), followed by films (11.7%), and fragments (5.8%). Blue was the most abundant color (42.8%), followed by green (23.8%), and transparent (9.5%); other colors presented percentages less than 5%. Finally, MPs polymeric composition corresponded to polyacrylonitrile and polyethylene terephthalate, and colorants such as indigo blue, acetoacetic arylide, and phtalocianine. Even though MPs concentrations were lower than expected considering their proximity to the North Pacific Garbage Patch, these results demonstrate for the first time the presence of microplastic particles, mainly synthetic microfibers, in the seabed of CCZ.

# Session 7: Tracking Primary Pollutants and Their Toxicologically Relevant Transformation Products

## 7A.O-Tu-1

### Butyltins and Phenyltins Concentrations in Gastropods and Sediments From the Colombian Caribbean Coast

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#### Abstract

Many studies worldwide have examined imposex and organotin compounds (OTC) contamination, focused on regions such as Europe, North America, Asia, North Africa, and South America. Surprisingly, OTC have not been quantified in Colombia. Therefore, this study evaluated, for the first time, the current status of OTC contamination in sediments and gastropods of areas under the influence of maritime activities (main port, shipyard, and marinas) in Cartagena Bay. Concentrations of Butyltins -tributyltin (TBT), dibutyltin (DBT), and monobutyltin (MBT)- collected in 16 sites in Cartagena Bay ranged from 1.1 to 164.4 ng g<sup>-1</sup>, with the highest levels found in a shipyard of Contecar (164.4 ng g<sup>-1</sup>), a small harbor of the Bahía de las Animas (118.1 ng g<sup>-1</sup>), in front of industrial company Argos (61.4 ng g<sup>-1</sup>) and a small port at Ciénaga de las Quintas (50.7 ng g<sup>-1</sup>). Conversely, low levels of Phenyltins -triphenyltin (TPhT), diphenyltin (DPhT), and monophenyltin (MPhT) were found, ranging from 1 to 6.9 ng g<sup>-1</sup>, with the highest levels detected in a small port at Ciénaga de las Quintas (6.9 ng g<sup>-1</sup>), in front of the industrial companies Ecopetrol (3.3 ng g<sup>-1</sup>) and Zona Franca (2.4 ng g<sup>-1</sup>). The degradation indices of butyltin compounds (BDI) in all sites showed values greater than 1, indicating that their contributions may be old or their degradation very rapid. However, in front of these companies and a small port of Ciénaga de las Quintas, the degradation indices of phenyltin compounds (PhDI) were less than 1, suggesting that the contributions of phenyltin to the sediments was new or their degradation has been slow. The gastropod *Strombus pugilis* collected from Cartagena Bay, showed concentrations of butyltin in females of 19.2 ng g<sup>-1</sup> and 17.0 ng g<sup>-1</sup> in males. For *Melongena melongena*, concentrations in two sites of Cartagena Bay were 114.2 ng g<sup>-1</sup> in females and 33.1 ng g<sup>-1</sup> in males in a small harbor of the Bahía de las Animas, and 35.1 ng g<sup>-1</sup> in females and 30.8 ng g<sup>-1</sup> in males in the Club Nautico marina. No phenyltin concentrations were found in any gastropod samples. According to OSPAR the concentrations of our sediments are classified as C and D, likely to cause effects after long-term exposure or compromise reproduction in gastropods. Our study provides insights into the concentrations of organotin compounds in Colombian coasts. These results could be valuable for policymakers to develop effective strategies to control and monitor these compounds in aquatic ecosystems.



## 7A.O-Tu-2

### Polycyclic Aromatic Hydrocarbons in a Natural Heritage Estuary Influenced by Anthropogenic Activities in the South Atlantic: Integrating Multiple Source Apportionment Approaches

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#### Abstract

Polycyclic aromatic hydrocarbons (PAHs) were analyzed in the sediments of one of the most well-preserved estuaries in South Brazil, the Paranaguá Estuarine System (PES), an ecosystem threatened by the activities of Brazil's largest bulk port and the pressure from the economic and political actions to install a series of port and oil infrastructure projects. The  $\Sigma$ PAH ranged from < DL to 125.6 ng g<sup>-1</sup> dw (dry weight) (average 29.9 ± 26.1 ng g<sup>-1</sup> dw), and the lowest levels detected were similar to those found in other protected areas of the world. In general, the PAH concentrations indicated excellent environmental quality for the entire estuary, but the highest concentrations of PAHs, observed in Antonina Bay, provide evidence of anthropogenic impacts on the Rio Cachoeira watershed resulting from the construction of an interconnection between large rivers and from years of intense deforestation in the Atlantic Forest. PAHs sources were investigated using several source apportionment tools. Principal component analysis (PCA) indicated that fine sediments and total organic carbon were the main factors controlling PAH concentrations in the PES. Multiple PAH sources were identified in the study area, and the predominant sources were related to biomass burning and fossil fuel combustion, as indicated by the analysis of PAH groups and diagnostic ratios. However, a considerable presence of petrogenic influences was also identified by PCA and Positive Matrix Factorization Model analysis (PMF). PMF identified the diagenetic process as the factor that contributes most to the total variance (36.5%), followed by recent petrogenic inputs (30.3%) probably related to the use of fuels for navigation, which results in chronic contamination from small oil spills. Fossil fuel combustion (22.1%) and petrol and paper industries (11.1%) were also identified as source factor profiles, demonstrating the model accuracy to identify even smaller sources, such as the paper industries. These results suggest that in the future, the PES, even in its less inhabited areas, may no longer be considered an environment that is isolated from human actions, and vulnerable regions such as the Paraná coast need socioenvironmental schemes that monitor, prevent and mitigate chemical stressor contamination and other environmental issues related to large oil infrastructure projects.

## 7A.O-Tu-3

### The Successfully Reducing the Risk of Imidacloprid in Australia's Great Barrier Reef Catchments

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#### Abstract

Due predominantly to the adverse effects of imidacloprid on arthropods, particularly species involved with plant pollination, there is mounting worldwide pressure to limit the use of neonicotinoid insecticides, which contributed to the 2018 European Union ban on all outdoor use of clothianidin, imidacloprid and thiamethoxam. Due to the physicochemical properties of imidacloprid, the potential for movement from agricultural land into waterways is high. In addition to the identified risks in Europe, there is new evidence about the risk of imidacloprid to surface waters and aquatic ecosystems. There is anecdotal evidence in Australia of increasing application of imidacloprid to agricultural land. Water quality monitoring has identified significantly ( $p \leq 0.05$ ) increasing imidacloprid concentrations and ecological risk over time in several major waterways discharging to the Great Barrier Reef lagoon. This study will discuss the monitoring results, data analysis and possible reasons for these increases in concentrations. More importantly recent results are indicating that detected concentrations are declining due to land management interventions, thus potentially reducing impacts to aquatic arthropod species. Whether or not there is ecosystem harm, the decreasing concentrations in measured imidacloprid is an important step in protecting the ecosystems of Australia's Great Barrier Reef catchments.

## 7A.O-Tu-4

### Preliminary Data on Internal Concentration of Per- and Polyfluoroalkyl Substances (PFASs) in a Marine Tropical Amphipod

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#### Abstract

Per- and polyfluoroalkyl substances (PFASs) are synthetic compounds used as surfactants since the early 1950s in cookware, cosmetics, military equipment, and fabrics. Many of these compounds present long half-life, classifying them as bioaccumulative. Perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are the most prevalent PFASs, and their toxic effects are related to metabolic alterations in lipid metabolism and decrease in growth, reproduction, and survival of many organisms. PFASs were already detected in distinct environments, even in polar regions. Despite the existing studies related to PFASs, environmental monitoring data are still few. *Parhyale hawaiiensis* is an epibenthic marine amphipod that has circumtropical distribution, presents sensitivity to pollutants and has been highlighted as an experimental model in several areas, including ecotoxicology. The hemolymph of these organisms was already used for the determination of other pollutants. An analytical method by liquid chromatography-tandem mass spectrometry for the analysis of PFOS and PFOA is being developed on the LCMS8060 (Shimadzu®, Kyoto Japan). Chromatographic separation is performed with an Acquity UPLC HSS T3 (1.8  $\mu$ m, 2.1 x 50 mm, Waters, Milford, MA, EUA) column. The mass spectrometer is equipped with an electrospray ionization source, operating in negative mode. A calibration curve of 0.1-100 ng/mL (LOQ/LOD = 0.1 ng/mL) was initially determined. Adult organisms (4,  $\leq$  8 months-old) were exposed to different PFASs concentrations (0, 10 and 100 ng/mL) in a 1:1 sex ratio for 96h. Then, the hemolymph of these organisms was collected with a thin glass needle and a pool with hemolymph from 4 organisms was diluted in 20  $\mu$ L of reconstituted seawater. Each pool was diluted with 180  $\mu$ L of ultrapure water (dilution of 1:10) to reduce salt concentration, and 10  $\mu$ L was injected into the LCMS system. The found concentrations of PFOA in the hemolymph were 13.3 and 188.5 ng/mL, following the respective exposure concentrations 10 and 100 ng/mL, and the found concentrations for PFOS were 15.1 and 239.1 ng/mL, respectively. The higher PFOS results is expected considering its higher bioaccumulation potential already seen in other species. The developed methodology seems promising as a monitoring tool, and more experiments will be performed to provide a better understanding of some parameters as bioconcentration factors for each PFASs in *P. hawaiiensis*.

## 7A.O-Tu-5

### Paraben Transport and Transformation and Bioaccumulation Natural Waters and Engineered Systems

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#### Abstract

Parabens are commonly used preservatives that are weakly estrogenic. Parabens enter wastewater treatment plants (WWTPs) in large quantities, and their presence in wastewater effluent is the greatest contributor of parabens in surface waters. Parabens are well removed in WWTPs, although little is known about paraben transformation product concentrations in wastewater effluent. We evaluated paraben transformation in WWTPs and release into the Brazos River (Texas, USA) throughout a calendar year. Paraben concentrations were quantified in upstream and downstream reaches of the river, and in resident fish. Influent concentrations and transformation rates varied seasonally at the two WWTPs. Para-hydroxy benzoic acid was present in greatest amounts in influent and effluent at both WWTPs. Parent paraben transformation rates were greater at plant 1. Dichlorinated transformation products increased as much as 10X in some cases. Shorter chained parabens were generally transformed more rapidly.

River water downstream of wastewater treatment outfalls had lower concentrations of methyl paraben. Para-hydroxybenzoic acid was the compound present in greatest concentration at most sites. Dichlorinated paraben concentrations increased downstream of WWTPs. Concentration increases indicate that effluent contains dichlorinated parabens in high enough concentrations to effect concentrations in the river. Dichlorinated species also persisted, with no significant decreases at sites further downstream. Methyl paraben concentrations decreased at the site furthest downstream while dichlorinated methyl paraben concentrations remained stable showing that the dichlorinated species degraded slower than their respective parent paraben. Due to the dichlorinated species being released in higher concentrations in effluent than parents and being more resistant to degradation, dichlorinated parabens are more likely to be environmentally relevant than are parent parabens. The nuances of chemical profiles indicate that sorption and bioaccumulation processes are important.

Parabens and their transformation products are differentially accumulated by the two evaluated fish species, smallmouth buffalo (*Ictiobus bubalus*) and gizzard shad (*Dorosoma cepedianum*). Parent parabens and mono and dichloro disinfection byproducts were found in fish. Uptake depends on chemical speciation and the biological species that is exposed. Assays to determine endocrine effects show minimal estrogenic activity.

## 7A.O-Tu-6

### Effects of Antimicrobials and their Transformation Products on Antibiotic Resistance

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#### Abstract

There are many biocides used in various applications, including health and beauty products that are discharged to wastewater treatment plants and ultimately surface waters. There is increasing awareness of the development of antibiotic resistant microorganisms. For that reason, in September 2016, the US FDA announced a ban on nineteen ingredients in consumer antibacterial soaps citing a lack of evidence for safety and effectiveness. However, due to the need for antimicrobials and insufficient information several are still allowed and used in relatively large amounts so bans on benzalkonium chloride (BKC), was deferred to allow ongoing studies to be completed. Here we focus on two of the most used biocides, BKC, which is used in bar soaps, and benzethonium chloride (BZC), which is used in liquid hand soaps. BKC is classed as a Category III antiseptic active ingredient by the United States Food and Drug Administration (FDA). Ingredients are categorized as Category III when "available data are insufficient to classify as safe and effective, and further testing is required". Among the data gaps is whether these two biocides can cause antimicrobial resistance. In addition, these two compounds are transformed during wastewater treatment and in the environment. Here we present information on pathways of degradation and products formed and mechanisms of toxicity of transformation products to microbes and whether exposure to these transformation products can result in microbial resistance. We surveyed concentrations of biocides in wastewater in Canada and measured antibiotic resistant bacteria in wastewater and surface water and conducted an assessment of risks posed by these two compounds relative to development of drug resistant bacteria.

## 7B.O-Tu-1

### Investigating Non-Lethal Approaches to Contaminant Assessment in Elasmobranchs: The Brazilian Guitarfish as a Case Study

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#### Abstract

Elasmobranchs are exceptionally prone to accumulate contaminants due to their life history strategies combined with high trophic positions. For this reason, this group has been pointed out as a good biomonitoring model. Parallely, sharks and batoids are amongst the most endangered vertebrate taxa nowadays, yet non-lethal approaches aiming to assess organic contamination in these organisms have scarcely been proposed. In this context, this study aimed to investigate the potential use of muscle and blood as non-lethal matrixes for Polycyclic Aromatic Hydrocarbons (PAHs) assessment. Sixteen PAHs were determined through gas-chromatography coupled to mass spectrometry in blood, liver, and muscle samples of the Brazilian guitarfish *Pseudobatos horkelii*. Correlations between tissue concentrations were assessed by Spearman's correlation test and Principal Component Analysis (PCA), whereas the Permutational Analysis of Variance (PERMANOVA) was used to test the influence of tissue on contaminant profiles. Liver presented the highest detection frequency for all analytes, whereas the detection frequency of PAHs was 7.7 – 100% in blood, and 35.3 – 100% in muscle. Naphthalene (100%), benzo[a]anthracene (92.3 – 100%), and chrysene (92.3 – 100%) were the most detected compounds in all tissues. Significant correlations were found for naphthalene concentrations in all tissues ( $r=0.565 - 0.882$ ). Correlations between blood and liver levels were found for benzo[a]anthracene ( $r=0.866$ ) and chrysene ( $r=0.784$ ). Finally, acenaphthylene levels in liver and muscle samples were positively correlated ( $r=0.570$ ). The PCA exploring relationships of PAHs in the three examined tissues showed a clear aggregation of blood samples, with modest overlap between tissues, suggesting that PAHs profiles were not similar. The PERMANOVA corroborated this hypothesis, as tissue was a determinant factor influencing contaminant profile ( $F=9.139$ ;  $p=0.001$ ). Liver is the main target organ for organic contaminants accumulation in elasmobranchs due to its high lipid content and biotransformation capacity. For this reason, this tissue is undoubtedly the more accurate matrix when assessing organic contaminant levels in elasmobranchs. Our results imply that, although non-lethal sampling should be preferred in studies using elasmobranchs as animal model, interpretation of contaminant levels assessed in blood and muscle biopsy samples must be cautious, since these tissues might not reflect hepatic loads.

## 7B.O-Tu-2

### Records of heavy metals in sediments of Concepción Bay, Central Chile: As, V, Cr, Mo, Co, Ni, Cu, Zn, Cd, Pb, Hg

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#### Abstract

In this study, we assess the environmental health status of Concepción Bay (36°40'S, 73°01'W), Chile, using sedimentary records. A sediment core was taken in the bay, cut in slices (1-25 cm) and analyzed for heavy metals (As, V, Cr, Mo, Co, Ni, Cu, Zn, Cd, Pb, Hg) (ICP-MS) and for radioisotopes (<sup>210</sup>Pb, constant rate supply dating). The core encompasses the years 1930-2014. Results showed the following concentrations (mg kg<sup>-1</sup>) inorganic chemicals levels were As (19±2), V (130±10), Cr (49±1), Mo (9.3±1), Co (9±0.5), Ni (19±0.6), Cu (37±4.3), Zn (81±6.3), Cd (3.6±0.6), Pb (24±3), Hg (0.1±1). Total organic carbon (TOC) was also measured (2.0±0.1%). The sedimentation rates, based on <sup>210</sup>Pb activities, were rather constant in the last 40 years (0.51±0.87 μg m<sup>-2</sup> yr<sup>-1</sup>). In general, the estimated fluxes showed two high values at the top of the sediment core (at 1 and 4 cm depth, corresponding to 1995). Except for some of the inorganic contaminants, these concentrations are low in comparison with historical organic contamination patterns of highly industrialized coastal environments in the northern hemisphere. Further research is needed to assess the implementation of national or international regulations i.e., the implementation of the Minamata Convention.

## 7B.O-Tu-3

### Glyphosate Dissipation in Soil and Flooded Water During Rice Production Cycle

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#### Abstract

Rice production has undergone a process of intensification which requires a comprehensive assessment of its environmental sustainability.

Rice is a crop that undergoes a flooded phase, thus the behavior of pesticides in both anaerobic and aerobic conditions is important as well as in the different crop rotations.

To address this, the National Institute of Agricultural Research (INIA) conducted a long-term experiment (ELP) in Paso de la Laguna, Treinta y Tres, Uruguay, analyzing the performance of rice rotations with varying intensity in land use. This study selected four rotations (continuous rice, rice-pasture, rice-soybean, and rice cropping followed by pasture) to investigate the behavior of glyphosate, a commonly used pesticide, as a fallow and weed control agent in rice cultivation using statistical modeling. Continuous rice is not a commercial practice, its established only as a reference.

The study involved analyzing glyphosate and its main metabolite (AMPA) in soil and floodwater samples taken from rice plots during the 2019/2020 production cycle. Glyphosate was applied twice in all rotations: the first application was used as a chemical fallow to eliminate cover crops, and the second application was made before rice emergence. The study analyzed the dynamics of glyphosate and AMPA concentration in soil and water separately by fitting exponential decay models at different time intervals from the first application to crop harvest, using rotations as covariates.

The study found that there were differences in the decay of glyphosate in soil between the aerobic and anaerobic phases of the crop. The first order exponential decay models had a good fit, and the decay of glyphosate differed between rotations based on the timing of the first application of glyphosate. In floodwater, the study observed a transfer of both compounds from soil to water and found no differences in the decay of glyphosate and AMPA between the different rotations.

These findings contribute to the understanding of the persistence and decay of glyphosate in soil and water in a system that alternate aerobic and anaerobic conditions.



## 7B.O-Tu-4

### Applications of Mass Spectrometry to Agroecosystem Conservation: Analysis of Glucosinolates in Broccoli (*Brassica oleracea* var. *italica*)

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#### Abstract

Glucosinolates (**GLS**) are natural products generated by Brassicaceae plants as a natural defense mechanism. GLS are degraded by enzymatic action forming, among other products, isothiocyanates for which nematocidal, fungicidal and bactericidal effects have been reported. This natural process is used in biofumigation, an agronomic practice with an agroecological approach for pest control, particularly of plant parasitic nematodes. Knowing the GLS doses used is fundamental to generate a comprehensive effect assessment. The GLS analysis methods reported in the literature mainly use two analysis approaches differentiated by the chemical structure of the molecule: on the one hand, intact GLS and on the other hand desulfo-glucosinolates (dsGLS) - products of enzymatic desulfation. The **objective** is to optimize, validate and compare different methodologies for the analysis of intact GLS and dsGLS on broccoli samples using tandem mass spectrometry in different working modes. Five analysis methodologies were developed: 3 for dsGLS and 2 for intact GLS. Extraction from broccoli plant material was performed on the previously lyophilized sample with a hot Methanol:H<sub>2</sub>O mixture. A portion of the extracts was diluted, filtered and used to analyze the intact GLS by LC-MS/MS (Alliance 2695, Quattro Premiere XE, Waters). Another portion of the extract was desulfated on an anion exchange resin with enzymatic action and analyzed for dsGLS by LC-MS/MS and HPLC-UV (Agilent 1100). All assays were evaluated against external standards for seven Sigma Aldrich and Extrasynthese reference GLS. Among the most relevant results, it was observed that all methods identify and quantify the principal GLS of broccoli samples and had a recovery of between 77 and 93%. The notable contribution of mass spectrometry tools in the intact GLS methods contributes to reducing the sample pretreatment processes without losing selectivity and sensitivity. In addition, the method of identification of intact GLS that was optimized with the specific loss of Sulfates in the QqQ system, allowed a qualitative profile of GLS of the plant material to be made quickly and without standards and to quantify those of interest.

## 7B.O-Tu-5

### Ozone and PM<sub>2.5</sub> Behavior in Small Cities in Southern Brazil

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#### Abstract

Air pollution is a significant global health issue, with the World Health Organization estimating that seven million people die annually due to pollutants from air pollution. In Brazil, less than 2% of over 5000 municipalities have continuous monitoring of atmospheric pollution, which can reduce life expectancy by up to three years depending on the region. However, few studies have evaluated the contribution of the transport of pollutants over long distances and from urban and/or industrial sources in small cities. The objective of this study was to evaluate the concentration of air pollutants in the five least populated cities in the state of Rio Grande do Sul, Brazil, and compare them with their reference cities and the state capital. We manually extracted atmospheric pollution (O<sub>3</sub> and PM<sub>2.5</sub> in  $\mu\text{g}\cdot\text{m}^{-3}$ ) and meteorological data from the Copernicus Atmospheric Monitoring Service between September 18, 2021, and October 17, 2021. Regarding O<sub>3</sub> concentration, the state capital, Porto Alegre, had four episodes in which the concentration was above the limits established in national legislation (100  $\mu\text{g}\cdot\text{m}^{-3}$ ), while all other cities remained below these limits. On the other hand, Porto Alegre did not exceed the PM<sub>2.5</sub> limits, while four of the five small cities and four of the five reference cities exceeded the limits established in the legislation (25  $\mu\text{g}\cdot\text{m}^{-3}$ ). The percentage of episodes in which small towns had higher daily O<sub>3</sub> concentrations than their reference cities varied between 23% and 80%, while the variation between these cities and the capital Porto Alegre was between 13% and 27%. The concentration of PM<sub>2.5</sub> was higher in small towns compared to the reference cities, ranging between 17% and 67%, and between 27% and 43% when compared to the capital Porto Alegre. Our findings demonstrate that, in the state of Rio Grande do Sul, the five less populated cities had an average concentration of O<sub>3</sub> and PM<sub>2.5</sub> similar to the reference cities in their micro-region and also comparable to the state capital, which has a population of over 1.5 million inhabitants. However, it is important to note that more extensive and continuous monitoring is necessary to fully understand the impact of air pollution on human health in these regions.

## 7B.O-Tu-6

### Forecast of Air Pollution Levels in Temperature Increase Scenarios Due to Climate Change

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#### Abstract

Climate change is an increasingly pressing issue, with the rise in emission sources and population growth majorly contributing to its impacts. The resulting greenhouse effect will have far-reaching implications for human populations, with unforeseen consequences in the coming years. One of the most significant health impacts of atmospheric pollution is respiratory illness, with pollutants such as ozone (O<sub>3</sub>) and particulate matter (PM<sub>10</sub> and PM<sub>2.5</sub>) causing negative health outcomes. Understanding the relationship between meteorological variables and these air pollutants is crucial in anticipating the effects of increased air temperatures caused by climate change.

This study sought to investigate the association between atmospheric pollutants and meteorological variables across different seasons of the year in seven municipalities in the state of Rio Grande do Sul, Brazil, jointly evaluating two scenarios considering temperature increases (+2 and +4 °C). Atmospheric pollution data was manually obtained from the Copernicus Atmospheric Monitoring Service (CAMS) and the meteorological variables (humidity, pressure, wind speed, UV radiation, among others) were obtained from the Brazilian National Institute of Meteorology (INMET). Pearson correlation analysis was performed to examine the relationship between meteorological variables and air pollutants. The analysis was conducted using an independent variable (temperature), dependent environmental variables (precipitation, pressure, humidity, wind speed, and UV index), and pollutants.

The results show that PM<sub>10</sub> and PM<sub>2.5</sub> followed constant values in all temperature scenarios, with observed values higher than predicted in most cases. O<sub>3</sub>, on the other hand, showed a significant increase in concentrations in stations with higher temperatures. The use of Machine Learning in the Statistica software (v.10.0) enabled these findings to be analyzed comprehensively. The study's findings suggest that climate change may result in increased concentrations of O<sub>3</sub> in areas with higher temperatures, exacerbating respiratory and cardiovascular complications. These findings have significant implications for policymakers and the broader society, highlighting the urgent need for proactive measures to mitigate the negative effects of climate change.

# Session 8: Environmental Mutagenesis in Human Populations

## 8.O-Tu-1

### Settleable Atmospheric Particulate Matter Harms a Marine Invertebrate: Integrating Chemical and Biological Damage in a Bivalve Model

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#### Abstract

Some atmospheric pollutants may affect aquatic ecosystems after settling, generating contamination, bioaccumulation, and threats to aquatic species. Metallurgical processes result in the emission of settleable atmospheric particulate matter (SePM), including metals and metalloids, along with rare earth elements (REE) that are considered emerging contaminants. SePM pollutes estuarine ecosystems, close to steel industries of southeast Brazil. We report the 30-days exposure of the brown mussel (*Perna perna*) to SePM from a polluted area, followed by a 30-days clearance period, looking to identify and understand the toxic potential of SePM to this model mollusk. Thus, the bioaccumulation of 28 elements and their sublethal effects on these aquatic invertebrates were evaluated. REE were found in SePM (Ce, Y and La). A significant bioaccumulation of eight metals (Fe, Ni, Cu, Zn, Rb, Sr, Cd and Ba) was observed in the bivalves exposed to SePM, which correlates with the observed cytotoxicity and genotoxicity in a dose-dependent mode, suggesting a pre-pathological condition that could lead to ecological disturbances over time. Conversely, the lack of lipid-peroxidation after the SePM exposure could indicate the effectiveness of the antioxidant system in protecting gills and digestive glands. The clearance period was not enough to successfully reverse the negative effects observed. So far, the current results enhance the comprehension on the negative role of SePM on the metal bioaccumulation and metal-induced toxicity to the aquatic biota. Thus, this report adds innovative findings on the role of SePM on the aquatic pollution in coastal areas affected by atmospheric pollution, which should be relevant for future public policies to verify and control the environmental pollution.

## 8.O-Tu-2

### Occupational Exposure of Farmers to Complex Mixtures During Tobacco Culture

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#### Abstract

The tobacco industry in southern Brazil is of significant socioeconomic importance, as many people are employed directly in this sector. Agricultural workers involved in tobacco cultivation are constantly exposed to large amounts of pesticides, as well as to nicotine present in raw tobacco leaves and tobacco-specific nitrosamines (TSNAs) in dry tobacco leaves. Pesticides are known to be potential chemical mutagens, as experimental data have shown that various agrochemicals possess mutagenic properties. Studies have also shown that nicotine absorbed through the skin can lead to a characteristic green tobacco sickness (GTS), an occupational illness reported by tobacco workers. Exposure to dried tobacco leaves has been linked to an increased risk of toxicity and respiratory illness due to the presence of nicotine and other chemicals, including TSNAs. Our studies aimed to evaluate the DNA damage and cellular mechanisms caused by exposure to tobacco growers during the application of pesticides, leaf harvest, and dry leaf classification process. Genotoxicity was evaluated using the alkaline comet assay (CA), micronucleus assay (lymphocytes and buccal cells), and measurement of telomere length (TL). We also measured the lipid peroxidation index (TBARS), nitric oxide metabolites (nitrates), and inorganic elements. We observed genotoxicity, including an increase in comet cells and micronuclei, and telomere reduction. Results showed a correlation with inorganic elements and oxidative stress. Principal component analysis (PCA) demonstrated discrimination between the groups (exposed and controls), and our data showed that tobacco workers exposed to pesticides, nicotine, and TSNAs presented DNA damage induced by different mechanisms, including redox imbalance. Financial support from FAPERGS, CAPES, and CNPq.

## 8.O-Tu-3

### Genetic Instability and Alteration of Expression Pattern of PPA1, AQP5, and MT-ATP6 Genes in Human Dental Fluorosis

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#### Abstract

Chronic exposure to fluoride, even in small amounts, when continuously ingested by the human population, can cause a serious public health problem called dental fluorosis. This condition can be aggravated by low body weight, skeletal growth rate, and periods of bone remodeling. The effects of fluoride on human health, as well as its potential to affect DNA, are not well understood. Our data demonstrate that individuals with dental fluorosis show a significant increase in micronucleus (MN) frequencies compared to non-fluorosis individuals. Results from the enzyme-modified comet assay suggest oxidation of purines in DNA as a result of fluoride exposure. We observed a decrease in telomere length (TL) and increased expression patterns of PPA1 (inorganic pyrophosphatase1) and AQP5 (Aquaporin 5) genes, as well as significant changes in cytokine release. There were significant correlations between the Thylstrup-Fejerskov index of fluorosis and age, and the levels of necrotic cells, fluoride, and MN, Visual score, and MT-ATP6 (Mitochondrially Encoded adenosine triphosphate Synthase Membrane Subunit 6) gene. Our data indicate the need to continue monitoring individuals with dental fluorosis to better understand DNA damage and the mechanisms that may be affecting genomic instability. Further studies in this area are still needed to support discussions about the necessity and regulation of drinking water fluoridation. FINANCIAL SUPPORT: CNPq, CAPES, FAPERGS.

## 8.O-Tu-4

### Effects of Contaminants of Emerging Concern on Avian Transcriptome: Understanding the Potential Impact of Perfluoro Alkyls Substances and Antibiotics on Wetlands Birds

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#### Abstract

Despite to be recognized as key ecosystems on earth, wetlands have been heavily altered worldwide. Mobilized by the water cycle, pollutants inevitably end on many wetlands affecting their ecological features including effects on biodiversity. Recently, contaminants of emerging concern (CECs), such as perfluoroalkyl substances (PFASs) and antibiotics have been detected in many wetlands, so is important to study their potential effects on biota. Because of their widespread use and environmental persistence, PFASs have been heavily detected in wetlands where Red Winged Blackbird (*Agelaius phoeniceus*) inhabits. In the other case, considerable amount of aquaculture, veterinarian and human used antibiotics have been released around Hudsonian godwit (*Limosa haemastica*) foraging areas. Using blood transcriptomics analyses as a non-lethal technique, we explore the effects temporal exposure of PFASs on Red Winged Blackbird and antibiotics on Hudsonian godwit. Sixteen godwits were temporarily kept inside indoor aviaries and exposed treatment birds to environmentally relevant concentrations (16.35 mg d<sup>-1</sup> bird<sup>-1</sup>) of florfenicol in drinking water using acute (2d) and chronic (78d) expositions. After RNA extraction from blood, 135,860 transcripts were De Novo assembled annotating 47,951. Total number of transcripts changed in the blood of birds exposed to florfenicol were 64 for acute exposure and 118 for chronic exposure. The most affected transcript was a coiled-coil domain-containing protein 117 associated with DNA damage which was upregulated in godwits chronically exposed to antibiotics. In the case of Red Winged Blackbird, 20 males were exposed to a PFASs treatment including 7 ug/kg of PFOS, 0.3 ug/kg of PFOA and 0.07 ug/kg of a mixture of 11 PFASs (Approximately 0.006 ug/kg of each PFASs). After RNA extraction from blood, we determined 90,222 sequences in RWB and annotated 16,491 genes. Within the 135 altered transcripts, we found genes related with lipid transport, glucose import and mitochondria respiratory chain. Peroxisome Proliferator Activated Receptor Delta (PPARD) was one of the main altered transcripts in PFASs exposed Red Winged Blackbird. The use of blood transcriptomics as non-lethal technique opens an avenue of environmental science research as it allows the examination of changes in physiological processes within wild animals across time, space, and major life-history challenges.

## 8.O-Tu-5

### Assessing the Impacts of Anthropogenic Stress Factors on Health Status of Wild Caiman Populations

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#### Abstract

Numerous studies have confirmed that the constant use of pesticides have negative impacts on the health of wild species and habitat fragmentation in combination with toxicological effects of pesticides could, in the long term, generate deep affections on natural populations dynamics of various wild species, including *Caiman latirostris*. In this sense, the objective of this work was to evaluate the effects generated by environmental exposure to pesticides in wild populations of *C. latirostris* that inhabit areas of Santa Fe (Argentina) environmentally exposed to pesticides, and compared with populations from a protected area., without direct exposure. Eggs were collected after the application calendar in the fields was completed, and adults (males and females) were sampled and immediately released. All animals were weighed and measured in total length, blood samples were processed for the analysis of genotoxicity (comet assay and micronucleus test), oxidative damage to lipids and DNA, modulation of antioxidant defenses, and the expression levels of genes encoding these enzymes. Besides, samples of water and soil from exposed and non-exposed natural environments were obtained for an environmental DNA study to analyze species diversity as an indication of environmental perturbation. The results indicated higher DNA damage, increased frequency of micronucleus, lipid peroxidation and alteration in antioxidant defenses in exposed populations (hatchlings and adults) with respect to those of the control areas. Taking into account the wide use of pesticide formulations in today's agricultural environments, early detection of their effects through an integral approach combining the analysis of biomarkers of different endpoints is of paramount importance. It is necessary to continue monitoring natural populations for the medium and long-term conservation of this wild species.



## 8.O-Tu-6

### Genetic Instability among Hitnū People Living in Colombian Crude-Oil Exploitation Areas

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#### Abstract

Oil exploration, drilling, transportation, and refining processes can generate a complex mixture of chemical compounds, including polycyclic aromatic hydrocarbons (PAHs), which may pose health risks to populations residing in the proximity of mining activities, known as the zone of influence (PZOI). To gain a better understanding of the effects of oil exploitation activities on cytogenetic endpoint frequency, we conducted a biomonitoring study on the Hitnū indigenous populations in eastern Colombia. The cytokinesis micronucleus cytome assay (CBMN-cyt) was employed to assess the impact of PAH exposure, while levels of urine 1-hydroxypyrene (1-OHP) were measured using high-performance liquid chromatography (HPLC). We also evaluated the relationship between DNA damage and 1-OHP levels in the oil exploitation area, as well as the impact of community health factors, such as Chagas infection, nutritional status, and consumption of traditional hallucinogens, tobacco, and wine from traditional palms. The CBMN-cyt assay parameters exhibited similar frequencies between individuals from PZOI and those living outside the zone of influence of mining activities (POZOI). However, a non-significant incremental trend was observed among individuals from PZOI for most of the DNA damage parameters. Similarly, levels of 1-OHP were found to be a risk factor for increased micronucleus (MN) frequency (PR = 1.20) compared to POZOI (PR = 0.7). Additionally, proximity to oil exploitation areas was also identified as a risk factor for elevated frequencies of nucleoplasmic bridges (NPBs) and APOP-type cell death. Our findings suggest that genetic instability and its potential effects among Hitnū individuals from both PZOI and POZOI could be modulated by multiple factors, including the levels of 1-OHP in urine, malnutrition, and certain traditional consumption practices.

# Session 9: Aquatic Ecotoxicology: Test System and New Approaches

## 9A.O-We-1

### Using Chemical Activity Assess the Mixture Effects on Phytoplankton

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#### Abstract

Hydrophobic Organic Contaminants (HOCs) impact the abundance and community structure of phytoplankton, which can have cascading effects on the aquatic food web. However, assessing the effects of chemical mixtures on aquatic ecosystems is challenging due to the non-additive nature of concentrations of different chemicals. Chemical activity, a thermodynamic concept based on the chemical's energetic state, provides a useful tool to overcome this challenge. The individual compounds' activities can be summed up to obtain the total chemical activity of a mixture. Polycyclic aromatic hydrocarbons (PAHs) are a type of HOC that accumulate in the phytoplankton primarily through partitioning to lipids. However, the role of lipids in algae sensitivity to chemical activity is understudied. This study utilized the chemical activity concept to assess the impact of a mixture of four PAHs on different species of algae and examine how lipids modulate the sensitivity across different species. Silicone rods loaded with the PAH mixture were used as donors in the toxicity test system (passive dosing), keeping the exposure media at a range of activities between 0.01 and 0.18 for 72 h. Growth inhibition and photosynthetic efficiency were assessed. LC and GC-MS methods were employed to confirm exposure in both medium and algae samples, respectively. Additionally, algae samples were collected to quantify the total lipid content and characterize lipid profiles. The results show that the chemical activity of the PAH mixture and growth inhibition follow a dose-response curve, with observed effects starting within the chemical activity range of 0.01-0.1, consistent with previous studies on baseline toxicity. PAHs negatively impact primary and secondary processes involved in photosynthesis, leading to a decrease in primary production and biomass. Specifically, chemical activity decreased the photosynthetic efficiency of the algae *Rhodomonas salina*, likely by reducing the relative efficiency of chlorophyll *a* to use the absorbed light in photosystem II. Preliminary results from the interspecies comparison showed that the sensitivity varied across species. *Prymesium parvum* was the most sensitive, followed by *Rhodomonas salina*, Picoplankton, *Monoraphidium minutum*, and *Phaeodactylum tricornum*, in descending order. Chemical activity offers a realistic and feasible approach to assess the PAH mixture effect on phytoplankton species.

## 9A.O-We-2

### **Oxidative Stress and Gene Expression Modulation Induced by Chronic Exposition to a Glyphosate-Based Herbicide in the Endangered Annual Killifish *Austrolebias charrua***

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#### **Abstract**

Annual killifishes are the most threatened group of fish in Brazil. The species *Austrolebias charrua* strictly inhabits temporary ponds that emerge in the rainy season from the extreme south of Brazil to the east of Uruguay. The main anthropic factor that threatens this species is extensive agriculture, which determines the loss of flooded areas and the pollution of ponds due to the irregular use of glyphosate-based herbicides, especially Roundup, in the Rio Grande do Sul state. This study aims to measure the toxic effects of Roundup Transorb® on *A. charrua*. Cytological and biochemical parameters in the erythrocytes affected by oxidative stress were evaluated. Moreover, expression of the antioxidant genes *cat*, *sod2*, *gsta*, *atp1a1*, *gclc*, and *ucp1* were evaluated in the brain, gills, and liver. For this, 24 adults were collected in nature under permit IBAMA/SISBIO 71072. The experimental design consisted of two groups: the “Control group”; and the “Roundup group” in which fish were exposed to environmentally realistic concentrations of the herbicide (5 mg. L<sup>-1</sup>) for 96 h. Blood samples were submitted for flow cytometry. The collected tissues were submitted to RNA extraction, followed by cDNA synthesis, molecular cloning, Sanger sequencing, and qPCR. The exposition of annual killifish to a glyphosate-based herbicide significantly increased ROS production and lipid peroxidation in the erythrocytes when compared to the control group. Similarly, Roundup Transorb® increased membrane fluidity and DNA damage in the erythrocytes of exposed fish. Flow cytometry analysis indicated that herbicide exposure generates systemic oxidative stress on *A. charrua* by negatively affecting fish physiology. Gene sequencing contributed to a better knowledge of the structural and functional genomics of Brazilian endangered wildlife. Furthermore, gene expression evaluation revealed that glyphosate exposure alters the relative expression of antioxidation-related genes across different tissues of exposed fish. Indeed, qPCR is a powerful tool to assess water quality through the quantification of genetic biological responses underlying toxicant exposure, using fish as bioindicators. Our results give rise to new insights into biochemical and genomic adaptive mechanisms of annual killifish in response to pollution. Since Brazilian annual killifishes strongly risk extinction, this study paved the way for the development of novel biotechnologies applied to biomonitoring and ecotoxicology assessment.

## 9A.O-We-3

### **Modulation of Liver Antioxidant System and Its Oxidative Effects in *Danio Rerio* (Zebrafish) Exposed to Leachate From a Closed Dump**

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#### **Abstract**

The final disposal of municipal solid waste (MSW) in dumps can create a large contamination zone. Therefore, the contamination of surface waters by leachate is a permanent concern during the disposal of MSW at these places. That's why, ecotoxicological assessments and monitoring show important relevance in determining the environmental impacts of MSW management. In this regard, the aim of this study was to evaluate the antioxidant system modulation of *Danio rerio* liver, exposed to leachate from a non-operational dump. For 96 h, groups of 50 fish were exposed to concentrations of 0, 5, 15, 30 and 50% of leachate. The fish exposure was carried out under license n. LW-49/19 CEUA/FIOCRUZ. Alterations in the antioxidant system were evaluated by determining the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST), and levels of glutathione (GSH) and metallothionein (MT). The determination of oxidative effects was carried out by analysis of lipid peroxidation (LPO) and protein carbonylation (PTC). The leachate showed levels of chlorides ( $2288.4 \text{ mg L}^{-1}$ ), electrical conductivity ( $8434.0 \text{ mS cm}^{-1}$ ) and ammoniacal nitrogen ( $19.0 \text{ mg L}^{-1}$ ) which characterizes the leachate as possibly toxic to aquatic organisms. SOD, CAT and GST activities were increased by 37, 67 and 39%, respectively, at 50% leachate concentration. The same was observed for GSH and MT, which increased respectively, 57 and 15% for the same concentration. No effect of lipid peroxidation was determined, observing a 73% reduction in LPO at the 50% concentration. On the other hand, an increase ranging from 3 to 53% in PTC was observed. The evaluation of antioxidant system showed an increase in enzymatic and non-enzymatic biomarkers, which prevented LPO, but were not prevented the PTC. Therefore, the leachate can be characterized as potentially harmful to the liver of zebrafish since there was protein carbonylation. Another point to be highlighted is that more than one oxidative effect analysis must be carried out in ecotoxicological evaluations, since if only the determination of the LPO were performed, we would have stated that the leachate did not present toxicity for the antioxidant system of the exposed fish.

## 9A.O-We-4

### **LC-HRMS-Based Metabolomics as a Powerful Approach to Elucidate Mechanisms of Action of Environmental Chemicals From Doce River in Brazilian Fish Embryos**

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#### **Abstract**

Mining and other essential economic activities have a long historical impact on aquatic environments, such as the Doce River Basin (DRB), in Brazil. High concentrations of metals combined with organic chemicals introduced from multiple sources of contaminants may trigger complex toxicity pathways which are difficult to interpret and distinguish. For unveiling mechanisms of toxicity, LC-HRMS metabolomics can be a powerful approach in field-scale monitoring studies, helping to properly comprehend the biological responses of environmental stressors. This study aimed to investigate mechanisms of toxicity of environmental chemicals from DRB using a comprehensive untargeted LC-HRMS metabolomics approach (data-independent acquisition of all ion-fragmentation mode), in fish embryos (*Rhamdia quelen*) exposed to chemical mixtures. To solve the problem of the complex mixtures generated by the large-scale datasets of mass spectra (MS/MS), the ROIMCR approach was applied to compress and resolute the data. Fish embryos exposed for 96h to 6 treatment groups showed a distinct pattern of responses when compared to controls, with downregulated essential metabolites, such as amino acids, as a main response, especially for metal exposure. Unexpectedly, the combination of organic contaminants extracted from sediments with inorganic elements showed reduced toxicity than isolated metal mixtures. The results helped to identify and distinguish the effects of different classes of environmental chemicals when combined as complex mixtures. HRMS combined with ROIMCR approach represents a suitable strategy for metabolomics-derived data to identify and distinguish the effects of different classes of environmental chemicals and could be applied not only for the DRB assessment but also in other aquatic ecosystems.

## 9A.O-We-5

### Toxicity Induced by the Pesticides Ametrine, Carbendazim, and Fipronil in the Early Stages of *Danio rerio* Development

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#### Abstract

Ametrine, Caberndazin, and Fipronil are pesticides frequently found in Brazilian surface freshwater and their environmental concentrations present a high risk to aquatic biota. The Zebrafish (*D. rerio*) is an organism consolidated as an experimental model in several areas of science and in Brazil the National Council for Animal Experimentation (CONCEA) recommends this fish species for toxicological evaluation of chemicals. The fish embryo acute toxicity test (FET) allows the assessment of acute toxicity or lethality of pesticides in Zebrafish embryonic stages. Although these pesticides have already been submitted to FET, there are still gaps regarding their toxicity in the early developmental stages of fish. Our objective was to investigate the lethality endpoints (coagulation of fertilized eggs; absence of somites formation; non-detachment of the caudal yolk sac bud; or lack of heart beats); in addition to developmental endpoints: developmental delay, body and eye pigmentation; yolk sac absorption; blood stasis; otolith formation; formation of the nervous system and closure of the neural tube; outbreak; edema; heartbeat; swim bladder filling; balance changes; among other variations or developmental malformations in zebrafish embryos exposed to a wide range of concentrations of these pesticides. The experiments were carried out according to the OECD protocol n° 236 (2013). Regarding the herbicide Ametrine, our data corroborate the international literature, confirming the effects mainly on survival, hatching and edema formation. However, we showed that Ametrine also causes bradycardia and blood stasis, effects not previously reported. The fungicide Carbendazim, presented results consistent with those found in the literature, however we were able to verify such effects in lower concentrations than those previously used. This may be directly related to the difficulty in solubilizing this fungicide in water, making co-dilution in DMSO necessary. As previously reported, the insecticide Fipronil had effects on survival, the appearance of edema, deformations in the spine, among others. However, we also evidenced the malformation of the eyes and head, effects still little explored in the literature. Based on our results, we suggest the need of evaluation of toxicity of mixtures of these pesticides. Grant 2022/03094-5, São Paulo Research Foundation (FAPESP)

## 9A.O-We-6

### Acute Toxicity of Water-Soluble Fraction (WSF) From the Oil of Mysterious Origin That Reached Brazilian Beaches.

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#### Abstract

In 2019, large amounts of oil from mysterious origins started to cover the Brazilian coast, especially along the Northeast region, causing immediate ecological and socioeconomic impacts. The oil spill was marked by the sighting of oil in more than 1000 locations, making it the most significant episode of this type among tropical oceans in terms of the length of coastline affected (2890 km). This study aimed to evaluate the acute toxicity of the water-soluble fractions (WFS) obtained from oil fragments collected at two beaches from the State of Bahia (Arraial da Ajuda and Massarandupio). The FSM stock solution was prepared after making an oil: water mixture (1:9) and collecting the supernatant portion after the mixture had spent 20h on the magnetic stirrer. Then, the following concentrations of WFS were made by dilution in filtered seawater: 0.01; 0.1; 1; 10; and 100% plus the control. The test system consisted of 4 replicates for each treatment in 15 ml glass test tubes. Each replicate received 10 ml of test-solution and ten nauplii of *Artemia sp.* After 24 and 48 h, the mortality rates were evaluated. Analyzing mortality, observed the “non-observed effect concentration (NOEC)” and the “lowest observed effect concentration (LOEC)” when possible. The concentrations of aliphatic hydrocarbons (AHs) and polycyclic aromatic hydrocarbons (PAHs) from the oil fragments were analyzed by Gas Chromatography coupled to a Mass Spectrometer (GC-MS). In the Massarandupio sample, light HAs (decalines (m/z 138, 152, 166), naphthalene (m/z 128, 142, 156)), and that of 1-methylpyrene (1-Mpy) and its by-product 2-methyl-pyrene (2-Mpy) were found. For 1-Mpy and 2-Mpy, their ratio suggested that the oil was not weathered by sunlight, indicating that its transport was subsurface. The WFS obtained from an Arraial da Ajuda oil fragment was toxic only at 100% concentration (LOEC-100% and NOEC-10%), whereas the sample from Massarandupio was not toxic to *Artemia sp* nauplii. Our results differ from other studies, which have reported higher toxicity of WSFs produced with other fuels such as diesel and bunker oil. This investigation provides initial data on the toxicity of WFS of the oil of mysterious origin that contributes to assessing the impacts caused by this incident. However, results with more samples and species are necessary for a detailed evaluation.

## 9B.O-We-1

### **Glyphosate Affects Biochemical and Locomotors Parameters of Newborns of the Fish *Jenynsia multidentata* at Allowable Concentration for Aquatic Bodies**

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#### **Abstract**

Glyphosate is the most widely used herbicides in the world to avoid weeds in agriculture. It has been constantly detected in the aquatic environments in Brazil and may cause harmful effects to non-target organisms residing in agricultural regions, as fish. The present study evaluated biochemical and locomotors parameters in newborns of *Jenynsia multidentata* exposed for 96 hours to 65 µg/L of glyphosate, environmentally relevant concentration and allowed aquatic bodies intended for the protection of aquatic communities in Brazil. *J. multidentata* is a neotropical fish widely distributed in South America that naturally inhabits streams close to agricultural areas. The herbicide did not affect the activity of the glutathione S-transferase, an enzyme involved in detoxification process, but increased the activity of the acetylcholinesterase (AChE) and the levels of lipid peroxidation (LPO). Indeed, glyphosate affected newborns locomotors parameters causing reduction in the distance traveled, velocity, activity and turn angle when compared to the control group. Locomotor alterations pose a risk to the survival of the animals, since several essential activities, such as foraging and escaping from predators, depend on good locomotor ability. Moreover, the increase in AChE activity can directly affect the swimming performance since it controls the neurotransmitter acetylcholine in the synapses, and the increase in LPO is an indicative of oxidative stress. Overall, results from this study highlight the importance of investigating the toxicity of xenobiotics in regional species and reinforces the importance of decision-making regarding the use and regulation of glyphosate considering preservation of aquatic ecosystems.



## 9B.O-We-2

### Monitoring Glyphosate and Chlorpyrifos Pollution and the Effects of Environmental Samples Exposure on Biomarkers of Oxidative Stress and Neurotoxicity in Freshwater Fish

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#### Abstract

Water pollution caused by agrochemicals is currently one of the most critical problems for the conservation of aquatic ecosystems. Glyphosate and chlorpyrifos have been commonly used pesticides in agricultural practices in the region. Due to their extensive use, it is crucial to understand their fate and potential toxicity to non-target species. To address this, a monitoring study was conducted in the Las Catonas sub-basin (Río Reconquista basin, Province of Buenos Aires, Argentina) which involved physicochemical analyses, quantification of metals and pesticides (glyphosate and chlorpyrifos), and ecotoxicological bioassays. The specific aim of this work was to evaluate the effect of exposure to environmental samples from two sites with different anthropic impacts, on antioxidant defenses parameters (catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH)), oxidative stress marker (lipid peroxidation, TBARS), the detoxifying enzyme glutathione-S-transferase (GST) and the neurotoxicity biomarker acetylcholinesterase (AChE) in a native teleost *Cnesterodon decemmaculatus*. Semi-static acute toxicity bioassays (96 h) were conducted under standardized conditions according to IRAM 29112-2008, exposing specimens to environmental samples from SA, an upstream site close to a residential area, and SC, downstream and bordering a horticultural area. After the exposure period, homogenates of the anterior, middle section, and muscle were prepared and the parameters (CAT, SOD, GST and AChE activities and GSH and TBARS levels) were determined. In SA, chlorpyrifos and glyphosate levels were not detectable, while in SC, the presence of these pesticides was identified and levels of other compounds exceeded the guideline levels for the protection of aquatic life. The results from the bioassays showed changes in the neurotoxicity biomarker and antioxidant defenses. Some of the effects on certain parameters could have low specificity, as they were altered by exposure to both SA and SC (SOD). On the other hand, a comparison of the effects produced by exposure to both sites, along with previous results from exposures to synthetic samples of chlorpyrifos, suggests that the concentration of this insecticide in SC could fully or partially explain the effects on the other biomarkers (AChE, CAT, GSH). Finally, it cannot be ruled out that part of the observed effects may be caused by the interaction of pollutants considering the complex mixtures found in the environment.

## 9B.O-We-3

### Assessment of Combined Toxicity of Aluminium and Tebuconazole on the Planarian *Schmidtea mediterranea* Using Behavioural and Developmental Biomarkers

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#### Abstract

In Bolivia, high metal content, such as aluminium, lead and chromium (VI), has been reported in aquatic environments due to bad wastewater management. Metals in surface water are part of complex mixtures that can change their properties and toxicity. Although there is rising concern about mixture toxicity worldwide, most studies and regulations are still focusing on individual compounds, leading to an underestimation of the effects of mixtures in aquatic ecosystems. Our study explored the interaction between the widely used fungicide in Bolivia, tebuconazole (TEB), and the metal aluminium, at environmentally relevant concentrations. We focused on the effect on developing organisms and used the regeneration capacity of the planarian *Schmidtea mediterranea* as a proxy to assess developmental effects. *Schmidtea mediterranea* is a benthic organism, hence, is exposed to higher amounts of precipitated pollutants. After decapitating, the planarians were exposed to 0.27 and 1.08 mg/L of Al alone and in combinations with a concentration of TEB close to found in rivers waters 0.02 mg/L and two orders higher, 2 mg/L, according to a factorial design 2<sup>2</sup>. The study showed a significantly increased pLMV in aluminium mixtures with 0.02 mg/L TEB compared with the aluminium single effect, indicating a strong effect on planarian behaviour. Moreover, the mixture of the lower concentrations of Al-TEB strongly affected the regeneration capacity. All the mixtures of Al-TEB induced neurodevelopmental effects, based on the of relative brain ganglia sizes after regeneration and incomplete and asymmetric brain development in *S. mediterranea*. An antagonistic effect of the Al-TEB mixtures on cell cycle dynamics was observed; the presence of TEB decreased the effect of aluminium on the proliferation of stem cells. Our study confirmed the neurotoxicity effects of aluminium reported for other organisms, such as zebrafish. The concentrations used in this study are remarkably close to the regulation limits of aluminium concentration in surface waters. In combination with other pollutants, such as TEB, aluminium can induce neurodevelopmental toxicity and behavioural changes in planarians, even at low concentrations. Considering that in natural water, mixtures exposure is the reality, the environmental risk assessment of aquatic ecosystems should focus on mixtures effects rather than on single compounds.

## 9B.O-We-4

### The Use of Eugenol at Different Concentrations in *Baryancistrus xanthellus* (Siluriformes, Loricariidae) Subjected to a Simulated Transportation

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#### Abstract

Eugenol is a phenolic compound belonging to the class of essential oils, found in various aromatic plants, and is considered a byproduct of clove oil. It possesses sedative properties that may result in minimizing stress and reducing mortality during and after the handling and transportation of animals. Recently, eugenol has been widely used as an alternative anesthetic in immersion baths for fish due to its easy availability, low economic cost, and perceived safety for both animals and operators. *Baryancistrus xanthellus* is the main ornamental species collected at Xingu River and widely exported to the international ornamental fish market. Many animals suffer from stress and physical injuries during transportation, resulting in a high mortality rate. The use of anesthetics at appropriate dosages can be an effective strategy to mitigate this issue. This study aims to analyze the efficiency of eugenol as a sedative for the transportation of *B. xanthellus*, suggesting the appropriate dosage for safe exportation. The animals were captured with SISBIO License No. 33558-1, and the study was approved by the Commission on Ethics in Animal Use (CEUA - UFPA 112-13). Eugenol was diluted in alcohol (70° GL) at a ratio of 1:10 (eugenol:alcohol). The experiments were conducted with 120 animals. The fish were subjected to simulated transportation, placed in plastic bags with water and oxygen, packed in thermal boxes, and exposed to doses of 2, 4, 6, 8, 10, and 14 mg.L<sup>-1</sup>, as well as two control groups: one with only water and another with water and alcohol (70° GL). The physicochemical factors of the water were monitored before and after the simulation, including pH, temperature, electrical conductivity, dissolved oxygen (O<sub>2</sub>), and total ammonia (NH<sub>3</sub>) levels. The difference in the relationship between treatments at different doses and controls, as well as the difference in physicochemical parameters of the water, was tested using a Two-way ANOVA. After 48 hours of transportation simulation, the survival rate was 100%, which may indicate the absence of short-term deleterious effects. The concentration that proved most suitable for the transportation of *B. xanthellus* was 6 mg.L<sup>-1</sup>, as at this concentration, after 48 hours, the fish did not emerge from the deep sedation stage, which is recommended for fish transportation. However, dissolved oxygen levels decreased, and ammonia levels in the treatments increased proportionally to the controls.

## 9B.O-We-5

### Importance of Age-Election in a Coastal Fish Used as a Bioindicator of Pollution

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#### Abstract

Different fish species have widely been used as effective bioindicators of contamination or degradation in marine environments. Jenyns' sprat *Ramnogaster arcuata* (Teleostei: Clupeidae) is a small pelagic zooplankton consumer that habits the southwestern Atlantic with a wide distribution in coastal zones. It has a short life cycle (age 0, age 1, age 2, and age 3) that can occur entirely circumscribed within the Bahía Blanca estuary (BBE) in Argentina. Due to this characteristic and its resident habits, this fish species has been proposed as an efficient bioindicator organism for the South American coastal environments. The purpose of this study was to demonstrate that the age of a marine coastal fish used as a bioindicator is important in being considered for evaluating the health of an aquatic environment. To this end, different journey samplings were performed over a year at different places in the BBE to obtain specimens of *R. arcuata* at different ages. Macromolecules levels (protein, glucose, triglycerides, and cholesterol), metabolic enzymes activities (Alanine aminotransferase, Aspartate aminotransferase, Lactate dehydrogenase, and Creatine Kinase), and oxidative stress (lipid peroxidation) were measured in muscular and liver tissues. Results showed that the most representative age of *R. arcuata* during an entire year in the BBE was the age of 1, followed by age 2; ages 0 and 4 being the least represented. Moreover, there was a variability of the biomolecules, metabolic enzymes, and oxidative stress in different tissues analyzed according to age. In conclusion, the more representative age of *R. arcuata* in the BBE to be used as a bioindicator is 1, and different biomolecules and enzymes could be used as biomarkers of contamination, especially lipid peroxidation in liver tissue.

## 9B.O-We-6

### Bile Analysis as a Biomarker of Abietic Acid Toxicity in Fish

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#### Abstract

Abietic acid is an acidic resin produced by conifers such as pine. Considerable amounts of acidic resins are produced and released in effluents from pulp and paper industries and released into rivers and water bodies. In this study, through the analysis of bile, the possible deleterious effects of abietic acid on fish (*Oreochromis niloticus*) were evaluated under laboratory conditions, exposed to concentrations in the range of 100 to 150 µg/L. The fish were kept in groups of 10 individuals, in 200L water boxes, fed with commercial feed. Bile samples were collected after a period of 7 days of exposure. Bile samples were extracted with different solvents and prepared for analysis by GC/MS-TOF, after derivatization with BSA or methylation in basic medium. The samples were further analyzed by fluorescence, monitoring emissions at 250 and 280 nm, with excitation at 356 and 458 nm. The results obtained showed changes in the profile of bile acids, especially cholic and deoxycholic acids. Fluorescence analysis showed significant changes in the emission spectrum of bile samples.

DRAFT

# Session 10: Understanding Contamination Threats to Amphibians and Reptiles of Latin America to Promote Their Conservation

## 10.O-Tu-1

### Assessing the Effects of Pesticide Overspray on Amphibians and their Impact on Skin Function and Structure

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#### Abstract

Amphibians are the most threatened group of vertebrates, and, since these animals often inhabit and migrate through agricultural lands, the impact of pesticide use is of major concern. However, research on the effect of current-use pesticides on amphibians is scarce and mainly limited to aquatic life stages. Moreover, amphibians are not generally included in the environmental risk assessment of pesticides, assuming that the evaluation conducted on other vertebrate taxa grants them protection. This assumption raises concern, considering the differences in structure and function of amphibian skin with respect to the integument of other groups. In this study, we investigated the toxicity of dermal exposure to current-use pesticides, on amphibian terrestrial stages, using Perez's frog (*Pelophylax perezii*) and the Iberian painted frog (*Discoglossus galganoi*) as model species. To do so, froglets of both species were reared in the laboratory. Juvenile frogs were exposed by overspray using a pump calibrated to simulate realistic pesticide application regimes. During the experimental period (7 or 21 days) we recorded survivorship and changes in body condition and analysed histopathological changes on dorsal skin. Besides, we collected samples from their skin secretions to investigate skin peptide bioactivity, and frog tissue samples to examine pesticide residue toxicity accumulation by LC-MS. Results from the assays with Perez's frog showed that overspray with alpha-cypermethrin-based insecticides and tebuconazole-based fungicides at realistic doses compromise survival. Also, exposure to insecticides can alter the skin epithelium of froglets, since the skin of exposed individuals presented an increased stratification and swelling of keratinocytes in the stratum corneum (the outermost layer of amphibian skin). A thicker epidermis could impact the permeability of the skin and affect skin functionality, possibly compromising animals' fitness. The amount of secreted skin peptides was unaffected by exposure to phytosanitary products. Ongoing analyses are focused on determining whether possible changes in peptide composition might influence their antifungal activity. Assays with Iberian painted frogs are currently in progress to be presented at the meeting. Expected results from these assays will allow us to study the effects of pesticide overspray on amphibians belonging to different families and will help in designing more robust tests to be used worldwide.

## 10.O-Tu-2

### Acute Toxicity and Impact on Anuran Metamorphosis of Environmentally-Relevant Concentrations of Neonicotinoid and Anthranilic Diamide Insecticides

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#### Abstract

In the agricultural region of the Argentine Pampa, the neonicotinoid (NEO) and anthranilic diamides (AD) families of insecticides are widely used on numerous crops, including soybean and corn. Both families of insecticides are persistent in the environment and can reach the aquatic environment by lixiviation and runoff. The objective of the present study was to analyze the impacts on amphibian species native to the Pampa region of the commercial formulations Actara and Confidor containing the NEOs, thiamethoxam (THIA) and imidacloprid (IMI), and the commercial formulations Coragen and Fortenza containing the ADs, chlorantraniliprole (CHLO) and cyantraniliprole (CYAN). Acute and chronic toxicity as well as impacts on development were examined in tadpoles of the toads *Rhinella arenarum*, *Rhinella fernandezae* and the frog *Scinax granulatus* at a range of concentrations. The lethal concentration 50 (LC50) values obtained after 96h of exposure were greater than 10 mg/L in all cases, the greatest toxicity being observed in the case of *Scinax granulatus* exposed to IMI, with a 96h LC50 of 11,28 mg/L. With regards to metamorphosis, the effects occurred mainly between stage 39 and 42. In general, NEO and AD acted differently at environmentally-relevant concentrations. In the case of NEO, exposure to THIA caused a delay in the time to metamorphosis of *R. arenarum* starting from the lowest concentration tested (5 µg/L), while the percentage of animals completing metamorphosis was not affected. For its part, exposure to IMI had no major effects on the development. Exposure to AD, CHLO and CYAN, altered the time to metamorphosis in a non-monotonic manner (increased or decreased depending on the concentration) and significantly reduced the percentage of animals completing metamorphosis but, in this case, with linear dose-response relationship.

## 10.O-Tu-3

### Lethal and Sublethal Effects of the Fungicide Difenoconazole (Active Ingredient and Formulation) on Larvae of *Ceratophrys ornata* (Anura, Ceratophryidae)

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#### Abstract

The amphibians are currently the most globally threatened group of vertebrates and have experienced a drastic decline in their populations. Many causes for this decline are established (e.g., habitat loss, increased susceptibility to disease, climatic changes) but pesticide contamination is considered a primary factor, especially in agricultural landscapes; where in aquatic spawning habitats, eggs and larvae may be repeatedly exposed during ontogeny. In recent years, fungicides have become a more important component of agricultural pest and disease management programs and new formulations are continually being developed to find more effective treatments that can overcome the speed at which pathogens acquire resistance. In this work we set out to compare the lethal and sublethal effects caused by the commercial formulation (CF) of difenoconazole (Janfry®) and its active ingredient (a.i.) on larvae of *Ceratophrys ornata*, a species of anuran native to the Pampas region of Argentina. The experimental design consisted of exposing organisms individually in 100 ml.-1 glass chambers. Eight concentrations were selected in the range of 0.01 and 5.00 mg.L-1. Tests included 20 replicates per concentration and 2 negative control groups: (1) test water and (2) dilution control, containing the highest concentration used in the dilutions (0.1% acetone). As endpoints, mortality, swimming alterations and effects on growth and external morphology of each organism were evaluated. Mortality and swimming alterations data were analyzed by log-probit regressions and significant differences (p-value <0.05) were found for FC toxicity and a.i. (LC50-96 h: 1.68 mg.L-1 and EC50-96 h: 0.09 mg.L-1 vs. LC50-96 h: 2.75 mg.L-1 and EC50-96 h: 0.13 mg.L-1, respectively). Using an ANOVA followed by Dunnett's test, the effects on growth were evaluated, finding a significant difference (p-value <0.05) between the total length of the controls and the organisms exposed for 96 h from concentrations greater than 1 mg.L-1. In addition, morphological abnormalities such as severe body edema and caudal fin flexion, curvature, breakage, or folding were found. These abnormalities were dependent on DFZ concentration, with an odd ratio of 5.08 [2.97 - 8.42] but with no differences between the a.i. and the CF Janfry®. From the results obtained, it can be concluded that DFZ in the CF evaluated has a higher toxicity than when present only as i.a. to the larvae of *Ceratophrys ornata*.



## 10.O-Tu-4

### The Integrated Biomarker Response in Three Anuran Species Larvae at Sublethal Concentrations of Cypermethrin, Chlorpyrifos, Glyphosate, and Glufosinate-ammonium

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#### Abstract

The aim of the present study was to evaluate the response in larvae of the anuran species *Rhinella arenarum*, *Rhinella dorbignyi* and *Odontophrynus americanus* exposed to glyphosate (GLY, 2.5mg L<sup>-1</sup>), cypermethrin (CYP, 0.013mg L<sup>-1</sup>), chlorpyrifos (CP, 0.1mg L<sup>-1</sup>) and glufosinate-ammonium (GLU, 15mg L<sup>-1</sup>) using two behavioral endpoints: mean speed (MS) and total distance moved (TD); and two enzymatic biomarkers: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). The tested concentrations of GLY, CYP and CP are considered environmentally relevant concentrations (ERC); GLU concentration was used as a positive control. In order to assess a global response and to determine the most sensitive species, an integrated biomarker response (IBR) index was calculated. Behavioral and enzymatic biomarkers were tested after 60 min exposure. The results showed that: (1) there were statistical differences between species in a series of responses in swimming behavior, and cholinesterase activities within the first-hour of exposure to CYP, GLY, and CP at ERC; (2) IBR determined that *Rhinella* species were the most sensitive of the species tested and (3) IBR provided a comprehensive assessment of the health status of species exposed to ERC of a wide variety of agrochemicals globally and frequently used.

## 10.O-Tu-5

### Development of an Ecological Interaction Model: Evaluation of Acute Infection of *Ceratophrys ornata* Eggs With Different Oomycete Species

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#### Abstract

The study of toxic effects on ecological interactions is becoming increasingly important in the field of ecotoxicology. It requires the development of a model that is more ecologically relevant than individual or subindividual models. Interactions between species can occur in several ways, one of which is parasitism. Oomycetes, also known as aquatic molds, are a group of microorganisms belonging to the Straminipila kingdom that are infectious parasites for some amphibian species. In the present study, the infection of *Ceratophrys ornata* (Anura: Ceratophryidae) eggs with 15 different species of oomycetes isolated from aquatic environments surrounding the city of La Plata (Buenos Aires Province - Argentina) was evaluated. Two acute infection assays were performed in multiwell plates. One with unfertilized eggs and the other with fertilized ones. In both assays eggs were exposed to spore suspensions of known concentration, without medium renewal and under controlled laboratory conditions ( $25^{\circ} \pm 2$ , photoperiod: 16:8 L:O). The results in the unfertilized eggs indicate that the albuminous structure covering *C. ornata* eggs is a favorable substrate for the development of all oomycete species tested. Significant effects were also observed on survival, growth, development and morphology of hatched *C. ornata* embryos from fertilized eggs. The oomycetes identified as more pathogenic were: *Achlya sp.*, *Brevilegnia sp.* and two *Saprolegnia spp.* In addition, the response to infection was found to differ among the different oomycete species, suggesting that future tests should be considered species-specific. The results obtained also suggest that infection of *C. ornata* eggs with oomycetes is a useful model for ecotoxicology to study the effects of contaminants on this ecological interaction. The next step will be to include a fungicide in the proposed model.

## 10.O-Tu-6

### How the Interactions in Pesticide Mixtures Affect Genotoxicity and Oxidative stress in *Caiman latirostris* Hatchlings.

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#### Abstract

*Caiman latirostris* is one of the two native species of crocodylians in Argentina which area of distribution covers a wide central-northern region of the country. In the last two decades, natural distribution of this species has been strongly overlapped by agricultural frontier expansion, which has caused great loss and degradation of its habitat. In this context, caiman populations are exposed to complex mixtures of different pesticides formulations, which represent a high risk for the development of embryos and neonates. The objective of this study was to determine the genotoxic effects and oxidative stress of glyphosate (GLY), 2,4-dichlorophenoxyacetic acid (2,4-D), imidacloprid (IMI) and chlorantraniliprole (CAP) individually and in mixtures (binary and quaternary combination) in *C. latirostris* embryos in order to assess the potential risk of *in ovo* exposure that this species is suffering in their natural habitat. Under controlled condition, an embryonic exposure to these pesticides was done at concentrations recommended for soybean crops. Treatments were: negative control, GLY, 2,4-D, IMI, CAP, mixture 1 (M1): GLY+2,4-D, M2: IM I+CAP and M3: GLY + 2,4-D + IMI + CAP. At hatching, blood samples were taken for the evaluation of genotoxicity, oxidative damage to lipids and DNA, the enzymatic activity of Catalase (CAT) and Superoxide dismutase (SOD), and the expression level of their corresponding genes (catalase: *cat* and superoxide dismutase: *sod*). It has been shown that IMI, M2 and M3 induced a significant inhibition of CAT activity while no effect was observed on SOD. In turn, lipid peroxidation was significantly higher in individuals exposed to IMI, and to all the mixtures. Besides, genotoxicity and oxidative DNA damage were observed in all exposed groups. The results of mRNA expression showed no difference at transcription levels. In the same way, no alterations in growth parameters were recorded at hatching. Regarding to the mixtures, we observed a potentiating action of IMI on M3 in lipid peroxidation as well as independent action on oxidative DNA damage and genotoxicity parameters. Our results indicate that the potential environmental risks of pesticides in reptiles should receive more attention, using these early warning markers to monitor natural populations under environmental stress.

# Session 11: Physiologically Based Pharmacokinetic Modeling: Applications in Biopharmaceutics, Precision Dosing and Toxicokinetic Predictions

11.O-We-1

## Development of a Physiologically Based Pharmacokinetic Modeling to Assess the Bioavailability of Gastroretentive Furosemide Formulations

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### Abstract

Furosemide is a potent diuretic widely used in the clinic, however it has a narrow absorption window restricted to the upper part of the gastrointestinal tract. In order to increase its oral bioavailability and diuretic effect, there is evidence that the best strategy is to develop controlled release gastroretentive systems of Furosemide. Computational Physiologically Based Pharmacokinetic (PBPK) models are being developed using PK-Sim® and MoBi® software with the goal of predicting the *in vivo* behavior of the gastroretentive formulations based on results obtained from *in vitro* studies. For the construction of the models, bibliographic data of *in vitro* and *in vivo* studies were collected and for model evaluation, data obtained from *in vitro* and *in vivo* bioequivalence studies were used. Despite the fact that it has been possible to obtain a model that explains the *in vivo* behavior of Furosemide after its oral administration, the model fits better in studies carried out in men than in women. Therefore, a deep understanding of pharmacodynamic-pharmacokinetic (PD-PK) relationships has been essential to achieve the adjustment of the *in silico* model, which in turn enables the development of successful furosemide dosage forms. Finally, the model was optimized to enable the acquisition of plasma and urinary excretion profiles of Furosemide based on the sex of the individual.

## 11.O-We-2

### In vitro – In vivo Extrapolation in Predicting Hepatic Drug Clearance: Theoretical Basis Updated

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#### Abstract

In vitro – in vivo extrapolation (IVIVE) of drug blood clearance ( $CL_B$ ) is essential in the early stages of pharmaceutical development in order to accurately establish doses that avoid toxicity or ineffectiveness in the individuals. However, two erroneous theoretical principles are causing little success in the prediction of hepatic blood clearance ( $CL_{B,H}$ ): 1) the hepatic blood flow ( $Q_H$ ) should not be its maximum value, but the cardiac output (CO), and 2) the hepatocyte/blood ratio of unbound drug concentrations ( $C_{uH} / C_{uB} = [Y \cdot \mu]_H$ ) should not equal 1. The Y factor is a ratio of tissue blood flow to tissue water content. The greater the fraction of cardiac output received by the tissue, the greater the tissue concentration of drug. The  $\mu$  factor is an adjustment factor that takes into account the effect of membrane transporters on tissue-blood exchange.

Deviation in the IVIVE of the hepatic blood clearance becomes successfully corrected using the updated equation here presented:

$CL_{B,H} = CO \cdot f_{uB} \cdot [Y \cdot \mu]_H \cdot CL_H / (CO + f_{uB} \cdot [Y \cdot \mu]_H \cdot CL_H)$  instead of the current equation:

$CL_{B,H} = Q_H \cdot f_{uB} \cdot CL_H / (Q_H + f_{uB} \cdot CL_H)$  Being  $f_{uB}$  the unbound fraction in blood.

Special attention should be paid to the  $\mu$  factor in the hepatic case, since hepatocytes exchange molecules not only with blood but also with the intestine (also a potential metabolizing tissue) and through it can return to the systemic circulation as well.

## 11.O-We-3

### **LiADME: An Open-Source Tool for the Prediction of Pharmacokinetically Relevant Properties of Small Molecules**

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#### **Abstract**

Early assessment of PK-related properties of small molecules is of great value to prioritize drug candidates, guide their molecular optimization and select dosing schedules during preclinical development and clinical trials. Furthermore, in silico predictions on ADME properties may be used as input for elaborate PK models.

Here, we report the development of an open-source machine learning-based webapp publicly available in our institutional website as a part of LIDeB tools ( [https://lideb.biol.unlp.edu.ar/?page\\_id=1076](https://lideb.biol.unlp.edu.ar/?page_id=1076)), capable of predicting a wide array of ADME properties, including aqueous solubility, log P, log D, tissue/blood partition coefficients, fraction bound to plasma proteins, total clearance, organ clearance, apparent volume of distribution, CYPs liability, and many others. Each predictor has been inferred and validated from a chemically diverse, representatively sampled curated dataset, and each prediction is associated with a reliability flag based on applicability domain assessment. In line with the open-source philosophy, our code can be assessed and modified for improvement, expansion, or to adjust to specific needs. We provide regular maintenance and free user support. Also noteworthy, our suite of predictors covers some frequently overlooked ADME properties, such as affinity for pharmacokinetically relevant SLC transporters and nuclear receptors associated with enzyme and transporter induction.

## 11.O-We-4

# The Evolution of Physiologically Based Pharmacokinetic Modeling and Its Current Impact on Pharmacology and Pharmaceutical Applications

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### Abstract

In the context of quantitative pharmacology, the use of Physiologically Based Pharmacokinetic (PBPK) models has become established as one of the primary tools applied within the model-informed drug development paradigm (MIDD), serving as a platform for the integration of information from various sources and using it to make mechanistic extrapolations, maximizing the use of available knowledge, experimental data, and reported data aiming at improve the quality, efficiency, and cost-effectiveness of decision making. PBPK has transitioned over the years to a regulatory necessity, with the potential of reducing clinical and preclinical experimentation. Interestingly, for many years the scientific framework and application of digital computation to create PBPK models of increasing complexity advanced through the study of risk assessment of environmental chemicals, as these models allowed the prediction of systemic exposure and tissue dosimetry of compounds after their entrance through different routes and particularly the extrapolation of animal to human toxicology by pharmacokinetic interspecies scaling often in the absence of human exposure data. Currently, PBPK models are being increasingly applied in the context of model-informed precision dosing (MIPD), aiming at delivering the right dose, to the right patient, at the right time by using a “virtual twins” approach. In this talk, the scientific background of PBPK modeling will be covered, along with the related software that has led to a noticeable increase in its application and realization of its value in pharmacology and pharmaceutical applications. Several examples will be provided, highlighting the potential of this approach to support knowledge generation in different areas of application. In particular, the talk will focus those applications that are being implemented in the context of Latin American research to improve the quality of medicines.

# Session 12: Tropical Ecosystem Health Assessment: Effects of Contaminants on Marine and Estuarine Ecosystems

## 12A.O-Tu-1

### Microplastics Characterization Using the Raman Spectroscopy Technique in the Coast Dune of the Mexican Caribbean

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#### Abstract

Microplastics (MPs) are fragmenting into smaller and smaller pieces due to different weathering mechanisms that act individually or together, such as photo-oxidation by ultraviolet rays, hydrolysis, wear due to abrasion with sand, among others. The Mexican Caribbean, due to its natural beauty and importance for marine life, is a priority site for attention and research regarding the effects of pollution by plastic waste, so the purpose of this work is to characterize MPs physicochemically by Raman spectroscopy accumulated and found in the beach between Mahahual and Xahuayxol, in the southern Mexican Caribbean.

Mainly, it was possible to identify the colored plastic fragments in the size range of 2 to 5 mm; however, this is difficult when the plastic particles have a size of 1 mm or less, so for the identification or quantification of PMs it is necessary to have more powerful techniques, such as Raman spectroscopy. In this sense, structures of sizes smaller than 5 mm were found, in various shapes and colors: foams and fibers, transparent or blue, to mention a few examples, identifying the main components as polyethylene (PE) present mainly in plastic bags, polypropylene (PP) the basic component of ropes, polyvinyl chloride (PVC) present in pipes and linings, and polyethylene terephthalate (PET) used in the manufacture of containers such as soda bottles. The Ministry of the Environment and Natural Resources of Mexico calculated a production of 9 billion PET plastic bottles per year, which is directly related to the results found on the beach in this study. As a consequence of the accumulation of these compounds, the decline of the populations of organisms that inhabit the coastal dunes can be observed, since they can ingest this type of compound and accumulate polybromodiphenyl ethers, which are persistent toxic chemical compounds in the environment.



## 12A.O-Tu-2

### **Distribution and Abundance of Microplastics on Beaches with Different Levels of Anthropization: Analysis of Sediment, Water and Different Tissues of *Stramonita brasiliensis* (Claremont & D. Reid, 2011)**

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#### **Abstract**

The production of plastics on a global scale is growing dramatically, surpassing 367 million tons in 2020, most of which are discarded after a single use. It is estimated that between 4.8 and 12.7 million metric tons of plastic end up in the world's oceans each year. One of the categories of plastic waste are microplastics (MPs), which by definition have a size between 0 and 5 mm and originate from two main sources: they can arise from the degradation of larger plastic debris or they can be produced in microscopic size. Such particles can be ingested by many marine species, leading to direct physical damage and potential toxic effects. In addition, they are bioaccumulated along the food chain and reach edible species, which can even cause risks to human health. Therefore, this work aims to compare the distribution and abundance of MPs found in seawater, sediment and different tissues of *Stramonita brasiliensis* collected on beaches subjected to different levels of urbanization and anthropic impacts. For this purpose, samples of sediment, water and specimens of *Stramonita brasiliensis* were collected at five sampling points along the coast of Espírito Santo - Brazil. All the material was processed considering the specificities of each matrix in order to extract the MPs that were later transferred to filter papers arranged in Petri dishes, where they were visualized in a magnifying glass, photographed, quantified, classified by type and the chemical composition of ten specimens was analyzed by Raman Spectroscopy. Statistical analysis was performed using GraphPad Prism 9.3.0. Significant values followed  $p < 0.05$ . The results show a directly proportional relationship between urbanization and the availability of MPs in the marine environment and reinforce the ubiquity of this contaminant in the ecosystem, since we found MPs in all samples analyzed. In addition, RAMAN analyzes identified three different types of polymers: polyurethane (PUR), polyethylene (PE) and polyester (PES). Furthermore, the gastropod *Stramonita brasiliensis* is consolidated as an efficient and reliable bioindicator for the analysis of contamination by MPs due to the high abundance of particles found in the organisms. Finally, understanding the relationship between the origins of contaminants and their distribution in the environment facilitates the construction of public management plans in order to minimize environmental contamination.

## 12A.O-Tu-3

### **Biomarkers in Pufferfish: Evaluating the Environmental Health of Brazilian Marine Protected Areas**

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#### **Abstract**

Marine Protected Areas (MPAs) are defined areas designed to conserve biodiversity and protect vulnerable species and ecosystems. This study aimed to assess the environmental quality of three Brazilian MPAs, based on the integrated analysis of biomarkers of different levels of biological organization in pufferfish (*Sphoeroides testudineus*). The MPAs are differentiated by the type and degree of anthropogenic impacts, as follows: Barra do Una Estuary sustainable reserve (JUR) is as a reference area due to its low levels of contamination and high species diversity. The Cananéia Estuarine System (CAN) has been recognized as a biosphere reserve by UNESCO, as well as a Ramsar wetland. Parts of this MPA are influenced by mining activities upstream, which results in the introduction of metals in the estuarine waters, and the discharge of untreated sewage. The São Vicente estuary (SSV) is a permanent protection area, but its vicinities lack proper sanitation infrastructure. After collection, the animals were euthanized, their soft tissues were removed, and multiple biomarkers were analyzed in the gills and liver: somatic biomarkers (condition factor-CF and hepatosomatic index-HSI), genotoxic biomarkers (micronucleus and nuclear abnormalities), biochemical biomarkers (Gpx, GST, GSH, LPO, DNA damage and muscular AChE) and morphological biomarkers (histopathological and morphological analysis). All data were analyzed using one-way ANOVA. A one-factor multivariate analysis was applied to evaluate the differences between the data sets, and the matrices were analyzed using PERMANOVA to test the "estuary" factor. The results were integrated using PCA with a cut value of 0.5 and also an integrated biomarker response (IBR) was calculated. PCA extracted four factors that explained 68% of the data. In general, SSV differed from CAN and JUR with highest IBR, followed by CAN. Organisms from SSV showed greater gill pathology, elevated AChE and LPO activity, and presence of micronuclei. CAN presented intermediate IBR, demonstrating more changes in liver pathologies. In fact, CAN indicated to be an intermediate environment between SSV and JUR, indicating not only the importance of the existence of MPAs for the preservation of environmental quality, but also the need for the maintenance and monitoring of the already established areas so that they remain environmentally preserved environments, since the contamination of aquatic environments is independent of physical barriers.

## 12A.O-Tu-4

### Nuclear Techniques for Reconstructing Microplastic Pollution Trends in Marine-Coastal Areas

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#### Abstract

Sediments are a significant repository of microplastics (MP), and undisturbed sediment cores are valuable environmental archives to reconstruct the history of their contamination, using radioactive chronometers for dating, and quantifying MP abundances. Through the International Atomic Energy Agency regional project RLA/7/025, the REMARCO network is implementing harmonized methods to obtain intercomparable data on long-term temporal trends of MP accumulation in the coastal zone, in Latin America and the Caribbean region. These are the results of a study case from Estero de Urias, an urbanized coastal lagoon in the southern Gulf of California, Mexico. Sediment cores analyzed for  $^{210}\text{Pb}$ ,  $^{226}\text{Ra}$ , and  $^{137}\text{Cs}$  by gamma-ray spectrometry, were dated using the constant flux (CF) model. Particles were extracted by density separation (NaCl solution,  $1.2 \text{ g cm}^{-3}$ ) and filtration ( $0.7 \mu\text{m}$  pore size GF/F filters), and counted under a stereomicroscope using Nile red staining. Particles' polymeric composition was determined by  $\mu\text{FTIR-ATR}$  spectroscopy. Results were analyzed by periods, i.e., pre-1950 (before mass plastic production), 1950-1990 (increasing global plastic production and consumption), and 1990-2021 (faster-increasing rate of plastic production to the year of sampling). Suspect MP abundances ranged from  $100 \pm 10$  to  $31,907 \pm 500$  particles  $\text{kg}^{-1}$ . Fibers (66-89% of total MP particles) predominated over the fragments (11-34%). No MP particles were detected before 1950. The gradual increase in MP abundances and fluxes towards the present was associated with the population growth of Mazatlán City. MP fluxes were lower in 1950-1980 than in 1990-2021, likely due to the discharges from four municipal wastewater treatment plants installed since 2008. The only synthetic polymers found in the cores were polyethylene terephthalate (PET, 12-14%), rayon (2-5%), and polypropylene (PP, less than 2%). This study case demonstrated the usefulness of nuclear techniques, particularly  $^{210}\text{Pb}$ -dating, for reconstructing long-term MP contamination trends in the coastal zone. It showed that the concentration of MPs in sediments at Estero de Urias Lagoon increased over time with population density and wastewater discharges, so MP contamination (and its environmental implications) may continue increasing in the coming years. Harmonized research methodologies, environmental education, and integrated coastal management programs are needed to protect coastal environments from continued MP pollution.

## 12A.O-Tu-5

### Imposex Prevalence in Colombian Caribbean Coast: 2014 - 2023

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#### Abstract

Coastal systems worldwide are facing pollution from shipping, agricultural, urban, and industrial activities that release organotin compounds (OTC), which adversely affect marine organisms and humans. OTC act as endocrine disruptors, causing gastropods to grow a pseudopenis in females, also known as imposex, which blocks their reproductive system. Despite having two coasts, Pacific and Caribbean, and a variety of marine activities, contamination by OTC have not been studied in Colombia. Some studies on imposex were conducted before the ban of OTC compounds in 2004, and a few more were carried out after the ban in 2018 and 2020 on the Caribbean coast, with none on the Pacific coast. Thus, our objective was to investigate the prevalence of imposex in meso and neo gastropods at different times, 2014 and 2023, after the global ban of tributyltin (TBT) in antifouling paints and try to explain imposex origin.

Our findings on the Caribbean coasts indicate that *Strombus pugilis*, a mesogasteropod, shows imposex prevalence in Cartagena Bay, with a slight decrease from 81% to 56% from 2014 to 2023. However, Barú island and Coveñas beach did not exhibit the phenomenon in both periods. *Melongena melongena*, a noegasteropod, exhibited a considerable increase in imposex in the Tolú marina from 7% to 61% from 2014 to 2023, respectively, due to high operation and repair of small vessels in the area. In the Tesca lagoon, imposex was absent in 2014 but appeared with a percentage of 36% in 2023. Similarly, the Caimanera lagoon experienced a slight decrease from 39% to 27% in imposex prevalence from 2014 to 2023, respectively. Moreover, the highest imposex levels were found in two sites of Cartagena Bay for *M. melongena*, with percentages of 52.9% and 87.5%, respectively, indicating strong contamination by organotin compounds in the area. Our findings suggest that contamination by OTC in the Caribbean coast was intense in the years before the ban.

Keywords: Organotin compounds, butyltin, phenyltin, antifouling paints, endocrine disruptors.

## 12A.O-Tu-6

### **Evaluation of Sediment Quality and its Influence on the Taxonomic Composition of Zoobenthos in the Río Portoviejo Estuary Biocorridor**

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Universidad Laica Eloy Alfaro de Manabí, Ecuador

#### **Abstract**

The objective of this study was to evaluate the influence of sediment quality on the taxonomic composition of zoobenthos. Two sampling stations were established in the Portoviejo River Estuary Biocorridor (Manabí-Ecuador), and were carried out in March, April and May 2022. A 0.50 x 0.50 m quadrat was used for sampling, placing 5 PVC tubes to take samples in the first 15 centimeters of depth. The samples were sieved with 2 mm, 1.5 mm and 1 mm sieves mesh size. The biological material obtained was preserved in a 5% formalin solution for later identification in the laboratory. The sediment characterization was carried out with the analysis of the following parameters: heavy metals (Ar, Cd, Hg and Pb), surfactants, organic matter, and organochlorine pesticides. Among the parameters analyzed, the only one that exceeded the permissible limits was Cd at the two sampling points during the months of March and April. The taxonomic composition of zoobenthos was represented by a total of 92 individuals, with a diversity of 15 species. The Multivariate Index-AZTI Marine Biotic Index (M-Ambi) determined that Las Compuertas station is in a poor ecological state, while the Desembocadura enjoys a very good ecological state. It is suggested that Cd could be influencing the composition of zoobenthos in the Biocorridor, and that taxonomic composition found could be used as a bioindicator.

## 12A.O-Tu-6

### **Biomonitoring of Guaraguaçu River, Paraná (Brazil), using biomarkers in the Neotropical fish, *Hoplias malabaricus* (Bloch, 1794)**

Hugo de Andrade Gonçalves dos Santos, Rafael Shinji Akiyama Kitamura, Luis Phelipe de Souza Miranda, Maritana Mela Prodocimo, Jean Ricardo Simões Vitule, Marta Margarete Cestari, André Andrian Padial, Helena Cristina da Silva de Assis  
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#### **Abstract**

The Guaraguaçu River, in the Atlantic Forest biome, is the main river of the Paraná coast (Brazil), due to its unique biodiversity, economic importance and water supply for the cities of Matinhos, Paranaguá and Pontal do Paraná. The literature shows that this river receives contaminants due to disorderly urban growth on its margins, release of domestic sewage and constant flow of ships. The aim of this study was to evaluate the influence of anthropic action on water quality using the analysis of biomarkers of environmental contamination in a top predator fish *Hoplias malabaricus* (BLOCH, 1794). The fish were collected in 5 mm mesh fyke net traps, in three sectors of the river considering the gradient of anthropic impact: sector 1 - pristine; sector 2 - impacted and sector 3 - less impacted. The fish were anesthetized, and blood was taken by caudal vein puncture for genotoxicity biomarker analysis. After euthanasia, gills, liver and posterior kidney were removed for analysis of antioxidant and histopathological biomarkers. Brain and muscle were collected for neurotoxicity biomarker analysis. The results showed that the activity of brain acetylcholinesterase decreased in sector 2 compared to sectors 1 and 3. In gills and liver, despite the increase of activity in antioxidant system (superoxide dismutase and glutathione peroxidase - GPx), lipoperoxidation damages were observed, mainly in sector 2 when compared to sector 1, indicating a possible oxidative stress. In the kidney, GPx and glutathione S-transferase activities increased in sector 2 and 3. These results suggest that a cholinesterase inhibitor can be present in sector 2, such as organophosphate or carbamate compounds or metal. Histopathological biomarkers showed different lesions in the liver and gills of fish from sector 2, when compared to other sectors, such as presence of hepatic necrosis and vacuolization of hepatocytes, the presence of aneurysm, hyperplasia with total lamellar fusion and increase in mucus cells in gills. For genotoxicity biomarkers, the presence of micronucleus occurred at least in one organism of each sector, in addition to the presence of other morphological alterations in the nucleus of erythrocytes, such as vacuolated, notched, binucleus, lobed and blebbed. In conclusion, the results highlighted the problems of the river water quality, creating database of the knowledge to the people that live in the region and use the river for survival and to the decisions- makers.

## 12B.O-Tu-1

### Microplastic Distribution and Accumulation in the Coastal Dune of the Southern Mexican Caribbean Sea

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#### Abstract

Microplastic accumulation in the ocean and coastal areas around the world is a major problem for human health and ecosystem stability. In the water, microplastics can be ingested by marine organisms and then they can be transferred to humans. In the coastal dune, the microplastic accumulation can produce an increase in the temperature that will affect the development of animals and plants. In the Mexican Caribbean Sea, macro and microplastics have been found as pollutants on beaches, but there is a lack of information on their abundance and distribution. To contribute to the knowledge of the impact of microplastics in the ecosystem, this work focused the activities on the identification of microplastics by shape and color, to evidence the accumulation and distribution in the coastal dune of the southern Caribbean Sea. Results showed the presence of fibers, particles, sponges, and films, all of them with a length between 3 to 5 mm. The blue/black fibers were the most abundant microplastics in the area (around 550fibers/100g sand), followed by blue/black particles (around 100partibles/100g sand). According to the vertical distribution from the beach berm to the frontal dune, sponges were found as the most abundant microplastic in the frontal dune. No differences were found in the horizontal distribution of microplastics in the dune. The abundance of microplastics can be a consequence of massive tourism and an inappropriate disposition of plastic trash. It was also observed the deposition of plastic in the dune from the marine circulation. Water and soda bottles with publishing marks from Trinidad and Tobago, Japan, Central America, and other countries were found in the water and in the dune. It is necessary to make conscience about the use and final disposition of plastic containers, they are affecting our health and our ecosystems.

## 12B.O-Tu-2

### DDTs in Water and Sediments from Espírito Santo Inner Shelf (Southern Atlantic) After Fundão Tailings Dam Failure: Water-Sediment Exchange and Spatial and Temporal Variations

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#### Abstract

In November 2015 the Fundão dam failure (FDF) released about ~34 million m<sup>3</sup> of tailings, creating a mud avalanche that wiped out about 15 km<sup>2</sup> of land area, reached the Doce River and flowed throughout it until reach the Espírito Santo inner shelf (ESIS). The tailing wave eroded the Doce River carrying a variety of compounds from the watershed to the marine environment, promoting an unexpected and hazardous increase in DDT levels on ESIS. The present study discussed the temporal and spatial distribution of DDT on ESIS, as well as the associated coastal dispersion patterns and water-sediment exchange trends, three years after the FDF. DDTs were assessed in water (n=231) and sediments (n=225) samples from five sectors of the ESIS (from north to south: Abrolhos, North, Mouth, Costa das Algas, and South; totaling ~ 4,904 km<sup>2</sup>), between October 2018 and November 2019. Costa das Algas had the highest  $\sum$ DDTs values in water ( $0.86 \pm 1.62$  ng L<sup>-1</sup>), followed by the North, Mouth and Abrolhos, indicating a southern dispersion. In sediments, the Mouth sector presented the highest  $\sum$ DDT values ( $1.03 \pm 1.88$  ng g<sup>-1</sup>;  $p < 0.05$ ), probably due to its proximity to the Doce River mouth and related factors as the local intense flocculation process, finer grain size, and fluid mud areas. No differences between wet and dry seasons were verified in sediments ( $p = 0.49$ ), while DDT levels were significantly higher for the wet group in water ( $p < 0.05$ ), which is related to the river flow increase. DDT water-sediment exchange was assessed by fugacity fraction analysis ( $ff$ ), that ranged from 0.20 to 0.47, indicating a DDT net flux from water to sediment. Therefore, the results suggest that DDTs were released from the Doce River, travelled to the south by the water column due to the prevalent N-NE winds and returned to the mouth region by northward sediment transport, where they accumulated. Intense rainfall in wet periods increased the input of DDTs to the ESIS. In addition, a net flux of DDTs from water to sediment indicates a predominant supply of DDT via the aquatic compartment, and that sediments act as a sink for DDT. These findings are important to understand temporal and spatial patterns of DDT availability to the biota; to highlight the importance of assessing the indirect impacts of large-scale land disasters, such as dam failures, on marine environments; as well as may be helpful in future interpretations of additional local trends and global inventories of legacy pollutants.



## 12B.O-Tu-3

### ***Parhyale hawaiiensis* as a New Model Organism in Marine Ecotoxicology**

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#### **Abstract**

Marine and estuarine environments are extremely vulnerable areas to pollution and their protection is imperative to allow the functioning of this ecosystem. In tropical regions due to a limited lack of model organisms, it is common to use temperate model organisms to assess the adverse effect of chemicals and environmental quality. We started to investigate the circumtropical marine amphipod *Parhyale hawaiiensis* in ecotoxicology because a considerable amount of knowledge has been published in the genetics, evolution/development fields. *P. hawaiiensis* was collected from a pristine area in Sao Paulo state shore, Brazil, in 2010 and since then has been successfully cultured in our laboratory in standardized conditions. Protocols for acute (96-h) and chronic toxicity tests (42-d), both using miniaturized approach, were successfully developed and applied to reference substances and environmental samples. The sensitivity of *P. hawaiiensis*, measured as 50% lethal concentration (LC50) and no observed effect concentration (NOEC), is in the same range as other amphipods. Protocols for the analysis of different endpoints based on genotoxicity as comet assay/micronuclei, gene expression and regenerative responses were developed and applied to test multiple toxicants. Methods for determination of internal concentrations of metals and organic substances in hemolymph were developed providing sources for the establishment of toxicokinetics models in aquatic amphipods. Characterization of the hemocytes from *P. hawaiiensis* was performed and published, increasing the knowledge for the investigation of immune responses. *P. hawaiiensis* has become an attractive model for ecotoxicology as well and a promising tool for risk assessment evaluations in marine tropical environments. Acknowledgments: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance code 001; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

## 12B.O-Tu-4

### Toxicity of PAHs-enriched Sediments on Meiofauna Communities Under Ocean Warming and CO<sub>2</sub>-driven Acidification Scenarios

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#### Abstract

Global changes have been decisive for the resilience of marine ecosystems. Persistent chemical contaminants, such as PAHs, also greatly affect the ecological quality of coastal marine environments. These pressures affect biota at different levels of biological organization, and little is known about the interactive effects of CO<sub>2</sub>-driven acidification, temperature rise and PAHs in sediments on biota, especially at the community level. The aim of the present study was to evaluate the relationship between CO<sub>2</sub>-driven acidification, temperature rise and PAHs toxicity on meiobenthic communities. Controlled microcosms were assembled in the laboratory, in full factorial experimental design: (i) PAHs (2 levels: acenaphthene + benzo(a)pyrene spiked sediments and negative control); (ii) temperature (2 levels: 26°C and 28°C); (iii) pH (2 levels: 8.1 and 7.6). After 14 days the community was characterized according to density, richness, diversity and evenness of the large meiofaunal groups (i.e. Nematoda, Copepoda, Ostracoda and Acoelomorpha). The Nematoda assemblage has been identified down to the genus level. Univariate analysis of meiofaunal taxa density indicated different responses to the tested factors. The temperature caused a decreasing effect on Copepoda and the nematodes *Pseudochromadora* and *Daptonema*, and an increased effect on *Sphaerotheristus* and *Sabatieria*. The increments, however, were limited to the scenario where there is no CO<sub>2</sub>-driven acidification. Acidification also had a decreasing effect on Copepoda and nauplii densities. The density of Ostracoda was increased in the acidified scenario. Contamination by PAHs caused a decrease in the density of *Daptonema*, but Acoelomorpha and certain genera of Nematoda, especially *Pseudochromadora*, showed an increase in PAHs enriched sediments. The results showed that changes induced by acidification, warming and PAHs contaminating sediments in benthic communities are driven by vulnerability and tolerance limits of each taxonomic group, in addition to indirect effects that occur in Nematoda assemblages.

## 12B.O-Tu-5

### **Effects of Sediments Contaminated by Metals in Future Scenarios of Acidification and Warming of the Marine Environment on the Production of Offspring of the Species *Nitokra* sp.**

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#### **Abstract**

Since the beginning of the Industrial Revolution, there has been a constant increase in the atmospheric concentration of CO<sub>2</sub>, which has also led to an increase in the planetary average temperature. The oceans are important sinks of CO<sub>2</sub> and heat on the planet, regulating CO<sub>2</sub> concentrations in the atmosphere, but the environmental and climate changes experienced in recent decades have been putting pressure on the resilience of these ecosystems. Allied to these environmental transformations, marine ecosystems are also affected by other stressors, including chemical contamination. The aim of the present study was to evaluate the effect of different levels of metal contamination (Cu, Pb, Zn and Hg) in the sediment under scenarios of warming and marine acidification on the copepod *Nitokra* sp. offspring production. The test organisms were exposed to sediments spiked in the laboratory with a mixture of metals (Cu, Pb, Zn and Hg) and respective negative controls, in combinations of three pH levels and 2 temperature levels. Acidification was carried out through the controlled injection of pure gaseous CO<sub>2</sub> into the experimental medium. The effect of the fixed factors pH, temperature and contamination, and interactions between them, on the fecundity responses of *Nitokra* sp. were evaluated using Permutational multivariate analysis of variance (PERMANOVA). The temperature of 27°C increased the offspring production in all treatments. The interaction of temperature at 27°C and moderate pH reduced the offspring production in sediments without contamination and in the intermediate contamination level (corresponds to 37.7% of TEL). In conclusion, acidified environments can delay the hatching of eggs, interfering with the production of copepod offspring. Temperature directly influences the increase in offspring production. Metals in sediments can potentiate their toxicity to organisms in acidification scenarios. Therefore, metals when combined, increase toxicity and the acidified environment reduces the offspring of the species *Nitokra* sp. The present study showed that the combination of pH, temperature and contamination factors has an effect on the organism's offspring production and subsidizes the discussion on the need to update the sediment quality guides.

## 12B.O-Tu-6

### **Metal-Specific Biomagnification and Trophic Dilution in the Coastal foodweb of the California sea lion (*Zalophus californianus*) off Bahía Magdalena, Mexico**

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#### **Abstract**

Trace metals concentrations along with stable isotopes ratios were measured in marine algae, sea grass, Porifera, Echinoderms, Mollusks, Crustaceans, Actinopterygii, and Chondrichthyes fish, and the California sea lion (*Zalophus californianus*), to assess the bioaccumulation potential and detect potential risks for top predators in Bahia Magdalena, Mexico. We assessed the trophic magnification factor (TMF) to determine the biomagnification potential for 11 trace metals. The concentrations of Fe and Zn were one order of magnitude higher than all other metals. Concentrations of As, Cu, Cd, Co, Cr, Fe, Mn, and Ni correlated negatively with trophic level, supporting trophic dilution (TMF<1, p>0.05), while Zn and Hg exhibited significant trophic magnification (TMF>1, p<0.05) when assessing only the benthic-pelagic foodweb. This research contributes to the baseline level of metals, metal-specific foodweb bioaccumulation, and biomagnification of mercury underscoring the key role of the macrobenthic community as biovectors for trophic transfer of Hg to the California sea lion foodweb. The findings of this study also have implications for metal pollution management to support ecotoxicological risk assessment and conservation of marine species and the regional ecosystem, as well as the public health of local communities heavily reliant on commercial fish and traditional seafoods.

# Session 13: Detection, Toxicity and Environmental Risk of UV Filters and Cosmetic Ingredients in Aquatic Ecosystems

## 13.O-Mo-1

### Developing Relevant, Defensible and Reliable Toxicity Assays in the Scleractinian Coral *Acropora cervicornis*

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#### Abstract

Coral reefs are economically and ecologically important and responsible for supporting a significant diversity of marine life in tropical regions. Coral reefs are directly or indirectly impacted by a combination of stressors including a diverse array of chemical contaminants. Recently, concerns have been raised regarding the impact of UV filter constituents contained in sunscreens and other products. However, clear assessment of the environmental risk of chemical contaminants to scleractinian corals is hampered as there are currently no standard test protocols for corals and it is unclear how representative other standard test species (i.e. marine invertebrates and algal species) may be, given the complexity of the host-symbiont-holobiont system. Toxicity tests with corals have been conducted but the lack of a standard methodology has limited comparisons between studies and highlighted concerns on data reliability and quality. We provide guidelines and discuss considerations moving forward on designing standard toxicity tests for scleractinian coral species using results from flow-through exposures using common contaminants (e.g., copper and diuron) and new emerging contaminants (e.g., UV filters). Factors discussed include choice of test species and life-stage, specific test designs and replication requirements, inclusion of appropriate parameters for quality control, timing of exposures, analytical verification, and the choice of appropriate biological endpoints for acute and chronic assessments to provide reliable and statistically appropriate measurements.

## 13.O-Mo-2

### Effects of UV Filter Benzophenone-3 and Heat Stress on the Gills of the Marine Bivalve *Amarilladesma Mactroides*

Fernanda Chaves Lopes<sup>1,2</sup>, Guilherme Toledo Alves Patrocinio<sup>1</sup>, Micheli Castro<sup>1</sup>, Camila de Martinez Gaspar Martins<sup>1</sup>

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#### Abstract

Benzophenone-3 (BP3) is a UV filter detected in water bodies, biota and sediment due to its high lipophilicity and low degradation, leaving aquatic organisms susceptible to contamination. However, if contamination occurs in the oceans, organisms deal with an additional stressor, the increase in temperature, which occurs as a result of global warming. The increase in temperature affects the physiological processes of animals, and may influence the toxicity of chemical products present in the environment. This study investigated whether exposure to BP3 and high temperature affect the antioxidant capacity of the yellow clam *Amarilladesma mactroides*. The animals were collected in southern Brazil and exposed to BP3 at an environmental concentration (1  $\mu\text{g/L}$ ) at 20 and 24 °C for 96 h. The animals were previously acclimated to the experimental temperatures. At the end of the experiment, the animals were euthanized, and their gills were collected for analysis of the biochemical biomarkers: glutathione s-transferase (GST) and 7-ethoxyresorufin-0 desethylase (EROD), glutamate cysteine ligase (GCL), glutathione reductase (GR), glutathione peroxidase (GPx) and reduced glutathione (GSH). It is expected an increase in the antioxidant defense in animal exposed to BP3, however the energy demand with metabolic processes at high temperature could reduce the amount of energy available for detoxification, inhibiting the antioxidant capacity in clam's gills. Such results have already been observed for the digestive gland of the *A. mactroides*. We would expect same for the gills since it is considered a main entry route for contaminants, and also have a high energy demand and detoxification capacity.

## 13.O-Mo-3

### **Spatial and Temporal Investigations on the Concentrations of Organic UV Filters in Seawater Surrounding Coral Reef Ecosystems**

Carys Mitchelmore, Andrew Heyes, Michael Gonsior  
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#### **Abstract**

Despite concerns regarding the impact of UV filters contained in sunscreens to corals there is limited spatial and temporal information available on the environmental concentrations of these chemicals in seawater near coral reefs. Previous studies from other locations have found spatial and temporal variations in concentrations, for example, lower concentrations with depth in the water column, distance from the shoreline and in times of lower recreational activity. It is hypothesized that concentrations are higher in the microlayer yet there is limited or no data to support this. To address these knowledge gaps seawater samples from multiple sites (i.e. beach and reef locations) in the Florida Keys and Hawaii, USA were analyzed for up to 13 organic UV filters. At recreational beach locations multiple sites were assessed for concentrations in the microlayer and surface seawater at two distances from the shoreline. Temporal assessments included samples collected early in the morning, mid-afternoon and late in the evening. At Florida reef locations both surface and seawater collected at coral depth were analyzed. UV-filters were measured using LC-ESI-MS/MS techniques in both the dissolved and particulate fractions. Numerous UV filters were detected in seawater with the highest concentrations present in the sites closest to the beach correlating with the level of recreational activity. UV filters were present at significantly lower concentrations in samples taken further from the beach and in early morning and late evening samples. A number of UV filters were present in higher concentrations in the microlayer samples compared with the corresponding surface water samples. A number of UV filters were detected in seawater collected both at coral depth and the surface, although both were at much lower concentrations compared to beach samples. This study provides insight as to the environmentally relevant concentrations in seawater samples at beaches and coral reefs locations. Furthermore, the spatial and temporal assessments provide data that are essential for developing probabilistic marine exposure models and environmental risk assessments.

## 13.O-Mo-4

### **Environmental Impact of Currently Marketed Sunscreens and Potential Human Impacts of Changes in Sunscreen Usage and Anticipated Research Needs**

Charles Menzie

Exponent, USA

#### **Abstract**

Concerns have been raised about the potential toxicity of sunscreens to a variety of marine and freshwater aquatic organisms, particularly corals. At the same time, there are concerns that people will use less sunscreen as a result of environmental concerns. An ad hoc committee of the National Academies of Sciences, Engineering, and Medicine reviewed the state of the science on the use of the active ingredients in sunscreens (UV filters) currently marketed in the United States. This review was conducted to provide information useful for future application in ecological risk assessments, by reviewing information on UV filter fates, exposure, and effects. The report also includes review of the potential human impacts that could result from changes in availability of certain UV filters for use in sunscreens, in order to inform management of both human and ecosystem health. This presentation will describe the committee's findings, conclusions, and recommendations related to this issue, as well as the priority knowledge gaps to fill to inform higher tiered risk assessments. The presentation will include discussion of the intersection of aquatic chemistry, ecotoxicology, ecology, and epidemiology to understanding the potential for risks from UV filters and implications to human health for changes in sunscreen use. An EPA workshop to help identify research needs took place in early 2023 and some highlights from that workshop will be presented along with related activities.

DRAFT



## 13.O-Mo-5

### Effect of Sunscreens With Microplastics on Artemia

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#### Abstract

Primary microplastics (MP) are used for industrial purposes, some of them are added to personal care products such as sunscreens which are leave-on products, that is, those that remain on the skin once applied. The objective of this work was to evaluate the toxicity of sunscreens with MP, commercialized in Mexico City and the State of Mexico, in the aquatic species *Artemia salina*. The toxicological test was carried out by adapting to the ISO/TS 20787:2017 guide. 10 sunscreens were used: eight that indicated the presence of some microplastic on their label, one with physical UV filters of titanium dioxide (TiO<sub>2</sub>) and zinc oxide (ZnO) and another that included only chemical filters. The test lasted 72 hours, it was done in quadruplicate, in 5 mL capacity flasks containing 5 brine shrimp, the blocker concentrations were 0.0001, 0.001, 0.01, 0.1, and 0.5 g/L. Every 24 hours the motility and survival of the artemias were checked. The results showed that no statistically significant relationship was found between the types of sunscreens (with UV and MP filters) and the toxic effects caused in artemias. This may be due to the diversity of ingredients in sunscreens, as sunscreens range from 16 to 52 ingredients.

DRAFT

# Session 14: Nanomaterials

## 14A.O-Mo-1

### Aquatic and Terrestrial Toxicity Evaluation of Microfibrillated Cellulose

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#### Abstract

Microfibrillated cellulose (MFC) is an attractive nanomaterial for industrial applications due to its excellent mechanical properties and green and biodegradability features. However, the potential environmental impacts of MFC still require investigation. Thus, ecotoxicity studies can provide hazardous information about MFC, supporting its safe application in the industry and helping develop eco-friendly products. Here, we evaluated MFC from bleached cellulose pulp of Eucalyptus sp. regarding its potential to induce toxic effects on terrestrial organisms (plants and earthworms) and fish. Fish acute toxicity was evaluated by a modified version of the OECD Test Guideline (TG) 249 with RTgill-W1 cell line (gill cells, *Oncorhynchus mykiss*). The modification in this protocol refers to exposure conditions to ensure the bioavailability of the test agent, avoiding death caused by deposition on cells and not due to proper cell uptake (static exposure or exposure under orbital shaking at 150 rpm). Terrestrial toxicity was evaluated by the seed germination and root elongation test (*Cucumis sativus*, *Allium cepa*), cyto-/geno-toxicity *A. cepa* test, and earthworm (*Eisenia foetida*) acute toxicity test (OECD TG 207). The results from plant test systems showed that MFC (100, 10, 1, 0.1, 0.01 mg/L) did not inhibit seed germination or root development or cause cellular and genetic changes in plant meristematic cells (100, 10, 1 mg/L). MFC did not cause lethal effects on earthworms (100, 10, 1, 0.1, 0.01 mg/L). For fish, MFC (1250, 625, 312.5, 156.25, 78.1, and 39.0 mg/L) also did not affect the cell viability of RTgill-W1 cells under both exposure conditions (static and orbital shaking), assigning this nanocellulose to the non-toxic category according to hazard designations for aquatic life of the United State Environmental Protection Agency (US EPA). Thus, MFC is not toxic to organisms with important ecological functions in terrestrial ecosystems, primary producers (plants), and soil engineers (earthworms), as well as does not seem to pose a concern for aquatic organisms, fish. Although a broader study is still required to truly determine the environmental safety of MFC, the present findings open good perspectives of MFC applications on greener and safer nanotechnology-based products.

## 14A.O-Mo-2

### Evaluations of Different Nanoparticles Tested *in vivo* and *in vitro*.

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#### Abstract

Nanomaterials continue to be used indiscriminately and without testing their effects at the molecular and tissue levels before being available on the market. For this reason, studies carried out with different nanoparticles (Nps) are justified. Several types of Nps demonstrated toxicity in different biomarkers including: genotoxic, cytotoxic, enzymatic and histological, when used *in vivo* and *in vitro*. Different tissues in aquatic organisms were analyzed, and demonstrated tissue specificity of nanoparticles (TiO<sub>2</sub>, Ag and ZnO). Nps (TiO<sub>2</sub> and Ag) are able to perform DNA-protein Crosslinks in cell culture. In addition, RTG2 *in vitro* was also observed. Thus, demonstrating why there is often a reduction in DNA damage when only the alkaline comet assay is used in living tissue. When testing the Nps TiO<sub>2</sub>, a verified change in the physical form of anatase to brookite was viewed when inside the organism. Furthermore, structural changes in the meristem cells of *Allium cepa* took place. Evidence from these studies, suggest that expanding studies with Nps is necessary to understand tissue specificities and possible structural changes within an organism.

DRAFT

## 14A.O-Mo-3

### Assessing the Toxicity of New Nanoengineered Antifouling Biocides to the Sun Coral *Tubastraea coccinea*

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#### Abstract

Biocides have been used in the formulation of antifouling (AF) paints to avoid marine biofouling. However, such substances can gradually leach, and some can persist for long periods in the marine environment, causing harmful environmental effects to non-target organisms. New techniques such as the use of nanoengineered materials containing AF compounds have been developed to control the release rate and reduce the toxic effects of biocides. They include the biocides encapsulation in mesoporous nanocapsules of silica (SiNC), in order to control the release of the biocide to the aqueous medium. This study aimed to evaluate the toxicity of the AF biocide DCOIT, in its free and nanoengineered forms, to *Tubastraea coccinea* (sun coral) colonies, and to observe sublethal effects on the animals tested. The tested compounds included the free form of the biocide, the empty SiNC, their respective nanoengineered forms (SiNC-DCOIT), and a version coated with silver (SiNC-DCOIT-Ag). These new nanomaterials are expected to be ecologically friendly alternatives to the free DCOIT, as in preliminary tests made with organisms from temperate regions indicated lower toxicity and good antifouling effectiveness. The target organism of this study, *T. coccinea*, is considered invasive along the Brazilian coast and can be an alternative test organism for evaluating the risks to native species of corals. The colonies were collected in the Alcatrazes Archipelago, SP, Brazil. After acclimation, they were individually exposed to different concentrations of the substances for a period of 96 h, in 500 mL flasks, kept with gentle aeration and at constant temperature of  $23^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and natural photoperiod (12h:12h light/dark). At the end of the experiment, no significant mortalities were observed, but some sublethal effects were observed, such as the limestone skeleton apparent in practically all concentrations and replicates (but absent in the controls), and embrittlement and whitening of the skeleton at higher concentrations. *P. coccinea* was little sensitive to the substances tested, and this higher resistance may indicate a greater adaptive tolerance to these antifouling biocides. We assume that such resistance to antifouling biocides may increase the species' capacity for proliferation, worsening the current problems associated with bioinvasion.

## 14A.O-Mo-3

### Nanopesticides: Effects on Two Neotropical Aquatic Species

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#### Abstract

The development of nanoparticles as release systems for pesticides seeks to improve their characteristics and to increase their efficiency. Thereby release systems for different pesticides would minimize the contamination of the environment, such as nanocapsules prepared with synthetic and natural materials. Besides, metal nanoparticles produced using microorganisms have promising agricultural use. However, for the safe use of these nanopesticides, studies investigating their toxicity are of great importance. In this sense we carried out some studies to analyze the effects of nanoencapsulated herbicides (atrazine) and biopesticides (neem oil), as well as metal (Ag, Fe and TiO<sub>2</sub>) nanoparticles synthesized from the fungus *Trichoderma harzianum*, on two different biological models: the fish *Prochilodus lineatus* and the bivalve *Anodontites trapesialis*, both freshwater and native species. A set of genotoxic, biochemical and physiological biomarkers were analyzed after exposure to the different types of nanopesticides. Our results indicated that the nanoencapsulated atrazine showed to be less toxic for *P. lineatus* in comparison to the free herbicide, since it did not promote increase in glycemia, alterations in the activity of antioxidant enzymes, glutathione content, carbonic anhydrase enzyme activity, and increase in the frequency of micronuclei and other nuclear erythrocyte abnormalities. On the other hand, in relation to the nanoencapsulated neem oil, we observed that the conventional neem oil caused milder effects to *P. lineatus*. The most relevant effects attributed to the nano-biopesticide should be associated with their greater resistance to photodegradation, provided by the nanocapsule. Concerning the effects of metal nanoparticles synthesized from the fungus, the bivalve *A. trapesialis* showed bioaccumulation potential, but was less sensitive to the nanoparticles than the teleost *P. lineatus*. Silver nanoparticles caused most effects, which suggests that they are more harmful than the other two (TiO<sub>2</sub>NP and FeNP) under the conditions evaluated. Taking together, our results show that depending on the pesticide, the nanoencapsulation may be a tool to reduce the damage to the environment and non-target organisms, nevertheless more studies are needed for the safe use of nanoparticles in agriculture.

## 14A.O-Mo-5

### Innovative Anti-Corrosion Nanomaterials: Environmental Behavior and Marine Hazard Assessment

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#### Abstract

Nanomaterials have been extensively used in a wide range of applications, including in protective coatings to mitigate maritime corrosion in immersed metallic structures. Most commercial anti-corrosion coatings rely on the use of efficient, but toxic corrosion inhibitors (CIs). In the framework of the NANOGREEN R&D project, the engineered nanoclays Zn-Al and Mg-Al layered double hydroxides (LDH) loaded with alternative CIs (e.g., benzotriazole) have been suggested as promising nanoadditives that can control the spontaneous and premature leaching of CIs in coatings with potential environmental benefits. Therefore, the present study aims to assess the environmental behavior, short-term effects, and marine hazard of nanostructured and soluble forms of benzotriazole (BTA vs. Zn-Al LDH-BTA and Mg-Al LDH-BTA). The environmental behavior of both LDHs was assessed in artificial saltwater (ASW) daily (up to 96 h), using two concentrations (1.23; 100 mg/L; n=3). The endpoints were the hydrodynamic size (DLS), stability (zeta potential), and dissolution (Mg, Zn, and Al determination; ICP-OES). Ecotoxicological standard tests were carried out using 14 marine temperate species from different trophic levels (concentrations ranged from 0.14 to 100 mg/L). The lowest no-effect concentration (NOEC) value was then divided by an appropriate assessment factor (AF=100) to derive the predicted no-effect concentration (PNEC).

The BTA exposure caused significant effects on the bacteria *Aliivibrio fischeri* and the sea-urchin *Paracentrotus lividus* (EC<sub>50</sub> = 42.4 and 52.6 mg/L, respectively). Regarding the nanostructured forms, Zn-Al LDH-BTA was very toxic to *P. lividus* (EC<sub>50</sub> = 0.21 mg BTA/L) while Mg-Al LDH-BTA was harmful (E/IC<sub>50</sub>=10–100 mg/L) towards *A. fischeri*, *P. lividus* and the microalgae *Phaeodactylum tricornutum*, *Isochrysis galbana*, and *Tetraselmis chuii*. From all tested species, *P. lividus* gathered the lowest NOEC for the three tested compounds (0.14 mg/L); thus, the estimated PNEC was 0.0014 mg BTA/L. Such findings may be linked to the chemical properties of BTA, but also of both LDHs dispersions. Larger aggregates tend to sink in the first 24 h (i.e., may closely interact with the benthic sea-urchin embryos) while the levels of Zn, Mg, and Al were above the baseline levels in ASW regardless of the concentration or timepoint. These results will foster the forefront of knowledge towards a new generation of efficient and truly eco-friendly anti-corrosion nanoadditives.

## 14A.O-Mo-6

### Comparing the Ecological Hazards of New Nanostructured Antifouling Biocides to Neotropical Marine Environments

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#### Abstract

Recent investigations have shown that the encapsulation of the antifouling DCOIT (4,5-Dichloro-2-octylisothiazol-3(2H)-one) in mesoporous silica nanocapsules (SiNC) reduced the biocide's toxicity to marine non-target species. The antifouling properties could be increased by impregnating the nanoadditive SiNC-DCOIT with silver (Ag), based on the antimicrobial properties of this metal. This novel antifouling nanoadditive has lower toxicity to temperate species, but there is no data on tropical marine species, which is key for a more realistic assessment of this new generation of materials. This study aimed at assessing and comparing the hazard of the soluble and nanostructured forms of DCOIT (SiNC-DCOIT and SiNC-DCOIT-Ag) on neotropical marine species. Toxicity tests were conducted with 13 species, considering bacteria (*Vibrio parahaemolyticus*, *V. aestuarius*, *Bacillus subtilis*), fungi (*Penicillium citrinum*, *P. clerotigenum*, *Aspergillus niger*), microalgae (*Chlorella minutissima*, *Thalassiosira pseudonana*), copepod (*Nitokra* sp.), brine shrimp (*Artemia salina*), mussel (*Perna perna*), sea urchin (*Echinometra lucunter*), and sand dollar (*Mellita quinquesperforata*). For each chemical and species, no effect concentration (NOEC) values were determined following an analysis of variance (ANOVA). These values were combined with NOEC values reported in the literature for tropical species in order to derive the hazard concentration to 5% species (HC5) based on the Species Sensitivity Distribution (SSD) method. The HC5 was then divided by an appropriated assessment factor in order to calculate the predicted no effect concentration (PNEC) for the hazard assessment. According to the probabilistic approach, the HC5 were estimated as low as 0.0001  $\mu\text{g L}^{-1}$  for the soluble DCOIT, 0.029  $\mu\text{g DCOIT L}^{-1}$  for SiNC-DCOIT, and 0.025  $\mu\text{g DCOIT L}^{-1}$  for SiNC-DCOIT-Ag. Then, the marine PNEC were established as 0.0001  $\mu\text{g DCOIT L}^{-1}$  for soluble DCOIT, 0.0097  $\mu\text{g DCOIT L}^{-1}$  for SiNC-DCOIT, and 0.0083  $\mu\text{g DCOIT L}^{-1}$  for the SiNC-DCOIT-Ag. The immobilization of the biocide in SiNC reduced its hazard in the tropical marine environment. SiNC-DCOIT-Ag presented slightly higher hazard than the SiNC-DCOIT, but still 83-fold lower than the soluble DCOIT. According the present findings, the encapsulation technique greatly reduces the DCOIT hazard to neotropical marine species, indicating the potential of these nanomaterials to substitute the current forms of the state-of-the-art antifouling biocides.

## 14B.O-Mo-1

### Effects of an Innovative Anti-corrosion Nanomaterial on Neotropical Species

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#### Abstract

Corrosion is a worldwide problem with direct yearly costs of 3.5% of the global Gross Domestic Product (GDP). Common practices to control this phenomenon include the use of corrosion-resistant metals and/or coatings with corrosion inhibitors (Cis) and cathodic protection. The 2-mercaptobenzothiazole (MBT) has been used as a corrosion inhibitor by the oil and naval industries, but environmental restrictions require the development of more effective and non-toxic CIs. The immobilization of such CIs in manufactured nanomaterials (NM) represents a promising alternative to reduce the associated environmental impacts. This study aimed at assessing if the immobilization of MBT in Layered Double Hydroxides (LDH), an engineered nanomaterial with exchange capacity, could reduce the toxicity of the active compound on the embryonic development of the sea urchin *Echinometra lucunter* and fecundity of the copepod *Nitokra* sp. Toxicity tests were performed with both species considering the soluble MBT, its forms immobilized in LDH (MgAl LDH-MBT and ZnAl LDH-MBT), and the unloaded nanomaterials (MgAl LDH and ZnAl LDH). The results were analysed by ANOVA, followed by the Dunnett's test, and the Effective Concentrations to 50% organisms (EC50) were calculated through a non-linear regression method. For *E. lucunter*, the results indicated greater toxicity of the soluble MBT (EC50 = 0.020 mg L<sup>-1</sup>), in comparison with the MgAl LDH MBT and ZnAl LDH MBT (respective EC50s = 0.055 and 0.054 mg L<sup>-1</sup>). The ZnAl LDH was more toxic to the embryos than MgAl LDH. The *Nitokra* sp. experiments did not exhibit statistical differences between MgAl LDH, ZnAl LDH, and MgAl LDH MBT. In its turn, the soluble MBT was more toxic than the ZnAl LDH MBT, with respective EC50 values of 0.23 and 0.52 mg L<sup>-1</sup>. Overall, immobilization on nanomaterials has been shown to reduce MBT toxicity for both test organisms. The present study showed that unloaded nanomaterials are no/low toxic for the tested tropical species and that the encapsulation process protected tropical marine species by decreasing at least 2-fold the toxicity of MBT. Globally, toxicity can be summarized as: MBT>LDH-MBT>>LDH. Therefore, LDH-MBT seems to be a promising anticorrosion nanomaterial with clear environmental benefits. Finally, this study also suggests that the most promising alternative is the use of MgAl-based nanoclays, as this nanomaterial was more effective in reducing the chronic toxicity of MBT to neotropical aquatic organisms.



## 14B.O-Mo-2

### Impact of Co-Exposure of Titanium Dioxide Nanoparticles and Cadmium on Skeletal Development of Zebrafish (*Danio rerio*)

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#### Abstract

Titanium dioxide nanoparticles (TiO<sub>2</sub> NPs) are emerging pollutants of great ecotoxicological importance. However, their interactive effects with metals on the skeletal development of aquatic organisms are still scarce. Thus, the present study evaluated the potential effect of TiO<sub>2</sub> NPs and Cd (alone or in co-exposure) on zebrafish (*Danio rerio*) embryos and larvae. Embryos were exposed at environmentally relevant concentrations of TiO<sub>2</sub> NPs (0.1, 1.0 and 10 µg L<sup>-1</sup>) and Cd (10 µg L<sup>-1</sup>), alone and in co-exposure, for 168 h. Chondrotoxicity and osteotoxicity were evaluated at the end of the experiment (168 h) through diaphanization and differential staining of cartilage and bones in zebrafish larvae. Isolated TiO<sub>2</sub> NPs do not alter cartilaginous and bone development in zebrafish after 168 h of exposure. However, Cd alone or in co-exposure with TiO<sub>2</sub> NPs affect cartilaginous and mainly bone development, where most structures were altered or not formed in 168 h. Furthermore, it was observed that the skeletal structures of the viscerocranium were more affected than the skeletal structures of the neurocranium. The present study confirmed the chondrotoxic and osteotoxic potential of Cd and its association with TiO<sub>2</sub> NPs. Furthermore, this is the first study to evaluate the effects of TiO<sub>2</sub> NPs in the zebrafish skeleton, and the association of TiO<sub>2</sub> NPs and Cd. The present results raise the question of the importance of studying the skeletal system as a biomarker for nanoecotoxicological studies, in addition to the importance of studies on the toxicity of mixtures, mainly at environmentally relevant concentrations.

## 14B.O-Mo-4

# Toxic Effects on the Photosynthetic Mechanism of *Chlorella vulgaris* (Microalgae) Caused by Relevant Environmental Concentrations of TiO<sub>2</sub> Nanoparticles

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### Abstract

The production of nanoparticles (NPs) has rapidly increased in recent years, causing an uncontrolled release into the environment. Due to their size of less than 100 nm, they allow direct Access to cells and interact with the different organelles that compose them. In Chile, there is a lack/absence of data focused on the amount of NP entry into environmental matrices and their effects.

Considering that primary producers maintain a balance in the quality of aquatic systems and any effect on them can cause metabolic imbalance. Currently, traditional ecotoxicity assays focus on the physical observation of organisms, ignoring the biochemical process in which NPs interact. Considering the concentrations at which they are found ( $\mu\text{g L}^{-1}$ ), the genetic expression of photosynthetic processes genes is a good tool for measuring sublethal effects as a first alert, providing an opportunity to integrate such analyses into a framework for assessing the impacts of nanoparticles in the aquatic environment.

This study proposes the analysis of 6 genes for the genetic expression in *Chlorella vulgaris* involved in the energy production of photosynthesis: *psaA*, *psaB*, *psaD*, *psbA*, *rbcL*, and *AtpB*. Through a previous study, TiO<sub>2</sub> NPs concentrations were estimated in the Biobío River located in south-central Chile. The samples were characterized and quantified by TEM and ICP-MS, respectively. These estimates revealed a TiO<sub>2</sub> concentration of less than  $17.6 \mu\text{g L}^{-1}$  in particle size ranges between 80-120 nm. These data were used to test the photosynthetic process of *C. vulgaris*.

Toxicity tests with *C. vulgaris* showed cell fragmentation and decrease cell sizes starting from  $50 \mu\text{g L}^{-1}$ , which allowed a higher number of cells in the count than in the control. According to these results, the photosynthetic process of *C. vulgaris* measured through the analysis of genetic expression, considering ranges  $<17.6 \mu\text{g L}^{-1}$  to reveal sublethal damages at actual environmental concentrations of NPs, will be presented. The hypothesis put forward in this experiment: Due to the interaction of NPS-TiO<sub>2</sub> with *C. vulgaris*, sublethal effects are evident at the photosynthetic level that are not observed morphologically.

This study contributes on a regional and international scale with data and observations made in real environmental matrices, highlighting the need to inform the nanotechnology industry about the risks and deterioration of aquatic ecosystems caused by NPs when they enter into the environment.

# Remediation of Endocrine Disruptive Compounds by Nanocomposite Polyvinylidene Fluoride Membrane

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## Abstract

The occurrence of the micro-pollutants in water is a matter of concern due to their persistent influence on aquatic and terrestrial ecosystems, even at low concentrations. The environment is exposed to these compounds' cocktails, which increases their impact and risk on the aquatic ecosystem. Their presence and effect have been reported worldwide in almost all environmental matrices such as food chains, soil, underground-surface water, plants, and animals. Therefore, there is a need for removal that leads to the development of advanced nanocomposite membranes to effectively remove endocrine disruptive compounds from water matrix. Graphene is one of the nanomaterials used in water treatment applications due to its excellent properties. We have derived graphene oxide from agricultural waste and used it to remove endocrine disruptive compounds, antibiotic compounds, and other organic contaminants, which has application in industrial effluent treatment. Polyvinylidene fluoride (PVDF) membrane was fabricated by solution casting method, in which different weight percentages of waste-derived graphene were incorporated during the membrane casting. Further membrane flux and permeability studies have been performed to understand the feasibility of the membrane for industrial application. Membrane performance studies have shown excellent rejection of endocrine disruptive compounds, antibiotic compounds, and other organic contaminants. Therefore it can be concluded that the developed nanocomposite membrane is promising in terms of remediation of micro-pollutants from water.

## 14B.O-Mo-5

### Characterization of Lyophilized-Sonicated Graphene Oxide (L-SGO) and Ecotoxicity Assessment on Biological Models

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#### Abstract

Graphene Oxide (GO) is a highly oxidized form of graphene and an emerging nanomaterial with a large surface area, excellent strength, and other mechanical properties, while maintaining high ductility, flexibility and hydrophilicity being widely used for technological and commercial uses. These unique properties facilitate the adhesion of GO to cell surfaces; L-SGO ecotoxicity is largely unknown. The current study provides a structural and chemical analysis of L-SGO by Optical Microscopy (OM), Scanning Electron Microscopy (SEM), RAMAN Spectroscopy and XPs Diffraction, and ecotoxicity assessment on 3 biological models. GO was synthesized by the modified Hummer method, from natural graphite powder and NaNO<sub>3</sub>, mixed with H<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>O<sub>2</sub> stirred for 5 h, then washed with HCL, deionized water, and ethanol until neutral pH. The solution was lysed and dispersed in water and sonicated for 3 h to obtain graphene oxides in the form of sheets. The potential toxicity of 0.1, 1.0, 10, and 100 mg L<sup>-1</sup> L-SGO and a control (without L-SGO) was previously evaluated on *C. vulgaris*, *L. sativa* and *A. cepa*. Graphene oxide showed a sheet structure (573.2 ±324 nm) with irregular folds and wrinkles on its edges and surface. In the L-SGO leaves predominated Carbon and Oxygen, corroborated by Raman Spectroscopy: D, G, 2D and D+D bands characteristic of GO. XPS measurements of L-SGO showed a 29.05% O and 70.95% C concentration. No significant differences were found in *C. vulgaris* density, cell diameter, biovolume, or pigment concentration between the control and all treatments with L-SGO. Still, there was inhibition of the growth rate at the highest concentration. SEM images in control show intact cells; in 0.1 and 10 mg L<sup>-1</sup> the cells remain undamaged, but a large number of L-SGO sheets were observed on cell surfaces. Conversely, in 100 mg L<sup>-1</sup> cells are destroyed and form bulks. Similar results were obtained from *L. sativa* imaging by SEM. *L. sativa* tests showed no significant differences (p>0.05) between the control and the concentrations tested in radicle elongation, only significant differences (p<0.05) were found between the highest concentration and the control in the % germination. *A. cepa*: only root length decreased significantly at the highest GO concentration (100 mg L<sup>-1</sup>) (p<0.05). These results are consistent with low L-SGO toxicity for *C. vulgaris*, *L. sativa* and *A. cepa*. However, this topic deserves further attention and further research has to be done on more biological models.

## 14B.O-Mo-6

# Graphene Based Nanocomposite Foam for Sorption of Organic Compounds from Sea for Environmental Remediation

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### Abstract

Pollution due to organic compounds, petrochemical products, and crude oil has significant adverse effects on the soil, water, aquatic ecosystem and humans. Oil spills, transportation and exploration activities significantly contribute to water pollution and resource loss. Therefore, there is a great need to not only clean the water bodies from spills but also, if possible, recover the spilled oil and organic solvents to incur minimal financial losses. This study explored an economical and scalable method for synthesizing oxidized graphene framework followed by developing graphene-based foam to recover oil and organic solvents. The systematic modifications conducted for the development of graphene-based foams with polymer to improve its physical, mechanical, and adsorptive properties. As-synthesized composite foam has been characterized to confirm its composition and applicability in terms of pollutant removal and resource recovery. Additionally, nanocomposite foam surface wetting studies conclude a super-oleophilic surface with water and motor oil contact angles of  $132 \pm 5^\circ$  and  $0^\circ$ , respectively. The sorption capacity of three different oils groups; vegetable oil, lubricants, and organic compounds, has been explored to demonstrate the versatility in application. The sorption capacity of motor oil, mustard oil, and DCM was 12.5, 12.1, and 26.3 gg<sup>-1</sup> respectively. The excellent sorption capacity for oils with ease in its recovery with 500 cycles of reusability defines the utility of developed foam to address environmental pollution globally.

# Session 15: Toxic Metal and Metalloids Pollution in Surface and Groundwater Hydric Resources

## 15A.O-Mo-1

### Metal Bioaccumulation, Antioxidant Responses and Histological Changes in Gill of Fat Snook Fish (*Centropomus Paralellus*) to Metallic Atmospheric Particulate Matter

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#### Abstract

Metallic smoke released by steel industries is constitute by a mixture of fine and gross particles containing metals, including the emerging ones, which sedimentation contaminates soil and aquatic ecosystems and put in risk the resident biota. This study determined the metal/metalloids in the settleable particulate matter (SePM) from a metallurgical industrial area and evaluated metal bioconcentration, antioxidant responses, oxidative stress and the histopathology in the gills of fat snook fish (*Centropomus paralellus*) exposed to different concentrations of SePM (0.0, 0.01, 0.1 and 1.0 g/L), for 96 h. From the 27 metals (Al, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Rb, Sr, Y, Zr, Nb, Mo, Ag, Cd, Sn, Ba, La, Ce, W, Hg, Pb, Bi) analyzed, 18 were quantified in SePM and dissolved in seawater. The higher (>200 µg/g) metal concentrations in SePM were Fe>Al>Ti>Mn>Zn while metals dissolved in water were Al>Zn>Ba>Fe. The metals having higher bioconcentration in the gills were Fe>Sr>Zn>Al. The activity of superoxide dismutase (SOD) decreased in the gills of fish exposed to 0.1 g/L, catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) as well the level of glutathione were unchanged. The unchanged levels of lipid peroxidation indicate that the antioxidant responses were efficient to avoid oxidative stress. Organ lesion indices were higher in the gills of fish exposed to 0.01 and 1.0 g/L SePM. Pavement cell hypertrophy on lamellar epithelium increased taken from 50 to 75% of epithelium and epithelial lifting were more frequent; changes in blood circulation were characterized by blood congestion in the pillar cell system (11-25%). Hyperplasia of filament epithelium resulted in total or partial lamellar fusion. Focal necrose (irreversible lesion) was identified in fish exposed to 0.1 g/L SePM at very low frequency (< 10%). Although at sublethal levels, the SePM seawater contamination affects fish gills. Cellular/organ morphology changes reduce the animal performance facing new environmental challenges and implies in energy expenditure to restored cell structure. *Financial support:* FAPESP Grant 2019/08491-0, CNPq Grant 306818/2020-5, FONCYT, PICT-2015-2819, SECyT-UNC, 411/18. Scholarship awards: R. Monteiro (CAPES, Finance code 1); FAPESP Pos-Doctoral fellowship I.C Souza (Grants 2016/025257-2) and M Morozesk (Grant 2021/02906-3).

## 15A.O-Mo-2

### Transcriptomic Alterations Related to Methylmercury (MeHg) Exposure in Wild Living Rainbow Trout, *Oncorhynchus mykiss*, from Chile.

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#### Abstract

In recent decades, the understanding of methylmercury (MeHg) toxicity in different forms of life has progressed substantially. Evidence have shown that MeHg bioaccumulation in fish can trigger alterations in the synthesis of various essential reproductive hormones by the alteration of, i.e., the expression of vitellogenin, a protein involved in egg formation in fish and a widely used biomarker of endocrine disruption. And nonexclusively, the interruption of energy metabolism, oxidative stress; also, alteration of the immune response and lipid metabolism, some of these important functions being performed in the liver. The evaluation of the molecular response to the presence of MeHg in model fish has not been carried out in Chile until now. Therefore, we pursue to evaluate the effect on gene expression in rainbow trout, *Oncorhynchus mykiss*, exposed to MeHg. This fish species has advantageous characteristics to be used as a bioindicator, first for its high position in the aquatic food web, second for its meat rich in lipid content that it is not only of interest for human consumption but also provides a prodigious media for MeHg bioaccumulation, and last the rainbow trout has a large distribution worldwide and along Chilean watersheds. Therefore, we first determined three natural sites where we found different MeHg bioaccumulation levels in rainbow trout muscle (low: 0.005-0.008  $\mu\text{g g}^{-1}$ ; middle: 0.034-0.136  $\mu\text{g g}^{-1}$ ; high: 0.168-0.310  $\mu\text{g g}^{-1}$ ). Of each site male and female rainbow trout were collected and the liver was processed for qPCR of six genes and Next Generation Sequencing (NGS) analysis for gene discovery, related to MeHg exposure. The qPCR showed that female trout with high exposure to MeHg have alterations in the expression of genes decoding for zona pellucida, estrogen receptor beta, metallothionein; meanwhile male trout showed an alteration in the expression of genes decoding for estrogen receptor alpha and beta, and metallothionein. The NGS data showed that from a total of 14,016 genes expressed 826 genes were differentiated expressed in the middle and high MeHg exposed female trout, when only 529 from 13,484 genes were differentiated expressed in middle and high MeHg exposed male trout. Accordingly, these preliminary results evidence a sex difference between the genes that are down or up regulated because of MeHg exposure in rainbow trout. This work was funded by FONDECYT Initiation Grant 11180914, E.V. was funded by ANID Scholarship 21220155.

## 15A.O-Mo-3

### Hematological Parameters in *Oreochromis niloticus* Collected in Lakes Under the Influence of Metallurgical Industries

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#### Abstract

The industrial revolution resulted in an intense urbanization process that generated anthropic impacts on the environment, such as the contamination of water resources. Brazil is one of the largest producers and exporters of iron in the world with high metallurgical activity and the largest metallurgical complex is the Tubarão Complex located in the Espírito Santo state, Brazil. The present study evaluates the physiological changes in the blood of *Oreochromis niloticus* collected in estuarine lakes under influence of metallurgical industries (Maemba –sites P1 and P2 and Carapebus) and a lake (Alegre), distant 150km from this industrial complex, as a reference site. *O. niloticus* has a worldwide distribution, it is abundant in Brazilian reservoirs and has a well-known physiology. Several biomarkers as cortisol; glucose; ions (chloride, sodium and potassium ions); osmolality; lysozyme; leukocyte burst; total plasma protein; total thrombocyte and leukocyte; differential leukocyte number (monocyte, lymphocyte, neutrophil, granular leukocyte PAS-positive and eosinophil); micronucleus; hematocrit; hemoglobin; erythrocyte number and hematological indices (MCV; MCHC and MCH) were analyzed to diagnose the environmental conditions in which they are living. Alegre fishes had a higher number of neutrophils, granular leukocyte and erythrocytes and lower leukocyte respiratory activity (burst) and MCV than the other lakes; Carapebus fishes had higher concentration of cortisol, chloride and sodium ions in plasma, eosinophils and micronuclei; Maemba fishes had higher concentration of lysozyme and total plasma protein, fishes from P1 site presented more number of leukocytes and MCV and lower osmolality, lymphocyte number, hemoglobin, MCHC and MCH compared to the other lakes and P2 site presented higher lymphocyte number and MCH compared to the other lakes and P1. The higher cortisol level in Carapebus indicated stress condition and the higher lymphocytes in fish from P2 site, in Maemba may indicate an immune response to inflammation. Therefore, *O. niloticus* from lakes under industrial influence presented stress responses suggest low water quality.

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## 15A.O-Mo-4

### Differential Metallothionein and Hypoxia-Induced Angiogenesis Gene Expression in Adult Zebrafish (*Danio rerio*) Exposed to Cd, Pb and As.

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#### Abstract

Metals are natural components of the biosphere. Although Cu, Fe, Ni and Zn are necessary for living beings, Cd, Pb and As are not essential biologically and accumulate in organisms. Once the metabolic, storage and detoxification capacity is exceeded in fishes living in waters with high concentrations of such metals, the risk of toxicity due to absorption through the liver, gills, muscles or intestinal wall increases. This study evaluated the temporal pattern of metal mediated-toxicity through MT, HIF-1 and VEGF gene expression in the liver and gills of adult zebrafish (*Danio rerio*) chronically exposed to sublethal concentrations of As, Cd, and Pb. This was done to assess their utility as metabolic pathway biomarkers of early exposure (MT gene) and subsequent effects such as oxidative stress and early angiogenesis (HIF-1 and VEGF genes respectively). We compared the pattern of basal expression of these genes and their temporal responses to waterborne metal exposure to concentrations similar to those from highly metal impacted freshwater ecosystems in northern Chile. Our results showed that such concentrations caused hypoxia-mediated angiogenesis in adult zebrafish livers. The metabolic pathway evaluated through the sequential response of initial biomarkers of exposure (MT gene expression) and subsequent biomarkers of effect (HIF-1 and VEGF genes expression) also revealed a protective effect of Cd-induced metallothioneins, hypoxia caused by As and early angiogenesis caused by Pb>As>Cd.

## 15A.O-Mo-4

### Protecting the Environment by Developing Aquatic Risk Assessment and Management Tools for Latin America

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#### Abstract

The first talks on pesticide risk assessment in Latin America date back to the 90s, but it was only until 2002 that the Andean community settled on a framework for performing environmental risk assessments within the registration process of pesticides. Since then both regulators and industry have spent efforts to improve the scientific basis for aquatic exposure assessments for plant protection products in the Andean countries. In 2017 CropLife Latin America engaged in the development of two aquatic models with support of Waterborne Environmental: The Andean Aquatic Screening Tool, and the Andean Pesticide Exposure Simulation Tool ANDES.

This presentation provides an overview of the collaboration between industry, the environmental agencies from Peru and Colombia and Waterborne when generating the local exposure scenarios for banana, plantain, potato, tomato, coffee, maize, asparagus, avocado, grapes, dry and paddy rice crops and implementation of mitigation measures within both tools.

Effective mitigation measures are critical to ensure the environmental safety of plant protection products, but it is also as important that regulatory Risk Assessment schemes consider such tools in order to reach the common goal of helping to protect the environment and improve agricultural practice in Latin America.

## 15A.O-Mo-5

### Use of the Burrowing Crayfish Parastacidae (Crustacea: Decapoda) as a Bioindicator of Groundwater Quality in South America

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#### Abstract

In groundwater-dependent ecosystems (GDEs), the infiltration of contaminants is often rapid and difficult to manage, which makes them particularly vulnerable and puts their ecological structure and function at risk. Ecotoxicological studies in subterranean environments are scarce and scattered, mostly evaluating the occurrence of trace elements in crustaceans of subterranean habitat. In this context, the use of biological indicators is a useful tool to monitor the health of these ecosystems and to evaluate the impact of human activity. In this review, we suggest the ability of the burrowing crayfish of the Parastacidae family, widely distributed in the region in GDEs, as a bioindicator of groundwater quality, due to their characteristics such as their low vagility, similarities between species of the same genus, and their ability to live immersed in a particular environment throughout their life cycle and being considered a key specie in the transfer of energy between different trophic groups, representing a good model to evaluate levels of bioaccumulation of trace elements and their subsequent effect on the food chain, food security and economy of the communities that depend on this resource. In this presentation we use crayfish in Chile to describe the measurements of different trace metals (TXRF) in its tissues and their relationship with site attributes used in different sites characterized by different anthropogenic impacts, in this way, the use of these burrowing crayfish is a useful tool for biomonitoring groundwater quality in places where they are distributed.

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## 15A.O-Mo-6

### Unravelling the Environmental Association of Total and Bioavailable Metal Burdens in Coastal Sediments With Artificial Freshwater Discharge

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#### Abstract

Sediments in sandy beaches can accumulate trace metals from freshwater discharge or oceanic inputs from natural or anthropogenic sources. Metal burden in sediments is an indicator of environmental quality, and depending on the bioavailability of metals, the potential trophic transfer of elements can occur. This work aimed to describe the accumulation of metals (Al, Ca, Fe, Ti, S, Ba, Mn, Sr, P, V, Zn, Pb, Cr, Cu, Ni, Cd, and Hg) in sediments generated by an artificial canal that discharges freshwater along with an unknown quantity of pesticides and suspended organic matter onto the SW extreme of the 22-km sandy beach fringe between La Coronilla and Barra del Chuy (Uruguay). We explored the association of the total and extractable (bioavailable fraction) metal burdens with environmental parameters such as salinity, temperature, pH, carbonate, and granulometry, using generalized additive mixed models. The models help to unravel the source, fate, and behavior of elements in the study system. None of the elements analyzed reached toxic levels. Regarding total concentrations, out of 17 elements, 8 were negatively associated with grain size (which decreased with distance from the canal, towards the sea) and 3 elements were positively associated with salinity and carbonates (increased towards the sea). In the case of the bioavailable fraction, 4 elements were negatively associated with grain size and 3 elements were positively associated with salinity and carbonates. These findings offer valuable insights into the degree of enrichment of various elements in sandy beaches, originating from both marine and terrestrial sources, and therefore can inform policies aimed at reducing their impact on sandy beach ecosystems.

## 15B.O-Mo-1

### Gill Histopathological Modifications in *Oreochromis Niloticus* Collected in a Lacustre Systems Under Metallurgical Industrial Influence

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#### Abstract

The demand for natural resources has been growing exponentially since the industrial revolution and increasing of human population. Consequently, the water resources have been under constant pressure as one of the main conditions in the development of the human population. Metals and metalloids occur naturally in the environment, however, due to the anthropogenic needs, these elements have been manufactured and their wastes released in the environment impacting atmosphere, soil, lakes, rivers, and reservoirs. Fish and their gills are suitable bioindicators as they are sensitive to changes in the environment. The gill functions, structure and direct contact with water facilitate the absorption of pollutants, especially under stress conditions. Histopathological analyses are a useful tool to identify the effects of contaminants in water. The present study evaluated histopathological alterations in the *Oreochromis niloticus* gills collected in two lakes located in industrial metallurgical areas in the state of Espírito Santo, Brazil, and a reference lake located more than 150km from this region. The main pathologies identified in *Oreochromis niloticus* gills collected in the reference lake were lamellar hypertrophy and congestion. However, lakes under influence of industrial contaminants had a higher incidence and diversity of lesions, such as lamellar hypertrophy, congestion, filament epithelium displacement, filament epithelium hyperplasia, mucosal cell proliferation, lamellar fusion, aneurysms, and necrosis. These gill pathologies are morphological changes resulting from physiological and biochemical changes caused by environmental conditions that can alter the normal function of the organ. Most injuries arise as defense mechanisms or compensatory mechanisms to adapt the organ to the stressor.

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## 15B.O-Mo-2

### **Scientometric Analysis of Contamination by Metals and Trace Metalloids in Sediment from the Coast and Estuaries of Argentina and Uruguay**

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#### **Abstract**

The present study is a scientometric evaluation of scientific publications on contamination by trace metals and metalloids in sediments from coastal environments of countries located in the Large Marine Ecosystem (LME) of the Patagonian Shelf (Argentina and Uruguay). Research articles on sediment assessments were quantified through systematic review, using the keywords (Metal\*) AND (Sediment\*) AND (Estuar\* OR Coast\*) AND (Urugua\* OR Argentin\*) in the Web of Science until the year 2022. Articles from other countries, which presented contamination data only in the biota, did not present contamination data in the sediment, did not present data in a way that it was possible to identify concentrations, and review works were discarded. The publications had their contents tabulated for the scientometric record according to the number of scientific articles (1) by country/province, (2) by year and (3) by analyzed element. Studies were found reporting concentrations of metals Al, Cd, Pb, Cu, Cr, Sc, Sn, Fe, Mn, Hg, Ni, V, Zn, and trace metalloids (or semimetals) As and Se. The oldest sediment assessments date back to 1993, with a total of 56 publications up to the year 2022 being included, resulting in 756 records. Most of the studies were carried out in Argentina (48 articles), while only 8 articles referred to Uruguay, Rio de La Plata (Buenos Aires and Montevideo), with 47.2%, and Bahia Blanca (Buenos Aires), with 31, 3% were the places with the most records. The number of publications remained between 1-4 articles per year, with a maximum in 2017, with 7 articles. Pb, Zn, Cu, Cr and Cd were the main metals evaluated in the sediment, appearing in 89.3%, 85.7%, 83.9%, 80.4%, 60.7% of the articles respectively. The percentages of reported values for each element that violate the Probable Effect Level (PEL) were Zn (7.1%), Pb (4.4%), Cu (4.1%), Cr (3.2 %), Hg (1.5%), Cd (0.5%), indicating attention to these contaminants, since values above those allowed by the sediment quality guidelines may present an ecological risk. All this information is valuable for researchers and policymakers interested in the topic.

## 15B.O-Mo-3

# Assessment of Arsenic Levels in Groundwater and Its Risk of Environmental Exposure in the Rural Community of San Antonio-Uruguay in 2021 and 2023

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### Abstract

Groundwater quality has been the focus of multidisciplinary Medical Geology research studies in Uruguay, with arsenic being one of their main concerns due to environmental health impacts in regions where arsenic levels are higher than WHO recommendations ( $<10 \mu\text{g/L}$ ). These geogenic arsenic levels in different aquifers in Uruguay have been reported only since 2007. However, there are no studies of lifetime health risks in the population exposed to low doses of arsenic.

In rural areas populations do not have access to regular water supply and drinking water sources are from their own wells, which cannot be properly controlled for arsenic among other elements, with potentially serious health risks for that consuming population.

The aim of this preliminary work is to investigate the association between arsenic exposure through drinking water from such wells and adverse health effects (dermatological manifestations and cancer) by a multidisciplinary Medical Geology approach in a rural population located in San Antonio, Canelones department.

During the first stage in 2021, water from 30 private wells in this area was sampled and analyzed.

In the second step, the survey uses a cluster sampling procedure to select private wells within this referred locality, where 10 samples have been studied so far, in addition to a targeted survey to find out about consumption habits.

The health risk was estimated with the frequency and duration of exposure to water from these well. The health risk was estimated based on the frequency and duration of exposure to water from these wells. Arsenic levels in the water were used to calculate the arsenic average daily dose(ADD). The risk was represented by the hazard quotient(HQ) and the cancer risk(CR).

HQ was estimated for dermatological manifestations, using the arsenic toxicity reference dose of  $0.0003 \text{ mg/kg/day}$ . A health risk situation was assumed when HQ levels were  $>1$ . Cancer risk (CR) was calculated using the cancer slope factor of  $1.5 \text{ mg/kg/day}^{-1}$ . Values between  $10^{-4}$  y  $10^{-6}$  are considered safe.

More than 95% of the samples tested had arsenic levels higher than the WHO, and initial results of the risk assessment showed that there is a risk of dermatological damage and cancer associated with arsenic exposure.

High levels of arsenic in private wells water were confirmed. There may be a health risk for the exposed population. There are no previous risk assessment studies on arsenic, so this may be the first one in Uruguay.

## 15B.O-Mo-4

### **Arsenic and Other Inorganic Pollutant Levels in Uruguayan Groundwater Intended for Human Consumption**

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#### **Abstract**

The state-owned company provides safe drinking water to a large portion of the Uruguayan population. However, in some rural areas, this "official" water supply does not exist. People in these places drink their own well water, which cannot be properly controlled with the potential health risks for those consumers. Arsenic, one of the concerns for environmental health in locations where levels are higher than WHO limits ( $10 \mu\text{g/L}$ ), has been the subject of research on medical geology in Uruguay. In addition, it is well known that drinking water containing high levels of nitrate, fluoride, and manganese has harmful effects on human health, and WHO recommends nitrate levels lower than  $50 \text{ mg/L}$ , fluoride lower than  $1.5 \text{ mg/L}$  and manganese lower than  $0.1 \text{ mg/L}$ . Based on our group's preliminary studies that investigated groundwater from all over the country, different areas with arsenic water levels above international regulations were identified. One of them was in Canelones department, where arsenic levels ranged from  $63.2$  to  $120.4 \mu\text{g/L}$ .

The objectives of this work were to perform more in-depth research in this zone by sampling groundwater from privately owned wells used for drinking water by residents and analyze arsenic and other pollutants. Studies on health risk assessment have also used this data as a starting point.

San Antonio, a tiny Uruguayan community in Canelones, with 1650 rural residents, the majority of whom have their own wells and utilize this water for drinking as well as for their agricultural and livestock activities was selected for this research. A cluster sampling procedure was used to select 30 private wells within this area, and water from 10 wells has been sampled and analyzed up to this point. Analytical determinations of arsenic were performed by hydride generation microwave-induced plasma atomic emission spectrometry, fluoride concentrations were measured using a combined fluoride electrode, manganese by electrothermal atomic absorption spectrometry, and nitrate by means of ion chromatography.

According to preliminary findings, 100% of the samples had safe fluoride and manganese levels, whereas 90% of the samples had arsenic levels above international norms and two samples had nitrate levels over the safe values.

Our findings indicate that arsenic levels in groundwater, as well as their relationship with other inorganic parameters, should be thoroughly investigated in our country to prevent long-term health effects on consumers.



## 15B.O-Mo-5

### **Arsenic and Its Inorganic Species in Boreholes for Human Consumption in Southeastern Kiyú, San José, Uruguay**

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#### **Abstract**

Arsenic (As) is a natural element of the earth's crust that in its inorganic forms can be toxic to humans. One of the main sources of exposure to arsenic is the consumption of untreated groundwater, an evident problem in certain Uruguayan aquifers, such as the Raigón Aquifer, where levels higher than the World Health Organization (WHO) recommended value of 0.01 mg L<sup>-1</sup> have been detected. This research was coordinated with the Groundwater Division of Obras Sanitarias del Estado (OSE) whose main objective was to preliminarily determine the levels of As and its inorganic species +3 and +5 in two time periods (winter and spring) in four boreholes that supply drinking water to the locality of Kiyú, department of San José, Uruguay. For this purpose, majority ions, As and its inorganic species and physicochemical parameters from eight groundwater samples were analyzed by standard methods (APHA). According to the results, groundwater is classified as Na-HCO<sub>3</sub> to Ca-Mg-HCO<sub>3</sub>, hard, neutral to slightly alkaline and oxidizing. Arsenic values between 0.012 and 0.019 mg L<sup>-1</sup> were detected in winter and between 0.014 and 0.017 mg L<sup>-1</sup> in spring. In the winter samples, As+5 values between 0.010 and 0.017 mg L<sup>-1</sup> and As+3 values between 0.001 and 0.002 mg L<sup>-1</sup> were detected. In spring, only the As+5 species was detected. Therefore, the predominant species detected corresponds to arsenate (As+5), which although on the toxicity scale is 10 times less toxic than arsenite (As+3), its presence in the human body could affect health. A priori, these differences found between the levels of As and its inorganic species could be due to changes in the chemical conditions of the groundwater (pH and/or redox potential levels variations) could produce the desorption of As from the mineral phases. This is a major breakthrough for the area as this finding will help OSE to develop a management plan for water resource use in the run-up to the 2023-2024 adoption of the WHO proposed guideline value. It will also contribute to future studies linking diseases associated with frequent water intake to the aforementioned levels.

## 15B.O-Mo-6

### Geochemistry and Variability of Arsenic Concentration in Groundwater: Kiyu Case, San Jose, Uruguay

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#### Abstract

The present research was oriented towards the lithological and chemical study of sediments from two wells of the Raigón aquifer and the chemical study of groundwater from four wells, with the objective of correlating the concentrations of arsenic in groundwater with the chemistry of the rock. The geochemical study was carried out on samples (extracted every meter) from a 42m-deep well, located on the property of Rural School No. 65, in Kiyú town, San José Department. The school had two disabled wells, due to high concentrations of arsenic (<0,020 mg/L) two more wells were drilled during this research, which were used for sampling groundwater and sediments. The sediments were processed and shipped to the ACTLAB Laboratory in Canada, as well as groundwater samples from permeable levels collected during drilling. According to the obtained results, the highest concentration of arsenic, 0,013 mg/L, was detected in a soil sample corresponding to a depth between 0 m and 1 m. The average value of arsenic concentrations in the entire profile traversed is 0,0065 mg/L, observing that the highest concentrations are found in the silt-clay levels and the lowest in the permeable sandy levels. Arsenic was correlated with Co, Cr and Sc, showing identical behaviors from base to top of the profile. Arsenic in groundwater at different permeable levels shows a variation from 0,0209 mg/L (20 to 22m) non-drinking water to 0,0134 mg/L (39 to 40m) drinking water according to the potability standard in force at the date in Uruguay. Analyzing the results of the chemistry of the rock samples, with the chemistry of the groundwater of the studied wells, it is concluded that: (a) there is a relationship between arsenic in rock and arsenic in water; (b) other toxic elements that have been identified in the rock and in groundwater are observed and have similar behavior, and are associated with As; (c) a high concentration of arsenic in the soil, which suggest that there are other sources of arsenic that are not exclusively from the rock.

# Session 16: Microplastics

## 16A.O-Mo-1

### Fate and Effects of Nanoplastics in Freshwater Ecosystems

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#### Abstract

Plastic pollution is a global environmental problem and freshwater ecosystems not only transport plastics to the ocean, but also transform it and act as sinks of plastic pollution. Nanoplastics (NPs, particle size  $<1 \mu\text{m}$ ) have been shown to be toxic to several freshwater organisms and to negatively affect ecosystem function. Most previous studies on NPs have been performed at the lab scale, in simplified experimental designs, and have contributed considerably to our understanding of transport and toxicity of NPs. However, little is known about the transport, uptake and effects of NPs in natural ecosystems. Therefore, we assessed the fate and effects of NPs in freshwater ecosystems at different levels of biological organization. We used wetland mesocosms and two different plastic nano-particles: gold-doped polystyrene NPs to explore the fate, and pure polystyrene NPs to explore the effects. We showed that 97% of the NPs were retained in the wetlands, mostly in the sediments, and were also taken up by biota, such as *Daphnia magna*, *Asellus aquaticus* and macrophytes. We found that the fate of the NPs was guided by distance from the addition point and that hetero-aggregation processes might have had a fundamental role in explaining the fate. Regarding the effects, we found significant effects on *D. magna*, and the phytoplankton community composition, but not on the other organisms studied. Organic matter decomposition rate was not affected in the time-frame assessed (70 days). Overall, our results suggest that the plankton food chain is more negatively affected by NPs than the detritivore one. Hence, our study advances our understanding regarding the fate and effects of NPs when they enter natural freshwater ecosystems.

## 16A.O-Mo-2

### Do Functional Traits Influence Microplastics Bioaccumulation In Marine And Estuarine Crabs?

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#### Abstract

Despite the growing discharge of plastic into the environment, few articles deal with the macroecological implications of microplastic (MP) bioaccumulation in organisms. In a novel approach, we propose the use of a meta-analysis, employing a database of MP accumulation in true crabs and pseudocrabs worldwide to determine if: I) functional traits influence the bioaccumulation of MPs in the different tissues of crabs; II) there are geographic regions where crabs accumulate more MPs than others; III) there is a tissue that can accumulate more MPs; IV) there is a possible ability to accumulate or sort particles according to size, color, shape, and type. We obtained twenty-eight articles from 2015 to 2022, with 50 organisms studied, corresponding to 15 families. Our results showed that functional traits might influence the accumulation of MPs. Among these traits, non-human-consumed crabs accumulated significantly more MPs than human-consumed ones; smaller crabs in size and weight and with shorter lifespans tended to exhibit more plastic particles. According to the environment, estuarine crabs from intertidal and muddy substrates held more MPs. Also, burrowers exhibited significantly more particles in the tissues and omnivorous crabs. Likewise, we found that crabs from parallels 0 to 20 presented more plastic particles, probably because of the mangroves' location that acts as traps for MPs. Considering the tissues, gills tended to accumulate more than the digestive tract, but without significant differences. Finally, colorless fibers of 1-5 mm of PA, PP, and PET were the predominant characteristics of MPs, probably indicating that crabs do not sort plastic according to color and accumulate denser types. Our analysis indicates that functional traits and latitude might influence the accumulation of MPs. As a result, it is essential to articulate the current bottom-up approach of ecotoxicology merged with a top-down macroecological approach that focuses on the ecological effects of MPs at large spatial scales and explores ecological systems as integral entities. This information can identify the most affected areas and define priorities for monitoring and implementing actions toward reducing plastic use globally.

## 16A.O-Mo-3

### Interactive Effects of Microplastics and Benzo[a]pyrene on Marine Invertebrates

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#### Abstract

The presence of microplastics in the ocean has become a major concern due to their wide distribution, persistence, the possibility of being ingested and accumulated by marine organisms, and ability to adsorb organic contaminants. The current research aimed to evaluate the toxicity of benzo[a]pyrene (B[a]P) and microplastics, alone and in mixture, at different concentrations (0.03, 0.3, 3, 12, 21, and 30  $\mu\text{g L}^{-1}$  of B[a]P; and 5, 50 and 500  $\text{mg L}^{-1}$  for microplastics), by measuring individual (embryo-larval development of *Echinometra lucunter* and mortality of *Mysidopsis juniae*) and sub-individual responses (DNA damage and lipid peroxidation in *M. juniae*). The hypothesis tested was that microplastics and B[a]P interact, modifying the toxicity of the isolated elements. Toxic effects of B[a]P with microplastics at environmental concentrations (5  $\text{mg L}^{-1}$ ) were not evident, but at higher concentrations of microparticles (50 and 500  $\text{mg L}^{-1}$ ) the toxic effects of B[a]P at both sub-individual (LPO and DNA damage) and at individual levels (embryo-larval development) was diminished. Concurrently, the chemical analyses showed that MPs can interact with B[a]P in water, slightly increasing B[a]P concentrations in water at low concentrations of B[a]P (<12.0  $\mu\text{g L}^{-1}$ ), but decreasing substantially B[a]P levels in water at higher concentrations (>21.0  $\mu\text{g L}^{-1}$ ). Microplastics interacted with B[a]P in seawater, reducing its toxicity, probably due to adsorption of B[a]P to the surface of plastic microparticles, consequently reducing the presence of the compound in the water. Although toxicity was reduced in both tested species, *E. lucunter* in its larvae stage were more sensitive to contamination. Assessing toxic effects using different organisms is relevant not only because it incorporates different sensitivities into the analysis, but also because different exposure pathways can be considered. However, further studies are needed to assess the interactive ecotoxicological effects of MPs and hydrophobic contaminants, preferably at environmentally relevant concentrations, for organisms that uptake the particles, either by ingestion or by simple attachment to epithelia. The results of the present study provide subsidies for the evaluation of the ecological risk caused by microplastics to marine biota, including their interactions with other stressors.

## 16A.O-Mo-4

### Glitter Trouble: Uncovering the Toxic Effects of Sparkles on Marine Life

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#### Abstract

Glitters (GL) are found in our daily lives and used in various products such as carnival, makeup, and beauty products. It is a small plastic particle formed of plastic polymers covered by thin metallic layers that is produced in different sizes, shapes, and colors. Its bright color which comes from their thin metallic layer, as different elements, such as Al, Fe, and Ti, produce a different color. This study aims to evaluate the toxicity of green and white GL leachates on the embryolarval development of sea urchins *Echinometra lucunter*, *Arbacia lixula*, and the mussel *Perna perna*. Moreover, the Toxicity Identification and Evaluation (TIE phases 1 and 2) approach was used to identify the possible compounds in GL that cause toxic effects on the larvae of *E. lucunter* for white GL and *A. lixula* for green GL. A GL (white and green) concentration of 500 mg/L was prepared with 0.05% alcohol, the concentrations used in the experiments were as follows: control and control ethanol (0.05%); 50; 100; 200; 300; 400 and 500 mg/L. White GL was analyzed using Pyrolysis Coupled with Gas Chromatography/Mass Spectrometry (Py-GC/MS), and its polymeric type was vinyl chloride-methyl acrylate copolymer - P(MA-VC). The effective concentration of green GL to 50 % embryos (EC50) after 24h was 72 (58.44-85.56) mg/L to *A. lixula*, while the EC50-24h values for *P. perna* were 38 (29.65-46.35) mg/L and 23 (20.17-25.83) mg/L of white and green GL, respectively. The EC50-36h values for *E. lucunter* were 118 (87.22-148.75) mg/L of green GL and 101 (89.26-122.74) mg/L of white GL. In the TIE for *A. lixula*, it was observed that the treatments aeration (removes volatiles), sodium thiosulphate (removes oxidants), and EDTA (removes metals), were the treatments that eliminated the possible causes of toxicity ( $p < 0.05$ ). The filtered leachate obtained of green GL was analyzed using Flame Atomic Absorption Spectrometry (FAAS), and the following metals were identified: Ag 0.01 mg/L, Zn 0.014 mg/L, Mg 0.25 mg/L, Ti 0.001 mg/L, Mn 0.008 mg/L, and Fe 0.018 mg/L. The leachates of both GL were toxic at higher concentrations. Based on the TIE, GL toxicity could be caused by dimethicone and capric triglyceride polymers (volatile compounds), and silver, magnesium carbonates, and zinc stearate (potentially chelated by EDTA and oxidized by thiosulphate). More studies should be necessary understand the toxicity of GL to aquatic organisms and the deleterious effects of their improper disposal in the environment.

## 16A.O-Mo-5

# Microplastics Role in Metolachlor Toxicity Modulation in the South American Decapod *Palaemon argentinus*: Bioaccumulation and Biochemical Responses in Sub-Chronic Bioassays

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### Abstract

Microplastics (MPs) are contaminants of emerging concern reported in most of the world ecosystems including aquatic ones. Due to their small size (1  $\mu\text{m}$ –5 mm), they can be ingested by a wide range of organisms. However, the information about their adverse effects on freshwater invertebrates remains limited. Moreover, MPs have been identified for their capacity to bind and transport other contaminants (such as pesticides). The main goal of the present study was to assess the toxicity of polylactic acid (PLA) MPs, alone and combined with an herbicide (metolachlor, MET), through sub-chronic bioassays using a South American native species (*Palaemon argentinus*). Organisms were collected in quasi-pristine site and acclimated to laboratory conditions for two weeks. Adults were exposed during 7 days to following experimental treatments (n=3): Control (CTL), MPs (5 mg/L), MET (7.5  $\mu\text{g/L}$ ) and the mixture (MPs+MET). The type of MPs polymer was confirmed using attenuated total reflection infrared spectroscopy and the size was characterized using optical microscope (fragments, 75-270  $\mu\text{m}$  mayor side). After exposure, the bioaccumulation as well as damage and defense biomarkers were measured in shrimps' cephalothorax (CTH) and abdomen (ABD). The MET concentration in the exposure media was measured by LC-MS. CTL and MPs were below the detection limit (1.8  $\mu\text{g/L}$ ); MET 6.70 $\pm$ 0.37  $\mu\text{g/L}$  and MPs+MET: 6.63 $\pm$ 0.29  $\mu\text{g/L}$ . The results showed a significant bioaccumulation of fragments in organisms, with higher values in CTH compared with ABD. In addition, significantly more particles were observed in CTH from MPs and MPs+MET treatments (22.25 $\pm$ 0.96 items) compared with CTL and MET conditions (3.25 $\pm$ 3.20 items). The content of carbonyl groups in proteins (CP) was quantified, giving a significant increase in CTH from MPs and MPs +MET treatments compared with CTL. Regarding enzymatic activities, significant effects were observed: MET inhibited CAT activity compared to CTL; MPs inhibited AChE activity compared to CTL and MET+MPs; while MET+MPs induced the GST activity respect to all other treatments. No significant difference in BChE activity was observed. The results of this study demonstrate that MPs can bioaccumulate and induces biomolecules damages and neurotoxicity, while MET affected oxidative stress and detoxification processes. Measured biomarkers were significantly modified by the co-exposure to MPs. Our results suggest the usefulness of *P. argentinus* as bioindicator of MPs exposure.

## 16A.O-Mo-6

### Plastic Pellets Make *Excirrolana armata* More Aggressive in Field Ecotoxicological Assays

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#### Abstract

Plastic pellets are a common form of microplastic (< 5 mm) found in high densities on beaches close to port regions. Acute and chronic toxicity has been observed in marine organisms exposed to similar pellets densities in laboratory assays. However, is this same toxicity pattern repeated in natural environments? This study investigates the ecotoxicological effects of beach-stranded plastic pellets on the macrobenthic population of the cirrolanid isopod *Excirrolana armata* under natural conditions. We also aimed to compare the toxicity of these microplastics in different *E. armata* populations, considering sediment characteristics (organic matter - OM, and grain size) and quality (contamination by hydrophobic chemicals). We used simulations of different real-world pellet densities on a beach free from pellet contamination, and laboratory experiments with two *E. armata* populations from beaches with different levels of pellet contamination. Experiments evaluated the behavioral responses (vertical displacement) and mortality of the organisms exposed. In the first experiment, ten individuals of the model species were directly exposed to different densities of pellets for a period of six hours. In laboratory we used one and five individuals from two *E. armata* populations from beaches with different levels of pellet contamination exposed to a fixed pellet density. The lowest density of pellets tested - with densities commonly found on beaches around the world - was sufficient to cause higher mortality in the exposed organisms. No effect was observed on vertical displacement. Populations that inhabit beaches without pellets show higher mortality than those inhabiting beaches with high pellet densities. The mortality of *E. armata* is higher when the exposure occurs in sediment with high OM, indicating that chemicals might be transferring from pellets to OM. Another hypothesis is that the population from the most pellet contaminated beach acquired resistance through natural selection. We also observed cannibalistic behavior in the exposed organisms, with lethargic and dead individuals being preyed upon by healthy individuals in a coordinated attack. Curiously, we did not observe mortality when the organisms were exposed individually to pellets, and the cannibalistic behavior occurred with greater intensity in plastic pellets treatments. This leads us to believe that, even with the species' innate scavenger habit, exposure to plastic pellets made them more aggressive.



## 16B.O-Mo-1

### Embryotoxicity of Polyethylene Microplastics and TiO<sub>2</sub> Nanoparticles Mixture in Bullfrogs Using the Frog Embryo Teratogenesis Assay (FETAX)

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#### Abstract

Amphibians are facing alarming declines worldwide, and many emerging pollutants contribute to that situation. Recently, scientists have increased their concerns about the ecotoxicological effects of microplastics (MP) and nanomaterials, such as TiO<sub>2</sub> nanoparticles (TiO<sub>2</sub> NPs), on aquatic biota. These materials are often added to cosmetics, used as vehicles for drugs and medical diagnostics, in electrical and daily purposes, or even originate from the degradation of plastic materials. Once discarded, the residues of these compounds reach effluents and water bodies, potentially affecting aquatic biological communities, such as amphibians. Natural environments often create mixtures of such pollutants; therefore, our hypothesis considers that a mixture of polyethylene MPs and TiO<sub>2</sub> NPs could exert synergistic toxicity to amphibians. Thus, this study aimed to assess the ecotoxicity of environmentally relevant concentrations of TiO<sub>2</sub> NPs (10 µg/L) and PE MPs (60 mg/L) (isolated or combined) on bullfrog (*Aquara catesbeiana*) embryos, using the Frog Embryo Teratogenesis Assay (FETAX). Embryos were individually positioned into 3,5mL wells of sterile cell culture plates, filled with FETAX solution and/or the experimental contaminants (MP/ NP/ cyclophosphamide as a positive control), and assembled in triplicates for each experimental group. The 96h experiment decreased the survival rate of the embryos exposed to the isolated NP (only 20% of embryos survived by the end of the experiment), as well as to the mixed exposure (MP + NP, 50% of survival); however, the isolated effect of NP was more pronounced. Similarly, the hatching rate of the embryos was also mainly reduced by the isolated MP, or the NP. Although the reduction in hatching rate was also observed in the mixture group, that effect was weaker than in the isolated contaminants. These results indicate that the interaction of MPs and TiO<sub>2</sub> NPs may have reduced the uptake and /or bioavailability of the nanoparticles, which increased the effects on the survival rate, as well as the hatching rate in the isolated groups. This study reinforces the need to understand how the interaction of different emerging pollutants can alter their ecotoxicity. We also highlight the use of the FETAX as an efficient method in amphibian ecotoxicological studies as well as for other animal models.

## 16B.O-Mo-2

### Identifying Potential Biomonitors for Microplastic Pollution in Sandy Beach Ecosystems

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#### Abstract

Microplastic contamination poses severe threats to the marine environment and, for this reason, global efforts have been undertaken for monitoring trends in microplastics distribution in different ecosystems. Identifying useful species to monitor microplastics environmental contamination is key to assess risks for ecosystems health. In beach environments, there is a general lack of information on how the biota interacts with microplastics to support monitoring and beach management. In this context, benthic biota inhabiting the intertidal zone are good candidates for biomonitoring. These are likely to be frequently exposed to microplastic uptake, owing to their feeding strategies and the constant resuspension of microplastics present on the sand with wave action. In this study, we assess the potential of the bivalves *Donax hanleyanus* and *Tivela mactroides*, two dominant intertidal species occurring on the Atlantic coast of South America, as microplastic biomonitors in sandy beaches. Differences in microplastic abundance were analyzed by collecting both species on three southeastern Brazilian beaches. Samples (n=5 pools of 5 individuals for each beach and species) were alkali-digested and vacuum-filtered following methods well established in literature. Visual inspection of putative microplastics was conducted under the stereomicroscope and chemical characterization is being completed by Fourier Transformed Infra-Red spectrometry (FTIR). Preliminary data indicate a total of 276 putative plastics recorded for *D. hanleyanus* (Mean =  $18 \pm 10.4$ ) and 289 putative plastics for *T. mactroides* (Mean =  $20 \pm 6.2$ ), which were categorized by color and shape. Confirmed microplastics data will indicate if both bivalves can be used as biomonitors for microplastics occurrence on sandy beaches. Further analysis will also indicate if both species can be used to monitor microplastic occurrence in beaches of different morphodynamic types and levels of human uses. Thus, we expect the results of this study to be relevant to large spatial scales, helping to identify potential hotspots and trends in microplastic pollution in sandy beaches using a standardized approach.

## 16B.O-Mo-3

### Spatio-Temporal Assessment of Microplastic Contamination in the Intertidal Mussel *Brachidontes rodriguezii* from Buenos Aires, Argentina

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#### Abstract

Microplastic (MP) accumulation has become a widespread social and scientific concern/problem in the last decade. Its ingestion has been documented over marine species from different trophic levels. Mussels are one of the most studied groups in marine contamination and have been proposed as suitable bioindicators for monitoring MP pollution. The present study evaluates the MP accumulation in the mussel *Brachidontes rodriguezii*, collected on the Southwestern Atlantic coast (Buenos Aires, Argentina). Individuals were collected in the intertidal, seasonally (every 3 months), during a year in three places with different anthropogenic impact: Camet, located next to a sewage treatment plant; Mar del Plata harbor, located in the most important fishing harbor in Argentina and, Las Brusquitas, a site far from urban centers. After the collection, individuals were placed in sealed plastic bags at  $-20\text{ }^{\circ}\text{C}$  until analysis. In the laboratory, samples were measured and dissected and the soft tissues were removed and weighed. Microplastics were extracted using a 10 % potassium hydroxide digestion method. The digested solutions were filtered on a cellulose nitrate filter ( $1.2\text{ }\mu\text{m}$  of porosity) and then the filters were dried at room temperature in a closed glass Petri. All laboratory procedures were carefully performed with the aim of preventing MP contamination. Visual identification and characterization were conducted under a stereomicroscope and MP accumulation was registered. Results showed that all the observed MP were fibers and were present in all the analyzed specimens. The whole abundance of MP in the sampling area was  $14.6 \pm 0.9$  items/individual and  $73.3 \pm 7.8$  items/g of wet tissue ( $n = 120$ ). According to the two-way analysis of variance, MP accumulation significantly changed with sampling sites and sampling sites\*season interaction. The highest mean abundance of MP was recorded for the individuals collected from Camet during the autumn season ( $21.5 \pm 4.1$  items/individual and  $112.4 \pm 24.2$  items/g) followed by the spring season ( $19.6 \pm 8.7$  items/individual and  $99.0 \pm 18.3$  items/g). Mar de Plata city is the major seaside resort of the country, so during summer and winter vacations the population increases, and in consequence, the sewage discharge increases. In conclusion, the sewage outfall of Mar del Plata could possibly impact the intertidal population of *B. rodriguezii* by releasing microplastics into the environment.

## 16B.O-Mo-4

### Seasonal Occurrence of Microplastics in Mangrove Mussels

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#### Abstract

Microplastic pollution is a growing environmental concern because these tiny plastic particles are present not only in the environment but also in consumer products and human fluids. Microplastics (MP) may be potentially harmful to the marine organisms. MP get into marine ecosystem via physical (sedimentation, accumulation), chemical (degradation and absorption) and biological (ingestion and biodegradation). The goal of this project was to study the presence and abundance of microplastics in mussels from a mangrove ecosystem of Ecuador. We dissected soft tissues from mussels, then a digestion process followed using different reagents to compare procedural efficiency and corrosion effects of chemicals on MP. Our preliminary results revealed that fibers and filaments were the most abundant types of MP, however the number of MP found in mussels found in this study are low when compared to previous research. No significant differences appeared for digestion and damage of MP by reagents. A qualitative evaluation showed that KOH as the most appropriate reagent for mussels' soft tissue digestion. These initial results demonstrate that MP are present in aquatic organisms of estuarine environments. Future steps aim to better understand a potential negative impact of MP in reproduction, growth, and behavior of aquatic species.

DRAFT

## 16B.O-Mo-5

### Reproducible Analytical Pipelines and Technological Readiness Levels in Decision-Making on Monitoring of Plastic Pollution

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#### Abstract

Research into environmental impacts of plastic pollution has exploded in the last decade, generating a plethora of research questions and approaches to target plastics of assorted sizes, in all imaginable environmental matrices. This has resulted in an overwhelming diversity in methods and protocols to analyze and monitor plastic pollution. Systematic validation and worldwide harmonization are urgently needed. Common steps of analysis, from survey design to data reporting must be critically assessed in a systematic manner to support the identification of reproducible methods for monitoring.

Based on the outcome of systematic review of methods and protocols for analysis of nano, micro and macroplastic in different environmental matrices we show that Reproducible Analytical Pipelines (RAPs) can be identified in plastic research and assessed for applicability using Technological Readiness Levels (TRLs) [1]. All methods used in plastic research can be broken into six fundamental steps for RAPs: Survey design, sample collection, sample preparation, analytical detection, quantification, and data reporting. The first two steps are only matrix dependent, the final three – only particle size dependent. None of the steps were found to be dependent on geographical location.

TRLs can be used to assess the status of every RAP step and are a promising tool to synthesize the status of methods, facilities, and technologies. Our concept establishes that robust and replicable information can be extracted by combining RAPs and TRLs. We believe that RAPs and TRLs provide a much needed and valuable approach to support the global assessment of plastic pollution. Readiness is not necessarily an indication of appropriateness, so TRL provides international expert groups, regulators and stakeholders with a decision-making tool for evaluating available methods, optimizing monitoring strategies and supporting policy implementation in plastic and other pollutants action plans.

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[1] Aliani, S., Lusher, A., Galgani, F. et al. Reproducible pipelines and readiness levels in plastic monitoring. *Nat Rev Earth Environ* (2023). <https://doi.org/10.1038/s43017-023-00405-0>

## 16B.O-Mo-6

### Identification of Synthetic Polymers

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#### Abstract

Microplastic (MP) pollution studies have been evidenced that they have negative impacts on ecosystems, organisms, and most important human health. MPs are ubiquitous, and their range of sizes, morphology, density, and type of synthetic polymer make their identification and quantification difficult. Different analytical techniques have been used to identify MPs chemical composition. The Fourier Transform Infrared with Attenuated Total Reflectance (FTIR-ATR) spectroscopy provides information about the chemical structure of the polymer by analyzing the absorption spectra of the sample. Red Nile dye is used to distinguish between natural and synthetic polymers, and UV and Royal Blue light provide fluorescence properties of the polymers. This study aimed to compare the identification of plastic particles quantitatively using the method mentioned above. Our results showed that FTIR-ATR spectroscopy is the most effective technique to identify synthetic polymers, and we used it to compare the other identification methods. UV light was 52% effective in identifying plastic particles (~5000 particles analyzed), Royal blue was effective in identifying anthropogenic particles from natural polymers (cotton, cellulose), and red Nile showed 57% falsely identified particles as microplastics (~400 particles).

DRAFT

## 16C.O-Tu-1

### **Analysis of the Presence of Contaminants “Microplastics” in Marine and Freshwater Environments in the World Heritage Site Cocos Island National Park, Costa Rica**

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#### **Abstract**

Microplastics (MPs) defined as ‘small’ pieces of plastic < 5 mm have been found everywhere scientists have looked: in deep oceans; in Arctic snow and Antarctic ice; in shellfish, table salt, drinking water and honey; and drifting in the air or falling with rain over mountains and cities. Nevertheless, considering that oceanic insular environments such as Cocos Island, are even more vulnerable to plastic pollution because they could retain plastics from the adjacent ocean currents by different meteoceanographic mechanisms; ecologically, they are unique ecosystems in terms of biodiversity and endemism, and, the limited human activity and its remoteness from the mainland, the question arises about the status of contamination in Cocos Island National Park. The purpose of the project was to analyze the presence of MPs in the marine and freshwater ecosystem by comparing samples of biota, sediments and water. Two phases have been carried out; in 2018 the first phase was carried out and the presence of the contaminant in both ecosystems was analyzed. The detection of MPs in the marine ecosystem samples was expected due to exposure by marine currents, tourism activities, and discarded fishing gear from illegal fishing activities; however, the presence in the freshwater ecosystem were not expected and the sources of contamination were unclear, also the first phase only focused the survey close to the human activity. In the second phase developed in 2022, an analysis was conducted around the island, with the intention of compare the areas with human presence and areas without human presence to determine if the presence of ranger parks was a significant source of contamination in the freshwater ecosystem and also to determine if the distribution of MPs is consistent around the island or if there are accumulation sites in the two ecosystems. The results obtained indicate that the distribution of MPs in the marine and freshwater ecosystem was consistent around the island; therefore, human activity by staff is not a significant source of MPs. These results prove that issue must be viewed as a planetary boundary threat, therefore, governance strategies must be implemented. Also, a Global Plastic Waste Partnership must be established to determine the path to move forward.

## 16C.O-Tu-2

### Degradation of Polyethylene Microplastics in Different Marine Environments: Evaluating Chemical and Physical Changes Using FTIR, EA and SEM Methods

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#### Abstract

Understanding the degradation of microplastics (MPs), plastics with sizes < 5 mm, is important to define the impact of these emerging contaminants on the marine environment. To evaluate the degradation of MPs we performed the following experimental study. We selected polyethylene (PE) pellets, and exposed it to in-situ environmental conditions. The MPs were exposed to degradation for 112 days at a depth of 2 meters in marine water at Guanabara Bay, Niterói, and at Cabo Frio Island, Arraial do Cabo, both located on the Brazilian coast to compare environments with different pollution loads. To evaluate MPs degradation, we used Fourier Transform Infrared (FTIR) Spectroscopy and Elemental Analyzer (EA) to calculate degradation indexes and compare the MPs changes in bond structures. We calculated previously proposed indexes (Carbonyl and carbon-oxygen bonds indexes) using FTIR interest peaks of changes in chemical bond structures (carbonyl groups, 1509 - 1780  $\text{cm}^{-1}$ , and carbon-oxygen, 950 - 1200  $\text{cm}^{-1}$ ). We used a scanning electron microscope (SEM) to evaluate the visual changes in MPs. Preliminary results show an increase in indexes for both experiments until day 56. After this point, on day 112, the indexes remained constant for samples from Arraial do Cabo. In Guanabara Bay, however, the indexes increased until day 112. This suggests that the indexes are effective addressing the duration of MPs exposure to the environment. To test whether the indexes were changing due to the MPs molecular composition changes or biofilm formation, we tested different organic matter digestion methods using MPs exposed to the marine environment for 112 days and control MPs. The MPs were digested for 24 and 120 hours in 30% HCl, and 10% KOH solutions. The results show a statistical difference for the same index before and after digestion. The indexes calculated from the degraded samples after both digestion treatments showed no statistical difference from the indexes calculated from the control MPs. Our preliminary results show an increase in MPs surface rugosity and yellowness, we could observe significant increases in degradation indexes and more negative values to  $\delta^{13}\text{C}$  along the experiment as well as the advancement of biofilm formation and clusters between MPs and foraminifera. With the advancement of this study, we hope to elucidate the relationship between biofilm and degradation indexes and better understand the factors acting at MPs degradation in the marine environment.



## 16C.O-Tu-3

### **Abundance, Distribution and Characteristics of Microplastics-MPs in Sandy Beaches of the Colombian Caribbean and Pacific**

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#### **Abstract**

Colombia has 1932 km of coastline in the Caribbean and 1599 km in the Pacific, where activities that generate waste are developed, which are mostly disposed of in open dumps or in natural bodies of water, where they are transformed into MPs that accumulate in the ecosystems generating negative effects on biodiversity, availability of resources and economy of communities.

Since 2017 INVEMAR through projects funded by the IAEA, began to strengthen its technical capacities for the sampling of MPs in coastal marine ecosystems; and has made progress in the regional harmonization of MPs monitoring protocols. Within the framework of the national monitoring network–REDCAM, MPs are being evaluated in sandy beaches of the Colombian Caribbean and Pacific; information for the annual report on the state of MP pollution at national and regional levels and for the indicator report of target 14.1.1.b of SDG 14.

To determine MPs in beach sand, a transect of 100 m was drawn in the high tide zone, where 5 quadrants of 50x50 cm were defined, and sand was collected in the area of each one. In laboratory, the samples were dried, sieved and observed directly to the stereoscope.

In 2017-2018, MPs on 43 beaches in the Caribbean and Pacific presented values of up to 1387 items/m<sup>2</sup> and 317 items/m<sup>2</sup> respectively. In 2021, MPs on 27 beaches in the Caribbean and Pacific reached values of up to 154 items/m<sup>2</sup> and 182 items/m<sup>2</sup> respectively. In 2022, the abundance on 38 beaches in the Caribbean and Pacific reached values of up to 4920 items/m<sup>2</sup> and 860 items/m<sup>2</sup> respectively. Most abundant types of MPs were fragments that come from inadequate waste management from urban and rural settlements; and product of fishing and tourism activities.

In the coastal areas of Colombia there is an inadequate management of plastic waste, a fact that represents a threat to the ecosystems of Colombia, which provide environmental goods and services, in addition to being the economic sustenance of populations that live from tourism. Therefore, Colombia must advance in the generation of technical-scientific support information as the main input for the development of policies, actions and implementation of mechanisms for sustainable management of solid waste that contribute to reducing pollution by plastics in the marine environment. This information is part of the regional standardized monitoring in Latin America and the Caribbean that aims to address the problem from a local, regional and global perspective.

## 16C.O-Tu-4

### **Five Years of Plastic Monitoring South of the 62°S: Micro and Mesoplastics Debris Stranded in a Beach of Fildes Peninsula (King George Island, Antarctica)**

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#### **Abstract**

Marine currents transport plastic debris over long distances, and several reports have evidenced their presence in remote areas far from their sources. Considering the distance and the presence of the Southern Hemisphere the Antarctic Circumpolar Current (ACC), in Antarctica, it would be expected that local sources related to logistic, scientific and touristic activities would be more feasible than external contributions from lower latitudes such as South America or Oceania.

However, since the first record of primary microplastics (resin pellets) on Antarctic beaches in 2019, we established an annual monitoring program to track stranded micro and mesoplastics through the AntarPLAST project. Using the same methodology employed when we found the pellets on the Klotz Valley beach (located on the coast of the Drake Passage at S 62.1582, W 58.9391, NW Fildes Peninsula - King George Island), we collected the same beach annual samples over the last five years. Specifically, we characterized the first 2 cm of sediment from 5 quadrants (50x50 cm) along 100 m of the highest strandline. We removed all debris from the sediment via flotation, identified each item with naked eye, classified them into main types, and weighed and measured them using the ZooScan and Image-J software.

Although the amount of research and results on plastic contamination in the Southern Ocean and Antarctica has grown significantly in recent years, knowledge of plastic dynamics and their impact in this remote but critical area of the planet is still scarce. The available information is usually punctual, both in time and space, so the development of long-term databases in such a remote area seems like an urgent need to address the current challenges faced by the Antarctic Treaty System regarding this issue.

## 16C.O-Tu-5

### Management of information on plastic pollution in Colombia for SDG 14 reporting

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#### Abstract

In 2001, Colombia created the REDCAM marine environmental monitoring program to monitor the environmental quality of marine and coastal ecosystems in Colombia's Caribbean and Pacific regions and to follow up on the activities and sources of pollution that affect them. REDCAM is an inter-institutional cooperation program, coordinated by the Institute of Marine and Coastal Research (INVEMAR), with the participation of the 12 environmental authorities of the departments with coastal areas, and supported by the Ministry of Environment and Sustainable Development (MinAmbiente). With a view to reporting data and periodic information on the state of pollution by microplastics and macroplastics (marine litter), as well as reporting on the indicator of target 14.1 of SDG 14, since 2017 the variables marine litter and microplastics were incorporated into the monitoring in more than 45 sampling stations.

REDCAM has positioned itself as an instrument for the generation of technical-scientific information for the monitoring of marine-coastal water resource conditions, of Colombia and the Latin American region, integrating itself to the monitoring of marine stressors of the REMARCO Network. Through its robust information system designed with ORACLE database processing standards under a computerized network, research actors and surveillance and control managers can access databases, Geographic Information Systems and Cartography, which contain information on environmental quality including plastic pollution in marine-coastal ecosystems of Colombia.

Currently, the REDCAM information system has geographic information from more than 45 marine debris and microplastics sampling stations, automated platforms to visualize the information, and data management from a SQL Developer computer language. Having a robust information system with a database and cartography, gives Colombia a position in information management for Latin America and the Caribbean, which will be articulated for the reporting of information to SDG 14.1 in the region through the REMARCO Network.

## 16C.O-Tu-6

### Export of Microplastics to Coastal Areas in Central Chile: The Contribution of BioBío River

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#### Abstract

Plastic particles were collected from Biobío river (and their tributaries) and Itata river (n = 13) of central Chile in 2 different campaign (Summer: January 2022, and winter: July 2022). The sampling zone was divided as rivers of the Upper, Middle and Lower area. Samples were collected using an plankton net with a circular mouth opening of 25 × 50 cm and 330 μm mesh size. Plastics were analyzed using Fourier-transform infrared spectroscopy for polymer identification and physical characterization. Plastic fibers were the abundant morphology (60-80%) in all three zones. Polyethylene terephthalate (PET) (40-80%) and Polypropylene (PP) (40-60%) were the most abundant polymer along the whole River. An average of 20 particles/m<sup>3</sup> along the whole sampling sites. In general, the lower zone of the Biobío River was the site with more variety of samples (size, colours and type of polymer). In winter (July 2022) we observed an increase of more than 50% of plastic particles compared to those detected in summer season. These findings are lower than those reported in previous studies of the North Hemisphere but higher than the ones detected in the southern countries.

## 16D.O-Tu-1

### Microplastic Contamination in a Mexican Lagoon

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#### Abstract

Lagoons are a type of freshwater body that has the purpose of storing and supplying water. However, these bodies of fresh water, like others, have been affected by different types of contamination, among which are contamination by microplastics (PM). The objective of this study was to quantify and classify the PM present in the sediments of the Zumpango lagoon, located in the State of Mexico, as well as to identify possible sources of contamination. Six sediment samples were randomly collected from the bottom of the lagoon, with the help of a Van Veen sampler. The samples were taken to the laboratory and processed, in triplicate, through a treatment train that included drying, digestion, flotation test, vacuum filtration, and visual identification under the microscope. Subsequently, the possible sources of PM contamination in the Zumpango lagoon were identified. The results showed a concentration range of 7.9 - 13.3 MP/L (average of 9.48 MP/L), the types of MP found were mainly fibers, followed by films, fragments and foams with proportions of 53.1, 25.4, 20.6 and 0.9%, respectively. The size interval of the MPs was between 0.06 and 5.00 mm. As possible sources of contamination, fed water from other bodies of water (canals and rivers), wastewater discharges and waste originating from tourist-recreational activities were identified.

DRAFT

## 16D.O-Tu-2

### First Assessment of Microplastic Contamination in Surface Waters of the Tietê River, São Paulo, Brazil

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#### Abstract

In recent years, microplastics (MPs) have become an emerging concern worldwide. These particles, with sizes between 1  $\mu\text{m}$  and 5 mm, can be found in all environmental matrices and inside the most diverse organisms, from the lowest trophic levels. Although MPs have already been studied in different aquatic environments in Latin American countries, studies in Brazil are still scarce, especially in rivers. Furthermore, there are few Brazilian studies that address the interaction between MPs and organic contaminants. Thus, the present study evaluated the abundance of MPs and the occurrence of polychlorinated biphenyls (PCBs) sorbed to these particles present in the surface water of the Tietê River, which is the largest watercourse in the state of São Paulo and has great economic and social relevance for the territory. For the analysis, MPs from surface water samples were collected during the flood and dry periods, and filtered in 0.106  $\mu\text{m}$  mesh. The MPs were identified by mid-infrared spectrophotometry using the Fourier transform (FTIR-ATR) and characterized by visual inspection. The presence and concentration of PCBs was assessed using gas chromatography coupled with mass spectrometry (GC-MS). The MPs were found in all sampled points, in greater abundances at high urban density sites, with concentration ranging from 6.67 particles  $\text{m}^{-3}$  to 1,530 particles  $\text{m}^{-3}$ , with a relative predominance of the polymers polyethylene (PE, 58.17%) and polypropylene (PP, 23.53%). The main morphological classes found were fragments (56.63%), fibers (28.42%), and films (13.06%), which evidences the presence of diffuse sources of MPs. The total PCBs concentrations in MPs ranged from 20.53 to 133.12 ng  $\text{g}^{-1}$ , indicating the ability of MPs to act as potential vectors of organic pollutants in the aquatic environment. The results obtained in this study are relevant for understanding the dynamics of MPs in freshwater environments and their interactions with organic contaminants. Furthermore, it is expected that these results can serve as an alert to the population and public agencies regarding waste management in the state of São Paulo.

## Microplastics and Emerging Concern Microparticles in Sediments from Buenos Aires Aquatic Environments: All in the Same Microplastics Analysis Bag?

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### Abstract

Coastal sediments are affected by contaminants from *in situ* and *ex situ* sources, including microplastic particles (MPs) derived from land-based and marine productive activities. However, due to the complexity of the matrix and the time-consuming/expensive methods available, there is little information on the MPs levels in this environmental compartment. In addition, the presence of emerging concern microparticles, such as paints/coatings and black rubbery particles commonly referred to as tire wear particles (TWPs), pose challenges for recovery due to their reported higher density and chemical complexity. Analysis of MPs, including paints and TWPs, in subtidal and riverine sediments from urban, peri-urban, and rural areas located in densely populated regions such as Buenos Aires Province revealed their occurrence. Paints and TWPs were found mainly at sites associated with stormwater runoff, bridges of major and minor roads/highways. Also it revealed its high contribution in the total MPs stock from low to impacted areas (Paints 0-36% and TWPs 0-90%). In addition to the complexity of chemical characterization, current separation methods rely on the use of dense floating solutions to separate MPs from sediment components. Many commonly used floating solutions result in underestimation of these emerging particles. Floating solutions that achieve higher densities are expensive or pose a risk to humans and the environment due to their toxicity. In addition, interactions with the matrix and biogenic aggregations can compromise the efficiency of density-floating methods. Based on methodology proposed for the analysis of high-density microparticles such as paints/coatings, we compared the use of two extraction methods for subtidal sediments impacted by stormwater discharges. Our preliminary results showed differences between density-based and non-density-based methods in MP abundance averaging 255% and 310% for paints and TWPs, respectively. Our preliminary approach to analyzing classic and emerging concern microparticles from impacted and non-impacted sediments demonstrated their contribution and the need to properly identify these microparticle groups. In addition, non-floating extraction techniques could provide a non-expensive, affordable, and more green friendly technology for microparticle assessment in environmentally relevant but complex matrices.

## 16D.O-Tu-5

### **Macro, Meso, Micro and Nanoplastics in Horticultural Soils in Argentina: Abundance, Accumulation and Fragmentation Mechanism**

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#### **Abstract**

The contamination of soils, rivers and seas caused by plastic waste is today a global concern in different forums. Although terrestrial ecosystems are the main source of plastic residues in water, there is limited knowledge about the physical and chemical processes that these polymers experience in soils. Between these processes, fragmentation is a very significant phenomenon in agricultural soils because a large amount of plastic film is used for covers and could potentially lead to secondary small plastic fragments. Our research group has reported in 2015 that the aforementioned plastic fragmentation process is already taking place in Argentine horticultural soils from Moreno, Buenos Aires, where macroplastics (macroPs) with mean sizes of  $8 \pm 13 \text{ cm}^2$  were found in amounts of around  $30 \text{ kg plastic} \cdot \text{ha}^{-1}$ . Noteworthy, this area was not used between 2015 and 2022 for any productive purposes and no horticultural plastic input occurred during this period. Therefore, this scenario represented a unique opportunity to understand plastic film fragmentation dynamics in horticultural soils. To the best of our knowledge, no such studies have been reported for Argentinean soils.

In the current study, during 2022, we quantified macro, meso, micro and nano plastics in two horticultural fields, originally studied in 2015. The objectives of this work were i) to compare the amount of macroPs found in horticultural soils and the evolution of its size in comparison with the 2015 scenario; ii) to determine the size distribution of meso, micro and nano plastics; iii) to propose a plastic fragmentation mechanism in soil based on the principles of geometric statistics and dynamic fragmentation.

The analysis showed that polyethylene black mulching was the main type of plastic found, considering either the fragment number (86 %) or the relative mass (61.2 %). No significant macroPs mass variation was observed between 2015 and 2022, which is consistent with no additional incorporation of mulching into the soil. A significant increase in the number of macroPs.m<sup>-2</sup> between 2015 and 2022 was found (2015:  $13 \pm 4 \text{ items} \cdot \text{m}^{-2} \text{ soil}$ ; 2022:  $36 \pm 11 \text{ items} \cdot \text{m}^{-2} \text{ soil}$ ). A linear correlation ( $R^2 > 0,9$ ) was found between plastic abundance and their sizes, which shows that a random dynamic fragmentation process occurs.

These results contribute to the information on Argentinean soil microplastic pollution and a better understanding of microplastic formation in soils.



## 16D.O-Tu-6

### Microplastics Interfere With the Sorption-Desorption, Leaching, and Mobility of Diflubenzuron, Fluazinam, and Metribuzin in Tropical Soil

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#### Abstract

Roofing systems with high-density polyethylene (HDPE) plastic films (mulching) are used with pesticides to control invasive pests. However, when exposed to weather conditions, they may suffer fragmentation and generation of microplastics (PMs), favoring the retention of pesticides in the soil and altering the dynamics of these products in the environment. Thus, as pesticides and PMs are contaminants that coexist in agricultural systems, this study aimed to investigate the behavior of diflubenzuron, fluazinam, and metribuzin pesticides under the influence of HDPE PMs in tropical soil. The sorption-desorption was analyzed using the "batch" equilibrium test, with the application of different concentrations of pesticides (1/4, 1/2, 1, 2, and 4 times), from the recommended in the field, and the MPs (0, 0.25, 1 and 4 % w/w). Leaching was analyzed in glass columns (200 mm in 6 hours) with the same concentrations of PMs and with the recommended field dose for each pesticide. The distribution of pesticides was verified in the 0-10 cm layer and the leachate. The mobility was checked by means of soil thin-layer chromatography under the same conditions as in the leaching study. At concentrations of 1 and 4 % PMs, there was a reduction in sorption of 40.79 % and 51.12 %, respectively, of metribuzin in the soil. For diflubenzuron with the addition of 4 % PMs, there was a reduction in sorption of 20.65 % in the ground. For fluazinam, no interference was observed in its environmental behavior. Desorption occurred for all pesticides. In the solution, 11 %, 71 %, and 68 % of the solution was sorption for metribuzin, diflubenzuron, and fluazinam, respectively. In that order, 36-93 %, 2-2.8 %, and 0.51-0.57 % of the metribuzin, diflubenzuron, and fluazinam were leached. At concentrations of 1 and 4 % of PMs, there was a reduction in the mobility of the metribuzin going from intermediate to slightly mobile. No significant differences remained immobile in the soil for fluazinam and metribuzin. Our findings showed that the addition of different concentrations of PMs to soil interferes with the sorption-desorption, leaching, and mobility processes of pesticides depending on particle concentration, and physicochemical properties, especially octanol-water partition coefficient ( $K_{ow}$ ) and pesticide doses. Through this study, it is possible to understand the behavior of the association between PMs and pesticides and the role of PMs as vectors of organic pollutants in the soil.

# Session 17: Biological Consequences of the Environmental Radiation Exposure

## 17.O-We-1

### Natural and Anthropogenic Radionuclides Distributions and Radiological Hazard Risks in Rocha, Uruguay

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#### Abstract

The activity concentration of  $^{238}\text{U}$ ,  $^{226}\text{Ra}$ ,  $^{210}\text{Pb}$ ,  $^{232}\text{Th}$ ,  $^{40}\text{K}$  and  $^{137}\text{Cs}$  radionuclides in different compartments (sand, soil and *Baccharis trimera*) of the strip coast of the Rocha were evaluated. The radiological hazards for both inhabitants and tourists were also assessed.

All samples were measured by gamma-spectrometry after reach secular equilibrium between  $^{226}\text{Ra}$  and  $^{232}\text{Th}$  and their short life daughters, with a High Pure Germanium Detector GMX35P4-76-RB, 35 % efficiency and 1,75 keV energy resolution for the  $^{60}\text{Co}$  photopeak.

Overall, the area showed a higher  $^{232}\text{Th}$  activity concentration than the worldwide mean for both soil and sand, while the  $^{226}\text{Ra}$  activity concentration was only higher than the worldwide mean for some sand samples. The  $^{238}\text{U}$  series shows disequilibrium for both sand and soil, with high concentration of  $^{238}\text{U}$  and  $^{210}\text{Pb}$ .

$^{210}\text{Pb}$ ,  $^{40}\text{K}$  and  $^{137}\text{Cs}$  transfer from soil to *Baccharis trimera*, although more determinations must be made in order to know the  $^{210}\text{Pb}$  fallout intake.

The Annual effective dose equivalente outdoors (AEDE) was evaluated using  $^{226}\text{Ra}$ ,  $^{232}\text{Th}$  and  $^{40}\text{K}$  activity concentrations and the conversion coefficients recommended by United Nations · Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). The excess lifetime cancer risk (ELCR) was evaluated for inhabitant using the calculated AEDE, the life expectancy in Uruguay (77.7 years) and the cancer risk factor per Sievert, which is stochastically determined by ICRP at 0.05 for the public.

The ELCR ranges from  $7.5 \times 10^{-5}$  to  $2.5 \times 10^{-3}$  with a mean value of  $3.2 \times 10^{-4}$ . The highest values were obtained for Aguas Dulces's sand beach. The mean ELCR value obtained for the region slightly exceeds the world mean reported by UNSCEAR ( $2.9 \times 10^{-4}$ ).

The radiological hazard indices for some regions' inhabitants were higher than the worldwide mean and recommended values. This may contribute to Rocha's higher standardized mortality ratio (SRM) value, although a direct correlation cannot be assured with the epidemiological information currently available. Social, medical, and anthropological studies will be conducted in the future to provide data and verify this correlation.

## 17.O-We-2

### Biological Effects of Low Doses of Ionizing Radiation in Human Health

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#### Abstract

Humans are constantly being exposed to one or the other kinds of radiation either from natural sources, such as sunlight or from man-made inventions. Ionizing radiation plays an important role in the daily life of human life such as the sources used in medical diagnosis, X-rays, and computed tomography scans for example. Most of hospitals around the world use radiation for either diagnosis or for cancer therapy. Industrial uses of ionizing radiation such as food irradiation and sterilization of medical and other equipment have major implications in our daily life. Historically, humankind has witnessed few nuclear accidents/incidents such as the Hiroshima and Nagasaki bombings (1945), Chernobyl (1986), Goiania (1987), and Fukushima (2011) where unintended exposures have occurred. Besides, Humans are exposed to cosmic radiation from space exploration and air travel. Therefore, become important to understand the biological effects of various types of radiation. While high doses of ionizing radiation have shown to be harmful to the exposed individuals, low doses of radiation may have long-term consequences. Nowadays, most of the people exposed to low doses of radiation come from populations living in areas with higher levels of natural background radiation or from medical fields, either radiation-exposed workers or patients. Most of the background radiation is originated from natural gamma radiation emitted by rocks, soil, and terrestrial radon. However, there is a certain percentage of background radiation coming from cosmic radiation. On the other hand, most radiation-exposed workers are radiologists or radiotherapists, who are exposed to protracted low-dose and low-dose rates of ionizing radiation. Although a lot of effort has been done in this respect, up to now, there is not sufficient statistical power to detect detrimental low-dose health effects in occupational radiation workers, because large numbers of exposed persons and long-term follow-ups are still required. In this respect, we have set up new modern cytomolecular methodologies to determine systematically the accumulated biological effect of exposure to low doses of radiation. Preliminary data on a group of interventional cardiologists who represent the group of radiation workers more exposed in Uruguay as we have determined previously by a retrospective study carried out at the University Cardiovascular Centre from the Faculty of Medicine (University of the Republic) will be presented.

## 17.O-We-3

### **Bi<sub>2</sub>S<sub>3</sub> Nanoparticles as Sensitizers to Enhance the Radiotherapy Efficiency - Study of Cell Death Mechanism and DNA Damage**

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#### **Abstract**

Radiotherapy has a central role in cancer treatment, but it is not specific to tumor cells, affecting also healthy tissues. To overcome this fundamental challenge, radiosensitizers were conceived as a novel and simple solution to this problem, increasing the amount of radiation that a cell can absorb. The radiosensitizer allows the use of a lower dose of radiation, leading to fewer secondary effects. This study investigates the use of biocompatible bismuth sulfide (Bi<sub>2</sub>S<sub>3</sub>) nanoparticles as radiosensitizers and their potential advantage over cell death. Using a hot injection method and oleylamine as ligand, we synthesized Bi<sub>2</sub>S<sub>3</sub> nanorods measuring on average 4.1 nm in width and 20.4 nm in length. Next, the ligand of the nanoparticles was successfully exchanged to polyvinylpyrrolidone (PVP) to improve their biocompatibility. We also examined the effect of PVP-coated Bi<sub>2</sub>S<sub>3</sub> nanoparticles on the response of breast cancer cell line MCF7 to a dose of 2 Gy Co-60 gamma radiation or 6 MeV X-rays. Their effectiveness raised up to 38% more cell death in comparison to only irradiated cells. We have also performed a comprehensive study of the mechanism of cell death using the triple-stain assay, which employs three fluorescent dyes: Hoechst 33342, propidium iodide and fluorescein acetate. This assay revealed the importance of the synergy between nanoparticles and irradiation in enhancing the effect of radiotherapy, resulting in a significant increase in the percentage of cell death by necrosis when cells are irradiated in the presence of nanoparticles. Additionally, we examined DNA damage using the micronucleus assay, which revealed chromosomal aberrations. We observed an increase in the frequency of micronuclei generated when cells were irradiated, particularly in the presence of nanoparticles. In conclusion, we have observed a more efficient radiotherapy treatment as a result of the use of nanoparticles as radiosensitizers, resulting in a greater amount of cell death, particularly by necrosis.

## 17.O-We-4

### Radionuclides in Food and Environment in Uruguay

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#### Abstract

Human beings naturally have radioactive elements in our constitution since we are a product of our environment and we ingest them every day from air, water and food.

Radionuclides of natural and anthropogenic origin are integrated into different ecosystems, as do other types of contaminants. Depending on their physical, chemical and biological behavior, they can migrate and be incorporated into different plant and animal species.

Since 1940, nuclear tests have been carried out around the world that has released radioactive material into the environment. Since 1986 there have been three major accidents that released radioactive particles into the atmosphere, which by different mechanisms have been distributed throughout the world.

Uruguay doesn't have nuclear power plant facilities and it does not have nuclear reactors of investigation or power, however for more than forty years, samples of goods (cattle meat, milk, powder milk, butter, cheeses, beans, etc.) and environmental samples such as soils, sediments, waters are analyzed by High Resolution Gamma Spectrometry in order to know the background radiation, natural radioactivity levels and artificial radioactivity levels in the country.

In March 2004, Uruguay started to execute an Environmental Radiological Monitoring Plan. In the frame of this work, environmental samples over the whole national territory are periodically analyzed and through a coordinated program, with the Ministry of Livestock, Agriculture and Fisheries, test samples of milk, dairy products and meat samples of Uruguay are analyzed by High Resolution Gamma Spectrometry. An automatic monitoring station for air particulate matter was installed in order to have real-time information.

The consumption of food containing radionuclides of anthropogenic origin increases the internal dose of radiation received by the population. Due to this, the International Organizations (FAO; WHO; IAEA) recommend restrictive values for the presence of artificial radionuclides in food as well as some countries set their own action limits.

Analytical results of the analysis of national food and environmental samples taken throughout the Uruguayan territory are presented.

## 17.O-We-5

### Examples of Environmental Radioactivity in Uruguay

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#### Abstract

The eastern region of Uruguay exhibits environmental natural radioactivity mainly due to the black sand deposits centered in the Aguas Dulces – Valizas area (which contain  $^{238}\text{U}$  and  $^{232}\text{Th}$  and their decay products). Also, anthropogenic radioactivity appears at least in the lacustrine systems. In order to perform a comprehensive study of the environmental radioactivity in the area, several studies have been carried out. Gamma emitters have been quantified around the black sand ores. A first result of these studies was related to the compliance (or not) of the secular equilibrium among the radionuclides belonging to the  $^{238}\text{U}$  natural radioactive series and its indirect hints of geochemistry behavior during the Holocene in the region. A second result refers to the transfer factors of the radionuclides from sand and soil to vegetables, especially those which contribute to the human intake (food, teas). A third result gives the radiological hazard indices due to the presence of the ore, resulting that for some regions' inhabitants the indices were higher than the worldwide mean and recommended values. Also,  $^{137}\text{Cs}$ , anthropogenic radionuclide, has been determined in the lagoons, showing contamination levels in agreement with worldwide values, and a very good temporal correlation with nuclear events which contaminate the Earth atmosphere. Furthermore, geochronological studies ( $^{210}\text{Pb}$  dating and  $^{137}\text{Cs}$  verification) for paleoenvironmental reconstruction on lagoon sediments have been done, and correlated with contaminants such as polycyclic aromatic hydrocarbons (PAHs). In addition, studies of  $^{210}\text{Po}$  – alpha emitter- as radiotracer permitted to evaluate the trophic chain in two lagoons, from phytoplankton to molluscs, crustaceans and fishes, and to correlate these trophic results with physicochemical properties of the lagoons. Finally, and in the framework of the study of normally occurring radioactive materials (NORMs), the presence of natural radionuclides in fertilizers used in Uruguay and their cumulative use on the cultivated soils, as far as their presence in the materials used for construction, are in current investigation. In conclusion, several aspects of environmental radioactivity have been studied in the region, opening the possibility of a lot of future work. Last but not least, some results led to ask the Uruguayan health system for a more subtle collection of data, or give a baseline for establishing rules about NORMS in the country.

## 17.O-We-6

### Effects of radiation exposure on offspring and next generations

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#### Abstract

Exposure to low dose radiation during pre- or post-conception result in both foetal and natal consequences. Cellular response to radiation, especially during organogenesis is manifold. Effects of pre-conceptional or in utero exposures to radiation may be transmitted to the next generation – the transgenerational effect, which is thought to result from epigenetic phenomenon. There is limited human-based evidence studying transgenerational effects of radiation which mainly focused on A-bomb data, Chernobyl, and similar radiation exposures. Both human and animal data will be reviewed in the presentation. In this regard, it is imperative to appreciate the pathways of metabolic and endocrine intrauterine effects exaggerated by environmental factors such as smoking, alcoholism, obesity, diabetes mellitus, polycystic ovarian syndrome, pharmacological agents, etc. These environmental and life-style factors can increase the sensitivity of foetal cells to radiation-induced damage. Exposure to environmental toxicants, pesticides, herbicides, endocrine disruptors, prior to and/ or during conception generate an intrauterine environment of high oxidative stress. Moreover, while determining the effects of radiation, it is critical to discuss the (multiple) exposures to routine diagnostic ultrasound during pregnancy. Under such circumstances, effective mitigation of DNA damage and commencement of repair processes may be hindered. Individual genetic susceptibility varies depending on the race, dietary pattern, lifestyle, ethnicity, socio-economic conditions etc. Further research should focus on combined exposures of both environmental and physical agents. The presentation will review the literature and critically evaluate the potential influence of environmental confounding factors in radiation-induced changes in developing embryos and/or offspring.

# Session 18: Development and Acceptance of New Methodological Approaches (NAMs) in Latin America

## 18.O-Mo-1

### The Importance of Case Studies in Establishing Scientific Confidence of New Approach Methodologies

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#### Abstract

The growing need for alternatives to animals testing has led to the development and evaluation of New Approach Methodologies (NAMs). NAMs are already recognized as part of integrated testing strategies and are playing a supporting role in environmental decisions. Key to their acceptance in regulations and risk evaluations, is a demonstration of their relevance and reliability, which are necessary to establish scientific confidence. One strategy that builds such confidence is the use of multiple case studies using the same NAM, but covering different contexts, information needs and cross-sector requirements. Outcomes from case studies provide a mechanism for an independent evaluation and validation of NAMs, and generate an in-depth and multi-faceted understanding of their applicability, strengths, and limitations, while providing direction for improvements. The objective of this presentation is to share findings from case studies for a single NAM, Interspecies Correlation Estimation (ICE) models, applicable to different chemicals and scenarios. These models leverage data and describe mathematical relationships between species pairs allowing toxicity predictions from surrogates to untested species. Despite having gained scientific acceptance for their potential in replacing animal testing, ICE models have yet to be used for regulatory purposes. In this work, attributes that make ICE models a reliable NAM are highlighted, and their utility is demonstrated through several case studies. These case studies included hazard assessments of chemicals with a shared mode of toxicity, produced water from multiple offshore platforms and chemical constituents in biocide formulations. These and previously published case studies collectively demonstrate that ICE models are a promising alternative to toxicity testing, and thus could be useful in reducing vertebrate testing and supporting hazard evaluations for chemicals with limited data. The latter has practical implications for regulatory purposes, including product registration. In closing, regulatory acceptance of NAMs could be achieved through outcomes from independent and collaborative case studies involving multiple stakeholders with a shared interest. The scientific community in Latin America is invited to participate in case studies around NAMs, which will contribute towards fostering international and multidisciplinary interactions, while advancing this field of research in the region.



## 18.O-Mo-2

### Development of a Computational Model to Simulate the Exposure of a Virtual Colony of *Caenorhabditis elegans* Worms to Different Concentrations of Benzo[a]pyrene

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#### Abstract

In silico models and computational techniques are constantly being developed to evaluate the toxicity profile of substances. It is important to use appropriate biological models so that in silico models are reliable and accurate. *Caenorhabditis elegans*, a free-living nematode, has been used as a model organism in various in silico and toxicity studies of pollutants due to its ease of cultivation, manipulation, genomic sequencing, and well-established physiological responses. In this study, a simulation model was adapted to design a virtual population of *C. elegans* that reproduces, competes for resources, and undergoes natural mortality when exposed to benzo[a]pyrene.

The in silico model was created as a 2D Gaussian Python object with functions that define the worm's development, including survival probability, movement, reproduction, and number of offspring. To simulate exposure, toxicological data from a real in vivo exposure of *C. elegans* to benzo[a]pyrene at concentrations of 0 (control), 25, 50, 75, 100, 120  $\mu\text{g/L}$  were obtained. A mathematical function was then created from the regression of the percentage of surviving animals and applied to the probability of death of adult animals. In addition, other concentrations not found in the literature were estimated by the in silico model: 150, 175, and 200  $\mu\text{g/L}$ .

As a result of a 72h exposure, the percentage of surviving animals at the end of the experiment in the literature and the model, respectively, was: control: (100%/100%), 25  $\mu\text{g/L}$  (86%/82%), 50  $\mu\text{g/L}$  (77%/69%), 75  $\mu\text{g/L}$  (57%/57%), 100  $\mu\text{g/L}$  (44%/30%), and 120  $\mu\text{g/L}$  (34%/20%). Considering that the values returned by the model are the average of 10 experiments, the variability between the value found in the literature and the value returned by the model is lower than that found in intralaboratory experiments, with different strains and animals cultivated for different periods. In conclusion, the model can be of great value for purposes such as estimating the LC50 of contaminants, as well as estimating their lethality at concentrations not yet tested by researchers, thus reducing costs and operational time and serving as a reliable and complementary tool for toxicity evaluation.

## 18.O-Mo-3

### Recent Advances in Animal Alternatives for Environmental Risk Assessment

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#### Abstract

The replacement of traditional whole animal tests with assays that reduce, refine, or replace (the 3-R's) animal use has historically been a long-term goal in toxicity testing, although its prominence in the field of ecotoxicology is more recent. Risk assessments of chemicals require ecotoxicity studies with vertebrate organisms such as fish, and in many countries, fish tests are also required on a routine basis to evaluate the toxicity of effluents. A number of approaches have been developed or are underway that have the potential to replace or reduce the use of traditional in vivo fish toxicity tests. These include in silico, in vitro, and modified in vivo approaches, as well as the accompanying frameworks that can be used to integrate various information streams in a weight of evidence approach. This presentation will provide an overview of recent developments related to fish toxicity testing, with particular emphasis on the Fish Embryo Toxicity (FET) Test (OECD TG 236), the fish gill cell line (RTgill-W1) assay (OECD TG 249), the fish in vitro biotransformation assay (OECD TG 319 A/B), and the threshold of ecotoxicological concern (ecoTTC) approach. Ongoing developments and recent research related to applications beyond fish testing, including birds and amphibians will also be highlighted.

DRAFT

## 18.O-Mo-4

### Advancing the 3Rs in Research through Intestinal Organoids: Some examples of this Promising Approach

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#### Abstract

Intestinal organoids are three-dimensional, self-organized structures that mimic the intestinal epithelium's morphology, cellular diversity, and physiology, providing a more relevant model than traditional immortalized cell lines.

We have implemented intestinal organoid culture from adult stem cells of murine and other mammalian species to partially reduce the use of laboratory animals. Organoids can be frozen, thawed and passaged like a cell line. These mini-organs provide a continuous source for modeling the intestinal epithelium without requiring additional animals. Moreover, they allow the development of species-specific *in vitro* models, which were previously unavailable for some species.

Furthermore, we have characterized and validated intestinal organoids obtained from a transgenic mouse reporter for the nuclear factor-kappa light chain enhancer of activated B cells (NF- $\kappa$ B) as an alternative method to explore the epithelial TNF- $\alpha$ -induced NF- $\kappa$ B contribution in the small intestine. When stimulated with TNF- $\alpha$ , jejunum-derived-reporter organoids, provided a useful model to evaluate the anti-inflammatory effects of natural and synthetic compounds while helping to reduce the use of laboratory animals.

Murine colon-derived organoids were also applied to understand the role of the intestinal epithelium in the infection and persistence of the *Trypanosoma cruzi* parasite. Two and 3 dimension organoid cultures were infected with *T. cruzi*. After 72 h post-infection, the parasite distribution and load were evaluated by confocal microscopy, showing that organoids are sensitive to *T. cruzi* infection, providing a valuable tool for exploring mechanisms of enteric disease.

Organoids can also contribute to the replacement of *in vivo* models. For example, *Toxoplasma gondii*'s sexual replication is restricted to the feline intestine, making it difficult to study. We have used "felinized" murine intestinal organoids to model this process, obtained by supplementing the cultures with linoleic acid and inhibiting the delta-6-desaturase enzyme. Ongoing studies are addressing the detection of pre-sexual and sexual stages of *T. gondii* after infections of these models.

In summary, our results highlight the potential of using intestinal organoids as valuable *in vitro* tools to model intestinal epithelium. The versatility of these models can contribute to the 3Rs in research by partially reducing the use of laboratory animals and paving the way for replacing *in vivo* models.

## 18.O-Mo-5

### ***Caenorhabditis elegans* as a Potential Model for the Study of Estrogen-Like Endocrine Disruptor Compounds**

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#### **Abstract**

Nowadays there is growing concern about the adverse effects that estrogen-like compounds (pesticides, plastics and personal care products) can cause, creating a demand for the rapid and predictive evaluation of their impacts on human health and the environment. Added to it, there are a tendency to reduce or eliminate the use of mammalian animals in experimentation. In this sense, *Caenorhabditis elegans* is an excellent alternative biological model whose use does not raise the ethical issues associated with vertebrate research and that offers several advantages to develop bioassays to estimate the potential risk of reproductive toxicity of xenobiotics. In this work, three pesticides suspected of being endocrine disruptor (atrazine, 2,4-dichlorophenoxyacetic acid and chlorpyrifos) were studied to assess the impact on the reproductive development of the nematode *Caenorhabditis elegans*. Nematodes in the L4 larval stage were exposed to different concentrations of pesticides for 24 h and the consequences on brood size, percentage of gravid nematodes, expression of reproductive-related genes and vitellogenin trafficking and endocytosis were measured. In addition, 17 $\beta$ -estradiol was used as an estrogenic control throughout the work. We found that atrazine induced a similar response that 17 $\beta$ -estradiol in all responses analyzed. Chlorpyrifos and 2,4- dichlorophenoxyacetic acid decreased the brood size, caused abnormal vitellogenin distribution and disturbed the expression of some reproductive-related genes like atrazine and 17 $\beta$ -estradiol, but to a lesser extent, suggesting these pesticides could also induce an estrogenic action. In conclusion, our work proved that *C. elegans* is a useful biological model to identify the effects of estrogen-like endocrine disruptor compounds and can be used to study a wide range of biological questions without the use of traditional vertebrate models.

## 18.O-Mo-6

### Endocrine New Approach Methods (NAMs): Moving from Traditional Validation to Scientific Confidence Frameworks

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#### Abstract

The regulatory science community is evolving away from traditional, time- and resource-intensive validation of new approach methods (NAMs) toward 'fit-for-purpose' validation using scientific confidence frameworks (SCFs). SCFs are flexible, can be adapted to any regulatory or product stewardship context, can be used for any toxicological endpoint, and can accommodate rapidly evolving NAMs technologies. The American Chemistry Council (ACC) has developed an SCF that is centered on explicitly applying the scientific method. ACC's SCF consists of 7 components: 1) problem formulation and hypothesis (explicit proposition that the NAM can be used to provide actionable information for a specific decision context) 2) the biological relevance & plausibility of the NAM; 3) assay performance (documentation of sensitivity, specificity, reliability, & domain of applicability of the NAM); 4) documentation of the performance of inference (prediction) models based on the NAM response – outcome response relationship; 5) dissemination of the data, inference models, etc. to support independent replication; 6) a narrative rationale making the case that there is / is not sufficient scientific confidence in the NAM to support the specific application for the chemistry domain of interest; and 7) verification through independent scientific peer review. ACC's SCF will be presented and an example SCF application will be shown through testing of the inference model component of the Human Relevant Potency Threshold (HPRT) approach for Estrogen Receptor alpha agonism.

# Session 20: Environmental Assessment of Pesticides for Soil Organisms in Latin America

## 20A.O-We-1

### Are Enchytraeids Relevant for Soil Protection Goals in Brazil?

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#### Abstract

The definition of specific protection goals including soil organisms is important for a more robust environmental risk assessment scheme. Considering the case of enchytraeids, a soil inhabiting animal, a thorough analysis was carried out with regular and grey literature (theses, dissertations, conference abstracts, technical reports, unpublished datasets) to know their occurrence and distribution in Brazil. The analyses comprised enchytraeid sampling of 106 terrestrial sites (15 studies) using ISO guideline and 259 sites (29 studies) using TSBF from 1997 to 2020. The sites of occurrence included crops, pastures, integrated production system, planted forests and natural vegetation sites. Enchytraeid density calculated for TSBF method, on average, varied from 2- 1500 ind.m<sup>2</sup> (individuals per square meter), with a single site surpassing 7.000 ind.m<sup>2</sup>, while for ISO method, density was 1300- 12600 ind.m<sup>2</sup>, with some sites surpassing 30.000 ind.m<sup>2</sup>. Highest average densities were found in native vegetation, pasture and crop sites. Five genera were identified in samples from Amazonia, Cerrado and Mata Atlantica biomes, among which Enchytraeus and Fridericia are more frequent and abundant in cultivated areas contrasting with the dominance of Guaranidrilus, and sometimes Hemienchytraeus, in native vegetation sites. At species level, overall, 42 valid species were recorded in Brazil with dozens more to be described. Sampling effort is clearly scattered with a concentration in Mata Atlantica biome, therefore allowing only gross general estimation of abundance and diversity. Overall mean abundance of enchytraeids could be expected to be around 10.000 ind.m<sup>2</sup> with contrasting genus composition between natural and cultivated areas. Considering the widespread occurrence of enchytraeids, their importance for bioturbation, nutrient cycling, food web and their susceptibility to pesticides and other anthropogenic stressors through oral and contact exposure as well the habitat threats, enchytraeids are relevant to be included in specific protection goals. However, it is still hard to establish an accurate effect magnitude in terms of abundance or diversity in Brazil due to the need for more studies both in-field and off-field areas.

## 20A.O-We-2

### The Collembola Species from Brazilian Agricultural Areas and the Representativeness of *Folsomia candida* in Pesticides Risk Assessment

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#### Abstract

To define appropriated organisms to be used in pesticide risk assessment for soil organisms, it is important to understand their representativeness in agricultural fields, their sensitivity to pesticide exposure, and how efficient it is to extrapolate the effects from one species to the community. The soil trophic web comprises several hundreds of different organisms, which play distinct roles in the processes of maintaining ecosystem functions and services. An important group of soil microarthropods is Collembola (Hexapoda), commonly present in natural and agricultural fields in significant abundance and diversity. Thus, the main goal of this study was to review the literature to search for available information on both Collembola species from agricultural fields in Brazil and the sensitivity of different species of Collembola to pesticides, in comparison to the standardized species *Folsomia candida* Willem 1902. In a systematic review, of 21 articles found, only five had a good taxonomic resolution and identified Collembola species from Brazilian agricultural areas. The most common sub-order found was Entomobryomorpha, with emphasis on the Entomobryidae and Isotomidae families (50% of all organisms identified). Although *F. candida* was not identified, it was observed that the genus *Folsomia* was present in agricultural fields from Santa Catarina and Espírito Santo. Besides, it was also observed that Isotomidae species were the most abundant in these sites and represented 28% of the total species identified. In terms of sensitivity, the results obtained in the review showed that *F. candida* is a very sensitive species to different pesticides, but it was not possible to conclude whether it is the most sensitive species due to little information about other species' sensitivities and methodological differences in the organisms' exposure. Furthermore, as Collembola morphological diversity is strictly related to their habitat, depending on where they live and how the pesticide is applied on crops, the organisms will be differently exposed to pesticides and may consequently present distinct sensitivities accordingly. Thus, though abundant, *Folsomia* species were only identified in two Brazilian states, hence a much greater effort is necessary to identify Collembola species. Also, testing different species of Collembola and ways of exposure is of crucial importance to better understand the reliability of the extrapolation of results obtained with *F. candida* species.

## 20A.O-We-3

### Regulatory Review and Update of the Soils Required in Brazilian Legislation for Environmental Assessment of Pesticides

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#### Abstract

The Brazilian Institute of Environment and Renewable Natural Resources (IBAMA), throughout Ordinance No. 84/1996, requires three soil types in pesticides behavior tests regarding environmental assessments: *Latossolo Vermelho Escuro*, dystrophic or acidic, A moderate, medium texture; *Latossolo Roxo* dystrophic or acidic, A moderate, clayey texture; *Glei Húmico*, Tb, A prominent, medium texture. However, the classification of these soils is outdated, concerning the Brazilian Soil Classification System (SiBCS), and its update is necessary to standardize results. Although, representing Brazilian agricultural lands by a minimum set of soils is a challenging task, due to national territory vastness and soil and climatic conditions diversity. According to SiBCS, the soils updated nomenclature in Ordinance No. 84/1996 is: *Latossolo Vermelho* Dystrophic, medium texture, A moderate; *Latossolo Vermelho* Dystrophic, clayey texture, A moderate; *Gleissolo Melânico* Tb Eutrophyc or Dystrophic, medium texture, A prominent. Thus, these *Latossolos* subtypes differ from each other basically in texture, resulting, in practice, in only two distinct soils: *Latossolo* and *Gleissolo*. Yet, for a proper environmental assessment, it is expected that the required soils should cover broader variations in pH, texture, and organic matter to elucidate pesticides' behavior in Brazilian soils. In this study, geoprocessing was used to overlay land use data (annual crops, perennials, pastures, or planted forests) with soil classes. As a result, observing contrasting overall properties, the predominant soil types in Brazilian agricultural areas were: *Latossolo Vermelho/Vermelho-Amarelo* Dystrophic typic; *Argissolo Vermelho-Amarelo* Dystrophic typic; *Neossolo Quartzarênico Órtico* typic; *Gleissolo Háptico/Melânico* Tb Dystrophic typic. This means the update of *Latossolo* and *Gleissolo* classes, and the inclusion of *Argissolo* and *Neossolo* classes. Furthermore, the update of the soil classes alone would represent 15.6% of Brazilian agricultural area. With the inclusion of *Argissolo* and *Neossolo*, this percentage increases to 30.7%. Moreover, adding the *Latossolo Vermelho* or *Vermelho-Amarelo* option raises this value up to 40.4% of the agricultural area, representing 13% of the national territory. Accordingly, regarding environmental assessment of pesticides, based on the proposed improvement, a broader comprehensive and realistic representation of the diversity of agricultural soils in Brazil is expected.



## 20A.O-We-4

### Soil and Groundwater Exposure Scenario Development for Sugarcane in Brazil

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#### Abstract

The development of pesticide groundwater leaching exposure scenarios that are representative of a range of conditions associated with a given agricultural region or crop are needed to accurately assess potential soil and groundwater exposure risks. Even across a single crop growing region, the variability in climate and environmental conditions that dictate the magnitude and likelihood of pesticide leaching can be substantial, making the availability of exposure scenarios representing local conditions important for efficiently conducting realistic risk assessments. Exposure scenarios derived in other regulatory jurisdictions, such as North American, can provide a useful starting point for estimating exposure, however, the use of scenarios and assessments from other regions may not be relevant for some compounds and use pattern because climate, soils, geology, and agronomic practices can be very different between those regions and Latin American agriculture. This study offers a framework for developing soil and groundwater exposure scenarios representing local conditions using the US EPA's PRZM model, with an example provided for the sugarcane growing areas of south-central Brazil. The scenario development process involved the following steps: (1) spatial identification of the high intensity sugarcane growing areas, (2) obtaining representative long-duration (30-year) daily climate records, (3) identification and characterization of the range in soil conditions associated with sugarcane growing areas, (4) determination of groundwater table depths for sugarcane growing areas, and (5) development of PRZM leaching scenarios and modeling for the range of conditions determined in the previous steps. Sensitivity analysis was conducted to understand the impacts of uncertainty in groundwater temperature, soil organic carbon in and below the root zone, and biological degradation depth on predicted soil and groundwater concentrations for a moderately mobile and slightly degradable compound. The modeling results provided a range of relevant expected concentrations showing substantial differences within the sugarcane growing areas of Brazil, with the sensitivity analysis providing important context regarding the impacts of parameter assumptions on the conservatism of the scenarios. Based on the desired regulatory protection level, appropriate screening level exposure scenario are readily extracted from the population of scenarios evaluated.

## 20A.O-We-5

### Case Study: A Screening Level Approach to Assess Risk of Combined Pesticide Exposure to Soil Organisms

Steven Levine

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#### Abstract

Pesticides used in crop protection are regulated and undergo a comprehensive environmental risk assessment prior to authorization. In addition, regulators in some world areas may require an environmental risk assessment for formulations containing more than one active ingredient or for specific combinations that are prescribed for tank mixes. However, environmental detection of pesticides has raised concern that regulatory processes may be insufficient to assess risks from exposure to pesticide mixtures. This talk will present a case study that uses the conservative Hazard Index (HI) approach to screen for potential long-term risk of combined pesticide exposure to soil organisms. The HI is a measure of cumulative risk and is based on the addition of risk quotients for individual components. A  $HI \leq 1$  indicates minor or no concern and a HI of  $> 1$  indicates potential risk and further analysis may be required. Exposure concentrations were drawn from Silva et al. (2019), that reported pesticide residues in soil cores ( $n = 317$ ) collected from the uppermost layer of soil in orchards and agricultural fields across Europe. 58% of the soil samples contained mixtures and they highlighted the need to assess these residues through the lens of a soil organism risk assessment. For pesticides registered at the time of sample collection, glyphosate, its primary soil metabolite AMPA, boscalid, epoxiconazole, and tebuconazole were detected most frequently and at the highest concentrations. Glyphosate and AMPA had the highest detection frequency, with max levels of 2.05 and 1.92 mg/kg soil, respectively. Median values for glyphosate and AMPA were 0.14 and 0.15 mg/kg soil, respectively. Tebuconazole had a median of 0.02 and max level of 0.19 mg/kg soil, epoxiconazole had a median level of 0.02 and a max of 0.16 mg/kg soil, and boscalid had a median level of 0.04 and max of 0.41 mg/kg soil, respectively. For conservatism, the exposure assessment assumed the highest measured concentrations for the 5 components and that all 5 components co-occurred at their max levels. The effect endpoint that was used for each component were the no observed effect concentrations (NOECs) from laboratory earthworm reproduction studies (OECD, 222). No single component had a risk quotient  $> 1$  and the cumulative HI ( $\Sigma HI$ ) did not exceed 1, indicating the low likelihood of long-term effects to earthworm populations under this conservative scenario.

## 20A.O-We-6

### Analysis of the Distribution and Vulnerability of Native and Exotic Earthworms in Brazil for Implementing Environmental Assessment of Pesticides

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#### Abstract

In pesticide Environmental Risk Assessment (ERA) focusing on earthworms, the ecological entity to be protected is the populations of different earthworm species. The present analysis was based on integrating the native earthworm richness of each Brazilian state with the amount of pesticides sold (tons/km<sup>2</sup>). States with high native species richness and high pesticide use were considered most vulnerable to local species loss. Only very toxic active ingredients to earthworms were considered. The richest states in native earthworm species were: São Paulo (69 species), Paraná (65) and Amazonas (60). The richest in exotic/cosmopolitan species were: São Paulo (31), Rio Grande do Sul (30), and Paraná (29). Native species have restricted geographic distribution and endemism rates, whereas many exotic species tend to be widely distributed throughout the country. The most widespread native species in Brazilian territory were *Urobenus brasiliensis* (10 states), *Andiorrhinus duseni* (5) and *Eukerria urna* (5). On the other hand, the cosmopolitan earthworm *Pontoscolex corethrurus* was found in 21 states and was often the most abundant species found in managed ecosystems. This preliminary diagnosis of the states' vulnerability to the local loss of native species indicates that the most susceptible states are Mato Grosso, Paraná and São Paulo, followed by Goiás, Mato Grosso do Sul, Rio de Janeiro, Roraima, Rio Grande do Sul e Santa Catarina. Acre, Ceará and Rio Grande do Norte showed the lowest vulnerability for this parameter. As native earthworm species have a more restricted distribution than aliens, their use as test organisms is not recommended in ecotoxicological studies when assessing conservative tiers of ERA. However, considering their ecological importance, the inclusion of native earthworms in ERA is necessary, and may be accomplished by extrapolation factors, designed through species sensitivity distribution (SSD) studies. The future refinement and integration of this vulnerability map into ERA scenarios can supply important information about the Brazilian regions that should be prioritized, aiming towards proper protection of soil organisms, such as earthworms, from the non-target effects of pesticides. Neglecting this fact could mean the local loss of Brazilian species biodiversity or even part of essential ecosystem functions and services provided by these organisms.

## 20B.O-We-1

### Ecotoxicity of Treated Wheat Seeds for Soil Organisms

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#### Abstract

The use of pesticides has been enhanced in agriculture, seeking to control pests and diseases that may affect the cultivation of agricultural crops. One of the most common ways of using pesticide molecules nowadays is in seed treatment; however, these molecules can also affect non-target organisms, mediators of functions relevant to soil ecology. In this sense, the aim of this work was to evaluate the ecotoxicological effect of wheat seeds (*Triticum aestivum*) treated with fungicides and insecticides together, by industrial treatment, in standardized laboratory tests of reproduction of springtails (*Folsomia candida*) and enchytraeids (*Enchytraeus crypticus*), and earthworm avoidance test (*Eisenia andrei*). The studies were carried out in Tropical Artificial Soil (TAS) and in a Natural Tropical Soil (Latosolo), where three seeds were added in each replicate. Three seed treatments and one control were evaluated: A (Piraclostrobin + Thiophanate-methyl + Fipronil), B (Flutriafol + Imidacloprid); C (Thiamethoxam + Iprodione + Azoxystrobin + Carbendazim) and D (seeds without treatment). The evaluations followed the recommendations of ABNT NBR ISO 17512-1 (avoidance of earthworms), ABNT NBR ISO 11267 (reproduction of springtails) and ABNT NBR/ISO 16387 (reproduction of enchytraeids). No escape of earthworms in any of the treatments was observed. The reproduction tests showed that the treated seeds caused a significant reduction in the reproduction of springtails in Natural Soil comparing the control ( $196.60 \pm 35.40$ ) with treatment B ( $99.80 \pm 48.79$ ), followed by A ( $100.20 \pm 64.62$ ) and C ( $106.20 \pm 42.59$ ). In TAS, differences were not detected. Reproduction of enchytraeids showed a significant difference between treatment A (Piraclostrobin + Thiophanatomethyl + Fipronil ( $482 \pm 151.80$  juveniles) and D (control,  $714.6 \pm 152.31$ ). This work indicates that treated seeds have potential to affect the reproduction of springtails and enchytraeids. Further studies must be carried out to quantify the concentration of active molecules in the seed, in addition to determining the degree of effects in situ.

## 20B.O-We-2

### **ERAMYC – Assessing the Sensitivity of Arbuscular Mycorrhizal Fungi to Chemicals in Soil in the Pre-symbiotic Phase**

Luís Caros Luñes Oliveria Filho<sup>1</sup>, Tiago Natal-da-Luz<sup>2,3</sup>, Patrícia Ferreira<sup>2</sup>, Tanel Vahter<sup>4</sup>, Pia Kotschik<sup>5</sup>, Silvia Pieper<sup>5</sup>, Susanne Walter-Rohde<sup>5</sup>, Sonia Purin Cruz<sup>6</sup>, Susana Rodríguez-Echeverría<sup>3</sup>, Matthias C Rillig<sup>7</sup>, Mari Moora<sup>4</sup>, José Paulo Sousa<sup>3</sup>, Osmar Klauberg-Filho<sup>1</sup>

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#### **Abstract**

Arbuscular Mycorrhizal Fungi (AMF) are organisms that live in symbiosis with most plant species, being responsible for supporting key soil functions and providing important ecosystem services. Due to their ecological relevance and sensitivity to chemicals, AMF have been indicated by the European Food Safety Authority (EFSA) as a potential group of non-target test organisms to be used in the risk assessment of Plant Protection Products.

The existing ISO protocol 10832 describes a method to evaluate the effect of chemicals on AMF, but considers only germinated spores (pre-symbiotic phase). Germ tube formation and asymbiotic hyphal growth are important phases of the fungal life cycle and are essential for establishing symbiosis. However, other processes like root colonization could be affected by chemicals in the soil and are not investigated when following the current test protocol.

ERAMYC is a project which integrates a consortium of experts with the aims to 1) improve the existing protocol for the pre-symbiotic phase by including additional AMF species, test conditions and procedures embracing the symbiotic phase of AMF species; 2) standardize the developed test protocol through a ring-test with different chemicals; 3) develop a draft OECD Test Guideline and evaluate experimental results in reference to the existing framework for the risk assessment of soil organisms exposed to chemicals.

To comply with these objectives, two phases of preliminary experiments were performed. In the first phase, experiments were conducted without chemicals to evaluate the performance/suitability of the selected AMF species in different soils under specific environmental conditions and selected host plants. The second phase included the use of test chemicals to assess how the different AMF species and respective parameters react to the selected chemicals.

The use of seeds pre-soaked in water and a total AMF colonization higher than 40% in controls have been required to validate the experiments. Results of previous studies have shown that Chlorothalonil had effects on both the pre-symbiotic and symbiotic phases of AMF *Gigaspora albida* and *Rhizophagus clarus*. In the symbiotic phase, arbuscular colonization was, in most cases, more sensitive when compared to total colonization and total extra-radicular mycelium length. However, this pattern can be different when testing an herbicide or/and a fungicide with a different mode of action.

## 20B.O-We-3

### Sensitivity of Standard and Alternative Species of Collembolans to Mancozeb and Imidacloprid in Tropical Natural and Artificial Soils

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#### Abstract

Collembola species have been used as bioindicators of soil quality. This group is very sensitive to abiotic and biotic changes in ecosystems, as well as to soil contamination. Although these organisms represent a small proportion of soil biomass and respiration, they have a crucial role in decomposition and nutrient cycling regulation in soil ecosystems. The Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) is the federal agency acting to improve the regulatory process for pesticide registration in Brazil, including their risk assessment to soil organisms. This work aimed to compare the sensitivity of the standard species *Folsomia candida* with the alternative species *Proisotoma minuta* and *Sinella curviseta*, in one natural tropical soil (Oxisol) and in tropical artificial soil (TAS), to answer the questions: a) Are the results from the sensitivity of *F. candida* species to pesticides enough to protect other Collembola species?; b) Are the pesticide effects in artificial soil similar to that in Oxisol? For this purpose, the active ingredients (a.i.) from the two most commercialized pesticides in Brazil were evaluated, Imidacloprid and Mancozeb. Reproduction assays followed the ABNT NBR ISO 11267, with adaptations for the alternative species: 20 organisms per replicate in tests with *P. minuta* and *S. curviseta* (sexual reproduction); juveniles of 20-23 days old for *S. curviseta*. Values of NOEC and EC50 were compared among species and soils for each a.i. For Imidacloprid, the species *F. candida* and *P. minuta* presented similar sensitivities and responses in both soils. For Mancozeb, *F. candida* was the most sensitive species, showing the lowest EC50 value in Oxisol (20,63 mg.kg<sup>-1</sup>, CI 5,38-35,89), as well as *P. minuta* (41,06 mg.kg<sup>-1</sup>, CI 29,13-52,99). *S. curviseta* was the least sensitive species. Results indicated that *F. candida* has the potential to represent the sensitivity of other Collembola species and that, depending on the species, protection values obtained with natural soil can be more restrictive than those obtained with artificial soil.

## 20B.O-We-4

### Applying a Tiered Environmental Risk Assessment Framework to Estimate the Risk of Pesticides to Soil Organisms in Latin America

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#### Abstract

This work investigates the application of a tiered risk assessment scheme for soil organisms based on the risk quotient (RQ) and the toxicity exposure ratio (TER). Forty-five pesticides registered in Latin America were chosen and the ecotoxicological endpoints for earthworms, Collembola, and microorganisms were collated. Tier I assessment was made on conservative assumptions in which no refinements were applied. There, 14 pesticides (31%) exceed the RQ regulatory trigger indicating unacceptable risk, whereas 27 (60%) indicate unacceptable risk on the TER approach. In a Tier II evaluation when refinement options such as foliar interception, field half-life, and the dissipation following the peak estimated environmental concentration are considered, eight (18%) pesticides indicate unacceptable risk based on the RQ, and 15 (33%) indicate unacceptable risk based on the TER. A nonmetric multidimensional scaling evaluation was performed to understand the relevant characteristics involved in how each pesticide poses a risk to soil organisms. Based on the outcome of this analysis, we observed that, for a given pesticide, the combination of high persistence, low or no crop interception, and high toxicity are likely to require higher tier risk assessment. Refinement options can consider either or both the exposure and/or the effect side of the framework. Exposure refinements are potentially simpler and can be conducted with data already available to risk assessors, whereas effect refinements involving further testing with the organisms potentially at risk are still under discussion for intermediate and higher tiers. A sensitive, simple, and logical environmental risk assessment framework can be used to adequately identify risks based on the relevant protection goals that, in turn, will help to protect the desired soil multifunctionality of the ecosystem. We encourage academia and industry to further investigate these topics to provide the most scientifically robust and evidence-based information to decision makers.

## 20B.O-We-5

### **Risk Values for Soil Organisms in Vineyards Exposed to PPP (Plant Protection Products) Spray Series: Evaluating the Efficacy of Different Risk Mitigation Measures**

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#### **Abstract**

Risk mitigation measures (RMM) are usually suggested to reduce the impact of Plant Protection Products (PPPs) towards non-target organisms. RMM can be related to the use of more target-specific or low-risk PPPs, the use of new spraying technologies, the development of precise Early Detection Systems (EDS) and of Decision Support Systems (DSS) that reduces PPP use by predicting disease outbreaks. These RMM were developed/tested in isolation and in combination in field trials at the H2020 OPTIMA project aiming to develop an optimized novel Integrated Pest Management (IPM) system targeting the goals of the EU Farm to Fork strategy. In this study, we assess the risk of PPP spray series used in vineyards to earthworms, where those RMM measures were adopted. The aim was to holistically assess the cumulative risk of each spray series and the effectiveness of the RMM (isolated or in combination) in reducing the risk to in-soil organisms. Six field trials (named Ref 0 to Ref 5) were conducted on vineyards, where the different RMM were tested. Each reference consisted of a spray series with 13 application events, where 19-20 active substances (a.s.) were used. Ref 0 was considered the baseline condition. The risk of the spray series from each reference was calculated deriving the daily cumulative ETR values (ETR<sub>sum</sub>) over the application period of 144 days based on the Concentration Addition model. The toxicity data (NOEC) were collected from Draft or Renewal Assessment Reports or derived from laboratory tests. PEC values were estimated using the PERSAM software. The a.s. degradation was assessed by using a first order kinetics reaction. The comparison of the overall risk was done by calculating the “area under curve” and estimating the risk reduction. Considering the IPM system, there was a risk reduction among all references, particularly visible on Ref 3 and Ref 5 (71.9% and 57.5%, respectively) where some of the synthetic PPPs were replaced by bio-PPPs and anti-drift nozzles were used. The adoption of other RMM also resulted in an overall risk reduction: 6.8% (Ref 1), 26.4% (Ref 2), and 34.6% (Ref 4). However, despite these values and the efficiency of mitigation measures, none of the references tested fell below the trigger value during most of the application period. Despite being conducted in Europe, results presented here can act as an exemplary case-study to be adopted in Latin America to test the risk of PPP spray series.



## 20B.O-We-6

### Field Study With Carbendazim in Brazil to Evaluate Effects on Local Earthworm Community

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#### Abstract

There is a growing interest of Latin American (LA) countries, such as Brazil, to adopt a tier-based environmental risk assessment (ERA) of plant protection products (PPPs) to soil organisms. Field studies are very important to calibrate lower tier ERA, but the vast majority of existing field studies has been conducted in Europe. Potential higher tier refinements in PPP ERA in LA countries, if needed, are available for European environmental scenarios. However, these are partially distinct from those typical of LA countries. As the sensitivity of local earthworm populations is unknown and local conditions (i.e. soil properties and climate conditions) in Europe are different from those in LA countries, it is crucial to understand at which point European studies can be used for an ERA in LA. Therefore, a field study is being conducted in an planted grassland area in Santa Catarina State, Brazil, aiming to 1) compare the sensitivity of earthworm field populations with those from European studies; 2) calibrate Brazilian lower tier risk assessment. Results of this study can as well be useful for development and validation of effect models for a Brazilian scenario, which might become an important tool in the future soil risk assessment in LA. A commercial formulation of Carbendazim is being used as model substance, a randomized block design and three carbendazim doses plus a control were used, with each treatment having 6 plots of 10m x 10m. Carbendazim was applied once at the beginning of the study by spray onto the soil surface. The study have a duration of 12 months. Four earthworm samplings were and will be performed in each plot, immediately before Carbendazim application (pre-sampling) and 1, 6 and 12 months after the start of the experiment. Additional samplings for residue analysis and climate data records will be performed during the study to monitor the exposure conditions.

Data from samplings before and 1 and 6 months after Carbendazim application will be presented and discussed and technical limitations will be pointed out. Six earthworm species belonging to four families (Glossoscolecidae, Ocnerodrilidae, Megascolecidae and Lumbricidae) were identified in the pre-sampling and showed a higher earthworm density compared to the sampling after 1 month. A heterogeneous distribution of earthworms was found, independently of the application rates. Samplings have evidenced that earthworms density is highly influenced by soil moisture and recent rain events.

# Session 21: Nature-Based Solutions for Remediation and Restoration of Water Quality

## 21A.O-Tu-1

### Phytoremediation of Two Tropical Soils Contaminated With Hexazinone and Amended With Rock Powder

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#### Abstract

Phytoremediation techniques, combined with soil rock powder, can contribute to better efficiency of soil decontamination by residual herbicides, such as hexazinone. The objective this study was to evaluate the influence of the rock powder in the phytoremediation process of two soils contaminated by hexazinone. 7 kg pots were filled with each soil, Oxisol, and Inceptisol and incorporated into rock powder according to the respective doses (0, 4, and 8 t ha<sup>-1</sup>). Then, *Canavalia ensiformis* (jack bean) was sown, and hexazinone was applied at doses of 0, 125, 250, 375, and 500 g a.i. ha<sup>-1</sup>. Biometric evaluations of *C. ensiformis* were collected at 70 days after emergence - DAE in Oxisol and at 120 DAE in Inceptisol for the variables height (HT), diameter (DM), trefoils number (TN), leaf area (LA), dry matter of the root (DMR) and shoot (DMS) and at 42 DAE the injury level (IL). After finishing the phytoremediation experiment, the soil was homogenized and separated for herbicide analysis in High-Performance Liquid Chromatography (HPLC) and bioassay with the bioindicator species (cucumber, *Cucumis sativus*) to evaluate the hexazinone presence. The IL and ADM evaluations of the bioindicator species were performed at 21 DAE. In Oxisol, a difference was found between herbicide doses and plant death with doses above 375 g a.i. ha<sup>-1</sup>. In Inceptisol, the increasing doses of rock powder influenced the increase in the variables HT, DM, and TN of *C. ensiformis*. On the other hand, only the dose of 4 t ha<sup>-1</sup> positively influenced the LA, DMR, and DMS variables. The analytical technique did not detect the presence of herbicide residues in both soils after 70 and 120 DAE. For this reason, the bioindicator did not show IL and difference in DMR compared to the treatment without hexazinone application. Overall, rock powder did not affect potentiating the phytoremediation of the two soils contaminated by the herbicide, but *C. ensiformis* could phytoremediate hexazinone at low doses.

## 21A.O-Tu-2

### Approaches on Biochar from Agricultural Waste: Soil Conditioner and Wastewater Treatment

Claudia Hitomi Watanabe Rezende<sup>1,2</sup>, Reginaldo Gomes Barbosa<sup>2</sup>, Kathlein Aparecida Alves Bernardo<sup>2</sup>, Wesley Wellington Alves Bernardo<sup>2</sup>, Junia Verginia de Souza Silva<sup>2</sup>, André Henrique Rosa<sup>1</sup>  
<sup>1</sup>Sao Paulo State University (UNESP), Brazil. <sup>2</sup>State University of Maringa (UEM), Brazil

#### Abstract

Biochar (BC) produced from agricultural waste is known to improve soil quality. Thus, the production of biochar has proven to be one of the most efficient strategies for managing biomass residues, generating added value and environmental value in the fight against global warming and climate change. Since biochar is considered a versatile bioproduct, the present work aimed to evaluate the potential of BC as a soil conditioner and its application in the treatment of effluents. To achieve this objective, BC was produced by biomass pyrolysis and characterized. To ensure agronomic quality, germination bioassays were performed to assess possible beneficial or phytotoxic effects, based on standardized phytotoxicity test protocols and previous studies. As a result, the biochar produced improves the germination of *Zea mays* seedlings, demonstrating its capacity as a soil conditioner. For the treatment of effluents, the presence of biochar promotes almost 100% removal of methylene blue (dye). Thus, it was possible to demonstrate that the reuse of agricultural residues, transforming them into biochar, can contribute both to improving soil quality and to a sustainable increase in crop productivity and as a good adsorbent for wastewater treatment.

DRAFT

**21A.O-Tu-3**

## **New Technologies to Facilitate Environmental Sampling**

Claudia Elizondo

Hidrodata, Uruguay

### **Abstract**

HidroData is a synergy of the latest technology, as it brings the technology of unmanned vehicles to the water together with the existing system of physical and biological sensors, which will remotely and autonomously allow water systems to be monitored from the comfort of an office.

It capable of moving to different pre-established points, increasing the control surface with a single system. The use of sensors for environmental parameters, send the information obtained in real time, which makes the value obtained more efficient and accurate, since there is no alteration of the parameters due to the sampling and waiting time to be analyzed.

DRAFT

## 21A.O-Tu-4

# Remediation of Soil Polluted With Toxic Metals and Alleviation of Oxidative Stress in Brassica Rapa Plant Using Nanoscale Zerovalent Iron Supported With Coconut-Husk Biochar

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### Abstract

Accumulation of toxic elements by plants from polluted soil can induce the excessive formation of reactive oxygen species (ROS), thereby causing retarded plants' physiological attributes. Several researchers have remediated soil using various forms of zerovalent iron; however, their residual impacts on oxidative stress indicators and health risks in leafy vegetables have not yet been investigated. In this research, nanoscale zerovalent iron supported with coconut-husk biochar (nZVI-CHB) was synthesized through carbothermal reduction process using Fe<sub>2</sub>O<sub>3</sub> and coconut husk. The stabilization effects of varying concentrations of nZVI-CHB and CHB (250 and 500 mg/kg) on cadmium (Cd) and lead (Pb) in soil were analyzed, and their effects on toxic metals induced oxidative stress, physiological properties, and antioxidant defence systems of the Brassica rapa plant were also checked. The results revealed that the immobilization of Pb and Cd in soil treated with CHB was low, leading to a higher accumulation of metals in plants grown. However, nZVI-CHB could significantly immobilize Pb (57.5-62.12%) and Cd (64.1-75.9%) in the soil, leading to their lower accumulation in plants below recommended safe limits and eventually reduced carcinogenic risk (CR) and hazard quotient (HQ) for both Pb and Cd in children and adults below the recommended tolerable range of <1 for HQ and 10<sup>-6</sup> – 10<sup>-4</sup> for CR. Also, a low dose of nZVI-CHB significantly mitigated toxic metal-induced oxidative stress in the vegetable plant by inhibiting the toxic metals uptake and increasing antioxidant enzyme activities. Thus, this study provided another insightful way of converting environmental wastes to sustainable adsorbents for soil remediation and proved that a low-dose of nZVI-CHB can effectively improve soil quality, plant physiological attributes and reduce the toxic metals exposure health risk below the tolerable range.

## 21A.O-Tu-5

### **Nature Based Gualaxo Do Norte River Restoration After the Fundão Dam Rupture: A Comparative Study Among Three Reaches of the River**

Sabrina Loise de Moraes Calado<sup>1</sup>, Tatiana Furley<sup>1</sup>, Kátia Chagas<sup>1</sup>, Douglas de Pádua Andrade<sup>1</sup>, Hana Dalila Fernandes<sup>2</sup>, Pedro Ivo Diogenes Belo<sup>2</sup>, Monique Evellin Bodart Reis Gottardo<sup>2</sup>, Carolina Heck<sup>2</sup>

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#### **Abstract**

In November 2015, Gualaxo do Norte River (MG-Brazil) was impacted by iron ore tailings from the Fundão Dam rupture. The deposition of tailings on the riverbed changed the hydrogeomorphological and ecological characteristics. The main of this study was to compare the results of the river restoration project over one year (hydrogeomorphological and ecological responses) among three reaches of Gualaxo do Norte River that were classified as: i) T6R – high presence of riparian vegetation and low impact by iron ore tailings in relation to other reaches, source of macroinvertebrates and fish populations <1 km away; ii) T7R – low presence of riparian vegetation and high impact by iron ore tailings in relation to other reaches, source of macroinvertebrates and fish populations >5 km away; and iii) T9R - medium presence of riparian vegetation and medium impact by iron ore tailings in relation to other reaches, source of macroinvertebrates and fish populations >10 km away. During the restoration project, 364 wooden structures were installed in three reaches of the river (T6R, T7R, T9R), totaling 4.1 km long following a BACI design. Upstream of Restored reaches there is a Reference reach (not impacted and source of macroinvertebrates and fish populations) and for each Restored reach there is a Control (impacted and without structures). The campaigns in the reaches were carried out one year after the restoration process. After one year of the woody installation was observed the enrichment of substrate diversity (T6R-43%; T7R-34,7; T9R-29%). The benthic macroinvertebrate community showed an increase in the total abundance (T6R – 110%; T9R-25%), including the most sensitive groups (T6R-124%; T7R-27%; T9R-530%). For fish, only T6R showed an increase of recruitment up to 81% (hand nets capture), and the abundance and the biomass of some species were also higher than the Control reaches, mainly in T6R. The better positive results of restoration were observed in the T6R, indicating that the reaches with riparian vegetation, nearby source populations and less impacted by iron ore tailings have greater potential for success in restoration studies.

## 21A.O-Tu-6

### High-Rate Algae Pond Coupled Electrochemical Process for Removal of Pharmaceutical Compounds From Real Urban Wastewater

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#### Abstract

Pharmaceutical compound residues are in the environment in concentrations between  $\mu\text{g L}^{-1}$  and  $\text{ng L}^{-1}$ . The presence of these compounds in water sources is associated with the urban, hospital, and pharmaceutical industry wastewater discharge because conventional wastewater treatments have low removal efficiencies of these compounds.

A continuous coupled bio-electrochemical treatment was evaluated at a pilot scale. This coupled treatment was formed by a high-rate algae pond-HRAP followed by an electrochemical process with three days and one hour of hydraulic retention time, respectively. The electrochemical process operated with a current density of  $29.7 \text{ mA cm}^{-2}$ . In addition, the effluent of HRAP had a concentration of total iron, dissolved oxygen, and conductivity of  $1.6 \text{ mg L}^{-1}$ ,  $9.06 \text{ mg L}^{-1}$ , and  $469.4 \mu\text{S cm}^{-1}$ , respectively. Therefore, chemical reagents and supply air were not added to the electrochemical process. Bio-electrochemical processes' removal efficiencies were above 80 % for compounds of hypolipidemic, analgesic, and anti-inflammatory drugs. Antiepileptic drugs had the lowest removal, between 36.8 y 69.1 %. However, carbamazepine and its metabolite 10,11-Dihidro-10,11-dihidroxicarbamazepine increased their removal five times in the coupled treatment concerning HRAP. Only lamotrigine increased the concentration in the effluent of coupled treatment associated with the possible inverse transformation of its glucuronide in the HRAP.

This study showed that the continuous coupled bio-electrochemical treatment is an efficient alternative for removing pharmaceutical compounds from real wastewater. In addition, the bio-electrochemical treatment is environmentally friendly because the electrochemical process requires low chemical reagents.

## 21B.O-Tu-1

### Cellulose Acetate Film Containing Bonechar for Removal of Metribuzin from Contaminated Drinking Water

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#### Abstract

Bonechar presents high sorption capacity for mobile herbicides retained in soil and water. However, its use in a granulated and/or powder form makes it difficult to remove water. The objective of this study was to produce a cellulose acetate film with bonechar as a viable alternative to remove metribuzin from water. The treatments were composed of 2 and 3 g of bonechar fixed on a cellulose acetate film, pure bonechar, and a control (no bonechar). The sorption and desorption study was carried out in the equilibrium batch mode with five concentrations of metribuzin (0.25, 0.33, 0.5, 1, and 2 mg L<sup>-1</sup>). The water used in the experiment was potable water. Herbicide analysis was performed by High-Performance Liquid Chromatography (HPLC). The addition of 2 and 3 g of the bonechar fixed on the acetate film sorbed 40% and 60%, respectively, of the metribuzin at the lowest concentrations (0.25, 0.33, and 0.5 mg L<sup>-1</sup>). For both additions, desorption was low, being 7% and 2.5% at 24 and 120 h, respectively. There are still no reports of the production of cellulose acetate film with bonechar for herbicide removal in water, considered an alternative of easy handling and indicated for water treatment plants.

DRAFT



## 21B.O-Tu-2

### Nature-Based Solution for a Safer World: Harnessing the Power of *Salvinia molesta* Plants to Prevent Toxicity from Ciprofloxacin for One Health

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#### Abstract

Aquatic macrophytes have demonstrated excellent potential as nature-based solutions (NbS) for the removal of antimicrobials from water. However, macrophytes with proven capacity to treat contaminated water, such as *Salvinia molesta*, may also produce compounds with allelochemical potential or release antimicrobial metabolites in water whose toxicity is not yet fully understood. Therefore, it is important to investigate the ecotoxicological effects of waters treated by phytoremediation. In this study, we evaluated whether the phytoremediation capacity of *S. molesta* prevents the toxicity of ciprofloxacin (Cipro) to the microalgae *Desmodesmus subspicatus* and the Neotropical catfish *Rhamdia quelen*. We also investigated whether the macrophyte reduces the risks of the antimicrobial to human health. The microalgae and catfish were exposed to water contaminated with Cipro (0, 1, 10  $\mu\text{g.L}^{-1}$ ) that was either untreated or treated by *S. molesta* for 96 hours. In microalgae, we investigated the effects on cell growth, respiration, photosynthesis, and biochemical biomarkers (catalase – CAT; ascorbate peroxidase – APX;  $\text{H}_2\text{O}_2$  – hydrogen peroxide; lipid peroxidation – LPO). In the catfish, we evaluated hematological parameters (number of erythrocytes, leukocytes, and thrombocytes) and LPO in the liver and posterior kidney. Additionally, we investigated the accumulation of Cipro in the muscle of fish to predict the risk to human health (target hazardous quotient > 1) after the consumption of contaminated animals. The results showed that *S. molesta* exhibited a greater phytoremediation capacity, ranging from 76 to 97% of Cipro removal. The microalgae exposed to 10  $\mu\text{g.Cipro.L}^{-1}$  showed inhibition of growth, a decrease in respiration and photosynthesis, and an increase in oxidative stress (CAT, APX, LPO and  $\text{H}_2\text{O}_2$  concentration). In *R. quelen*, Cipro (1 and 10  $\mu\text{g.Cipro.L}^{-1}$ ) reduced hematological parameters and increased LPO in the liver and posterior kidney. The treatment of contaminated water with *S. molesta* prevented the toxicological effects of Cipro on both *D. subspicatus* and *R. quelen*. Moreover, the phytoremediation prevented the accumulation of the antimicrobial in the muscle of fish, favoring safe consumption by humans and preventing the risk to human health caused by Cipro. The phytoremediation capacity of *S. molesta* is an efficient NbS to prevent toxicity from Cipro to One Health approach (environmental, animal, and human health).

## 21B.O-Tu-3

### Multifunctional Approach to Evaluate the Efficiency of Urban Wastewater (UWW) Treatment from San Justo city (Santa Fe, Argentina)

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#### Abstract

Urban wastewater treatments (UWTs) transform biodegradable material into nontoxic products, to remove nutrients, and contaminants from different sources. The aim of the study was to know: (1) the physicochemical characteristics, including the presence of hormones, of UW during periods of high and low temperature and rainfalls; (2) the ecotoxicity of UW using *Chlorella sorokiniana*, and (3) the detection of SARS-CoV-2 in raw and treated UW. The UWT consists of (i) two anaerobic ponds; (ii) two primary facultative ponds; (iii) two secondary facultative ponds (SFPs); and (iv) a chlorination chamber. Raw UW and treated UW in SFPs were collected for three winter months (WIN - 2021 and 2022), and three summer months (SUM - 2021, 2022, and 2023), for physicochemical and hormonal analysis. Estrogenic/anti-estrogenic and androgenic/anti-androgenic activities were evaluated in UW samples at both seasons. For the ecotoxicological tests, different dilutions of UW from the SFPs were used in algal growth inhibition assays. The % removal of chemical oxygen demand (COD) (WIN 64% - SUM 52%), biological oxygen demand (BOD<sub>5</sub>) (WIN 81% - SUM 79%), total suspended solids (TSS) (WIN 28% - SUM 25%), ammonium (WIN 36% - SUM 27%), and phosphorus (WIN 52% - SUM 12%) were higher in WIN. Higher % removal was only obtained in SUM for sulfide (SUM 97% - WIN 79%). Only COD and ammonium exceeded slightly the values proposed in the current provincial regulations. The decrease of ammonium is related to the biological processes of nitrification and its conversion to nitrate and nitrite by oxidation reactions. The biodegradability index registered in the raw UW was very similar between seasons (0.45-0.46), but decreased in the treated UW, being slightly higher in WIN (0.24 and 0.19). Estrogenic activity was detected only in SUM samples. A dilution of 90% UWW inhibited the algal growth (50%) of *C. sorokiniana*. According to the risk classification system for wastewater discharged into the aquatic environment, the UW presented an Acute Toxicity Class III. Of the total positive samples, 77% correspond to untreated UW, 23% to pre-chlorination, and no SARS-CoV-2 RNA was registered at the post-chlorination sampling site. This work could contribute to building a network for wastewater-based epidemiology. The results can be used to put management policies into practice, with the purpose of contributing to the river ecosystem's integrity.

## 21B.O-Tu-4

### Clearing the Way for a Cleaner Tomorrow: Testing a Novel Green Technology for Removing Antimicrobials and Antimicrobial-resistant Bacteria from Wastewater Treatment Plant Effluent

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#### Abstract

Antimicrobials are often present in the effluent of wastewater treatment plants (WWTP), contributing significantly to antimicrobial resistance – one of the biggest health problems of the 21st century. To address this issue, we developed an integrated green system composed of three modules that utilize macrophyte species capable of removing antimicrobials from water. The modules are connected, and the effluent passes through a first 1000 L tank planted with the emerging macrophyte *Sagittaria montevidensis*, followed by a second 500-L tank containing a mix of the floating macrophytes *Salvinia minima* and *Lemna gibba*. Finally, the effluent passes through a third 500-L tank containing the emerging macrophyte *Canna indica* in a continuous flow of 500 ml/min. We evaluated the ability of the system to ameliorate physicochemical parameters (pH, conductivity, color, total soluble solids, dissolved oxygen, and phosphate concentration), and to reduce antimicrobials (sufamethoxazole, sulfadiazine, ciprofloxacin, enrofloxacin, norfloxacin, levofloxacin, oxytetracycline, tetracycline, azithromycin, amoxicillin, meropenem and gentamicin) and antimicrobial-resistant bacteria (ARB) from the WWTP's effluent. After an initial period of two months for in situ acclimatization of the plants, evaluations were conducted. The system reduced conductivity, total soluble solids, color, and phosphate concentrations and increased dissolved oxygen in the effluent. Likewise, significant reductions were observed in the concentrations of all the investigated antimicrobials. Moreover, there was a reduction in the number of colonies of ARB. Our results indicate that the composed-green system is efficient in preventing water contamination and antimicrobial resistance. In addition to its efficiency, this new green technology is cheap, easy to operate and manage, and requires low energy. It must be considered as an alternative for tertiary treatment in WWTPs around the world. Given the availability of this technology, we encourage governments to start establishing limits for antimicrobial release from effluents.

### Assessment of Chromium Tolerance and Removal Using Constructed Wetlands Planted With Emergent Macrophyte (*Eleocharis sp.*)

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#### Abstract

Industrial development worldwide leads to metal pollution of aquatic ecosystems and water deterioration. Therefore, it is necessary to develop environmentally friendly strategies for treating industrial wastewater. In this study, constructed wetlands planted with *Eleocharis sp.* were exposed during 30 days to a culture medium with three different levels of chromium contamination (T1: 5 mg L<sup>-1</sup>; T2: 20 mg L<sup>-1</sup>; T3: 50 mg L<sup>-1</sup>) typically found in tanneries, as well as a control treatment. The objectives of this research were to assess the capacity of the wetlands for removing Cr and the physiological response of the macrophyte to metal exposure. At the end of the experiment, the removal efficiency was over 94% for all treatments, but only T1 and T2 achieved Cr values below 1 mg L<sup>-1</sup>, as established by the guidelines for effluent discharge into surface water bodies of Cordoba, Argentina. The adsorption of Cr in sediments, determined by a sequential chemical extraction, depended on the metal concentration of the culture medium and significantly differed between fractions in the order of FIII > FV > FIV > FI + FII. The accumulation of the metal in the FI + FII fraction of sediment was less than 5%, while it was around 75-85% in FIII for all treatments. These results indicate that coprecipitation with Fe is a significant pathway for Cr removal, and in these sediments, Cr is nearly unavailable to aquatic organisms. For this reason, metal accumulation in the plant is likely related to the concentration of Cr in water and in FI and FII of sediments. The accumulation of Cr in the shoots, rhizomes, and roots of the emergent aquatic plant depended on the metal concentration of the culture medium, and in general, there were no significant differences found between different exposure times. The accumulation of metal varied among the macrophyte tissues and was ranked as follows: roots > rhizomes > shoots. Furthermore, aquatic plants exposed to Cr in the constructed wetlands did not exhibit visible damage, and there were no significant differences in the survival of the macrophyte and in the relative growth rate compared to the control treatment. Additionally, the relative growth rate increased in all treatments over time. In conclusion, based on the removal of Cr, the bioavailability of the metal in sediments, and the ability of the macrophyte to tolerate and accumulate Cr, the constructed wetlands could be scaled up to assess its feasibility for real wastewater treatment.

## 21B.O-Tu-6

### Use of Marine Microalgae Biomass Residue From Lipid Extraction for the Biosorption of the Female Hormone 17 $\alpha$ -Ethinylestradiol

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#### Abstract

Among the synthetic hormones most commonly used by women, 17 $\alpha$ -ethinylestradiol (EE2) stands out, and each year it has been found in increasing concentrations ( $\mu\text{g/L}$ ) in different water bodies in Brazil. This situation is due to the low rates of sewage treatment in Brazilian cities and is further aggravated by the fact that the conventional technologies applied to the treatment of domestic effluents in Brazil were not originally designed to remove hormones and other endocrine disruptor chemicals (EDC). The aim of the present study was to evaluate the EE2 biosorption by a green biosorbent from the marine microalga *Nannochloropsis oculata*, derived from the residual biomass of lipid extraction. The effect of applying a tannin-based polymer (Tanfloc SG) for *N. oculata* biomass harvesting was also evaluated to verify whether it would alter the biochemical composition compared to harvesting the biomass by centrifugation. This natural-based flocculant is used to reduce microalgae production costs since separating biomass from the culture medium is one of the biggest challenges for large-scale microalgae production. The use of residual microalgal biomass after lipid extraction can be considered a sustainable alternative to be applied in the removal of EDC, also having the advantage of producing value-added products. Assays were carried out in 125 mL Erlenmeyer flasks containing 50 mL of EE2 solution ranging from 5 to 50  $\mu\text{g/L}$ . The variables used were: 30 mg of dry microalgal biomass; 24 h of contact time; a pH of 7; 150 rpm of orbital agitation; and a 25  $\pm$  2°C temperature. When comparing the treatment efficiencies of the two types of biomass evaluated, the biomass without extraction promoted the removal of EE2 in the range of 52–74%, while the residual biomass extracted from lipids removed between 42 and 64%. The model that best described the adsorption process for both conditions was the Freundlich isotherm. However, in the future, a biomass treatment can be carried out to optimize its surface properties and increase the EE2 adsorption capacity. The results also demonstrated that, although Tanfloc SG may have affected the lipid extraction of the biomass (36  $\pm$  1.1%) compared to centrifuged biomass (58.8  $\pm$  1.4%), it did not significantly alter the fatty acid composition of *N. oculata*. Thus, considering the high flocculation efficiency (99.4  $\pm$  0.7%), the use of Tanfloc SG can be considered an efficient and low-cost method for harvesting *N. oculata* biomass.

# Session 23: Planetary Health: Chemical Pollution as Driver for Loss of Ecosystem Services and Biodiversity

## 23A.O-Mo-2

### Chemical Pollution as the Under-Investigated Threat to Ecosystem Integrity and Global Biodiversity: The Way Forward

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#### Abstract

Anthropogenic drivers such as climate change, changes in land use, introduction of invasive species, and chemical pollution play a central role in the global degradation of ecosystems and loss of biodiversity. Despite the urgency of this crisis, our understanding of these drivers is often too rudimentary to adequately guide mitigation strategies. To compare the research conducted on chemical pollution with research on the other major drivers of global biodiversity loss, we searched the scientific literature published between 1990 and 2021 and examined the journals in which studies on these topics have been published.

Our analysis revealed that while hundreds of thousands of scientific papers on chemical pollution have been published, the majority of them have appeared in a rather narrow selection of scientific journals. The sharp

contrast between the high number of papers produced on chemical pollution and the limited spectrum of journals in which those papers have been published suggests a high degree of encapsulation of the field. In contrast, research on climate change, habitat loss, and invasive species has been published in a much more diverse array of journals. Furthermore, research on these three drivers and biodiversity loss itself has often addressed all of them together, and even when treated separately, individual studies are published in the same journals, indicating connections among the topics. Lastly, ecological perspectives are commonly used to guide research on these three drivers. In contrast, research on chemical pollution has been predominantly conducted in isolation and disconnected from ecology. Consequently, its results have been published in specialized ecotoxicological journals that rarely publish papers on other drivers of biodiversity loss or biodiversity loss itself.

Despite substantial progress fueling advances in chemical pollution science and policy, research on chemical pollution has remained predominantly technical, isolated from other biological disciplines, and surprisingly disconnected from the biodiversity loss perspective. In this presentation, we will further analyse the potential causes underlying the disconnection between chemical pollution and ecological research and propose concrete actions to remedy this situation.

DRAFT

## 23A.O-Mo-3

### Scientometric Assessment of the Effects of Chemical Pollution on Biota on Estuaries From Argentina in the Last 30 Years

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#### Abstract

Estuaries are highly impacted by human activities, receive pollutants from various sources and have a strong tendency to accumulate pollutants. Several scientific papers are published, but these documents are scattered in scientific journals and reports from environmental agencies in a non-complementary way, making more in-depth studies difficult and challenging the initiative of developing larger-scale assessments. Thus, this study aims to synthesize the knowledge about the biological effects caused by contaminants on marine and estuarine organisms along the coast of Argentina through scientometric analysis, in addition to developing a database with this information. Published scientific articles containing information on biological responses to pollution in estuaries and associated ecosystems in Argentina were reviewed and analyzed. The keywords (Argentina\*) AND (estuar\*) AND (pollut\* OR contamina\*) AND (response\* OR biological effects OR endpoint\* OR toxic\* OR ecotoxic\* OR biomarker OR bioaccumulation), considering a 30-year period, were searched in the Web of Science. Only studies reflecting in situ conditions were included, i.e., laboratory manipulations were excluded. The information was categorized by sampling site, contaminant type, bioecological effect types, level of biological organization and taxon/group studied. 160 articles retrieved from the search and 66 fitted the inclusion criteria. 2019 showed the highest number of publications/year (17). Studies with metals and organic contaminants related to bio/ecological responses had the largest numbers of records (228 and 96, respectively). The most studied classes of organisms were Actinopterygii (180), Malacostraca (63) and Bivalvia (53), in addition to planktonic communities such as mesozooplankton (13). The most representative response level was individual (59%), with the highest number of records over the years, occurring in almost all year classes (3-year), being absent only between the 1990-1992 and 2021-2022 classes. The most reported effect was bioaccumulation (53%), followed by changes in abundance (12%) and changes in community structure (7%). Considering the data mentioned, is highly important to conduct and share studies like this, which synthesize the available data about biota, environment, contaminants, and their relationships, so that it can contribute to preventive management actions.



## 23A.O-Mo-4

### Exploring the Relationship Between Pollution and Parasites in *Corbicula fluminea*

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#### Abstract

In aquatic ecosystems, chemical pollution and parasites can cause organisms' tissue damage and physiological alterations (*e.g.*, changes in the condition index and respirometric rates). Despite the fact that the majority of aquatic organisms exposed to pollutants are also infected with at least one parasite, studies combining the effects of these two stressors are scarce. The present study compares the infection rates and effects of the oligochaete parasite *Chaetogaster limnaei* on introduced *Corbicula fluminea* clams in two areas of the Río de la Plata estuary with contrasting pollution conditions: Parque de los Niños (PN) influenced by urban pollution and Punta Lara (PL) away from this influence. Detailed physicochemical datasets of each site were obtained from databases published by monitoring programs of the Argentine Ministry of Environment and Sustainable Development. The prevalence of the parasite was calculated and the body condition index (*i.e.*, dry tissue weight/shell length), a number of histological alterations, and the respiration rate were used as biomarkers to determine the parasite effect. PN was the most contaminated site associated with higher concentrations of hydrocarbons (average concentration 211 mg l<sup>-1</sup> compared with 0.12 mg l<sup>-1</sup> in PL) and coliforms (106,196 NMP 100 ml<sup>-1</sup> compared with 16,432 NMP 100 ml<sup>-1</sup> in PL). In association with this, clam condition index and parasite prevalence were significantly lower in PN than PL. Histological analyzes revealed that the presence of the parasite was not associated with clam gill damage and changes in the respiratory rate in PN. Contrastingly, in PL, infected clams showed gill damage, which was more severe at high parasite loads; and the respiration rates of infected clams were higher than those of uninfected conspecifics. Our results suggest the existence of differences between the parasite load and the effects of those parasites on host clams in sites with different degrees of chemical pollution. Toxicological and parasitological studies should be integrated to better understand the influence of pollution on symbiotic interactions.

## 23A.O-Mo-5

### Exposure to Inorganic Elements of the Snow Goose and the Green-winged Teal During their Winter Stay in the Santiaguillo Lagoon

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#### Abstract

The Santiaguillo Lagoon located in Durango state, Mexico is an Important Area for the Conservation of Birds, it is a resting and feeding refuge for resident and migratory waterfowl. Waterfowl is the only group of birds (41 species) with a population increase of 56.0% (34.9 millions of net abundance change) in North America in the period 1970-2017; however, various activities carried out by industry, agriculture and urban development have given rise to innumerable accidents that have contaminated terrestrial, atmospheric and aquatic resources with toxic materials. Heavy metals and metalloids are a growing environmental problem worldwide as a result of this development, for this reason many potentially harmful chemicals are found contaminating the aquatic environments used by ducks and geese. The aim of this work was to determine the concentration of metals and metalloids in liver tissue of snow goose (*Anser caerulescens*) and green-winged teal (*Anas crecca*) during their winter stay in Laguna de Santiaguillo, Durango, Mexico. For the above, we collected 27 green-winged teals and 30 snow goose specimens during the 2021-2022 hunting season. Liver tissue samples were collected and analyzed using the voltammetry technique to determine the elements Zn, Cd, Pb, Cu, Cr, Sn, Al, As, Ni, and Hg. We obtained the hepatic concentrations (mg/kg dry basis, mean, standard deviation, minimum and maximum) and prevalence of each inorganic element for both species. Zn, Cu, Cr, Sn, Al, As and Ni concentrations were similar in both species (Kruskal-Willis test  $p > 0.05$ ), but Cd, Pb were higher (Kruskal-Willis test  $p < 0.05$ ) in the snow goose; Hg was higher (Kruskal-Willis test  $p < 0.05$ ) in the green-winged teal. Al was the element with highest concentration (average) in both species and the lower concentration determined were Pb for green-winged teal and Cd for snow goose. Consistently in both species, we observed that the average levels of metals and metalloids are low, however, some individuals have high concentrations that in some cases can compromise their health. The present study provides a first insight into the contamination with metals and metalloids of two aquatic bird species wintering at this important site. Further studies should increase the sample size to assess metal and metalloids levels and age or sex related differences in concentrations.

## 23A.O-Mo-6

### Effects of Municipal Wastewater Discharges on Microbiomes of Aquatic and Riparian Hosts

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#### Abstract

Microbial communities on or within a host, better known as microbiomes, affect an organism's health due to their roles in nutrient accessibility, immune system function, etc. and shifts in these communities are increasingly linked to diseases. These communities of microorganisms can be affected by a host's diet, environmental conditions and chemical exposures, including pharmaceuticals. Effluents from wastewater treatment plants (WWTPs) contain diverse antimicrobials and bacteria that have the potential to affect microbiomes of aquatic species, with consequences for the health of aquatic species. In addition, waterborne contaminants are transferred to riparian spiders with potential impacts on their microbiomes. We collected mussels, several species of macroinvertebrate larvae and adults, and riparian spiders from sites upstream and downstream of many WWTPs on the Grand River in southern Ontario and Bow River in Alberta, Canada. Bacterial genomic DNA was extracted from the gut contents of mussels as well as whole larval and emergent insects and spiders, followed by the nested PCR amplification of the bacterial signal and sequencing using the V3-V4 hypervariable region of the 16S rRNA genetic barcode. Changes in the relative abundance of major bacterial phyla were observed among sites in most aquatic invertebrate microbiomes. The Phyla Cyanobacteria often increased and Proteobacteria often decreased in taxa from the downstream sites, and alpha diversity commonly decreased in hosts from the downstream sites. However, these trends varied among taxa. Some among-site differences in endosymbiont bacteria were observed in Tetragnathid spiders including increases in *Rickettsiella* (affects reproductive success) downstream of some WWTP-impacted sites. Up to one third of aquatic species at each impacted site contained effluent-associated bacteria including ones used in wastewater treatment and opportunistic pathogens. In addition, spiders also contained bacteria present in the human digestive tract and human pathogens. Overall, these results demonstrated the transport of WWTP-derived bacteria through aquatic ecosystems to riparian predators, and that bacterial DNA is a novel indicator of municipal wastewater exposure in aquatic-riparian food webs. Additionally, the microbiome of invertebrates differed from upstream to downstream of WWTPs, which has implications for host health.

## 23B.O-Mo-1

### Neonicotinoid Insecticides and the Great Barrier Reef: An Ecosystem Unprotected?

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#### Abstract

Poor water quality associated with land-based runoff is one of the primary pressures impacting the health and resilience of Australia's Great Barrier Reef, with land-based runoff of pesticides counted as one of the top three risks to the Great Barrier Reef and coastal ecosystems. The neonicotinoid imidacloprid is one of the most frequently detected pesticides in the Great Barrier Reef catchment area as it is registered for, and highly relied upon, in a diverse range of crops including cereals, cotton, pastures, stone fruit, sugar cane, bananas and various vegetable crops. Other neonicotinoids, such as acetamiprid and clothianidin, are also detected.

Since the most recent Great Barrier Reef Scientific Consensus Statement, new research has indicated the potential for some neonicotinoid insecticides to bioconcentrate, meaning the combined ecosystem impact may be greater than that estimated previously. This presentation will provide a revised risk assessment based on pesticide concentration data from 28 long term monitoring sites across 22 unique river basins that drain to the Great Barrier Reef lagoon, as well as an updated method for including time-dependant toxicity of neonicotinoids into pesticide mixture risk assessment. The spatial and temporal drivers and potential impact of neonicotinoid bioconcentration on fresh water and marine (Reef) ecosystems will also be discussed. The results indicate that effective land management and risk mitigation measures are urgently needed to reduce the aquatic ecosystem risks arising from agricultural insecticide use.

## 23B.O-Mo-2

### Exploring Niche Size in Contaminant Exposure Assessments: An Experimental Evaluation With Amphipods

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#### Abstract

Little is known about the background variability in physiological and biochemical traits involved in wild populations exposed to stress. Recently, stable isotope metrics were proposed as population-level markers of stress that could complement existing ecotoxicological markers. Here, we explored this idea by exposing the benthic invertebrate species *Monoporeia affinis* to a sediment spiked with PAHs and PCBs and evaluating the amphipods responses related to feeding (added diatoms), growth, neurotoxicity (acetylcholinesterase [AChE] inhibition) and stable isotope ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) composition. The amphipods were sampled from two close sites from the Stockholm archipelago (Sweden), living in sediments characterized by: (1) relatively low organic carbon content and low contamination level (Low, hereafter), and (2) relatively high organic carbon and high contamination level (High). We expected to find more adverse growth and neurotoxic responses in the exposed treatment for the Low compared to the pre-exposed High amphipods as an indication of local adjustment (or possibly adaptation) to contaminant stress in the latter. With regard to the stable-isotope responses, a higher inter-individual variability and fractionation were expected to occur in the exposed amphipods, which would translate into a wider isotope niche. In all amphipods exposed to the spiked sediment, AChE inhibition was observed. However, both controls and exposed amphipods from the High site had higher survival, nutrient uptake and condition status than the Low amphipods, which did not feed on the added diatoms as indicated by their isotope values. A niche expansion was found in the exposed High amphipods but, contrary to our expectation, the exposed Low amphipods showed a significant isotope niche compression. We found no signs of local adaptation to contaminants with regard to neurotoxicity and growth status but geographic variability and metabolic status were important factors for interpretation of niche isotope metrics, which instead proved useful in assessing in stress responses at the population level.

## 23B.O-Mo-3

### High-Throughput Sequencing of Ovarian MicroRNA in Annual Killifish Exposed to Roundup Transorb®: Bringing Genomics Into Wildlife Ecotoxicology

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#### Abstract

MicroRNAs (miRNAs) are short noncoding RNA molecules, 22 – 25 nucleotides in length, which regulate gene expression at the post-transcriptional level. Environmental situations (e.g., pesticide pollution) can shape miRNA biogenesis and its target genes expression. Therefore, these molecules have recently been studied as biomarkers of environmental stress in ecotoxicological studies. Annual killifishes are the most endangered group of fish in Brazil. Among them, the species *Austrolebias charrua* endemically inhabits temporary wetlands from the extreme south of Brazil to the east of Uruguay. The main anthropic factor that determines the species imminent extinction is extensive agriculture since *A. charrua* inhabits ponds adjacent to rice and soybean monocultures dependent on the use of glyphosate-based herbicides, mainly Roundup. This study aims to prospect the global profile of miRNA expression in the ovary of the annual killifish *A. charrua* following exposure to Roundup Transorb®. Hence, 12 females were collected in nature under permit IBAMA/SISBIO 71072. In the laboratory, fish were divided into couples throughout two experimental groups: the “Control group”; and the “Roundup group” (5 mg. L<sup>-1</sup> for 96 h). Fish gonads were collected after chronic exposure to the herbicide, followed by RNA extraction, library preparation, and miRNA sequencing. The assembly of the transcriptome was achieved based on deposited genomes of fish species phylogenetically close to *A. charrua*. Annotation of miRNA-seq data was performed from zebrafish miRNAs deposited in miRBase. Thus, miRNA transcriptome sequencing of ovarian tissue from *A. charrua* revealed expressions of 205 mature miRNAs. This study is a pioneer in sequencing miRNA in annual killifish exposed to environmental pollution and increases the knowledge about the (epi)genomics of a species that compose the endangered Brazilian fauna. From future perspectives, based on the prospection of the ovarian microRNAome of *A. charrua*, the analysis of differential expression will enable the identification of miRNAs related to herbicide toxicity in this endangered species. Since miRNAs are being used innovatively as biomarkers in ecotoxicology, this study encourages the development of new molecular tools based on the measurement of epigenomic factors for the biomonitoring of annual killifishes and ecotoxicology assessment.

## 23B.O-Mo-4

### **Biomonitoring of Aquatic Invertebrates Using Biomarkers to Assess the Environmental Quality of the San Luis River (Argentina)**

Leandro Rodriguez<sup>1</sup>, Miriam Roxana<sup>2</sup>, Patricia Laura Colombetti<sup>2</sup>, Camila De Giuseppe<sup>2</sup>, Patricia Gonzalez<sup>2</sup>, Cesar Almeida<sup>2</sup>, Juan Manuel Perez Iglesias<sup>2</sup>

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#### **Abstract**

Currently, urban aquatic environments are highly affected by different actions of anthropic origin, mainly due to the contribution of sewage effluents, industrial discharges and agrochemicals from agricultural and livestock areas on these bodies. In particular, the rivers that cross urban and peri-urban centers are characterized by anthropic impacts that generate alterations in water quality and therefore in biodiversity. Therefore, the monitoring and control of river quality is an essential and unavoidable step in water resources management. The present work aims to evaluate the effects of water quality on biomarkers of macroinvertebrate bioindicators, combining biomonitoring strategies, to understand how anthropogenic pressure could harm the aquatic biodiversity of the San Luis River. To carry out the study, 5 sites were established along the San Luis River (S1 to S5) where water and sediment samples were taken and macroinvertebrates of the Chironomidae family were sampled with Surber net with 90 cm. Water and sediment sampling allows physicochemical characterization and subsequent calculation of the water quality index (WQI). From macroinvertebrate samples collected at each site, biochemical biomarkers such as catalase (CAT), cholinesterase (ChE) and malondialdehyde content (TBARS) were assessed. The results showed that water quality is "excellent" in S1, "intermediate" in S2, "acceptable" in S3 and S4 and "unacceptable" in S5. In agreement with these chemical results, the enzymes evaluated showed significant increases for CAT in S2 and inhibition of lipid peroxidation in S2 and S3. While no significant differences were observed in the determinations of ChE. It should be noted that no macroinvertebrates or aquatic vertebrates were detected in S5. In conclusion, it is important to mention that these results are the first in the region to combine water quality biomonitoring with biomarkers in macroinvertebrates. In addition, and as a novelty, we were able to determine enzymes related to oxidative stress in these bioindicator species. Finally, we highlight that, although it is necessary to further study these methodologies applied to new species, the integration of biomarkers with biomonitoring in invertebrates resulted in useful early warning tools to detect the degradation of aquatic ecosystems due to anthropogenic activity.

## 23B.O-Mo-5

### **Biomonitoring of the Water Quality of the Santana River (Rio Grande Basin) in the Areas of Influence of a Century-Old Hydroelectric Plant, in Different Climatic Seasons**

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#### **Abstract**

The water quality of rivers and tributaries is an important global issue, as it is vital for the survival and maintenance of various ecosystems and Public Health, especially in areas under pressure from the implementation of large infrastructure projects, such as hydroelectric plants, mining and industries. The toxicological evaluation of water is an important tool for determining this quality, in this context, biomonitoring is an efficient study method. The zebrafish is an animal model usually used in this type of test, due to its favorable characteristics for laboratory management. In this context, the study of the impact of the rupture of the dam in Brumadinho on the rivers is of extreme environmental and social importance, so the present work aims to evaluate the toxicity of three points (upstream, powerhouse and upstream of the Hydroelectric Generating Center) of the rio Santana (Rio Grande Basin) in dry and rainy seasons, through in vivo assays using zebrafish embryos. During the evaluations, the embryos were characterized as alive, dead, with or without teratogenic alterations, in addition to evaluating the heart rate (HR) for a period of 144 hours post fertilization (hpf). Bioassays with zebrafish revealed that there is an increase in embryonic mortality downstream of the CGH Santana ( $p < 0.05$ ), with a higher mortality rate being observed in the rainy season. In the other points there was no significant mortality ( $p > 0.05$ ) regardless of the weather season. Embryos exposed to samples collected in the dry season did not show significant teratogenic changes ( $p < 0.05$ ) when compared to the control group and other treatments. Embryos exposed to samples collected downstream and upstream of CGH Santana showed a high rate of teratogenicity ( $p < 0.05$ ) during the rainy season when compared to the control group. In the HR evaluation, no alterations were observed in the HR in the embryos exposed to the samples collected in the dry season ( $p > 0.05$ ). However, the HR of the embryos exposed to the samples collected in the rainy season showed a decrease in HR at all points, when compared to the control group ( $p < 0.05$ ). Low survival, teratogenic and CF alterations may be associated with increased water volume, mainly downstream of the CGH, during the rainy season, as there may be leaching of waste contained on the banks to the river bed, thus altering the quality from water.

Keywords: anthropization, embryotoxicity, aquatic toxicology, zebrafish



## 23B.O-Mo-6

### Current Scenario and Future Prospects of Effluent Toxicity Assessment for Environmental Regulation in Latin America

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#### Abstract

For more than 60 years, ecotoxicity testing has been used to assess potential ecological impacts, and particularly used for effluent quality assessment. Several developed countries have included ecotoxicity testing in their regulatory framework to complement physical and chemical analysis. In Latin America, only Brazil included those tests in their regulations for effluent discharge and for water quality characterization. To update environmental regulations, it is essential to promote a dialogue among government, academia and industry. SETAC LA, organized a four-day Workshop on Effluent Ecotoxicity in November of 2022 in Buenos Aires, Argentina, entitled "Setting the basis for future regulation in Latin America". Its main objective was to share different perspectives from the academy, industry, and government representing Latin American countries regarding the processes of updating current environmental regulations. Brazil was invited to share the experience, success and failures during the implementation of ecotoxicity testing in its environmental regulation. The idea behind was to contribute to the process of strengthening the environmental regulations of other countries from the region. The Workshop embraced a wide governmental, academics and industrial visions regarding effluent quality, effluent treatment technologies, advances in regulations and analytical capacities. Objectives of quality and environmental protection levels, selection of the most suitable tests were other aspects discussed. A discussion session using the "World Coffee" method was organized and important key messages were summarized as, I) the current environmental regulatory framework in several Latin-American countries require urgent improvement to guarantee environmental protection, especially regarding the control of industrial and municipal discharges. II) A political support is far the most relevant premise and a science-based decision-making process need to be encouraged in governmental sector, for what a better dialogue between research scientists and governmental agents needs to be implemented. III) The need for proper training in the procedures and concepts were identify among all sectors. And IV) the role and compromise of industrial sector demonstrated to be crucial. As a conclusion, further actions are needed to advance into a more compromised environmental regulation in Latin America to ensure wildlife and human health protection

# Session 24: Impacts of the Expansion of Anthropogenic Activities on Water Quality and Biota in the Amazon and Pantanal Biomes

## 24.O-We-1

### Arsenic Bioaccumulation and Speciation in Phyto- and Zooplankton of the Brazilian Pantanal

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#### Abstract

The Pantanal is the largest wetland in the world. In Nhecolandia subregion, thousands of lakes, ranging from freshwater to soda lakes (pH up to 11) occur in close proximity. Those lakes are a natural hotspot of arsenic concentrations up to 3,000  $\mu\text{g/L}$ . The biological community present in these lakes is believed to play an important role on the As availability for the water column. No data is available on the role of organisms at the base of the food web on As speciation in the Pantanal. The effects of different eutrophication status on the accumulation and speciation of As in these organisms are so far unknown. The objective of this work was to assess As accumulation and speciation in plankton collected in soda lakes under a salinity gradient and under contrasting environmental conditions. Sampling campaigns were conducted in five well-characterized lakes in different stages of eutrophication (eutrophic turbid (ET), oligotrophic turbid (OT) and clear vegetated oligotrophic lakes (CVO)) in 2017 (dry year) and 2018 (wet year). Total As analysis was carried out by hydride generation atomic fluorescence spectrometry coupled to an on-line UV reactor (UV-HG-AFS). Arsenic speciation was analysed by high-performance liquid chromatography (HPLC) coupled to UV-HG-AFS. A suspect screening of organoarsenicals in plankton samples was carried out by ultra high-performance liquid chromatography (UHPLC) coupled to high-resolution mass spectrometry (UHPLC-HRMS). The capacity of the resident planktonic community to bioaccumulate and biotransform As depended on lakes' hydrological/limnological conditions and season. The content of dissolved organic matter was an important limiting factor of As availability for the biological community by forming poorly labile As complexes. Similarly, salinity stress may reduce As uptake by plankton in these alkaline lakes. Arsenic speciation patterns showed the importance of Cyanobacteria in the As cycle, with more abundance of species in the more eutrophic lakes. UHPLC-HRMS revealed important organoarsenicals involved in detoxification processes in plankton. Therefore, limnological lake conditions, regulated by an evapoconcentration process ongoing in the Pantanal, controls As availability and uptake by the plankton community and then the plankton community composition controls the occurrence of organic species to the water column. No biomagnification was observed, although diet seemed to be a relevant way of As uptake by zooplankton.

## 24.O-We-2

### Environmental Risk Assessment and Human Exposure to Pesticides at the Amazonian Agricultural Frontier

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#### Abstract

The use of pesticides has become increasingly frequent in Brazilian agricultural production, a fact that has inserted the country in the first positions of the largest consumers of chemicals in the world. In addition to deforestation, IBGE data indicate an increase in the consumption of pesticides in these states. In the Amazon, the rate of pesticide consumption increased about 677% between the years 2000 and 2018. Given this, the main purpose of the study was to evaluate the environmental and human health risks caused by these products in the region. The study population was that of pesticide applicators, living in the municipalities of Apuí, Boca do Acre, Lábrea, Humaitá and Manicoré. A total of 89 interviews were conducted in 2020 and 2021. The Spearman Rho test and the Cochran test were performed to determine the correlation between health and environmental protection behavior. To calculate the occupational exposure of farmers, the algorithm of Dosemeci et al. (2002) and the OHRI Indicator were used. The environmental risk assessment was performed using the PRIMET version 3.0 model. We found 22 types of active ingredients, with the preponderant use of Glyphosate, Difenconazole, Thiamethoxam and Chlorpyrifos. Although 88% of respondents report that pesticides cause damage to health, most of them use PPE incomplete or do not use any form of protection, store pesticides improperly, dispose of packaging incorrectly and do not receive technical guidance. Most farmers showed risk behavior related to health. The behavior of farmers to health protection is related to income and label reading. The behavior related environmental protection, with income, gender and technical assistance. Males have higher cumulative exposures than females. Most of the pesticides used in the municipalities surveyed present risk scenarios in the environmental compartments evaluated, and half of them are not approved for use in the European Union because they are considered harmful to the environment and humans. It is essential to implement public policies aimed at raising awareness and educating producers, with programs that stimulate good agricultural practices to reduce exposure and more sustainable agricultural policies regarding the use of pesticides in the region.

## 24.O-We-3

### Metals in Fishes from an Historically Contaminated Amazonian Oil Field

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#### Abstract

More than fifty years of poor industry practices during the exploration and exploitation of petroleum in northeastern Peru has left a legacy of contamination in the headwaters of the Amazon River. This remote region is home to several indigenous ethnic groups which rely on the natural environment to provide them with food, fiber, fuel, and medicines. These populations live in fear that they are chronically exposed to hydrocarbons and heavy metals that are affecting their health. To address this concern, a landscape-level cross-sectional assessment of blood and urine concentrations of metals, and potentially associated factors, in the region's residents was conducted by Peru's national health institute. The study also included evaluation of metals in fish tissues consumed by the local indigenous inhabitants. Fish species representing four trophic levels were collected from rivers and lakes utilized for subsistence fishing. Metals associated with produced water and oil spills (arsenic, barium, cadmium, lead, and mercury) were analyzed in offal separately from the carcass. The carcasses of the majority of the fish had concentrations of arsenic, cadmium and lead at detection levels. Except for mercury, all metals were higher in offal compared with carcass and benthic species tended to have greater tissue concentrations of all metals than mid-water species. Spatial differences in fish tissue metal results were detected and, in some cases, could be associated with previously reported results of metals in people consuming those fish. This study has provided a unique opportunity to examine fish as a dietary exposure pathway connecting a human subsistence population to areas contaminated by petroleum exploitation.

## 24.O-We-4

### Arsenic in Areas Under the Influence of the Belo Monte Hydroelectric Power Plant, Xingu River, Amazon, Brazil

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#### Abstract

Arsenic (As) is a potentially toxic element (PTE) of great environmental concern because its inorganic forms can induce DNA mutations and impact animal metabolism. This metalloid can result from anthropogenic actions, such as the construction of hydroelectric dams. This study aimed to quantify the concentrations of As in water, sediment, and two fish species (*Cichla melaniae* and *Baryancistrus xanthellus*) of the Xingu River in regions of influence of the Belo Monte Hydroelectric Plant. Environmental samples (water and sediment) and tissues (gills, liver, and muscle) from the two species (15 animals per species from each point) were collected at eight strategic points of influence of the dam. Collections were conducted in the Amazon drought (October 2020), under permits: SISBIO 71763-1 and Ethics Committee on Animal Use, CEUA 8166251119. The quantification of As was done by inductively coupled plasma mass spectrometry. In water, As was higher in the area of the river diverted for the construction of the hydroelectric power plant and with a history of gold mining (Volta Grande do Xingu, VG); however, the values found were below the maximum limit established by Brazilian legislation (up to 0.14  $\mu\text{g}\cdot\text{L}^{-1}$ ). In sediment, even below the concentrations allowed in Brazil (15  $\text{mg}\cdot\text{Kg}^{-1}$ ), As was higher in the reservoir. *Cichla melaniae* presented the highest concentration of this PTE; in both fishes, the gills were the organ with the highest amount of As. As had a higher concentration in the tissues of both species in the reservoir, and only in the gills of *C. melaniae* this metalloid was more incident in the VG. There was no correlation between growth and accumulation of As in *C. melaniae*; in *B. xanthellus*, however, this correlation was negative. The values of As in the tissues were below the limit allowed in Brazil (up to 1  $\text{mg}\cdot\text{Kg}^{-1}$ ). The mining history and the natural presence of this PTE in the Amazon may influence the concentrations found in this study. The results raise the importance of monitoring the affected area and questioning the continuity of hydroelectric projects in the Amazon, which destroy the biodiversity of this biome.

## 24.O-We-5

### Copper Deficiency in Areas Under the Influence of the Belo Monte Hydroelectric Plant, Xingu River, Amazon

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#### Abstract

Copper is an essential element for the organic composition of organisms and is required to perform vital cell functions. Copper deficiency can cause changes in oxidative metabolism, decrease the immune response, and cause problems in the reproductive process. Therefore, this study aimed to evaluate the copper concentration in tissues of *Baryancistrus xanthellus* in areas under the influence of the Belo Monte Hydroelectric Plant (UHEBM). Biological samples (gills, liver, and muscle) were collected at four points in the river from 15 animals from each site in October 2020 (Amazon drought), with SISBIO 71763-1 and Animal Use Ethics Committee 8166251119 permits. In the laboratory, copper concentrations were analyzed by inductively coupled plasma mass spectrometry. Comparison of this metal between tissues was made by the Kruskal-Wallis test, followed by Dunn's post-hoc, Bonferroni-adjusted test. A Two-Way ANOVA was used to test the difference in copper in the tissues between the collection points, followed by the marginal means analyses, with Bonferroni adjustment. Among the organs analyzed, the liver had the highest amount of copper, which may be related to its storage and detoxification function of that metal. The gills and muscle showed no significant difference between them; the low concentration of copper may be related to its absence of bioaccumulation in organisms and its chemical composition in the environment. Regarding the sampling points, the gills showed higher amounts of copper in the reservoir area, the liver in the stretch of the river that was diverted for the construction of UHEBM and the muscle in the site used as a reference for the study. The results of copper found in the tissues are in concentrations below the maximum limits of tolerance of fish (1500 to 5000  $\mu\text{g}\cdot\text{kg}^{-1}$ ), which may be related to the changes caused in the habitat of these organisms in recent decades. This scenario leads to problematic reproduction and survival of the species since copper deficiency has implications for cellular metabolism and reproductive disorders. Given this, further investigations are needed to understand the effects of copper on fish in impacted areas, and it is also necessary to verify the possible impacts that the construction and permanence of UHEBM caused and might causing to the environment and its biodiversity.

## 24.O-We-6

### PFAS Contamination in Water, Macrophytes and Fish from the Amazon Region

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#### Abstract

Perfluorinated alkyl substances (PFASs) are receiving increasing worldwide attention due to their persistence, toxicity, and widespread occurrence. This study provides the first large-scale assessment of the occurrence and distribution of these contaminants in the Amazon River, the largest drainage basin in the world. A monitoring campaign covering more than 1500 km of the Brazilian Amazon River was carried out in November-December 2019. Thirty-nine water surface samples were taken in urban streams (Manaus, Santarem, Macapá, Belém), the Amazon River and three major tributaries. In nearby areas, 17 aquatic macrophyte samples were collected and analysed separately considering aerial (leaves) and submerged (stems and roots) parts. Likewise, 53 fish samples were acquired at local markets in the sampled areas. These belonged to 14 different species including carnivorous, omnivorous, herbivorous and detritivores fish. Identification of 27 individual PFAS including carboxylates, sulfonates, phosphonic, propanoic and ethanoic acids, fluorotelomers and phosphates, and also Gen X was performed by HPLC-QqQ. In the water, 12 compounds were identified for the first time in Amazon River, being PFPeA, PFOS and PFHxS the most abundant ones, and being all short chained. *P. rotundifolia* bioaccumulated significantly more PFAS than the other two species (*Eichhornia crassipes*, *Eichhornia azurea*). Short-chain PFCAs were significantly more bioaccumulated on aerial parts of this species. Significant differences were also found for different PFASs families and chain lengths between plant parts. As for fish, PFOS and PFOA concentrations were lower than those found in Europe and Asia, although the concentration of compounds such as PFHxA, PFBA, PFHPA, FPNA y PFHxS were higher than expected. This study shows that PFAS are widespread contaminants in the region, with urban areas being a major discharge point into the Amazon river network.

# Session 25: Toxic Cyanobacterial Blooms: Causes of a Growing Problem

## 25A.O-Mo-1

### Monitoring Cyanobacterial Harmful Algal Blooms by Drone-Base Remote Sensing in Shallow Water Reservoirs

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#### Abstract

The spatial distribution of Cyanobacterial Harmful Algal Blooms (CyanoHABs) may change rapidly due to variations in wind, water currents, and population dynamics. This means that water toxicity can vary from hour-to-hour and day-to-day and it can remain toxic even after a bloom has disappeared. Traditional sampling methods may not be enough for an effective local risk assessment. Thus, water sources should be monitored frequently for outbreaks and establish assessment programs.

Satellite-based remote sensing is a broadly used method for water quality assessment and CyanoHABs monitoring because of its specialized sensors and availability of historical data of the water body. However, the spatial and time resolution may not be enough in case of outbreaks in small water sources, for example, on-farm reservoirs. On the other hand, Unmanned Aerial Vehicles (UAV) emerge as a cost-effective alternative with high spatial and temporal resolution. In this session, a comparison between satellite and UAV remote sensing approaches for monitoring CyanoHABs in a small and shallow water reservoir is presented.

Unmanned aerial vehicles rise as a reliable complement to satellites thanks to their superior temporal and spatial resolution. Unmanned aerial vehicles can gather data at appropriate spatial and temporal resolutions, with full control over data collection timing and rapid result availability. We show that data can be correlated with chlorophyll ( $r^2 = 0.79$ ) and cyanobacteria ( $r^2 = 0.77$ ) concentration at the water surface, leading to spatial distribution maps capable of spotting cyanobacteria patches in water bodies precisely.

In addition, climatic change may benefit various species of harmful cyanobacteria by increasing their geographic distributions and modifying population dynamics. Thus, new methods with a high spatial and temporal resolution for monitoring blooms come in handy under changing climate scenarios.



## 25A.O-Mo-2

### Toxic Cyanobacteria Blooms: Alert Systems and Health Risks in Uruguay

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#### Abstract

Toxic cyanobacterial blooms are a worldwide problem. Contact with cyanotoxins during recreation affect health causing acute and chronic illness. Monitoring programs and alert systems based on cyanotoxins guidance values (GV) are used to establish risk levels and protect public health. However, GV are applied without analyzing population heterogeneity neither ecosystems and catchments characteristics, nor considering local perceptions and historical context. Here, we carried out a critical review on the generation and application of these GV in Uruguay. We conducted a literature review and analyzed a large cyanotoxins data set from riverine and estuarine beaches of Uruguay river (UR) and Río de la Plata estuary (RP). A 5 % of the monitoring instances in the UR showed microcystin concentrations higher than the GV defined by OMS (24  $\mu\text{g/L}$ ), while this increased to 76 % in the Montevideo coast. Further, local populations characteristics (age, body weight) were used to estimate the microcystin dose per person. With cyanobacterial foam, children specially with low weight (first percentil) had doses (58 to 64  $\mu\text{g}$  microcystin/kg body) comparable with the LD 50 for experimental animals. These results are discussed within the framework of increasing toxic blooms due to global changes in land use and climate.

## 25A.O-Mo-3

### **Aluminum Compounds as a Management Tool for Eutrophic Lakes: State of the Art and Potential for Application in Subtropical Urban Lakes.**

Guillermo Goyenola<sup>1</sup>, Virginia Fleitas<sup>1</sup>, Lucía Gaucher<sup>1</sup>, Giannina Pinotti<sup>1</sup>, Claudia Fosalba<sup>1</sup>, Sofia Rehermann<sup>1</sup>, Mariana Meerhoff<sup>1</sup>, Lucia Boccardi<sup>2</sup>, Yemira Zabala<sup>2</sup>, Gustavo Saona<sup>3</sup>, Leticia Iribarne<sup>3</sup>, Milagros Valverde<sup>3</sup>, Valentina Amaral<sup>4</sup>, Maite Burwood<sup>1</sup>

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#### **Abstract**

As a result of various significant anthropogenic pressures, urban lakes typically experience a rapid increase in their internal nutrient load and therefore, reducing the external load is often not enough to improve their environmental quality in a significant manner.

Different interventions are often used to modify the internal nutrient load, reducing productivity and, therefore, the magnitude and frequency of occurrence of eutrophication symptoms.

This study provides an intensive review of the applications of aluminum compounds for the rehabilitation of eutrophic lakes worldwide, complemented with an experimental approach considering six urban lakes from Uruguay.

The results indicate that aluminum compounds have proven effective in reducing the internal phosphorus load and preventing the occurrence of cyanobacterial blooms. Different chemical forms had been used globally, but based on our review, Polyhydroxy Aluminum Chloride (PAC) seems to present several comparative advantages, such as greater buffering capacity and flocculation efficiency, as well as avoiding the addition of sulfates and interference in sulfur cycles. Dosing method, temporality, influence of meteorological conditions, efficiency range and use of adjuvants to improve the efficiency of treatments, were also reviewed.

The highest doses used for experiments reached 8 mg/L of total Al applied as PAC. As expected, the reduction in total phosphorus did not show a proportional relationship with the Al dose applied, and the treatment efficiency varied between lakes. Al concentrations resulting from the most severe tested applications remained mostly below the strict ecotoxicological limits established by EPA (also presented at SETAC 2023 by Fleitas et al.). Bioassays did not show detectable effects (also presented at SETAC 2023 by Saona et al.).

Our results contribute to planning environmentally safe applications of aluminum in eutrophic lakes and to ruling out inconvenient projects in advance. As with any other chemical treatments, Aluminum treatments must be lake-specific, and the results of the characterization of each lake and their potential ecological response to an Al application must be taken strictly into account. We have also identified aspects that require further research (particularly ballast).

## 25A.O-Mo-4

# Is Polyhydroxy Aluminum Chloride Toxicologically Safe for Managing Internal Phosphorus Load in Urban Lakes in Uruguay? Application of 2018 Aluminum Criteria from the US Environmental Protection Agency

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### Abstract

Managing the internal phosphorus load in urban lakes is a challenging task. Aluminum compounds are commonly used, but ecotoxicological risks must be considered.

A collaboration between Universidad de la República, Intendencia de Montevideo and Intendencia de Canelones (Uruguay) explored the use of Polyhydroxy Aluminum Chloride (PAC) to control the phosphorus load in urban lakes (also presented at SETAC 2023 by Goyenola *et al.*). The criteria for aluminum in freshwater published by the U.S. Environmental Protection Agency (EPA) in 2018 was used to guide the experimental approach conducted with water from six urban lakes.

At the laboratory, 8 mg Al/L were applied to a sample of each lake, while lake water without treatment was included as a control. Each set comprised three replicates. After a week of controlled temperature conditions, the supernatants were characterized.

Acute and chronic aluminum exposure limits are suggested by EPA based on pH, dissolved organic carbon (DOC) and total hardness. The recommended acute exposure criterion (criteria maximum concentration, CMC) is associated with average exposures over one-hour periods, while the criterion for chronic exposures (criteria chronic concentration, CCC) is based on average exposures over four days. This criterion is highly restrictive and is based on a wide sample of the aquatic community.

At the end of the experiment, the pH ranged from 7.36 to 9.39, the DOC from 6.48 to 18.52 mg/L and the total hardness from 85 to 230 mg CaCO<sub>3</sub>/L. For these values, EPA establishes a limit of 2800 µg Al/L of CMC and a range from 780 to 1700 µg Al/L of CCC. The majority of the samples treated with PAC were below the lower limit, being the highest 570 µg Al/L.

The results conclude that the use of EPA 2018 criteria can contribute to establish objective criteria to define safe applicability of PAC in urban lakes. To scale up this approximation to lakes, more knowledge about water quality variability must be generated. Automatic monitoring of potentially highly variable parameters (such as pH) should be implemented. It is also important to note that most of the model species used by EPA are not present in Uruguayan ecosystems. To complement this approach, bioassays with native species *C. decemmaculatus* (Poeciliidae) and *D. magna* (Crustacea) were conducted (also presented at SETAC 2023 by Saona *et al.*).

## 25A.O-Mo-5

### First Report of Anatoxin and Saxitoxin in Negro River, South America, Uruguay

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#### Abstract

Cyanobacterial blooms are observed in several Uruguayan aquatic ecosystems. Particularly, in Negro river over 70 species of cyanobacteria were registered, many of which can produce cyanotoxins such as microcystins (MCs), anatoxin-a (ATX-a) and saxitoxin (STX). MCs and STX were previously reported in Uruguayan ecosystems, however, are not regularly tested. Biological monitoring in Negro river was carried out between October 2020 and March 2023 framed in the baseline studies of a new pulp mill located in the middle section of the river. This is the first time that three cyanotoxins were tested using ELISA assays in freshwater samples during routine studies. Surface water samples were collected in the middle section of Negro river, between Bonete and Baygorria reservoirs during 22 sampling campaigns in 14 sites distributed along the study area. Samples were tested for MCs, STX and ATX-a, and the cyanobacterial community was studied. MCs was the most quantified cyanotoxin (105 positive results from a total of 273 analyses), followed by ATX-a (79 out of 260) and STX (3 out of 267). The maximum MCs (304  $\mu\text{g/l}$ ) was detected in March 2022 in Bonete reservoir under the presence of  $4.5 \times 10^5$  cel/ml of *Microcystis aeruginosa*. The detected ATX-a was associated with various cyanobacterias, including *Dolichospermum uruguayense*, *D. viguieri*, *D. circinale*, *D. crassum*, *D. planctonicum*, *Planktolyngbya limnetica* and *Gleitlerinema* sp. The maximum ATX-a concentration registered was 4.2  $\mu\text{g/L}$ , detected in March 2021 also in Bonete reservoir associated with  $3.8 \times 10^3$  cel/ml of *D. circinale*, *D. crassum* and *D. uruguayense*. Our study highlights the importance of including ATX-a in regular analyses of cyanobacterial blooms in Uruguayan ecosystems. This is particularly important considering the expansion of distribution of many cyanobacterial taxa which could potentially produce this cyanotoxin, especially where global trends show increased eutrophication and rising temperatures.

## 25A.O-Mo-6

### Population Specific Responses to Toxic Cyanobacteria in Benthic Amphipods

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#### Abstract

The Baltic Sea has a latitudinal gradient in bloom composition and magnitude, which vary from north to south, with cyanobacterial blooms being more common in the south. In this study, populations of the deposit feeder amphipod *Monoporeia affinis* originating from either the northern Baltic Sea (Bothnian Sea: BoS) or southern (Baltic Proper :BP) basins were exposed to feeding regimes representing seasonal phytoplankton blooms using a common garden experimental design. We hypothesized that growth responses of the amphipods differ between the basins, with animals from BoS being specialists relying on the diatom bloom, and those from BP being generalists with a more heterogeneous diet. Consequently, when exposed to a diet containing toxin-producing cyanobacteria, the animals from the BoS were expected to decrease their feeding, growth and body condition; moreover, they were expected to develop toxic responses due to their lower capacity to cope with cyanotoxins. By contrast, the amphipods from BP were expected to be more equipped to handle cyanobacteria-rich diet and benefit from addition of cyanobacteria. Stable isotopes were used to measure incorporation of C and N of diatoms and cyanobacteria in amphipods, physiological and toxic endpoints to measure diet effects. Amphipods from both populations incorporated nutrients of diatoms and cyanobacteria in similar quantities. Contrary to our expectations, both populations maintained or improved growth and body condition in treatments where diet was dominated by cyanobacteria. However, the degree of neurotoxicity was highest in the BoS population exposed to the treatment with highest cyanobacteria addition. These results suggest that cyanobacteria are important as supplemental food source despite its toxicity and, hence, the increase in cyanobacterial blooms observed in the BoS during the last decades might have beneficial effects on the amphipods in this basin, although negative effects on higher trophic levels due to the increased cyanotoxin transfer cannot be ruled out.

## 25B.O-Mo-1

### Primary Screening of Cyanobacteria Biomass From a Tropical Reservoir: Biological Activity and Metabolomics Analyses of Crude Extracts

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#### Abstract

Cyanobacteria harmful algal bloom events (CyanoHABs) can produce natural toxins that risk ecosystems and human health worldwide. Nevertheless, the potential risk of many cyanobacterial metabolites remains unknown. To date, only the microcystins class has been studied intensively to produce a magnitude of evidence on toxicity and exposure concentrations, leading to their inclusion in risk management and water quality regulations. However, cyanobacteria produce an incredible diversity of cyanopeptides beyond the class of microcystins that have received little or no attention regarding their potential human and ecological risk. Information about exposure concentrations in surface waters, co-occurrence and synergy, and toxic effects of cyanopeptides are still poorly explored. The present study aims to identify the metabolic profile of environmental cyanobacterial biomass collected in a Brazilian eutrophic reservoir through LC-MS-based metabolomics and to correlate the presence of different cyanopeptides with the acute toxicity - of extracts and fractions - observed against the microcrustacean *A. salina* using the web-based platforms NP Analyst and Data Fusion-based Discovery (DAFdiscovery). The metabolomics revealed the presence of four different cyanopeptides classes: microcystins, microgynins, aeruginosins, and cyanopeptolines. The bioinformatics tools show that not only microcystins are responsible for the toxicity observed. Compounds of cyanopeptolines class showed high bioactivity correlation scores in both tools, sometimes even higher than those attributed to microcystins. The presented results shed light on the urgent need to evaluate further the (eco)toxicological risks of the different classes of cyanopeptides found in environmental blooms, considering their potential for exposure and synergistic action between them. The present work also shows the possibility of doing a risk assessment using a less time-and-reagent-consuming approach through LC-MS/MS-based metabolomics and bioinformatics tools.

## 25B.O-Mo-2

### **Blooms of Potentially Toxic Cyanobacteria in Laguna del Sauce (Uruguay): Drivers and Impacts**

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#### **Abstract**

Laguna del Sauce (34° 43' S, 55° 13' W) is an eutrophic shallow lake and Uruguay's second drinking water source. This ecosystem suffers recurrent blooms of cyanobacteria which are potentially toxic, mostly during summertime. The phytoplankton of this ecosystem has been monitored since 2015, as part of a vigilance monitoring program. In this study, we analyze the occurrence, persistence, and toxicity of cyanobacteria blooms in Laguna del Sauce from 2015 to 2023. During this period seven N-fixing cyanobacteria blooms (>10 mm<sup>3</sup>.l<sup>-1</sup>) were registered in the Laguna del Sauce subsystem, composed either by Dolichospermum, Raphidiopsis, Cuspidothrix, and Aphanizomenon. The blooms with the highest persistence were the ones of *R. raciborskii*, which lasted 3 to 4 months each and started at the onset of autumn. All other N-fixing cyanobacteria blooms occurred in summer and lasted for up to 1 month. In this period, two non-N-fixing cyanobacteria blooms also happened (*Microcystis aeruginosa* complex). *Raphidiopsis raciborskii* blooms occurred in a particular combination of environmental conditions: dry periods with clear water conditions (low turbidity and watercolor), a combination only observed 3 times in the historical record (2003-2023) of the drinking supply company. Other Cyanobacteria blooms developed in non-extreme situations of water level and transparency (turbidity and color), *Dolichospermum* and *Microcystis* occurred in similar environmental conditions and/or together. Knowing the key climatic and limnological drivers of the Cyanobacteria blooms allow for anticipating and adapting the water treatment facilities to decrease the potential risks due to toxins in water detected: saxitoxin (*Raphidiopsis* blooms) and microcystin (*Dolichospermum* and *Microcystis* blooms).

## 25B.O-Mo-3

### A Fast Method to Detect Toxic *Microcystis* in Water Samples by Loop-Mediated Isothermal Amplification (LAMP)

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#### Abstract

The presence of high *Microcystis* biomass in aquatic ecosystems causes severe intoxication problems in humans and animal death. Current strategies to detect *Microcystis* spp. lack the sensitivity needed to provide early warnings, as they are based on chlorophyll concentration, cell abundance, or time-consuming analytical methods to determine toxicity. Molecular biology-based methods allow early detection and quantification of target organisms or genes, being extremely specific, sensitive, and feasible to implement in real-time. Among these, loop-mediated isothermal amplification (LAMP) stands out as a simple and powerful tool of exceptional specificity in the case of sequence-specific amplification. Moreover, LAMP-based techniques are suitable for on-site implementation, since they do not require specific or expensive equipment. In this work, we developed a colorimetric LAMP method for the *in situ* detection of toxic *Microcystis* spp. using the *mcy* genes (involved in microcystin synthesis) as targets. The method was implemented using DNA obtained from environmental samples having different abundances of toxic *Microcystis* and from an axenic culture (NIES-843 strain) at different concentrations. Amplification by quantitative real-time PCR was used as gold standard for validation and to establish the detection limit of the developed method. The LAMP primers showing the highest specificity produced positive results in less than one hour and at very low concentrations of *Microcystis* cells ( $\leq 100$ /mL).



## 25B.O-Mo-4

### Aquatic Ecotoxicity Test Of Three Chemical Agents Used To Reduce Cyanobacterial Biomass In Eutrophic Lentic Systems

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#### Abstract

Globally, various strategies are used to reduce cyanobacterial blooms and/or internal loads in freshwater ecosystems. In this study, we evaluated the effectiveness of different treatment agents to reduce cyanobacterial abundances in lentic systems. In the first stage, we assessed the efficiency of the different methodologies, such as coagulation-flocculation of cyanobacteria, oxidation of organic matter, and phosphate adsorption, at a laboratory-scale. For the ex-situ experiments, we selected a hypereutrophic urban lake located in Paso Carrasco, Canelones, Uruguay, which has experienced blooms of different cyanobacterial species including *Planktothrix cf. suspensa*, *Raphidiopsis raciborskii*, and *Raphidiopsis mediterranea*. Based on the results obtained, we identified the treatment agents (and their corresponding doses) that showed the highest efficiency in terms of cyanobacteria removal and phosphate adsorption. Subsequently, in order to evaluate the possible ecotoxicological effects of the selected compounds, acute toxicity bioassays were conducted using the cladoceran *Daphnia magna* and the fish species *Pimephales promelas*. In this phase, lake water samples were exposed to the selected treatments under controlled conditions, and after 24 hours of exposure, bioassays were carried out with the mentioned species. The *Daphnia magna* bioassays were performed using the Daphtoxkit F™ kit, and inhibition of organism motility was evaluated at 24 and 48 hours. The potential toxicity of hydrogen peroxide (2 mg.L<sup>-1</sup>), Polyhydroxy Aluminum Chloride (PAC; Greenpac 3940L; 6 mg Al.L<sup>-1</sup>), lanthanum-modified bentonite (Phoslock®, 400 mg.L<sup>-1</sup>) and combination of treatments such as PAC with Phoslock®, and hydrogen peroxide with PAC and Phoslock®, were tested. In addition, potassium dichromate (a standard toxicant) was tested at doses ranging from 0.32 to 3.2 mg.L<sup>-1</sup>. The fish bioassays were conducted only with the combined treatments, and the survival of the organisms was evaluated. Each set of bioassays was comprised of three replicates, while lake water without treatment was included as a control. The results of the bioassays for both species did not indicate toxic effects for the doses tested, which allow us to proceed to the next phase, where the effects of the different treatments at the ecosystem level will be determined through controlled experiments in the lake.

## 25B.O-Mo-5

### DNA-Based Strategies to Identify Cyanobacteria in a Reservoir of the Río Negro in Uruguay- Rincón Del Bonete

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#### Abstract

Cyanobacterial blooms have serious impacts on ecosystems, public health, and productive activities. Both domestic and wild animals ingest water from reservoirs, usually with high concentrations of cyanotoxins, which has led to cases of poisoning and mortality reported throughout the world. To date, a significant challenge in the effective management of cyanobacterial blooms has been to detect and differentiate toxic from nontoxic blooms, also using molecular techniques. The objective of this study was to identify potentially toxic species in the reservoir using a broad spectrum of both classic and DNA-based techniques to deeply analyze cyanobacteria communities of soil and sediment near different uses of the land (forestry, agriculture, and livestock production), and a native forest. Using qPCR, cyanobacterial DNA was detected in most of the samples; however, toxin genes were only detected near forestry, livestock and near the native forest. Compared to classical light microscopy approach, more genera and species were identified in sediment through 16S rRNA gene sequencing. In addition, a potent neurotoxic, anatoxin-a, was detected in water samples in all sites using ELISA technique. DNA-based techniques resulted effective for the detection and identification of cyanobacteria DNA in the sediment as a complement of light microscopy to monitor or anticipate cyanobacterial blooms.

## 25B.O-Mo-6

### Challenges of the First Two Consecutive Years of Continuous Monitoring with Multiparameter Sondes in Negro River

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#### Abstract

The Negro river is characterised by having good to regular water quality. However, it presents a mesotrophic/eutrophic trophic state due to its high nutrient content. During the summer, associated with higher temperatures, frequent cyanobacterial blooms were observed. In November 2020, three HYDROLAB HL7 multiparameter sondes were installed in the middle section of the Negro river, between Bonete and Baygorria dams, for baseline studies of a new pulp mill. The multiparameter sonde has seven sensors that measure temperature, conductivity, pH, dissolved oxygen, turbidity, chlorophyll-a and phycocyanin. A standardised protocol for sondes operations was established based on USGS guidelines, to obtain the most accurate and complete possible record. The protocol included maintenance of monitoring stations and equipment, periodic verification of sensor calibration, and steps for data corrections (i.e. the maintenance frequency was adjusted to a monthly frequency to minimise errors due to the rate of fouling of the sensors). Applying data corrections allows to adjust the recorded data that was inaccurate as a result of environmental or instrumentation effects, such as instrument calibration drift or fouling errors. Data correction is necessary when the combined absolute values of calibration drift and fouling error exceed the water quality criteria. The water quality parameters are sampled every 30 minutes, transmitted to a control centre in LATU and submitted to the Environment Authority in real-time. Considering each analysed parameter, the recovery of valid data ranged between 92.7% for turbidity and 97.7% for dissolved oxygen. The trends observed in the data acquired and validated for the present study were also reflected in the results obtained during the discrete monitoring and satellite images. The execution of discrete and continuous monitoring together allows an evaluation of the environmental conditions of the system at different scales since continuous monitoring detects rapid changes in the river conditions with automatic detection of potential conditions for the formation of phytoplankton blooms, while discrete monitoring integrates more variables which generates complementary information about the state and spatiotemporal dynamics of the water quality conditions of the Negro river.

# Session 26: Cutting-Edge Monitoring Technologies for Health Protection

## 26A.O-Mo-1

### Antioxidant Effects of the Phytocannabinoid Cannabidiol (CBD) in the Brain of Chlorpyrifos-exposed Goldfish (*Carassius auratus*)

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#### Abstract

Pharmacological and therapeutic properties of phytocannabinoids present in *Cannabis sativa* L. (Linnaeus) are of increasing interest worldwide. In the present study, a group of goldfish (*Carassius auratus*) were used to test the antioxidant effects of cannabidiol (CBD) in the brain of *in vivo* chlorpyrifos (CPF)-exposed fish. CPF, an organophosphate insecticide, is known for its pro-oxidant effects and irreversible inhibition on cholinesterase (ChE) enzymes. The experimental fish (n=22) (22.86 ± 1.15 g body weight, 8.33 ± 0.16 cm total length) were randomly distributed in 4 different treatments: A. Control fish (n=6), B. CPF-exposed fish (n=5), C. CPF-exposed + IP injected vehicle (n=5), and D. CPF-exposed + IP-injected CBD (5 mg/Kg) (n=6). Fish were humanely sacrificed after 96 h of the experimental time and brains were processed to measure F2-isoprostanes (F2-IsoPs) as a biomarker of oxidative stress response (ELISA OxiSelect™ 8-iso-PGF2a). ChE activity was measured in fish blood plasma as a biomarker of CPF exposure. Mean values of F2-IsoPs and ChE were used as statistics for comparison among treatments. Neither of the fish treatments showed signs of acute poisoning after the CPF exposure. However, plasma ChE activity showed inhibition effects in the corresponding groups after the 96-h exposure to CPF. The lowest F2-IsoPs value (mean ± SE, pg/ml) was found in the CPF-exposed + CBD-injected fish (281 ± 45) followed by the controls (396 ± 90). The two highest values of F2-isoprostanes were present in the CPF-exposed (728 ± 236) and the CPF-exposed + vehicle-injected fish (566 ± 90) (ANOVA, 95%, p < 0.05; Fisher least significant difference). Three types of functional groups within the CBD structure are considered a key factor to explain the prevention of oxidative stress: limonene, phenol and aliphatic group. These groups, particularly the phenol, transfer electrons and hydrogen atoms to avoid the lipid peroxidation in the tissues affected by pro-oxidant agents. Results in the present work showed that CBD protected of the oxidative stress caused by CPF in the brain of goldfish. This neuroprotective action of CBD against oxidative stress promotes further research to explore pharmacological and therapeutic applications.

## 26A.O-Mo-2

# Mercury and Selenium Levels in a Small-Scale Gold Mining Community in Colombia: The Importance of Se:Hg Molar Ratio as a Biomarker for Exposure and Risk Assessment

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### Abstract

Mercury (Hg) is an extremely toxic metal that is widespread in the environment and highly bioaccumulative, mainly as methylmercury (MeHg). MeHg is a neurotoxic compound that can lead to irreversible neurological disorders and other adverse health effects. Selenium (Se) may bind to Hg through the selenol groups, and its antioxidative properties can help eliminate reactive oxygen species induced by Hg. The Se:Hg molar ratio in blood could be a good biomarker of exposure because Se has a protective effect against Hg toxicity by inhibiting oxidative damage caused by Hg. San Martin de Loba town in Colombia is a small-scale gold mining area that was selected for this study. Total mercury (T-Hg) and selenium (Se) in blood samples were determined for 154 volunteers, including 85 men and 69 women, aged 1-87, who were enrolled in April 2022. Samples were analyzed using a cold vapor atomic absorption spectrometer for T-Hg and a graphite furnace atomic absorption spectrometer for Se. This mining community had different exposure pathways to mercury, including the consumption of fish as the main route to MeHg exposure for the entire population, while elemental mercury used in mining activities is another important route for miners. For all participants, concentrations, on average (mean  $\pm$  SD), were  $12.5 \pm 14.1$   $\mu\text{g/L}$ , a median of  $8.8$   $\mu\text{g/L}$ , and ranged from  $0.01$  to  $84.5$   $\mu\text{g/L}$  for T-Hg, while for Se, the concentrations were  $239 \pm 75.3$   $\mu\text{g/L}$ , a median of  $227$   $\mu\text{g/L}$ , and ranged from  $72.7$  to  $528$   $\mu\text{g/L}$ . The Se:Hg molar ratio was considered as a biomarker of risk assessment, suggesting that a Se: Hg molar ratio above 1 is essential for protection against Hg adverse health effects. In this study, the Se: Hg molar ratios in human blood were, on average,  $1926 \pm 4752$  and ranged from 10 to 30444. All samples had a Se: Hg molar ratio above 10, indicating less MeHg neurotoxicity. Selenium may form a strong complex with MeHg than cysteine, making MeHg less available and less likely to cross the blood-brain barriers. The Se: Hg molar ratio as a function of Hg molar concentration ( $\mu\text{mol/L}$ ) in human blood was positively correlated ( $R^2 = 0.88$ ,  $p < 0.01$ ). In conclusion, our results showed that the Se: Hg molar ratio is an important biomarker for Se:Hg interaction, and Se could attenuate the adverse effects of Hg exposure.

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## 26A.O-Mo-3

# Biomonitoring of Glyphosate and AMPA Exposure in an Agricultural Population From the Province of Córdoba for Risk Assessment via Estimated Daily Intake Calculation

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## Abstract

The widespread use of the herbicide glyphosate (GLY) has raised concerns about the possible adverse effects on human health. Gaps in conclusions among international agencies show up the need for new studies conducted to provide relevant information on exposed populations. Biomonitoring is an important tool for the assessment of the internal dose of exposure to pesticides. It involves the determination of pesticides and/or their metabolites in human matrices and is essential to perform risk assessment in different scenarios of exposure.

The actual toxicokinetic knowledge refers that most of the absorbed GLY is eliminated mainly as the parent compound in urine. Therefore, biomonitoring is focused on the determination in urine of GLY, and to a lesser extent on its metabolite aminomethylphosphonic acid (AMPA) to perform risk assessment and to evaluate estimated daily intakes (EDI). The obtained EDI will be finally compared with the acceptable daily intake (ADI) established by regulatory agencies.

The aim of the study was to perform a risk assessment of the exposure to GLY by using the concentration of GLY and AMPA in the urine of different populations of one of the most important agricultural areas of the province of Córdoba (Argentina) to calculate the EDI and contribute to the risk management.

The exposure to GLY was assessed in two populations with different scenarios of exposure: a- terrestrial applicators of pesticides (subjects occupationally exposed to pesticides), and b- a control group (subjects with environmental exposure to pesticides). The sum of the urinary concentration of GLY and AMPA, the urinary volume excreted per person per day of 1.5 L, the recent knowledge of the human urinary excretion rate (UER) of 1 % of GLY, and the individual body weight were used to calculate the EDI. Then, the results of the EDI were compared with the ADI established by several regulatory agencies.

The general media and median values of calculated EDI for both groups of subjects were below the 1 % of the lowest ADI (0.5 mg/kg BW /day) established by the regulatory agency European Food Safety Authority. No statistical differences in EDI median values were found between the two groups of subjects.

According to the obtained results, the internal dose of exposure to GLY and AMPA does not represent a risk to the health of the population under study.

DRAFT

## 26A.O-Mo-4

### SARS-CoV-2 Surveillance-Based on Municipal Solid Waste Fresh Truck Leachate

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#### Abstract

Fresh truck leachate is the liquid produced in the basin of municipal solid waste (MSW) collection trucks. MSW can be a source of microorganisms due to remains of bandages, disposable diapers, toilet paper, secretions, etc. Solid waste leachate-based epidemiology (SWLBE) is proposed as a cutting-edge monitoring tool for viral epidemiological tracking, applying the monitoring of fresh leachate from collection trucks as an environmental matrix for early warning of health protection. This study aimed to investigate the potential of SARS-CoV-2 surveillance-based on solid waste fresh truck leachate. Twenty truck leachate samples were collected from the truck basin from November 2020 to January 2021 at a Waste Transfer Station (WTS) located in Rio de Janeiro, Brazil. Samples were ultracentrifuged and nucleic acid extracted with QIAamp Fast DNA Stool Mini kit<sup>®</sup>. Taqman<sup>®</sup> Real-Time RT-qPCR was applied for gene quantification of SARS-CoV-2 N1/N2 with specific primers and probes. SARS-CoV-2 viral isolation, inference of variants of concern (VOC), and whole genome sequencing were also performed. SARS-CoV-2 was detected in 40% (8/20) of fresh truck leachate samples, with concentration varying from 2.89 to 6.96 Log<sub>10</sub> RNA 100 mL<sup>-1</sup>. Attempts to isolate SARS-CoV-2 and recover the whole genome were unsuccessful, however, positive samples were characterized as possible pre-VOC lineages, VOC Alpha (B.1.1.7) and a variant of interest (VOI) Zeta (P.2), compatible with the molecular epidemiological scenario. This approach proved to be a cutting-edge monitoring tool to infer the occurrence of SARS-CoV-2 in the environment, especially in places that do not have a sewage collection network, but have implemented a MSW collection system. This innovative proposal can help in surveillance epidemiology in municipalities and public health protection decision-making.

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## 26A.O-Mo-5

### Assessing Mercury Concentrations in the Urban Atmosphere in Quibdó City: Implications for Human Health

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#### Abstract

The objective of this study was to evaluate atmospheric mercury concentrations in the urban area in Quibdó City, Colombia, because high toxicity of mercury as a heavy metal contaminant for human health. This assessment focused on artisanal gold miners who sell their gold to shops located in the city center. To measure mercury levels, a portable mercury analyzer Lumex RA 915+ was used, measuring for six days at different points in the urban area, including gold shops and nearby residential areas. The results showed that mercury concentrations ranged from 2 to 62,915 ng/m<sup>3</sup>, with the highest levels near gold shops. In these sites, higher concentrations were observed in the afternoon (40,000 - 62,915 ng/m<sup>3</sup>), compared to the morning hours (20,000 -30,000 ng/m<sup>3</sup>). These levels exceeded the guideline values established by the WHO, indicating a significant risk to the human health of people living, working, and transiting near these shops. Individuals exposed to these extreme concentrations of mercury vapor may suffer neurological and renal deficiencies. Therefore, it is critical to regulate and control the use of mercury in artisanal gold mining activities, as well as to monitor and enforce regulations to minimize the harmful effects of mercury contamination on human health and the environment. The results obtained can serve as a tool for decision-making by the corresponding authorities, ensuring the integrity of the city's inhabitants and the environment in which they live.

## 26A.O-Mo-6

# Improving Lead and Arsenic Environment Health Risks Exposure Assessment and Management in Uruguay, Through New Trends of a Collaborative Multidisciplinary Approach

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### Abstract

Recent advances in scientific knowledge of “Environmental sciences” incorporate new technologies and concepts. Then, multidisciplinary approaches aroused worldwide, not only for scientific research studies but also for official decisions and actions.

We reviewed these collaborative emerging disciplines of major relevance and focus on our experience in Uruguay for environmental lead and arsenic exposure health risks assessment and management.

The Medical Geology approach was incorporated by Environmental Toxicology and Geochemistry scientists for the studies of health risks exposure to environmental geological factors such as the arsenic levels in the groundwater of Uruguay. This problem has been first approached by geoscientists, chemists, toxicologists, and epidemiologists’ research teams. from 2007 on. This discipline is a recognized area both at the University of the Republic(UDELAR) and the Geosciences Area of PEDECIBA (Basic Science Development Programs).

In addition, Ecohealth is a transdisciplinary approach displayed worldwide for human health studies, from an eco-systemic view, and includes support from affected communities. This has been widely relevant in Uruguayan lead pollution research issues as well. Consequently, jointly official actions related to health and environmental issues towards lead in children have been carried out to improve their health surveillance.

Environmental Sciences research also incorporates new WHO concepts such as “One Health” which is now also focused on chemical-related illnesses in animals and their relationship to detecting and preventing human illness. This approach to chemical exposures is very useful in environmental health and toxicology studies or public health surveillance if common environments and food sources shared by humans and animals are considered. In Uruguay, dogs showed to be useful tools as sentinels to assess and prevent lead risks for children when environmental data are not available.

“Health Impact Assessment(HIA)” has also been included in “Environmental Impact Assessment” studies dealing specifically with human health environmental impacts while. “Exposome” refers to all complex exposures that humans are subjected to throughout their lives including diet, lifestyle factors, and social influences.

So, we highlighted the importance of this multidisciplinary collaboration among health and environment disciplines, to work on a common basis for people's welfare in their general environment.

## 26B.O-Mo-1

### Exposure to Environmental Contaminants and Stunting Prevalence in Infants Living in the Lake Atitlán Watershed, Guatemala

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#### Abstract

Guatemala has the highest child stunting prevalence in the Americas and is ranked sixth internationally. This pilot project aims to explore the relationship between stunting prevalence and exposure to environmental contaminants among infants. We completed a cross-sectional study (n=80 mother/baby dyads) with 20 mothers/baby dyads from each of four communities: San Juan la Laguna, San Pedro la Laguna, Panajachel, and Santiago Atitlán. We administered a survey based on the language preference of the mother with the help of a community translator. We collected data about parity, baby's age, parents' occupational status, use of pesticides, and diet. We measured the length of each infant to assess stunting (based on age). The WHO establishes that stunting is a value of height-for-age  $<-2$  SD. The mean age of mothers was 29 years. Mothers in our sample from San Pedro had the highest educational attainment. Eighty percent of mothers in our sample from Santiago were currently working, which is more than the mothers from the other communities. Fourteen mothers reported that they used pesticides. Of the 80 babies, 57.5% were males and 42.5% were females, and the average age was 131 days. The mean z-score for stunting was -1.39. Babies from Panajachel had a higher prevalence of stunting than did babies from the other three communities. We collected breastmilk samples from each mother and examined the breastmilk for concentrations of toxic metals and metalloids, along with elements key to nutrition. Analysis of pesticides is underway. Stunting results will be compared with concentrations of contaminants to assess if these contaminants play a role in stunting. This study also provides background information on contaminant concentrations in breastmilk of women from the Atitlán watershed, which we relate to our prior results of contaminant concentrations in subsistence foods.

## 26B.O-Mo-2

### Fish Consumption Preferences and Human Total Mercury Levels in Hair and Urine Samples from the Puerto Hondo Community

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#### Abstract

Mercury is a heavy metal considered one of the most dangerous environmental pollutants, not only because it may cause diseases in humans but also it may accumulate along natural food chains. Mercury exposure through fish is a wide known public health problem. Evaluating this exposure is important for health risk assessment, especially in communities that relies on mangroves ecosystems for feeding and living. Herein, we evaluated Hg exposure in Puerto Hondo community by assessing consumption preferences via questionnaires. In addition, we measured Total Hg (THg) in fish, seafood and human hair and urine. For quality control and quality assurance, we used standard references materials (DORM4 = 107.34%), duplicated measurements and blanks ( $0.0047 \pm 0.0070$ ). A total of 292 persons were interviewed. From this total, 62% depend on the mangrove ecosystem for food. Further, from those who consumed fish, 39% ate fish 3 or 4 times per week, and 9% reported eating fish more than 5 times per week. Preliminary results showed a THg median concentration (n=65) of 1.46 mg/kg (range 0.30 - 4.43) in hair and  $0.63 \pm 0.92 \mu\text{g/L}$  (range 0.001 - 3.4) in urine. Total Hg in human hair was positively associated with fish consumption. Higher THg concentrations were present in hair when people consumed fish more than 3 times per week than when people consumed less fish per week. Our data suggest that Hg absorption and accumulation in the food chain may build up into humans via fish consumption. These results of this project will allow regulatory institutions to take appropriate measures to prevent Hg pollution and the generation of health problems in the community of Puerto Hondo.

## 26B.O-Mo-3

### **Intracellular Incorporation of Environmental Atmospheric Metallic Nanoparticles From Three Different Sources in Epithelial and Fibroblast Human Lung Cells**

Iara Souza<sup>1</sup>, Mariana Morozesk<sup>2</sup>, Elson Galvão<sup>3</sup>, Steven Mutsaers<sup>1</sup>, Cecilia Prêlee<sup>1</sup>, Alex Vieira<sup>4</sup>, Douglas Lougon<sup>4</sup>, Martin Saunders<sup>1</sup>, Jeremy Bougoure<sup>1</sup>, Silvia Matsumoto<sup>3</sup>, Magdalena Monferrán<sup>5</sup>, Daniel Wunderlin<sup>5</sup>, Michael Elliott<sup>6</sup>, Marisa Fernandes<sup>2</sup>, Peta Clode<sup>1</sup>

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#### **Abstract**

The present study demonstrates the cellular internalisation of environmental settleable atmospheric particulate matter (SePM) and PM10 from urban areas subject to the major influences of the steel industry, iron pelletizing and civil construction in the bronchial epithelial (16HBE) and fibroblast (CCD19) human lung cells. High resolution transmission electron microscopy (HRTEM), nanocrystallography, electron energy loss spectroscopy (EELS), nanoscale secondary ion mass spectrometry (NanoSIMS) and inductively coupled plasma mass Spectrometry (ICP-MS) were applied to characterise the SePM, including nanoparticles, at the subcellular level. Elements and nanoparticles uptake from PM10 were higher compared with those from SePM and, those from metallurgical sources were more incorporated than from civil construction/vehicular sources. Fibroblasts showed higher metal incorporation than epithelial cells after SePM exposure. The internalisation of Al, Si, Ti, Fe and Zr was observed in free nanoparticles into the nucleus and membrane-bound particles/clusters into the cytoplasm of cells. Chemical and ultrastructural analyses demonstrated that titanium, iron, and zirconium nanoparticles were mainly associated with metallurgical sources and, secondly, with vehicular emissions. This study raises concerns about the PM releasing in metalliferous areas, requiring stricter environmental regulations of atmospheric quality to protect human health.

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## 26B.O-Mo-4

### Health Impacts of PM<sub>2.5</sub>-Bound Metals and PAHs in a Medium-Sized Brazilian City

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#### Abstract

Rio Grande is a significant industrial medium-sized city in the southernmost region of Brazil, which has been previously studied and shown to be contaminated by metal(loids) and polycyclic aromatic hydrocarbons (PAHs) in water, soil, sediment, and the atmosphere. Despite the recent incorporation of PM<sub>2.5</sub> monitoring in Brazilian environmental legislation (2018), there is still a shortage of studies in developing countries.

This study aims to investigate the PM<sub>2.5</sub> levels in both the industrial and urban areas of Rio Grande, determine the concentration of metal(loids) As, Cd, Cu, and Pb and 16 PAHs in PM<sub>2.5</sub> samples, conduct a Human Health Risk Assessment for these contaminants, and perform a Health Impact Assessment for two potential scenarios of reducing PM<sub>2.5</sub> levels.

Our findings indicate that the levels of PM<sub>2.5</sub> in Rio Grande exceeded the limits established by Brazilian legislation, and by current and former World Health Organization parameters, in both the industrial and urban areas. Furthermore, all PM<sub>2.5</sub> samples contained metal(loids) that posed both noncarcinogenic and carcinogenic health risks. However, no carcinogenic risks were found for the assessed PAHs. Our study also identified that the proposed reduction scenarios in the Health Impact Assessment could potentially reduce up to 22 deaths annually and decrease health-related expenditures.

The results of this study provide a solid foundation for the development of public health policies aimed at improving air quality, supporting health surveillance, and directing future studies towards a more holistic approach to this issue. This study's comprehensive findings highlight the need for increased monitoring and regulation of industrial and urban areas to mitigate the harmful health effects of air pollution in developing countries such as Brazil.

## 26B.O-Mo-5

### Investigation of Vapor Intrusion with Multiple Lines of Evidence for Health Risk Assessment of Inhabitants in a Residential Area in Brazil - São Paulo

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#### Abstract

Vapor Intrusion (VI) is defined as the migration of volatile organic compounds (VOC) from the contaminated subsurface (soil and/or groundwater) into overlying buildings (houses) through subsurface soil or preferential pathways (e.g., floor cracks and underground utilities). These VOC have the potential to impact indoor air (IA) quality inside the affected buildings and thus, human health. Given the absence of national procedures and standards for conducting the investigation of intrusive vapor, this study case developed a protocol, using multiple lines of evidence investigation methods to assessing main pathways of intrusive vapors and whether the receptors were in a potential risk situation. To evaluate the vapor intrusion in 17 residences located in a contaminated area by chlorinated solvents from a former laundry, an investigation was held through 3 stages based on multiple lines of evidence. The 1st stage aimed to identify the possible preferential pathway of vapors mass flow through in situ measurements using a portable photoionization detector. The 2nd stage consisted of semi-quantitative analysis with a portable chromatograph at locations where relevant VOC readings were found, to verify if these occurrences were the compounds of interest (COI - chlorinated solvents). The last stage consisted of sampling in the utilities and indoor air where COIs were identified or in the most 'sensitive' points (with higher occupancy time and lower air exchange rate) in order to quantify the risks through analysis in laboratory with active (TO-15) and passive (TO-17) methods. For the 17 houses evaluate, only 5 indicate the occurrence of vapor intrusion through preferential pathway, and only 1 residential building presented vapor inhalation risk for residents. Furthermore, it allowed to identify different patterns of vapor intrusion occurrence, for example, in some residences floor cracks and openings in door jambs act as preferential pathways, while others vapor intrusion were through utilities such as sink drainers, underground pipes and electrical outlets. The protocol developed for these investigations provide dynamic, agile and precise decision-making on-site, enabling through multiple lines of evidence the identification of residences with a potential risk to human health. This optimized the field work time in the residences, minimizing discomfort to the residents, and directed mitigation actions effectively, as the vapor intrusion pathway was adequately mapped.

## 26B.O-Mo-6

### Molecular Signatures Related With Mitochondrial Damage After Diuron Exposure

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#### Abstract

Diuron, 3-(3,4-dichlorophenyl)-1,1-dimethylurea, is a worldwide used herbicide whose biotransformation gives rise to the metabolites, 3-(3,4-dichlorophenyl)-1-methylurea (DCPMU) and 3,4-dichloroaniline (DCA). Previous studies indicate that diuron and/or its metabolites are toxic to the urothelium of the Wistar rats urinary bladder where, under certain conditions of exposure, they may induce successively urothelial cell degeneration, necrosis, hyperplasia and eventually urothelial tumors. The hypothesis was raised that the molecular initiating event (MIE) of this Adverse Outcome Pathway (AOP) is the mitochondrial toxicity of those compounds. This study aimed to investigate in vitro the metabolic alterations resulting from exposure to diuron and its metabolites DCPMU and DCA on urothelial mitochondria and hepatic mitochondria isolated from male Wistar rats. A non-targeted metabolomic analysis showed discriminative clustering among groups and alterations in the frequency of 3 membrane-associated molecules phosphatidylcholine (PC), phosphatidylinositol (PI) and phosphatidylserine (PS), in addition to 3-dehydro-L-gulonate and 2-methylhexanoyl-CoA, all of them involved in critical mitochondrial metabolism. And monitoring of functional parameters of liver mitochondria. Collectively, these data indicate the mitochondrial dysfunction as a MIE that triggers cellular damage and death observed in previous studies.



# Session 27: Advances and Challenges in the Analysis of Toxicity and Environmental Data: Statistics, Models, Databases, Index, and Risk Assessment

27.O-We-1

## Pesticides, Pharmaceuticals and Personal Care Products in Tres Arroyos Basin (Buenos Aires, Argentina): Sources, Occurrence and Ecological Risk Assessment

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### Abstract

Human activities, both in rural or urban environments, are sources of chemical pollution mainly for aquatic ecosystems. Moreover, the presence of chemicals' mixtures including compounds with different physical-chemical characteristics and mechanisms of action (pesticides, pharmaceuticals and personal care products (PPCPs), among others) has become a topic of interest not only for scientists, but also for regulatory authorities. In this context, the present study aimed on evaluate the presence, and possible sources of pesticides and PPCPs in different streams of Tres Arroyos basin, and to perform an ecological risk assessment. For this purpose, two sampling events (spring, summer) were carried out in five sites of three streams with different land use. Surface water samples were collected in triplicate for chemical analysis. Samples were filtered and solid phase extraction was performed. Chemical analysis was performed by high resolution liquid chromatography mass spectrometry. Risk assessment was performed using the Risk Quotients (RQ) approach, and SRQ was calculated both for pesticides and PPCPs. A total of 20 pesticide molecules were quantified, encompassing fungicides, herbicides, insecticides and metabolites, being atrazine and its metabolites, the fungicide carbendazim and the insecticide imidacloprid the most ubiquitous. The highest Spesticides concentrations were found in a fully rural environment (Primer Brazo, Claromecó stream) with extensive crop production (corn, wheat, sunflower), and without a clear temporal trend. Regarding risk assessment related to pesticides,  $\Sigma$ RQ showed a potential high risk for all the sites and sampling seasons, despite the land use. On the other hand, 18 PPCPs were quantified in the basin, with detection in all the sites and both seasons, but with a clear peak in Parque Cabañas site, related to wastewater treatment plant (WWTP) discharge. The main findings were the ubiquity of caffeine, the high concentrations of acetaminophen and ibuprofen, the detection of the plasticizer bisphenol A, and the first report of parabens in aquatic ecosystems in Argentina. Regarding risk of PPCPs,  $\Sigma$ RQs have shown the same pattern than quantified concentrations, with a clear peak in the site close to the WWTP. This study is the first step in the evaluation of chemical risk assessment in the basin. Next step must involve evaluation of effects on aquatic organisms *in situ*.

## 27.O-We-2

### Effect of Streamflow on Water Quality in the Mediterranean Zone of Central Chile: Spatial and Temporal Analysis of the Mataquito River Basin

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#### Abstract

The loss of water quality can have a significant impact on ecosystems, disrupting biodiversity and ecosystem services. Climate change studies in the Mediterranean zone of central Chile, specifically in the Mataquito River basin, have forecasted a 40% reduction in runoff and a 30% decrease in precipitation for the 2070-2099 period under the RCP8.5 climate scenario. This would have significant impacts on ecosystems and the population. Furthermore, the impact on water quality due to climate change is unknown. Using observational data, the purpose of this research was to relate streamflow with water quality in the Mataquito River basin. Water quality and streamflow data were collected over a period of 40 years (1980-2019) at a total of five and three stations, respectively. These data were obtained from the National Water Directorate (DGA) of the Government of Chile. An aggregated water quality index (WQI) was calculated for temporal and spatial comparisons. The data were grouped by stations of the annual cycle and by wet and dry periods of the year according to the Walter-Lieth climate chart. An exploratory data analysis was conducted to verify the existence of relationships between water quality parameters and station location. For this purpose, a principal component analysis (PCA) was applied, and non-parametric Pearson correlation was used to evaluate the relationship between the different parameters. In addition, a Mann-Kendall trend test and Sen's method were performed to verify the existence of trends and the degree of significance of the water quality parameters. A logarithmic linear relationship was also employed to verify the relationship between WQI and streamflow. Among the evaluated water quality parameters, pH, electrical conductivity, calcium, magnesium, sodium, total hardness, and SAR showed a significant increasing trend across all study stations. On the other hand, the temporal trend of the WQI was decreasing and significant in all stations. The linear regression presented evidence of the relationship between the WQI and streamflow in the high, middle, and low zones of the Mataquito River basin. Observational data demonstrates a decrease in water quality in recent decades that can be related to, among other factors, a decrease in streamflow that can be associated with climate change impacts, but also with the water management at the basin scale. Thanks to ANID/FONDAP 15130015 and CONICYT/Doctoral Fellowship/2020-21201107.

## 27.O-We-3

### Qualitative Analysis of the Most Locally Relevant Runoff and Erosion Parameters for Constructing Brazilian Scenarios

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#### Abstract

Estimating exposure is one of the most important steps in an environmental risk analysis of crop-protection products to nontarget organisms. Regulatory agencies such as the US Environmental Protection Agency (USEPA), Pest Management Regulatory Agency (PMRA), and European Food Safety Authority (EFSA) all use mathematical exposure models in their regulatory assessment process. Brazil has been discussing the adoption of the Pesticide in Water Calculator (PWC) to be applied in aquatic pesticide risk assessment. Therefore, a qualitative sensitivity analysis (Morris OAT method) was performed to understand which are the most important local parameters in the PWC to estimate environmental concentrations in surface water (EECSW). In addition, an exercise made up of two corn scenarios in two Brazilian regions was developed (Uberlândia [UDI] and Arapoti [ARA]). Two herbicides with different soil-binding properties and modes of action were selected to estimate the EECSW. The results demonstrated that the parameters of importance were different for each site, probably the result of different soil characteristics and meteorological patterns. This outcome suggests that regulatory agencies should consider developing more than one scenario to account for different agricultural regions. For Herbicide 1, the EECSW for UDI were similar to US scenarios, whereas for ARA they were lower. For Herbicide 2, the EECSW for the UDI site was higher than most of the US scenarios, whereas at the ARA site, EECs were similar to four US scenarios and lower than the other six. Local data were used as a refinement, resulting in the decrease in the EECSW for both herbicides in the UDI site. For the ARA site, Herbicide 1 displayed a similar EECSW value, whereas for Herbicide 2, it was lower after the refinement. Overall, these results demonstrated the importance of developing local scenarios to provide more realism to estimate pesticide exposure from its agricultural use and may help regulators to determine and recommend mitigations regarding the use of crop-protection products

## 27.O-We-4

### **Assessing the Ecological Impact of Aquatic Pollution in a Rural Environment: Risk Assessment, Indicators or Multivariate Statistics?**

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#### **Abstract**

Rural aquatic ecosystems are simultaneously subjected to multiple sources of contamination such as pesticides and nutrients associated with agriculture, as well as bacteriological contamination associated with livestock and sewage effluents discharge. In the present study, pesticide, metal, and bacterial aquatic contamination were evaluated together with the trophic state in the Pergamino River (Buenos Aires province, Argentina) in order to study the anthropogenic impacts on the water quality of the river. The Pergamino River flows through productive fields dedicated mainly to extensive crop production and livestock pastures. The river also crosses through the City of Pergamino. Four sites were sampled along the river in November 2021 and March 2022. Two of the sites were located upstream from the City of Pergamino, while the two other sites were located downstream from the city, so as to compare the impacts of agriculture and urbanization on water quality. Forty-four different pesticide molecules were detected in surface water samples, the herbicides saflufenacil and atrazine, and the fungicide carbendazim were detected in all water samples tested. Other frequently detected pesticides were triadimefon, tebuconazol, and cyproconazol, which were present in more than 80% of the samples. The Trophic State Index had values above 80 in all sampling sites, indicating hypertrophic conditions. The water concentrations of Cu, Pb, Hg, Mn, Cr, and Al frequently surpassed water quality criteria's. Biological and chemical oxygen demands as well total coliforms values were greatly elevated in sites downstream from the municipal wastewater outlet. The potential impacts of these findings on aquatic life are evaluated according to different approaches such as environmental risk assessment, indicators or multivariate statistics. Overall, results obtained point out the pervasive impact of the anthropogenic activities (agriculture and urbanizations) on the water quality of the Pergamino and the potential negative effects on the aquatic life.

## 27.O-We-5

### FAO Pesticide Index vs. Ecotoxicological Footprint: Which Better Expresses the Environmental Impact for Uruguay?

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#### Abstract

Within the framework of sustainable development goal 2 -focused on achieving zero hunger- and FAO policies to develop a sustainable intensification of agriculture on arable land, the tools to assess the environmental impacts of intensification in a standardized way become a priority. And also the means to allow comparison between technological options (i.e., crop rotation) and food supply chains.

Among the most relevant environmental impacts of agricultural intensification is the use of pesticides. For this reason, FAO developed the Pesticide Index (PI), a ratio calculated for each country between the annual mass of pesticides used and the corresponding crop area — an estimated index since 1999 for each country in FAOSTAT.

This paper analyzes and discusses the possible inconsistencies and confusion that the use of IP can produce. First, it does not guide local/international environmental public policies because the IP needs to include information on the toxicological quality of the pesticides involved. This problem could be solved by using the Toxicology Unit, which standardizes the mass of pesticide used by dividing it by its corresponding LD50. Secondly, the PI seems different from the standardization strategies of environmental impacts recommended by SETAC, FAO, ISO, and different eco-label standards, which suggest a life cycle analysis approach (i.e., ecotoxicological footprint).

This paper reviews the limitations and methodological inconsistencies of the FAO IP through the analysis of a database of 210 pesticides imported by Uruguay since 1999. This article compares the changes in the FAO IP for Uruguay with the corresponding annual toxicological units. For 1999-2018. This work analyzed the databases of crops and pesticides used in Uruguay from FAOSTAT, Ministry of Livestock, Agriculture, and Fisheries (MGAP, Uruguay), and University of Hertfordshire Toxicology Database.

## 27.O-We-6

# In Silico Prediction of Chemical Toxicity on Multiple Avian Species Using Explainable Deep Learning Ensemble Models: An Artificial Intelligence Approach

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### Abstract

A comprehensive safety evaluation of chemicals should require toxicity assessment in both the aquatic and terrestrial test species. Due to the application practices and nature of chemical pesticides, avian toxicity testing is considered an essential requirement in the risk assessment process. Understanding the costly and time-consuming characteristics of experimental assays, *in silico* models have emerged as a practical solution to be employed as screening tools and within integrated testing strategies to avoid animal testing as well as reduce cost and chemical waste. In this study, multitask quantitative-structure activity relationship (QSAR) models were developed to predict the chemical toxicity of pesticides against four avian species (*Anas platyrhynchos*, *Colinus virginianus*, *Coturnix japonica*, *Phasianus colchicus*[BJN1] ) following OECD guidelines. Initially, a dataset comprising 683 compounds with experimental LC50 values was collected from various sources. Then, ensemble decision-forest single-task models and a multitask learning model were developed using molecular fingerprints (ECFP4). As a result, single-task models significantly outperform the multitask approach, with acceptable average test RMSE values ranging between 0.32–0.54 and *r* values between 0.62–0.78. Additionally, the Continuous Ranked Probability Score (CRPS) and Negative Log-Likelihood methods were employed to gauge how confident each prediction was. Since the "black-box" nature of deep learning can make mechanistic interpretation of predictions difficult, feature contributions to toxicity were assessed following SHapley Additive exPlanations (SHAP) values. The local SHAP value calculations yield quantitative feature contributions that support potency toxicity against four avian species. More specifically, the models indicated that moieties formed by carbonyl, chlorine, phosphate, and sp<sup>2</sup> hybridized carbons positively weighted the predicted potency across the three tasks. In summary, the approach developed here represents a new computational tool for assessing the toxicity of pesticides against four bird species.

**POSTER  
PRESENTATIONS**

**DRAFT**

# Session 1: Environmental Risk Assessment

1.P-Mo-001

## Ecological Risk Assessment Based on Passive Sampling for Chlorpyrifos Presence in a Tropical Mountain Reservoir

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### Abstract

The presence of organophosphate pesticides in aquatic systems raises concerns, yet their degradation processes often lead to their transformation into less toxic compounds, resulting in their unnoticed presence. Passive sampling of organic compounds has proven to be an effective tool for detecting trace and sub-trace levels of these substances. In our study, we focused on chlorpyrifos and its primary degradation product, 3,5,6-trichloro-2-pyridinol, and utilized passive sampling to estimate average concentrations and bioavailable fractions that may impact the ecosystem's health. We established six sampling points in the body of water to monitor chlorpyrifos contamination events based on the presence of its degradation product.

We evaluated chlorpyrifos's risk through the acute toxicity units method and the chronic exposure hazards index. We discovered that chlorpyrifos poses a moderate risk for acute exposure and a risk for chronic exposure for the organisms present in the water and sediment of a small tropical mountain reservoir. Although the degradation product exhibited higher concentrations, it presented no risk for either acute or chronic exposure. Sediment risk assessment revealed no acute exposure risk for the two compounds analyzed, but chronic exposure was high for both of them.

Finally, the human health risk assessment suggested that the compounds pose no risk.



## 1.P-Mo-002

# Environmental Risk Assessment of Emerging Contaminants in Protected Coastal Lagoons of Uruguay

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### Abstract

Emerging contaminants (ECs) are substances with a widespread occurrence in the environment found many times at trace levels and with potentially detrimental effects to both aquatic ecosystems and human health. These pollutants are not commonly included in routine monitoring programmes but may be candidates for future regulation.

ECs include a wide range of substances, such as pharmaceuticals, personal care compounds, drugs of abuse and pesticides. Environmental risk assessment of ECs in the aquatic environment is usually based on effect and exposure assessment. The ability to evaluate the individual and interactive effects of ECs on aquatic ecosystems is critical, particularly as the frequency of their occurrence continues to rise in natural waterbodies around the world. However, the limited availability of toxicity data leading to the lack of regulatory or screening values for most ECs makes difficult to understand the ecological significance of occurrence data.

Here we characterized the environmental risk of a number of ECs along Laguna de Rocha and Laguna de Castillos basins, both declared national protected areas and belongs to the Ramsar Convention for wetlands protection sites. Samples covered at a gradient from low to high-impacted urban regions. The risk quotient (RQ) approach was used by relating the measured environmental concentrations (MECs) in water with published toxicity data to different aquatic organisms and trophic levels. We combined this information with land use analysis to identify vulnerable sites to be considered in environmental management. In addition, a scoring and ranking system was used for prioritization based on occurrence frequency, spatial distribution, persistence, toxicity, bioaccumulation and endocrine disruption potential to aquatic organisms. The analysis revealed different types of ECs with high and extremely high risk. Of special concern are hormones (17- $\beta$ -estradiol and 17- $\alpha$ -Ethinylestradiol) derived from inefficient wastewater treatment plants (WWTP). Furthermore, 15 critical compounds were identified for which further monitoring and re-evaluation is highly recommended. Our results suggest the need for implementation of management policies at basin scale including efficient in WWTP and special regulation on agricultural activities within and nearby internationally recognized sites for conservation.

## 1.P-Mo-004

### Pharmaceutical Compounds in Wastewater and Natural Water Bodies in Peru and Their Risks to Aquatic Organisms

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<sup>1</sup>Universidad Nacional Agraria la Molina, Peru. <sup>2</sup>Universidad Nacional del Altiplano, Peru

#### Abstract

The presence of emerging contaminants in wastewater and water sources has become a major concern worldwide due to the possible negative environmental and health impacts, which has led, for example, to the inclusion of pharmaceutical compounds such as antibiotics, anti-inflammatory drugs, anti-epileptics and hormones in the environmental regulations of countries such as the United States, Australia and the European Union. In Peru, the discharge of untreated wastewater into natural receiving bodies can increase the potential adverse effects of pharmaceutical compounds on aquatic organisms and the environment, and there is little information on the presence and concentrations of these compounds, which prevents an effective assessment of this problem. The objective of this research was to determine the concentration of pharmaceutical compounds in domestic wastewater and natural water bodies (Río Rímac, Lima-Peru; Laguna Espinar, Puno-Peru) to assess the risk to aquatic organisms. Applying liquid chromatography coupled to mass spectrometry (LC/MS-MS), were analyzed 22 pharmaceutical compounds, including antiepileptics, analgesics, antipyretics, anti-inflammatories, antirheumatics, hypolipidemic agents and tranquilizers. At least 2 compounds were found in all the samples analyzed. The concentrations obtained were compared with those reported in research where ecotoxicological tests were carried out on model organisms for aquatic ecosystems at different levels of the trophic chain, such as bacteria, microalgae, crustaceans, fish and amphibians. The compounds with the highest concentrations in all the samples were Naproxen, Ibuprofen and Diclofenac, drugs that belong to the group of non-steroidal anti-inflammatory drugs (NSAIDs), which are commonly used in the treatment of acute pathologies and can be acquired without a medical prescription in Peru. Another compound detected in relevant concentrations was carbamazepine, an anticonvulsant drug of controlled sale and with a high persistence in aquatic ecosystems. It can be concluded that the concentrations found mainly of NSAIDs may indeed represent an environmental risk for aquatic organisms, mainly amphibians, not only because of their pharmacological effect and toxicity but also because of their potential for bioaccumulation and biomagnification.

## 1.P-Mo-005

### **Toxicokinetic-Toxicodynamic Modelling – Examples and Available Freeware-Tools**

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#### **Abstract**

Successful uses and validation of toxicokinetic-toxicodynamic modelling approaches have increased exponentially since 2011 in which the General unified threshold (GUTS) model of survival was published as a generic framework to describe and predict lethal effects. Recently the BeeGUTS model was published demonstrating the power of the approach to evaluate different tests with different exposure route in a holistic way. For sublethal effects toxicokinetic-toxicodynamic models based on the dynamic energy budget theory have proven their predictive power for various of species. In this poster we will summarize the potential use of these models for evaluation of standard ecotoxicological test systems and in environmental risk assessment, as well as providing an overview of the necessary tools which are open access and free to use in different software environments like stand alone software, R or Matlab.

DRAFT

## 1.P-Mo-006

### Heavy Metal Concentrations in Some Commercially Important Fishes in a Coastal City in Colombia and Potential Risk to Human Health

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#### Abstract

Heavy metals constitute a group of pollutants that generates the most negative impacts on aquatic ecosystems, a situation that has generated an increase in public concern worldwide, due to its toxicity, high persistence, bioaccumulability, biomagnification, and non-biodegradability in food chains. In this work, the concentrations of heavy metals (Zn, Cr, Pb and Cd) were evaluated in the muscle tissue of several relevant fish species (*Prochilodus magdalenae*, *Arius proops*, *Lutjanus synagris*, *Caranx crysos*, *Haemulon plumierii*, *Oreochromis niloticus*, *Oreochromis sp* and *Eugerres plumieri*), which are highly commercialized in Riohacha, a city located on the Caribbean coast of Colombia. Between fourteen and twenty units of each species were collected and analyzed using atomic absorption spectrophotometry. The highest concentrations for Zn ( $24.64 \pm 0.43 \mu\text{g/g}$ ), Cr ( $0.72 \pm 0.16 \mu\text{g/g}$ ), Pb ( $0.22 \pm 0.06 \mu\text{g/g}$ ) and Cd ( $0.13 \pm 0.02 \mu\text{g/g}$ ) were registered in the species *Prochilodus magdalenae*, *Oreochromis sp*, *Eugerres plumieri* and *Lutjanus synagris*, respectively. The estimation of hazard quotient (HQ) associated with fish consumption indicated that none of the species studied in the present study exceeded the threshold  $\text{HQ} = 1$ , however, chromium presented the greatest potential risk. This risk did not exceed the allowed limit, it was very close to one, mainly for *Lutjanus synagris* (0.93). For this reason, it is recommended that consumption of all species to be moderate, as long bioaccumulation periods of exposure to these metals in the human body could trigger various affectations in the organism, having repercussions on food safety and public health of the population of the study area.

## 1.P-Mo-007

### Case Study: Ecological Risk Assessment Developed in a Mining Site

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#### Abstract

The Ecological Risk Assessment (ERA) is the process for evaluating the adverse effects to an ecosystem as a result of exposure to anthropic activities. Currently, the main methodologies to conduct an ERA are the TRIAD (Chapman, 1989; ISO 19204, 2017) and the procedure proposed by USEPA (1997). These methodologies are based on three lines of evidence (chemical, ecological and ecotoxicological) and propound different approaches for consolidating the results and concluding about the relevance of the observed effects.

In Brazil, the conduction of the ERA is established by a federal law (Resolution CONAMA 420/2009), but, at this moment, there is no national methodology standard defined for ERA studies. Recently, in December/2022, Sao Paulo's environmental agency (CETESB) published its procedure (Technical Standard CETESB P4.001) defining the procedures for ERA.

This case study aims at the development of the ERA in a mining site in Brazil, located on a region of natural ecosystems and water resources under anthropic influence. Geologically, the area is placed on a carbonatite complex, where metals levels are naturally anomalous compared to environmental standards.

At this point, the study is in the early stage, when the existing information was gathered in order to formulate the problem, identifying the stressors of concern and the assessment endpoints. This step included the evaluation of chemical results (surface soil, sediment, and surface water) and biota secondary data.

Based on the initial evaluation, a Preliminary Ecological Conceptual Site Model (Eco-CSM) was formulated, and a Sampling / Analysis Plan was proposed to subsidize the next steps of ERA, comprising the three lines of evidence (chemical, ecological and ecotoxicological) for both terrestrial and aquatic ecosystems. For this purpose, the study area was subdivided into Decision Units – DU (ITRC, 2020) considering the environment characteristics, the land use, and the chemical anomalies distribution.

Considering that the area is in a mineralized zone and, therefore, with natural geochemical anomalies, the main challenge of the study is the definition of the reference area to be used as background / baseline for results and effects evaluations compared to each Decision Units.

The results of the next steps of ERA will enable the revision of the Eco-CSM and the definition of the risk management actions to protect the biota and to promote the environmental restoration, if necessary.

## 1.P-Mo-008

### Assessment of Risk, Impact, and Disease Burden Due to Exposure to Fine Particulate Matter in the City of Córdoba, Argentina

Iván Tavera Busso, Ana Carolina Amarillo, Matías Reinaudi, Carina Inés Argañaraz, Ana Paula Varón, Daniela Tinunin, Hebe Carreras  
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#### Abstract

Air pollution caused by suspended particulate matter poses a serious threat to human health. Fine particulate matter (PM<sub>2.5</sub>) is especially toxic due to its composition, which includes polycyclic aromatic hydrocarbons (PAHs). In this study, we used the WHO AirQ+ 2.1 software to investigate the short- and long-term impacts of PM<sub>2.5</sub> on the health of people living in the city of Córdoba, Argentina. We found that various meteorological factors such as temperature, wind speed, and precipitation, affected PM<sub>2.5</sub> and PAHs levels, with high concentrations during autumn and winter. The impact analysis showed that deaths attributable to PM<sub>2.5</sub> exposure accounted for between 1.04% and 1.44% of total deaths in the short term, and up to 1 ‰ inhabitants in the long term. However, the burden of disease analysis indicated that other factors, such as the chemical composition of PM<sub>2.5</sub> could contribute to deaths. We also found that the risk of developing cancer due to PM<sub>2.5</sub> exposure was highest during the cold seasons, but only for certain concentration ranges (23-33, 48-58 and >73 µg m<sup>-3</sup>). This pattern would be related to different environmental conditions of the city: the first range could be associated to regular vehicular traffic levels; the second range could be attributable to high pollutant level events due to thermal inversions and no rain periods; and the third one with extreme events, such as forest fires which have increased their frequency during the last years, in the vicinity of the city. The findings of the present study highlight the need to address the new WHO guidelines for PM<sub>2.5</sub> in order to reduce the number of deaths as well as the lifetime lost due to exposure to air pollutants.

## 1.P-Mo-009

### Ecological Risk Assessment in an Industrial Area - Conceptual Model

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#### Abstract

The industrial areas are often located near assets to be protected, such as preservation areas, parks, and other green areas. To assess the likelihood of adverse ecological effects, an Ecological Risk Assessment (ERA) must be prepared especially in cases of known contamination in a physical compartment, such as soil or groundwater. This study presents the Conceptual Model and the Work Plan to be applied in an industrial area, along with the evaluation of the flow and transport model of groundwater contamination plumes. The study was structured following the three ERA stages: (i) problem formulation, based on the integrated assessment of relevant and available information about the sources of stress and their characteristics, leading to the problem formulation, conceptual model, and analysis plan; (ii) analysis phase, by characterizing the exposure and the ecological effects on the target compartments. The target compartments were identified by evaluating and interpreting the historical monitoring data and other studies already conducted in the area. The conceptual model considered the industrial area as a stressor or source of contamination; the discharge of effluents into a receptor body and the plume of groundwater contamination as a source or transport route; the physical compartments of groundwater and soil as a source or means of exposure to risk; as exposure method, we identified the absorption and exposure and, the entomofauna and local vegetation as receptors. Considering the Conceptual Model, we prepared the Work Plan for the exposure characterization phase to characterize a complete hydrological cycle, for which (four) quarterly monitoring campaigns are proposed. In this stage, each campaign for the terrestrial environment should include (i) chemical analysis with soil samples; (ii) ecotoxicological tests in soil with animals of the Oligochaeta group for the characterization of soil harmfulness for edaphic fauna by bioassays; (iii) chronic ecotoxicological tests in underground water with *Ceriodaphnia* spp (Crustacea, Cladocera); (iv) analysis of the invertebrate community and (v) analysis of the vegetation cover. After the four campaigns have been performed, the chemical, ecotoxicological, and ecological lines of evidence will be analyzed, as well as their integration.

# Session 2: Research and Management Approaches to Achieve a Balance Between Food Production and Nature Conservation – How to Conserve Biodiversity in an Agricultural Context?

## 2.P-Tu-001

### Ecotoxicological Evaluation of the Herbicide Imazamox for Neotropical Organisms

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#### Abstract

For the use of herbicides and algaecides, in the aquatic environment, to control aquatic plants and algae, it is necessary to assess the acute and chronic ecotoxicity, in addition to the possible sublethal effects for non-target organisms of the application. Thus, ecotoxicological studies are necessary, with anthropogenic bioindicators, of these products so that those that do not cause damage or harm the health of the environment are selected. Thus, the objective of the study was to evaluate the acute ecotoxicity of imazamox for the snail (*Pomacea canaliculata*), for the bushfish (*Hyphessobrycon eques*) and the freshwater prawn (*Macrobrachium acanthurus*). Each organism, with an average weight of  $1.2 \pm 1.5$  g, was acclimatized in bioassay rooms for 10 days in boxes with continuous aeration, static system, temperature at  $25.0 \pm 2.0$  °C, photoperiod of 16 hours light, a sensitivity test was performed for all organisms, with the reference substance potassium chloride (KCl) to assess the health of the batches. The organisms were transferred to an aquarium with three liters of water, the tested concentrations were: 0.1; 1.07; 3.5; 11.16; 36.5; 118.0 mg L<sup>-1</sup> and a control, with three replicates each. For the snail, five animals were used per repetition, for the Mato Grosso fish and the Tupi freshwater shrimp, three animals were used per repetition. The evaluation of immobility/mortality of organisms was performed at 24 and 48 hours. In the acute ecotoxicity assays for snails and for Mato Grosso fish, no mortalities occurred at any of the tested concentrations. The 50% lethal/effective concentration (LC50/EC50;48h) was greater than 118.0 mg L<sup>-1</sup>, being classified as practically non-toxic for the two test organisms. For the acute ecotoxicity test for shrimp, only 100.0% mortality occurred at the concentration of 118.0 mg L<sup>-1</sup>. The LC50;48h of was 44.3 mg L<sup>-1</sup>, with a lower limit of 23.31 mg L<sup>-1</sup> and an upper limit of 84.17 mg L<sup>-1</sup>, classifying it as slightly toxic. It is concluded that aquatic organisms should not be used in the evaluation of the ecotoxicity of imazamox, as they were tolerant to high concentrations of the herbicide in standardized exposure tests.



## 2.P-Tu-002

### Arsenic in Vegetable Crops of a Horticultural Center of the Southeast Pampean Plain

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#### Abstract

Intensive crops involve the application of fertilizers providing the nutrients for their growth. The prolonged use of these agrochemicals, mainly phosphates, is strongly linked to the accumulation of metals and arsenic (As) in soils. Previous results demonstrated the presence of As in phosphate fertilizers ( $\approx 25$  mg/kg) used in one of the most important of Buenos Aires province horticultural centers, located in nearly Mar del Plata city. Therefore, we hypothesize that the prolonged use of them could be contributing As to the soils, which can be assimilated by the crops. In this way, vegetable samples (edible parts) from conventional crops (with application of fertilizers) and agroecological crops (without chemical fertilization) were taken. Samples of tomato (*Lycopersicon esculentum*), lettuce (*Lactuca sativa*), spinach (*Spinacia oleracea*), bell pepper (*Capsicum annuum*), beetroot (*Beta vulgaris*), brucella cabbage (*Brassica oleracea var. gemmifera*), broccoli (*Brassica oleracea*), corn (*Zea mays*) and potato (*Solanum tuberosum*) were dried at 60°C and stored in the freezer. The quantification of total As was carried out by Atomic Absorption Spectrophotometry with a hydride generation. Data were expressed in mg/kg dry weight. The limit of detection was 0.005 mg/kg. The As concentrations in the analyzed samples ranged from  $< 0.005$  to 0.181 mg/kg. The highest levels were observed in fertilized beetroot leaves (0.181) and lettuce (0.148) while the lowest were in potato (0.006) and corn ( $< 0.005$ ). The concentrations of agroecological tomato and lettuce were between  $> 0.005$  - 0.011, while those fertilized varied between 0.020 - 0.148. In turn, the agroecological vegetables presented lower concentrations compared to the fertilized ones. These results revealed that fertilization practices influence As content in vegetables analyzed in the study area. Arsenic concentrations in all studied vegetables did not exceed the maximum limit allowed by the Argentine Food Code (1 mg/kg, fresh weight); thus, the vegetables grown in the study area are currently safe for consumption. However, the accumulation of As in crops must be monitored periodically until fertilization practices not be modified.

## 2.P-Tu-003

### Use of Poultry Litter as Fertilizer: Can an Agroecological Practice Pose a Risk to the Environment?

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#### Abstract

During chicken meat production, rice and peanut husks or wood shavings are spread on the breeding shed floor to absorb the excrement of the birds and maintain an appropriate and dry environment for them. Once the growing period has finished, this material, which consists of a mixture of feces and bedding material known as poultry litter (PL), is removed from the sheds and used as fertilizer since it is rich in nutrients and organic matter. Although this practice is widespread in Argentina, where more than 5 million tons of PL are generated each year, it is carried out without knowing the physical-chemical composition of this residue. This study aimed to: i) perform an elemental analysis on PL samples from 3 farms located in Entre Ríos, Argentina; and ii) perform an elemental analysis on clean bedding material (BM) and chicken feed (CF) samples. The content of 30 elements was assessed: Li, Be, B, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, Ag, Cd, Sb, Ba, Hg, Tl, Pb, Bi, and U. The samples were digested in a mixture of H<sub>2</sub>O<sub>2</sub>, HCl, and HNO<sub>3</sub> and analyzed through Inductively Coupled Plasma Mass Spectrometry. The metal content in PL samples presented the following trend: Ca > K > Mg > Na > Al > Fe > Mn > Zn > Sr > B > Cu > Ba > Rb > V > Ni > Mo > Pb > Cr > As > Li > Se > Co > Ag > Hg > U > Sb > Cd. The metal content in BM and CF samples followed the same trend for most elements analyzed. Be, Bi, and Tl were not detected in any of the evaluated matrices. The metal content determined in BM samples was significantly lower than the metal content found in PL samples for all the analyzed elements, except for Cr, which showed no significant differences (LSD Fisher,  $p > 0.05$ ). On the other hand, the metal content in CF samples presented intermediate values to those found for BM and PL samples. Given these results, we can affirm that the use of PL as fertilizer or soil amendment represents a potential source for the entry of inorganic contaminants to terrestrial ecosystems, from where they can be transferred to the food produced there or reach surface or underground water bodies. In addition, the results obtained for the CF samples would indicate that they are one of the possible sources by which the metals reach the PL through the chicken manure.

## 2.P-Tu-004

### Remediation of Pb-polluted Agricultural Soils by Application of Biochar Amendments: Effects on Yield and Toxicological Risk Parameters in Soybean Crops

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#### Abstract

The present work evaluated the use of biochar amendments made from peanut industry residues, using 3 different pyrolysis temperatures, to remediate lead-contaminated agricultural soils. Biochar is a type of organic amendment consisting of a stable, carbon-rich material obtained by the thermal decomposition of organic matter. To evaluate its use for remediation in agricultural soils, an experiment was conducted under controlled greenhouse conditions with contaminated soils, under 4 different treatments: (1) without amendment, (2) with 5% biochar produced at 300°C, (3) with 5% biochar produced at 400°C and (4) with 5% biochar produced at 500°C. A complete growth cycle of soybean (*Glycine max* (L.) Merrill) was carried out in 9 L pots. The response variables determined included: Pb accumulation in plant organs (acid digestion and atomic absorption spectrometry measurement), morphological state of the plants (length and plant dry weight), and yield of the produced grains (number of seeds and pods, weight of 1000 seed, aborted pods). Moreover, toxicological risk index (target hazard quotient, THQ) was evaluated for Argentinian and Chinese consumers, with the latter being one of the main destinations of Argentinian soybean.

The results showed that soybean plants grew better in amended soils, especially regarding plant length and root dry weight. The highest plant length was found in treatment (2), and the highest root dry weight was found in treatment (3). At the yield level, plants from all the amended treatments showed a greater number of seeds than the not amended one, although the seed dry weight per plant and the weight of 1000 seeds did not show differences. Pb accumulation in organs was mainly concentrated in the roots, with no remarkable differences among the treatments analyzed. It is noteworthy that the seeds showed Pb concentrations that exceeded the limit values of international legislation for soybean consumption (circa 1.5 ppm), a situation that was not reversed by the use of the amendment. In this sense, the evaluation of the risk index for consumers showed elevated values for Chinese consumers, although without an effective risk.

In conclusion, the use of amendments made from peanut industry residues improved soybean development and yield, but it did not prevent metal accumulation in soybean seeds. For this reason, it is recommended to evaluate amendments from other sources for soybean production in agricultural soils contaminated with Pb.

## 2.P-Tu-005

### Characterization of Urban and Horticultural Pesticide Profiles in Surface Water Bodies Impacted by Horticultural and Urban Activities in the Greater La Plata Area

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#### Abstract

The increased use of chemical products to control the wide variety of pests in crops has resulted in their presence becoming more frequent in multiple environmental matrices. This situation is evident in the water courses immersed in the horticultural region of the La Plata area that flow into the La Plata River. The objective of this study was to analyze different pesticides in 7 streams associated with both agricultural and urban activities. For this purpose, two monitoring campaigns were carried out in which samples of surface water were collected, filtered by 0.45 $\mu$ m, and extracted by sonication, while the soluble fraction was extracted using SPE. The extracts were analyzed by LC-MS/MS using 4 isotopic tracers. Out of a total of 70 pesticides studied, 39 compounds were detected in water (1 synergist, 16 herbicides, 14 insecticides, and 8 fungicides) in a concentration range between LD to 27.09  $\mu$ g mL<sup>-1</sup> during the first campaign, and 21 were detected in suspended particulate matter (1 synergist, 6 herbicides, 6 insecticides and 8 fungicides) in a concentration range between LD to 2.06  $\mu$ g mL<sup>-1</sup>. For the second campaign, there was a decrease in the pesticides present, with 29 compounds detected in water (1 synergist, 12 herbicides, 8 insecticides and 8 fungicides) in a concentration range between LD to 16.45  $\mu$ g mL<sup>-1</sup>, and 18 detected in suspended particulate matter (1 synergist, 4 herbicides, 4 insecticides, and 9 fungicides) in a concentration range between LD to 5.86  $\mu$ g mL<sup>-1</sup>. The most commonly detected compounds include the herbicides Metolachlor and 2,4-D, the insecticides Imidacloprid and Chlorpyrifos Ethyl, and the fungicide Azoxystrobin, which were associated with the water column and present in all 7 streams studied. In suspended particulate matter, the insecticides Chlorpyrifos and Imidacloprid were most commonly found (found in 100% and more than 57% of streams respectively), along with the herbicide Prometryn, present in more than 70% of the water bodies. The number of pesticides detected is similar in all streams, however, the concentrations found in streams with agricultural influence for many of these compounds are higher compared to streams that only pass through urban areas.[Ja1] This study contributes to expanding the information on the presence and amount of these compounds associated with surface water sources and the impact generated by horticultural activities mainly in the increase of environmental concentrations.

## 2.P-Tu-006

### The Efficacy of Essential Oil of *Triphasia trifolia* Berm. From Colombia Against Maize Weevils *Sitophilus zeamais* (Coleoptera: Curculionidae)

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#### Abstract

*T. trifolia* is an aggressive invasive plant with the capacity to grow in sunny open areas, is a glabrous shrub or small tree, belongs to Rutaceae family. Is native to South-East Asia, Malaysia, and Christmas Islands. It was introduced as an ornamental and as a “hedge or boundary plant” in many tropical and subtropical countries and currently it can be found in Africa, USA (Florida, Hawaii, and Texas), Mexico, Central America, South America, the West Indies, and the Pacific islands. *T. trifolia* is also used in traditional medicine, the plant is considered antifungal and antibacterial and used to treat colic, diarrhea, and skin afflictions. Fruits are used for coughs and sore throats. The other hand, in order to assure food security, proven postharvest pest management tools are needed to secure grains and processed food from insect infestations. Plants and plant products have over the years been proven to possess great insecticidal properties.

The objective of this work was to get the chemical profile from the leaf essential oil of *T. trifolia* and its insecticidal potential against one stored product pests as is the *Sitophilus zeamais*. Essential oils from the leaves of *T. trifolia* collected from one location in Bolivar town (Colombia) were isolated by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). Fifteen compounds were characterized, with b-pinene as the major component (59.0%) in leaf essential oils, follows of D- limonene (9.5%) and *p*-cymene (2.6%).

The fumigant activity of *Triphasia trifolia* essential oil, and three pure monoterpenes against *S. zeamais* were determined, according to the results of the *probit* data analysis. The mean lethal concentration (CL<sub>50</sub>) of the oil was 115.0 µg.mL<sup>-1</sup> air; followed by *p*-cymene (CL<sub>50</sub> =121.6 µg mL<sup>-1</sup> air), D- limonene (CL<sub>50</sub> =187.5 µg mL<sup>-1</sup> air), and b-pinene (CL<sub>50</sub> = 210.2 µg mL<sup>-1</sup> air).

Our work demonstrated that essential oil from *T. trifolia* has the potential for the development of natural biocides.

## 2.P-Tu-007

### **Biochar From Soybean Straw Reduces Heavy Metals Mobility in Contaminated Agricultural Soils While Enhances Its Quality**

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#### **Abstract**

The use of biochar (BC) as an organic amendment to remediate soils contaminated with heavy metals (HMs) is projected as an efficient, profitable and ecological remediation tool, since it is obtained from residual organic matter and improves soil quality due to its capacity to immobilize HMs, improve physical-chemical properties and favor the development of microbiological communities. These properties depend on the BC production conditions and the raw material used.

The aim of this work was to evaluate the effect of BCs produced at different temperatures on agricultural soils contaminated with HMs. For this purpose, BCs produced from soybean straw at 300 °C (BCI), 400 °C (BCII) and 500 °C (BCIII) were used. The contaminated agricultural soils (CAS) were collected in the vicinity of an open dump of industrial waste in the province of Córdoba, Argentina, and presented elevated concentrations of Pb, Cd, Cu and Zn. The collected soils were dried, homogenized and distributed in 7 treatments (n=8): Control; CAS with no BC; CAS amended with BCI; CAS amended with BCII; CAS amended with BCIII; Mixture; and Mixture amended with BCIII. The "mixture" treatments correspond to CAS soils with a 10 % addition of proximate soils which present a higher degree of contamination. This mixture was performed to simulate the future dispersion of the HMs. All the soils were incubated in darkness at 65% water-holding capacity with samples from each replicate being collected every 3 months.

The response variables determined for all samples were: concentration of Pb, Cd, Zn and Cu in five soil fractions through sequential extraction (I, II, III, IV and V), with fractions I and II being considered as bioavailable; soil physicochemical parameters (pH, electrical conductivity (EC), organic matter content (OM)); general microbiological activity by hydrolysis of Fluorescein Diacetate (FDA).

The results revealed that BC application significantly reduced the HMs concentration in the bioavailable fractions compared to CAS and increased the physicochemical parameters (pH, EC, % soil OM and WRC). There were no significant differences among the treatments with different BCs. Microbiological activity was not affected by BCs application.

With these results we can conclude that, in the short term, the use of BCs produced from soybean straw is efficient for HMs immobilization as well as for improving the physicochemical properties of agricultural soils.

## 2.P-Tu-008

### Are the Problems Involving Acephate Already Resolved? a Scientometric Review

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#### Abstract

Acephate is an organophosphate pesticide used to control agricultural and domestic pests. Due to its toxicity, its use is restricted in several countries, such as the European Union. Acephate performs the bioactivation of metamidophos, a highly toxic metabolite that inhibits the enzyme acetylcholinesterase in the nervous system of target and non-target organisms. As the persistence of acephate in the soil is moderate, it increases the probability of contamination in groundwater and aquatic environments, compromising the flow of energy and the health of the ecosystem. Studies are reporting the toxicity of this pesticide in microorganisms, invertebrates, fish, birds, and mammals. In mammals, acephate contamination can cause neurocognitive, reproductive system, and DNA damage, in addition to neurodegenerative and respiratory diseases and cancer. The objective of this study was to carry out a scientometric analysis on the state of the art of acephate, aiming to analyze trends and identify gaps for better targeting of future research. The search was carried out in the Web of Science database, selecting all databases and all years until 2021. The search term used was TS= acephate, resulting in 1,993 records, after refinement, 1,042 documents were used. The dataset was analyzed in Microsoft Excel and CiteSpace software. The countries that most published about acephate were the USA, China, India, Brazil, and Japan, concomitantly they are world powers in agricultural production. When checking the research objectives of the documents, it was observed that the main ones were to evaluate the effectiveness of acephate, its toxicity, and its analysis methodology. Keywords show us the search trends on the subject. Pesticide, toxicity, residue, and pesticide residue were the most used keywords. It was possible to identify gaps regarding the lack of studies related to chronic toxicity, genotoxicity, and cytotoxicity. In addition, a scarce number of studies on metabolic and biochemical pathways, and genes related to the action and biodegradation of the pesticide were observed. With the global analysis, we noticed that many questions about acephate still need to be clarified. Therefore, it is suggested that future studies investigate the pesticide's mechanism of action and defense pathways, as well as toxicity at different levels of cellular organization and efficient degradation methods.

## 2.P-Tu-009

### Environmental Risk Assessment: How Precision Application Could Contribute and Be Beneficial

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#### Abstract

Agriculture as a whole and specifically farming practices are currently undergoing a major transformation globally focusing on the sustainable use of crop protection products as an important component of the paradigm change. This transformation is expected to be based on technological advancements and innovations, many of them related to digital farming. Emerging technologies such as satellite imagery, data analytics, sensors, drones/UAVs (unmanned aerial vehicles), robotics, and artificial intelligence are key components. For weed management (e.g., post-emergence application of herbicides) remotely gathered data by UAVs can be used by equipping ground sprayers with a dedicated patch spraying setup or can be paired with a targeted UAV application. In fact, many precision application technologies are already used by growers without the use of digital farming approaches (e.g. band treatment, drip or drench applications). Overall, digital farming solutions can help farmers to apply the right amount of the right pesticide, at the right time, in the right place, allowing to reduce the volume of pesticides used. Accordingly, precision application has financial benefits for the farmer but is also expected to be beneficial for the environment by preserving biodiversity and fostering sustainability (e.g., less drift, run-off, and groundwater entry). Data gathered by digital farming and precision application can be useful to inform the risk assessment of the future.

Our presentation aims at better understanding precision application options and tools. We will be focusing on the increasing knowledge about the positive environmental contributions of precision application and on how to find ways to address them within the risk assessment schemes of the future. Current global standard risk assessment are mostly not fit-for purpose to assess the risks resulting from precision application methods. Indeed, the approach is unrealistic, sometimes resulting in over-protective risk mitigation measures. Related to this, the options explored are: (i) a harmonized inclusion of precision applications in the current risk assessment and (ii) developing a field specific risk assessment/ on-field risk diagnosis approach in order to leverage the benefits into the risk assessment. Awareness will be raised for a critical assessment of precision ag-related benefits (e.g., which areas/organisms may face a change in exposure/effects under realistic conditions).



## 2.P-Tu-010

### Meta-Analysis of the Effects of Atrazine on Animal Mortality

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#### Abstract

Atrazine (ATZ) is one of the most widely used herbicides in the world, due to its high accessibility and effectiveness in controlling weeds, being widely used in corn and sugarcane plantations. Applied directly to the soil or sprayed on crops, it is also frequently detected in surface and groundwater due to its long half-life; thus, it can progressively accumulate in environmental compartments and affect non-target organisms. The aim of the present study is to evaluate the lethal effects of ATZ in different animal species, based on a meta-analytical review addressing studies with environmental concentrations of ATZ. The search was carried out in the Web of Science, Scopus and PubMed databases and after the selection process was completed, 49 datasets were generated and analyzed. The global effect indicated a significant increase in the mortality rate recorded for animals exposed to ambient concentrations of ATZ compared to the control in the experiment (non-exposed animals). The segregation of animals into taxonomic categories showed that amphibians, fish and insects had higher mortality rates after exposure to the investigated substance. The present meta-analysis indicated that in the life stages, larval and juvenile animals were more susceptible than adult individuals and embryos exposed to different concentrations of ATZ. Organisms that were exposed to ATZ by immersion showed an increase in mortality. The animals submitted to the commercial and pure formulation also had a higher mortality rate. Thus, ATZ can indeed increase the mortality rate in animals, but it depends on the sensitivity of the species, the life stage and, the way of exposure. This is the first meta-analytical study evaluating the mortality rate after ATZ exposure in various animal species.

## 2.P-Tu-011

### Evaluation of Pesticides in Conventional Agricultural Soils With Controlled Applications and Their Scope to Neighboring Agroecological Systems. Case of INTA, Oliveros, Santa Fe

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#### Abstract

Productive activities in Latin America have expanded accompanied by the technological package based on GMOs, direct sowing, and the use of agrochemicals in an increasing way. Agroecological systems emerge, as an alternative for sustainable production, on account of the environmental, social, and productive problems caused by the conventional system, since they promote technologies based on processes without the use of pesticides. However, as being geographically surrounded by conventional practices, they are often affected by the entry of pesticides from neighboring fields. In this context, the objective of this study is to evaluate the presence and concentration of pesticides in soils with an agroecological approach, in coexistence with the conventional system, under Integrated Pest Management (IPM). The study sites of both systems are located in the Agricultural Experimental Station of INTA Oliveros, in Santa Fe province, Argentina. Soils of 35 sites were sampled, 20 from agroecological system fields and 15 from conventional ones. 46 pesticides were analyzed, 24 were herbicides and metabolites, 13 were insecticides, and 9 were fungicides. Glyphosate (GLI) and its environmental metabolite AMPA, were extracted by employing a borate buffer and derivatized with FMOC-Cl, all other pesticides were extracted by QuEChERS methods, and analyzed by LC-MS/MS. In the agroecological samples only 1 fungicide was detected (<LQ), and for insecticides two analytes were found with a concentration <2µg/kg; while for the conventional sample soils, 9 analytes of these two pesticide families were detected. In the case of herbicides and metabolites, GLI, AMPA, atrazine, and hydroxy atrazine, were the analytes with the highest detection frequency and range concentration in both agroproductive systems. For example, detection frequency for AMPA was >90% in both system soils, the median and maximum range (µg/kg) were 11.54-52.59 in agroecological soils, and 187.61-503.13 for conventional samples. These results show the entry of pesticides into soils with agroecological production, surrounded by conventional fields with controlled applications, which can generate an adverse impact on the structure and function of the agroecosystem. This information should be taken into consideration in future studies focused on agricultural biodiversity, a fundamental pillar of agroecology, due to the importance of biological control and nutrient recycling.

## 2.P-Tu-012

### Atrazine Bioconcentration in *Cnesterdon decemmaculatus*

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#### Abstract

The pollution of the aquatic environments due to the agricultural expansion is a growing concern since the last few decades. Many pesticides have been banned as a result of numerous studies showing its toxicity and persistence. Atrazine is an herbicide from the triazine family that has been proven to cause endocrine disruption, malformations and mortality in different aquatic organisms. Even though it has been prohibited by the European Union, it is still used by several countries, including Argentina, and it can be found in multiple water bodies. *Cnesterodon decemmaculatus*, also known as ten spotted live-bearer, is a fish that can be commonly found in aquatic environments, and it is used as a test species due to its sensitivity and availability. The goal of this study was to evaluate the bioconcentration of atrazine in *C. decemmaculatus*. To achieve this, semistatic standardized laboratory bioassays were carried out on adult fish, which were exposed to 4 concentrations between 0.5 and 10 mg active ingredient (a.i.)/L of a commercial formulation of atrazine (Atramy 90 WG<sup>®</sup>) for a subchronic period of time (336 h). Each treatment was performed by triplicate and simultaneous controls with dechlorinated water were maintained. Once the exposure period finished, the organisms were sacrificed, homogenized and processed to measure the concentration of herbicide in their tissues using HPLC coupled with a tandem quadrupoles mass spectrometer. Atrazine levels in the control group were below the detection limit. Within the exposure range, the concentration of herbicide in the tissues increased with the exposure concentration, showing a significant growth in 10 mg/L regarding the lowest treatment (0.5 mg/L). The coefficient between the amount of atrazine in the tissues and its availability in the exposure tanks was higher than 1, meaning that the organisms kept incorporating the contaminant with each water renewal. Further studies must be held in order to be able to determine the bioconcentration factor, which can later be used to establish a biomagnification potential. This is relevant because the accumulation of pesticides in lower levels of the food webs can impact and biomagnify in upper levels, causing a risk for the populations of the aquatic organisms.

## 2.P-Tu-013

### **Pesticide-Induced Alterations in Zebrafish Behavior, Histology, and Gene Expression: An Integrated Approach**

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#### **Abstract**

Pesticides, after being applied, are distributed in the environment and can be retained, degraded and/or transformed, or transported outside the application site. Chronic exposure of organisms to low concentrations of these compounds can have cumulative sublethal effects, influencing metabolism, fitness, and reproductive success, hence producing effects at the population level. The impact of pesticides on natural populations is often underestimated or unknown. To evaluate the effect of the herbicides glyphosate and 2,4-D and the insecticide imidacloprid, individually and through the mixture of them, under environmentally relevant concentrations, adult zebrafish (*Danio rerio*) were used. Bioassays were performed exposing the animals for 96 hours with daily changes of the solutions. Analysis of the behaviour, as well as histology of the gills and expression of genes involved in the pathways of oxidative stress and cell apoptosis were performed. In behavioral studies, it was found a significant lower mean swimming speed ( $V_{med}$ ) in animals exposed to the mixture of products, compared to the controls. The histological study showed significant differences in the tissue structures. Exposure to imidacloprid [20%] produced mild (G1) lesions, whereas to glyphosate [0.5 mg/L] it produced moderate (G2) to severe (G3) lesions. Regarding the analysis with the herbicide 2,4-D [0.8 mg/L] and the mixture of all the compounds, severe lesions were observed (G3). Gene expression studies showed an overexpression of the genes involved in the oxidative stress and the cell apoptosis pathways, while in all cases there was evidence of a decrease in the expression of the *ogg1* gene (involved in the DNA repair). These results indicate that the pesticides cause strong behavioral, morphological, and molecular changes, being able to trigger cell apoptosis, in the gills of zebrafish exposed to environmental concentrations. There is a clear need to continue this type of research on the ecological consequences of these compounds in the environment.

## 2.P-Tu-014

### Are the Recommended Concentrations of Metals in Rice Sufficiently Protective?

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#### Abstract

The contamination of food with heavy metals is a growing problem. Heavy metals can cause a range health effects, including cancer. Rice is one of the main sources of food around the world. It is widely consumed in some countries in Asia, and consumption in Latin America is also high. Contamination of rice with arsenic, cadmium and lead has been reported in different countries. To protect human health the FAO/WHO has set maximum allowable concentrations for different metals. In the current study, the presence of lead (Pb), cadmium (Cd) and arsenic (As) in rice in Colombia was determined, and both cancer and non-cancer risks were calculated. Although the concentrations of these three metals in rice were below the maximum allowable limits recommended by FAO/WHO, the threshold for non-cancer risk for both As and Cd was exceeded for children, and the recommended limit for cancer risk was also exceeded for the general population, raising the question if whether the FAO/WHO recommended limits adequately protect health. In the case of Pb, the results for most samples were below the detection limit, and the health risk could not be assessed because of the lack of reference values in the literature. Thus, a different analytical technique may be needed to better understand the health risk resulting from the presence of Pb in rice.

DRAFT

## 2.P-Tu-015

### Poultry Litter Land Application: Does This Practice Represent a Threat to Hydric Resources? A Preliminary Study on Poultry Litter Leachate

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#### Abstract

During chicken meat production, a residue known as poultry litter (PL), composed of bedding material (rice and peanut husks, wood shavings) and bird dejections, is generated. After the animal's growing period has finished, this material is piled outside the breeding sheds until it is removed by agricultural producers, who use it as fertilizer since it is rich in nutrients and organic matter. These residues contain inorganic pollutants, and their land application can lead to the accumulation of these elements in the soil. In addition, due to the action of rain, leachate could be generated that would allow the entry of these pollutants into different water resources. This work aimed to: i) generate PL leachates at a laboratory scale, testing different pH and time extraction; ii) evaluate the levels of Li, Be, B, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, Ag, Cd, Sb, Ba, Hg, Tl, Pb, Bi, and U in these leachates; and iii) compare the content of elements in the residues before (PL) and after (EPL) aqueous extraction. Leachates (L) were generated from an aqueous extraction using 1 g of PL, 10 mL of H<sub>2</sub>O, and stirring at room temperature. Two pH (4.5 and 6.5) and 4 extraction times (1, 2, 4, and 24 h) were evaluated. PL and EPL samples were digested with a mixture of H<sub>2</sub>O<sub>2</sub>, HCl, and HNO<sub>3</sub>. All the determinations were made through Inductively Coupled Plasma Mass Spectrometry. Be, Bi, and Tl were not detected in any of the evaluated matrices. Although the content of all the elements analyzed in the L samples was significantly higher than the content present in the control water, no significant differences were observed at the different pH values evaluated ( $p > 0.05$ ). However, differences were observed due to the extraction time. The metal content in the L samples increased as the extraction time increased for most elements analyzed. The contents of B, Na, Mg, K, Ni, Rb, and Mo presented significant differences between PL and EPL samples, being higher in PL samples ( $p > 0.05$ ). These results show that water is an agent capable of extracting these contaminants from the PL matrix. Thus, the pile-up or application of PL as fertilizer represents a potential source for the entry of inorganic pollutants not only into terrestrial ecosystems but also into underground or surface water resources. Therefore, further studies about the behavior of these pollutants in these complex matrices are needed to propose safer disposal methods for these residues.

## 2.P-Tu-016

### **Morphological, Histochemical, and Morphometric Analysis of the Sublethal Effect of Fipronil on Cells of the Hepatonephrocytic System of *Bombus atratus***

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#### **Abstract**

Fipronil is an insecticide that targets postsynaptic neuronal receptors with inhibitory action, causing damage to all cognitive abilities in bees. The effects may impact *Bombus* bees, a species susceptible to environmental stress. Thus, the work analyzed the action of the pesticide fipronil at an estimated field dose of 2.5 ppb to 3.5 ppb on the cells that make up the hepatonephrocytic system (HNS) of *Bombus atratus* workers. The HNS cells analyzed were pericardial cells, trophocytes, and oenocytes. *B. atratus* were collected and taken to the laboratory under standardized conditions (26°C, 70% relative humidity) for 96 hours. Bioassays were performed, offering sucrose solution (70%) to the control and experimental groups. Concentrations of 2.5 ppb and 3.5 ppb of fipronil were offered to the exposed group. Morphological, morphometric, and histochemical analyses were performed. Results of morphological analysis indicated that pericardial cells and trophocytes were highly impacted by fipronil. The pericardial cells showed nuclear pyknosis, and the trophocytes showed vacuolization of the cytoplasm and chromatin defragmentation. Oenocytes were the cells least affected by the contaminant. Histochemical analysis demonstrated that pericardial cells and trophocytes were again the most affected, with exposure of 3.5 ppb leading to the formation of reddish or opaque nuclei, indicating chromatin fragmentation. Morphometric results indicated that there was a difference between the pericardial cells between the control group and those exposed to 3.5 ppb, and between the groups exposed to 2.5 ppb and 3.5 ppb. Regarding trophocytes, there was a difference in area between the control group and those exposed to 2.5 ppb and 3.5 ppb. However, there was no difference between those exposed to 2.5 ppb and 3.5 ppb. Regarding oenocytes, there was a significant difference between the control areas and those exposed to 2.5 ppb and 3.5 ppb; however, there was no difference between the groups exposed to 2.5 ppb and 3.5 ppb. The research concluded that the concentrations of fipronil found in the field cause damage to HNS cells, and have the potential to contribute to the decline of bee populations of the genus *Bombus*.

## 2.P-Tu-017

### Evaluation of the Biochemical and Genetic Effects of Organic and Conventional Diets: A Randomized Clinical Trial Focusing on Insecticide Exposure

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#### Abstract

Insecticides are a group of pesticides widely used around the world to control insects in crops and to control vector-borne diseases in public health. Therefore, it is necessary to understand the relationship between exposure and health effects, including biochemical and genetic effects. The purpose of this study was to conduct a Randomized Clinical Trial (RCT) to analyze the health effects of dietary exposure in healthy adults. A double-blind RCT was conducted with a 14-day intervention involving a group of conventional food consumers and a group of organic food consumers. Participants' urine was collected for quantification of insecticides and blood for assessment of effects by biochemical markers and DNA damage at the beginning and end of the intervention. The 148 study participants were evenly distributed between the conventional (n=70) and organic (n=78) groups. Initially, 24 insecticides were detected in the urine of the participants and, after the intervention period, there was a reduction in both groups: conventional (32.5%) and organic (89.5%). Specifically, pyrethroid insecticides decreased from 4.6µg/L to undetectable levels in the organic group and increased from 0.18µg/L to 0.34µg/L in the conventional group. After the intervention, all biochemical markers were within established normal limits. Genetic damage was observed for both groups after the intervention, and the percentage of DNA damage repair by BER and NER mechanisms was not statistically significant for either group after the intervention. The data obtained in this study suggest that the predominant consumption of food obtained by conventional cultivation may be associated with higher urinary levels of insecticides, mainly pyrethroids. Long-term intervention studies are needed to determine dietary exposure to pesticides and ensure the Food and Nutritional Security of the population.



## 2.P-Tu-018

### Acute Toxicity of the Herbicide Clomazone to the Apple Snail *Pomacea* sp.

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#### Abstract

Clomazone is a herbicide used in the control of grasses and weeds in rice crops. In Uruguay, is used generally in combination with glyphosate and others agrochemicals. Pesticides applied in crops are transported by runoff to nearby water-bodies, affecting the associated fauna. The apple snail *Pomacea* sp. inhabits freshwater bodies in Uruguay, it is an invasive species in USA and it is used as bioindicator. The aim of this study was to assess acute toxicity of Clomazone in the apple snail. Organisms between 4 and 10 g were exposed individually to 3,75, 7.5 and 15 mg/l (nominal concentrations, treatments A, B, C respectively) of Clomazone for 96 hours, at temperatures between 18.4 and 19.8°C. Each treatment had 10 organisms used as replicates. Mortality at the end of the trial was 0 in control and treatment A, 1 in B and 5 in the C treatment. Behavior of the snails was registered daily and oxygen consumption of 3 organisms was registered at the beginning and at 48 hours which suggested that sub-lethal effects are present at all Clomazone concentrations. The 96-h LC50 of Clomazone is near 15 mg/l; we suggest that the apple snail *Pomacea* sp. is a good bioindicator species for studies of risk assessment in freshwater ecosystems.

DRAFT

## 2.P-Tu-019

### Determination of Acute Ecotoxicity of the Herbicide Dicamba for Zebrafish (*Danio rerio*)

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#### Abstract

The zebrafish (*Danio rerio*) is an excellent biological model for ecotoxicological assessment of pollutant effects and for environmental risk assessment. Thus, the aim of this study was to determine the acute ecotoxicity (LC50;48h) of dicamba for zebrafish (*D. rerio*). For this purpose, the fish were acclimatized in a bioassay room with a temperature of 25.0 to 27.0 °C and a photoperiod of 12 hours of light, for ten days, in a 250.0 L box, with a continuous aeration system promoted by a blower. of air and fed, once a day, with commercial feed with 28% of crude protein. For the quality control of the batch of fish, sensitivity tests were performed (LC50; 48h) with the reference substance potassium chloride (KCl), with a purity content of 99.9%. The evaluated concentrations were: 0.01; 0.10; 0.50; 1.00; 1.55 and 2.44 g L<sup>-1</sup> and a control (0.0 g L<sup>-1</sup>), with LC50;48h of 1.26 g L<sup>-1</sup> without confidence interval. Next, the assay was performed with dicamba, at concentrations of 0.1; 0.32; 1.05; 3.43; 11.15; 36.25 and 117.8 mg L<sup>-1</sup> (dilution factor 3.25 mg L<sup>-1</sup>), with three replicates and three fish per concentration and one control (0.0 mgL<sup>-1</sup>), at a maximum density of 1, 0 g L<sup>-1</sup>, in static system. The evaluation of mortality and water quality (electrical conductivity (EC), dissolved oxygen (O.D), temperature (T) and hydrogen potential (pH)) was performed at 24 and 48 hours after exposure with the removal of dead organisms from the containers . The 50% lethal concentration (LC50;48h) was > 117.84 mg L<sup>-1</sup> and the herbicide was classified as practically non-toxic to zebrafish (*D. rerio*). After 48 hours of exposure, there was no mortality at any of the tested concentrations and there was no change in the water quality standard with T of 25.40 ± 0.24 °C, O.D of 4.59 ± 0.25 mg L<sup>-1</sup>, C.E of 250.0 ± 20.0 μS cm<sup>-1</sup> and pH of 7.64 ± 0.25. Thus, it can be concluded that *D. rerio* cannot be used as a bioindicator organism for dicamba, as its ecotoxicity response was low, indicating that this fish is not sensitive to the herbicide.

## 2.P-Tu-020

### Ecotoxicological Effects of Rice Husk Ash on Seed Germination: Implications for Soil Health and Agriculture

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#### Abstract

The rice culture (*Oryza sativa*, L.) has high demand, playing an indisputable role in providing staple food in both the Brazilian and global scenarios. The grain is a daily dietary staple for over 3.5 billion people, corresponding to 19% of global food energy. The State of Rio Grande do Sul is the largest Brazilian producer, accounting for almost 71% of the crop. The rice processing generates waste, including rice husks, which represents 20% of the grain weight, equivalent to approximately 1.8 million tons per harvest. Improper disposal of rice husk waste releases harmful greenhouse gases like methane and carbon monoxide. Husks are mainly burned for thermal energy, leaving behind only ash. Due to its easy access and viability, its incorporation into agricultural soils is common, due to the benefits this practice brings, such as soil pH correction, reducing the risk of compaction, and it can also reduce the occurrence of fungi in crop of interest. Silicon dioxide is the main component of rice husk ash. Despite demonstrated benefits of adding it to soil for crop performance, few studies have assessed potential effects on non-target organisms. Therefore, this research aims to evaluate the ecotoxicological effects of rice husk ash through germination tests of two plant species, lettuce (*Lactuca sativa*) and arugula (*Eruca sativa*). The rice husk ash was collected from a rice processing mill in the municipality. In this mill, rice husks are burned in a boiler that supplies the rice parboiling process. The germination assay was performed by exposing the seeds of both plant species to different concentrations of rice husk ash elutriate, ranging from 100% to 3%, as well as positive (1% ZnSO<sub>4</sub>) and negative (distilled water) controls. The parameters evaluated were radicle length (R) and hypocotyl length (H) elongation. Two pilot germination assays were conducted. Preliminary results indicated a 15% and 18% reduction in radicle length at concentrations of 100% and 75% elutriate, respectively, compared to the negative control in the lettuce seed assay. In the assay with arugula, inhibition of radicle elongation was observed at a 25% elutriate concentration. No differences were observed in hypocotyl elongation. Soils are of utmost importance to the agricultural sector and provide essential environmental services, therefore, their conservation and preservation are of great economic and environmental importance.

## 2.P-Tu-021

### Application of the True Diversity Index in an Agroecosystem for the Evaluation of Food sustainability

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#### Abstract

Cochineal agroecosystems play a crucial role in agriculture in Mexico and other countries, where the cochineal insect is cultivated to produce natural red dyes. Grana cochinilla, also known as cochineal or carmine, is a natural dye obtained from the cochineal insect (*Dactylopius coccus*), which is native to Mexico and Central America. As consumers increasingly seek out natural and healthier products, cochineal can provide an advantage as a natural alternative to artificial colorants. One benefit of grana cochineal is its resistance to heat and light, making it an ideal choice for foods that are exposed to high temperatures or stored for extended periods. However, sustainable agricultural practices are necessary to maintain the health and productivity of the agroecosystem. The vegetation of a cochineal farm in Oaxaca was measured using manual methods over a 30-year period, applying the true diversity index to compare changes in diversity over time, providing valuable data for farmers and stakeholders to make informed decisions about sustainable agricultural practices. By reducing water use and promoting biodiversity, sustainable development can be achieved while preserving traditional knowledge and cultural practices associated with cochineal cultivation. Overall, the use of grana cochineal in food production can promote sustainability by reducing the reliance on artificial colorants and encouraging the use of sustainable agricultural practices that protect natural resources and traditional cultural practices.

## 2.P-Tu-022

### Acute Toxicity of Mining Environmental Waste on *Bulnesia retama* and *Plectrocarpa tetraantha* Seedlings

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#### Abstract

Native species of shrubs from arid environments disperse seeds in the surroundings of sites contaminated with mining waste. During the germination period and the first days of seedling development, numerous physiological processes occur in which the presence of toxic substances can interfere with the plant survival and normal development. The objective of this study was to evaluate the acute toxicity of soils contaminated with mining waste on seeds of *Bulnesia retama* (*Br*) and *Plectrocarpa tetraantha* (*Pt*), two native species from the Monte region (La Planta, San Juan, Argentina). Seeds were collected in the surroundings of La Planta and soil samples were taken in two sites: contaminated (S1) and reference sites (S2). Seeds were exposed to 6 increasing concentrations of soil mixtures from Site 1 and 2: 0, 10, 25, 50, 70, and 100% (v/v), where 0 corresponds to S2 soil and 100% to S1 soil. Experimental unit consisted of 20 seeds placed on wet soil into a Petri dish, reaching a total of 5 repetitions per treatment. A pre-germination treatment was applied to the seeds to ensure the seedling emergence. Toxicity tests were carried out in a germination chamber in darkness at 25±2 °C. Due to differences in germination time of each species, exposure of *Br* and *Pt* lasted 12 and 7 d, respectively. Mean Germination Time (MGT), Metal Tolerance Index (ITM), Germination Index (GI) and IC50 of root (r) and hypocotyl (h) length were estimated. For *Br*, a significant decrease was observed from 50% S1 concentration in ITMr, ITMh, and GI ( $p < 0.001$ ). The estimated values of IC50r and IC50h in *Br* were 14.7 and 28.4%, respectively. For *Pt*, seed germination inhibition of 100% was observed in 100% S1 concentration. Statistical differences were observed between the values of ITMr from 50% concentration of S1, and the values of ITMh and GI from 25% S1 concentration ( $p < 0.001$ ), while MGT showed no significant differences. The estimated values of IC50r and IC50h in *Pt* were 16.1% and 21.9%, respectively. In both species, no differences between MGT values were observed ( $p > 0.05$ ). The values of the toxicity endpoints, the phytotoxicity indexes and metal tolerance index showed that these native species are more tolerant to contaminated soil of La Planta than horticultural species evaluated in previous studies (IC50r < 1% S1 concentration). In future studies, chronic effects should be evaluated to determine the underlying physiological mechanisms of the metal(loid)s tolerance in *Br* and *Pt*.

## 2.P-Tu-023

### Evaluation of the Effects of Oral Exposure of Stingless Bees and Honey Bees in the Larval Stage to Lambda-Cyhalothrin and Spinetoram

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#### Abstract

Brazil has the greatest diversity of bees in the world and makes intensive use of pesticides. Therefore, our aim was to evaluate the effects of Lambda-Cyhalothrin and Spinetoram at realistic field concentrations (FC) on larvae of the native species *Scaptotrigona postica* compared to honeybees exposed through a contaminated diet. The bioassays were carried out using the in vitro larval rearing method for both species. The tests were conducted with two concentrations, namely "maximum FC" and "minimum FC", both recommended by the product. To estimate the maximum and minimum FC, we followed the recommendations in our country for application on citrus crops and plotted the values in the BeeRex table. The reference item "Dimethoate" was used and the mortality reached the expected level. The control group showed no mortality during the entire test period. From the analyses carried out, it was observed that both the "maximum FC" of both AIs were statistically significant when compared to the control, with a mortality rate of 25-29% for honeybee larvae and 31-35% for stingless bee larvae. Extrapolating this scenario to the field could have serious consequences for the bee population in general. Weakened bees in every generation could, in a short time, cause the death of the entire colony. Thus, it is essential that public policies for bee protection be periodically reviewed and discussed by academia, industry, and government.

## 2.P-Tu-024

### Evaluation of the Effects of Contact and Oral Exposure of Stingless Bees and Honey Bees to Lambda-Cyhalothrin and Spinetoram

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#### Abstract

Among pollinating insects, bees are known to stand out as pollinators in natural environments and agroecosystems. In Brazil, in addition to the exotic species *Apis mellifera*, we have species of stingless bees, among them *Scaptotrigona postica*. In Brazil, there is intensive use of pesticides, therefore, our objective was to evaluate the effects of Lambda-Cyhalothrin and Spinetoram at maximum and minimum realistic field concentrations (FC) on individuals of the native species *Scaptotrigona postica* compared to honeybees. Two distinct tests were performed, the first test exposing the bees through a contaminated diet (OECD 213) and the second exposing the bees through contact (OECD 214). The doses used in the tests were named "maximum FC" and "minimum FC". In both tests, the reference item "Dimethoate" was used, and mortality reached the expected value of over 50%. Both control groups did not show mortality throughout the test period. From the analyses performed, it was observed that at both tested concentrations there was statistical significance when compared to the control, the same was observed for the oral and contact tests. This shows that even at doses considered safe for use in the field, there can be mortality in individuals, which can lead to an imbalance in the colony of both species. Therefore, we can consider that the results brought in this study can be useful in monitoring bees to elucidate the action of pesticides on these pollinators, and help with studies for risk assessment, contributing to the generation of adequate information to support conservation strategies for these species.

## 2.P-Tu-025

### Global Trend and Prospects in Imidacloprid Research: A Scientometric Review

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#### Abstract

Imidacloprid is a widely used insecticide that is commonly used to control pests in various crops, as well as in ornamental plants, turf, and home gardens. However, due to its potential impacts on human health and the environment, particularly its effects on non-target organisms such as bees and other pollinators, imidacloprid has been the subject of extensive research. This scientometric analysis aimed to provide an overview of studies that detect and analyze the effects of imidacloprid on biota, using scientific publications obtained from the Web of Science™ with the keyword "imidacloprid" as the search topic (TS), without language or year restrictions. Selected articles were manually screened for titles, abstracts, and materials and methods, and further analysis was conducted using Microsoft Office Excel, VosViewer, and R programs with the 'Bibliometrix' package. The analysis included a total of 5,221 articles authored by 15,246 authors from 113 countries. These articles were cited a total of 127,486 times, with an H-index of 122, indicating a significant impact factor for this research topic. The findings of the analysis revealed that in the first decade of research, the focus was on the mode of action and efficacy of imidacloprid against different groups of insects. However, in the second decade, the keyword "toxicity" emerged as a prominent topic, with studies investigating the presence of imidacloprid in pollens and its bioavailability to bees in the field, particularly at concentrations that correspond to sublethal effects on bees. Other frequent topics included toxicity, pesticides, and exposure. This increased concern about imidacloprid's impact on pollinators may have contributed to the adoption of severe restrictions by the European regulatory body in 2013 and the subsequent ban on the use of this active substance in open areas in May 2018 due to its adverse effects on bees. The study emphasizes the need for continued investment in research and technologies to better understand the effects of imidacloprid on non-target organisms, both in isolation and in association with other pesticides, as imidacloprid is often used in combination with other chemicals. Furthermore, it highlights the importance of remediating the presence of imidacloprid and other pesticides in surface waters, soils, and sediments to minimize chronic exposure to plants, animals, and humans.



## 2.P-Tu-026

### Industry (UAPASTF) Response to "State of the Knowledge" Review of Unmanned Aerial Vehicle Use for Pesticide Application: Update on the Development and Field Testing of an Off-site Movement Protocol

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#### Abstract

The Organisation for Economic Co-operation and Development Working Party on Pesticides (OECD WPP) was formed in 1992 with one of its goals being to harmonize data and methods used to test and assess pesticide risks. Following a June 2019 seminar on “Evolving Digital and Mechanical Technologies for Pesticides and Pest Management”, the OECD WPP formed a Drone/UAV Subgroup that, in 2021, published a “state of the knowledge” report on pesticide application using UAVs[1]. Of the many recommendations from this report, one focused on “...a clear and urgent need for a set of standard testing protocols to be agreed upon for the assessment of UASS,” in order to ensure that any new data generated to describe off-site movement (i.e., spray drift) is of sufficient quality to draw appropriate conclusions on UAV applications. In response to this, the pesticide registrant industry formed the UAPASTF in Summer 2022. One goal of this task force was to develop a generic protocol that ensures the quality of data generation for assessing off-site movement and downwind deposition of pesticides applied by UAVs. This protocol has been completed in 2022. Input and comments by OECD member states and external experts were considered and incorporated into the protocol. A non-GLP field trial was initiated in early 2023 to test the protocol under field conditions, with GLP field trials currently being planned across regions including Latin America. In this presentation, we will discuss the protocol development and recommendations, results of protocol testing, ongoing field trial work, and the next steps for data generation from the UAPASTF.

[1]OECD (2021), Report on the State of the Knowledge – Literature Review on Unmanned Aerial Spray Systems in Agriculture, OECD Series on Pesticides, No. 105, OECD Publishing, Paris.

## 2.P-Tu-027

# Ciprofloxacin Residues in Irrigation Water and Their Effects on Alfalfa (Medicago Sativa) Carbon Fixation, Number of Root Nodules and Root Length

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## Abstract

### Introduction

Antibiotic compounds are ubiquitous in pharmaceutical, industrial and domestic industries effluents originating from wastewater treatment plants (WWTPs), due to inefficient treatment, resulting from wastewater discharges following conventional WWTP treatment, following transferral to water bodies. In this sense, municipal waste discharges and their use in crop irrigation comprise a significant concern, as they result in soil contamination. Negative effects in this regard have been reported for some plant species grown in CPX-contaminated soils. In this context, the present study evaluated the effect of irrigation using water containing CPX residues on alfalfa (*Medicago sativa*) carbon fixation, number of root nodules and root length.

### Methods

The experiment was conducted employing a complete randomized design consisting of 12 pots distributed in three treatments comprising three repetitions with three controls. About 4 kg of previously prepared soil were poured into 20 X 16 X 15 cm. Thinning was carried out in all treatments, leaving only one plant per experimental unit. Treatments were carried out in a vegetative growth chamber at the CENA/USP Ecotoxicology Laboratory.

Carbon determination in alfalfa leaves and stems

The ground leaf subsamples were placed in tin capsules and loaded into an Thermo Quest-Finnigan Delta Plus isotope ratio mass spectrometer connected to an elemental analyzer to determine total carbon contents, expressed as percentage (%). The method principle lies in the isotopic ratio mass spectrometry.

Root nodule determinations

After 40 days of seed sowing, the alfalfa plants were harvested to determine the number of root nodules. This process was carried out using plastic trays to separate the roots from the soil. Root nodule counts were performed under a cold light lamp using a magnifying lens.

Root length determinations

Plants were carefully separated from the soil in plastic trays and root lengths were measured with a ruler, from the beginning of the root (plant base) to the end.

## Conclusion

CFX in irrigation water reduces carbon fixation in alfalfa stems and leaves in up to 8.9%. This antibiotic also significantly reduces the number of alfalfa root nodules and root elongation, the latter inversely proportional to CFX concentrations.

DRAFT

## Session 4: Wildlife Ecotoxicology

### 4.P-Tu-029

#### Blue vs. Humpback Whales, Frenzy for Food or Contaminants? Trophodynamics of Mercury in Patagonia

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#### Abstract

Chilean Patagonia is considered a remote ecosystem, which is characterized by oceanographic, topographic and geological conditions that, together with the large contributions of fresh water in the sector (rain, melting of glaciers and rivers), allow the existence of a complex and dynamic marine ecosystem that produces one of the largest estuarine systems in the world, characterized by a high primary productivity that supports a great diversity of organisms of commercial and ecological importance. The northern sector of Chilean Patagonia is described as a feeding site for large cetaceans such as blue whales (*Balaenoptera musculus*) and humpback whales (*Megaptera novaeangliae*), due to the large existing populations of their main preys, krill (*Euphausia* sp.) and squat lobster (*Munida* sp.) respectively. Mercury (Hg) is a global pollutant of concern. It transforms to an organic form known as methylmercury (MeHg) in aquatic ecosystems, which is one of the most toxic forms of Hg due to its potential to bioaccumulate in tissues and its efficient trophic transfer through marine food webs (biomagnification), which implies a higher risk for species with higher trophic levels, particularly for cetaceans like baleen whales. The aim of this study was to assess the trophic interactions between two baleen whale species and different zooplanktonic organisms, and trophodynamics of Hg in *B. musculus* and *M. novaeangliae* in the Fjords of the Chilean Patagonia. Prey items were collected through net trawls and whales were sampled by collecting skin and blubber biopsies. Stable isotope analysis of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  helped to determine that krill was the main prey item for blue whales, and for humpback whales it was the squat lobster. Humpback whales also presented a higher trophic level than blue whales. MeHg was detected both in prey items and whale species, being significantly higher in whales than in preys. The biomagnification factor (BMF) calculated, indicate that diets generate a significant contribution of MeHg through biomagnification processes which was also supported by positive values of trophic magnification factor (TMF) that help us conclude that besides both whales are feeding in the same area, due to different diets, they accumulate different concentrations of MeHg.

## 4.P-Tu-031

### **Evaluation of Mercury Levels in South American Sea Lion Pups (*Otaria byronia*) During Their First Weeks of Life**

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#### **Abstract**

Mercury is a global pollutant and long-range. In Chile, numerous mines (mainly gold and copper) but also, other human activities are important sources of mercury (Hg). Accumulation of Hg in sediment on the bottom of the ocean and coastal ecosystems, in biota, and it is transferred to upper trophic levels. Marine mammals are useful bioindicator species for monitoring metal contamination in aquatic ecosystems because they are crucial for determining the potential risk of contaminated food consumption. Pinnipeds are excellent valuable indicators of pollutants accumulated in this environment due to their biological and ecological characteristics: their top position in the food chain, their long-life span and their capacity to accumulate high levels of chemicals., and as top predators. The bioaccumulation of this element could be a systemic toxicant and can induce organ damage, especially in young animals, due to prenatal and early exposure. The current study estimates the levels of Total Hg (THg) in blood samples of South American Sea lion (*Otaria byronia*, SASL) pups during their first weeks of live, in a SASL colony in Cobquecura using a Direct Mercury Analysis System (DMA-80evo, Milestone). Preliminary results showed a range of THg between 8,356 - 285,429 (mg/kg ww), with an average of 109,42± 61.56 mg/Kg ww of Hg. These results are higher to those found in serum from fur seal pups (*Arctophoca australis* ssp.) from Perú ( $1.8 \times 10^{-8} \pm 7 \times 10^{-9}$  mg/g ww), and to those found in kidney (0.15ug/g ww (0.7 dw ± 0.1)) and liver (0.57 ug/g ww (2.2dw ± 1.2)) in SASL from Chile. Our results confirm the early exposure of pups to Hg sources, and even the vertical transfer (intraplacental) of THg from mother to pup.

This research is possible thanks to Fondecyt 1221348 and Fondecyt 11200302.

## 4.P-Tu-034

### My Mom Gives Me Hg: Evaluation of Mercury Contamination in Fur Seal Pups (*Arctophoca australis*)

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#### Abstract

In Chile, numerous mines (mainly gold and copper) but also, other human activities are important sources of mercury (Hg), a global pollutant and object of long-range transport. The discharges from mines and metal-related industries flow into rivers and the ocean, where this metal accumulate in sediment deposits on the bottom of the ocean, in biota, and it is transferred from lower to upper trophic levels. Marine mammals, as pinnipeds, are useful bioindicator species for monitoring metal contamination in aquatic ecosystems because their biological and ecological characteristics (their top position in the food chain, their long-life span and their capacity to accumulate high levels of chemicals.). They are crucial for determining the potential risk of contaminated food consumption in remote and non-evaluated areas. In pinnipeds, the accumulation in high concentrations of Hg is toxic and can induce clinical disease or with neurological manifestations. The current study estimates bioaccumulation and compares the levels of Total Hg (THg) in blood samples of fur seal adult females and their pups during the first days of life, in the largest breeding colony of South American fur seals (*Arctocephalus australis*) during the breeding season (December to march, in Guafo Island, a remote oceanic island in the South of Chile. THg, was analyzed using a Direct Mercury Analysis System (DMA-80evo, Milestone). The main objective of this study is to try to evidence the horizontal transfer of this metal. Preliminary results in females showed a range of THg 618.24-15.65 mg/kg ww, with an average of 201.5 mg/kg ww. In pups, showed a range of THg between 358.77-6.54 mg/kg ww, with an average of 77.54 ± 6.71 mg/kg ww. These preliminary results are higher to those found in serum of fur seal (*Arctophoca australis* ssp.) from Perú (levels found in females 0.0028 ± 0.0021 ug/g ww, and 0.018 ± 0.007 ug/g ww in pups). Our results confirm transfer of THg from mother to pup. This research is possible thanks to Fondecyt 1221348 and Fondecyt 11200302.

## 4.P-Tu-036

### Reproductive Parameters Affected by Atrazine Exposure in Males of the Eared Dove (*Zenaida auriculata*)

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#### Abstract

Reproduction is fundamental for species survival and enables physiological processes, associated behaviors and structures involved in the next generation production. Thus, it is important to include reproductive endpoints when conducting ecotoxicology studies, taking into account the current global wildlife crisis. Atrazine (ATZ) is an herbicide of the triazine family widely used in the world. In Argentina, it is the second most used herbicide, associated with corn and sorghum crops. Its effects as an endocrine disruptor in amphibians are widely documented, with known cases of feminization and intersex gonads in exposed individuals. In birds, some effects on gonads size and circulating steroid hormones are known in ATZ exposed individuals, however, there are no studies involving bird native species. In this context, the aim of this study was to evaluate ATZ effects on reproductive tissue in males of the native bird *Zenaida auriculata*. Eighteen organisms distributed in three groups (0, 25 and 250 mg ATZ/kg) were assayed with 6 replicates each. ATZ diluted in corn oil was administered by intermittent gavage during 15 days, being administered only corn oil (without ATZ) in the control group (0 mg ATZ/kg). Once the experiment was finished, body mass of each organism was recorded and birds were dissected (prior anesthesia). Gonad mass was recorded for Gonadosomatic Index (GSI) determination. Subsequently, the testes were fixed in bouin solution, embedded in paraffin, and serially cut 6  $\mu$ m thick for histological analysis of the germinal epithelium. Seminiferous epithelium was classified in VII categories according cellular maturation and association, being stage I-VII of growing maturity. To contrast body mass and GSI between groups, one-way ANOVA was performed (Tukey post-hoc test,  $p < 0.05$ ). Data that failed to meet ANOVA assumptions were analyzed by Kruskal-Wallis test. Seminiferous epithelium stage VII were recorded only in 250 mg ATZ/Kg exposed birds, with mature stages of spermatid and residual bodies at the lumen of seminiferous tubules. In addition, GSI resulted higher in birds exposed to 250 mg ATZ/Kg ( $p < 0.05$ ) and no effects were observed in body mass ( $p > 0.05$ ) between treatments. These results suggest that there is an increase in germinal epithelium development in birds exposed to high concentrations of ATZ. Further studies evaluating steroid hormone levels in doves exposed to atrazine are needed to confirm an endocrine disruption effect.

## 4.P-Tu-037

### Pesticide Exposure Alters Lipid Contents in *Caiman latirostris* Egg Yolk

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#### Abstract

Lipid study is of special interest because they are essential components of biological membranes, extra and intracellular signaling processes, hormone precursors, signal transducers and amplifiers of regulatory cascades. Particularly, in crocodylians, lipids are the main source of energy for reproduction, growth, and embryonic development. Therefore, lipid homeostasis is crucial during the development of a healthy embryo. In Argentina, wild populations of *Caiman latirostris* are exposed to successive applications of pesticides used to optimize agricultural production. In the present study, composition of fatty acids (FAs), cholesterol (C) and tocopherols (T) concentrations were evaluated in *C. latirostris* yolk remainder at the moment of hatching after *in ovo* exposure to pesticide formulations widely used in extensive crops in Argentina. Pesticides were applied by spraying on nest material at the beginning of the incubation period, at concentrations applied on soybean crops for each of the compounds. Treatments were: negative control, spread with tap water (NC), a formulation of glyphosate (GLY; 2%), of 2,4-dichlorophenoxyacetic acid (2,4-D; 0.12%), of imidacloprid (IMI; 0.2%), of chlorantraniliprole (CAP; 0.03%) and a mixture (M) of all pesticide formulations at half concentration (GLY 1; 2.4D 0.06; IMI 0.1 and CAP 0.015%). The analysis of the composition of FAs in egg yolk showed the presence of 25 FAs, where polyunsaturated FA showed changes in its concentration in hatchlings exposed to GLY compared to the NC (Dunn Test  $p < 0.05$ ). In addition, the results showed a significant lower level of T in animals exposed to the M, compared to the NC and to GLY treatment (Dunn Test  $p < 0.05$ ). Finally, the levels of C in *C. latirostris* yolk remainder were significantly lower in hatchlings exposed to CAP with respect to NC; and CAP compared to the M (Dunn Test  $p < 0.05$ ). The present study provides information on the negative effect produced by pesticides on the lipid contents in egg yolk of a native caiman species of South America. These alterations could be crucial for the development of a healthy embryo, and in the long term, for the conservation of this species.

Keywords:– crocodylian – environmental contamination-embryonic development - metabolism



### First Assessment of Arsenic in Magellanic Penguin from Argentina

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#### Abstract

Arsenic (As) is one of the most important global environmental pollutants. Although human activities contribute little to the As increase of the open ocean, they may be important in estuaries and coastal waters receiving As-contaminated drainage from the land. Penguins have been used as monitors of the state of the environments. The Magellanic penguin (*Spheniscus magellanicus*) is the most important seabird as a tourist resource on the Argentine coast and many populations are affected by fishing activities, chronic pollution and global climate change. The aim of this study was to determine the concentrations of total As in liver, kidney and muscle of Magellanic penguins. During the years 2020 and 2021, 20 individuals were found dead on the beaches between Miramar and Mar del Sud in Buenos Aires province, Argentina. The quantification of total As was carried out by Atomic Absorption Spectrophotometry with a hydride generation. The limit of detection was 0.005  $\mu\text{g/g}$ . Data is expressed in  $\mu\text{g/g}$  wet weight. The detection percentage was 40% for liver and kidney and 70% for muscle. The As concentrations in the analyzed samples ranged from < 0.005 to 0.039  $\mu\text{g/g}$  for liver, < 0.005 to 0.190  $\mu\text{g/g}$  for kidney and < 0.005 to 0.20  $\mu\text{g/g}$  for muscle. In order to perform statistical analysis, a value equal to half of the detection limit was arbitrarily assigned for those samples that showed values below this limit. Mean As values in muscle and kidney tended to be slightly higher than those found in liver, but this difference was not statistically significant. A relationship was found between body weight and As concentrations in muscle ( $R^2=0.74$ ,  $p<0.01$ ), possibly indicating an accumulation of the metalloid in that tissue. No relationship was found between As concentrations in tissues and total length. The concentrations found in the internal tissues of the Magellanic penguin are low. This is to be expected given the mainly ichthyophagous diet of the species (priority item *Engraulis anchoita*) in its non-breeding season. It is important to highlight that this is the first report of As in the species, so it is necessary to continue monitoring this element over time in Magellanic penguins. It will also be interesting to know the dynamics of this element in other tissues such as feathers, which are usually a detoxification route for As in birds, or bone, which is an accumulative tissue.

## Persistent Organic Pollutants (POPs) Along A Marine Food Web From Patagonia

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### Abstract

Persistent organic pollutants (POPs), include polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OCPs), are toxic compounds that pose a serious threat to human health and the environment due to their persistence, bioaccumulation, and potential for long-range transport. They are regulated by the Stockholm Convention, which aims to worldwide reduce and eliminate their production, use, and release. There is an increasing concern about the presence of POPs in marine organisms because POPs can be accumulated in the food chain, potentially reaching high concentrations in top predators. The aims of this work was to study the POPs levels in a marine food web from a harbor area in Atlantic Patagonia and to understand the relationship of contaminants among trophic levels. This objective is part of a larger project that involve the evaluation of POPs in a trophic web that includes species of commercial interest. Fishes (*Pinguipes brasiliensis*, *Odontesthes smitti* and *Callorhynchus callorhynchus*) were obtained from recreational anglers at the harbor, while invertebrates (crabs, bivalves, echinoderms, polychaetes and gastropods) were collected by diving in the harbor area. Trophic levels and benthic-pelagic reliance were determined by stable-isotope ratios of Carbon and Nitrogen. POPs concentrations were determined by GC-ECD and reported as  $\mu\text{g}\cdot\text{kg}^{-1}$  wet weight. On average, POPs were most accumulated in fish liver (<LOD-120) with a bioaccumulation pattern PCBs>OCPs>PBDEs. In prey species (<LOD-110) the pattern was OCPs>PCBs>PBDEs. The main OCPs bioaccumulated were dioxins, endosulfans and DDTs. For PCBs the main compounds bioaccumulated were 4-CB, 5-CB and 6-CB. Stable isotope analysis showed a trophic level of 3.4, 3.2 and 3.1 and 65 %, 50 % and 75 % benthic dependence for *P. brasiliensis*, *O. smitti*, *C. callorhynchus* nutrition, respectively. These results are consistent with the known feeding habits and behavior of these fish and indicate that POPs are incorporated from the diet, mainly for crabs and gastropods. This work is part of the first record of POPs for edible species in the Patagonian area of Golfo Nuevo. This fact is relevant given the importance the harbor areas for local recreational anglers and potential risks associated with the consumption of contaminated seafood and environmental health. In this sense, monitoring of POPs in marine organisms is necessary to generate regulatory measures in relation to food safety

4P-Tu-032

## Potential Oral Exposure of the Antillean manatee (*Trichechus manatus manatus*) to Pesticides in an Estuarine System in the Caribbean Region of Costa Rica

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### Abstract

Agricultural activity is highly relevant to Costa Rican economy, which is associated with an intensive pesticide use, leading to environmental contamination of water bodies, specifically coastal marine ecosystems like Madre de Dios Lagoon (habitat of the herbivorous marine mammal Antillean Manatee), located in the Caribbean of Costa Rica. This estuarine system receives the influence of intensive agricultural practices, mainly banana plantations. The presence of pesticides in the lagoon poses a possible situation of chronic exposure of the manatee. Therefore, in order to contribute to a better understanding of the levels of exposure, the assessment of the manatee's potential oral exposure (intake of food and water) to pesticides was carried out by the implementation of an indirect method. Pesticide residue concentrations were determined in water and macrophytes samples (manatee's food) taken from the lagoon, subsequently, the average daily dose (ADD) was determined using an exposure model. Up to 22 and 31 pesticides were found in water and macrophytes samples (58% fungicides), primarily associated with banana plantations. The macrophyte species with the highest concentration of pesticides were *Eichornia crassipes*, *Ludwigia helminthorrhiza* and *Hydrocotyle ranunculoides*. The highest bioconcentration was found in roots (47.6%), followed by leaves (30.6%) and stems (24.0%). The ADD estimated was higher for food intake than for water intake, which reaffirms the ability of macrophytes to bioaccumulate pesticides. Manatee is potentially most exposed to imazalil, fenpropimorph, diuron, azoxystrobin and spiroxamine.

## 4P-Tu-033

### **ARODs Activities in Brazilian *Pontoporia blainvillei*: An Approach Between Sex, Age and PAHs Levels**

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#### **Abstract**

It is well established that anthropogenic activities have a significant impact on ecosystems worldwide. Oceans, which are home to millions of species, are particularly vulnerable to various contaminants such as untreated sewage, medication, residual sunscreen and beauty products, plastics, organic pollutants, trace metals, polychlorinated biphenyls and petroleum-derived compounds such as polycyclic aromatic hydrocarbons (PAHs). These xenobiotics are known to be harmful to marine biota. Biomarkers, which are commonly used in environmental monitoring programs, provide a means of assessing the potential harm caused by these chemicals. The cytochrome P450 family is primarily responsible for the biotransformation of petroleum, and therefore, their measurement and characterization are important tools for evaluating possible exposure of wildlife in the oceans. The Franciscana dolphin (*Pontoporia blainvillei*) is the most endangered cetacean in the Southwest Atlantic. Due to their small size, they are often caught in fishing nets. The degradation of their natural habitat from pollution and boat traffic further hinders the conservation of this species. In this study, we measured Alcoxy Resorufin O-deethylases activities (MROD, BROD and EROD) in microsomes obtained from the liver of 21 Franciscana dolphins collected during the sampling efforts of the Projeto de Monitoramento de Praias da Bacia de Santos – PMP-BS (in English, “Beach Monitoring Project of Santos Basin”), carried out by PETROBRAS to satisfy environmental constraint of the federal environmental licensing of PETROBRAS’ activities of production and outflow of oil and natural gas in Santos Basin, conducted by IBAMA. The ARODs activities were quantified using enzymatic assays with 7-MR, 7-BR, 7-ER as substrates and with NADPH as cofactor at concentrations ranging from 1,2  $\mu$ M to 2,25  $\mu$ M and from 0,5 mM to 1 mM, respectively. We compared the ARODs activities between males and females, different age groups, and the presence or absence of PAHs using unpaired t-tests, but no significant differences were found. These results may be related to the biotransformation profile of Franciscana dolphin, but further analyses are required to draw more definitive conclusions. Understanding the profiles and differences that affect these biomarkers in organisms collected from natural environments is important for the accurate interpretation of ARODs data in biomonitoring programs.

## Gene Expression Profiles as Potential Biomarkers in *Caiman latirostris* Hatchlings Exposed to Pesticides

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### Abstract

For several years, caiman populations have been facing one of the main problems affecting many wild species: habitat loss as a consequence of land being used for agricultural, and potential exposure to pesticides used in such settings. Alterations in mRNA patterns could offer new insights into the role of genes in the context of toxicity. In this sense, the altered gene expression could detect biological toxicity and/or monitor the impact of different xenobiotics in natural environments. Therefore, this study investigates the toxicity of pesticides in blood of *Caiman latirostris* hatchlings by using a particular set of genes involved in detoxification pathways, DNA damage repair and cell cycle regulation [i.e. *p450*, *gst*, *parp1*, *p53*, *bax* and *bcl2*]. Pesticides concentrations employed were equivalent to those recommended in agricultural practices for application in soybean crops: glyphosate (GLY -2 %), 2,4-dichlorophenoxyacetic acid (2,4- D – 0.12 %), and the binary mixture (M: GLY+2,4-D). The applications were made on the nest material in contact with the eggs at the beginning of the incubation period. After hatching, total RNA was isolated from blood of all caiman, and expression levels were analyzed through PCR. The mRNAs levels were visualized in agarose gel and registered using a digital camera. Bands intensity of each row, internal control, and target gene, were quantified by ImageJ software, and intensity ratios were calculated. The results showed downregulation in the expression of *p53* and *bax* genes in GLY and M compared to the controls. In turn, an overexpression was found in *bcl2*, *p450*, and *gst* in GLY as well as in *bcl2* in animals exposed to the M. This study proved that the use of pesticide formulations at low concentrations generates modifications in expression pattern of genes encoding proteins that participate in detoxification pathways, DNA damage repair and cell cycle regulation in this species. Overall, the present study is the first to investigate these genes as potential biomarkers and their related biological pathways under pesticide exposure, in blood of the broad snouted caiman. Overall, the results from this work suggest the potential application of the expression profiling of these genes for the biological monitoring of pesticide exposure in caimans.

4P-Tu-038

## Mercury Concentration and Risk Assessment for Consumption of Fish of Different Trophic Levels in Buenaventura Bay, Colombian Pacific

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### Abstract

The Bay of Buenaventura presents a high fish diversity exploited by hand and is exposed to different sources of anthropogenic contamination, derived mainly from the dumping of wastewater, the mouth of rivers affected by mining activities and port activities that include periodic dredging. To assess possible effects of mercury contamination on the ecosystem, total mercury concentrations (dry weight) were measured in fish of 10 species, collected with an artisanal trawl net and widely consumed locally, making a risk assessment based on the hazard index proposed by the EPA. The species with the highest concentration of mercury was *Haemulopsis nitidus* ( $0.431 \pm 0.152 \mu\text{g/g}$ ), it was also the species with the highest trophic level. However, *Urotrygon rogersi* presented the highest percentage of methyl mercury (80.3%) compared to accumulated mercury. The average levels of mercury exceed the values for the vulnerable population. For the Ariidae family, the highest concentrations of mercury were found in *Ariopsis simonsi* ( $0.339 \pm 0.141 \mu\text{g/g}$ ), followed by *Cathorops multiradiatus* ( $0.300 \pm 0.149 \mu\text{g/g}$ ) and the lowest in *Notarius troschelii* ( $0.290 \pm 0.109 \mu\text{g/g}$ ). The risk assessment suggests that daily intakes above 22 g of *A. simonsi*, 25 g of *C. multiradiatus* and 27 g of *N. troschelii* could pose risks to human health. It is recommended to implement monitoring of this contaminant in order to prevent risks associated with the accumulation of mercury in the local population.

# Session 5: Challenges and Strategies for Linking Adverse Effects to Endocrine Activity to Identify Endocrine Disruptors

5.P-Tu-039

## Alterations in the Mouse Mammary Gland Promoted by Exposure to the Neonicotinoid Imidacloprid

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### Abstract

Imidacloprid (IMI) is a neonicotinoid insecticide widely used in agriculture worldwide, posing great public health concern. IMI is an agonist of the nicotinic acetylcholine receptor (nAChR) and an endocrine disruptor, enhancing estradiol secretion in breast cancer cells. Since the mammary gland development is largely regulated by the endocrine system, we hypothesize that IMI exposure produces alterations in the mammary gland that favor tumorigenesis. We have previously found that IMI (10  $\mu$ M) increases the migration ability of the mammary epithelial cell line NMuMG as well as the metalloproteases (MMP) 2 and 9 activity. Furthermore, IMI increases the expression of G protein-coupled estrogen receptor (GPER) at the same dose. Herein, we set out to delve into the IMI mechanism of action to promote NMuMG cell motility. In addition, we aimed to investigate whether the pesticide induces alterations in the mammary gland, so we exposed pre-pubertal BALB-c mice to IMI (0.01, 0.1 and 10 mg/kg/day) orally for 4 weeks. The whole mammary gland was mounted for morphological analysis, while hematoxylin-eosin stained tissue section were examined for histological studies.

First, we analyzed the NMuMG cell migration (wound healing assay) and the MMP2 and 9 activity (gel zymography), in the presence or the absence of the GPER specific inhibitor, G15 (1  $\mu$ M). Results showed that IMI (10  $\mu$ M) stimulates both processes in a GPER-dependent manner ( $p < 0.001$ ). Taking into account that  $\alpha 7$ -nAChR is expressed in the mammary gland, we studied its protein levels by western blot (WB) and found an enhancement at 10  $\mu$ M (190%  $p < 0.001$ ). Next, we analyzed c-Src activation, a kinase downstream of GPER and  $\alpha 7$ -nAChR, by WB. IMI (10  $\mu$ M) increases c-Src phosphorylation after 1, 2 and 4 h of exposure (150%, 80% and 60% respectively,  $p < 0.01$ ). *In vivo* assays showed IMI (10 mg/kg/day) to enhance ductal hyperplasia (140%  $p < 0.01$ ) and the number of terminal end buds (TEBs, 140%  $p < 0.05$ ). IMI (0.1 mg/kg/day)-treatment significantly induced ductal growth (46%  $p < 0.05$ ), quantified as the distance between the lymph node and the mammary gland final edge, but reduced branch density (33%  $p < 0.05$ ).

Our findings show that IMI increases mammary epithelial cell motility through GPER and alters mammary branching morphogenesis, likely leading to preneoplastic lesions and retaining TEBs. Taken together, these data support the notion that IMI may represent a risk factor for breast cancer development.

## 5.P-Tu-040

### Endocrine Disrupting Environmental Pollutans Hexachlorobenzene and Chlorpyrifos Induce Endometriosis Associated Angiogenesis in Aryl Hydrocarbon Receptor Dependent Manner

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#### Abstract

Endometriosis is a common gynecological disease suffered for reproductive-age women, which is defined by the growth of endometrial tissues outside the uterine cavity. It often causes dysmenorrhea, chronic pelvic pain, and infertility, affecting profoundly women's quality of life. Angiogenesis is critical in endometriosis progression, is a complex process involving endothelial cell migration, proliferation, tube formation, and survival. Vascular endothelial growth factor (VEGF) is a powerful angiogenic factor. Endocrine-disrupting environmental pollutants are thought to play a role in the development of this disease. Hexachlorobenzene (HCB) is an organochlorine pesticide that increases microvessel density and VEGF levels in a rat endometriosis model. Chlorpyrifos (CPF) is an organophosphate insecticide that acts as an Endocrine Disruptor. Both are weak Aryl Hydrocarbon Receptor (AhR) ligands and promote proliferation and migration in endometrial stromal and breast cancer cells. The present study examined the effect of HCB and CPF on endometriosis angiogenesis in vitro. T-HESCs cells (endometrial stromal cell line) were exposed to HCB (0.005, 0.05, 0.5 and 5  $\mu\text{M}$ ), CPF (0.05, 0.5, 5 and 50  $\mu\text{M}$ ) or vehicle (ETOH) for 48 h, and the conditioned media (CM) were then used to stimulate EA.hy926 endothelial cells. The results showed that HCB (0.005, 0.05, 0.5  $\mu\text{M}$ ) and CPF (0.5, 5, 50  $\mu\text{M}$ ) induced VEGF secretion ( $p < 0.05$ ) in T-HESCs. Moreover, the CM of HCB treatment enhanced the endothelial cell proliferation (PCNA expression and MTT assay) (0.005-0.5  $\mu\text{M}$ ) ( $p < 0.05$ ), migration (scratch motility assay) (0.005-0.5  $\mu\text{M}$ ) ( $p < 0.05$ ) and tube formation (tube-like structure formation in Matrigel assay); increasing total tube length (0.005  $\mu\text{M}$   $p < 0.05$ ; 0.5  $\mu\text{M}$   $p < 0.01$ ) and the number of branching points (0.5  $\mu\text{M}$   $p < 0.05$ ). The assays with CPF CM showed an increase in endothelial cell proliferation (0.05-50  $\mu\text{M}$ ) ( $p < 0.01$ - $p < 0.001$ ), migration (5  $\mu\text{M}$ ) ( $p < 0.05$ ), and tube formation, enhancing total tube length (5  $\mu\text{M}$   $p < 0.05$ ). In addition, we evidenced that the enhancement in angiogenesis induced by HCB (0.5  $\mu\text{M}$ ) and CPF (5  $\mu\text{M}$ ) exposure was mediated by AhR signaling pathway. Our results demonstrated that HCB and CPF exposure induces VEGF secretion in human endometrial cells triggering angiogenesis in endothelial cells, a critical event for the endometriosis progression.



## 5.P-Tu-041

### Possible Obesogenic Effect of Carbaryl Insecticide on 3T3-L1 Preadipocytes

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#### Abstract

3T3-L1 mouse preadipocyte cell line is one of the most widely used in vitro models for the study of adipogenesis and in recent years it began to be used to evaluate the cytotoxic and genotoxic effects of environmental contaminants. Even more, the induction of adipocyte differentiation in vitro makes it possible to find potential obesogens, to later be confirmed as obesogens by in vivo assays. Obesogens or obesogenic endocrine disruptors have the ability to alter hormonal balance through a change in lipid homeostasis and promotion of adipogenesis. Endocrine disruptors differ from other toxics because they can act at very low concentrations, often lower than known environmental concentrations. Carbaryl is a carbamate insecticide used in Argentina, whose main mechanism of action is the inhibition of acetylcholinesterase. The aim of this work was to evaluate the cytotoxic and genotoxic effects of carbaryl (active principle) on the proliferation and differentiation processes of adipocytes in the 3T3-L1 and the potential obesogenic effect of this insecticide. Cells were exposed 48 h to carbaryl (0.05-500  $\mu\text{g/ml}$ ) and viability using MTT assay and genotoxic effects using the test of micronucleus were evaluated. For the induction of adipogenesis differentiation mixture (DM), containing insulin, dexamethasone, and 3-isobutyl-1-methylxanthine were added to post-confluent 3T3-L1 cells. During the differentiation process cells were exposed to different concentrations of carbaryl. Lipid accumulation was quantified by staining triglycerides with Oil-Red-O and reading at 490 nm with a plate reader. Preadipocytes viability decreased significantly compared to the solvent control (acetone) and the medium control from 5  $\mu\text{g/ml}$ . No genotoxic effects were observed with the concentrations used. Regarding differentiation, a significant increase in adipocytes was obtained with respect to the controls (DM+solvent and DM) at lower concentrations than those that decrease viability in pre-adipocytes (0.5  $\mu\text{g/mL}$  and 0.1  $\mu\text{g/mL}$ ). Environmental pollutants are being indicated as one of the possible factors that favor the development of obesity and consequently the related pathologies Our results indicate that carbaryl is a potential obesogen, which would have to be corroborated with in vivo studies.

## 5.P-Tu-042

### Breast Cancer Cells Exposed to Pesticides Enhance Vascular Endothelial Growth Factor Secretion and Activate Endothelial Cells Promoting Angiogenesis

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#### Abstract

Chlorpyrifos (CPF) is one of the most widely used organophosphate pesticides in agriculture. Hexachlorobenzene (HCB) was used as a fungicide in the past and is still released into the environment as a byproduct from industrial processes. CPF and HCB bind weakly to the Aryl Hydrocarbon Receptor (AhR), a transcription factor involved in vascular development and tumor progression. Breast cancer is the most frequently occurring malignancy in women, and in recent years the exposure to pesticides has gained importance as a risk factor acting like endocrine disruptors. CPF and HCB promote cell migration and invasion in breast cancer cells, as well as mammary tumor growth. Hypoxia Inducible Factor-1 $\alpha$  (HIF-1 $\alpha$ ) induces vital genes in tumor survival, such as Vascular Endothelial Growth Factor (VEGF), Nitric Oxide Synthase-2 (NOS-2) and Cyclooxygenase-2 (COX-2). Our objective was to evaluate the CPF and HCB action on proangiogenic factors expression like HIF-1 $\alpha$ , VEGF, NOS-2 and COX-2 in triple negative breast cancer cells MDA-MB-231. In addition, we examined whether conditioned medium (CM) of pesticides-treated MDA-MB-231 could activate the endothelial cells. Tumor cells were treated with CPF (0.05; 0.5; 5 and 50  $\mu$ M) or HCB (0.005; 0.05; 0.5 and 5  $\mu$ M) at 1, 6 and 24 h. Endothelial cells were exposed to CM from tumor cells treated with pesticides (0.05  $\mu$ M) for 6 h, and cell proliferation and migration were assayed. Results in MDA-MB-231 cells showed that CPF and HCB (0.05  $\mu$ M) enhance HIF-1 $\alpha$  protein content (28%,  $p < 0.05$ ) at 6 h and 1 h, respectively (Western blot, WB). VEGF secretion was increased by both pesticides (0.05  $\mu$ M) (56%,  $p < 0.001$ ) at 6 h (WB). Besides, the compounds (0.05  $\mu$ M) raise VEGF expression in MDA-MB-231 cell lysate through AhR and HIF-1 $\alpha$  pathways ( $p < 0.05$ ). In addition, the NOS-2 (230%) and COX-2 (85%) protein levels were enhanced with CPF and HCB (0.05  $\mu$ M) at 6 and 24 h respectively ( $p < 0.01$ ) (WB). Moreover, CM from tumor cells exposed to CPF or HCB (0.05  $\mu$ M) for 6 h, increases endothelial cell proliferation evaluated by PCNA content (70%,  $p < 0.05$ ). Endothelial cell migration was enhanced with CM-HCB (22%,  $p < 0.05$ ) without changes with CM-CPF. In conclusion, CPF and HCB induce an enhancement in proangiogenic factors, while increasing VEGF expression via AhR and HIF-1 $\alpha$  signaling. Besides, the pesticides activate endothelial cells through CM from tumor cells, contributing with angiogenesis processes.

## 5.P-Tu-043

### Effect of Endocrine Disrupting Pollutants Hexachlorobenzene and Imidacloprid on the Regulation of Liver Cell Growth

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#### Abstract

Some anthropogenic compounds like a pesticide Hexachlorobenzene (HCB) or insecticide Imidacloprid (IMI) can cause serious pathologies such as hepatocellular carcinoma (HCC), as occurs with the hepatitis B virus which, through the expression of the HBx protein, deregulates cell growth. HCB is a hepatic tumor promoter, its commercialization is currently prohibited, however it continues to be released into the environment. IMI is classified as moderately toxic and unlikely to be carcinogenic by the WHO. HCB is an endocrine disruptor (ED), alters thyroid hormones (TH), deregulates cell growth and develops liver tumors. Some research suggests that IMI could be ED, but its mechanism of action is unclear.

Objective: To study the effect of HCB and IMI on proliferative and apoptotic processes, as well as on key molecules in tumor development in vitro. HCB (0.05, 0.5 and 5  $\mu$ M), IMI (0.02, 0.2 and 2  $\mu$ M) dose curves were performed in Hep-G2 and Huh-7 cells whether or not they express HBx.

Cells were treated with HCB or IMI for 24hs. Experiments were carried out with pre-treated exogenous T3 (10<sup>-9</sup>M, 10<sup>-7</sup>M and 10<sup>-5</sup>M), or with TGF- $\beta$ 1 inhibitor (SB-431542) or conditioned medium (CM) from cells treated with HCB or IMI, for 24hs.

Statistic, ANOVA two way, \* p 0.05 and post test Tukey-Test. Evaluated: 1-proliferation: PCNA, Westernblot (WB) and 5<sup>3</sup>BrDU incorporation (IH). 2-apoptosis: caspase-3 and cytochrome-c, (WB). 3-Growth regulators: TGF- $\beta$ 1 and p-21, RT-PCR. 4-COX-2 (WB). 5-pERK and pJNK, (WB). 6-Response to HT.

Results: HCB: PCNA increased (134 and 169%) for (0.5 and 5  $\mu$ M); caspase-3 (28, 51, 73%) and Cit-c (23, 46, 71%). TGF  $\beta$ 1 and JNK increased dose-dependently (67, 178, 314%) and (37, 58, 64%). pERK increased (47 and 61%) and COX-2 (23 and 52%) at high doses. T3 at 10<sup>-7</sup>M normalized PCNA increases. MC on untreated cells increased PCNA 41%. Pre-treatment with SB-431542 prevented the increase in PCNA generated by HCB or MC.

IMI: PCNA decreased (25 and 46%) for high doses, caspase-3 (20, 36, 67%), Cyt-c (31, 46, 83% at 24h. TGF- $\beta$ 1 increased (23, 37, 41 %), pJUK (18, 30, 45%) and COX-2 (31, 53%) at high doses. In Huh-7 / HBx, PCNA and TGF- $\beta$ 1 increased 66% and 71% (p <0.01). In Huh-7 / HBx with 5  $\mu$ M HCB, PCNA increased 120% (p <0.001. Conclusion: HCB as well as IMI deregulate cell growth. TGF $\beta$ 1, JUNK and COX-2 could be involved in its mechanism of action. Toxic treatment on cells expressing HBx potentiates the proliferative effect.

## 5.P-Tu-044

### **Ongoing Development of a Framework for the Population Relevance Assessment of Endocrine Disruptors in Mammalian Non-Target Organisms.**

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#### **Abstract**

European Commission Regulation 2018/605 sets out scientific criteria for determining endocrine disruptor (ED) substances subject to Regulation 1107/2009 for plant protection products. These criteria include the 3 established ED attributes, as defined by WHO (2002): (1) endocrine activity, (2) adversity, and (3) the causal link between them. If the ED criteria are met, the next step for non-target organisms (NTOs) is to determine if the ED-mediated adverse effects are relevant at the (sub)population level, however this level is not quantified. For mammalian NTOs, adverse effects on apical endpoints of reproduction, development, growth and survival within in vivo mammalian toxicity studies are considered potentially population relevant because they can interfere with the maintenance of wild mammal populations. Yet, there is no single study protocol that provides a holistic view of endocrine-mediated adversity, endocrine activity, and population relevance together. Therefore, the NTO ED determination is based on the weight of evidence analysis which assembles different information into lines of evidence and then assesses it to determine an outcome. The adverse outcome pathway (AOP) approach can support the ED assessment by investigating links between specific biological perturbations and adverse outcomes; however, AOPs do not always address adversity at the population level. Currently, there are no regulatory guidance for assessing the population relevance of EDs in mammalian NTO. We recognize that this knowledge/methodological gap needs to be addressed, and the determination of the population relevance/non-relevance of endocrine-mediated adverse effects still needs further consideration. These considerations could include the analysis of potentially vulnerable species, their life history traits and population dynamics, determination of population relevant endpoints and thresholds of effects at the individual and population level. Possible approaches include exploring field studies or population modelling methods, such as individual based models and dynamic energy budget models. It is also important to distinguish mammalian endpoints that might be considered adverse for human health assessments, but not relevant for mammalian NTO populations. While these approaches are based on EU legislation, the scientific concepts are expected to be protective of mammalian NTO populations occurring in global agricultural landscapes in different regions, including LATAM.

## 5.P-Tu-045

### **Current Methodological Approaches to Evaluate the Endocrine Disruption of Pharmaceuticals Compounds on Thyroid Axis of Aquatic Animals: A Short Systematic Review**

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#### **Abstract**

Pharmaceutical compounds (PCs) in aquatic environments have been measured worldwide and despite the specific pharmacological roles for human or animal uses, adverse endocrine disruption (ED) to non-target aquatic species have been elucidated, especially reproduction endpoints. Nonetheless, thyroid ED by pharmaceutical exposures is still limited and novel methodologies are emerging for new outcomes. To address this concern, a systematic review of the literature was conducted in order to give insights to the recent data regarding thyroid ED and evaluations protocols. Using PubMed and Scopus databases advanced search tools for the following filters were applied: 1) key words: “pharmaceuticals” AND “thyroid” AND “endocrine disruption” AND “water”, 2) years of publication range: 2006- 2023 and 3) language: English, and 4) types of journal articles: reviews, original articles, clinical trials, short notes. In total, 35 articles were obtained. Review articles and original research accounted for 23% and 77%, respectively. According to the data, approximately 62% of the animal models are teleost fishes and 37% amphibians, where *Xenopus laevis* was the major species tested. Geographically, most of the studies were conducted in Asia and Europe, lacking data in other regions including Latin America. Regarding the evaluated pharmaceuticals, the investigations focus on anticonvulsants, NSAIDs, inhibitors, antibiotics, antiarrhythmics and antidepressants, and the adverse effects described were downregulation of thyroid genes, decrease of hormone content and morphological alterations on thyroid hormone tissues. The larger part of the research aimed to more chronic time of exposure which includes development at early life stage of the studied species. Finally, the major methodological approach includes development, key genes for thyroid functions and morphological pathologies. This review indicated a potential of thyroid to identify endocrine disruptors, nevertheless the number of investigations concerning pharmaceuticals, thyroid ED mechanisms and aquatic species is still low or inexistent in some regions including Latin America.

## 5.P-Tu-046

### **Bisphenol A Affects Thyroid Follicular Morphometry During Larval Development of the Freshwater South American Cichlid Fish *Cichlasoma dimerus***

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#### **Abstract**

Bisphenols are chemical compounds widely used in plastic manufacture. They can reach superficial and coastal waters by effluents discharge from urban and industrial centers. Bisphenol A (BPA) shares a structural resemblance with thyroid hormones (T3 and T4), and could therefore act as an endocrine disruptor, posing a threat to fishes due to its presence in the aquatic environment. The aim of this work was to investigate possible alterations of the thyroid axis, with emphasis on thyroid follicles morphology, caused by exposure to BPA of larvae of the freshwater South American native fish *Cichlasoma dimerus*. Subchronic exposure experiments were conducted with larvae from the day of hatching (0) until day 16 post-hatching. Three sublethal concentrations of BPA were tested (0.1, 10 y 1000  $\mu\text{g/L}$ ), alongside a 6-propyl-2-thiouracil treatment (PTU, 10  $\mu\text{g/L}$ ), a known antithyroid drug, as a positive control. During the exposure experiment, animals were sampled at two-day intervals and fixed in Bouin's solution for 24 hours for histological processing. Slides were stained by the periodic acid- Schiff reaction (PAS) followed by a hematoxylin counterstain. Morphometry and number of thyroid follicles were studied in microphotographs, analyzed by ImageProPlus software. In this species, thyroid follicles were found dispersed in the subpharyngeal region adjacent to the ventral aorta. A decrease in the number of thyroid follicles was found in animals exposed to PTU and the highest concentration of BPA (1000  $\mu\text{g/L}$ ), when compared to the control treatment. Furthermore, average colloid area per larvae was greater in fish exposed to PTU and 1000  $\mu\text{g/L}$  BPA than in the control, suggesting follicular hypertrophy, which would be indicative of thyrocyte hyperplasia due to decreased hormone synthesis. Our results suggest that the exposure to BPA during early life stages of *C. dimerus* induces alterations of normal thyroid follicles development, with effects comparable to those caused by a hypothyroid drug. This study expands existing knowledge of the potential role of BPA as a thyroid disruptor to a South American native species.

## 5.P-Tu-047

### Effects of Bisphenol A, Benzophenone 2 or Benzophenone 3 on Autophagy Protein Markers in Immortalized Gonadotropin-Releasing Hormone (GnRH) Neurons

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#### Abstract

Benzophenones (BP) and Bisphenol A (BPA) are endocrine disrupting chemicals (EDC). BPA is a monomer of polycarbonate plastics and BPs are UV filters. Previously, we showed that the *in vitro* exposure to EDC inhibited kisspeptin-10-induced GnRH gene expression, and altered expression of inflammatory markers in GT1-7 cells; it also modified gene expression of autophagy markers in GnRH-expressing cell lines. In this study, we evaluated the effects of the exposure to BPA, BP2 or BP3 ( $1 \times 10^{-7}$  or  $1 \times 10^{-9}$  M) on protein expression of autophagy markers, LC3 and p62.

GnRH-expressing cell lines, GT1-7 or GN11 cells, were plated in 6-well plates, in DMEM high glucose, with 10% FBS and antibiotics. Twenty-four hours later, media were replaced by phenol red-free DMEM with 10% charcoal-stripped FBS and antibiotics, and cells were exposed to BPA, BP2 or BP3 ( $1 \times 10^{-7}$  or  $1 \times 10^{-9}$  M), alone or in combination with Chloroquine (CQ, an inhibitor of the degradation of the autophagosome content), for 12 or 24h. Media were removed and plates were frozen at  $-70$  C for western blot analysis. Cells were lysed in RIPA buffer with protease inhibitors and protein concentration measured by Bradford. Proteins were separated in polyacrylamide gels and transferred to PVDF membranes. LC3, p62, and tubulin were detected with specific antibodies. Results were expressed as Mean $\pm$ SEM and analyzed by Repeated Measures Two-way ANOVA or by ANOVA (Statistica).

In GT1-7 cells, CQ increased the lipidated form of LC3 (LC3-II,  $p < 0.001$ ) and p62 ( $p < 0.05$ ) after 24h treatment. Regarding the EDCs, BP2  $1 \times 10^{-9}$  M decreased LC3-II relative to control values [LC3-II (AU) C:  $1.1 \pm 0.1$ . C-CQ:  $1.9 \pm 0.3$ , BP2-7:  $1.3 \pm 0.2$ , BP2-7-CQ:  $1.8 \pm 0.3$ , BP2-9:  $0.6 \pm 0.2$ , BP2-9-CQ:  $0.8 \pm 0.2$ ; Repeated Measures Two-way ANOVA: Main Effect Treatment with the EDC: BP2-9, BP2-9-CQ different from C, C-CQ,  $p < 0.05$ ; Main Effect treatment with CQ: different from conditions without CQ,  $p < 0.001$ ;  $n = 4$ ]. Exposure to the EDC for 24h did not significantly modify p62 protein expression. In GN11 cells, CQ increased LC3-II ( $p < 0.01$ ) and p62 ( $p < 0.05$ ) after 24h treatment; in this cell line none of the EDC had a significant effect at this time point. The effects after 12h exposure to the EDC are being currently analyzed.

Our results show that exposure to EDC alters autophagy proteins in immortalized GnRH neurons. More experiments are underway to further explore the observed effects.

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## 5.P-Tu-048

### **Nuclear Receptor Superfamily Structural Diversity in Pacific Oyster: *In Silico* Identification of Estradiol Binding Candidates**

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#### **Abstract**

The Pacific oyster, *Crassostrea gigas*, is a filter-feeding, sessile oyster species subject to environmental changes and exposure to contaminants. The inhabiting sites of the oyster, such as the estuarine and coastal environments, are constantly exposed to organic and inorganic pollutants discharged into their waters as a consequence of anthropogenic and industrial activities. The nuclear receptor superfamily, due to the structural characteristics of binding to ligands that inhibit or activate gene transcription, are frequent targets of xenobiotic compounds. The present work aims to understand the potential interaction between the emergent contaminant estradiol and the nuclear receptors of *C. gigas*. In the Pacific oyster, the nuclear receptor homologous to the vertebrate estrogen receptor is not able to bind to estradiol, as it does in vertebrates. Possibly, another receptor exhibits responsiveness to estrogen-like molecules and derivatives. In order to identify potential binding candidates with estradiol, within the nuclear receptor superfamily, our approach consisted in the combination of high-performance *in silico* methodologies, such as three-dimensional modeling, molecular docking and atomistic molecular dynamics. Through this perspective, it was possible to predict structural properties for the nuclear receptor superfamily of the Pacific oyster and to select ligand candidates with the endocrine disrupting contaminant of interest, in a non-model invertebrate organism. Candidates of most favorable interaction with the molecule of interest, among the *C. gigas* nuclear receptor superfamily, were found belonging to the NR0B, NR1P, and NR2E nuclear receptor groups. The application of this computational methodological strategy demonstrated high performance in the virtual screening of candidates for binding with the target xenobiotic molecule, and can be employed in other studies in the field of Ecotoxicology.



# Session 6: Emerging Contaminants and Environmental Remediation: Monitoring of Effects by Ecotoxicological Bioassays

6.P-Mo-010

## Long-term Exposure of the Red Cherry Shrimp *Neocaridina davidi* to Diclofenac: Impact on Reproductive Potential

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### Abstract

Pharmaceutically active compounds are an important group of emerging pollutants that represent a serious threat to freshwater and marine ecosystems worldwide. Non-steroidal anti-inflammatory drugs (e.g., diclofenac) occupy the first place in environmental presence, compared to other pharmaceuticals. The current study was aimed at studying the long-term effects of diclofenac (DCF) on *Neocaridina davidi*, concerning its reproductive output especially across successive generations. Both ovigerous females and males of this species were exposed to 0 (control), 0.1, or 1 mg/L of DCF. The experiment comprised 63 days, in order to allow embryo development until hatching in a first reproductive cycle, a subsequent ovarian re-maturation, followed by mating, embryo development, and a second hatching. Shrimps were daily inspected to record egg loss, hatching and new spawning. Recently hatched juveniles were counted and the lecithotrophic index (area of the hepatopancreas occupied by lipid droplets in mm<sup>2</sup>/total area of cephalothorax in mm<sup>2</sup>) was quantified. After this, all juveniles were fixed in formalin 5% to analyze morphological abnormalities. At the end of the assay, shrimps were fixed in Bouin, and ovaries were dissected. All oocytes were counted, classified as initial (pre-vitellogenic) or advanced (vitellogenic) and the oocyte's area was measured. At the highest concentration, the percentage of females with a second spawn, observable from day 45 (full re-maturation takes place approximately 45 d after a previous spawning) increased significantly, while the time between spawns was significantly reduced at both concentrations assayed. However, the ovaries showed a significantly lower proportion of advanced oocytes in females exposed to 1 mg/L, as compared to control. Concerning hatching, the percentage of ovigerous females that had successful hatching was reduced at 1 mg/L of DCF, especially for the first spawn. For the second spawn, the low number of juveniles hatched from females exposed to 1 mg/L showed a significantly higher incidence of morphological abnormalities, such as hydropsy and underdeveloped appendages. Taken together, these results showed that even when DCF was able to produce earlier spawns, the reproductive output of each spawn was reduced.

## 6.P-Mo-011

### Validation of Analytical Methodology for Determination of Glyphosate and Study of Photodegradation With Silver Arsenate

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#### Abstract

The growing demand for food, due to constant population growth, has led to the exacerbated use of pesticides to combat pests, aiming at increasing productivity and crop quality. Herbicides are a class of pesticides widely used in agriculture to combat weeds and are potentially toxic to the environment and human health. Glyphosate is the most commercialized herbicide worldwide, as it has a broad post-emergence spectrum and is non-selective for the control of undesirable plants. Due to the extensive use of this herbicide and its toxicity, the present study aims to optimize and validate a method for determining glyphosate in water with pre-column derivatization with the reagent 9-fluorenylmethyl chloroformate and to evaluate the degradation capacity of this compound using the photocatalyst  $\text{Ag}_3\text{AsO}_4$  synthesized with analysis by high performance liquid chromatography with fluorescence detection. The chromatographic analysis was performed in isocratic mode, obtaining an excellent retention time of 2.3 min. Method validation showed satisfactory results, with a correlation coefficient of 0.9995, detection limit of  $0.0324 \mu\text{g L}^{-1}$  and quantification limit of  $0.0324 \mu\text{g L}^{-1}$ , variation coefficients for repeatability and intermediate precision lower than 6.41% and 9.22%, respectively, and recovery levels between 96.83% and 103.73%. The photocatalytic potential of  $\text{Ag}_3\text{AsO}_4$  was verified, obtaining a removal rate for glyphosate of 43%. New studies will be carried out to optimize the parameters of the degradation method.

## 6.P-Mo-012

### Evaluation of the Ecotoxicological Effects of Three Quinolone Generations on *Chlorella vulgaris*

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#### Abstract

Quinolones are antibiotics widely used in human and veterinary medicine to treat different types of infections. They enter into the environment, especially aquatic systems, by infiltration, runoff, or by effluents scarcely or poorly treated before they are discharged into the receiving watercourse. Despite this, there is a gap in legislation regarding environmental contamination by pharmaceuticals, probably because chronic toxicity studies are insufficient to infer the risks of long-term exposure of pharmaceuticals and their metabolites to wildlife. Algae were chosen as test organisms to evaluate the ecotoxicological effects of quinolones because they belong to the first level of the trophic chain, so any disturbance in their dynamics might affect the ecosystem's higher levels and they are also very sensitive to changes in their environment. The tests with unicellular organisms show greater reproducibility, reliability, and robustness than multicellular organism tests. In this study, algae growth inhibition assays (96 h) of *Chlorella vulgaris* exposed to quinolones were performed according to OECD (2011). The microalgae were cultured modified Johnson's medium and the conditions were fixed to 6000 lux of intensity,  $23 \pm 2$  °C, and initial concentrations of  $10^4$  cell mL<sup>-1</sup>. A control and six treatment assays with three quinolone generations (ciprofloxacin -second-, levofloxacin -third-, and moxifloxacin -fourth-) were evaluated in concentrations ranged from 0.26 to 62.5 mg L<sup>-1</sup>. The endpoints were cell density, growth rate, and mean effective concentration (EC<sub>50</sub>), determination of pigments (chlorophyll a, b, total, carotenoid, and pheophytin), body store (lipids, carbohydrates, and proteins), biovolume, enzymatic biomarker (catalase, and glutathione S-transferase activities), and lipid peroxidation (malondialdehyde assay, biomarker of oxidative stress). As a result, the EC<sub>50</sub> obtained was 7.4, 10.6, and 1.4 mg L<sup>-1</sup> for ciprofloxacin, levofloxacin, and moxifloxacin, respectively. Cell densities, growth rate, and pigment contents were lower than the control ( $p < 0.05$ ) and significantly lower with increasing generation ( $p < 0.01$ ). The biochemical compositions and enzymatic biomarkers were altered and lipid peroxidation was detected in the presence of quinolones compared to the control response. These results show that the toxicity of quinolones on *C. vulgaris* depends on its generation whereas the fourth-generation quinolone is more toxic than the second-generation one.

## 6.P-Mo-013

### Toxicological Bioassays on the Native Microalga *Dictyosphaerium* sp. as a Possible Phycoremediator Against Relevant Pesticides

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#### Abstract

Input of xenobiotics into the aquatic environment can cause an imbalance by altering the communities that inhabit. Microalgae are the producer community and this is why they are the first to be exposed to environmental changes and respond to this. On the other hand, it is known that in neotropical regions the most widely used pesticides are the herbicide glyphosate (GLY) in a cocktail with new-age insecticides such as chlorpyrifos (CLO) and/or imidacloprid (IMI). In this sense, this work aimed to evaluate the response of a native algae (*Dictyosphaerium* sp.) against the exposure of three widely used pesticides: GLY, IMI and CLO in a range of five concentrations between 5 to 100 µg GLY/L, 1 to 20 µg IMI/L and 10 to 100 µg CLO/L. Acute toxicity bioassays (96h) were carried out placing an initial cell density of a microalgae strain of  $1.6 \times 10^6$  cells/mL, by triplicate. As toxicity endpoints, algal growth (by Neubauer chamber), chlorophyll-a and catalase activity were evaluated. The results obtained showed that the microalgae cultures had an average increase in growth of 18%, 16% and 15% for GLY, CLO and IMI, respectively. No significant differences were observed in chlorophyll-a concentrations between the treatments and the control. Catalase activity revealed significant increases in the highest concentrations of insecticides. In the case of GLY, catalase activity decreased with increasing herbicide concentrations. It should be noted that, for the first time, different physiological biomarkers are evaluated in native algae isolated from pure strains from arid regions in the presence of contaminants such as pesticides. On the other hand, in previous studies carried out by the working group, we demonstrate the ability of this algal species to reduce the presence of these pesticides was demonstrated in bioassays on a pilot test. In this context, and adding these new results where algal growth and enzymatic response are observed, this work demonstrates the potential phycoremediation use of a native algae against possible contamination by widely used pesticides.

## 6.P-Mo-014

### Anaerobic Reactor Reduces the Toxicity of Synthetic Domestic Sewage Enriched With Organic Micropollutants on Tropical Freshwater Invertebrates

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#### Abstract

One of the problems of emerging contaminants in natural environments is their complex removal from water and wastewater samples, highlighting a gap in the potential toxic effects in organisms present in aquatic systems. In this context, this study aimed to investigate the lethal effects of synthetic domestic wastewater (500 mgDQO/L) contaminated with ibuprofen (1.2 ng/L), diclofenac (0.40 ng/L), and caffeine (92 ng/L), treated by anaerobic sequencing batch reactor containing immobilized biomass (AnSBBR, 5 L, agitation of 200 rpm), on the tropical invertebrate species *Allonais inaequalis* and *Chironomus sancticaroli*. Three treatment configurations were used, varying temperature and feeding time (E1=15 min feeding time and 30°C; E2 = 15 min feeding time and 25°C; E3 = 2 hr feeding time and 30°C), in 6 hr feeding cycles. Acute toxicity assays (96h) were performed using *C. sancticaroli* larvae in 500 mL beakers containing 250 mL of the test solution and 50 g of muffled sediment. Tests using *A. inaequalis* were performed in 100 mL beakers containing 60 mL of the test solution and 10g of muffled sediment. Dilutions (100; 50; 25; 12.5, and 6% for affluent and effluent samples) were prepared using dechlorinated tap water, performing three replicates and a control sample. The tests were maintained at  $25 \pm 2$  °C, with a controlled 12-hour light/dark photoperiod and feeding at the beginning of the tests. The R software was used to calculate the concentration of lethal effects in 50% of the population (LC50). The significant differences among the responses of dilutions and control were checked using the Past software. The bioassays using *C. sancticaroli* indicated that treatment E1 was responsible for the lowest mortality rates, demonstrating complete removal of toxicity for the 100% sample (undiluted) (p-value of 0.1); treatment E3 was also responsible for a reduction of toxicity, having an LC50 of 77%, followed by sample E2, with an LC50 of 45.38%. For *A. inaequalis* specie, the toxic effect observed was similar to that observed using the Chironomidae, with treatment E1 (LC50 of 92.4%) being much more efficient in reducing acute toxicity compared to the other samples, followed by treatment E3 (LC50 of 34.9%) and E2 (LC50 of 25.4%). Overall, it was possible to show that the treatments in anaerobic systems with high temperatures were able to mitigate the toxicity to aquatic invertebrates of the samples containing micropollutants.

## 6.P-Mo-015

### **Assessment of the Impact of Ceftriaxone on the Functional Profile of the Soil Microbiota Using the Biolog EcoPlate™**

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#### **Abstract**

Undoubtedly, antibiotics are an indispensable tool in the treatment of infectious diseases. However, over the past decade, there has been a significant increase in discussions about the potential impacts that the use of these compounds can cause, both on the environment and on the economy and human health. When excreted, antibiotics can remain unchanged or be metabolized into more complex compounds, and their presence can exert toxicity by contaminating different environmental compartments, including soil.

Soil is especially relevant due to the multiple functions it performs, as it directly affects the communities of microorganisms, plants, and animals that make it up, exerting influence on the health and productivity of these living beings. The functional profile of soil microbiota is a promising tool for evaluating soil quality. The aim of this study was to evaluate the functional profile of soil microbiota and the gut of Californian worms in soil contaminated with ceftriaxone, using Biolog EcoPlate.

Thus, soils contaminated with different concentrations of ceftriaxone (0, 1, and 10 mg/kg) were incubated for 14 days in the presence or absence of the *Eisenia andrei* worm. After exposure, the physiological profile of the soil microbiota and the intestinal microbiota of the worms were evaluated using Biolog EcoPlate.

The results of our study suggest that even at concentrations as high as 1 mg/kg and 10 mg/kg of ceftriaxone, the physiological profile of the soil microbiota remained largely unchanged. Although it is known that the presence of worms is important for maintaining soil quality, our results did not reveal any significant correlation between changes in microbial communities and the presence of worms. These observations underline the complex nature of soil microbiota and the need for more research to fully understand the underlying mechanisms of these interactions.

## 6.P-Mo-016

### Remediation of 2,4-D in Soil Percolated Water: Potential Use of Biochar as a Retaining Layer

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#### Abstract

Due to the increased use of pesticides, such as the herbicide 2,4-D, many impacts are caused, including soil, surface water bodies, and groundwater contamination. Some alternatives are studied to mitigate pesticide pollution and reduce their environmental risks. In line with this, biochar – a carbonaceous material produced by pyrolysis – has the potential for the sorption and remediation of organic substances. Thus, this study aimed to analyze the potential retention of 2,4-D in soils with a layer of biochar produced from sugarcane straw.

For this, four treatments were prepared ( $n = 3$ ) in PVC tubes (15 cm diameter) containing a layer (20 cm) of natural sandy soil. Two of these treatments received an intermediate layer (2 cm) of biochar (B). Two treatments - soil with (D+B) and without biochar (D) - were contaminated with 2,4-D ( $14 \text{ mg L}^{-1}$ ), and the others were maintained as untreated controls (C and C+B). After contamination, three rain events (350 mL of deionized water each) were simulated: (I) on the day, (II) four, and (III) seven days after contamination. The water percolated in the soil column was collected and used in experiments assessing the germination, root, and shoot elongation with the species *Eruca sativa* L. The bioassays were carried out in acrylic plates, using sterilized filter paper, 10 seeds per plate, 2 mL of percolated water, and 3 replicates by each unit. Laboratory control was prepared with distilled water. The tests were kept in the dark for 96 h at  $20^\circ\text{C}$ . Responses were analyzed by Two-way ANOVA followed by the Tukey post-hoc test.

The results showed differences in the germination rates between treatments 'D' and 'D+B' for rains 1 and 2 ( $p < 0.05$ ), thus demonstrating that the biochar liner reduced the toxicity of 2,4-D in the percolated water. The growth of shoots and roots showed differences between treatments 'C' and 'C+B' ( $p < 0.05$ ) after exposure to the water from the three rain simulations, indicating an improvement in water properties to plant growth after percolating by the biochar layer. Although not statistically significant ( $p > 0.05$ ), the 'D+B' treatment presented a slight increase in growth compared to 'D'. It was concluded that the biochar layer in the soil reduced the toxicity of the herbicide 2,4-D in the percolated water. Future studies may verify the adsorption capacity of biochar with other layer thicknesses and raw materials to ensure greater effectiveness in its use in the remediation of contaminated soils.

## 6.P-Mo-017

### Degradation of Oxytetracycline by Coupled Biological and Photo-Fenton Processes

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#### Abstract

Intensification of livestock production entails an increase of biosecurity threats due to the fact that fattening in huge concentrations of animals per surface unit favor the transmission of infectious diseases. Antibiotics such as oxytetracycline (OTC) are widely used to prevent the spread of diseases caused by overcrowding of animals. However, they are poorly metabolized by the body, so a high percentage of them is excreted in feces and urine, entering the environment through industrial effluents. The presence of OTC and other antibiotics in the environment has the potential to induce the proliferation of resistant microorganisms, which represents a severe public health problem.

In this work, said pollution problem was addressed by the isolation of bacteria capable of degrading OTC and the subsequent development of a coupled biological-chemical process. Effluent samples were taken from a pig farm in the town of Marcos Paz, Buenos Aires, Argentina. Microorganism was enriched and selected until an *Acinetobacter gernerii* strain, with great ability to degrade OTC, was isolated. This bacterium was used on the biological treatment carried out on a bioreactor with 3 L of porcine effluent with 400 mg L<sup>-1</sup> of OTC. After 13 days, a photo-Fenton treatment was applied on 100 mL of the supernatant during 8 hours. Total organic carbon was analyzed throughout the coupled-process and toxicity assays using *Raphanus sativus* seeds were performed at the beginning and at the end of each treatment. With this degradation strategy, it was possible to mineralize 37 % of OTC concentration and to reduce the toxicity of the aqueous medium by 52 %.



## 6.P-Mo-018

### Cytogenotoxic Responses in *Allium cepa* L. Roots Exposed to Water Samples From Three Rivers of the Iguaçu River Basin (Paraná, Brazil)

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#### Abstract

Water is a natural resource that is indispensable for living beings, it is directly related to biological cycles and to the balance of the environment, it is also an indispensable element in agricultural and industrial activities, these activities generate a wide range of residues and pollutants that, once in the environment, can generate various health risks. One way to evaluate such risks is through biomonitoring programs with bioindicators. Cytogenetic studies with higher plants provide important answers about the effects of pollutants on cells, even when exposed to them in the short term. Among the higher plants, the bioassay with the common onion, *Allium cepa* L. is widely used in the analysis of cytotoxic, genotoxic and mutagenic effects of pollutants present in the environment. In this sense, this work aimed to evaluate changes in the mitotic index and index of chromosomal aberrations in the bioindicator exposed to water collected from three rivers of the Iguaçu River basin. A sampling point located in the urban perimeter and with a high degree of pollution and riparian forest degradation was selected (P1), a sampling point close to extensive agricultural activities and with little presence of riparian forest (P2) and, finally, a point far from urban centers and with more preserved riparian forest in relation to the other points (P3). They were also submitted to a negative control that contained only reconstituted water. The bioassay had an experimental time of 168 hours. Evaluation of cytogenetic damage occurred at the end of the experiment from the collected root material. Our results showed that, in relation to the cell cycle, the mitotic index, that is, the index used as an indicator of adequate cell proliferation showed mitotic changes in P1 ( $p=0.0152$ ) and P3 ( $p=0.0423$ ) in relation to the negative control, thus raising the hypothesis of the presence of compounds that interfere in the mitotic cycle of *Allium cepa* L. at these sampling points in the Iguaçu River. The analysis of chromosome aberrations revealed a statistically superior difference only in P3 when compared to P1. The damage to the bioindicator occurring in P3 may be due to several causes, including altered rainfall in the region in question and application of chemicals in nearby farms. However, active and constant biomonitoring is of utmost importance, because environmental pollutants alone or in synergy can present deleterious effects to several non-target organisms, including humans.

## 6.P-Mo-019

### Phytoremediation of Microcystins Prevents Alterations in Hematological Parameters of Neotropical Catfish

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#### Abstract

Microcystins are hepatotoxins not completely removed by conventional water treatment. Phytoremediation can be an alternative solution that seems to be suitable and efficient for this cyanotoxin removal. However, the evaluation of prevention of adverse effects in non-target organisms is needed. Despite the hepatotoxicity of microcystins, the use of hematological biomarkers can be an alternative to evaluate sublethal effects, due to the faster detection of physiological alterations and it is a low-invasive technique to collect samples. Thus, the study aimed to evaluate phytoremediation efficiency in reducing sublethal effects of microcystins in the hematological parameters of *Rhamdia quelen*. In the first experiment, *R. quelen* were exposed to 1 and 10  $\mu\text{g/L}$  of microcystins ( $n=12/\text{group}$ ), obtained from *Microcystis aeruginosa* aqueous extract. Secondly, 4 tanks of 15 L containing test concentrations of microcystins were prepared for phytoremediation using *Myriophyllum aquaticum* at 10 g/L, for 7 days. Then, treated water was used to expose a new group of *R. quelen* for 96 h, at the same conditions as the first experiment. After 96 h of each experiment, fish were anesthetized and blood was collected for hematological analyses: number of erythrocytes, leukocytes, and thrombocytes, measurement of hemoglobin and hematocrit. The results indicated that microcystins caused a decrease in the number of erythrocytes, thrombocytes, and leukocytes when compared to the control group. In addition, the concentration of 10  $\mu\text{g/L}$  of microcystins decreased the hemoglobin concentration and hematocrit percentual. These results can indicate difficulty in oxygen transporting, a possible anemic condition, and a reduction of fish immunity. However, after phytoremediation, no changes were observed for hematological parameters when compared to the control group, suggesting the efficiency of water treatment in preventing adverse effects on hematological parameters. Although phytoremediation efficiency varied from 7 to 12%, these results indicate a good perspective on the use of *M. aquaticum* to prevent hematological alterations. Furthermore, are needed integrated assessment with other biomarkers to better understand microcystins exposition effects and removal from water sources.

## 6.P-Mo-020

### Evaluation of the Insecticide Chlorantraniliprole by Ecotoxicological Tests With Non-Target Organisms

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#### Abstract

Currently, Brazil is among the countries that have the most pesticides in agriculture and its southeast region is responsible for the consumption of large volumes of this product. Based on the traditional method of cultivation with pesticides, the state of São Paulo is considered the largest national producer of sugarcane. In this intensive monoculture, many different molecules are applied for different purposes, such as the active ingredient chlorantraniliprole, aimed at controlling the sugarcane borer. Despite being proven to be effective and recommended as a more modern insecticide, little is known about its ecotoxicity. Thus, the present study aimed to evaluate the ecotoxicological effects of environmental concentrations of chlorantraniliprole on non-target organisms, such as the animal bioindicator *Artemia* sp. (brine shrimp) and the plant bioindicator *Allium cepa* L. (common onion). Initially, seven different concentrations of the insecticide were evaluated by testing with *Artemia* sp.. The recommended concentration for field application was tested and used to define the others, extrapolating one above and five below, considered residuals. The tests were conducted according to the test method established by the Brazilian Association of Technical Standards (ABNT), identified as ABNT NBR 16530/2016. Then, based on the results obtained in this and other tests, with another test organism (not presented here), four concentrations were selected and tested through bioassays with *A. cepa* L.. The field concentration, one higher, and two lower concentrations were used. The tests were carried out with onion seeds, germinated directly in samples of chlorantraniliprole, as recommended by Organisation for Economic Cooperation and Development (OECD), based on guideline number 208. The result with brine shrimp test demonstrated that the insecticide was harmful and induced acute toxicity to the species. Significant lethality rates were evidenced for all tested concentrations. In the test with the plant bioindicator, genotoxic and mutagenic damages were observed, with an aneugenic action being suggested by the active ingredient chlorantraniliprole. Through this study, it was possible to better elucidate the effects of this pesticide on non-target organisms. The results demonstrate that pesticides need to be widely evaluated, considering that their persistence or drift can contaminate the environment and impact other species.

## 6.P-Mo-021

### Effects Of Diazepam And Sertraline On Plant Bioindicators

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#### Abstract

Water resources have been the fate of numerous contaminations in recent years and much of this contamination comes from domestic and industrial sewage. The treatment of these sewage, when applied, are not always efficient in removing some contaminants. Among these, emerging contaminants, such as drug residues, have rivers as their main destination, putting aquatic organisms and other living beings at risk. Over the years, the consumption of anxiolytic and antidepressant drugs has intensified, a fact also seen during the COVID-19 pandemic. Thus, the objective of the present study was to evaluate the ecotoxicity of two pharmaceuticals, Sertraline and Diazepam, on the bioindicator *Lactuca sativa* L. (lettuce), in order to estimate the possible impacts on terrestrial and aquatic ecosystems. The plant bioassays were conducted based on the guideline number 208, established by Organisation for Economic Cooperation and Development (OECD). Lettuce seeds were exposed to the different concentrations of the pharmaceuticals, based on those found in Brazilian aquatic environments. Diazepam is reported to be found at 0.763 µg/L, and five concentrations were tested: C1=75.0 µg/L; C2=7.5 µg/L; C3=0.75 µg/L; C4=0.075 µg/L; C5=0.0075 µg/L. Sertraline is reported to be found at 0.16 µg/L, and five concentrations were tested: C1=15.0 µg/L; C2=1.5 µg/L; C3=0.15 µg/L; C4=0.015 µg/L; C5=0.0015 µg/L. After the exposure, seed germination rate, hypocotyl and radicle length were measured to assess phytotoxicity. Diazepam did not induce changes in seed germination rate. However, for the measured lengths, C2, C4, and C5 promoted significant inhibition of hypocotyl development, while only C5 inhibited the radicle development. Comprising the total length of the seedling, C2 and C5 had a significant impact on the plant bioindicator, inhibiting its growth. Sertraline also did not change seed germination rate. Regarding the measured lengths, C4 inhibited the development of the hypocotyl, while C2, C3, C4, and C5 significantly altered the development of the radicle, also impacting the total length. These results show the harmful effect of the pharmaceuticals on the test organism, despite there was no dose-response relationship, as could be expected. Thus, these preliminary data need to be confirmed, suggesting the need of other tests.

## 6.P-Mo-022

### Effects of Diclofenac and Caffeine on Reproduction of *Astyanax lacustris* Males

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#### Abstract

Currently, the dumping of substances has been growing in water bodies, among these substances, there are some compounds that do not have current legislation, however, carry the potential to cause harm to aquatic animals, being called contaminants of emerging concern. Among them are drugs, which arrive in the environment either by incorrect disposal or by metabolites from humans and animals. Once in the environment, drugs can interfere with the reproduction of organisms, for example in animals that are produced in commercial fish farms. *Astyanax lacustris* is a species of Neotropical fish that has commercial importance because it is easy to cultivate, and can be used for direct consumption, industrialization in the form of preserves, as fodder food in the creation of carnivorous fish, and can also be sold as bait live for sport fishing. The objective of this study was to analyze the effect of two drugs, diclofenac, and caffeine, isolated or combined, in subchronic exposure, on the reproduction of *A. lacustris* males (CEUA-IB n°366-2020). The animals were exposed in duplicates in 4 treatments: Control (CTRL- without drug addiction), Diclofenac (DCF, 0.4 µg/L), Caffeine (CAF, 27.5 µg/L), and Mixture (DCF + CAF) for 14 days. After exposure, the animals were anesthetized, and blood was collected for measurement of gonadal steroids: 17-β estradiol (E2), testosterone (T), and 11 ketotestosterone (11 KT). Then the animals were euthanized, the testicles removed, fixed in “Karnovsky” and processed for light microscopy. For the analysis of gene expression, we analyzed the *fsH* and *lh* genes produced by the hypophysis using the RT-qPCR method, using two reference genes (*rpl7* and *ef1a*). Exposure to DCF reduced the plasma concentration of T and exposure to CAF reduced the plasma concentration of 11-KT. The analysis of the concentration of E2 in the plasma indicated that there were no significant alterations in the different treatments. Analyzes of testicular histology showed a lack of pattern in the reproductive stages, suggesting that after 14 days of exposure to drugs, the duration of spermatogenesis was altered, which could impair the reproduction of this species. And regarding gene expression data, statistical analyzes did not show significant changes in any of the treatments, which may indicate that there were no changes at the gene level, but at post-transcriptional levels.

## 6.P-Mo-023

### Sewage Sludge Detoxification for Agricultural Use by Biostimulation and Bioaugmentation: Evidence of Phytotoxicity Reduction in *Allium cepa*

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#### Abstract

Sewage sludge (SS) is a residue resulting from the wastewater treatment process. Due to its increasing production and toxic composition, its correct management is essential to avoid harmful effects on the environment. Among the possible alternatives for a more sustainable destination, there is its use as an organic fertilizer due to the possibility of reusing its nutrients, since it is rich in organic matter, phosphorus, nitrogen, and micronutrients. However, the presence of toxic substances can make this practice unfeasible. The aim of this work was to propose low-cost technologies for the SS detoxification, allowing its use as an agricultural additive. For this, SS samples were submitted to the biostimulation process, using rice husk as a bulking agent, and to the bioaugmentation process, through the incorporation of the lignolytic fungus *Pleurotus ostreatus* (shimeji). The sludge was diluted in the following volumetric proportions for the experimentation: SS (*in natura*), SS + soil (3:1); SS + soil + rice husk (3:1:1); SS + soil + *Pleurotus ostreatus* (3:1); SS + soil + rice husk + *Pleurotus ostreatus* (3:1:1). These treatments were evaluated at the initial time (T0) and after 40 days of bioremediation (T1) for phytotoxicity with the test organism *Allium cepa*. Physicochemical characterization was also performed to verify alterations in the series of solids, pH and conductivity along the detoxification process. At T1, there was an increase in solids content for all treatments and a slight decrease in organic matter when compared to T0, suggesting that the stabilization process is occurring. The obtained pH values, in both times, remained close to neutrality. The electrical conductivity parameters remained close in both periods, not presenting significant drops. For phytotoxicity, a complete inhibition of germination was observed at T0, for all samples. At T1, an improvement was observed, especially for the sample Sewage SS + Soil. However, the results of the germination index classified the samples as highly toxic, even after 40 days of the process. Thus, it is expected that longer bioremediation periods of 3 and 5 months will lead to a decrease in phytotoxicity and the stabilization of organic matter, contributing to solving the problem of SS disposal. This will transform the waste, which has an environmental impact, into a material with added value for agricultural purposes that is guaranteed to be environmentally safe.

## 6.P-Mo-024

### The Influence of Environmental Concentrations of the Neuropharmaceutical Carbamazepine Associated With Calcium, on Sperm Quality Parameters of *Astyanax lacustris*

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#### Abstract

Carbamazepine (CBZ) is of interest in ecotoxicological studies, as it is present in water bodies in high concentrations and is resistant to biodegradation. CBZ can interfere with the biology of aquatic organisms by altering several biological processes, such as spermatogenesis, and consequently compromising sperm parameters *i.e.*, kinetics in fish spermatozoa. The physical and chemical characteristics of the water added to the presence of pollutants can influence the toxicity of drugs to aquatic organisms, but this relationship is scarcely studied. However, it is not understood whether the presence of calcium (Ca), an ion that can affect drug availability, in the water interferes with CBZ toxicity and even if the co-exposure between both can influence sperm parameters in fish. Therefore, this study aims to evaluate the effect of different environmental concentrations of Ca and CBZ in water, as well as co-exposure, on sperm kinetics of *Astyanax lacustris*. Briefly, males were exposed, for seven days, to the different experimental groups: Dimethyl sulfoxide (DMSO (vehicle) – control group); 16 mg L<sup>-1</sup> Ca (Ca16); 250 ng L<sup>-1</sup> CBZ (250CBZ); 500 ng L<sup>-1</sup> CBZ (500CBZ); 16 mg L<sup>-1</sup> Ca + 250 ng L<sup>-1</sup> CBZ (Ca16/250CBZ); 16 mg L<sup>-1</sup> Ca + 500 ng L<sup>-1</sup> CBZ (Ca16/500CBZ), after this exposure period, they were induced with carp pituitary extract (5 mg Kg<sup>-1</sup>) for semen sampling. Motility (MOT), curvilinear (VCL), linear (VSL), mean velocity (VAP), and straightness (STR) of spermatozoa were analyzed using the CASA (Computer Assisted Sperm Analysis) system (ISAS® Integrated Semen Analysis System, Proiser, Valencia, Spain) coupled to a phase contrast microscope UB200i (UOP/Proiser) with a 10x negative phase contrast objective. The results showed that the 250CBZ and 500CBZ groups had lower percentages of MOT compared to DMSO. In VCL, VSL, VAP, and STR, the 250CBZ, 500CBZ, and Ca16/250CBZ groups were significantly different from the DMSO group. In this context, these preliminary results show that exposure to the isolated drug CBZ, as well as co-exposure to Ca+CBZ can influence the sperm kinetics of *A. lacustris* spermatozoa, suggesting that they can impair the reproductive success of the species. Additionally, other sperm parameters are being studied in our group, as well as analysis of the reproductive axis, to verify the action of CBZ as possibly an endocrine disruptor in the biology of aquatic organisms, especially in teleost fish.

## 6.P-Mo-025

### Acute and Chronic Effects of Silver Nanoparticles on a Native Argentinian Cladoceran

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#### Abstract

Silver nanoparticles (AgNPs) are increasingly used in various fields, consequently are being manufactured and released into aquatic ecosystems, and their potential toxicities in aquatic organisms are a matter of increasing concern. The present study assessed the acute and chronic toxicity of AgNPs in a native Argentinian water flea: *Simocephalus vetulus*. The AgNPs were obtained by chemical synthesis, characterized by Dynamic Light Scattering and spectrophotometry. The culture and assays were in a culture chamber under constant conditions (APHA medium,  $22 \pm 2$  °C, 16L:8O). For the acute test, neonates (<24 h) were exposed to 6 concentrations of AgNPs: 4.4, 8.8, 17.5, 35, 70, and  $140 \mu\text{g L}^{-1}$  (5 neonates per replicate, 4 replicates per treatment and negative control). The EC<sub>50</sub>-48h was calculated by linear interpolation. For chronic test 1 neonate of *S. vetulus* (<24 h) was used per replicate (21 days, 10 replicates per treatment and negative control, fed 3 times per week -*Chlorella vulgaris*-). The sublethal concentrations used were: 3.12 (C1), 6.25 (C2), and  $12.5 \mu\text{g L}^{-1}$  (C3). The number of live organisms, the number of offspring, the age at first reproduction, and the number of molts were recorded to assess survival, fecundity, and growth. Data were analyzed using ANOVA. The AgNPs showed an absorbance peak at  $\lambda_{\text{max}} = 395$  nm, an average size of  $47 \pm 3$  nm, and a zeta potential of -14 mV. In APHA medium, the nanoparticles showed a  $314 \pm 118$  nm average diameter and -13 mV zeta potential. The EC<sub>50</sub> was  $25 \mu\text{g L}^{-1}$ . In relation to the chronic assays, the survival of *S. vetulus* showed significant differences at the three highest concentrations with respect to the control ( $p < 0.001$ ). Survival decreased progressively with increasing AgNPs concentration from 28.5% to 70% with an average survival of  $5 \pm 4$  days at C3. Significant differences in growth were also detected with respect to the control at C1, C2, and C3 ( $p < 0.001$ ). The number of molts decreased from  $9 \pm 1$  in control to  $2 \pm 2$  at C3. Regarding fecundity, significant differences were found between the control and all treatments ( $p < 0.001$ ). No hatches were recorded at C1, C2, and C3. The presence of aborted eggs was recorded at C1. *Simocephalus vetulus* was found to be highly sensitive to nanosilver exposure. Low concentrations of AgNPs generate critical sublethal effects in *S. vetulus* demonstrating that significant population effects can occur in aquatic systems at environmentally relevant exposure levels.



## 6.P-Mo-026

### Biochemical Effects on Immature *Chironomus sancticaroli* (Diptera: Chironomidae) Exposed to Gadolinium, a Technology-Critical Element

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#### Abstract

The enrichment of Rare Earth Elements (REEs) has been reported in natural environments as a result of the input from hospital and/or industrial effluents. Gadolinium (Gd) is a Technology-Critical Element (TCE) used as a contrast agent for magnetic resonance imaging that can reach concentrations in water compartments up to two orders of magnitude above baseline levels. Despite its frequent use, there is little information on its effects on aquatic benthic macroinvertebrates, therefore, this study aimed to evaluate the impact of Gd on the physiology of *Chironomus sancticaroli* immatures. For this, pools formed of fourth instar larvae were exposed to Gd at 0.03; 0.3; 3.0; 30.0 and 120.0  $\mu\text{g L}^{-1}$  for 48 h. The endpoint biomarkers were glutathione S-transferase - GST (antioxidation), esterase  $\alpha$  and  $\beta$  - Est- $\alpha$  and Est- $\beta$  (biotransformation) and acetyl cholinesterase - AChE (neurotoxicity). After analysis of variance, mean values were compared by Tukey's test at 5% significance ( $p < 0.05$ ). The results showed an increase in AChE activity of larvae exposed to all Gd concentrations when compared with larvae exposed to the control. Similarly, Est- $\alpha$  and Est- $\beta$  activity increased in larvae exposed to Gd; the increment of Est- $\alpha$  activity of larvae exposed to 0.03  $\mu\text{g L}^{-1}$  was 1.6 times higher than the one measured in control larvae; likewise, Est- $\beta$  activity of larvae exposed to 0.3  $\mu\text{g L}^{-1}$  was 2.5 times higher than the one obtained in control larvae. Nevertheless, GST activity of larvae exposed to 120  $\mu\text{g L}^{-1}$  was inhibited three times compared to the control larvae. Despite the fact that Gd concentrations did not cause mortality in larvae during the acute exposure, biomarkers of neurotoxicity and biotransformation increased, while that of antioxidant activity decreased even at the lowest Gd concentrations. The results indicate that low levels of stress activate defense strategies of the organism to avoid damage and may even influence the development and reproductive success of *C. sancticaroli*. It is noteworthy that this study is pioneering in showing the biochemical effects of ETCs on insects. The information obtained indicates the impact and risk of Gd when disposed in lentic and lotic freshwater environments.

## 6.P-Mo-027

### Assessment of Phytotoxic Potential of a Slow-Release Multicomponent Vitreous Fertilizer on *Allium cepa* L.: A Preliminary Study

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#### Abstract

Soil fertility is a crucial factor for plant development and agricultural production. Brazil, one of the world's largest food producers, is also among the biggest consumers of fertilizers globally. However, obtaining these inputs related to soil fertilization has been challenging due to the ongoing war situation involving the nations from which most of this input is imported and the increasing value of the dollar. As a result, the development of more efficient national inputs, such as slow-release fertilizers, which aim to improve the uptake of nutrients by plants through the progressive release of nutrients, has gained emphasis. However, little attention has been paid to the ecotoxicological potential of these new fertilizers and their environmental implications. Thus, the present study aimed to evaluate possible phytotoxic alterations of a slow-release multicomponent vitreous fertilizer through the bioassay with the test organism *Allium cepa* L. The fertilizer, with a granulometry between 0.8 and 1 mm, was obtained by the traditional melting-cooling method, which has phosphorus, silicon, and boron as the basis for its formation. Seeds were germinated in individual Petri dishes containing the soil and one of the five concentrations tested (0.317 g/L; 0.633 g/L; 1.267 g/L; 2.534 g/L; 5.063 g/L). Negative and positive controls were performed with sandy soil (with 70% of its water holding capacity) and zinc sulfate (0.05 M), respectively. The plates were incubated at 22°C, with a photoperiod of 12h, for 5 days. Then, germination rate, radicle size, and germination index were assessed. The data were statistically compared with ANOVA. So far, the results have shown that the germination rate and radicle size induced no statistically significant variations when compared to the negative control, demonstrating that the concentrations of agriglass tested did not lead to phytotoxic effects in *A. cepa*. Regarding the germination index (IG%), all the concentrations were classified as not toxic, presenting values above 80%. Although complementary analysis, such as cytotoxicity, genotoxicity, and mutagenicity, must be performed, the preliminary results point to the vitreous fertilizer as a promising material to promote more sustainable agriculture.

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## 6.P-Mo-028

### **Preliminary Analysis of the Effects of Triclosan on Brain of Foraging Workers of *Bombus atratus***

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#### **Abstract**

Triclosan (TCS) is an antimicrobial used widely in pharmaceutical and personal care products. TCS has been listed as an emerging micropollutant due to its prevalence in water bodies and bioaccumulation properties. However, there is still not enough information about its risk to terrestrial organisms, such as bees. Studies of TCS in bees have not been reported in the literature so far. Thus, the aim of this study was to analyze the effects of TCS at two safety concentrations of 0.1% and 0.3% (EPA/FDA) on the brain of foraging workers of *Bombus atratus*. *B. atratus* workers were collected in the field and transferred to the laboratory, where they were kept in a controlled environment (26 °C; 70% relative humidity) for 96 hours. Bioassays were performed by offering sucrose solution (70%) for the control, and two different solution concentrations of TCS for the experimental group, 1000 ppm and 3000 ppm respectively. In addition, an acetone control group was performed. Groups were fed *ad libitum*. After exposure, workers were anesthetized (4°C; 20 min) and their brains were dissected and prepared for morphological and morphometric analysis. Preliminary data showed that the concentration of 1.000 ppm of TCS did not induce morphological effects on the brain. Kenyon cell nucleus areas did not differ from the control. However, a mischaracterization of the topography of the Kenyon cells inside the neuropile can be observed. At 3000 ppm TCS, we observed a change of the Kenyon cells, with swollen intercellular spaces between the cells and pyknotic nuclei. There was also an increase in the area of Kenyon cell nuclei in exposed bees at a concentration of 3000 ppm TCS, which indicates chromatolysis. Additionally, morphometric data performed by the Kruskal-Wallis non-parametric statistical test, followed by the post-test of Dunn's multiple comparisons, showed a significant difference between the experimental and control groups ( $p < 0.001$ ) for inner noncompact and inner compact Kenyon cells. These cells are responsible for the orientation memory capacity of bees. Therefore, these preliminary data suggest that the TCS at 3000 ppm may affect the performance of the foraging workers of bumblebees.

## 6.P-Mo-029

### **Evaluation of Anti-inflammatory Drug Diclofenac Effects on Growth, Energy Reserves and Osmolarity of the Crayfish *Cherax quadricarinatus***

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#### **Abstract**

The presence of emerging contaminants in ecosystems has an increasing global concern. Diclofenac (DCF), a non-steroidal anti-inflammatory drug, is one of the most widespread pharmaceutical compounds found in freshwaters. Because of the inadequate wastewater management, this compound is incompletely removed, so it can reach the water bodies impacting the aquatic life. The aim of this study was to evaluate the effects of DCF, at sublethal concentrations, on growth, energy reserves and osmolarity in juveniles of the crayfish *Cherax quadricarinatus* as a model species of higher crustaceans. For this purpose, a 90 days in vivo assay was carried out. Male and female juveniles weighing from 0.8 to 1.2 g were isolated in glass containers and randomly assigned to one of the following treatments: Control (freshwater), DCF 0,1 mg/L and DCF 1 mg/L. The assay was conducted under controlled conditions of feeding, photoperiod, aeration, and temperature ( $26 \pm 2^\circ\text{C}$ ); the water in all containers was completely replaced every 72 h. During the assay animals were periodically weighted and molting was registered. At the end of the assay hemolymph of each juvenile was withdrawn, and then they were sacrificed by cryoanesthesia to dissect hepatopancreas and muscle for determination of lipid and protein levels. Besides, the gills were fixed in Bouin solution for histological analysis. No significant differences were observed between control and groups exposed to DCF neither in weight gain nor in hepatosomatic index or energy reserves levels ( $p > 0.05$ ). The percentage of molting as well as the time between molts did not show significant differences in exposed groups compared to the control group ( $p > 0.05$ ). However, the osmolarity in hemolymph was significantly lower ( $p = 0.021$ ) comparing both DCF concentrations to the control. Moreover, the percentage of gills presenting melanization significant increased ( $p = 0.011$ ) in both DCF concentrations, with respect to control. Together, these results suggest a probably gill disfunction caused by diclofenac, which had been barely reported previously. We are currently looking for other pathologies on *C. quadricarinatus* gills, also analyzing the hemolymphatic components involved with the change in osmolarity.

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## 6.P-Mo-030

### Evaluation of the Response of *Hyalella curvispina* from a River in Norpatagonia Exposed to Caffeine

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#### Abstract

The world population is constantly increasing as well as the development of socioeconomic activities. This situation causes an uncontrolled increase of environmental pollutants. Many of these reach water bodies through the discharge of industrial, municipal, and agricultural effluents. Caffeine (CF) is an organic compound present in consumer beverages and it is used as a psychostimulant, analgesic, and bronchodilator. Moreover, it is considered an efficient pollution indicator of anthropogenic activity due to its widespread use in the population and its frequently detected, mainly in aquatic environments.

Neuquén is the second province with the higher population growth rate in the last 12 years, with an average of 40 inhabitants/day. This population growth represents a potential pollutant risk in water bodies that cross urbanized areas. The aim of this work was to evaluate and compare the response of laboratory and field populations *Hyalella curvispina* from the Neuquén River. Acute toxicity tests were carried out at 24, 48 and 72 hours with *Hyalella curvispina* exposed to CF at different concentrations. For each CF concentration range (100-998 mg L<sup>-1</sup>) at least 4 replicates were performed with their respective controls per experimental unit. The tests were static and without feeding during their development. Mortality (%M) was recorded when no movement of the pleopods was observed. Within the range of concentrations tested, only 100 %M was obtained at 72h. The concentration-response relationship as a function of the log of the concentration showed a different behavior than expected. The results showed that it did not follow logarithmic behavior. However, it was observed that laboratory organisms show a higher %M to CF at all exposure times than field population, suggesting a greater sensitivity to CF. According to these results biochemical studies will be carried out. It should be noticed that these studies are in a preliminary stage to evaluate toxicological parameters and the risk factor calculation.

## 6.P-Mo-031

### Bisphenol *a* Toxicity in Five Species of Cladocerans

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#### Abstract

Bisphenol *a* (BPA) is a compound present in plastics type 3 and 7, present in water bottles, baby bottles, disposable tableware, toys, and coatings on food cans, mainly. This compound is harmful because it acts as an endocrine disruptor. Since the information on the sequelae of this compound in aquatic organisms is scarce, the objective of this work was to evaluate the effects of Bisphenol *a* on the survival, growth, and reproduction of five species of cladocerans: *Daphnia magna*, *Ceriodaphnia dubia*, *Moina macrocopa*, *Simocephalus* sp. and *Chydorus* sp. Initially, a 48-hour acute bioassay was performed, where 5 concentrations of BPA were tested, to determine the LC<sub>50</sub> (Lethal Concentration 50). Afterwards, a sublethal bioassay (21 days) was carried out with 2 sublethal concentrations (LC<sub>50</sub>/100, LC<sub>50</sub>/10) where the following were evaluated: time of the first molt, number of moults, time of the first spawning, number of spawnings and the growth rate. The LC<sub>50</sub> values obtained in the tests varied from 5.4 to 12.1 mg/L, the most sensitive species was *Moina macrocopa*. Regarding the time in which the first molt occurred and the time of the first spawning, significant differences were observed between the organisms exposed to BPA and the control group ( $p > 0.05$ ). An increase in the time was observed for the cladocerans to have their first molt and spawn. The number of spawnings in the organisms exposed to BPA was lower compared to the control group. In addition, a decrease in the growth rate of between 1.5% and 44.9% was observed. These data confirm that in sublethal concentrations BPA affects cladocerans.

## 6.P-Mo-032

### Toxic Effects of Two Antiparasitic Drugs in Aquatic Organisms of Different Trophic Levels

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#### Abstract

Drugs have become major sources of contamination of aquatic systems, because they are substances used in large quantities, which enter aquatic systems through the discharge of wastewater. Mebendazole is an anthelmintic that has embryotoxic and teratogenic effects in mice. And Metronidazole is a drug used as a microbiocide and antibacterial that has genotoxicity effects. Since the sequelae of these compounds in aquatic species are not completely known, the objective of this work was to evaluate the toxic, genotoxic and neurotoxic effects of the drugs Metronidazole and Mebendazole in organisms of three trophic levels: the microalgae *Monoraphidium pusillum*, the cladoceran *Ceriodaphnia dubia* and the zebrafish *Danio rerio*. Acute bioassays were performed with a duration of 96 hours for the microalgae and fish and 48 hours for the cladocerans where the LC<sub>50</sub> was determined. Subsequently, sublethal tests were carried out with the LC<sub>40</sub> and LC<sub>10</sub> concentrations for the microalgae where the concentration of chlorophyll, carotenes, phenols and degree of lipoperoxidation were evaluated. In the tests with cladocerans and fish, LC<sub>10</sub> and LC<sub>1</sub> were tested and the degree of lipoperoxidation, the activity of AChE, the content of macromolecules and the genetic damage were evaluated. Toxicity tests indicated that the organism most sensitive to drugs was microalgae. In sublethal tests it was observed that both drugs alter chlorophyll concentrations, increase the production of carotenes and phenols. Mebendazole had an oxidative effect on the microalgae. In the bioassays with cladocerans and fish, it was observed that both drugs cause an increase in the levels of lipoperoxidation. Mebendazole caused a neurotoxic effect in cladocerans and fish. Both drugs caused genotoxic damage but acumulative effect was observed with Metronidazole test. The 2 drugs had deleterious effects at sublethal concentrations.

## 6.P-Mo-033

### The Effects of the IM1-12Br Ionic Liquid and Oxytetracycline Mixture on Selected Marine and Brackish Microorganisms

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#### Abstract

The widespread presence of emerging contaminants is a potential factor that contributes to the deterioration of freshwater and marine ecosystems. Currently, risk and hazard assessments are based mainly on the evaluation of the effects of individual chemicals. However, ecosystems are exposed to multiple-component pollutant mixtures. Interactions between these pollutants and their continuous presence in the aquatic environment can increase the overall threat to organisms and result in unexpected negative effects.

Pharmaceutical residues, including antibiotics, are commonly detected in all types of water. Ionic liquids (ILs) are a class of organic salts that have gained a great deal of attention in recent years because of their potential applications in industrial areas. However, because of their unique physicochemical properties and resistance to biodegradation, they are considered a threat to the aquatic environment.

The purpose of the presentation is to provide a general overview of the challenges and potential risks associated with the presence of micropollutant mixtures in the aquatic environment, including information on the effects of mixtures of ionic liquids and other organic micropollutants. The presented results include the investigation of chronic exposure of microorganisms present in brackish coastal waters and the open Baltic Sea basin, including the green algae *Chlorella vulgaris*, the cyanobacteria *Microcystis aeruginosa*, and the diatom *Phaeodactylum tricorutum* to antibiotic oxytetracycline (OTC) and imidazolium-based ionic liquid 1-dodecyl-3-methylimidazolium bromide (IM1-12Br) mixtures. Standard measurements (e.g. optical density, pigments concentration) were combined with the evaluation of photosynthetic parameters. Variations in chlorophyll fluorescence kinetics defined as the O, J, I, and P steps of the redox states of the PSI and PSII directly related to electron transfer efficiency were quantified. In addition, the impact on the luminescent marine bacterium *Vibrio fischeri* has been evaluated. Growth, photosynthesis process, and pigment ratios were affected in all target microorganisms. The concentration addition (CA) and independent action (IA) mathematical models followed by the Model Deviation Ratio (MDR) evaluation allowed for the identification of mainly synergistic interactions in the studied mixtures. The results of the study provide insight into the effects of ionic liquids and other organic micropollutants.



## 6.P-Mo-034

### **Microcystin-LR Cause More Damage to Liver Tissue of Fish *Poecilia reticulata* in Co-exposure with Iron Oxide Nanoparticles**

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#### **Abstract**

Microcystin-LR (Mic-LR) is a cyanotoxin that occurs in eutrophicated aquatic environments and may be harmful to human and environmental health. The use of iron oxide nanoparticles (IONPs) for adsorption of Mic-LR may be an alternative for mitigating the effect of these cyanotoxins in the environment, however, it is necessary to understand the toxicity of these agents for the purpose of developing safe environmental remediation practices with IONPs. In this regard, female fish *Poecilia reticulata* were exposed for 7 days, organized into: A) control group; B) iron ions 0.3 mgFe/L (IFe); C) IONP 0.3 mg/L; D) Mic-LR 1.0 µg/L; E) Mic-LR 1.0 µg/L +IONP 0.3 mg/L. Biometric (condition factor, hepatosomatic index) and histological (liver histopathology) biomarkers were analyzed. Fulton condition factor (KFulton) and hepatosomatic index (HSI) did not vary between treatments ( $p>0.05$ ), which corroborates good experimental conditions and inability of treatments to cause nutritional impacts in 7 days of exposure. On the other hand, in the histopathological evaluation the livers of animals treated with IFe, Mic-LR and IONP+Mic-LR showed intense occurrence of steatosis and presence of necrotic areas. In all treated groups, including IONP, circulatory disturbances, leukocyte infiltration and formation of melanomacrophage sites were noted. In conclusion, exposure of *Poecilia reticulata* to Mic-LR and IONP for 7 days is potentially capable of compromising the homeostasis of the animals, so that more severe effects are related to the presence or association of Mic-LR. Thus, even at regulated concentrations Mic-LR is a problematic environmental contaminant and studies such as this one with longer time intervals should be conducted.

## 6.P-Mo-035

### Removal of Emerging Contaminantes (Endocrine Disruptors) Using Ag<sub>3</sub>AsO<sub>4</sub> and Detection by High Performance Liquid Chromatography (HPLC).

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#### Abstract

Currently, one of the biggest problems in the world is environmental pollution with the contamination of soil, air and water, which is an essential resource for the maintenance of life. Among several types of emerging contaminants, there is the release of endocrine disruptors 17 $\beta$ -estradiol (E2) and 17 $\alpha$ -ethinylestradiol (EE2) in sanitary sewage and consequently in water bodies, since conventional treatments do not remove this type of pollutant. In view of this, it is necessary to develop studies and technologies for the removal/degradation of these organic contaminants from water bodies. The present study aims to evaluate the ability to remove the endocrine disruptors 17 $\beta$ -estradiol (E2) and 17 $\alpha$ -ethinylestradiol (EE2) in water by the photocatalytic activity of the compound Ag<sub>3</sub>AsO<sub>4</sub>. Silver arsenate was synthesized and characterized, the quantification of hormones E2 and EE2 by high performance liquid chromatography with fluorescence detector went through a validation process and some preliminary tests of photodegradation of hormones using the Ag<sub>3</sub>AsO<sub>4</sub> catalyst were performed. As partial results, the validation was performed and reached satisfactory results:  $r^2=0.9976$  (E2),  $r^2=0.9969$  (EE2), recovery of 100.15% (E2) and 100.31% (EE2). In photodegradation studies, Ag<sub>3</sub>AsO<sub>4</sub> showed different behavior in the presence of light for each hormone. In solution with E2, it reached a removal rate of approximately 35% of the hormone under LED light, acting as a photocatalyst. While with EE2, it reached a removal rate of approximately 96%, but for this hormone, it presented behavior of adsorbent material and photocatalyst. Given this, the studied material showed great potential to act in environmental remediation processes.

## 6.P-Mo-036

### Electrochemical Sensors for the Detection of Pollutants in Water Using Laser-Induced Graphene Electrodes: A Case Study of Advanced Oxidation Processes of Amoxicillin With the $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ Fenton Reaction

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#### Abstract

In this work, a simple, rapid, sensitive and inexpensive method using laser-induced graphene electrodes (LIGEs) for chronoamperometric (CA) determination of amoxicillin (AMX) in laboratory-prepared samples and river water samples before and after advanced oxidation treatment is presented. The CA sensor method is based on the advanced oxidation of AMX catalyzed by the electrochemical oxidation of potassium ferricyanide in the presence of hydrogen peroxide. The delta of the current measured before and after the electrochemical oxidation of AMX showed a linear dependence on the concentration in three linear ranges. The first range was between 0.5 and 500 ppb ( $\text{ng ml}^{-1}$ ), the second range was between 0.500 ppm and 30 ppm ( $\mu\text{g ml}^{-1}$ ), and the third range was between 30 and 2000 ppm. The detection limit (LOD) for the determination of AMX was 0.5 ppb. The developed electrochemical method was validated using high performance liquid chromatography in combination with a mass detector (HPLC-MS). In addition, the method HPLC-MS was used to investigate the electrochemical mechanism of the detection method. The method was validated with a pharmaceutical product and an enriched well water sample. Very good agreement was obtained between the detected and theoretical concentrations of branded pharmaceuticals, with recoveries ranging from 99.5% to 104.8% for pharmaceutical and well water samples, respectively. Interference with other samples containing diclofenac was investigated, and the sensor can be used to determine total emerging contaminants as a criterion for drinking water quality.

## 6.P-Mo-037

### **Biomarker Responses in *Piaractus mesopotamicus* Following Oxytetracycline Treatment at Therapeutic Doses**

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#### **Abstract**

Antibiotics are commonly used in aquaculture, although their overuse or misuse can have negative effects on fish health. Oxytetracycline (OTC), is the principal member of the tetracycline group used in aquaculture worldwide, however, the ecotoxicological effects is still not fully understood. In this study, the pharmacological treatment of the freshwater fish *Piaractus mesopotamicus* with diets containing OTC pure (ATB1) and commercial form (ATB2) was carried out for 10 days with a withdrawal period of 21 days. After 0, 0.25, 3, 10, 11, and 31 days, alterations in morphological, hematological, plasma biochemical parameters and the enzymatic activity in different tissues (CAT, GST, APA, and LDH) were evaluated. The result showed that OTC did not generate morphological changes in individuals, but the fish fed with ATB2 suffered a decrease in hematocrit and an increase in glucose levels. In general, an increase in CAT activity is observed in the liver, gills and muscle of individuals treated with OTC, especially with ATB2. A similar trend was observed in the GST activity of the brain, gill and muscle. On the contrary, APA activity in the liver decreased in the groups fed with ATB1 and ATB2 when compared to the control. Moreover, only ATB2 induced LDH after 1-day depuration in fish muscle.

Principal components analysis (PCA) revealed that most enzymes (except for FAL and GST in the liver), glucose, MCH and MCHC were associated with fish-fed ATB2. Conversely, hemoglobin, hematocrit, RBC, MCV, cholesterol, triglycerides, total protein, FAL and GST liver were associated with fish-fed ATB1 and the control group. These results demonstrated that the dietary therapeutic dose of OTC particularly that issued in commercial formulation caused adverse effects in *P. mesopotamicus*.

## 6.P-Mo-038

### Assessment of Biotransformation of the Pesticide Acephate Using the Zebrafish Animal Model

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#### Abstract

Acephate is one of the best-selling pesticides in Brazil. Studies relate acephate and your major metabolite methamidophos with symptoms of acute toxicity, genotoxicity, neurotoxicity and reproductive toxicity. The European Union prohibits the use of acephate in food crops and tolerates the detection of both in drinking water at a maximum concentration of 0.1 ug/L. However, in Brazil, the use of acephate is allowed in food cultures, with an acceptable ADI of 0.0012 mg/Kg and, in drinking water, the maximum allowed value (MMP) of this compound and its metabolite was recently defined in 7 ug/L. It is noticed that there is a need for studies that analyze the chronic toxicity of this compound, since, in Brazil, acephate is widely used, and there are few studies reporting the effects of its chronic exposure and there was a recent definition of MMP for residues in potable water. Thus, the present work evaluates the biotransformation in Zebrafish to the acephate, through the metabolomic analysis of aquarium water. The maintenance and exposure of the animals to the selected pesticides was performed as described in OECD protocol No. 305. The biotransformation assay was carried out for 7 days, with single application of the acephate solution at first, at a concentration of 200  $\mu\text{g/L}$ . The evaluation of the metabolites generated was performed with 3 different groups. In the first, called stability, there was only water and the acephate solution. In the second, called treatment, Zebrafish fish were used to analyze the biotransformation of acephate in the organism of these animals. And the third, the negative control. For the analysis of the study, aliquots of water from the tanks were collected at 0h, 1h, 3h, 6h, 8h, 12h, and 24h from all the tanks on the first day; and every 24h until the 7th day. Samples were analyzed on an HPLC to a mass spectrometer with electrospray ionization source and orbitrap-type analyzer. The same metabolites are observed in the treatment and stability groups. However, the increased formation of the metabolites is exacerbated by the metabolism of the Zebrafish animal model. Furthermore, from statistical analyses of the collected data, it was possible to define the indicator metabolites for acephate exposure. So far, this work is the only one to present the compounds related to the biotransformation and degradation of acephate in water, which can serve to monitor acephate in aquatic environments, aiding in its detection.

## 6.P-Mo-039

### Ecotoxicity of the Active Herbicide Ingredient Nicosulfuron on Aquatic Bioindicators

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#### Abstract

Environmental contamination by pesticides is of great concern and herbicides are the most applied pesticides in agriculture. One of the most widely used herbicides in the world is based on the active ingredient nicosulfuron, a sulfonylurea compound applied mostly in maize fields to post-emergent weed control, such as annual and perennial grasses and some broad-leaf weeds. The nicosulfuron molecule is relatively mobile and could be bioavailable in soil and water, representing a toxicological risk to the non-target organism. Hence, this study aimed to investigate the effects of nicosulfuron (1) on the population growth rate of the microalgae *Raphidocelis subcapitata*; (2) the mobility of the microcrustacean *Daphnia magna*; and, (3) the reproduction of the rotifer *Brachionus calyciflorus*. Nicosulfuron stimulated the population growth of *R. subcapitata* after 72 h of exposure. In contrast, the highest concentration applied in *D. magna* completely inhibited the mobility of the individuals after 48 h of exposure and the highest concentration applied to *B. calyciflorus* totally inhibited their reproduction. Therefore, we demonstrated that the active compound nicosulfuron is ecotoxic in the aquatic medium. All in all our study advanced in the knowledge of nicosulfuron effects in non-targeted organisms and the risk of its presence on the environmental.

# Session 7: Tracking Primary Pollutants and Their Toxicologically Relevant Transformation Products

## 7.P-Tu-049

### Trace Elements in Bituminous Coal in Colombia: Environmental Health Issues

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#### Abstract

Coal extraction is one of the most important economic sources in several countries, including Colombia. Despite the benefits to the gross domestic product, its extraction is related to the emissions of dust particles, which have the capacity to adsorb trace elements, e.g., metals and polyaromatic hydrocarbons (PAHs). Metal analysis in bituminous coal dust with particle sizes up to 38  $\mu$ m was developed using inductively coupled plasma mass spectrometry (ICP-MS) and a RA-915M Zeeman mercury analyzer with RAPID software (Lumex, Russia) with a pyrolysis unit (PYRO-915+) from the open-pit coal mine El Cerrejón, La Guajira (Colombia). In addition, the coal particles were submitted to hydroethanolic extraction in order to detect the presence of HAPs and organic compounds, using gas chromatography with mass spectrometry (GC/MS). Results show that the coal extracted from this open-pit coal mine presented forty four trace metals and metalloids: Li, Be, Hg, Sc, V, Cr, Co, Ni, Cu, Ga, Ge, As, Se, Rb, Sr, Y, Zr, Nb, Cd, Sn, Sb, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ta, Tl, Pb, Bi, Th, and U, and Seventeen organic compounds were identified: undecane, pentadecane, dodecane, undecane, 4-methyl, dodecane 2,6,11-trimethyl-, Heptane, 2,3-dimethyl-, Octane, 4-methyl-, Nonane, 4,5 dimethyl, Tetradecane, 2,6,10-trimethyl, cis-Aconitic anhydride, Glycerin, Ethyl iso-allocholate, Octanoid acid, 1H pyrrole-2,5 dione, Estra-1,3,5(10)-trien-17 $\beta$ -ol, Benzo[a]anthracene, 6,12-dimethyl-1,2,3,4-tetrahydro- and Neoquassin. These findings suggest that chemicals present in coal dust are biologically active and may interfere with key biochemical processes in living organisms. Also, these trace elements have major concern in toxicology due to their relationship with oxidative stress in several organisms and human tissues, including the human population, which is chronically exposed, as well as metabolic alterations, histopathological changes in macroinvertebrates of soil and mitigation of the reproductive capacity in mammals and detritivores.

## 7.P-Tu-050

### Degradation Dynamics of Commercial Formulations of Postharvest Fungicides Fludioxonil and Pyrimethanil under Laboratory Conditions and in Degradation Pools in North Patagonia, Argentina

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#### Abstract

Commercial formulations of the postharvest fungicides fludioxonil (FLU) and pyrimethanil (PIR) are employed for conventional production of pears and apples in North Patagonia. During the productive season, a considerable volume of fungicide-contaminated water is generated, which is subjected to photolytic degradation in pools. However, their residence time in the pools is uncertain, as well as the efficacy of the treatment. Objectives: to determine if FLU and PIR are being degraded by photolysis in the sampled pools, and to establish their degradation kinetics under laboratory conditions. Methods: Water samples were taken from the input and output of degradation pools belonging to two different companies. FLU and PIR were identified and quantified in the samples after liquid-liquid extraction and injection into HPLC-UV. To evaluate the degradation kinetics under laboratory conditions, the commercial formulations Scholar® 23SC (Syngenta; FLU 23% w/v) and Penbotec® 40 SC (Janssen Pharmaceutica; PIR 40% w/v) were used to prepare stock solutions of known theoretical concentration, from which working solutions (10 mg/l) were then prepared. Aeration was evaluated as a variable in the assays. The glass trays containing the solutions were covered with cling film and were kept in a room with temperature control and photoperiod. The degradation kinetics was studied for 30 days. Samples were taken at different intervals, and were analyzed by HPLC-UV. Physico-chemical parameters were monitored. At the end of the analysis period, the trays that contained FLU were rinsed with 10 ml of methanol and these samples were analyzed. Results: FLU was detected in all water samples collected from the degradation pools, which coincides with its usage during the productive season. PIR was also detected, even though it was not actively being used. The concentrations measured in the output of degradation pools were superior to the concentrations of input. A decrease of FLU concentration was observed under laboratory conditions in the sampled water, but it was adsorbed to the sediment that settled on the bottom of the trays. PIR concentrations did not change. Conclusions: The photolytic degradation pools analyzed are not able to remove FLU and PIR from the effluents that are then discharged into the authorized receptors, that ultimately reach rivers or wastewater treatment plants. No degradation of PIR and FLU was observed under laboratory conditions.



## 7.P-Tu-051

### Agricultural Organochlorine Pesticides Residues In Surface And Groundwaters of the Negro River Basin (North Patagonia)

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#### Abstract

In the present research, we studied the occurrence of a total of 16 organochloride pesticides (OCs) forbidden by the Convention, including  $\alpha$ -HCH,  $\beta$ -HCH,  $\gamma$ -HCH,  $\delta$ -HCH, heptachlor, aldrin, heptachlor epoxide, dieldrin, endrin, endrin aldehyde, pp'-DDT, pp'-DDE, and pp'-DDD, as well as endosulfan and its metabolites ( $\alpha$ -endosulfan,  $\beta$ -endosulfan, endosulfan sulfate) in the surface and ground waters of the Negro River basin in seven sites with different land uses. Our results concluded that the physicochemical parameters determined that groundwater was unsuitable for human consumption due to high pH and low DO. The mean value of the sum of the concentrations of the OCs analyzed for dissolved pesticides in groundwater samples was 2.35 ng L<sup>-1</sup>, and only aldrin was detected at the outlet of the river area in the order of 5.44 ng L<sup>-1</sup>. In contrast, no pesticides were detected in waters characterized by mixing processes with seawater. As a result, chlorinated cyclodiene insecticides, like heptachlor,  $\beta$ -endosulfan, and aldrin, were detected in groundwater samples. Only aldrin was detected in the surface waters of one site (the river outlet). However, most of these samples' OCs were <DL. Some OCPs (aldrin, heptachlor, and  $\beta$ -endosulfan) could have entered the groundwater through the filtration of layers of sand, clay, and adjacent strata. These OCPs might come from local agriculture and horticultural practices in the most productive area of Patagonia. Aldrin and  $\beta$ -endosulfan were the most frequent OCPs in all the sites, except for the estuarine waters, where all OCPs were <DL, probably because of a dilution factor. The PCA analysis showed endosulfans and heptachlors better-explained groundwater samples and cyclodienes surface waters. The correlations between the physicochemical parameters of groundwaters and  $\beta$ -endosulfan might imply that this chemical comes from recent applications in the area. Health risk assessments demonstrated that groundwaters might not pose carcinogenic risks, but aldrin values were above the recommendations for drinking waters, and these values should be further monitored. Nonetheless, chronic exposure to these contaminants might still be risky for human health. Thus, the anthropogenic contaminants are associated with the geographical locations of wells and economic activities. Therefore, future review policy options or management could be necessary to protect the aquifer and the river used as a water source for local communities.

## 7.P-Tu-052

### Accumulation of Current-Use Pesticides in Fish From an Agricultural Region of the Argentine Pampa

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#### Abstract

Modern agricultural systems are highly dependent on pesticides. An estimated 3 to 4 million tons of pesticides are applied annually to agricultural lands at the global scale. Such an intensive use of pesticides has resulted in a widespread contamination of air, water and soil worldwide. The objective of this study was to evaluate the presence of pesticides in fish of the species *Cnesterodon Decemmaculatus* from a sample site located near the end of the Pergamino River (Buenos Aires, Argentina), which crosses an area of intensive agricultural use. Adults females *C. decemmaculatus* were collected on 21 September and 7 November 2021, when corn and soybean sowing were, respectively, in progress. In November, the mortality of many fish species was observed in concurrence with the sampling. The weight and standard length of the fish were evaluated to calculate body condition. Pesticide residues in fish tissues were determined following extracción by the QueChers technique and posterior analysis by High Resolution Liquid Chromatography (HPLC) and Gas chromatography (GC). A total 43 current use pesticide were determined of which 18 were herbicides, 17 fungicides and 8 insecticides. 35 of the 43 pesticides were detected in sampled fish: Imazetapir, cyantraniliprole, chlorothalonil, fludioxinil, myclobutanil and atrazine were the pesticides with the highest detection rates, being present in more than 50% of the individuals, up to 75% of the individuals in the case of Imazetapir. Thirteen pesticides (S-metolachlor, acetochlor, tebuconazole, flurochloridone, triadimefon, cyproconazole, trifloxystrobin, saflufenacil, paraquat, chlorantraniliprole, thiophanate methyl, carboxine, thiametoxam) were detected in 30 to 50% of the fish examined. No significant difference was observed between the body condition of the fish captured in September and November.

## 7.P-Tu-053

### Gas Analyser Calibration for Reliable Air Pollutants Measurement and Minimization of Environmental Risk

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#### Abstract

Carbon dioxide and carbon monoxide are substantially polluting gases that are released into the atmosphere mainly by automotive vehicles and by industry-related activities. The presence of these contaminants at high concentration levels contributes negatively to air quality, affecting human health and the future of our planet. Carbon dioxide is the most important greenhouse gas, absorbs and radiates heat causing global temperature to rise. On the other hand, carbon monoxide has a short lifespan and eventually reacts with oxygen to form carbon dioxide. The reliable measurement of air pollutants allows the correct evaluation of the environmental impact associated with obtaining an inaccurate result. One way to achieve this, is by using gas analysers that were calibrated against certified reference materials of gas mixtures to ensure metrological traceability. In this study, a flue gas analyser was calibrated in carbon dioxide and carbon monoxide in the range of measurement of the instrument, quantifying the error with its uncertainty at each calibration level. This process was performed in the frame of the Quality Management System according to ISO/IEC 17025. The correction of the measurements carried out by the gas analyser led to trustworthy results to support reliable decisions regarding health and environmental protection, and to the compliance with current regulatory requirements.

DRAFT

## 7.P-Tu-054

### **Mercury Pollution in the North of Colombia: Relationship Between Mercury Concentrations in Sediments From the San Martin De Loba Gold Mining Area and Cartagena Bay Connected Through Magdalena River**

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#### **Abstract**

Bay of Cartagena is located in the north of Colombia, it is one of the major ports due to its strategic location in the Caribbean Sea and privileged natural conditions. Activities such as loading and unloading ports, national and international maritime traffic, industry, and the mouth of continental waters are the main source of pollution. The Cartagena Bay contemplates the highest percentage of freshwater discharge through the Canal del Dique and Magdalena River watershed receiving mercury pollution from the gold mining sites located in the south of the country in the Bolivar and Antioquia Departments. Sediment samples were collected from San Martin de Loba, Cartagena Bay, and their surrounding places in the dry and rainy seasons. The freeze-dried and sieve (< 60) sediments were analyzed by thermal decomposition in an atomic absorption spectrometer with Zeeman-effect background correction technique, RA 915M, and PYRO 915+ (Lumex Instruments). The LOD (0.0025 mg/kg), LOQ (0.0085 mg/kg), calibration curve ( $R \geq 0.995$ ), accuracy and precision of the CRMs PACS-3: Marine Sediment Certified Reference Material (NRC, Canada) were 97.9% and CV% of 7,07%, respectively, were assessed as quality control of the measurements. On average, the concentrations of total mercury in sediment were (mean  $\pm$  SD):  $0.244 \pm 0.362$  and ranged from 0.037 to 1.352 mg/kg for Cartagena Bay and  $0.107 \pm 0.075$  mg/kg ( $0.036$  mg/kg –  $0.295$  mg/kg) for waterbodies close to San Martín de Loba town. These T-Hg concentrations were higher than the estuaries and/or coastal systems of Scheldt, Belgium (0.14 – 1.80 mg/kg); Mira, Portugal (0.005 – 0.081) and in the Bay of Fundy in Canada with results less than 0.024 mg/kg. But in turn lower than the concentrations of the bays or coastal areas of Wuli, China (44 - 64 mg/kg), Minamata Bay, Japan (1400 - 4300 mg/kg), and Tagus of Portugal (0.99 – 29 mg/kg). Therefore, the bay of Cartagena and its surrounding places are considered the sink for mercury from artisanal and small-scale gold mining in the Antioquia and Bolívar Departments which are transported through the Canal del Dique and the Magdalena and Cauca rivers.

## 7.P-Tu-055

### **Weekend Effect of Air Pollutants in Small and Medium-Sized Cities: The Role of Policies Stringency to COVID-19 Containment**

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#### **Abstract**

The COVID-19 pandemic has led to a significant reduction in human mobility and economic activities, resulting in a notable decrease in air pollutant levels. However, the effect of these changes on the weekend, a period that is typically associated with lower pollutant levels due to reduced economic activity, has received little attention in previous studies.

This study aims to fill this gap by investigating the levels of five common air pollutants, namely O<sub>3</sub>, NO<sub>2</sub>, SO<sub>2</sub>, PM<sub>2.5</sub>, PM<sub>10</sub>, and the PM<sub>2.5</sub>/PM<sub>10</sub> ratio, before and after the COVID-19 pandemic in four cities in the state of Rio Grande do Sul, Brazil. The study also evaluates the impact of local policies on the so-called "weekend effect," which refers to the difference in pollutant levels between weekdays and weekends.

The results of this study indicate that there is a positive weekend effect for almost all criteria air pollutants in the four cities, which means that the pollutant levels are higher on weekends than on weekdays. Additionally, the study finds an intrinsic relationship between the weekend effect and the restriction measures adopted in the different regions. Specifically, the data suggests that a negative weekend effect was observed when less restrictive policies were in place, while more stringent measures led to higher intensities of positive weekend effect.

The study's findings suggest that the COVID-19 pandemic has had a complex and varying impact on air quality, depending on the level of restrictions implemented in different regions. The results also highlight the importance of considering the weekend effect when designing and implementing air quality policies. These insights could help guide responsible authorities in devising future strategies and policies for air quality control, with the ultimate goal of improving public health and environmental sustainability.

## 7.P-Tu-056

### Distribution and Exposure to Alternative Plasticizers in Indoor Dust From Three Cities in Colombia

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#### Abstract

Plasticizers are chemical additives added to polymers for the manufacturing of plastics to provide special features of durability, elasticity, and softer and more flexibility. Plasticizers are not chemically bonded to the materials and they can easily be released from products into the indoor environment, in which people spend most of their time. Plasticizers may be part of a dust particle, contributing to human exposure, and pose a risk to human health because they are suspected to be endocrine disruptors with many negative health outcomes. A group of alternative plasticizers were investigated in the present study in dust collected from cars, houses, and offices in three Colombian cities: Bogota, Medellin, and Cartagena. Samples were collected using a household vacuum cleaner and placed in solvent-rinsed aluminum foil, sealed in plastic zip lock bags, and stored at  $-20\text{ }^{\circ}\text{C}$ . Target compounds were analyzed using an Agilent GC coupled to an Agilent 5973 MS operated in electron ionization mode (EI). The GC system was equipped with an HT-8 column ( $25\text{ m} \times 0.22\text{ mm}$ ,  $0.25\text{ }\mu\text{m}$ ) electronic pressure control and a programmable-temperature vaporizer (PTV) inlet. The mass spectrometer was run in SIM mode with 2 characteristic ions acquired for each analyte and the IS. Matrix correlation was calculated using the concentrations of individual and total plasticizers relative to characteristics of the indoor environment (e.g. car, house, and office). Correlation coefficients were calculated to evaluate a possible relationship between two variables of the detected concentrations of individual and total plasticizers. A total of 10 alternative plasticizer compounds (ATBC, ATEC, BTHC, CDPHP, DBS, DEHA, DIBA, DINCH, THTM, and TOTM) were detected. The compounds detected more frequently in cars include Bis(2-Ethylhexyl) adipate (DEHA) y Tris-2-ethylhexyl trimelitate (TOTM) with a median (range) concentration of 22432 (9594 – 84659) and 35504 (13308 – 175203), respectively. The houses were found to contain Acetyl tributyl citrate (ATBC) (median: 16983; range: 4572-109267) and Bis(2-Ethylhexyl) adipate (DEHA) (median: 19136; range 5285 – 158595). In offices, the compounds found included TOTM (median: 64879; range: 17318 – 244797) and Bis(2-Ethylhexyl) adipate (DEHA) (median: 55069; range: 7589 – 88426). The plasticizers detected and most abundant in cars houses and office environments are DEHA, TOM, and ATBC. These compounds were prevalent in all three environments.

## 7.P-Tu-057

### Analysis Of Warfarin And Degradation Products In Uruguayan Waters

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#### Abstract

Warfarin, 4-hydroxy-3-(3-oxo-1-phenylbutyl)-coumarin is an anticoagulant drug that acts by inhibiting the synthesis of vitamin K-dependent coagulation factors (VII, IX, X, II). Factor VII is the most rapidly affected and II the least sensitive. It has no effect on already formed thrombi. It is useful to prevent the development of new thrombi and the extension of the clot. It is used in the treatment and prophylaxis of venous thrombosis and its complications (pulmonary embolism), and in cases of heart valve replacement.

Water samples were collected from different sites in Montevideo and its surroundings.

To carry out the analysis, the water sample is extracted with ethyl acetate (3 extractions). The organic layer is dried with anhydrous sodium sulfate and the solvent is evaporated under reduced pressure. The residue is analyzed by thin layer chromatography, nuclear magnetic resonance and mass spectrometry.

The drug was detected in the sample. Also, hydrolysis products were observed (benzalacetone and 4-hydroxycoumarin). Products resulting from the reaction of these compounds with chlorine (from sodium hypochlorite used as a disinfectant) are also observed.

## 7.P-Tu-058

### Analysis Of Enalapril And Degradation Products In Uruguayan Waters

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#### Abstract

Enalapril is used to treat high blood pressure and heart failure. The usefulness of angiotensin-converting enzyme (ACE) inhibitors as antihypertensives was first demonstrated with teprotide (a nonapeptide isolated from snake venom). Enalapril is a prodrug: it has little pharmacological activity, but the ester group is hydrolyzed in the liver to form enalaprilat (active molecule).

Water samples were collected from different sites in Montevideo and its surroundings.

To carry out the analysis, the water sample is extracted with ethyl acetate (3 extractions). The organic layer is dried with anhydrous sodium sulfate and the solvent is evaporated under reduced pressure. The residue is analyzed by thin layer chromatography, nuclear magnetic resonance and mass spectrometry.

The drug was detected in the sample. Also hydrolysis products and a diketopiperazine were observed. Products resulting from the reaction of these compounds with chlorine (from sodium hypochlorite used as a disinfectant) are also observed.



## 7.P-Tu-059

### Analysis Of Paracetamol And Degradation Products In Uruguayan Waters

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#### Abstract

Paracetamol is an analgesic and antipyretic and is used to treat fever and mild pain. It is available as a generic drug and is low cost. The compound have the amide functional group, so can undergo hydrolysis under certain conditions, giving rise to the formation of inactive or toxic products.

Water samples were collected from different sites in Montevideo and its surroundings.

To carry out the analysis, the water sample is extracted with ethyl acetate (3 extractions). The organic layer is dried with anhydrous sodium sulfate and the solvent is evaporated under reduced pressure. The residue is analyzed by thin layer chromatography, nuclear magnetic resonance and mass spectrometry.

The drug were detected in the sample. Also, hydrolysis is observed, forming 4-aminophenol. Products resulting from the reaction of these compounds with chlorine (from sodium hypochlorite used as a disinfectant) are also observed

## 7.P-Tu-060

### Analysis Of Nimesulide And Degradation Products In Uruguayan Waters

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#### Abstract

Nimesulide is an anti-inflammatory with antipyretic and analgesic action. It has been linked to liver toxicity. The compound have the amide functional group, so can undergo hydrolysis under certain conditions, giving rise to the formation of inactive or toxic products.

Water samples were collected from different sites in Montevideo and its surroundings.

To carry out the analysis, the water sample is extracted with ethyl acetate (3 extractions). The organic layer is dried with anhydrous sodium sulfate and the solvent is evaporated under reduced pressure. The residue is analyzed by thin layer chromatography, nuclear magnetic resonance and mass spectrometry.

The drug were detected in the sample. Also, hydrolysis of the active ingredient is observed, forming 2-phenoxy-4-nitroaniline. Products resulting from the reaction of these compounds with chlorine (from sodium hypochlorite used as a disinfectant) are also observed.

## 7.P-Tu-061

### Analysis Of Diclofenac And Degradation Products In Waters Of Uruguay

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#### Abstract

Diclofenac is a compound that is used as an analgesic and antirheumatic. The global consumption of diclofenac is estimated to be 940 tons per year. Diclofenac enters the aquatic environment by direct discharge or human or animal excretion. Since removal in water treatment plants is incomplete, diclofenac is often detected in natural waters. Diclofenac has been shown to harm freshwater fish at low concentrations. The use of diclofenac for animals is controversial due to its toxicity when ingested by carrion birds that eat dead animals. The use of diclofenac in animals has been reported to cause a decline in vulture populations in India and Pakistan. Toxicity may be due to either causing renal failure or inhibiting uric acid excretion.

Water samples were collected from different sites in Montevideo and its surroundings.

To carry out the analysis, the water sample is extracted with ethyl acetate (3 extractions). The organic layer is dried with anhydrous sodium sulfate and the solvent is evaporated under reduced pressure. The residue is analyzed by thin layer chromatography, nuclear magnetic resonance and mass spectrometry.

The drug was detected in the sample. Degradation products are carbazols and aromatic amines. The main degradation product was found to be 2-(8-chloro-9H-carbazol-1-yl)acetic acid. Products resulting from the reaction of these compounds with chlorine (from sodium hypochlorite used as a disinfectant) are also observed

## 7.P-Tu-062

### Analysis Of Ibuprofen And Degradation Products In Uruguayan Waters

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#### Abstract

Ibuprofen is a non-steroidal anti-inflammatory, used as an antipyretic, analgesic and anti-inflammatory. It is used for the symptomatic relief of fever, headache (headache), and mild to moderate pain. It acts by inhibiting cyclooxygenase. This enzyme converts arachidonic acid into cyclic endoperoxides, which are transformed into prostaglandins and thromboxanes, mediators of inflammation. The inhibition of the synthesis of prostaglandins and thromboxanes by ibuprofen is responsible for its therapeutic activity.

Water samples were collected from different sites in Montevideo and its surroundings.

To carry out the analysis, the water sample is extracted with ethyl acetate (3 extractions). The organic layer is dried with anhydrous sodium sulfate and the solvent is evaporated under reduced pressure. The residue is analyzed by thin layer chromatography, nuclear magnetic resonance and mass spectrometry.

The drug was detected in the sample. Also, the following products were detected : 4-isobutylacetophenone, isobutylbenzene and phenols.

Products resulting from the reaction of these compounds with chlorine (from sodium hypochlorite used as a disinfectant) are also observed

## 7.P-Tu-063

### Analysis Of Sulfamethoxazole And Degradation Products In Uruguayan Waters

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#### Abstract

Sulfamethoxazole is a sulfonamide-type bacteriostatic antibiotic generally used in combination with trimethoprim. It is generally used in combination with trimethoprim in a 5:1 ratio (cotrimoxazole). Its primary activity is against susceptible strains of *Streptococcus*, *Staphylococcus aureus*, *Escherichia coli*, *Haemophilus influenzae*, and oral anaerobes. It is frequently used for the treatment of urinary infections. It can also be used to treat toxoplasmosis and pneumocystosis. Trimethoprim is a bacteriostatic antibiotic derived from trimethoxybenzylpyrimidine and used almost exclusively in the treatment of urinary tract infections.

Water samples were collected from different sites in Montevideo and its surroundings.

To carry out the analysis, the water sample is extracted with ethyl acetate (3 extractions). The organic layer is dried with anhydrous sodium sulfate and the solvent is evaporated under reduced pressure. The residue is analyzed by thin layer chromatography, nuclear magnetic resonance and mass spectrometry.

The drug was detected in the sample. Also, hydrolysis is observed, forming 4-aminosulfonic acid and an oxazole derivative. Products resulting from the reaction of these compounds with chlorine (from sodium hypochlorite used as a disinfectant) are also observed.

## 7.P-Tu-064

### Analysis of Amoxicillin and Degradation Products in Uruguayan Waters

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#### Abstract

Amoxicillin is a semisynthetic antibiotic derived from penicillin. It acts against a wide spectrum of bacteria, both Gram-positive and Gram-negative. For this reason it is often used as the first drug in infections of different severity, both in human and veterinary medicine.

Water samples were collected from different sites in Montevideo and its surroundings.

To carry out the analysis, the water sample is extracted with ethyl acetate (3 extractions). The organic layer is dried with anhydrous sodium sulfate and the solvent is evaporated under reduced pressure. The residue is analyzed by thin layer chromatography, nuclear magnetic resonance and mass spectrometry.

The drug was detected in the sample. Also, hydrolysis is observed, forming 2-amino-2-(4-hydroxyphenyl)acetic acid and a penicillanic acid derivative.

Products resulting from the reaction of these compounds with chlorine (from sodium hypochlorite used as a disinfectant) are also observed.

## 7.P-Tu-065

### **Trophic Transfer of Human Pharmaceuticals in Fish From Wastewater-Impacted Sites: A Case Study in the Uruguay River, South America**

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#### **Abstract**

Occurrence of Human Pharmaceuticals (HPs) in surface water bodies provides evidence of the urban impact on water resources. After consumption, wastewater effluents are the main source of input of HPs to surface water bodies. HPs such as atenolol (ATE), carbamazepine (CBZ), diclofenac, and ibuprofen were frequently detected in wastewater and receiving water bodies in Argentina, at higher levels than in North America and Europe. ATE, CBZ, enalapril, and sildenafil have been detected in the range of 28-92% in the muscle of fish species of commercial interest in the region. Distinctive accumulation patterns among species with different feeding habits have been demonstrated in the Uruguay River for ATE and CBZ. The Uruguay River, a boundary between Argentina and Uruguay, is a major river of one of the most important basins in South America, the Rio de La Plata basin, and is essential for commercial and recreational fishing. Research questions related to potential biomagnification or trophic dilution of HPs through aquatic food webs have been identified as a global research concern to be assessed in a SETAC international workshop related to pharmaceuticals in 2011 and then again as a key research need to be considered in Central and South America in 2017. We critically examined the refereed literature on this topic, which largely has been performed in Eastern Asia, North America, and Europe, highlighting research needs and data gaps in Latin America. Results reported for these studies showed a trophic dilution of HPs, including CBZ and ATE, across aquatic food webs. This project aims to assess the influence of trophic structure in the bioaccumulation of HPs in fish of different trophic levels inhabiting sewage receiving waters in the Uruguay River. Fish and water samples are collected from 4 riverine sites within the Uruguay River: Mocoretá, Puerto Yerúa, Concepción del Uruguay, and Villa Paranacito. Fish species are selected based on the representativeness of different trophic levels, and availability in the field. Fish samples are immediately ice-cold anesthetized. Water and fish samples are stored at -80°C, until international shipment. Pharmaceuticals, glucocorticoids, and antipsychotics, more commonly prescribed in the region and worldwide, are then studied using isotope dilution LC-MS/MS. The results obtained in this study are then critically compared with previous results reported for aquatic systems in other regions with different trophic structures.

## 7.P-Tu-066

### Occurrence and Ecological Risk Assessment of Pesticides in a Brazilian Estuary

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#### Abstract

Contamination of aquatic environment by pesticides has been related to adverse effects on aquatic ecosystems and human health. This study investigates the occurrence and potential risks posed by 8 pesticides (alachlor, atrazine, chlorfenvinphos, diuron, DCPMU, irgarol, isoproturon and simazine) in water samples from 12 sites in an estuarine area in the Northeast Brazil. Samples were collected in 2018 and 2019. The analysis by liquid chromatography coupled to mass spectrometry revealed the presence of all pesticides analysed, detected in at least one of the sampling campaigns. In 2018, herbicides were the most found, with diuron detected in 100% of the samples, followed by irgarol (90%) and atrazine (18%). The highest concentration was measured for irgarol (13.6 ng L<sup>-1</sup>), followed by diuron (10.0 ng L<sup>-1</sup>). Alachlor, isoproturon, and simazine were not detected in any samples. The detection frequency of pesticides was higher in 2019, with atrazine, isoproturon and a diuron degradation product (DCPMU) detected in 100% of the samples. The average pesticide concentration increased nearly 7 times from 2018 to 2019 and spatial differences between sampling sites were more evident in 2019, suggesting regular inputs since they were collected in the same season. In 2019, the organophosphate chlorfenvinphos had the overall highest concentration (48.6 ng L<sup>-1</sup>), followed by isoproturon (44.6 ng L<sup>-1</sup>). The potential risks to aquatic organisms were assessed by calculating risk quotients (RQ) based on the measured environmental concentration of the individual pesticides (MEC) and their corresponding predicted no-effect concentration (PNEC) towards marine water biota. Irgarol posed the highest risk, with RQ > 1 in all sites for both sampling years. Chlorfenvinphos and diuron presented RQs up to 4.9 and 2.4, respectively. Atrazine and DCPMU represented as low risk, while isoproturon showed medium risk in two sites from the 2019 samples. The combined toxic effect (RQ<sub>mix</sub>) was higher than 1 in all sampling sites, indicating a significant impact on aquatic ecosystems. This study highlights the presence and potential risks of pesticides in water samples from this area, with irgarol, chlorfenvinphos, and diuron posing high risks to the aquatic biota. The increase in pesticide concentrations and detection frequency from 2018 to 2019 emphasizes the importance of continuous monitoring and management strategies to mitigate the potential impacts of these contaminants on aquatic ecosystems.



## 7.P-Tu-067

### Polycyclic Aromatic Hydrocarbons in the Atmosphere of Antarctica

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#### Abstract

The presence of polycyclic aromatic hydrocarbons (PAHs) in the Antarctic continent has been attributed mostly to long-range atmospheric transport. Nonetheless, recent studies consider local activities as the main source of these compound emissions. The aim of this study was to compare the concentrations of several PAHs in the Chilean stations Arturo Prat and Yelcho in Antarctica. To do this, atmospheric samples were collected from both stations during a month in summer 2022 and using polyurethane foam (PUF)-passive air samplers. The samples were extracted using a Soxhlet extraction process to then be fractionated in silica columns, concentrated in an extra-pure nitrogen flow, and finally analyzed in a gas chromatograph with a flame ionization detector. Potential emission sources were identified using molecular diagnostic ratios. The results showed a higher concentration of PAHs in the samples taken at both stations, compared to points at more than 2 km away, which indicated that the research stations were the main emission sources. The Arturo Prat station showed higher concentrations compared to the Yelcho station, being this attributed to the presence of a garbage incinerator. Fluoranthene was the predominant PAH at both stations, with 25.7 ng m<sup>-3</sup> in Prat and 22.1 ng m<sup>-3</sup> in Yelcho. Chrysene showed a significantly higher concentration in Prat (12.9 ng m<sup>-3</sup>) compared to Yelcho (0.9 ng m<sup>-3</sup>), as well as Retene, with concentrations of 4.9 ng m<sup>-3</sup> in Yelcho and 9.1 ng m<sup>-3</sup> in Prat. On the other hand, the diagnostic molecular ratios showed that the contamination at both stations comes from a pyrogenic origin, associated with biomass burning.

**7.P-Tu-068**

## **Substituted Polyurethane Substrates to Capture Atmospheric Polycyclic Aromatic Hydrocarbons**

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### **Abstract**

Understanding the complexity of chemical pollution requires the development of effective sampling methodologies that could cope with the vast number of contaminants present in urban and rural environments and the analytical challenges they represent. Passive air sampling has emerged as a relevant technique for capturing this complexity in atmospheric samples, simultaneously reducing costs, and improving spatial resolution, compared to active methods. However, most of the passive sampling methods use polyurethane foams or other substrates that capture chemicals with a large range of polarities. This complexity is not always needed when the focus of the analysis is a selected group of chemicals that can be used as markers of emission sources or transformation processes in the atmosphere. In this study, we developed a modified polyurethane substrate with aromatic substituents to capture polycyclic aromatic hydrocarbons from the atmosphere using the electrospinning technique. The final product was compared to the original polyurethane foam in a field experiment in urban centers of Chile.

DRAFT

## 7.P-Tu-069

### Measuring the Oxidative Potential of Atmospheric Samples Collected with Passive Samplers

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#### Abstract

The oxidative potential (OP) has been extensively used to evaluate the ability of atmospheric particulate matter samples (PM) to produce reactive oxygen species or to oxidize a target molecule. It is well understood that finding the components of the PM that are correlated to higher OP could help in the ongoing efforts to improve air quality and to reduce its impact on human health. However, very few studies have focused simultaneously on both the PM-bound and gas-phase components of air samples collected using alternative methods to active sampling. The objective of this study was to optimize a method for the estimation of the OP of the water-soluble fraction of samples collected using polyurethane foam based passive air samplers in urban environments of Chile. Passive air samples were collected in three urban centers in the northern, center, and southern regions, which are known to have different emission sources (mining related industry in the north, heavy urban emissions in the center, and woodburning emissions in the south). The samplers were deployed for 3 months in at least 2 locations in each urban center. They were later extracted using Soxhlet extraction, and an aliquot of the extract was used to estimate the OP of the water-soluble fraction using the dithiothreitol method (DTT). The remaining extracts were used to quantify a selected list of polycyclic aromatic hydrocarbons after fractionation with silica columns and sample concentration. Field and laboratory blanks were used to estimate the effect of the substrate on the final OP estimation. The results showed that the polyurethane foam samplers used contributed with some OP to the final estimation but were low enough to allow to distinguish all urban centers. These results could improve the spatial resolution of OP measurements, lowering costs, and generating valuable data for policy makers.

## 7.P-Tu-070

### Evaluation of Pharmaceutical Products in an Urban Wastewater Treatment Plant in Argentina

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#### Abstract

Pharmaceuticals are one of the most important emerging pollutants because they are massively consumed by the population. They are discharged into urban wastewater and end up in receiving water bodies or are treated in treatment plants, which are not designed to remove pharmaceuticals, so in some cases, these compounds are not completely removed and are still present in the effluents even after being treated. In Argentina, the pharmaceutical products more frequently detected in aquatic environments are antibiotics (32%), analgesics (14%), antidepressants (14%), antihypertensives (11%), and anxiolytics (3%). The classical analytical methods of pharmaceutical detection in environmental samples are based on liquid chromatography techniques. Alternatively, electrochemical methods do not require expensive instrumentation and supplies, trained personnel, and can be used in situ. The objective of this work was the development of electrochemical methods to detect pharmaceuticals in urban wastewater in the treatment plant in San Justo city (Santa Fe, Argentina). The analgesic acetaminophen was selected as a model of pharmaceutical in this study. The electrochemical measurements were performed on Dropsens screen printed electrodes (SPE) modified with 50% iron nanoparticles (FeNPs) of 30 nm diameter (90% Fe and 10% FeO) provided by Nanomateriales ([www.nanotek.ws](http://www.nanotek.ws)). The electrochemical technique selected was the square wave voltammetry in the range from 0.2 to 0.8 V, in which the analgesic had an oxidation peak at 0.416 V vs. reference electrode (Ag/AgCl). In order to elaborate the calibration curve of acetaminophen, standard solutions (0.05 to 2.00 mg L<sup>-1</sup>) in Britton-Robinson buffer 0.1 mol L<sup>-1</sup> and NaCl 0.1 mol L<sup>-1</sup> at pH = 4.0 were assayed on SPE modified, obtaining a detection limit of 0.5 mg L<sup>-1</sup>. Since concentrations in the effluents were lower than expected, pre-concentration steps of 100 or 1000 times by solid-phase extraction had to be applied before the analysis. The samples before and after the treatment process in the plant were analyzed and the results were expressed as % removal efficiency  $[(IC-EC)/IC] \times 100$ , where IC and EC were the acetaminophen concentrations in the influent and the effluent, respectively. Finally, the environmental risk associated with its effluents when are dumped into the Salado River was calculated.

## 7.P-Tu-071

### Assessment of Air Pollution in Greater Vitória-ES by Evaluating the Pigment Content in *Tradescantia pallida*

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#### Abstract

The region of Grande Vitória/ES, Brazil, suffers great interference in air quality due to emissions of atmospheric particulate matter (APM) contaminated with heavy metals from the mining and steel industry. Plants are very sensitive to environmental pollution, which has direct effects, especially on the leaves, and can relocate in the intracellular spaces causing reduction or stimulation of growth, changes in gas exchange, photosynthetic rates and antioxidant metabolism. The objective of this work was to evaluate the effects of atmospheric pollution on the leaf pigment contents (chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and anthocyanins) of *Tradescantia pallida* (Rose) D. R. Hunt var. purpúrea Boom, collected from flowerbeds in Grande Vitória. *T. pallida* leaves were collected from two points in the municipality of Vitória (Ilha do Boi and Jardim Camburi), one point in Vila Velha and one point in Governador Lindenberg, which is not affected by metal contaminated particulate material. The analyses were performed with fresh leaves, 0.4 g of plant material was macerated with 15 ml of 95% ethyl alcohol. The homogenate was incubated for 24 h at 4°C in the dark, centrifuged for 20 min at 4°C, and the supernatant collected for the analyses. The chlorophyll and carotenoid contents were quantified by spectrophotometer reading at wavelengths 470, 648, and 664 nm. The reaction for anthocyanin content was performed in the dark for 24h at 4°C with 1.5 mL of ethanolic extract in 1% HCl, readings were taken at 535 nm. The data were submitted to Tukey's mean test ( $P < 0.05$ ). The results found show that there was no statistical difference in the content of anthocyanins between the points. But there was a significant reduction in the chlorophyll content (a, b and total) in relation to the point Ilha do boi compared to Vila Velha. As well as, when analyzing the carotenoids, where the Ilha do Boi point presented a significant reduction when compared to Vila Velha and Jardim Camburi points. The data indicate that the physiological alterations evidenced in this study, especially at the Ilha do Boi point, were caused by the proximity to the polluting source and the wind direction which intensifies the dispersion of the particulate matter to this point, causing a reduction in the photosynthetic processes and antioxidant metabolism of the plants analyzed, possibly due to the excess of heavy metals present in the atmospheric particulate matter emitted into the air.

## 7.P-Tu-072

### **Fine Aerosol Particulate Matter and Their Sources Applied as Potential Quality Indicator: The Study Case of a South Atlantic Estuarine Environment Under Massive Harbour Activities**

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#### **Abstract**

Fine particulate matter (PM<sub>2.5</sub>) is one of the major atmospheric pollutants associated with human health problems, including respiratory and cardiovascular diseases. Their emissions are primarily caused by the burning of fossil fuels in several activities, such as power generation, industrial processes, residential heating, and urban transportation. Paranaguá city hosts the largest port in South Brazil, leader in bulk transport of soybean production in Latin America. Conversely, Paranaguá is within one of the largest continuous areas of preserved Atlantic Forest, classified as one of the most biodiverse biomes in the world. The local sociobiodiversity context allowed the area to be listed as a World Heritage Site and Biosphere Reserve by UNESCO. The purpose of this study is to examine the concentrations and sources of PM<sub>2.5</sub> in this important port region in the South Atlantic by conducting the most extensive and rigorous sampling to date in Paranaguá city. The PM<sub>2.5</sub> mean concentration was  $15.26 \pm 7.5 \mu\text{g m}^{-3}$ , with a range from 0.7 to  $41.0 \mu\text{g m}^{-3}$ , exceeding both World Health Organization target thresholds 3 and 4. Notably, 10% (n = 34) of the samples overtook Brazil's final threshold for PM<sub>2.5</sub> 24-hour mean ( $25 \mu\text{g m}^{-3}$ ), which is significant since the guidelines recommend a maximum of 3-4 exceedance days per year. Bivariate plots with meteorological data and Positive Matrix Factorization were employed to estimate the sources of PM<sub>2.5</sub> from soluble ions, trace and major trace elements composition. The findings suggest that truck activity in a nearby parking lot is the primary source of fine particulate matter, while the presence of a transportation structure linked to grain transportation serves as a secondary source. This study identified several cases of high short-term and a high annual mean for PM<sub>2.5</sub> in the Paranaguá port region. The observed levels are alarming for several health problems compared to the WHO guidelines and are poorly monitored in Brazil. These results evidenced the PM<sub>2.5</sub> as environmental quality indicator to estuarine human impact areas and highlight the urgent need for effective measures to mitigate the negative impacts of port activities on air quality and public health in Brazil. Further, these results indicate an urgent need to address the impact of the predominant mode of production on human health and biodiversity in the region, given the context of excessive PM<sub>2.5</sub> concentrations and the presumable risks they pose.

## 7.P-Tu-073

### Acaricides Monitoring in Soils of the Northern Area of Uruguay

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#### Abstract

In Uruguay, one of the main sources of foreign exchange comes from the exportation of meat, which is why livestock farming plays an important role in the economy of this country. Therefore, any problem affecting this production is of great importance, one of those many problems is parasitosis. In particular, the common cattle tick, *Rhipicephalus B. microplus* (*R. microplus*), is a vector for many diseases that cause millions of dollars in losses for the agricultural sector. Because of this, in the affected area, various treatments are carried out on cattle with acaricides belonging to different chemical groups. Consequently, the residues of this type of insecticides can end up in the soil, causing damage to the environment and biodiversity. In this work, a multi-residue methodology was developed for acaricides by liquid chromatography coupled to tandem mass spectrometry in soil. Thirty-four acaricides belonging to different chemical families (pyrethroids, organophosphates, macrocyclic lactones, benzoylureas, carbamates, amidines and phenylpyrazoles) were selected from among the most widely used in the territory. Extraction of the compounds were performed using a modified QuEChERS method. The validation parameters were evaluated according to the acceptance criteria of SANTE Guidelines at three concentration levels of 10, 25, and 100 µg/kg. The recoveries percentage obtained for 100 µg/kg were between 70 and 120% with relative standard deviation below 20%. The compounds showed a linear behavior in the range 10 to 250 µg/kg in matrix, with percentage of residuals ≤ 20% and a correlation coefficient of  $r^2 \geq 0.99$ . This method allows the determination of acaricides in soil samples obtained from establishments in where *R. microplus* is being controlled. Amitraz, ethion, chlorpyrifos, coumaphos, ivermectin, and fipronil were detected in the analyzed samples.

## 7.P-Tu-074

### Validation of a Methodology of Pesticides Metabolites in Biomixture

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#### Abstract

Biobeds have shown a great performance in degrading pesticides, but to assess the real efficiency of these bioreactors is necessary to also evaluate the generation and subsequent degradation of metabolites of toxicological significance. As a first approach to evaluate in depth this situation, in this work, the metabolites selected to be evaluated were those included in the Maximum Residue Limit definition of the parent pesticides. The aim of this work was to adjust and validate an analytical methodology for the simultaneous determination of 21 pesticides' metabolites included in the MRL-EU definitions and their precursors in biomixture.

The final analytical methodology was an acetonitrile extraction with 2 g of sample, a salting out step with NaCl and MgSO<sub>4</sub> and for the instrumental analysis an LC-MS/MS with the optimized metabolite transitions adjusted using the multi reaction mechanism acquisition mode. The method was validated following the SANTE document 2021 guidelines for both, the parent compounds, and their metabolites. The whole parameters were studied at 3 concentration levels (50, 100, 200 mg/kg), yielding recovery values between 58-128 % with %RSDs lower than 20 %. Matrix effects were calculated, and the quantification performed using matrix matched calibration curves. Then, the presence and behavior during a productive cycle of the pesticides and the degradation metabolites determined in established biobeds was studied. Biobeds installed in farms were evaluated and the presence of degradation metabolites of boscalid and saflufenil were detected. This approach will contribute to assess the safety biobeds degradation process for the final disposal of the biomixture when the bioreactors are no longer active.



## 7.P-Tu-075

### Surface Runoff and Decay of Glyphosate in Experimental Rice Fields

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#### Abstract

The sustainability of natural resources is a growing concern worldwide, and in our region, agriculture is considered one of the main sources of diffuse contamination of rivers, lakes, and other bodies of water. Glyphosate (GLY) is applied in fallows and pre-emergence to control weeds in rice cultivation areas.

The objective of this study was to evaluate the runoff loss of GLY and its main metabolite, aminomethylphosphonic acid (AMPA), during the rice pre-flooded period and their dissipation in water throughout the flooding period in two rice-based rotation systems. A field experiment was conducted during the 2022/2023 rice season, in a representative flat, poorly drained, and medium-low fertility soil of the Laguna Merin basin. Two rice rotation alternatives were tested: a) Rice-rice-3-year pasture rotation, b) Continuous rice (1 year). The rainfed monitoring period included chemical fallow, planting, pre-emergence and emergence until the V5 growth stage (60 days) () and the rice flooded period covered from V6 to pre-harvest drainage (118 days).. Glyphosate-based herbicides were applied 1 and 2 times during the study period for continuous rice and rice-pasture treatments, respectively, using conventional doses. Concentrations of GLY and AMPA were measured in the collected water samples. Rainwater and irrigation water (Rio Olimar) samples were also analyzed.

Preliminary results showed the presence of both analytes in the study samples. For surface runoff water in the continuous rice treatment, GLY values ranged from 4 to 800  $\mu\text{g/L}$ , while AMPA values ranged from 15 to 170  $\mu\text{g/L}$ . For the rice-pasture rotation treatment, the ranges fluctuated between 10 and 350  $\mu\text{g/L}$  for GLY and 15 and 85  $\mu\text{g/L}$  for AMPA. In the analysis of the floodwater samples in the continuous rice treatment, GLY values ranged from not detected (ND) to 30  $\mu\text{g/L}$  and AMPA from ND to 60  $\mu\text{g/L}$ . Meanwhile, in the rice-pasture rotation treatment, GLY values ranged from ND to 24  $\mu\text{g/L}$  and AMPA from ND to 150  $\mu\text{g/L}$ . GLY and AMPA contents in the paddy field peaked in the first week after flooding and were going down until being not detectable 60 days after flooding. In addition, maximum GLY concentrations of 2.3  $\mu\text{g/L}$  and AMPA concentrations of 1.3  $\mu\text{g/L}$  were detected in rainwater samples, while freshwater inputs to the system showed maximum concentrations of 1.3  $\mu\text{g/L}$  for AMPA, without quantifiable GLY residues.

## 7.P-Tu-076

### Determination of the Volatility of the Herbicides Dicamba and Fluroxypyr-Meptyl+tricypr in Laboratory Conditions

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#### Abstract

Dicamba is very effective in controlling many species of dicotyledonous (broadleaf) weeds. The major environmental limitation of the application of this herbicide is its potential for volatility and the occurrence of drift damage to non-target crops. Thus, the aim of the study was to determine the volatility of dicamba and fluroxypyr+tricypr under laboratory conditions. For that, three tests were performed to guarantee the experimental quality, with 5 replicates each test. Thus, in a glass beaker with a capacity of 250.0 mL containing 10.0 grams of activated charcoal, previously dried in an oven at 100.0 °C for 30 minutes. A cellulose filter paper (55.0 mm in diameter with a grammage of 100.0 +/- 20.0 g m<sup>-2</sup>) was attached with a clip to a Petri dish with an internal diameter of 90.0 mm and the set was weighed. on an analytical scale (plate + filter paper), obtaining an initial weight of the set between 50.0 and 53.0 grams. After this process, 0.2 mL of each herbicide was transferred to the filter paper and the set was weighed again. Then, the set was placed on a beaker with activated charcoal and transferred to a crystallizer, maintained at a temperature of 25.0 ± 5.0 °C. At 1 and 7 hours after the assembly of the system, the weight of the assembly was obtained again. In the 1 hour evaluation for dicamba (test 1) 37.11% m/m of evaporated material occurred and in 7 hours 37.72%. In test 2 in 1 hour there was 36.43% m/m and in 7 hours 37.42% m/m and for test 3 in 1 hour 37.20% m/m and in 7 hours 37.39%. For fluroxypyr+tricypr for the 3 tests in 1 hour, the evaporated material was 26.79; 26.50 and 26.45% m/m and for 7 hours 32.78; 32.40 and 32.14% m/m. Thus, it is concluded that both herbicides, under laboratory conditions, can be considered practically non-volatile.

## 7.P-Tu-077

### Levels and Distribution of Organochlorine Pesticides, Polychlorinated Biphenyls, and Polybrominated Diphenyl Ethers in Salt marsh Sediments from Bahía Blanca Estuary, Argentina

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#### Abstract

Salt marshes are intertidal wetlands that provide various ecosystem services, including shelter for migratory birds and commercial fish species, and sequestration of carbon and pollutants. However, salt marshes are threatened by human activities that release pollutants such as persistent organic pollutants (POPs). Here we report the levels of several POPs in salt marsh sediments from the Bahía Blanca estuary (BBE), Argentina, including organochlorine pesticides (dichlorodiphenyltrichloroethane - DDT - and hexachlorocyclohexanes - HCHs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs). This study aimed to identify sources and patterns of distribution in relation to the tidal range and the presence of plant species (*Spartina alterniflora*, *Spartina densiflora*, and *Sarcocornia perennis*). Composite sediment samples were collected in both bare and vegetated sediments by the 3 mentioned species in 6 salt marshes of the BBE in February 2020. The concentration of  $\Sigma$ HCHs,  $\Sigma$ DDTs,  $\Sigma$ PCBs, and  $\Sigma$ PBDEs varied widely across the 6 salt marshes, ranging from 0.055 to 0.8, 0.065 to 25.09, 0.285 to 14.005, and <0.01 to 0.15 ng/g dry weight, respectively. The salt marsh adjacent to urban sewage consistently exhibited the highest values, particularly for DDTs and PCBs, with significant differences compared to the other sites, which did not significantly differ among them, despite salt marshes adjacent to industrial discharges and port areas were assessed. The content of organic matter (OM) varied between 2.9 and 14.76 % and correlated with the content of POPs, especially DDTs and PCBs. Higher values of POPs were generally found in the high marsh and in vegetated sediments, suggesting that plants could promote the accumulation of certain POPs in sediments; however, further study is needed to confirm this trend. This is the first study that evaluated the presence of POPs in Bahía Blanca salt marshes and the potential contribution of plant species to their distribution. Further investigations will analyze root uptake and translocation to leaves of different species of salt marsh plants.

# Session 8: Environmental Mutagenesis in Human Populations

8.P-Tu-078

## Chromosomal Alterations in Plant Cells of *Allium cepa* Exposed to Pesticide Residues

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### Abstract

In recent decades it is undeniable the increase in the use of pesticides in Brazil, being the agribusiness sector the main responsible for their management. Thus, it is strictly necessary to develop actions aimed at evaluating the environmental impacts caused by these products, as well as the search for ecologically safer products. To determine the effects of pesticides on the cell cycle, analyses were performed on the bioindicator organism *Allium cepa* (common onion) after exposure to soil contaminated with pesticide residues collected at the farm of the Federal Technological University of Paraná in Dois Vizinhos, using the Biobed elimination method. The analyses presented here focus on the mutagenic and cytotoxic potential of pesticide residues for plant cells of the analyzed bioindicator organism, which was exposed to pesticide residues used on the UTFPR-Dois Vizinhos farm, in order to evaluate the rate of abnormalities and chromosomal aberrations in *Allium cepa* plant cells. It was concluded, on a statistical basis, that there were changes in the rate of mitotic changes and chromosomal delays.

## 8.P-Tu-079

### Toxicogenetic Evaluation of the Restinga Soil of Espírito Santo, Brazil After the Collapse of the Fundão Tailings Dam (Mariana/MG, Brazil)

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#### Abstract

In 2015 Brazil's worst socio-environmental disaster occurred, the collapse of the Fundão tailings dam in the state of Minas Gerais. A large volume of ore tailings flowed into the Doce River to its mouth in the Atlantic Ocean in the state of Espírito Santo, where the sediments spread for hundreds of kilometers along the coastal zone and affected the coastal vegetation both by wave deposition and by marine aerosols. Metal pollutants from the sludge can accumulate in the soil and be incorporated into the local food chain, causing imbalances in the ecosystems. Thus, the main objective of this study was to analyze the toxicogenic potential of solubilized sandbank sediments indirectly affected by mining tailings, through bioassay with *Allium cepa* L. and *Lactuca sativa* L. The solubilized was prepared from the sediments of six sampling stations of the restinga. The experiment was prepared with the seeds of the plant models, the six treatments of the solubilized, ultrapure water as a negative control, and methyl methanesulfonate as a positive control. Phytotoxicity was evaluated by analyzing germination and root growth in *L. sativa*, and cytotoxic, genotoxic, and mutagenic potential by analyzing the cell cycle in *A. cepa*. The data were evaluated by the Shapiro-Wilks test to check for normal distribution of the means. Kruskal Wallis test ( $P < 0.05$ ) was used to analyze phytotoxicity, cytotoxicity and genotoxicity, and Tukey's test ( $P < 0.05$ ) was used for mutagenicity analysis. The results showed that there was no phytotoxic action of any solubilized, because there was no reduction in root length or germination, compared to the negative control. There were no significant chromosomal and nuclear alterations observed in the cell cycle when compared to the negative control. However, there was an increase in the chromosomal alterations (lost chromosome, c-metaphase and delay) at stations E1 and E3, while the nuclear alterations occurred in higher numbers at E7 and E3 (nuclear budding and micronucleus). The effects observed in this study may be related to the metals from the sludge tailings, considering that they can impact the metabolism and physiology of plants, besides acting in the breakdown and loss of genetic material. This causes changes in the phases of cell division of the organism and makes it impossible to follow the process of division or renders the cell unviable and generates cell death. Funded by PMBA/FEST – UFES and FAPES.

## 8.P-Tu-080

### **Application of Comet Assay to Assess Genotoxicity in Fish of the Species *Psalidodon bifasciatus* (Characiformes) Present in Affluents of the Iguaçu River (Brazil)**

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#### **Abstract**

The growing urbanization and demand for agricultural productivity is proving to be a worrying factor especially for aquatic animal species, since the final destination of a wide range of pollutants are the water bodies. For this reason, it is necessary to constantly monitor the quality of rivers and streams, both urban and rural. One of the most efficient means of monitoring is through bioindicator species, and fish can be very important tools for ecotoxicological monitoring. In this context, the objective of our research was to evaluate possible genotoxic effects through alkaline comet assay in fish of the species *Psalidodon bifasciatus* present in three affluents rivers of the Iguaçu River (Brazil) in the city of Dois Vizinhos (Paraná), which stands out for the highest rates of pesticide consumption in the state of Paraná. The comet assay is a technique developed in gel electrophoresis to measure DNA breaks in eukaryotic cells and provides classification of DNA breaks in 5 categories ranging from 0 damage (no apparent damage to genetic material) to 4 damage (state of cell apoptosis). For the performance of the technique blood, kidney and liver cells were used from the collection of 20 fish of the bioindicator species in three rivers, being a river with preserved environmental characteristics, that is, with the presence of preserved riparian forest and considerably distant from urban centers (Point 3), a river in the urban perimeter and with anthropized characteristics (Point 1) and another river in a rural region and susceptible to contamination by agricultural residues (Point 2). The results obtained were not significant for kidney ( $p=0.82315$ ) and liver ( $p=0.1478$ ) cells between the points, however, when analyzing the blood cells we observed a statistical difference ( $p=0.0252$ ) between point 1 and point 2, and the location with agricultural interference showed more DNA damage compared to the urban point, this result highlights the genotoxic effects of pesticides, since a large part of the residues flow into the water and affect non-target organisms such as fish. Through the results obtained, it is concluded that environmental monitoring is extremely important, even more so in the city of Dois Vizinhos, since the city is predominantly agricultural. More tests and active monitoring are extremely encouraged, since the ecotoxicological effects of various pollutants can cause deleterious and irreversible effects to all living beings.

## 8.P-Tu-081

### **Sublethal Effects on Mangrove Oyster (*Crassostrea rhizophorae*) During a Long Exposition of Settleable Atmospheric Particulate Matter and After a Clearance Period**

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#### **Abstract**

Air pollution in small diameter particle is considered more dangerous than larger sizes. Mining and steel industries result in the emission of settleable atmospheric particulate matter (SePM) which comprises several contaminants and harms aquatic ecosystems. SePM is considered a source of pollution in estuarine ecosystems in southeast Brazil (Espírito Santo, State) affecting the local biota. We investigated sublethal effects on mangrove oyster (*Crassostrea rhizophorae*) during a long exposition (30 days) of SePM and after a clearance period (deuration of 30 days), looking to assay the toxic potential of SePM to this model bivalve. We observed that organisms exposed to SePM showed higher cytotoxicity (neutral red assay) and genotoxicity (DNA-Strand Break) effects in a dose-dependent mode. A significant increase of cytotoxicity was recorded in exposed organisms over time after 4 and 7 days and a higher genotoxicity after 30 days of exposure to SePM. Lipid peroxidation (LPO) were slightly higher in the digestive glands compared to those observed in gills, however, there were no significant differences between the different SePM concentrations treatments and exposure times. There was not successfully recovery of the cytotoxicity and genotoxicity effects after 30 days of deuration (T-60) since the exposed animals kept physiological and DNA damage even after exposure them to water free of contaminants. These findings are innovative and highly relevant for future decision-making in processes related to SePM contamination and still neglected by Brazilian environmental regulations.

## 8.P-Tu-082

### Chloramine T Causes DNA Abnormalities in Adult Zebrafish

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#### Abstract

Aquaculture is a food production practice, and it is necessary to use means to maintain aquatic organisms, such as disinfectants use. These products are shown to be cytotoxic and/or genotoxic at low concentrations. Chloramine T (CL-T) is an N-halogeno-N-metalloarylsulfonamides and is used as a disinfectant in fish farms and aquariums to treat bacterial infections in gills and fish skin of different species. Although its effect on combat pathogens is effective, the effects on the genetic material and histopathological changes of *Danio rerio* (zebrafish) are poorly studied. For this purpose, micronucleus and other nuclear abnormalities were chosen as genotoxicity markers and carried out the measured integrated optical density (IOD) of nuclei erythrocytes cells. The treatment was conducted for 96h at 70, 140, and 200 mg/L concentrations. The results showed that CL-T did not cause changes in the micronuclei frequency, however, our results show that nuclear abnormalities increased, and the changes were greater at a sublethal concentration of 200 mg/L of CL -T, suggesting cytogenotoxic potential to zebrafish adults. In addition to the previously mentioned findings, it was observed that CL -T can act on the chromatin of peripheral erythrocytes, due to the increase in IOD. Thus, it is suggested that this effect may influence the condensation, organization, position, and renewal of chromatin and/or mitotic spindles. Overall, the results indicate the importance of evaluating the effects of exposure to CL-T, providing a perspective on the risk of use for zebrafish.



## 8.P-Tu-083

### **Evaluation of the Relationship Between Contaminants Occurrence and Acute Toxicity and Mutagenicity in the Sediments From the Upper Estuary of the Santos Port Channel, Brazil**

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#### **Abstract**

The upper estuary of the Santos Port Channel, Brazil, was severely anthropized during industrialization from the 1950s onwards. Strict control measures conducted in the 1980s resulted in a significant decrease in contaminant inputs to the estuary. However, the inherited contamination remained in the sediments, especially in sedimentation areas adjacent to the navigation channels. This is a comprehensive study on the interrelation between the concentrations of contaminants with toxic potential and the results of acute toxicity and mutagenicity tests in the sediments from the upper estuary of the Santos Port Channel. The evaluation considered a database of 102 samples of surface sediments obtained between May/2020 and May/2022. The samples were composed of 03 subsamples collected with van Veen grab samplers. In each sample, total metals and semi-metals, PAHs, PCBs, total organic carbon (TOC), nutrients, granulometry, as well as acute toxicity tests for the amphipod *Leptocheirus plumulosus* and mutagenicity tests with *Salmonella typhimurium* (Ames Test) were analyzed, totalizing more than 7,000 analytical results. The procedures were performed by laboratories accredited by NBR ISO/IEC 17.025. Overall, sediments with fine granulometry (clay and silt) and rich in the organic matter were predominant, some metals like Cu, Cr, Hg, Ni and Zn were quantified and the PAHs, with sums above 4,000 ug/kg (level 1 of Res. CONAMA 454/12) in 18 samples (17.6%) with a maximum of 115,935 ug/kg, indicating a history of untreated industrial discharges. Despite this scenario, of the 19 samples (18.6%) presenting acute toxicity, 06 showed PAH above 4,000 ug/kg. Similarly, of the 09 samples presenting mutagenicity, only 04 had Hg contents above 0.13 mg/kg, and none showed HPA above 4,000 ug/kg. Complementary tests of linear correlation showed no significant relationship between the concentrations of contaminants and the occurrence of toxicity and/or mutagenicity, suggesting that these contaminants may be present in sediments in non-bioavailable forms. They do not have the potential to cause adverse effects on aquatic communities, including bioaccumulation.

## 8.P-Tu-084

### Evaluation of the Genotoxic Effect of 4-Octylphenol, Using Native Fish *Rhamdia quelen*

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#### Abstract

Alkylphenol compounds are considered emerging contaminants and therefore have been deserving special attention, as their emissions into water bodies are not regulated by any legislative apparatus. 4-Octylphenol (OP) is considered an emerging contaminant and its use has intensified in the production of emulsifying surfactants for domestic and industrial use. The treatment process with effluents of this compound does not present total effectiveness in the removal, which ends up leaving traces of the contaminant in the water bodies. The use of bioassays involving bioindicators is of great importance because, through them, it is possible to predict the consequences that a particular ecosystem is subject to. Bioassays help in decision-making so that the environment is preserved to guarantee quality of life. The native fish of the Americas, *Rhamdia quelen*, is a bioindicator widely used to predict and evaluate the quality of the aquatic environment. This is due to its high tolerance to contaminants and its sensitivity to stress induced by harmful agents. The use of genetic biomarkers is intended to assess damage to genetic material in certain tissues that can be caused by mutagens, and one of the most used is the comet assay. This study aimed to evaluate the damage caused to the DNA of *Rhamdia quelen*, via trophic assay, by the contaminant 4-octyl phenol at different concentrations (5.8, 58, and 580  $\mu\text{g/g}$ ), for 60 days having an interval of 72 hours for each dose of OP. Phenol was tested from an environmentally relevant concentration found in the Iraí reservoir. To perform the comet assay, first the fishes were anesthetized and euthanized by medullary section, after the euthanasia blood and liver tissues were used to perform the technic. The results showed no statistically significant difference ( $p < 0.05$ ) between the treatment and control groups. From these results, it is possible to make some inferences about the non-presentation of results. The first point discussed is that the concentration of 4-octylphenol used may not have been sufficient to cause DNA damage in tissue cells. As a second hypothesis, the liver may have been efficient in metabolizing this emerging contaminant. Thus, further studies are needed to understand the action of the contaminant and what concentration can be supported by the bioindicator.

## 8.P-Tu-085

### Relative Telomere Measurement as a Molecular Biomarker in Fish *Ancistrus* sp.

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#### Abstract

*Ancistrus* sp. is a detritivores fish species that feed plant and animal remains and is an excellent bioindicator of environmental quality. The increasing amount of pollutants coming into contact with water environments are a major source of concern in scientific circles, as the individual effects as well as their possible synergistic effects contribute to telomere shortening. The telomeres are the chromosomes end, which have the function of maintaining the integrity of their structure, and their shortening causes cellular aging, and when totally shortened they can lead to the cells death. Therefore, the aim of our research was to test a protocol to measure the relative size of telomeres in the fish *Ancistrus* sp. 5 individuals were collected from the tributary of the Iguaçu River (Paraná State/Brazil). Afterward, we used the Wizard Promega® Kit to extract the genetic material and then performed the qPCR technique to prospect the relative telomere measurement. Relative measurement occurs based on the measurement of a constitutive gene, (GAPDH gene). The standardization of times and temperature annealing was successful. The average telomere Cts showed a value of 22.9 cycles, while the Cts of the GAPDH gene was 28.7 cycles, so the telomere region was detected around 5.7 cycles earlier than the GAPDH gene showing a  $\Delta\Delta Ct$  value of 1.911, in future research, values below 1.911 may signify telomere shortening. The application of this method can mean a major advance in the field of ecotoxicology, since a wide range of pollutants can cause telomere shortening and give rise to some kind of telomere alteration, and thus some interference in the quality of life of the species, so active monitoring of the aquatic ecosystem is of great importance for human and animal welfare.

Keywords: *Ancistrus* sp.; Measurement; Telomeres;

## 8.P-Tu-086

### Evaluation of Genotoxic Effect of the Alkylphenol 4-Nonylphenol, Using Native Fish *Rhamdia quelen* (Catfish)

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#### Abstract

There has been growing concern in recent decades about the effects of human and animal exposure to environmental micro-contaminants. One such contaminant that has drawn attention of the scientific community is 4-nonylphenol. Due to its recalcitrant xenobiotic nature, this contaminant tends to accumulate in sediments, which facilitates its transport over long distances. The widespread use of phenols by industry enables their dispersion and consequent pollution in different environments. 4-nonylphenol has been found in various products, such as bottled water, and even breast milk. According to the Water Framework Directive of the European Union, 4-nonylphenol is one of the substances of greatest significant danger. Thus, to test its mutagenic potential, the Comet Assay technique was used, using 96 fish separated into seven groups. Three of these groups served as controls (positive, negative, and solvent) and four as the actual test, with each testing a different concentration of the contaminant. After trophic contamination for 60 days with a 72-hour interval, the animals were anesthetized, euthanized, and their gonadal and renal tissues were collected. These tissues were subjected to the Comet Assay technique, resulting in slides individualized by tissue and specimen. After analysis, a statistical test was performed, resulting in significant values in three tissues. In the gonads and posterior portion of the kidney, the medium and high concentration groups showed statistically significant results. In the anterior portion of the kidney, the groups that presented higher significant values were of lower and medium concentrations. This occurred mainly due to the function of each organ. The contaminant, upon contact with the tissues, regulated the response differently, intensifying or not its mutagenic capacity. Therefore, it is of great importance to prohibit the use of this contaminant in order to reduce its final concentration in the most diverse environmental media. Its action can be easily replaced by other compounds already tested and approved for industrial use, such as ethoxylated sugar ester. Research using this alkylphenol still proves necessary until it is no longer used and there are means to remove it from the environment.

## 8.P-Tu-087

### Development of Telomere Length Analysis Caused by Pollution of the Iguaçu River-Brazil in Fish of the Species *Corydoras* sp. (Order Siluriform)

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#### Abstract

Bioindicators are characterized as organisms that indicate the quality of an environment and the changes undergone by it over the time. With the action of several pollutants, telomeres, which are structures present at the chromosomes ends, which act in the protection of DNA and in the regulation of cellular senescence, can suffer shortening, causing this way the eventual loss of gene regions coding at each cell division. Thus, seeking to analyze possible genetic alterations caused by pollution, fish of the species *Corydoras* sp. (Order Siluriforme) were collected in the Iguaçu River, which has great importance in this aquatic ecosystem, and can be classified as a bioindicator. This way, the objective of this research was to standardize a method to measure the telomere length of *Corydoras* sp., using an existing telomere measurement protocol, which was successful used in the species of *Rhamdia quelen*. The extraction of the genetic material took place using the Wizard Promega® extraction kit, then we performed the DNA amplification and used the qPCR technique to perform the relative measurement of telomeres. The relative size is possible through the measurement of an endogenous gene, and in our research, we used the GAPDH gene. After testing this method, it was possible to successfully standardize both telomeres and the GAPDH gene, with a mean cycle threshold (Ct) of 18,829 and 26,384, respectively. With the average Ct value, it was possible to calculate the  $\Delta Ct$  value, which provides the difference in the number of molecules, in cycles, between the target region and the endogenous gene. The difference between the telomere region of *Corydoras* sp. and the endogenous GAPDH gene was 7,555, that is, 7,555 more cycles are required to initiate the amplification signal of the GAPDH gene. From obtaining this value, it can be concluded that, in future ecotoxicological tests, values below 7,555 may mean an alteration in telomeres, a result of oxidative stress caused by one or more pollutants present in the environment in which the bioindicator is exposed. Subsequently, this study will collaborate in future tests in ecotoxicology by inferring the relative size of telomeres exposed to the most varied contaminants released into the environment, in addition to being able to make future comparisons in different species and regions of aquatic ecosystems.

## 8.P-Tu-088

### Genotoxic Damage in Blood and Liver Cells of *Rhamdia* sp. in Southwest Paraná, Brazil

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#### Abstract

Pollution in rivers come from different sources (e.g., cities, agriculture, industry), and can have a variety of toxic effects on the living organisms at the site. Genetic damage is a biomarker for the body's response to different stressors that affect its homeostasis. In order to compare the genotoxic effect of different sources of pollution on aquatic organisms, 20 fish were collected in 3 sampled sites with different characteristics: 1) an urban site; 2) a rural site; 3) a more pristine local in a ~ 40,000 people city. Blood and liver of *Rhamdia* sp. underwent Comet Assay test, where tissues are disaggregated, cells were embedded in low melting point agarose, placed on slides with normal agarose, and lysed. Subsequently, the cells were electrophoresed in pH > 13 buffered solution and stained with ethidium bromide for analysis under a fluorescence microscope. In the analysis, the effects were scored as damage 0 (no damage), one, two, three and four, and later statistical analysis was performed in the RStudio. The genotoxic damage in the blood showed a statistical difference between the groups by the Kruskal Wallis test (p-value 0.017). By Dunn's test with Bonferroni-adjusted p-value, fish from the urban site showed a higher rate of genotoxic damage than fish from the rural zone (p = 0.013), but statistically significantly higher than the most pristine point (p = 0.86). The Comet Assay for liver cells showed no statistically significant difference between rivers by ANOVA test (F = 0.51; p = 0.60). The results point that the site theoretically more pristine, not is preserved. Possibly the riparian forest and the conditions at this site are still not sufficient to preserve the organisms or can receives pollutant discharges along its path. This pollution is evident in the genotoxicity data for the liver, the body's main detoxification organ. The urban-influenced stream caused higher genotoxic damage in the erythrocytes of *Rhamdia* sp. It is important to perform environmental monitoring from chemical and biological analyses, especially in the aquatic fauna of streams, because besides their ecological niche, they serve as food for nearby communities. For a better analysis of the effects of streams on aquatic fauna more biological and chemical analyses are needed.

## 8.P-Tu-089

### Genotoxicity of Emerging Pollutants in the Zebrafish *Danio rerio* (Hamilton, 1822)

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#### Abstract

Emerging contaminants are chemical compounds, pharmaceuticals, and personal care products, which have been detected in concentrations from 0.05 to 3,500 µg/L in natural systems and in water from treatment plants. There is limited information on the effects that these contaminants have on aquatic organisms, for this reason, in this study the genotoxic effects of 17 products were evaluated (five non-steroidal analgesics, six detergents, two toothpastes, two mouthwashes and two fabric softeners) in zebrafish. Bioassays were carried out with duration of 10 days where the fish were exposed to a sublethal concentration (LC<sub>1</sub>) of these compounds. At the end of the exposure period, the degree of lipid peroxidation (Tbars) in the gills and the genetic damage (frequency of micronuclei) in peripheral blood were evaluated. The most toxic product was a mouthwash (LC<sub>50</sub> = 0.56 mg/L) and the least toxic was a toothpaste (>1000 mg/L). In the results obtained, it was observed that there are significant differences in the degree of lipid peroxidation and genetic damage between the exposed organisms and the control group (p <0.05). Detergents and drugs had the greatest oxidative effect. In the genotoxicity evaluation, the highest frequency of micronuclei was observed in fish exposed to paracetamol (0.74%) (control 0.071%). Results indicated that all tested products caused deleterious effects in fish at sub-lethal concentrations. The concentrations of detergents and drugs tested in this study have been recorded in the waters of treatment plants, therefore, the presence of these substances in natural waters constitutes a risk to fish populations.

## 8.P-Tu-090

### Assesement in Vitro on the Expression of *Bax* and *Bcl-2* in Bovine Cell Culture Exposed to Chlorpyrifos

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#### Abstract

Chlorpyrifos (CPF) is the most widely applied organophosphate to prevent and control external parasitic diseases in cattle, associating it with various toxicological effects (nephrotoxic, mutagenic and teratogenic). Programmed cell death (PCD) is a mechanism that can be induced and regulated by external factors. The *Bcl-2* family of proteins is related to the intrinsic pathway (mitochondrial pathway), where *Bax* is characterized as a pro-apoptotic protein and *Bcl-2* as an anti-apoptotic protein. The proportion in the expression of both is an important determinant of cell susceptibility to apoptosis. The balance of these genes ultimately results in cell survival or apoptosis. The objective of this work was to determine the gene expression of *Bax* and *Bcl-2* in Madin-Derby Bovine Kidney (MDBK) cells exposed to different concentrations of CPF to evaluate its effect on the regulation of PCD "apoptosis". MDBK cell cultures (third passage) were grown in 6-well plates by triplicate at a concentration of  $3 \times 10^5$  cell/ml and incubated at 37 °C in a 5% CO<sub>2</sub> atmosphere. Before performing the assays, confluent monolayers were cells were exposed to CPF concentrations of 72.5 ppm (CL<sub>50</sub> determined in previous tests) and 10 ppm. The mRNA expression levels of *Bcl-2* and *Bax* genes in cell cultures were determined by RT-qPCR. *Bax* expression was increased (24h =  $14.44 \pm 0.358$  and 48 h =  $14.11 \pm 0.358$ ) with respect to *Bcl-2* (24 h =  $4.69 \pm 0.292$  and 48 h =  $4.24 \pm 0.310$ ), relativized to the endogenous gene (GADPH) and to the negative control. This significant difference ( $p < 0.01$ ) in the expression of the *Bax* gene with respect to *Bcl-2* (3 times higher), is observed independently of the concentration of CPF and the time of exposure to it. Since *Bax* is a pro-apoptotic gene, it can be inferred that CPF acts as an inducer in PCD signaling pathways. The results of this preliminary study provide relevant information on the in vitro behavior of CPF in cell cultures. These findings may be useful for the design of future in vitro experiments to investigate the role of CPF in different established and primary cell culture lines. Observing that CPF is an apoptosis-inducing agent, it is necessary to continue this line of research to elucidate its role in all the pathways involved in the PCD process.



## 8.P-Tu-091

### Genetic Effects of $\lambda$ -Cyhalothrin and Imidacloprid Based Insecticides in Different Tissues of *Danio rerio*

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#### Abstract

Brazilian agricultural production is built on the intense use of the land and the constant application of pesticides. Among them, the pyrethroid  $\lambda$ -cyhalothrin (LC) and the neonicotinoid imidacloprid (IMI). These insecticides are used worldwide and commonly applied in combination to control insect populations in different crops. The uncontrolled use of these products can lead to the contamination of surface waters and directly threaten aquatic organisms. Thus, this work aimed to evaluate the genetic effects of LC ( $0.15\mu\text{g L}^{-1}$ ) and IMI ( $100\mu\text{g L}^{-1}$ ), isolated and mixed (MIX), in different tissues of the teleost *Danio rerio*, after 96 h of exposure. The exposures were carried out in 10 L aquariums, containing four animals per aquarium, in sextuplicate ( $n=6$ ) per experimental group. The experimental medium (50%) was renewed every 24 h. Both insecticides, either alone or in the mixture, caused DNA damage in at least one tissue of *D. rerio* and increased erythrocyte nuclear alterations. Fish exposed to the LC, IMI, and MIX groups showed an increase in DNA damage scores in the whole body, blood, and liver. The animals exposed to IMI also showed an increase in the score of DNA damage in the gills in relation to the other groups. The blood of animals in the LC, IMI, and MIX groups showed a significant increase in the frequency of total erythrocyte nuclear changes and kidney-shaped nuclei compared to the control group. The animals of the LC group also showed a significant increase in the relative expression of the *ogg1* DNA repair gene in the gills. As a consequence, the gills of animals exposed to LC did not present DNA damage after 96 h. The brain was the only tissue that did not show any significant change in the evaluated biomarkers, possibly due to blood-brain barrier. The different organs showed distinct responses to the insecticides studied, probably due to the characteristics of each tissue and their biological functions. Even at low concentrations, both LC and IMI showed to be harmful to *D. rerio*, therefore, further studies are needed to determine safe levels of these insecticides in the aquatic ecosystem.

## 8.P-Tu-092

### **Thiamethoxam Induces Oxidative Damage in DNA Pyrimidine Bases in *Agelaioides badius* (Passeriformes, Icteridae) Evaluated by the Comet Assay**

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#### **Abstract**

Birds' populations have steadily decline in the last decades with agriculture as one of the main causes, and pesticides are considered the most common driver. Among most employed insecticides we can include neonicotinoids -such as thiamethoxam (TMX), imidacloprid and acetamiprid-, which are applied on extensive crops like soybean, corn and wheat, as well as for seed treatments. TMX has been reported to induce several adverse effects including physiological, behavioral and reproductive changes and oxidative stress, among others. The aim of this study was to evaluate the chronic sublethal genotoxic effects exerted by the neonicotinoid commercial formulation Actara® (25% TMX) in circulating blood cells of the passerine grayish baywing *Agelaioides badius* specimens after 21 days of exposure to treated seeds. Adult specimens were feed with peeled millet previously treated with 0.05, 0.5 and 5 g TMX/kg seed, concentrations that falls within the range recommended for coating seeds in the Pampasic Region of Argentina. Negative (non-treated seeds) and positive (50  $\mu$ M H<sub>2</sub>O<sub>2</sub>) controls were conducted and run simultaneously. The alkaline single cell gel electrophoresis (comet) assay was employed as endpoint for genotoxicity. In addition, this assay was modified including incubation with formamidopyrimidine DNA glycosylase (Fpg) lesion-specific endonuclease, which detect oxidized pyrimidine bases. Results demonstrated a significant enhancement of the genetic damage index (GDI) by increasing the frequency of damaged nucleoids after 0.5 and 5 g TMX/kg seed treatments ( $P < 0.01$  and  $P < 0.001$ , respectively). Moreover, results revealed that after incubation with Fpg a significant increase in the GDI was observed for those peripheral blood cells of *A. badius* specimens exposed to TMX compared with control values (enzyme buffer treated cells) ( $P < 0.05$ ). Our results demonstrate that this neonicotinoid insecticide increase DNA single strand breaks and induces oxidative DNA damage at pyrimidines bases on *A. badius* adult specimens after being exposed to environmentally relevant concentrations. Our results indicate that an appropriate evaluation of genotoxic risk associated with the use of the insecticide TMX in agricultural practices is imperative in order to reduce the negative impact exerted, not only on living species but also on the environment.

## 8.P-Tu-093

### Toxicogenetic Analysis *allium cepa* L. Roots Exposed to Water Samples From Coastal Lakes Under Metallurgical Influences (BRAZIL)

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#### Abstract

Urban and industrial regions present a variety of pollutants, such as inorganic substances as metals and metalloids. Once in the aquatic environment, these contaminants can reach the biota causing several biological damages. The objective of this work was to evaluate the quality of water samples from Carapebus (ES), Maembá (ES) coastal lakes and in Alegre artificial lake located in the Federal Institute of Espírito Santo (IFES) based on toxicogenetic studies on *A. cepa*. The experiment was carried out in a completely randomized design, using five Petri dishes per treatment, containing water samples collected at three different sampling stations in each lake, using distilled water as a negative control and trifluralin as a positive control. Analysis of germination, root growth (phytotoxicity) and assessment of cytogenotoxic potential were carried out. Data were analyzed using the Shapiro-Wilks test to check their normality. Tukey's test ( $P < 0.05$ ) was used for phytotoxicity analysis and the Kruskal Wallis test ( $P < 0.05$ ) was used for cell cycle analysis. Inhibition of seed germination was observed at Point 1 and point 3 of the Carapebus lagoon and reduced germination at point 2, as well as a reduction in root growth at point 2 of the Carapebus lagoon. For cell cycle analysis, an increase in the rate of nuclear and chromosomal alterations was observed in Carapebus P1. In view of the tests, phytotoxicity was observed at Points 1, 2 and 3 of the Carapebus lagoon due to the inhibition of the germination index and root growth, and genotoxicity and mutagenicity at point 1 of the Carapebus lagoon due to the increase in the occurrence of chromosomal and nuclear alterations.

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## 8.P-Tu-094

### Analysis of the Polymorphism in the *ugt2b7* Gene (RS7438135) In Breast Cancer Patients Exposed and Not Exposed to Agrochemicals

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#### Abstract

Female breast cancer is the second most prevalent cancer in Brazil. In the southwest region of the state of Paraná, specifically, due to its economy concentrated in agriculture and agribusiness, it was found that women occupationally exposed to agrochemicals have tumors with a worse prognosis. Xenobiotics such as agrochemicals are metabolized in the human body in a two-phase cellular process; the first is performed mainly by enzymes of the cytochromes P450 family (CYPs) and the second by conjugation enzymes such as those of the UDP-glucuronosyl transferase family (UGTs). The UGT2B enzymes are essential in the detoxification of a variety of endogenous and exogenous compounds, including agrochemicals. They participate in the elimination of estrogen, through glucuronidation, which, when deficient, can be a critical factor for the development of breast cancer, as this hormone is involved in its pathogenesis. Thus, the presence of polymorphisms in their genes can modify the metabolism of agrochemicals, making them carcinogens, and directly influencing the susceptibility and/or prognosis of cancers related to occupational exposure. Therefore, to evaluate a possible association between the single nucleotide polymorphism rs7438135 in the UGT2B7 (G>A) gene and breast cancer, a retrospective and prospective, quantitative, exploratory study was carried out. For this purpose, peripheral blood samples from voluntary patients with breast cancer exposed and not exposed to agrochemicals were collected between May 2017 and December 2021, at the Hospital do Câncer de Francisco Beltrão. Genotyping of the polymorphism was performed using the real-time PCR technique using the TaqMan probe. Quantification of nitric oxide was performed using high-sensitivity chemiluminescence to assess oxidative stress. Binary and linear logistic regression statistical tests were applied using the IBM SPSS Statistics 23 program. Statistical analyzes did not indicate a significant association with the parameters, chemoresistance (sig=0.491), and nitric oxide concentration (Sig= 0.496), however, there was a significant association with recurrence (Sig=0.02; Exp (B)= 2.779). Therefore, patients with the homozygous rare AA genotype are 2.8 times more likely to have a recurrence, when compared to those with at least one G allele. However, does not seem to be associated with parameters evaluated in the present analysis, which does not rule out further studies in larger samples and other populations.

## 8.P-Tu-095

### Toxicity Evaluation of Pesticide Mixtures Through Oxidative Stress and Genotoxic Damage in *Palaemon argentinus*

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#### Abstract

Aquatic organisms are exposed to multiple stressors, including the insecticide chlorantraniliprole (C), the fungicide epoxiconazole (E), and the herbicide metolachlor (M), which enter water bodies by drift or runoff. The risk associated with exposure to complex mixtures may be underestimated if only the individual toxicity of each of the stressors is taken into account without considering the possible interactions between them. The aim of this work was to expose the native decapod, *Palaemon argentinus*, to binary and ternary mixtures of C, E and M under laboratory conditions to evaluate the antioxidant activity through catalase activity (CAT), the oxidative damage through carbonylated proteins content (CP) in cephalothorax and abdomen, and establish genotoxic damage using a DNA damage index (DI) measured by comet assay in the decapod hepatopancreas. Individuals were exposed for 96 h (n=8 (CAT), n=6 (CP), n=15 (DI)) to two environmentally relevant concentration levels, low (LC) and high (HC), in equitoxic units: C: 0.002 and 0.04  $\mu\text{gL}^{-1}$ ; E: 1.3 and 26.07  $\mu\text{gL}^{-1}$ ; M: 3.5 and 70.5  $\mu\text{gL}^{-1}$  and a control condition (CTL): 0  $\mu\text{gL}^{-1}$ . The results in cephalothorax showed a significant increase ( $p < 0.05$ ) with respect to CTL, for CAT: C+M at HC; CP: C+E and C+E+M at LC, as well as for the ID: E+M and C+E+M at HC. In addition, significant differences ( $p < 0.05$ ) were observed for CAT in C+M between HC and LC. The results in abdomen at LC presented a significant increase ( $p < 0.05$ ) with respect to the CTL for CAT: C+E and CP: E+M and C+E+M, and a significant decrease was observed at HC ( $p < 0.05$ ) with respect to the CTL for CP: C+E, E+M, C+E+M. In addition, significant differences ( $p < 0.05$ ) were observed for CAT between C+E and E+M at HC and LC. CAT was not affected by the LC and HC mixtures, with the exception of C+M (HC) and C+E (LC), suggesting an underlying mechanism for energy saving at the expense of protein and DNA structural and functional damage. Depending on the combination of pesticides, CP increased at LC, while the DI increased at HC, which can be related to a higher protection barrier for DNA. The interactions in the mixtures that showed the highest frequency at LC and HC were antagonism and potentiation, followed by additivity. These findings highlight the value of further research into the toxic effects of stressor complex mixtures on native aquatic organism when present at environmental concentrations.

# Session 9: Aquatic Ecotoxicology: Test System and New Approaches

## 9.P-We-001

### Effects of Bifenthrin Application on Oxidative Stress and Neurotoxicity Biomarkers in Fish: A Cage Experiment

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#### Abstract

Integrated rice–fish farming implies the concomitant production of grain and animal protein on the same piece of land. However, the use of pesticides in an attempt to boost rice productivity could generate effects on fish. The present study aimed to assess the potential effects of the insecticide bifenthrin to fish (*Piaractus mesopotamicus*) using a caging experiment. Juveniles ( $n = 32$ ;  $44.5 \pm 10.1$  g;  $14.2 \pm 1.0$  cm total length) were obtained from a local farm and were transported to a rice field (San Javier, Santa Fe, Argentina). Fish were divided into two sites: control (C) and bifenthrin-exposure (BF). These sites were irrigation canals next to paddy fields with a separate water supply but under similar environmental conditions. Two cages ( $n = 8$  fish/cage) per site were placed separately at each irrigation canal. BF application (Seizer®) was carried out with coastal spraying equipment, following the BF recommendation dose for rice culture ( $100 \text{ cm}^3/\text{ha}$ ). After 72 h, fish were retrieved and rapidly transported to the laboratory for sample processing. Gills, liver, brain and muscle were dissected for biochemical analyses. The following oxidative stress biomarkers were measured: glutathione S-transferase (GST) and catalase activity (CAT), and lipid peroxidation levels (TBARS). Brain and muscle tissues were also investigated to assess acetylcholinesterase activity (AChE). The application of the insecticide caused an increase in the antioxidant enzymes CAT and GST activities in the liver and muscle tissues. Lipid oxidative damage was only observed in muscle. Furthermore, BF exposure significantly reduced AChE activity in fish muscles. Overall, our results demonstrate that the current use of bifenthrin in a rice field induces oxidative damage and neurotoxic effects in fish, which indicates potential risks to freshwater fish cultured in integrated rice–fish farming systems.

## 9.P-We-002

### Toxicity of Coastal Waters on *Perumytilus purpuratus* From Quintero Bay, Región De Valparaíso, Chile

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#### Abstract

The aim of the study was to evaluate the cytotoxic and genotoxic effects on *Perumytilus purpuratus* of the coastal waters of Quintero bay that receives industrial residues. Individuals were collected from five sites in the bay and in a control site in the coastal area of Tinquén. The stability of the lysosome membrane using Neutral Red retention time (NRRT) and the frequency of micronuclei (MN) were determined in hemocytes and branchial tissue. The concentrations of the trace elements Cu, As, Zn and Cd were measured in the tissue. The results show significantly lower NRRT and greater frequency of MN in the individuals of Quintero Bay compared to the control zone. The order of the trace element concentrations in tissues was  $Zn > Cu > Cd \geq As$ . The individuals with the highest concentrations of copper in the tissue had the lowest retention times for neutral red and the highest frequencies of micronuclei. In conclusion, the water of the study area in Quintero Bay has chemical agents that produce cytotoxic and genotoxic effects in the individuals of *P. purpuratus* present in this ecosystem. The use of these biomarkers in *P. purpuratus* is proposed as early warning tools for the biomonitoring of the environmental quality of coastal areas affected by anthropic activities.

DRAFT

## 9.P-We-003

### An Approach to the Effects of Chlorpyrifos on *Jenynsia lineata* Exposed to Environmentally Relevant Concentrations: Biomarkers of Oxidative Stress, Neurotoxicity and Genotoxicity

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#### Abstract

Chlorpyrifos (CPF) is a common organophosphate insecticide used for pest control in agriculture and non-agricultural settings, acting as an acetylcholinesterase (AChE) inhibitor. It is highly toxic to aquatic life and can persist in the environment (half-life: 81 days in surface water). The aim of the present study was to evaluate effects of oxidative stress and damage, neurotoxicity and genotoxicity in *Jenynsia lineata* exposed to environmentally relevant concentrations of CPF during 96 h. Five tanks (30 L) were set up, using females of *J. lineata* (n=6 per treatment; standard length: 4.31 ± 0.50 cm; total weight: 1.67 ± 0.51 gr), one as a control and four with CPF concentrations of 0.05, 0.5, 5, and 50 µg/L. Liver, gills, and brain were dissected and immediately frozen with liquid nitrogen and stored at -80°C until biomarkers analysis. The blood was extracted and smear were done and fixed with methanol for further analysis. During the course of the experiment, three individuals exposed at 50 µg/L and one exposed at 5 µg/L died at the first 24 hs indicating that this concentration is closed to the LC50. While the remaining three individuals exposed at 50 µg/L were euthanized because they looked moribund, and were used for measuring biomarkers. An increase in the activity of the enzymes catalase and glutathione-S-transferase in brain, and of superoxide dismutase in gills was observed at 0.05 µg/L (p<0.05). There were no significant differences in the activity of AChE between the control and the lowest concentration (0.05 µg/L) (p>0.05), while inhibition of AChE was observed at 0.5 µg/L (29.51%) and 5 µg/L (49.53%) (p<0.05). No significant differences were observed for micronuclei or other nuclear aberrations (p>0.05). At 50 µg/L, increased activity of CAT in liver and a 77.6% inhibition of AChE was observed (p<0.05). The results of this study demonstrate that environmentally concentrations of CPF can induce oxidative stress at concentrations lower than those at which AChE inhibition occurs. Although AChE is the target biomarker for organophosphates, it may not be as efficient early warning system as antioxidant enzymes, highlighting the approach of not always use the mode of action-biomarkers. Taking into account that CPF values of the lethal concentration 50 (LC50) for other fish species are very high compared to the concentrations used in this experiment, next steps will include the evaluation of the LC50 in *J. lineata*.



## 9.P-We-004

### Effects of Ivermectin, an Antiparasitic Widely Used in Animal Production, on a Main Inland Fisheries Species

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#### Abstract

Ivermectin (IVM) is a widely used antiparasitic drug used in animals and humans. Most of the IVM is excreted unaltered in the feces by the treated animal being the main path of entrance into the environment. The sábalo *Prochilodus lineatus* (Valenciennes, 1836), is a migratory characiform fish present in numerous South American rivers. The sábalo has a detritivorous diet and it is the most abundant fish resource in the Cuenca del Plata. The extensive exploitation of livestock in the wetland area of the lower basin constitutes a potential risk for the fish communities associated with this environment. The reproductive strategy of the sábalo involves the presence of larvae and juveniles in floodplain lakes of the rivers where they coexist with the intense cattle activity on the islands, where IVM is commonly applied. In this context, the environmental impacts of the antiparasitic IVM was evaluated using a physiological, ecological and behavioral approach in *P. lineatus* larvae and juveniles using experimental designs in the laboratory (acute and subchronic exposures). The exposure of juveniles (in water and sediments) with environmental relevant concentrations of IVM affected the biotransformation processes and promoted oxidative damage in the liver, gills and brain; and impacted swimming behavior (escape velocity, routine swimming speed, and general activity). Measurable levels of IVM were detected in the study area. Based on the effects on swimming behavior, using a virtual population analysis, a potential impact on the sábalo population was observed.

## 9.P-We-005

### **Antioxidant System Modulation and Oxidative Effects in the Brain of *Danio rerio* (Zebrafish) Exposed to Leachate From a Closed Dump**

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#### **Abstract**

Leachate is an effluent of complex and varied composition, capable of contaminating surface and groundwater, which creates a large contamination zone around the dumps. Therefore, ecotoxicological assessments and monitoring in these areas are of great relevance to measure the environmental impacts of municipal solid waste management. The objective of this study was to evaluate the modulation of antioxidant system and its oxidative effects in *Danio rerio* brains exposed to leachate from a non-operational dump. Groups of 50 fish were exposed for 96 h to concentrations of 0, 5, 15, 30 and 50 % of leachate. The exposure was carried out under license n. LW-49/19 CEUA/FIOCRUZ. To determine antioxidant system changes, the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) were measured, as well as the levels of reduced glutathione (GSH) and metallothionein (MT). The oxidative effect was determined by the quantification of lipid peroxidation (LPO) and protein carbonylation (PTC). The leachate showed levels of chlorides (2288.4 mg L<sup>-1</sup>), electrical conductivity (8434.0 mS cm<sup>-1</sup>) and ammoniacal nitrogen (19.0 mg L<sup>-1</sup>) characterized as possibly toxic to aquatic organisms. Regarding antioxidant system, no changes were observed in SOD and no levels of CAT activity were detectable. A 58 % GST reduction was observed at 30 and 50 % exposures and a 24 % GSH increase at 5 and 15 % exposures. MT levels increased with increasing leachate concentration, reaching levels 120 % above control at 15 % concentration. Analysis of oxidative effects showed a concentration-dependent reduction in LPO levels, reducing by up to 42 % at 30 % leachate concentration. PTC was significantly increased by 39 and 28 % for concentrations of 15 and 30 %, respectively. Thus, it is concluded that the leachate generated an antioxidant response in exposed fish, modulating the brain antioxidant system, which was able to prevent LPO. On the other hand, antioxidant system was unable to avoid PTC, showing that the leachate can lead to oxidative effect, which would not be observed if only the LPO analysis was performed. Therefore, the importance of evaluating different oxidative effects in ecotoxicological evaluations is highlighted.

## 9.P-We-006

### Erythrocyte Nucleometry and Topography in *Astyanax lacustris* Exposed to the Pyrethroid Lambda-Cyhalothrin

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#### Abstract

In recent years, new computational systems and analytical methods have improved the morphometry, stereology, static, flow cytometry, and three-dimensional reconstruction disciplines. Among these potentialities, nucleometry or quantitative nucleology has become an alternative tool for genotoxic studies in fish once that nuclear variation in size determines chromatin instability. We assess the effects of lambda-cyhalothrin (LTC), a type II synthetic pyrethroid, on the erythrocyte's nuclear plasticity from the *Astyanax lacustris*, a fish widespread in the Neotropical region. Fish (n=10) were exposed to 10,3  $\mu\text{g/L}$  and compared to respective controls (n=5) for 1, 3, 6, and 12 days. Twenty-five erythrocyte nuclei were randomly and digitally evaluated per specimen from blood smears stained by May Grunwald-Giemsa-Wright in bright field microscopy. Nucleometry was estimated by integrated optical density (IOD), nuclear volume ( $\mu\text{m}^3$ ), and roundness (0-1) using the ImageJ software. Nuclear topography was evaluated through atomic force microscopy (AFM) in vibrating mode, using a Workshop TT-2 scanning probe microscope. Images were processed in the Gwyddion data analysis software. Nuclear volume and roundness explained 55% of the IOD variation of the control samples and 46, 83, 85, and 78% of the 1-, 3-, 6- and 12-day exposition, respectively, by linear regression analysis. Twenty-nine profiles of the control nucleus were compared with 31 nucleus profiles of the fish exposed for the topography assessment. The analysis revealed that the erythrocyte nucleus of the *A. lacustris* is concave (a central depression is noted) which is variable according to depth and width (nm). There is a significant variation in the ratio between the height of the cell and the height of concavity. The ratio was around 23% for the control (160.3/684.0 nm) and 47% for the 12 days-exposed samples (418.3/896.8 nm). Dispersion of 8% was found in both groups. Our results suggest that nuclear format and profound morphological changes occur in *A. lacustris* exposed to LTC, which may represent ruptures in the organization of chromatin and compaction of nuclear structures, usually not observed in conventional microscopy techniques. The topographic analysis allows us to explore new morphological aspects associated with the effects of pyrethroids on fish.

## 9.P-We-007

### Acute Toxicity of Anthracene In Adult Individuals of the Amphipod *Hyalella curvispina*

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#### Abstract

One of the main economic activities of North Patagonia is oil and gas field exploitation. The region is also characterized by large urban settlements, established along natural and artificial watercourses. Economic and anthropogenic activities generate pollutants that can reach aquatic resources, so their quality might be at risk. Anthracene is a polycyclic aromatic hydrocarbon (PAH) considered potentially harmful, whose environmental analysis and monitoring is considered essential. In the last decades, there has been an increasing concern about the presence of PAH in aquatic ecosystems due to their wide distribution and potential adverse effects on living organisms, even at low concentrations. This study analyzed the acute toxicity of anthracene in the native amphipod *Hyalella curvispina*, possible behavioral alterations, and the dynamics of anthracene in the exposure media. Individuals were collected from a reference site in Mari Menuco Lake, Neuquén, Argentina. The amphipods were maintained and raised under laboratory conditions. Groups of 10 adults were exposed to a range of anthracene concentrations in 250 ml of filtered and dechlorinated water, during 96 hours, at constant temperature of  $23 \pm 1^\circ\text{C}$  and 16:8 h (L:D) photoperiod. Bioassays were carried out keeping equal proportions of adult male and female individuals per experimental unit. The concentrations tested were 0.1, 0.5, 1, and 2 mg/l. Each concentration was tested by triplicate and each exposure was repeated at least three times. Mortality and behavioral alterations were employed as endpoints to determine toxicological parameters. Anthracene concentrations throughout the assay were determined by direct fluorometry. No significant differences were observed between control and anthracene-exposed individuals with regards to mortality, so the LC50 could not be calculated. However, the observation of behavioral alterations allowed us to calculate a 24h-EC10 of 0.09 mg/l. Anthracene concentration decreased significantly in the presence of individuals, with a T1/2 of 36.5 hours. Surprisingly, our results suggest that the studied concentrations of anthracene are not lethal for the amphipod *H. curvispina*. Nevertheless, it generates behavioral alterations such as changes in reaction speed and stillness. This study is of great relevance because the progress made in the research of anthracene toxicity is scarce when compared to its ubiquitous presence in the environment.

## 9.P-We-008

### Effect of Phenanthrene on the Behavior and Oxidative Metabolism of *Epinephelus marginatus* (Perciformes: Serranidae) Juveniles

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#### Abstract

Polycyclic aromatic hydrocarbons (PAHs), such as phenanthrene (PHE), are the main crude oil components (achieving 98% of its composition) and are toxic to fish, acting as endocrine disruptors (ED). PHE is one of the 129 contaminants of overriding concern by USEPA (United States Environmental Protection Agency) due to their distribution and toxicity. The dusky grouper *Epinephelus marginatus* is a protogynous hermaphrodite fish listed on the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN), as “vulnerable”. Previous studies demonstrated that PHE exposure reduced 11-ketotestosterone levels in *E. marginatus* juveniles, suggesting that this compound can cause changes in steroidogenesis in this species. We evaluated the effects of PHE on animal behavior, cortisol plasma profile, and enzymatic antioxidant defense after acute exposure (96h). Briefly, *E. marginatus* juveniles were divided into four experimental groups: PHE exposure at two concentrations (0.1 mg/L (PHE1) and 1 mg/L (PHE2)); exposure to the vehicle (ethanol, 0.004%) and control group (sea water). During the experimental exposure, we video recorded the aquarium for further qualitative and quantitative behavior analysis. The animals were video recorded for 1h at the following exposure times: 0, 6, 10, 24, 30, 34, 48, 54, 58, 72, 78, 82, and 95 h. After 96h of exposure, the animals were anesthetized, and the blood was collected for cortisol plasma profile analysis by enzyme-linked immunosorbent. After this procedure, animals were euthanized, and liver and gills were sampled for analysis of enzymatic antioxidant defense, superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST). Preliminary analyzes indicate that PHE alters the behavior of *E. marginatus*, mainly in the PHE2 group, in which changes in equilibrium were observed. Additionally, high mucus production was observed in the groups exposed to PHE and the dilution vehicle. Plasma cortisol concentration was higher in the animals exposed to PHE compared to the control and vehicle groups. In the liver, SOD, CAT, and GST activity did not change. However, in the gills, SOD reduced its activity in the animals from the PHE1 group compared to the control one. Therefore, PHE can be considered ED, due to the increase of plasma cortisol profile, triggering the first response of enzymatic antioxidant defense, normally promoted by SOD.

## Toxicological Effects of Atrazine in *Cnesterodon decemmaculatus*

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### Abstract

Atrazine is one of the most used herbicides in the last decades, and it is frequently detected in different water bodies, both shallow and underground. Toxicity assessments in non-target species are important to evaluate unintended risks in the environments. Many studies have shown the potential risks of atrazine in aquatic organisms, such as endocrine disruptive effects, mortality and neurotoxicity. The purpose of this study was to evaluate the lethal and sublethal effects of this herbicide in *Cnesterodon decemmaculatus*, a native fish species that can be found in many water bodies in South America. To achieve this, a series of semistatic standardized bioassays were performed during acute (96 h) and subchronic (336 h) periods of time. Adult fish were randomly distributed between 8 concentrations between 0.5 and 100 mg a.i./L of a commercial formulation of atrazine (Atramyl 90 WG®) in 1 liter tanks with 10 individuals in each. Each assay was conducted by triplicate and controls with dechlorinated water were simultaneously maintained. During the exposure periods, ethological changes were recorded daily for each concentration. The LC-50s obtained were 77.46, 4.03, 56.77, 45.96 and 13.61 for 24, 96, 192, 264 and 336 h respectively. Mortality was reported at 24 h in 100 mg/L (LOEC-24h). At 96 hours, 60 mg/L was the highest concentration in which there was no observable effect (NOEC-96h). From 192 to 264 h, the NOEC value was 40 mg/L, and at 336 h it was 10 mg/L. Regarding behavioural alterations, these were observed from 10 mg/L and included lethargy, lack of equilibrium or erratic swimming and natation in the surface of the tank. These results further accentuate the toxicological effects of atrazine in aquatic organisms, both lethal and sublethal. Additionally, these findings highlight the importance of strict regulations regarding the usage of atrazine and other pesticides near water bodies, in order to avoid unintended effects in the ecosystems.

## 9.P-We-010

### Effects of Roundup® Original DI measured by markers of metabolism and oxidative balance in *Parastacus promatensis* (Crustacea, Decapoda, Parastacidae)

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#### Abstract

Pesticides have a potential for contamination of water resources and fixation to soil and sediment. In Brazil, glyphosate is present in more than 130 commercial formulations and its toxic effects have already been studied in different species to understand its impact on the biota. However, the number of studies with decapod crustaceans is still very small, especially if we consider Brazilian native species. Decapod crustaceans are widely used as experimental models due to their biology, their sensitivity to pollutants and their convenience of collection and maintenance in the laboratory. *Parastacus promatensis* is found at 850m above sea level, in a conservation area in São Francisco de Paula, Rio Grande do Sul, Brazil. The objective of this study was to evaluate the metabolic variations in the hemolymph and in the oxidative balance in the hepatopancreas and in the gills of *P. promatensis* against exposure to Roundup®, to establish physiological markers of susceptibility that can be used to understand the impact of this herbicide on biodiversity. The crayfish were captured in the Garapiá stream located within the area of the Center for Research and Conservation of Nature Pró-Mata. After capture, the animals were transported to the aquatic animal culture room at the Center for Experimental Biological Models at PUCRS. The animals were kept in aerated aquariums, in the temperature-controlled room ( $22\pm 2^{\circ}\text{C}$ ) with a light-dark cycle (12h:12h) and fed with lean beef. After 10 days of acclimatization, crayfish were exposed to different concentrations of glyphosate (0, 65, 260, 520 e  $780\mu\text{g/L}$ ). After the exposure period (seven days), the animals were weighed, measured, and tissues removed and frozen. Our results showed there was a significant variation in glucose levels and total proteins, showing a decrease of glycemic suggesting a greater uptake of this carbohydrate by the tissues and/or a decrease in glucose from the gastrointestinal tract. There was a significant increase in the enzymatic activities in the hepatopancreas and in the gills. Tissues showed antioxidant enzyme activity after glyphosate exposure that differed from the baseline activity observed in the group that was not exposed to glyphosate. The results of this study made it possible to understand and describe more clearly aspects of the toxicokinetic and toxicodynamic of glyphosate on a native and non-target species of this herbicide, evidencing a negative impact on the species.

## 9.P-We-011

### Immunological Response of *Biomphalaria straminea* (Gastropoda, Pulmonata) Exposed to Water Pollution From an Urban Lake

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#### Abstract

Lugano Lake is located in an Ecological Reserve of Buenos Aires City, Argentina. The water comes from rainfall and surface runoff, however, has been receiving raw sewage for a long period. Its study is essential due to the functions it performs, as a space for recreational and educational activities, and as a refuge for biodiversity. *Biomphalaria straminea* is a native hermaphroditic aquatic gastropod inhabiting freshwater bodies in Argentina. The immune defense of gastropods is simple, being the circulating hemocytes that can recognize foreign bodies, perform encapsulation responses, phagocytosis, and cytotoxic reactions. Thus, immunological responses have been proposed as biomarkers to environmental pollutants exposure. The aim of this work was to assess the viability and proportion of hemocytes population following a seven-day exposure to water samples from Lugano Lake (L1, L2, and L3) during four seasons over a year. Physicochemical parameters of the water samples were also analyzed. A control group was included, using tap water (TW). Dissolved oxygen varied seasonally (5.8 mgL<sup>-1</sup> in summer to 20.2 mgL<sup>-1</sup> in spring), conductivity was high which is usually indicative of contamination (max: 1741 µScm<sup>-1</sup> in winter), and pH was persistently alkaline (8.5 - 10.4). Regarding nutrients, a concentration peak was observed for ammonium in winter (6.3 mgL<sup>-1</sup>) and phosphates in spring (8.7 mgL<sup>-1</sup>), while dissolved chemical oxygen (COD) ranged between 90 mgL<sup>-1</sup> in winter to 504 mgL<sup>-1</sup> in summer. Ammonium, pH, and COD exceeded aquatic life protection guidelines. Three types of hemocytes were identified: granulocytes, granulocytes with pseudopodia, and hyalinocytes. In autumn, spring, and summer, granulocytes and granulocytes with pseudopodia represented the higher % of the total hemocytes in snails exposed to L1, L2, and L3 with a maximum of 70% and 74%, respectively, while the % of hyalinocytes was lower. During winter, only organisms exposed to L2 showed a higher % of granulocytes (63%) and a lower % of hyalinocytes (31%). In TW, the hemocyte population comprised 55-71% hyalinocytes, 9-12% granulocytes, and 26-42% granulocytes with pseudopodia. No significant differences in non-viable hemocytes were recorded. Our results suggest that exposure to pollutants in the lake may compromise the immune system of *B. straminea*. This could be because granulocytes play a dominant role in the phagocytosis, while hyalinocytes are primarily involved in early inflammatory responses.



## 9.P-We-012

### Non-Lethal Biomarkers Response in Fish Caged in a Rice Field During a Bifenthrin Application

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#### Abstract

There is a growing need to develop and improve non-lethal biomonitoring of fish, capable of providing information regarding stress response. Moreover, native fish inhabiting rice fields are exposed to multiple stressors like the exposure to current-used pesticides, extreme temperatures, and hypoxic conditions. The aim of the present study was to assess metabolic and immunological biomarkers in plasma and skin mucous of *Piaractus mesopotamicus* caged in a rice field during a bifenthrin (BF) application. Fish from a local farm ( $n = 32$ ;  $44.6 \pm 10.1$  g;  $14.2 \pm 1.0$  cm total length) were randomly divided into a control group and a bifenthrin-exposed group. Each was placed in different irrigation canals next to a paddy field (San Javier, Santa Fe, Argentina). These sites had separate water supplies but were under similar environmental conditions. Two cages ( $n = 8$  fish/cage) per site were placed separately at each irrigation canal. BF application (Seizer®; 100 cm<sup>3</sup>/ha) was carried out with coastal spraying equipment. Fish were removed 72 h afterwards for blood and mucous biochemical analyses. Glucose (Glu), triglycerides (Tg), total proteins (TP), and albumin (Alb) concentration were measured as metabolic biomarkers in plasma, together with alkaline phosphatase (AP) and lysozyme (Lys) activities as immunological endpoints in this sample. Mucous was investigated for TP concentration and for protease (P), AP, and Lys activities. The insecticide application caused changes in plasma metabolic and immunological profiles: AP activity and Glu, TP, and Alb concentration increased while Tg level and Lys activity decreased. Furthermore, in skin mucous, BF exposure significantly increased AP and P activities. These results evidenced the current use of BF in rice fields might alter the metabolic and immunological status of fish inhabiting rice fields. Our results also show that blood and skin mucous are very promising matrices for the development of non-lethal biomarkers to assess fish health in a stressed environment.

## 9.P-We-013

### Population Rescue of Microalgae in the Face of Glyphosate Contamination

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#### Abstract

Glyphosate-based herbicides are the most widely used in the world. This toxic compound reaches aquatic ecosystems and can affect populations of microalgae, which are the basis of the trophic web and the main primary producers in these environments. The present study tested the sensitivity of two species of green algae (Chlorophyta) to a glyphosate-based herbicide (Roundup Transorb) and the occurrence of population rescue. Strains of *Desmodesmus communis* and *Pseudopediastrum boryanum* were tested in isolation and in co-occurrence in a two-step experiment, lasting 72 hours each. In step 1, the populations of the two microalgae were exposed to Roundup Transorb at 0, 100, 500, and 1000  $\mu\text{g.L}^{-1}$  glyphosate. Aliquots of each treatment were then transferred to a new media where 20000  $\mu\text{g.L}^{-1}$  glyphosate was added (step 2). Growth inhibition of microalgae population at the end of step 1 was dose-dependent, regardless of the species of algae and whether they were isolated or combined. Rescue after exposure to 20000  $\mu\text{g.L}^{-1}$  glyphosate for 72 hours was more successful for populations previously exposed to intermediate concentrations of 100 and 500  $\mu\text{g.L}^{-1}$  glyphosate. It seems that these concentrations were able to trigger some process that increased algae tolerance to glyphosate. Additionally, neither the species nor the fact that they were isolated or combined influenced the glyphosate toxicity, at least for the concentrations tested in this study.

## 9.P-We-014

### **Waterborne Exposure to Diclofenac, a Nonsteroidal Anti-inflammatory Pharmaceutical, in the Freshwater Cichlid Fish *Cichlasoma dimerus* (Cichliformes, Cichlidae)**

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#### **Abstract**

In the last decades, concern over the contamination of water bodies has been increasing. Most of this pollution is due to pharmaceutically active compounds, that are increasingly present and are expected to rise over time. One of the most ubiquitous pharmaceuticals found in the aquatic environment and widely prescribed is diclofenac (DCF). It belongs to the family of the nonsteroidal anti-inflammatory drugs and inhibits the enzyme cyclooxygenase 1 (COX-1), in order to decrease the levels of prostaglandins and relieve pain during the inflammatory response. It reaches water bodies mostly through poorly treated sewage effluents and its impact on aquatic organisms is poorly understood. The aim of this study was to analyze the impact of waterborne exposure to DCF on biochemical and hematological biomarkers in the freshwater cichlid fish *Cichlasoma dimerus*. Adult fish were exposed to 1, 10 or 100  $\mu\text{g/L}$  DCF for 14 days under a semi-static renewal system. Biomarkers assessed included: hematological parameters, glycemia, and lipid peroxidation (TBARs), glutathione-S-transferase (GST) activity and antioxidant capacity (ACAP) in liver, gills and brain. Even though glycemia increased in exposed animals, no significant differences were observed on these parameters due to DCF exposure. However, multivariate analysis showed oxidative stress damage in gills and hematological parameters. Since pharmaceuticals have become the focus of emerging concern compounds, it is imperative to perform environmental risk analysis on non-target species such as fish.

## 9.P-We-015

### Biochemical Evaluation of the Pharmaceutical Carbamazepine in the Liver of *Astyanax lacustris* (Teleostei: Characidae)

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#### Abstract

Contaminants of emerging concern (CECs), such as pharmaceuticals and personal care products, have been constantly detected in freshwater ecosystems worldwide. Since most of those compounds present a low degradation rate, they remain bioavailable to organisms for a further period of time, impacting directly their physiology and health. Among those CECs, carbamazepine (CBZ) is a drug widely used for the treatment of epilepsy and bipolar disorder, and due to its wide usage and release in ecosystems, fish and other organisms have been subjected to its possible toxicological effects. Considering that little is known about the toxicity of CBZ, the present study aimed to evaluate the influence of this neuropharmaceutical on biochemical biomarkers of the teleost *Astyanax lacustris*. Sexually mature females were randomly separated into four experimental groups, as follows: 1. control (with the vehicle, dimethyl sulfoxide), 2. 250 ng L<sup>-1</sup>, 3. 500 ng L<sup>-1</sup>, and 4. 1250 ng L<sup>-1</sup> of CBZ. After 7 days of exposure, fish were anesthetized with eugenol and euthanized for tissue collection. Samples of liver were weighed and immediately stored at ultra freezer -80 °C until further analysis. Total lipid and protein content, lipoperoxidation, as well as the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) were evaluated in liver samples. Statistical analysis was performed through one-way ANOVA followed by Tukey post-hoc test, considering  $p < 0.05$ . The results demonstrated that CBZ did not influence total lipid and protein content in the liver of the fish. Also, the drug was not capable of influencing the activity of SOD, CAT, and GST in the liver. Nonetheless, female fish exposed to 500 ng L<sup>-1</sup> and 1250 ng L<sup>-1</sup> of CBZ reduced the levels of lipoperoxidation in the tissue when compared to the control group. As demonstrated, CBZ, in the concentrations and exposed period tested, was not capable of altering most of the biochemical biomarkers in *A. lacustris* females. Typically, this species inhabits clean and polluted waterbodies, being adjusted to adverse conditions and presenting phenotypic plasticity. In this context, CBZ is not impacting the liver of *A. lacustris* females, however, fish are subjected to this compound in ecosystems and the environmental risk might remain since there is still no regulation towards CBZ release and presence in aquatic waterbodies.

## 9.P-We-016

### Gene Co-expression Network Analysis for the Selection of Candidate Early Warning Indicators of Heat and Nutrient Stress in *Posidonia oceanica*

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#### Abstract

The continuous worldwide seagrasses decline calls for immediate actions in order to preserve this precious marine ecosystem. The main stressors that have been linked with decline in seagrasses are 1) the increasing ocean temperature due to climate change and 2) the continuous inputs of nutrients (eutrophication) associated with coastal human activities. To avoid the loss of seagrass populations, an “early warning” system is needed. We used Weighed Gene Co-expression Network Analysis (WGCNA), a systems biology approach, to identify potential candidate genes that can provide an early warning signal of stress in the iconic seagrass *Posidonia oceanica*, anticipating plant mortality. Plants were collected from both eutrophic (EU) and oligotrophic (OL) environments and were exposed to thermal and nutrient stress in a dedicated mesocosm. By correlating the whole transcriptome expression after 2-weeks exposure with the shoot survival percentage after 5-weeks exposure to stressors, we were able to identify several transcripts that indicated an early activation of several biological processes (BP) including: protein metabolic process, RNA metabolic process, organonitrogen compound biosynthetic process, catabolic process and response to stimulus, which were shared among OL and EU plants and among leaf and shoot apical meristem (SAM) in response to excessive heat and nutrients. Our results suggest a more dynamic and specific response of the SAM compared to the leaf, especially the SAM from plants coming from a stressful environment appeared more dynamic than the SAM from a pristine environment. A list of potential molecular markers is also provided that can be used as targets to assess field samples.

## 9.P-We-017

### **Evaluation of Germination and Root Size in Bioindicator *Allium cepa* L. Exposed to Water From Three Tributary Rivers of the Iguaçu River (Paraná, Brazil)**

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#### **Abstract**

The biomonitoring of aquatic ecosystems is extremely important, because through it we can obtain an environmental diagnosis of our rainwater environments. One way to perform this monitoring is through exposure bioassays with bioindicators to the medium or pollutant that is to be studied. In this sense, bioassays involving the common onion *Allium cepa* L. have a remarkable prominence, since these plants provide a reliable result quickly and efficiently. Industrial and agricultural activity results in a wide range of pollutants and residues that can prove toxic to various organisms. Therefore, the objective of our research was to evaluate on *Allium cepa* L. roots the effect of three tributary rivers of the Iguaçu River (Paraná, Brazil) that is characterized as the second most polluted river in Brazil and the region of Dois Vizinhos where the study was done, stands out for the highest rates of pesticide consumption in the state of Paraná. The selected rivers had different characteristics, point 1 was located in the urban perimeter and is characterized by the absence of riparian forest and intense pollution; Point 2 is located near agricultural properties and has no riparian forest; Point 3 has its original characteristics more preserved than the other points. A negative control was also exposed, containing only reconstituted water. The bioassay lasted 168 hours, germination was evaluated daily, and root length was measured at the end of the experimental period. Our results showed initial root germination in 72 hours for all treatments, a value considered normal for the species. Regarding the length of the radicle, there was no significant difference between the experimental groups ( $p=0.195$ ). When we evaluated the germination rate of seeds per point, a statistically significant reduction in the germination of seeds from point 3 in relation to the other points was observed ( $p=0.00028$ ), however, the relationship between time and point of study did not differ significantly in the germination rate ( $p=0.492$ ). Despite being characterized as a point with more preserved characteristics, point 3 presented the worst germination rates, this may have occurred due to several factors, such as higher rainfall rates in the region or even the application of chemicals in agricultural productions located near the river. Continuous monitoring, even in different seasonal seasons, is highly recommended in future ecotoxicological research.

## 9.P-We-018

### **Survival of the Embryo-Larval Stage of *Rhamdia quelen* (Siluriformes) Exposed to the Water of Tributaries of the Iguaçu River (Brazil) in Different Contexts of Use and Occupation**

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#### **Abstract**

With the growing industrialization and demand for agricultural productivity, the load of pollutants that come into contact with the environment also increases, and one of the ecosystems most affected by pollution is the aquatic ecosystem. In this sense there is a need to constantly verify the possible effects of the most diverse pollutants on various organisms present in the aquatic biota, especially non-target individuals. The objective of our research was to evaluate if the water from rivers with similar characteristics, but in different contexts regarding land use and occupation, affects the survival of the embryo-larval stage of the biological model *Rhamdia quelen*. For this purpose, we used larvae of the bioindicator in 24-well plates, analyzing mortality daily for a period of 7 days after fecundation. The rivers were selected based on their characteristics, geographical location and surrounding land use, Point 1 is characterized by the absence of riparian forest, bad odor, proximity to the urban region, Point 2 is characterized by the proximity to extensive agricultural production with steep slope that favors the entry of contaminants in the water body, as well as the presence of a road connecting an important community with the urban perimeter of the municipality of Dois Vizinhos with considerable traffic of trucks and agricultural vehicles, finally, Point 3 has well preserved riparian forest and is considerably distant from points of extensive production and an urban center. Our results showed that Point 3 was the treatment with the highest survival rate compared to the other collection points and controls, highlighting the importance of maintaining riparian forest around rivers and streams. There was no significant difference between P1, P2, and Negative Control, however, all were statistically different from Positive Control, except for P1, which showed no significant difference with Positive Control, and both recorded the worst survival rates, which shows the size of the negative impact of urban and rural pollutants on the early stages of *Rhamdia quelen*. Through our results it is evident the need for preservation of our water bodies to reduce the toxicity indices on the aquatic biota, the active monitoring and investigation of the xenobiotics present, as well as their possible synergistic effects in these rivers are extremely important in order to preserve the natural resources that inevitably affect all living beings, including us, humans.

## 9.P-We-019

### **Histopathological Assessment in Gills and Digestive Gland to Evaluate the Health Status of *Mytilus platensis* (Bivalvia, Mytilidae) From a Seaport Area of North Patagonia Argentina.**

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#### **Abstract**

Trace metals may bioaccumulate in soft tissues of aquatic biota and cause toxic effects. Bivalve gills regulate gas exchanging and particulate filtering besides functioning as a barrier for pathogens and pollutants, while digestive glands are involved in intracellular digestion and detoxification. We studied the histopathological impact of metal pollution in *M. platensis* in two sites at the north coast of Patagonia, Argentina. Trace metal concentrations were quantified in water of the Port of San Antonio Este, and a reference site, Isla Mejillón, also a common locale of mussel extraction. Metal concentrations in water and organs were measured using atomic absorption spectrometry. To observe effects histologically, gills and digestive glands were dissected, fixed and prepared for routine techniques and lipofuscin detection with the Nile blue method. Histological and morphometrical alterations were registered. Higher concentrations of chromium, nickel and zinc in water were registered in the harbor, along with copper and chromium in gills and digestive gland of *M. platensis* in this site. Mussels from the harbor area had dilated hemolymphatic vessels with numerous hemocytes in the gill filaments. Fused filaments were also found. Morphometric analysis of the gill filaments showed an enlargement in the epithelial thickness in areas responsible for interfilament union and gas exchange. Furthermore, the area whose dominant cells are mucocytes showed a marked reduction of the epithelial thickness, likely due to metal expulsion with mucus. Digestive glands of port mussels significantly accumulated lipofuscin granules within the absorptive cells, accounting for lipid and protein peroxidation; these granules were not found or were scarce in the digestive gland tubules of mussels collected at the reference site. The metal accumulation results found in both organs along with the histopathological alterations observed evidenced its negative effects on individuals of *M. platensis* inhabiting the harbor area of Port San Antonio Este. Therefore, we recommend the use of the histopathological essays applied in this study as biomarkers to improve the evaluation of metal accumulation effects over the health status of *Mytilus platensis* in aquatic environments under intense human activity.



## 9.P-We-020

### Development of the Embryo-Larval Phase of *Rhamdia quelen* Exposed to the Water of the Lower Iguçu Rivers

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#### Abstract

Fertilizers and other chemical substances used in the management of agricultural pests have a high potential for toxicity and may accumulate in non-target organisms, as well as affect their development and physiology. The objective of the study was to evaluate whether the development of the embryo-larval phase, of the biological model *Rhamdia quelen*, is affected by exposure to water from rivers with different environmental situations. In the bioassay, water was used in an urban stretch of river (P1), a rural stretch (P2), and a more preserved location (P3). The water was collected every 48h and kept in an amber bottle at 4°C up to one hour before use. Additionally, a negative control (CN) and a positive one (CP) were established in reconstituted water. For the latter, a mixture of glyphosate, 2,4-D, and bifenthrin was made at the maximum concentrations allowed for class 2 rivers, according to Brazilian environmental legislation. Freshly fertilized, viable *R. quelen* eggs were placed in 7 24-well plates, with 5 individuals per well. The embryos were exposed in a semi-static manner, with an exchange of 50% of the volume of water/well every 24h. Sampling was carried out 96 hours after fertilization, with 6 replicates of 5 individuals/each per treatment being collected and kept in 5% formalin. Body area (mm<sup>2</sup>) and morphological deformities were evaluated under a stereomicroscope with a camera attached. The damages were categorized as axial, in the fins, craniofacial and thoracic-abdominal. From the sum of damages, an index of deformities was established. CN and P3 registered the lowest incidence of deformities. Also, in P3, larvae with a larger body surface area were observed, statistically differing from the CP that contained pesticides known to be genotoxic ( $p=0.02$ ). In P2, with rural influence, the larvae with the highest rate of body deformities were obtained ( $p=0.03$ ). The most recurrent damage was axial damage, involving lordosis, kyphosis, scoliosis, or atrophy. With intensive agricultural systems in their surroundings, P2 is more likely to be hit by pesticide and fertilizer releases. The embryotoxicity test applied to river water proved to be responsive and sensitive in identifying the stretches with the greatest influence of chemical substances with teratological effects. The preservation of riparian vegetation at P3 corroborated with the lowest incidence of deformities and the largest size of *R. quelen* larvae, demonstrating its environmental relevance.

## 9.P-We-021

### The Use of Eugenol as an Anesthetic for the Amazonian stingray *Potamotrygon Leopoldi* (Myliobatiformes, Potamotrygonidae)

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#### Abstract

*Potamotrygon leopoldi* is a freshwater stingray of the Potamotrygonidae family, and it is widely collected in the wild and sold in the ornamental fish trade. An alternative for the conservation of the species and preservation of its natural populations is the adaptation of technologies for ex-situ reproduction. The first step is establishing an appropriate anesthesia protocol to achieve good management. Also, the handling of this species must be highly cautious since they have toxins and can cause injuries to people when mishandled. This work aims to evaluate the anesthetic induction and recovery time in *Potamotrygon leopoldi* submitted to different doses of eugenol, an anesthetic considered reliable in adequate dosage for fish anesthesia. The experiments were approved by the Ethics Committee on Animal Use CEUA No. 4751220822, where three dosages 20, 25, and 30 mg.L<sup>-1</sup> of eugenol diluted in alcohol (70° GL) at a 1:9 ratio (eugenol:alcohol) were tested in 9 specimens. Anesthetic induction and recovery were evaluated in four stages (1, 2, 3, and 4). Specimens were anesthetized and reanesthetized 15 days later at the same concentrations to evaluate the differences between dosages and assays. The calculation of the eugenol concentration necessary for anesthesia of the species was done with a Permutational Analysis of Variance (PERMANOVA), considering as dependent variable the concentrations of eugenol, and the time of anesthesia and recovery as independent variable. The time of exposure and animal size was included as co-variables in our analyses. After an observation period of 72 hours post-experiments, the survival rate was 88.9%. The specimens take longer in the second trial to reach the state of anesthesia, demonstrating that priming of the organism on the chemical compound occurred, thus giving a greater response to the drug. In both assays the appropriate dosage to reach the stage of total immobilization was 30 mg.l<sup>-1</sup>. Eugenol has been widely used as an anesthetic in fish, however, the effects of the anesthetic on animals that have undergone multiple exposures are still unknown. The present study is a pioneer in investigating a second exposure of eugenol in Chondrichthyes, providing a possibility of safe management of freshwater stingrays.

## 9.P-We-022

### Acute Toxicity of the Herbicide Imazamox for Aquatic Plants

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#### Abstract

One of the ways of evaluating the environmental risk and the ecotoxicology of pesticides is the use of bioindicator organisms in standardized tests (acute or chronic ecotoxicity) or the sublethal effects, through measurable behavioral or metabolic reactions, which indicate changes in the balance of the environment. Thus, the objective of this study was to evaluate the acute ecotoxicity (LC<sub>50</sub>h;7d) of the herbicide imazamox for the aquatic bioindicator plants *Azolla caroliniana*, *Lemna minor* and *Wolffia brasiliensis*. The plants were cultivated in boxes containing organic substrate, soil type oxisol, sand (1:1:1; vv<sup>-1</sup>). After the vegetative growth (*A. caroliniana* and *L. minor*) they were transferred to crystallizers containing Hoagland's medium in a bioassay room at a temperature of 25.0 ± 2.0 °C, with a photoperiod of 12 hours of light, for four days. For *W. brasiliensis*, collections of plants contained in 0.3 mm in diameter were used, for *L. minor*, 4 plants with 3 fronds each were used in each experimental unit, and for *A. caroliniana*, 5 plant samples were used per test container. In the acute ecotoxicity tests, concentrations of 0.10; 1.07; 3.44; 11.16; 36.40; and 118.0 mg L<sup>-1</sup> and a control, with three replicates each. The evaluation of plant mortality was carried out on the 3rd, 5th and 7th day and an increase in the number of fronds, chlorosis and necrosis were observed. For *W. brasiliensis* at a concentration of 11.16 mg L<sup>-1</sup>, 17.0% of mortality occurred 36.40 and 118.0 mg L<sup>-1</sup>, 20.0% and 23.0% of mortality, respectively. The 50% lethal concentration (LC<sub>50</sub>;7d) was > 118.0 mg L<sup>-1</sup>, being the herbicide classified as practically non-toxic. For *L. minor* at 11.16 mg L<sup>-1</sup>, 33.0% of mortality occurred and at concentrations 36.40 and 118.0 mg L<sup>-1</sup>, 43.0% and 66.0% of mortality occurred, respectively. Acute ecotoxicity (LC<sub>50</sub>;7d) was 42.9 mg L<sup>-1</sup>, with a lower limit of 27.81 mg L<sup>-1</sup> and an upper limit of 66.18 mg L<sup>-1</sup>, with imazamox being classified as slightly toxic for this bioindicator. For *A. caroliniana*, at 36.40 mg L<sup>-1</sup>, 9.0% of plant mortality occurred and at 118.8 mg L<sup>-1</sup>, 14.0% of mortality occurred, respectively. The LC<sub>50</sub>;7d was greater than 118.0 mg L<sup>-1</sup>, being classified as practically non-toxic. Thus, it is concluded that aquatic plants should not be used in the evaluation of the ecotoxicity of imazamox, as they were tolerant to high concentrations of the herbicide in standardized exposure tests.

## 9.P-We-023

### Behavioral Alterations Produced by Exposure to the Antidepressant Fluoxetine in Two Fish Species of the Family Poeciliidae

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#### Abstract

Chemical pollution from pharmaceuticals is increasingly recognized as a major threat for aquatic wildlife. Among the wide variety of pharmaceuticals, fluoxetine (FLX) is one of the most widely prescribed antidepressants, often detected in aquatic systems that receive sewage and pharmaceutical effluents. Since FLX is designed to modulate human behavior and its action pathways are conserved in vertebrates, this compound could affect the behavior of non-target species such as fishes. In this regard, behavior is an ecologically relevant indicator of exposure to neuroactive compounds as it can directly affect the fitness and survival of organisms. In turn, behavioral biomarkers are gaining increasing interest as an early warning system in ecotoxicological studies. In this study, female *Cnesterodon decemmaculatus* and *Gambusia holbrooki* (Teleostei, Poeciliidae) were exposed to nominal concentrations of 0 (control), 1, 5, 25, and 50 µg/L FLX for 14 days, under a semi-static design with daily renewal of test solutions (n=5 per aquarium, in duplicate). Following exposure, aggressive interactions between individuals in each aquarium were recorded by video for 10 min, before and after being fed *Artemia* sp. nauplii. From the video records, the latency to emergence of aggression, as well as the number and duration of aggressive interactions in which each individual participated, were quantified. Next, each fish was transferred to an individual aquarium, and after 90 min, swimming activity was recorded for 10 min. Videos were analyzed using Ethovision software to quantify locomotion parameters (distance traveled, average speed, time in motion, static time, time in each third of the water column). The results showed differential effects of FLX in both species. In *C. decemmaculatus*, exposure to higher concentrations of FLX resulted in an increase in aggressive interactions between conspecifics in the presence of food, as well as an alteration of swimming activity. In *G. holbrooki*, a marked attenuation of swimming activity was evident with increasing FLX concentrations, whereas the effects on aggression were less evident. For both responses, the effects were significant at concentrations higher than those of environmental relevance. The results of this study contribute to the assessment of the impact of pharmaceuticals on fish fauna.

## 9.P-We-024

### Preliminary Data on the Development of Toxicity Tests to Evaluate Aquatic Safety of Gels with Biocidal Capacity

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#### Abstract

In Brazil, two invasive species of sun coral were disseminated by vessels and have been spreading rapidly along the coast, causing impacts on biodiversity and socioeconomic activities. Alternative to manual removal of the coral, gels with biocide agents seems a promising control method. These gels can be applied directly to the surface of coral colonies, and studies about the application using underwater vehicles remotely operated are under development, as well as different types of biocides. However, the use of these products can also affect non-target species from the region; therefore, it is important to assess their toxicity using representative species. The objective of the present study was to develop protocols to evaluate the toxicity of these gels and their components using the marine amphipod occurring in Brazilian coast, *Parhyale hawaiensis*. For toxicity evaluation of the biocides that are under evaluation and the gel components, acute toxicity tests were performed with neonates ( $\leq 7$  days old) using 96-well microplates at  $24 \pm 2^\circ\text{C}$ , 12h/12h light and dark, during 96h of exposure. A device was developed for the evaluation of the gel, consisting of a nylon bag containing 1 g of gel exposed to 100 mL seawater containing 5 neonates, in the same conditions previously mentioned. The 50% lethal concentration (LC50) of the biocides were 2.7 mg chlorine/L for sodium hypochlorite and 168 mg/L for acetic acid. The toxicity of the gel components presented LC50 of 1980 mg/L for calcium chloride and 189.9 mg/L for ammonium persulfate. Using the nylon bag device, the gel without the biocide was not toxic, but when it contained sodium hypochlorite, mortality of 100% of the organisms was observed. More tests will be performed using the gel containing the other biocides. The developed protocol was successfully used in the evaluation of the gel. The next step will be to determine the required dilution to predict where no toxicity is observed. This will help to determine the expected impact zone during the field application of those gels to control this invasive coral.

Acknowledgments: Engr. Carlos Speglich; Petr leo Brasileiro S.A.; Laboratory of Ecotoxicology and Genotoxicity.

## 9.P-We-025

### Effect of the Interaction Between *Cnesterodon decemmaculatus* and Biofilm Grown With and Without Arsenic

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#### Abstract

Arsenic (As) contamination is a worldwide problem. Biofilm communities play an important ecological role in freshwater ecosystems, actively influencing metal/metalloid sorption, desorption and transformation. The aim of this work was to evaluate the ecotoxicological response of the fish *Cnesterodon decemmaculatus*, against chronic As(III) exposure, in microcosm experiments. For this, biofilm was collected in the Naveira stream (belonging to the Lujan river basin) and cultured in the laboratory for 25 days in aquariums (8L) with ground glass plates (10 x 05 cm). One week before finishing the culture, 20 µg As(III)/L was added to one of the aquariums. At the end of the period, a bioassay was carried out with *C. decemmaculatus*, during 21 days of exposure to As(III) (20 µg As/L) in glass aquariums (2L) inside incubation chambers with controlled photoperiod and temperature (16: 8 h L:D/ 22 ± 1°C), and constant aeration. Five fish and four biofilm plates per aquarium were used; and media with (As) and without As(III) (tap water-TW) were established, with biofilm cultivated with and without As(III), forming 4 treatments: TW-Bio(TW), TW-Bio(As), As-Bio(TW) and As- Bio(As). Tap water passed through an As filter was used as control. During the entire bioassay fish had free access to the plates to feed with biofilm, in addition to being fed with balanced food of known composition (2% of the fish biomass). After the exposure, they were dissected and the following were extracted: livers, for the measurement of the enzymatic activity of catalase (CAT) and glutathione-S-transferase (GST); and muscles, for content of lipids, carbohydrates, proteins and activity of the electron transport system (ETS), with which an integrated index of Cellular Energy Allocation (CEA) was developed. The main significant differences in the fish were observed in the type of culture of the biofilm. Fish exposed to biofilm cultivated with As(III) showed a variation on their response in CAT, GST, carbohydrates, lipids and ETS. Furthermore, energy reserves was the most affected parameter. This is a preliminary test, so more studies are required to obtain conclusive answers.

## 9.P-We-026

### Embryotoxicity of Stream Water on the Neotropical fish *Rhamdia quelen*, in an Agricultural Region of Paraná

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#### Abstract

Aquatic environments are routinely affected by chemical substances used in soil fertilization and pest control in productive systems. These pollutants have toxic potential and, consequently, can affect the health of non-target organisms. The objective of this work was to evaluate whether water from streams with different conditions in their course can affect the survival and embryo-larval development of catfish *Rhamdia quelen*. A bioassay was conducted by collecting water from streams in a rural area in southwest Paraná, being: P1- a creek with protected springs with little agricultural influence, whose waters were clear; P2- a stream with great influence of agricultural activities, with little riparian vegetation on the banks; P3- an area of confluence of the waters of the previous points. Reconstituted water was used as a negative control (CN) and, as a positive control (CP), a mixture was prepared with the pesticides glyphosate, 2,4-D, and bifenthrin, at the maximum concentrations allowed by Brazilian legislation. The water was collected every 48 h and kept in amber bottles at 4°C until one hour before use. Freshly fertilized and viable eggs of *R. quelen* were placed in 15 plates of 24 wells, with 5 individuals/well. Survival was evaluated every 24 h for 7 consecutive days and morphological deformities and body area of the larvae (mm<sup>2</sup>) after 96 hours of fertilization. For this last analysis, 30 larvae were collected per treatment, which was distributed in 6 tubes soaked in 5% formaldehyde for preservation. Exposure took place by the semi-static method with water renewal every 24 h of 50% of the volume of the wells. Survival was significantly reduced in CP, being similar in the other groups. Deformities and body area were analyzed using a stereoscope with a camera attached and showed no significant difference between treatments. The mixture of pesticides used in the CP and widely disseminated in agricultural management caused a toxicological effect (survival), even in amounts allowed by law. However, the water from the sampling points did not show a visible effect, and the analysis of biomarkers is recommended for a better understanding.

## 9.P-We-027

### Analysis of the Toxicity of the Iguaçu River Water on Two Fish Species

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#### Abstract

The Iguaçu River Basin is located in the State of Paraná, Brazil, covering 111 municipalities (28 percent of the total area of the State of Paraná) with an estimated population of 4,405,882 inhabitants. Among Paraná rivers, the Iguaçu River is the largest river basin, which is known for its high endemism, estimated at 69.7 percent of the total species. This basin is strongly impacted by several factors such as urbanization, agriculture, dam construction, and the introduction of non-native species, which can cause damage to native species, considering the modification of the feeding habits of non-native species, leading to a competition for food resources with native species.

In Brazil, pesticide sales have increased significantly in the last 10 years, with herbicides, fungicides, and insecticides being the best-selling use classes. These active ingredients act on non-target organisms, such as fish, which have a delayed metabolic action, which contributes to significantly increase their susceptibility to toxicity of these compounds.

In order to measure the genotoxicity of the water, 10 organisms from each point were collected from the fish *Ancistrus* sp., which feeds on algae and sediment debris, and is considered a detritivore. The species was collected in three different cities along the river: Cascavel, Parque Nacional do Iguaçu, and Dois Vizinhos. Blood analysis was performed using the comet assay, where cells were homogenized and mixed with low melting point agarose, placed on slides with agarose and then lysed. After this, the cells were electrophoresed in buffer solution of pH > 13. The slides were stained with ethidium bromide for analysis under a fluorescence microscope. In the analysis, effects are divided into: damage 0 (no damage), damage 1, 2, 3 and 4. According to the Kruskal-Wallis test, with  $p=0.45$ , there were no statistical differences in the damage rates among the three groups observed, that is, there were no significant damage rates among the points in the three cities analyzed, which means that the water from the Iguaçu River, at these points, does not present toxicity to the blood cells of *Ancistrus* sp. This fact probably occurs because fish have a defense mechanism that acts to repair DNA damage, but they are susceptible to possible contamination by anthropic actions. Still, more studies are needed with other species and also with different tissues for environmental monitoring of rivers for public supply.



## 9.P-We-028

### Metabolic And Histological Biomarkers Reveal Anthropogenic Impact On Fish In The Reconquista River

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#### Abstract

The Reconquista River belongs to one of the main basins of the Buenos Aires Metropolitan Area and is the second most polluted river in Argentina. The native viviparous freshwater fish *Cnesterodon decemmaculatus* habits much of this basin. The objective of this work was to compare metabolic, morphometric, and histological biomarkers in females from the upper and middle basins of the Reconquista River and a control population reared in the laboratory (Ci).

Adult specimens of *C. decemmaculatus* were captured in the upper basin of the Reconquista River (R1) and 18 km downstream (R2) -in a site more impacted by industrial and urban activity. The animals were sexed under a magnifying glass and from the selected females were extracted brain, muscle and gonads for different determinations: acetylcholinesterase activity (AChE) and energy metabolism: lipids (LIP), carbohydrates (CHO), proteins (PROT), and the electron transport system (ETS). In addition, cellular energy allocation indices (CEA), condition factor, hepatosomatic index (IHS) and gonadosomatic index (IGS) were calculated. Histological analysis was performed by fixing whole fish with Bouin's solution and sections were stained with Masson's Trichrome.

Regarding the biomarkers determined in muscle, a decrease in lipid content was found in R1, and protein content for R1 and R2. The CEA that integrates all the metabolic parameters showed a reduction in the animals of R1 and R2 compared to Ci. AChE activity was inhibited in brain and muscle for both populations. The histological analysis of gonads revealed the presence of vitellogenic and previtellogenic oocytes in Ci and R2, but only previtellogenic oocytes were found in ovaries from R1 females. The morphometric indices showed an increase in the IHS and a decrease in the IGS for these females. Historically, the upper basin of the Reconquista River (R1) has been considered a site of low anthropic impact. However, in this work we realize that the health condition of these fish, the analyzed biomarkers that showed a level of oxidative stress with changes in the availability of energy reserves, neurotoxicity and alterations in reproductive stages, prove that the anthropic impact has progressed in ecosystems that were considered low impacted.

## 9.P-We-029

### Development of Enzymatic Endpoints in *Hyalella curvispina* as Future Biomarkers in Evaluations With Acute Sediment Bioassays

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#### Abstract

Anthropic activities such as industrial, agricultural and urban activity can be a source of pollution gradients being proposed as a cause of habitat fragmentation. The Conlara River located in the northeast of San Luis province suffers different environmental pressures along its course, from urban to agro-industrial areas. Our previous work has shown that the presence of environmental stressors in the watershed negatively affects native fish. In this context, the objective of this work was to develop enzymatic endpoints in *Hyalella curvispina* as future biomarkers in evaluations with acute sediment bioassays. Sampling was carried out in four sites along the basin from S1 (upstream) to S4 (downstream). Water and sediment samples were taken from each site to carry out the bioassays on the bioindicator *H. curvispina*. Sediment samples were transferred to the laboratory and subsequently used for acute sediment bioassays (96 h exposure). In the samples, heavy metals and physicochemical parameters were determined. Biochemical parameters such as catalase (CAT), cholinesterase (ChE) and lipid peroxidation (TBARS) were evaluated after 96h of exposure to the sediments. For enzymatic determinations, 500 mg of *H. curvispina* was taken and 500uL of phosphate buffer was added. Then, they were homogenized and centrifuged at 10000 rpm for 12 min. The supernatant was then extracted for enzymatic determinations. Kruskal Wallis no parametric test was used to detect differences among sites. The biomarkers showed no significant differences in ChE ( $p=0,20$ ) and CAT ( $p=0,20$ ) after acute exposure, while in TBARS ( $p=0,10$ ) enzymatic activity showed a gradient from site 1 (minor contamination) towards site 4 (higher contamination), indicating a stressful situation at the impacted site. Although the results showed no significant differences, there was a trend, probably due to the low number of replicates, suggesting an increase in the number of replicates in future test. It should also be noted that the samples were within the protein calibration curve, without the need for prior dilution of the amphipod pool. It is important to mention that these results are the first in semi-arid regions. Finally, although it is necessary to deepen these methodologies applied to native species of semi-arid zones, we reinforce the use of biochemical biomarkers in their ability to provide an early warning system, at the organismal level, before changes occur at the level of ecological organization.

## 9.P-We-030

### The Use of Eugenol as an Anesthetic for *Myloplus rubripinnis* (Characiformes, Serrasalminidae)

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#### Abstract

*Myloplus rubripinnis* is a species of the Serrasalminidae family that is an important food source for the Amazonian people. The species is heavily targeted in artisanal fishing and faces uncertainties regarding the dynamics of its natural populations in the face of anthropogenic environmental changes. An alternative for the conservation of the species and preservation of its natural populations is the adaptation of technologies for *ex-situ* reproduction. To achieve a good management, the first step is establishing an appropriate anesthesia protocol. Eugenol, a phenolic essential oil derived from clove oil, is commonly used as an anesthetic for fish sedation. Therefore, this study aims to evaluate the optimal dosage of eugenol for sedation of *Myloplus rubripinnis* at different stages. The research was conducted with the authorization of the Committee on Ethics in Animal Experimentation (CEUA No. 4751220822). The experiments were performed using 135 specimens. Eugenol was diluted in alcohol (70° GL) in a ratio of 1:9 (eugenol:alcohol). The fish were divided into five groups with nine specimens in triplicate, tested at concentrations of (10; 12.5; 15; 17.5; 20 mg.L<sup>-1</sup> of eugenol). The data were logarithmized based on 10, as they did not meet the tests' assumptions. The difference in the relationship between dosage and induction, and recovery times at each stage was tested with Two-way ANOVA. After the experiments, the specimens were observed for 96 hours, and the survival rate was 100%, indicating a possible absence of short-term lethal effects. The dosage of 20 mg.L<sup>-1</sup> was effective in achieving complete immobilization (surgical stage), which is important for hormonal induction and gamete extraction in *ex-situ* reproduction. At 10 and 12.5 mg.L<sup>-1</sup>, the fish did not progress beyond the stages of deep sedation and narcosis, respectively, which are important for handling and transportation of these animals. Eugenol proved to be effective at a dosage of 20 mg.L<sup>-1</sup> for use with *Myloplus rubripinnis* and can be used for safe handling of these animals.

## 9.P-We-031

### **Anesthetic for Amazonian Ornamental Fish *Baryancistrus xanthellus* (Siluriformes, Loricariidae)**

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#### **Abstract**

*Baryancistrus xanthellus* is a species of the Loricariidae family endemic to the Xingu River basin, widely traded in the aquarium market worldwide. The species is among the ten most exported in Brazil, raising a great demand for care and improvement in handling and transport to prevent stress and physical injury to these animals. The testing of anesthetics in different dosages is essential for improving such care. This study aims to evaluate the best dosage of eugenol for anesthesia in *B. xanthellus*. Animals were captured with SISBIO license number 33558-1, and the study was approved by the Ethics Committee on Animal Use (CEUA -UFPA 112-13). The eugenol was diluted in alcohol (70° GL) in a 1:10 ratio (eugenol:alcohol). The experiments occurred in two distinct periods within two months, using 40 fish. In both assays, 2 mg L<sup>-1</sup> of eugenol was added every 2 minutes, and the stages of anesthesia were recorded. Permutational Analysis of Variance (PERMANOVA) was used to calculate the concentration of eugenol necessary to anesthetize the species, considering the stages of anesthesia and recovery as the dependent variable and the concentrations of eugenol as the independent variable. The time of exposure and animal size was included as co-variables in our analyses. After an observation period of 72 hours post-experiments, the survival rate was 100%, indicating the possible absence of short-term lethal deleterious effects. The specimens reached complete immobilization with a lower dosage in the second trial, and the recovery time was the same in both periods. In the first period, the dosage to reach the immobilization stage was  $21.69 \pm 3.38$  mg.l<sup>-1</sup>, in the second stage the dosage was  $14.50 \pm 3.20$  mg.l<sup>-1</sup>. Eugenol is a natural compound and has been widely used as an anesthetic in fish and other animal species. However, the effects of eugenol on fish that have been subjected to multiple exposures are still unknown. The present study is pioneering by investigating a second exposure to eugenol in fish, which will aid in the management of wild fish species by providing less stress and less harm to the animals.

## 9.P-We-032

### Morphological Damage in the Embryo-Larval Stage of *Rhamdia quelen* (Siluriformes) Exposed to Realistic Cd Concentrations

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#### Abstract

Cadmium (Cd) is an extremely toxic heavy metal, being soluble in water and therefore dangerous for aquatic organisms. To monitor its potential effects, fish in the embryo-larval stage may represent a sensitive and responsive tool to toxicity due to the lack of protection mechanisms and systems in formation. The present study investigated the effects of exposure to different concentrations of Cd on the development of the embryo-larval phase of *Rhamdia quelen*. The concentrations were established based on: 1- the maximum value allowed by Brazilian legislation in surface waters ( $5 \mu\text{g L}^{-1}$ ); 2- a realistic value measured in rivers of Brazil ( $50 \mu\text{g L}^{-1}$ ); 3- a value 10x higher than that recorded in rivers, considering a perspective of increase in the future ( $500 \mu\text{g L}^{-1}$ ). A negative control group was also established in reconstituted water. For the embryotoxicity test, 5 fertilized eggs were allocated in each well of 24-well microplates containing 2.5 ml of the test solutions and incubated in B.O.D at  $25^\circ \text{C}$ . Each microplate presented all experimental groups and 1 ml of the test solutions were replaced every 24h. The effect on the embryo-larval development was evaluated by the set of morphological deformities and by the body area ( $\text{mm}^2$ ). About 30 individuals/group were collected and fixed in 5% formaldehyde at the times of 48 and 96 hpf. Morphometry and morphological deformities were evaluated under a stereomicroscope with an attached camera, and damage was scored and classified into damage categories (axial, fin, craniofacial, and thoracic-abdominal). From ANOVA, it was observed that in 48hpf all tested concentrations induced negative effects on larval development, with a gradual increase in the rate of deformities as the concentration of Cd. The body area was also affected, with a significant reduction at concentrations of 50 and  $500 \mu\text{g L}^{-1}$ . The concentration of  $5 \mu\text{g L}^{-1}$  did not differ statistically from the negative control. For 96hpf time only the control and  $5 \mu\text{g L}^{-1}$  Cd larvae were analyzed, due to the lethality of higher concentrations. The index of deformities and the size of larvae were drastically affected by exposure to the lower Cd concentration. It is concluded that permissive and realistic concentrations of Cd showed severe effects on the early stages of the species, posing a high risk to the maintenance of populations in case of bioavailability.

## 9.P-We-033

### Realistic Cd Concentrations and Their Potential Toxic Effects on *Allium cepa* L.

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#### Abstract

High concentrations of heavy metals, including Cadmium (Cd), are increasingly present in natural environments due to mining and farming activities, posing a serious problem to organisms due to potential toxic and bioaccumulative effects. In this scenario, plants are widely used in bioassays to detect mutagenic and cytogenotoxic alterations, because they present reliable and low-cost results. The present study evaluated whether realistic concentrations of Cd promote phytotoxic and cytogenotoxic damage in *Allium cepa*. The concentrations were established based on: 1- the maximum value allowed by Brazilian legislation in surface waters ( $5 \mu\text{g L}^{-1}$ ); 2- a realistic value measured in rivers of Brazil ( $50 \mu\text{g L}^{-1}$ ); 3- a value 10x higher than that recorded in rivers, considering a perspective of increase in the future ( $500 \mu\text{g L}^{-1}$ ). In addition, a negative control (ultrapure water) and a positive control (methyl methanesulfonate (MMS) at 10 mg/L) were used. Twenty-five seeds of *A. cepa* cv. Baia Periforme (Isla®) were distributed in Gerbox boxes lined with two layers of germiest paper. The test solutions (groups) were pipetted in a volume of 2.5 times the weight of the paper layers, and incubated at 20° C in B.O.D with photoperiod (12/12h). After 72 hours, germination was evaluated, and after 96 hours, the rootlets were collected and fixed for genotoxic analysis. The bioassay was conducted for 240h and, in the end, root length was determined. Germination was significantly lower at all tested Cd concentrations when compared to the negative control. The radicle length did not show any variation between the groups. The Chromosomal Aberrations Index showed a significant increase in cytogenotoxic damage in all concentrations of Cd tested, with  $5 \mu\text{g L}^{-1}$  having the greatest effect. The damage that presented the highest frequency in all concentrations tested was C-metaphase, followed by chromosome breakage. The lowest concentration of Cd caused the highest incidence of adhesions, anaphase bridges, and C-metaphase. It is suggested that the lower concentration of Cd was more easily absorbed by plant cells, making it more available and thus inducing a higher occurrence of chromosome abnormalities. According to the results obtained, the phytotoxic and cytotoxic potential of Cd can be reaffirmed, even at low concentrations.

## 9.P-We-034

### Ecotoxicity of the Insecticide Deltamethrin for Zebrafish (*Danio rerio*)

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#### Abstract

Insecticides can cause serious effects on the fauna and flora not targeted for application, mainly with the contamination of water or other compartments of the environment. Thus, the objective of this study was to evaluate the acute ecotoxicity (LC<sub>50</sub>;48h) of the insecticide deltamethrin, in the Decis® formulation for zebrafish (*Danio rerio*). For this purpose, the fish were acclimatized in a bioassay room with a temperature of 25.0 to 27.0 °C and a photoperiod of 12 hours of light, for ten days, in a 250.0 L box, with a continuous aeration system promoted by a blower. air and fed, once a day, with commercial feed with 28% crude protein. For the quality control of the batch of fish, sensitivity tests were performed (LC<sub>50</sub>; 48h) with the reference substance potassium chloride (KCl), with a purity content of 99.9%. The evaluated concentrations were: 0.01; 0.10; 0.50; 1.00; 1.55 and 2.44 g L<sup>-1</sup> and a control. The 50% lethal concentration (LC<sub>50</sub>;48h) of KCl was 1.14 g L<sup>-1</sup> with a 95% confidence interval between 1.45 and 1.85 g L<sup>-1</sup>. Next, the definitive test was carried out with the insecticide at concentrations of 0.0000585; 0.000234; 0.000937; 0.00375 and 0.015 mg L<sup>-1</sup> (dilution factor 0.25) and a control with three replicates and three fish per concentration and a control (0.0 mgL<sup>-1</sup>), at the maximum density of 1.0 g L<sup>-1</sup>, in static system (3 L of dilution water). The evaluation of mortality and water quality (electrical conductivity (EC), dissolved oxygen (O.D), temperature (T) and hydrogen potential (pH) was performed at 24 and 48 hours after exposure with the removal of dead organisms from the containers. The lethal concentration of 50% (LC<sub>50</sub>;48h) for zebrafish was 0.001011 mg L<sup>-1</sup>, with a lower limit of 0.0006391 mg L<sup>-1</sup> and an upper limit of 0.00160 mg L<sup>-1</sup>, with a coefficient of determination of 0.89 (R<sup>2</sup>). At concentrations 0.0000585 mg L<sup>-1</sup> and 0.000234 mg L<sup>-1</sup>, no mortality occurred. At 0.000937 mg L<sup>-1</sup>, 44.0% occurred and at 0.00375 and 0.015 mg L<sup>-1</sup>, mortality was 100.0%. The evaluated water quality variables were: (T) 25.4 °C; (C.E) 240.0 μS cm<sup>-1</sup>; (O.D) 4.71 mg L<sup>-1</sup> and pH of 8.5, without changing the initial pattern. The insecticide has been classified as extremely toxic to zebrafish. The zebrafish organism (*D. rerio*) was considered extremely sensitive to the insecticide deltamethrin and can be used in biomonitoring programs and environmental risk assessment.

## 9.P-We-035

### Potential Eco-Friendly Antifouling: Influence of Aquatic Macrophytes Extracts on Bacterial Biofilms

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#### Abstract

The initial stage of the biofouling process is represented by bacterial colonization of hard substrates, forming a biofilm that changes the physical and chemical characteristics from the surface. This initial microbial colonization is one of the main conditioning factors for the subsequent fouling of other organisms such as fungi, algae and invertebrates. When associated with made-man surfaces, biofouling can cause several economic losses. This damage can be magnified when it is associated with colonization by invasive species such as the golden mussel *Limnoperna fortunei*, which has been associated with the obstruction of water pipes in hydroelectric plants. A common strategy against these organisms is the use of antifouling paint, which are known to cause environmental damage due to their high toxicity. In this way, there is a growing interest on natural antifouling alternatives, that would display greater biodegradability and lower toxicity against non-target organisms when compared to conventional antifouling. Aquatic macrophytes are plants capable of influencing the growth of bacteria, however, their antifouling activity has not yet been investigated. The aim of this work was to evaluate the ability of aquatic plant extracts to inhibit bacterial biofilm. For this, 25 different aqueous extracts of aquatic macrophytes were prepared, according to the plant species and organ. For each extract, 300 mL of limnic water was mixed with 6 g of dry plant material. Mixing resulted in a 100x stock solution. This was diluted with sterile limnic water to obtain treatments 0, 5, 10, 20 and 40x. To obtain a bacterial consortium, limnic water was collected and filtered (7 µm) to remove phytoplankton and zooplankton organisms, maintaining the bacterial community, without distinction of species. The bacterial consortium resuspended in nutrient broth was used for the inhibition assay (initial bacteria density of 1 x 10<sup>6</sup> bacteria mL<sup>-1</sup>). Out of all 25 extracts tested, 9 extracts showed capacity of biofilm inhibition ≥ 60%, being them: the extracts of *Eichhornia crassipes*, *Salvinia herzogii*, *Schoenoplectus californicus*, *Typha domingensis*. These of the extracts that stood out were the extracts of *E. crassipes* - root and *S. herzogii* - leaf that inhibited ≥ 70% of bacterial biofilm. These results point to the biotechnological potential of aquatic plants for incorporation in antifouling coatings, as long as its ecotoxicological potential is evaluated.



## 9.P-We-036

### Assessment of the Health Status of *Crassostrea palmula* in Five Localities in the Southern Part of Bahía de la Paz BCS

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#### Abstract

*Crassostrea palmula* is a bivalve found in the La Paz Bay, this organisms is not considered a resource, due to its small size, but they can be used as a bioindicator species. The objective of this work was to determine the Health Status of *C. palmula* using the BRI Index (Biomarker Response Index) and to evaluate the use of these oysters as a reliable tool in environmental biomonitoring studies. Thirty adult oysters ( $3.15 \pm 0.58$  cm) were collected in winter and summer (2018 and 2021) in 5 locations with different degrees of pollution, located in the southern part of La Paz Bay. An evaluation of 4 biomarkers was carried out in the samples: Condition Index, oxidative stress (Tbars), AchE inhibition (neurotoxic effect) and genetic damage (frequency of micronuclei). The results indicated that there are significant differences between the sites. The biomarkers presented values similar to the control (oysters kept under controlled conditions in the laboratory), in the oysters collected in the area without the influence of human activities. The BRI values were lower in the organisms collected in areas with urban influence and were related to the concentrations of the metals Cd, Cu, Ni, Pb and V. The season in which the organisms presented severe alterations was in summer due to the fact that metal concentrations increase. According to the data obtained, this bivalve could be used in environmental biomonitoring studies.

## 9.P-We-037

### Determination of the Ecotoxicity of the Insecticide Deltamethrin for the Neotropical Bioindicator Mato Grosso (*Hyphessobrycon eques*)

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#### Abstract

Insecticides can cause various damages to non-target organisms at different trophic levels such as algae, microcrustaceans, molluscs and fish. The objective of this study was to evaluate the acute ecotoxicity (LC50;48h) of the insecticide deltamethrin, in the Decis® formulation for the bioindicator fish Mato Grosso (*Hyphessobrycon eques*). For this purpose, the fish were acclimatized in a bioassay room with a temperature of 25.0 to 27.0 °C and a photoperiod of 12 hours of light, for ten days, in a 250.0 L box, with a continuous aeration system promoted by a blower of air and fed, once a day, with commercial feed with 28% of crude protein. For the quality control of the batch of fish, sensitivity tests were performed (LC50; 48h) with the reference substance potassium chloride (KCl), with a purity content of 99.9%. The evaluated concentrations were: 0.10; 0.56; 1.00; 1.56; 2.44 and 3.00 g L<sup>-1</sup> and a control with a confidence interval. Next, a definitive test was carried out with the insecticide at concentrations of 0.0000585; 0.000234; 0.000937; 0.00375 and 0.015 mg L<sup>-1</sup> (dilution factor 0.25 and a control with three replicates and three fish per concentration and a control (0.0 mgL<sup>-1</sup>), at the maximum density of 1.0 g L<sup>-1</sup>, in a static system (3 L of dilution water).

The evaluation of mortality and water quality (electrical conductivity (EC), dissolved oxygen (O.D), temperature (T) and hydrogen potential (pH) was performed at 24 and 48 hours after exposure with the removal of dead organisms from the containers. The lethal concentration of 50% (LC50;48h) for Mato Grosso (*H. eques*) was 0.000637 mg L<sup>-1</sup>, with a lower limit of 0.004813 mg L<sup>-1</sup> and an upper limit of 0.0000844 mg L<sup>-1</sup> and determination coefficient of 0.94 (R<sup>2</sup>). At the concentration of 0.0000585 mg L<sup>-1</sup>, no mortality occurred. At 0.000234 mg L<sup>-1</sup> and 0.000937 mg L<sup>-1</sup>, 33.33% mortality occurred; for 0.00375 mg L<sup>-1</sup> occurred 77.78% and at 0.015 mg L<sup>-1</sup> mortality was 100%. The evaluated water quality variables were: (T) 25.6 °C; (C.E) 100.0 µS cm<sup>-1</sup>; (O.D) 2.38 mg L<sup>-1</sup> and pH 7.1, without changing the initial pattern. The insecticide was classified as extremely toxic to the bush (*H. eques*). It is concluded that the mato-grosso fish (*H. eques*) was considered extremely sensitive to the insecticide deltamethrin and can be a bioindicator for environmental monitoring and environmental risk assessment.

## 9.P-We-038

### Response Kinetic of EROD in Pejerrey (*Odontesthes bonariensis*) Exposed to a Model Polycyclic Aromatic Hydrocarbon

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#### Abstract

Cytochrome p450 plays an important role in xenobiotic compounds detoxification. In particular, the induction of the *cyp1a1* gene is regulated by the aryl hydrocarbon receptor (AhR), which is activated by agonist ligands represented by organic aromatic compounds, such as PCBs, dioxins, and polycyclic aromatic hydrocarbon (PAHs). The activity of *cyp1a1* encoded enzyme, the 7-ethoxyresorufin-O-deethylase (EROD), has been successfully used as a biomarker to assess exposure to the mentioned pollutants. The aromatic PAH  $\beta$ -naphthoflavone (BNF) is a proven AhR agonist model. The pejerrey, *Odontesthes bonariensis*, is a native fish from the southern sector of the Del Plata Basin, highly appreciated as a game fish and the quality of its flesh, and very sensitive to aquatic pollution. This study aimed to evaluate the response kinetic of EROD activity in juveniles of *O. bonariensis* exposed for a duration to a given concentration of BNF and followed by a depuration period. Fish were exposed to a concentration of 100  $\mu\text{g/L}$  BNF for 0 (preexposure), 24, 48, 72, and 96 h (exposure period) and then switched to clean water the following 120, 144, 168, and 192 h (depuration period). The assay was run by quintuplicate, under laboratory conditions, and fish were daily fed, 1 h before media renewal. The enzymatic activity was measured by a fluorometric method. The EROD activity was significantly increased compared to the control group (13 fold) at 24 h of exposure. The highest difference (27 fold) was observed at the end of the exposure period (96 h). In the depuration period, the EROD activity begins to decrease at 120 h, becoming significantly lower than the maximum at 144 h. However, it remained higher than the control (9 fold) even at the end of the experiment. We can conclude the EROD activity is quickly induced by BNF and show a strong response in exposed juveniles of *O. bonariensis*. The activity is reduced during the depuration period but the recovery is slow remaining significantly induced 96 h after exposure was retired.

## 9.P-We-039

### **Biomonitoring of the Water Quality of the Piracanjuba River Near the Main Surrounding Cities**

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#### **Abstract**

Water quality has always been one of the main subjects debated between researchers and public agencies, as in recent decades it has been affected due to increased urbanization, the emergence of industries, livestock and plantations. The Piracanjuba River is an important tributary of the Paranaíba River, which is impacted by five major cities along its course. The evaluation of the water quality of this is important, due to the capture of water for urban supply and use for production, in this context, biomonitoring is an efficient method of study. The zebrafish is an animal model usually used in this type of test, due to its favorable characteristics for laboratory management. Thus, the present work aims to evaluate the toxicity of four quadrants (Bela Vista, Piracanjuba, Rio Quente and Água Limpa) in addition to the source, through in vivo assays using zebrafish embryos. During the evaluations, the embryos were characterized as alive, dead, with or without teratogenic alterations, in addition to the evaluation of the heart rate (HR) for a period of 120 hours post-fertilization (hpf). The bioassays with the embryos revealed an increase in the mortality rate from 24 hpf in the Piracanjuba and Água Limpa quadrants ( $p < 0.05$ ), when compared to the control group and other points. In the Rio Quente quadrant, the mortality rate was significantly lower when compared to the other quadrants ( $p < 0.05$ ), but there was no difference in relation to the control group. In the assessment of teratogenesis, no significant differences were found ( $p > 0.05$ ), comparing the quadrants with each other and with the control group. When the HR was evaluated, the embryos from the Água Limpa and Piracanjuba quadrants showed an increase in HR ( $p < 0.05$ ), when compared to the control group and other concentrations. The other quadrants did not show changes in HR when compared to the control group ( $p > 0.05$ ). The low survival rate and the increase in RH in the Piracanjuba and Água Limpa quadrants may be related to the decrease in riparian forest, agricultural exploration and leaching of pollutants in the river bed. The high survival rate in the Rio Quente quadrant is related to the section of the river where the riparian forest is in a better state of preservation. Thus, we can observe that riparian forest has a great impact on improving water quality and can minimize the environmental impact of production.

Keywords: urban supply, embryotoxicity, aquatic toxicology, zebrafish

## 9.P-We-040

### Metabolic Changes In Fish Exposed To Acid Resins Characterized By Metabolomic Analysis

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#### Abstract

Acidic resins are diterpenoids produced by conifers, such as *Pinus*. Such resins are largely released in soils in areas used for planting *Pinus*. The pulp and paper industries, when producing cellulosic pulps, produce large amounts of effluents as by-products, which can contain, on average, from 50 to 250 ug of resins per L. Both the resins released in soils and those produced in effluents can be carried to water bodies, contaminating them. In this study, the possible deleterious effects of abietic acid, one of the acidic resins, on fish (*Oreochromis niloticus*) were evaluated under laboratory conditions, exposed to concentrations in the range of 100 to 150 ug/L. The fish were kept in groups of 10 individuals, in 200L water boxes, fed with commercial feed. Samples of blood, liver, gonads and gills were collected after a period of 7 days of exposure. The blood plasma obtained was analyzed for TGO, TGP and alkaline phosphatase. Gonads, liver and gills were homogenized, extracted with solvent (MTBE) and analyzed by GC/MS-TOF, in addition to the analysis of total carbohydrates and lipids. The results obtained indicated significant changes in the total concentration of lipids and carbohydrates, as well as changes in the profile of lipids (fatty acids and cholesterol) and carbohydrates analyzed by GC. The level of plasma enzymes were greatly altered, with a significant increase in enzyme activity. Abietic acid proved to be toxic to the analyzed fish.

## 9.P-We-041

### Chronic Tests with *Pimephales promelas* and Biomass Differences to Evaluate Water Quality in Negro River

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#### Abstract

*Pimephales promelas* is a fish species sensitive to a wide range of chemicals, and it is used in toxicity testing and research by several environmental regulatory agencies worldwide. Ecotoxicity studies with *Pimephales promelas* (chronic tests) were performed for the baseline studies of the future pulp mill that will be located in the middle zone of Negro river in Uruguay. For that purpose, between October 2020 and October 2022 17 sampling campaigns took place, and a total of 116 integrated water samples (from bottom to surface) were collected between Bonete and Baygorria reservoirs. Water toxicity was assessed through ecotoxicity studies with *Pimephales promelas* (chronic tests). The chronic toxicity tests with *Pimephales promelas* were based on the reference standard EPA-821-R-02-013, short-term methods for estimating the chronic toxicity of effluents and receiver waters to freshwater organisms, where the same control batch was used for each sampling campaign. Also, to compare between sites and campaigns, the difference in biomass with respect of the control was also evaluated (expressed as percentage). It is observed that in general, when the percentage of variation between the biomass of the organisms exposed to a concentration of 100 % with respect to their control is close to or less than -20 % (there is a decrease in biomass), significant differences are observed. Similarly, when the biomass percentage increase is close to or greater than 20 %, significant differences are also observed. In some specific samples, an important mortality of *Pimephales promelas* was observed (ie: April and October 2022). In some cases, mortalities coincided with the presence of cyanobacteria blooms, but other ecotoxicological (water and sediment tests) and potential contaminants analyses complied with the applicable regulations, highlighting the importance of the ecotoxicology analyses and the biological matrix when evaluating water quality in aquatic ecosystems.

# Session 10: Understanding Contamination Threats to Amphibians and Reptiles of Latin America to Promote Their Conservation

10.P-Tu-096

## Genotoxic Effects Induced by the Mixture of Microplastics and Carbendazim in *Hypsiboas pulchellus* Tadpoles (Anura, Hylidae)

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### Abstract

Microplastics (MPs, < 5mm) are ubiquitous pollutants present in all environmental matrices such as air, water, soil, and sediments. MPs pollution has become a worldwide concern in the last decades. To date, scarce information is available on the interaction of MPs and other contaminants like pesticides as well as on their deleterious consequences on aquatic organisms. At the present, the simultaneous exposure of living species to these mixtures is one of the most dangerous threats to the biota. Carbendazim (CBZ) is a systemic fungicide employed in agriculture and veterinary medicines to control fungal diseases and as a preservative in textile, papermaking and leather industries, among others. It is known that its extensive and repeated use induces several toxic effects. The present study was designed to investigate the genotoxic effects exerted by MPs (polyethylene, size 34-50  $\mu\text{m}$ ) in combination with CBZ (Fungoxan<sup>®</sup> 50% CBZ) on *Hypsiboas pulchellus* tadpoles (stage 36) after an acute treatment (96 h). Experiments were performed employing realistic concentrations of 60 mg/L of MPs and 5 mg/L of CBZ and negative (dechlorinated tap water) and positive control (40 mg/L cyclophosphamide) were conducted and run simultaneously. The micronuclei (MN) frequencies and the comet assay were selected as endpoints. Tadpoles exposed to 60 mg/L of MPs or 5 mg/L CBZ did not showed a significant increase in MN frequency regarding control values ( $P > 0.05$ ). Oppositely, the mixture of MPs plus CBZ increased the MN frequency ( $P > 0.01$ ) indicating a synergistic pattern. After treatment, comet assay revealed a significant increase of the genetic damage index (GDI) regardless of the compound assayed ( $P > 0.01$ ). The combination of MPs plus CBZ did not induce significant increases in the GDI ( $P < 0.05$ ) in regard to control values. This study represents the first experimental data of genotoxicity exerted by MPs of an amphibian species native to Argentina as *H. pulchellus*. Similarly, our results showed the induction of MN and primary DNA breaks by this fungicide on the species. The results contribute valuable baseline data toward realistic exposure scenarios where amphibians are exposed to these chemicals and their mixtures.

## 10.P-Tu-097

### Effects of Neonicotinoid and Anthranilic Diamide Insecticides on the Metamorphosis of the Common Toad *Rhinella Arenarum* Individually and in Pairwise Combinations

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#### Abstract

In the agricultural region of the Argentine Pampas, the neonicotinoids (NEO) and anthranilic diamides (AD) insecticide families are widely used on crops such as soybeans and corn. NEO act by binding to acetylcholine receptors in target organisms, causing a nervous disorder that can cause paralysis or death. AD are the latest generation of insecticides that act by binding to ryanodine receptors (RyR), altering calcium release and disrupting coordinated muscle contraction in target organisms. Both insecticides are relatively persistent and mobile in the environment, reaching aquatic environments by lixiviation and runoff. The objective of the present study was to evaluate the impact of pairwise combinations of the NEOs, thiamethoxam (THIA) and imidacloprid (IMI) and the AD, chlorantraniliprole (CHLO), in their commercial formulations actara 25%, confidor 20% and coragen 20%, respectively, on the metamorphosis of the common toad *Rhinella arenarum*. Tadpoles at stages 34-37 (E34-37) (according to Gosner 1960) were exposed to the nominal concentration of 50 µg/L of THIA, IMI and CHLO, individually and pairwise combinations of 25 ug/L of each pesticides : THIA-IMI, THIA-CHLO, and IMI-CHLO, until completion of metamorphosis in a semistatic system. The effect on metamorphosis development was determined by evaluating the time required for 50% of individuals to reach stage 39 (S39), stage 42 (S42) or to complete metamorphosis (MET) and the proportion of individuals reaching each stage. Body weight and length, as well as the jumping ability of the individuals that reached the end of development were also evaluated. The effects observed occurred principally between S39 and S42. All insecticide exposures, individually and in pairwise combinations, caused a similar delay in development and a decrease in metamorphic success without affecting the weight and length of the metamorphs. The exception was the exposure to a mixture TIA-IMI, where metamorphic success where similar to controls but metamorphs had significantly lower weights. Evaluation of the jumping ability of the metamorphs revealed that individual exposures to CHLO and THIA and those exposed to the pairwise combinations of THIA-IMI and CHLO-THIA significantly reduced jumping ability compared to the control group and the group exposed to IMI.



## 10.P-Tu-098

### Toxicological Evaluation in Sediments From a Section of the Bebedero Basin Using *Rhinella arenarum* and *Odontophrynus americanus* (=Occidentalis) As Bioindicator Species

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#### Abstract

Previous studies on the Bebedero basin have shown through physicochemical and biological indices that, in final sections, the quality of the water is unacceptable and some environmental stressors exceed the permitted values. However, there are no studies on the toxicological status of the basin, making it impossible to know the risk to mountain aquatic ecosystems and human health. Within the basin, the San Luis River (RSL) is of major importance since it crosses the cities of Juana Koslay and San Luis, being used as a water and tourist resource by the population. In this context, the objective of this study was to characterize the toxicity of sediments, of a section of the Bebedero basin with anthropic impact, in tadpoles of two local species (*Rhinella arenarum* and *Odontophrynus americanus*), evaluating and comparing lethal and sublethal effects. Sediment and water sampling was carried out at 5 sites arranged from upstream (headwaters) to the final surface section of the basin (downstream): Los Molles river-S1; Las Chacras stream: after the Potrero de los Funes reservoir-S2; RSL after Juana Koslay city-S3; RSL in recreational activities area-S4; and RSL after industrial discharge and sewage effluents-S5. The samplings were carried out during the low water period and 10 physicochemical parameters were evaluated for the subsequent calculation of the water quality index (WQI) and 5 metals of toxicological interest. Toxicity bioassays were carried out with sediments during 10 days of exposure, with feeding and renewal of the test water. Mortality was recorded during exchanges and at the end of the bioassay tadpoles were weighed, photographed and sacrificed to determine individual and cytogenetic biomarkers (micronuclei-MNs) in both species. The results showed that there are differences between the species: *R. arenarum* had mortality of 5%, 15%, 20% and 10% in S2, S3, S4 and S5, respectively; while no alterations were observed in the biomarkers evaluated. On the other hand, *O. americanus* showed mortality of 8.3% and 16%, in S4 and S5, respectively; and observed increases in MNs in S4 and S5, compared to S1. Finally, the results according to the WQI show that the water quality is "excellent" in S1, "intermediate" in S2, "admissible" in S3 and S4; while it is "inadmissible" in S5. From these results we can infer toxicological risk in the final stretch of the basin for the integrity of aquatic ecosystems and for human health.

## 10.P-Tu-099

### Xenometabolomics in Plasma of Anurans Inhabiting Sites Affected By Sewage Discharges

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#### Abstract

A thorough assessment of organisms exposed to complex chemical mixtures such as sewage effluents can benefit from analytical non-target approaches, which do not require pre-selection of target compounds. Most studies in the field of biomonitoring of aquatic organisms use invasive techniques that sacrifice them. In recent years, priority has been given to techniques that aimed to replace, reduce and/or refine practices (the 3Rs approach). In this regard, biofluid extraction, such as plasma, is a less invasive approach because individuals can be safely returned to their environment after sample collection. The aim of this study was to perform a non-target xenometabolomics study on the plasma of adult individuals of *Leptodactylus luctator* (Anura: Leptodactylidae) inhabiting wastewater effluent channels. Two sites with apparently different levels of anthropogenic disturbance were selected in northeastern Buenos Aires Province (Argentina): a reference site in Punta Indio and a site with direct discharge from a secondary wastewater treatment plant in Chascomús. Adult individuals of both sexes (n = 12) were collected at both sites in December 2022. Individuals were anesthetized and blood samples were collected through the femoral vein with heparinized syringes and then centrifuged at 7000 rpm for 15 min to collect plasma. Each plasma sample was divided into two replicates and deuterated atrazine was added (final concentration in plasma 50 µg/L). Proteins were then precipitated with acetonitrile (3:1, v/v) and centrifuged at 7000 rpm for 15 min. Procedural controls were prepared in duplicate with 0.7% saline solution. Instrumental analysis was performed using LC-MS Orbitrap, operating both in positive and negative mode. Preliminary results indicate a clear separation of individuals from both sites. This study will provide valuable information on the health status of the organisms, as plasma contains many endogenous metabolites that may be altered by exposure to xenobiotics, as well as xenobiotics and their metabolites that may be accumulated and distributed to other tissues in exposed organisms. This is the first non-target xenometabolomics study in adult anurans environmentally exposed to various contaminants.

## 10.P-Tu-100

### Trace Metals in Juvenile *Chelonia mydas* from Buenos Aires Province, Argentina

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#### Abstract

Green turtle (*Chelonia mydas*) is one of the three endangered species of sea turtles that are regularly found in the coastal waters of Argentina. Juvenile specimens find feeding areas in argentine estuarine and coastal areas, reporting an omnivorous diet, where the consumption of jellyfish is predominant. The presence of chemical residues, including trace metals, in sea turtles tissues is of global concern. Therefore, the aim of the study was to determine the concentrations of cadmium (Cd), lead (Pb), chromium (Cr) and manganese (Mn) in liver, kidney and muscle of *C. mydas* from the province of Buenos Aires, Argentina. Seven green turtle specimens were found stranded or entangled on the north coast of the province. All turtles were juveniles and the average curved carapace length was  $35.5 \pm 2.18$  cm. An acid digestion of the samples was performed and the quantification of metals was carried out by Flame Atomic Absorption Spectrometry for Cd, Cr and Mn, and with Graphite Furnace Atomic Absorption Spectrometry for Pb. The limit of detection (LOD) was  $0.02 \mu\text{g/g}$  for Cd, Cr and Mn, and  $0.004 \mu\text{g/g}$  for Pb. Data is expressed in wet weight. The tissue distribution of Cd was: kidney (mean $\pm$ SD=  $16.29 \pm 5.9 \mu\text{g/g}$ ) > liver ( $4.52 \pm 1.9 \mu\text{g/g}$ ) > muscle (< $0.02$  for all turtles). Lead concentrations ranged from  $0.03$ - $1.3 \mu\text{g/g}$  in kidney,  $0.46$ - $1.66 \mu\text{g/g}$  in liver and < $0.004$ - $1.7 \mu\text{g/g}$  in muscle. Those of Cr were similar between liver ( $0.58 \pm 0.21 \mu\text{g/g}$ ) and muscle ( $0.41 \pm 0.31 \mu\text{g/g}$ ). Manganese levels ranged < $0.02$ -  $0.055 \mu\text{g/g}$  in liver, and were below LOD in muscle and kidney. This is the first report of trace metal concentrations in internal tissues of *C. mydas* in Argentina. This information provides an essential background to conduct further studies, to increase knowledge for its conservation.

## 10.P-Tu-101

### Lipid Assessment of *Rhinella arenarum* Larvae Exposed To Copper Oxide Nanoparticles And $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ Salt

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#### Abstract

Copper oxide nanoparticles (CuO-NPs) are increasingly used in various industry sectors for their unique properties, which make these materials very promising and efficient, but are also responsible for their higher reactivity towards other molecules. These reasons make it necessary to know its possible toxic effects. Aquatic environments are the main final disposal of these NPs that can reach them during their life cycle (production, use and disposal) and they have generated concerns related to the conservation of more vulnerable groups, such as amphibians. The aim of this work was to evaluate the toxicity of the CuO-NPs and its respective salt ( $\text{CuCl}_2 \cdot \text{H}_2\text{O}$ ) in the lipid content (neutral lipids, fatty acids and phospholipids) of larvae of the common South American toad, *Rhinella arenarum*. In this sense, standardized bioassays were performed up to 21 days of exposure to different sublethal concentrations: 5 and 10 mg/L CuO-NPs; 0.1 and 0.5 mg/L  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ . The bioassays were semi-static with replacement of the solutions every 48 h, treatments were done by triplicate, and a control group without exposure was simultaneously maintained. After exposure, the pool of individuals of each treatment was weighted, then they were homogenized and the lipids were extracted by a mixture of solvents (chloroform:methanol 2:1). The phospholipids quantification was performed by Rousser et al. (1970) method, neutral lipids and free fatty acids were separated by thin layer chromatography (TLC) on silica gel plates and detected with iodine vapors and sulphuric acid treatment, and triglyceride levels were preliminary determined by an enzymatic reactions kit (TG color GPO/PAP AA, Wiener lab, Argentina). The results show that the weight of individuals exposed to 0.5 mg/L salt was significantly lower than 0.1 mg/L salt. Although there were no significant differences in the amount of phospholipids between the control and the exposed treatments, a decreased tendency was observed at highest concentration of salt (0.5 mg/L). As phospholipids are the major lipids in biological membranes, this decrease may be due to delayed development in these exposed individuals, which is also related to the lower weight observed. The TLC separation of lipids shows no significant differences depending on the concentration of salt or nanomaterials, in the different bands of all acylglycerols and free fatty acids, between the treatments and the control.

## 10.P-Tu-102

### Gill and Liver Biochemical Biomarkers of *Aquarana catesbeiana* Tadpoles After Exposure to Atrazine

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#### Abstract

Atrazine (ATZ), a widely used herbicide in Brazil, has been detected in surface waters causing toxic effects to non-target organisms. Amphibians, specifically those of the Anura order, constitute one of the vertebrate classes most severely impacted by agricultural activities because their larval stage is restricted to water, maintaining direct contact to contaminants. Therefore, the present study investigated the effects of a 24-h exposure to ATZ on biochemical biomarkers of American bullfrog tadpoles (*Aquarana catesbeiana*). For the experiment, *A. catesbeiana* tadpoles (Gosner stage 25-30) were obtained from a frog farm, acclimated in lab conditions for 7 days and then divided into two groups (n = 8 aquaria/group; 2 tadpoles/aquaria) and exposed for 24 h to: dechlorinated tap water with ethanol 0.01% (CTR) and dechlorinated tap water with 10  $\mu\text{g}\cdot\text{L}^{-1}$  of ATZ dissolved in ethanol 0.01% (ATZ). Then, the tadpoles were anesthetized and euthanized by medullary section to remove the gills and liver for the analysis of glutathione (GSH) content, occurrence of lipoperoxidation (LPO) and enzymatic activities of superoxide dismutase (SOD) and catalase (CAT). A significant decrease in GSH content was found in the liver of ATZ tadpoles compared to the CTR group. Conversely, the GSH content in the gills of ATZ tadpoles was significantly higher than in CTR animals. However, there were no significant differences in SOD and CAT activity in the liver and gills of both groups. Animals exposed to ATZ exhibited a significant increase in LPO in their gills compared to the CTR group. On the other hand, no significant differences were observed in LPO in the liver. In conclusion, this study provides evidence that short-term exposure to atrazine at a concentration of 10  $\mu\text{g}\cdot\text{L}^{-1}$  can elicit significant effects on biochemical biomarkers, especially in the gills, an important tissue that is often neglected in ecotoxicology studies using tadpoles. Therefore, this research provides valuable information on the potential effects of atrazine exposure on bullfrog tadpoles and emphasizes the need for continued research and regulation of pesticide use to protect the health and well-being of aquatic organisms and the ecosystems they inhabit.

## 10.P-Tu-103

### Skin and Liver Morphological Biomarkers in Frogs as a Tool for Environmental Monitoring

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#### Abstract

Morphological and histological features are good biomarkers of intermediate responses to contaminants, because they integrate biochemical and physiological responses. Morphological changes in organs and tissues can be defensive or compensatory responses to maintain tissue and organ functions against degenerative changes. However, few studies have evaluated how urbanization have changed multiple skin features of tropical frogs. Here, we tested how urbanization affected multiple morphological biomarkers of skin and liver in adults of *Leptodactylus podicipinus* (Anura: Leptodactylidae) collected in rural and urban sites. We compared the thickness of the epidermis (EP), stratum spongiosum (SS), and the Eberth-Kastschenko layer (EK) between populations. We also quantified melanomacrophage (MM) area, and area and volume of hepatocytes. We then used simple t-tests to test the effect of site (urban vs rural) on each variable separately. All layers were smaller in urban animals, while the total thickness and the stratum compactum did not differ between urban and rural sites. Our results demonstrate that environmental changes associated with urbanization can consistently change skin histometric characteristics. As a result, morphofunctional characteristics of the skin can be altered, including gas exchange during respiration. Therefore, the skin can be used as a biomarker in environmental monitoring. There was an increase in the area occupied by melanomacrophages and a decrease in the area and volume of hepatocytes in the urban population. Increases in MM area can be associated with damages in the hepatic tissue resulting from oxidative stress and hepatocyte morphologic alterations, demonstrating possible cytotoxic effects experienced by urban frogs. In summary, morphological biomarkers are important to infer about the overall health of frogs in the face of environmental stressors.

## 10.P-Tu-104

### Third Update of the Ecotoxicology of Amphibians and Reptiles textbook - Recent Advancements in Amphibian and Reptile Ecotoxicology

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#### Abstract

Prior to 2000 and the first edition of the book *Ecotoxicology of Amphibians and Reptiles* by Sparling et al., ecotoxicological research focusing on amphibians and reptiles was very limited relative to that on other vertebrate classes. In 2010, the second edition of the book reported significant increases in number of studies and on the expanded range of species models. However, the editors reiterated the need for additional information and a greater understanding of exposures and responses of these two classes of organisms. Members of SETAC's Interest Group on Ecotoxicology of Amphibian and Reptiles are now seeking to update the book and publish a third edition. For the second edition, Sparling et al. performed a literature review covering the years 1996-2008 showing that ten years after publication of the first edition, amphibians and reptiles remained underrepresented in the ecotoxicology literature relative to other vertebrates. For the updated edition, we conducted a literature review for the years 2006-2022. Although the number of citations increased tremendously since the second edition, preliminary results show that amphibians and reptiles still remain less represented in the literature. Of the 127,894 citations examined (7-fold increase in comparison to 1996-2008), 57% were for fish, 23% for mammals, and 13% for birds. Since the second edition, the number of citations related to amphibians increased from 3.8% to 4%. For reptiles, the relative growth was more important, the number of citations increasing from 0.8% to 3%. Nevertheless, the number of contaminant-related publications for amphibians and reptiles was stable or somewhat decreased. Further evaluations on topics such as main contaminant classes, annual publication numbers, most studied taxa geographical representation and emerging stressors such as climate change and diseases, are ongoing and detailed results will be presented at the conference.

## 10.P-Tu-105

### Effects of Atmospheric Particulate Matter Exposure on Bullfrog Tadpoles

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#### Abstract

Anthropogenic activities such as industrial processes, biomass burning and the increased use of motor vehicles are some of the causes of the increase in atmospheric pollution by atmospheric particulate matter (APM). Being the APM responsible for some types of damage to the soil and water, modifying the ecosystem and thus being able to cause damage of different levels in the organisms that live there. Monitoring, evaluating and recognising the effects that APM cause on aquatic organisms, helps to predict their adverse effects on the environment, therefore the objective of this work is to evaluate how the effects of APM affect bullfrog tadpoles, *Lithobates catesbeianus* by biomarkers biochemicals in different organs, liver, muscle, kidney and brain. For this, the tadpoles were exposed to APM (1mg/L, 96h) and then their organs were collected for analysis of protein carbonyl concentration (PCO), lipoperoxidation (LPO), metallothionein (MT) and acetylcholinesterase (AChE). The results obtained from the analyses of protein and LPO in the liver, muscle and kidney did not show any significant difference in relation to the control and exposed groups. However, in relation to PCO, there was an increase of 66% in the liver, of the exposed group, which may indicate oxidative stress, due to oxidation catalysed by metals, which are components of APM. With regard to MT, there was a decrease between the control and the exposed groups, which may indicate a lack of bioavailability of metals to bind with this protein, in compensation AChE increased in both organs, brain and muscle. Thus, the results contribute to the understanding that the action of APM has a deleterious effect on the aquatic environment, negatively affecting the bullfrog tadpoles, in different ways and levels in relation to the analysed organs.



## Male-Transmitted Transgenerational Effects of the Herbicide Linuron on DNA Methylation Profiles in *Xenopus tropicalis* Brain and Testis

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### Abstract

Human activities are driving the ongoing loss of biodiversity, and amphibians are among the most threatened groups. The herbicide linuron has been shown to cause endocrine disrupting effects in *Xenopus tropicalis* frogs, and these effects can be transmitted across generations, even to offspring that were never exposed to the contaminant. However, the mechanisms underlying these transgenerational effects require further investigation. Here, we conducted a study examining the transgenerational effects of linuron on DNA methylation patterns in the brain and testis of *X. tropicalis*. Tadpoles were exposed to an environmentally relevant concentration of linuron during development (45 µg/L) until metamorphosis, and adult males were then mated with naïve females to obtain the F1 generation frogs. Adult males from linuron lineage F1 were mated with control F1 females to obtain F2 generation and follow the paternally inherited transgenerational effects. We used reduced representation bisulfite sequencing (RRBS) to assess DNA methylation patterns in the brain and testis of the adult male F2 generation. Our analysis revealed numerous differentially methylated regions (DMRs) in the brain (3060 DMRs) and testis (2551 DMRs). Gene sets were over-represented in pathways related to synaptic plasticity in the brain and epigenetic regulation in the testis. We found that ancestral linuron exposure caused DMRs in genes important for DNA methylation (*dnmt3a*), somatotropic (*gh1*, *irs2*, *igfbp4*, *igfbp5*), thyrotropic (*trhr3*, *trhde*, *dio1*, *tg*), and GnRH signalling (*kiss2*, *prkag2*, *prkar1b*) in the brain of F2 adult males. In the testis, DMRs were observed in genes essential for spermatogenesis, meiosis, and germ cell development (*piwil1*, *mael*, *spo11*, *ddx4*) as well as DNA methylation (*dnmt3a*, *mbd2*) and histone modifications (*ep300*, *elp3*, *kat5* and *kat14*). These findings are consistent with previously observed phenotypical alterations and suggest that developmental exposure to linuron can cause transgenerational alterations in DNA methylation patterns that affect growth, metabolism, reproductive function, and epigenetic regulation in amphibians. Our study contributes to the understanding of potential mechanisms underlying transgenerational effects of pollution in amphibians and emphasizes the need for further research to elucidate the long-term effects of environmental contaminants on wildlife populations.

## 10.P-Tu-107

### Biomarkers in Neotropical Anurans of Two Species From Riparian Forest Fragments With Different Levels of Conservation

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#### Abstract

Surface runoff is known to be the main route of pesticide transport from agricultural fields to surface waters, and the lack of riparian vegetation favors the large-scale transport of compounds into water bodies. Moreover, the availability of pesticides in water can be influenced by environmental conditions and by the complex mixtures containing different compounds in the same place, which potentially increase the effects on aquatic biota. Since amphibians are highly sensitive to different pollutants and depend on water for their reproduction and development, this study evaluated the health status of anurans of two species (*Proceratophrys avelinoi* and *Dendropsophus nanus*) from riparian forest fragments (Tibagi River basin) surrounded by agricultural fields in the North of Paraná State, Brazil. These forest fragments present different integrity levels where adult anurans were collected for the analyzes of biochemical, neurotoxic and genotoxic biomarkers. Three areas were selected for each species, each one classified into high, moderate and low levels of integrity. The concentration of the non-enzymatic antioxidant glutathione in the liver of *D. nanus* differed between high and low integrity sites. The glutathione-S-transferase activity in the liver of *P. avelinoi* showed higher activity at the low integrity site. The occurrence of lipid peroxidation in the liver of *D. nanus* indicates a difference between the high and moderate integrity areas when compared with the low integrity area. Acetylcholinesterase activity in muscle and brain of both species showed a difference between the high and moderate integrity areas when compared to the low integrity area in muscle tissue. Individuals of *P. avelinoi* from the low integrity area showed higher occurrence of DNA damage while individuals of *D. nanus* from the moderate integrity site showed lower occurrence of DNA damage. A higher number of erythrocyte nuclear abnormalities were observed in specimens of *D. nanus* from the low integrity site. All these biomarkers depicted significant alterations in at least one of the studied populations from the moderate and low integrity sites, showing that the characteristics of the environment directly influence the health status of these anurans. Thus, the use of amphibians as bioindicators for environmental monitoring may be efficient in identifying the effects of habitat fragmentation and pollution in aquatic environments, water quality and the preservation of biodiversity.

## Biomarkers in Sea Turtles: What do They Indicate? A Systematic Review

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### Abstract

Sea turtles are charismatic and threatened vertebrates. Population trends for all species for which data is available are decreasing, posing a risk of extinction for these organisms. Pollution is considered one of the main threats, and understanding its effects, including at the molecular level, is a conservation priority. Biomarkers can signal early exposure and possible damage caused by contaminants; therefore, they represent an important tool for environmental risk assessment. This systematic review aimed to identify biomarkers and studies on their associations with contaminants in sea turtles. We searched for articles in six databases (Web of Science, Scopus, Pubmed, Embase, SciELO and Lilacs) and Google Scholar, obtaining 832 peer-reviewed research articles. After removing duplicate records and conducting two screening steps using selection criteria, we retained 78 studies for subsequent analysis. Most studies have been published in the last 10 years and the most researched species were *Chelonia mydas* and *Caretta caretta*. All species, except for *Natator depressus*, have been studied. Blood was the main biological material used for biomarker analysis, followed by skin cells and liver. The biomarkers were related to biotransformation, cito/geno/imuno/neurotoxicity, endocrine disruption, kidney and liver damages, metal exposure and transport, carbohydrate metabolism, muscle metabolism, oxidative stress, and general health parameters. Among contaminants, organic xenobiotics were the most studied, and many researchers focused on polycyclic aromatic hydrocarbons and organochlorines compounds. The biomonitoring studies established a baseline for some biomarkers in pristine areas in Costa Rica and Brazil and showed that contaminants may have harmed the health status of sea turtles in important nesting areas in Mexico and Colombia. The studies also revealed that environmental pollutants might be involved with the development of fibropapillomatosis in sea turtles sampled in Brazil. Finally, our review identified gaps in the current state of knowledge about biomarkers evaluated in sea turtles, proposed guidelines for future research efforts (including biomonitoring studies), and suggested public policies aimed at the conservation of sea turtles.

## 10.P-Tu-109

### Effects of Haloxyfop p-methyl and its Formulated KATANA 54® on *Ceratophrys ornata* Larvae

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#### Abstract

The amphibians are characterized by having a critical aquatic life phase where differentiation processes and development of cells, organs and systems occur. The use of pesticides is one of the main hypotheses explaining the global decline of amphibian populations. In agricultural practice, pesticides are used as commercial formulations (CF). These CFs consist of combinations of one or more active ingredients (a.i.) and other substances that are added to improve dilution, stability, absorption and action of the a.i. In this work, we compared the lethal and sublethal effects of haloxyfop P-methyl (HPME) and its CF KATANA54® on larvae of *Ceratophrys ornata*. The experimental design consisted of exposing organisms individually in 100 ml glass chambers. Ten concentrations were selected in the range of 0.01 and 7.00 mg.L<sup>-1</sup>. The tests included 20 replicates per concentration and 2 negative control groups: (1) test water and (2) dilution control, containing the maximum concentration of solvent used in the dilutions (0.1% acetone). Mortality, swimming alterations and effects on growth and morphology were evaluated as endpoints. The results indicate that there are no significant differences (p-value <0.05) between CF toxicity and a.i. when assessing both mortality and swimming alterations (LC50 at 96 h of 7.16 mg.L<sup>-1</sup> and EC50 at 96 h of 0.43 mg.L<sup>-1</sup> vs. LC50 at 96 h of 8.79 mg.L<sup>-1</sup> and EC50 at 96 h of 0.59 mg.L<sup>-1</sup>, respectively). To analyze the effects on growth, an ANOVA (p-value <0.05) followed by Dunnett's test was performed, and a significant difference was observed between the total length of the exposed organisms and that of the controls after 96 h. Morphological abnormalities were found (in the body: ventrally concave body, asymmetric body, hydrops or several edema; and in the caudal fin: tail flexure, curvature, breakage, and folding) depending on the concentration of HPME, but without differences between a.i. vs CF; indicating that for each unit of mg.L<sup>-1</sup> that our concentration increases, we have a probability twice as high of finding abnormalities in the exposed larvae. We can conclude that CF does not present a differential toxicity to a.i. in the endpoints evaluated.

## 10.P-Tu-110

### Evaluation of Environmental Quality Using *Boana pulchella* Larvae in Sediment and Field Bioassays

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#### Abstract

Sediment evaluations often require multiple lines of evidence to determine their dangerousness, one of which is toxicity bioassays in the laboratory, which have limitations when extrapolating results to more complex study systems (ecosystems) or making predictions at higher ecological levels. Another approach is in situ evaluations of representative organisms from these ecosystems. Using both lines of evidence allows for comparisons and provides information that corroborates predictions from one level to another and grants new elements to validate laboratory bioassays. The objective of this study was to analyze the effects on *Boana pulchella* larvae using two approaches: laboratory bioassays, evaluating larvae exposed to sediment samples, and field studies with larvae obtained from the same sites. Sediment samples and larvae were collected from sites with a prevalence of agricultural and industrial land use and a reference site. Larvae were exposed to complete sediments using standardized protocols, analyzing mortality, growth inhibition, development, and frequency of external abnormalities. Field larvae were measured, weighed, and preserved to analyze the presence of external and internal abnormalities and calculate the hepatosomatic index. Data were analyzed using ANOVA-Dunnnett and Kruskal-Wallis ( $\alpha$ : 0.05). From the bioassay results, it was observed that the industrial site presented the most toxic sediments, causing significant effects in mortality, weight, and prevalence of abnormalities in the larvae. As for the agricultural site, only significant differences were observed in the prevalence of abnormalities. When comparing these results with those obtained in field larvae, it can be observed that both agricultural and industrial sites had significantly higher hepatosomatic indices and high prevalence of internal abnormalities. On the other hand, the prevalence of external abnormalities was 99% for the industrial site, 41% for the agricultural site, and 2.5% for the reference site. These results are congruent to the effects observed in the bioassays, demonstrating the sensitivity of the larvae in response to pollution associated with industrial and, to a lesser extent, agricultural use. This study provides elements to consider field and laboratory studies with anuran larvae as a useful tool that provides relevant and complementary information applicable to the diagnosis of complex local environmental problems.

## 10.P-Tu-111

### Effects of Anthropogenic Disturbance on White Blood Cell Differential Count of *Rhinella Arenarum* at the Limay-Neuquén-Negro River Basins

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#### Abstract

The main productive activities in North Patagonia are oil and gas exploitation and fruit culture. Such activities may generate pollutants that can reach natural and artificial watercourses, affecting aquatic life. In this context, hematologic biomarkers are relevant to assess health risks and environmental exposure to pollutants. Also, they are important for the development of measures that serve as early warning signals in polluted areas. The aim of our work was to evaluate leukocyte profile in adult individuals of *Rhinella arenarum* in aquatic environments affected by different anthropic activities. 10 sites were selected, 2 reference sites with low anthropic impact and 7 with different types and magnitudes of productive activity, among which is fruit culture production, oil and gas exploitation, mixed activities and urbanization. At each site, until 15 individuals were collected and blood was extracted from the femoral vein. Smears were prepared on clean slides, fixed, and stained with May Grönwald/Giemsa dye. The stained blood films were examined with an optic microscope under 1000 X with oil immersion for the identification and counting of leukocytes. At least 100 cells were counted or 150 fields were examined. Statistical analysis on cell type frequencies was carried out by the non-parametric Kruskal-Wallis test. Lymphocytes showed the highest frequencies (59,9%), followed by neutrophils (20,6%), and in lower proportion basophils (8,5%), eosinophils (5,5%) and monocytes (5,3%). The *R. arenarum* individuals inhabiting urbanized sites presented significant differences in the proportions of basophils and monocytes with respect to those inhabiting reference, petrochemical and fruit culture sites. Adults from urbanized and mixed sites presented less proportions of lymphocytes and differed statistically respect to petrochemical sites. No significant differences were found for eosinophil and neutrophil proportions. Our results suggest that individuals from urbanized sites may be exposed to greater amounts of xenobiotics, due to the high proportion of basophils and monocytes. On the other hand, reduction in lymphocyte counts has been reported associated with an increase in glucocorticoids in plasma, which may result from the stress situation of individuals. Thus, the reduced lymphocyte proportion in white blood cell differential count would result in a good biomarker of stress in the common toad adults from Río Negro, Limay and Neuquén basin.

# Session 12: Tropical Ecosystem Health Assessment: Effects of Contaminants on Marine and Estuarine Ecosystems

12.P-Tu-112

## Differential Transcription Analysis of Oysters *Crassostrea rhizophorae*: Environmental Contamination Reveals Potential Biomarker Candidates

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### Abstract

The native mangrove oyster *Crassostrea rhizophorae* can be found along the entire Brazilian coast. Like several other mollusk bivalves, *C. rhizophorae* has biological mechanisms that allow it to tolerate the pollution found in Brazilian estuaries and mangroves due to anthropogenic and industrial activities. Therefore, it is important to understand the biochemical and molecular mechanisms by which the species responds to the environment changes for use in aquaculture and ecotoxicology. To identify possible metabolic pathways affected by pollution, this study aims to analyze and validate transcriptomic data from a *C. rhizophorae* population that has been chronically exposed to sanitary sewage discharges in the environment. Oysters were collected in March 2019 at two sites in Florianópolis: a polluted and a reference site, and the differential transcriptional profile was evaluated in gills by Paired-end strand-specific RNA-seq analysis. Upregulated annotated genes found are involved in oxidation-reduction, superoxide metabolic, reactive oxygen species metabolic, growth processes and lipid metabolism. On the other hand, some cytochrome P450 isoforms and monooxygenases-related genes are among the downregulated genes. Thus, we used qRT-PCR analyzes (n=10) from 11 genes to validate the transcriptome. The expression quantification of each gene was evaluated using the 2<sup>-ΔCq</sup> value, normalized by the cDNA concentration of each sample and relativized by quantification of gene expression in reference samples. Our qRT-PCR results validated the transcriptome, since the upregulated genes like Acyl-CoA-like, Fatty Acid Synthase-like, FABP-like, Kunitz-type protease inhibitor-like, Extracellular Superoxide Dismutase-like and Xanthine Dehydrogenase-like were also upregulated in qRT-PCR analyzes. Meanwhile, the downregulated genes such as Cytochrome P450 1A5-like and Flavin Monooxygenase-like were also downregulated in the qRT-PCR analysis. Further qRT-PCR analysis is being conducted to validate additional metabolic pathways, with the goal of finding and characterizing new pollution biomarkers for the native mangrove oyster, *C. rhizophorae*, in the future.

## 12.P-Tu-113

### **A Temporal Assessment of Anthropogenic Marine Debris on Sandy Beaches from Ecuador's Southern Coast**

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#### **Abstract**

Anthropogenic marine debris (AMD) cause different effects on human health, marine life, economy, and the environment. The AMD can contaminate the shorelines, mangrove areas, sea surface, and beaches. For this reason, monitoring marine debris on shorelines is fundamental to developing public policies and management strategies to reduce the frequency, amount, and type of litter. The purpose of this study was to analyze the composition, abundance, size, and temporal distribution of AMD on three sandy beaches in Guayas province, Ecuador. The field samplings were assessed using transects from December 2018 to February 2020. A total of 12,362 items of AMD were counted in all the sites, and the abundance average was 1.95 macro-litter items/m<sup>2</sup>. Plastic items (91.8 %) were the most common AMD, followed by cloth and wood (1.9 %), while cigarette butts were found exclusively on village beaches. Consequently, the village beaches showed the highest AMD abundance because they are close to fishing communities and small coastal towns. In addition, the AMD abundance also showed a temporal trend, being slightly higher at the beginning of the dry season than in the rainy season. For that, it is urgent to put forward effective solid waste management and environmental education campaigns. Finally, more research is needed to find anthropogenic sources and other factors related to AMD abundance on sandy beaches.



## 12.P-Tu-114

### **Sun Corals a Resistant Invader: Assessment of the Acute Toxicity of Contaminants Associated With Petroleum and Petrochemical Activities on the Species *Tubastraea coccinea***

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#### **Abstract**

The oil and petrochemical industry are an important source of pollution to the marine and coastal environments. Tropical and subtropical regions are particularly susceptible to the environmental impacts from oil spills, including possible effects to reef ecosystem, which are abundant within these areas. Brazil harbors a coral fauna considered unique in the world, which has been suffering from numerous environmental pressures, with many species currently threatened. Corals of the genus *Tubastraea* (Sun corals) are non-indigenous species on the Brazilian coast, which are becoming adapted to the local ecosystems. This study evaluated biological hazards of water soluble fractions (WSF) of fuel oil, marine diesel, samples of spilled oil in the NE, and the Aqueous Film Forming Foam (AFFF)-Agefoam 2133 on the species *Tubastraea coccinea*, through acute toxicity tests. The tests took place in triplicates, in which colonies with 9-30 polyps were exposed to contaminants for 96 hours. Organisms from the controls and most of the test-dilutions presented 100% survival, except for the highest concentrations of substances AFFF-Agefoam 2133, WSF from marine diesel, and the oil sample from the Northeast spill in 2019. The highest concentrations of AFFF and WSF of marine diesel showed significant effects compared to the control, being determined as the lowest-observed-effect concentration (LOEC) and the no-observed-effect concentration (NOEC), which were, 1% and 100% respectively. Other substances (WSF from NE oil slicks and bunker C fuel oil) did not cause significant deleterious and sublethal effects. The study provided the first information on the effects of these compounds on scleractinian corals in Brazil.

## 12.P-Tu-115

### Embryo-Larval Development of the Sea Urchin *Echinometra lucunter* Exposed to the Soluble Fraction of Spilled Oil in Northeastern Brazil

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#### Abstract

Between August 2019 and March 2020, oil slicks appear in more than a thousand Brazilian seashore locations in the Atlantic Ocean. More than 55 marine and coastal Conservation Unities (UC) were affected. One of the UCs reached by the oil slicks was the Environmental Protection Area (EPA) of Costa dos Corais (APACC). The present study evaluated the embryo-larval development of the sea urchin (*Echinometra lucunter*) exposed to the soluble fraction (WSF) of this oil recovered on the Brazilian coast. The oil WSF was quantified and qualified for its composition of metallic elements and polycyclic aromatic hydrocarbons (PAHs). The sea-urchin were exposed for 42 h to the WSF, and at the end of the test, the EC50- 42 h (effective concentration); NOEC (no observed effect concentration); OEC (observed effect concentration) were determine. Of the 16 analyzed PAHs, phenanthrene; anthracene; and dibenz[a,h]anthracene were found above 1 µg/L. The benzo[a]anthracene; benzo[a]pyrene, benzo[b]fluoranthene, chrysene, dibenzo[a,h]anthracene and indeno[1,2,3-c,d]pyrene were above the limits allowed by the Brazilian legislation. Regarding the metallic elements, 7 of the 28 have their concentrations regulated by the Brazilian agency (Cd; Cr; Cu; Ni; Pb; Se; Zn; Au), and, except for Au, all these elements showed concentrations above the legislation. The oil WSF at 100% showed high concentrations of emerging elements (Rb, Ti, Ba, Bi, Mo, Nb, Sn, Sr, V, Y, La, Ce, W, Zr). Compared to the WSF 100%, the fractions 35.01; 45.5; 59.17; and 76.9% caused delayed embryo-larval development. The EC50 – 42 h was 39.45%; the NOEC 0% and the OEC 35.01%. The data obtained showed a high toxic potential of the oil WSF, showing malformations of the embryos in their final stage of pluteus larvae. The oil WSF showed a high concentrations of the PAHs and metals present, which explain the abnormality founded during the embryos development.

## 12.P-Tu-116

### Assessing the Biochemical Biomarkers and the Bioaccumulation of Polycyclic Aromatic Hydrocarbons in Fish Species From a Threatened Marine Ecosystem

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#### Abstract

In 2019/2020, oil slicks appear in more than a thousand Brazilian seashore locations in the Atlantic Ocean, where the oil slicks affect several Unit Conservations (UC), such as the Environmental Protection Area (EPA) of Costa dos Corais (APACC). To assess the potential impacts of the oil slicks in APACC, 2 fish species, representing two trophic guilds, omnivorous and herbivorous, were collected: *Eugerres brasiliensis* (site 1) and *Mugil curema* (site 2), respectively. White muscle and sediment samples were collected for polycyclic aromatic hydrocarbons (PAHs) concentration, the gills and liver samples for TBARs, DNA strand breaks and carbonyl proteins, and the gallbladder for BaP-, Pyrene- and Naphthalene- type metabolites in the bile. The concentrations of PAHs of low molecular weight (LMW), high molecular weight (HMW) and  $\Sigma$ PAHs in sediment samples from site 1 and 2, were 26.3, 16.57, 99.86; and 73.55, 28.18, 44.75 ng g<sup>-1</sup> (dry weight), respectively. These values are below the regulatory limits established in Canada (Canadian Environmental Quality Guidelines) and Brazil agencies (CONAMA 454/2012). The PAHs concentration of LMW, HMW and  $\Sigma$ PAHs in white muscle samples (dry weight) were 26.71 ± 1.72; 63.17 ± 11.48; 89.91 ± 10.7, and 24.28 ± 0.56; 58.07 ± 10.46; 82.36 ± 6.56 ng g<sup>-1</sup> for *E. brasiliensis* and *M. curema*, respectively. Regarding the limits for fish consumption established by the European Union (Regulation n° 835), the sum of the PAHs: benzo[a]pyrene, chrysene, benz[a]anthracene and benzo[b]fluoranthene (12 ng g<sup>-1</sup>), were about 4 times higher for both *E. brasiliensis* (44.88 ng g<sup>-1</sup>) and *M. curema* (40.18 ng g<sup>-1</sup>). The diagnostic indexes calculated for PAHs in sediment and white muscle indicated a mixed PAHs source, petrogenic and pyrolytic. Correlations were found between the TBARs in liver and DNA strand breaks in gills with the total PAHs concentration in white muscle. Our results demonstrate that the APACC sediments and studied fish species are contaminated with PAHs. The white muscle presented worrisome concentrations of the most toxic PAHs for human consumption. Also, according with the correlation analyses, these PAHs bioconcentrated in white muscle are related with damages in DNA and lipids of liver and gills, respectively.

## 12.P-Tu-117

### Pesticide Levels in Marine Sediments of the Bay of Cartagena, Colombia

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#### Abstract

The distribution of sediments in the Bay of Cartagena is greatly influenced by the Canal del Dique, a man-made channel that connects the bay with the Magdalena River, an important river as it is the largest in Colombia. It is well documented that pesticides can be carried into ecosystems by surface runoff, erosion, and wind, adding to agricultural pollution. Sediments are known to become sinks for pollutants. Depending on environmental conditions, contaminants in sediments can be released into the water column and circulate in ecosystems. Among them, pesticides.

Families of pesticides such as organophosphates, triazines, thiocarbamates, and chloroacetanilides have been introduced as alternatives to organochlorine pesticides because these compounds are less persistent. Notwithstanding some molecules of this, new families of pesticides have also been found to be toxic to biota.

Ten sampling stations for sediment collection were defined along the Cartagena Bay (S1 to S10). A multi-residue method was used for the analysis of semi-volatile organic pollutants in marine sediments from the Bay of Cartagena. For the determination of pesticides, the QUECHERS extraction technique and gas chromatography-triple quadrupole mass spectrometry (GC-MS/MS) were used.

Thirty of the forty-nine target pesticides were detected in at least one of the sediment samples. Total pesticide concentrations ranged from 0.83 to 33.67 ng/g dry weight, with the highest levels found at the mouth of Canal del Dique (S1, S2, S3 and S4), and at S9. Consequently, these locations exhibited the highest amount of detected pesticides with a significant contribution from herbicides such as atrazine, bromacil, and butachlor. Organophosphates (OPP) and OCPs were the main contributors to the total pesticide concentrations detected at the remaining locations.

Chlorpyrifos (0.28–1.43 ng/g), and diazinon (< 0.08–0.27 ng/g) were detected in all sediments sampled.

## 12.P-Tu-118

### Ecotoxicological Monitoring of Industrial Effluents in Brazil: A Study Case

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#### Abstract

The effluent monitoring, performed by industries in Brazil, must comply with the CONAMA Resolution 430/11 on discharge standards. This resolution establishes that the effluent should not cause toxic effects on the receiving water body. The receiving water body is also monitored according to the CONAMA Resolution 357/05 on surface water quality. We present results from 29 campaigns from the case study of an industrial plant monitored since 2008 and located on the coast of São Paulo state. The monitoring includes two effluents (E1 and E2) and 3 points in the receiving water body (PA1, PA2, and PA3). Initially, the effluents were assessed by chronic toxicity testing with sea urchin (*Lytechinus variegatus*); chronic toxicity testing with seaweed (*Skeletonema costatum*), and acute toxicity testing with mysidaceans (*Mysidopsis juniae*). *L. variegatus* was chosen for continued monitoring of the effluent and receiving water body, and it is performed semi-annually to the present day. From May 2016 on, *L. variegatus* was replaced by *E. lucunter*. Chemical parameters are also analyzed every six months, and the effluent dispersion study is performed every two years. At the beginning of the monitoring, the effluent flow ranged between 5,000 and 10,000 m<sup>3</sup>/h, forming a large dispersion plume. The highest toxicity of the effluent was observed between 2008 and 2015, with NOEC (No Observed Effect Concentration) values <20%, leading to studies for toxicity identification evaluation (TIE) in 2012 and 2014. The first study showed reduced toxicity after sample filtration, metal complexation, and removal of ammonia by *Ulva lactuca*, indicating ammonia to be the main cause of toxicity. The second study showed an almost 100% reduction in ammonia after zeolite filtration, and toxicity was eliminated. In 2015, changes in the industrial production processes led to a huge reduction in effluent generation, with current flows of 15 to 45 m<sup>3</sup>/h. The effluents showed reduced toxicity, with NOEC values >65%. In the receiving body, of the 66 samples tested with *E. lucunter*, only 18 samples showed toxicity. The modeling study performed on October/22 indicated that the effluent disperses completely near the discharge and demonstrates that under current conditions the effluent does not have the potential to confer a toxic effect on the receiving body.

## 12.P-Tu-119

### Mercury Bioavailability in Sediments and Bioaccumulation Potential on Fish in the Upper Estuary Region of the Santos Port Channel, Brazil

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#### Abstract

The upper estuary of the Santos Port Channel, Brazil, was severely anthropized during industrialization from the 1950s onwards. Strict control measures conducted in the 1980s resulted in a significant decrease in contaminant inputs to the estuary. However, the inherited contamination remained in the sediments, especially in sedimentation areas adjacent to the navigation channels. These areas are commonly the habitat of benthic and demersal-benthic organisms, which interact with the sediment and its contaminants, especially mercury, which presents a potential for bioaccumulation. The study evaluated the bioaccumulation potential of the mercury present in the sediments of the upper estuary of the Santos Port Channel. The database obtained between May/2020 and May/2022 was composed of 102 surface sediments samples (102 of total mercury and 16 of methyl mercury analyses) and 60 samples of fish tissue for the following species: parati (*Mugil curema*), carapeba (*Diapterus rhombeus*), siri (*Callinectes danae*), sea bass (*Centropomus* sp.), croaker (*Micropogonias furnieri*) with analyses of total mercury. The analysis procedures were performed by laboratories accredited by NBR ISO/IEC 17,025. In sediments (n=102), total Hg was observed in 51 samples (50%), with levels above 0.13 mg/kg (Threshold Effect Level (TEL) - CCME 2002 guideline value) in 50 samples (49.0%), with a maximum of 3.35 mg/kg. Among them, methylmercury analyses were performed in 16 samples with total Hg contents above TEL (0.230 to 0.591 mg/kg), with results below the method's quantification limit (QL) in all samples, indicating the sediment as a no significant source of methylmercury for the aquatic biota. Additionally, in fish samples (n=60), total Hg was found in only 06 samples (10%) and at trace levels (0.09 to 0.28 mg/kg), below the maximum tolerable limit (MTL) defined by ANVISA Normative Ruling 88/2021, for human consumption (0.50 mg/kg). It corroborates the results obtained for the sediments showing that the total mercury levels recorded in the sediments, despite being above TEL (CCME 2002), have a low potential for bioaccumulation.

## Does the Increase in Nitrate Concentration Combined With Thermal Stress Affect Coral-Dinoflagellate Symbiosis?

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### Abstract

Nitrate ( $\text{NO}_3^-$ ) is the main polluting nutrient from land runoff. Its release into the coastal waters has affected coral health and growth. Such effects may be intensified when exposure to nitrate is combined with increased water temperature, leading to a disruption of symbiosis (bleaching) between corals and photosynthetic micro-algae. Coral bleaching concerns the degradation of photosynthetic pigments and/or the expulsion of endosymbionts by the coral, which are responsible for providing more than 90% of fixed carbon by the hosts. Thus, bleaching can cause energy imbalance and affect coral physiological processes. Most of studies relating the effects of nitrate and temperature in corals are concentrated in Indo-Pacific and Caribbean species, thus representatives from tropical, oligotrophic waters. However, average nitrate concentrations in subtropical, South Atlantic reefs are significantly higher. Thus, we investigated whether nitrate combined with high temperature affects the symbiosis between the Brazilian coral *Mussismilia hispida* and its symbionts. Colonies (N=8) sampled from the Alcatrazes Archipelago, SP/Brazil, were acclimated for 21 days until being fragmented. Afterwards, coral nubbins were exposed to an open-water system that reflects the natural oscillations of the surrounding seawater of São Sebastião channel for 14 days at 26° C prior to the experiment: control and  $\text{NO}_3^-$  enrichment (+30  $\mu\text{M}$  above ambient levels) at 26° C and 29.5° C (IPCC 2023, + 3.5° C) for also 14 days. We analyzed: symbiont density (Sd), chlorophyll-a concentration ( $\text{Chl}_a$ ) and photosynthetic maximum quantum yield (Fv/Fm). In summary, higher temperature promoted coral bleaching, reducing Sd,  $\text{Chl}_a$  and Fv/Fm compared to the control condition (ambient  $\text{NO}_3^-$ , 26° C). Interestingly, the combination of temperature with  $\text{NO}_3^-$  +30  $\mu\text{M}$  had a synergistic effect, showing a higher reduction in the aforementioned traits. As expected, global warming is harmful to corals and its negative effects can be higher in the presence of high nitrate concentration, typical of eutrophic environments.

## 12.P-Tu-121

### Evaluation of the Systemic Effects of Settleable Atmospheric Particulate Matter on Aquatic Organisms Through Biomarkers in the Hemolymph

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#### Abstract

The Tubarão harbour complex located in the municipality of Vitória, Espírito Santo, Brazil, has proven to be an important contributor of settleable atmospheric particulate matter (SePM) to the local coastal zone, due to its various activities, including a plant for processing, storage and disposal of iron ore, in which the material is exposed to the open sky and is carried by the wind, being deposited along the entire coastal region, including aquatic ecosystems, becoming a source of contaminants such as metals and metalloids for these environments and biota associated. Based on these facts, the study aimed to evaluate the toxicity of SePM in estuarine organisms *Crassostrea rizophorae* and *Ucides cordatus* through an assessment of the systemic health of the organisms using biomarkers in the hemolymph of the species. Thus the organisms of the different species could be evaluated, they were submitted to an experiment lasting 30 days with four treatments: control; 0.01g.L, 0.1g.L, 1g.L of SePM, with the hemolymph being sampled in periods of 2, 4, 7, 15 and 30 days after the start of exposure, where it was evaluated using biomarkers of lipid peroxidation (LPO), DNA damage, cholinesterase (ChE) and neutral red retention time (NRRT). The results in the hemolymph showed a dose-dependent cytotoxic response. From the evaluation of the hemolymph, it is possible to have a real state of systemic health of the organisms exposed to SePM, since the tissue has contact with the organs exposed to the environment, such as the gills, as well as being an important transport route of the contaminants to other tissues, potentially compromising the health status of organisms exposed to SePM.



# Session 13: Detection, Toxicity and Environmental Risk of UV Filters and Cosmetic Ingredients in Aquatic Ecosystems

13.P-Mo-040

## Effluents Generated in A Beauty Parlor After Using Brown Hair Dye - Ecotoxicity on Aquatic Organisms

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### Abstract

The aquatic environment is highly complex and rich in diversity, comprising various types of compartments such as rivers, lakes, estuaries, seas and oceans. In recent years, some studies have shown the presence of hair dyes ingredients in aquatic ecosystems, such as precursors, couplers, and pigments. Thus, hair dyes constitute an environmental threat due to the indiscriminate disposal of these substances, among which some have persistent, bioactive compounds with bioaccumulative potential, becoming a potential risk for living organisms. Therefore, the objective of this study was to evaluate the in vivo effects of two types of effluents generated in beauty parlors after hair dyeing with brown coloration. Hair dye effluents associated with shampoos and conditioners (EA) and effluents not associated with these products (ENA) were evaluated, using ecotoxicity tests with the bioindicators *Artemia salina*, *Daphnia similis* and *Danio rerio*. The bioassays were selected in order to assess acute toxicity on a broad scale, considering the impact of these effluents on the water bodies and its biota. The treatments were carried out with the pure sample (100%) and the dilutions of 50.00%, 25.00%, 12.50%, 6.25%, and 3.13% in the bioassays with *A. salina* and *D. rerio*, for both effluents. In the bioassay with *D. similis*, the treatments were performed with the pure sample (100%) and the dilutions 50.00%, 25.00%, 12.50%, 6.25%, 3.13%, 3.00 %, 1.00%, and 0.50% for the ENA and pure sample (100%) and the dilutions 50.00%, 25.00%, 12.50%, 6.25%, 5.00%, 3.13%, 3.00%, and 1.00% for the EA. Bioassays with *D. similis* and *A. salina* were carried out with 48 h of exposure, while *D. rerio* bioassay was follow-up for 24 h, 96 h and 144 h, after egg fertilization (hpf). The results showed that both effluents were toxic: EC50 values of 3.43% for EA and 0.54% for ENA (*D. similis*); LC50 values of 8.327% for EA and 3.874% for ENA (*A. salina*); and the LC50 values of 4.93% (24 hpf), 3.90% (96 hpf and 144 hpf) for EA, and 6.59% (24 hpf and 96 hpf) and 6.37% (144 hpf) for ENA (*D. rerio*). Among the test organisms exposed to effluent samples, *D. similis* showed the highest sensitivity. Given these results, we can infer that hair dyes, even in residual concentrations, have a high toxic potential in relation to aquatic biota, as they have induced different intensities of deleterious effects to all studied organisms.

## 13.P-Mo-041

### Evaluation of the Bioconcentration Factor (BCF) of Parabens in the Zebrafish *Danio rerio* (Cypriniformes, Cyprinidae)

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#### Abstract

Different personal care products (PCPs) such as creams, toothpastes, sunscreens, contain chemical agents to achieve their compositional stability, including preservatives, antimicrobials, and fragrances. As a bactericide of these formulations, the use of parabens is frequent. These compounds, classified as contaminants of emerging concern, reach the aquatic environment as a consequence of poor wastewater treatment, negatively affecting biota, bioaccumulating and generating estrogenic effects on it. Previous studies from our group have quantified them in urban rivers and streams (2.7 µg/L). The objective of this work was to determine the bioconcentration factor (BCF) of the methyl-, ethyl-, propyl-, and butyl-parabens (MePar, EtPar, PrPar, and ButPar) in the species *Danio rerio* (Zebrafish). After acclimatization of one week, a 96-hour to waterborne exposure in glass aquariums (T°25°C; light:dark 14:10) to each parabens (solution concentration 1 mg/L) under semi-static conditions with daily renewal of test solutions and one organism per container was performed. Before and after renewal, water samples were taken (n=5 for each compound and control group). After the exposure, bioconcentration in total homogenate of each specimen was analyzed using QuEChERS method. The obtained extracts were filtered and analyzed by LC-MS/MS (Waters Alliance-ESI-Quattro Premier XE). The results show that the BCF presented the following behavior: PrPar>ButPar>EtPar>MetPar, where the PrPar is the most bioconcentrated in the studied species. A different behavior of the parabens than expected is highlighted, since the bioconcentration was not directly proportional to the number of carbons present in the molecules. Possible mechanisms associated with its incorporation and elimination will be evaluated. It is concluded that these contaminants of emerging interest are being released into the environment with a high bioaccumulation potential in aquatic biota, negatively affecting biodiversity.

## 13.P-Mo-042

### Analysis of UV Filters and Their Degradation Products in Uruguayan Waters. Part I: Avobenzone

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#### Abstract

Moderate exposure to sunlight has several beneficial effects on human health. However, excessive exposure to UV radiation causes various damages to the body, including burns, photoaging and skin cancer. The first commercially-available sunscreen appeared in 1928, it contained benzyl salicylate and benzyl cinnamate. Currently, these substances are, not only incorporated in sunscreens, but also in a large number of cosmetics for skin and haircare. There are several important aspects to be taken into account, such as the photostability of the sunscreen, its toxicity in humans, and its final disposal into the environment. Currently, one of the few UVA filters approved in the United States and Europe is tert-butylmethoxydibenzoylmethane (avobenzone). However, this compound is unstable from a photochemical point of view and cannot be used in combination with certain sunscreens. Recent studies show that the irradiation of avobenzone causes a breakdown of the molecule in radicals, this generates compounds, such as arylglyoxals and benzyls, or react with other sunscreens.

Water samples were collected from different sites in Montevideo and its surroundings.

To carry out the analysis, the water sample is extracted with ethyl acetate (3 extractions). The organic layer is dried with anhydrous sodium sulfate and the solvent is evaporated under reduced pressure. The residue is analyzed by thin layer chromatography, nuclear magnetic resonance and mass spectrometry.

The compound was detected in the sample. Also, degradation products were detected (benzoic acids, acetophenones and benzyls). Products resulting from the reaction of these compounds with chlorine (from sodium hypochlorite used as a disinfectant) are also observed.

13.P-Mo-043

## Analysis Of UV Filters And Their Degradation Products In Uruguayan Waters. Part II: Benzophenones

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### Abstract

Some benzophenones are currently used as UV filters, although it is known that have estrogenic and antiandrogenic activities.

A important problem related with UV filters is environmental pollution. Because coastal tourism is rapidly growing, sunscreen is now considered an emerging pollutant. Sunscreens are formulated to resist being washed out while bathing, however, it is estimated that after 20 min of immersion, 25% of the ingredients are released into the water. Several ecological problems have been reported in recent years.

Water samples were collected from different sites in Montevideo and its surroundings.

To carry out the analysis, the water sample is extracted with ethyl acetate (3 extractions). The organic layer is dried with anhydrous sodium sulfate and the solvent is evaporated under reduced pressure. The residue is analyzed by thin layer chromatography, nuclear magnetic resonance and mass spectrometry.

The compounds was detected in the sample. Also, degradation products were detected (rearranged benzophenones, benzoic acids and phenols).

Products resulting from the reaction of these compounds with chlorine (from sodium hypochlorite used as a disinfectant) are also observed.

## 13.P-Mo-044

### Sunscreen's: How Protecting Ourselves From the Sun Harms the Ocean

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#### Abstract

Sunscreens are personal care compounds responsible for protecting human skin from UV rays burns; they are based on UV-filters as their active ingredients, which include oxybenzone, titanium dioxide, zinc oxide, octisalate, octinoxate, octocrylene, avobenzone, and homosalate. Sunscreens are among the most used personal care products in the world, and the rise of global tourism is causing the amount of sunscreen detected in coastal and ocean waters to be significantly increased. This study aimed to compare the toxicity of commonly used sunscreen brands in Brazil by conducting ecotoxicological tests on the larval development of sea urchin (*Echinometra lucunter*), mussel (*Perna Perna*), sand dollar (*Mellita quinquesperforata*), and on the mortality of brine shrimp *Artemia salina*. Two sunscreens were used: Neutrogena Sun Fresh (NEU) and Sundown Praia e Piscina (SUN), both containing the chemical UV filter octocrylene. The concentrations tested were confirmed using UV spectrophotometry. Thus, the lethal (LC) and effective (EC) concentrations to 50% of the organisms were based on actual concentrations. NEU was found to be more toxic than SUN because it caused more deformities in larvae at lower concentrations. The toxicity of both sunscreens was similar for sea urchin species NEU EC50-36 h 7.20 (6.018-8.547) mg/L and SUN 9.2 (7.58-11.13) mg/L, for mussels NEU was more toxic, with EC50-24 h 3.94 (2.73- 6.06) mg/L and SUN 24.36 (11.8-131.42) mg/L. Sand dollars were found to be highly sensitive to sunscreens, with an EC50 48 h of 3.13 (2.56-3.82) mg/L for NEU and 2.31 (1.90-2.81) mg/L for SUN. *Artemia salina* was less sensitive, with an LC50 48 h of 23.3 (18.47-29.38) mg/L for NEU and 53.25 (38.69-97.7) mg/L for SUN. Comparing these results with those found in the waters of Atlantic Ocean beaches (10-96.7 mg/L sunscreen), it was observed that sunscreens are causing deformities in echinoderm and mussel larvae, and causing the death of microcrustaceans, as higher concentrations were found in the environment than the LC and EC50 calculated in this study. Therefore, greater attention should be given to sunscreens as emerging contaminants, and other forms of protection against the sun should be used to prevent the environment from becoming contaminated with these pollutants.

## 13.P-Mo-045

### Evaluating The Potential of Cosmetic Preservatives in Inducing Acute Toxicity in Algae and Fish

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#### Abstract

Although cosmetics are extensively evaluated regarding human health effects, there is a gap in knowledge regarding their impacts on the environment, especially aquatic environments, which are the final destiny of several cosmetic ingredients. Preservatives added to many cosmetic products aim to prevent microorganism growth and prolong the product's lifetime; however, preservatives are a cosmetic ingredient category of current concern to the environment. Thus, in this study, we evaluated the potential of aquatic toxicity of two preservatives commonly used by cosmetic industry, the benzyl alcohol and ethylhexylglycerin. For this evaluation, the acute toxicity test with algae (Organization for Economic Co-operation and Development Test Guideline - OECD TG 201) and permanent fish cell line RTgill-W1 (OECD TG 249) and zebrafish cell line ZFL, as well as *in silico* tools were used. Physicochemical data collection in Comptox Chemicals Dashboard (United States Environmental Protection Agency) and *in silico* predictions with VEGA QSAR platform (version 1.2.0) showed that benzyl alcohol is not persistent in water while ethylhexylglycerin is persistent, and that both preservatives have low bioaccumulation potentials due to their molecular structures (an aromatic alcohol and a glycerol ether). For both preservatives, inhibition of algae growth was verified, and benzyl alcohol showed greater toxicity potential compared to ethylhexylglycerin. Contrary to results in the algae test system, in the *in vitro* assays, a greater potential for ethylhexylglycerin toxicity was preliminarily observed, although the range of concentrations used in the test is currently under adjustment. Such results may indicate a difference in the sensitivity of plant cell walls and animal cell membranes to the preservatives tested, and for that, further research on the mode of action of benzyl alcohol and ethylhexylglycerin is suggested. It is noteworthy that the scope of this work does not consider actual exposure to environmentally relevant concentrations of the tested preservatives; therefore, risk assessments must be conducted to understand the possible harmful effects of these substances on aquatic biota.

## Session 14: Nanomaterials

14.P-Mo-046

### Silica Nanoparticles Induce Mortality and Oxidative Stress in *Ceriodaphnia reticulata* (Cladocera)

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#### Abstract

Nanotechnology industry has been extensively developed in the recent years due to the unique properties of nanoproducts and their diverse applications. Nevertheless, such development has not been accompanied by ecotoxicological assessments, especially considering that aquatic systems are their main final sinks. Silica nanoparticles (SiNP) are currently one of the most produced nanomaterials under the assumption of their safety. However, the ecotoxicological studies are still incipient and their toxicity may vary according to different SiNP properties such as their size. We analyzed the effects of two sizes of SiNP (50 and 300 nm) on mortality and oxidative stress of *Ceriodaphnia reticulata* (Cladocera). SiNP were synthesized through Stöber method by modifying the reagents concentration according to the desired particle size. *C. reticulata* neonates were exposed to five concentrations (10 – 500 µg/ml) of each SiNP size and mortality was recorded at 24, 48, and 72 h. *C. reticulata* adults were exposed to two sublethal concentrations of each SiNP size during 72 h to assess lipid peroxidation (LPO) and the activity of antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST). The lethality of the smaller SiNP (LC50 72 h: 105.5 µg/ml) was higher than the larger ones (LC50 72 h > 500 µg/ml). The 50 nm-sized SiNP increased SOD and inhibited GST activities, while larger SiNP did not exert oxidative stress under the tested concentrations. SiNP caused mortality and oxidative stress in *C. reticulata* despite several reports have assumed their safety. As SiNP ecotoxicity depended on their size, being the smaller ones more toxic, this study highlights the relevance of assessing the effects of different nanoparticles intrinsic properties in sensitive non-target organisms such as cladocerans. This information is crucial for regulatory purposes and contributes to the develop of safe-by-design nanoproducts to ultimately guaranteed the environment protection.

## 14.P-Mo-047

### Biogenic Metal Nanoparticles Impact in Freshwater Systems

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#### Abstract

Silver (Ag), gold (Au) and titanium dioxide (TiO<sub>2</sub>) nanoparticles (NP) are among some of the most promising metal nanoparticles (MNP) regarding antimicrobial properties. Biogenic MNPs are becoming more interesting since their synthesis is considered environmentally friendly, presenting low toxicity for aquatic and terrestrial organisms. Here, we evaluated the ecotoxicity of biological AgNP (size 37.4-67.4 nm), AuNP (size 13.3-20.3 nm), and TiO<sub>2</sub>NP (size 69.80 nm), immobilized in calcium alginate in 4 freshwater species belonging to three different trophic levels: a primary producer – the microalgae *Chlorella fusca*, a primary consumer – *Palaemon pandaliformis*, and 2 secondary consumers – *Danio rerio* and *Oreochromis niloticus*.

Organisms were exposed to 17.5 g of silver nitrate (AgNO<sub>3</sub>), tetrachloroauric acid (III) (HAuCl<sub>4</sub>.4H<sub>2</sub>O), titanium dioxide (TiO<sub>2</sub>), AgNP, AuNP, and TiO<sub>2</sub>NP, immobilized in calcium alginate. The inhibition growth assays of *C. fusca* were carried out in 50 mL at 1.0×10<sup>4</sup> cells/mL. The toxic response was assessed at 680 nm, followed daily for 72 h, and performed in triplicate. Routine metabolism tests (dissolved oxygen and ammonia excretion) were performed in *P. pandaliformis*, *D. rerio*, and *O. niloticus*. Organisms were randomly separated into 4 groups of 10 individuals, and each group was placed in 2 L of filtered (0.25 μm mixed cellulose ester membrane) dechlorinated tap water (pH=6.5). The aquaria were constantly aerated, and temperature was controlled and kept stable at 20.5 ±0.5 °C. The organisms were exposed for 72 h to the sublethal concentration: 0,01 mg/L (AgNP, AgNO<sub>3</sub>) or calcium alginate. The control group received only dechlorinated filtered tap water. Statistical analysis verified data normality.

For all organisms studied, the MNP toxicity was lower than their metal precursor. Alginate did not compromise the growth of *C. fusca* which presented a survival rate greater than 85%. Regarding the results obtained for routine metabolism, there were no significant changes, except for *O. niloticus* (ammonia excretion) when exposed to AgNO<sub>3</sub>, which was statistical different (ANOVA p>0.05) in comparison to the other compounds tested. In view of the results obtained, the immobilized MNP show promising potential for their use in freshwater systems decontamination, especially against pathogenic microorganisms, making it possible to reduce deleterious impacts on the ecosystem and enabling the conservation and management of this natural resource.



## 14.P-Mo-048

### Exotoxicology Assessment on the Adverse Effects of Zinc Oxide Nanoparticles in a Model Native fish, *Cnesterodon decemmaculatus*

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#### Abstract

The use and production of nanoparticles (NPs) in recent years has grown exponentially due to their multiple physical and chemical properties. In particular, zinc oxide (ZnO) NPs have several applications for medicinal use, among which we can mention: anticancer treatment (photodynamic, photothermal, and sonodynamic therapy), theragnostic (bioimaging), drug delivery, tissue engineering, radiation filter (insunscreens, cosmetics), antimicrobial agents (toothpaste, dentistry implants, food packaging, food additive). Due to diverse applications and daily use, they are widely distributed and can easily reach bodies of water. Also, it has been detected in aquatic environments generating invertebrate and vertebrate mortality from 3.2 mg/L ZnO. Added to this, sub-lethal effects have been reported on zebrafish model: immunotoxicity, developmental alterations, abnormalities neurotoxicity, reproductive, cardiovascular, hepatotoxicity and genotoxicity. However, in our regions, studies on the toxicity of ZnO NPs are scarce. For example, there are no evaluations on the adverse effects of ZnO NPs on native fish. It is important to note that studies using native species allow a better understanding of ecosystem functioning, so their use has great advantages for carrying out ecotoxicological bioassays. Environmental monitoring showed ZnO NPs in surface waters worldwide being detected in concentrations between 0.001 to 0.1 mg/L. In this context, the objective of this work was to evaluate the response to different relevant sublethal concentrations of ZnO NPs in a local fish *Cnesterodon decemmaculatus*. Acute toxicity bioassays were carried out using sublethal concentrations between 1 and 10 mg/L of ZnO NPs based on LC50 values from the literature. Biochemical biomarkers were studied: catalase (CAT), cholinesterase (ChE) and inhibition of lipid oxidation (TBARS) after 96h of exposure to the xenobiotic. The results suggest that the evaluated concentrations do not produce mortality in *C. decemmaculatus*. The biomarkers showed significant differences in ChE for fish exposed to 1 mg/L, while in CAT no significant differences were observed in the antioxidant response after acute exposure. Finally, TBARS activity decreased significantly compared to the control group at the highest concentrations (5 and 10 mg/L). This study shows that the presence of ZnO NPs in water bodies can represent a risk for the fauna that inhabit them and for human health.

## Ecotoxicity Assessment of Silica Nanoparticles Combined with Cooper in *Gambusia holbrooki* Fish

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### Abstract

Despite high-tech nanoproducts have gained importance in the last decades, there is a lack of regulation in terms of the amounts of production and release into environments, whereas the final sinks are the water bodies that already suffer from other pollutants such as metals. As silica nanoparticles (SiNP) are one of the most produced and applied nanomaterial worldwide, we aimed to assess SiNP ecotoxicity, alone and in combination with an environmentally relevant concentration of Cu in *Gambusia holbrooki* freshwater fish. We exposed adult females to 0 (control), 10, and 100 mg SiNP/L (Sigma®, <50 nm-sized), with or without 0,25 mg Cu/L. After 96 h, dermal mucus of each fish was isolated to quantify, after 24 h of incubation, the amount of colony forming units (CFU) to assess the antibacterial effect of the treatments. Then, fish were dissected and oxidative stress markers (catalase -CAT-, glutathione-S-transferase -GST- antioxidant activities, and lipid peroxidation levels -LPO-) were measured in liver, gills, muscle, and brain. Neurotoxicity in terms of acetylcholinesterase activity was also measured in brain and muscle, and tissue damage markers (alkaline phosphatase -AP-, and transaminases: glutamate-pyruvate transaminase -GPT-, and glutamic oxaloacetic transaminase -GOT- enzymatic activities) were determined in liver. The amount of CFU decreased in all treatments where the Cu was present in the media. Hepatic CAT activity increased in the case of Cu exposure, alone and in combination with both SiNP concentrations when compared to the control; meanwhile GST activity increased only in the case of 10 mg SiNP/L. In gills and muscle, GST activity also augmented at 10 and 100 mg SiNP/L+Cu, respectively. LPO increased in liver of fish exposed to 100 mg SiNP/L+Cu, but decreased in brain at 10 mg SiNP/L+Cu. When no neurotoxicity was registered, regarding hepatic tissue damage, AP activity decreased at 10 mg SiNP/L+Cu, and at 100 mg SiNP/L, meanwhile GPT one decreased in the case of Cu exposure alone. We concluded that the presence of Cu may enhance the ecotoxicity of SiNP with emphasis on oxidative stress and being the liver the target organ, whereas the nanomaterial may not be highly toxic by its own. Further research is encouraged to achieve a more complete overview of the ecotoxicology of these poorly studied yet widely applied nanoparticles.

## 14.P-Mo-050

### Acute Toxicity of Magnetite Nanoparticles Coated With Oleic Acid In The Amphipod *Hyalella curvispina*

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#### Abstract

The main productive activities in North Patagonia are oil and gas exploitation and fruit culture. Such activities may generate pollutants that can reach natural and artificial watercourses, affecting aquatic life. Due to the high quality of regional water resources, there is an environmental preservation requirement from different social actors. In this context, the presence of organic contaminants on aquatic environments require effective solutions and there is a worldwide growing necessity of techniques that minimize their concentration. Nowadays, the development of environmental remediation technologies using nanomaterials has emerged as an interesting alternative. However, prior to their application, it is necessary to carry out an initial toxicity evaluation of these materials. Our aim was to evaluate the acute toxicity of magnetite nanoparticles coated with oleic acid (NPOA), developed and synthesized by our group for the remediation of hydrocarbons-contaminated water. This study analyzed NPOA acute effects in adult individuals of the native amphipod *Hyalella curvispina*. Individuals were collected from a reference site in Mari Menuco Lake, Neuquén, Argentina. The amphipods were maintained and raised under laboratory conditions. Groups of 10 adults were exposed to a range of NPOA concentrations in 250 ml of filtered and dechlorinated water, during 96 hours at constant temperature of  $23 \pm 1^\circ\text{C}$  and 16:8 h (L:D) photoperiod. The concentrations tested were 1, 5, 10, 25, 50, and 100 mg/l. Mortality and behavioral alterations were employed as endpoints to determine toxicological parameters. Significant differences were observed at 96 hs only between control and 5 mg/l with regards to mortality. The analysis of behavioral alterations show significant differences between control and all the NPOA concentrations tested, reaching up to 58% of exposed individuals. NPOA-exposed individuals showed alterations such as changes in reaction speed, locomotor behavior and increase in ventilation frequency. Our results suggest that the lethal effects of the NPOA could vary according to their aggregation state in the medium. NPOA aggregation starts at 10 mg/l, and is more visible as concentration increases. Hence, mortality does not show a linear relationship with concentration, while behavioral alterations do. Thus, behavioral alterations become more relevant in amphipod health status than direct lethal effects driven by NPOA exposure.

## 14.P-Mo-051

### Toxicity of Copper Oxide Nanoparticles and $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ Salt on Embryo-Larval development of *Rhinella arenarum*

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#### Abstract

The release in environment of emergent contaminants as nanoparticles (NPs) and the respective salts use in their synthesis, has drawn considerable attention which has become an eminent area of research to understand the potential risk related to their short- and long-term toxicity. In this sense, the aim of this work was to evaluate the toxicity of copper oxide NPs (CuO-NPs) and compare it with its respective salt ( $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ) in the early development of a native amphibian, *Rhinella arenarum*, by acute and chronic standardized bioassays (AMPHITOX). Lethality and sublethal effects such as developmental, morphological and ethological alterations were assessed in embryos and larvae exposed to different concentrations in the range: 0.001-100 mg/L CuO-NPs, and 0.001-10 mg/L  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  up to 21 days. The bioassays were semi-static with replacement of the solutions every 48 h; treatments were done by triplicate, and a control group without exposure was simultaneously maintained. The results show that, in both developmental periods, the salt caused higher toxicity than CuO-NPs with LC50-96 h values of 0.080 and 1.26 mg/L for embryos respectively. The difference in toxicity between NPs and salt could be due to both structural and physicochemical characteristics that influence on bioavailability and toxicity. Moreover, toxicity increased with exposure time in all cases (NOEC-504 h for larvae=0.5 mg/L for  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  and 10 mg/L for CuO-NPs), with a greater sensitivity during the embryonic period (NOEC-504 h for embryos= 0.01 mg/L for  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  and 0.1 mg/L for CuO-NPs). Regarding sublethal effects, exposed embryos from 0.001 mg/L to both substances exhibited several alterations such as persistent yolk plugs, cell dissociation, developmental delay, edemas, severe malformations, underdeveloped gills and tails, agenesis and/or curvature of tails and microcephaly. In the chronic and subchronic exposure periods, embryos and larvae presented spasmodic contractions and weak movements. Taking into account the exponential growth in production and use of these substances it is expected that their levels of contamination by these chemical agents increase considerably and that wildlife, particularly amphibians, will be more exposed and their populations will eventually be at risk.

## 14.P-Mo-052

### Assessment of *Chlorella vulgaris* Death Induced by Silver Nanoparticles

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#### Abstract

Microalgae are the basis of food webs and a potential target of silver nanoparticles (AgNP) in aquatic ecosystems. The aim of the study was to clarify the effects of AgNP on *Chlorella vulgaris*. The surface plasmon of citrated-capped AgNP was determined in a spectrophotometer; zeta potential and electrophoretic mobility were measured in a Nano Zetasizer. *Chlorella vulgaris* was growing in Bold's Basal Medium (BBM) and exposed to 1440  $\mu\text{gL}^{-1}$  AgNP. For Optical Microscopy (OM) the cells were stained with Methylene blue (MB), Lugol (L), and Congo red (CR). An *in situ* attenuated total reflectance-Fourier transform infrared (ATR-FTIR) technique was used to study the oxidation of phosphatidylcholine liposomes formed of egg yolk phosphatidylcholine (EYPC) as a model for the lipid fraction of algae. The AgNP showed an absorbance peak at  $\lambda_{\text{max}} = 415$  nm. In BBM the AgNP had an average size of  $144 \pm 22$  nm. Zeta potential and electrophoretic mobility were  $39 \pm 2$  mV and  $3.06 \pm 0.16$  m.cm/Vs. In MO the control cells stained with MB, L, and CR showed intact green algae cells, no stain was observed inside the cells. Microalgae treated with AgNP and stained showed disruptive effects in exopolysaccharides, starch-like substances, and in membrane integrity. The highly pro-oxidant generation of  $\text{OH}\cdot$  by Fe-citrate with hydrogen peroxide promoted significant FTIR spectral changes consistent with lipid oxidation. The exposure of EYPC liposomes to AgNP promoted a discrete increase in the band  $\approx 1740$   $\text{cm}^{-1}$ , assigned to the stretching vibrations of the carbonyl groups, a biomarker of lipid oxidation. Oxidation of the allylic carbon of a lipid acyl chain triggers free radical propagation, producing aldehydes (methylglyoxal, malonaldehyde, acrolein). Acrolein was also a compound that could contribute to a band increase at  $1664$   $\text{cm}^{-1}$  resulting from the C=C stretch in alkenes. AgNP could promote the oxidation of lipid acyl chains via the formation of carbonyls. Aldehydes and ketones are targets of homolytic and heterolytic cleavage by peroxidases and cytochromes, resulting in the generation of free radicals, electronically excited triplet species, and singlet oxygen. All structural and functional disturbances observed in *C. vulgaris* exposed to AgNP were related to cellular and organelle membrane changes. Microalgae are key environmental organisms; gaining knowledge on the effects of AgNP on cell damage will contribute to understanding the impact of nanosilver and preventing ecosystem imbalances.

## 14.P-Mo-053

### Ultrastructural and Biochemical Changes in the Gills of Guppy, *Poecilia reticulata*, After Exposure to Iron Oxide Nanoparticles ( $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>) in the Treatment of Water Contaminated With Glyphosate

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#### Abstract

Iron oxide nanoparticles functionalized with citrate (IONP) have high potential for remediation of different environments. However, toxicological studies are needed for IONP safe using in aquatic environment remediation. This study evaluated the gill, a multifunctional organ, having direct contact with water, large surface area which favors interaction with xenobiotics. Guppy, *Poecilia reticulata*, were exposed, for 21 days, to 5 treatments: IFe (Fe<sup>3+</sup> = 0.3 mg/L); IONP, [IONP  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> free (Fe = 0.3 mg/L)]; NR1, IONP+Roundup (GLY a 0.65 mg/L); NR2, IONP+Roundup (GLY a 0.65 mg/L); GLY (ONP +GLY = 0.65 mg/L); C, control (reconstituted water without ONP and GLY). The gills were sampled for scanning electron microscopy and analyzes of enzymes involved in the ionic homeostasis. IFe and ION induced alterations in the pavement cell (PVC) surface and loss of regular microridges, NR1 and NR2 induced PVC peeling and microridges changes, PVC hyperthrophy occurred after exposure to NR2 and, mucous cell and muco releasing increased in fish exposed to GLY. Na<sup>+</sup>/K<sup>+</sup>-ATPase activity unchanged after 7 days exposure to all treatments, decreased after 14 days exposure to GLY and increased in fish exposed to NR1, after 21 days, compared to the controls. H<sup>+</sup>-ATPase activity was unchanged after 7 days, increased after 14 days exposure to GLY and was unchanged after 21 d. Carbonic anhydrase activity decreased after 7 days exposure to IFe<sup>3+</sup> and NR1 and, 14 days exposure to IONP and R1. After 21 days, CA activity increased in the treatments: NR1, NR2 and GLY. IONPs showed be toxic to the gills of fish, but the transitory changes in the activity of Na<sup>+</sup>/K<sup>+</sup>-ATPase, H<sup>+</sup>-ATPase and carbonic anhydrase evidenced responses to maintain ionic homeostasis although, it may imply in energetic cost for fish. *Financial support*: FAPESP Grant 2019/24180-4, FAPEG Grant 201710267001261 and 2019011000085; CNPq Grant 306818/2020-5, CAPES Finance Code 001 and post-doctoral fellowship, FAPESP Grants 2016/025257-2 (ICSouza) and 2021/02906-3 (MMorozesk).

## 14.P-Mo-054

### Susceptibility of *Aquarana catesbeiana* tadpoles to Nanoencapsulated Geraniol

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#### Abstract

Geraniol (GER) is a monoterpene produced by aromatic plants and used as an active ingredient in some pesticides, such as fungicides for grape crops in Europe. Being an organic molecule, GER is more easily degraded than synthetic compounds, reducing the risk of toxicity to non-target organisms. However, amphibians, particularly those of the Anura order, are highly vulnerable to the negative impacts of agricultural activities due to their dependence on water during their larval stage, which exposes them directly to contaminants. To enhance GER properties, it can be encapsulated in nanocapsules of zein protein extracted from corn. Thus, this study aimed to evaluate the susceptibility of American bullfrog tadpoles (*Aquarana catesbeiana*) to nanoencapsulated geraniol (nGER) by testing the activity of the animals exposed to different concentrations of nGER (1, 5, 10, and 20 mg·L<sup>-1</sup>). Tadpoles at appropriate developmental stage (Gosner stage 25-30) were obtained from a frog farm, acclimated for 7 days, and exposed to different treatments for 96 hours with an 80% medium renewal at 48 h. The treatments included dechlorinated tap water (CTR), water with Pluronic F-68<sup>®</sup> solvent 0.1% (PLU), and nGER at concentrations of 1, 5, 10, and 20 mg·L<sup>-1</sup> (nGER 1, 5, 10, and 20). The tadpoles activity was assessed at 6, 24, 48, and 96 h by agitating the water for 15 s and observing their swimming for 45 s. Results showed that tadpoles exposed to nGER 20 and nGER 10 were lethargic after 6 h of exposure and remained so throughout the whole experiment, exhibiting no swimming response to water agitation. Tadpoles exposed to nGER 5 showed a decrease in the swimming response after 48 h, but no loss of responsiveness was noted. Meanwhile, CTR, PLU, and nGER 1 tadpoles remained unaffected throughout the experiment. The results of this study demonstrate that nGER can cause lethargy in American bullfrog tadpoles, with nGER 20 and nGER 10 displaying similar results. These findings highlight the potential environmental impact of pesticides containing GER, and the need for further research to understand the effects of nGER on aquatic ecosystems.

## 14.P-Mo-055

### Nanomaterials Containing Natural Products to Control Leaf-Cutting Ants and Application Methodologies

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#### Abstract

The development of nanomaterials to control pest leaf-cutting ants is very important for Brazilian agriculture. Nanocapsules, nanospheres and nanoemulsions containing natural products, extracts of plants and/or microorganisms, that in general, have low stability and low solubility in water, have been developed by the Natural Products Group of the Chemistry Department of the Federal University of São Carlos - SP – Brazil to control pests. These products that in general have low stability and low solubility in water, are obtained from Brazilian plants and microorganisms from the nest of *Atta sexdens rubropilosa*. The objective of this communication is to present the methodology developed using nanomaterials containing extracts and natural products of the plant *Hymatatus articulatus* (sucuuba) and extracts of *Phialophora attae*, obtained from monoculture and co-culture with microspore of *Escovopsis*. Thus, nanospheres and nanocapsules containing extracts and pure compound of *H. articulatus* in three biodegradable polymers (sugarcane lignins, gelatin and PCL) and nanoemulsions containing the active extracts of *P. attae* and *E. micropore* were developed using the inversion of phases method. It was composed of the non-ionic surfactants sorbitan monooleate and PEG 40 hydrogenated castor oil, soybean oil as the oil phase and distilled water as the aqueous phase. The nanoemulsion had particle size of 87 nm, a polydispersion index of 0.1 and a zeta potential of -9.84 mV, and it was stable after accelerated stability assay by thermal stress and centrifugation. These data suggest that the development of nanoemulsified system containing *P. attae* and *E. microspora* extract can be an alternative to control leaf-cutting ants thoroughly its symbiont fungi *Leucoagaricus gongylophorus*. *H. articulatus* is characterized by the classes of compounds such as iridoids, triterpenes, flavonoids and indole alkaloids. The ethanolic extract of the barks of this species was evaluated and its fungicidal property against the ant symbiotic fungus *L. gongylophorus* was evaluated and shown completely inhibition. Six lactonic Iridoides class were isolated from the bark of the plant and evaluated against the ant symbiotic fungus. It was observed total growth inhibition response indicating a promising fungicide. To confirm the effectiveness in field of the new products, future studies using leaf-cutting ant nest models should be carried out. Acknowledgement: FAPESP, CNPq, CAPES



## 14.P-Mo-056

### Review on the Impacts of Nanoparticles, In Particular Iron Oxide and Their Implications for the Conservation Biology of Teleosts

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#### Abstract

Iron nanoparticles (NOFs) have reached a wide field of applications in nanotechnology, especially in the medical and environmental areas, as described in the literature. The release of nanomaterials in the aquatic environment is evidenced in all stages of manipulation (production, application and disposal). It is necessary to survey the possible impacts of nanomaterials on teleost fish biomonitoring species.

teleosts, as well as to relate their reproductive capacity with the conservation events of the species, since there is a direct relationship between the maintenance of the number of individuals and the perpetuation of these teleosts. Collaborating with data that evaluate the environmental impact of nanoparticles (NPs), providing subsidies to understand the behavior, transformation, as well as their actions on cells and tissues in order to assess toxicological effects on the reproductive system of fish, embryonic and larval development. The data survey was performed on the ISI Web of Science site using the following keywords: Nanopart\* AND Fish AND Gonad\* OR Reproduct\* OR Ovar\* OR Embry\* OR Teratogenes\* AND Develop\* AND Tox\*. The parameters analyzed were: (1) year of publication; (2) geographical distribution (location of the corresponding author); (3) keywords; (4) abstract; (5) experimental condition under which it was considered (in vitro, in vivo and ex vivo tests); (6) developmental stage (embryo, larva, juvenile and adult); (7) establishment of the developmental stage that was used in the experiment; (8) female reproductive system. Excluding records, reviews, technical reports, protocols, abstracts, and academic theses. A total of 127 articles from indexed journals were selected. Iron oxide nanoparticles in fish were studied by five groups, with publications between the years 2013 to 2020. Adopting embryotoxicological tests in *Danio rerio* and *Oryzias latipes*. Three of the countries that make up the BRICS had summed the highest percentages in the world scenario of publications that with silver oxide nanoparticles. However, regardless of the type of NPs used these studies had outstanding scientific productivity in nanotechnology in the countries involved. *Danio rerio* is considered a bioindicator model of excellence due to internationally accepted and standardized parameters, external development of easy observation, which confer high reliability to the evaluations performed.

## 14.P-Mo-057

### Toxicity of ZnWO<sub>4</sub> Nanoparticles on the Green Microalga *Raphidocelis subcapitata*: Evaluating Growth and Physiological Parameters

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#### Abstract

Nanoparticles (NPs) have great potential in areas of environmental remediation, human health, cosmetics, sensor fabrication, agriculture, food industry. According to studies, the ZnWO<sub>4</sub> NP's have potential for water decontamination and, since these NPs will eventually end up in aquatic ecosystems, it is important to test their toxicity on aquatic organisms. As far as we know, there are no studies regarding ZnWO<sub>4</sub> toxicity to aquatic biota. In this context, we aimed to test the toxicity of ZnWO<sub>4</sub> (initial size of 23.8 ± 6.9 nm) to the green microalga *Raphidocelis subcapitata* (synonym of *Pseudokirchneriella subcapitata*), a widely used test-organism in ecotoxicology assays. We analyzed, after a 96 h exposure, the parameters of growth, obtained by flow cytometry; and photosynthetic parameters obtained by PAM fluorometry: maximum ( $\Phi_M$ ) and effective ( $\Phi_M'$ ) efficiency of the photosystem II, efficiency of the oxygen evolving complex ( $F_0/F_v$ ) and non-photochemical and photochemical quenchings. Initially we assessed a range-finding test with concentrations between 0.025 and 100 mg/L. The NPs presented aggregation in the algal culture medium, with a size of 1431.33 ± 148.33 nm at the highest concentration tested. Based on cell growth, our preliminary results indicate toxic effects at high doses, with preliminary 96h-IC50 of 55.22 mg/L. Regarding photosynthesis, after 96h the  $\Phi_M$  and  $\Phi_M'$  decayed ( $p \leq 0.001$ ) about 10% at 100 mg/L. The  $F_0/F_v$  was negatively affected ( $p < 0.05$ ) by 30% at 100 mg/L. There was also an increase ( $p = 0.014$ ) of about 30% of the non-photochemical and non-regulated quenching Y(NO) at 100 mg/L, indicating an increase in passive energy dissipation. Although our preliminary findings indicate toxicity only at high NPs' levels, we will evaluate additional endpoints in order to better understand the possible hazards of ZnWO<sub>4</sub> NPs to *R. subcapitata*.

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## 14.P-Mo-058

### Using Physiological and Morphological Endpoints to Evaluate the Effects of Nickel Tungstate (NiWO<sub>4</sub>) Nanoparticles for *Raphidocelis subcapitata*

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#### Abstract

The increasing production and use of nanoparticles (NPs; < 100 nm) of nickel tungstate (NiWO<sub>4</sub>) in environmental and technological areas contributes to the presence of these contaminants in aquatic ecosystems. The effects of NiWO<sub>4</sub> on freshwater microalgae are unknown. In light of this, the present study investigated the effects of NiWO<sub>4</sub> NPs on the freshwater microalga *Raphidocelis subcapitata*, at cellular level and photosynthetic activity. This species makes up the base of aquatic food chain and the contact of these organisms with the NPs can result in bioaccumulation and transfer of contaminants to higher trophic levels. To synthesize the NPs (size of 19.7 ± 4.6 nm) we used the coprecipitation method in an aqueous medium, followed by microwave irradiation. The algal cells were exposed during 96 h to different concentrations of NiWO<sub>4</sub> NPs (10, 20, 50, 70 and 100 mg L<sup>-1</sup>). To investigate the toxicity, we used flow cytometric analysis to access the cellular complexity, cell size and growth. To evaluate photosynthetic activity we used PHYTO-PAM. Our results evidenced that NPs aggregated in the culture medium, especially at 100 mg L<sup>-1</sup>, with a size of 463.77 ± 93.98 nm. Regarding ecotoxicity tests, preliminary data showed that NP did not inhibit the growth of the algal cells. On the other hand, algal cell size and cellular complexity were affected (p<0.05) by the NP. Also, we found changes in some photosynthetic parameters, specifically in the maximum quantum yield in the first hours of exposure at 70 and 100 mg L<sup>-1</sup> (p<0.05). After 96h of exposure, at 100 mg L<sup>-1</sup> we observed an increase in passive energy dissipation, demonstrated by the increase in Y(NO) (non-photochemical, non-regulated quenching) (p=0.04), but the photoprotection mechanism was not compromised, corroborated by Y(NPQ) (non-photochemical, regulated quenching) (p=0.069). Therefore, our preliminary results highlight the sensitivity of the endpoints used to evaluate the effects of NPs on algal cells, but we emphasize that damages to this trophic level affect the aquatic trophic web, causing imbalance throughout the ecosystem.

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## 14.P-Mo-059

# Drinking Water Disinfection By Immobilized Mycogenic Silver Nanoparticles In A Fixed Bed Reactor And Their Ecotoxicological Effect

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### Abstract

Contamination of drinking water (DW) by pathogenic microorganisms is a worldwide problem that urgently requires the creation of innovative and low-cost decontamination processes. The use of nanomaterials in this type of approach has been extensively researched, especially with the incorporation of commercial silver nanoparticles (AgNP). With the aim of reducing potential deleterious effects to the environment, the use of biogenic AgNP has been presented as a more ecologically and economically viable alternative. There are no resultant residues during the synthesis and they are less aggressive to the environment.

In this study, we evaluated: (a) the efficiency of a fixed bed reactor (FBR) operated in continuous mode, by filling 100% of the bed with mycogenic AgNP (size 37.4-67.4 nm; concentration 10µg/L), produced by the fungus *Aspergillus niger* IBCLP20 and immobilized in calcium alginate, with a volumetric feed rate of 4.0 mL/min ( $1 \times 10^3$ - $1 \times 10^6$  CFU/mL *Escherichia coli* IPT245), at 37 °C for 8 h; and, (b) cell growth inhibition of in the microalgae *Chlorella fusca* exposed to immobilized AgNP (17.5 g, mass corresponding to the bed filled in the FBR). Cell growth inhibition tests were done in 50 mL, containing the inoculum ( $1.0 \times 10^4$  cell/mL) in Bold's Basal Medium. The toxic response was evaluated at 680 nm and monitored daily for 96 h. For both experiments, silver nitrate (AgNO<sub>3</sub>) immobilized in calcium alginate (10 µg/L) was used as a positive control and the immobilizing agent as a negative control. Assays were performed in triplicate.

The results obtained indicated 100% efficiency regarding the antimicrobial activity of FBR against *E. coli* IPT245 at a concentration of  $1.00 \times 10^3$  CFU/mL. At concentrations between  $1.00 \times 10^4$  and  $1.00 \times 10^5$  CFU/mL, the efficiency of the system was 85%; whereas, at a concentration of  $1.00 \times 10^6$  CFU/mL, no antimicrobial activity of immobilized AgNPs was detected. Regarding the inhibitory effect on cell growth of *C. fusca*, a growth inhibition of 10% was observed for the microalgae. In view of the results obtained, we consider the use of FBR for the decontamination of DW in concentrations of up to  $1.00 \times 10^5$  CFU/mL of *E. coli* IPT245 efficient. Furthermore, this meets the Brazilian and international environmental regulatory standards, presenting low toxicity for organisms belonging to the first trophic level of the food chain.

## 14.P-Mo-060

### Effectiveness of Green Nanostructured Corrosion Inhibitors on Marine Microorganisms

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#### Abstract

Due to the increasing exploitation of the marine environment, the economic impact triggered by corrosion (CO) has become increasingly evident. The degradation of metals causes: equipment failure, delays in various daily activities, damages in infrastructure, and accidents. About 20% of CO processes are associated with the microorganisms. Microbial induced CO implies a synergistic effects of several species. Corrosion inhibitors (CI), such as 2-mercaptobenzothiazole (MBT) and sodium gluconate (SG), are widely used in protective anti-corrosion coatings, but are released in early life stages of such coatings. To overcome this, CI have been immobilized in stimuli-responsive engineered nanomaterials (ENM), such as layered double hydroxides (Mg-Al LDH or Zn-Al LDH).

Here, we evaluated the toxicity of such novel compounds and their precursors was against 4 bacteria (*Bacillus pumilus* PSBp15, *Bacillus cereus* PSBc15, *Vibrio aestuarinus* PSVa15 and *Vibrio parahaemolyticus* PSVp15), 3 filamentous fungi (*Aspergillus niger* IB-CLP20, *Penicillium polonicum* IB-CLP2 and *Trichoderma harzianum* IB-CLP34) and 1 microalgae (*Chlorella minutissima* 26a), isolated from the marine environment. Microorganisms were exposed to 7 compounds: the innovative ENMs loaded with CIs (Zn-Al LDH-SG, Zn-Al LDH-MBT, Mg-Al LDH-MBT), the CIs (MBT, SG), and the raw ENMs (Zn-Al LDH, Mg-Al LDH), using the concentrations 1.23, 3.7, 11.1, 33.3, and 100 mg/L, for 48/72 hours. The minimal inhibitory concentration (MIC) and minimal bacterial/fungicidal concentration (MBC/MFC) were determined for each species and chemical. Microalgae growth inhibition was determined at 570 nm, every 24h, and values for 50% growth inhibition (EC50) were calculated. All experiments were performed in triplicate.

MBT was the most toxic compound for all microorganisms tested. At 100 mg/L, it presented a bacteriostatic/fungistatic effect on PSBc15, PSVp15 and IB-CLP20, and EC50(72h) of 0.0438 mg/L for 26a. None of the compounds showed bactericidal/fungicidal activity. Only Zn-Al LDH-MBT promoted bacteriostatic effect at concentrations  $\geq 33.3$  mg/L against PSBc15 and PSVp15. In contrast, all compounds were toxic to the microalgae 26a (EC50 (72h): Zn-Al LDH 1.0673; Zn-Al LDH-MBT 0.0737; Mg-Al LDH 2.2049; Mg-Al LDH-MBT 0.5529). These show that nanotechnological-based approaches reduce the negative environmental impact of conventional ICs on marine microorganisms. However, further studies on other microorganisms are still needed.

## 14.P-Mo-061

### Effects of Geraniol and Nanogeraniol on the Bivalve *Anodontites Trapesialis*: Biochemical Biomarkers

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#### Abstract

As an alternative to synthetic pesticides, geraniol has been studied as a potential natural fungicide, and combined with the nanoencapsulation technique that allows a gradual and efficient release of pesticides, the transport of these products into water bodies can be reduced. In this context, the aim of this work was to evaluate, by using biochemical biomarkers, the effects of geraniol and nanoencapsulated geraniol on the neotropical bivalve species *Anodontites trapesialis*. The bivalves were separated in individual beakers and exposed to 4 treatments (n=8 each): control, water containing the surfactant Pluronic F-68® (PLU - 0.0005%), zein nanocapsules, without active compound (NANO - 5 mg L<sup>-1</sup>), nanoencapsulated geraniol (NGER - 5 mg L<sup>-1</sup>) and geraniol (GER 5 mg L<sup>-1</sup>). The animals were exposed for 96 h, with total water renewal after 48 h. After the exposure period, the animals were anesthetized on ice, and killed by sectioning the adductor muscles. Tissues from the gill, mantle, and digestive gland were collected for the analyzes of antioxidant defenses (superoxide dismutase – SOD, glutathione-S-transferase - GST, reduced glutathione GSH) and oxidative damage (lipoperoxidation - LPO). In the digestive gland, it was possible to observe a significant increase in SOD in the GER group relative to the NANO group, and a significant decrease in LPO in the nGER and GER groups relative to the PLU and NANO groups. In the gills, there was a significant decrease in SOD in the nGER group relative to the other groups and a significant increase in LPO in the NANO group relative to the other groups. In the mantle, a significant increase in LPO was noted in the NANO group relative to the nGER and GER groups, and a significant decrease in the GER group relative to the PLU and NANO groups. It was found that geraniol provided a higher activity of enzymatic antioxidant defense in the digestive gland, thus preventing damage to membrane lipids. A trend of increased antioxidant defense was also observed in nGER. The animals exposed to the NANO group, in an opposite way, showed an increase in LPO in the gills and mantle, which may be related to the contact of the nanoparticle. Therefore, it is concluded that nanoencapsulated geraniol has similar properties to geraniol and that the nanocapsule without an active ingredient may be harmful to *A. trapesialis*.

## 14.P-Mo-062

### Do Environmentally Relevant Concentrations of Polyethylene Nanoplastics Induce Developmental Toxicity and Behavioral Changes on Zebrafish?

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#### Abstract

Plastics have been widely produced, leading to their release from the most diverse sources into the aquatic environment. Once in water bodies, plastics can undergo transformations that lead to the form of micro and nanoplastics, which can interact with aquatic organisms, such as fish. The zebrafish (*Danio rerio*) is recommended by the OECD as a model species and is also widely used in various studies. However, little is known about polyethylene nanoplastics (PE-NPs) at environmental concentrations and their effects on zebrafish embryo-larval development and behavior. In this sense, the present study aimed to evaluate the ecotoxicity potential of PE-NPs in zebrafish by evaluating developmental and behavioral biomarkers. PE-NPs (size = 339 nm) were obtained manually from 5g of PE microplastics. The tested concentrations of PE-NPs used were 0.04 and 34 ng/L, considering relevant environmental concentrations, in addition to the negative (reconstituted water) and positive control (3.7 mg/L of 3,4-dichloroaniline). Zebrafish embryos and larvae were analyzed during 144 h of exposure using a multibiomarker assessment (survival rate, hatching rate, spontaneous contraction frequency, and morphological changes and behavioral biomarkers such as average speed, maximum speed, and total distance travelled). Results showed that the static exposure of zebrafish to PE-NPs at environmentally relevant concentrations did not cause significant effects on mortality, hatching rate, or spontaneous contraction frequency in comparison with unexposed group. Also, zebrafish larvae exposed to PE-NPs showed similar behavior (average speed, maximum speed, and total distance traveled) compared to unexposed ones. Despite the previous studies reporting the developmental toxicity of PE-NPs at high concentrations, the current study demonstrated that static exposure to PE-NPs at environmentally relevant concentrations induced no developmental toxicity in zebrafish.

## 14.P-Mo-063

### Biochemical Changes in *Chlorella vulgaris* Exposed to Silver Nanoparticles, Ciprofloxacin, and the Mixture

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#### Abstract

The mixture of silver nanoparticles (AgNPs) with antibiotics such as ciprofloxacin (CIP) is considered an alternative to combat microbial multidrug resistance bacteria. However, the toxicity of both biocides could affect non-target organisms. Microalgae are relevant in aquatic ecosystems since they are the first trophic link. A key issue is to elucidate the effect of AgNPs and CIP when acting together and to study agonistic, synergistic or independent effects. The aim of the present study was to evaluate the biochemical changes in the microalgae *Chlorella vulgaris* exposed to AgNPs and CIP, both individually and in a mixture. The AgNPs were obtained by chemical synthesis, characterized by Dynamic Light Scattering and spectrophotometry alone and in the mixture in the synthetic medium. Microalgae were exposed to 6 concentrations of AgNPs: 58 – 1,85  $\mu\text{g L}^{-1}$ ; of CIP: 0.64 – 62.4  $\text{mg L}^{-1}$  and of the mixture: 49 – 1,59  $\mu\text{g L}^{-1}$  (AgNPs:CIP ratios 1:1). All assays were performed in triplicate and with negative control. The assays (96 h) were performed under controlled conditions (modified Johnson's medium, 6000 lux;  $23 \pm 2$  °C; initial concentration =  $10^4$  cells  $\text{mL}^{-1}$ ). Effects of AgNPs, CIP, and the mixture on protein (Coomassie brilliant blue method), carbohydrate (Dubois's method), and total lipid content (sulfo-phospho-vanillin reaction) were investigated. Catalase (CAT), glutathione S-transferase (GST), and the level of lipid peroxidation (malondialdehyde, MDA) were determined. The AgNPs showed an absorbance peak at  $\lambda_{\text{max}} = 395$  nm, an average size of  $47 \pm 3$  nm, and a zeta potential of  $-14$  mV. In the synthetic medium, the nanoparticles alone and in combination with CIP showed a  $108 \pm 31$  and  $135 \pm 53$  nm average diameter and  $-7,4 \pm 1$  and  $-12 \pm 3$  mV zeta potential, respectively. *Chlorella* cells exposed to AgNPs showed, at all concentrations, an increase in the presence of lipids and carbohydrates ( $p < 0.001$ ,  $p < 0.05$ ) as well as changes in the activity of CAT and GST ( $p < 0.001$ ) and a significant increase in the presence of MDA ( $p < 0.001$ ), an indicator of lipid peroxidation. *Chlorella* exposed to CIP showed only an increase in the amount of lipids ( $p < 0.001$ ) but no change in MDA and an alteration in both enzymes ( $p < 0.001$ ). However, in the mixture, no significant changes were found in any of the parameters analyzed. The mixture was antagonistic. Further studies are required to evaluate these emerging contaminants alone and in combination on different non-target aquatic organisms.



## 14.P-Mo-064

### Insights Into the Toxicity of Carbon Nanofibers in Developing Zebrafish (*Danio rerio*)

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#### Abstract

Carbon-based nanomaterials (CNMs) are applied in several sectors of society, mainly in manufacturing electronic equipment, antifouling paints, and drug delivery. Among the CNMs, carbon nanofibers (CNFs) stand out due to their use in energy storage, biomedicine and the environment. However, knowledge of the ecotoxicological potential of CNFs, especially for aquatic vertebrates, is still limited. Thus, the present study aimed to evaluate the ecotoxicity of CNFs during the early developmental stages of zebrafish (*Danio rerio*). The zebrafish embryo-larval toxicity test and cell death assessment were performed after semi-static exposure to five concentrations of CNFs (6.12, 12, 25, 50 and 100 mg/ L) for 144 hours, jointly with negative and positive control groups. CNFs reduced survival and induced neurotoxic (reduced frequency of spontaneous contractions) and cardiotoxic (increased heart rate) effects, while no significant effect was observed on the hatching rate. Also, CNFs induced morphological abnormalities in zebrafish embryos and larvae, including pericardial and yolk sac edema, tail and vertebral column changes. Zebrafish larvae exposed to CNFs (100 mg/L) showed an increase in cell death, indicating the cytotoxic potential of these nanomaterials. The current study pioneers analyzed the potential ecotoxicological risk of CNFs for the health of aquatic vertebrates, especially during the early developmental stages.

# Session 15: Toxic Metal and Metalloids Pollution in Surface and Groundwater Hydric Resources

15.P-Mo-065

## Isotopic Analysis of Cadmium Present in *Mytilus chilensis* and Sediments of Chiloé Island, Southern Chile

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### Abstract

Chile has become the world's second-largest producer of cultured mussels, developed intensively in the Chiloe Island. The mussel industry faced the occurrence of high concentrations of Cd in the tissues of mussels, with the main source being unknown. Cd can be of geogenic or anthropogenic origin. The former can come from the soil, volcanic activity, or mineralization, while the latter is produced by hazardous wastes, mining, smelting, crude oil refining, coal combustion, waste incineration, agricultural activity, etc. The purpose of this study is to identify whether there are differences in the isotopic signals of cadmium present in sediments and the tissue of *Mytilus chilensis* to determine the possible origin of Cd present in mussels. For this purpose, sampling was carried out in 5 mussel farming areas, and muscle and stomach samples were extracted for Cd isotope analysis ( $\delta^{114}\text{Cd}$ ), which represents 41% of the seven stable cadmium isotopes present in nature, using an ICP MS Multicollector. The results show differences in the isotopic signal of the sediment and mussels, indicating that this is probably not the main source of Cd entry into the organisms. Isotopic signals can also be distinguished at different sampling sites, indicating that Cd incorporation processes may come from other sources in the environment, which are probably not the sediments, and show Cd fractionation in the mussel. The Cd isotopic signature ( $\delta^{114}\text{Cd}$ ) varies between -0.524 and 0.253 in mussels, while it is almost stable in sediments with values between -0.020 and -0.141, which should be considered the same value according to the analytical error. In mussels, there is a strong effect of Cd concentration on  $\delta^{114}\text{Cd}$ : the relationship between Cd concentration and  $\delta^{114}\text{Cd}$  follows an exponential law. This is probably due to the fractionation of Cd during bioaccumulation and the complexation of Cd in mussel bodies. The  $\delta^{114}\text{Cd}$  is not influenced by the position of mussels in the water column or by the age of the mussels. Clearly, the Cd concentration in mussels is controlled by the Cd in solution in seawater, which varies, very likely, between the different sampling sites. Also, as the Cd concentration in sediments is low compared to that of mussels, it is very likely that Cd in seawater is present in inorganic form. The concentration of Cd in sediments is in the range of uncontaminated sediments. Supported by ANID Millennium Science Initiative Program ICN 2019\_015.

## 15.P-Mo-066

### Trophic Dynamics of Selenium in the Alkaline Omak Lake, Washington, USA

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#### Abstract

The role of selenium (Se) as an essential micronutrient that in excess is toxic has been amply studied. Se toxicity in aquatic food webs is related to bioaccumulation leading to biomagnification. This is mainly dietary, but Se cycling is another essential factor. Selenite and selenate are species produced when alkaline and oxidizing conditions prevail in watersheds facilitating uptake. Omak Lake is the largest alkaline lake in Washington state and its Se content is unknown. Hence, this study aimed (i) to investigate Se in Omak Lake's food web, (ii) determine if food web biomagnification occurred (iii) inspect fish feeding, age, and habitat influencing Se uptake, and (iv) compare aquatic and terrestrial prey sources to determine if Se concentrations between prey differ. In tissue, Se was assayed via ICP-MS, and  $\delta^{15}\text{N}$  was used to assign trophic positions; then the trophic magnification factor model was used to determine if biomagnification occurred. We found that Se concentrations were below the toxicity thresholds for all fish and invertebrates analyzed. However, evidence of biomagnification was only present in the apex predator, Lahontan Cutthroat Trout (LCT), and prey fish. Sculpin spp. had the highest Se concentrations detected. Se content was not significantly different when analyzing LCT by age nor all fish habitats. Although Se concentrations in aquatic and terrestrial invertebrates were not significantly different, they suggested terrestrial prey could be a significant supplemental Se source. Further examination of littoral and benthic aquatic invertebrates suggested that littoral prey might be an important source. Overall, the effect of alkaline water over Se cycling and biomagnification was observed as expected. However, it was apparent that Se dietary sources are the main driver of biomagnification.

## 15.P-Mo-067

### Evaluation of Physiological and Genotoxic Effects of Arsenic on Rainbow Trout Fingerlings (*Oncorhynchus mykiss*)

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#### Abstract

Arsenic (As) is a natural contaminant widely distributed in surface waters either as AsIII or AsV, which may be toxic to aquatic such as the rainbow trout (*Oncorhynchus mykiss*), a farmed fish with commercial importance worldwide. The aim of this work was to evaluate the effect of AsIII on *O. mykiss* fingerlings. We performed *in vivo*, *ex vivo*, and *in vitro* assays using 240 fingerlings of  $25.37 \pm 0.5$  mg and  $1.84 \pm 0.48$  mm from the Center for Applied Ecology of Neuquén (CEAN). For the *in vivo* assays, we sorted the fingerlings in 4 glass flasks with 15 individuals each (2 control flasks and 2 flasks with  $3.85 \mu\text{M}$  AsIII). After 48 h, we extracted the blood to determinate micronuclei (MN) and nuclear aberrations (NA) in erythrocytes. Also, we dissected gills, liver, foregut, posterior intestine, and caudal muscle, to determine the As bioaccumulation. For the *in vitro* assays, we used suspensions of isolated enterocytes pooled from 15 individuals. The enterocytes were incubated for 1 h at AsIII increasing concentrations from 0 to  $7.7 \mu\text{M}$  AsIII. We analyzed the lysosomal membrane stability by recording the Neutral Red Retention Time of 50% of the cells observed (NRRT50). We evaluated the effect of AsIII over ATP-binding cassette transporters using the calcein efflux technique in intestine strips. For this purpose, the middle intestine was extracted, weighed, and incubated in glass tubes with AsIII increasing concentrations from 0 to  $77 \mu\text{M}$ , plus  $0.25 \mu\text{M}$  calcein-AM. As a result, we did not obtain differences for MN and NA between the two treatments at 48 h. We observed As bioaccumulation only in gills ( $0.46 \mu\text{g As / g tissue}$ ) and muscle ( $2.77 \mu\text{g As / g tissue}$ ). From a non-linear regression model NRRT50 vs. log AsIII concentration, we observed a concentration-dependent effect of AsIII on lysosome membrane stability, with an EC50 of  $0.37 \mu\text{M}$  AsIII. We observed an AsIII concentration-dependent effect (IC50 =  $5.48 \mu\text{M}$  AsIII) over the calcein transport rate in a non-linear regression model, which indicate a competitive inhibition of ABC transporters. All these results indicated that a) AsIII probably enters the *O. mykiss* organism through the gills, b) AsIII is also capable of entering the intestine cells since it causes deleterious effects observed in lysosomal membrane stability of enterocytes, c) no detection of As in the intestine probably reflects detoxification by ABC toward the external medium or systemic circulation, and d) AsIII is accumulated in the muscle.

## 15.P-Mo-068

### Effects of the Settleable Atmospheric Particulate Matter on the Osmoregulatory Responses of *Perna perna* Mussels

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#### Abstract

The precipitation of the metals from the settleable atmospheric particulate matter (SePM) in the aquatic environment can harm such environment, being able to affect the aquatic organisms' physiology. The mussel species *Perna perna* has been widely used as bioindicator due its wide distribution and feeding habits. The aim of this study was to analyze the effects of three different concentrations of SePM (0.01; 0.1; 1 g L<sup>-1</sup>) on the activity of enzymes involved in osmoionoregulatory processes (Na<sup>+</sup>, K<sup>+</sup> ATPase, H<sup>+</sup> ATPase, and carbonic anhydrase) in the gills, through acute time (T0, T2, T4 and T7 days) and chronic (T15 and T30 days) exposures, in addition to a 60-day depuration period. The results showed that the carbonic anhydrase enzymes exposed to SePM varies between time and concentrations, where the 0.01 g L<sup>-1</sup> caused a decrease in T7 in relation to control. The higher SePM concentration caused an increase in the Na<sup>+</sup>, K<sup>+</sup> ATPase in relation to 0.1 g L<sup>-1</sup> in T4, while 0.1 g L<sup>-1</sup> caused a reduction for T4 in relation to the other exposure times. The H<sup>+</sup> ATPase enzyme showed changes just for the 1 g L<sup>-1</sup> SePM, where decreases in T30 were observed in relation to T4. In aquatic molluscs, the gills are a key interface for the absorption of metals dissolved in water. Several metals appear to target specific ion absorption channels in the gills, probably using these channels as an entry route through "ionic mimicry". Through this mimicry, metals can reduce the absorption of an essential nutrients, that could be compensate by the alterations of the osmoionoregulatory enzymes, as demonstrated in the present work. Financial support: FAPESP Grant 2019/08491-0.

## 15.P-Mo-069

### Use of Oxidative Damage Biomarkers to Evaluate the Effects of Iron in Different Species of Brazilian Corals

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#### Abstract

Coral reefs are subjected to climate change and are severely impacted by human activities. Iron (Fe) is an essential metal but in excessive concentrations, it can increase the production of highly reactive and toxic species causing a range of biological damage. The ecological relevance of parameters measured at biochemical or cellular level is now considered an extremely important feature in environmental studies, and can be used as early warning signs of environmental degradation. In this context, the effects of acute (96h) and chronic (28 days) exposure to Fe were assessed on maximum photochemical efficiency of zooxanthellae (Fv/Fm) and the response of biomarkers related to oxidative damage [lipid peroxidation (LPO) and carbonyl protein (PCN)]. The corals *Mussismilia harttii*, *Siderastrea stellata* and *Millepora alcicornis* were exposed to different concentrations of dissolved Fe (0, 100, 300, 900  $\mu\text{g}\cdot\text{L}^{-1}$ ) using two different experimental approaches: a laboratory closed system and a marine mesocosm system. Fv/Fm values were not affected by exposure to Fe in any of the exposure systems. However, in the laboratory closed system a significant increase in LPO was observed in *M. harttii* exposed on the highest concentrations. PCN significant increase were also observed in *M. harttii* and *M. alcicornis* on lowest concentrations. These findings indicate that exposure to dissolved Fe increases susceptibility to bleaching, however the species tested showed a high capacity for physiological regulation, given the absence of damage in the chronic experiment.

## 15.P-Mo-070

### Effect of Biochar Amendment on Pb-Polluted Agricultural Soils in a Mid-Term Experiment

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#### Abstract

Heavy metal contamination of soils is one of the most serious environmental problems today. Recently, numerous studies have focused on the use of biochar (BC) as an amendment of polluted soils to prevent the migration of contaminants to crops. BC is a carbon-rich product obtained from biomass by pyrolysis. Its effects depend on the starting organic matter, pyrolysis temperature, soil characteristics, and the contaminant. In addition, these effects may vary over time with BC aging.

This work aimed to study the effect of BC application, produced at different temperatures from peanut industry residues, in an Pb polluted agricultural soil, following its evolution for 36 months. For this purpose, the soil was collected from a polluted site in Córdoba province. It was dried, homogenized, and distributed among 4 treatments: polluted soil without amendment (Pb); polluted soil with BC produced at 300 °C (BC300); at 400 °C (BC400); and at 500 °C (BC500). The soils were incubated (darkness and 70% of their water-holding capacity) for 18 months, with sampling every 9 months. They were then subjected to agricultural use, soybean crop, for two cycles, with soil samples taken after each harvest. Sequential extraction was performed on the samples to determine the concentration of Pb in different soil fractions: (i) exchangeable; (ii) bound to carbonates; (iii) bound to Fe and Mn oxides; (iv) bound to organic matter; (v) pseudo-residual. Fractions (i) and (ii) are interpreted as bioavailable.

Regardless of the time course, BC application produced significant effects on the Pb concentration in most fractions. The 3 BCs caused an increase in fraction (i) and a decrease in fractions (ii) and (iii), BC300 and BC400 also caused a decrease in fraction (v). It is important to note that the decrease in fraction (ii) was smaller than the increase in fraction (i), so that, in contrast to what was expected, the bioavailability of Pb was increased with the amendment. The Pb distribution in soil fractions evolved over time, with sustained increases observed in fraction (i) for treatments BC300 and BC400, in fraction (v) for all experiments, and in fraction (iii) during incubation for all treatments. Fraction (ii) showed a gradual reduction over time for all treatments.

In conclusion, the application of BC produced from peanut shells modified the distribution of Pb in the soil fractions, but these changes are not optimal to reduce the Pb bioavailability for the crops grown there.

## 15.P-Mo-071

### Mercury in Fish Collected From the Gold Mining Area of the San Martín De Loba and Its Great Impact in the Bay of Cartagena in Colombia

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#### Abstract

Mercury (Hg) is a heavy metal with a high environmental impact given its toxicity and biomagnification capacity. In sediments, Hg is methylated by bacteria to methylmercury, which is its liposoluble specie with the ability to accumulate in organisms, allowing its transfer through the trophic web until it reaches humans. Artisanal and small-scale gold mining (ASGM) is one of the largest sources of anthropogenic mercury emissions in the world. In Colombia, ASGM are located mainly in the departments of the Antioquía and Bolívar which discharges Hg near the Cauca and Magdalena rivers transporting Hg pollution through the Canal del Dique to the Bay of Cartagena. Carnivorous and non-carnivorous fish were collected near ASGM in the San Martín de Loba and the Cartagena Bay. The samples were analyzed by thermal decomposition in an atomic absorption spectrometer with Zeeman-effect background correction technique, RA 915M, and PYRO 915+. The LOD (0.0025 mg/kg), LOQ (0.0085 mg/kg), calibration curve ( $R \geq 0.995$ ), accuracy and precision of the CRMs DORM-4: Fish Protein Certified Reference Material (NRC, Canada) were 99.6% and CV% of 6,40%, respectively assessed as a quality control of the measurements. On average, the total concentrations of mercury (T-Hg) expressed in wet weight for the fish collected in the municipality of San Martín de Loba were, mean  $\pm$  SD (range),  $0.159 \pm 0.138$  (0.003 – 0.735) mg/kg for all fish and for carnivorous fish were  $0.200 \pm 0.113$  (0.003 – 0.735) mg/kg while for fish collected in the Bay of Cartagena and surrounding areas were  $0.187 \pm 0.185$  (0.009 – 0.771) mg/kg and Carnivores  $0.197 \pm 0.184$  (0.036 – 0.771) mg/kg. T-Hg concentrations in this study were similar to those determined in fish from other ASGMs in Latin America, such as in the Tapajós River in Brazil had  $0.412 \pm 0.321$  (0.032 – 2.25) mg/kg and Puerto Maldonado in Peru  $0.2716 \pm 0.3543$  (0.0013 – 1,128) mg/kg. In conclusion, part of the mercury pollution produced by mining is received in the Bay of Cartagena through the Canal del Dique, affecting the marine ecosystem and being a problem for communities with direct exposure to this metal through the fish consumption.



## 15.P-Mo-072

### Metals and Metalloids in *Eruca Sativa* Produced Under Different Conditions and Their Potential Risk to Human Health

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#### Abstract

Wastewater, as well as organic and inorganic fertilizers used in agricultural activities, can contain a variety of pollutants that can accumulate on plants of food interest and pose a health risk to consumers. This study aimed to evaluate the effect of irrigation with contaminated water and the application of manure on horticultural crops, in comparison to conventional fertilizers, regarding the content of Mg, Ca, V, Cr, Mn, Zn, Fe, Co, Ni, Cu, As, Se, Rb, Sr, Mo, Ag, Cd, Ba, Hg and Pb. For that purpose, *Eruca sativa* (arugula) was grown under different treatments in the laboratory (n=3). In control arugula grew in commercial soil and was irrigated with potable water. In treatments 1 and 2 arugula grew in a substrate amended with 10% of manure and 2% of urea respectively, and both were irrigated with residual water + metals (max. levels allowed in irrigation water): Pb (200  $\mu\text{g L}^{-1}$ ), Cr (100  $\mu\text{g L}^{-1}$ ), As (100  $\mu\text{g L}^{-1}$ ), Cd (10  $\mu\text{g L}^{-1}$ ) and Hg (1  $\mu\text{g L}^{-1}$ ). Finally, in treatments 3 and 4 arugula grew in a substrate amended with 10% of manure and 2% of urea respectively, but were irrigated with potable water + metals: Pb (100  $\mu\text{g L}^{-1}$ ), Cr (50  $\mu\text{g L}^{-1}$ ), As (50  $\mu\text{g L}^{-1}$ ), Cd (5  $\mu\text{g L}^{-1}$ ) and Hg (0.5  $\mu\text{g L}^{-1}$ ). Irrigation water, soil/manure and harvested plants (2 months) were analyzed for multi-elemental content using ICP-MS. The human health risk associated with the consumption of arugula produced was assessed using estimated daily intake. The residual water presented a higher concentration for most of the elements than the potable water, except for the intentionally added metals that met their nominal concentration. Overall, the multi-elemental profile of the substrates did not differ significantly between treatments. However, different patterns of element accumulation were observed in the plants according to the treatments. In particular, toxic elements such as Cr (2>1>3 and 4>C), As (1>2>3> 4 and C) and Cd (2>1>3> 4>C), were found to accumulate at higher concentrations in plants irrigated with the highest concentrations of these elements. Mercury was accumulated in vegetables grown under the different exposure treatments, except in controls. Based on the results obtained from estimated daily intake calculation, the use of contaminated water in the irrigation of arugula, as well as the application of synthetic fertilizers and manure, do not represent a risk to the health of the consumer concerning the content of inorganic pollutants, under these conditions.

## 15.P-Mo-073

### Complexation and Precipitation Mechanisms During Heavy Metal Adsorption on a Variety of Biochars

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#### Abstract

Since the industrial revolution, pollutant emissions have increased exponentially, generating significant environmental problems in nature. Nowadays, there are several methods for air, water and soil remediation. Biochar (BC) is a material obtained by pyrolysis of organic matter, rich in recalcitrant carbon, with a high cation exchange capacity and a large surface area. BC has great potential for the remediation of soils and water contaminated with heavy metals (HMs) because it immobilizes them by adsorption on its surface. The adsorption mechanisms vary from one BC to another, and may be different for each HM.

In this work, two adsorption mechanisms (complexation and precipitation) of HMs (Cd, Cu, Pb and Zn) were studied on BCs produced at 3 pyrolysis temperatures (300, 400 and 500°C) from soybean straw, peanut husks and brewer's malt. Each of the BCs was loaded with each of the HMs separately by batch adsorption assays with aqueous solutions. To study the complexation mechanism, FTIR spectra of BCs with and without HMs were compared. To study the precipitation mechanism, X-ray diffraction technique was used.

The results obtained by FTIR show that the richness of functional groups decreases with increasing production temperature. That is why the mechanism of complexation with functional groups was more important in BCs produced at lower temperatures. The functional groups with the highest participation in the adsorption of HMs were C=O, C-O, C=C, COOH and COO<sup>-</sup>. Soybean straw, peanut husks, and their respective biochars showed participation of the complexation mechanism for all 4 HMs; malt BCs did not show this mechanism although un-pyrolyzed malt did.

The results obtained by X-Ray diffraction showed that the precipitation mechanism of HMs is influenced by both the raw material used and the pyrolysis temperature. In the soybean BC produced at 400 °C only Pb precipitation occurred, while in the one produced at 500 °C Cd and Cu were also precipitated. In the peanut shell BC no precipitation phenomenon was observed. In the malt BC, precipitation was observed only for Pb, being more noticeable with increasing production temperature.

In conclusion, soybean BCs must be recommended for soil and water remediation considering that they presented the highest adsorption efficiency for the 4 HMs and showed a greater participation of the complexation and precipitation adsorption mechanisms, both important for immobilization stability.

## 15.P-Mo-074

### **Metal Bioaccumulation in Muscle and Liver of the Migratory Grey Mulletts (*Mugil liza*, Valenciennes, 1836) From Southern Brazil**

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#### **Abstract**

Metal contamination stands out for concerning due to their potential of bioaccumulation and biomagnification in aquatic food chains, which depends on the physical and chemical nature of the metal, and the ecological parameters of the aquatic species. Fish is an important source of proteins and high quality nutrients for population in southern Brazil, especially during the migratory season of grey mullet (*Mugil liza*). Therefore, the assessment of bioaccumulated levels of metals in fish tissues allows the establishment of quality parameters for fish consumption besides their bioavailability in the ecosystems. The present study determined the levels of cadmium (Cd), lead (Pb), zinc (Zn), and copper (Cu) in muscle and liver samples of grey mullets from the southern fish stock (shared by Argentina and Brazil), which has a catadromous life cycle. Fish were collected by net-casting fishers at Laguna Estuarine System, located in Santa Catarina state, southern Brazil. 27 specimens of grey mullets (mean weight: 847 g) were collected during the 2022 artisanal mullet season (May–June); 17 were male and 10 female. Analysis of metals were performed in an atomic absorption spectrophotometer and our primary analysis in muscle samples showed that the concentrations of Cu and Zn were above the detection and quantification limits, while Cd and Pb were below the analytical capacity. In liver tissue, all samples were above these limits, except for Pb, which was excluded for further analysis. The metal trend pattern observed in the liver samples was Cu > Zn > Cd, with the highest values found in mg.Kg<sup>-1</sup> wet w. being 3.081.64 for Cu, 144.17 for Zn and 3.21 for Cd. Overall, our data showed that the mean concentration of all metals in liver exceeded the maximum permissible levels allowed by the Brazilian Health Regulatory Agency Guidelines (Anvisa 1965; 2013), which is 50 mg.Kg<sup>-1</sup> for Zn, 30 mg.Kg<sup>-1</sup> for Cu and 0.1 mg.Kg<sup>-1</sup> for Cd. Contrariwise, muscle showed the lowest concentrations of metals (mean Cu 0.43 mg.Kg<sup>-1</sup> and 5.21 mg.Kg<sup>-1</sup>) and were within set limits for human consumption. Although the liver is an important tissue of metal detoxification, long-term metal exposure can overcome the fish's ability to regulate and excrete metals, resulting in higher loads for natural top predators, such as marine mammals. Thus, the use of migratory grey mullets is of real interest to increase our understanding of ecotoxicological effects and potential risks to wildlife and aquatic environments.

## 15.P-Mo-075

### **Mercury Distribution in Tissues of Layard's Beaked Whale, *Mesoplodon layardii* (Gray, 1865)**

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#### **Abstract**

Mercury input into the ocean is determined by both anthropogenic activities and natural geological sources. This biomagnifies in the trophic chains as a product of its bioaccumulative nature of toxic concentrations, close to organisms at higher levels. Among the key species that can be used to assess the level of bioavailability of these pollutants in ecosystems are marine mammals and, in particular, small cetaceans, since they are considered sentinel species for ocean health thanks to its biological and ecological characteristics. Also, exposure to mercury coupled with a limited ability to metabolize and eliminate contaminants, results in large accumulations in the tissues of exposed organisms. This exposure to the metal can be assessed through its concentration in the organs and tissues in which it accumulates, such as the liver. In this sense, odontocetes cetaceans such as beaked whales could represent ideal bioindicator species for persistent compounds in the Chilean marine ecosystem, due to their deep diving and the consumption of high trophic level prey. The beaked whales are one of the groups of odontocetes whales that currently have less information and in fact, it is classified as an insufficiently known species by the International Union for Conservation of Nature (IUCN), this is because their sightings are limited and their encounters are a special or fortuitous occasion. Furthermore, the information on heavy metals in the tissues of this species is quite scarce. Therefore, the objective of this study is to determine the distribution of mercury in tissues of a specimen of *Mesoplodon layardii* using a Direct Mercury Analyzer (DMA-80). Here, high concentrations of mercury are evident in nine tissue samples from the specimen, where the liver was the significantly more important accumulated organ with a value of 427 mg/kg dw and 243 mg/kg ww, followed by the kidney and lung. These results exceed the values found for other cetacean species and exceed reported liver toxicity levels, providing new baseline information on mercury exposure in *Mesoplodon layardii*. There is a large information gap on the levels of contaminants in marine mammals around the world, especially along the Chilean coast, and continuous tracing of their populations is recommended, monitoring the presence of contaminants in individuals and their exposure with the intention of developing strategies for its conservation.

15.P-Mo-076

## Atmospheric Conditions in the Region of Vitória-ES, Brazil, Impacted by Metallurgical Industries Using *Tradescantia pallida*

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### Abstract

The capital of Espírito Santo, Vitória/ES, has been impacted by atmospheric particulate matter contaminated with metals and metalloids. The bioaccumulation of these metals into the cell affects several cellular functions. Based on this issue, this study aimed to evaluate the effects of metals and metalloids accumulation from atmospheric pollution in the cities of Vitória-ES and Governador Lindenberg-ES, in *Tradescantia pallida* cells. For this, collections of *T. pallida* leaves were carried out at two points in the city of Vitória, Ilha do Boi (I.B) and Enseada do Suá (E.N), impacted by metallurgical activities, with the E.N also impacted by vehicular sources, and at a point in the city of Governador Lindenberg (G.L), located in the Northwest region of the state of Espírito Santo, which suffers little industrial and vehicular influence. The metals internalization in the leaves was analyzed with ICP-MS and qualitative analysis by scanning electron microscopy (SEM). The biological effects were analyzed by the quantification of chlorophyll a, b, total pigments, carotenoids and anthocyanin and by DNA strand breaks (genotoxicity effects). The results demonstrate a significant internalization of Fe and Y in the I.B and of the metals Cr, Se, Y, La, Ce and Pb in the E.N compared to the GL station - metals released mainly by the metallurgical industries. With regard to biological damage, a significant decrease in chlorophyll b, total and carotenoid pigments was observed in the two points of Vitória-ES. The anthocyanin increase and genotoxic damage showed no significant difference. However, multivariate correlation analysis showed a positive correlation between the presence of metals and increased anthocyanin and genotoxic damage. Thus, these results indicate that atmospheric pollution from metallurgical sources affects the accumulation of metals in the leaves of *T. Pallida*, causing changes in photosynthesis and genetic material.

## 15.P-Mo-077

### **Spatial Distribution and Pollution Risks by Heavy Metals and As in Bottom Sediments of Uruguayan Coastal Lagoons**

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#### **Abstract**

Heavy metals and As that reach bottom sediments of coastal lagoons are of great environmental concern and a growing problem worldwide; being human activities (e.g.: industry and agriculture) the main ways that these elements enter into the ecosystems. Therefore, the study of the geochemistry, distribution and quantification of metallic elements in aquatic sediments provide information on environmental changes related to anthropic activities, denoting the risk associated and the anthropogenic impact on natural environments. The concentration of some anthropic metallic elements (As, Cd, Cr, Cu, Ni, Pb, Zn) were analyzed in bottom sediments of Uruguayan coastal lagoons of the Atlantic coast (Laguna Garzón, Laguna de Rocha and Laguna de Castillos). The goal of this work was to evaluate, for the first time, the possible heavy metals pollution in their bottom sediments by applying several geochemical index methods (e.g.: potential ecological risk (PER), contamination factor (CF), pollution load index (PLI) and index of geoaccumulation (Igeo)) together with multivariate ordination and classification methods. The relative spatial distribution of most heavy metals was similar, with highest concentrations in the central zone of the lagoons, while the lowest values were found southern and northerly. The average of heavy metal concentrations followed the descending order: Zn > Cr > Cu > Ni - Pb > As > Cd. Significant positive correlations were found between heavy metals, total organic matter and mud. Furthermore, we found significant differences between stations, denoting some areas which exhibit any degree of pollution risks. Using pollution indicators such as CF, Igeo and PLI, most of the stations were unpolluted. Also PER values for analyzed metals in almost stations showed low and moderate ecological risk, but some areas of Rocha lagoon showed values denoting warning, where an incipient significative ecological risk exist, indicating progressive deterioration of the sediment quality. In summary preliminarily, the extent of pollution by heavy metals in bottom sediments of Uruguayan coastal lagoons implies that the environmental condition is relatively stable, but attention is required in order to prevent the increment of the metal enrichment in these ecologically important coastal aquatic environments.-

## 15.P-Mo-078

### Arsenic High Levels in Raigon Aquifer at Kiyu, Libertad and Santa Regina Localities

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#### Abstract

According to O.S.E. (Obras Sanitarias del Estado) data base (2010-2020 period) provided in terms of the Law number 18.381 to access to public information, arsenic (As) concentrations in Raigon Aquifer available data, for consuming water were analyzed and mapped about Raigón Aquifer System, showing detected levels of As above the maximum value recommended by WHO (0,01 mg/L) and in some cases above the UNIT limit (0,020 mg/L), with maximum values of 0,042 mg/L in Kiyú. The objective of this research is to understand and concientizise the population about the risk of these values that are above the standards limits.

In Santa Regina (Colonia) values above 0,010 mg/L but below 0,020 mg/L are encountered.

In Kiyú (San José) most of the wells arise As values above 0,010mg/L, and only two wells with values above 0,020 mg/L. but one of them is above 0,040 mg/L. At Libertad (San José), all values are between 0,010 and 0,020 mg/L.

Although surface distribution had not interpretable results, diferencial values in respect to well depth was encountered. From another point of view it was not observed a quantitative correlation between As and other trace elements: Pb, Cr, Cd, Se y Cu, which are toxic too.

This situation diagnosis implies that there might continue the studies about toxic elements in groundwater sources which are used in human consumption and try to give a solution to the exposure risk to them.

## Session 16: Microplastics

16.PA-Mo-079

### Microplastics in the Diet of *Hypostomus Ancistroides* (Ihering, 1911) in a Sewage Treatment Effluent (ETE) Stream, Paraná III Basin

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<sup>3</sup>A Universidade Tecnológica Federal do Paraná - Campus Dois Vizinhos, Brazil

#### Abstract

Microplastics are ubiquitous in aquatic and terrestrial ecosystems. Plastic products originate from petroleum-derived resins and are characterized by their easy dispersion, high durability, resistance to high pressure and radiation. These products have been improperly discarded and reach the most varied environments around the globe, becoming a major threat to biodiversity and public health. In the last decade, the number of studies that report the occurrence and impacts of microplastics in different organisms and ecosystems has increased exponentially. In this sense, this study aimed to evaluate the incidence of these polymers in the diet of *Hypostomus ancistroides*, a fish of the Loricariidae family. This species has a detritivorous feeding habit, obtaining algae, insect larvae and detritus through the behavior of scraping the substrate. Therefore, we hypothesize that this habit makes the species more susceptible to the accidental consumption of microplastics, since these particles tend to be deposited in the beds of water bodies. Fish were collected in a stream that receives effluents from a water treatment plant (ETE) in the municipality of Cascavel, Paraná, Brazil, in March 2017, using the electric fishing technique. Through the volumetric method, the gastrointestinal content was analyzed by investigating the occurrence of synthetic particles and diet analysis with the aid of optical microscopy. 32 gastrointestinal contents were analyzed, which resulted in the registration of 152 plastic waste classified into categories (fibers and particles) and by the corresponding color (transparent, blue, red, black, white and silver). We verified that 84% of the individuals analyzed ingested plastic polymers. Fibers were more abundant (24.34%) than particles with 9.87%. These results reveal that the stream that receives effluent from the sewage treatment plant is affected by microplastics and the fish present there ingested these particles along with their food. These findings represent a major concern considering that large-scale microplastics can endanger all biodiversity.



## 16.PA-Mo-080

### Monitoring Microplastic Pollution in Callao Bay, Peru: A Low-Cost Approach using LADI Trawl

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#### Abstract

Microplastic contamination is a growing problem that threatens various species in various environmental compartments at both the micro and macro levels. The purpose of this study is to quantify the presence, distribution, and characteristics of microplastics in the surface waters of Callao Bay, located on the central coast of Peru in South America, where more than 100 particles per cubic meter were identified. A cost-effective, homemade Low-Tech Aquatic Debris Instrument (LADI) trawl was used for sampling, costing approximately USD 150 to manufacture. The LADI trawl was modified to use anti-aphid nets with a size of 300-micron pore size (#50 mesh).

Samples were collected from a 2 km stretch of surface water, parallel to the shoreline, at a speed of 1.5 to 2 knots. The measurements were made in a single day in mid-February 2023. At the beginning of each sampling, pH, conductivity, and dissolved oxygen measurements were taken. The six collected samples were stored, separated, filtered, dried, and analyzed by visual inspection and weighing of the particles, which presented various shapes and colors.

The findings highlighted the mouth of the Chillón River as the area with the highest incidence of particles, with 4.4 million particles per square kilometer or 82 particles per cubic meter. This research not only improves the understanding of microplastic contamination in the surface waters of Callao Bay but also underscores the urgency of addressing this pressing environmental challenge. The use of a low-cost homemade LADI trawl net demonstrates the potential for affordable and cost-effective solutions to monitor and investigate microplastic contamination, furthering a greater understanding of this critical environmental issue.

## 16.PA-Mo-081

### Identification of Additives and Plasticizers in Marine Litter, Gulf of Arauco, Central Chile: Polyethylene (PE), Polypropylene (PP), Macroplastics (MCPs)

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#### Abstract

Macroplastics (MCPs) play an important role in the availability and mobility of additives as they fragment in the environment, and consequently can be a source of these contaminants, among the most used plasticizers are phthalates and adipates used to optimize the properties and useful life of plastics. In this study we report the presence of plastics additives, in microplastic samples from the Gulf of Arauco (Spring 2021 and summer season 2022) in central Chile. Samples were analyzed using the technique of Fourier transform infrared spectroscopy (FTIR) and attenuated total reflectance (ATR) (Jasco Brand Model FT/IR-4600LE) together with a NICODOM brand IR library of polymers and additives for the identification of molecular structures present in samples of MCPs. Preliminary results indicate that the plasticizer most frequently detected is dioctyl phthalate, corresponding to 33.33% (n=10) of the samples identified. In addition, the most frequent polymers analyzed were polyethylene (PE) and polypropylene (PP). The presence of these additives and plasticizers in the environment can pose a risk to organisms and represent a threat to higher trophic levels, including people. Further research is needed to assess additives as indicators of the traceability of the presence of the polymer in the environment.

## 16.PA-Mo-082

### Passive samplers to trap microplastics in the atmosphere of Concepcion city in Central Chile: HYSPLIT (Hybrid Single Particle Lagrangian Integrated Trajectory Model)

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#### Abstract

Nowadays, microplastics have been detected in the atmosphere of urban and remote areas, suggesting possible long-range atmospheric transport, and therefore, there is growing interest in understanding and analyzing the fate, transport and effect of atmospheric microplastics in the environment. In this study we used passive air sampler to detect microplastic particles in the Tres Pascualas campus of the Universidad San Sebastián in Concepcion city. The samples obtained at different sampling points (areas of high and low flow of people) and were analyzed by means of a physical characterization according to the color, shape, and size of plastic particles. Samples were identified through optical microscopy and a chemical characterization through Fourier transform infrared spectroscopy (FTIR). In addition, possible sources were identified through retrospective air mass modeling in the sampling area with the HYSPLIT program. The results showed that the occurrence of plastic particles most frequently corresponds to the type of plastic fibers of size between 5mm to 0.001mm, and 25% of the total samples analyzed were plastic (polyester) and the predominant color is blue (30%) of the total occurrences of particles. HYSPLIT model showed a prevalence of mixed air masses, coming from the north and the south (Pacific Ocean). Further is needed to improve the air sampling technique and the potential for atmospheric transport effects of microplastics for human health.

## 16.PA-Mo-083

### Presence and Characterization of Microplastics and Additives in Canned Bivalves of Commercial Interest Through Infrared Spectrometry

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#### Abstract

Contamination by microplastics (MICPs) in marine organisms is an environmental problem with implications for the safety of the aquatic ecosystem and human health. Among marine organisms, bivalves are considered environmental indicators and a possible route of human exposure to contaminants such as microplastics. It is estimated that the annual per capita consumption of seafood in Chile is approximately 14.9 kilos in 2019. The objective of this study is to evaluate the presence of (MICPs) in canned food samples of bivalves (clams and mussels), and the composition and possible associated plastic additives were evaluated, using the analytical technique of Fourier Transform Spectroscopy (FTIR-ATR). The results evidenced the presence of microplastics in clam samples, with a total of 4 microplastic particles in 37.5 g (wet weight), the predominant shape detected was fiber type. In the case of mussels, plastic fibers were the most abundant and polyethylene (PE) was the most frequent type of polymer detected in the canned clam sample. Further research is still ongoing to assess the environmental sources and to motivate authorities to improve water quality systems in direct contact with natural ecosystems (rivers, lakes, sea). The additives and plasticizers identified in samples of commercial clams correspond to Dioctyl Phthalate, Stearic Acid and Phenolic Resin associated with the identified microplastic particles.

## 16.PA-Mo-084

### Time-Dependent Biochemical Responses of the Zoanthid *Zoanthus* Spp. Exposed to Polyvinyl Chloride (PVC) Microplastics

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#### Abstract

Microplastics (MPs, < 5mm) pollution can be considered as an emerging threat to coral reefs due to their complex interactions such as tissue necrosis, bleaching and photochemical efficiency. The present study investigates if the polyvinyl chloride (PVC) MP size ranging from 79-149  $\mu\text{m}$  and concentration of 10 mg/L could cause any time-dependent responses of the MP absorption and biochemical responses (GST, TBARS, DNA strand breaks) for *Zoanthus* spp. during acute (7 days) and chronic (14 days) exposures. For the role experiments 72 mini colonies of *Zoanthus* spp. were used in three experimental groups with triplicate. Monitored parameters were temperature  $26 \pm 0.4^\circ\text{C}$ , salinity  $33 \pm 0.6$ , and pH  $8.2 \pm 0.2$ . Significant alterations on *Zoanthus* spp. were observed after acute and chronic exposure to PVC MPs. For lipid damage (TBARS), a two times higher concentration was found only for the acute exposure. The GST activity decreases, while the DNA damage increases during the chronic exposure. This study concludes that the exposure time had a direct influence under the PVC MPs absorption, which consequently contributed for time-dependent alterations on GST activity, TBARS concentration and DNA damage, bringing new insights of how exposure time to MPs could influence biochemical responses in corals.

16.PA-Mo-085

## Plastic Pollution Solution: Mechanical Recycling of Marine Plastic Debris From a Brazilian Beach

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### Abstract

Plastic is a versatile material present in human daily life. However, marine plastic pollution is a growing global threat to biodiversity, economy and human health. Plastic waste abandoned on beaches and oceans causes negative impacts on the landscape and water quality, further to being responsible for the death of animals. In this sense, there is growing interest in the potential use of the oceans to improve human well-being, as well as encourage environmental awareness through outdoor sports. Previous studies have shown that outdoor sports, such as surfing, favor pro-environmental behavior and more sustainable living. Our research objective was to carry out the mechanical recycling process of marine plastic waste found on Brazilian beaches to manufacture a bodysurfing handboard. We collected 160 caps from high-density polyethylene (HDPE) plastic bottles on Boqueirão beach, located in the city of Praia Grande, on the coast of São Paulo, Brazil. After washing, the material was crushed into smaller portions, put in a metal mold and taken to the oven at 120 degrees Celsius during 60 minutes. Then, we took the marine plastic still in the metal mold to a universal press to be compressed during 5 minutes. After cooling, we took the marine plastic board and use a laser cutter to design the handboard. We conclude that it is possible to recycle marine plastic polymers, in this case HDPE plastic bottle caps, demonstrating a smart solution to plastic waste found on beaches. As the plastic is manufactured on a large scale and persists for long in the environment, recycling should be encouraged to contribute to the reduction of economic, environmental, and social impacts. In particular, the 100% handboard made from recycled plastic found on beaches, without the application of plastic additives, can be a way to adding value to plastic and also promoting an active life style through sport.

## Open-Air Dumps in Cordoba, Argentina: A Study on Macro-, Meso-, and Microplastic Pollution

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### Abstract

Anthropogenic activities are increasingly causing harmful impacts on ecosystems where plastic garbage is persistent. Open-air dumps are common in Latin-American and can affect nearby freshwater ecosystems. To address this problem, the aim of this study was to characterize the distribution of open-air dumps soil samples as well as riverbank soil and river sediment and water samples from nearby areas. Regarding the size, the plastic debris was termed micro, meso or macro-plastic and the abundance and plastic mass was calculated for each site. Macro- and meso-items were classified taking account of their functional origin (e.g. food wrappers, packaging, bottles, bags, etc.), type (hard, foamed, film, fiber) and color. Polymers types were identified using Attenuated Total Reflection Infrared Spectroscopy. Results show that subsurface dump-soils presented macro- and meso-plastics concentrations of  $4.9 \pm 1.8$  items/kg and  $27.22 \pm 7.5$  items/kg, respectively. Regarding origin and color, bags and films as well as white and black predominated in the samples. The main polymer in macro was polystyrene and polypropylene prevailed in meso. In riverbank sediments,  $82 \pm 2.3$  macro-plastic items/m<sup>2</sup> were counted with predominance of food wrappers and film, white color, and polypropylene polymer. For meso-plastic on riverbank, most of the items were film and hard type with white color while no origin was defined. Polypropylene and polyethylene terephthalate were the main polymers identified. Similarly, this is the case for meso-plastic found in river. Finally, blue cellulosic fibers were the most abundant items in water river samples ( $4.5 \pm 3$  items/L) with a mean size of  $0.42 \pm 0.30$  mm. Our results show the presence of macro- and meso-plastic in open-air dumps and rivers located nearby (including the control site) as well as microplastic in river water samples. This demonstrates the presence and persistence of plastic in different sizes due to the use mainly of bags and food wrappers with polymers applied in the manufacture of these containers. High plastic consumption in cities and inadequate dumps treatment could be responsible for the high presence of plastic in the ecosystems. Considering these results, a deep evaluation of the negative effects on the aquatic and terrestrial biota is needed. Also, environmental public policy actions should be taken.

## 16.PA-Mo-087

### Plastisphere Role on Microplastics Density

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#### Abstract

Plastic pollution is one of the major environmental problems discussed nowadays. The main sources of these contaminants are related to human activities on the continent. In aquatic environments, plastics undergo chemical, physical and biological processes, including biofouling, which is defined as the colonization of micro and macroorganisms on hard substrate. When this colonization is formed on plastic waste, it is called Plastisphere. The environmental consequences of Plastisphere are the introduction of alien species, increase of the plastic palatability, changes in microplastics (MPs: plastic  $1 \mu\text{m} \geq 5 \text{mm}$ ) density, and consequent intervention in the vertical transport of these particles. In this context, this work aims to investigate the role of the Plastisphere in the density of MPs of different polymers and exposure times in an estuarine environment. The experiment was carried out with MPs based on: Polystyrene (PS), Polypropylene (PP) and Ethylene Vinyl Acetate (EVA) polymers. The MPs were submerged in an estuarine environment for 1, 5, 15 and 30 days during the winter and summer seasons. Particle density was evaluated using a pycnometer ( $\text{g}/\text{cm}^3$ ) and a precision scale. With the results obtained, an increase in density regarding to exposure times was observed for all polymers evaluated for both winter and summer seasons. Particles with 1 day of exposure showed little variation in relation to plastics without any fouling. PS showed an increase of density from 0.09 to 0.11  $\text{g}/\text{cm}^3$  for both winter and summer in 1 day of exposure, original EVA density from -0.27 to -0.04 (winter) and 0.04 (summer)  $\text{g}/\text{cm}^3$  at 1 day. At 5 days was observed the greatest difference in density for PS polymer from 0.09 to 2.09  $\text{g}/\text{cm}^3$  during the winter and from 0.09 to 2.18  $\text{g}/\text{cm}^3$  during summer. After 15 days of exposure, the polymer that showed the greatest density variation was PP from 0.95 to -2.75  $\text{g}/\text{cm}^3$ , being recorded a reduction in density during the summer. In winter, the greatest difference in density was observed after 30 days of exposure to PP from 0.95 to 3.09  $\text{g}/\text{cm}^3$ , while in summer, the difference for this polymer at 30 days was from 0.95 to 1.51  $\text{g}/\text{cm}^3$ . The density of EVA at the end of winter was 0.003  $\text{g}/\text{cm}^3$ . These changes were related to Plastisphere, which showed preference for PP over PS and EVA polymers. These changes corroborate the fact that MPs with a density originally lower than that of water are found in sediments, affecting their position in the water column.



16.PA-Mo-088

## Ingestion of Plastic Particles by the Shortnose Guitarfish *Zapteryx brevirostris* (Müller & Henle, 1841) in Southeast-South Brazil

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### Abstract

Due to improper disposal, inadequate or insufficient management, as well as increased industrial demands, there has been a significant increase in the amount of plastics in the oceans. This has caused direct impacts, such as ingestion by marine animals, as well as indirect impacts, such as the adsorption of persistent organic pollutants that bind to plastic particles and can affect the health of all marine fauna. The shortnose Guitarfish *Zapteryx brevirostris* is a benthic species found in unconsolidated bottoms up to 50 m deep, which feeds mainly on shrimp Penaeidae and Polychaeta. Thus, it represents a suitable model to monitor the presence of plastics in the marine substrate. The aim of this study was to analyze the ingestion of plastic particles by *Z. brevirostris* on the Southeast-South coast of Brazil (São Paulo to Santa Catarina), in order to understand the relationship between benthic elasmobranchs and plastic pollution. Sixty individuals (31 females and 29 males) were analyzed, totaling 549 plastic items in the stomachs and intestines of 100% of the specimens. Synthetic fibers were the most common typology, with colorless (49%), blue (28%) and black (12%) being the most representative. No significant differences were observed in the amount and size of fibers between genders. Correlation analyzes showed a significant relationship between the amount of plastic particles and the weight of the stomach and intestine contents, as well as between the number of particles in the stomachs and intestines. Our results can help establish protocols for monitoring contamination of the marine environment and provide information for public policies aiming the conservation of this and other species of elasmobranchs.

## 16.PA-Mo-089

### High Density Polyethylene Plastic in Civil Construction: a Sustainable Prototype of German Tile

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#### Abstract

Plastic waste can take anywhere from 20 to 500 years to decompose, and even then, it never completely disappears. The presence of microplastics in human blood, breast milk, oceans, air and even uninhabitable places on planet Earth has already been proven. In this sense, new ways of reusing plastics have been studied, including in civil construction, aiming to reduce the impact on the environment and successively provide a more sustainable economy. The objective of this study was to produce a tile prototype using 280 high-density polyethylene plastic bottle caps. The plastic material used came from household disposal in the region of the Brazilian coast of São Paulo. After collection, the plastics were washed with soap and water to remove impurities and then dried. In this way, the plastics were fragmented into smaller portions using an industrial shredder. Thus, the plastics were placed in a non-stick aluminum mold and heated in the laboratory oven at 200 degrees Celsius for 40 minutes. Afterwards, the plastics were inserted into a non-stick aluminum mold that had the design of a German tile made with AutoCad software. Then, the mold was compressed in a universal press to remove air for 15 minutes. A tile prototype with an excellent aesthetic appearance and great design was obtained, with stiffness and lightness above expectations. Finally, we can transform plastic waste that would be inappropriately disposed of in nature into sustainable materials, thus reducing the impacts that these plastics could cause on our ecosystem. From the preliminary results we conclude that it is possible to make a tile with 280 plastic bottle caps, generating a priori quality product from household waste.

## 16.PA-Mo-090

### Interaction of Microplastics and Pesticides and Their Implications for Aquatic Invertebrates of Different Feeding Habits

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#### Abstract

In conventional farming, particles of microplastics (MPs) will eventually interact with the pesticides applied to the crops. Interactions among MPs and pesticides likely impact the environmental fate of these materials and the impacts that they have on organisms in aquatic ecosystems. In this way, this research aims to assess the interactions between MPs and pesticides and how this interaction interferes with the life cycles of aquatic invertebrates from different feeding habits. For these experiments, the insecticide imidacloprid and the fungicide carbendazim and MP polymers of polyethylene and polypropylene have been selected because they are among the most frequently encountered in the freshwater environments in Brazil. We hypothesize that (i) degraded MPs have a high adsorption capacity for pesticides and decreases in pH, comparable to the digestive tract of invertebrates, increases the desorption of pesticides and the risks to organisms; (ii) environmentally realistic concentrations of microplastics do not cause lethal effects to invertebrates; however, short-term (growth and development) and long-term sublethal effects will be evidenced; (iii) different feeding habits reflect the rates of MP ingestion, being filter feeders, collectors and predators the feeding groups whose have the high uptake rates; (iv) the ingestion of MPs particles decreases energy reserves due to false satiety and changes in food processing (v) the combined effects of MPs and pesticides are synergistic; thus, greater effects are expected for those groups occurring together in the environment. The results will expand the discussion about the MPs pollution in agricultural fields, whose effects are still poorly understood by the scientific community.

## 16.PA-Mo-091

### Ocurrence of Microplastics in Estuarine Sediments of the Biobío Region, Chile: Influence of Nearby Anthropogenic Activity

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#### Abstract

The different human activities located in the coastal area have triggered contamination by microplastics (MP) in different regions of the planet. Estuaries are important ecosystems of the coastal area that have a crucial role as sites of reproduction, development, and growth of marine species. Despite of such role, estuaries are also classified as MP hotspots, because its dynamics and density structure favor the accumulation of MP in its sediments. However, the status of these contaminants in estuarine sediments in Chile, is unknown. Hence the aim of the present study is to determine the concentration of MP in sediments of the Lenga and Tubul-Raqui estuaries in the Biobío region. The Lenga estuary has suffered a strong industrialization accompanied by considerable economic development, while the Tubul-Raqui estuary is part of a small watershed with low anthropogenic influence.

Eighteen sampling points per estuary were collected using a dredge during the spring 2022. 50 ml of sediment were analyzed for MP determination. Additionally, organic matter (%) in the sediments was determined to evaluate its influence on the abundance of MP. By means of the density separation method using sodium chloride (NaCl), the supernatant was analyzed, and the quantity, color and shape of the MP were observed under a stereomicroscope. In total, 142 MP particles were found in the Lenga estuary and 85 particles in the Tubul-Raqui estuary. In both cases fibers predominated with an abundance of 89% and 85%, respectively. Significant differences in fiber abundances were found between the estuaries ( $p < 0.05$ ). In addition, a positive correlation was verified between the abundance of fibers and organic matter ( $cor = 0.132$ ,  $p = 0.038$ ). Currently, complementary Fourier transform infrared spectroscopy (FT-IR) analysis are being carried out on the identified particles and characterize particle size and pH in sediments to assess their eventual influence on the abundance of MP in surface sediments of marine estuaries. Because estuaries are ecosystems that have not been given enough attention in Chile, this study is key to begin to have an overview of the situation of these ecosystems and begin to develop plans for their control. This study was supported by ANID–Millennium Science Initiative Program–Code ICN2019\_015.

## Global Patterns on Fish Ingestion of Microplastics: A Scientometric Review

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### Abstract

The billions of tons of plastic released into the environment, for the most part, are not biodegraded, but are fragmented into smaller particles that reach rivers and oceans. These microplastics (MPs) are considered emerging contaminants and have high adsorption capacity of chemicals that interact synergistically and can produce changes in aquatic organisms. Studies on the effect of MEPs on fish have increased considerably in recent years. Thus, this study aimed to review and quantify published data on the abundance of PMs ingested by fish, through a scientometric analysis. A search was performed in the Web of Science database, using a combination of keywords (microplastic, nanoplastic, plastic, fish) and Boolean operators. Based on prism guidelines, research articles have been identified, selected, and summarized, including studies of natural and controlled environments. This search resulted in the registration of 787 documents that addressed the ingestion of plastics, microplastics and/or nanoplastics by fish. Data were analyzed using Microsoft Excel and CiteSpace. The results indicated an increase in knowledge production after the year 2010, reporting a diversity of colors, shapes and materials of debris distributed throughout the world. The highest proportions of studies were conducted in China (24.77%), followed by those in the USA (10.67%). The most influential journals were Environmental Pollution and Marine Pollution Bulletin. The top ten authors represent 18.9% of the total number of studies evaluated here. The main institutions were the Chinese Academy of Sciences, followed by the Spanish Institute of Oceanography. The keywords most used by the researchers were Ingestion, fish, marine environment and plastic debris. Our results show that studies with PMs in fish show an increasing trend in relation to the toxic effects that these debris can produce when attributed to ingestion, the evidence still shows that most of the studies are in fish of seas and oceans, but the growth of research carried out on fish in rivers and streams is already evident. This article provides a comprehensive review of the abundance of PMs in river and sea fish around the world over the past two decades and contributes to future research in the area.

## 16.PA-Mo-093

### Environmental Perception of Brazilian Beach Users Regarding the Presence of Plastics

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#### Abstract

The exponential increase in the human population associated with the behavior of contemporary society, characterized by an accelerated routine and the culture of immediacy, consequently influence consumption habits and pollution rates. Thus, the production and disposal of waste has reached large proportions on beaches and in the oceans. In this sense, environmental perception is essential to understand the way human beings act in relation to the environment. However, the environmental perception of Brazilian beachgoers regarding the marine debris problem is still little explored, while the focus of investigations is on the causes and consequences of the presence of marine debris. To bridge this gap, we conducted a survey of 100 individuals, both male and female, aged 18 years or older, who visit Brazilian beaches at least once a week. The survey included 50 participants who engage in sports activities on the beach and 50 who do not. Participants were asked about the types of marine debris they observe on the beach. Our findings show that plastic waste is the most frequently observed type of debris, significantly more prevalent than metal, paper, organic waste, and glass, for both sport practitioners ( $X^2 = 31.40$ ,  $df = 4$ ,  $p < 0.01$ ) and non-practitioners ( $X^2 = 26.28$ ,  $df = 4$ ,  $p < 0.01$ ). We also compared the types of sport activities and the number of plastic waste types observed by each group. Diving and swimming were found to have a higher number of plastic waste types observed in their perception than other sports. By detecting an overview of the environmental perception of users of Brazilian beaches, we can recommend sustainable alternatives for beach conservation, working as a support for environmental management and the elaboration of public policies aimed at waste management

## 16.PB-Tu-122

### Microplastics Consumed by *Poecilia reticulata* in Urban Streams: Raman Spectroscopy as an Identification Tool

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#### Abstract

Plastic origin materials have a high fragmentation capacity when exposed to a combination of environmental factors, being able to remain for long periods in the aquatic ecosystem and likely to be ingested by the ichthyofauna. Knowing the composition of synthetic microparticles can help to understand what are their main sources in the environment, as well as to examine their environmental impacts. However, one of the great challenges for the characterization of plastic materials is the chemical changes caused during the degradation process, which make the spectroscopic correspondence and, therefore, the identification difficult. The objective of this work was to identify the types of microplastics found in the gastrointestinal tract content of *Poecilia reticulata* in streams under intense anthropic pressure by using Raman microspectroscopy. For this, fish were collected with electric fishing equipment in the city of Cascavel, PR, Brazil. The gastrointestinal content of each individual was removed, analyzed and the registered microplastics were stored in 70% alcohol for analysis. Raman microscopy (830 nm excitation, 1 s exposure time, 100 mW laser power) was used in order to obtain the probable chemical composition of the 12 analyzed polymers, since it is an efficient tool in the analysis of microparticles (< 5mm). The results indicated the presence of five types of post-consumer plastic fragments, with polyethylene terephthalate (PET), polypropylene (PP) and high-density polyethylene (HDPE) being identified in the gastrointestinal contents of *P. reticulata*, while some fragments could not be identified but contained artificial pigments. Our results suggest that these environments are strongly impacted by the contribution of microplastics and that these particles have different chemical compounds, which may have different effects on the individuals ingesting them.

## 16.PB-Tu-123

### Presence of Microplastics in the Native Crustacean *Palaemon argentinus* from a Shallow Pampean Lagoon

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#### Abstract

Microplastics (MPs) are one of the emerging contaminants in aquatic environments around the world. The effects of plastic pollution on environmental ecosystems and organisms have been mainly analyzed from marine environments, however, studies for freshwater environments are scarce. The shrimps of the genus *Palaemon* (Crustacea: Decapoda: Caridea) are considered good bioindicators of environmental pollution due to their sensitivity to a wide variety of pollutants, including MPs. *Palaemon argentinus* is an abundant freshwater shrimp with wide distribution in South America, being found in most of the Pampean lagoons. According to their trophic and ecological habits, each species presents a particular situation with respect to MPs. This study aimed to evaluate the presence and abundance of microplastics in *P. argentinus* from a shallow lake in Buenos Aires Province, Argentina. Shrimp were obtained from Los Padres lagoon (37°57'S, 57°44'W), a typical shallow Pampean lagoon, which has a tributary that crosses a large expanse of horticultural fields and an effluent that drains through a gate regulating the level of the lagoon. Likewise, it is close to a residential area with a commercial and tourist center. Shrimp (n=3, 15 individuals in each pool) were collected using a hand net and immediately transferred to the laboratory to they were sacrificed and the exoskeleton was removed. Simultaneously, samples of water (n=3, 5 L. each) were taken with a bucket and filtered with a net of 36 µm mesh size. All samples were digested with hydrogen peroxide (30%) and filtered with nitrocellulose filters (0.45 µm). Each piece was photographed, and information about the maximum length (mm), shape, and color was collected to classify the MPs into four categories: fibers, films, fragments, and pellets. To discard airborne contamination, Petri dishes with control filters (blanks, n=3) were left exposed to air during all procedures; and it was considered to be negligible (0 to 1 MPs found). Both shrimp and water samples presented MP with a clear dominance of fibers followed by fragments and films. The fibers present in water were generally longer than those found in shrimp tissues, and the dominant color was black. Pellets were found in 33.3 % of the water samples, but not in shrimp tissues. The findings confirm the presence of MPs in the natural habitats of *P. argentinus* and indicate that shrimp can ingest them.



## 16.PB-Tu-124

### **A Multiparameter Analysis of Microplastic Ecotoxicity in Developing Zebrafish (*Danio rerio*)**

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#### **Abstract**

Plastics are already a pollutant of worldwide concern, among them microplastics (plastic particles within the 100 nm–5 mm size range), which are considered a major threat to the biota of the most diverse environments, due to their wide distribution and permanence in different compartments. With that, it becomes urgent to study the toxicity of these compounds in model organisms aiming at the protection and health of the environment. Therefore, the present study aimed to evaluate the polyethylene microplastic (PE MP) ecotoxicity in *Danio rerio* embryos and larvae, an important biological model in Ecotoxicology. For this, the zebrafish embryos were exposed statically for 144 hours to concentrations of 0.5, 5.0 and 50 mg PE/L (size = 600 $\mu$ m), in which spontaneous contractions, heart rate, hatching rate, survival rate and morphological changes and behavioral biomarkers such as average speed, maximum speed and total distance covered were evaluated. Among the results found, a decrease in heart rate (cardiotoxicity) was determined, with a significant difference from the control in the highest concentration, as well as a delay in the hatching rate also in this concentration (50 mg PE/L). For the other evaluated criteria, survival, spontaneous movement and morphological changes, there was no significant difference from the control. Data related to behavioral biomarkers are still being evaluated. Overall, it is possible to identify that aquatic organisms may be at risk when exposed to PE MPs due to their effects during the early developmental stages.

## 16.PB-Tu-125

### Assessment of Degradation and Toxicity of Spray Paint Microplastics

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#### Abstract

A study on the effect of ingestion of pristine and leached microplastics (MPs) is being conducted in foraging workers of *Bombus atratus* using the Luxens® brand's "glossy black" paint, which has Chromium (Cr) in its composition. The objective of the study is to verify whether MPs can be toxic to the bees when they are ingested by them. The first step of the research was to validate if these MPs would release Cr after being exposed to ultraviolet radiation (UV-C), simulating a weathering scenario. In a second moment, after the ingestion of the particles by the bees, the possible impacts of these paint MPs on their internal organs will be verified. The current study produced pristine MPs particles from this paint and evaluated different features caused by UV-C radiation in two different exposure times, in order to understand their toxicity and behavior. The MPs were produced by spraying the paint in a closed environment, followed by decantation of droplets and aerosols. After this process, the particles were collected, sanitized, and separated by granulometric fraction (25 to 53 µm). These particles were classified as pristine, and their characteristic shape is spherical. To evaluate the photooxidation rate, four working groups were created: 1) one UV-C lamp for 24 hours; 2) one UV-C lamp for 48 hours; 3) three UV-C lamps for 24 hours; 4) three UV-C lamps for 48 hours. After the exposure time, all groups were analyzed using a Scanning Electron Microscope (TM300–Hitachi®) and their areas, perimeter and sphericity were compared using the Kruskal-Wallis test. Only group four showed a significant difference in particle area ( $p=0.0266$ ) when compared to the pristine material, in addition to the formation of nanoplastics. There was no significant difference in perimeter between all groups and the control ( $p=0.0722$ ). The sphericity was significantly altered in groups two and four ( $p<0.0001$ ), indicating deformation and change in particle shape. Finally, initial semi-quantitative analyzes using a Dispersive Energy Detector were carried out in the different exposed groups and a gradual increase in the availability of Cr in the samples was detected. Photooxidation may release nanoplastics and higher doses of Cr into the environment, making the particles more potentially toxic to bees and other animals. In addition, this data brings greater attention to the study of the environmental impact of spray paint microplastics.

## 16.PB-Tu-126

### Toxicology and Microplastics: A Scientometric Review

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#### Abstract

Plastic products have been widely used in contemporary society. A good part of the waste from these products is disposed of improperly. These plastic wastes are broken down into smaller particles by a series of natural processes. Those particles less than 5 mm in size are considered microplastics (PMs), a class of emerging contaminants widely distributed in marine and freshwater environments around the world. In this context, a scientometric analysis was carried out in order to identify patterns and trends in research and demonstrate the main gaps in the field of knowledge. Data were obtained from the Web of Science (WoS) platform. Search terms were All Fields = microplastic\* OR "microparticles plastics" OR "nanoparticles plastics" OR mesoplastic\* OR "waste plastics" OR "plastic debris" OR "plastic microfiber" OR nanoplastic\*. The research was carried out considering the period from 2010 to 2022, and 2010 was the year that had the first publication of PMs in the field of toxicology. The publications were refined, the total resulted in 606 references. For each publication, the type of document, year, journals, country, publication author and publication category were identified. Results were analyzed using Microsoft Office Excel and Citespace. Articles were the predominant types of documents (77.88%). The leading country in number of publications in the area was China, followed by Italy and Germany, respectively. With Citespace it was possible to analyze categories, journals and authors, co-occurrence and analysis of keywords and terms used in publications. In addition, we note that some of the authors worked collaboratively, with concentrated efforts, allowing important advances in this field of research. Studies on the toxicity of PMs were published in 57 journals. The most cited source was *Ecotoxicology and Environmental Safety* (Impact Factor 7,129) with 107 records. The keyword that appeared most frequently in newspapers was 'marine environment'. A strong association with the words 'research', 'toxicity', 'oxidative stress' and 'accumulation' is emphasized. The results presented in this study provided useful information on research that related the toxicological effects of PMs, allowing inferences about worldwide trends in the field and providing a guide for future efforts. In conclusion, this study provides the use of an efficient method to quantitatively visualize information about the development of this area of study.

## 16.PB-Tu-127

### Influence of Polyethylene Microspheres on the Activity of Calcifying Enzymes of the Yellow Clam (*Amarilladesma mactroides*)

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#### Abstract

Currently, a problem present in the world's oceans is the occurrence of microplastics. These particles are capable of generating effects in several animals exposed to them, ingestion being one of the main ways of contact with this emerging contaminant. Special emphasis is given to bivalves since they are filter feeders susceptible to the ingestion and accumulation of these particles. In this regard, variations in the activity of important enzymes involved in the biomineralization process, such as  $\text{Ca}^{2+}$ -ATPase and carbonic anhydrase, have been recorded by some studies, after exposing calcifying individuals to these materials. Given these aspects, the present study investigated the effects of acute exposure (96 h) to polyethylene (PE) microspheres (20  $\mu\text{g/L}$ ) on the functioning of enzymes responsible for calcification in the mantle and gills, as well as the hemolymphatic concentration of calcium ( $\text{Ca}^{2+}$ ) from the marine bivalve *Amarilladesma mactroides*. Our analysis revealed that after the interval of exposure to polyethylene microspheres, the enzymes carbonic anhydrase and  $\text{Ca}^{2+}$ -ATPase showed a reduction in activity when evaluating the mantle tissue. On the other hand, the activity of both enzymes was not affected in the gill tissue, after the period of exposure to the described contaminant. The same was verified for the concentration of  $\text{Ca}^{2+}$  ions in the hemolymph, which did not vary between experimental conditions. Considering the purpose of the enzymes analyzed in the gill tissue for the uptake of essential ions for the biomineralization event in shellfish, our findings suggest that these proteins were not targets of the effects of the microplastic, since the hemolymphatic concentration of the  $\text{Ca}^{2+}$  ion was not altered. On the other hand, our results demonstrate that the effect of the microplastic on the enzymes evaluated in the mantle indicates that there may be significant damage to the calcification process, especially concerning the direct functioning of these proteins aimed at  $\text{Ca}^{2+}$  renewal and deposition in the shells of yellow clams.

### Scientific Knowledge About Micro and Nanoplastics: A Scientometric Review

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#### Abstract

Microplastics and nanoplastics (MNPs), plastic particles smaller than 5 mm, are omnipresent emerging contaminants. This scientometric review points out trends, patterns, and possible gaps in knowledge, listing the countries and categories that publish the most on the subject, the hotspots and research fronts. The Web of Science database was searched in 2022, 09, may, by all years and all fields for the descriptors: microplastic\* OR "microparticles plastics" OR "nanoparticles plastics" OR mesoplastic\* OR "waste plastics" OR "plastic debris" OR "plastic microfiber" OR nanoplastic\* to select articles that studied NPMs directly or indirectly. 13,142 publications were grouped and after refinement, 8,015 indeed relate to NPMs remained. They were analyzed by CiteSpace and Bibliometrix softwares. Publications started to increase in 2010 and the H-index was 291, representing the relevance of the topic in the scientific context. The countries that publish the most are were: China (2,144), the USA (822), Germany (675) and, Italy (533). The outstanding categories were Environmental Science (5,733), Freshwater Marine Biology (1,317) Environmental Engineering (1,292) and Toxicology (606). The research hotspots are pollution (1,796) marine-environment (1,654), plastic debris (1,272) and ingestion (1,269) and there is a change of trend on the research fronts, from water, exposure and toxicity for hydrogen-production, efficiency and east China. Countries with most publication are economic powers, and therefore large consumers of plastics. There is significant representation in the environmental sciences, but microplastic pollution is a complex topic and involves sources, transport, distribution, removal, associations with other pollutants and pathogenic organisms, and synergistic or non-synergistic effects on fauna, flora, and the environment. The marine environment is the most studied, but MNPs have even been reported in human tissues and cells. Currently, research is more focused on the removal efficiency of this contaminant, the effects of MNPs pollution on hydrogen production by biomass, and China as a major research front on MNPs. An arduous search is needed for ways to control MNP pollution, removal, and long-term effects, Biotechnology and Ecotoxicology are areas that can contribute largely to these gaps.

## 16.PB-Tu-129

### Sublethal Effects in *Centropomus undecimalis* Fish Exposed to Polyethylene Microplastics Contaminated With Triclosan

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#### Abstract

Microplastics (MPs) and personal care products (such as the bactericidal triclosan (TCS)) interact in the marine environment and cause impacts on the biota. However, the evaluation of the combined effects of MPs and TCS on fish species from tropical estuaries are still scarce. This study aimed to evaluate the effects on *Centropomus undecimalis* exposed to polyethylene MPs (150 to 250  $\mu\text{m}$ ) contaminated with the bactericide TCS (MPs: 250 mg/L; TCS: 1 mg/L) at two exposure times (3 days and 7 days). Therefore, biomarker responses (GST, GPx, GSH, LPO and DNA damage) were evaluated in different target tissues (gills, liver, muscle, intestine and brain) in the organisms exposed to three treatments: (I) control (C); (II) virgin microplastics (MP); and (III) triclosan-spiked microplastics (MPT). The transfer of TCS from microplastics to water and organisms was confirmed through chemical analysis. Triclosan was observed both in the water ( $1.034 \mu\text{g.l}^{-1}$ ) and in the body ( $0.0313 \mu\text{g.g}^{-1}$ ) of the organisms after 7 days. The organisms showed, at 3 days, DNA damage in the liver, neurotoxicity in the gut, and lipid peroxidation and neurotoxicity in the brain associated with exposure to MPT treatment. In a longer period of exposure (7 days), fish exposed to microplastics and TCS showed an induction in the antioxidant defenses of the gills, liver and intestine. Lipid peroxidation was observed in the liver and intestine, while DNA damage occurred in the gills, intestine and brain. When considering the treatment exposed to virgin microparticles, an activation of the antioxidant enzymes of the gills, liver, intestine and brain was observed. Once present in the water column, MPs can have adverse effects on fish through the desorption of substances associated with their structure, direct contact and through ingestion, triggering responses in various organs. The results from the current research showed that microplastics can be carriers of PPCPs, being harmful to *C. undecimalis*. Furthermore, over a longer period of exposure, virgin microplastics produced adverse effects in the model organism. Our results demonstrate the potential of these contaminants to impact tropical estuarine fish, which can affect the fitness of this species.

## 16.PB-Tu-130

# Study of the Interactions Between Pesticides and Bioplastic Coatings Used in Horticultural Production Systems

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### Abstract

An environmental problem derived from the use of plastic mulches in horticultural systems is the incorporation of plastic fragments in soils. These fragments, after being abandoned, fragilize and decompose easily to form microplastics. An alternative to this situation is the use of biodegradable plastic mulches. Their potential advantage lies in the fact that it is not necessary to remove and dispose of the plastic after use because it can degrade in the soil itself or be composted at the end of the season. The biodegradability of these new plastic mulches in soils depends on several environmental factors, some biodegradability studies conducted in long term field conditions resulted in mechanical fragmentation of the plastic films and subsequent formation of microbioplastics.

It is of vital interest to study the interactions that can occur between biodegradable plastic mulches and pesticides to compare these interactions with those occurring with polyethylene black mulching. The present study analyzes the migration and sorption processes between trifluralin, chlorpyrifos and procymidone and commercial bioplastic and polyethylene mulches. For this purpose, different tests were carried out at laboratory scale. Soil-plastic- atmosphere microenvironments were used to study the influence of temperature and initial soil moisture on the migration process of pesticides from biodegradable plastic or polyethylene to the soil and the atmosphere.

On the other hand, to determine whether biodegradable plastic or polyethylene residues buried in horticultural soils could act as potential pesticide collectors, pesticide mobility tests were carried out in soil columns.

The tests indicate that temperature and initial soil moisture affect the dynamics of migration. In addition, they showed that pesticide retention in plastic matrices is significantly higher in biodegradable plastic mulch fragments than in polyethylene plastic mulch fragments.

Our results contribute to a better understanding of pesticide distribution dynamics in the environment and the role of plastic mulches in horticultural production systems.

## 16.PB-Tu-131

### Screening of Marine Filamentous Fungi for Polyethylene Microplastic Degradation

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#### Abstract

Microplastics (MPs) accumulation on the environment has been a bottleneck for scientific society. Several approaches are proposed to reduce MPs in aquatic and terrestrial ecosystems, however, most of them are not environmentally correct. The use of free or immobilized filamentous fungi (Ff) cells are currently a promising solution for MPs degradation. The purpose of the present work was to evaluate six Ff from Mangrove sediments for their capability to biodegrade polyethylene (PE) MPs. Briefly, before biodegradation study, PE MPs were characterized according to their size and morphology. All strains were identified by morphological analysis. Screening for biodegradation of PE MPs was performed in Erlenmeyer flasks containing 50 mL of minimal liquid medium. Cell growth and biodegradation of PE MPs were determined after insertion of 5 culture discs (6 mm in diameter) of each strain and 0.05 g of PE MPs in Erlenmeyer flasks incubated in the rotary shaker at 30°C and 150 rpm for 28 days. The most promising strain was evaluated for changes in the composition of the MPs by Fourier transform infrared spectroscopy (FTIR) and laccase enzyme activity. The granulometry of the PE MPs ranged from 500 to <63 µm, whose size of 250 µm was the most representative. Three morphologies of PE MPs were detected: microfiber, microfragment and microsphere, the latter being of greater proportion. After morphological analysis, the strains were identified as belonging to the genera *Aspergillus* (MQ1C and AQ3A), *Penicillium* (MQ1A), *Rhizopus* (AQ2A) and *Trichoderma* (MQ1B and MQ2A). Ff AQ3A showed the most promising results with a PE MPs reduction rate of 55% and biomass formation of 0.0840 g·mL<sup>-1</sup>. The MQ1B and AQ2A strains presented a similar profile of 38 and 36% reduction of PE MPs and biomass formation of 0.0505 and 0.0510 g·mL<sup>-1</sup>, respectively. Complementary studies with *Aspergillus* spp. (AQ3A) using FTIR highlighted changes in the molecular structure of PE MPs. The laccase activity was not identified in the extracellular extract, suggesting the use of other enzymes by Ff. These results indicate that Ff can contribute to the biodegradation of PE PMs. However, other parameters need to be explored, mainly associated with the enzymes that are involved in this biodegradation process.



## 16.PB-Tu-132

### Analysis of Anthropogenic Fibers Using Royal Blue Light

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#### Abstract

Plastics have been a significant problem in our environment since their introduction. Plastics, especially microplastics that are found in the seas, lakes, and rivers, are causing harm to natural habitats. Extensive research has been conducted in identifying microplastics that are scattered across the water. The main goal of this research is to easily distinguish anthropogenic fibers from microplastics from samples using a combination of Royal Blue fluorescent light, and a Fourier Transform Infrared spectroscopy (FTIR). Royal blue light (450-465 nm) has been shown to make microfibers fluorescent, which are normally hard to detect with a white background. The FTIR is used to minimize problems such as false positives and negatives that could potentially interfere with the integrity of the result. By quickly identifying which type of fibers are present in the water, we can produce solutions faster to minimize damage to the environment. Using the Royal Blue fluorescent light, we found that fluorescent fibers that were transparent or white were 92% likely to be anthropogenic fibers like cotton or cellulose. Fibers that were blue and fluorescent had a 33% chance of being anthropogenic. Blue fibers had the most false negatives. None of the black or purple fibers had any fluorescence, so it would be best to use Royal Blue light when analyzing white or transparent fibers.

DRAFT

## 16.PB-Tu-133

### Microplastics in Bottled Waters in the Santiago Metropolitan Region of Chile

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#### Abstract

Bottled water has emerged as a possible healthier alternative due to concerns about the quality of drinking water sources. However, recent studies have detected worrying concentrations of environmental contaminants in bottled water, including microplastics. Therefore, it is an emerging need to quantify their concentrations in local suppliers, which could differ among countries and regions. In this work, we used fluorescence microscopy with Nile Red for the identification and quantitation of microplastics in twelve brands of bottled water distributed in the Santiago Metropolitan Region of Chile. The average concentration of microplastics was  $391 \pm 125$  p L<sup>-1</sup>, while the highest concentration observed was  $633 \pm 33$  p L<sup>-1</sup>. Microplastics between 5 to 20  $\mu$ m were the major contributors, a size fraction that has been reported to be susceptible to accumulate in the digestive tract or generate potential alterations in the lymphatic and circulatory systems. The estimated daily intake (EDI) value for per capita was estimated to be 229 p kg<sup>-1</sup> year<sup>-1</sup> for people weighing 65 kg and 198 p kg<sup>-1</sup> year<sup>-1</sup> for those weighing 75 kg.

DRAFT

## 16.PB-Tu-134

### Presence of Microplastics in the Veracruz Reef System National Park (VRSNP)

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#### Abstract

Background: Plastic production has increased with deterioration in recent decades, having a production in 2019 of 368 million tons, where a considerable amount ends up discarded in the environment where it wears out and breaks down into microplastics. Methods: This study conducted a test of sand quadrats on the Veracruz coast, more specifically in La Mancha, Chachalacas, Chalchihuecan, Antón Lizardo and El Sendero, in the municipalities of Actopan, Úrsulo Galván, La Antigua and Alvarado respectively. Plastic particles were acquired after drying and sieving the sand, and quantified and characterized by FTIR and SEM. Results: In the zone of influence of the national park of the Veracruz reef system, a total of 180 pieces of plastic were found (4.5 pieces/m<sup>2</sup>). The beaches of the trail and chachalacas presented the largest number of plastic pieces. Of the total particles in the system, 92.35% appeared irregularly, the rest were fibers, "pellets" and films. Regarding the type and predominance of plastic polymers found on the beaches, the three main materials found were high and low density polyethylene (26.67 and 27.22% respectively) and polypropylene (23.33%). Conclusion: The presence of plastic pieces was detected in the 5 beaches of the zone of influence of the national park of the Veracruz reef system. Most microplastics are low and high density polyethylene and irregular in shape. the three main materials found were high and low density polyethylene (26.67 and 27.22% respectively) and polypropylene (23.33%). Conclusion: The presence of plastic pieces was detected in the 5 beaches of the zone of influence of the national park of the Veracruz reef system. Most microplastics are low and high density polyethylene and irregular in shape. the three main materials found were high and low density polyethylene (26.67 and 27.22% respectively) and polypropylene (23.33%). Conclusion: The presence of plastic pieces was detected in the 5 beaches of the zone of influence of the national park of the Veracruz reef system. Most microplastics are low and high density polyethylene and irregular in shape.

## 16.PB-Tu-135

### Appraisal of Microplastic Degradation in a Salt Marsh Environment From Southern Brazil

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#### Abstract

Salt marshes are one of the least studied estuarine ecosystems in terms of impacts caused by the presence of microplastics (MPs). Although recent investigations showed that this environment act as a sink and a source of MPs for the ocean, studies about how plastic degradation occur are scarce. Thus, the present study evaluated the environmental degradation on two types of plastic pellets (low density polyethylene-LDPE and polycarbonate-PC) exposed for 12 months in a salt marsh (Patos Lagon Estuary, southern Brazil). Three cylindrical samplers with 30 units of pellets were fixed at the Mudflat (MUF), Low (LOW) and Middle (MID) zone of the salt marsh. Changes in physical and chemical properties of MPs were assessed. Surface degradation (cracks, holes, and depressions) and the presence of organisms attached in MPs samples were assessed by Scanning Electron Microscopy. The surface of the pellets was more deteriorated, showing holes and cracks with increasing exposure time, suggesting that the PC pellets deteriorated less than the PE pellets. Presence of diatoms and bacteria in surface samples can intensify the degradation in regions where photodegradation cannot act as well as cause a “shield” and decreasing the effect of photodegradation in dry zones. Crystallinity fraction (CF) was assessed by Differential Scanning Calorimetry. For LDPE was possible observe a significative decrease in CF, when compared with virgin pellets, after four months ( $59.9 \pm 17.5$  % in MUF,  $36.6 \pm 17$  % in LOW, and  $61.3 \pm 3.5$  % in MID), while in PC after two months was already observed a significative decrease in CF ( $44.7 \pm 21.8$  % in MUF,  $12.8\% \pm 8.4$  % in LOW, and  $11.2 \pm 6.2$  % in MID). However, over the time, the degree of crystallinity of the samples fluctuates, mainly due to the crosslinking phenomena that occur within the polymers. Comparing differences between the polymers, was observed a significant mainly in the LOW and MID. The difference is mainly due to the intrinsic characteristics of each polymer. The amorphous part of the polymer is preferentially degraded during weathering, thus increasing the crystalline generation. Therefore, polymers with more amorphous area, like PC, are degraded more intensively under UV radiation. These physical and chemical changes may occur through an interaction between abiotic and biotic factors, being photodegradation probably the most relevant mechanism in areas with lower flooding rate.

# Session 18: Development and Acceptance of New Methodological Approaches (NAMs) in Latin America

18.P-Mo-094

## Eye Damage Reversibility in an in Vitro Model of Bovine Cornea to Replace the Draize Test Completely

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### Abstract

One of the requirements for the registration of substances such as agrochemicals is to provide evidence about their potential eye damage. The Draize test performed in rabbits allows the products to be classified into four categories, considering both the severity of the lesions produced in the animal's eye as well as its healing time. The available alternative methods to this live animal test do not allow documenting the damage reversibility, nor the time necessary for such reversibility to occur, as required by the UN GHS classifications.

Our proposal is to complement the in vitro model that uses the bovine cornea as a substrate to predict whether a substance is irritating or non-irritating (BCOP), with a strategy that allows predicting if the observed irritation is reversible and the time it takes to revert. This is necessary to finally replace the Draize test completely.

Limbal stem cells are known to play an important repairing role in corneal injury; therefore we isolated these cells from bovine cornea and used them to evaluate the cell sensitivity to reference products. A wound healing assay was also performed to study whether these products differentially affect the replication and migration capacity of the cells. Furthermore, a tissue explant and an organotypic cornea culture model were implemented to study if the chemical exposure alters cell's replication, migration and overall wound healing differentially.

In conclusion, a combination of the approaches used have been proven effective to detect the four categories of GHS reference products

## 18.P-Mo-095

### Acute Oral Toxicity Assessment of Pesticides

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#### Abstract

Agencies worldwide have attempted to develop nonanimal approaches to predict acute systemic toxicity, but it remains a challenge yet. In Argentina, the rodent acute oral lethal dose (LD50) is demanded for products registration. However, local regulatory organisms and industries are willing to replace the in vivo assay. Our goal is to develop an integrated approach for acute oral toxicity assessment of pesticides. The BALB/3T3 Neutral-Red Uptake Cytotoxicity Assay (OECD 129) was used to estimate the in vitro LD50 values of 20 pesticide formulations, which were provided by ATANOR S.C.A and GLEBA S.C.A companies along with their in vivo LD50 doses. In vitro/in vivo datasets were classified as Extremely Hazardous (EH:<50mg/Kg), Moderately Hazardous (MH:50-2000mg/Kg) or Unlikely Hazardous (UH:>2000mg/Kg). None of the pesticides was classified as EH for both datasets. With the in vivo data, 80% was classified as UH while 20% as MH. In the in vitro assay 10% was classified as UH while 90% as MH. The concordance was 30%, none of the in vitro LD50 values were underestimated and 70% was overestimated. In vitro classification is more conservative than in vivo one, most of the pesticides were categorized as UH with the in vivo data unlike the in vitro data that fell into the MH level. In addition, I am currently working with different softwares such as SwissADME, ICE (Integrated Chemical Environment) and QSAR toolbox to predict the physicochemical descriptors as well as the ADME parameters of the pesticide active ingredients. We expect to integrate the in vitro results with the in silico data in order to develop an acute oral toxicity prediction model for pesticides, which would represent a first step to replace the in vivo assay

## 18.P-Mo-096

### Implementation of an *In Vitro* Methodology for Phototoxicity Evaluation in 3T3 Cell Line

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#### Abstract

Phototoxicity is an acute response induced by light, which occurs when photoreactive chemicals are activated by sunlight and transformed into cytotoxic products against cells of the skin and/or eye. *In vivo* methods using pigmented guinea pigs, mice or rats are used to simulate real human phototoxicity scenarios. Animals are exposed to chemicals topically or systemically and irradiated with an appropriate dose of ultraviolet A (UVA) light. However, the animal sacrifices, expense, and time required to perform the test pose many problems, especially in the era of widespread awareness of animal welfare and ethics. Non-animal phototoxicity tests are gaining popularity these days to overcome these problems. The 3T3 *in vitro* phototoxicity assay can replace the use of experimental animals in the study of substances with phototoxic potential.

The purpose of this work was to implement an *in vitro* test following the OECD guidelines No. 432 for the evaluation of the safety of multiple substances and products such as cosmetics, phytosanitary products, medical devices, drugs, effluents and industrial waste, among others, with in order to predict the potential effect of acute phototoxicity.

Methodology: 3T3 cells were used and the cytotoxicity of different test substances was evaluated both in the presence and in the absence of UVA. Concentration-response curves (CRC) were performed using chlorpromazine (CPZ) as a positive control. To evaluate the response of the system, substances with a positive and negative response were evaluated, such as Norfloxacin and Sodium Lauryl Sulfate, respectively. Finally, the relative reduction in cell viability was evaluated and the inhibitory concentration 50 (IC<sub>50</sub>) was calculated, which allows calculating two indices: the photoirritation factor (PIF) and the photomedian effect (MPE).

Results: The CRC for CPZ gave an IC<sub>50</sub> of  $23.04 \pm 0.09 \mu\text{g/mL}$  (mean  $\pm$  standard deviation) for the condition without UVA radiation, meeting the acceptance criteria of the methodology (7.0 to 90.0  $\mu\text{g/mL}$ ). In turn, under UVA irradiation the acceptance criteria were also met (0.1 to 2.0  $\mu\text{g/mL}$ ) since the IC<sub>50</sub> was  $0.76 \pm 0.01 \mu\text{g/mL}$ . On the other hand, the indices calculated for CPZ were PIF > 5 and MPE > 0.15, revealing its positive phototoxic response.

It is essential to have an effective *in vitro* test method to detect substances and products with phototoxic potential after application to skin, eyes and other external epithelia exposed to light.

## Ex Vivo Gill Assays as an Alternative Approach to Fish Experimentation

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### Abstract

Fish acute toxicity studies are one of the most frequently conducted vertebrate ecotoxicology tests where death is the intended endpoint, therefore, opportunities to Replace, Reduce, and Refine Regulatory of those tests has become of increasing importance. Addressing the need for alternatives, sub-organismal responses proved to be useful to assess chemical stress and were referred to biochemical/physiological changes (biomarkers) measured in vivo or by in vitro bioassays using isolated cell lines exposed to extracted and enriched surface water. Interestingly the freshwater fish gills are a focal point for studies that seek to understand deleterious effects of various contaminants of emerging concern (EC). In this context, predicting fish acute toxicity of chemicals by means gill ex vivo assay is an alternative method to the conventional approach in line with the 3Rs ideas. The aim of this study was to assess the adverse response of selected oxidative stress biomarkers in different model fish species after ex vivo gill exposure to ECs (fipronil, Fp; triclosan, Tcs; nanosilver, AgNPs and ivermectin, Iv). Alternative studies were conducted in order to compare: Fp in vivo and ex vivo responses; the mitigation effect of humic acids on the toxicity of NPs and the photolytic degradation of TCS. These short-term assays were conducted with native (*Prochilodus lineatus*, *Cyphocarrax voga*, *Piaractus mesopotamicus*, *Corydoras paleatus*) and standardized species (*Cyprinus carpio*) as a preliminary screening approach where environmental representative concentrations of ECs were assessed. Briefly, four fish were used and four pairs of gill arches were obtained that were randomly assigned to four treatment groups (n=5): freshwater fish saline solution (Ctrl); methanol in saline solution (CtrlSv); and two nominal concentrations of C1 and C2 prepared from a methanol stock solution of the EC. After incubating in glass containers for 1h at 25°C, branchial arches were removed and kept at -80 °C until biochemical analysis. Results indicated that biomarker responses were dependent on fish species and the assayed ECs and alternative assays gave rise to valuable information. The ex vivo model provided a versatile way of exposure with a reduced number of fish and generate rapid information from a key target organ of toxicity. Besides, native test species were able to generate responses with several ECs showing response capacity even under environmentally relevant concentrations.



18.P-Mo-098

## Assessing the Environmental Safety of Sunscreens: A Novel Approach Methodology for Evaluating Coral Toxicity

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### Abstract

In the development of sunscreens, it is imperative to ensure their efficacy, human and environmental safety, particularly with respect to the preservation of coral reefs. Research suggests that various ingredients utilized in the cosmetic industry may have adverse effects on corals. However, formulating coral-safe sunscreens may prove to be technically challenging given the absence of an official protocol for this type of testing. This study aimed to propose a New Approach Methodology (NAM) to assess the toxicity of sunscreen products on coral based on their final composition. Notably, this protocol does not fall under the definition of animal testing under Directive 2010/63/EU and Brazilian Resolution n°58/2023. Five samples of sunscreen containing chemical filters with different Sun Protection Factors (SPF) were tested for acute toxicity on coral fragments (*Seriatopora hystrix*). The fragments were exposed to concentrations of the products that were higher than those expected in the natural aquatic environment, for 48 and 96 hours. The results were assessed based on the extent of fragment retraction and bleaching to determine the Lowest Observed Effect Concentration (LOEC) and the No Observed Effect Concentration (NOEC). To quantify coral bleaching during the exposure period, an analysis of the RGB (Red, Green, and Blue) color pattern of coral images was performed using Photoshop 23.5.1 software. An increase in the range of RGB values indicated greater bleaching. Statistical analyses were carried out using the TOXSTAT 3.5 program. The experimental results revealed that none of the evaluated products displayed any adverse effects on the coral fragments, as evidenced by the absence of polyp retraction or bleaching. Furthermore, a quantitative analysis using RGB data was conducted, which supported the visual observations and allowed for a comparative analysis of the coral fragments at 48 and 96 hours after exposure to the sunscreens, with the initial state being taken as a reference. This study demonstrates the importance of determining a product's specific toxicity to corals not solely by excluding potentially harmful substances from the formula but by considering the entire product. This approach leads to a more robust, quantitative, and reproducible methodology for claiming a product as "reef safe." Therefore, this NAM provides an effective pathway to develop safe and effective sunscreens that meet the demands of consumers for trustworthy products.

## 18.P-Mo-099

### Emtricitabine (FTC) Metabolite Enhances Animal Metabolic Disruption *in silico*

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#### Abstract

*In silico* approach arose during pandemics more impactful than in the 90's. Outcomes seem to be superior in prior approaches on ecotoxicology concerns as well as novel and in use drugs assessment for resistance, targets and metabolism. Specially in Latin America, resources are extremely limited and diverse interests may conflict with researchers aims. Computer-based research on chemicals and neglected contaminants, such as antiretrovirals (ARV) used for Prophylaxis Pre-Exposure for HIV (PrEp) and effective treatment for HIV infected individuals. We aimed to understand if emtricitabine, most present in both forms of HIV treatment and prophylaxis, can be acute or chronic - genotypic, proteomic or metabolic – toxic for *Chordata*, emphasized fishes and/or humans; if only for non-human species, we asked about indirect impact in human health. As material, was disposed emtricitabine first passage metabolite designed within PubChem according to pharmaceutical and pathology international guidelines; SMILES used was Nc1nc(=O)n(cc1F)C1CSC(O1)COP(=O)(OP(=O)(OP(=O)(O)O)O)O and its chemical activity was evaluated in SwissTargetPrediction, by Swiss Institute of Bioinformatics, defaulting *Homo sapiens* as target species and ToxTree 3.1.0, open source software. Results were analyzed through significance in populations bigger than 1.000 individuals (n>1000) and coincidence of finds among both softwares and National Library of Medicine of National Center for Biotechnology Information. Our results evidenced emtricitabine metabolite targets two main sites or vias: in genome, binds to ENPP1 gene product, phosphodiesterase family enzymes with special binding to ectonucleotide pyrophosphatase with probability of occurrence of 0.116, which are homologue to other *Chordata* species and main animal lineage; emphasized three classes of metabolic disruption, occurring, respectively, S-oxidation, amine hydroxylation and aliphatic hydroxylation mainly in hepatic cells of animals, including fishes and humans. All enzymes and metabolic sites associated to CYP450 are insulin dependent proteins in cell membrane. Validation *in vitro* and *in vivo* is mandatory as further research, although is quite possible to red flag ARV and PrEp drugs as contaminants of emerging concern (CEC). Brazil and other latins are highly susceptible to health damage, probably indirectly, due to fish contamination and loss of nutritional value and, eventually, insulin resistance and mutations within the enzyme targeted.

## 18.P-Mo-100

### **Development, and Regulatory Acceptance of Alternative Methods to the Use of Laboratory Animals in Safety Studies of Products for Human Use and Agrochemicals in Latin America and Selected Countries**

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#### **Abstract**

The development and regulatory acceptance of alternative methods to animal testing in safety studies of products for human use and agrochemicals in Latin America and selected countries is still limited. Currently, the legislation for the registration of these products and the regulatory authorities responsible for their authorizations, require toxicological studies carried out in animals and the acceptance of studies carried out by alternative methods is limited. In this context, we will review the current methods available, the different technologies under development in the short, medium or long term that would allow us to replace, reduce or refine the current toxicological methods. Additionally, we will analyze the legislation in Latin America regarding the toxicological evaluation of products and their acceptance by the regulatory authorities. This review is a useful tool to exchange knowledge, disseminate progress and experiences of the different countries and promote the acceptance of alternatives to animal testing in toxicological studies of products in Latin America. This information is of great interest of the representatives of the industry, academy and government, in order to develop different policies and promote regulatory changes in their countries and regions.

## 18.P-Mo-101

### New Approaches Methodologies to Assess the Dermal Toxicity of an Alternative Flame Retardant

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#### Abstract

Organic flame retardants (FR) are chemicals applied in the manufacture of various products to prevent or reduce the flammability of polymers and fabrics. Recent epidemiological studies have shown that the dermal route of exposure is also relevant in FR human contamination, but there is a gap in dermal toxicity related to FR exposure. This study evaluated the dermal toxicity of the alternative FR aluminum diethylphosphinate (ALPI) combining in vitro and in silico approaches. Three non-cytotoxic concentrations of ALPI were exposed to human keratinocytes (HaCaT cell line) and were measured the potential of ALPI to alter the DNA using flow cytometry techniques. Was quantified the intracellular reactive oxygen species (ROS) using the H2DCFDA probe, the histone variant  $\gamma$ -H2AX to analyze DNA damage, and 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) was quantified to determine changes in global DNA methylation. Effects in the inflammatory cascade was also measured using the Cytometric beads array (CBA) kit. The results showed that ALPI exposure increases ROS levels in a concentration-dependent manner. No DNA damage was observed after exposure to ALPI, and no changes in global DNA methylation patterns were observed. Cytometric beads array (CBA) showed no effects on the cytokines IL-6 and IL-8 after exposure in HaCaT cells. ALPI was run into OECD QSAR Toolbox for mechanistic profilers, and neither profiler has shown the presence of alerts. Although DNA damage was not verified,  $\gamma$ -H2AX is a biomarker for double-strand breaks, and a chemical can damage DNA by other genotoxic modes of action not covered by the  $\gamma$ -H2AX quantification. For 5mc and 5hmc, although no changes in global DNA methylation pattern were shown, the occurrence of changes in specific gene regions can not be excluded and still need to be elucidated. Regarding to inflammatory effects, IL-6 and IL-8 in HaCaT cells are more related to sensitivity effects, which agrees with the results found in the in silico analysis. In summary, our results showed that that ALPI did not cause changes in the genetic material of human keratinocytes and not affect the inflammatory cascade; however, a broader investigation covering different modes of action still needs to be performed to ensure their safety. Furthermore, this study highlights the importance of the multi-biomarkers strategy, covering different modes of action.

# Session 20: Environmental Assessment of Pesticides for Soil Organisms in Latin America

20.P-We-042

## Development of a Soil Exposure Assessment for Plant Protection Products in Brazil: Requirements, Options, and Recommendations

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### Abstract

The Brazilian Environmental Authority (IBAMA) has recently identified soil organisms as a priority area for risk assessment development for plant protection products. This includes consideration of which species in soil fauna are important to be tested for risk assessment purposes, which ecotoxicological tests should be used, and which exposure models would be suitable, considering Brazilian soils, local agricultural practices, and local biodiversity. In this brief communication, we present proposals and recommendations for the selection of appropriate regulatory exposure assessment techniques to support initial or foundation tier assessment.

DRAFT

## 20.P-We-043

### Ecotoxicological Review: Enchytreids and Earthworms Exposed to Pesticides in Brazil

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#### Abstract

The purpose of this study is to provide information on the current Brazilian scenario regarding the use of pesticides and the consequences of the toxic potential of insecticides (Imidacloprid, Lambda Cyhalothrin, Chlorpyrifos, Fipronil), herbicides (2,4-D, Atrazine, Clethodim, Glyphosate) and fungicides (Chlorotalonil, Mancozeb, Picoxystrobin, and Thiram) to non-target organisms (focusing on oligochaetes) and in different types of Brazilian soils. The keywords used in the searches were: oligochaetes, earthworms, enchytreids, pesticides, insecticides, herbicides, fungicides, biocides, Brazil, tropical and subtropical, in 2 languages (Portuguese and English). A total of 76 articles were found, with 31 articles being considered of interest reporting the toxic effects of pesticides on oligochaetes in Brazilian soils and TAS (tropical artificial soil). In these articles of interest, 62 studies were identified, of which 68% were conducted in tropical and subtropical Brazilian natural soils. Insecticides were the most evaluated compounds (46%), followed by fungicides (31%) and herbicides (23%). Tests with earthworms represent 76% of the studies and the species most used in the tests were *Eisenia andrei* and *Eisenia fetida*. Only one species of enchytreids (*Enchytraeus crypticus*) was used in studies with Brazilian soils. The ecotoxicological effects survey will support the risk assessment of pesticides, and point out gaps and future perspectives.

## Relevant Edaphic Parameters for Constructing Environmental Risk Assessment Scenarios for Soil Organisms in Brazil Based on the Toxicity of Pesticides to Microarthropods

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### Abstract

In order to advance the regulatory process for pesticides in Brazil, with the implementation of environmental risk assessment (ERA) of pesticides for soil organisms, it is pertinent to define national exposure scenarios by delimiting agricultural areas, as well as to define labiotic factors which may influence the vulnerability to pesticides toxicity to these organisms. Thus, a literature review about the ecotoxicological effects of ten different pesticides on edaphic microarthropods (Collembola and Acari) was carried out. Environmental parameters significantly influenced the sensitivity of these microarthropods to pesticides, hence could be considered for use in the ERA scenarios for soil organisms. The parameters were: temperature, moisture content, and soil type - whether natural or artificial, including differences in texture and organic matter content. Studies with chlorpyrifos, imidacloprid, lambda-cyhalothrin showed that higher temperatures tend to increase acute and chronic effects of the pesticides on some Collembola and Acari species, both in natural and artificial soils. There is also a rising trend of the toxicity of imidacloprid and lambda-cyhalothrin to Collembola species as soil moisture decreases. Furthermore, chlorpyrifos, fipronil and imidacloprid showed to be highly toxic to *Folsomia candida* in all soils tested. However, when tested with natural soils, a greater toxicity was observed for Fipronil and Imidacloprid. Also, a variance in toxicity between natural soils (Entisol and Oxisol) was observed for fipronil and mancozeb, probably due to differences in cation retention and in soil texture. Regarding organic matter (OM), the relationship between toxicity and OM content was not clear in the observed studies. In studies with imidacloprid, OECD soils with 10% OM content were less toxic than soils with 5%, while soils with about 12% OM content showed similar toxicity when compared to OECD soils with 5% OM. Then, this environmental parameter needs to be better studied to prove its relevance in the construction of ERA scenarios. Therefore, it is assumed that environmental parameters such as temperature, moisture content, and soil type must be considered for the establishment of these scenarios, aligned with the Brazilian Specific Protection Goals for soil fauna, due to their observed direct influence on the toxicity of pesticides for edaphic microarthropods.

## 20.P-We-045

### Does Fertirrigated Sugarcane Vinasse Cause Ecotoxicological Effects on the Reproduction of the Potworm *E. crypticus*?

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#### Abstract

Vinasse is a byproduct of the sugar or ethanol industry that has been used as a fertilizer in crops, thus preventing direct discharge into water bodies. Although vinasse fertilizer is considered to contribute to a circular economy by aggregating value to this subproduct, it is important to assess the safety and sustainability of continued recycling of vinasse as a fertilizer for the soil environment. This study evaluated the potential adverse effects of sugarcane vinasse on an important soil model organism, the potworm *Enchytraeus crypticus*. The tests were conducted over 21 days using both in natura sugarcane vinasse and sugarcane vinasse biodigested in a methanogenic reactor, in LUFA 2.2 soil, and exposed to six different vinasse concentrations (0.23, 0.92, 3.59, 14.38, 57.50 and 230 ml/kg dry soil, with the highest concentration corresponding to the recommended field dose). The results showed that the concentration of vinasse did not significantly affect the survival or reproduction of the enchytraeids ( $p = 0.273$  and  $0.489$ , Two-Way ANOVA, factor A). However, the type of vinasse (in natura or biodigested) did have a significant impact on the ecotoxicological response ( $p < 0.05$ , Two-Way ANOVA, factor B), with higher survival and reproduction observed in exposures to biodigested sugarcane vinasse. However, a similar increase was also observed in the control group. These findings suggest that in natura sugarcane vinasse may alter the chemical characteristics of the soil but has a low impact on the life cycle of *E. crypticus*. Nevertheless, it is recommended to further investigate the potential effects of continued use of sugarcane vinasse on other species or soil organisms and assess its effects on aquatic organisms considering potential runoff in crops field.



## 20.P-We-046

### Sorption Process of Diclosulam in a Tropical Soil Amended With Raw Feedstock and Olive Stone Biochar

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#### Abstract

Diclosulam (2',6'-dichloro-5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide) is a soil applied sulfonamide herbicide and any addition of organic compounds to the soil, such as biochar, can interfere with the effectiveness of weed control. Thus, this study aims to evaluate the sorption of diclosulam in a tropical soil amended with raw feedstock and olive stone (*Olea europaea*) and pyrolysed biochar at two temperatures (BC300 and BC500°C). The soil was classified as an Oxisol. The soil was amended at an application rate of 10% (w w<sup>-1</sup>) representing 100 Mg ha<sup>-1</sup>, assuming a soil density of 1 g cm<sup>-3</sup> and incorporation depth of 0.10 m. The control treatment was without biochar addition (unamended soil). Sorption isotherms were performed by the equilibrium bath method. The quantification of diclosulam was by a High Performance Liquid Chromatography (HPLC). The Freundlich sorption coefficient (K<sub>f</sub>) of diclosulam ranged from 0.65 (unamended soil) to 2.27 mg<sup>1-(1/n)</sup> (g<sup>-1</sup>) L<sup>1/n</sup> (amended-BC300 soil), with the amended-BC500 and -raw feedstock soils sorbed similar to unamended soil. The BC300-amended soil showed the highest sorption of diclosulam. The sorption capacity of diclosulam was different for the treatments, which can be explained by the different bonds between the matrices of the soil and soil-biochar mixtures with the herbicide.

## 20.P-We-047

### Effect of Herbicides Used in Sugarcane Cultivation on the Avoidance Behavior of *Eisenia andrei*

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#### Abstract

The intensive use of long-residual herbicides can directly influence terrestrial ecosystems. In this sense, the objective of this work was to evaluate the avoidance behavior in *Eisenia andrei* after exposure to different concentrations of the herbicides indaziflam, sulfentrazone and clomazone. This experiment was conducted in accordance with the ISO 17512-1 (2008) standard and the tests were performed separately for each of the herbicides, in which the following sublethal concentrations were used: 1, 10, 25, 50 and 75 mg a.i. kg<sup>-1</sup> dry soil for clomazone and 1, 10, 50, 75 and 100 mg mg a.i. kg<sup>-1</sup> dry soil for sulfentrazone and indaziflam. A dual control test containing only distilled water on both sides was also performed. After 48 hours, the cardboard divider was reintroduced between the treated and untreated soils and the number of earthworms on each side was counted and the median evasion concentration (AC<sub>50</sub>) after 48 hours of exposure was determined using the statistical method of Trimmed Spearman-Kärber and significant differences in the distribution of earthworms were analyzed using Fisher's exact test (p<0.05). For clomazone and indaziflam AC<sub>50</sub> was estimated at concentrations of 17, 50 and 29.66 mg a.i. kg<sup>-1</sup> of soil, respectively. Sulfentrazone provided a high percentage of leakage regardless of the concentration used, and from a dose of 1 mg i.a. kg<sup>-1</sup>, an average leak of 40% was observed, therefore, it was not possible to estimate the AC<sub>50</sub>. While for clomazone and indaziflam, higher percentages of leakage were observed at concentrations of 50 and 75 mg a.i. kg<sup>-1</sup> and 75 and 100 mg a.i. kg<sup>-1</sup>, respectively. Therefore, it is concluded that although an escape behavior was observed for all the studied herbicides, mainly in the highest concentrations, none of them provided >80% and cannot be considered toxic.

## 20.P-We-048

### Biochar from Sugarcane Straw Reduces the Ecotoxicity of the Pesticides 2,4-D and Fipronil to the Earthworm *Enchytraeus crypticus*

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#### Abstract

The contamination of soil by pesticides is one of the main threats to the environment due to their effects on non-target organisms, with consequences for biological assemblages and ecosystem functioning and services. In this sense, this study applied biochar, produced from sugarcane straw, to reduce the ecotoxicity of soils contaminated with the pesticides fipronil and 2,4-D, alone and in a mixture. Tests at the microcosm level (28 days) evaluated the influence of biochar on the survival, reproduction, and feeding rates of the terrestrial worm *Enchytraeus crypticus*. Thereunto, non-toxic (Copaza®) cylindrical polypropylene containers (15 cm diameter, 12 cm height, 1 kg capacity) were used. Each microcosm was filled with 800 g of moistened soil and the proportion of biochar tested in the treatments was 3% (w:w). All results were analyzed using two-way ANOVA followed by Tukey's pos-hoc test using the "Desc Tools" package in R Studio®. In the microcosms without biochar, fipronil and pesticide mixture caused decreases in the survival of adults compared to the control ( $p < 0.05$ ). Fipronil and 2,4-D also decreased the reproduction of *E. crypticus* in microcosms, with emphasis on fipronil toxicity in both scenarios (alone and in a mixture). Biochar reduced the toxicity of fipronil in soils, increasing the reproduction of *E. crypticus* by 43% compared with soils without biochar. However, the control with biochar showed a reduction of 23% in reproduction when compared with the control without biochar, indicating possible toxicity. Regarding the feeding activity of *E. crypticus*, all food in bait-laminas was consumed by the organisms, which lead to no statistical difference between the treatments ( $p > 0.05$ ). According to the literature, the effects of pesticides on *E. crypticus* are attributed to their mode of action. In addition, the flexible body and permeable skin of the species facilitate the contact with pesticides, thus affecting the assessed endpoints. The biochar efficiency can be attributed due to its high specific surface area (SSA) and developed micropore structure that increases its sorption potential, reducing the pesticides ecotoxicity on non-target organisms. Even with the reduction in the reproduction of *E. crypticus*, biochar was still able to attenuate the toxic effects of pesticides, given all the other beneficial effects when applied. Therefore, biochar amendments showed high potential in reducing the ecotoxicity of pesticides to the studied species.

## 20.P-We-049

### Behavioral Pattern of Edaphic Cyanobacteria Community in Relation to Atrazine and Nitrogen Use

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#### Abstract

The morphological and physiological adaptability of cyanobacteria allows them to be pioneers in terrestrial ecosystems. However, the intensification of herbicide and fertilization practices has not considered the possible impacts on these communities. In this work, the behavior of cyanobacteria was evaluated in relation to two agrochemicals used in direct sowing: atrazine and nitrates (N-NO<sub>3</sub>). After field trials applying four treatments plus their control: with atrazine (CA), with nitrogen and atrazine (CNA), with nitrogen (CN), without nitrogen or atrazine (SNA) and control (Nativo), soil samples were taken, and cyanobacteria cultures were grown in the laboratory under controlled conditions of light and temperature. A total of 38 taxa were identified by optical microscopy. The generalized procrustes analysis, using treatments as classification criteria, allowed us to determine that the variables studied for the soil, such as the presence/absence of species, were equivalent, since a Consensus = 0.926 was obtained for both groups of variables. From the analysis of the minimum path tree lines, it can be seen that the CN and Nativo treatments differ from the remaining CA, CNA and SNA treatments. It should be noted that this analysis shows inverse variation between treatments with atrazine (0.27) and without atrazine (-0.27), for the association of the variable presence/absence of cyanobacteria and soil variables. The differences between the communities are related to the presence of common and exclusive species; the highest number of common species was detected between the native forest soil and the CN treatment with 7 species and the lowest number of common species was observed when comparing the native soil with the atrazine treatments with 1 and 2 species in common respectively. In relation to the species that developed exclusively, 2 species were observed in the soil with CA treatment and 8 in the native soil. This work provides valuable information about the impact of atrazine and nitrate use on the edaphic cyanobacterial community, causing at least a reorganization of the community structure analyzed in terms of dominant species, structural and functional diversity of the community.

## 20.P-We-050

### Use of Edaphic Cyanobacterial Consortium to Assess Atrazine Toxicity

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#### Abstract

Atrazine is the second most used herbicide in Argentina and, although there are studies on the impact on some soil microorganisms, the effect on edaphic cyanobacteria communities is unknown. In this work, the effect of atrazine exposure on a soil cyanobacterial consortium was evaluated. From soil samples, with no history of agriculture, cyanobacteria were cultured under controlled light and temperature conditions. The trials consisted of exposing the crops to 0.10, 0.01 and 0.001 mg/kg of the commercial formulation atrazine. The response variables were % of dry matter (DM), yield (R) and chlorophyll a (Cla). When evaluating DM, average values between 2.651% and 6.847% were obtained for the concentration of 0.001 ppm with 24 h of exposure to the herbicide and the Control, respectively. In the case of the variable R, the minimum average value of 1.041 mg/cm<sup>2</sup> was obtained for the concentration of 0.001 ppm with 96 hours of exposure and the maximum average value of 4.836 mg/cm<sup>2</sup> in the Control. Regarding Cla the minimum average value was obtained with the concentration of 0.01 ppm with 5.003 mg/kg at 24 hs of exposure, while the maximum average value resulted in the Control with 17.425 mg/kg. The analysis of variance showed that for the DM variable, significant differences were found both between exposure times (F=4.91; p=0.0064) and between the concentrations applied (F=3.03; p=0.0434). The times 24 and 72 h (3.86 and 3.95 ± 0.33, respectively) were significantly different from the Control (5.42 ± 0.33). In the case of the variable R, significant differences were found between atrazine exposure times (H=16.98, p=0.0007). In this case, the value obtained for the Control (2.98 ± 1.19) was significantly higher than the remaining exposure times, of which the minimum was obtained at 24 h of exposure (1.41 ± 0.61). For Cla, significant differences were found between exposure times (H=11.66; p=0.0086); the 24-h group (6.24 ± 1.90) was significantly lower than the Control (10.74 ± 4.66). It can be concluded that the observed effects are maximum at 24 hs of exposure to atrazine and decrease at 72 and 96 hs. Therefore, it is inferred that atrazine produced at least a temporary toxicity on the studied consortium, with significant changes in the cyanobacterial community.

## 20.P-We-051

### **Influence of Soil Properties on the Sensitivity of the Collembolan Species *Folsomia Candida* to Neonicotinoids**

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#### **Abstract**

Soil is the basis of life and terrestrial organisms depend directly or indirectly on it. Among edaphic organisms, microarthropods stands out (especially mites and springtails) due to their abundance and diversity in soils, in addition to their sensitivity to chemical and physical changes in the environment. They are commonly considered excellent bioindicators of soil quality. Among the activities with the potential to change environmental systems are traditional agricultural crops, which are heavily dependent on the use of pesticides to combat harmful organisms. When applied to the crops, pesticides become contaminants in soil, which is its main receptor. As the action of the pesticides is not exclusive to target organisms, negative effects can also be observed in non-target individuals, as is the case of neonicotinoid insecticides (developed from the nicotine molecule) for springtails. In addition to the mode of action of pesticides, different physicochemical properties of the soil (eg, organic matter, pH, texture and porosity) can influence pesticides toxicity to soil organisms, since clayey and sandy soils result in different levels of pesticide availability due to their molecules adsorption in a greater or lesser extent. Therefore, this study aimed to evaluate the toxic effects of the neonicotinoid insecticides CRUISER 350 FS (thiamethoxam as the active ingredient) and INSIDE FS (clothianidin as the active ingredient) on the survival and reproduction of *Folsomia candida* springtails when exposed to two types of soil: Tropical Artificial Soil (TAS) (75% sand, 20% kaolinite and 5% coconut fiber) and Natural Soil (NS) collected in a preserved area and characterized as a red oxisol. For each test, TAS and NS, 10 adult individuals (10 to 12 days) were exposed to increasing concentrations of the insecticides, plus a control treatment (without contamination). At the end of the exposure (28 days) the organisms were significantly sensitive to both pesticides, with clothianidin being more toxic than thiamethoxam. The results also showed a greater sensitivity of *F. candida* when exposed to artificial soil, indicating the influence of soil properties on the toxicity of these compounds. Tests with other types of natural soils are crucial to understand the influence of soil properties on the toxicity of pesticides. Besides, it may help to understand the effectiveness of the extrapolation of the results obtained using artificial soils to natural soils.

## 20.P-We-052

### Evaluation of the Effects of the Insecticide Thiametoxan on the Relationship of Predator-Prey Interaction of Soil Invertebrates

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#### Abstract

The growing demand for food and energy, sustained by human needs, causes an increase in the consumption of agricultural inputs, such as pesticides. Among the most used classes of pesticides are insecticides. One of the great representatives of this class of pesticides are the neonicotinoids, such as thiamethoxam, an insecticide widely used to eliminate sucking and chewing pests in several agricultural crops. However, though widely used, little is known about its effects on non-target edaphic organisms. By acting on the nervous system of arthropods, neonicotinoids may imbalance the ecosystem by altering the trophic relationships between organisms and consequently affecting the continuity of populations. Therefore, the aim of this study was to evaluate the effects of the insecticide thiamethoxam on the trophic relationship of edaphic invertebrates in two types of soil (natural and artificial soil). The natural soil was collected in the Environmental Protection Area (APA) of Lajeado, in the municipality of Palmas, Tocantins, in an area of red latosol. The Tropical Artificial Soil (TAS) was produced in the laboratory and composed of 75% fine sand, 20% kaolin and 5% powder of coconut fiber. The test substance used was the pesticide CRUISER® 350 FS, whose active ingredient (i.a.) is thiamethoxam. The organisms tested were the predatory mite species *Hypoaspis aculeifer* and the springtail *Folsomia candida*. Adult organisms with known ages of both species were used to perform the assays, being 28 to 35 days for *H. aculeifer* and 10 to 12 days for *F. candida*. For each assay, seven increasing concentrations of thiamethoxam were used, based on the sensitivity of the organisms and the recommended dose for application of the insecticide in sunflower crops. With the results, it was possible to observe differences in sensitivity between the species, with *F. candida* being more sensitive than *H. aculeifer*. When compared with individual trials, a tendency of higher sensitivity of the species *F. candida* when exposed together with mites was observed. Results showed the importance of observing joint effects on organisms of different trophic levels. In addition, it was possible to observe the influence of soil properties on the sensitivity of the species tested and the importance of using distinct soils for better extrapolation of the results in the environment and conservation of the species.

## 20.P-We-053

### **Influence of Soil Properties on the Sensitivity of the mite *Hypoaspis aculeifer* to Neonicotinoids**

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#### **Abstract**

Pesticides are chemical or biological substances used to eliminate harmful organisms in agricultural crops. Neonicotinoids act on the nervous system of arthropods, reaching both target and non-target organisms, and causing imbalance in essential soil processes, such as nutrient cycling and organic matter decomposition. In addition to the mode of action of pesticides, the physicochemical properties of the soil (organic matter content and texture) may influence the sensitivity of the exposed species. In this sense, the objective of this work was to evaluate the effects of the insecticides clothianidin and thiamethoxam, used in seed treatment, on the reproduction of the mite *Hypoaspis aculeifer* in two types of soil: Tropical Artificial Soil - TAS (75% fine sand, 20% kaolin and 5% coconut fiber powder) and Natural Soil - NS. Reproduction tests with the mite *H. aculeifer* followed OECD standard 226. The tested concentrations of clothianidin and thiamethoxam in both soils varied between 10 and 200 mg.kg<sup>-1</sup> and from 1 to 400 mg.kg<sup>-1</sup>, respectively. The data obtained in the tests were submitted to unidirectional analysis of variance, aiming to evaluate the significance of the results when compared to the control treatment, and consequently to obtain the lowest (LOEC) and the non-observed effect concentrations (NOEC). Obtained data were also fitted to non-linear regression models for the calculation of the effective concentrations needed to reduce *H. aculeifer* reproduction by 50% (EC50) and 20% (EC20). The results found indicate that the neonicotinoids clothianidin and thiamethoxam caused significant effects on the reproduction of the predatory mite *H. aculeifer*, both in TAS and the oxisol soils, with clothianidin being more toxic than thiamethoxam. Although the influence of soil properties on the toxicity of compounds was expected, organisms showed similar sensitivities on both soils. The low influence of these soil properties on the sensitivity of the mite *H. aculeifer* can be explained by the way these organisms are exposed to soil contaminants. Because they are predators, they end up ingesting only the non-contaminated food, hence not ingesting soil particles as many other terrestrial invertebrates. Besides, they have a low cuticle permeability, which makes them less susceptible to the contaminated water in the soil. Thus, more researchs on different soils and ways of exposure are essential to better understand the mechanisms of action of pesticides on mites.



## 20.P-We-054

### **Risk Assessment of Pesticides for Soil Organisms Following the Ecosystem Services Concept**

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#### **Abstract**

Plant protection products (PPPs) are widely used in agriculture to control pests, weeds, and diseases and thereby contribute to increasing yields worldwide. A robust environmental risk assessment for PPPs is necessary to protect non-target organisms and ecosystems from unacceptable side effects. In agricultural landscapes soil organisms are playing an important functional role by contributing to e.g. organic matter degradation, nutrient cycling, carbon sequestration, water infiltration and soil structuring, moreover also by acting as a critical component in a complex soil food web. Hence, a PPP risk assessment for soil organisms and their major functions is useful from both, an ecological and agronomical point of view. We present an outline proposal on a risk assessment and management framework for soil organisms and functions based on the Ecosystem Services concept. Different Ecosystem Services are provided by different parts of the landscape. Therefore, we propose a spatial differentiation of soil risk assessment by focusing on the most relevant services which are provided by the respective landscape elements. Targeted risk mitigation and management measures can help to minimize side effects of PPPs uses and ensure that environmental protection goals are met, and crop yields are maximized in a sustainable way.

## 20.P-We-055

### **Review of the Brazilian Soil Macro- and Meso-fauna: Earthworms, Enchytraeids, Springtails and Soil Mites**

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#### **Abstract**

Brazil is the 5th largest country in the world, with close to 846 million hectares (ha) of land. Agricultural production and livestock production cover 284 and 220 million ha, respectively (i.e. 34 and 26% of the total area). In terms of environmental conditions, Brazil ranges from equatorial rainforests in the north, to temperate coniferous forests in the south. This diversity in environmental conditions and land occupation has a major effect on the country's fauna and flora, with a great variety of species populating distinct habitats. Among the organisms living in the different ecosystems, soil macro- and meso-fauna are two of the groups being most affected by changes in land occupation and environmental conditions. Soil ecosystems are highly complex and dynamic and provide a multitude of key environmental, economic, social, and cultural ecosystem services. Essential soil functions that contribute to ecosystem services, such as organic matter degradation, nutrient cycling, and water infiltration, are to a certain extent mediated by soil organisms (including soil microorganisms). Soil functions and functionally relevant soil organism groups should therefore be protected from unacceptable side effects from intensive agriculture and live-stock production. Soil organisms in general can show a great species diversity, abundance and seasonality depending on several abiotic parameters such as the use of land for agriculture/live-stock production, rainfall/humidity levels or temperature. With this in mind, Specific Protection Goals (SPGs) in context of environmental risk assessment will be developed for pesticides (e.g., data requirements, acceptable effect magnitude and duration). SPGs should aim to be realistic, workable, and relevant for the agronomic environment, and their definition should take into account the usual fluctuations of soil organisms and functions in agricultural ecosystems, i.e. the 'Normal Operating Range'. The results of a literature review on soil biodiversity regarding earthworms, enchytraeids, springtails and soil mites will be presented, evaluating the effects of agricultural practices, location, regional climate conditions, soil type and covering vegetation on the 'Normal Operating Range' for these species in terms of diversity, density, distribution, and variability across Brazil. This evaluation is considered to be a stepping stone for the definition of SPGs for Brazil.

## 20.P-We-056

### Use of the Non-Standard Collembola Species *Onychiurus yodai* in Ecotoxicological Assays

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#### Abstract

Soil biodiversity performs extremely important functions for the quality of the ecosystem. Among the most abundant edaphic organisms are representatives of the mesofauna, with Collembola being one of the most diverse group. With the increasing anthropic use of soils, ecotoxicology becomes one fundamental tool for assessing the effects of contaminants from different origins that reach this compartment. Using standard species, contaminant concentrations that cause harmful effects on the biota are investigated. However, it is not well understood whether results obtained to standardized species may be extrapolated to any species, due to possible differences in their sensitivities to contaminants. Thus, the objective of the present study was to use the ISO 11267 guideline, described for the species *Folsomia candida*, and study necessary adaptations to enable the use of the species *Onychiurus yodai* as a test organism. Hence, three different assays with organisms aging from 21 to 29 days were performed. The test substance used was boric acid, and the substrate was the tropical artificial soil - TAS (75% fine sand, 20% kaolin, 5% coconut fiber). Boric acid concentrations varied according to the available information regarding the effect caused. In the first test, a range of 1 to 1000 mg of boric acid/kg of soil was used, obtaining no significant effects up to the concentration of 100 mg/kg, but at the concentration of 1000 mg/kg all organisms died. Thus, at the second assay concentrations varied between 35 and 491 mg/kg, and effects in the reproduction of the organism were observed at 170 mg/kg. In the first two tests, 10 adult organisms were used per replicate, but the number of juveniles in the control situation was a lot variable. Hence, a third assay was carried out with concentrations ranging between 45 and 512 mg/kg, using 20 organisms per replicate, which proved to be more appropriate for the species under study. The proposed adaptation is justified by the fact that the species *O. yodai* has sexual reproduction, while *F. candida* reproduces asexually. The adaptation proved to be efficient, fulfilling the validation criteria stipulated for the control group in the ISO guideline. We conclude that the species *O. yodai* may be used in ecotoxicological assays using *F. candida* guideline with little adaptations. This will favor the understanding of the accuracy in the extrapolation of results from *F. candida* to other Collembola species.

## 20.P-We-057

### Physical Attributes of Collembola Species and Their Relationship With the Organism's Sensitivity to Boric Acid

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#### Abstract

Collembola is microarthropods that have three pairs of legs (hexapods), a pair of antennae, a furca, and no wings. Although present in different ecosystems, they are of greater abundance and diversity in soils and are considered bioindicators of soil quality, due to their importance and sensitivity to changes in the environment, in addition to their fundamental role as microbiological regulators. According to their vertical distribution in soils, Collembola species will present different morphological characteristics (eg, size of legs, antennae and furca, and presence or absence of hair and color, etc.), which defines their way of exposure to different contaminants and may influence their sensitivity. Thus, when evaluating the effect of contaminants on springtails, morphologically distinct species shall be tested to compare the differences in terms of sensitivity and better understand whether the results of only one standardized species may be extrapolated to other Collembola species. Thus, the goal of this study was to assess the sensitivity of two springtail species (*Sinella curviseta* and *Protaphorura fimata*) to boric acid and the relationship between their sensitivities and physical characteristics. The studied endpoints were survival and reproduction in tropical artificial soil (SAT), produced in the laboratory (75% fine sand, 20% kaolin and 5% coconut fiber), tested according to the protocol established for the standard species *Folsomia candida* (ISO 11267, 2014), with minor adaptations. For both species, reproduction was the most sensitive parameter, with reductions of 50% (EC50) at the concentrations of 131 [117 – 145] and 190.5 [142 – 238] mg of boric acid/kg of SAT, for *S. curviseta* and *P. fimata*, respectively. The results, therefore, showed a trend of greater sensitivity of *S. curviseta*, which is a surface species with high mobility and the presence of hair and eyes, when compared to *P. fimata*, a species that lives buried and has low mobility and absence of hair and eyes. This result was surprising since greater sensitivity was expected to *P. fimata* since it has a more permeable cuticle and is in direct and constant contact with the contaminated soil. Studies with more species and metabolic biomarkers are needed to understand the modification of boric acid toxicity on these organisms.

20.P-We-058

## Ecotoxicological Profile of the Herbicide Zethamaxx in Soil, Evaluated by the Test Organism *Allium cepa*

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### Abstract

Due to the growing demand for food, the world consumption of pesticides has grown considerably in recent years in an attempt to reduce losses caused by pests and increase agricultural productivity. Brazil, one of the largest agricultural producers and exporters, is one of the five countries that most consume pesticides in the world. Of these, Zethamaxx is a herbicide widely used in soybean cultivation, of which Brazil is the largest producer in the world. Despite the significant economic gains from the use of pesticides, this practice has resulted in intense environmental degradation of different environments (soil, water, and atmosphere), compromising ecosystem quality and the health of organisms exposed to them. In this sense, this study aimed to evaluate the phytotoxicity of the herbicide Zethamaxx, in 3 different concentrations (1%, 1.5%, and 2%), on the test organism *Allium cepa*. The concentrations were chosen based on the manufacturer's recommendations for field application. Soil field capacity was set to 50%, and seed germination was carried out directly on the soil in Petri dishes. The pesticide was applied with a sprayer considering an application rate of 200 L/ha. Negative and positive controls were carried out with soil and zinc sulfate (0.05 M), respectively. The plates were adequately covered and sealed with plastic film to avoid evaporation and subsequently incubated in BOD at 22 °C for a period of 6 days, with 12-hour photoperiod. In the phytotoxicity test, although no significant effect was observed on the percentage of seed germination, yellowing of the radicles and a decrease in root growth were observed in all tested concentrations. Additionally, the results of the germination index (IG%) allowed the classification of all three concentrations as moderately toxic, indicating their environmental hazard. However, other parameters such as genotoxic and mutagenic effects must be assessed to better understand its mode of action in the DNA of non-target organisms and amplify comprehension regarding the ecotoxicological effect of this pesticide.

## 20.P-We-059

### Obtaining Biological Parameters of Native Cerrado Collembola Species for Ecotoxicological Assessment

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#### Abstract

The Collembolas are apterous arthropods of the edaphic mesofauna. They participate in the processes of fragmentation and decomposition of organic matter, nutrient cycling and population control, especially of fungi. Due to the abundance and diversity of these organisms in the soil, they are used as indicators of quality and assist in studies of environmental impacts, especially in relation to chemical substances, such as pesticides. Currently, the species of Collembola standardized for ecotoxicological tests are from temperate climate environments, and there are still no tests with tropical climate organisms in Brazil. Therefore, this study aims to cultivate at least one species of collembola collected in the Cerrado biome, in the State of Tocantins, and to identify biological parameters about its growth and reproduction, in order to make it available for ecotoxicological tests. To this end, two collections were carried out in different Forest Agricultural Systems, to obtain nativespecimens. Intact soil samples were collected, following the ISO 23611-2 standard. For the extraction of the organisms, a system adapted from the Berlese-Tullgren funnel was used for 5 days. The cultivation procedures were adapted based on the ABNT NBR ISO 11267 standard. With the extraction, it was possible to collect 17 species, while of these, 3 species are currently being cultivated in the laboratory. With the help of the Collembola.org identification key, three different families were identified, Entomobryidae, Isotomidae and Turbegiidae. In the family Isotomidae, the genus called Archisotoma was identified. Based on the previous results of the monitoring of the life cycle of Archisotoma, it was identified that this species takes about 16 days to reach reproductive age and its reproduction is sexualized. The eggs take about 3 days to hatch and reproduction is most successful in containers with a larger number of organisms. The results obtained indicate success for the cultivation of collembola native to the Cerrado. In addition, these results are of paramount importance for the adaptation of the protocol used with the standardized species of springtail *F. candida* (ISO 11267) and the correct evaluation of the ecotoxicological effects of various substances on native species of collembola.

## 20.P-We-060

### Toxicogenetic Effect of the Herbicide Clethodim on the Test System *Allium cepa* L.

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#### Abstract

Pesticides are compounds that have a variety of chemical substances or biological products developed in order to enhance a biocide action. Their use is correlated with the world economy and with the increase in agricultural productivity, but the risks of their use cause damage to health and the environment, damaging the existing biota. Clethodim is a post-emergent and systemic herbicide of graminicidal action, highly selective for cotton, coffee, onion, carrot, soybean, among others. The objective of this work was to evaluate the toxicogenetic effects of the herbicide, using the plant model *A. cepa*. The experiment was carried out in an entirely randomized design, with six Petri dishes per treatment, containing different concentrations (g/L) of the herbicide according to the concentration recommended (C3) by the product manufacturer: C1 (1.92), C2 (0.96), C3 (0.48), C4 (0.24), C5 (0.12), C6 (0.06). Analyses of germination, root growth and cytogenotoxic potential were performed using cell cycle analysis. The data were analyzed by the Shapiro-Wilks test to check their normality. For phytotoxicity analysis (germination and root growth) the Tukey test was used ( $P < 0.05$ ) and for cell cycle analysis and mitotic index the Kruskal Wallis test ( $P < 0.05$ ). The results reveal that the herbicide inhibited the rate of germination and root growth of the roots. No significant changes were observed in the cell cycle such as chromosomal changes, condensed nucleus formation, DNA fragmentation, and alteration in the mitotic index. The phytotoxic potential of the product was observed at concentrations 1, 2 and 3 respectively 1.92 g/L, 0.96 g/L and 0.48 g/L. Although Clethodim herbicide caused the inhibition of the germination index and root growth, no significant difference was observed in the mitotic index. Regarding the phases of the cell cycle, no significant difference was observed between the concentrations with the negative control. However, an increase in the frequency of micronuclei was observed at concentration 1 (1.92 g/L) compared to the negative control. The tests showed that the herbicide was phytotoxic by inhibiting the germination rate and root growth, and mutagenic in some concentrations by increasing the occurrence of nuclear alterations.

## 20.P-We-061

### **Pesticide Residues Determination Aiming to Assess the Effect of Herbicide Mixtures on Different Soil Functions in Uruguay**

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#### **Abstract**

Latin America is the region with the largest soybean area planted in Argentina, Brazil, Bolivia, Paraguay and Uruguay. Among the pesticides used for this extensive production, herbicides are the most used with the highest rates. There is little data available about the levels, environmental fate and impacts on ecosystemic services of herbicides and its metabolites in Latin American soils. In an effort to fill this gap, in Uruguay a field experiment was conducted aiming to assess the effects of frequently used herbicide mixtures on different soil functions. Mixtures of flumioxazin, fomesafen, metolachlor and clethodim were applied at the label recommended dose, following a real application scheme, previously and during the soybean crop. Different combinations of the herbicides were tested: a total of 8 treatments plus control (n=3). Soils were sampled four times during the 8 months crop season, from the first application until the soybean harvest. The whole experiment was repeated in two consecutive years. The first step was to develop and validate an adequate analytical methodology, which is a chemical challenge. Five different QuEChERS based sample preparations were evaluated to analyze the herbicides together with the metabolites clethodim sulfone, metolachlor OA and metolachlor ESA by LC-MS/MS. Finally, an appropriate multiresidue methodology was validated according to SANTE/12682/2019 guidelines. This methodology is useful for the analysis of a total of 82 analytes including acidic and polar pesticides. Limits of quantitation (LOQ) range from 5 to 10  $\mu\text{gkg}^{-1}$ . Recoveries from 70 to 120 % with RSDs <20 % were obtained. Linear calibration curves with BCC <20% were obtained in the range 5-100  $\mu\text{gkg}^{-1}$  or 10-100  $\mu\text{gkg}^{-1}$  depending on the analyte. Matrix effects were <20% for most of the compounds but the 10% showed higher values. The analysis of the samples showed that the herbicides and metabolites under study ranged from <LOQ to 483  $\mu\text{gkg}^{-1}$  depending on each analyte and field dose. Each herbicide and/or its metabolites remained detectable in the subsequent samplings following applications. The analytical methodology proved to be fit for purpose and the obtained results are valuable data needed for the environmental assessment of pesticides in Latin American soils.



## 20.P-We-062

# Deltamethrin Photoproducts: Determination and Quantification by Gas Chromatography Mass Spectrometry (GC-MS) and Study of Their Ecotoxicological Impact in Soil Using *Eisenia fetida* as Bioindicator .

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### Abstract

In recent times, agriculture in Argentina has had its yields increased through the application of pesticides and the use of polyethylene (PE) sheeting coverings (mulching) which are employed in greenhouse construction and as regulators of soil moisture and weeds. After being used, they accumulate on site, leading to environmental problems. Additionally, a fraction of the applied pesticides remain deposited on these coverings, which further aggravates the situation. Under laboratory conditions, these pesticides could have the ability to migrate from the PE to the soil, modulating the fate of these substances in the environment.

The objectives of this work were to study the photochemical transformation reactions of the pyrethroid insecticide deltamethrin that may occur on PE sheeting and to evaluate the ecotoxicological effect these photoproducts could have on soil biota .

To study the photochemical degradation of deltamethrin, an analytical method was developed for identification and quantification of pesticide and photoproducts. For this purpose, 9 cm<sup>2</sup> fragments of black (25  $\mu$ m) and crystal (100  $\mu$ m) PE, previously spiked with deltamethrin (58.2  $\mu$ g/cm<sup>2</sup>), were irradiated for 30 minutes. Then, an extraction with cyclohexane was performed and derivatized with N,O-Bis(trimethylsilyl)trifluoroacetamide for analysis by GC-MS. The percentage of original deltamethrin was reduced by around 50% in both types of films. The presence of decamethrinic acid (CPCA), 3-phenoxybenzaldehyde, and 3-phenoxybenzoic acid, as photoproducts was determined.

To evaluate the acute toxicity of the photoproduct CPCA, lethal toxicity tests were carried out using the contact paper technique for both deltamethrin and CPCA. It is interesting to note that there is no reported ecotoxicological information on the toxicity of CPCA in earthworms *Eisenia fetida*. LC50 was estimated following international standards (OECD 207, 1984). According to classification of toxicity proposed by Robert and Dorough (1984), deltamethrin was relatively non-toxic while CPCA was categorized as very toxic in *E. fetida*.

The results obtained constitute a starting point for studying the migration processes of CPCA that may occur from PE to the soil. The quantitative determination of CPCA formed from photodegradation and/or biodegradation processes from different amounts of deltamethrin will be of great interest as it will allow for a more precise evaluation of the ecotoxicological impact on agricultural soils.

# Session 21: Nature-Based Solutions for Remediation and Restoration of Water Quality

21.P-Tu-136

## Evaluation of *Salvinia minima* for Phytoremediation of Veterinary Drugs in a Dairy Farm Wastewater

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### Abstract

Antibiotics and their metabolites are ubiquitous emerging pollutants present in water bodies. Due to the frequent use of these compounds in dairy farms, either for therapeutic or growth-promoting purposes, there is concern about the promotion of antibiotic resistant bacteria and their release into the environment. A significant amount of pharmaceutically active compounds (PhACs) including antibiotics administered to animals are not fully metabolized and are eliminated in urine, and/or faeces. Therefore, treatment strategies are required for the removal of these emerging contaminants from animal wastewater. In this sense, phytoremediation is a sustainable, low-cost alternative with high applicability for the rehabilitation of environments affected by natural and anthropogenic contamination. In this context, phytoremediation trials using *Salvinia minima* were carried out for the treatment of dairy farm wastewater (DFWW) in order to remove veterinary PhACs and thus achieve a higher quality effluent for its subsequent use within the farm or to reduce contaminants loads for its final disposal into the environment. To do this, firstly, the presence of PhACs in wastewater from dairy farm of Tucumán, in North of Argentina was evaluated. In the screening of veterinary drugs from crude DFWW (before phytotreatment) ciprofloxacin, tilmicosin, flunixin, diclofenac, furosemide, acetaminophen, ibuprofen and bezafibrate were identified. Here, the concentrations were very low, except that acetaminophen was 4217 ng/L. The removal efficiency of *Salvinia minima* was studied by cultivation in DFWW spiked with a mixture of penicillin G, ofloxacin, ciprofloxacin, azithromycin, clarithromycin, oxytetracycline, sulfamethazine, sulfadiazine, sulfapyridine and diclofenac at 10 µg/L during 7 days and in controlled conditions. The compounds showing the highest removal during the phytoremediation process were ofloxacin, ciprofloxacin, clarithromycin, sulfamethazine, sulfadiazine, sulfapyridine and diclofenac with more than 76% reduction. Therefore, *Salvinia minima* is a promising macrophyte with high potential for phytoremediation studies of veterinary drugs in dairy farm waters.

## 21.P-Tu-137

### **Assessing a New Passive Sampler and Remediation Device for Contaminated Water: A Case Study in Lagoa Da Conceição, Florianópolis, Brazil**

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#### **Abstract**

The presence of emerging contaminants in surface waters has raised concerns among researchers and health authorities worldwide. In Latin America, limited access to wastewater treatment leads to high concentrations of pollutants in the water of densely populated cities. In 2019, the city of Florianópolis (Santa Catarina, Brazil) experienced an incident of overflow from an evapo-infiltration pond, in which water from a sewage treatment plant broke through and reached the Conceição Lagoon, one of the sources of water for treatment and potabilization in the municipality of Florianópolis. This incident had serious consequences for the ecosystem of this lagoon. Over the past few years, several projects have been developed in partnership with universities and companies responsible for water treatment and distribution in the region to mitigate, prevent, and remediate the effects of this accident. Within this context, we present a floating prototype capable of simultaneously removing contaminants from water and concentrating them in absorbent material, which can be easily removed and transferred to a laboratory for the purpose of analyzing and quantifying the presence of these contaminants in the equipment. Similarly, this approach also allows for determining the flow of water treated by the system. The prototype has been produced and tested in the Conceição Lagoon as part of the REACQUA project.

To test the prototype, contaminants in sewage treatment water, Conceição Lagoon, sediments, and biota were analyzed. Thirty-five emerging contaminants were discovered, and the five most frequent (diclofenac, sertraline, caffeine, nimesulide, and clindamycin) were chosen for evaluating the equipment in the laboratory and on a real scale. These substances were added to samples of ultrapure water and water from the lagoon. The solar-powered device was placed over a 25-liter aquarium containing these compounds, and the efficiency of contaminant removal was evaluated.

The prototype (2m<sup>2</sup>) was placed in the lagoon near the treatment plant to check for the presence of these pollutants in the water samples from the location. The results showed that the prototype has great potential for decontaminating and remedying water.

## 21.P-Tu-138

### Multiple Barrier Technologies to Remove Disinfection Byproducts for Water Reuse in Hydroponic Farming to Promote Food Safety and Reduced Environmental Impact

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#### Abstract

Water is indispensable for all life forms. It is a limited resource that must be sustainably managed to prevent and not contribute to its scarcity. The aim of this study was to recycle the water employed during washing and disinfection (W&D) of hydroponically grown vegetables through a proper treatment to obtain drinking water quality.

The water used for W&D was potable, but ulterior addition of 0.5-1.0 ppm chlorine dioxide generates disinfection byproducts when in contact with organic matter. Spent water, initial (SWi) was recovered and treated at pilot scale. SWi was flocculated with ferric chloride until turbidity <11 NTU. Supernatant was subjected to a system containing two "Y" strainers placed in series (750 and 149  $\mu\text{m}$ ), followed by a filter of pressed activated carbon and a thread winding filter of 1  $\mu\text{m}$ . Next, the sample went through a ceramic filter (0.9  $\mu\text{m}$ ). Finally, UV light at 253 nm was applied. The final treated sample was named SWf. The process was followed up through microbiological and physicochemical analyses for SWi and SWf.

The system reduced counts of aerobic microorganisms and total coliforms. No impact was observed on fecal coliforms and *Escherichia coli* counts that were initially below the limit of detection (LOD). All counts in SWf were below LOD and no *Pseudomonas aeruginosa* was detected, complying with national regulation.

Regarding physicochemical parameters, reduction of turbidity, total organic carbon, adsorbable halogenated organic compounds, soluble and total phosphorous, free and total chlorine and iron were observed, while pH, conductivity and chloride increased during processing. However, SWf still complied with current regulations of this parameters. Different trends were observed for chlorites ( $\text{ClO}_2^-$ ) and chlorates ( $\text{ClO}_3^-$ ), but final values were not below the regulatory standards and high concentrations of these disinfection byproducts can compromise consumer's health. Therefore, two different experiments were carried out (laboratory scale), using ascorbic acid and an ion exchange resin (Lewatit® TP 106). Individual results demonstrated the effectiveness of each stage for the reduction of  $\text{ClO}_2^-$  and  $\text{ClO}_3^-$ , respectively. Both processes should be optimized and scaled up.

The results highlight the importance of implementing multiple barrier technologies to remove toxic disinfection byproducts in water used for W&D. This allows the reuse of processing water, reducing environmental impact without compromising food safety.

21.P-Tu-139

## A Multi-Objective Optimization Tool to Select Water Treatment Technologies in Chilean Rural Areas Aiming for Water Reuse

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### Abstract

Water scarcity is one of the main problems worldwide and millions of people do not have access to safe drinking water. Because of this, in recent decades, wastewater has become a valuable resource. Wastewater could not only alleviate the effects of scarcity through water reclamation but also provide a source of energy and fertilizer. Currently in Chile, the water governance system is dispersed in 56 public agencies, and decisions are made at the central level, which has not allowed for solving the accumulated problems of each sector over the years, causing narrow circumstances in rural sanitation services, such as lack of water availability, lack of safe drinking water, lack of wastewater collection and sanitation. Because the selection of viable technologies for wastewater reclamation is challenging, software tools using artificial intelligence have been developed in recent years. With this background, the objective of this work is to develop a software tool to help decision-making in the implementation of technologies in wastewater reclamation in rural areas. The problem is modeled as a multi-objective optimization search the minimizing costs of inversion and operation. To find the Pareto frontiers of a multi-objective problem, a resolution framework based on an adaptation of an elitist non-dominated sorting genetic algorithm (NSGA-II) is provided for the problem. The tool's focus is the design of a wastewater treatment plant, to reuse water in certain use cases appropriate to the user's needs. Therefore, this tool is intended to be used in the initial design phase of a wastewater treatment plant construction.

## 21.P-Tu-140

### Urban Wastewater: Bioremediation with *Chlorella vulgaris* and Reuse as Irrigation Water

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#### Abstract

The treatment of urban wastewater (UWW) can vary depending on the geographical region and on technological and economic capabilities. In this sense, bioremediation emerges as an alternative to complex and expensive decontamination systems. A load of contaminants can be reduced by bioremediation processes obtaining a treated effluent that can be used for different applications. The objective of this work was to know the capacity of *Chlorella vulgaris* to bioremediate UWW of San Justo city (Santa Fe, Argentina) and to evaluate the reuse of treated UWW as irrigation water. UWW were collected from facultative lagoons and pretreated by filtration and centrifugation. From preliminary tests, it was found that *C. vulgaris* could grow at 100% UWW. In the definitive test, *C. vulgaris* was cultured in triplicated 1 L flasks containing 100 % UWW (inoculum  $10^5$  cells mL<sup>-1</sup>). Culture conditions were: continuous lighting (8000 lux), temperature (24±2°C), aeration and constant agitation. The chemical and biological oxygen demand (COD and BOD), nutrients (nitrates, nitrites, ammonium, total phosphorus) and fecal coliform bacteria were determined three times (day 1, day 4 (T1) and day 7 (T2)), to determine the microalgae removal efficiency (%). Next, the potential of treated UWW as irrigation water was evaluated at T1 and T2 by a 120-h *Lactuca sativa* test. The concentrations tested at each time (T1, T2) were: 6.2, 12.5, 25, 50 and 100% of treated UWW and negative control (reconstituted water) for triplicate. The evaluated endpoints were: % germination (G), radicle length (RL) and hypocotyl length (HL) (mm). In T1, *C. vulgaris* showed removal of nutrients greater than 90%, reduction of BOD (73.20%), COD (39.70%), and bacteria (99.9%). In T2 an increase in BOD and COD was registered, which could be due to the release of organic compounds by the microalgae. The results showed that *C. vulgaris* reduced the parameters analyzed in UWW. In the *L. sativa* test, the highest %G was 88% at T2 (100% UWW treated). Significant differences ( $p < 0.05$ ) were observed between the control and treatments for T1 and T2 in RL and HL. These differences could be due to the presence of compounds that were not removed by *C. vulgaris* and that cause toxic effects in *L. sativa*. The results obtained show: 1) the potential of *C. vulgaris* to bioremediate UWW; 2) the need to carry out broader characterizations of UWW, evaluate different UWW treatment processes and carry out risk assessments when reusing treated UWW.

## 21.P-Tu-141

### **Constructed Wetlands as a Solution for Sustainable Sanitation in Valdivia, Chile: An Integrative Solution Among Climate Change, Resilience and Circular Economy**

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#### **Abstract**

About eighty percent of wastewater worldwide is discharged into the environment untreated. Many challenges are decelerating the global sanitation problem, such as financial limitations and lack of technical capacities. Parallel to this, our country is facing a growing demand on their limited water resources during periods of drought. Higher water demand and limited availability leads to over abstraction and deterioration in the availability and water quality resources. Therefore, there is a growing interest in finding low-cost, easy to operate and sustainable sanitation solutions. Constructed wetlands (CWs) in recent years have proved their capability in the sanitation sector as an appropriate sanitation system in different context, improving the water quality and effectively reducing contaminants of emerging concern. Many benefits and facts, such as low costs for construction and operation, low energy demand, and less operational requirements. All these advantages have raised the interests in CWs as a water treatment technology. On this research, we present the first comprehensive data set on water quality, contaminants of emerging concern and water quantity from three constructed wetlands in Valdivia, Chile. We analysed water quality (physicochemical properties, nutrients, heavy metals and contaminants of emerging concern) of three CWs with contrasting hydrological regimes; two of the CWs were fed from grey water, and the other one fed from both grey and sewage water. This work also focused on the improvement in water quality and the potential use for irrigation. Results demonstrated that water quality consistently improved in all three CWs. The majority of the nutrients, heavy metals and contaminants load decreased significantly, demonstrating that CWs are an efficient technology for diffuse pollution and improve water quality from wastewater.

## Structures and Dynamic of Planktonic Assemblages in Urban Wastewater Treatment Ponds (San Justo, Argentina)

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### Abstract

The generation of Urban Wastewater (UWW) is increasing as a consequence of demographic expansion. These include greywater, sewage, and wastewater from domestic activities. In San Justo city (Santa Fe, Argentina), UWW is treated by a system of biological treatment ponds followed by a chlorination process and then discharged into the Salado river. In these ponds, the organisms of the zooplanktonic (ZA) and phytoplanktonic (FA) assemblage play a key role in the purification of the effluent. The aim of this work was to study the structure and composition of the ZA and its relationship with the FA in the secondary facultative ponds (SFPs) of the UWW treatment plant. Six samples have been collected, three in winter (WIN - June, July, and August) and three in summer (SUM - November, December, and February). Temperature (T), dissolved oxygen (DO), conductivity (CO), and pH were measured *in situ*. Qualitative and quantitative ZA samples were collected (45 µm net, Van Dorn bottle, and 4% formol + erythrosine). Quantitative samples of FA (20 µm net, 1% Lugol + glacial acetic acid, Utermöhl method) were collected. SFPs showed T: 11.6-27.3 °C; OD: 1.7-15.4 mg O<sub>2</sub> L<sup>-1</sup>; CO: 437.1-1246 µS cm<sup>-1</sup>; pH: 7.0-9.6. A total of 13 taxa were recorded: (8) Rotifera -ROT-; (3) Cladocera -CLA-; (2) Copepoda -COP-. Total zooplankton density (TZD - ind. L<sup>-1</sup>) was higher in SUM. In both seasons, ROT contributed the highest density. In WIN the most abundant taxa were *Brachionus calyciflorus* (8,997 ind. L<sup>-1</sup>), *B. angularis* (4,696 ind. L<sup>-1</sup>), and *B. urceolaris* (89 ind. L<sup>-1</sup>); Order Cyclopoida (521 ind. L<sup>-1</sup>) and *Alona* sp. (11 ind. L<sup>-1</sup>). In SUM were *B. angularis* (26,859 ind. L<sup>-1</sup>), *B. urceolaris* (12,814 ind. L<sup>-1</sup>), and *Filinia terminalis* (7,107 ind. L<sup>-1</sup>); Order Cyclopoida (648 ind. L<sup>-1</sup>) and *Moinodaphnia macleayi* (2,419 ind. L<sup>-1</sup>). The relationship between the two assemblages was inverse. When TZD was maximum (29,228 ind. L<sup>-1</sup>), total phytoplankton density (TPD) was low (109,333 ind. mL<sup>-1</sup>); whereas, when TZD was minimum (103 ind. L<sup>-1</sup>), TPD reached its maximum (1,136,205 ind. mL<sup>-1</sup>). The ZA was dominated by rotifers, which is in agreement with that reported for this type of system. The species recorded are bioindicators of effluent quality and play a key role in the removal of bacterial load, nutrient cycling, and control of algal biomass prior to discharge to the receiving watercourse.



## 21.P-Tu-143

### **Evaluation of the Adsorption and Desorption Capacity of Two Natural Materials used as Support Media for Constructed Wetlands for the Treatment of Water Contaminated with Pesticides**

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#### **Abstract**

Constructed Wetlands (CW) are promising alternatives for the treatment of contaminated water from agricultural activities, due to their ecological benefits and low operation and maintenance costs. The pollutants elimination occurs through physical, chemical and biological processes such as sedimentation, volatilization, adsorption, microbial decomposition and plant uptake. Among the CW components, the support medium or substrate plays an important role in the growth and development of plants and microorganisms; however, very little information is available on its influence on the elimination of contaminants. In order to select the most efficient substrate to removal of pesticides by CW, the adsorption and desorption of two of the most used natural materials in these systems (Gravel and Pometine) were evaluated, using the insecticide Imidacloprid (IMD) as a model. The IMD removal capacity of both substrates was assessment through insecticide concentration and the ecotoxicity of the solution measured before and after treatment. Determination of imidacloprid in water and soil samples was performed by HPLC-DAD system, and the ecotoxicity of the samples were assessment using the crustacean *Daphnia magna* as a standard test species. Each treatment consisted in 500 mL glass flasks where 150 g of substrate were placed with 200 mL of IMD solution and left on a shaker at 150 rpm and 20°C for 24 h. The evaluated IMD solution concentrations were 36 and 180 mg/L that correspond with the lethal concentration 50 and the maximum recommended doses for use in the field, respectively. The adsorption capacity was determined at different time intervals; aliquots of the aqueous phase were taken at 15, 30 min, 1, 6 and 24 h. Once this assay was completed, desorption was determined for the IMD solutions at 24 h time. None of the evaluated substrates significantly reduced the IMD concentration, so there was no evidence of the insecticide in the desorption samples. However, it was observed that the treatment with pometine reduced the toxicity of IMD by 73% and no differences were found in the attenuation of toxicity with the use of gravel as a substrate. The findings demonstrate that, although the adsorption capacity of pometine against IMD is low, this substrate modifies its bioavailability, making it a good potential material for the treatment of pesticides in CW compared to gravel.

## 21.P-Tu-144

### Synthesis and Characterization of Bamboo Biochar for Microcystin-LR Removal

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#### Abstract

The contribution of nutrients from agricultural practices and the discharge of wastewater into bodies of water, together with specific climatic conditions, are the main factors that lead to the proliferation of cyanobacteria. More than 70 cyanotoxin variants have been identified, of which microcystin-LR (MC-LR) is the best known. Cyanotoxins are released into surface waters and are considered toxic to humans and the aquatic ecosystem. Conventional wastewater treatment or water for human consumption usually uses activated carbon in large quantities to remove these contaminants. However, it is a material that must be imported into Uruguay. Therefore, creating a more economical and sustainable national material that guarantees drinking water supply is of great interest. This work developed and characterized a national biochar from bamboo (supplied by the Uruguayan company Bambú del Este) to remove MC-LR. The best biochar was obtained by carbonizing the raw material in a tube furnace at 600°C for 30 minutes under an inert atmosphere. The resulting material comprises 85% carbon, 1.7% hydrogen, 0.8% nitrogen, 7.2% oxygen, and 5.2% ash. Mercury porosimetry showed a macroporosity of 55.8%. The specific BET surface obtained by nitrogen adsorption isotherm at 77K is 64m<sup>2</sup>/g. The removal percentage of MC-LR in aqueous solution in a fixed bed was 92%, while the column removal percentage of a sample contaminated with MC-LR was 99.5%. These excellent results are mainly due to the electrostatic attraction between the MC-LR molecule and the functional groups of the biochar. Biochar, basic in nature (zero charge point 8.7), is positively charged at the pH of the contaminated effluent (close to 7). At the same time, the MC-LR molecule is in its anionic form under the same conditions. Consequently, adsorption by electrostatic attraction is maximized. This work further proves that Uruguay has the necessary biomass and knowledge for developing sustainable materials applicable to remediation processes for contaminated water.

## 21.P-Tu-145

### Preliminary Studies on the Use of *Salvinia minima* in Ibuprofen Phytoremediation of Aquatic Environments

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#### Abstract

The main emerging contaminants present in water include drugs for human and/or veterinary use. The presence of drugs such as ibuprofen, amoxicillin and paracetamol in continental waters has been frequently detected in many countries including Argentina; these cause alterations in the biota at different levels could alter the dynamics of aquatic ecosystems. The study of emerging contaminants in water and the application of new technologies for its elimination is crucial to carry out a good management of the few water resources that need to satisfy a growing demand for water, and whose quality is seriously affected by anthropic activities; in this context and in search of ecological alternatives and the application of environmentally friendly phytotechnologies, the use of plants such as species of the *Salvinia* genus, widely studied in phytoremediation, could be a strategy for the elimination of drugs in aquatic environments. For this work, *Salvinia minima* plants were placed for 7 days in trays containing a 4 mg/L solution of commercial IBU. This concentration was chosen based on previous studies where it was observed that 4 mg/L was the highest concentration at which they were obtained. exposed plants without showing macroscopic alterations. The results obtained showed a decrease in the IBU content in the water in the presence of the plants of around 80%. In parallel, some physiological parameters related to the primary metabolism and the oxidative response of *S. minima* in the presence of IBU were analyzed; the content of photosynthetic pigments did not show great differences between the control plants and those exposed to IBU, only a slight decrease in the *chl b* content, the soluble sugars did not show negative effects as a result of IBU exposure, with slight increases in sucrose and glucose content being observed; in consistent with these results, the biomass production was not affected, observing a slight increase in weight in the exposed plants with respect to the control plants; regarding the oxidative response, the MDA (malondialdehyde) content was slightly increased, while the hydrogen peroxide content did not show significant differences. Considering the marked decrease in the IBU content of the medium in the presence of *Salvinia minima* together with few physiological effects produced as a result of exposure to the drug, we can conclude that this species would be a promising candidate for phytoremediation of emerging contaminants such as ibuprofen.

## 21.P-Tu-146

### Design of a Preliminary Priming Treatment in *Salvinia rotundifolia* to Improve Its Performance During Vinasse Remediation

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#### Abstract

During the process of obtaining bioethanol, the sugar-alcohol industry generates a residual liquid called vinasse. Physicochemical characteristics of this effluent make it a potential contaminant of water resources, so it is important to seek and implement treatments to reduce its pollutant load and high volume of production.

To improve the performance of *Salvinia rotundifolia* during the remediation process and, in turn, obtain a more suitable effluent for subsequent uses and/or dumping, a priming treatment was carried out on *S. rotundifolia* using manganese sulfate at low concentrations during 24 hours. First, vinasse was treated by bioadsorption, using agro-industrial wastes, then remediation was carried out for 7 days using 1:10 diluted vinasse and the primed plants. Vinasse samples were taken at 0, 2 and 7 days and pH, conductivity, sucrose, reducing sugars and phenolic compounds was determined. In plant tissues, we proceeded to quantify the content of H<sub>2</sub>O<sub>2</sub>, phenolic compounds and malondialdehyde (MDA) in samples taken at the beginning and at the end of the phytoremediation treatment.

The results showed a 50% decrease in the content of phenolic compounds in the effluent treated with plants with priming, compared to the effluent treated with plants without priming. However, this effect was not observed in the levels of reducing sugars and sucrose. Regarding the plants, although macroscopic signs of damage were not observed, the content of hydrogen peroxide and MDA showed significant increases on day 7.

Based on the results obtained, we can conclude that priming with Mn improved the performance of the plant, favoring the reduction of phenolic compounds present in the vinasse, which could be related to a stimulation of Mn-peroxidase. Likewise, it cannot be ruled out that Mn has stimulated both endogenous mechanisms of the plant and the activity of other microorganisms present in the rhizosphere. The stress indicator parameters showed that oxidative processes were activated in the plant, which could harm the remediation process in the long term. For this reason, more studies are necessary to adjust the Mn dose, the incubation time and the addition of some factor that favors the antioxidant mechanisms of the plant.

## 21.P-Tu-147

### Detoxification of Filter Cake through Biostimulation with Rice Husk: a Sustainable Solution for Agro-industrial Waste

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#### Abstract

Brazil, one of the largest agricultural producers globally, generates a significant amount of agro-industrial waste, including filter cake (FC), which is usually disposed of incorrectly. FC is a mixture of decantation sludge and sugarcane bagasse, containing high levels of organic matter and minerals. However, when used as a biofertilizer in natura, it is toxic and causes environmental damage. Thus, this study aimed to develop a sustainable, efficient, and low-cost technology for detoxifying FC through a biostimulation process with rice husk (RH). The effectiveness of the process was evaluated through a phytotoxicity bioassay with *Allium cepa* (Amaryllidaceae). FC was diluted in the following volumetric proportions for experimentation: FC (in natura), FC + soil (3:1); FC + RH (3:1); FC + soil + RH (3:1:1). These treatments were kept in stainless steel vats and evaluated at the initial period (T0) and after 2 months of biostimulation (T2) for seed germination rate. Physicochemical characterization was also performed to verify alterations in moisture content, organic matter, and pH along the detoxification process. All treatments presented slightly basic pH values (~8.5) at T0, which decreased after 2 months, especially for FC (7.6). Additionally, at T2, the dry weight increased in all the treatments, being more pronounced in that without RH. A small decrease was observed for the amount of organic matter, indicating that more biostimulation time is required for its stabilization. At T0, the germination rate indicated values above 88% for all treatments, except for FC + RH, which showed a significantly lower rate (9%). However, after 2 months, this value increased and became similar to the negative control (94%). Although other parameters require verification, it is expected to obtain a material with better nutritional properties and greater added value, contributing to environmental sustainability and the management of agro-industrial waste.

## 21.P-Tu-148

### **Anionic Resin: Extractor With Higher Predictive Power of P in Soil for a Sustainable Management**

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#### **Abstract**

Regarding the different sources of P, the careful selection of a soil phosphorus extractor is crucial for minimizing negative environmental impacts. Excessive phosphorus fertilization can cause its accumulation in soil, leading to various environmental issues such as eutrophication, reduction of biodiversity, and greenhouse gas emissions. The objective of this work was to contribute to the advancement of sustainable agriculture by presenting relevant information about the main phosphate fertilizers and their extractors, their environmental impacts, and strategies to minimize them. To investigate this, an experimental design was adopted in a factorial scheme 5X2, that included the combination, four fertilizer sources (organomineral, struvite, thermophosphate, and triple superphosphate), a control (without P addition), and two successive corn (*Zea Mays*) crops of 45 days each, in two independent soils with contrasting textures. At the end of the 1st crop period, 45 days after sowing (DAS), in the sandy soil, we found that the addition of P resulted in higher soil available P when the TSP source was applied, as determined by the Mehlich-1 extraction method. Also, when the resin extraction method was used to access the available P, the TSP source led to high amount of P, however did not differ from the OM source addition. Still in the sandy loamy soil, after 2nd crop at the end of the experiment, it was observed that the fertilization with TSP along with ThermoP resulted in the highest levels of P in the soil, but concomitantly with lower P contents in the plants. In this study, in terms of magnitude, Resin was the extractor that quantitatively extracted the least amount of available P in the soil in both soils and crops. However, as this extractor showed in general the highest strength of correlation coefficient with plant P, it can be stated that, under the conditions of the study, it is the extractor with the greatest potential to predict available soil P, mainly in the sandy loamy soil. In our study the Mehlich-1 and Mehlich-3 extracted more available P than the plant can absorb which can lead to a misinterpretation of phosphorus availability in the soil. Consequently, this could seriously influence a wrong future fertilizer recommendation for crops.

# Session 22: Eco-Economy: New Economies for Sustainability

22.P-Mo-102

## Unraveling the Challenges of Chlorinated Paraffins in Recycled Polymers from Three South American Countries

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### Abstract

Recycling is widely promoted as an essential measure for effective plastic waste management and environmental protection. However, establishing a safe circular economy for plastics poses significant challenges. Research has identified over 13,000 different chemicals used in plastic production, with more than 3,200 of them considered hazardous to the environment and human health. Among these chemicals, chlorinated paraffins (CPs) are synthetic compounds commonly used as additives in polymers, serving as plasticizers, flame retardants, and lubricants. For academic and regulatory purposes, CPs are classified into three subgroups based on their carbon chain length: short-chain (SCCPs: C<sub>10</sub>–13), medium-chain (MCCPs: C<sub>14</sub>–17), and long-chain (LCCPs: C<sub>>18</sub>) CPs. SCCPs are globally restricted under the Stockholm Convention on Persistent Organic Pollutants (POPs) due to their persistence, bioaccumulation, and toxicity in the environment. Recently, MCCPs have also been proposed for listing as POPs and inclusion in the Restriction of Hazardous Substances Directive of the European Union. Consequently, the presence of CPs in recyclable polymers poses a significant challenge in implementing these restrictions and achieving a safe circular economy. To address this challenge, our research aims to measure the content of 74 CP homologs, covering SCCP, MCCP, and LCCP chain length groups, in polymers recycled in Argentina, Brazil, and Chile. Samples of polymer shreds, pellets, and recycled products were collected from recycling facilities in these countries from 2020 to 2023. The polymer samples were dissolved in toluene, purified, and then analyzed using liquid chromatography–electron spray ionization–tandem quadrupole mass spectrometry to measure CP levels. The results will be presented and discussed, taking into consideration the CP contents in different types of polymers, their potential sources, and the implications for implementing CP restrictions and promoting safer circular economies in the studied countries.

## 22.P-Mo-103

### **Sustainable Urea Production Using Green Hydrogen and Carbon Capture: Towards a Circular Economy in the Carbon Usage**

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#### **Abstract**

Food production is expected to double by 2050 for satisfying the increasing world's population. Fertilizers play a key role on this goal. Urea is the fertilizer most used worldwide; in Uruguay, urea imports are about 230.000 tons/year. Nitrogen fertilizers are produced from ammonia; particularly, urea is produced from the reaction between carbon dioxide and ammonia. Ammonia is synthesized from the reaction between nitrogen and hydrogen, where the latter is usually produced through steam methane reforming, generating a large amount of carbon emissions. Considering the urgency in decarbonizing the economy, industrial process reevaluation is needed to mitigate the worse environmental effects. The reduction in renewable energy prices is opening the possibility of generating carbon neutral hydrogen from water electrolysis using electricity from renewable energy sources. With this in mind, green hydrogen can decarbonize the ammonia production, and even consider the urea synthesis with the aim of utilizing carbon captured from other industrial processes.

Worldwide, the cement industry is between the hardest to abate sectors of the economy, mainly due to the intrinsic carbon emissions released on the decarbonation process of the raw material. This work aims to explore the synergy between urea and cement production.

On the other hand, considering the local availability of biogenic carbon dioxide coming from the chimneys of pulp mills, this work proposes to compare the urea production using biogenic carbon from a pulp mill against the carbon captured from a cement plant.

In this way, carbon emissions could be used to produce low-carbon or even carbon-neutral fertilizers, which is a clear example of circular economy applied to climate change mitigation.



## 22.P-Mo-104

### False Claims Related to Single-Use Plastic Utensils Sold in Brazil: Out of the Frying Pan Into the Fire

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#### Abstract

The increase in world population and changes in society's lifestyle have led to a large production and consequent inadequate disposal of solid waste in natural environments. Plastic polymers, mainly derived of single-use utensils, are currently recognized as the most abundant and frequent residues found in natural environments inducing deleterious effects on live organisms. In this regard, replacing single-use plastic with so-called biodegradable ones have been emerged as part of a suitable actions to reduce global environmental impacts. However, issues related to false claims and improper certification of single-use plastic utensils claiming biodegradability have been described in scientific literature. Despite this, greenwashing incidence has not been systematically evaluated so far, while may generating useful information to guide a proper legislative framework on the sector. Therefore, this study aims to quantitatively estimate the greenwashing incidence among single-use utensils offered for sale in the Brazilian market. The incidence of greenwashing was assessed in supermarkets of four Brazilian cities, including the most populous (Rio de Janeiro and São Paulo), and in two less populated towns (Santos and Peruíbe). At least ten supermarkets were selected in each city, and labels of single-use plastic utensils claiming biodegradability were carefully inspected. Were found 49 different products including cups (16.3%), cutlery (22.5%), straws (16.3%), plates (24.5%), trays (6.1%), tablecloths (2.0%), lids (2.0%), balloons (2.0%), party ware (4.1%) and jars (4.1%). In addition, 93.8% claimed to contain pro-oxidant additives (able to accelerate biodegradation processes in natural conditions), being categorized as oxo-biodegradable materials, and 6.2% of the products were made of regular polymers with no additive. Considering that the biodegradability of oxo-polymers has been widely refuted by scientific literature, our results indicates that none of the products claiming biodegradability sold in Brazilian supermarkets, are in fact biodegradable. Moreover, the European Parliament issued a directive, prohibiting sales of oxo-degradable utensils in all member states due potential risk of accelerated microplastic formation making these materials more hazardous than conventional plastics. These results indicate that companies producing and selling single-use plastic utensils need to abandon this greenwashing practice.

## Botanical Extracts as Biopesticides: Molle (*Schinus johnstonii*) as a Sustainable Alternative to Control the Spotted-Wing Fly *Drosophila suzukii* (Diptera: Drosophilidae)

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### Abstract

*Drosophila suzukii* is a global pest with a high incidence in Argentine Patagonia, which affects fine fruit production. Thus, environmental friendly alternatives to pest control is an important issue to consider in order to maintain fruit quality and sustainable production. The objective of the present work was to evaluate the efficiency of different extraction methods of the molle (*Schinus johnstonii*) extracts and its lethal effects on *D. suzukii*. Botanical extracts from leaves were obtained considering different variables: type of solvents (hexane or ethanol), plant-solvent proportion (1:1.5; 1:4; 1:6), dry or fresh leaves and maceration periods (24, 48, 72 or 168 h). Adults of 5-7 days old from a susceptible laboratory strain were exposed for 24 and 48 h to these plant extracts. Bioassays were carried out in glass flasks coated with each extract to determine lethal effects. Ethanol or hexane was considered, in each case, as a control. No mortality was observed in hexanic extract exposures. On the other hand, ethanolic extracts induce mortality in flies. The highest mortality values were recorded for dry leaves-72 h maceration extracts, observing 88.8% (24 h) and 94.7% (48 h) mortality. Extracts prepared with fresh leaves-48 h maceration showed 96.7% at 48 h of exposure. Preliminary tests suggest the bioinsecticide potential of *S. johnstonii* ethanolic extracts on *D. suzukii*. In this sense, botanical extracts of *S. johnstonii* could be a sustainable alternative to be incorporated in integrated pest management in Argentine Patagonia.

## Botanical Insecticides to Sustainably Control Pests: Lethal Effects of Essential Oils and Hydrolates on Adults of Spotted-Wing Flies

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### Abstract

Fine fruits production such as strawberries and blueberries represents one of the most important economic activities in the North Patagonia (Argentina). Hence, pest control is relevant to maintain fruit health and quality. Particularly, the spotted-wing fly *Drosophila suzukii* represents an incipient threat to production performance of fine fruits. Despite the integrated pest management, conventional insecticides are usually the most applicable ones to control this pest. Therefore, the incorporation of botanical products such as essential oils, would be a sustainable option. The objective of this study was to evaluate the lethal effect of different botanical insecticides on the spotted wing fly.

Adults of 5-7 days-old from a laboratory susceptible strain were exposed for 24 h to different hydrolates (HT) and essential oils (EO). EO of flowers of available lavender variety (*Lavandula hybrida*), rosemary (*Salvia rosmarinus*), and a regional species pichana (*Baccharis aphylla*), and their HT were obtained by steam distillation and assayed. Expositions were performed in glass flasks in which a filter paper disc of 5 cm diameter soaked in 300  $\mu$ l EO or HT was placed, acetone alone was considered as control. Lethal concentration 50 (LC50) was determined by probit analysis and chemical composition was performed by GC-MS.

The results obtained showed that undiluted HT lethality were 13.14% for lavender, 14.80% for rosemary and 10.64% for pichana. On the other hand, EO manifested greater toxicity than their respective HT, obtaining LC50 values of 1.26% (95% CI: 0.774% -2.059%) for lavender, 1.50% (95% CI: 1.183 - 1.924) for rosemary and 2.55 % (95% CI: 1.999% - 3.264) for pichana. Relative abundance revealed that linalool (53.37%) and camphor (19.29%) were the most abundant terpenes in both lavender HT and EO. Rosemary HT and EO composition consisted mostly in  $\beta$ -myrcene (25.79%) and camphor (17.81%) while pichana was constituted mostly by limonene (48.54%) and caryophyllene (26.81%).

These results indicate that EO have greater toxicity than their respective HT, which could result in a sustainable alternative to control *D. Suzukii*. In this sense, lavender showed higher toxicity than rosemary and pichana. It is important to continue with toxicity tests considering other effects such as deterrence or delay in hatching of eggs, among others, which may allow these potential botanical insecticides may be used as a sustainable alternative to be applied to a larger scale.

## 22.P-Mo-107

### Valorization of Urban Wastewater (UWW) for Microalgae-based Biotechnology

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#### Abstract

Microalgae-based biotechnology can contribute to the bioeconomy through the production of valuable metabolites. The objective of this work was to know the biochemical composition of *Chlorella vulgaris* in mixotrophic medium composed of Urban Wastewater (UWW). These were collected from the treatment plant of San Justo city (Santa Fe, Argentina), with a system of stabilization ponds and a chlorination chamber, before discharging in the Salado River. Samples were taken prior to the chlorination chamber and were filtrated and centrifugated, before performing a 72 h algal growth inhibition test. *Chlorella vulgaris* grew at a maximum concentration of 100% UWW, selected to carry out a 100% UWW Mixotrophic Test (MT) (in triplicated, 1 L flask, inoculum of  $10^5$  cells mL<sup>-1</sup>, 24:0 light:dark 8000 lux at  $24 \pm 2$  °C, pH =  $7.0 \pm 0.5$ , aeration and continuous magnetic stirring). The growth curve of *C. vulgaris* was made and an intermediate time (T1, exponential phase) and a final time (T2, decline phase) were determined. At each time, carbohydrates (C) (Dubois phenol-sulphuric acid method), proteins (P) (alkaline hydrolysis method and quantification by the Bradford method), lipids (L) (sulpho-phospho-vanillin reaction) and fatty acid profile (FAP) (gas chromatography) were determined. For C the difference was significant ( $p < 0.001$ ), with the highest accumulation obtained at Control Test (CT)-T2:  $39.50 \pm 4.26$  %. For P the difference was significant ( $p = 0.025$ ) between MT-T2 and CT-T1, when the highest accumulation of  $5.22 \pm 1.73$  % was obtained, and in MT-T2:  $2.30 \pm 0.56$ ; but there was no difference between MT-T1 and T2 ( $p > 0.05$ ), nor between MT and CT-T2. The highest accumulation of L was achieved at MT-T2:  $17.45 \pm 3.27$  %. FAP obtained was similar in MT and CT mainly composed by fatty acids of 16 and 18 carbon atoms; palmitoleic and oleic acids: 3.20% and 11.47% (T1); 1.39% and 21.70% (T2). Linolenic acids reached a maximum of 17.33% in MT-T1. Linoleic acids are relevant for aquaculture diets because microalgae consumers cannot synthesise them. The cultivation of microalgae in UWW is a tool of the new Circular Bio-based Economy. Where the UWW are valued as raw material to produce biomass of sustainable origin with high value in the marking of bioproducts.

# Session 23: Planetary Health: Chemical Pollution as Driver for Loss of Ecosystem Services and Biodiversity

23.P-Mo-108

## Biochemical and Molecular Changes in *Chironomus sancticaroli* exposed to Multiple Pollutants in Sediments From the Rio Doce Basin

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### Abstract

Natural environments, such as the Doce River Basin (DRB), are vulnerable to the constant impact of multiple sources of pollution from human activities. When reaching water bodies, pollutants can affect exposed organisms. Among the benthic organisms, the Chironomidae represent an important role in the food web of freshwater ecosystems, however, they are subject to a wide diversity of organic and inorganic contaminants causing different biological responses. Faced with the need to understand the impact of different contaminants present in the sediments of the DRB on a benthic organism, this study aimed to evaluate and integrate the responses of different biochemical (acetylcholinesterase,  $\alpha$ -Esterase,  $\beta$ -Esterase, glutathione-S-transferase, superoxide dismutase, catalase, lipid peroxidation and DNA damage) and molecular biomarkers (gene expression of hemoglobins (HBs) and metallothionein (MT)) of *Chironomus sancticaroli* larvae exposed to sediments of the DRB, after the event of the rupture of the iron ore tailing dam. Sediment collections were carried out in six locations along the upper (Gualaxo do Norte, UHE Candonga), middle (Naque, Referencia, Governador Valadares) and lower (Aimorés) Doce river, in July and August of 2019. The bioassays consisted of exposing immature *C. sancticaroli* for a period of eight days to sediments (river and control), under controlled conditions. Biomarker results were integrated using the Integrated Biomarker Response Index and related to sediment chemical contaminants and larval bioconcentrates using principal component analyses. The research results demonstrated that the sediments from the middle Doce River were the ones that most affected the enzymatic biomarkers of the exposed larvae, mainly in the biotransformation enzymes of organic compounds and oxidative stress. This segment of the basin is influenced by mining and receives domestic and industrial effluents with a lack of adequate sewage treatment, recurrent problem in water resources in Brazil. The highest HBs and MT gene expressions occurred in the upper Doce River, which may be related to the presence of metals, which are predominant in this river segment. It was possible to verify that the biomarkers were efficient in responding to the effects of the different contaminants in the sediments of the river and that through the integration of the biomarkers it is possible to better observe the effects of these multiple contaminants on the organisms.

## 23.P-Mo-109

### Contribution of the Biological Pump to the Flux of Persistent Organic Pollutants in the Water Column in Antarctic Coastal Ecosystems

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#### Abstract

Antarctica has been considered one of the last pristine places on the planet, but during the last century the industrial development has caused a deep impact in Antarctic ecosystems, by releasing persistent organic pollutants (POPs) to the atmosphere. These POPs are ubiquitous in the environment, semi volatile, and they can be transported long distances through the long – range atmospheric transport (LRAT) and reach the polar areas. They can also bioaccumulate and biomagnify through trophic webs and have toxic effects in biota. Few studies have considered studying oceanographic processes in the water column, such as the biological pump, and their relationship with the dynamics and cycling of POPs in polar environments, and there is fewer information for Antarctic. The study of the biological pump process is important due to the role played by phytoplankton in the cycling of POPs in the water column and in food webs. A good representative of these pollutants is the polybrominated diphenyl ethers (PBDEs), substances widely used, both commercially and domestically, as flame retardants, and its presence in Antarctic biota of lower trophic levels has been scarcely studied. This study was focused on detecting the contribution or facilitation of the biological pump to the availability of POPs in the water column of Antarctic coastal ecosystems. Through the collection of water samples, water column organic matter, phyto and zooplankton, the concentration of 10 PBDE congeners were detected. When evaluating the accumulation process in organisms, it was lower than expected based on their physicochemical properties and water temperature. Stable isotopes of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were used to assess the food web structure in the water column confirming a clear predatory behavior of consumers (zooplankton) over primary producers (phytoplankton), and in the case of sinking particles we detected a wide range of  $\delta^{13}\text{C}$  values, indicating a wide source of organic matter sinking to the sediments. The biomagnification factor indicated that the PBDE congeners with higher LogKow are being magnified to higher trophic levels, but the process only occurs for some congeners. The combination of all data collected in this study allowed us to conclude that PBDEs could be sinking associated with organic matter to the sea floor, so contrary to what was hypothesized, the biological pump won't be making POPs more bio available to biota that inhabits in the water column of Antarctic coastal ecosystems.

## 23.P-Mo-110

### Biomonitoring of the Pioneiro, São Camilo and Santa Fé Rivers, Palotina – Pr.

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#### Abstract

Biomonitoring is an important tool for assessing impacts on water quality and consequent damage to the biota of aquatic systems. The aim of this study was to monitor water quality and fish health in the Pioneiro, São Camilo and Santa Fé Rivers, located in the municipality of Palotina, western Paraná, using biomarkers of neurotoxicity in the native fish species *Hypostomus ancistroides*. Levene and Shapiro-Wilk tests were used to test assumptions of homogeneity of variances and normality, respectively. Data were submitted to one-way ANOVA tests followed by Tukey's test, for normal data, and Kruskal-Wallis followed by Dunn's test, for non-normal data. The decision rule was  $p < 0.05$ . Fish, water and sediment samples were collected throughout the winter of 2022. Three sampling points were selected in each river: P1 (upstream), P2 (urban perimeter) and P3 (downstream), in which water quality parameters were evaluated and some metals in the sediment. In fish, muscle, and brain acetylcholinesterase (AChE) activity was evaluated. In the Pioneiro River, was observed significant differences only in muscle AChE activity between points P1 and P3, and between P2 and P3 ( $H=17.0962$ ;  $gl=2$ ;  $p=0.0002$ ). The fish from P3 present lower activity compared to P2 and P1. In the sediment, the P3 showed higher concentrations of Co (0.28 mg/kg), Cu (0.89 mg/kg), Zn (1.36 mg/kg), and lower DO (3,85 mg/L) in the water. In the São Camilo River, significant differences were observed in muscle AChE activity between points P2 and P3 ( $f=3.47$ ;  $p=0.0424$ ). The activity was lower in P3 in relation to P2. The P3 showed higher concentrations of Mn (9.15 mg/kg), Ni (0.53 mg/kg), nitrate (24.64 mg/L), nitrite (0.0044  $\mu\text{g/L}$ ) in the sediment, and ammonia (0.36 mg/L), COD (94.22 kg/mL) in the water. In the Santa Fé River, it was not observed a significant difference in muscle AChE activity. Therefore, in the brain was observed between points P1 and P3 ( $H=8.52$ ;  $gl=2$ ;  $p=0.0141$ ). The P3 presented lower activity related to P1. Point P3 showed higher concentrations of Cu (3.24 mg/kg), Ni (0.48 mg/kg), Pb (0.19 mg/kg), in the sediment, and nitrate (43.21 mg/L), nitrite (0.0063  $\mu\text{g/L}$ ) and ammonia (0.42 mg/L) in the water. In the P3 points downstream of the three rivers water quality parameters was changed and some anticholinesterase agents can be influencing the decrease in the AChE activity, demonstrating the need to adopt mitigation measures.

## 23.P-Mo-111

### Altered Spermatogenesis in *Patagonotothen tessellata* Collected From Areas With A High Degree Of Anthropic Impact In The Beagle Channel

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#### Abstract

*Patagonotothen tessellata* is one of the most abundant notothenioid fish in the Beagle Channel. Gonads of fish collected from Bahía Ushuaia (Tierra del Fuego, Argentina) accumulate persistent organic pollutants associated with human activity. In addition, feminization of *P. tessellata* was recently reported, supported by an upregulation of vitellogenin in male fish. The aim of the present work is to assess tissue damage in testes of this species, in order to determine the degree of pollution in Bahía Ushuaia and surrounding areas of the Beagle Channel.

Fishes were sampled in Tierra del Fuego National Park (reference site) and Bahía Ushuaia (anthropic activity site) during 4 seasons from 2016 to 2019. Histological sections of testes were stained with Masson's Trichrome and examined under a photomicroscope. Digital images were obtained from 3 areas of each gonad: center (close to the efferent duct), middle and periphery. A scale bar and a grid with quadrants of 100  $\mu\text{m}^2$  were applied to each photo; 10 quadrants were randomly selected and cells within them counted and identified as: spermatogonia A and B, spermatocytes I and II, spermatids, free spermatozoa in lumen, Leydig cells and Sertoli cells. Feret diameter of nuclei measurements were obtained using ImageJ software.

No significant differences were found as regards the size of cells between sites, but the frequency of one cell type was significantly reduced in fish from Bahía Ushuaia in comparison to the reference site. While the rest of the germ cell types showed no differences in their frequency between sites, the polluted one showed between 45.3% and 86.1% less amount of spermatids. In this context, two possible explanations arise:

- 1) Since in fishes during spermiogenesis, spermatids show steroid receptors that enable the maturation to spermatozoa, these cells might be the target of water pollutants that could be altering the timing process and therefore leaving testes with less spermatids.
- 2) The coordination of spermiogenesis and spermiation within cysts is regulated by somatic cells and hormones. Pollutants in the aquatic environment could be targeting Sertoli or Leydig cells, leading to an early release to the lumen of immature germ cells, such as spermatids, and hence to a reduction in their amount.

These findings are of relevance and set a precedent that water pollution in Bahia Ushuaia has a negative impact on the fish reproductive biology and could be extended to marine wildlife of the area.



## 23.P-Mo-112

### Biomarkers in Two Neotropical Freshwater Teleosts for Environmental Monitoring

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#### Abstract

Different contaminants, generated by many human activities, are discharged into the aquatic environment by multiple pathways, specially continental aquatic ecosystems, affecting a great diversity of organisms, including fishes. Thus, this work compared the health status of streams located in northern Paraná (Brazil), under different types of anthropic interference, by analyzing a set of biomarkers in the benthopelagic fish *Geophagus brasiliensis* and the demersal fish *Hypostomus ancistroides*. For this, *G. brasiliensis* (n = 24) and *H. ancistroides* (n = 19) were collected in four different streams representing a conservation area (CONS), an agricultural area (AGR), and an urban area (URB). Following the capture, fish were transported to the lab with local water and proper aeration, where they were anesthetized and euthanized by medullary section. The liver and gills were removed for the analyzes of glutathione (GSH) and the occurrence of lipoperoxidation (LPO), and the brain and muscle were removed for acetylcholinesterase (AChE) activity analysis. GSH content, both in gills and liver of *G. brasiliensis*, was not significantly different among the streams. However, the gills of *H. ancistroides* from AGR showed higher concentration of GSH than those from CONS, while in the liver, the GSH content was higher in animals living in URB compared to CONS. LPO was higher in the liver of *G. brasiliensis* from AGR compared to those from CONS, but no difference was detected in the gills. On the other hand, in the gills of *H. ancistroides* LPO was higher in URB compared to AGR, which was higher than CONS. Moreover, the occurrence of LPO in the liver of *H. ancistroides* from URB was higher than in the other streams. Brain AChE activity did not show any significant differences in neither of the two species, among any of the streams. However, AChE activity in the muscle was higher in *G. brasiliensis* from AGR compared to other locations, and lower in *H. ancistroides* from URB compared to other streams. Thus, we can conclude that the urban and agricultural streams appear to be the most harmful for *H. ancistroides* and *G. brasiliensis*, respectively, and that neurotoxic biomarkers and those linked to oxidative stress in *G. brasiliensis* and *H. ancistroides* are important tools for environmental monitoring.

## 23.P-Mo-113

### **Histological and Biochemical Evaluation on Liver from Two Natural Population of *Cnesterodon decemmaculatus* (Cyprinodontiformes, Poeciliidae) from the polluted Reconquista River, Argentina**

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#### **Abstract**

The Reconquista river is an ecosystem conditioned by the anthropogenic activity. It is highly polluted by industrial and domestic outputs from point and diffuse sources. The liver of fish is a target organ for xenobiotics. The aim of this work was to compare the freshwater fish *Cnesterodon decemmaculatus* from polluted sites of the Reconquista river to a laboratory raised group (control, Ci) using biomarkers. Water samples and fish of both sexes were collected from the upper basin (Roggero dam, R1), and middle basin (San Francisco park, R2) of the Reconquista river. Fish were then fixed using Bouin's solution and stained with Masson's Trichrome through routine histological analysis for conventional histology. Histopathological parameters of the liver were studied, and a reaction index was calculated taking into account: circulatory damage, regressive and progressive changes, and inflammatory processes. In addition, histochemical changes were evaluated by glycoconjugates and proteins stains. Morphometrical measurements of hepatocytes and their nuclei were also obtained using ImageJ 1.54d software. We also evaluated liver oxidative stress biomarkers such as catalase activity (CAT), glutathione-S-transferase (GST) and glutathione content (GSH). The liver histopathological study from Reconquista river fish populations showed differences: an increased amount of regressive changes, being hydropic and lipid degeneration and pyknosis of hepatocytes the most frequent alterations. The cell size was greater in R2 and the nuclear size was greater in R1. Regarding the oxidative stress biomarkers, R1 and R2 showed a decreased CAT activity while GST activity increased, in relation to the Ci population. Glutathione content was lower only from R1 fish group. We observed poorest landscape of the Reconquista river downstream, with high values of ammonium, phosphorus, chlorides, BOD and low DO levels. These results showed an important condition of severe stress in the water quality of the Reconquista river, which is reflected in the responses of histopathological and oxidative biomarkers of the fish from the studied sites.

## 23.P-Mo-114

### Assessing and Comparing Toxicity of Industrial Effluent by Using the Nematode *Caenorhabditis elegans* and the Lettuce Seed *Lactuca sativa*

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#### Abstract

Industrial activities are responsible for the discharge of liquid effluents containing a large amount of persistent and toxic chemical compounds. Industrial effluents are therefore a major source of threat to the integrity of aquatic ecosystems. Even though there should be treatment plants designed to remove polluting compounds from effluents, they are not always implemented, nor do they work properly, resulting in poor quality treatment that does not comply with current environmental standards. In addition, there are many industries that do not have treatment plants and their discharges are clandestine.

In Argentina, environmental regulations only require periodic determinations of physicochemical and bacteriological parameters, resulting in a short-sighted, static and limited analysis of effluent quality and their probable impact on the environment. It has been shown that some industrial effluents, although their quality is sufficient to comply with regulations, induce toxic effects. The aim of this study was to evaluate the toxicity of effluents from multiple industries located in the Matanza-Riachuelo river basin (Buenos Aires province, Argentina) and to compare the biological responses of two standard ecotoxicological tests: the nematode *Caenorhabditis elegans* and the lettuce seed *Lactuca sativa*.

Twelve different types of effluents representative of the textile, ceramics, petrochemical, food, paper, automotive, mechanical, pharmaceutical and construction industries were analysed. Physicochemical parameters such as pH, electrical conductivity, chemical oxygen demand, turbidity among other ions, organics and metals.

The results indicated that toxicity is expressed differently between the biological tests with the nematode being more sensitive than vascular plant seeds. In addition, highly toxic effects were revealed in the petrochemical, ceramic and construction industries, even when 1:50 dilutions were tested, pointing to the need to carefully review the toxic properties of complex effluent mixtures and matrices.

Results, indicated that toxicity is a parameter that bring important information to be considered by environmental regulation in order to better protect the environment and human health.

## 23.P-Mo-115

### **Arsenite and Arsenate Toxicity in the Earthworm *Eisenia andrei* (Bouché 1972) in Natural Soil and Tropical Artificial Soil**

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#### **Abstract**

Arsenic, in the inorganic forms of arsenite (As (III)) and arsenate (As (V)), is highly toxic and commonly present in soil. The toxicity of these compounds can have detrimental effects on key ecological processes and organisms, such as earthworms. This study aimed to assess the toxicity of arsenite and arsenate on *Eisenia andrei*, a Californian earthworm, in natural and artificial tropical soil. The assessment involved avoidance testing, acute toxicity testing, and sublethal concentration testing to analyze biochemical parameters. The results showed that arsenite had a significantly higher impact on the avoidance behavior of earthworms in natural soil, as well as on antioxidant capacity, glutathione levels, lipid damage, and DNA damage in animals exposed to arsenite, without differences regarding the types of soil. On the other hand, exposure to As (V) resulted in increased activity of enzymes related to glutathione metabolism. The LC50 values for arsenite were 21.27 mg/kg in natural soil and 19.0 mg/kg in TAS, while for arsenate, the values were 76.18 mg/kg in natural soil and over 120 mg/kg in TAS. These findings emphasize the adverse effects of Arsenic exposure on the health of *E. andrei*, particularly in the form of arsenite. It also highlights the need for public authorities to consider soil properties and the different chemical species of contaminants when establishing legal limits. Further studies should investigate the role of soil composition in the toxicity of Arsenic and other contaminants on other toxicity parameters, including differences between natural and artificial soils and their compositions.

## 23.P-Mo-116

### Histopathological Biomarkers in *Danio rerio* Used in Biomonitoring of Urban Streams

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#### Abstract

The aquatic biota is constantly exposed to many toxic substances resulting from human activities that have been altering the physical-chemical and biological processes of water sources. This contributes to reducing environmental quality and can compromise the animal's health in these ecosystems. In this sense, biomonitoring based on aquatic organisms makes it possible to detect the effects of toxic compounds that may be present in this environmental compartment. This study aimed to evaluate the water effects from urban streams in Campo Grande, Mato Grosso do Sul, Brazil. For that, the *Danio rerio* model was used in a passive biomonitoring system, comparing the temporal dynamics of liver histological markers exposed for 3, 6, and 12 days in three streams (Bandeira, Anhanduí, and Lagoa) which drain well-populated areas. Histological alterations were present in fish exposed to water from the three streams and at all exposure times. Pyknosis, karyolysis, karyorrhexis, and megalocytosis were the most common hepatocellular alterations in the three exposure times, with predominance at 12 days-exposition. Hypertrophy, lipidosis, and necrosis were observed in individuals exposed to the three sampled streams, occurring more frequently after 12 days. Circulatory alterations such as vascular hyperemia, sinusoidal edema, as well as diffuse leukocyte infiltrate were found in individuals exposed to the Bandeira stream. Inflammatory changes were found in individuals exposed to the Anhanduí and Lagoa streams. The degree of tissue changes (DTC) was higher in all periods (3, 6, and 12d) in Bandeira ( $4.2 \pm 0.60$ ;  $6.4 \pm 1.3$  and  $8.0 \pm 0.1$ ) and Lagoa ( $4.7 \pm 0.9$ ;  $7.1 \pm 1.4$  and  $13.5 \pm 1.6$ ), respectively. W1 and W2 changes, which represent distinct degrees of tissue reversibility, retained 64 and 70 % of the variation in CP1 (57.9%) and were more critical in the Anhanduí and Lagoa streams. W1 retained 91% in CP 2 (32.9%), respectively individualizing the Bandeira stream. The employed model allows us comparing the temporal dynamics of different health markers, using the zebrafish as an environmental bioindicator. Future studies may improve this model for other aquatic environments to support new approaches to biomonitoring studies.

## 23.P-Mo-117

### Toxicity of Biorational Insecticides in Adults of *Drosophila suzukii* (Diptera: Drosophilidae)

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#### Abstract

The spotted-wing fly, *Drosophila suzukii*, is a recent pest in Argentina that affects a variety of fine fruit crops. Worldwide, the intense use of conventional insecticides has a negative impact on the environment and adverse effects on non-target organisms. Biorational insecticides began to be used as an alternative which is more selective and safer. Nevertheless, there is evidence of resistance development to these insecticides in *D. suzukii* in other regions of the world which show lower resistance to cyantraniliprole under continued selective pressure compared to spinosad. The aim of this study was to evaluate the toxicity of cyantraniliprole and spinetoram using commercial formulations in adults of a laboratory susceptible colony (LSC) of *D. suzukii* to establish their susceptibility baselines. Spotted-wing flies 5-7 days old were exposed for 24 h in glass flasks internally treated with 5 concentrations of cyantraniliprole between 8 and 200 mg/L; and 6 concentrations of spinetoram between 10 to 210 mg/L. The control groups were exposed to flasks treated with solvent alone (acetone). The results were used to calculate values of Lethal Concentration 50% and 95% (LC50 and LC95, respectively) with their respective Confidence Intervals 95%. These parameters were calculated using the software PriProbitNM. The CL50 and CL95 for cyantraniliprole were 60.79 (23.28-125.21) and 369.72 (64.12-280.77) mg/L, respectively; and for spinetoram, 88.48 (76.79-100.83) and 351.95 (161.63-13,023) mg/L, respectively. These preliminary results suggest that LSC adults are more susceptible to cyantraniliprole than to spinetoram. In the future, the LSC baseline will be compared through LC50 of field populations and determine the potential development of resistance in Argentina.

## 23.P-Mo-118

### Co-exposure of Iron Oxide Nanoparticles and Microcystin-LR Induces Histological Damage in Nervous Tissue of Fish *Poecilia reticulata*

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#### Abstract

Microcystins LR (Mic-LR) are cyanotoxins widely found in freshwater cyanobacterial blooms, their production is frequent in eutrophication processes. The contamination of the aquatic environment by Mic-LR can impact the environment, the ecosystem and human health since the toxin can lead to the bioaccumulation process. In this regard, this work presents citrate functionalized iron oxide nanoparticles (IONPs) as an alternative for remediation, since it has the potential to absorb organic molecules. Neurotoxicity was assessed using tissue biomarkers. Thus, 3 fish of the species *Poecilia reticulata*, from each group, were exposed for 7 days in tanks to the following treatments: A) control with reconstituted water; B) Mic-LR (1.0 µg/L); C) IONPs (0.3 mg/L); D) Mic-LR (1.0 µg/L) + IONPs (0.3 mgFe/L); and E) iron ions (0.3 mg/L) (IFe). The animals' encephalons were fixed in Karnovsky solution, dehydrated in increasing concentrations of ethanol, embedded in methacrylate glycol, sectioned at 3µm thickness, stained with 1% Toluidine Blue and evaluated to identify possible histopathological changes in the region of the *tectum opticum* (*To*). The study considered only the *stratum album centrale* (*Sac*) to identify the following changes: inflammatory response, edema, areas of possible cell loss in the layers of the *To*. The control group showed no abnormalities. Group B, exposure with Mic-LR alone had zones of cell loss in the *Sac* layers and edema. In contrast, groups C and D showed inflammatory responses, edema, and regions of probable cell loss. Group E showed inflammatory responses, edema, areas with possible cell loss of the layers of the *To*, edematous area between the *Sac* and the remaining layers of the *To* generating structural damage in nervous tissue, which in a later study will be quantified, because the changes identified in the *To*, which is an essential structure for vision processing, are relevant and becomes an object of analysis with great potential to contribute with data to evaluate the toxic effect of various materials including nanoparticles and algae toxins.

## 23.P-Mo-119

### **The *P. argentinus* Shrimp as a Potential Indicator of Water Quality: Characterization of a Reference Site and Physiological State of the Organism**

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#### **Abstract**

*Palaemon argentinus* is an important representative of the communities in the Pampean streams and has been proposed as an indicator of environmental quality and a model for ecotoxicological studies. The objective of this work was to characterize the collection site and the physiological status of *P. argentinus*. Adult shrimp were collected from a freshwater stream located in the Magdalena district, Buenos Aires province (35°7'44.50" S, 57°41'51.95" W), frequently used as a reference site. To contextualize the sampling site, information on soil type (1:50,000 soil maps), coverage and available use (image classification) from the National Institute of Agricultural Technology (INTA) was contrasted. Physicochemical parameters of the water were also recorded in situ, and sediment samples were taken for granulometric analysis, organic matter and pesticide, hydrocarbon (HC), and metal content. For the biochemical analyses, the hepatopancreas and the muscle of the shrimps were dissected after collection. Total protein content (TP), protein carbonyls, and triacylglycerides (TAG) were determined in the hepatopancreas, as well as the activities of the enzymes catalase and glutathione S-transferase. In muscle, TP, glycogen, lactate, TAG, and the activity of lactate dehydrogenase enzyme were determined. In general, it was observed that there was no extensive agricultural activity in the area. HC analysis showed a predominance of organic matter derived from higher plants, and only trace concentrations (<1 ng/g) of Endosulfan and its metabolite Endosulfan Sulfate were detected among the pesticides, coinciding with a low level of anthropic disturbance in the site. The concentrations of Zn, Pb, Cd, Cr, and Ni were all below the reference levels and adverse effect thresholds for the protection of aquatic life. The biochemical results represent the first characterization of the baseline oxidative and energy metabolism of this shrimp species at the study site. The results of this study contribute to the description of the reference site and the physiological status of *P. argentinus* to enable its use in future ecotoxicological studies in the region.



## 23.P-Mo-120

### The Life Cycle of the Free-living Nematode *Caenorhabditis tropicalis* and its Implications for Future Ecotoxicological Studies

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#### Abstract

In experiments to assess the effect of contaminants, preference should be given to the use of species that occur in the climatic zone under study and/or native species. However, for the implementation of experimental assays with these species, especially if their biology is not well known, it is recommended to collect data on their different biological dimensions, such as data on their life cycle and development time. This study aims to describe the life cycle of *Caenorhabditis tropicalis*, a species widely found in the tropical region, and discuss the implications of its life cycle in future ecotoxicological studies. The wild-type JU1428 strain of *C. tropicalis* was provided by the *Caenorhabditis* Genetics Center. The experiments were carried out at 25°C, the substrate temperature where these animals were collected, in Nematoda growth medium (NGM) seeded with the bacterium *Escherichia coli* (OP50 strain). Under these conditions, the egg hatching time was 11.3 h  $\pm$  0.5, and the hatching rate was 70%. The body length of the individuals increased linearly until the first egg-laying, but from 27 h after hatching the male's body length was shorter than that of the hermaphrodites. Moreover, from 29 h, the male showed a greater body width in the cloaca region, which became the fan/copulatory bursa after a few hours. *C. tropicalis* underwent four molts, all occurring after hatching, and the first egg was laid by the hermaphrodites 48 h  $\pm$  1 after the hatching of the initial eggs, completing the life cycle. The life cycle is short and quite similar to that of *C. elegans*. The easy cultivation, ability to self-fertilize, occupation of little space in the laboratory, short life cycle and easy acquisition from the *Caenorhabditis* Genetics Center or by isolating from the natural environment makes *C. tropicalis* a species of high potential as a model organism in experimental studies, particularly in bioassays with specific environmental conditions of tropical ecosystems.

23.P-Mo-121

## Ecotoxicological Monitoring Using Integrative Tools: a Case Study in Southern Brazil

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### Abstract

Mirim Lagoon is a watershed located in Southern Brazil close to the border between Brazil and Uruguay. It has been degrading over the years, mainly due to the use of pesticides and fertilizers in the surrounding areas characterized by intense agricultural activity. Water from Mirim Lagoon is collected for rice crops irrigation and before harvesting it is pumped again into the lagoon. The evaluation of water resources using bioindicator organisms are recommended for effective environmental monitoring, complementing water physical-chemical conditions and contamination. This study evaluated the water quality of Mirim Lagoon from an ecotoxicological point of view through toxicity tests and biomarkers and using multivariate analyzes and integrative tools. Tests were performed with neonates of *Daphnia magna* and embryos of *Danio rerio*, exposed to surface water samples collected at different sites of the watershed, in three different periods (January, March, and June 2022), chosen based on agricultural dynamics. Biomarkers analyzed were behavioral (distance traveled and average speed), Acetylcholinesterase (AChE) and Glutathione S-transferase (GST) activities. The Integrated Biomarker Response (IBR) index showed that the water collected in March caused greater effects on the behavioral performance of both *D. magna* and *D. rerio*. March is characterized by the drainage of water used for the rice irrigation back to the lagoon before grain harvest, carrying the agricultural residues as the pesticides and their metabolites as well as fertilizers. Redundancy Analysis (RDA) indicated a correlation between the behavioral biomarkers and abiotic parameters, mainly nutrients (total phosphorus and total nitrogen), fecal coliforms, total solids, and turbidity. The evaluation of the influence of physical-chemical factors on biomarkers is important, as fluctuations in these parameters can also alter the physiological parameters of organisms. Multivariate analyses and the IBR index proved to be good tools for assisting the monitoring of Mirim Lagoon waters, allowing a spatial and temporal overview of the effects.

## Transcriptomic Changes at the Reproductive Level of Catfish (*Trichomycterus areolatus*) Associated with Sewage Effluents in Greater Santiago, Chile

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### Abstract

The *Trichomycterus areolatus* is a Chilean native freshwater catfish, it has benthic habits, and it is widely distributed between central and southern Chile, areas highly urbanized. Santiago, with the highest urban density, it is in the central zone of Chile. This province has two wastewater treatment plants (WWTPs) that handle almost 90% of discharges, both of which flow into the Mapocho River. Worldwide, it has been observed that fish exposed to sewage discharges show changes at different levels: at the population level, alterations have been recorded in the proportion of females and males; at the individual level, changes in size and the presence of intersex have been observed. In addition, modifications in gene expression at the transcriptomic level have been observed. In the case of catfish, information is scarce regarding transcriptomic changes in habitats subjected to sewage discharges. The purposes of the study are to compare biological parameters (condition factor (K), gonadosomatic index (GSI) and hepatosomatic index (LSI)) among the different study sites and to quantify by qPCR gene expression changes at the sexual level that may be affected by sewage effluents. The collection sites were determined according to their anthropic pressure and presence of *T. areolatus*, two reference sites (Estero el Arrayan and Clarillo river), two urban sites (Estero las Hualtatas and Club de Golf) and two sites downstream WWTPs (Trapiche and El monte). The collection of individuals was carried out during the months of October and November 2022. A total of 91 individuals (M= 37 and F = 54) were collected at five sites; at the golf club there was no collection of *T. areolatus*. The genes of interest are androgen receptor; estrogen receptor subtype 1, vitellogenin, aromatase, estrogen receptor beta, spermatogenesis associated protein 4 and as control genes elongation factor 1 alpha and elongation factor 1 gamma. Laboratory analyses are in process. This study would be the first to include changes in gene expression of *T. areolatus* in environments exposed to sewage discharges.

# Session 24: Impacts of the Expansion of Anthropogenic Activities on Water Quality and Biota in the Amazon and Pantanal Biomes

24.P-We-063

## Effects of Land Use on Water Quality and Pesticide Risks in Freshwater Ecosystems of the Ecuadorian Amazon

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### Abstract

The Ecuadorian Amazon has experienced a significant land use change due to the demographic increase and the expansion of the agricultural frontier. Such changes in land use have been associated to water pollution problems, including the emission of untreated urban wastewater and pesticides used in intensive agriculture. In this study, we evaluated the influence of different land uses (African palm oil production, corn production, urban areas and nature areas) on water quality parameters, pesticide contamination and on the ecological status of Amazonian freshwater ecosystems. We monitored 19 water quality parameters, 27 pesticides, and the macroinvertebrate community in 40 sampling locations of the Napo River basin (northern Ecuador) and assessed pesticides risks using a probabilistic approach. The results of our study show that urban areas and areas dominated by African palm oil production have a significant influence on water quality parameters, affecting macroinvertebrate communities and biomonitoring indices. Pesticide residues were detected in all sampling sites, with carbendazim, azoxystrobin, diazinon, propiconazole and imidacloprid showing the largest prevalence (>80% of the samples). We found a significant effect of land use on water pesticide contamination, with residues of organophosphate insecticides correlating with African palm oil production and some fungicides with urban areas. The pesticide risk assessment indicated organophosphate insecticides (ethion, chlorpyrifos, azinphos-methyl, profenofos and prothiophos) and imidacloprid as the compounds posing the largest ecotoxicological hazard, with pesticide mixtures potentially affecting up to 26-29% of aquatic species. Ecological risks of organophosphate insecticides are more likely to occur in rivers surrounded by African palm oil plantations, while imidacloprid risks were identified in corn crop areas as well as in nature reserves. Future investigations are needed to clarify the sources of imidacloprid contamination and to assess its effects for Amazonian freshwater ecosystems.

## 24.P-We-064

### **A Systematic Review on Metal Contamination due to Mining Activities in the Amazon Basin and Associated Environmental Hazards**

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#### **Abstract**

Metal contamination associated with mining activities is a reality for the Amazon region. The overall magnitude of metal exposure in water and sediments in relation to background levels is, however, still unknown. This review assessed 53 papers and reports published between 1989 and 2020 describing environmental concentrations of different metals and metalloids (As, Hg, Mn, Fe, Cd, Cu, Cr, Pb, Ni, and Zn) in water and sediments of mining and non-mining areas in five geographic regions of the Amazon basin. Concentrations were compared with sediment (CCME) and water (CCME and USEPA) quality standards set for the protection of aquatic life. Concentrations in non-mining areas were below the established standards, with the highest background levels being found for Cd, Cu, Hg, Mn, and Zn in the Southern Amazon region. Significant enrichments of As, Cu, Fe, Hg, Mn, and Pb in sediments and Cd, Cr, Fe, Hg, Mn, and Zn in water samples were observed in mining areas. The highest prevalence of enrichment for sediment comprised Pb (67% of samples), As (76%) and Mn (92%), and for water, Fe (83%), Hg (86%), Mn (100%) and Zn (100%), detected at 10 to 100-fold higher concentrations than mean background values. Cd exceeded the TEL quality standard, while Fe and Hg exceeded the short-term standard, and Zn the long-term quality standards. Therefore, the findings of this study indicate that mining activities significantly contribute to water and sediment contamination in the Amazon basin, posing a hazard for freshwater ecosystems and potentially having human health implications.

## 24.P-We-065

### Emerging Organic Micropollutants: The Reality of Urban Rivers in the Amazon Region

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#### Abstract

The emerging micropollutants are classified as a group of chemicals that are present in very low concentrations in the environment (from ng/L to µg/L). Some of these are considered priorities due to their continuous input in environment from multiples sources, such as organochlorines, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, hormones, phthalates, and others. The aim of this study was to identify and quantify different groups of emerging micropollutants in urban rivers in the city of Belém, PA, the largest urbanized area in Eastern Amazonia. Water samples were collected in rainy season (ebb tide) along two urban channels of Belém, the Tamandaré (1°27'24"S 48°29'45") and Tucunduba (1°28'34"S 48°27'14") channels. Sampling occurred in triplicate at three different points in each of the channels, considering that there are anthropic and tide influence along the two studied areas. The Tamandaré channel proved to be more contaminated than the Tucunduba channel because the tidal influence is higher in the last one, which makes it less susceptible to the accumulation of these contaminants. Our analyses demonstrated the presence of organochlorines, polycyclic aromatic hydrocarbons and phthalates in the Amazonian urban rivers studied. These findings indicate the need of a continuous monitoring of this kind of pollution in Amazonian water bodies since these contaminants can cause effects such as neurotoxicity, apoptosis, genotoxicity, reproductive system damage, neoplasms, endocrine disruption, developmental toxicity, mutagenic and teratogenic characteristics, among other damages, both to human health and to aquatic organisms.

## 24.P-We-066

### Identification and Quantification of Antimicrobials in Water Bodies Near Wild Cat Habitats in Costa Rica

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#### Abstract

Antimicrobial resistance (AMR) threatens human survival and will be the leading cause of death in the world by 2050, surpassing cancer and traffic accidents. AMR is the ability of bacteria to resist the effects of an antibiotic to which they were previously sensitive. Bacteria become resistant due to exposure to antimicrobials, causing genetic mutations and the acquisition of resistance genes (ARGs). The main sources of contamination are hospitals and veterinary clinics, where bacteria are exposed to high concentrations of antibiotics, as well as households, treatment plants, and agricultural activities. The disproportionate use of these products could explain the finding of ARGs in felines in Costa Rica. Angulo et al., 2023 quantified sixteen ARGs in feline feces and detected at least one ARG in all samples. Objective: This work aims to demonstrate the presence of antimicrobials in conserved areas of Costa Rica, specifically in rivers close to feline habitats, by identifying and quantifying antimicrobials in river water samples in which contact with felines has been confirmed. Sampling: 22 water samples were taken from rivers where feline sightings and feces have been previously reported and collected. The study area includes Braulio Carrillo National Park, Los Quetzales National Park, and surrounding farms. Analysis of antimicrobial residues A targeted analysis was performed by the direct injection method, using UPLC-MS/MS. Results Antibiotics were detected in 47% of the samples, with ciprofloxacin and norfloxacin being the most frequent. All antibiotic concentrations were reported to be below 8 ng/L. The following antimicrobials were reported: Erythromycin (6.0-2.3 ng/L), Roxithromycin (3.8-2.8 ng/L), Ciprofloxacin (8.2-2.1 ng/L) Norfloxacin (7.5-5.1 ng/L) Sulfamethoxazole (3.8-1.4 ng/L) Trimethoprim (1.5-0.8 ng/L) and Furaltadone (2.2-1.7 ng/L). In Braulio Carrillo National Park, a protected area known for the presence of felines, the following were reported: Ciprofloxacin, Norfloxacin, Erythromycin, and Sulfamethoxazole. Conclusions The analytical method is so sensitive that it allowed levels as low as 0.8 ng/L to be reported. Antibiotics were reported in rural areas with anthropogenic influence and in national parks. Although the concentrations reported are relatively low, they could be sufficient to generate resistance, further threatening an endangered species such as felines.

## 24.P-We-067

### Risks to Human Health Associated With the Consumption of Fish From the Pantanal Sul-Mato-Grossense Contaminated by Metals

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#### Abstract

As observed in other regions of Brazil, the Pantanal Biome has suffered serious environmental impacts resulting from the contamination of aquatic ecosystems by toxic metals. The bioaccumulation of toxic metals in fish native to the Pantanal Sul-Mato-Grossense is a concern both from an environmental and public health point of view. In this context, our objective was to quantify the metals Cd, Cu, Ni, Pb, and Zn bioaccumulated in the muscle tissue from two native fish species (*Hypostomus regani* and *Brycon hilarii*) and to evaluate the risks to human health arising from their consumption. Fish samples were collected in November 2020 in the Aquidauana River, Pantanal Sul-Mato-Grossense, Brazil. To quantify metals in muscle tissue of fish samples, inductively coupled plasma optical emission spectrometry was used. For risk assessment, we used the risk quotient (RQ) approach, which was obtained by the ratio between the concentration of each metal present in fish muscle tissue samples, and their maximum limits established by Brazilian legislation for metals in fish intended for human consumption. For Cd and Pb, RQ values were > 1 for both *H. regani* and *B. hilarii*, indicating risks to human health due to the consumption of these contaminated fish. For Cu, Ni, and Zn, RQ values were < 1, indicating no risk to human health. In this way, contamination by metals in the Brazilian Pantanal is an important threat to biodiversity, in addition to causing risks to human health due to the consumption of fish contaminated by toxic metals.

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## 24.P-We-068

### Bioaccumulation of Inorganic Elements in Different Tissues of Native Fish Species From Pantanal Sul-Mato-Grossense, Brazil

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#### Abstract

The Pantanal Biome holds a vast ichthyofauna, in addition to being considered an important refuge and nursery for several species of fish. However, the biodiversity of the Pantanal Sul-Mato-Grossense is constantly being threatened by the expansion of the agricultural frontier and urban areas. Such activities generate high amounts of solid and liquid waste that can reach water resources and consequently affect the aquatic biota. In this sense, our objective was to determine the bioaccumulation factor of the inorganic elements Al, As, Cd, Cu, Fe, Mn, Ni, and Pb in the muscular and hepatic tissues of four native fish species. The fish species *Hypostomus regani*, *Prochilodus lineatus*, *Brycon hilarii*, and *Mylossoma duriventre* were sampled in November 2020 in the Aquidauana River, Pantanal Sul-Mato-Grossense, Brazil. The quantification of inorganic elements in fish muscle and liver tissue samples was performed using inductively coupled plasma optical emission spectrometry. The bioaccumulation factor (BAF) was calculated by the ratio between the concentrations of each inorganic element present in the muscular and hepatic tissues and its concentration in the Aquidauana River waters. In total, 5 individuals of *H. regani*, 7 *P. lineatus*, 11 *B. hilarii*, and 4 *M. duriventre* were sampled. BAF values were greater than 100 only for liver tissue samples from the four fish species. Cd showed BAF > 100 only in the liver tissue of *P. lineatus*; Cu in liver tissue of four fish species; and Fe in the liver tissue of *P. lineatus*, *B. hilarii*, and *M. duriventre*, indicating that these elements were bioavailable in the water of the Aquidauana River. The highest values of BAF obtained for the liver tissue of all fish species can be explained by the function of the liver, which includes the biotransformation of toxicants and eventually its detoxification. It is important to emphasize that the contamination of water resources in the Pantanal Sul-Mato-Grossense, by inorganic elements, is a serious environmental concern, as these contaminants can seriously affect biodiversity, threatening several native species. We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Process 160827/2019–1) for the scholarship of the first author and Universidade Federal da Grande Dourados for support.

## 24.P-We-069

### **Risks to Human Health Arising From the Consumption of Two Species of Amazonian Fish of Commercial Interest Collected in the Araguari River, Amapá State, Brazil**

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#### **Abstract**

The Araguari River comprises a vast aquatic biodiversity, and its waters are used as a source of fresh water for riverside populations. The riverside and indigenous communities that live on its banks also practice subsistence fishing. The Araguari River is not exempt from this influx of contaminants, which can lead to losses in local biodiversity and risks to human health. Thus, the present study aimed to investigate whether the concentrations of cadmium (Cd) and lead (Pb) present in the muscle tissue of two species of Amazonian fish represent risks to human health due to their consumption. Sampling was carried out between September and October 2018. *Ageneiosus inermis* and *Hoplias aimara* were sampled using cast nets and nets with meshes. Metal concentrations in fish samples were determined by an atomic absorption spectrophotometer. For risk assessment we use the risk quotient (RQ) approach. The RQs were calculated by the ratio between the concentration of each bioaccumulated metal in the muscle tissue of both fish species and their respective maximum limits established by Brazilian legislation. For two species of fish analyzed, we observed values of  $RQ > 1$  for the metals Cd and Pb, indicating that the daily consumption of both species poses risks to human health. The risks of prolonged consumption of fish with high concentrations of these metals can lead to food poisoning and other chronic diseases such as cardiovascular and liver diseases, in addition to genotoxicity and neurological problems.

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## 24.P-We-070

### Mercury Concentration in Water, Sediment, and Two Species of Fish in Areas Under the Influence of the Belo Monte Hydroelectric Power Plant, Xingu River, Amazon, Brazil

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#### Abstract

Mercury (Hg) is among the top 10 chemicals of global concern, according to the World Health Organization (WHO). In the Amazon, the construction of hydroelectric dams has a strong relationship with this Potentially Toxic Element (PTE). This metal affects human health and impacts fish homeostasis. Therefore, the objective of this study was to verify the concentration of Total Hg in water, sediment, and two fish species from Xingu River (*Cichla melaniae* and *Baryancistrus xanthurus*) from areas under the influence of Belo Monte Hydroelectric Plant (UHEBM). Environmental (water and sediment) and biological (gills, liver, and muscle) samples were collected at eight points in October 2020 during the Amazon drought (Permits: SISBIO 71763-1; Ethics Committee on Animal Use, CEUA 8166251119). In the laboratory, all samples were subjected to acid digestion, heating on an electric plate at 200° C, and analysis in an atomic absorption and cold vapor spectrometer. In all water samples collected, the quantification of Hg was above the maximum limit established by Brazilian legislation (up to 0.2  $\mu\text{g.L}^{-1}$ ). As for the sediment, although Hg was quantified below the limit allowed (0.5  $\text{mg.Kg}^{-1}$ ), the highest values were found in the area of the stretch of the river diverted for the construction of the hydroelectric dam and with a history of gold mining (Volta Grande do Xingu, VG). Among the species, *C. melaniae*, a carnivore, showed the highest concentrations of this metal. Among the fish tissues, the liver showed the highest amount of Hg. At the collection points, Hg in gills and muscle was higher in the reservoir for both species, *C. melaniae* showed higher concentrations of Hg in the liver in the VG region. The relationship between growth and Hg accumulation was positive only for *C. melaniae*. The concentrations of this PTE in fish are within the limits of the Brazilian legislation (1  $\text{mg.Kg}^{-1}$  for predator fish and 0.5  $\text{mg.Kg}^{-1}$  for non-predator species). The results found in water and sediment may be related to the organic matter accumulated in the reservoir and the history of mining in the region. In fish, the concentrations reflect the trophic position of the species, with the liver standing out due to its detoxification function. In this sense, it is necessary to investigate the viability of hydroelectricity, even in a run-of-the-river regime, to establish a clean energy matrix that preserves the Amazon, its people, and its biodiversity.

# Session 25: Toxic Cyanobacterial Blooms: Causes of a Growing Problem

25.P-Mo-123

## Evaluation of the Effect of Glyphosate-Based Herbicides on the Photosynthetic Response in Benthic Cyanobacteria from Tropical Lotic Environments

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### Abstract

The cyanobacteria (Filo Cyanobacteria) can be seriously considered as test organisms in aquatic environmental biomonitoring programs, especially when the main objective is to assess the potential effects of specific chemicals/environmental conditions on aquatic ecosystems. In the context of aquatic ecotoxicology, glyphosate-based herbicides can produce severe negative physiological effects on plants and bacteria, some of them specifically on photosynthetic metabolism. In this sense, this type of herbicide acts, as a rule, in draining carbon from the Calvin Cycle for the synthesis of shikimate and in reducing the number of photosystem II reaction centers caused by the inhibition of tyrosine production as considering that the presence of glyphosate-based herbicides in water bodies around agricultural areas has been widely reported worldwide and, at the same time, that benthic cyanobacteria are one of the most important primary producers in tropical lotic ecosystems, studies assessing the effects of these chemical compounds on different physiological aspects of cyanobacteria have become imperative for a better understanding of the ecological dynamics of these organisms and, in a broader sense, of the community of streams primary producers as a whole. Given this scenario, the present study was carried out aiming to evaluate whether glyphosate-based herbicide residues can affect photosynthetic responses, evaluated by specific parameter (effective quantum yield of PSII, quantum yield of non-regulated energy dissipation, and quantum yield of regulated energy dissipation) from the technique of chlorophyll fluorescence and by net photosynthetic and dark respiration rates from the technique of evolution of dissolved oxygen, of three species of lotic benthic cyanobacteria. Our results revealed that, in general, there were no statistical differences for most of the photosynthetic parameters from both techniques when the samples exposed to the glyphosate-based herbicide were compared with those of the control groups for the three cyanobacteria species investigated. These results confirm literature data that suggest that cyanobacteria have some mechanisms for regulating the photosynthetic apparatus capable of modulating damage to photosynthetic efficiency eventually produced by exposure to glyphosate-based herbicide.

## 25.P-Mo-124

### Validation of a Simple and Rapid Liquid Chromatography Coupled to Tandem Mass Spectrometry (LC-MS/MS) Assay for the Quantification of Cyanotoxins in Freshwater

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#### Abstract

Eutrophication of aquatic environments promotes the proliferation of cyanobacteria, which produce a wide variety of highly toxic compounds, cyanotoxins. In Uruguay, the main aquatic systems (Río Negro, Río Uruguay and Río de la Plata) are seriously affected by cyanobacterial blooms. Cyanotoxins can be structurally diverse, ranging from cyclic peptides to alkaloids. Among the cyclic peptides are the microcystins and the nodularins. The alkaloids include anatoxin-a, cylindrospermopsin, and saxitoxins. These compounds interfere with the water main uses: drinking water production, recreation, agricultural production, fishing, among others. To assess and manage the associated risks, rapid and validated methods are required, which make their determination feasible at low cost. Bioassays and biochemical assays are often used to determine cyanotoxins, but they are nonspecific, so they can be only used as screening methods. Instrumental analysis using liquid chromatography has several advantages providing very good specificity and sensibility. Different detectors can be used, ranging from simple ultraviolet-spectrometry to various types of mass spectrometry. In this work, a method was developed and validated for the determination of 4 cyanotoxins (selected by their presence in water bodies in Uruguay) in natural waters using liquid chromatography coupled to a tandem mass detector. First, the detection of cyanotoxins (saxitoxin, nodularin, microcystin-LR and cylindrospermopsin) was evaluated by direct injection, obtaining a sensitivity limit between 0,2 and 0,5  $\mu\text{g/L}$ , depending on the toxin. To improve sensibility, two sample pretreatments were evaluated, solid phase extraction and freeze-drying of the samples prior to detection. The freeze-drying methodology improved detection levels, reaching 0,01  $\mu\text{g/L}$  for cylindrospermopsin, microcystin-LR and nodularin. Both methodologies were validated following all the quality guidelines of the laboratory and the SANTE 2021 guide, complying with all the proposed acceptance criteria, and ensuring the reliability of the analyses. Therefore, both methodologies for the determination of cyanotoxins are being used in routine analysis.

## 25.P-Mo-125

### Bioassays in Samples of Water From Urban Lakes Treated With a Flocculant Compound (Polyhydroxy Aluminum Chloride)

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#### Abstract

Control of total phosphorus load and phytoplanktonic biomass in urban lakes is difficult to manage. The application of chemical compounds is an adequate tool, but it is not exempt from ecotoxicological risks.

The objective of this study was to establish if the concentrations of Polyhydroxy Aluminum Chloride (PAC) selected to control the total phosphorus load in urban lakes may present this risk for native fish and planktonic crustaceans. The project was developed in collaboration between Intendencia de Montevideo, Intendencia de Canelones and the Universidad de la República (Uruguay).

The experimental design consisted in carrying out bioassays with *Cnesterodon decemmaculatus* (Poeciliidae) and *Daphnia magna* (Cladocera), in water treated with PAC. The study was carried out using water from six Uruguayan urban lakes: Rodó, Cachón and Prado (Montevideo) and Jardín, Javier and Shangrilá (Canelones). At the laboratory, 8 mg/L of total Aluminium (Al) was applied as PAC. After 12 hours, supernatant water (Al concentrations range from 78 to 1610 µg/L) was used for ecotoxicological tests.

The *C. decemmaculatus* bioassay (96h and static) was carried out following the guidelines of Saona *et al.* (2015) with two adaptations: the test temperature was 25°C and the untreated water from each lake was used as control medium and for dilutions. The bioassay of *D. magna* (48h and static) followed the protocol of the ISO 6341 standard and the recommendations of the WaterTox network.

The 6 bioassays with *C. decemmaculatus* did not present significant differences ( $p > 0.05$ ) between the treated sample and the negative control. In addition, a dose-response relationship was not observed when considering the mortality of all the dilutions made. A relevant observation was the mortality recorded with the fish assay in the negative controls in a range of 3.3 to 26.7%.

The *D. magna* assays were non-toxic for all the lakes, with no mortality in the treated samples or in the negative control.

It is concluded that the used concentrations of Al do not produce relevant lethal effects for the fish or crustaceans studied. The results are robust and are supported by lakes with heterogeneous properties.

The observed mortality in the negative controls versus the results observed in controls with the standard culture medium are discussed.

The bioassays used could constitute a minimum set of ecotoxicological techniques recommended as preliminary tests before the direct application of PAC in lakes.

DRAFT

## 25.P-Mo-126

### Project Ecophysiometer: Updates and New Features.

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#### Abstract

Environmental inequality is exacerbated by the unavailability of environmental monitoring technologies for low income communities. The shortage of resources leads to incomplete databases with only a fraction of the relevant environmental variables being monitored. As both policy making and scientific discovery depend on the availability of high quality long term, uninterrupted data, any shortage on this end may lead to poorly managed ecosystems. As an attempt to solve this issue, the Ecophysiometer project started as an open-science initiative aiming to provide a versatile instrument capable of measuring multiple variables with a single equipment, and enabling users to develop and share customised methods and data. This work presents the improvements made to the instrument. We have developed a custom Printed Circuit Board (PCB) that interfaces with a Raspberry pi. Software was also improved by developing a series of python packages that ease users developing custom routines. A graphical user interface was created to easily find optimal conditions and monitor samples' spectra during method development or to use the ecophysiometer as a bench-top instrument. We have redesigned the instrument enabling it to measure the visible spectrum of absorbance and the emission spectra of fluorescence, at eight different excitation wavelengths (275 nm, 365 nm, 395 nm, 440 nm, 470 nm, 530 nm, 600 nm & 660 nm). The optical path-length of the absorbance measurements was enlarged to increase the sensitivity in optically thinner samples. Lastly, an auto-sampler was included in the new design. To explore the capacity of the equipment to measure several variables a series of methods were developed. Firstly, a method to measure turbidity (nephelometric turbidity units) was developed using the formazin standard proposed in SMEWW 2130 ( $n = 11$ ,  $p\text{-val} < 2 \cdot 10^{-16}$ ,  $R^2 = 0.9997$ ). To explore the capacity of the instrument to distinguish between algae groups, pure cultures of *Scenedesmus* spp. and *Dolichospermum* spp. were measured. The resulting spectra were then clustered with hierarchical cluster analysis, resulting in a clear separation of each sample group. Encouraged by these results, we are currently working on a method to quantify chlorophyll-a and another to quantify phytoplankton divisions' abundance.



25.P-Mo-127

## Comparative Analysis of the Seasonal Conformation of Planktonic Communities in Relation to the Physicochemical Parameters of Three Urban Lakes in Montevideo (Uruguay)

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### Abstract

The eutrophication of water bodies represents the main pollution problem worldwide, leading to the deterioration of water quality, ecosystems, its aesthetics, and a loss of values for conservation. Urban lakes are small and shallow artificial aquatic ecosystems, built in parks for aesthetic and recreational purposes, and key in regulating the microclimate of cities. Due to the strong anthropogenic influence they have, they are susceptible to eutrophication; therefore, the loss of clarity of the system's water is expected, as well as an increase in phytoplankton biomass and the potential development of cyanobacterial blooms. Many studies indicate that climate change will aggravate this situation, altering precipitation patterns, affecting river flows and increasing temperatures and periods of drought, posing a threat to the ecological integrity of bodies of water. Due to this, the analysis of the composition and biomass of the planktonic community is crucial for environmental monitoring. The present work had as main objective the comparative analysis of the changes of plankton species, to determine if they are the result of the interaction between the physicochemical and/or biological properties of a lake. The samples were taken in three urban lakes in the city of Montevideo (Uruguay): Rodó (LROD), Jardín Japonés (LJJ) and Rivera (LR) during the summer and non-summer seasons of the year 2022. Measurements of T (° C), pH, salinity (ppt), turbidity (NTU), conductivity ( $\mu\text{S}/\text{cm}$ ), chlorophyll-a ( $\mu\text{g}/\text{L}$ ), dissolved oxygen (mg/L), percentage saturation (%) and nutrients: PT, NT and PO<sub>4</sub> (mg/L), and a survey of the plankton species present was carried out. The results evidenced the presence of cyanobacteria in the three lakes, mainly in the summer period, and in autumn. LROD showed a marked dominance of Cyanophyceae (genus *Microcystis*), with high levels of chlorophyll a in all sampling months. LJJ presented the best water quality, with the highest transparency and the lowest plankton biomass, dominated by Chlorophyceae in the summer months, and Acanthocystidae in the non-summer months. On the other hand, LR was dominated by Chlorophyceae in all sampling months. This work shows that the comparative analysis of the seasonal variation of the plankton composition is relevant for the monitoring and conservation of urban lakes.

## Metallic Nanoparticles as a Remediation Alternative to Eutrophic Aquatic Systems in the Ecuadorian Andean Region: A Critical Review

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### Abstract

Eutrophication can lead to a body of water becoming enriched with nutrients, such as phosphorus and nitrogen, leading to overgrowth of aquatic plants and algae, which can accelerate the proliferation of harmful algal blooms (HABs) in aquatic ecosystems. HABs produce cyanotoxins that can harm aquatic life, impair water quality, and increase the risk of food poisoning in seafood consumers. Lakes and lagoons are particularly susceptible to HABs due to factors such as nutrient pollution, high temperatures, and lentic water conditions. In Ecuador, lentic aquatic ecosystems are found in the Andean region, where Indigenous communities and the public use them as a source of drinking water and recreational activities.

Unfortunately, a combination of stationality climate factors, agricultural runoffs and sewage discharges have led to the eutrophication in these ecosystems. Eutrophication has been reported in the “Yahuarcocha” lagoon, which is one of the major aquatic lotic ecosystems in the Northern Ecuadorian Andes. Traditionally, copper sulfate, hydrogen peroxide, and potassium permanganate have been used to precipitate HABs. However, chemical treatments have been associated with unfavorable outcomes such as metal pollution and anaerobic conditions. Alternatively, metallic nanoparticles including copper, silver, zinc, and metal oxide-based nanoparticles such as TiO<sub>2</sub> and the Fe<sub>2</sub>O<sub>3</sub>/TiO<sub>2</sub> nanocomposite have been specifically tailored to eradicate persistent biofilms, including HABs, in aquatic systems by inhibiting or interrupting essential pathways, including the electron transport chain, or by disrupting the cell membrane in the algae cells and bacteria associated with HABs. This critical review analyzes nanotechnology-based remediation technologies to mitigate eutrophication, and therefore HAB events, in freshwater ecosystems. We focused on eutrophic aquatic ecosystems in the Ecuadorian Andean region because to our knowledge, no efforts have been made to explore the application of nanotechnology to mitigate HABs. We extensively reviewed literature on nanotechnologies applications, eutrophic remediation methods and experimental designs using Google Scholar, ResearchGate, Web of Science, ScienceDirect, and public repositories from Ecuadorian universities from the last decade (2013-2023). The implications of the application of nano-remediation techniques in the Ecuadorian Andean region are discussed in the context of their technical and economic feasibility.

## 25.P-Mo-129

### Cyanobacterial Blooms in Urban Wastewater Treatment Ponds (San Justo, Argentina)

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#### Abstract

Urban Wastewater (UWW) is generated by human metabolism and domestic activities. San Justo city (Santa Fe, Argentina) has a treatment plant (TP) for UWW which is treated by a system of stabilization ponds (biological stages) and subsequent chlorination. These ponds are aquatic environments rich in nutrients, with long hydraulic retention times that favor the blooming of cyanobacteria. The aim of this work was to detect cyanobacterial blooms, potential toxin producers in secondary facultative ponds (SFPs) of the TP. Monthly sampling was carried out in June, July, and August (winter - WIN) and November, December, and February (summer - SUM). Temperature (T), dissolved oxygen (DO), conductivity (CO), and pH were measured *in situ*. Nutrients were determined according to standardized protocols. Qualitative and quantitative phytoplankton samples have been collected and analyzed (20 µm net, 1% Lugol + glacial acetic acid, Utermöhl method). A bloom was considered when the density of cyanobacteria was greater than 5,000 cells mL<sup>-1</sup>. SFPs showed T: 11.6-27.3 °C; DO: 1.7-15.4 mg O<sub>2</sub> L<sup>-1</sup>; CO: 437.1-1246 µS cm<sup>-1</sup>; pH: 7-9.6; Nitrate: 3.8 - 27.1 mg L<sup>-1</sup> N; Nitrite: 0.02 - 6.4 mg L<sup>-1</sup> N; Ammonium: 4.15 - 45.3 mg L<sup>-1</sup> N; Phosphorus: 1.4 - 7.0 mg L<sup>-1</sup> P. Episodes of mono- and polyspecific blooms were detected in WIN and SUM and in both SFPs. The species recorded were: *Aphanocapsa* sp., *A. delicatissima*, *Merismopedia tenuissima*, *Oscillatoria* sp.1, and *Oscillatoria* sp.2. The genus *Aphanocapsa* is one of the most widespread in Argentina; the species are sensitive to low resource supply, however, nutrient concentrations were sufficient to sustain their development. Due to the large biomass, they can reach, they are placed in the category of notoriously nuisance algae. From the point of view of effluent and receiving watercourse quality management, the cyanobacteria can become potentially hazardous if they produce toxins and form surface scums, this puts them in the category of notorious nuisance algae. *Microcystis aeruginosa* was also detected, blooms of this species have been recorded under conditions of T, CO, and pH similar to those of the SFPs studied. Due to the potential to generate blooms and the possible toxicity of the identified species, it is suggested to monitor them during the whole treatment process and to control them before discharge into the receiving watercourse.

## 25.P-Mo-130

### Potentially Toxic Cyanobacterial Blooms in Leachates of Urban Solid Waste (USW) Treated in the Environmental Complex of Santa Fe City (Argentina)

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#### Abstract

Cyanobacterial blooms usually occur in aquatic environments, generating sanitary problems and ecosystem deterioration; however, little is known about the species that could generate blooms in leachate treatment ponds. Santa Fe city has a USW leachates treatment plant, composed by a primary decanter, two biological treatments, ending in physicochemical and disinfection treatments. The objective was to identify potentially toxic cyanobacterial taxa and possible blooms in the aerobic leachate treatment pond and their relationship with environmental variables in contrasting periods of temperature and precipitation. Seasonal samplings were carried out in summer (SU) and winter (WI). The following variables were measured: pH, temperature (T, °C), conductivity (CO, mS cm<sup>-1</sup>), dissolved oxygen (mg L<sup>-1</sup>) and nutrients (mg L<sup>-1</sup>; nitrite NO<sub>2</sub><sup>-</sup>; nitrate, NO<sub>3</sub><sup>-</sup> and ammonium, NH<sub>4</sub><sup>+</sup>). Qualitative phytoplankton samples (20 µm mesh, 4% formaldehyde) and quantitative samples were taken using the Utermöhl method. Taxonomic keys were used to identify the species. Flowering was considered when the density of the same taxon was equal to or greater than 5000 ind. ml<sup>-1</sup>. Of the 13 recorded Cyanobacteria taxa, 7 caused blooms in both seasons, from highest to lowest abundance (ind.ml<sup>-1</sup>): *Romeria hieroglyphica*, *Aphanocapsa incerta*, *Aphanocapsa delicatissima*, *Coelosphaerium kuetzingianum*, *Aphanocapsa nobilum*, *Aphanothece* sp. and *Gloeocapsopsis crepidinum*. *R. hieroglyphica* was the most abundant species, recorded in pH ranges: 6-9; T: 16-29.5 °C and CO: 8.16-16.33 mS cm<sup>-1</sup>. The second most abundant species was *A. incerta*, developed in pH ranges: 6-8.3; and: 16-27 °C and CO: 8.16-15.96 mS cm<sup>-1</sup>, followed by *A. delicatissima* which was recorded at pH: 6-8.3; and: 16 - 27 °C and CO: 8.16 - 16.33 mS cm<sup>-1</sup>. The following nutrient ranges were recorded in the aerobic pond: NO<sub>2</sub><sup>-</sup>: 37.51 - 106.78 mg L<sup>-1</sup>; NO<sub>3</sub><sup>-</sup>: 633.6 - 951.32 mg L<sup>-1</sup> and NH<sub>4</sub><sup>+</sup>: 255.5 - 202.2 mg L<sup>-1</sup>. *Aphanocapsa* has microcystins that generate liver and neurological damage. In SU, temperature was the variable that most favored the development of cyanobacteria, although it was not a limiting factor in WI. High T values together with high pH values and nutrients acted favoring cyanobacterial blooms. The registered species have the potential to release cyanotoxins into the environment, making it necessary to control their blooms to avoid health risks in the treatment plant and in the receiving watercourse, the Salado River.

25.P-Mo-131

## Evolutionary and Ecological Strategies of the *Microcystis aeruginosa* Complex that Explain its Success and Global Expansion: A Decade of Studies

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### Abstract

Eutrophication by nutrient enrichment is a serious contamination problem affecting aquatic ecosystems worldwide. An alarming consequence of eutrophication is the emergence of toxic cyanobacterial blooms, among which those caused by *Microcystis aeruginosa* complex (MAC) are highly frequent. Despite extensive research on this topic, the mechanisms promoting the synthesis of toxic metabolites by these cyanobacteria are still under discussion. Here, we will summarize the results of ten years research aiming to elucidate which are the main environmental drivers of toxicity in MAC and to predict which environmental conditions impose a greatest risk to human, animal, and environmental health. The main ecosystems studied were the Uruguay River and the Río de la Plata estuary. We explored MAC community diversity at the specific and intra-specific levels using different approaches (microscopy, amplicon sequencing, genotyping and machine learning techniques), analyzed the toxin patterns under a gradient of environmental conditions (transcription and microcystin variants analysis) and explored the role of the microbiome associated to the colonial mucilage on the survival, growth and toxicity of MAC (metagenomics). The gathered knowledge allowed us to develop detection methods (qPCR, remote sensing, LAMP) and prediction (machine learning modeling using environmental and meteorological variables) of the occurrence of toxic MAC blooms.

# Session 26: Cutting-Edge Monitoring Technologies for Health Protection

## 26.P-Mo-132

### Association Among Pesticide Drinking Water Contamination, Cancer Incidence and Cancer Deaths in Nine Rural Municipalities From Paraná State, Brazil

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#### Abstract

Brazil is one of the leading pesticide consumers worldwide. A recent report from Water Quality for Human Consumption Vigilance Information System (SISÁGUA, Brazil) released in 2018 showed the presence of 27 pesticides in drinking water, of which eleven are probably, possibly, or proven carcinogens. Based on this, we assessed this data for nine municipalities in Paraná state Southwest, a landscape characterized by extensive use of pesticides and focused on by our group due to the high rates of cancer documented across the last decade. We selected data from proven, probable, or potentially carcinogenic pesticides reported by SISAGUA (alachlor, aldrin-dieldrin, atrazine, chlordane, DDT-DDD-DDE, diuron, glyphosate-AMPA, lindane- $\gamma$ -HCH, mancozeb-ETU, molinate, and trifluralin) and crossed with incidence and mortality cancer rates for the nine selected municipalities, obtained from the Cancer Hospital Record Information (IRHC), by the Brazilian National Cancer Institute (INCA). We found high contamination in almost all the nine analyzed cities for the eleven selected pesticides. Diuron, glyphosate-AMPA, and mancozeb-ETU were the main contaminants (10-200 ppb). We collected data about cancer registers for the same period of the SISAGUA report concerning the incidence and mortality rates for ten topographies (breast, prostate, colon and rectum, lung, trachea and bronchi, cervix, stomach, thyroid, oral cavity, esophagus, and bladder). It was observed that a rise from 94% to over 1.300% compared to national and state averages, especially for breast, prostate, colon and rectum, and esophagus malignancies. All mortality rates have also presented substantial increases for every neoplasm considered, emphasizing the rise of over 1,800% for prostate cancer and 1,075% for lung cancer for some localities, which appears to be the most lethal. Significant correlations were found among the drinking water levels of all currently allowed pesticides and persistent organic pollutants. Significant strong correlations were observed among pesticide contamination, cancer incidence, and cancer death for all pesticides, except alachlor, aldrin, and molinate. Lindane and glyphosate showed the highest correlations, and breast, prostate, and esophagus were the main correlated topographies. Our findings highlight significantly increased incidence and mortality in rural populations and correlate this public health concern to pesticides in their drinking water.

## 26.P-Mo-133

### Ethnic and Racial Disparities in Exogenous Poisoning Cases in Brazil

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#### Abstract

Ethnic and racial disparities are pervasive in Brazil, obstructing access to goods, services and opportunities, including health services. While studies in other countries have demonstrated ethnic-racial disparities in medication and drug abuse poisoning, researches examining pesticide poisoning are non-existent. Ethnic-racial disparities can impede access to health information and services, even causing general exogenous intoxication. These disparities can impede access to health information and services, resulting in general exogenous intoxication. The present study sought to investigate the relationship between race/ethnicity and general exogenous intoxication from medication, pesticides, and drugs of abuse in all Brazilian states. Using data from the Information System for Notifiable Diseases (SINAN) and the Brazilian Institute of Geography and Statistics (IBGE), we collected notifications of exogenous intoxication from medication, pesticides (for agricultural, domestic, public health, rodenticide, and veterinary use), and drugs of abuse from all Brazilian states for the years 2017 to 2019. Data were categorized into whites and non-whites (including black, brown, and indigenous peoples) and calculated notification percentages and population statistics for ethnic-racial groups. Also, the intoxication ratios between whites and non-whites were calculated. Our study showed that the ethnic-racial disparity of general intoxications by drugs and pesticides was highest in Pernambuco, while Paraíba had the highest rate of drug abuse. Non-white people in the North and Northeast regions had higher

relative risk values for all types of poisoning. Furthermore, a high percentage of unknown race was found in Amazonas, the Federal District, and states in the Northeast and Southeast regions. Our findings sheds light on the existence of ethnic and racial disparities in exogenous poisoning cases in Brazil, highlighting the urgent need for targeted interventions to reduce these inequalities. It also reveals a lack of data on ethnic-racial identity in some regions, which impedes the formulation of effective interventions.

DRAFT



26.P-Mo-134

## Alterations in the Neuronal Microenvironment Caused Metastatic Melanomas in *Mus musculus* Mouse

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### Abstract

Recently, the focus of the cancer research it's about the causes, however, little has been studied in relation to their development based on certain food items. This study aimed to evaluate the effects of canola and corn oil intake on the progression of tumor cells in the brain of mice subjected to a model of lung metastasis by melanoma. All this is according to the organ's morphology, somatic index, oxidative stress markers, and histology. The model species corresponds to the house mouse, *Mus musculus* strain C57BL/6, while the tumor cells belong to the B16 F10 of melanoma. The experimental design contrasts the effects of both lipids separately and under the comparison of a control group and another with only these cells tumors. The quantitative, univariate, and multivariate results showed that the groups of melanoma and melanoma with canola oil supplementation had similar behaviors. However, the qualitative results for the histology of the four groups, showed different levels of lesion in the brain tissue. According to these observations, it is concluded that melanoma, even without invading the central nervous system, alters its structure and biochemistry. Likewise, canola oil promotes metastasis, but not via oxidative stress in this organ. These results support understanding a new phenomenon of the effects of a rich fatty acids diet on metastasis in the central nervous system.

## 26.P-Mo-135

### Assessment of Pollutants in the Gaseous and Particulate Phase According to Vehicular Flow and their Impact on Health in Córdoba (Argentina)

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#### Abstract

In recent times, rapid urban and industrial development has caused significant environmental deterioration, especially in developing countries. The exposure of urban populations to high levels of atmospheric pollutants are responsible for increasing health risks. On the other hand, numerous studies have identified vehicular traffic as the main source of air pollution in Cordoba city, but this hypothesis has not been properly tested. In this study, PM<sub>2.5</sub> samples were collected at 10 sampling sites in Córdoba city, Argentina. These sites were classified in 3 categories according to their vehicular flow (Slow, Medium and Fluid), and the concentration of Polycyclic Aromatic Hydrocarbons (PAHs) was determined. In addition, values of O<sub>3</sub>, NO<sub>2</sub>, SO<sub>2</sub>, HCHO and CO, were obtained from the Copernicus Sentinel-5P satellite and atmospheric variables from NASA's MERRA-2 service. The results indicate that the highest levels of PAHs and NO<sub>2</sub> were found in the Slow traffic category. In contrast, PM<sub>2.5</sub> concentration showed the highest values in Fluid traffic category, emphasizing the importance of determining the composition of particles to understand their hazardousness. A similar behavior was observed between PAHs and NO<sub>2</sub>, identifying traffic as the primary source of these pollutants. Furthermore, the study estimated additional cases of lung cancer associated with population exposure using the Toxic Equivalent Concentration for Benzo[a]Pyrene (TECB(a)P) index employing AirQ+ software (WHO). According to Cordoba's regulations, the allowed value of B[a]P in the air was exceeded several times and the highest risk values of TECB(a)P were found in the Slow traffic category. Moreover, outliers' values were observed for NO<sub>2</sub> and PM<sub>2.5</sub> coinciding with a nearby forest fire event, highlighting the fact that these pollutants are reliable biomass burning markers. In conclusion, different levels of vehicular flow would have a different impact on PM<sub>2.5</sub>, PAHs, and gaseous pollutants concentrations, where Slow flow category would be the most dangerous. This emphasizes the need for thorough traffic regulation, not only because of the levels of emitted pollutants, but also due to their influence on human health.

## 26.P-Mo-136

### Biological Response to Air Particulate Matter From Different Sources: An *In Vitro* Lung Cell Model.

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#### Abstract

Although it is well known that air pollution (gases and particulate matter-PM) compromises respiratory health, epidemiological and clinical studies show different impacts depending on the pollutant involved. Acute inhalation studies in humans have shown that particles of industrial origin (Residual Oil Fly Ash-ROFA) are more aggressive than those of vehicular origin (Diesel Exhaust Particles-DEP). Particles from natural origin such as volcanoes also affect the exposed population health. Studying *in vitro* models helps to understand cellular mechanisms underlying the respiratory clinical picture related to PM exposure. In this study we evaluated the impact of vehicular (DEP), industrial (ROFA) and natural (Puyehue volcano-Pv), three PM from different sources of emission with dissimilar physicochemical characteristics on two human cell lines: human lung epithelial cells (Calu-3 ATCC® Cat HTB55) and monocytes differentiated to Macrophages (THP1 ATCC® Cat TIB-202). All PM were morphochemically characterized by Scanning Electron Microscopy (SEM) coupled to X-ray Dispersion Spectroscopy (EDX). Both cell lines were exposed to DEP, ROFA or PV at 1, 5, 10, 50 and 100  $\mu\text{g/ml}$  for 24h. Cell viability was assayed by a colorimetric method (MTS-CellTiter 96®, Promega) and the inflammatory response through the release of IL-6 (Human IL-6 ELISA Set, BD Biosciences). Morphochemical analysis revealed that DEP is composed of small homogeneous spherical ultrafine particles with no metallic traces while ROFA and Pv, proved to be heterogenous both in size and shape. ROFA EDX showed 90% of inorganic material rich in C, O, S, V, Na, Si, Al and Ca while Pv showed 70% Si. Calu-3 treatment with DEP, ROFA or PV had no effect on cell viability whereas with ROFA, THP-1 showed a decrease in cell viability. Regarding the inflammatory response, only ROFA induced IL-6 release in a dose-response manner on cultured Calu-3 cells while none of the PM assayed on THP1 triggered cytokine secretion. Consideration must be given to the fact that variations in cellular response could be attributed to PM intrinsic physical-chemical properties as well as to exposure times. These *in vitro* models employing epithelial and phagocytic lung cells could contribute to a better understanding of environmental pollution effects on the respiratory system.

## 26.P-Mo-137

### Buenos Aires Wetland Particulate Matter Wildfire Modifies Rat and Human Immune Cell Function In Vitro

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#### Abstract

Natural landscape fires maintain the ecological health of the land, yet adverse health effects associated with exposure to emissions from wildfire (WF) generate public health concerns. Climate change is a key factor in increasing the frequency and severity of WF. Numerous studies found a positive association between exposure to smoke or WF particulate matter (PM) with increased risk of respiratory diseases. Ultrafine WF-PM can penetrate deep into the lungs altering cell function. Herein, we investigated Buenos Aires WF-PM morphochemical characteristics, and evaluated on cultured primary rat alveolar macrophages (rAMs) and human monocytic cells (THP-1) differentiated to macrophages, its cytotoxicity and inflammatory response. WF-PM was characterized by scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX) and X-ray powder diffraction (XPD). Rat AMs and THP-1 cells were grown at a density of 125.000 cells/ml in RPMI-1640 medium supplemented with 10% FBS and antibiotics. THP-1 cell were differentiated to macrophages with PMA (10ng/ml) during 24hs. THP-1 and rAMs were exposed for 24h to 10-500  $\mu\text{g/ml}$  WF-PM. Cell metabolism was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) test. Pro-inflammatory cytokines IL-6 and TNF $\alpha$  were determined using specific enzyme-linked immunosorbent assay. SEM and EDX of WF-PM showed a heterogenous morphology, rich in C, O and Si. Low contents of Na, K, Ca, Mg and Cl were also detected. XPD analysis mainly showed the presence of amorphous compounds, possibly SiO<sub>2</sub> and carbon. In addition, some low-intensity Bragg peaks were observed, that can be attributed to quartz (SiO<sub>2</sub>), calcite (CaCO<sub>3</sub>), halite (NaCl) and sylvite (KCl). We found a high content of Si/dry weight (11.50  $\pm$  0.89 %) possibly due to the large number of burnt grasslands. Both cell types elicited a dose-response decrease ( $p < 0.05$ ) in cell viability with no IL-6 production. TNF $\alpha$  pro-inflammatory cytokine level increased in rAM ( $p < 0.05$ ). We conclude that in both phagocytic cells, regardless the specie, Buenos Aires WF-PM induces functional alterations that could compromise the exposed population immune response.

## 26.P-Mo-138

### Quantitative Microbiological Risk Assessment of Human Mastadenovirus in Solid Waste Workers

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#### Abstract

Municipal solid waste (MSW) workers involved in the waste collection are exposed to different occupational risks. Quantitative microbiological risk assessment (QMRA) estimates the probability of infection and/or disease to apply in risk interventions and establish risk communication for decision-making in health protection. This study aimed to evaluate the exposure of MSW workers to human mastadenovirus (HAdV) by inadvertent ingestion of truck leachate to estimate the risk of developing gastroenteritis (GI). From March to December 2019, 9 samples of fresh truck leachate were collected from the solid waste truck basin of a Waste Transfer Station located in Rio de Janeiro, Brazil. Samples were ultracentrifuged and inoculated into A549 cell line to determine HAdV infectivity, and results expressed as 50% tissue culture infecting dose per milliliter (TCID<sub>50</sub> ml<sup>-1</sup>). The QMRA approach was performed to estimate the probability of developing GI due to inadvertent oral ingestion of truck leachate by two different mechanisms: direct splash into the oral cavity or by hand-to-mouth contact (with and without gloves). HAdV infectivity ranged from 17 to 667 TCID<sub>50</sub> ml<sup>-1</sup>, and these concentrations were used in the QMRA. In hand-mouth contact, based on a hypothetical model in which workers did not use gloves as personal protective equipment (PPE), there was a higher probability of developing GI (risk of 67%). When compared to the model where workers wore gloves, there was a decrease in probability to 33%. On the other hand, by the splash route, the probability of developing GI was 58%. The results of the risk analysis evidenced the two routes, hand-to-mouth contact and splash route, as relevant for the risk analysis, with a high risk for workers who did not use gloves and the importance of continuing training on the correct use of PPE. This is the cutting-edge study to reveal infectious HAdV in truck leachate, indicating a potential occupational risk for MSW workers and reinforcing the importance of PPE to health protection.

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26.P-Mo-139

## Occupational Exposure to Pesticides and Poor Prognosis Breast Cancer in Brazilian Women: Epidemiological Data and Molecular Mechanisms Associated

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### Abstract

The participation of women in the agricultural chain is poorly discussed. In countries whose economy is based on agriculture, female rural workers are at constant risk of developing chronic pathologies, such as cancer, due to frequent environmental exposures to harmful substances such as pesticides. Here we characterized how pesticides contaminate women during their occupational exposure, their impact on breast cancer (BC) aggressiveness profile, and the molecular mechanisms enrolled. The study was conducted in Paraná state Southwest, a Brazilian landscape characterized by intense use of pesticides and family-based agriculture. We found that this area has higher diagnoses and mortality than Brazilian mean rates and a pesticide trade reaching six times more than the national average. Also, we identified that the main route of contamination of such women is during equipment decontamination and clothes washing. After these procedures, most investigated BC women had positive urine for glyphosate, atrazine, or 2,4-D. Analysis of non-tumoral mammary tissue obtained from women without BC exposed to pesticides showed levels of mediators enrolled in oncogenesis as PPAR- $\gamma$  and TNF- $\alpha$  compared to samples from unexposed women. We also investigated the impact of pesticide exposure on systemic and tumor profiles in a subset of BC patients characterized by poor prognosis. Impaired systemic immune responses (reduced interleukins 1 $\beta$ , 12, and 17), redox disbalance, and high expression of tumor-promoting molecules (TGF- $\beta$ 1 and CTLA-4) were found only in the BC-exposed patients. These findings suggest that occupational exposure to pesticides represents a real risk for developing aggressive BC, associated with a poor prognosis signature linked to immune response impairment. Considering the severity of our findings, we started an educative program aiming to aware rural women about pesticide exposure-related risks.

## 26.P-Mo-140

### Assessment of Drinking Groundwater Quality and Pesticide Pollution in Avia Terai, Chaco Province, Argentina

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#### Abstract

The occurrence of pesticides in drinking water is an issue of great concern in rural populations surrounded by agricultural areas. In Chaco Province (Argentina), around 40% of the population does not have access to a drinking water distribution system. Groundwater is the main source of drinking water and is supplied by runoff water. Different factors could affect the presence of these compounds in groundwater, such as drift, leaching, or runoff. Additionally, arsenic is ubiquitous in the environment in these areas. The aim of this study was to monitor the drinking water quality in a rural population of Chaco Province, specifically Avia Terai. For this purpose, the different sources of drinking water for the population were surveyed through a socio-environmental survey. Selected sampling sites included the four public boreholes and two private boreholes that sell water in Avia Terai. Two sampling events were carried out during 2017-2018: one in the pre-sowing period and another in the post-sowing period. Physicochemical determinations, heavy metal and arsenic, and pesticides analyses were performed on these samples. Regarding the results obtained from the two samplings of the public boreholes (S1, S2, S4 and S8), three of the four boreholes surveyed exceeded, in some case, the limits of the of the Argentine Food Code (CAA) for drinking water, showing high levels of parameters such as sulfate, chloride, hardness, or turbidity. In addition, some pesticides were identified in these boreholes, including glyphosate and DEET in S1, chlorpyrifos, paraquat, and glyphosate in S2, and glyphosate in S4. Moreover, all four boreholes showed arsenic (As) concentrations above 10 ppb, which is the new CAA limit recommended by the World Health Organization (WHO), and two of them (S1 and S2) even exceeded 50 ppb in one of the sampling periods. High concentrations of aluminum (Al), iron (Fe) and chromium (Cr) were also determined in S2. The private boreholes that sell water also exhibited high levels of As (greater than 50 ppb in both sampling periods) and presence of some pesticides. Based on these results, it is evident that the quality of groundwater as a suitable drinking water source in Avia Terai is not guaranteed. Therefore, there is a critical need for regular monitoring and provision of alternative water sources for the inhabitants.

## 26.P-Mo-141

### Individuals' Perception on the Use of Chemical Products in Gardening and Pest Control

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#### Abstract

The personal perception of the use of pesticides and other substances for gardening and pest control in domestic environments is often inadequate due to individuals' lack of knowledge about these products. Purchasing can now be considered a risky activity, both for humans and the environment. This study aimed to understand the perception and motivation behind the use of pesticides and products for home gardening, as well as to understand the existence of an awareness focused on self-protection and risk prevention. A questionnaire was designed and applied to 38 individuals who voluntarily participated in the study. The instrument aimed to determine if the participant had purchased any product, the name of the product, the type of product, how it was applied, the branch of the company where it was purchased, how it was acquired, if they read the label/indications before purchasing, the benefits, harms, and what was most important in purchasing the product, and if aspects such as low price, easy application, effectiveness, risks to plants/animals, the environment, health, accidents, and the need for personal protective equipment (PPE) were relevant or not. Among the 38 participants interviewed, approximately 63.2% had already acquired some chemical product for domestic use, with fertilizers/fertilizers being the most purchased (54.2%), followed by insecticides/larvicides (41.7%). Regarding the method of use, 45.8% of the participants diluted the product to apply it, and 75% of the items were purchased in supermarkets or agricultural stores. An aggravating fact is that 58.3% of the people responded that they do not read the labels and, therefore, do not have correct information about the item purchased, its indications, and potential risks. Additionally, 58.3% of the participants considered the benefits of the products to be the most relevant aspect when making their decision, even though there are associated harms. On the other hand, the risk to plants/animals, human health, accidents, and environmental risk only affected the choice of 62.5%, 54.2%, 50%, and 41.7% of the participants, respectively. Our findings show that pesticides and other chemical products have a significant commercialization, at the same time that people's perception proves to be inadequate. We highlight the risks associated with acquiring these substances without proper knowledge, increasing the risk of accidents, suicide attempts and improper disposal of packaging.



## 26.P-Mo-142

### Rat Liver Mitochondrial Toxicity of Diuron and Its Metabolites

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#### Abstract

Diuron, a herbicide derived from urea, can induce urinary bladder urothelial tumors in rodents. Therefore, the U.S. Environmental Protection Agency (USEPA) considers it a “known/likely” human carcinogen. At our laboratory, successive studies on the Diuron carcinogenic mode of action (MoA) have indicated that this herbicide is cytotoxic to the urinary bladder urothelium. Continuous provision of relatively high Diuron doses induces urothelial necrosis, regenerative hyperplasia and, in the long term, tumors. Thus, Diuron and its metabolites have been hypothesized to exert toxicity by damaging mitochondria (mitotoxicity). We have developed studies to evaluate how Diuron and its metabolites influence mitochondria isolated from the urothelium and the liver, the organ where most Diuron is biotransformed. Here, we present and discuss data regarding mitochondria isolated from Wistar rat liver exposed to Diuron or its metabolites 3,4-dichlorophenylurea (DCPMU) or 3,4-dichloroaniline (DCA) at concentrations ranging from 0.5 to 500  $\mu$ M in vitro. At 100 and 500  $\mu$ M, the tested chemicals uncoupled oxidative phosphorylation, as evidenced by mitochondrial membrane potential dissipation and basal oxygen consumption. At 500  $\mu$ M, DCA caused mitochondrial swelling, a morphofunctional indicator of severe organelle damage. These results indicate that Diuron and its metabolites DCA and DCPMU should also be classified as mitotoxic to liver cells, as judged from the severe mitochondrial dysfunction they cause

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# Session 27: Advances and Challenges in the Analysis of Toxicity and Environmental Data: Statistics, Models, Databases, Index, and Risk Assessment

27.P-We-071

## Pesticides and Sugarcane Vinasse Environmental Levels Effects on Benthic Macroinvertebrates: Assessment of Ecological Indices, Functional Traits and Taxonomic Diversity

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### Abstract

Pesticides reach aquatic ecosystems and cause negative impacts on several non-target organisms. Sugarcane is one of the main monocultures in Brazil, and the herbicide 2,4-D and the insecticide fipronil stand out as one of its main active ingredients used. In addition, vinasse stands out being extensively used in this plantation. These compounds occurring simultaneously in the aquatic environment can potentiate the deleterious effects on organisms. Thus, the objective of this study was to evaluate the composition, abundance and functional diversity of the benthic macroinvertebrate community, as well as its ability to reestablish itself in the face of environmental contamination by the pesticides Regent® 800WG (active ingredient - i.a. fipronil) (F) and DMA® 806BR (a.i. 2,4-D) (D) and vinasse (V), alone and in mixtures: of pesticides - M and of the three contaminants - MV. The study was conducted using outdoor mesocosms. The macroinvertebrate community was monitored through colonization structures, and the effects of contaminants were evaluated over the exposure time in 1, 7, 14, 28, 75 to 150 days. Environmental contamination was simulated with the application of recommended doses. Over time, changes were observed in the composition of the community. The family Chironomidae and subclass Oligochaeta were more sensitive to the treatment V and MV, while individuals from the families Phoridae, Ephydriidae and Sciomyzidae were occasionally found in this treatment. The insects were sensitive to treatments F and M, disappearing in these mesocosms after contamination, reappearing only after 75 days. Regarding functional food groups, the macroinvertebrate community was characterized by the highest proportion of collectors-gatherers. Predators, composed of insects, disappeared in treatments F and M. On the other hand, in V and MV, the disappearance of collectors-gatherers was observed, which later reestablished. In this realistic scenario of contamination, ecosystem functioning can be affected, given the role of benthic macroinvertebrates in linking organic matter and nutritional resources from lower to higher levels of the food chain.

## 27.P-We-072

### Environmental Data and Machine Learning: Case Study of Honey Bees and Aquatic Macroinvertebrates as Pesticide Residues Biomonitor

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#### Abstract

Pesticide residues are often detected in different environmental matrixes all over the world. Particularly, the ubiquitous presence of pesticide residues in honey bees and beehive products (wax, pollen, honey) was reported during the past decade. In Uruguay, honey bees and their beehives have been assessed as pesticide residues biomonitor of agroecosystems since 2014. Pesticide residue analysis in surface waters is challenging: representative sampling and very low detection limits should be accomplished. To evaluate the aquatic environment, during 2019-2020 three selected aquatic macroinvertebrates (AM): Corbicula, Aegla and Odonate nymphs were also assessed as biomonitor of pesticide residues. Beehive products and AM were sampled in six opportunities in eight sites of the Queguay river basin. Multiresidue pesticide analysis methodologies using LC-MS/MS and GC-MS/MS were validated including relevant pesticides for these agroecosystems. Acceptable recoveries (70-120 %) and RSDs  $\leq 20$  % were obtained for 80 to 100 compounds, depending on the matrix. Limits of quantitation ranged 0.0001 mg kg<sup>-1</sup> - 0.05 mg kg<sup>-1</sup>. A total of 287 samples were analyzed, and 46 different compounds were detected. Pesticides profile found in the apiaries and AM reflected the land use within its ecosystem. But still the question if and/or how are these findings affect honey bees and the environment is unsolved. Aiming to contribute to answer, machine learning prediction models have been explored. The models evaluated are Support Vector Machines (SVM) and Neural Networks (NN). These are supervised classification models which make predictions based on the variables defined and need to be trained and validated. The two classes were defined as "affected" and "non-affected" landscapes based on the pesticide load according to their land use. The variables used were biological (such as colony strength for apiaries), pesticide number of detections and indexes constructed with hazard quotient's structure. The selection and combination of different variables allows making various models which can be compared. From the total number of samples, a set (90%) is used for training and the other to validate the models. Accurate predictions were obtained with 4 of the 8 SVM and NN models compared. These are promising results which indicate that machine learning models can help to interpret pesticide residues data obtained from biomonitor such as honey bees and aquatic macroinvertebrates.

## 27.P-We-073

### **Pesticides, Nutrients, Pharmaceuticals and Personal Care Products as Possible Drivers of *Jenynsia lineata* Biomarkers Responses: a Field Study in Tres Arroyos Basin (Buenos Aires, Argentina)**

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#### **Abstract**

Chemical pollution could lead to a biodiversity loss in aquatic ecosystems, one of the components of the current triple planetary crisis. Since organisms are exposed to chemical mixtures from different anthropic sources, assessment of effects in biota must be tackled from an integrated approach. In this sense, this study aimed on assessing biomarkers associated to neurotoxicity, oxidative damage and genotoxicity in the fish *Jenynsia lineata* in Tres Arroyos Basin (Buenos Aires, Argentina) and on understanding if the presence of pesticides, pharmaceuticals, personal care products (PPCPs) and nutrients in the water could be drivers for this effects. For this purpose, two sampling events (December/ February) were performed in five sites of the basin. Samples of surface water were collected for chemical analyses, and fish for biomarkers evaluation. Acetylcholinesterase (AChE) was evaluated in brain, malondialdehyde (MDA) in liver and gills, and micronuclei and nuclear abnormalities in blood red cells. Principal Component Analysis (PCA) biplot was performed with morphometric parameters, biomarkers responses and chemical variables ( $\Sigma$ Pesticides,  $\Sigma$ PPCPs, total nitrogen, total phosphorous). Moreover correlation among variables was tested. PCA results showed that sites associated to rural activities are explained by morphometric measures and sites related to urban pollution by chemicals' concentration and nuclear abnormalities. Significant correlations were observed between fish morphometric measures and AChE activity (positive) and MDA content in gills (negative). Among biomarkers nuclear abnormalities were correlated with AChE activity and MDA in liver ( $p < 0.05$ ). It is noteworthy that  $\Sigma$ Pesticides and  $\Sigma$ PPCPs are positively and significantly correlated with MDA in gills ( $p < 0.05$ ), highlighting the usefulness of this biomarker in gills for this type of environmental studies. Regarding relations among chemicals, both total nitrogen and phosphorus were positively correlated with  $\Sigma$ PPCPs ( $p < 0.05$ ). Hence, taking into account these results we could conclude that despite morphometric variables could be confounding factors in biomarkers response, lipid peroxidation in gills of *J. lineata* was evidently associated to contaminants presence, and nutrients. Moreover, the  $\Sigma$ PPCPs correlation observed could be useful for future studies. Finally, these results would be interpreted in a comprehensive analysis with particular chemical identification of pesticides and PPCPS.

## 27.P-We-074

### Fourier Transform Infrared (FTIR) Spectroscopy and Multivariate Analysis to Identify Fish Exposure to Synthetic Pyrethroid

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#### Abstract

Agro-industrial production systems employ a wide array of natural or synthetic chemical compounds to assist in controlling and preventing pests and diseases. In this context, pyrethroids assume a prominent position as they are the most commercialized insecticide compounds in the world. Of the currently available compounds, lambda-cyhalothrin (LCT), a synthetic insecticide class II for fish, is widely used in soybean, cotton, and corn crops and in the urban environment. Elevated LCT concentrations have been detected in Paraguay, Argentina, and Brazil rivers. In the present assay, *Astyanax lacustris* (n=10) were exposed to 1.5µg/L for 12 days in a static system. Immediately after euthanasia, a sample (1mm) of the ventral fin was collected, dried (48h/50°C), and evaluated by FTIR spectroscopy (Perkin-Elmer, model spectrum 100). The patterns obtained were then compared with a control group (n=10). The fish's FTIR spectrum was collected using the Attenuated Total Reflection Fourier Transform Infrared (FTIR-ATR) in a range of 4000 to 600 cm<sup>-1</sup>. The data analysis was performed by Principal Component Analysis (PCA), and discriminative tests, such as Support Vector Machine (SVM) and k-Nearest Neighbor (K-NN), were prepared for sample classification. Our results showed that it is possible to differentiate the exposed fish from the control group from discriminative tests with 90% accuracy in the 3200 – 2700 cm<sup>-1</sup> range. The results suggest that this discrimination is mainly assigned to CH<sub>3</sub> vibrational bands related to protein molecules. Although our data are still partials, our results demonstrate that the FTIR applicability may be a valuable tool for analyzing fish samples under suspicion of environmental pyrethroid contamination.

## 27.P-We-075

# Concentration of Heavy Metals in Surface Water and Sediments in the Middle-Lower Ctlamochita River Basin, Córdoba, Argentina: Geoaccumulation Index and Human Health Risk Assessment

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### Abstract

Surface water is one of the most important resources in Córdoba province (Argentina) and receives a complex mixture of natural and anthropogenic contaminants. This study aimed to analyze the concentrations of As, Cd, Cr, Hg and Pb in surface water and sediments in the middle-lower basin Ctlamochita river basin, Córdoba; estimate the geoaccumulation index of elements in sediments; and assess the human health risk associated with water consumption. Water and sediment samples were collected at 14 sites in the Ctlamochita river during November 2021 and elements concentrations were determined by inductively coupled plasma-mass spectrometry. The geoaccumulation index (I<sub>geo</sub>) of elements in sediments was estimated. The non-carcinogenic human health risk related to water consumption was assessed by estimating the hazard quotient (HQ) and hazard index (HI). The elements with the highest concentrations were Pb and As in surface water (6.78 to 14.7 µg/L and 1.65 to 38.8 µg/L, respectively) and Pb, Cr and As in sediments (0.73 to 13.2 µg/g; 0.28 to 1.57 µg/g and 0.16 to 3.20 µg/g, respectively). The I<sub>geo</sub> showed high pollution levels for Cr and Pb classifying from "heavily polluted" to "extreme pollution" at monitoring sites. As presented the greatest variability ranging from "unpolluted" to "heavily polluted". Both, Cd and Hg, showed I<sub>geo</sub> values lower than 0 in all sites indicating "non-polluted" sediments. The HQ and HI indices, for all elements and sites (except for HQAs in three sites and HI in four sites) suggest no risk for the population (HQ and HI < 1). Specifically, HQAs was 1.53 in Bell Ville city, 2.28 for Bell Ville rural drainage channel and 4.58 for San Marcos Sud town. The HI (total risk) was higher than 1 in Morrison town and in the sites mentioned above, suggesting a non-carcinogenic risk for the water consumption for the general population. At sites where HI was higher than 1, the major HQ index contributors was As. This study area is one of the largest regions in the world with high As concentrations in groundwater, however this metalloid may occur in surface water as a consequence of both natural contamination and anthropogenic activities. The assessment of risks consumption associated with levels of heavy metals in surface water of Córdoba is essential for the agencies that control hydric resources and health surveillance to develop guidelines on safe consumption, strategies for removal methods, and implementation of long-term monitoring programs.

## A Novel Algorithm Based on Random Forest to Improve Prediction of Imbalanced Data Sets Applied to Harmful Algal Blooms

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### Abstract

Predicting the occurrence and intensity of harmful algal blooms based on easy to measure environmental data is a hard task. Most of the data from monitoring programs have a large number of cases in which there is no bloom alert (green status), some cases in which there is a moderate alert (yellow status) and only a minor fraction of bloom alerts (red). When the proportion of classes is widely different, these data sets are termed imbalanced. Most machine learning methods fails to predict the rare class in this type of data sets. In this presentation, we present a new algorithm based on random forest that increase prediction of rare classes without losing much capacity to predict majority class. The algorithm combines sequentially multiple random forest changing the cutoff parameter to predict majority class making an informed balance of the data set. We evaluated the algorithm using synthetic data sets and two real data sets of alerts in Montevideo and Salto Grande reservoir. The algorithm improves the prediction of the red class while it does not implies a reduced capacity to predict green class.

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