



# PROGRAMME AND ABSTRACT BOOK

**SETAC 12<sup>TH</sup> YOUNG ENVIRONMENTAL SCIENTISTS MEETING**

28 AUGUST–1 SEPTEMBER 2023 | LANDAU, GERMANY

*"SCIENCE THROUGH CRISES"*

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## Sponsors

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# Welcome from SETAC Europe SAC

Dear Students of SETAC,

I am honoured to write these opening lines for this year's Young Environmental Scientists Meeting. Time flies fast, and though we have long been looking forward to hosting the YES meeting here in Landau in der Pfalz, it feels incredible that the week has finally arrived. Together with the Local Organising Committee, past and current SAC members, the invaluable SETAC staff, motivated trainers and speakers, uncounted helping hands, and, of course, the generous financial support from many sponsors, we bring you an exciting week of conference experiences.



But most importantly, having received a record number of over 150 submitted abstracts this year, without participants, meaning *you*, this conference would be pointless. The YES meeting is about connecting students who are at the beginning of their scientific careers, kick-starting a broad network, discovering new opportunities for further education, meeting future colleagues, and collaborating while making new friends from all over the globe. A conference *organised by students – for students!*

With its slogan, *Environmental Quality Through Science®*, SETAC has a long-standing tradition of fostering understanding and communication alongside the comprehensive mitigation of environmental issues in a global community and across every sector of our society. Promotion of the young membership segment is key to SETAC. I encourage every one of you to watch out for opportunities to join and become engaged in the society, its committees, interest groups, or regional and global student networks, like the Student Advisory Council. There are plenty of opportunities that can shape and contribute to SETAC, opportunities that will provide unique experiences.

After two challenging years of a global pandemic, this YES meeting might be, for many of you, the first in-person scientific conference; for others, the very first conference ever attended. Take a plunge and make the most of this invaluable experience by connecting to others. Throughout the years to come, there is a significant chance that many of you will meet each other again and again. The world of environmental science is a small place, and I firmly believe that scientific communities will continue to cross borders, and value cooperation over competition, while supporting free, equal and inclusive access to education and information to tackle and overcome the multiple crises we all are and will be confronted with.

Having said this, I wish all of you a wonderful meeting here in Landau. Don't be shy; talk to each other and immerse into the YES meeting's unique by-students-for-students atmosphere with other young environmental scientists at the same or a similar stage of their early career. We are all equally curious and passionate about science! Most importantly, have fun, and enjoy Landau at the best time of the year!

Cheers, and wishing you all the best,

## **Markus Schmitz M.Sc.**

*Chair of the SETAC Europe Student Advisory Council  
Student representative at SETAC Europe Council*

# Programme Committee

## Scientific Committee

Markus Schmitz (SAC Chair), *Goethe University Frankfurt am Main, Germany*

Andreas Eriksson (SAC Outgoing Chair), *Jyväskylä University, Finland & University of Lethbridge, Canada*

Frederik Meyer, *RPTU Kaiserslautern-Landau, Germany*

Tobias Schmitt, *RPTU Kaiserslautern-Landau, Germany*

Sophie Oster, *RPTU Kaiserslautern-Landau, Germany*

Huiting Liu, *Karlsruhe Institute of Technology, Germany*

Marina Veseli, *University of Zagreb, Croatia*

Bidemi Green, *University of Portsmouth, United Kingdom*

Kristi Weighman, *Ghent University, Belgium*

Carolina Rocha, *University of Coimbra, Portugal*

Walter Cristiano, *University of Torino and Italian Institute of Health, Italy*

## Local Organising Committee

Frederik Meyer (Chair), *RPTU Kaiserslautern-Landau, Germany*

Tobias Schmitt (Co-chair), *RPTU Kaiserslautern-Landau, Germany*

Sophie Oster (Co-chair), *RPTU Kaiserslautern-Landau, Germany*

Markus Schmitz, *Goethe University Frankfurt am Main, Germany*

Bianca Dechent, *Goethe University Frankfurt am Main, Germany*

Micha Wherli, *University of Applied Sciences and Arts, Northwestern Switzerland*

Marius Schmitt, *Ghent University, Belgium*

# About the Student Advisory Council

The Student Advisory Council (SAC) of SETAC Europe was established in 2006 during the annual meeting in The Hague. The SETAC Europe Student Advisory Council represents the students' voice of and within SETAC and, therein, 25% of the membership within SETAC governance and decision-making. The main aim of the SAC is to improve communication among students within SETAC and to offer opportunities for students to get in contact with senior members of the Society. This includes advising the SETAC Europe Council (SEC) on student and early career interests and communicating feedback on relevant issues identified by the SEC back to the membership. Most importantly, we work on identifying the essential needs of students and early career scientists and work closely together with other councils to resolve these needs on the SETAC governance level. Last but not least, we see it as our responsibility to build the foundation to provide a step for young scientists into SETAC's broad network, to enable young environmental scientists to connect and start building their own network around the globe.



Organising the Young Environmental Scientists Meeting, aka YES Meeting, is the major outcome of this mission, next to a variety of activities organised by the SAC at SETAC annual meetings and throughout the year. We believe that these events and especially the YES meeting will be an unforgettable experience for all of you attendees, where you will meet new friends, take the first steps toward or expand your international scientific network and, finally, connect to future colleagues accompanying your early scientific career.



# About Landau and the Venue

## About Landau

Landau is located in the sunny southern Palatinate between the Rhine and the Palatinate Forest. The independent city with its 46,000 inhabitants is one of the largest wine-growing communities in Germany. The city has had a historically eventful history and is characterised by a well-preserved old town and fortifications. Today, Landau is a vibrant university town. Especially the grape harvest and the still summery temperatures attract many visitors to Landau in late summer and autumn.

## About the Venue

The iES Landau, Institute for Environmental Sciences, is a flagship of the Landau campus (approx. 8,300 students) of the RPTU (University of Kaiserslautern-Landau). One research focus of the iES Landau is on anthropogenic stressors in coupled ecosystems.

Transition areas between ecosystems are very important (e.g. high biodiversity, “hotspots” for biogeochemical processes, provision of ecosystem services), but at the same time are considered particularly vulnerable to environmental change. The aim of the iES Landau is to investigate these interactions in cooperation between the biological, chemical, physical, geoscientific and social science-oriented research groups.

### **Institute for Environmental Sciences**

RPTU Kaiserslautern-Landau  
Fortstrasse 7  
76829 Landau, Germany



# Welcome to Landau

Welcome to the 2023 YES Meeting in Landau,

Environmental pollution, biodiversity, water, energy and climate crisis! These are among the topics you are about to discuss during the SETAC 12th Young Environmental Scientists Meeting in Landau. In my view, the YES meeting series is one of the key scientific initiatives in the area of environmental sciences. It is so important that you, as young scientists and as future scientific leaders in academia, industry or government, put your heads together critically and come up with solutions to the multitude of problems former generations have left you with.



As an ecotoxicologist, you may think: “Luckily there is pollution cause otherwise I would not know what to do!” I can assure you: The problem of environmental chemicals occurring all over the world in every single ecosystem, whether it is close or even very far away from any anthropogenic source, will remain. It will, with regard to some chemicals, even persist forever.

YOU are the future generation to come up with solutions (other than dilution) for these problems and, even more importantly, to contribute to transforming the global society into one dealing with chemicals in a more responsible manner than has been dealt with in the past. Action is required NOW! Therefore, it is great news that many of you convene again at a YES Meeting to discuss this issue and develop strategies for the future! I sincerely thank you for this and wish you all the best for your future careers. This is your meeting, make the most out of it!

## **Ralf Schulz**

*RPTU Kaiserslautern-Landau*

# About SETAC

The Society of Environmental Toxicology and Chemistry (SETAC), with offices in North America and Europe, is a not-for-profit, worldwide professional organisation composed of more than 16,000 researchers, students, and expert practitioners from universities, institutions, governmental authorities, businesses, and nongovernmental organisations as well as 85 partner organisations 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 programme
- Organise 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, visit [setac.org](http://setac.org) or contact us at [setac@setac.org](mailto:setac@setac.org).



# SETAC Global Partners

Thank you to the SETAC Global Partners for helping ensure our goal of Environmental Quality Through Science®.

Interested in becoming a SETAC Partner? Visit [www.setac.org](http://www.setac.org) for more information or contact Barbara Koelman at [barbara.koelman@setac.org](mailto:barbara.koelman@setac.org).



# Practical Info

## Badges

Badges must be worn to gain access to the meeting.

## Emergencies and First Aid

If you need medical attention, ask any of the local volunteers. For emergencies, call 112.

## Wifi Information

Wifi: yesmeeting

Password: YesMeeting/2023

## SETAC Policies

SETAC provides open, safe forums for the purpose of exchanging ideas and information on the study, analysis and solution of environmental problems, the management and regulation of natural resources, promotion of scientific research and the development of strong environmental education.

Attendees of SETAC meetings are expected to adhere to all SETAC policies, including SETAC Participant Policies.

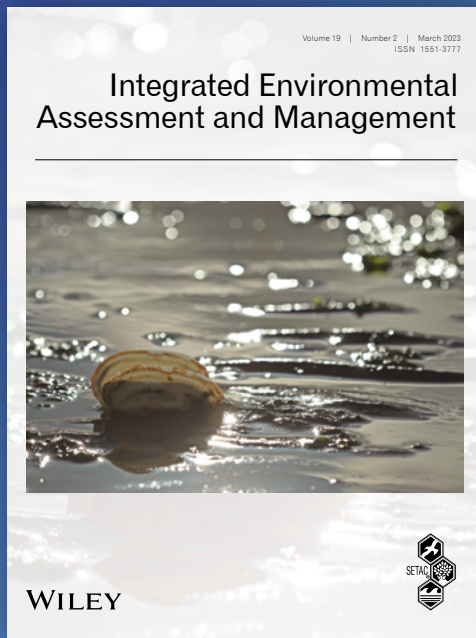
Learn more at [www.setac.org/learn-about-setac/policies.html](http://www.setac.org/learn-about-setac/policies.html).



## Conduct

Participants in SETAC activities are expected to adhere to the highest standards of integrity and professionalism and comply with the SETAC Code of Conduct. Attendees are reminded to observe SETAC's principles and values and to maintain an atmosphere of civil and constructive scientific exchange.

# Publish with **SETAC Journals**



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on open access fees!

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# Programme Overview

	Monday	Tuesday	Wednesday
08:30			
08:45			
09:00			
09:15			
09:30			
09:45			
10:00			
10:15		Workshops	
10:30			Poster Sessions & Coffee Break
10:45			
11:00			
11:15			Career talk
11:30			
11:45			
12:00		Lunch Break	Lunch Break
12:15			
12:30			
12:45			
13:00		Sessions (2 & 6)	Sessions (1 & 7)
13:15			
13:30			
13:45			
14:00			
14:15		Poster Sessions & Coffee Break	Poster Sessions & Coffee Break
14:30			
14:45			
15:00		Sessions (2 & 6)	Sessions (1 & 5)
15:15			
15:30			
15:45		Poster Sessions & Coffee Break	Poster Sessions & Coffee Break
16:00			
16:15		Expert Talk	Expert Talk
16:30			
16:45			
17:00	Opening Ceremony & Opening Keynote		
17:15		SAC Café	Fitness (Group I)
17:30			
17:45			
18:00			Fitness (Group II)
18:15			
18:30			
18:45			
19:00			
19:15			
19:30			
19:45			
20:00		Karaoke Schiller Pub	Fitness (Group III)
20:15			
20:30	Pub Quiz		
20:45			
21:00			
21:15			
21:30		Party	
21:45			
22:00			

# Programme Overview

	Thursday	Friday
08:30		
08:45		
09:00	Sessions (2 & 3)	Career talk
09:15		
09:30		
09:45		
10:00		
10:15	Poster Sessions & Coffee Break	Sessions (4 & 5)
10:30		
10:45		
11:00	Career talk	Lunch Break
11:15		
11:30		
11:45	Lunch Break	Closing Ceremony & Closing Keynote
12:00		
12:15		
12:30		
12:45	Sessions (2 & 3)	
13:00		
13:15		
13:30		
13:45		
14:00	Poster Sessions & Coffee Break	
14:15		
14:30	Expert Talk	
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15:15	JKI Excursion/Zoo	
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## Sessions

1. Analytical Chemistry
2. Aquatic Ecotoxicology
3. Computational Ecotoxicology & Alternative to Animal Testing
4. Mechanistic Ecotoxicology
5. Microplastics
6. One Health
7. Terrestrial Ecotoxicology

# NOMINATE SOMEONE FOR THE SETAC/ICA CHRIS LEE AWARD!



The **International Copper Association (ICA) Chris Lee Award for Metals Research** is sponsored by ICA and was initiated by SETAC to recognize the leadership and technical contributions of the late Chris Lee.

The award provides up to US\$ 5,000 to a **graduate student or recent graduate** who has focused on **research related to the fate and/or effects of metals in the environment**. A key consideration for this award is that the recipient will continue research on metals-related environmental issues, and funding will be provided by ICA for the recipient's ongoing research.

Know someone who deserves this award?

**Stay tuned for applications to open in September!**



Learn more about the awards programme and view this year's winners.

Time (CEST)	Programme	Location
17:00-19:00	Welcome address by former and outgoing SETAC President Mirco Bundschuh and the SAC/LOC, followed by the opening plenary: <b>Henner Hollert</b>	Audimax
19:00-22:00	Pub Quiz	C I Conference Room

## Plenary Speaker

### Environmental Impacts of Chemicals – Beyond the Planetary Guardrails?

#### Henner Hollert

*Goethe University Frankfurt am Main, Germany*

Potent stressors such as the degradation of habitats, the introduction of non-indigenous species, and climate change pose an unprecedented threat to the integrity of ecosystems and biodiversity on our planet. In the last decades, the chemical risks have mounted alarmingly following an unabated increase of the global production and release of chemicals into the environment. Last year, Persson and colleagues quantified for the first time the planetary boundary for novel entities. The results were alarming and showed that humanity is already outside the safe operating space for novel entities (Persson et al. 2022, ES&T). Soon afterwards, scientists highlighted the potentially massive link between biodiversity loss and chemical pollution, and the fact that both phenomena have often been studied within subdisciplines but have been rarely considered jointly and across subdisciplines (Groh et al. 2022, ES&T). Consequently, a group of international scientists has recently expressed support for the demand of the International Panel on Chemical Pollution (IPCP) initiative and several countries to establish a “World Chemicals Council” (Brack et al. 2022, ESEU). The scientific-political body, conceived after the Intergovernmental Panel on Climate Change (IPCC) and the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES), is to act as a global body to assess the consequences and promote management and regulatory action on chemicals and waste. At the fifth UN Environment Assembly in Nairobi on 3 March 2022, a resolution was passed to launch the “World Chemicals Council”.



In this keynote talk, the impact of chemicals from the perspective of planetary boundaries and the biodiversity crisis will be discussed. Although the detrimental effects of chemical pollution on humans and the environment have long been recognized, many problems have been identified only by chance and after the pollution events happened. A comprehensive understanding of the interactions between chemical pollution and biodiversity decline is needed to achieve a predictive assessment of the interactions between these components of ecosystem degradation and derive options for action and levers for transformation. This talk will also introduce the RobustNature excellence initiative. This scientific consortium led by the Goethe University Frankfurt has been established in close collaboration with Germany-wide (the Rhein-Main-Universities, Senckenberg (SGN), the Helmholtz Centre for Environmental Research (UFZ), ISOE, SAFE FhG-IME and RWTH Aachen University) as well as selected international partners (UoS Canada, ETHZ and Stockholm University) to investigate the impact of chemical pollution and more generally novel entities on the decline of biodiversity from an inter- and transdisciplinary perspective.

# Schedule | Tuesday, 29 August

Time (CEST)	Programme	Location
8:30–12:00	Workshops	To be announced
12:00–13:00	Lunch Break	CV
13:00–14:00	Session: Aquatic Ecotoxicology Session: One Health	B I 001 C I Conference Room
14:00–14:45	Poster Session & Coffee Break	CV
14:45–15:30	Session: Aquatic Ecotoxicology Session: One Health	B I 001 C I Conference Room
15:30–16:15	Poster Session & Coffee Break	CV
16:15–17:00	Expert Talk: <b>Patrick Baudy-Groh</b>	B III 040
17:45–18:15	SAC Café	To be announced
19:45–20:45	Karaoke Schiller Pub	Schiller Pub, An 44 20, 76829 Landau
21:00–5:00	Student Party	LOGO, Xylanderstraße 2, 76829 Landau



Society of Environmental Toxicology and Chemistry

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## Expert Talk

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### Aquatic Toxicity Testing of 'Difficult Test Chemicals' for Regulatory Purposes

#### Patrick Baudy-Groh

*BASF SE Ludwigshafen, Germany*



Dr. Patrick Baudy-Groh works at BASF's test facility 'Experimental Toxicology and Ecology' as ecotoxicologist. He is responsible for the scientific monitoring of ecotoxicological studies contracted to external laboratories and deals mainly with chemicals registered under REACH and are 'difficult to test'.

In his expert talk, we will delve into the challenges of testing the aquatic toxicity of 'difficult test chemicals' for regulatory purposes. We will discuss the difficulties that can arise in performing these tests, such as low solubility, degradation, adsorption or chemicals with a complex / an unknown composition (UVCBs) and talk about current approaches and methods to overcome these difficulties.

# Workshop Descriptions

## Transfer of Anthropogenic Stress From Aquatic to Terrestrial Ecosystems – Perspectives From Young Researchers

### SystemLink

The close connection between aquatic ecosystems and adjacent terrestrial land has been a long-standing paradigm of ecological research. Existing concepts, however, mainly focus on the terrestrial input into freshwater ecosystems and the consequences for aquatic life. The flux of resources and pollutants from aquatic to terrestrial systems or the role of invasive species in this context has been less studied. Recently intensified research revealed that material fluxes mediated, e.g., by emerging insects and flood events, are of high ecological relevance, particularly for the receiving ecosystem. In addition, these fluxes are increasingly affected by anthropogenic and climatic stressors, such as micropollutants, invasive species, and hydrological alterations. The workshop invites contributions from all aspects of resource and stressor transfer from aquatic to terrestrial ecosystems and the ecological responses in the terrestrial recipient ecosystem. Although the workshop particularly aims to bring young researchers (Master or PhD students) in this area of research together, we also welcome participation by researchers at advanced career steps.

## Data Science, Machine Learning, and Artificial Intelligence and Their Potential for Environmental Protection and Nature Conservation

### Jochen Zubrod

Technological progress and digitization are ever advancing, and with them the speed of creation and the volume of data that we generate. The mountain of data is growing, and entire professional fields have formed around it. These are constantly generating new infrastructures and methods to collect and analyze previously inaccessible data treasures.

“Data is the new oil.” This statement from 2017 impressively shows the (above all economic) importance that data is now ascribed to in our society. However, the development described is not only perceived positively. For example, the immense resource consumption of the digital economy and the associated disadvantages for nature and the environment lead to a negative connotation of terms such as big data. In addition, the rapid rise of artificial intelligence tools creates fears related inter alia to widespread misinformation and black box decision-making processes. Nevertheless, this progress creates enormous potentials, also in environmental protection and nature conservation. New developments enable, for example, resource-conserving production, intelligent energy management or effective monitoring of endangered species.

In this course we will...

... get an overview of terminology and professional fields in the context of collecting, processing and analyzing data.

... get to know modern approaches in data science (i.e., machine learning and artificial intelligence).

... discuss some negative aspects associated with big data and artificial intelligence.

... explore the potential of these approaches for environmental protection and nature conservation using examples.

## Build Your Digital Brand – Scientist Edition

### Pranoti Kshirsagar

As a scientist, you are more than just the papers you publish and the degrees you collect. We have put together the perfect brand-building-toolkit for scientists like You so that you can establish a solid digital presence. This session is designed to get you started with communicating science. You will learn how to get started and then make the most out of Twitter/Insta/TikTok/LinkedIn. We will take a deep dive into:

- Different SciComm platforms & formats
- Content crafting
- Feature opportunities to grow your network
- How to start your own podcast

## Stable Isotopes in Ecotoxicology – An Introduction to Concepts, Methodology and Applications

### Eric Bollinger

Stable isotope analysis has been used for decades in ecology given that it offers a wide range of information in one sweep (e.g., diet composition, niche size, and trophic position). However, its use in a chemical stress-related context is capable of improvement. Given that “ecotoxicology becomes stress ecology” (Van Straalen, 2003), basic knowledge about stable isotope ecology is key for the future ecotoxicologist. In this course you will learn fundamental concepts of stable isotope ecology, the methods used to analyze stable isotopes and its main applications.

# Tuesday Platform Presentations

## Afternoon Sessions I

### Aquatic Ecotoxicology | B I 001

- 13:00-13:15 **01 - Toxicity of Antineoplastic drug mixtures to Danio rerio larvae**  
Daniel Bruno, *University of Aveiro*
- 13:15-13:30 **42 - Extrapolation of Cytotoxic Masked Effects from Building Materials in Planar in Vitro Assays**  
Timothy Rosenberger, *Federal Institute of Hydrology*
- 13:30-13:45 **03 - Effects of a Nanomaterial and an Antineoplastic Agent in Zebrafish**  
Diana Carneiro, *CESAM & University of Aveiro*
- 13:45-14:00 **04 - Are We Making Zebra Mussels Happier by Exposing Them to Psychoactive Drugs? Study of the Sublethal Effects of Sertraline**  
Clara Baldacci, *LIEC Laboratoire Interdisciplinaire des Environnements Continentaux*

### One Health | C I Conference Room

- 13:00-13:15 **05 - Geo-Environmental Implications of the Progression of Chronic Kidney Disease of Unknown Etiology (CKDu) In the Dry Zone of Sri Lanka**  
Navodya Dhananjalee, *University of Peradeniya*
- 13:15-13:30 **06 - Profiles Of Selected Antibiotic Residues and Resistomes in Urban Aquatic Systems Of Lusaka, Zambia Using High-Throughput Quantitative PCR Analysis**  
Pius Kinoti Kairigo, *University of Jyväskylä*
- 13:30-13:45 **07 - Tire Components and Rising Temperatures Accelerate Aging Pathways and Neurodegeneration**  
Annette Limke, *IUF - Leibniz Research Institute*
- 13:45-14:00 **08 - The Influence of Bio-Based Fertilizers on the Sorption Behaviour of Pharmaceuticals in Soil**  
Yan Dong, *University of Amsterdam*

# Tuesday Platform Presentations

## Afternoon Sessions II

### One Health | C I Conference Room

- 14:45-15:00 **09 - Germination as a Carbon Reduction and Nutrient Enhancing Solution: A Life Cycle Assessment on Novel Cultivation Method for Plant-based Meat**  
Nichole Lalas, *Tokyo City University*
- 15:00-15:15 **10 - Pesticide Exposure Assessment in (Migratory) Birds**  
Aafke Saarloos, *Wageningen University*
- 15:15-15:30 Discussion

### Aquatic Ecotoxicology | B I 001

- 14:45-15:00 **12 - Does Light Pollution Affect Zebrafish Embryos Response to the Anti-neoplastic Drugs?**  
Maria Costa, *CESAM - University of Aveiro*
- 15:00-15:15 **13 - Impact of Salt of the Toxicity in Textile Dyes in Neurotoxic Endpoints in Danio rerio and Genotoxicity on Cell-Based Level**  
Jan Halaunia, *Goethe University Frankfurt*
- 15:15-15:30 **14 - Integrated Toxicological Analyses of Marine Micro- And Nanoplastic Particles Using in Vivo and in Vitro Bioassays**  
Clara Kempkens Palacios, *Goethe University Frankfurt*

# Schedule | Wednesday, 30 August

Time (CEST)	Programme	Location
10:15–11:15	Poster Session & Coffee Break	CV
11:15–12:00	Career Talk: <b>Clara Mendoza</b>	B III 040
12:00–13:00	Lunch Break	CV
13:00–14:15	Session: Terrestrial Ecotoxicology	B I 001
13:00–14:00	Session: Analytical Chemistry	C I Conference Room
14:00–14:45	Poster Session & Coffee Break	CV
14:45–15:45	Session: Microplastics	B I 001
15:00–15:45	Session: Analytical Chemistry	C I Conference Room
15:45–16:15	Poster Session & Coffee Break	CV
16:15–17:00	Expert Talk: <b>Elena Adams and Daniel Faber</b>	B III 040
17:30–20:30	Social Event: Fitness Workout (divided in 3 groups)	Max-von-Laue-Straße 8, 76829

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## Get Involved in Three Easy Steps:

1. Identify yourself as a mentor on your SETAC profile, or search the membership directory to find a mentor
2. Report to SETAC when you've found a match
3. Follow the framework outlined in the handbook

Learn more at [setac.org/career-development/mentoring](https://setac.org/career-development/mentoring)

## Career Talk

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### A gender-biased Tale of Flow and Nutrients

**Clara Mendoza**

*RPTU Kaiserslautern-Landau, Germany*



Jun.-Prof. Dr. Elisabeth Berger leads a BMBF (German Ministry for Education and Research) funded, interdisciplinary junior research group on the topic of freshwater salinization using the Draa river Basin in southern Morocco as a case study. She will talk about the perks and challenges of academic careers in interdisciplinary settings from her own experience and that of her network of transdisciplinary scholars from the Robert Bosch Academy of transformational leadership and BMBF Social-Ecological Research Groups.

## Expert Talk

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### Reducing Vertebrate Testing in Pesticide Risk Assessment

**Elena Adams and Daniel Faber**

*Bayer AG, Germany*



Dr. Elena Adams and Dr. Daniel Faber work in the Environmental Safety department of the CropScience Division of Bayer AG. Elena is an environmental risk assessment expert, specializing in terrestrial and aquatic organisms. She is on the editorial board of the SETAG IG for amphibians and reptiles which is updating the textbook for Ecotoxicology of Amphibians and Reptiles. Daniel is an experienced aquatic ecotoxicologist and the lab leader of the aquatic experimental unit specialized in aquatic vertebrate studies. In his role he takes care of animal alternatives in the context of fish testing, working in different teams at Bayer and supporting for example the SETAC IG for Animal Alternatives.

Together with their colleagues, Elena and Daniel focus on alternatives for (non-) vertebrate testing methods in the context of environmental risk assessment of plant protection products. In their shared expert talk, they will give an insight into case studies for potential refinement, reduction and replacement of vertebrate testing for both the aquatic and terrestrial vertebrate risk assessment.

# Wednesday Platform Presentations

## Afternoon Sessions I

### Terrestrial Ecotoxicology | B I 001

- 13:00-13:15 **15 - Pharmaceuticals and Personal Care Products; Ecological and Environmental Concerns for Soil Fauna**  
*Judith Ehigie, Helmholtz Centre for Env Res - UFZ*
- 13:15-13:30 **16 - Toxicity of Arsenic-Contaminated Soils to *Folsomia candida* After a Short-Term and Long-Term Exposure**  
*Kamila Kluczek, Goethe University Frankfurt & Wroclaw University of Environmental and Life Sciences*
- 13:30-13:45 **17 - Effect of Deoxynivalenol on Soil Nitrification**  
*Sven Korz, RPTU, iES Landau*
- 13:45-14:00 **18 - Fungicide Reduction Enhances Beneficial Arthropods in Grapevine**  
*Jo Reiff, RPTU Kaiserslautern-Landau*
- 14:00-14:15 **19 - Metal Transfer from Insects to Bats in a Metal-contaminated Environment**  
*Olha Timofieieva, Jagiellonian University*

### Analytical Chemistry | C I Conference Room

- 13:00-13:15 **20 - Eco-Friendly Elimination of <sup>14</sup>C-Phenanthrene by the Newly Isolated Marine-Derived Fungus, *Mucor Irregularis* using Response Surface Methodology**  
*Paul Bankole, University of Granada*
- 13:15-13:30 **21 - Study of Short- and Medium- Chain Chlorinated Paraffins (S/MCCPs) in indoor dust using High-Resolution Mass Spectrometry (HRMS) in Negative Chemical (NCI) and Electron Impact (EI) ionization modes**  
*Idoia Beloqui Ezquer, Linköping University*
- 13:30-13:45 **22 - Contamination Patterns and Sources of Heavy Metals in a Typical Urban Zone**  
*Muhammad Faisale, Dalian Maritime University*
- 13:45-14:00 **23 - Into the Unknown: Examining Analytical Techniques for Monitoring Hydrocarbon Oxidation Products in Ballast Water Treatment**  
*Maxwell Harsha, University of New Orleans*



# Wednesday Platform Presentations

## Afternoon Sessions II

### Microplastics | B I 001

- 14:45-15:00 **24 - Effects of Microplastics on the Gut Microflora, Behavior & Histology of Zebrafish**  
Nawal Al Jabri, *Sultan Qaboos University*
- 15:00-15:15 **25 - Assessing Risks Associated With Microplastic Exposure: Cytogenetic Evaluation On Different Ontogenesis Stages Of Salmonid Fish**  
Agne Bucaite, *Nature Research Centre*
- 15:15-15:30 **26 - In-Vitro Toxicity of Highway Runoff Samples Using Mechanistic Reporter-Gene Assay**  
Sarah Cüpper, *Goethe University Frankfurt*
- 15:30-15:45 **27 - The Suitability Of Standard Soil Assays To Study The Biodegradation Of Microplastics**  
Grace Davies, *University of Birmingham*

### Analytical Chemistry | C I Conference Room

- 15:00-15:15 **28 - Pesticide Residues Over the Course of a Year in Soil and Vegetation of Fields and Meadows**  
Carolina Honert, *iES Landau, RPTU*
- 15:15-15:30 **30 - Sewage Analysis as an Effective Tool for Monitoring Contaminants of Emerging Concern, Including Drug of Abuse and New Psychoactive Substances, in Tunisia**  
Bilel Moslah, *CAMU*
- 15:30-15:45 **31 - A novel Accelerated Solvent Extraction Method for Comprehensive Pollutant Profile Characterization in Sewage Sludge**  
Rhayn Werz, *Örebro University & Ragn-Sells*

# Schedule | Thursday, 31 August

Time (CEST)	Programme	Location
9:00–10:15	Session: Aquatic Ecotoxicology Session: Computational Ecotoxicology & Alternative to Animal Testing	B I 001 C I Conference Room
10:15–11:15	Poster Session & Coffee Break	CV
11:15–12:00	Career Talk: <b>Elisabeth Berger</b>	B III 040
12:00–13:00	Lunch Break	CV
13:00–14:00	Session: Aquatic Ecotoxicology Session: Computational Ecotoxicology & Alternative to Animal Testing	B I 001 C I Conference Room
14:00–14:30	Poster Session & Coffee Break	CV
14:30–15:15	Expert Talk: <b>Leonie Müller</b>	B III 040
15:30–17:30	Social Event: Julius Kühn Institute (JKI) Excursion (divided in 2 groups) Zoo Landau as alternative	Geilweilerhof, 76833 Siebeldingen

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## Career Talk

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### Sustainability Science

#### Elisabeth Berger

*RPTU Kaiserslautern-Landau, Germany*

As biogeochemist and system ecologist mainly working in streams and rivers, Clara Mendoza-Lera's research focuses on interactions among physical, chemical and biological elements organizing stream biogeochemistry across scales (mainly Nitrogen and Carbon). Currently she is Research Associate and Stream Biogeochemistry Team Leader at the RPTU. Furthermore, she is part of the Gender and Science AIL group since 2014, recognized with the prize 'Inclusion and diversity in academia' in 2021 by the AEET (Spain).



## Expert Talk

---

### Policy and Environmental Science – Crossroads, Chances and Challenges

#### Leonie Müller

*RWTH Aachen University, Germany and Alvertox Academy, Belgium*

Dr. Leonie Mueller currently works as policy officer at Alvertox Academy and Postdoc at the RWTH Aachen University. Her academic research focused on mechanistic toxicity of environmental pollutants in the aquatic environment and how in vitro and non-animal in vivo methods can be applied and advanced for chemical risk assessment. For her work at Alvertox Academy she focuses on the translation of science on non-animal methods for hazard and risk assessment in the context of chemical regulation in the EU. Leonie is currently the co-chair of SETACs Science and Risk Communication Interest Group.



# Thursday Platform Presentations

## Morning Sessions

### Aquatic Ecotoxicology | B | 001

- 09:00-09:15 **32 - Exploring The Combined Effects of Emerging Contaminant and Elevated Water Temperature on Aquatic Moss**  
Iva Kokotovic, *University of Zagreb, FoS*
- 09:15-09:30 **33 - Acute Toxicity of Four Pesticide Formulations in Binary Mixtures on *Daphnia magna***  
Jonas Nelles, *University of Bremen*
- 09:30-09:45 **34 - Effects of Di-n-butyl Phthalate (DBP) on Life History Traits of *Daphnia Magna*: Comparison of Two Exposure Windows**  
J r mie Ohanessian, *LIEC- universit  de lorraine*
- 09:45-10:00 **35 - Why is pH an Important Factor When Testing Rare Earth Elements Toxicity?**  
Edith Padilla, *University Federico II*
- 10:00-10:15 **36 - Early Developmental Effects of Copper (II) Sulfate Pentahydrate on the Green Sea Urchin *Strongylocentrotus droebachiensis***  
Simon Rodriguez Satizabal, *NIVA*

### Computational Ecotoxicology & Alternative to Animal Testing | C | Conference Room

- 09:00-09:15 **37 - Assessing Molecular Initiating Events in Vitro to Replace Animal Testing** Hans Thomas Allner, *GOBIO*
- 09:15-09:30 **38 - Transcriptomic Profiling of Clobetasol Propionate Induced Immunosuppression During TLR-7-Dependent Immune Challenge in Zebrafish Embryos**  
Benedikt Luckner, *Fraunhofer IME*
- 09:30-09:45 **39 - Development and Evaluation of Simplified Machine Learning Models for the Prediction of Sorption Coefficients for Ionisable Pharmaceuticals in Soils and Sludge**  
Nahum Ashfield, *University of York*
- 09:45-10:00 **40 - Performance and Robustness of General Unified Threshold model of Survival (GUTS) Models on Acute and Chronic Stressor Datasets**  
Leonhard B rger, *Osnabr ck University*
- 10:00-10:15 **41 - Temporally Extended Occurrence of Pesticides Based on Monitoring Databases – Knowledge From Europe**  
Larissa Herrmann, *iES, RPTU Kaiserslautern Landau*

# Thursday Platform Presentations

## Afternoon Sessions

### Aquatic Ecotoxicology | B | 001

- 13:00-13:15 **02 - Ecotoxicological Implications on Releasing Reverse Osmosis-Concentrates and Antiscalants into the Environment**  
Carolin Bertold, *GU Frankfurt*
- 13:15-13:30 **43 - Aquatic Ecotoxicity of Safe and Sustainable by Design Organophosphate Flame Retardants**  
Bianca Stadelmann, *University of Amsterdam*
- 13:30-13:45 **44 - Direct and Indirect Effects of a Chemical Mixture towards Periphyton and its Grazer *Cloeon dipterum***  
Mugil Vannan, *RPTU Kaiserslautern Landau*
- 13:45-14:00 **45 - Transgenerational Behavioral Effects of Perfluoroalkyl Substances (PFAS) in Zebrafish**  
Jonas Zetzsche, *Örebro Universitet*

### Computational Ecotoxicology & Alternative to Animal Testing | C | Conference Room

- 13:00-13:15 **46 - Environmental Residue Behavior of Imidacloprid in Peanut Cultivation System and Its Dietary and Ecological Risk Assessment**  
Abdul Kaium, *Sher-e-Bangla Agricultural Univ.*
- 13:15-13:30 **47 - From Facades to Groundwater – Modeling of Biocides at District Level**  
Felicia Linke, *University of Freiburg*
- 13:30-13:45 **48 - Comparing Simple Fate Model Estimates to Data from Surface Water Monitoring for the Identification of Important Input Parameters**  
Paula Scharlach, *Osnabrueck University*
- 13:45-14:00 **49 - Multivariate Time-Series Analysis for Sensitive Evaluation of Behavior Data From the Light/Dark Transition Test With Zebrafish Larvae**  
Katja Schröder, *RWTH Aachen University*

## Schedule | Friday, 1 September

Time (CEST)	Programme	Location
9:00-9:45	Career Talk: <b>Marissa Kosnik</b>	B III 040
9:45-10:45	Session: Microplastics	B I 001
9:45-10:45	Session: Mechanistic Ecotoxicology	C I Conference Room
11:15-12:15	Lunch Break & Poster Session	CV
12:15-13:45	Closing Ceremony & Closing Plenary: <b>Jutta Paulus</b>	Audimax

## Career Talk

---

### Going With the Flow and Enjoying the [Unexpected] Journey

#### Marissa Kosnik

*Eawag, Swiss Federal Institute of Aquatic Science and Technology*



Marissa Kosnik is a new tenure-track Group Leader in Systems Biology in the Department of Environmental Toxicology at Eawag, where her group researches the multi-level biological impacts of stressors on aquatic ecosystems. She will discuss how 'going with the flow' has helped her through setbacks and has carved the path for her career up to this point. She'll talk about things she would do differently now, things she wishes she'd known sooner, and being willing to follow a moving target.

## Plenary Speaker

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### Between Healthy and Toxic – From Nature Restoration Law to REACH – an Unfiltered Update From the European Parliament

#### Jutta Paulus

*Member of European Parliament for GREENS/EFA*



European environmental policy is often the decisive tailwind that drives environmental and species protection forward. At the same time, the pressure from lobbyists against strong environmental protection in the EU is like a storm. Both of my heartfelt issues, the Nature Restoration Law and REACH, are central ships that need to be brought ashore with scientific expertise, good cooperation and a lot of persistence. An update from the raging ocean of European environmental policy.

# Friday Platform Presentations

## Morning Sessions

### Microplastics | B | 001

- 9:45-10:00 **50 - The Inclusion of Supplementary Analysis Within a Standardized Biodegradability Testing Framework Has the Potential to Provide Enhanced Comprehension of Microplastic Degradation**  
Eva-Maria Teggers, *INVITE GmbH/Fraunhofer IME*
- 10:00-10:15 **52 - Plastic Microfibres in Remote Scottish Soils: What Influences Their Distribution and Abundance?**  
Tereza Pavlíková, *Masaryk University*
- 10:15-10:30 **54 - Microplastics in Wastewater Can Affect Nutrient Removal in Constructed Wetlands**  
Ula Rozman, *University of Ljubljana*
- 10:30-10:45 **55 - High Levels of Small Microplastics (>40 µm) in Compost Samples From Scandinavia: An Important Contributor to Soil Toxicity?**  
Kevin Ugwu Hernandez, *MTM Örebro University*

### Mechanistic Ecotoxicology | C | Conference Room

- 09:45-10:00 **56 - Exploring Lysine Acetylation and Phosphorylation in Adipocyte Differentiation and their Exposure to Emerging Plastic Additives**  
Alix Sarah Aldehoff, *Helmholtz UFZ Leipzig*
- 10:00-10:15 **57 - Combining Multi-Omics Approaches to Reveal the Response of a Microbe-Poplar Holobiont to a PAH Contamination Gradient**  
Nicolas Gallois, *LIEC - CNRS/UL - Nancy*
- 10:15-10:30 **58 - Eye Development Effects of Antidepressants Amitriptyline and Metabolite Nortriptyline in Zebrafish on Multiple Levels of Organisation**  
Marwin Jafari, *University of Stavanger*
- 10:30-10:45 **59 - Exploring the Complexity of Antidepressant Effects in Ecotoxicology: Low Dose and Non-Monotonic Effects of Fluoxetine on Caenorhabditis Elegans Behaviour**  
Merel van der Most, *Wageningen University*



# Poster Presentations

## Aquatic Ecotoxicology

**P01 - Thyroid hormone disruption of Hexa-bromobenzene(HBB) in embryo-larval and adult zebrafish(Danio rerio)** | Lee Yura, *Seoul National University*

**P02 - Thyroid Disruption Effects of Binary Mixture of Major Organic UV Filters in Zebrafish (Danio rerio)** | Juyeon Choi, *Seoul National University*

**P03 - Interactive effects of nitrate and heat-waves on the survival, growth, and reproduction of Daphnia Magna** | Sabiha Akter, *Ecosphere, University of Antwerpen*

**P04 - Exposure of Dreissena polymorpha and Dreissena r. bugensis to Polluted Resuspended Sediments: Effects on Biological Responses** | Bain Manon, *LIEC - Université de Lorraine*

**P05 - Ecotoxicity of novel biosurfactants and corrosion inhibitor polymers as green alternatives for industrial applications** | Marlene Baum, *Goethe University Frankfurt*

**P06 - Impairment of Sensory Organ Development in Petroleum-Exposed Zebrafish Embryos – Lateral Line System and Motor Behaviour Reaction** | Alischa Becker, *Goethe University Frankfurt*

**P07 - Toxicity of a Novel Bio-Based Nanomaterial with Anti-Corrosion Properties on Different Tropical Marine Species** | Mariana Bruni, *University of Aveiro*

**P08 - SensoryTox – The Impact of Crude Oil on Early Sensory Organ Development in Zebrafish** | Bianca Dechent, *Goethe University Frankfurt*

**P09 - Metabolic and endocrine responses on Astyanax lacustris (Teleostei: Characidae) females after diclofenac and ibuprofen waterborne exposure** | Filipe Godoi, *USP*

**P10 - Effects of plastic additives on precopulatory pairing behaviour and male infertility of a marine amphipod** | Bidemi Green-Ojo, *University of Portsmouth*

**P11 - Impacts of the cumulative effect of copper exposure and ocean acidification on cold-water octocoral Viminella flagellum – a transcriptional approach** | Beatriz Mano, *University of the Azores*

**P12 - Investigating priority effects in the response of benthic biofilm communities to the stress of artificial light at night** | Jan Scheu genannt Mecker, *RPTU Landau*

**P13 - Does Benzophenone-3 Cause Any Oxidative or Estrogenic Effects in Juveniles of Octopus maya?** | Gissela Moreno-Ortiz, *PCMyL - UNAM*

**P15 - Barrier Function of the Marine Medaka (O. melastigma) Chorion** | Jakob Pfefferle, *Goethe University Frankfurt*

**P16 - Ecotoxicological Characterization of Peracetic Acid in Freshwater and Marine Water.** | Francesca Provenza, *University of Trieste*

# Poster Presentations

**P17 - Personal Care Products Ecotoxicity in Marine Environments: Different Formulations and Global Change Scenario** | Francesca Provenza, *University of Trieste*

**P18 - Identification of Frequent Co-Exposure Chemical Combinations in Adult Women and Assessment of Their Effects on Thyroid Hormone Disruption in Embryo-Larval Zebrafish (*Danio rerio*)** | Hyesun Seok, *Seoul National University*

**P19 - Aquatic Ecotoxicity of Safe and Sustainable by Design Organophosphate Flame Retardants** | Bianca Stadelmann, *University of Amsterdam*

**P20 - Combined Effects of Chemical Mixtures and Temperature on *Daphnia magna*** | Sophie Steigerwald, *SU, Dep. of Environmental Science*

**P21 - Toxicity of Individual and Combined Effect of Mefenpyr di-Ethyl Safener and its Co-Herbicide, Fenoxaprop-P-Ethyl, to *Daphnia magna*** | Bunmi Femi-Oloye, *University of Saskatchewan*

## Terrestrial Ecotoxicology

**P22 - Nanozeolite Interactions with Earthworms and N-Cycling Soil Microbial Communities** | Jessica Chadwick, *University of Birmingham*

**P23 - Heat Waves Have an Impact on the Toxicity of the Fungicide Fontelis 20 EC on *Folsomia candida*** | Sara Coordes, *UFT, Bremen University*

**P24 - Exposure to Sublethal Concentrations of Imidacloprid, Pyraclostrobin and Glyphosate Harm the Behavior and Fat Body of the Stingless Bee *Scaptotrigona postica*** | Cliver Farder-Gomes, *Federal University of São Carlos*

**P25 - Effects of Temperature and Moisture on the Ecotoxicity of Metal-Based Fungicides Towards Earthworms** | Hussain Kaka, *North-West University*

**P26 - Insects Pesticide Exposition in Flower Strips at the Agricultural Field Areas in Landau in der Pfalz and Surrounding Areas, Germany** | María José Lazo-Hernández, *RPTU*

**P27 - Depth- and Time-Dependent Fate and Transfer of Current-use Pesticides in Different Agricultural Soils** | Lukas Reinhard, *iES Landau, RPTU*

**P28 - Investigation of Pesticide Accumulation in *Osmia bicornis* During Larval Development by Pollen Supply and Loamy Brood Cell Walls** | Katharina Wifling, *iES Landau, RPTU*

## Environmental Impact Assessment

**P29 - Parental Exposure to Atrazine Induces Chromosome Instability in Somatic and Germ Cells of Untreated Progeny of *Drosophila melanogaster*** | Estefania Arroyo Jilote, *Posgrado Ciencias Biológicas, UNAM*

**P30 - Environmental safety of two potential antifungal candidates: VT-1161 and T-2307** | Angela Maione, *University of Naples Federico II*

# Poster Presentations

**P31 - Influence of Spent Engine Oil-Contaminated Soil on Two Hydrolytic Soil Enzymes (Lipase and Urease): A Comparative Study.** | Dr Chigoziri Osuji, *Gregory University Uturu Abia State*

**P32 - Toxicological effects of silver nanoparticles (AgNPs) under localized surface plasmon resonance (LSPR) stimulation** | Lucas Queiroz, *University of São Paulo*

## One Health

**P34 - Interactive Effects of Pesticide Contamination, Waterlogging and Invasive Plant Species on Riparian Foodwebs – a Greenhouse Mesocosm Experiment** | Elyssa Dubois, *RPTU*

**P35 - Curcumin Promotes Animal Health and Antioxidant Protection in Pacific Oysters** | Heloísa Gabe, *UFSC*

**P46 - Effect of Hydrological Fluctuations on Aquatic-Terrestrial Food-Web Interactions** | Stephane Mutel, *RPTU Kaiserslautern-Landau*

## Analytical Chemistry

**P36 - Combined Adsorption and Photodegradation for Efficient Elimination of Ciprofloxacin by Sandwich-Like Covalent Triazine Frameworks -Titania Heterostructures** | Omoyemen Ehimatie, *University of KwaZulu-Natal*

**P37 - Aquatic-Terrestrial Pollutant Transfer in Riparian Plants via Flooding** | Franziska Fiolka, *RPTU Kaiserslautern-Landau*

**P39 - Deposition of Polycyclic Aromatic Hydrocarbons in the Selected Parks in Warsaw, Poland.** | Anita Murawska, *Warsaw University*

**P40 - Heavy metals in agriculture soils and associated ecological and health risk assessment** | Muhammad Saleem, *University of North Dakota*

**P59 - Fatty Acid Composition of Palaemon Adspersus (Decapoda, Palaemonidae) Muscle Under Barium Chloride Exposure** | Zeineb Khila, *University of Lorraine*

## Environmental Modeling & Computational Ecotoxicology

**P41 - The effect of food level on individual and mixture toxicity of prochloraz and benzalkonium chloride in Caenorhabditis elegans: Confirming the applicability of a dynamic energy budget model** | Charlotte Schmidt, *University of Copenhagen*

**P42 - Automated Whole Slide Image Analysis Methods for Detection, Quantification, and Characterization of Liver Histological Lesions in Marine Teleosts Species** | Valentin Geslin, *University of Stavanger*

## Mechanistic Ecotoxicology

**P43 - The endogenous aspects of mechanistic toxicity** | Andreas Eriksson, *Uni. of Lethbridge, & Jyväskylä*

**P44 - Water Accommodated Fractions of Crude oil impact the Marine Bivalve, Cerestoderma edule in a Tidal and Stagnant System** | Edith Etor, *Wageningen University & Research*

# Poster Presentations

**P45 - Investigation of mechanism-specific toxicity of water and particle samples from flooded areas in Stolberg (North Rhine-Westphalia) after the flood event in July 2021 using an in vitro biotest battery** | Dominik Nerlich, *Goethe-University Frankfurt*

## Microplastics

**P47 - Transport Mechanisms of Microplastics and Their Effects on Soil-Plant Systems** | Zhangling Chen, *University of Leeds*

**P48 - Accumulation and distribution of microplastics in Uludağ Ski Resort** | Fatma Nur Eraslan, *Eskisehir Technical University*

**P49 - Environmental transport of microplastics; mitigation strategies and relevant factors** | Zia-Melchior Hoseini, *Wageningen University & Research/TNO*

**P50 - Sorption behaviour of two Tetrachlorobiphenyl Homologs to High-Density Polyethylene (HD-PE) Microplastics** | Isaac Kudu, *University of KwaZulu-Natal*

**P51 - Microplastics, phthalates in sediment and water matrix of the urban aquatic system: Sukhna lake** | Ajay Kumar, *Indian Institute of Science Education and Research Mohali*

**P52 - Transport Mechanisms of Microplastics and Their Effects on Soil-Plant Systems** | Zhangling Chen, *University of Leeds*

**P53 - Chironomus sanctiparoli larvae acting on the biofragmentation of polystyrene microplastics** | Lucas Queiroz, *University of São Paulo*

**P54 - Biofragmentation of polystyrene microspheres by the amphipod Hyalella azteca** | Bárbara Rani Borges, *University of São Paulo*

**P55 - Microplastics in coral reefs in remote South Atlantic islands** | Bárbara Rani Borges, *University of São Paulo*

**P56 - Roadway to Linking Exposure and Effects of Highway Stormwater Runoff and Particulate Matter – First Case Study Results from a Highly Frequented Highway in Germany** | Markus Schmitz, *Goethe University Frankfurt*

**P57 - Interactions of Microplastics with Heavy Metals in Freshwater Environment: Assessing Toxicity and Oxidative Stress Effects on Daphnia magna** | Marisa Spampinato, *University of Naples Federico II*

**P58 - Pharmaceutical Contamination in European Aquatic Environments: Implications for Fish and Shrimp Health** | Ekeminin Okon, *Ghent University, & Universitat Autònoma de Barcelona*

# ABSTRACTS



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## Session 1: Analytical Chemistry

20

### Eco-friendly elimination of <sup>14</sup>C-phenanthrene by the newly isolated marine-derived fungus, *Mucor irregularis* using response surface methodology

Paul Olusegun Bankole<sup>1</sup>, Kirk Taylor Semple<sup>2</sup>, Elizabet Aranda<sup>1</sup>

<sup>1</sup>University of Granada, Spain. <sup>2</sup>Lancaster University, United Kingdom

#### Abstract

The teratogenic and genotoxic effect that polycyclic aromatic hydrocarbons exert on humans has raised serious health concerns and the need to eliminate them from environmental matrices is of great interest to researchers. This study investigated the removal of <sup>14</sup>C-phenanthrene degradation efficiency of a marine-derived filamentous fungus, *Mucor irregularis*. Response Surface Methodology (RSM) using Box–Behnken Design (BBD) was successfully deployed in the optimization of process parameters (pH-5, temperature-30°C, substrate concentration-30 mg L<sup>-1</sup>, and dry weight-2.0 g) resulting in 99.50% phenanthrene degradation on 5th day. The design and regression model was found to be statistically significant, adequate, and appropriate with  $p < 0.0001$ , F value = 412.19 and predicted coefficient of determination ( $R^2=0.978$ ). Optimization of the vital constituents of the mineral salt medium (MSM) used for the study using RSM-Central Composite Design (CCD) resulted in 99.90% phenanthrene degradation rate. Enhanced phenanthrene degradation efficiency (99.70%) was recorded when the optimized process variables were subjected to growth-linked validation experiments. The enzyme assay experiments revealed 98%, 86%, and 55% induction of laccase, manganese peroxidase and lignin peroxidase respectively. Metabolites obtained after the experiment were characterized and confirmed with GC-MS analysis. The findings revealed the promising potential of fungi in green remediation technology.

## Study of Short- and Medium- Chain Chlorinated Paraffins (S/MCCPs) in indoor dust using High-Resolution Mass Spectrometry (HRMS) in Negative Chemical (NCI) and Electron Impact (EI) ionization modes

Idoia Beloqui Ezquer<sup>1,2</sup>, Bo Yuan<sup>3</sup>, Thanh Wang<sup>1</sup>

<sup>1</sup>Linköping University, Sweden. <sup>2</sup>Örebro University, Sweden. <sup>3</sup>Norwegian University of Science and Technology, Norway

### Abstract

Chlorinated paraffins, CPs, are persistent, bioaccumulative and toxic. Even so, they are produced in high volumes, used in various products and applications, and added to a broad range of household products. Consequently, CPs leach into indoor environments.

The analysis of CPs is a challenge due to the presence and analytical interferences of other organic pollutants, and the high complexity of CP mixtures that consist of thousands of individual congeners with varying numbers of chlorine atoms and different chain lengths. HRMS such as gas chromatography (GC)-Orbitrap-HRMS can resolve homologs yielding ions, reduce background interference, study in-source fragmentation, thermal degradation of CPs, and improve detection accuracy. NCI is the most applied ionization mode for CPs. This “soft” ionization method enhances the sensitivity of the dominant fragment ion which is useful in the analysis of CP homologs with complex composition. Alternatively, “hard” ionization modes as EI, yield high fragmentation and it is often difficult to find homolog-specific ions with sufficient intensity for quantification. However, NCI usually has low sensitivity to the low chlorinated CPs ( $\leq$  Cl<sub>5</sub>), while EI produces higher sensitivity fragment ions for these homologs.

The present work studied the distribution of the different congener groups in technical mixtures and their sensitivity to accurately describe the CP mixtures that could be applied for further quantification methods of several indoor matrices. Different congener group distribution was found for the same standard mixtures depending on the used ionization mode. The obtained results showed the relevance of the complementary use of EI and NCI.

Indoor environments are a potentially important source of human exposure to pollutants, especially dust, still, the available information on CPs in interior settings is limited. In the present work, 12 dust samples were analyzed using GC-Orbitrap-HRMS in NCI and EI modes. The concentration ranged from 0.40 to 31.05  $\mu\text{g/g}$  and 0.85-68.50  $\mu\text{g/g}$  for the congeners with  $\geq$  Cl<sub>6</sub>, while 0.03-21.21  $\mu\text{g/g}$  and 0.05-8.06  $\mu\text{g/g}$  for the ones with  $\leq$  Cl<sub>5</sub>, SCCPs and MCCPs respectively. The obtained results suggested the accumulation of CPs over the years, a possible influence of cleaning routines in CP levels, and different descriptions of indoor CPs depending on the buildings and sampled areas. This project will give insight into the extent of CPs contamination and help to minimize indoor exposure.



## Contamination Patterns and Sources of Heavy Metals in a Typical Urban Zone

Muhammad Faisal<sup>1</sup>, Zai-Jin You<sup>1</sup>, M. Zuhaib Akram<sup>1</sup>, Muhammad Naeem<sup>2</sup>

<sup>1</sup>Dalian Maritime University, China. <sup>2</sup>Chinese Academy of Sciences, China

### Abstract

Exposure to heavy metals found in road dust poses a serious threat to human health. In the course of this research, the concentrations, patterns, and sources of eight potentially dangerous heavy metals (Cr, Ni, Cu, and Zn; As, Cd, Pb, and Hg) that were found in the street dust of Zhengzhou city in the People's Republic of China were explored. Three risk assessment strategies, including the geo-accumulation index (Igeo), the potential ecological risk assessment (RI), and the Nemerow synthetic pollution index (PIN), were applied to 87 samples of road dust. Hg and Cd contents were found to be 12 and 5 times higher than their respective baseline values, respectively. Igeo displayed the potential for contamination on a spectrum from minimally polluted (Cr, Ni) to highly polluted (Hg, Cd). RI proposed a scale of contamination severity, from very low (Cr, Ni, Cu, Zn, As, and Pb) to very high (Cd, Hg). According to the PIN, the danger of contamination ranged from being completely harmless (in the cases of Cu, As, and Pb) to being quite high (in the cases of Cd and Hg). PIN's findings highlighted the staggering danger posed by Cd and Hg in the urban environment. Positive matrix factorization was utilized in order to locate the many contaminated material sources. The contribution of factors 1 (vehicle exhaust), 2 (coal combustion), 3 (metal industry), and 4 (anthropogenic activities) to total heavy metal pollution was, in descending order, 15%, 33%, 38%, and 14%, respectively. The presence of metals in the city's air pollution poses a direct threat to human health and necessitates prompt and efficient pollution control and preventive measures.

## Into the Unknown: Examining Analytical Techniques for Monitoring Hydrocarbon Oxidation Products in Ballast Water Treatment

Maxwell Harsha<sup>1</sup>, Danielle Verna<sup>2</sup>, Yanila Salas-Ortiz<sup>1</sup>, Ed Osborn<sup>1</sup>, Eduardo Turcios Valle<sup>1</sup>, Patrick Tomco<sup>3</sup>, David Podgorski<sup>1</sup>

<sup>1</sup>University of New Orleans, USA. <sup>2</sup>Prince William Sound Regional Citizens' Advisory Council, USA. <sup>3</sup>University of Alaska Anchorage, USA

### Abstract

Hydrocarbon oxidation products (HOPs) are an emerging pollutant of concern formed by the degradation of spilled oil in aquatic ecosystems. HOPs are widely present in marine environments due to their high solubility in water, facilitating their rapid diffusion throughout the water column. Understanding the characteristics, fate, and transport of spilled oil in high latitude regions is crucial given the growing petroleum development and shipping activities, yet regulatory policies do not currently encompass HOPs. The characterization of HOPs poses challenges with traditional analytical methods due to their complex composition as a mixture of large, polydisperse, and polar compounds. This work explores analytical techniques to detect HOPs and the application of these methods to monitor HOPs at a ballast water treatment facility, which treats oil contaminated water before discharging into a marine ecosystem. Over the course of one year, sampling was conducted opportunistically at different stages of the treatment process, encompassing untreated water to treated effluent. Untargeted analytical techniques, such as non-volatile dissolved organic carbon analysis, fluorescence excitation-emission matrix spectroscopy, and high-resolution mass spectrometry were employed for quantification and characterization of HOPs. Results demonstrate that the treatment process effectively removes benzene, toluene, ethylbenzene, and xylene (BTEX) compounds, while HOPs remain. Optical analyses offer valuable insights into the composition and transformation of hydrocarbon oxidation products (HOPs), revealing a shift towards increasingly oxygenated and complex compounds throughout the treatment process. High-resolution mass spectrometry analysis is currently underway to characterize the molecular compositional shifts through the treatment process. Findings from this study offer valuable insight and implications for environmental monitoring and risk assessment in the context of ballast water treatment, highlighting the importance of comprehending and mitigating the impacts of contaminants derived from petroleum on aquatic ecosystems. Furthermore, the reported techniques have been identified as potential techniques in environmental monitoring and risk assessment.

## Pesticide Residues Over the Course of a Year in Soil and Vegetation of Fields and Meadows

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### Abstract

Abstract In recent decades, awareness of the potential impact of pesticides on the environment has increased. Pesticides are widely used in agriculture to control pests and diseases and to increase crop yields. However, they can also have negative impacts on the organisms in the terrestrial environment. The decline of insects in agricultural landscapes is now well documented but the magnitude of the pesticide impact is still under debate. To gain a better understanding of the presence and seasonal variations of current use pesticides (CUPs) in agricultural landscapes and its habitats, a comprehensive study was conducted to understand the exposure of insects

The main objective of this study was to record pesticide residues in fields and adjacent meadows over a period of one year and to analyse the occurrence of different CUPs. For this purpose, monthly soil and vegetation samples were taken from different agricultural crops (vineyards, orchards, arable fields) in the fields and at three points of increasing distance (1 m, 5 m, 20 m from the field) in adjacent meadows. Flower and water samples were also taken during the summer months. The analyses for residues of 98 different CUPs was carried out by HPLC-MS/MS to ensure accurate qualification and quantification.

The results of our study show that CUP residues are widespread in the fields and meadows studied. We detected a variety of CUPs in different concentrations and mixtures of up to 25 substances in vegetation and up to 32 CUPs in soil, with residues of some substances (fluopyram, boscalid) being detected in almost all samples. In addition, seasonal variations were found, with pesticide concentrations being highest at the time of applications and the composition of pesticide cocktails varying throughout the year. However, soils revealed a low concentration, chronic presence of mixtures of up to 20 CUPs.

The available data provide insights into the long-term fate of CUPs in the environment and address the potential exposure of insects in agricultural landscapes. These results help to understand the risks and develop targeted measures to reduce pesticide exposure and protect the environment.

## **Sewage Analysis as an Effective Tool for Monitoring Contaminants of Emerging Concern, Including Drug of Abuse and New Psychoactive Substances, in Tunisia.**

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Center Mahmoud Yaccoub for Urgent Medical Assistance, Tunisia

### **Abstract**

Many studies attest to the pollution of wastewaters by organic molecules including drug of abuse (DAs) residues and new psychoactive substances (NPS) at trace levels. The occurrence of these emerging micropollutants in influent wastewaters (IWW) from three Tunisian Wastewater Treatment Plants (WWTPs) located in north-eastern Tunisia was assessed. Influent wastewater composite samples (24 h) were collected over 7 consecutive days in November 2019. The determination and quantification of 11 drug of abuse or their metabolites was performed by the application of an optimized multi-residue method liquid chromatography tandem mass spectrometry (LC-MS/MS). All relevant method validation information's including limits of detection and quantification, recovery, linearity and matrix effects for the quantitative method were examined. MDMA, THC and benzoyl ecgonine (major metabolite of cocaine) were the most detected DAs across the three sewage plants investigated. A new approach namely wastewater-based epidemiology (WBE), was applied in this study to provide an estimate of community drug consumption. This innovative approach was used to calculate and to assess collective drug consumption of illicit drug at a community level, based on the concentration of selected drugs or their major metabolites in influent wastewater. The average MDMA consumption found in the selected cities ranged between 35,8–1531,1mg/day/1000 inhabitants and increased during the weekends. Cocaine consumption ranged from 24.5 to 179.8 mg/day/1000 inhabitants.

Complementary qualitative investigation of new psychoactive substances was monitored for the first time for an African country, examining the occurrence of 50 NPS in wastewaters. Out of 50 totals screened NPS across all sampling sites, 21 were tentatively identified with this approach. The 21 detected NPS were distributed on most representatives NPS classes; synthetic opioids, synthetic cathinones and synthetic cannabinoids. The highest detection frequency was recorded in the weekend particularly for synthetic cathinones and amphetamines derivatives (alpha-PHPP, mephedrone, pentedrone, pentylone, ethylone, MDA, MDEA), although synthetic cannabinoids (JWH-250, JWH-073, CP47497, HU-210) were much higher across the sampling week than other detected NPS in this study. This seems consistent that mainly stimulant drugs have been found, as music festivals and nightlife venues are more prone to the use of this category of NPS during weekend.

## A novel Accelerated Solvent Extraction Method for Comprehensive Pollutant Profile Characterization in Sewage Sludge

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<sup>1</sup>Örebro University, Sweden. <sup>2</sup>Ragn-Sells Treatment & Detox AB, Sweden

### Abstract

Sewage sludge - a byproduct of wastewater treatment - is commonly applied as fertilizer on agricultural fields due to its favorable phosphorous, organic matter, and nitrogen content. This practice also contributes to closing corresponding nutrient loops. However, the presence of heavy metals and a diverse range of (emerging) micropollutants, originating from industry, hospitals, and society at large, has sparked public debate. Examples of pollutant groups detected in sludge include pharmaceuticals, Per- and polyfluoroalkyl substances (PFAS), microplastics, phthalates, flame retardants, and industrial chemicals. While optimized extraction and chemical analysis methods exist for individual pollutant groups, there is a pressing need for a comprehensive and more cost-effective method. Namely, regulators, environmental agencies, and operators of wastewater treatment plants commonly lack the resources to monitor this multitude of pollutants.

My research focuses on developing such a characterization method using Accelerated Solvent Extraction (ASE). The investigation includes different polarity fractions, which are assumed to affect compound-dependent adsorption behavior and extraction efficiency.

To evaluate this method, compound mixes containing native standards for pharmaceuticals, PFAS, siloxanes, phthalates, organophosphate flame retardants, polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) as well as dioxins and furans (PCDD/Fs) are spiked onto freeze-dried sludge samples. Subsequent alkaline and acidic digestion should liberate neutral anions and cations respectively. Two "extraction pathways" are then explored, using polar solvent (methanol) and apolar solvent (n-hexane / dichloromethane, 8:2 (v/v)). Polar extracts undergo further cleanup using solid phase extraction (SPE) cartridges, while columns with deactivated silica gel are employed for apolar extracts. Respective undigested replicates and extraction blanks serve as controls. Lastly, chemical analysis of the extracts is performed using an LC-QTOF-HRMS or GC-orbitrap-HRMS instrument, depending on the pollutant group. First results and an assessment of the efficiency of the method will be presented at the conference.

Ideally, this method enables both comprehensive screening of sludge as well as more in-depth analysis of specific compound groups, facilitating the monitoring of pollutants in sewage sludge before agricultural application.

## Combined Adsorption and Photodegradation for Efficient Elimination of Ciprofloxacin by Sandwich-Like Covalent Triazine Frameworks -Titania Heterostructures

Omoyemen Ehimatie, Brenda Moodley

University of KwaZulu-Natal, South Africa

### Abstract

Covalent triazine frameworks (CTFs) with crystalline, mesoporous and nitrogen-rich structures are currently applied in photocatalysis, energy storage, and adsorption. However, synthesizing CTFs with intrinsic properties remains a challenge. This study provides a new route for fabricating CTFs using a binary molten salt template (CTF-BS250) at low temperatures and scalable conditions while maintaining the desired properties. Furthermore, novel CTF-BS250-TiO<sub>2</sub> composites were prepared by sandwiching TiO<sub>2</sub> particles on CTF-BS250 by wet impregnation method for improved performance in adsorption and photocatalysis. Characterization reveals that the prepared composites exhibited visible light absorption, narrow band gap, improved adsorption capacity, and reduced recombination rate of electrons and holes. Adsorption and photodegradation efficiency of the as-prepared materials were conducted using Ciprofloxacin (CIP) as a model pollutant. Adsorption result obtained indicates that the sorption of CIP was dependent on electrostatic and hydrophobic interaction. The maximum adsorption of CIP obtained is 30.30 mg/g and 13.61 mg/g for CT-1 and CT-2, respectively. Furthermore, our results show that based on the improved properties, the highly efficient CT-2 was able to degrade 96.7 % of CIP within 20 min of visible light irradiation in the presence of H<sub>2</sub>O<sub>2</sub> at optimum pH of 6. These results provide meaningful direction for solving ubiquitous compounds in the ecosystem.

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## **Aquatic-Terrestrial Pollutant Transfer in Riparian Plants via Flooding**

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### **Abstract**

Climate change will significantly alter and impact many ecosystems. One of these ecosystems is the riparian zone, which will be impacted even more in the future by recurring floods and droughts. Flooding can have a negative, but not yet fully investigated, impact on riparian communities, by transfer of micropollutants dissolved in water or bound to sediment to these riparian areas. The goal of this study was to determine the extent to which riparian vegetation communities were contaminated by organic pesticides through flooding events.

Plant communities were sampled from adjacent flooded and non-flooded riverbanks at five streams in the Upper Rhine catchment, which is an area strongly impacted by pesticide input from viticulture and fruit growing. The pesticide concentrations were determined in the rootzone soil, roots, stems, and leaves of five plant species. Therefore, the quantitative and qualitative differences in pesticide exposure between floodplain and non-floodplain sites were investigated. As a result, the question of species-specific uptake of pesticides with varying physicochemical properties can be addressed. Furthermore, the presence of organic pesticides in the photosynthetic active parts of the plant can be compared to the organic pesticides found in the roots, helping to understand the risk for relevant terrestrial consumers.

## Deposition of Polycyclic Aromatic Hydrocarbons in the Selected Parks in Warsaw, Poland.

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### Abstract

Increased contamination of urban ecosystems with polycyclic aromatic hydrocarbons (PAHs) has a significant impact on the citizens, as well as on urban ecosystems and semi-natural and natural ecosystems within the city.

The purpose of this study was to assess PAHs' contamination of soil and pine needles (*Pinus sylvestris* and *P. nigra*) and to use these matrices as bioindicators for seasonal (pine needles) and long-term (soil) contamination of the selected parks in Warsaw, Poland. Samples of soils and needles were collected in October/November 2021 and May/June 2022, and their contamination with 13 PAHs was analyzed using GC-MS. Additionally, in soil samples basic soil features were measured (pH, EC, and C, N, P contents). Moreover, we compared the collected data with records of PM<sub>10</sub>, PM<sub>2.5</sub>, and benzo(a)pyrene (in PM<sub>10</sub>) concentrations in ambient air, obtained from the Chief Inspectorate Of Environmental Protection of Poland.

According to the preliminary results of the study, the basic features of the studied soils differed significantly between the parks. The soils also showed significant differences in PAHs' contamination between the parks, both in October/November 2021 (for 3- and 5+6-rings PAHs) and in May/June 2022 (3-, 4-, 5+6-rings PAHs). In the soil samples collected in October/November 2021 mean total PAHs' concentrations ranged from 67.2 to 525.4 µg/kg. The mean total concentration of PAHs for all the studied parks was 241.1 µg/kg. The highest concentration was found in the southern part, and the lowest concentration was in the northern part of Warsaw. In the soil samples collected in May/June 2022 mean total PAHs' concentrations ranged from 106.5 to 705.2 µg/kg. The mean total concentration of PAHs for all the studied parks was 291.3 µg/kg. The highest concentration was found in the central part, and the lowest concentration was in the north-western part of Warsaw. In the soil samples collected in May/June 2022, the heaviest PAHs (5+6 rings) strongly dominated, which can be a result of the specific sources of contamination and/or of the short transport distance typical for heavy compounds. Obtained results show that the mean concentration of each compound in each park did not exceed the limit values featured in Polish law regarding contamination of soil. However, the maximum result value in one case of a sample from October/November 2021 and five cases of samples from May/June 2022 exceeded the limit values.



## Heavy metals in agriculture soils and associated ecological and health risk assessment

Muhammad Saleem

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### Abstract

Heavy metals soil pollution is increasingly becoming a problem and has become of great concern worldwide. The current investigation evaluates the selected metal levels and risks associated with these metals in agriculture soils. Composite surface soils samples were examined for As, Cd, Co, Cr, Cu, Hg, Fe, Mn, Ni, Pb, Tl and Zn by using microwave-assisted acid digestion and inductively coupled plasma mass spectrometry (ICP-MS) analysis. The pollution indices (enrichment factor, contamination factor, geo-accumulation index, ecological risk and potential ecological risk index) were used for ecological risk assessment. The metal levels in agriculture soils showed the following decreasing order: Fe > Mn > Zn > Ni > Cr > Cu > As > Pb > Co > Cd > Tl > Hg. Based on average levels, all studied metals except As and Cd were lower than guideline limits set by the international agencies. Principal component analysis (PCA) indicated the lithogenic origin of most of the studied metals. The Igeo results exhibited moderately polluted soil by As and Cd, and based on EF results, As and Cd exhibited significant enrichment. Contamination factor revealed that Zn and Pb showed moderate contamination, Hg exhibited low-moderate contamination, while As and Cd showed significant contamination in soil. Comparatively higher risk was noted in children than adults, and overall, As was the major contributor in non-carcinogenic risk assessment. Carcinogenic risk assessment revealed that As, Cr and Ni exhibited significant risk to populations associated with this agriculture soil. Lastly, this study showed moderate contamination by As, Cd, Pb and Hg and should be regularly monitored for pollution controlling authorities.

**Fatty acid composition of *Palaemon adspersus* (Decapoda, Palaemonidae) muscle under barium chloride exposure**

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**Abstract**

Pollution of the aquatic environment by barium chloride (BaCl<sub>2</sub>) becomes a major problem since this element is widely used in industrial manufacturing; aluminum refining, production of chlorine and soda, metals heat treatment, tanning of leathers, photographic stationery and textile pigments and dyes.

Since there are no data concerning the probable toxicity of barium chloride (BaCl<sub>2</sub>) in the marine environment, where the information available is limited to fresh water, terrestrial sediments, soil and wastewater, our study is therefore based on the choice of three concentrations lower than the predictable no-effect concentration recorded in wastewater. The objective of the present study is to assess, for the first time, the adverse effects of BaCl<sub>2</sub>, at different concentrations of 20, 40 and 80 mg/l, on fatty acids profile of *Palaemon adspersus* muscle.

Fatty acids, the regulator of lipid metabolism, has functional roles in the cellular physiological processes and they are considered as an early sensitive tool to assess xenobiotic contaminations in aquatic organisms.

The exposure for 5 days indicated, in the muscle, increased level of saturated fatty acids (SFA), decreased contents of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). Furthermore, alterations in arachidonic (C20: 4n-6, ARA), eicosapentaenoic (C20: 5n-3, EPA) and docosahexaenoic (C22: 6n-3, DHA) acids were observed in muscles treated with BaCl<sub>2</sub>.

Overall, this study confirmed the utility of fatty acids in the assessment of barium chloride toxicity, in order to indicate a mechanism of toxicity at the cellular level of this metal.

## Session 2: Aquatic Ecotoxicology

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### Toxicity of Antineoplastic drug mixtures to *Danio rerio* larvae

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#### Abstract

Millions of patients receive worldwide chemotherapy, and predictions indicate a considerable growth in cancer cases leading to the increased use of anticancer agents (AAs). Following their administration to patients, AAs and respective metabolites are released into the aquatic environment mainly through wastewater treatment plants effluents resulting in many AAs being identified and quantified in surface waters.

The increased use of AAs and consequent release into the environment leads to a growing concern about AAs and their environmental impact. Under real exposure scenarios, AAs can be found in mixtures with other AAs or compounds in the aquatic environment.

In this work, we assess the potential effects of three AAs, Trabectedin (TRAB), Doxorubicin (DOX), and Oxaliplatin (OXA), commonly used in cancer treatment patients jointly or in sequence, thus increasing their possibility to be part of mixtures. Embryos of the model organism *Danio rerio* were exposed to the three AAs individually and in combination following the OECD 236 Fish Embryo Acute Toxicity Test. Fertilized eggs were exposed to varying concentrations of each compound for 96 hours to evaluate acute toxicity and developmental alterations of the embryonic stages of zebrafish promoted by the AAs. Then the comet assay was performed using no-effect concentrations to evaluate DNA damage after 96 hours of each compound exposure. Finally, a full factorial design was used to predict their joint toxicity pattern, and embryos were exposed to various combinations of the three AAs and control treatments for 96 hours. The MIXTOX model predicted the joint toxicity effects and deviations from the reference models (Concentration Addition and Independent Action).

The single acute toxicity tests revealed that TRAB, DOX and OXA caused mortality and malformations in zebrafish embryos/larvae, including pericardial edema, yolk sac absorption and tail deformities. TRAB is the most toxic AA, followed by DOX and OXA, with values ranging from  $\mu\text{g/L}$  to  $\text{mg/L}$ .

DNA damage was also observed in larvae exposed to all three AAs at concentrations without observed apical effects, showing that AAs can promote cellular alterations at sub-lethal concentrations. Results of the mixture exposure suggest a Dose Ratio interaction between TRAB and DOX, while the toxicity of DOX and TRAB to zebrafish larvae follows an increasing pattern with increasing OXA concentrations.

## Ecotoxicological Implications on Releasing Reverse Osmosis-Concentrates and Antiscalants into the Environment

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### Abstract

Modern membrane process techniques like reverse osmosis (RO) and nanofiltration (NF) are constantly applied and optimized to ensure high drinking water quality. During the water purification process, concentrates are produced that contain salts, antiscalants and partly micropollutants. Subsequently, these concentrates are discharged either directly or indirectly into surface water. The potential impact of these discharges has not yet been fully investigated. Antiscalants (AS) mostly phosphonate-based are added to RO systems for problem-free membrane operation. Once leaked into the environment, their high complexation ability could lead to sediment sealing and enhance the migration of heavy metals. Also, AS show a low biodegradability and could aggravate phosphate, contributing to eutrophication. In the KonTriSol project, various AS active substances, AS technical products and RO-concentrates (RO-C) were tested to be supplied with a more precise statement on the effect of the RO-C discharge on the environment. The KonTriSol project aims to develop a comprehensive bioassay battery for the ecotoxicological assessment of AS and concentrates, evaluate the effects of AS active ingredients in complex mixtures and concentrate treatment strategies. Additionally, RO-C discharge conditions should be optimized. For a comprehensive ecotoxicological evaluation, AS active ingredients, AS technical products, RO-C, and treatment strategies were tested in a biotest batterie with acute (on daphnids, fish and algae) and mechanism-specific endpoints (genotoxic, endocrine activity). We found that AS and AS products have no endocrine nor genotoxic effects but exhibit acute toxic effects towards daphnids, especially algae. As there are toxic effects already present in potentially environmentally relevant concentrations, AS discharge could have a potential harm on the environment. The first results show that RO-C containing AS can exert an (low) acute toxic effect on daphnids and fish and algae. It must be considered that the concentrates are diluted when discharged into the environment, which will lower the effects. With the knowledge about the ecotoxicological properties of RO-C and AS, discharge conditions into the environment can be optimized, preventing potential damage to the aquatic environment. The Project is funded by the German Federal Ministry of Education and Research (BMBF) and co-financed by the German Technical and Scientific Association for Gas and Water (DVGW).

## Effects of a Nanomaterial and an Antineoplastic Agent in Zebrafish

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### Abstract

Layered double hydroxides (LDH) are a group of nanomaterials with a wide range of applications in various fields, from catalysis, water treatment, cosmetics, energy storage and drug delivery. The potential for use in various industries increases the possibility of ending up in the aquatic environment. Another emerging contaminant group (EC) present in the aquatic environment is the antineoplastic agents (AAs), which were already detected at low concentrations in the environment. Nevertheless, AAs are known for their ability to affect organisms through biological pathways, even at low concentrations. Since the LDH can occur in the aquatic compartment and interact with dissolved chemicals (e.g., drugs), through adsorption into the surface's layer or anionic exchange, and, can be also used as nanocarrier for drug delivery, it is important to determine their single toxicity and if their combined toxicity can be additively predicted. This study aimed to firstly evaluate the single ecotoxicity of Mg-Al LDH and Epirubicin in *Danio rerio*.

The zebrafish *Danio rerio* is a well-established freshwater model organism widely used in the ecotoxicological assessment of numerous contaminants. *Danio rerio* eggs were exposed to Mg-Al LDH (13 to 100 mg/L) and Epirubicin (11.46 to 87 mg/L) according to the OECD 236 FET protocol, where mortality, malformations and hatching delay were documented daily during 96h. The neurotoxicity and oxidative-stress related biomarkers, as well as DNA damage (comet assay), were assessed at the end of 96h exposure to sub-lethal concentrations of Mg-Al LDH (13 to 100 mg/L) and Epirubicin (88 to 670 µg/L). Embryos were also exposed to sub-lethal concentrations for 120h, and their locomotory activity was tracked using the ZebraBox (Viewpoint, Lyon, France).

No mortality or malformations were observed in the 96h exposure to Mg-Al LDH concentrations. Epirubicin showed a LC50 of 56.06 mg/L (CI = 52.8 – 60.4) and a NOEC for malformation of < 11.5 mg/L after 96 h of exposure. In the behaviour assay, the overall movement time significantly increased upon Mg-Al LDH exposure during the two dark cycles. For Epirubicin exposure, a significant increase in overall movement time was observed in the second light and dark cycles at the highest concentration tested compared to the control.

The findings from this research and future studies on their combined exposure will contribute to the near-future environmental risk assessment of these emerging contaminants.

## Are we making zebra mussels happier by exposing them to psychoactive drugs? Study of the sublethal effects of sertraline.

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Université de Lorraine, France

### Abstract

The consumption of antidepressants has increased by more than 60% in the last decade. Their presence in the environment, especially in freshwater ecosystems, is now a known fact. Sertraline, a selective serotonin reuptake inhibitor (SSRI) antidepressant, blocks the serotonin reuptake transporter in the presynaptic neuron, leading to an accumulation of extracellular serotonin, the “happiness” hormone. These molecular targets are evolutionarily conserved which raises questions about its potential effects on non-target organisms. Physicochemical properties of sertraline indicate a potential for bioaccumulation by aquatic organisms. Due to their capacity for inducing bivalve spawning or disrupting their oxidative status during short-term exposure, SSRIs in the environment are particularly interesting regarding the assessment of long-term effects on aquatic organisms and associated fitness trade-offs.

The objective of our study was to assess the biological responses of *Dreissena polymorpha*, a freshwater bivalve with strong bioaccumulation abilities, exposed to low concentrations of sertraline during 28 days. A targeted set of biomarkers was chosen, related to the functioning of the neuroendocrine and antioxidant defense systems, energy metabolism, hemolymph osmolality and the filtration of bivalves. These functions are expected to be disrupted in *D. polymorpha* according to what we already know for humans or other non-target organisms.

The results show differences in biological responses between males and females, with significant higher levels in males for the activities of the neuroendocrine system. Monoamine oxidase, an enzyme of the neuroendocrine system, catabolizes endogenous monoamines such as serotonin. A decrease in the activity of this enzyme has been observed in females, which may result in more serotonin in circulation. Is that enough to say that they are happier? This is not sure. At the level of energy metabolism, a disturbance in the functioning of mitochondria at the cellular level has been observed. Moreover, at the individual level, a hormetic effect on the scope for growth was measured, suggesting that energy allocation would be focused on reproduction, despite other vital functions of individuals. This study provides new insights into the sublethal effects of sertraline on non-target organisms over longer exposure times and points out that the general health of *D. polymorpha* is likely to be impacted and should be considered.

## Does light pollution affect zebrafish embryos response to the antineoplastic drugs?

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<sup>1</sup>Aveiro University, Portugal. <sup>2</sup>Biology Department, Portugal. <sup>3</sup>CESAM & Biology Department, Portugal

### Abstract

With most of the global population residing in urban areas, urban aquatic ecosystems are under considerable stress from various factors including light and chemical pollution. Artificial light at night (ALAN) is a significant contributor to multistressor scenario and has been shown to disrupt biological rhythms and reduce biological fitness of several aquatic species through changes in melatonin levels. In addition to light pollution, urban aquatic ecosystems are also exposed to increased concentrations of human pharmaceuticals from sources such as hospitals, wastewater treatment plants, and industries. Mycophenolic acid (MPA), a widely used cytostatic drug in cancer treatment, is a priority cytostatic compound due to its high-volume use, resistance to wastewater treatments, and presence in surface waters.

The combination of light pollution and antineoplastic drugs is of great concern for urban aquatic ecosystems, and their impact on early life stages of fish is not well understood. Since melatonin plays a central role in regulating circadian rhythms, antioxidant activity, immune and metabolic processes, it is essential to investigate the mechanistic relationships that determine how changes in lighting conditions affect early life stages of fish and their resistance to antineoplastic agents.

This study aims to investigate the impact of environmentally relevant levels of ALAN on mycophenolic acid toxicity in zebrafish embryos. A set of ecotoxicological approaches will be employed, manipulating both ALAN (using light emitting diodes) and exogenous melatonin to counteract the expected ALAN-induced reduction of nocturnal melatonin synthesis, as well as sub-lethal concentrations of MPA. Fish embryo development, heart rate, behaviour, oxidative stress biomarkers, and melatonin levels will be analysed to assess the extent of ALAN-induced effects on zebrafish embryos, the counteracting effects of exogenous melatonin, and the influence of ALAN on the toxicity response of fish to MPA exposure.

## Impact of Salt of the Toxicity in Textile Dyes in Neurotoxic Endpoints in *Danio rerio* and Genotoxicity on Cell-Based Level

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### Abstract

Only 3% of the water on earth is freshwater and therefore a limited resource. In addition, factors such as the growing population or pollution from agriculture and industrial wastewater are leading to an increased scarcity of freshwater resources. The textile industry is one of the main producers of industrial wastewater worldwide. In addition, textile wastewater is particularly difficult to treat due to the high number of different chemicals (e.g., reactive azo dyes) and high salt concentration. Wastewater of textile dyeing fabrics affect the physico-chemical parameters of water bodies and cause adverse effects in organisms. Even at low concentrations, effects such as neurotoxicity, genotoxicity or influence on fish hatching are known. Furthermore, due to the high variability and complexity of textile wastewater, mixture effects may occur leading to changes in toxicity. A synthetic textile wastewater is produced in the laboratory based on data from the wastewater of the textile industry MS/Rohini, Erode in Tamil Nadu, India. The synthetic wastewater contains the five dyes Red GDN, Navy GDG, Red ME3B, Yellow GD3R, Black GDNN [2.9 g/L] and a salt mixture [7 g/L] of NaHCO<sub>3</sub>, Na<sub>2</sub>SO<sub>4</sub>, NaCl, CaCl<sub>2</sub> and MgCl<sub>2</sub>. The neurotoxic potential and mixture effects will be investigated in *Danio rerio*. First results show an inhibition of *Danio rerio* hatching with no visible morphological effects below the EC<sub>10</sub> value (EC<sub>10</sub> = 0.6 g/L dye mix and 1.64 g/L salt mix). Less than 10 % of the fish hatched, whereas more than 90 % showed no visible morphological effects at 0.3 g/L dye mix and 0.81 g/L salt mix. This high level of hatching prevention could not be observed for the dye mix and salt mix individually, which could be an indicator for mixture effects. In addition, the synthetic textile wastewater caused an inhibition of Acetylcholin-Esterase, which indicates neurotoxicity. Therefore, specific tests for neurotoxicity such as the spontaneous tail coiling and the light-dark transition test will be carried out. Furthermore, the micronucleus assay will be performed using Chinese hamster lung fibroblasts (V79) to investigate the genotoxic potential of the synthetic textile wastewater as well as of the dye mix and salt mix individually. The test will also be performed using a rat liver S9 fraction containing phase I and phase II enzymes to simulate the metabolic process in organisms. Thus, the genotoxic potential of the metabolites can also be determined.



## Integrated toxicological analyses of marine micro- and nanoplastic particles using *in vivo* and *in vitro* bioassays

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### Abstract

Nano- and microplastics have been detected in organisms at the base of the food chain, posing a potential threat to marine ecosystems but also raising concerns about potential impacts on humans due to adsorbed chemicals and additives exhibiting toxicity. Target-, suspect, and non-target screening are used to identify the chemical composition of microplastics. However, due to the vast number of microplastics-related-toxicants and the cocktail effect caused by the mixture of chemicals, chemical analyses are insufficient for hazard assessment. The combination of different *in vitro* to *in vivo* Bioassays provides an accurate characterisation of the toxicity of bioactive chemicals.

This study is associated with the European research project “Response: Towards a Risk-Based Assessment of Microplastic Pollution in Marine Ecosystems” with the objective to develop a monitoring approach for marine plastic pollution. To identify possible accumulation zones in European coastal ecosystems Response analyses the abundance and type of micro- and nanoplastics detected in marine species, and how plastic affects the health of species and food webs. In this context, the present study aims to identify molecular and cellular toxicological effects in different *in vitro* and *in vivo* models after exposure to marine and beach plastic samples. For this purpose, the samples were cleaned and extracted with different organic solvents. Subsequently, the extracts of the polymers were analysed by different types of bioassays for agonistic and antagonistic activities. For the detection of endocrine disruption responses in nuclear hormone receptors we used estrogenic (ER) and androgenic (AR) CALUX<sup>®</sup> bioassays. For the detection of aryl hydrocarbon receptor-mediated activity the DR CALUX<sup>®</sup> bioassay was used. In addition, Cell Painting assay and different *in vivo* tests including developmental, behavioural, and gene expression analyses were performed using zebrafish embryos (*Danio rerio*).

This study demonstrates the importance of an integrated study design composed of different *in vitro* to *in vivo* bioassays and Cell Painting, to investigate and understand the toxic effects of micro- and nanoplastics at different trophic levels.

## Exploring The Combined Effects of Emerging Contaminant and Elevated Water Temperature on Aquatic Moss

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### Abstract

Anthropogenic activity has numerous negative effects on freshwater biota. Even though emerging contaminants (ECs) like pharmaceuticals (PhACs) and endocrine disrupting compounds (EDCs) are not monitored in water systems they can cause chemical stress in freshwater organisms. Moreover, elevated water temperature, as a result of climate change, can lead to thermal stress. Aquatic moss is an important bioindicator in freshwater ecosystems as it plays a crucial role in oxygen production and nutrient cycle. In this study, we investigated the effects of elevated water temperature and pollution with PhACs and EDCs on primary producers and first level consumers in freshwater ecosystems. We conducted a microcosm experiment with a simplified freshwater food web consisting of moss (*Cinclidotus aquaticus* and *Rhynchostegium riparioides*) and caddisfly larvae from an intermittent river. The experiment was conducted in a factorial design with four treatments: control, elevated water temperature for 4 °C compared to the natural temperature regime of the river, PhACs and EDCs mix at a pseudo-constant concentration of 500 ng/L, and a multiple stressor treatment. Analyses such as total protein content, total lipid content, metabolome and lipidome profiling were performed. The overall results showed that moss exhibited a mild response to the stressors and their combination. More specifically, changes in metabolome and lipidome response were related to temporal changes rather than to particular treatment whereas there were no changes in total protein and lipid content. This study suggests that aquatic moss of intermittent rivers is tolerant to emerging contaminants at environmentally relevant concentrations and mild increase in temperature.

## Acute Toxicity of Pesticide Formulations in Binary Mixtures on *Daphnia magna*

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### Abstract

Pollution of surface water bodies with pesticide mixtures in agricultural landscapes is problematic because tank mixtures applied to fields often contain several pesticide formulations. Formulations are mixtures of active ingredients and adjuvants in undisclosed composition that alter bioavailability and therefore toxic effects of the product. Although pesticide formulations are more realistic, toxicity data is mostly lacking, partly because most exposure experiments are conducted with active ingredients instead of formulations. The goal of these experiments is to generate novel data of pesticide formulation mixture toxicity to tackle the data gap and gain further insight into interactions between formulations and their constituents. A miniaturized version of the *Daphnia* sp. acute toxicity test was used, allowing for reduced numbers of neonates needed per concentration. Four different pesticide formulations were chosen based on these criteria: proven persistency, used in high amounts, identified previously in mixtures with synergistic effects, or frequently used in tank mixtures. Active ingredients in the formulations were the neonicotinoid acetamiprid (ACE), the herbicide glyphosate (GLY) and the fungicide prochloraz (PRO). Funguran Progress (FP) is a product relying on the fungicidal activity of  $\text{Cu}(\text{OH})_2$  in nanoparticle form. All formulations were tested separately and in binary mixtures with fixed concentration ratios. Compared to toxicity values given by manufacturers, toxicity test results for PRO were similar, ACE was more toxic, but GLY and FP were less toxic. Binary mixtures with GLY showed antagonistic effects. The mixture of ACE and PRO acted, as the concentration addition model suggested. However, mixtures of ACE and PRO with FP showed synergistic toxicity. In case of ACE and FP, the mixture was twice as toxic as expected from the additive model. Chemical analysis of test solutions is currently in progress. In summary, mixtures of pesticide formulations can cause unexpected acute toxicity that warrant further investigation into the mechanisms of the interactions. For this purpose, experiments to measure activities of metabolic enzymes and stress proteins are planned, as well as chronic toxicity tests. The results also show the need for more research and better legislation incorporating possible mixture effects of product formulations.

## Effects of di-n-butyl phthalate (DBP) on life history traits of *Daphnia magna*: comparison of two exposure windows

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### Abstract

Phthalates are compounds used as additives in the synthesis of plastics to increase their flexibility and strength. Among them is di-n-butyl phthalate (DBP), which has been described as an endocrine disruptor capable of causing reprotoxic effects in mammals. Although DBP is ubiquitously detected in freshwater environments at concentrations of the order of ten ng/L to  $\mu\text{g/L}$ , it remains little studied in ecotoxicology. To date, the few studies on the effects of DBP on *Daphnia* have not addressed the potential effects of exposure at low concentrations ( $< \text{mg/L}$ ) and in the absence of a solvent.

Our work focuses on the chronic effects of DBP on life history traits (survival, growth, reproduction) according to two exposure windows in the freshwater model species, *Daphnia magna* (Crustacea). Organisms were exposed at two exposure windows: I) from early embryonic development or II) adult stage from the third brood onwards, when most energetic investments in growth are completed. Our experimental design included nine exposure concentrations: from 0.5 to 2000  $\mu\text{g/L}$ .

The results obtained show that the DBP exposure window is an essential parameter for studying the effects of the contaminant on *Daphnia*'s life history traits. No significant response was observed in organisms exposed at the adult stage, while impairments in survival, growth and reproduction were observed from 10  $\mu\text{g/L}$  in organisms exposed at the embryonic stage. The results also showed that exposure to a concentration gradient of DBP leads to different dose-response profiles depending on the life history traits measured. Exposure from embryonic stage resulted in unexpected impacts on survival. The observed dose-response relationship was non-monotonic, with greater juvenile mortality at the intermediate concentrations (100, 280 and 500  $\mu\text{g/L}$ ) than at the highest concentrations (1000 and 2000  $\mu\text{g/L}$ ). As for effects on growth and reproduction, the organism responses showed classic monotonic dose-response relationships.

These data are essential for assessing and modeling the potential deleterious effects of DBP on life history traits, individual fitness and population dynamics of *Daphnia*. They will also provide a phenotypic anchoring for characterizing and understanding the dose- and time-dependent effects of DBP at the transcriptional level, which will be our next objective.

## Why is pH an important factor when testing Rare Earth Elements toxicity?

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### Abstract

Rare Earth Elements (REEs) are elements naturally found in the environment, available in trace quantities. Over the last decades, their use has increased in modern technology, becoming highly demanded chemicals, causing also an increase in their environmental concentrations. Concerns have been raised as there are many gaps in the knowledge related to their behavior in the environment and their related toxicity.

This work is focused on the individual toxicity of REEs to three aquatic organisms of different groups of the trophic level (*Daphnia magna*, *Raphidocelis subcapitata* and *Aliivibrio fischeri*). Because there are many physicochemical factors that influence the bioavailability of REEs and therefore, their toxicity, three of these elements were chosen based on their atomic number, regarded as “light” (La), “medium” (Gd), and “heavy” (Lu). The tests were performed by exposing the organisms to a range of concentrations diluted with the organisms’ culture media, and following the guidelines of chemical testing of each test species (OECD 202, OECD 201, and ISO 11348), with a modification to assess also the effects under acidic conditions, for this, the tests were performed under pHs of 6.3 and 5.3 ( $\pm 0.2$ ).

Preliminary results indicate that the test species *D. magna* was the most sensitive species under acidic conditions. While for *R. subcapitata* and *A. fischeri* results obtained indicate that some concentrations can have a hermetic effect. With the nominal concentrations, LC50 estimates and standard error for the pH 5 exposure of *Daphnia* to La, Gd, and Lu, correspond to 3.26 (0.43), 3.24 (0.32), and 2.82 (0.23) mgL<sup>-1</sup>. Moreover, to evaluate further the relationship between the toxicity and the bioavailability of the REEs, dose-response curves will be re-calculated based on the measured concentrations of REE in solution by ICP-MS.

Along with the laboratory tests, the behavior of the REEs was further evaluated by the modeling of the species present under the exposure conditions of each species. Previous studies have demonstrated that under acidic conditions, REEs are more likely to be present under their trivalent form (Ln<sup>3+</sup>) while at higher pH conditions, they complexate with other chemical species such as phosphates, sulfates.

Further results will help us get a better overview of how the different factors - such as chemical speciation - influence toxicity. This work will potentially inform the standardizing of toxicity tests for REE-complexes.

## Early Developmental Effects of Copper (II) Sulfate Pentahydrate on the Green Sea Urchin *Strongylocentrotus droebachiensis*

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### Abstract

Copper is used in the aquaculture industry as an antifoulant on net pens and its release into the marine environment could have effects on non-target organisms. The main objective of this study was to assess the early developmental effects of Copper (II) sulfate pentahydrate in the green sea urchin. *Strongylocentrotus droebachiensis* is one of the most widely distributed sea urchin species and it is found from the intertidal zone to more than 200 m depth. The use of embryonic studies with echinoderms include endpoints such as developmental time and morphometric analysis to determine adverse effects of chemical stressors. Sea urchins were sampled in the Oslofjord (Drøbak, Norway). Organisms were transferred to a climate room (10 °C) and kept in two tanks with filtered natural sea water (FNSW) on a flow-through system and fed ad libitum with kelp. Spawning was induced by an intracoelomic injection of KCl (0.5 M) and gametes were collected in FNSW. Gametes from 3 males and 3 females were pooled and the sperm stock solution was added to the egg stock solution. Egg shape, absence of fertilization, sperm mobility and gamete compatibility were used as quality criteria before starting the tests. One hour after fertilization 300 fertilized eggs were transferred to glass vials (30 ml) containing six concentrations of copper (II) sulfate pentahydrate (5, 9, 15, 28, 50 and 90 µg/L) along with a negative control. Four replicates were used for each concentration. The glass vials were incubated at 12 °C with a photoperiod of 16 h light:8 h dark for 120 h. At 120 h, test replicates were fixed with paraformaldehyde (4%) to prevent further embryo/larvae development. The numbers of unfertilized eggs, and embryonic development (i.e., blastula, gastrula, prism and pluteus) were assessed (n = 50 per vial x 4 replicates per condition). Arrested development in the prism stage was recorded in the highest concentrations (50 and 90 µg/L) which caused a reduced (50 µg/L) or total absence (90 µg/L) of pluteus larvae at 120 h resulting in a calculated IC50 of 35.6 µg/L. These results are part of the ANTIVENOM project, which is assessing the effects of antifoulants and veterinary medicinal products used in the salmon fishing industry in Norway.

## Extrapolation of Cytotoxic Masked Effects from Building Materials in Planar in Vitro Assays

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### Abstract

Planar biotests combine High-Performance Thin Layer Chromatography (HPTLC) and in vitro assays such as the planar Yeast Estrogen Screen (p-YES). The p-YES results in circular fluorescence signals on the surface of the HPTLC-plate indicating the presence of estrogen receptor agonists. Beside the p-YES, a number of further in vitro assays for the detection of e.g. androgenic or genotoxic compounds were successfully coupled with HPTLC. These techniques can be used to identify toxic sample fractions and support effect directed analysis, as they are able to tentatively assign single substances via their retention behavior and sum effects in parallel. Although, planar biotests can simultaneously identify cytotoxicity and specific end points, unlike the usual in vitro assays, many authors report cytotoxic masked effects. This indicates a lack of a method to quantify these affected signals. Regarding our work with eluates from building materials, we were often confronted with this incident. We developed an evaluation program using python to generate chromatograms along the sample tracks from HPTLC images. The program converts pixel values of detected fluorescence signals to grey values that are processed to chromatograms. By this means, signals affected by cytotoxicity result in double-peaks. Therefore, we investigated if double-peaks can be used as data basis to extrapolate the actual signals by fitting a peak function. The precision of the modelling was first validated based on 42 ideal peaks from estrogenic model compounds and second using the same peaks that were mathematically transformed to affected peaks. Finally, we applied the program to p-YES results from a building material eluate, used inter alia in water engineering. The program was able to provide data for generating adequate dose-response relations for cytotoxically affected signals, that would normally not suffice for adequate results. The developed method bridges the lack of the quantitative evaluation of cytotoxic affected signals in planar biotests and offers the possibility of application in other fields.

## Aquatic Ecotoxicity of Safe and Sustainable by Design Organophosphate Flame Retardants

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### Abstract

Organophosphate flame retardants (OPFRs), common polymer additives, were introduced as replacements for polybrominated diphenyl ethers following concerns about environmental and human health effects. However, today OPFRs have established themselves as notorious environmental contaminants. Tris(2-chloroethyl) phosphate (TCEP), a widely used OPFR, is frequently detected in a range of environmental matrices, especially fresh water, meets classification as very persistent and exerts toxicity to aquatic organisms.

To mitigate these adverse effects from the very start of the chemical's life cycle, TCEP has been structurally redesigned as a case study of putting the Safe and Sustainable by Design concept into practice. The alternative design is based on a computer-aided framework developed in house. The aim of the redesign was to retain the flame-retardant properties of the compound but minimize adverse environmental effects, taking persistent, bioaccumulative, mobile and toxic properties into consideration. A multicriteria analysis of millions of structures similar to the original chemical generated in silico, yielded a set of structures predicted to be the most benign, informing the design of a structural alternative for TCEP.

To assess the environmental safety of the newly designed compound, as well as the viability of the computer-aided design approach, the alternative chemical was synthesized and together with the original compound, subjected to a series of aquatic ecotoxicity tests. To gain possible clues about the relationship between ecotoxicological effects and molecular properties of OPFRs, we also tested the structural intermediate tris(ethyl) phosphate, which is currently used as a polymer additive. In this presentation the acute and chronic effects of this set of structurally related OPFRs, tested on three aquatic model organisms: the alga *Raphidocelis subcapitata*, the daphnid *Daphnia magna* and larvae of the insect *Chironomus riparius*, will be shown. The results were determined following OECD test guidelines. Current results indicate the predictions employed during the alternative compound design (US EPA EpiSuite Ecosar), are reliable, as in both acute and chronic tests, the proposed alternative compound is less toxic to all test organisms. The findings of this study support the Safe and Sustainable by Design concept and precautionary approach including extensive testing of additive chemicals to prevent adverse effects on aquatic ecosystems.



## Direct and Indirect Effects of a Chemical Mixture towards Periphyton and its Grazer Cloeon dipterum

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### Abstract

The application of pesticides has already crossed 2 million tonnes globally and is expected to increase rapidly in the future. Since 2007, herbicides are considered to be the highest-selling pesticides in USA and are predominantly detected in surface waters in Europe and the USA. The applied herbicides can enter freshwater bodies easily via different sources, for instance, run off events or drainages, and can cause harmful effects on the aquatic food chain. Therefore, ecotoxicological studies on herbicides in the aquatic environment are very essential. However, the range of chemical stressors in the realistic freshwater environment is broad. Multiple chemical stressors such as detergents, antibiotics, nanomaterials, etc., could be present in an aquatic body at once. This background raises a prominent question: what are potential effects of chemical stressors in freshwater ecosystems? Until now, most studies have focused on direct effects of single chemical stressors, but a knowledge gap exists on potential indirect and combined effects. Therefore, within this study direct and indirect effects of an herbicide combined with an antibiotic were investigated. Particularly, this study focused on investigating direct and indirect effects of the herbicide propyzamide and the antibiotic ciprofloxacin on freshwater periphyton communities and its grazer *Cloeon dipterum*. Periphyton was sampled, cultured in an indoor microcosm facility, chronically contaminated with chemical stressors (14 days) and finally fed to mayflies to detect potential dietary effects. Direct effects on the periphyton were analyzed via biomass endpoints such as ash free dry weight (AFDW) and pigment analyses. Indirect effects were analyzed in terms of the fatty acid composition as fatty acids represent the most important energy storage in invertebrates. It was hypothesized that 1) the chemical stressor would exert a stress on the periphyton, thus reducing the growth of the periphyton biomass and inducing a shift in the community structure (direct effects); 2) this could indirectly affect the feeding rate of the mayflies and therefore have a potential impact on the physiology of the animals. The data is currently being analysed supporting a coherent interpretation of potential bottom-up effects among trophic levels.

## Transgenerational Behavioral Effects of Perfluoroalkyl Substances (PFAS) in Zebrafish

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### Abstract

Poly- and perfluoroalkyl substances (PFAS) have received worldwide attention due to their persistent, toxic, and bioaccumulative properties. PFAS were used extensively in firefighting foams, textiles, and paper products and can frequently be detected across all environmental matrices. Among PFAS, perfluorooctane sulfonic acid (PFOS) is the most studied compound. Present research found that PFOS accumulates in the brain, hinders neuronal growth, and causes behavioral alterations in many different vertebrate species including zebrafish. Manufacturing trends have shifted from legacy PFAS towards short-chain replacement products, such as perfluorobutane sulfonic acid (PFBS), considered as less toxic and bioaccumulative. However, our preliminary results question this assessment, and knowledge of effects at low concentrations is incomplete for both compounds; even less is known about potential multi- and transgenerational effects. Therefore, the major aim of this work is to investigate the behavioral alterations caused by PFOS and PFBS in zebrafish (*Danio rerio*), at different early life stages and through multiple generations. Zebrafish embryos were exposed from 2-hours post fertilization (hpf) to 28 days post fertilization (dpf) at two different concentrations of PFOS or PFBS. The zebrafish model was used to analyze behavioral alterations at 120 hpf using a larval photo-motor response analysis (LPMR) and a vibrational stimulus (VS) test. At adult stages, the anxiety response was analyzed with a novel tank diving test (NTT). Larvae behavior represented the most sensitive endpoint. Overall, interesting dynamics in behavioral responses were observed between larvae and adult fish, as well as sex-specific differences. Behavioral alterations after direct PFAS exposure persisted into the F1 generation and transgenerationally affected the F2 generation. Moreover, PFOS consistently induces a reduction in fish mobility when faced with a stimulus mimicking the presence of a predator (light off). For the NTT, results suggest a more severe impact on female fish in comparison with male fish from the same exposure condition. Our results show that both PFOS and PFBS alter the behavioral responses of zebrafish. Altogether, the results presented in this work will help to further decipher the understanding of the toxicity of both chemicals and their adverse effects through multiple generations, possibly due to transgenerational epigenetic inheritance.

P01

## Thyroid hormone disruption of Hexabromobenzene(HBB) in embryo-larval and adult zebrafish(*Danio rerio*)

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### Abstract

Novel brominated flame retardants(NBFRs)are chemicals that have recently been used as substitutes for conventional brominated flame retardants(BFRs). NBFRs has a different structure from conventional brominated flame retardants, but have most of the physical-chemical properties. and NBFRs has been detected as a BFR substitute in a wide variety of environments, products and biotics. Among the NBFRs, HBB is a substance with a high log Kow value and has recently been reported to be associated with genes involved in thyroid regulation in aquatic organism. However, despite these potential toxicities, studies on endocrine toxicity by HBB exposure are very limited. Therefore, the purpose of this study is to determine the mechanism of thyroid toxicity by developmental stage of the aquatic zebrafish(*Danio rerio*). In embryo-larval zebrafish exposure experiment, changes in thyroid hormones and associated gene expression are analyzed after 7 days of exposure of HBB. Larval zebrafish's whole-body thyroid hormone measurements showed that the total form of T3, T4 increased, and the free form of T3, T4 and Thyroid-stimulating hormone(TSH) also increased. In addition, central regulatory genes(*crh*, *trh*), metabolism-related genes(*dio1*, *dio3a*, *dio3b*) and hormone synthesis genes(*nis*, *tg*, *tpo*) were all decreased. HBB was exposed to male adult zebrafish at 6 months of age for 21 days to analyze the changes in hormone and gene transcription related to Hypothalamus-Pituitary-Thyroid(HPT) axis. Thyroid hormone in the plasma of adult zebrafish was measured, and total form of T4, TSH tended to increase. In this study, exposure to HBB, a novel brominated flame retardant, resulted in thyroid disrupting response at different life stages in zebrafish. These finding suggests that further toxicity studies should be conducted on new brominated flame retardants that replace conventional brominated flame retardants.

P02

## Thyroid Disruption Effects of Binary Mixture of Major Organic UV Filters in Zebrafish (*Danio rerio*)

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### Abstract

Diethylamino hydroxybenzoyl hexyl benzoate (DHHB), octocrylene (OC), and avobenzene (AVB) have been widely used as UV-filters in many products. Despite often being used as a combination in products, previous studies exposing to UV-filters have been focused on exposure to single chemicals. Studies defining the joint interaction of a binary exposure to UV-filters are limited and lack toxicological information, especially thyroid hormone disruption.

In the present study, combined thyroid-disruption toxicities of DHHB, OC and AVB were assessed using embryo-larval zebrafish (*Danio rerio*). Following 120 h exposure, the whole-body contents of total T4, free T3, and thyroid stimulating hormone (TSH) were measured and the transcription levels of thyroid hormone-related genes were analyzed.

Individual substances decreased thyroid hormone levels in larval zebrafish. And some mixture combination caused significantly more decrease in thyroid hormone levels than single substances. Additivity was observed in thyroid hormone levels, where there was no statistical evidence of synergistic or antagonistic effects. After mixture exposure, several genes which are important for thyroid hormones changed in an additive fashion from individual exposure. The alteration of the genes related deiodinases could explain the response due to their metabolic activity to the hormones. The genes related thyroid hormone receptors and transporter (*trβ*, *mct8*) were up-regulated, and it can be considered as the secondary response for compensation. These gene alterations might have led to additive thyroid hormone decrease seen after mixture exposure.

In conclusion, these results suggest that mixtures of UV-filters can produce additive effects on thyroid hormone homeostasis in zebrafish of early life stage. Moreover, these results indicate the need to evaluate mixture toxicity and demonstrate the importance of considering mixture toxicity in risk assessment schemes.

## Interactive effects of nitrate and heatwaves on the survival, growth, and reproduction of *Daphnia Magna*

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### Abstract

stressors such as nitrate. Additionally, climate change causes more intensive and frequent heatwaves, which have a tremendous impact on all trophic levels of the ecosystem. Any change in the lower phytoplankton trophic level also introduces stress to the higher trophic levels such as to the crustacean *Daphnia magna*. Individual effects of heatwaves, high nitrate concentrations, and changing food quality have been studied on *Daphnia magna*, but less is known about their interactive effects. In this study, a 3x3x2 factorial design was used where 48h old *D. magna* were exposed to combinations of ecologically relevant nitrate concentrations (0, 50, or 200 mg/L) and different heatwave scenarios (no, short, or long). They were either fed with a control diet or an experimental diet of microalgae that were exposed to the same conditions as the daphnia. Throughout the 45-day-long experiment, the interactive effects of nitrate, temperature, and feed on mortality, maturation, number of offspring, and body size were evaluated. Although it was suspected that exposing *D. magna* to a long heatwave scenario in combination with a high nitrate concentration would result in an overall low performance, this was not the case. Results show that daphnia cultured in the absence of nitrate had a longer maturation time, higher mortality, smaller body size, and a lower number of offspring, regardless of the temperature or the type of feed. The restricted continuous proliferation of microalgae at the nitrate-limited condition (0 mg/l) reduced the food availability causing this higher impact on *D. magna* life history traits. Heatwaves shortened the lifespan of the daphnia. Daphnids cultured in high (200 mg/L) nitrate with control feed performed better than with experimental feed, which indicates that at the high nitrate condition, the experimental phytoplankton was either unable to meet the energy requirements or it introduced extra stress to the daphnia. Interestingly, the quantity and quality of the feed had a higher impact on daphnids than nitrate concentration or temperature. Nevertheless, nitrate and temperature cannot be dismissed as stressors for *D. magna* as they impact the algae that *D. magna* feeds on.

## Exposure of *Dreissena polymorpha* and *Dreissena r. bugensis* to Polluted Resuspended Sediments: Effects on Biological Responses

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### Abstract

Dreissenid mussels are freshwater model bivalves frequently used for the monitoring of the aquatic environment. As they are filter-feeders displaying especially high filtration rates, they absorb and accumulate contaminants what makes them good biomonitors of water contamination. Accordingly, mussels may be precious tools to study the effects of polluted sediment resuspension often caused by operations of rehabilitation such as the opening of dams. In this context, we used Dreissenids to monitor the effects of dam opening in the Orne river, that has been strongly marked by the steelmaking industry for decades, even if steel factories are now closed. The restoration operations and specific hydrological events, such as floods, have led and still lead to resuspension of contaminated sediments previously accumulated during the period of industrial activity. During three years, caged mussels were exposed in the Orne river trying to cover different hydrological seasons (low or high water, flooding events) and then different situations of sediment resuspension. On the whole, we observed only weak but significant biological effects in the mussels. The aim of the present work was to confirm in the laboratory the results obtained in the field by exposing mussels to resuspended sediments of the Orne river under controlled conditions. We chose an environmentally realistic exposure scenario and we took care to select particles whose size was compatible with filtration capacity of the mussels. Both species of Dreissenids were exposed 7 days to sediments weakly, highly or not contaminated. The biological responses were measured on digestive gland and gills and were targeted to anti-toxic and anti-oxidant defenses, to cellular toxic effects and to energy metabolism. We observed slight significant variations of biological responses indicating that resuspended sediment particles may have been filtered by the mussels. Indeed, toxic effects (apoptosis and lipid peroxidation), anaerobic metabolism and anti-toxic defenses (glutathione-S-transferase) tended to decrease in the weakly contaminated sediment compared to the highly contaminated one. The level of responses differed between species and organs, but the overall trends remained the same. With the present work, we show that polluted sediments were able to modify the response of mussels reinforcing the relevance of their use in watercourse biomonitoring programs for assessing the health status of the biological compartment.

## Ecotoxicity of novel biosurfactants and corrosion inhibitor polymers as green alternatives for industrial applications

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### Abstract

The bioeconomy strategy pursues the goal of developing biobased and sustainable products as well as intermediates for the chemical industry. The replacement of synthetic chemicals by suitable natural products from biochemical routes is of great importance as synthetic compounds with potential harmful effects for the environment and often based on fossil resources are still widely used. One promising group of biobased products for several fields of application are biosurfactants, which have already been intensively studied and seem to be promising candidates with environmentally more friendly characteristics than the chemical counterparts. Another group of increasing interest but with far less knowledge on environmental consequences are polymer-based products. However, to enable industrial application, further steps such as a comprehensive ecotoxicological assessment must be taken. Due to the high chances for successful industrial process development and the existing industrial demand, the present study focuses on the ecotoxicity of novel biosurfactants and novel polymer-based products. A special field of application for novel biobased materials could be corrosion protection. Corrosion of metal surfaces and machine parts is a global problem. One way to provide temporary corrosion protection is through self-assembled monolayers. However, some are problematic due to their low solubility in aquatic systems and suspected adverse environmental and health effects. As an alternative, work is being done on polymers and biodegradable biosurfactants that cause less harm. Hence, the present study investigates the aquatic ecotoxicity of two novel biosurfactants for different applications named SerrawetinW1 and Liamocin, and novel corrosion protection materials as well as corrosion removers, which include an anionic surfactant and polymer-based materials. Those substances were investigated by means of the aquatic triad (Acute fish embryo toxicity test, Acute immobilization test, Freshwater algae growth inhibition Test). Besides the acute toxicity also selected mechanism-specific toxicity test were performed by using in vitro-based effect-based methods. Preliminary results show that the biobased and biodegradable biosurfactants and the corrosion inhibitors are less toxic than synthetic compounds. In addition, it was found that polymers with lower molecular weight have a stronger toxic effect than polymers with higher molecular weight.

## Impairment of Sensory Organ Development in Petroleum-Exposed Zebrafish Embryos – Lateral Line System and Motor Behaviour Reaction

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### Abstract

Although we are at a turning point in the transformation of the global energy sector, we continue to observe an increasing demand for crude oil in modern society. Through steadily increasing oil production, consumption, transportation, and rapidly moving exploration, huge and small diffuse oil spills still pose a severe risk to the aquatic environment. Fish early life stages exposed to crude oils were found to be highly sensitive. Recent studies provide evidence that in addition to well-known cardiotoxic effects, neurotoxicity also plays a role. In this respect, oculotoxicity induced by oil exposure has been shown, leading to the hypothesis that other sensory organs may also be affected. In this context, the present study aimed to investigate whether water-accommodated fractions (WAF) of native and chemically dispersed crude oil impact the development of the lateral line system (LLS) of developing zebrafish embryos. The involvement of the LLS in a multitude of behavioral traits makes it crucial for survival. We expected a sensitive response to WAF exposure due to the direct contact of LLS hair cells with the surrounding medium. A set of morphological and behavioral endpoints was examined in the transgenic zebrafish line *cldnb:lyn-eGFP*, visualizing the lateral line primordium and neuromast cells. This in turn facilitates tracing of *in vivo* LLS development. Zebrafish embryos were exposed to different WAFs at sublethal effect concentrations that do not induce visible morphological malformations ( $\leq EC_{10}$ ). LLS assessment was conducted by analyzing the number of neuromasts along one side of the embryo's trunk (48-72 hpf). Potential impairment of neuromast structure was quantitatively investigated by DAPI (nuclei)/DASPEI (hair cells) staining (96 hpf). First results indicated a delay in neuromast development and a significantly reduced number of neuromasts. As an endpoint of developmental neurotoxicity spontaneous tail coiling was investigated (24 hpf), resulting in significantly reduced tail coils of oil-exposed zebrafish embryos. To better understand oil-induced effects on embryonic motor behaviour, development of primary and secondary motoneurons was examined using a double-transgenic zebrafish line (24-48 hpf), highlighting caudal primary motoneurons (*nrp1a:eGFP*) in a pan-neuronal reporter background (*xl.a.tubb:DsRed*). Overall, the present results will contribute to a deeper insight into neurodevelopmental toxic mode of actions of petroleum exposure.



## Toxicity of a Novel Bio-Based Nanomaterial with Anti-Corrosion Properties on Different Tropical Marine Species

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### Abstract

The metallic corrosion is the main cause of degradation of materials immersed in seawater. This process is minimized through the application of protective coatings containing efficient corrosion inhibitors (CI). However, such molecules are toxic to the biota requiring their replacement by eco-friendly alternatives with good anti-corrosion efficacy, such as sodium gluconate (SG), a bio-based CI. In the framework of the NANOGREEN project, Zn-Al layered double hydroxides (LDH) have been used to immobilize SG, which can reduce the early leaching of CIs and extend the corrosion protection of metallic immersed substrates over time. Since LDH-SG is a novel anti-corrosion nanomaterial, the present study aims at assessing the toxicity of LDH-SG and its free counterparts (SG; LDH) on ecologically relevant tropical marine species of the sand dollar *Mellita quinquiesperforata*, the mussel *Perna perna*; and the copepod *Nitokra* sp. Organisms were exposed using standard international guidelines with minor adaptations for *M. quinquiesperforata*, and for *P. perna*, in both cases to determine the normal embryonic development, and to determine the effects over the copepod's spawn of *Nitokra* sp. Five exposure concentrations were tested ranging between 0.41 and 33.3 mg/L for *M. quinquiesperforata*, and 1.23 and 100 mg/L for the other tested species (dilution factor=3), plus a negative control (only artificial seawater). The three tested compounds gathered a NOEC of 1.23 mg/L for *M. quinquiesperforata*, and <1.23 mg/L for *P. perna* embryos. Nevertheless, for the copepods the NOEC was 33.3 mg SG/L, 11.1 mg LDH/L and <1.23 mg SG/L (LDH-SG). EC50 values of 37.12 mg SG/L, 5.76 mg LDH/L and 1.37 mg SG/L (LDH-SG) were estimated for *M. quinquiesperforata*, and 3.79 mg SG/L and 0.26 mg LDH/L for *P. perna*. IC50 values of 40.4 mg SG/L, 15.04 mg LDH/L and 0.94 mg SG/L (LDH-SG) were determined for *Nitokra* sp. These results contradict the pattern found for several temperate invertebrate species, i.e., soluble CIs are more toxic than the nanostructured forms. Further studies are advised to confirm the present findings and provide sound information to support the development of truly environmentally friendly CIs suitable for all major marine compartments.

**SensoryTox – The Impact of Crude Oil on Early Sensory Organ Development in Zebrafish**

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**Abstract**

The risk emerging from oil releases to the aquatic environment is constantly given since the demand for crude oil based products such as plastics, chemicals or pharmaceuticals is steadily rising. Substances released to the water phase partition rapidly, thus the immediate application of appropriate measures after an oil spill is of high relevance. One method used is chemical dispersion to increase the transport of oil components into the water column for enhanced biodegradation, however, this method is known to increase acute toxicity to exposed aquatic species. Beyond the well examined acute crude oil toxicity leading to effects on the cardiovascular system of various fish species, recent studies with zebrafish also found evidence for potential neurotoxicity and, in particular, oculotoxicity at much lower concentrations. Within the SensoryTox project we address the impact of crude oil and chemical dispersants on the early sensory organ development in transgenic zebrafish. Therefore, zebrafish embryos were exposed to sublethal effect concentrations (EC<sub>5</sub>) of water accommodated fractions of (I) crude oil, (II) chemically dispersed crude oil, and (III) pure chemical dispersant for up to 120 hours post fertilization (hpf). Immunohistological analysis of zebrafish retina revealed a significant reduction of red-light sensitive photoreceptors and opsins per photoreceptor. Currently the impact on further photoreceptor types is investigated. We also found the lateral line system of exposed zebrafish to be affected. This distant-touch sensory system is involved in a multitude of behavioral traits and consequently crucial for survival. We found a significant decrease in the number of neuromasts after 48 hpf which was recovered after 72 hpf. Additionally, fluorescent staining of the neuromast hair cells revealed an increase in the area and in the number of hair cells per neuromast. The assessment of different behavioral endpoints showed severe effects in the spontaneous tail coiling assay at 24 hpf and in the touch-evoked response assay at 72 hpf. Ongoing work addresses underlying mechanisms of these neurotoxic pathways by focusing on the outgrowth of primary motoneurons and the optic tectum indicating impairments of neurodevelopment and visual perception. The improved understanding of the neurotoxic mode of action of environmentally relevant concentrations of petroleum products contributes to a more realistic projection of threat deriving from crude oil pollution.

## Metabolic and endocrine responses on *Astyanax lacustris* (Teleostei: Characidae) females after diclofenac and ibuprofen waterborne exposure

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### Abstract

The most prescription NSAIDs Ibuprofen (IBU) and diclofenac (DCF) have been detected in aquatic ecosystems and investigations have reported effects on non-targeted aquatic species. Nevertheless, studies on native South American species are scarce, thus the endocrine disruption assessments remain unclear. The scope of this study was to evaluate the effects of IBU and DCF on gene expression, hormones, and energetic substrates related to the reproduction and metabolism of *A. lacustris* females. Fish were exposed to 3.08 mgL<sup>-1</sup> DCF and 13.7 mgL<sup>-1</sup> IBU, based on previous toxicity tests. A total of 96 females were used, 4/aquarium, 4 groups: control (CTL), DCF, IBU, and MIX (DCF+IBU) during 24 and 96 hours. Plasma concentration of 17 $\beta$ -Estradiol (E2), testosterone (T), thyroxine (T4), and triiodothyronine (T3) were measured, pituitary gene expression, as well as the biochemical content of ovaries and liver, were evaluated. The T4/T3 ratio decreased in females exposed to IBU and MIX groups after 96h, but not after 24h of exposure, T/E2 ratio did not display any significant alterations among treatments and exposure periods. Expression of *pomc* and *fsbh* genes were upregulated in the IBU treatment compared to control after 24 h, however, such alterations were not observed on *lhb* and *tshb* genes, neither at 96h exposure when gene expression was not altered comparing treatments against CTL. Regarding the biochemical content, females exposed to DCF and mixture treatments after 24 h showed a decrease in muscle protein content; and an increase in protein liver content when compared to the control. Liver lipids content increased in MIX treatment when compared to control after 96 h, but such responses were not observed after 24h. The lipid and protein content were not affected in the ovaries after 24 or 96h, which was also observed in the liver and muscle protein content after 96 h of exposure. The present data suggests that native teleost species could respond biologically to acute NSAIDs exposure, additionally these responses could be related to endocrine disruption activity of NSAIDs on *A. lacustris* females. Thus, the present investigation displays relevant endpoints to assess NSAIDs endocrine disruption on South American species and risk assessment tools for more investigations regarding the pharmaceuticals toxicology to non-target species.

## Effects of plastic additives on precopulatory pairing behaviour and male infertility of a marine amphipod

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### Abstract

Plastics contain hundreds to thousands of additives reported to be neurotoxic, immunotoxic and endocrine disruptors. Most are not chemically bound and are commonly known to leach into the environment. Triphenyl phosphate (TPHP) is a replacement plastic additive that has now been placed on the international watchlist for evaluation due to its suspected effect on the environment. This study aims to assess the impact of these TPHP on reproductive behaviour and male fertility in the marine amphipod, *Echinogammarus marinus*. We assessed reproductive behaviour by exposing 20 precopulatory pairs of *E. marinus* to varying concentrations of the test compound. Male fertility was investigated by counting sperms of *E. marinus* after 14 days of exposure. High throughput optimization of the precopulatory pairing behaviour was developed, and repairing success was recorded at 15mins and daily for 96 hours. Repairing success was impacted by the presence of the test compounds, even at low concentrations. Animals in the exposed group took a long time to reform pairs, with significant differences in the exposed group for contact time and repairing time. It was observed that an environmentally relevant concentration of 0.5 -5 ug/l significantly impacted preparing time while some of the animals did not reform pairs within the 15mins of initial observation. The percentage of repairing success was 100 % in the control group and ranged between 25 - 80% in the exposed group. Sperm count declined after 14 days of exposure, showing a dose-response relationship. Overall, this study provides evidence that TPHP can impact reproductive mechanisms and sperm count of amphipods even at environmentally relevant concentrations, thus, demonstrating the potential of this endpoint in toxicity testing and its implications on population-level effects.

## Impacts of the cumulative effect of copper exposure and ocean acidification on cold-water octocoral *Viminella flagellum* – a transcriptional approach

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### Abstract

Deep-sea mining is one of the potentially most threatening activities for the marine environment. The mechanical and toxic effects originated by the sediment plumes are described as highly deleterious for the deep-sea organisms[1]. In parallel, the excessive amounts of CO<sub>2</sub> that have been absorbed by the oceans are leading to a decrease in the pH of seawater, named ocean acidification (OA). This phenomenon is expected to severely affect seawater chemistry (e.g., trace metal speciation) restricting the calcification of marine organisms and favouring erosion of habitats created by marine calcifiers, such as Cold-Water Coral (CWC) species[2]. Characteristics of long living and slow growth make CWC particularly vulnerable to anthropogenic impacts[3]. The octocoral *Viminella flagellum* is one of the most important structuring species in the Azores[4]. The polymetallic sulfide (PMS) particles originating from mining activities plumes have adverse consequences for *V. flagellum* communities, such as the polyps smothering and clogging and the toxic effect of copper (Cu) which is one of the most released metals from the PMS particles[5]. How can vulnerable CWC overcome the cumulative effect of Cu exposure, under a mining activities scenario, and OA? The results show a Differential Gene Expression (DGE) between treatments. Our experimental results indicate that is Cu exposure that modulates *V. flagellum* gene expression rather than seawater acidic conditions. Also, the cumulative effect of both seems to suppress most of the genes expressed under non-cumulative treatments. In this work, we discuss the molecular effects of the interactive effects of anthropogenic activities on CWC and the importance of identifying genetic biomarkers of physiological stress for future studies of environmental risk assessment[6].

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## Investigating priority effects in the response of benthic biofilm communities to the stress of artificial light at night

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### Abstract

Light pollution has emerged as an escalating problem in contemporary society. Microalgae, critical for primary production and freshwater health, are vulnerable to this issue. While numerous studies aim to enhance our understanding of biofilm succession and community composition, the processes involved in benthic freshwater biofilm community assembly and maintenance remain poorly understood. In this study, we investigated the potential influence of artificial light at night (ALAN) on community composition. To explore this, we allowed one set of ceramic tiles to colonize under natural conditions and while another set of tiles got subjected to light stress (ALAN treatment) in eight flumes at a stream mesocosm site (RSM) in Landau, Germany. After a colonization phase, we switched half of the tiles between the treatments. Over a 28-day sampling period, we examined changes in the community composition of total cyanobacteria, diatoms, and green algae using a BenthosTorch (bbe moldaenke, Germany) to measure total Ch-a (chlorophyll-a) in  $\mu\text{g per cm}^{-2}$ . We hypothesized that light stress would impact the initial community composition due to the differential responses of the three taxa to light stress. Additionally, we anticipated the occurrence of priority effects, particularly in the group transitioning from light stress to the control. Our results revealed significant temporal effects and suggested the presence of priority effects. However, due to the limitations of the BenthosTorch method and its inability to measure species-level variations, we want to use DNA-metabarcoding for frozen samplings to gain deeper insights into the effects at a species level. Biofilm communities offer valuable insights into fundamental ecological theories. And yet, to the best of our knowledge, no studies have examined priority effects in stressed biofilm communities. Overall, more research is needed to comprehensively understand the structure and ecology of biofilms. This study provides an initial investigation into priority effects in stressed biofilm communities, offering a basis for further examination. Understanding these communities contributes to the verification of foundational theories in community ecology, with biofilms serving as excellent models due to their complex composition within a confined space.

## Does Benzophenone-3 Cause Any Oxidative or Estrogenic Effects in Juveniles of *Octopus maya*?

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### Abstract

Benzophenone-3 (BP-3) is an active ingredient which is widely used in sunscreens, different types of personal care products, as well as in different types of plastics due to its UV absorption properties. However, the ecotoxicity of BP-3 and its threats to wildlife have recently garnered worldwide attention and concern, because of its lipophilic ( $\log K_{ow}=4.0$ ), bio-accumulative, and photo-stable potential. Consequently, this compound has frequently been detected in the environment and biota.

Due to the intensive use of BP-3 aquatic and marine environments are easily polluted. Previous studies reported estrogenic and oxidative effects in some aquatic organisms. However, there is a lack of information about the effects on marine animals such as octopus. In the southeastern of Mexico, one of the most economically and ecologically important octopus specie is *Octopus maya* which is in the currently affected by climate change.

In this study we evaluated the estrogenic effects through the expression of vitellogenin (VTG) as a sensitive biomarker to test estrogenic properties of xenobiotics. Oxidative effects through the measurements of different biomarkers and the activity of Esterases in juveniles of *O. maya*. To carry out this experiment, animals were exposed to control (marine water), control solvent (0.01% DMSO), positive control (estradiol, E2) and 5, 50 and 500  $\mu\text{g/L}$  of BP-3 during 48 h. After that time, we obtained muscle to evaluate oxidative stress and Esterase's activities and hepatopancreas to evaluate estrogenic effects.

In this work, we found that BP-3 does not show estrogenic effects in contrast with E2 ( $p < 0.05$ ) which induce the VTG expression. We found that BP-3 cause oxidative stress in 50 and 500  $\mu\text{g/L}$  treatments ( $p < 0.05$ ) (nominal concentrations). In the Esterases activities measurements, we did not find significative differences ( $p > 0.05$ ) in any of the treatments.

Oxidative stress was reported as the leading cause of BP-3 induced damage in corals, marine bivalves, and freshwater fish. The findings in this work provide valuable ecological information for this important species. Because it has been reported that the load of oxidative stress is inherited from mothers to their offspring and it is expressed with low survival, depressed routine resting and high metabolic rates which can be exacerbated with the exposure to BP-3 in their natural environment.

**Barrier Function of the Marine Medaka (*O. melastigma*) Chorion**

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**Abstract**

The marine medaka, *Oryzias melastigma*, has been identified as a promising model species for marine ecotoxicology and shares many advantages for early life stage testing with the well-established freshwater model species zebrafish (*Danio rerio*). Therefore, the standardized Fish Embryo Acute Toxicity (FET) test has been successfully adapted to *O. melastigma* within previous work. To allow a comparison to other model species, different species-specific aspects such as development, metabolism and sensitivity have to be considered. In this context, the present study focused on the chorion barrier function. The protective role of the chorion as a physical barrier has already been identified as an important factor for the uptake of chemicals and thus the resulting toxicity. As the chorion of medaka is about 10x thicker compared to zebrafish, this might be an indication for an increased barrier function resulting in deviating uptake kinetics. In a first experiment the influence of solvents (DMSO) on the chorion permeability and thus, the compound uptake, was investigated in *O. melastigma* and *D. rerio* using fluorescent dyes. Our results confirmed previous findings that solvents increase the chemical uptake and supported the hypothesis that the medaka chorion provides a stronger barrier compared to zebrafish. Furthermore, structural properties of the chorion were analyzed using scanning and transmission electron microscopy (SEM, TEM) with particular interest on the property of the pore canals and the overall chorion surface conditions after the commonly applied removal of villi as a pretreatment step within FET. In this context, also the molecular weight as a relevant criterion for chorion passage was evaluated. To identify the molecular weight threshold for the transport of compounds through the pore canals, medaka embryos were exposed to solutions of polyethylene glycols (PEG) with different molecular weights. Solutions were prepared in hyperosmotic concentrations, resulting in an immediate loss of fluid and shrinkage of the chorion. Eggs exposed to PEG solutions containing glycols  $\leq 4000$  Da were able to regain the original shape and equilibrium by uptake of surrounding solution. Hence, the higher molecular weight PEG solutions were most likely not able to pass the chorion. Additionally, an enzymatic dechoriation procedure was optimized to further investigate the role of the chorion and to extend the potential for early life stage testing with the marine medaka.



## Ecotoxicological Characterization of Peracetic Acid in Freshwater and Marine Water.

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### Abstract

Peracetic acid (PAA) is a powerful oxidant compound, widely used as bactericidal, fungicidal and sporicidal. Due to its characteristics, such as stability and oxidant capacity, it is largely used in disinfection of wastewater treatment plants effluents. One of the main advantages of using PAA is the absence of persistent toxicity and of toxic by-products. PAA is usually commercialized as technical mixture, frequently associated with acetic acid (CH<sub>3</sub>COOH) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as boosters to enhance disinfectant action and stabilizers. The present study aims at evaluating the ecotoxicological effects of a technical mixture of PAA (Sanacet®) and investigating the contribution to toxicity of its single components in fresh and marine water. A battery of marine and freshwater bioassays was used for the assessment of ecotoxicity, as follows: inhibition of bioluminescence in *Aliivibrio fischeri* (marine and freshwater protocol), inhibition of growth rate on microalgae (freshwater: *Raphidocelis subcapitata*; marine water: *Dunaliella tertiolecta*), acute toxicity test on freshwater crustacean *Daphnia magna*, acute and chronic toxicity test on marine water crustacean *Artemia salina* and spermioxicity and inhibition of larval development tests in the serpulid polychaete *Ficopomatus enigmaticus*. For each assay, EC/LC<sub>10</sub>, EC/LC<sub>50</sub>, NOEC and LOEC values were calculated. Sanacet® and PAA as single component showed more pronounced toxic effects with freshwater organisms than marine ones; EC<sub>50</sub> values of for almost all organisms, with the exception of *A. salina* (Sanacet® and PAA), *D. tertiolecta* (Sanacet®) and *R. subcapitata* (PAA), resulted below the threshold of 1 mg/L, which represents the Italian threshold of chemical solvents in wastewaters discharges. Toxicity of the other single components (H<sub>2</sub>O<sub>2</sub> and CH<sub>3</sub>COOH) resulted of scarce relevance, indicating PAA as the main component contributing to the overall toxicity of the technical mixture. An HPLC method together with a spectrophotometric one were used to assess the degradation kinetics of PAA in the technical mixture both in natural freshwater and marine water. Both methods showed a rapid degradation kinetic of PAA in both tested matrices. The results supported the applicability of this compound for the potential direct use in natural water bodies in order to control microbiological load deriving from urban wastewater, or other point sources of fecal contamination.

## Personal Care Products Ecotoxicity in Marine Environments: Different Formulations and Global Change Scenario.

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### Abstract

Each of us uses personal care products (PCPs) every day. Two of the most used products are shampoo and conditioner. There are thousands of these products on the market, varying in composition, fragrance or purpose. Some manufacturers produce different lines of the same product, such as the international brand considered in this study (here reported as anonymous trademarks), which produces shampoo and conditioner from the same cosmetic line in vegan and non-vegan forms. Ecotoxicological effects of these products could be significantly different and global change could affect species' responses due to changing of environmental drivers (i.e., pH, salinity, etc.). Four PCPs are analyzed to determine the impact on aquatic trophic webs (*Aliivibrio fischeri*, a bacterium, *Phaeodactylum tricornutum*, a producer; *Paracentrotus lividus*, a consumer) under standard and modified environmental conditions. PCPs tested were dissolved in different seawater matrices at different pH and salinity (S) conditions representative of standardized and changed values testing at all five different conditions (pH 8.0; S = 34 PSU, S = 36 PSU, and S = 38 PSU; and S = 34 PSU, pH = 7.8; pH = 7.5) simulating the effect of global change on pH and salinity. Concentration tested were 0.001 mg/L, 0.1 mg/L, and 1.0 mg/L. The objectives of this study were to define: i) the potential toxicity of PCPs in marine ecosystems in the current scenario; ii) the potential toxicity of PCPs in marine ecosystems in the global change scenario; iii) the difference in marine ecotoxicity between the "old" formulation of PCPs and the "new ethically sustainable" formulation.

## Identification of Frequent Co-Exposure Chemical Combinations in Adult Women and Assessment of Their Effects on Thyroid Hormone Disruption in Embryo-Larval Zebrafish (*Danio rerio*)

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### Abstract

Currently, numerous chemicals are used in consumer products, resulting in simultaneous exposure to multiple chemicals through various routes. However, research on the toxicity of chemical mixtures is limited, as most studies have focused on individual substances. One of the crucial issues in mixture research is identifying combinations of chemicals to be investigated. Since the number of possible chemical combinations is overwhelming, a process of finding crucial chemical combinations by identifying chemical exposure patterns is needed to make them manageable.

To address this challenge, our study aimed to identify frequently co-exposed chemicals in humans. As quantity alone does not determine the health effects, the study proposed to apply toxicological criteria to identify toxicologically significant frequent combinations. Furthermore, the study aimed to experimentally verify the toxicity of selected combinations.

Using data from the National Health and Nutrition Examination Survey (NHANES) program, we converted chemical concentration in urine samples from adult women into daily intake amounts. Subsequently, thresholds based on the reference dose were used to discretize the daily intake. Association rule analysis was conducted using discretized data, considering toxicological criteria, to generate a list of toxicologically significant frequent combinations of chemicals.

Among 298 premenopausal women aged 20-44 years in the NHANES 2015-2016 program, a total of 17 target biomarkers were analyzed, including environmental phenols (BP3, BPA, BPS, PrP, MeP), phthalates (MCNP, MCOP, MECPP, MHiBP, MBP, MEP, MEHHP, MiBP, MEOHP, MBzP), and flame retardants (BPP, BDCPP). After adjusting the indicators from the association rule result, a combination of tris(1,3-dichloro-2-propyl) phosphate (TDCPP) and diisononyl phthalate (DiNP) was selected for experimental validation.

Concentrations for preparing the mixture were determined through individual experiments and the mixture and single chemicals were exposed to zebrafish embryos to evaluate thyroid hormone disruption effects. The mixture, including each chemical, significantly affected thyroid hormones including TSH and related genes. However, it was too vague to identify interactions between the individual chemicals within the mixture.

In conclusion, our study proposes a novel approach for selecting mixtures considering toxicity and is expected to contribute to future research on mixture toxicity and epidemiology.

## Aquatic Ecotoxicity of Safe and Sustainable by Design Organophosphate Flame Retardants

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### Abstract

Organophosphate flame retardants (OPFRs), common polymer additives, were introduced as replacements for polybrominated diphenyl ethers following concerns about environmental and human health effects. However, today OPFRs have established themselves as notorious environmental contaminants. A widely used OPFR, tris(2-chloroethyl) phosphate (TCEP) is frequently detected in a range of environmental matrices, especially fresh water, meets classification as very persistent and exerts toxicity to aquatic organisms.

To mitigate these adverse effects from the very start of the chemical's life cycle, TCEP has been structurally redesigned as a case study of putting the Safe and Sustainable by Design concept into practice. The alternative design is based on a computer-aided framework developed in house. The aim of the redesign was to retain the flame-retardant properties of the compound but minimize adverse environmental effects, taking persistent, bioaccumulative, mobile and toxic properties into consideration. A multicriteria analysis of millions of structures similar to the original chemical generated in silico, yielded a set of structures predicted to be the most benign, informing the design of a structural alternative for TCEP.

To assess the environmental safety of the newly designed compound, as well as the viability of the computer-aided design approach, the alternative chemical was synthesized and together with the original compound, subjected to a series of aquatic ecotoxicity tests. To gain possible clues about the relationship between ecotoxicological effects and structural trends of OPFRs, we also tested the structural intermediate tris(ethyl) phosphate, which is currently used as a polymer additive. This poster will present the acute and chronic effects of this set of structurally related OPFRs on three aquatic model organisms: the alga *Raphidocelis subcapitata*, the daphnid *Daphnia magna* and larvae of the insect *Chironomus riparius*, which are being determined following OECD test guidelines. Results gathered thus far show the predictions employed during the alternative compound design (US EPA EpiSuite Ecosar), to be reliable, with the proposed alternative compound being less toxic to all test organisms. These findings support the Safe and Sustainable by Design concept and precautionary approach to include extensive testing of additive chemicals to prevent adverse effects on aquatic ecosystems.

## Combined Effects of Chemical Mixtures and Temperature on *Daphnia magna*

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### Abstract

In the environment, chemicals are often present as mixtures and act in concert with additional stressors, such as temperature and other physicochemical factors, which may influence the chemical exposure effects. However, assessing the toxicity of chemical mixtures is a challenging task, particularly, when the interactive effects of the chemical exposure with other stressors are of interest. Here, we used a novel combination of the chemical activity concept and degree-days method to evaluate interactions between the chemical exposure and thermal stress in model organism *Daphnia magna*. The chemical activity approach simplifies the determination of mixture toxicities for a mixture of organic contaminants, with single activities being additive in the mixture and narcosis occurring between activities of 0.01 and 0.1. We hypothesized that in the acute toxicity test with a chemical mixture in this activity range and under different temperatures: (1) lower median lethal activity (La50) occurs at a higher temperature if the exposure time is defined in calendar days (72 h) because of the temperature effects on the metabolic rate and time-to-toxicity, and (2) there is no temperature effect when the exposure time is defined in degree-days (60 DD) representing the physiological time at different temperatures. A mixture of four polycyclic aromatic hydrocarbons (acenaphthene, fluorene, fluoranthene, and phenanthrene) was tested using a passive dosing system and two temperature regimes (20 and 25°C) in an acute toxicity test with *D. magna*. The experimental results will serve to develop recommendations for the implementation of the chemical activity approach in the hazard assessment of organic contaminants in environments with variable thermal regimes.

## Toxicity of Individual and Combined Effect of Mefenpyr di-Ethyl Safener and its Co-Herbicide, Fenoxaprop-P-Ethyl, to *Daphnia magna*

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### Abstract

Herbicides and safeners have been formulated together to help protect crop plants from the injurious effects of herbicides while maintaining the ability of the herbicides to selectively remove the targeted weeds. Safeners are classified as inert for regulatory purposes, and despite their presence in surface water and their ability to move around, there is limited data regarding their toxicity to non-target organisms in aquatic environments. Thus, the study aimed to assess the toxicity of Mefenpyr diethyl (MEF), an emerging safener, and its co-herbicide Fenoxaprop-p-ethyl (FEN), used in pre- and post-emergence of grains, on *Daphnia magna*, a non-target cladoceran invertebrate. The evaluation of these chemicals is conducted both individually and in combination using an in vivo method. This allows for the assessment of acute, sublethal, and chronic effects. The studies were carried out with true replicates for each concentration, with every daphnid exposed to a 25ml chemical solution in a glass vial. The experiments were conducted for four days to assess acute impacts, with survival being the primary endpoint. Meanwhile, a 21-day exposure was observed for sublethal and chronic effects to consider the influence on reproduction and its related endpoints. Results from the study showed sublethal and lethal effects on the organisms exposed to MEF and FEN singly and in a mixture at environmentally relevant concentrations. A dose-dependent decrease in neonates and broods per adult was observed. The results highlight the potential dangers of these substances in the environment. The analysis reveals a biphasic trend in the reproduction of *D. magna* exposed to MEF, suggesting that MEF could potentially disrupt the endocrine system as a chemical. Mixture studies showed intra-additive reactions on endpoints, including survival. The mixture effects provided relief from adverse effects, indicating protection for the exposed animals. This study discovered that while safeners help reduce the negative effects of herbicides on animal models, they still have sublethal effects that are cause for concern. Additionally, safeners previously deemed inert should be re-evaluated for their potential toxicity. This study helps to further advance the study of mixture toxicities.

## Pharmaceutical Contamination in European Aquatic Environments: Implications for Fish and Shrimp Health

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### Abstract

There is widespread pharmaceutical contamination in the aquatic environment, raising concern over aquatic health. Fish and shrimps are key inhabitants of aquatic ecosystems and are particularly vulnerable. They can accumulate these compounds in their tissues through water, dietary and sediment uptake, leading to potential effects on humans who consume them. Therefore, this study examines the status of pharmaceutical contaminants in European aquatic environments, focusing on their major sources, hotspots, impacts on fish and shrimp, and potential mitigation strategies. This study employed a multidisciplinary approach, integrating findings from field surveys, laboratory analyses, and data syntheses to assess emerging pharmaceutical contaminants' status in European aquatic environments. This study indicates that hospital discharge, sewage and wastewater treatment plants (WWTP), and pharmaceutical effluents constitute the main sources of contamination. River catchments of Spain, Bulgaria, Luxembourg, the United Kingdom, Belgium, Northern Ireland, Germany, and Austria indicate higher pharmaceutical contamination than in other European countries. Furthermore, findings indicate that pharmacological exposures can alter fish and shrimp metabolism, reduce the immune-mediated response, alter the functioning of the endocrine system, and affect growth, reproduction and behaviour. Alternative add-on systems to conventional wastewater treatment facilities are being developed to mitigate these impacts of pharmaceuticals and reduce their effects on fish and shrimp. Moreover, bioremediation systems such as mycoremediation have been proposed, which offer the great benefit of chemical transformation of pollutants rather than shifting them from one environment to another. However, their effectiveness varies with the type of pharmaceuticals, their concentration, the microbial community of the system, and environmental conditions. Thus, it is necessary to consider redesigning pharmaceuticals to increase their metabolism in the body and minimise their toxicity in aquatic environments. Legislative strategies through policy development by the European Commission to reduce the entry of pharmaceuticals into rivers will advance sustainable management practices and protect these vulnerable ecosystems.

## Session 3: Computational Ecotoxicology & Alternative to Animal Testing

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### Assessing Molecular Initiating Events *in vitro* to replace animal testing.

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#### Abstract

Concerns about endocrine disruption due to exposure to xenobiotics have led to the introduction of effects-based substance testing in the field of environmental legislation. These methods (OECD 229, 230, 234, MEOGRT) use fish as vertebrate models. The utility of testing strategies that require both the MIE (molecular initiating events) and the corresponding AOP (adverse outcome pathway) for individual test substances is questionable. Based on comparative AOP studies in laboratory model fish and in riverine and marine animals with longer life spans and more pronounced abilities for tissue regeneration and repeated gonad recruitment, we identified a cell type that appears to be critical for healthy seasonal gonad development and tissue regeneration. Using a targeted procedure, we isolated non-tumorigenic, self-renewing cell lines from the brain of *Cyprinus carpio*. A constitutive histone2B-GFP fusion protein-expressing transgenic variant of these cell lines behaved like regenerating gonadal cells. Based on transcriptome data, the cells correspond to mammalian mesenchymal stem cells. They are therefore suitable for providing initial insights into the mechanisms of action, carcinogenicity and cytostatic effects of the test substance used. These initial data can be used to replace inaccurate animal testing methods. With a database of over 40 000 annotated genes, various molecular biological endpoints can be studied. The H2B-GFP transgenic variant is used for micronucleus-based genotoxicity assessment as a surrogate for OECD 487. A battery of transgenic variants with different mechanisms of action is currently under development. For example, with respect to the endocrine effects of estrogens, a transgenic variant that transactivates an NLS-GFP fusion protein driven by an estrogen response element shows promise. This construct allows visualization of estrogen receptor activation *in vitro* at the single cell level. The technique allows monitoring of exogenous activation of the intrinsic estrogen receptor using high-throughput live cell imaging. Alternative test systems only provide *in vivo* insights and are therefore no longer up to date under the concept of the three Rs. The comprehensive characterization makes the cell lines ideal for MIE imaging, contributing to the replacement of animal experiments.



## Transcriptomic Profiling of Clobetasol Propionate Induced Immunosuppression During TLR-7-Dependent Immune Challenge in Zebrafish Embryos

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### Abstract

To safeguard human and environmental health the approval of new compounds is subject to strict regulations and an extensive toxicological testing to predict its hazard potential. Although a correlation between environmental contaminations and an impaired immunocompetence of wildlife populations is confirmed by numerous studies, the detection of immunomodulative modes of action (MoA) is currently not covered in this regulatory framework. This is not least due to the complexity of the immune system and a lack of standardised methods and validated biomarkers.

In this study, the transcriptomic profiles of zebrafish embryos in response to the immunosuppressive compound clobetasol propionate (CP), a synthetic glucocorticoid, or / and the immunostimulatory compound imiquimod (IMQ), a TLR-7 agonist, were thoroughly analysed with the goal to validate reported and identify novel biomarkers for immunotoxic MoA. Based on the gathered data, the suitability of previously proposed genes such as *slc16a9*, *lyve2*, *nr1d1*, *socs3*, *nfkbia*, *anxa1c*, *fkbp5* and *irg1l* as possible biomarkers for immunotoxicity was substantiated. Likewise however, several of these genes were unmasked unsuitable to reliably distinguish between a suppressive and stimulatory fashion of action. Based on a differential regulation in opposite directions in response to both tested compounds, *krt17*, *rtn4a*, *and1*, *smhyc1* and *gmpr* were identified as novel potential biomarker candidates with said power to differentiate.

With IMQ as a reference substance, known for its potential to induce psoriasiform effects, another main goal of this study was to evaluate the suitability of the zebrafish embryo as a more 3R conform alternative for the IMQ-induced psoriasis mouse model. Observed IMQ-induced alterations in the expression of genes associated with inflammation and the pathogenesis of psoriasis such as *krt17*, *nfkbia*, *mpeg1.2*, *irg1l*, and *anxa1c* substantiate the suitability of the zebrafish embryos as a said model. Elevated IMQ-induced expression of *cyp1a* further provides novel insights corroborating the translatability of IMQ-induced effects between zebrafish and human as an important aspect in the prediction of psoriatic adverse effects during the development of new pharmaceutical agents.

These findings will hopefully assist in the development of reliable adverse outcome pathways to predict the immunotoxic hazard potential of chemicals, usable in the process of regulatory decision making in the future.

## Development and Evaluation of Simplified Machine Learning Models for the Prediction of Sorption Coefficients for Ionisable Pharmaceuticals in Soils and Sludge

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### Abstract

Pharmaceutical compounds have been repeatedly identified in the environment, many of which have the potential to be considered contaminants of emerging concern, with a limited understanding of their environmental toxicity or fate. The focus of pharmaceutical fate research thus far has primarily been the aquatic environment, however, there are concerns over the potential risks in the terrestrial compartment through exposure to sludge as a soil amendment and reclaimed water irrigation. At present there is a lack of reliable predictive fate models for ionisable organic compounds, including pharmaceuticals, in terrestrial systems. This study aims to fill this knowledge gap by developing existing machine learning models for the sorption of pharmaceuticals to sewage sludges as well as soils. These models include random forests and artificial neural networks, which have demonstrated powerful predictability for the sorption of ionisable pharmaceutical compounds in soil, improving on regression-based approaches. This study, will build on these models and apply them to model sewage sludge sorption, thus refining exposure pathway predictions for pharmaceuticals in the terrestrial environment. Sludge sorption studies, including pharmaceutical compounds identified through the application of a framework in a previous prioritisation exercise, will be exploited to train new simplified machine learning approaches. Priority compounds cover a broad range of physicochemical properties including acids, bases and neutrals as well as zwitterionic compounds, several of which have been previously identified in the terrestrial environment such as, carbamazepine, metformin, diclofenac and ibuprofen. Model outputs for priority compounds will be used to inform further work on pharmaceutical fate in soils, as well as uptake into soil dwelling organisms and the potential for secondary poisoning at higher trophic levels.

## Performance and Robustness of General Unified Threshold model of Survival (GUTS) Models on Acute and Chronic Stressor Datasets

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### Abstract

Mechanistic effect models help to understand the effects of stressors, like pesticides, on the behaviour and survival of affected species. The General Unified Threshold Model of Survival (GUTS) of Jager et al. (2011) managed to unify many existing toxicokinetic-toxicodynamic (TKTD) models into a consistent framework to analyse stressor impacts on individual survival based on laboratory test data. Since 2018 it is recognized and adopted by the European Food Safety Authority (EFSA) as a regulatory risk assessment method for aquatic species. The GUTS model family consists of multiple model variations fit for different purposes. Commonly used are two reduced (GUTS-RED) variations relating the death mechanism to either stochastic death (SD) or individual tolerance (IT).

Performance of the two variations is thus compared between different datasets with acute and chronic exposures based on model accuracy and parameter robustness. Parameter robustness is here interpreted as reproducibility of fitted parameter values on comparable datasets. It is calculated using leave-one-out cross validation (LOOCV) by comparing the spread of parameters values during the calculation.

Accuracy results show both variations performing comparably in chronic (aquatic) exposure scenarios, while the SD model outperforms the IT model in acute (terrestrial) scenarios. Parameter robustness seems to vary considerably between chronic and acute datasets, suggesting other model variations may be required when working with acute exposure datasets.

## Temporally extended occurrence of pesticides based on monitoring databases – Knowledge from Europe

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### Abstract

Pesticides - entering surface water bodies through a variety of routes, e.g., runoff, spray-drift or leaching - can pose significant risks to non-target aquatic organisms. While these risks are typically assessed either on acute or chronic bases, knowledge about pesticide exposure patterns in aquatic ecosystems and influences of pesticide applications and environmental behavior is critical for defining and validating assumptions within ecotoxicological testing. Large-scale monitoring data offers a broad base for the assessment of aquatic exposure through the analysis of sequential concentrations, which can serve as reference for the exposure behavior of pesticides through the inclusion of common assumptions and knowledge about their environmental behavior (e.g., persistence). We therefore explored the probability of re-occurrence (POR) of pesticides in European streams over short- (4 - 7 days) and longer-term (8 - 30 days) periods, in other words, their propensity to occur at measurable concentrations over periods of time exceeding the acute exposure duration of 96 hours (4 days). Publicly accessible European pesticide monitoring data were compiled and processed to derive pesticide concentration sequences, forming the foundation for substance-specific PORs for about 360 substances, ranging from < 1 - 100 %. By comparing short- and long-term PORs, three categories were found, describing a majority of pesticides: (1) occasionally, (2) repeatedly and (3) continuously reoccurring substances. Fungicides were dominating repeatedly reoccurring substances, while neonicotinoid insecticides and legacy compounds were among pesticides continuously reoccurring. Challenging existing frameworks of persistence and effect assessments, PORs are linked to substance-specific factors (e.g., physico-chemical properties, application recommendations and regulations), determining the nature of pesticide exposure of aquatic environments. While future research needs to further resolve influencing mechanisms at underlying scales (e.g., geographic level), this study highlights the necessity to assess pesticide exposure in consideration of consecutive concentrations, advancing the comprehension of realistic exposure potentials of aquatic ecosystems on a substance basis.

## Environmental Residue Behavior of Imidacloprid in Peanut Cultivation System and Its Dietary and Ecological Risk Assessment

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### Abstract

Imidacloprid, a neonicotinoid insecticide with high effectiveness, broad spectrum, and low toxicity, is widely employed in crop pest control. Improper use of imidacloprid may result in excessive residues, posing a safety risk to human health and causing high toxicity to environmental organisms like bees. In these studies, imidacloprid FS 600 was used as a seed treatment for peanuts to control soil pests and protect the plants during the vegetative stage from sucking pests, with special attention paid to protecting nontarget organisms like bees. Field experiments investigated the residue behaviour of imidacloprid in the peanut fields in Shandong, Henan, and Anhui Provinces in China. In the residue behaviour experiment, imidacloprid was applied once at a high dose (1.5 times the recommended dose), and samples were collected at different time points for residue determination. The results showed that the first-order degradation kinetic model described the dissipation dynamics of imidacloprid in peanut field soil. The half-lives of imidacloprid in peanut plants and soil were 21.0 ~ 46.2 days and 10.3 ~ 30.1 days, respectively. In the final residue experiment, low dose (recommended dose) and high dose (1.5 times the recommended dose) were applied once. Peanut and soil samples were collected at harvest time to detect the final residues. The results showed that the average residue of imidacloprid in peanuts was less than 0.05 mg/kg. Peanuts' highest average residue of its major metabolite, 6-chloronicotinic acid, was 0.344 mg/kg. Furthermore, the maximum average imidacloprid and 6-chloronicotinic acid residues in peanut field soil were 0.370 and 0.716 mg/kg, respectively. The results of the dietary risk assessment showed that the chronic dietary risk quotient (RQ) of imidacloprid for the general population in peanuts was 39.82%, which was less than 100%, indicating that imidacloprid would not pose an unacceptable risk to human health. This study used different earthworm species as ecological risk indicators. The ecological risk of imidacloprid was measured using the ecological risk quotient (RQ) and the toxicological exposure ratio (TER). The results showed that imidacloprid posed a low-to-moderate risk to earthworms in the soil of peanut fields in China. Therefore, special care should be taken when using imidacloprid in soil because it is vulnerable to non-target soil organisms.

## From facades to groundwater – modeling of biocides at district level

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### Abstract

Biocides used in paints and renders wash off from facades during wind driven rain events and end up in the aquatic environment including groundwater where they have adverse effects on organisms. However, little is known about the entry of biocides into groundwater at the district level, although this knowledge is important to evaluate potential environmental impacts. The aim of this study is to quantify biocide inputs to groundwater, preferential input locations, transport and degradation of biocides in a district of the city of Freiburg, Germany. The study area (38ha) contains residential buildings built within the last two decades and connected to a sustainable urban drainage system. Several groundwater monitoring wells exist due to a chlorinated hydrocarbon (CHC) contamination site. Three selected biocides (terbutryn, diuron, octylisothiazolinone) and four transformation products of terbutryn and diuron were measured in low ng/L concentrations on nine occasions between 2015 and 2022. Other available data include more than a decade of continuous groundwater level data and biannual measurements of CHC concentrations in the groundwater. These measured data form the basis of our modeling approach, which combines three models: a biocide emission model, a rainfall-runoff-model and a groundwater model. The modeled biocide emissions and the groundwater recharge form the input to the groundwater model, which calculates biocide leaching and transport to groundwater, calibrated with CHC data. Terbutryn is chosen as the model compound because it has been continuously detected in groundwater. The model results confirm that terbutryn enters the groundwater via stormwater infiltration systems as well as other entry pathways such as soils adjacent to facades or permeable pavements. Modeled and measured data for biocides in groundwater are in good agreement when limited retention in stormwater infiltration systems is assumed, confirming experimental data. The model provides insight into the potential reduction of biocides by calculating scenarios for different levels of biocide application. The model allows to determine diffuse losses due to sorption, which are difficult to measure. Although stormwater infiltration systems are designed to retain pollutants, they do not work for all substances. Therefore, biocides should be explicitly considered in the design of swales. Reduction at source is necessary to avoid potential environmental impacts.

## Comparing Simple Fate Model Estimates to Data from Surface Water Monitoring for the Identification of Important Input Parameters

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### Abstract

Predicted environmental concentrations (PEC) resulting from mathematical fate models can be used to estimate the quantities of occurrence of pesticides and other chemicals in the environment, for example in surface waters. There are plenty of chemical fate models that differ, amongst others, in the detail of their process descriptions. The FOCUS TOXSWA model, as the standard model used in the EU for pesticide registration, is using a high level of complexity for the process descriptions, specifically concerning the sediment compartment. In contrast, more parsimonious models with less detailed process descriptions exist that need less input parameters. An example for such simple models is the PEC-CKB model developed by the Swedish University of Agricultural Sciences. It calculates chemical concentrations in surface water based on a single equation and is, therefore, much simpler than TOXSWA as a dynamic process model. Using the simple estimation approach, PEC derived from the PEC-CKB model fitted well to measured environmental concentrations (MEC) from a Swedish monitoring dataset. Still, the PEC-CKB model need to be validated with MEC from an independent study. We compared predictions of the PEC-CKB model to MEC from a lowland stream monitoring study in Germany (Kleingewässermonitoring) to investigate the suitability of the model under German conditions. In addition to testing the original model, modifications and the impact of substance- and site-specific parameters such as Koc values, application doses and periods, and landscape characteristics, were analyzed concerning their impact on the PEC calculations. With this work, we aim to refine our understanding of the relationship between process details and predictive accuracy, and to identify relevant processes and parameters.

## Multivariate Time-Series Analysis for Sensitive Evaluation of Behavior Data From the Light/Dark Transition Test With Zebrafish Larvae

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### Abstract

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Behavior is the result of the complex integration of information from an organism's internal state and its environment. Biotests that utilize behavior endpoints are often especially sensitive to detect environmental toxicants, which is why the Light/Dark Transition test with zebrafish larvae has been increasingly popular in ecotoxicology research. One mayor challenge for a more standardized application in ecotoxicology is replicability of the results. As the data generated in this test are multivariate time-series, statistical analysis is not straight forward.

Data from untreated fish was analyzed in order to increase the understanding about control variability and propose validity criteria that ensure comparability and repeatability of the results. Therefore, an analysis algorithm was developed that allows for consideration of multiple aspects of behavior simultaneously, while preserving information about their development over time.

First results indicate that this can increase sensitivity of the statistics and facilitate the differentiation of different types of behavior changes. This could increase the tests usefulness for applications like biological early warning systems and allow for a development towards an application in effect directed analysis.



## The effect of food level on individual and mixture toxicity of prochloraz and benzalkonium chloride in *Caenorhabditis elegans*: Confirming the applicability of a dynamic energy budget model

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### Abstract

Understanding mixture toxicity and multiple stressor effects is one of the great challenges that ecotoxicologists are facing. Next to classical dose-response analyses and EC50 values, state of the art toxicokinetic/toxicodynamic (TKTD) models and research on their potential application in prospective environmental risk assessment (ERA) are on the rise.

One of these models, DEBtox, has been identified by EFSA as having “great potential for future use in prospective ERA for pesticides”. Dynamic energy budget (DEB) models provide a comprehensive framework that considers the energetic processes and resource allocation in organisms. By incorporating TKTD information, DEB models can assess the combined toxicity of multiple chemicals. Furthermore, DEB models can incorporate sublethal endpoints which provide information on the physiological, biochemical, and cellular processes that may be affected by stressors. This mechanistic understanding is beneficial when trying to comprehend how pollutants interact with biological systems.

The aim of this study was to confirm the applicability of a dynamic energy budget model while characterizing the effect of food level on individual and mixture toxicity of prochloraz and benzalkonium chloride in *Caenorhabditis Elegans*. Several time-resolved 72-hour experiments were performed in which *C. Elegans* were exposed to varying concentrations of the fungicide and endocrine disruptor prochloraz, the disinfectant benzalkonium chloride and food stress as an environmental stressor. Mixture toxicity effects were tested by exposing the organism to different combinations of the two chemicals and the environmental stressor. Both mortality and the sublethal endpoints of offspring number, offspring size, offspring length, mother size, mother length and total biomass were monitored over time.

Next to classical dose-response modelling, the data will be used to test the applicability of the DEBtox model. The DEB approach will make it possible to model growth, reproduction, and survival simultaneously over time and thereby enable a more complete assessment of the toxicity of prochloraz and benzalkonium chloride. Former results have shown the potential for DEBtox models to be utilized for accurate predictions, specifically for differing food levels. Hence a main goal of the mixture study is to determine whether there is a distinct threshold beyond which lack of food drastically increases toxicity.

## Automated Whole Slide Image Analysis Methods for Detection, Quantification, and Characterization of Liver Histological Lesions in Marine Teleosts Species

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### Abstract

Histopathology is the cornerstone between whole organism and subcellular endpoints, and it remains one of the most reliable, sensitive, and comprehensive methods to identify and characterise diseases. However, traditional histopathological diagnostic methods remain subjective and poorly reproducible as profoundly relying on pathologist experience. Moreover, scoring systems used to assess pathology severity are often qualitative and there is a general lack of homogeneity among scoring systems, which makes it difficult to compare results. The scarcity of histopathologists together with the fact that performing rigorous histological diagnostics is time-consuming, stresses the need to improve diagnostic methods for marine teleost species.

Automated whole slide image analysis methods could help to improve the detection, quantification, and severity assessment of histological lesions. An automated method to detect and quantify melano-macrophage aggregates (MMA) and steatosis from digitized slides of liver from different marine teleost species has been developed. MMA detection method is based on a color threshold applied to previously normalized images. Detected objects are filtered based on different shape factors (circularity, Feret diameter, roundness). Steatosis detection is based on the "Hough Circle Transform" plugin from ImageJ. It allowed to consistently detect circular shapes in images. The total area per sample affected by the two pathologies can be calculated using pixel dimension stored in each image metadata. These automated methods have been tested against drawing contours of affected areas by human operators made with QuPath. No significant difference was found in the area affected by pathology for each image. It implies that these two methods are reliable and could potentially be used as routine diagnostic methods. The results obtained with these automated methods haven't been analyzed against the level of contaminants yet but that is the next step of this project.

These methods allow a fast, consistent, and unbiased analysis of multiple images. Pathology severity is assessed quantitatively which gives more information than qualitative data, makes it easier to compare results, and could lead to refine biological endpoints. Ultimately it could lead to a better understanding of host-pollutant interactions, participate in a global homogenization of histopathological assessment, and reduce diagnostic time for histopathologists.

## Session 4: Mechanistic Ecotoxicology

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### Exploring Lysine Acetylation and Phosphorylation in Adipocyte Differentiation and their Exposure to Emerging Plastic Additives

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#### Abstract

Obesity and overweight are the most severe global public health challenges that are affecting most of the European population. Concurrently, humans are exposed to an increasing variety of environmental pollutants. Chemicals with endocrine disruptive traits, such as phthalates, are considered relevant contributors to the obesity pandemic as they interfere with the regulation of adipogenesis. Restrictions on use of leading phthalates have favoured their replacement towards substitutes, like 1,2-cyclohexanedioic acid diisononyl ester (DINCH). A mechanistic understanding of substitutes mode of action has not yet been fully established but is of great relevance to counteract this aspect of the obesity pandemic. Since biological processes are controlled at protein level and dynamically regulated by post-translational modifications (PTMs), we are interested in the involvement of PTMs in the occurring effects. Besides protein phosphorylation (PP), we focused on lysine acetylation (AcK) as a direct link between cellular metabolism and signalling. We hypothesize that phthalate substitutes modify intracellular processes in adipogenesis by causing alterations in PTM profiles.

We elucidate temporal dynamics of AcK and PP in adipogenesis, using liquid chromatography-tandem mass spectrometry (LC-MS/MS) in combination with PTM-enrichment strategies on chemical-exposed human (SGBS) and mouse (3T3-L1) adipocyte cell culture models.

Both cell lines showed unique temporal AcK- and PP-proteome profiles throughout adipogenesis. Across differentiation, a large set of AcK/PP-sites was significantly regulated and enriched for pathways associated with central metabolism and specifically in SGBS cells PPAR signalling. Multiple AcK sites located on proteins involved in precursor and fatty acid synthesis and transport, such as ACLY, ACAA2, FASN, FABP4/5 (human), Acsl1 or Taldo1 (mouse), were determined to be key drivers of the changes across adipogenesis. Differentiating adipocytes exposed to MINCH displayed distinct PTM profiles and showed reduced sirtuin signaling and gluconeogenesis compared to control adipocytes. PP data is still being analyzed.

We provide detailed mechanistic information on the regulation of human and mouse adipocytes by PTMs, ie. AcK and PP, during adipogenesis and exposure to MINCH. This data contributes to a better understanding of the modes of action of plastic additives, which is vital for developing AOPs and regulatory risk assessment.

## Combining Multi-Omics Approaches to Reveal the Response of a Microbe-Poplar Holobiont to a PAH Contamination Gradient

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### Abstract

Polycyclic aromatic hydrocarbons (PAHs) are pollutants that can be found worldwide, in particular in industry-impacted soils, and have negative effects on both the environment and human health. Phytoremediation, the use of plants to remove contaminants from soil, is an effective and affordable way to clean up PAH-contaminated soil. Previous research has mainly focused on the role of microorganisms in the rhizosphere, the soil surrounding the roots of plants, but recent studies have shown that PAHs can be absorbed by plants and transported within their tissues. Endophytes, microorganisms that live inside plants, may play a crucial role in how the plant responds to the toxicity of internalized PAHs.

To better understand the complex biological processes involved, an integrative approach that combines data from various levels, such as the transcriptome, proteome, and metabolome, can be implemented. This approach can help determine the physiological response of the plant-microorganisms holobiont to PAH contamination and reveal key molecular mechanisms involved. The objective of this study was to investigate the response of endophytic microbial communities and *Populus canadensis*, the host plant, to varying levels of PAH contamination in soil.

An aged PAH-contaminated soil from a former coking plant in France, was chosen and spiked with increasing concentrations of phenanthrene (a three-cycle model PAH). After four weeks, plants were harvested and root and leaf samples were taken for transcriptomic, metabolomic, and proteomic analyses. The data from each analysis were studied independently and then integrated using the mixOmics R package. The data revealed that there was a different response between the highest and lowest concentrations of phenanthrene, with a tipping point at 700 mg.kg<sup>-1</sup>. Several metabolic pathways were identified in both concentration classes, including phenylpropanoid biosynthesis and alpha-linolenic acid metabolism for the high concentration, and inositol phosphate metabolism and glycerophospholipid metabolism for the low concentration. The data collected allow us to propose scenarios for the molecular mechanisms involved in the microbe-plant system's response to PAH contamination.

## Eye Development Effects of Antidepressants Amitriptyline and Metabolite Nortriptyline in Zebrafish on Multiple Levels of Organisation

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### Abstract

A multitude of active pharmaceutical ingredients (APIs) are finding their way into marine ecosystems, in which they may cause non-target effects on marine biota that are not fully understood. The antidepressant amitriptyline, as well as its metabolite nortriptyline, were both shown to affect the behaviour of zebrafish embryos in assays such as the photomotor response assay. The pathway through which these APIs cause behavioural effects is not fully understood. This study aims to investigate alterations to eye development as a potential source of the behavioural effects.

In order to test the hypothesis that amitriptyline and nortriptyline cause adverse development of the eyes, a series of endpoints on different levels of biological organization were measured. Differential gene expression was measured via mRNA sequencing at timepoints of 48, 72 and 96 hpf for concentrations of 3 and 300 µg/L ami- and nortriptyline. The histopathology of the eye, including retinal pigment epithelium thickness, was observed at a timepoint of 120 hpf for concentrations ranging from 0.3 to 300 µg/L ami- and nortriptyline, and the optokinetic response assay was performed at the same timepoint and concentrations in order to test eye function. Next to the antidepressants, a solvent control of 0.01% DMSO and a positive control of 3.5 µM phenanthrene were included.

RNA sequencing results show 107 differentially expressed genes for amitriptyline and 48 for nortriptyline, of which 28 were shared. Gene ontology term enrichment revealed camera type eye development to be the 4th most enriched pathway amongst differentially enriched genes. Results of histopathology and optokinetic response assay will show, if the substances also cause effects on higher levels of biological organization, and potentially reveal a pathway that through altering eye structure and function causes behavioural effects in zebrafish larvae.

## Exploring the Complexity of Antidepressant Effects in Ecotoxicology: Low Dose and Non-Monotonic Effects of Fluoxetine on *Caenorhabditis Elegans* Behaviour

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### Abstract

As the global amount of antidepressant prescriptions continues to rise, there is a growing concern about the potential impact on nontarget organisms exposed to residues of these drugs in the environment. Antidepressants are neuroactive compounds that therefore have the ability to induce behavioural effects. What is particularly interesting is that these antidepressants often exhibit non-monotonic dose-response relationships, wherein lower concentrations of the drugs results in a stronger response compared to higher concentrations. In this study, we utilized the nematode *Caenorhabditis elegans* as a model organism to investigate its behavioural and other biomarker responses to the antidepressant fluoxetine.

Our findings from behavioural experiments with *C. elegans* have revealed that exposure to fluoxetine concentrations as low as ng/l can bring about effects on chemotaxis and activity. These effects were more pronounced at lower concentrations (ng/l) compared to higher concentrations (mg/l). To gain further insight into the underlying mechanisms responsible for these effects, we conducted experiments using knockout mutant strains. The results suggest that that desensitization of the G-protein coupled receptor likely plays a role in modulating the toxic effects, thereby contributing to the observed non-monotonic dose-response curve. Additionally, we are currently investigating other feedback mechanisms involved in serotonin production, metabolism, and release.

The outcomes of this study emphasize the importance of considering a wide range of concentrations when evaluating the effects of psychopharmaceuticals, as neglecting lower concentrations may lead to a severe underestimation of their toxicity. Understanding the mechanistic basis of these responses is crucial for interpreting their relevance in a more ecologically relevant context, accounting for scenarios involving peak exposures and co-exposures with other compounds. Drawing on our experience with studying non-monotonic dose-responses, we can provide important experimental considerations for investigating these complex response patterns.

## The endogenous aspects of mechanistic toxicity

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### Abstract

In the field of environmental toxicology, we focus on the potential causative links between exposure to xenobiotics and subsequent adverse outcomes. This is the fundament, and therefore well established from an empirical perspective. However, the cause-and-effect relationship between exposure to a xenobiotic and the development of subsequent toxicity is often very complex, as multiple factors must be considered: e.g., factors related to toxicodynamic, toxicokinetics, organ, and in relation species specificity. A small but growing body of data have highlighted that exposure to certain xenobiotics can cause the accumulation of endogenous metabolites that in turn can aggravate and contribute to toxicity. One such metabolite is 6-Formylindolo(3,2-b)carbazole (FICZ): a tryptophan metabolite, FICZ is a known aryl hydrocarbon receptor 2 (AhR2) agonist, which in turn can activate a myriad of molecular functions, e.g., cytochrome p450 (Cyp1a).

In this study, we co-exposed rainbow trout alevins (*Oncorhynchus mykiss*) to the polycyclic aromatic hydrocarbons retene and fluoranthene, either alone or as a mixture; both being AhR2 agonists, while fluoranthene is a Cyp1a antagonist. We observed that exposure to this mixture caused a synergized Blue Sac Disease index (a common apical endpoint when assessing developmental toxicity in fish larvae) and the expression of *cyp1a* compared to those exposed to the individual components and in relation to control. These synergises coincided with the detection, quantification, and temporal fluctuation of endogenously derived FICZ following exposure to the binary mixture (but not to the components). It is plausible that accumulation of FICZ influences multiple biological processes and pathways; potentially linked to increased oxidative stress, which promotes the formation of FICZ. Additionally, liver maturation, contesting affinity for AhR2 by FICZ and the PAHS, alongside antagonism and inhibition of Cyp1a by fluoranthene, is likely to contribute to the temporal fluctuations in body burden and developmental toxicity. Therefore, we propose that accumulation of FICZ is likely to contribute to overall and AhR2 mediated toxicity in developing rainbow trout alevins. By extension, in vivo studies investigating the empirical effect(s) of simple and complex mixtures should quantify the temporal variations of endogenously derived FICZ, as a potential contributor to toxicity, AhR2 dependent and independent.

## Water Accommodated Fractions of Crude oil impact the Marine Bivalve, *Cerastoderma edule* in a Tidal and Stagnant System

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### Abstract

The continuous existence of an organism at the individual level relies on its capacity to screen its environment for stressors. A corresponding response is also expected in behaviour, biochemical or physiological effects. This study aims to investigate the sublethal reaction of the marine invertebrate mechanistically, the common cockle (*Cerastoderma edule*), upon exposure to the water-accommodated fractions (WAFs) of crude oil (1: 40, o: w): 0%, 0.41%, 3.7%, 11.11%, 33.33%, and 100%. Experiments were performed under stagnant and tidal conditions as co-stressors at 12°C. The effects of WAFs on the survival and behavioural activities (burrowing, siphoning, and t-half of the filtration rate) were analyzed during the 72 h of exposure at 1,5 h, 24 h, 48 h and 72h. *C.edule* were fed with *Nannochloropsis oceanica* (DW = 28.29%) at 72 h. At the end of the exposure period, no mortality and no significant effect were observed for burrowing in both systems. However, a change was observed in the siphoning activity during the first 1.5 h of the tidal and stagnant system and the t-half filtration rate in both systems. Lipid peroxidation was determined on the gland tissues using a thiobarbituric acid reactive substance (TBARS). Lipid peroxidation in gland tissues of cockles in the tidal system was higher than in the stagnant condition. The outcome of this study suggests that *C.edule* used avoidance to escape the acute toxicity of the water accommodated fractions in a stagnant system but becomes a challenge under the tidal condition.



## Investigation of mechanism-specific toxicity of water and particle samples from flooded areas in Stolberg (North Rhine-Westphalia) after the flood event in July 2021 using an *in vitro* biotest battery

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### Abstract

Climate change, rapid land use transformation, and anthropogenically altered water bodies increase the frequency and intensity of flood events globally. The extreme flood from July 13 to 16, 2021 led, amongst others, to the flooding of industrially used areas and contaminated sites in the catchment area of the rivers Inde and Vicht around Stolberg (North Rhine-Westphalia, Germany) and remobilized historic pollutant sources. Due to the historical settlement of the metal processing, chemical, and pharmaceutical industries, the soils and sediments are among the most polluted in Germany. In this context, especially particulate phases as long-term reservoirs of accumulated pollutants are potential so called “chemical time bombs”.

This study aims to evaluate the consequences of the flood at the toxicological level and elucidated the toxicity contributions of the matrices water and suspended particulate matter. For the analysis, water samples were collected at various flooded areas around Stolberg and subsequently filtered and the water phase was extracted by solid phase extraction. The filter residues were processed separately in a similar approach using pressurized liquid extraction to co-consider the particle-bound pollutants. All organic sample extracts were then tested for their mechanism-specific toxicity. For this purpose, an *in vitro* bioassay battery of different reporter gene assays was used. This bioassay battery includes the neutral red retention assay (cell toxicity), various CALUX<sup>®</sup> bioassays (ER $\alpha$ , AR, anti-ER $\alpha$ , anti-AR, GR, PR) to investigate the (anti-)estrogenic, (anti-)androgenic, glucocorticoid and progestogenic activity, the  $\mu$ EROD bioassay (dioxin-like potency), and the Ames fluctuation assay (mutagenicity). Preliminary results indicate that the toxicity is more likely to be due to particle-bound contaminants. Accordingly, the most significant measured effects of increased dioxin-like potency are found in suspended particulate matter in the Inde River basin.

The work is part of a SynergyFund of the excellence initiative RobustNature of the Goethe University and a DFG-funded project. Within the project, the consequences of the flood are further assessed by an interdisciplinary team combining the results from (eco)toxicological and chemical analyses with social processes, discourses, and the potential impact on nature connectivity in the affected regions in the context of the hydraulic engineering situation.

## Session 5: Microplastics

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### Effects of Microplastics on the Gut Microflora, Behavior & Histology of Zebrafish

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#### Abstract

Microplastic pollution has emerged as a new and widespread phenomenon. At present, the direct and indirect impacts of microplastic on animal health are poorly understood. Fish can ingest microplastics during feeding where they will pass through the gastro-intestinal tract, affecting its structure, function and associated microbiome. The gut microbiome is an important link between the external and internal environment. Little is known about the effects of microplastics on the microbiome and how this translates into impacts at higher levels of biological organization. The aim of this study was to measure the effects of two types of polyethylene microplastics on the gut microbiome, histology, stress markers and behavior of zebrafish.

Zebrafish were exposed for 28 days to one of five treatments: 10 µg/L or 1000 µg/L of high or low density polyethylene microplastics mixed with their food plus a microplastic-free control. There were three replicates for each treatment, with each replicate having six fish (3 males & 3 females). All fish were individually tagged and behavioural responses were measured before and after exposure. At the end of the experiment, two biomarkers of oxidative stress were measured: Superoxide dismutase (SOD) and catalase (CAT). Additionally, one fish from each treatment was preserved for histopathological analysis.

Zebrafish exposed to high-density polyethylene microplastics at both 10 and 1000 µg/L displayed lowered boldness and less anxiety, but were also more social than controls. There was no effect of microplastic exposure on either SOD or CAT. Fish exposed to microplastics showed evidence of liver necrosis and kidney damage. There was high variability in the gut microbiome between treatments and a significant difference between controls and the high density polyethylene 1000µg/L.

In conclusion, MPs caused subtle changes in zebrafish behavior and caused tissue damage to the liver and kidney that might affect normal biological functions. Our study demonstrates that low levels of microplastics can effect fish health and that high density polyethylene was more toxic than low density microplastics.

## Assessing Risks Associated With Microplastic Exposure: Cytogenetic Evaluation On Different Ontogenesis Stages Of Salmonid Fish

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### Abstract

Although microplastic (MP) pollution is frequently considered as a single entity, the term "microplastic" really refers to a wide range of materials with different chemical compositions, additives, and sizes. Black rubber, often known as tire particles (TP), is one of the most frequently observed MPs in environmental samples. Tires are typically comprised of rubber polymer (e.g., styrene butadiene), fillers such as carbon black, and inorganic and organic compounds (e.g., oils, vulcanization agents) added to help in manufacturing and to improve specific characteristics like elasticity, durability, and resistance to degradation. The aim of this work is to evaluate genotoxic and cytotoxic effects of TP on rainbow trout (*Oncorhynchus mykiss*) early-life stages and juveniles. Therefore, we exposed *O. mykiss* embryos to TP (size  $\leq 0.250$  mm) for one month and juveniles to TP (size  $\leq 0.500$  mm) for 3.7 months. Since *O. mykiss* larvae do not feed exogenously exposure of early-life stages was through water but juveniles were exposed through contaminated food. Our hypothesis was that the impact of TP genotoxicity on juveniles would be more significant due to the fact that exposure occurs through feeding, as compared to waterborne exposure, given the protective role played by the chorion during embryonic development. Cytogenetic analysis was carried out using erythrocytic nuclear abnormalities assay. The formation of micronuclei (MN), nuclear buds (NB), nuclear buds on filament (NBf), blebbed nuclei (BL) cells were assessed as genotoxicity endpoints, as well as 8-shaped, fragmented-apoptotic (FA) and bi-nucleated (BN) cells as cytotoxicity endpoints.

A significant increase in genotoxic and cytotoxic endpoints was observed in juveniles exposed to TP. Meanwhile, only genotoxicity increased in TP-exposed embryos. In conclusion, we observed significant adverse effects of TP on *O. mykiss* at different ontogenetic stages. Understanding the impact of TP genotoxicity on aquatic organisms is crucial for assessing the potential risks associated with the disposal of tire debris in aquatic environments and developing effective mitigation strategies.

Study was funded by the Research Council of Lithuania (LMTLT) through the project S-MIP-22-51 (ARFA) and project no. S-SV-23-95 (P-SV-23-46).

## In-vitro toxicity of highway runoff samples using mechanistic reporter-gene assays

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### Abstract

As the number of vehicles in use constantly rises globally, so does the amount of road- and traffic-related pollutants released into the environment. At the same time, awareness and knowledge about road runoff toxicity and tire and road wear particles (TRWPs) are rapidly increasing. However, the fate and ecotoxicological effects of road runoff are yet to be understood. Additionally, the possibilities of interactions between TRWPs with other road runoff pollutants are rarely known. As a master's thesis, the presented study is embedded in the project RoadTox, which aims at a comprehensive understanding and quantitative ecotoxicological risk assessment of stormwater runoff sampled from highly frequented urban, country, and highway roads in Germany.

In brief, samples of different runoff scenarios were collected, e.g., precipitation events after a long dry period and consecutive events at the federal highway/Bundesautobahn BAB4 in Aachen, Germany. The samples were filtered (0.3-0.5 µm) across a glass fibre filter and loaded onto Solid Phase Extraction (SPE) cartridges, which were then eluted with a final relative enrichment factor (REF) of 2000. For use in the in-vitro assays, aliquots of the extracts were transferred to DMSO. To assess the mechanism-specific toxicity, an in vitro-biotest battery with several reporter gene assays is used to investigate dioxin-like activity, (anti-)estrogenic and -androgenic activities, respectively. Prior to the measurement of the mechanism-specific endpoints, the neutral red assay was performed for all used cell lines to determine the cytotoxicity of each sample as range-finding experiments.

In previous in-vitro trial studies, estrogenic, anti-androgenic and Ethoxyresorufin-O-deethylase (EROD) activities were already detected for selected samples. For the samples used in the current study, extensive in-vivo data obtained from fishembryo toxicity assays and chemical data are available. Hence, the aim of the presented master's thesis is to provide corresponding mechanism-specific in-vitro data based on specific road runoff scenarios. The study is still in process, and obtained data will be available for the presentation. The results will contribute to a better understanding of road runoff ecotoxicity and identifying possible toxicity drivers.

The project is funded by the Ministry for Environment, Nature and Transport of the State of North Rhine-Westphalia (MUNV), Germany.

## The Suitability Of Standard Soil Assays To Study The Biodegradation Of Microplastics

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### Abstract

New restrictions proposed by the European Chemicals Agency will limit intentional addition of microplastics to the environment. A derogation states that biodegradable polymers are exempt from this restriction. It is likely that increasing amounts of biodegradable polymers will be used in soil applications (e.g., plastic mulching films, controlled release fertilisers, plant protection products). International standard specifications are required for testing the biodegradability of plastics in soils, coupled with clear and robust standard test methods to verify complete biodegradation within a specific timeframe under controlled environmental conditions.

Standard tests exist for measuring biodegradation of plastic under varying conditions, e.g., ISO 17556:2019(E) for the aerobic biodegradation of plastic in soils, no method exists specifically for measuring the biodegradation of microplastics.

In this work, the suitability of ISO 17556 for determining the biodegradation of microplastics is investigated via quantification of CO<sub>2</sub> evolution and O<sub>2</sub> demand. The percentage biodegradation of the positive reference materials, cellulose and PHB (poly(r)-3-hydroxybutyrate), was measured via CO<sub>2</sub> evolution. Duplicate soil samples were removed at specific timepoints, for analysis of the parent polymer in the soil sample. To determine the reproducibility of the test method across soil types, cellulose degradation was compared in three different soils; two natural soils (UK, Switzerland) and a standard soil (specified in ISO 17556).

Additionally, an OxiTop<sup>®</sup>-IDS system was used to measure O<sub>2</sub> demand. The system was validated for use with soil matrices following an OECD 301F test with sodium benzoate. After optimisation, the biodegradation of cellulose and PHB was monitored.

The use, accuracy, and reproducibility of data obtained from both methods was compared. The suitability of ISO17556-style tests for measuring microplastic degradation is discussed, highlighting the significant knowledge gap in identifying and quantifying polymer degradates.

## The inclusion of supplementary analysis within a standardized biodegradability testing framework has the potential to provide enhanced comprehension of microplastic degradation

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### Abstract

To improve product performance microplastics are often intentionally added e.g. in cosmetics, fertilizers, plant protection products or paints. During application and use they enter the environment in various ways. Potential impacts on environmental and human health have raised concerns, therefore intentionally added microplastics are expected to be restricted by the European Union soon. Exempt from this restriction are biodegradable polymers.

The proposed test methods to classify microplastics as biodegradable comprise several standardized test methods in a tiered approach. These proposed methods all target ultimate degradation (mineralization, i.e., organic C is converted to CO<sub>2</sub> or O<sub>2</sub> consumption) as defined biodegradation pass criterion. However, the overall degradation of the polymer is not only the result of the complete mineralization to CO<sub>2</sub> but also other processes such as abiotic degradation (e.g., hydrolysis) or fragmentation (mechanical or biological) and moreover is dependent on several microplastic particle characteristics (shape, size and surface area).

This PhD project is about integrating microplastic-specific analyses into a standardised biodegradability test system to get a more accurate picture of microplastic degradation. The focus of this work is on degradation in the OECD TG 301B test (aqueous medium, readily degradation) and in the OECD TG 307 (soil, simulation test).

The biodegradation of a polyurea capsule suspension was tested by performing a modified OECD 301B CO<sub>2</sub>-Evolution test. Since the polymeric component of the suspension comprised <1%, <sup>14</sup>C-radiolabeling of the polymer was a pre-requisite to distinguishing the degradation to <sup>14</sup>CO<sub>2</sub> from other components of the suspension.

This year, the aim is to find a suitable separation method for extracting the microcapsules from the soil (pristine and weathered) as part of a master's thesis. Since the polyurea capsules have a high hydrophobicity and initial density separation attempts were not promising, the focus is on oil extraction and magnetic extraction methods. The developed separation method will ideally be used for MP analysis within the framework of an OECD TG 307 (Aerobic and Anaerobic Transformation in Soil) to investigate primary degradation products or fragmentation of the microcapsules.

## Plastic Microfibres in Remote Scottish Soils: What Influences Their Distribution and Abundance?

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### Abstract

Microplastics pollute all types of environments, with fibres being (one of) the most dominant shape. However, a small number of studies quantifying microplastics in soils exist, covering mostly highly populated areas such as Germany or China. Not only we do not have enough data on how much are different soils worldwide polluted, but we even do not know what influences the abundance of microplastics in soils.

To investigate the factors affecting the amount of plastic microfibres in soils, a total of 63 topsoil samples were collected on the Isle of South Uist (Scotland) and analysed. South Uist is located west of the Scottish mainland in the Atlantic Ocean. With a small population and minimal industry, it is an ideal place to detect the near-natural processes that influence the distribution of plastic microfibres in soils. Samples were collected along the west coast and in four west-east transects to mirror the prevailing western wind direction.

An optical microscope was used to quantify plastic microfibres. In the inland samples, a median of 36,900 fibres/L was found, while only 3,580 fibres/L in the coastal samples. The huge difference is caused by the difference in these two environments.

As there is only a minimal direct human impact, we assume that the Atlantic Ocean in the west, specifically the sea spray, is the source of microplastics in the soils. Microplastics enter the terrestrial environment through the west coasts, which are sandy in texture and with only sparse vegetation (samples were collected on the edge of the vegetation). It is relatively easy for the wind to deflate the microplastics (along with the other sediment) and transport them east. Right next to the sandy beaches is machair (a low-lying, predominantly flat area) with a sandy loam texture and low, medium-dense vegetation. Machair soils are less susceptible to wind erosion than the beaches, so more plastic microfibres deposit there, but it is still possible for the wind to deflate the microplastics. Contrary, the inland soils are loamy, with dense and high vegetation, and often peaty. Once plastic microfibres deposit there, they accumulate and are not transported further because of the high wind abrasion resistance.

Soil susceptibility to wind erosion is the driving factor for the abundance and distribution of plastic microfibres in remote soils. It should be considered in other areas with dominant atmospheric sources and wind transport of microplastics.

## Microplastics in wastewater can affect nutrient removal in constructed wetlands

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### Abstract

Wastewaters are known to be one of the most important pathways for microplastics (MPs) into the environment. Therefore, researchers have studied the removal of MPs in conventional wastewater treatment plants and the impact of MPs on their treatment efficiency. However, it is not yet known whether the presence of MPs affects the treatment performance of small, domestic wastewater treatment plants, such as constructed wetlands. Constructed wetlands are often used for the treatment of municipal wastewater at locations where population cannot be connected to the sewerage system. In this context, the aim of the study was to investigate the treatment efficiency of the horizontal sub-surface flow laboratory constructed wetland (LCW) for wastewater treatment in the presence of MPs. Two types of MPs commonly found in wastewater were used: polyethylene fragments extracted from a cosmetic product and polyacrylonitrile fibres from a synthetic clothing. The LCW operated for 257 days, the first 78 days without MPs and the following 179 days with a weekly addition of MPs. The presence of MPs in wastewater did not affect the removal efficiency of organic matter (evaluated as dissolved organic carbon, chemical oxygen demand, biochemical oxygen demand) and the removal of ammonium and nitrate. On the other hand, the removal efficiency of nitrite increased, and the removal efficiency of orthophosphates decreased. This suggests that the accumulation of MPs in constructed wetlands (99.99% of all MPs were retained in the LCW during the experiment), which are used as a small wastewater treatment system, may cause secondary problems by affecting nutrient removal from wastewater. This could have a significant impact on water quality, especially in terms of eutrophication of natural ecosystems.



## High levels of small microplastics (>40 µm) in compost samples from Scandinavia: An important contributor to soil toxicity?

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### Abstract

Microplastics (MPs) have become a relatively relevant environmental issue in recent years. However, information on their occurrence, sources and effects in soils is very limited and poorly known. Compost has become one of the main alternatives for providing nutrients in agriculture and for biogas production. However, domestic biowaste, which is a common substrate for compost production, often contains large amounts of microplastics from packaging. In turn, some composting plants use sludge, which has also been described as a source of microplastics. The main objective of the current project was the quantification of MPs, as well as to determine spatial and temporal differences in MP concentrations between biogas plants. Also, the analytical method was optimized for µFTIR imaging of microplastics. According to this, we examined the microplastic content in compost from two biogas plants in Scandinavia, during two time periods 4 weeks apart. A total of 15 samples were analyzed in two size fractions: Small MPs (40-300 µm) and large MPs (>300 µm). The larger fraction was analyzed using microscopy, ATR-FTIR and gravimetrically. The smaller fraction was analyzed using a Perkin Elmer imaging FTIR and the Simple software. Our preliminary results show concentrations ranged between 20-59 microplastics/g compost (d.w). In general, the concentrations of the larger microplastics > 300 µm were higher compared to the smaller fraction. On average the dry weight of microplastics made up 0.8% of the samples dry weight. The contribution of microplastics >2 mm to the samples dry weight exceeded 0.3%, which is the limit in the EU for microplastics size >2 mm. These results confirm compost as an important source of MPs in soil, due to the tons of compost used on arable land each year in Scandinavia. Consecutively, the toxicity of these particles, as well as of plastic additives that may be stored and not degraded during the composting process, could have a negative impact on soil health and soil biota.

## Accumulation and distribution of microplastics in Uludağ Ski Resort

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### Abstract

Microplastics (MPs), which are released from the natural breakdown of plastic waste in the environment or interactions caused by humans, can travel great distances via atmospheric events (wind, rain, snow, etc.) and accumulate on the surface with rain and snowfall in remote areas. Eight snow samples were collected from two different regions: the mountainside (number 5) and its summit (number 3) of a popular mountain Ski Resort, Uludağ in Bursa, Türkiye. The distribution of MPs at the summit and the mountainside was also determined. Samples were photographed using a stereomicroscope. Subsequently, ImageJ software was used to determine the size distributions and counts of the potential MPs. The most frequently observed form of MP is fiber. The MPs were frequently observed to be transparent, red, blue, yellow, and purple colors. It is shown that MPs released from ski clothes and equipment on remote mountain spots can be transported by meteorological conditions.

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## **Environmental transport of microplastics; mitigation strategies and relevant factors**

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### **Abstract**

A "tragedy of the commons" has been created in our terrestrial environment, oceans, seas, and waterways as a result of the massive increase in plastics production, the shift to single-use, disposable plastics, and delays in implementing mitigation strategies. The role of four factors, namely human involvement, climate change, navigation of plastics, and the relative abundance of any scientific and voluntary evidence looking for the footprint of microplastics in the environment. The aim of the research is to gather evidences to identify a pattern for the environmental transport of microplastics in social perspective. By using the theory of conditional probability and continuing the work of Schwarz et al (2022), the research tries to estimate the loss of microplastics in eighteen end point compartments within nature. The results compare the amount of microplastics in four countries to achieve a set of mitigation and adaptation policies on a global scale.

## Sorption behaviour of two Tetrachlorobiphenyl Homologs to High-Density Polyethylene (HD-PE) Micropoplastics

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### Abstract

Microplastics have been documented to adsorb chemical pollutants from water and sediment/soil matrices in the environment. Sorption behaviour of hydrophobic organic compounds (HOCs) to microplastics is influenced by hydrophobicity of the HOC and the plastic polymer type. Polychlorinated biphenyls (PCBs) are comprised of different congeners with varying molecular structure and properties which have the potential to influence their sorption to microplastic particles. Even though production and use of PCBs have been prohibited through the Stockholm convention, concentrations are still being measured in aquatic ecosystems across the globe. The study aims to investigate the sorption behaviour of two PCB homologs: 2,2',5,5'-tetrachlorobiphenyl (PCB 52) and 3,3',4,4'-tetrachlorobiphenyl (PCB 77) onto high-density polyethylene (HD-PE) microplastics.

Model microplastics were mechanically produced by grating HDPE plastic bottles with a file. The plastic fragments were sieved through a 600  $\mu\text{m}$  sized mesh to obtain a uniformly sized fraction. Instrumental techniques, such Fourier transform infrared spectroscopy (FTIR), X-ray powder diffraction (XRD), scanning electron microscopy (SEM) and Brunauer-Emmett-Teller (BET) analysis were employed to characterize and determine the physico-chemical properties of the plastic particles produced. The microplastics produced were confirmed to be polyethylene polymer and semi-crystalline in structure with a surface area of 0.1166  $\text{m}^2/\text{g}$ . Batch adsorption experiments were carried out to characterize the kinetic and thermodynamic mechanisms underlining the sorption of PCB 52 to microplastics. The adsorption process is best described by the pseudo-second order kinetic model and occurs spontaneously as indicated by a negative Gibbs free energy value. The microplastics had a higher adsorption capacity for PCB 77 than PCB 52 at all the temperatures studied. There was no significant impact of solution pH on the adsorption process. Findings from this study will contribute to understanding the mechanisms behind the interactions between organic compounds and microplastics. The findings will also be useful for evaluating the exact risks posed by microplastics and their associated chemicals to aquatic organisms and humans.

Key words: Microplastics, polychlorinated biphenyls, high-density polyethylene, sorption

## Microplastics, phthalates in sediment and water matrix of the urban aquatic system: Sukhna lake

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### Abstract

In the last two decades, worldwide microplastic and associated contaminant studies got immense attraction from the scientific community. Microplastics and emerging contaminants have been discussed in freshwater ecosystems, but still, the need of the hour is to be more studied on a particular topic. The pollution/contamination in these systems is primarily driven by anthropogenic influence due to various environmental activities. Tracing these pollutants has become increasingly critical in the environment to overcome all these challenges. Microplastics identified in our study based on Sukhna Lake (Urban Freshwater Lake) are dominated by Polyethylene, polypropylene and polystyrene polymers. The abundance of microplastics in sediment samples is 170-2320 n/kg; in water samples, it ranges between 05-75 n/litre. Different morphologies, fibre, fragment, foam, film, and pellets, were observed in the lake sediments, which also signify the physiochemical and hydrological conditions on or near the lake system. On the other hand, Phthalates and additives in plastics were also investigated in the lake; diethyl phthalate (DEP), dibutyl phthalate (DBP) and Bis (2 – ethylhexyl) phthalate were found in the sediment samples. A significant correlation between MPs and PAEs was observed in lake samples suggesting the contaminants input from a common source, where PAEs concentration can be used as a proxy to MPs presence or vice-versa. The presence of MPs and PAEs and their correlation advances the knowledge that the system shows a significant contamination level and needs proper management policies to ensure the sustainability of Sukhna Lake. Also, Data from the research will be utilised as a baseline for further studies in freshwater ecosystems.

## Transport Mechanisms of Microplastics and Their Effects on Soil-Plant Systems

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### Abstract

Microplastics (MPs) are an emerging pollutant that has been receiving global attention due to uncertainty around their occurrence, fate and lack of quantitative information on potential environmental and human health risks. While research on the impact of MPs on aquatic systems is growing, there are few studies on their impact on terrestrial systems. Agricultural soil is a dominant sink for MPs; however, the transport mechanisms of these ubiquitous particles and how they would affect soil-plant ecosystems remains largely unknown. This study investigated the effect of various microplastics on soil quality and plant performance. Soil-incubation experiments were conducted, involving polyester fibers, polyethylene terephthalate fragments, and polystyrene microspheres as targeted microplastics, and lettuce and radish as biological models. Plants were cultivated in glass jars containing fluorescent microplastics which had been mixed with the soil. The location of microplastics was visualized using EVOS Auto FL 2 fluorescence microscopy and imaging. Soil endpoints revealed that all microplastics had a positive effect on the formation of water-stable aggregates, but microfibers and microspheres reduced water holding capacity, and micro-fragments increased it. There were no significant differences in bulk density, carbon, or nitrogen content among the different microplastic treatments. For plant endpoints, all microplastics inhibited root length and biomass, and chlorophyll a concentrations was more affected than chlorophyll b in the presence of microplastics. Microfibers were detected in both lettuce and radish roots and stems, and MP fluorescence was observed in lettuce leaves at high concentrations. Microspheres were detected in radish roots and stems at low concentrations but with limitations to enter fruit and leaf parts. The large size of micro-fragments prevented their absorption by any vegetables. Microplastics have multifaceted effects on soil-plant ecosystems, influenced by factors such as particle shape, size, concentration, surface physical chemical properties and plant species. Some plant roots could absorb microscale MPs and transport them to above-ground parts. This research provides scientific insights into the impact and pathway of different microplastics in soil-plant ecosystems, with significant implications for the toxicity effect of higher terrestrial plants and the risk assessment of food safety.

## Toxicological effects of silver nanoparticles (AgNPs) under localized surface plasmon resonance (LSPR) stimulation

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### Abstract

The widespread use of AgNPs inevitably results in AgNP pollution in aquatic ecosystems because these environments are the final destination of industrial, domestic, and agricultural residues. The entry of AgNP in aquatic environments can represent a risk to the inhabiting organisms. A particular characteristic of AgNPs is their sensitivity to light stimulation. The Localized Surface Plasmon Resonance (LSPR) promotes a collective oscillation of electrons on the interface of AgNPs after the incidence of light on their surface in a specific wavelength. The present study aimed to investigate the potential of plasmonically stimulated AgNPs to induce mortality and oxidative stress on *Daphnia similis*. AgNPs of different shapes were synthesized using chitosan as a biocompatible capping agent. Neonates (6 - 24h) were exposed to different concentrations of AgNPs (0 - 80  $\mu\text{g}\cdot\text{L}^{-1}$ ) and different light conditions (dark, white, red, green, and blue light) for 48 h at 22°C. The results demonstrated that under light conditions that promote plasmonic stimulation on the AgNPs, the mortality was significantly increased. For the tested AgNPs, the plasmonic stimulation increased the toxicity by 61.45 and 45.45% as function of the NPs' shape compared with dark condition. The treatments using white light conditions also showed an increase in the mortality for both AgNPs, compared with dark conditions. The results demonstrate the toxicological potential of AgNPs and the increase of this potential under specific plasmonic stimulation conditions. Particularly, under white light conditions we can obtain information about the impacts of the presence of these nanoparticles in natural environments. Also, the present work brings a new perspective on the luminosity adopted in regular studies investigating the effects of metallic nanoparticles sensitive to plasmonic stimulation. Additional laboratory studies will be carried out to investigate the sublethal effects on biomarkers of oxidative stress.

**Biofragmentation of polystyrene microspheres by the amphipod *Hyalella azteca***

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**Abstract**

Microplastics (MPs) are a pervasive form of pollution in the environment, posing a significant global problem. These particles are mainly originated from the breakdown of larger plastic debris and undergo continuous fragmentation. It is crucial to comprehend the pathways of fragmentation since they affect the fate of the particles and their toxicity. Amphipods have been suggested to play a role in inducing the fragmentation of plastic debris. To investigate this further, we exposed *Hyalella azteca* to different concentrations (540, 2700, 5400 items/L) of 24.5 µm polystyrene MPs (PS-MP) for seven days and observed particle size reduction and mortality rates. Exposure via food was prepared with a 5 g/L suspension of flaked fish feed (TetraMin). The fish feed suspension (100 µL) was added to the MPs suspension at each of the concentrations tested, and the resulting mixture was dried in porcelain containers to form a pellet. The porcelain container was placed on the bottom of each test flask. After each time point, the medium was completely filtered through a vacuum system in glass fiber filters. The filters were investigated under a stereomicroscope to measure particles (n = 20 per replicate) and to check if there was a reduction in mean size. During exposure, the flasks were monitored for amphipod mortality at 48, 96, and 168h. While we observed successful ingestion of the particles, we did not find any negative effects on *H. azteca* survival. However, we noted that the PS-MP underwent intense fragmentation throughout the ingestion and egestion processes, resulting in a final size up to 25.3% smaller than their initial size. This fragmentation was observed over different time points (24, 72, 120, 168h) and showed a constant reduction in average particle size, indicating that *H. azteca* can indeed induce PS-MP fragmentation. Scanning electron microscopy analysis of the exposed samples revealed visible signs of physical changes such as cracks, fissures, and irregular edges on the PS spheres. These modifications in the MPs morphology suggest that the organisms are interacting with the particles and causing damage to their structure. The changes in the surface and structure of the particles may have occurred due to the exposure to mechanical forces, gut enzymatic processes, the intestinal microbiome, or a combination of all three. The reduction in particle size over time could potentially represent a significant route for trophic transfer.



## Microplastics in coral reefs in remote South Atlantic islands

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### Abstract

The increase in oceanic pollutant entry has led to the degradation of coral reefs, making it essential to understand the patterns of transport and fate. Corals can ingest microplastics (MPs) unintentionally, leading to physiological changes, necrosis, and decreased of photosynthetic capacity. This study quantified and investigated the physical (size, color and morphology) and chemical characteristics of MPs and their distribution in four coral species (*Favia gravida*, *Montastrea cavernosa*, *Mussismilia hispida*, and *Siderastrea stellata*) from Brazilian coral reefs from remote South Atlantic islands: Trindade and Martim Vaz. The aim was to reveal the extent of MPs distribution in the coral reef environment and compare their capacity to adhere and accumulate MPs. Samples (n = 30) from 3 sampling stations were collected by autonomous diving (SCUBA). For analysis of adhered MPs, samples were sonicated and MPs extraction was performed by density separation (30% NaCl). To recover internalized MPs, soft tissue digestion was employed (30% KOH for 48h followed by 30% H<sub>2</sub>O<sub>2</sub> for 48h). The concentration of MPs was given in items per g<sup>-1</sup> of coral and items per g<sup>-1</sup> of soft tissue, respectively. Our study found MPs both adhered and ingested by the four coral species, across all sampling stations. For adhered MPs, *M. cavernosa* had the highest number of adhered particles, followed by *S. stellata*, *F. gravida*, and *M. hispida*:  $3.4 \pm 1.9$ ,  $2.5 \pm 2.1$ ,  $2.1 \pm 2.6$ , and  $1.7 \pm 0.7$  items g<sup>-1</sup> of coral, respectively ( $p > 0.05$ ). More than half of the total MPs adhered in coral surface were found within the size range of 100-300 µm, with fibers being the predominant morphology. The species with the highest quantity of ingested microplastics was *M. hispida*, followed by *S. stellata*, *M. cavernosa* and *F. gravida*:  $32.3 \pm 19.1$ ,  $28.8 \pm 12.7$ ,  $26 \pm 10.9$ , and  $11.1 \pm 2.45$  items g<sup>-1</sup> of soft tissue, respectively ( $p > 0.05$ ). The size of particles showed significant differences ( $p < 0.05$ ), with *F. gravida* being the species that ingested larger MPs ( $439.1 \pm 434.8$  µm) and *M. hispida* the smallest ( $366.6 \pm 325.6$  µm). The most predominant type of polymer were polyethylene (45%) and rayon (18%). The study reveals long-distance transportation of MPs from the continent to remote islands in South Atlantic, affecting coral species. MPs present in corals prompt biological responses, underlining the significance of assessing risks at individual and population levels to predict potential ecosystem impacts.

## Roadway to Linking Exposure and Effects of Highway Stormwater Runoff and Particulate Matter – First Case Study Results from a Highly Frequented Highway in Germany

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### Abstract

Awareness and knowledge about road runoff toxicity and tire and road wear particles (TRWPs) are rapidly increasing. Yet, the fate and ecotoxicological effects outside of communal wastewater treatment systems are insufficiently understood paired with a lack of data about how TRWPs interact with other road runoff pollutants. Consequently, aquatic environmental risk assessment of TRWP polluted runoff faces the following challenges: **(1)** Due to structural and chemical variability of TRWP, no standardized sample preparation protocols for (bio-)chemical assessment are available; **(2)** The lack of environmentally relevant effect data complicates a priori decisions on endpoints of interest to investigate. Therefore, comprehensively investigating the ecotoxicity of TRWP demands a bottom-up scientific approach generating a broad knowledge base covering both chemical and biological effect information for different environmental model scenarios.

The project RoadTox aims for a quantitative ecotoxicological risk assessment of stormwater runoff sampled from highly frequented urban, country, and highway roads. Here we present the current project state with a special focus on results obtained from an extended fish embryo toxicity assay battery on *Danio rerio* embryos combining several sublethal endpoints. Presented data includes standard sublethal effects complemented with spontaneous tail coiling, heartbeat-tracking, light-dark-transition responses and *in-vivo* EROD-activity. Additionally, supporting *in-vitro* data (e.g., EROD, estro- and androgenicity), chemical data, and road site biofilm community information from metabarcoding will add to the discussion.

Current results strongly indicate that road runoff toxicity is largely driven by particulate-bound contamination. Applied sublethal non-standard endpoints in zebrafish (e.g., light-dark transition response and *in-vivo* EROD) were able to detect effects in low concentration ranges ( $\leq EC_{10}$ ). Chemical profiles strongly depend on preceding weather conditions but display a mutual composition pattern between different samples obtained from the same site. Both PAHs and metals concentrations in parts reached concentrations which may impact fish health. However, at this stage, no general conclusion is possible yet. The project is funded by the Ministry of the Environment, Nature and Transport of the State of North Rhine-Westphalia (MUNV), Germany.

## Interactions of Microplastics with Heavy Metals in Freshwater Environment: Assessing Toxicity and Oxidative Stress Effects on *Daphnia magna*

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### Abstract

Microplastic pollution is a pervasive environmental issue, posing potential risks to aquatic ecosystems. This study investigates the interactions between microplastics and heavy metals in freshwater environment. The toxicity and oxidative stress effects of these interactions were evaluated using *Daphnia magna* as model organism. Microplastic samples subjected to an aging process were analyzed to simulate real-world conditions. The aged microplastics were then exposed to heavy metals commonly found in aquatic environments, including lead, copper, zinc and cadmium. To assess the toxicological implications of microplastic-metal interactions, *Daphnia magna* was exposed to various concentrations of microplastics and heavy metals. The endpoints evaluated included mortality rates, reproductive success, and biomarkers of oxidative stress. Acute toxicity experiments were performed to determine the acute lethal toxicity of microplastic and heavy metals alone or in mixture on *D. magna*. All details of the acute toxicity test using daphnids were according with OECD 211. The amount of ROS produced in *D. magna* was measured with the probe H2DCFDA by fluorescence (excitation wavelength of 350 nm and emission wavelength of 600 nm). Twenty exposed and nonexposed daphnids were homogenized in sucrose buffer and centrifuged. Enzymatic activities of superoxide dismutase and catalase were measured in the supernatant using a commercial kit.

Results revealed that microplastics can act as an absorbent for heavy metals, facilitating their accumulation in the aquatic environment. The aging process has increased the absorbency of microplastics for heavy metals, suggesting that long-term exposure may intensify metal pollution. Additionally, significant toxic effects have been observed on *Daphnia magna*, including increased mortality rates and reduced reproductive success. Furthermore, elevated levels of oxidative stress markers were observed in both organisms, indicating the potential oxidative damage caused by microplastic-metal interactions.

These findings highlight the importance of considering the combined effects of microplastics and heavy metals on freshwater ecosystems. Further research is warranted to better understand the mechanisms underlying the observed toxicity to develop effective strategies for mitigating the impact of microplastic pollution on aquatic environments.

## Session 6: One Health

05

### Geo-environmental implications of the progression of *Chronic Kidney Disease of unknown etiology* (CKDu) in the dry zone of Sri Lanka

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#### Abstract

In certain geographical regions in tropical countries, including Sri Lanka, *Chronic Kidney Disease of undetermined origin* (CKDu) has been causing a progressive kidney disease among farming communities, members of which lack of the 'traditional' risk factors for kidney disease (i.e., Severe diabetes or Obesity). The key objective of this study is to investigate the link between drinking water quality and CKDu in the dry zone region of Sri Lanka. Drinking water samples were collected from the CKDu hotspot of Wilgamuwa (n=59) and compared with the non-endemic region of Dodamwela (n=15) in the wet zone region, from a farming community with similar work and living environments. Samples were analyzed for major anions, cations (Na, K, Ca, Mg, Si), and trace elements. pH of CKDu endemic region varied from 5.02 to 8.81. Total alkalinity and total hardness ranged from 38 to 538 mg/L and 35 to 505 mg/L, respectively in the CKDu region. In contrast, the groundwater samples of the non-prevalence region showed significantly lower pH (mean 4.84), total alkalinity (36 mg/L), and total hardness (56 mg/L). In the CKDu hotspot, 64% of the groundwater samples fall on hard water (120–180 mg/L) and very hard water (>180 mg/L) categories. The major ions in CKDu groundwater varied in the order  $\text{Si}^{4+} > \text{Ca}^{2+} > \text{Na}^+ > \text{Mg}^{2+} > \text{K}^+$  while anions varied in the order  $\text{HCO}_3^- > \text{Cl}^- > \text{SO}_4^{2-} > \text{NO}_3^- > \text{F}^- > \text{PO}_4^{3-}$ . The non-CKDu groundwater showed higher  $\text{PO}_4$  and  $\text{NO}_3$  values and lower  $\text{SO}_4$  values than CKDu hotspot groundwater. Furthermore, most water samples from the CKDu region were Ca-Mg- $\text{HCO}_3$  type, while alkaline earth exceeds alkali elements.  $\text{F}^-$  content in groundwater samples in Wilgamuwa was 0.03–3.83 mg/L with a mean value of 0.70 mg/L, which exceeds the admissible capacity imposed by the WHO for tropical countries, while the maximum  $\text{F}^-$  content in the wet zone was 0.13 mg/L. Moreover, silicon (as  $\text{Si}^{4+}$ ) content in groundwater in the Wilgamuwa area (range: 15.85 - 64.58 mg/L; mean - 44.60 mg/L) was higher than that of the non-endemic region (range: 7.36 – 15.47 mg/L; mean – 11.06 mg/L). Overall, a considerably higher total alkalinity and Total hardness, Silicon, Bicarbonate, Fluoride, and Sulphate contents were observed in the CKDu region than in the wet zone where CKDu is non-prevalent. The synergetic effect of the hydro geochemistry of the groundwater and other environmental pollutants may play a significant role in the pathogenesis of CKDu in Sri Lanka.

## Tire components and rising temperatures accelerate aging pathways and neurodegeneration

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### Abstract

Rising temperatures and traffic-related air pollution are among the world's leading environmental health risks and have been linked to neurodegenerative diseases like Alzheimer's disease (AD) and Parkinson's disease (PD). However, nanoparticles such as nano silica have been produced for decades and remain widely applied in products like car tires. The underlying molecular pathways and the link to non-chemical exposome factors such as ambient temperature remain elusive.

To investigate long-term effects of the tire wear component nano silica, we monitored neurotoxicity in AD and PD reporter worms of the top animal model *C. elegans*. Age-resolved analysis showed significantly reduced locomotory fitness in *C. elegans* wild-type, AD and PD models. The most vulnerable to nano silica were AD reporter strains that express the peptide amyloid beta<sub>1-42</sub>. In the PD reporter model, the decline in locomotion correlated with an increase in neurodegeneration of dopaminergic neurons. Nano-silica induced neurodegeneration manifested as beading along the dendrites and axons of single dopaminergic neurons which is a commonly observed hallmark preceding axonal atrophy in PD. In line with this, rising temperatures aggravated nano silica-induced neurodegeneration and reduced locomotion fitness, especially in middle-aged cohorts which were the most vulnerable, temperature sensitive age-group.

Our results represent a starting point for the analysis of neural decline pathways by non-exhaust pollutants in *C. elegans* AD and PD models. To characterize individual resilience pathways targeted by nanoparticulate tire wear nano silica and rising temperature, we started to perform single worm analysis. In addition, we want to grasp the impact on soil organisms by isolation and cultivation of nematodes from contaminated sites and compare their fitness with worms from unpolluted habitats.

## The influence of bio-based fertilizers on the sorption behaviour of pharmaceuticals in soil

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### Abstract

The sorption process of pharmaceuticals in biological matrices has received much research attention as it controls the fate in the environment and risks to human health. The sorption behaviour of pharmaceuticals in soil can be affected by the agricultural application of bio-based fertilizers (BBFs), which are derived from various origins such as manure and sewage sludge to help close nutrient loops and serve as sustainable alternative for synthetic fertilizers. However, limited research has been conducted on the influence of BBFs on the subsequent sorption of pharmaceuticals in soils, via the interaction of the pharmaceuticals with the BBF matrix. To address this research gap, as part of the EU funded LEX4BIO project we conducted batch sorption experiments following OECD guideline 312 (OECD, 2004). The experimental setup included eight pharmaceuticals, four types of BBFs (ash-based, plant residue-based, manure-based, and animal-based), and two agricultural soils (Finland and Spain). The sorption data, including concentrations in soil or BBFs and concentrations in water solution, were fitted using Freundlich isotherms, which yielded good correlations ( $r^2$  from 0.66 - 0.99). The linearity factor ( $n$ ) indicated that most compounds exhibited nonlinearity in sorption. The Freundlich constant ( $K_f$ ) and the sorption coefficient ( $K_d$ , L/kg) showed the sorption capacity to soil/BBFs. Significant differences in  $K_d$  values were observed among the four BBFs and the two soils. Specifically, the compounds exhibited stronger sorption to plant-based BBFs ( $K_d$ : 13.6-473.4) and animal-based BBFs ( $K_d$ : 7.4-377.1), while manure-based BBFs ( $K_d$ : 1.90-44.18) and ash-based BBFs ( $K_d$ : 1.22-17.08) showed little difference compared to the two soils ( $K_d$  in Spain soil: 0.65-10.03;  $K_d$  in Finland soil: 5.77-31.68). Therefore, plant-based BBFs and animal-based BBFs exhibited a significantly higher sorption capacity for pharmaceuticals in soil, while ash-based BBFs and manure-based BBFs had minimal impact on their sorption. To further investigate this hypothesis, we plan to conduct sorption experiments using amended soil with these four BBFs. BBFs have the potential to influence the behavior of pharmaceuticals in soil through their strong sorption to BBFs matrices, which can reduce pharmaceutical bioavailability in soils, plant uptake (and consequently human health risks), as well as risks for soil fauna, and their potential spreading through leaching in the environment.

## Germination as a Carbon Reduction and Nutrient Enhancing Solution: A Life Cycle Assessment on Novel Cultivation Method for Plant-based Meat

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### Abstract

Several social issues surround the consumption of beef such as the extinction of animals, deforestation, climate change, wastage and consumption, human rights and land grabbing, ethics of animal slaughter and abuse, labor, safety, and health risks. Despite the arising issues, the growing global demand for beef continues to increase at 6% CAGR year-on-year. And with every business gearing towards sustainability, industry players are now pushed to innovate towards producing alternative options to lessen their environmental impact without compromising economic profitability. In Japan, a recent survey study reveals a significant market of generous consumers willing to pay more for organic and healthy beef products and novelty accepters that prefer plant-based and cultured meat causing the market size of plant-based to significantly increase in 2020 by 39%.

With this growing trend on plant-based meat, this research, therefore, aims to determine the environmental impact of plant-based meat (germinated soy and pea) throughout its product lifecycle. The research utilized life cycle assessment analysis on a patented germination method for soybeans and peas. The method utilizes the metabolic explosion of germinated plant seeds and aims to produce similar tasting and nutritional value meat products. Based on the initial results of this research, 1 kg of plant-based meat decreases GHG emissions from a minimum of 30% up to a maximum of 50% if compared to conventional cattle-based production. The plant-based meat has also been proven to have a higher protein content as compared to conventional. Plant-based can go up to three times higher protein content than conventional meat and up to six times higher than common soybean. The study further aims to provide insights and recommendations to help the business better strategize its environmental sustainability initiatives and strategies. For future research, this paper aims to also investigate the prospective life-cycle assessment of cultured meat. The application of lifecycle sustainability assessment to assess the economic and social impact will also be explored.

## Pesticide Exposure Assessment in (Migratory) Birds

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### Abstract

Migratory birds are often of sensitive conservation status. One of the stressors that may result in their declining populations, is that birds depend on areas that are often contaminated with a range of contaminants. Especially the lack of knowledge on birds' potential exposure to legacy and current-use pesticides is striking and possibly hampering efficient conservation of their populations. Therefore, the aim of this project is to quantify to which pesticides birds are exposed. Our research is divided into two parts: 1) a pesticide exposure assessment in the breeding habitats of the meadow bird *Limosa limosa*, and 2) an exposure assessment of pesticides in tissues of birds with different feeding traits to assess the potential impacts of different diets on exposures.

For the first part, soil and earthworm samples (=one of the main food item of *L. limosa*) were collected from 26 different agricultural fields in an essential breeding area of *L. limosa*, the province Friesland (Netherlands). The sampled plots consisted of 8 intensive grassland fields, 7 corn fields, 4 bulb fields and 7 old-grassland fields. For old-grassland, as far as known, no other crops than grass have been cultivated on the plot. The sampled plots belong to 8 different landowners. On each plot, a total of 15 soil samples, that were distributed throughout the plot, were collected from the topsoil (0-5 cm deep). The 15 samples obtained from one plot, were pooled into 3 composite samples per plot (5 samples per composite). Each composite sample was processed and the extract screened for the presence of 648 pesticides in a qualitative way. The 46 detected pesticides in soil are currently being quantified.

For the exposure assessment of pesticides in birds itself, it is desired to determine if there is a difference between the type and amount of pesticides detected in livers from healthy birds (birds shot by hunters) and potentially unhealthy birds (birds found dead are seen as potentially unhealthy). Furthermore, differences between species based on their feeding traits (granivore, herbivore) will be assessed and linked to the mode of application of the pesticides (e.g. foliar spray versus seed dressing).

By combining these two assessments, novel insights on pesticide exposure of birds with different feeding traits under different land use regimes will be obtained, which is valuable for the optimization of habitat quality management for a better conservation of (migratory) birds.



## **Interactive Effects of Pesticide Contamination, Waterlogging and Invasive Plant Species on Riparian Foodwebs – a Greenhouse Mesocosm Experiment**

Elyssa Dubois, Kai Riess

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### **Abstract**

Riparian foodwebs are subjected to a multitude of stressors such as pesticide contamination, waterlogging and invasive species. But in most cases, only the effects of single stressors on isolated levels of the foodweb are studied.

The aim of our study is to draw a holistic picture of the response of riparian foodwebs (including soil and root microbiota, plants, herbivorous insects and arthropod predators) to the three stressors mentioned above.

The mesocosms used to study these questions comprised riparian soil, a riparian native plant community and aphids that were fed to predators. The system was waterlogged either only with tap water, or with a mix of fungicides, herbicides and insecticides at environmentally relevant concentrations. Additionally, the effect of the invasive plant *Impatiens glandulifera* was observed separately, and in combination with (pesticide) waterlogging.

The growth and phenology of plants was assessed over an eight week period. Plant biomass as well as weight and reproduction of aphids were assessed after (pesticide) waterlogging events. Analyses on microbial community, activity and arthropod predators are still ongoing.

We hypothesize that (i) plants have reduced growth and delayed phenology due to the invasive plant species and pesticides, but are not be affected by waterlogging, (ii) herbivores show plant species specific patterns in reproduction and weight and are particularly negatively affected by pesticides (iii) the combination of stressors has more than additive effects on the foodweb.

This study contributes to the understanding of stressors that are likely to become more present in riparian ecosystems in the future. This is especially important in the current context, where studies including multiple stressors are lacking, even though these scenarios often occur together in nature.

## Curcumin Promotes Animal Health and Antioxidant Protection in Pacific Oysters

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### Abstract

The aquatic environment is subject to a variety of stressors, whether they are man-made, such as pollution, or natural, such as variations in temperature, salinity, and pH. As a result, aquatic organisms respond to the oxidative stress caused by environmental conditions, especially in dynamic environments like coastal habitats. In recent years, interest in the molecular mechanisms that govern antioxidant systems in aquatic animals has grown, particularly those associated with the Nrf2 pathway, which is a master regulator of antioxidant responses and a booster of animal health.

Curcumin (CUR) and other flavonoids have been used to activate the Nrf2 pathway and explore the dynamics of antioxidants in aquatic species. We exposed adult oysters to 30  $\mu$ M CUR for a period of two weeks. CUR increased antioxidant levels in tissues that come into direct contact with seawater, like as the gills and mantle, but not in internal tissues like the digestive gland. The glutathione system, an active Nrf2 pathway, and cellular defense against oxidative stress were all associated to this amplification. This type of interaction is a critical first line of defense in tissues that come into direct contact with seawater.

A second set of studies currently uses CUR to investigate the relationship between Nrf2 pathway, antioxidant protection, immunological function, and energy metabolism in oyster blood cells (hemocytes). CUR increases antioxidant capacity but has little effect on the kinetics of free radical generation in cells. Such effects did not demonstrate enhanced protection against well-known environmental stressors such as anti-fouling coatings and polycyclic aromatic hydrocarbons. Other environmental stresses, however, will be examined to verify the relationship between Nrf2 and cellular protection in bivalve cells. Assays measuring mitochondrial function and bioenergetics will also determine if the Nrf2 pathway may promote enhanced mitochondrial resilience to stress as well as gains in energy efficiency or output.

Our findings suggest that Nrf2 activators might be used to study the dynamics of endogenous antioxidants in marine bivalves, which could aid in understanding the molecular processes underpinning their lives in dynamic coastal and intertidal habitats. This also provides possible molecular targets and natural compounds that might help the aquaculture business in the future by improving animal health, metabolism, and resilience within a sustainable blue economy.

## Effect of Hydrological Fluctuations on Aquatic-Terrestrial Food-Web Interactions

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### Abstract

Assessments of effects that stressors originating from aquatic ecosystems can elicit on riparian communities seldom consider top-down and bottom-up effects across terrestrial food web trophic levels. For example, only few observational studies established a direct relationship between hydrological fluctuations and their impact on the terrestrial ecosystem beyond the first recipient level. While it has been observed that floods can favour tolerant aquatic insect larvae negatively impacting riparian vegetation, there is a paucity of information about how these effects can cascade on the terrestrial consumers, such as herbivore arthropods or carnivorous beetles and spiders, feeding on the vegetation and emerging aquatic insect subsidies.

In this project, as part of the Research Training Program SystemLink, we aim to investigate direct and indirect (top-down and bottom-up) effects that flooding can cause on riparian arthropod communities. In particular, we aim to assess the taxonomical and functional response of terrestrial arthropod communities analysing structural community metrics (e.g., abundance, community composition) as well as functional traits (e. g., size, reproductive abilities, movement capacity, feeding guild, trophic level) of ground-dwelling consumers.

The experiment will take place in 16 Riparian Stream Mesocosms (RSMs) in which channels are paired with adjacent terrestrial area, both colonised by local aquatic and terrestrial vegetation and invertebrates. The experiment is designed as a fully-replicated block with 3 treatments (n=4) varying in flooding duration, and a control (no flood). Biotic and abiotic samples will be collected from spring to summer 2023 before and after each flooding event. Insects in the terrestrial areas will be collected using various trapping approaches such as vacuum, pit fall traps, air-window traps from May until September 2023.

## Session 7: Terrestrial Ecotoxicology

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### Pharmaceuticals and Personal Care Products; Ecological and Environmental Concerns for Soil Fauna

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#### Abstract

Pharmaceuticals and personal care products (PPCPs) have increased in recent years due to rising populations globally. Many studies have extensively investigated their occurrence and impact on the aquatic environment and wastewater treatment systems. However, their effects on soil communities need more research to elucidate the concerns they pose. This work uses originally published research articles to assess the primary sources, classes, fates, and effects of PPCPs in soils. This study also outlines the ecological and environmental impact of PPCPs on soil fauna, as different PPCPs are associated with different impacts. Due to its high organic matter content, the primary source of PPCPs in soils was highly valued sewage sludge. Other significant pollutants sources were antibiotic accumulation from agricultural activities, irrigation with recovered water, and biosolids application. The study also identified significant pollutant classes of PPCPs, including steroids, analgesics, antibiotics, antidepressants, illicit drugs, drugs of abuse, psychiatric drugs, ultraviolet (UV) filters, polycyclic musk compounds (PMC), parabens, bisphenols, benzophenones, and alkylphenol ethoxylates. Soil nematodes, earthworms, and microbial communities were some of the soil fauna studied, of which earthworms were the most investigated. Effects of PPCPs reported include inhibition of feeding activity and growth rate, mortality, abundance reduction, and ecotoxicity. Therefore, there is a need to intensify measures to monitor and reduce pollution, including policy interventions. This is highly essential for soil health, as their impacts on soil interactions and communities consequentially affect organisms higher in the food chain.

Keywords: Pharmaceutical, Personal Care Products, Soil Fauna

## Toxicity of Arsenic-Contaminated Soils to *Folsomia candida* After a Short-Term and Long-Term Exposure

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### Abstract

Arsenic is a naturally occurring element in the environment. However, the presence of arsenic compounds in many ecosystems has increased due to human activities. With regard to the intensification of arsenic occurrence in different chemical forms (speciation) it is important to provide information to diagnose the concern. In order to assess the potential impact of contaminants in a broader perspective, it is relevant to develop new additional methods to verify potential toxicity to terrestrial organisms.

The aim of this study was to assess the toxicity of soil contaminated with different forms of arsenic, using *Folsomia candida* (*Collembola*) as a standard model species in ecotoxicology. Agricultural soil was spiked with arsenic(V) or arsenic(III). The exposure of the test organism was performed using two well-known available bioassays and an additional one proposed as a new alternative tool. For the assessment of short-term (acute) toxicity, an avoidance behaviour test was performed according to the ISO standards (ISO 17512-2:2011). For the determination of long-term (chronic) toxicity, the reproduction test was performed according to the OECD Guideline No. 232 (OECD Test 232, 2016) and an alternative growth inhibition test was performed based on previous studies and the above-mentioned OECD Guideline with modifications.

The avoidance test showed that increasing concentrations of As(V) affected the response of collembolans, resulting in a progressive avoidance of contaminated soils. The concentration that caused an effect in 50% of the organisms tested was EC<sub>50</sub> of 25 mg kg<sup>-1</sup> soil<sub>dw</sub>. A significantly reduced reproductive potential of *F. candida* was shown in the reproduction test even at low exposure concentrations (EC<sub>50</sub> of 8,22 mg kg<sup>-1</sup> soil<sub>dw</sub>) of As(V). In the growth inhibition test As(III) was found to be more toxic (EC<sub>50</sub> of 15,20 mg kg<sup>-1</sup> soil<sub>dw</sub>) in comparison to As(V) (EC<sub>50</sub> of 27,61 mg kg<sup>-1</sup> soil<sub>dw</sub>).

Arsenic was found to have a noticeable toxic effect on *F. candida* at different concentrations tested in the bioassays. The results also verified that the potential toxicity of arsenic as a soil contamination can be determined by the ability of *F. candida* to show responses in the proposed alternative growth inhibition test. Thus, the growth inhibition test can be considered as a new tool in ecotoxicology. However, further research is needed to evaluate and optimise the new method using other commonly occurring soil contaminants.

## Effect of Deoxynivalenol on Soil Nitrification

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### Abstract

Deoxynivalenol (DON) is a toxic secondary metabolite produced by the fungal genus *Fusarium*, such as *F. graminearum*. These species are phytopathogens, that are specialized in the infestation of flowering grasses, including crops. Therefore, DON is mainly regarded as a food contaminant. Since secondary metabolites also serve important biological functions in, e.g., microbial interactions, there is growing concern in DON as a soil pollutant, due to *in situ* production or when it is washed off the infested plants entering the soil. Despite its well-known toxicity to humans and livestock, the biogeochemical role of DON in the soil is far from being entirely understood, even though there are indications of the molecule's bactericidal properties.

Soil nitrification is the process of biological oxidation of ammonium (N-NH<sub>4</sub>) to nitrite followed by the oxidation of the nitrite (N-NO<sub>2</sub>) to nitrate (N-NO<sub>3</sub>). This process allows for the balance in the N-cycle in soils, which is relevant for soil functionality and productivity as well as plant nutrition. Secondary metabolites of plants, i.e., Resveratrol and procyanidins are known to affect this process in soil. Therefore, the question arises whether secondary metabolites of fungi may also affect nitrification since fungal growth and fitness in soil depend on N species and availability.

In this study, we investigated DON for its effect on the potential nitrification rate (PNR). The PNR of soil samples was determined by measuring the microbial oxidation of excess ammonium to nitrate over 24 hours in an aqueous phosphate buffer suspension. Soil samples were taken from a Riparian area in the east of the Landau in Rhineland-Palatinate. A soil subsample was treated at a level of 3 µg DON (g dry soil)<sup>-1</sup> and the results were compared to a non-spiked control group. The test was carried out in quadruplicates. Preliminary data shows that DON significantly increased the nitrification rate (N-NO<sub>3</sub> over N-NH<sub>4</sub>) in the tested soil by averagely 28 ± 1 % compared to the control group (t-test, t=6.8382, df=5.7913, p<0.001, n=4).

This observation provides evidence of the effect of DON on soil nitrifying bacteria or its enzymes and therefore potentially new understanding *Fusarium* phytopathogenicity and its control over the nitrification process. In further studies, we aim to validate these results, including soils with different land use and DON treatment in different concentrations as well stable N isotope analysis.

## Fungicide reduction enhances beneficial arthropods in grapevine

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### Abstract

Pesticides are considered one of the main causes for arthropod decline which in turn may affect ecosystem services such as natural pest control. Grapevine is one of the most pesticide depending crops, typically receiving ten fungicide sprayings in three months of growing season in the Palatinate region, Germany. Sprayings contain several plant protection products of varying toxicity towards non-target organisms in both organic and conventional viticulture. However, the cultivation of fungus resistant varieties allows reductions of fungicide applications by around 70%.

We studied effects of varying fungicide application frequency and toxicity in organic vs. conventional viticulture planted with susceptible vs. fungus-resistant grape varieties on non-target arthropods. Macro-arthropods were sampled with beat-sheets from the grapevine foliage. We further took leaf samples to assess densities of vineyard pest mites and their natural enemies.

Sprayings were drastically reduced under fungus-resistant varieties resulting in less toxic spraying regimes. Realized pesticide savings were higher in organic than in conventional vineyards. Reduced sprayings highly promoted predatory macro-arthropods, particularly spiders as well as several beneficial mite taxa. By contrast, pest mites were enhanced under frequent fungicide sprayings. We conclude that fungus-resistant varieties offer a great potential to reduce toxic inputs in vineyards. This fosters arthropod natural enemies and should consequently enhance the resilience of this agroecosystem to pest attack.

## Metal Transfer from Insects to Bats in a Metal-contaminated Environment

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### Abstract

All European bats are insectivorous and occupy the highest food web level. Because bats are strictly insectivorous top predators, the hazard from metal poisoning is enhanced by the process of biomagnification, i.e., the increase of metal concentrations along food chains. The main goal of this research was to study the transfer of Cd, Cu, Pb and Zn through the terrestrial trophic chain from insects to bats as top predators.

To assess metal transfer from insects to bats in a metal-contaminated environment, samples of soil, river sediments, insects and bat carcasses were collected in the small town of Sławków (Poland), located close to "Bolesław" zinc-and-lead smelter and mine. I took 25 random samples of topsoil (upper 5 cm) and 20 samples of water sediments in the area located nearby the roosting colony of *Myotis emarginatus*. Insects were sampled using light traps close to Biała Przemsza river in Sławków, Bat carcasses (15 individuals) and bat guano (20 mixed samples) were collected in a church (Sławków, city center), where the bats have the colony. All samples were analyzed for concentrations of Pb, Cd, Cu, and Zn using atomic absorption spectrophotometers AAnalyst 200 and PinAAcle 900Z (Perkin Elmer).

Concentrations of all four metals in bat guano were significantly higher than in insects, bat fur, bones and wing membrane, while concentrations of Cd, Cu and Pb in insects did not differ significantly from those in bat tissues. Concentrations of Zn in insects were higher than in wing membrane and bones. Concentrations of Zn in bats did not differ significantly between the tissues.

The higher concentrations of metals in guano than in bat tissues, together with the lack of differences in concentrations between insects and bat tissues in most cases, and Zn concentrations in bats even lower than in insects, indicate effective metal decontamination in bats. The lack of metal biomagnification in bats is generally positive information but further studies are needed to tell if the metals do not accumulate with age as the lifespan of *M. emarginatus* can exceed 20 years in the wild. Also, it would be interesting to study the possible fitness costs of metal decontamination.



## Nanozeolite Interactions with Earthworms and N-Cycling Soil Microbial Communities

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### Abstract

Nanomaterials have been shown to be useful in agriculture, with examples proving efficacy as pesticides, nanofertilisers or fertiliser additives. Nanomaterials unique properties such as large surface area to volume ratio increase their reactivity, with some nanomaterials subject to quantum effects depending on composition. While there is huge potential for nanomaterials to be used in agriculture, complete assessment of their long-term implications on soil health, and wider impact on ecosystem functioning, is yet to be fully understood. Earthworms are known as soil and ecosystem engineers, aiding in soil aeration, decomposition and mineralisation of litter, and therefore influencing resource availability for soil microorganisms. This study looked at two nanozeolites in particular, looking at the dose-dependent response of earthworms (*Eisenia fetida*) to their presence in soil. Nanozeolites are porous which enables high loading of nitrogen (N) or urea, allowing specific or non-specific interactions and potential to act as catalysts for different N containing compounds. This means they are good candidates for use as fertilisers, permitting slow nutrient release and minimising N pollution. Interrogating the impacts they have on nitrogen cycling is essential to understanding their role holistically in soil. The same nanozeolites as in the earthworm study were also used for growing lettuce with conventional fertiliser additions, with the focus on N cycling and soil microbial community responses to their presence/absence. Initial results indicate that differing nanozeolites can have very different interactions with earthworms and N-cycling soil microbes, potentially due to differing pore sizes.

## Heat Waves Have an Impact on the Toxicity of the Fungicide Fontelis 20 EC on *Folsomia candida*

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### Abstract

The ongoing climate change causes with increased mean temperature, heat waves, drought, cold snaps and extreme rain severe changes in the environment and biodiversity. These extreme climate events can presumably change the effects of agrochemicals on soil organisms. However, the changing climate is not accounted for in the standard soil ecotoxicological tests.

As the soil fauna takes an important part in most soil ecosystem services, the monitoring of these climate-induced stressors is significant. Thus, there is a need for more ecotoxicological studies outside of standard conditions, to provide a more comprehensive hazard assessment. We are interested in the effects caused by an interaction between heat waves and agrochemicals on the soil fauna. Good indicators for risks to soils as ecosystems are collembola, which play a central role in the ecological functioning of soils. Here, we work with the collembolan species *Folsomia candida* which is a model organism and well-established in ecotoxicological tests.

There is already research on the topic of increased temperature-induced stress for *F. candida*, but the number of studies including toxic substances is scarce. We chose Fontelis 20 EC as our test substance, a fungicide which is frequently used in the EU. The active ingredient is penthiopyrad, a succinyl dehydrogenase inhibitor, which has not yet been examined for its toxicity on the soil fauna and little is known of the toxicity on other organisms.

Our study aims to add heat waves as an extra stressor into the miniaturized *F. candida* reproduction test (OECD 232). Additionally, relative growth, a recovery phase, egg number, egg volume, and reproductive investment will be included in our analysis. I hypothesise that heat waves and the resulting heat shocks increase the adverse effects of Fontelis 20 EC on *F. candida* or change trade-offs between growth and reproduction. In conclusion, these results can highlight the importance of short heat waves not only as potential stressors but as synergistic stressors in combination with agrochemicals.

As of now, we do not have any results yet but will be able to present the first results of the heat wave experiment during the conference.

## Exposure to Sublethal Concentrations of Imidacloprid, Pyraclostrobin and Glyphosate Harm the Behavior and Fat Body of the Stingless Bee *Scaptotrigona postica*

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### Abstract

The use of pesticides in agriculture is one of the most critical threats to nontarget insects, such as bees. Most studies on their effect on pollinators have relied on the honeybee *Apis mellifera* as surrogate species for pesticide risk assessment for other bees. Considering the ecological and economic relevance of native bees, such as *Scaptotrigona postica*, and the scarcity of studies on the effects of these products on the behavior and physiology of these insects, it is paramount to improve the current knowledge about this issue. Therefore, this study aimed to investigate the sublethal effects of imidacloprid, pyraclostrobin and glyphosate, alone and in combination, on the stingless bee *S. postica*. The parameters investigated were the behavior analysis, the histological changes, immunofluorescence bioassays of caspase-3, TLR4 and heat-shock proteins 90 (HSP90) in the fat body of orally exposed workers using light, fluorescence and confocal microscopy. Experimental groups were divided as follows: Syrup control-CL; acetone control-ACT; imidacloprid-IMD (0.3 ng/μL); pyraclostrobin-PYR (1.5 ng/μL); glyphosate-GLY (7 ng/μL); imidacloprid and pyraclostrobin-IMD+PYR; imidacloprid and glyphosate-IMD+GLY; pyraclostrobin and glyphosate-PYR+GLY. Ingestion of the pesticides, alone and in combination, decreased the bees' walking distance and mean velocity compared to the control and solvent control groups. The bees exposed to the agrochemicals, alone and in combination, showed extensive cytoarchitecture disruption in the fat body, such as vacuolization and shape changes in oenocytes and altered nuclei morphology in trophocytes. Moreover, pesticide exposure increased the number of atypical oenocytes and altered trophocytes. The caspase-positive labeling showed a significant increase in all groups of exposed bees. On the other hand, TLR4 labeling significantly decreased in the exposed groups compared to the control groups (CL and ACT). Meanwhile, there was a significant increase in the HSP90 immunolabelling in all exposed groups compared to the control. These findings reinforce the importance of research on the sublethal effects of low concentrations of different pesticide on native bees and provide evidence that these toxic substances can impair the detoxification and immune defense of key neotropical pollinators.

## Effects of Temperature and Moisture on the Ecotoxicity of Metal-Based Fungicides Towards Earthworms

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### Abstract

The aim of this study was to determine if varying soil moisture contents and different temperatures will influence the toxicity of single and binary mixtures of the fungicides, copper oxychloride and mancozeb, (in different concentrations) towards earthworms (*Eisenia andrei*) as bioindicators of soil quality. Tests were done using artificial soil in accordance with the OECD guidelines under two different temperatures viz. 20°C and 25°C and two different moisture contents viz. 30% and 50%. The concentrations for copper oxychloride (CuOx) were 200, 500 and 1000 mg/kg and mancozeb (MnZn) 44, 850 and 1250 mg/kg in single and binary mixtures. Endpoints measured included metal analyses, comet assays (DNA analysis) and soil enzymatic analyses. DNA damage increased as the concentrations of the treatments increased. Significant differences ( $p < 0.05$ ) were found between the different temperatures and soil moistures although the different experiments produced different results. Body metal analysis test corresponded with the comet assay test wherein there was an increase in body metal concentration as the concentrations of the treatments increased ( $p < 0.05$ ). Significant differences ( $p < 0.05$ ) were found in the copper and manganese concentrations although no significant differences were found in the zinc concentrations. Soil enzymatic analysis indicated significant differences in the alkaline phosphatase and glucosidase tests for the single and binary (copper oxychloride and mancozeb) treatments and at the 25°C 50% exposure it was the most significant. It is evident that fluctuating temperatures and soil moistures had varying effects on the single and binary mixtures of copper oxychloride and mancozeb. It was concluded that varying temperatures and soil moistures would have a significantly different result on the effects of metal-based fungicides towards earthworms.

Keywords: Alkaline phosphatase; Body metal analysis; Climate change; comet assay; Copper oxychloride; *Eisenia andrei*; Glucosidase; Mancozeb; Moisture; Temperature

## Insects Pesticide Exposition in Flower Strips at the Agricultural Field Areas in Landau in der Pfalz and Surrounding Areas, Germany

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### Abstract

In Germany flower strips are conservation strategies implemented as ecological infrastructures that help to increase farmland biodiversity, as well as pollen and nectar sources, helping to increase the beneficial insects in the agricultural areas. But in the neighboring areas complex mixtures of pesticides are applied for agricultural production, being a problem for the flower-visiting insect that feeds on the areas. Therefore, the main objective of this research was to Determine the pesticide mixtures present in flowers from the flower strips of different crop areas around Landau in der Pfalz, Germany, from May till September, to establish a realistic pesticide mixture exposition for flower-visiting insects. The project also aims to compare the mixture compositions between the flower material coming from different crops, type of flowers and time of the year.

For the methodology, the flower material will be collected from flower strips placed close to different crops in Landau in der Pfalz and surroundings. Starting from the end of May till September. For the data collection liquid extraction of pesticides and quantification using Liquid chromatography–mass spectrometry will be made. The main hypothesis is that flower strips present pesticide exposition, having complex mixtures of active ingredients depending on the crops, type of flowers and application periods. This information helps to improve conservation strategies for visiting pollinators in agricultural areas, and decision making in neighboring conservation zones. Also helps to understand the realistic exposure scenario for those insects.

## Depth- and Time-Dependent Fate and Transfer of Current-use Pesticides in Different Agricultural Soils

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### Abstract

Current-use pesticides (CUPs) are widely used in agriculture to control pests and diseases, but their widespread use can lead to contamination of soil and water resources. When examining CUP residues in soil, most studies focus on the first 30 cm of soil. This is because this layer contains the highest concentrations of CUPs, the root system and most biological activity. However, the fate and transfer of CUPs below this depth is not well understood, and there is a need to assess their potential environmental impact and pathways to groundwater. In addition, CUPs in deeper soil layers may be mobilized by plowing later in the season. Soil studies for regulatory purposes are limited to near-surface depths and also restricted to a very limited number of soil types. However, soil characteristics such as humus content affect the sorption and therefore also the half-life of CUPs. To answer the question of how CUPs with different half-lives are transferred or metabolized as a function of soil depth and soil type (sandy, loamy, humic), a time series experiment is being conducted. Three fungicides (bixafen, fluopyram, prothioconazole) and one insecticide (pirimicarb) will be applied simultaneously to three winter wheat fields of different soil types in close proximity to each other (within 5 km) in the Palatinate region of Germany. On the day of application (day 0) and on days 1, 2, 4, 8, 16 and 32 after application, as well as after a rain event, soil samples will be collected to a depth of up to 1 m. Samples will be analyzed for these CUPs and their metabolites by HPLC-ESI-MS/MS. We hypothesize that the fate and transfer of these CUPs will be influenced by soil type, with higher mobility and potential for leaching in sandy soils, whereas loamy soils may retain the CUPs longer. The results of this study can be used to improve pesticide risk assessment by considering the exposure of soil organisms at different depths, and to guide the development of sustainable agricultural practices that help maintain healthy soils and ecosystems.

## Investigation of Pesticide Accumulation in *Osmia bicornis* During Larval Development by Pollen Supply and Loamy Brood Cell Walls

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### Abstract

The use of pesticides in agriculture affects not only target organisms but also other organisms such as pollinators. The use of pesticides is known to be a major cause of declines in insect populations, including pollinators such as honeybees, wild bees and bumblebees. Current policies do not provide adequate protection for pollinators, which play a vital role in the food supply. This project investigates the extent to which pesticide residues potentially accumulate in the developing wild bee *Osmia bicornis* through uptake from stored pollen and contact with the soil cell walls. Nesting aids were placed in the field (Rhineland-Palatine) and collected after a period of time. The female bee provided pollen in each brood cell, which was potentially contaminated with agriculturally used pesticides from the neighboring field. The bees were hatched under laboratory conditions and frozen immediately. After lyophilization the bee matrix was extracted and analyzed by HPLC/MS-MS. We developed an extraction method to individual bees and pollen stores by HPLC-MS/MS. Pollen residues and *Osmia* bees emerging from corresponding brood cells were analyzed and compared. We hypothesize that *Osmia bicornis* are contaminated with pesticides by pollen ingested as larvae and that the pesticides detected in brood cells can also be found in adult *Osmia bicornis*. The results of this study will improve our understanding of how pesticides affect pollinators in their development, especially solitary bee species such as *Osmia bicornis*.

## Session 8: Environmental Impact Assessment

P29

### Parental Exposure to Atrazine Induces Chromosome Instability in Somatic and Germ Cells of Untreated Progeny of *Drosophila melanogaster*

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#### Abstract

Atrazine is an herbicide that is widely used in some regions of the world which, in addition to its high persistence in soil and water, has been classified as an endocrine disruptor, thus representing a potential risk to the environment and organisms. However, information on the long-term impact of atrazine on the offspring of exposed organisms is scarce. The ability of atrazine to alter somatic and germ cell chromosome stability of unexposed offspring from treated female *Drosophila melanogaster* was evaluated. Wild type, third instar larvae were fed with instant medium enriched with atrazine (GESAPRIM®Calibre90) [7.45E-08 at 80 ppm] or distilled water (negative control). When the adults emerged, the number and sex of flies recovered were recorded. Thirty individual matings of treated wild type females with mutant, unexposed males carrying recessive sex-linked genes for white eyes (*w*) and miniature wings (*m*) were done. The number and sex of the F<sub>1</sub> (Filarial 1) were recorded. Individual F<sub>1</sub>xF<sub>1</sub> matings were made to obtain the F<sub>2</sub> (Filarial 2) in which four phenotypes are recovered: two parental and two recombinants. To evaluate the effect on the somatic cells of the progeny, the frequency of spots in the eyes of the *w/w+* heterozygous F<sub>1</sub> females was recorded. Loss of heterozygosity can occur by mutation, deletion, or recombination, among other causes. In F<sub>2</sub>, the impact of atrazine on germ cells was assessed by the change in recombination frequency between the recessive genes used. Atrazine treatment affected the average survival of treated flies and the number of F<sub>1</sub> and F<sub>2</sub> progeny recovered. Parental treatment with atrazine affected the survival of treated flies. The average amount of F<sub>1</sub> and F<sub>2</sub> progeny was modified, too. The frequency of spots in the eyes of *w/w+* females of the F<sub>1</sub> progeny, was higher at the lowest concentrations and at some of the highest concentrations assayed. In the F<sub>2</sub> progeny, the frequency of recombination between the *w* and *m* genes increased at the highest concentrations. In *Drosophila*, parental exposure to atrazine affected the chromosome stability of both somatic cells and germ cells of two consecutive untreated progeny. These results suggest that further studies of the long-term effect of atrazine in other biological models are required. Acknowledgments: Adriana Muñoz-Hernández, Hugo Rivas-Martínez. Biological Sciences Graduate Program, UNAM. CONACYT-630455, PAPIIT-UNAM IN226923, *Drosophila* Stock Center Mexico, UNAM.



**Environmental safety of two potential antifungal candidates: VT-1161 and T-2307**

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**Abstract**

Antifungal drugs are a class of pharmaceuticals that are widely used to treat fungal infections in humans, animals and plants. However, these drugs can also have unintended environmental impacts, as they can enter the environment through various pathways, such as human and animal excretion, improper disposal of unused medications, and agricultural runoff. Once in the environment, antifungal drugs can have toxic effects on aquatic organisms, plants, and microorganisms.

VT-1161, a novel tetrazole and the arylamidine T-2307 are two antifungal agents that are currently being studied for their effectiveness in treating various fungal infections. While there is limited information available on the specific environmental impacts of these drugs, it is important to consider the potential effects that they could have on the environment.

In order to complement toxicological information, this study applied a battery of bioassays to evaluate, for the first time, the effects of VT-1161 and T-2307 on a suite of bioindicators including *Aliivibrio fischeri* (Gram-negative bacterium), *Raphidocelis subcapitata* (green alga), and *Daphnia magna* (microcrustacean), and assessed the median effective concentration (EC50) values for the tested species.

Toxicity tests were performed according to ISO, namely ISO 11348:2007 for the bioluminescence of *A. fischeri*, ISO 8692:2012 for the algal growth inhibition of *R. subcapitata* and ISO 6341: 2012 for immobility of *D. magna*.

Preliminary results indicated both VT-1161 and T-2307 did not exhibit significant toxicity towards any of the tested organisms within the tested concentration range (0.1 mg/L to 100 mg/L). This suggests that these compounds have a low potential to cause adverse effects on non-target organisms and consequently may not pose significant risks at environmentally relevant concentrations. However, it is important to note that the effects of VT-1161 and T-2307 on other organisms or under different exposure conditions may vary, and further research is needed to fully understand their potential ecological impacts.

## **Influence of Spent Engine Oil-Contaminated Soil on Two Hydrolytic Soil Enzymes (Lipase and Urease): A Comparative Study.**

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### **Abstract**

Ecosystems being widely subjected to growing manmade stresses, and the question of the acclimation capacities of soil organisms to chemical pollution is of major concern. The effect of spent engine oil on soil pH as well as activities of selected enzymes (lipase and urease) was studied. The activities of soil lipase and urease were ascertained following customary procedures. Across a twenty-eight-day period (Days 0, 7, 14, 21, and 28), the activities of the soil enzymes were measured as a function of time and spent oil concentrations at 5 g (10 g / g), 10 g (20 g / g), 15 g (30 g / g), and 20 g (40 g / g). The results of the study showed a significant increase ( $p < 0.05$ ) in soil lipase activities in a concentration-dependent manner from 396.273 to 475.202  $\mu\text{g pNp}/(\text{gx}10\text{min})$  for 0 and 5g concentration of spent engine oil respectively on day 0. The soil lipase activity continued to increase significantly with time, as the concentrations of the spent engine oil increased. Then, rose to its peak on day 14, recording (790.19  $\mu\text{g pNp}/(\text{gx}10\text{min})$ ) and thereafter declined. However, it was observed that spent engine oil had an inhibitory and to a little extent, stimulatory effect on soil urease activity with respect to concentration and time. Soil urease activity was observed to fluctuate on day 0, across all concentrations while a significant decrease ( $p < 0.05$ ) in enzyme activity was recorded sequentially on day 7 in a concentration-dependent manner. Soil urease activities continued to decrease significantly ( $p < 0.05$ ) with time till day 21, and thereafter witnessed a slight increase on day 28. Spent engine oil also caused a slight change though not very significant ( $p > 0.05$ ) in soil pH relative to the control. Howbeit, the outcome of the present study suggests that spent engine oil alters soil biochemistry. These findings would be useful in ascertaining soil profile and subsequently ensuring a balanced ecosystem via adequate control schemes.

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