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BETWEEN GRINDING GEARS - STUDENTS AND EARLY CAREER SCIENTISTS UNDER PRESSURE

ABSTRACT BOOK

Abstract Book

SETAC 13th Young Environmental Scientists Meeting

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In an urban landscape, wastewaters containing chemicals such as pharmaceuticals, personal care products and biocides are a primary source of contaminants to freshwater ecosystems. Ideally, wastewater is treated prior to its release by wastewater treatment plants (WWTPs). Nonetheless, certain contaminants are hardly retained during conventional treatment steps, potentially adversely affecting freshwater organisms and the ecosystem processes these organisms perform. Against this background, the present study quantifies the impact of urban wastewater on the ecosystem level process of leaf litter decomposition, which represents a vital source of energy for aquatic food webs. Therefore, nine streams within the upper Rhine valley receiving WWTP effluents were studied in January 2025. To determine the microbial decomposition rate of leaf litter, fine-mesh bags filled with leaves of black alder or beech trees were deployed upstream and downstream of the WWTPs for three weeks. Different plant species produce litter that can differ greatly in terms of chemical composition, leading to variation in the microbial colonization and degradation of leaves. We consequently hypothesize that alder litter decomposes at faster rates than beech litter, and the decomposition rates are lower downstream of WWTPs effluents, which usually contain a diversity of contaminants including detergents, pharmaceuticals and personal care products. The samples are currently being processed for metabarcoding analysis to shed the light on changes in the fungal communities over the course of the study. As data collection and analyses are ongoing, the on-site presentation will critically reflect on the hypotheses we specifically tested.

2 | **Keeping It Real: Investigating the Impact of Neuroactive Pharmaceuticals on the Functional Role of a Freshwater Ecosystem Engineer, Gammarus pulex**
Joseph D'Souza, Cardiff University; Charles Tyler, Exeter University; Fredric Windsor, Cardiff University

An ever-increasing number of Pharmaceuticals and Personal Care Products (PPCPs) are being detected in freshwaters globally, continually released through wastewater discharges. While environmental concentrations are typically below toxic levels for aquatic biota, exposure to neuroactive PPCPs - substances that primarily invoke neurological responses - is of particular concern due to the myriad of behavioural effects they may induce. Research investigating the behavioural impacts of PPCPs at environmentally relevant concentrations is however scarce, with many unknowns shrouding their true ecological impact. Gammarus pulexplay a vital role in shredding coarse leaf litter, driving decomposition and nutrient cycling in rivers - key processes underpinning freshwater ecosystem function. To investigate how neuroactive PPCPs might impair ecosystem functioning via behavioural change, we exposed G. pulex to two neuroactive PPCPs - carbamazepine (anti-epileptic) and venlafaxine (anti-depressant) - in a 7-day acute exposure experiment. Individuals were dosed with either a single PPCP or a combination, at concentrations based on mean and maximum values from UK river monitoring data. Changes in behaviour (e.g., mobility rate, average speed and time spent frozen) as well as leaf litter shredding rates were measured and compared to a non-dosed control. These behavioural changes may represent underlying mechanisms (e.g., reduced foraging rate) through which shredding rates decline.
Behavioural observations showed high variability across all conditions, as expected in a wild-caught population. However, a significant decrease in mobility and average speed, and increase in time spent frozen, were observed in response to ‘high’ carbamazepine concentrations (500 ng/L). Shredding rates showed no significant differences between treatments and controls, suggesting that altered behaviour either does not translate into reduced feeding, or that these behavioural changes occur prior to any observable impacts on feeding. Long-term exposures may hence be required to detect impacts on functional endpoints such as leaf litter shredding. This study highlights the value of assessing sub-lethal and functional endpoints under realistic PPCP exposures, supporting a deeper understanding of their ecological risks and indicating potential pathways through which freshwater biodiversity and ecosystem functioning could be impacted.

3 | **From Biochemistry to Demography: New Prospects for Aquatic Biomonitoring**
Chloé De Vernisy, University of Lorraine (UL); Manon Bain, Université de Lorraine/LTSER France; Claire Caillard, Université de Lorraine/LTSER France; Simon Devin, Université de Lorraine/LTSER France; Sandrine Pain-Devin, Université de Lorraine/LTSER France

Pollution of aquatic environments is a major threat to biodiversity and the ecosystem services they provide. In this context, monitoring the state of aquatic environments is essential to anticipate the risks associated with anthropogenic pollution and to protect the health of these ecosystems. Analysing the effects of pollutants on organisms by studying biochemical biomarkers provides an opportunity to develop early detection tools, which are essential for anticipating environmental impacts. This study focuses on the implementation of in situ biomonitoring based on the combination of biochemical biomarkers measurements and demographic parameters obtained by modelling population dynamics on a sentinel species, the freshwater mussel Dreissena polymorpha. Thanks to its wide geographical distribution, its sessile character and its ability to bioaccumulate pollutants, this bivalve is an interesting model for assessing pressures on aquatic environments.

The biochemical biomarkers measured in this study are subcellular biochemical responses related to energy metabolism, antioxidant defences and cellular damage. Although they are often presented as early indicators of alterations at higher levels of biological organisation, we still need to establish a link between these biomarkers and individual or population level indicators. We are therefore implementing a multi-scale approach to interpret biochemical biomarkers in the light of individual growth-at-length parameters. Previous work on Dreissena polymorpha has shown a correlation between biomarkers and growth parameters, suggesting that exposure to different contaminants may affect individual growth. Indeed, energy allocation strategies vary according to the size or age of the organism, between reproduction, maintenance of physiological functions and adaptation to stress. The preliminary results of our study explore the relationship between individual size and the measured biomarkers. They show variations in antioxidant defences and cellular damage depending on the size of the organisms, explaining the positive or negative correlations observed. Future campaigns will confirm and complete these results, taking into account annual seasonal variations. This innovative approach will not only help to improve our understanding of the impact of pollutants on populations, but will also reinforce existing biomonitoring protocols, making them more accurate and better adapted to the management and conservation of aquatic ecosystems.

4 | **Testing the SPEARpesticides Index for Pesticide Risk on Macroinvertebrates in Swiss Streams**

Anthony Fow Esteves, Swiss Federal Institute of Aquatic Science and Technology (Eawag); Christiane Ilg, VSA - Swiss Water Association, Platform Water Quality; Nele Schuwirth, Swiss Federal Institute of Aquatic Science and Technology (Eawag); Matthias Liess, Helmholtz Centre for Environmental Research – UFZ; Anne Dietzel, VSA - Swiss Water Association, Platform Water Quality

Micropollutants in streams threaten aquatic ecosystems, with macroinvertebrate communities serving as a key bioindicator of water quality. This study assesses whether the pesticides effects indicated by the SPEARpesticides index correlates with the risk assessment based on chemical measurements in Swiss streams.
The SPEARpesticides index is based on pesticide-sensitive taxa and is designed to indicate pulsed exposure regimes of pesticides in streams. Since 2005, it has been applied in many European countries, as well as America and Australia, and showing a significant relationship with toxic units (TUs) from event driven samplings.
In Switzerland, chemical risk assessment based on the Swiss National Surface Water Quality Monitoring Programme (NAWA) uses chronic and acute risk quotients (RQs); the biological status is assessed, among others, by monitoring macroinvertebrates and calculating the Swiss Biological Index (IBCH) and the SPEARpesticides index.
Previous Swiss studies have shown a negative correlation between the SPEARpesticides index and external land use indicators, such as insecticide application rate or the proportion of agricultural area in the catchment.
However, until recently, consistent chemical measurements of pesticides have been lacking at most of the biomonitoring sites. To address this, we integrated the macroinvertebrate biomonitoring data with the corresponding pesticide data from the NAWA (2018-2023).
First analyses of the correlation between the SPEARpesticides index and the RQs did not show clear patterns. Therefore, our main focus is to investigate different ways of quantifying the chemical risk and potential adaptations between SPEARpesticides index values and their evaluation to quantify the ecological status.
Our analysis considers RQs and adapts the concept of TUs to be consistent with our 14- and 3.5-days composite samples, guiding the ecotoxicological endpoint selection in our risk metrics. This approach enables the comparison of the performance of RQs and TUs in explaining the evaluation by SPEARpesticides. Further steps will include environmental variables to refine our understanding of these relationships.
Finally, by analysing the SPEARpesticides together with the chemical risk in Swiss watercourses, this research aims to enhance the knowledge about the impact of pesticides on macroinvertebrate communities, to improve the assessment of mixture toxicity based on Swiss national wide data.

5 | **Alteration in Biomarker Response Pattern and Target Gene Expression in Zebrafish (Danio rerio) Embryos Exposed to River Water Extracts from the Holtemme River, Germany**

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Aquatic environments are facing continuous release of anthropogenic compounds, leading to growing concerns about human impact on environmental health. In addition, to diffuse sources of anthropogenic contaminants, wastewater treatment plants (WWTPs) are considered main point sources of chemical release into aquatic systems. WWTP effluents contain a broad range of chemicals, and therefore, their impact on aquatic organisms is difficult to assess. This study aims to investigate the responses of multiple biomarkers in zebrafish (Danio rerio) after exposure to water extracts from different sampling sites along the Holtemme River in Central Germany. This is supplemented by an investigation of target gene expression in exposed zebrafish larvae using a reverse transcriptase quantitative poly chain reaction (RT-qPCR). The sampling locations are marking a gradient of anthropogenic impact from a reference site to a site impacted by urban/industrial runoff and a site downstream of a WWTP. 21-day composite samples were taken at these locations using large-volume solid phase extraction. To determine the biomarker response of zebrafish, embryos were exposed to the extracts from the sites mentioned above for 120 h,

afterwards, homogenates and post-mitochondrial supernatants for every exposure group were prepared. Among others, the activity of ethoxyresorufin-O-deethylase, acetylcholinesterase, glutathione reductase, glutathione-S-transferase and catalase were determined. Regarding the target gene analysis, expression fold changes of 14 genes related to oxidative stress, biotransformation, general stress response and the endocrine system were investigated. Results indicate alteration of enzymatic activities in zebrafish larvae exposed to extracts from the anthropogenically impacted sites compared to the reference site, which is also reflected in the gene expression patterns. This could be explained by higher concentrations of chemical compounds in these samples and indicates considerable similarity with the biomarker responses and gene expression patterns measured in a field experiment with wild brown trout from the same sampling sites. Further analysis of the sensitivity and applicability of the genes investigated will provide valuable insight into the comparison between biomonitoring data and laboratory derived data on toxicological effects in fish and will strengthen the knowledge of suitable gene candidates.

6 | **Current Regulatory Approaches for Assessing Aquatic Community Level Studies**

Eilidh Garden, Chemicals Regulation Division, Health & Safety Executive; Isabel Navarro Law, University of York

Aquatic risk assessments for plant protection products (PPPs) follow a tiered structure, starting with the least complex data and the lowest levels of ecological realism. If an unacceptable level of risk is demonstrated at first tier, there are options to refine the risk by increasing the complexity and realism. There are a variety of options for higher-tier aquatic refinements including: geometric means and species sensitivity distributions (both incorporate toxicity data from multiple species), modified exposure studies, and toxicokinetic-toxicodynamic (TKTD) models. One of the highest investment options, both in terms of time and cost, is to conduct a community level study, for example a mesocosm.

Aquatic mesocosms are outdoor experimental ecosystems that represent a tier three refinement. When used in PPP risk assessments, they are designed to simulate the freshwater bodies found on the edge of agricultural fields. The design, conduct, and assessment of a mesocosm is labour-intensive and high cost, and it is therefore important that they are as reliable and representative as possible. One specific issue for use in PPP assessments is matching the exposure profile in the mesocosm study with the predicted exposure following use of the product.

The results of a recently conducted review of macrophyte mesocosms submitted for regulatory risk assessment in GB or EU will be presented. This review will summarise the current regulatory practice for using data from a mesocosm to refine the ecotoxicological aquatic risk assessment in Great Britain and Northern Ireland. Our evaluation approach will be discussed, alongside some common pitfalls that can reduce the applicability and reliability of the results. Using case studies, this work will summarise how HSE approach the evaluation of these studies and provide suggestions on best practice in conducting mesocosm studies for use in PPP regulation.

7 | **Tracking Metal Handling Responses in Liver's Subcellular Fractions Across Five Estuarine Fish Species Under Metal Exposure**

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Understanding how trace elements are handled at the subcellular level provides insights into their potential toxicity. However, most studies focus on freshwater species, analyse only a few metals, and rarely compare across multiple species. Here, we address these gaps by assessing the subcellular distribution of 16 elements in the liver of five estuarine fish species (Ariopsis felis, Archosargus probatocephalus, Centropomus undecimalis, Sciaenops ocellatus, Sphyrna tiburo) from the Indian River Lagoon (Florida, United States of America). We applied a subcellular partitioning protocol optimized for each species to obtain sensitive (mitochondria, lysosomes + microsomes, heat-denatured proteins) and detoxified (granules, heat-stable proteins) fractions. Mercury and methylmercury (MeHg) were primarily associated with sensitive fractions (>50%) in all species. Other class B metals such as cadmium (Cd) and copper (Cu), usually detoxified, were largely found in sensitive compartments in A. felis, C. undecimalis, and S. ocellatus (35–58%). Only A. probatocephalus showed Cd (62%) and Cu (57%) mainly as detoxified forms. Among Class A metals, praseodymium (Pr) and neodymium (Nd) (36–50%) were mostly present in the sensitive compartment in A. probatocephalus, C. undecimalis, and S. ocellatus, while rubidium (Rb) was consistently in detoxified fractions (>75%) across species. The borderline metals, zinc (Zn) (45–60%) and manganese (Mn) (36–57%), predominated in the sensitive fractions, and cobalt (Co) and vanadium (V) were distributed similarly in both compartments. Other metals (no class) such as arsenic (As) (51–79%), molybdenum (Mo) (31–46%), and sulphur (S) (37–50%) were mostly detoxified, while selenium (Se) was consistently in the sensitive compartment. While metal partitioning largely followed distributions based on their chemical class, interspecific differences among fishes emerged exhibiting distinct subcellular handling strategies. For instance, A. probatocephalus showed enhanced detoxification of Cd and Cu compared to other fish species, likely related to its high hepatic bioaccumulation of these elements. This may be related to its feeding behaviour, as herbivorous and detritivores species are known to ingest more sediment-bound or plant-associated trace elements. Such metal-handling differences seem to reflect fishes' particular ecological traits, highlighting the importance of multi-species approaches to unravel metal dynamics in marine environments.

8 | **Challenges in Regulating Botanical Active Substances in the UK: An Ecotoxicology Perspective**
Harriet Kelynack, Health And Safety Executive

Botanical Active Substances (BAS) are regulated according to Regulation (EU) 1107/2009 and utilising SANCO 2012 guidance, which defines BAS as active substances obtained from plant origin. The 2025 UK Pesticides National Action Plan aims to reduce pesticide risks, encouraging uptake of biopesticides including BAS. Although of natural origin and perceived as 'low risk', the underlying chemistry of plant extracts is the basis for many contentious substances. For example, the highly effective pyrethroid insecticides (similar to natural pesticide pyrethrum, produced by chrysanthemum flowers) are very toxic to aquatic invertebrates, fish, non-target arthropods and bees. To encourage submissions regulatory challenges must be better understood. Ecotoxicological challenges are explored further here.
The ecotoxicology assessment is complicated by difficulties establishing substance identity. Confirming identity is critical to ensure a suitable test item is used in ecotoxicology tests. BAS are often classed as Unknown Variable composition Complex reaction products of Biological origin (UVCB). BAS can be difficult to separate into identifiable individual components and due to natural plant variation test item batches may have different compositions. Aquatic toxicity tests require measurements of test item in solution. Problems associated with confirming identity make analytical methods difficult to establish. It is therefore difficult to accurately measure test item concentrations and confirm test organisms are exposed to required doses.
While standard chemical data requirements (283/2013) apply often BAS ecotoxicology dossiers contain incomplete data sets, using literature studies and reasoned cases to justify data gaps. Literature studies can be evaluated for relevance and reliability but rarely follow standard guidelines or provide endpoints in a suitable format for risk assessment. Often uncertainties remain as to the appropriateness of species used, test design or relevance of the test item and there is currently no guidance on how they are considered in evaluation. Reasoned cases (e.g. the substance is derived from food items) are evaluated based on expert judgement and weight of evidence (WoE). Consideration of uncertainties in WoE is critical to make an informed decision.
The unique nature of BAS brings challenges to each evaluation; establishing substance identity, ensuring reliable analytical methods and assessing WoE cases are key to regulatory acceptance.

9 | **Optimizing Passive Sampling of PFAAs in Wastewater: Sorbent Selection and Evaluation**
Kristina Mraz, University of Chemistry and Technology Prague (UTC); Antonin Halek, University of Chemistry and Technology Prague; Veronika Svobodova, University of Chemistry and Technology Prague; Vaclav Janda, University of Chemistry and Technology Prague; Jana Pulkrabova, University of Chemistry and Technology Prague; Jakub Hejnic, Prague Water Supply and Sewerage Company; Martin Srb, Prague Water Supply and Sewerage Company; Darina Dvorakova, University of Chemistry and Technology Prague; Vojtech Kouba, University of Chemistry and Technology Prague

Per- and polyfluoroalkyl acids (PFAAs) are a subgroup of the highly persistent per- and polyfluoroalkyl substances (PFAS), which frequently enter the aquatic environment via wastewater treatment plants (WWTP). As they are highly toxic to the environment and human health, monitoring regulations have been introduced under the EU Urban Wastewater Directive. Starting 2027, PFAS must be monitored in the inflows and outflows of wastewater treatment plants ≥ 10,000 PE. To keep these levels to a minimum, identifying the source can be beneficial. By using passive monitoring with polar organic chemical integrative samplers (POCIS), PFAA can be detected in low concentrations. In wastewater (WW), various matrix parameters can influence the sorption on POCIS and compromise the quantification accuracy. The aim of our research was to identify a suitable POCIS sorbent for WW and evaluate the parameter effects. Five ion exchange sorbents were selected for Batch tests, which were carried out using PFAA-spiked synthetic WW and deionized (DI) water. We found that Oasis HLB (HLB) (Waters, USA) performed well for long-chain PFAAs (C8-C11) but less well for short-chain (C5-C7), compared to Oasis WAX (WAX) (Waters, USA), which performed consistently well for all chain lengths (C5-C11). The sorption performance in the WW was significantly less efficient than in DI, which can be attributed to the complex matrix. For further investigation of the WW parameters, WAX was selected. Batch tests with pH (5, 8, 12), NaCl (7, 50, 1000 mg/L), humic substances (0, 10, 1000 mg/L COD) and organic substances (0, 50, 100 % true WW) are ongoing. In the standard range (6-9), the pH value has little influence on the speciation of PFAS; however, anion exchangers show a lower sorption capacity at higher pH values. Salinity can influence the sorption of PFAS by salting out, which increases sorption. High salinity concentrations, humic substances and organic substances can act as competing ions and compounds due to their polarity and interfere with the sorption. This experiment allows us to determine the optimum conditions for PFAA sorption in WW. Deviations from these conditions can thus be considered for in-situ sampling campaigns and extend the applicability of passive samplers to complex water matrices.

10 | **Glacial Meltwater as a Source of Semi-Volatile Organic Contaminants to High-Altitude Alpine Lakes**
Evah Peard, Utah State University; Anna Shampain, Utah State University; Kimberly Hageman, Utah State University

Semi-volatile organic contaminants (SVOCs) are a class of chemicals that tend to persist, bioaccumulate, pose health risks, and become widely distributed in the environment through natural processes. SVOCs are found in a wide range of products and materials, including insecticides, coolants, petroleum products, and more. SVOCs accumulate in glaciers due to enhanced deposition at colder temperatures. Increased glacial melting can lead to greater concentrations of stored SVOCs being released to the environment. SVOC persistence poses long-term risks to aquatic ecosystems and human health through bioaccumulation and

biomagnification in food webs. SVOCs have been detected in glacial meltwater and sediment in Europe, confirming the role of alpine glaciers as temporal contaminant sinks. The Western Airborne Contaminant Assessment Project (WACAP) found SVOCs in multiple environmental matrices, including sediment, collected between 2003 and 2006 from U.S. National Parks. However, no study has thoroughly investigated the relationship between glacial meltwater and SVOC accumulation in alpine lakes within these parks. Therefore, the objective of this project is to determine whether glacial meltwater contributes to SVOC deposition in alpine lake sediments within U.S. National Parks.

Surface sediments were collected from glacial and non-glacial lakes within Glacier and Grand Teton National Parks. Non-glacial lakes were chosen at similar elevation, size, and proximity to the glacial lakes to isolate the influence of glacial meltwater. The sediments were sectioned by depth and dated to evaluate trends in contaminant deposition over time. The target analyte list of 62 SVOCs includes pesticides, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs). Sediments were extracted using Energized Dispersive Guided Extraction (EDGE) with in-cell cleanup to minimize matrix interference. Analytes were quantified using gas chromatography tandem mass spectrometry (GC-MS/MS). PAHs were detected in all samples analyzed to date, and distribution patterns align with emissions from a nearby aluminum smelter.

We hope our findings will improve understanding of glacier melt's contribution to the contaminant load to glacier-fed lakes and identify other potential sources of SVOCs to alpine lakes. If glaciers are releasing SVOCs, we expect to observe higher contaminant concentrations at lower depths, especially for historic-use compounds, due to recent glacial melting.

11 | **Differences in the Bioconcentration Kinetics of Human Pharmaceuticals Among Two Fish Species With Different Tolerance to Environmental Pollution**

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The occurrence of human active pharmaceutical ingredients (HAPIs) in aquatic environments is well-established worldwide. Thus, aquatic biota are exposed to these emerging contaminants that are susceptible to bioaccumulation. This study aimed to comparatively evaluate the bioconcentration kinetics of seventeen HAPIs (alprazolam, atenolol, bromazepam, caffeine, carbamazepine, carvedilol, clonazepam, diclofenac, diphenhydramine, enalapril, fluoxetine, lorazepam, propinox, salbutamol, and sildenafil) between and environmental pollution tolerant and sensitive fish, respectively, Cnesterodon decemmaculatus and Odontesthes bonariensis. Juveniles of both species were exposed to a mixture of waterborne 0.05 µM of each HAPIs during 14 d, with media renewal, food administration, and fish and water sampling every 48 hours. Fish were anesthetized in ice-cold water, sacrificed, homogenized, and processed for HAPIs liquid-liquid extraction, followed by hexane cleanup. Fish extracts and water samples were analyzed by HPLC MS/MS. Whole-body concentrations were used to model the bioconcentration kinetics of each compound. Results showed differences in the bioconcentration pattern between species. All evaluated compounds reached maximum concentration during the tested time in C. decemmaculatus, with an average half-time bioconcentration of 14 hours. On the other hand, O. bonariensis exhibited marked differences in the bioconcentration kinetic depending on the HAPIs. Whereas some HAPIs quickly arrived at a maximum concentration (average half-time 33 hours), others kept a linear concentration and time ratio, far away from the asymptotic value, after 14 d of exposure. A significant linear correlation between the log of the octanol/water partition coefficient (log KOW) and the log of the bioconcentration factor (log BCF) was observed in both species. A significant linear correlation between the log KOW and the half-time and the log of the elimination constant (k2) was observed only for O. bonariensis. Also in this species, a significant relationship was found between the molecular weight (MW) and the two foreword kinetic parameters. In conclusion, bioconcentration kinetics varies between fish species, being HAPIs accumulation slower and higher in the more sensitive species. Also, molecular properties influence the HAPIs accumulation differently depending on the species.

12 | **Sources and Fate of Nitrate in a Tropical Crystalline Basement Aquifer: An Assessment Using Environmental Isotopes, Hydrochemistry and a Bayesian Mixing Model**

Louisa Preko Agyekumwaa, IHE Delft Institute for Water Education / Ghana Atomic Energy Commission; Tibor Stigter, IHE Delft Institute for Water Education; Abass Gibrilla, Ghana Atomic Energy Commission; Samuel Yao Ganyaglo, Ghana Atomic Energy Commission

Groundwater nitrate (NO_3^-) contamination has emerged as a crucial problem in many regions across the globe, particularly in areas with intensive agricultural activities. This study investigated the sources and factors affecting NO_3^- concentrations in groundwater within the Vea catchment of Ghana. The dual isotope approach ($\delta^{15}N$-NO_3^-, $\delta^{18}O$-NO_3^-) was employed to trace NO_3^- sources and transformation processes. A Bayesian mixing model in R (MixSIAR) was used to differentiate masked or overlapping sources and quantify the proportional contributions of NO_3^- from the various sources. Crucial factors such as land use, crop management practices, recharge, aquifer characteristics and flow paths were assessed, alongside biogeochemical processes including volatilization, nitrification, and denitrification, to better understand NO_3^- migration, transport, and dynamics in groundwater systems. Generally, NO_3^- concentrations ranged from 0.84 mg/L to 194.92 mg/L, with mean concentrations of 34.20 mg/L and 38.40 mg/L for boreholes and hand-dug wells, respectively. Approximately 13% of the samples exceeded the WHO drinking water limit of 50 mg/L, primarily in groundwater beneath densely built-up areas. Strong correlations were found between NO_3^- and Cl^- (R^2 = 0.88) and NO_3^- and SO_4^{2-} (R^2 = 0.77), whereas principal

component analysis indicates high positive loadings of NO_3^- (0.78), SO_4^{2-} (0.88), Cl^- (0.87), Na^+ (0.71), Ca^{2+} (0.95), and Mg^{2+} (0.89) on the first axis (PC1), and high positive loading of HCO_3^- (0.92) opposing a moderately high negative loading of NO_3^- (-0.52) on the second axis (PC2). These results pointed towards anthropogenic contamination from domestic wastewater and manure, enhanced by evapoconcentration (PC1), with relatively low residence time (PC2). Clay-rich regolith layers with low permeability created favourable conditions for ammonia volatilization and denitrification in the presence of organic matter. Conversely, preferential flow paths within saprolites enhanced NO_3^- transport through rainfall-induced recharge, without substantial attenuation. Source discrimination revealed that approximately 63% of the samples contained NO_3^- contamination emanating from manure and/or septic waste, contributing to approximately 40% of the total NO_3^- load in groundwater. Stable water isotopes ($\delta^{18}O$-H_2O, δ^2H-H_2O) revealed mainly depleted isotopic signatures in groundwaters, suggesting recent aquifer recharge. These findings underscore the vulnerability of groundwater to NO_3^- pollution from anthropogenic activities, emphasizing the need for regular water resources monitoring, sustainable agricultural practices, proper wastewater management systems, effective remediation measures and mitigation strategies, and increased public awareness.

13 | **Reproductive Effects of Arsenic-Contaminated Natural Diet in Zebrafish (Danio rerio) during Chronic Exposure**

Sravan kumar Putnala, Univeristy of Saskatchewan; Mahesh Rachamalla, University of Saskatchewan; Owen Luo, University of Saskatchewan; Som Niyogi, University of Saskatchewan

Understanding the reproductive effects of arsenic in aquatic organisms is critical, yet limited, particularly for chronic dietary exposures that better reflect environmental scenarios. The current study investigated the reproductive toxicity of environmentally relevant chronic arsenic exposure via an oligochaete worm (Lumbriculus variegatus) in adult zebrafish. Worms exposed to waterborne arsenic (0, 2.5, and 5.0 mg/L as arsenite) for 14 days had body burdens of 0.3 (control), 35.9 (low), and 78.0 (high) µg/g dry weight. For 60 days, experimental fish were fed either of these arsenic-contaminated worms at 3.5% body wet weight twice daily. Fish reproductive performance was evaluated after exposure by assessing reproductive behavior, gonadosomatic index (GSI), fecundity, fertilization and hatching success, larval survival, and deformities. In addition, brain, liver, gonads, and blood were collected to assess the relative expression of genes associated with the hypothalamic-pituitary-gonadal-liver (HPGL) axis and circulating sex steroid levels in males and females. Gonads (testes and ovary) were collected for histopathological analysis to examine the structural integrity of reproductive tissues. Finally, male fish sperms were collected to assess the effect of arsenic on sperm abundance and motility. Chronic dietary arsenic exposure showed impaired reproductive behavior, decreased fecundity, GSI, fertilization rate, hatching success, larval survival, and increased larval deformities in a dose-dependent manner. Gene expression analyses suggested chronic arsenic exposure disrupted the HPGL axis irrespective of gender. Arsenic also reduced sperm density and motility dose-dependently. Gonadal histopathology showed a significant decrease in gamete frequency both in ovaries and testes and the plasma sex steroid concentrations of 17β-estradiol (E2), 11-keto testosterone (11KT), and vitellogenin (VTG) were disrupted in females and males. Overall, our findings imply that arsenic is a potent endocrine disruptor and causes reproductive toxicity in zebrafish by disrupting the HPGL axis. This study also highlights the importance of incorporating dietary exposure into aquatic risk assessments.

14 | **Assessing the Environmental Hazard of Synthetic Phenolic Antioxidants**

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Synthetic antioxidants are contaminants of emerging concern due to their widespread use in a variety of applications, including personal care products, plastics, synthetic fibers, and even food. Consequently, these substances are now frequently detected in diverse environmental matrices, such as natural waters, sediments, and biota. While previous studies have shown that some synthetic antioxidants can be toxic to living organisms, data on the environmental fate and (eco-)toxicity of most compounds in this large class remain limited. This project aims to address this knowledge gap by investigating key environmental hazards posed by synthetic antioxidants, focusing on novel endpoints that could be incorporated into the early stages of chemical design and/or into regulatory hazard assessments. Many antioxidants function by being oxidized themselves and can thus be classified as reducing agents. Others prevent the formation of reactive oxygen species by absorbing light and dissipating the energy as thermal energy. A third group acts as chelating agents, sequestering metal ions that catalyze oxidation reactions. Reducing antioxidants have been highlighted as problematic due to the formation of toxic transformation products (TPs), such as quinones, upon fulfilling their antioxidant activity, a process that is currently poorly covered in regulatory frameworks. Quinone TPs, however, are known to induce various hazardous effects in vivo, including acute cytotoxicity and carcinogenicity. In this project, we assess the environmental persistence of approximately 80 phenolic antioxidants and investigate the formation of TPs using aquatic and activated sludge degradation assays. To this end, we developed a targeted LC-MS analytical method for the detection of a broad range of antioxidants. As a next phase, we will develop methods to couple (bio)degradation assays with animal-free in vitro bioassays to evaluate a variety of cellular toxicity endpoints. By coupling persistence and toxicity assessments, we aim to further explore a recently proposed alternative hazard assessment framework based on novel endpoints, i.e., the cumulative and persistent toxicity equivalents (CTE/PTE concept). In this approach, the CTE indicator quantifies the toxic effects of a sample, whether a single chemical or a mixture, while the PTE captures the residual toxicity after degradation, allowing for the identification of key

hazards associated with both parent compounds and their TPs.

15 | **Toxicity of Green Marine Fuels on Lower Trophic Levels: Preliminary Findings From a Mesocosm Experiment With Ammonia**
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The shipping industry's transition from fossil fuels to green fuels promises significant climate benefits. With maritime trade accounting for approximately 3% of global greenhouse gas emissions, the industry is actively exploring low- or zero-carbon fuel alternatives - such as ammonia, which is expected to become the leading green marine fuel by 2050. However, the potential environmental risks associated with the large-scale use of ammonia as a ship fuel remain underexplored.
This study investigates the in situ degradation and toxicity of ammonia in coastal marine waters using a mesocosm approach. Experimental toxicity tests will be conducted on lower trophic levels of coastal marine plankton (< 90 µm) over a 14-day period in May 2025, to assess ecosystem impacts following a potential ammonia spill from a ship. Our mesocosm setup involves 1,000 L containers submerged outdoors in a Danish fjord system, where the effects of varying ammonia concentrations on community composition, biomass, and growth responses of bacteria, phyto-, and microzooplankton collected from the fjord will be quantified using flow cytometry. The use of a marine mesocosm setup can capture more realistic conditions and population-level responses than laboratory single-species bioassays, which is particularly relevant when examining complex nitrogen speciation and degradation pathways.
These results will provide important information about the acute and chronic toxicity effects of ammonia on coastal marine plankton communities. This knowledge will enhance the understanding of the potential environmental impacts following an ammonia spill as well as support future environmental risk assessments of ammonia as a marine fuel.Disclaimer: The PhD of the presenting author is part of a larger research project (MAT-Fuels: https://projekter.au.dk/en/marine-transition-fuels) supported by Dampskibsselskabet NORDEN A/S.

16 | **The Effect Of Endocrine Disrupting Compounds On Neurodevelopment In Zebrafish Early Life Stages**
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Global interest in studying the effects of endocrine disrupting compounds (EDCs) on neurodevelopment, causing developmental neurotoxicity (DNT), has grown. EDC-induced impaired neurodevelopment has been identified as an adverse outcome (AO) targeting cochlear function, cognition, learning and memory in mammalian research. However, research on fish typically focuses on other adverse effects (e.g. swim bladder inflation, eye development). The present study therefore aims to determine the impact of EDCs on zebrafish early life stages by analyzing the mammalian AOs, cochlear function and learning and memory. Cochlear function was evaluated via the lateral line (LL) system, a neurosensory system positioned along the body of the fish which senses water motion and pressure gradients. The functional units of the LL system are neuromasts that contain hair cells (HCs), which are similar to HCs in the cochlea. Zebrafish embryos were exposed to model EDCs (thyroid hormone system disruptors and estrogen disruptors) and a positive control from 1 hour post fertilization to 5 days post fertilization, after which the number of HCs and total neuromasts were counted. Targeted exposure windows and gene transcript levels were used to gain more detailed mechanistic understanding of observed effects. Additionally, the adverse effect of EDCs, particularly thyroid hormone system disruptors, on learning and memory was studied via a forced turn larval T-maze behavioral assay. Zebrafish larvae exhibit spontaneous alternation behavior (SAB, i.e. the animal's tendency to alternate turn direction in consecutive turns). By closing one arm of the T-maze, the animal should choose to explore the other arm on the next trial. This behavior requires proper memory. Zebrafish embryos were exposed to a positive control and EDCs, after which SAB was assessed. Furthermore, other DNT-related endpoints (spontaneous tail coiling, touch evoked escape response, light/dark transition test) and gene transcript levels were evaluated to acquire more comprehensive mechanistic insights into the effects. Overall, this work aids in determining to which extent EDCs can affect neurodevelopment in zebrafish.

P1 | **Addressing Emerging Contaminants in Sewage Sludge: Anaerobic Digestion Challenges and Remediation Strategies**
Ogemdi Chinwendu Anika, De Montfort University/University of York/Yorkshire Water Services Ltd

As the wastewater sector shifts toward circular economy models, anaerobic digestion (AD) has become a central technology for converting sewage sludge (SS) into renewable energy & nutrient-rich biosolids for agricultural use. While AD is effective in reducing pathogen loads & many organic pollutants, environmental challenges persist, particularly the fate of microplastics, per- and polyfluoroalkyl substances (PFAS), pharmaceutical residues, & antibiotic resistance genes (ARGs) in treated SS. These contaminants pose long-term risks when biosolids are applied to land, raising regulatory, ecological, & public health concerns. The UK's regulatory framework for SS reuse has historically focused on pathogen control & heavy metal limits, with less emphasis on emerging contaminants. This review critically examines the current state of AD-based SS-to-energy & fertilizer systems in the UK and explores the potential of novel post-treatment processes to mitigate these challenges. By integrating policy perspectives & future research priorities, this work aims to provide a comprehensive overview of the remediation landscape for SS-derived biosolids, identifying knowledge gaps & technological opportunities to support safer, more sustainable resource recovery.

Advanced anaerobic processes & innovative post-treatments are showing potential in addressing these gaps. Thermophilic AD and thermal hydrolysis pretreatment (THP) have been increasingly adopted to enhance SS breakdown, boost biogas yields, & achieve higher levels of pathogen & ARG reduction. Co-composting, particularly with green or woody waste, has been shown to reduce pharmaceutical residues by up to 90% under optimal thermophilic conditions. Addressing persistent organic pollutants & microplastics in SS, will require not only its inclusion in the Environmental Permitting framework of the UK's regulatory guideline but also adopting advanced approaches pre and post AD. These techniques can degrade microplastics, pharmaceuticals, and PFAS, & produce biochar with potential agronomic benefits. However, further innovation is needed to address concentrated waste byproducts & improve scalability. In conclusion, AD remains central to SS treatment in the UK, its integration with advanced post-treatment processes is essential to address persistent contaminants. Combined strategies offer a path toward safer, cleaner biosolids, though trade-offs in energy demand, cost, & scalability remain.

P2 | **Individual and Binary Exposure of Short- And Long-Chain Phthalate Esters Decreases Viability and Inhibits Cellular Respiration in Human Lung Cells**

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Phthalate esters (PAEs) such as dimethyl phthalate (DMP), diethyl phthalate (DEP), bis-2(ethylhexyl) phthalate (DEHP), and di-n-octyl phthalate (DOP) are synthetic chemicals used as solvent stabilisers and plasticizers in commercial and industrial products. Emerging literature suggests that semi-volatile PAEs (e.g., DEHP) could be toxic to human lung cells, but atmospheric concentration regulations on PAEs remain limited. PAEs have been detected in inhalable particles such as PM2.5, however, their individual and combined effects on human lung cells beyond 48 h exposure remain unclear. This work evaluated the individual and combined toxicity of prolonged exposure to environmentally relevant concentrations of DMP, DEP, DEHP, and DOP (10 μM – 1 mM) on Calu-3 human sub-bronchial gland cells. Individual exposure to the tested PAEs yielded the following maximal inhibitory concentration (IC50) at 24 h (4.46 mM DMP, 2.05 mM DEP), 72 h (3.07 mM DMP, 927 μM DEP), and 168 h (418 μM DMP, 106 μM DEP, 343 μM DEHP, 400 μM DOP). The concentration addition model predicted additive effects at 72 h exposure with the DMP-DEP combination. Cellular oxygen (O_2) consumption was measured via high-resolution respirometry. Exposure of live cells to individual or binary mixtures of PAEs inhibited cellular O_2 consumption in a concentration specific manner, indicating direct inhibition of mitochondrial respiratory flux. The results of this work raise a concern on potential long-term adverse effects of atmospheric levels of DMP, DEP, and DOP on the human respiratory system. To the best of our knowledge, this is the first report on the cytotoxic effects of prolonged exposure to DMP, DEP, and DOP and the first to assess the toxicity of binary mixtures of DMP-DEP and DEHP-DOP in a human lung cell line. The results also raise a concern about whether current workroom air quality regulation on DMP, DEP, and DEHP are safe for human health.

P3 | **Disentangling the Web: The Impacts of Binary Chemical Mixtures on Consumer-Resource Feeding Interactions**

Dylan Asbury, The University of Sheffield; Amy Ockenden, University of Sheffield; Andrew Beckerman, University of Sheffield; Lorraine Maltby, University of Sheffield

Current evaluation of the impacts of chemicals and their mixtures on freshwater ecosystems relies heavily on single- and multi-chemical effects on individual species. However, species do not exist in isolation. and a mechanistic understanding of how chemicals and their mixtures impact species interactions is essential if we are to extrapolate from single-species single-chemical studies to assessing the impact of multiple chemicals on multispecies assemblages in ecosystems. Consumer-resource interactions, which represent fundamental building blocks of the food webs that drive biodiversity, may be impacted by chemicals through changes in abundance (e.g., lethal effects) or through sub-lethal effects such as changes in feeding behaviour, escape response or mobility. The chemicals in a mixture may affect consumer-resource interactions by affecting either the resource, the consumer, or both the resource and the consumer to the same or different extents. Here we demonstrate the use of a consumer-resource system to begin to disentangle the complex effects of chemical mixtures on ecological communities. We present the results from a series of crossed-design binary-chemical consumer (grazer)-resource (primary producer) interaction experiments using the freshwater snail, Potamopyrgus antipodarum and the freshwater diatom, Nitzschia palea. The chemical mixtures consisted of a biocide (copper) and an insecticide (azoxystrobin) in the first instance, and copper and a herbicide (Triallate) in the second. The experiments addressed three questions: (1) How are the impacts of binary mixtures altered depending on whether they are acting directly on an organism or indirectly through the consumer-resource interaction? (2) What are the consequences of differential sensitivity to binary mixtures for consumer-resource interactions? (3) Do the combined effects of individual chemicals within binary mixtures exert their impacts additively or otherwise in the case of consumer-resource interactions?

P4 | **Effect-based Ecotoxicological Risk Assessment of Hazardous Chemicals Entering Surface Waters from Water Recycling Centre and Constructed Wetland Treatment**

Joseph Beaney, University of Bath; Emma Vaughan, John Bagnall, Daniel Read, Charles Tyler, Ed Feil, Barbara Kasprzyk-Hordern

Constructed wetlands (CWs) are increasingly used to mitigate contaminants from wastewater, yet their efficiency in removing bioactive pollutants remains uncertain. While traditional chemical monitoring provides valuable insights, it often overlooks the biological effects of complex mixtures. Effect-directed analysis (EDA) offers a more comprehensive approach by integrating bioassays with chemical analysis to identify fractions contributing to toxicity. This study applies EDA to assess the ecotoxicological risks of CW-treated wastewater, combining algal growth inhibition assays with targeted chemical screening of over 300 priority contaminants.
Water samples were collected from the inflow of a water recycling centre (WRC), CW inflows and outflows. Samples were extracted using solid-phase extraction (SPE) and fractionated into chemically distinct groups to isolate toxic components. The freshwater alga Raphidocelis subcapitata was used to evaluate growth inhibition, providing a sensitive measure of sublethal toxicity. Following the exposure period, esterase activity staining and flow cytometry were employed to assess cellular health and metabolic function, offering further insight into mechanistic effects. Targeted chemical analysis was conducted to identify and quantify persistent contaminants within toxic fractions.
Preliminary results indicate that while CWs contribute to an overall reduction in toxicity, certain chemical fractions retain biologically active compounds that negatively impact algal viability. This study underscores the importance of integrating bioassays with targeted chemical screening to improve CW performance assessments. Identifying residual toxic fractions provides crucial information for optimising wetland treatment strategies and refining regulatory guidelines for wastewater reuse. A better understanding of bioactive contaminant fate within CWs will help inform future designs for more effective contaminant removal and enhanced environmental protection.

P5 | **Toxic Legacies: A Blueprint for Multilevel Ecotoxicological Investigation of Phenylarsenical Chemical Warfare Agents and Their Transformation Products**
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Around 50,000 tons of chemical warfare was dumped in the Baltic Sea after the World Wars - considered the most efficient and safest disposal method at the time. Progressive corrosion of metal shells and casings containing chemical warfare agents (CWAs) result in the release of toxic contents into the environment. This poses a significant threat to aquatic ecosystems and human health. Current environmental risk assessment of CWAs is primarily based on a limited number of organismic studies and modelling-based analysis, with a predominant focus on parent CWAs. However, emerging evidence highlights that transformation products can be considerably toxic, which has been largely overlooked. As a result, an in-depth examination of long-term environmental risks associated with CWA contamination is necessary. To bridge these gaps, it is essential to develop a tailored, multi-level bioassay battery for a holistic ecotoxicological assessment of CWAs and their transformation products. Among the most common dumped CWAs are phenylarsenic-containing agents; consequently, this project aims to evaluate the ecotoxicological effects of the CWA-related phenylarsenicals Clark 1 and phenyl(dichloro)arsine and their three methylated transformation products methyldiphenylarsine oxide, methylphenylarsinic acid and dimethylphenylarsine oxide, all previously detected in Baltic Sea sediments, a known dumping hotspot. A diverse range of model organisms spanning different trophic levels are used, including the well-established freshwater model species Danio rerio and Gammarus fossarum as well as representative brackish water model organisms (Oryzias melastigma and Gammarus oceanicus). By extending beyond traditional freshwater test species, this approach ensures a more ecologically relevant toxicity profile for marine and brackish ecosystems. Building on initial acute toxicity analyses, the focus is directed toward postulated neurotoxicity of CWAs. Systemic identification of neurotoxic pathways is performed by linking key events at the biochemical, tissue and organismal level with state-of-the-art techniques. Expected first outcomes, after establishing toxicity effect thresholds, include novel insights into neurobehavioral effects, particularly locomotor impairments (light/dark transition test) in early-life stages of D. rerio. Ultimately, this research seeks to enhance ecosystem-based risk assessments and drive more effective environmental management strategies for sea-dumped CWAs.

P6 | **Mechanism-Specific Toxicity of Road Pollutants in Snow: A Case Study of Norwegian Roads using a Zebrafish Embryo Multi-Endpoint Bioassay Battery**
Laura Behnstedt, Goethe University Frankfurt; Laura Behnstedt, Goethe-University Frankfurt; Sarah Johann, Goethe-University Frankfurt/Fraunhofer Institute for Molecular Biology and Applied Ecology (FhG-IME); Martin Wagner, Norwegian University of Science and Technology (NTNU) Trondheim; Fabian Weichert, Goethe-University/Fraunhofer Institute for Molecular Biology and Applied Ecology (FhG-IME); Henner Hollert, Goethe-University Frankfurt/Fraunhofer Institute for Molecular Biology and Applied Ecology (FhG-IME)

Micro- and nanoplastics, as well as associated chemicals have become an increasingly important topic in ecotoxicological research. One of the largest sources of plastic particles is tire wear released to the environment through road abrasion. Alongside other road pollutants, such as de-icing salt, vehicular fluids and exhaust gases, this runoff is transported into aquatic systems causing adverse effects in various organisms. Many studies focus on major emission events like storms and snowmelt during spring, but fewer analyze the effects of contaminated snow before it melts. However, snow samples have great potential for ecotoxicological impact assessment as they allow sampling of road pollutants before they are transported further to aquatic systems. This allows for a more targeted approach to pinpoint potential point sources and hot spots of pollution. For the

purpose of this study, we sampled snow near roadsides of Norwegian roads to obtain all potential pollutants and particles that it may contain. The roads are the European route E6, the road with the highest traffic intensity in Norway, and a more rural road, to compare the toxicity of pollutants associated with different road types and traffic intensities. To gain a comprehensive understanding of the potential toxicity mechanisms, we applied a bioassay battery with zebrafish (Danio rerio) embryos, consisting of a number of assessed in vivo endpoints, such as acute toxicity and behavioral effects. Additionally, we analyzed specific enzyme activity as biomarkers for neurotoxic substances and other xenobiotics, such as polyaromatic hydrocarbons. Because of the complexity of road pollutants and toxicity pathways we expect effects across a number of endpoints. Likely, a higher traffic intensity will lead to a higher toxicity within the samples. So far, pollutants extracted from our snow samples affect the cardiovascular system of the zebrafish embryos, leading to the formation of edema and a reduction of heartbeat. Currently, further endpoints are being evaluated to achieve a comprehensive understanding of the potential toxicity of road-associated pollutants in snow from Norway and to provide insights into adequate sampling and toxicity testing methods using zebrafish.

P7 | **Towards Harmonised Test Methods for Ecotoxicity Testing of Nanomaterials: Adaptations of the OECD Test No. 202**

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The evolving field of nanotechnology raises concerns for human and environmental health. Standardisation of Guidance Documents (GD) and Test Guidelines (TG) that address the challenges of nanomaterials (NMs) ecotoxicity testing are needed to ensure compliance with European regulations like REACH and for the adequate environmental risk assessment of NMs. The OECD GD No. 317 on aquatic and sediment toxicological testing of NMs provides recommendations for adapting existing OECD TGs, such as optimising dispersion stability during testing. The present study aimed to adapt the OECD Daphnia sp., Acute Immobilisation Test (No. 202) for assessing NM ecotoxicity. The objectives included: (1) evaluating dispersion efficiency of ZnO, TiO_2, bentonite NMs, and multi-walled carbon nanotubes (MWCNTs); (2) follow the OECD No. 202 to assess the ecotoxicity of these NMs to Daphnia magna using different experimental setups to determine the influence of container material (plastic versus glass) and depth (plastic tube versus plastic petri dish) on NM behaviour; and (3) characterising NM behaviour over time in ISO exposure medium. Results showed that stock dispersions of ZnO, TiO_2, and bentonite NMs were reasonably stable in Milli-Q water, with TiO_2 showing the highest stability over 48h (ζP: ~30 mV). However, in ISO medium, ζP values indicated unstable colloids (within ±30 mV), and hydrodynamic sizes varied, with higher stability observed for bentonite (percentage variation: -9.43 - 14.40), and lower stability for ZnO NMs (percentage variation: -8.92). Transmission Electron Microscopy analysis suggested that MWCNT dispersion efficiency improved with natural organic matter (NOM). Toxicity tests revealed that D. magna exhibited higher sensitivity to ZnO NMs (IC_{50}: 1.05 - 4.59 mg/L) and MWCNTs (IC_{50}: 58.13 - 257.91 mg/L), while TiO_2 and bentonite showed no toxicity (IC_{50} > 500 mg/L). Plastic test tubes were found to be preferable for ZnO NM toxicity testing, but no clear recommendations could be made for TiO_2, bentonite, or MWCNTs. Overall, this work provides key recommendations that are being included as annexes in the OECD GD No 317, supporting harmonisation of test methodologies for assessing NM hazards in D. magna.

P8 | **Ecotoxicological Assessment of Groundwater in a Key Region of Drinking Water Supply**

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The ‘gwTriade’ project combines chemical and ecotoxicological assessment with groundwater (GW) fauna analysis. This triad approach, successfully applied to surface waters and sediments, enables a comprehensive evaluation of GW status, identification of indicator substances, and a sound risk assessment. For ecotoxicological evaluation, GW samples are analysed using a biotest battery adapted from the EU project ‘SOLUTIONS’ and optimized for GW testing.In a recent cooperative sampling campaign, a monitoring station of the ‘WaRM’ project in the Hessian Ried, a key region for the drinking water supply of the Frankfurt metropolitan region, was sampled. This monitoring station consists of multi-level wells installed near the river Landgraben, a receiving water for municipal WWTP effluent.
Four samples were collected from two wells located at 2.4 m and 11.3 m from the Landgraben, at depths of 1.7 m and 4.7 m, respectively. One additional grab sample was taken directly from the river. Solid phase extraction (SPE) was used for sample preparation. Extracts were tested using the CALUX® assay for estrogenic (ER), androgenic, anti-estrogenic, and anti-androgenic (AR) activity, the micro-EROD assay for dioxin-like activity, the AMES test for mutagenicity, and the umu assay for genotoxicity.All samples showed strong effects in the anti-AR CALUX® assay, with bioanalytical equivalents (BEQ) exceeding the proposed effect-based trigger value (EBT) for drinking water proposed by Been et al. (2021) (4.8 µg Flutamide/L). Samples from 4.7 m depth and the Landgraben also showed strong effects in the ER CALUX® and micro-EROD assays, with one well-sample even exceeding EBTs for surface waters (0.1 ng 17β-estradiol/L and 50 pg TCDD/L). No effects were observed in the other assays.The observed effect patterns provide evidence of the infiltration of WWTP-impacted surface water into GW, with the strong effects in samples

from greater depths suggesting no attenuation during sediment filtration. Given the region's key role in drinking water supply, this indicates a potential risk to local GW resources and demonstrates the importance of incorporating effect-based tools into GW monitoring.
The samples will be further subjected to organism-based assays with algae, daphnia, and early life stages of zebrafish (Danio rerio).

P9 | **Temperature-Dependent Bioconcentration of Metals in Aquatic Organisms**
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Temperature can influence the chemical accumulation in aquatic organisms; however, it remains unclear whether these effects on bioconcentration are consistent across various species and metals. This study employs a mechanistic modelling approach to explore how temperature impacts the bioconcentration factor (BCF) of metals in aquatic species. The goal is to determine whether consistent temperature-BCF relationships emerge across species and metals, and whether such patterns can inform more targeted ecological assessments.
We addressed this by applying the Arrhenius equation. First, data were grouped by identical species, metal, and laboratory source, then progressively generalised into broader categories, including shared species across studies, taxonomic groups, and the combination of all available data. This tiered strategy was employed to progressively assess the impact of experimental variability on the observed temperature-BCF relationships.
No clear pattern emerged between temperature and BCF at the most generalised level. However, more specific groupings revealed distinct trends. For instance, in fish, BCFs for lead, vanadium, selenium, and copper generally increased with temperature, while arsenic, zinc, and nickel showed no significant change. In crustaceans, BCFs for mercury, arsenic, and zinc tended to decrease with temperature, while cadmium remained unaffected. Molluscs exhibited a variety of responses depending on the species and metal.
This study emphasises the significance of chemical-specific and species-specific contexts when assessing temperature effects on metal accumulation. The observed variability indicates that general assumptions regarding temperature-driven changes in bioconcentration may not be reliable without considering the organism and the chemical. By identifying where statistically significant patterns emerge—and where they do not—this work contributes to recognising when temperature adjustments are appropriate in predictive models. These insights highlight the need for context-dependent modelling approaches in risk assessment frameworks, particularly in light of climate change.

P10 | **First Flush and Seasons: The Influence on Dioxin-Like Activity and Endocrine Potential in a Small Urban Stream**
Jan Halaunia, Goethe University Frankfurt; Henner Hollert, Goethe University Frankfurt; Sabrina Schiwy, Goethe University Frankfurt

Chemical pollution of the environment is one of the big challenges of our time. Especially water bodies are particularly affected by chemical pollution. Despite the implementation of the EU Water Framework Directive (EU-WFD) in 2000, which aims to improve the chemical status of all water bodies in Europe, none of the water bodies in Germany has yet achieved a good chemical status. The Urselbach, a small stream in Frankfurt is one of the water bodies with a poor chemical status. Various sources along the Urselbach (e.g. a wastewater treatment plant and drainage from the A5 highway) discharge pollutants (e.g. pesticides and pharmaceuticals) into the receiving stream. Along the Urselbach, 11 sampling points will be investigated to determine the impact of the various sources of pollutants on the Urselbach. In addition to the water phase, the sediments will also be investigated. Sediments bind lipophilic pollutants, which can result in a higher concentration of pollutants in the sediment than in the water phase. The Urselbach is sampled at different times of the year to detect seasonal influences such as the increased need for medication in winter or the discharge of pesticides in spring. In addition, water samples are taken at selected sampling points during rain events using automated samplers. Samples are taken at the beginning of the rain events and one hour later to detect any 'first flush' effects, which can lead to a sudden increase in toxicity. These samples were taken once after a short dry period and once after a longer dry period. To prevent cytotoxicity from masking the mechanism specific effects, the cytotoxicity of the samples is determined in the neutral red retention test. All samples are tested for their dioxin-like activity in the microEROD assay using H4IIE cells. Furthermore, the endocrine potential of the samples will be investigated in various assays of the CALUX® test systems ((anti -) estrogenic and (anti)-androgenic) using the U2OS cell line. First results show that the sediments in the winter samples have a higher cytotoxicity and dioxin-like activity in H4IIE cells than the water samples. For the rain event after a short dry period, slight 'first flush' effects were observed in the microEROD assay. Until the conference, all sediment and water samples from winter and spring as well as the samples from the two rain events, will be investigated for their dioxin-like activity and endocrine disrupting potential.

P11 | **Spatiotemporal Patterns of Lead Exposure in English Wildlife: A Comparative Study of Otters and Buzzards**
Holly Hulme, Cardiff University; Glória dos Santos Pereira, UKCEH; Lee Walker, UKCEH; Frank Hailer, Cardiff University; Elizabeth Chadwick, Cardiff University

Aquatic and terrestrial ecosystems are increasingly threatened by chemical pollution, including legacy contaminants like lead. The toxic effects of lead have become well recognised, and despite efforts to limit its use, continues to harm ecosystems at all levels. This includes impacts on top predators like Eurasian otters (Lutra lutra) and Common buzzards (Buteo buteo).
In this study, lead concentrations were obtained from otter and buzzard liver samples, collected as part of the Environment Agency's H4 indicator program, which monitors chemical exposure in English wildlife under the

UK Government's 25-year Environment Plan. Our study aims to investigate the differences in concentrations of lead between the species using predictive modelling, and conduct time trend analyses while controlling for spatial variables. The study hypothesises that key pathways differ between species due to otters and buzzards differing diets and habitats. Liver lead concentrations for both species are expected to be correlated with environmental variables including; stream sediment lead and calcium, soil lead and calcium, rainfall, and wind speed.
The findings highlight the importance of considering spatial variability when evaluating environmental contaminants in both aquatic and terrestrial systems. The study also highlights the suitability of using otters and buzzards as sentinels for monitoring chemical contaminants over time.

P12 | **Specific Endpoints of Textile Wastewater at the Cellular and Organism Level**
Stella Jennes, Goethe University Frankfurt; Mira Goßen, Goethe University Frankfurt;
Anju Anna John, Indian Institute of Technology Madras; Indumathi M. Nambi, Indian Institute of Technology Madras; Sabrina Schiwy, Goethe University Frankfurt; Henner Hollert, Goethe University Frankfurt

Textile wastewater is one of the main factors for surface water pollution, especially in central textile-producing countries such as India. If untreated or insufficiently treated wastewater enters the environment, it can have adverse effects on aquatic organisms, such as acute toxicity, neurotoxicity, or endocrine activity. Therefore, further ecotoxicological test systems are needed to assess the complex mixture of components contained in textile effluents. Within the scope of this master thesis, raw textile wastewater, as well as fractions and treated wastewater from India, were assessed further to investigate specific endpoints such as neurotoxicity and endocrine activity. The fractionation into a mostly organic and a mostly inorganic fraction will later help to identify possible effect drivers. In addition to the first set of samples, another raw wastewater sample, treated wastewater samples, one post- chlorination and one treated with a lab-scale electroperoxone reactor, were obtained. Those samples were extracted using SPE. For further in-vitro testing, cytotoxicity of the raw textile wastewater, fractions and treated wastewater was tested on a cell-based level using the Neutral Red assay. The results show the highest cytotoxicity for raw wastewater (RawWW_N), followed by the non-binding-fraction (NB-fraction) and the raw wastewater extract (RawWW_E1), while the chlorinated (CLWW) and electroperoxone-treated wastewater (EPWW) showed no cytotoxicity. In the subsequently performed CALUX® Assay, effects were observed in the anti-AR CALUX® for the RawWW_N, raw wastewater extracts (RawWW_E1 and E2) and wastewater after the chlorination step in the treatment plant. Furthermore, organism-based tests with Danio rerio were carried out in the biomarker measurements of AChE and EROD. No significant results were obtained in both of these assays, but trends could be observed for AChE activity in the NB-fraction and RawWW_N indicating neurotoxic potential and for the EROD activity in the RawWW_N and RawWW_E1 indicating dioxin-like potential in the samples. In addition to that swimming behaviour was measured in the light-dark-transition assay in Danio rerio, which showed significant results for the samples RawWW_N, RawWW_E1 and NB-fraction.

P13 | **Development of a Semi-Static Algal Growth Inhibition Test Method**
Georgia Lees, Labcorp; Dominic Sacker, Labcorp

The OECD 201 Algal Growth Inhibition test guideline was developed to follow a static test design; however, many chemicals of interest (COI) being submitted for testing are unstable over the 72-hour test period. The types of issues experienced when conducting static tests with unstable COI include declining measured concentrations over the test duration and the potential build-up of breakdown products which are not necessarily monitored during the test and the unknown impact of these products on the overall toxicity. In other ecotoxicology testing such as the Daphnia sp. Acute Immobilisation Test (OECD 202), unstable COI would be tested using a semi-static test design to ensure concentrations of the COI are maintained close to the intended concentration. Therefore, development of a semi-static test design for use in the Algal Growth Inhibition Test would be highly beneficial.
The purpose of the current work is to investigate if it is possible to develop a semi-static algal test design which produces accurate and reproducible results and passes the required OECD 201 control validity criteria. It is anticipated that such a modification would require the development of methods for removing algal cells from test solutions and resuspension in fresh media without significant loss of or damage to the algal cells; in addition to preventing any adverse impact upon algal growth rate.
Semi-static algal growth inhibition tests would be conducted using the freshwater alga Raphidocelis subcapitata, with separation of the algal cells from the test solutions by centrifugation at 24- and 48-hours, with resuspension in fresh test media. Cell density determinations would be performed pre-centrifugation and post-resuspension to enable accurate algal growth rates to be calculated as well as quantifying algal cell losses during the transfer procedures. Furthermore, the impact of repeated COI dosing during a semi-static design would be investigated by assessment of reference chemicals for comparison of results with standard algal growth inhibition positive control tests.
If successful, a semi-static algal growth inhibition test would enable more accurate representation of the effects of COI, impacting significantly upon regulatory classifications.

P14 | **Assessing Environmental and Endocrine Risks of Diclofenac Liposome Encapsulation and Its Byproducts**
Carolina Machado, University of Aveiro (UA); Iria Naveira-Souto, Laboratorio Reig Jofre; Madalena Vieira, CESAM/University of Aveiro; Patrícia V. Silva, CESAM/University of Aveiro; Maria D. Pavlaki, CESAM/University of Aveiro; Elisabet Rosell-Vives, Laboratorio Reig Jofre; Susana Loureiro, CESAM/University of Aveiro

The increase of pharmaceuticals in the environment, mainly due to inadequate disposal and inefficient removal in wastewater treatment plants, has raised concerns about their potential effects on ecosystems and human health. To approach this problem, new methodologies, such as the encapsulation of compounds that allow a more controlled release at the site of action, are being developed to reduce their environmental impact across their life cycle. This study aimed to evaluate the effects of a novel liposomal encapsulation of diclofenac, as well as the impact of production residues, namely diafiltration waste, on different organisms, while also testing a commercial diclofenac solution for comparison. To this end, an acute toxicity test with Aliivibrio fischeri (Microtox), a Yeast Estrogen Screen Assay (YES) and an acute immobilisation test with Daphnia magna (OECD 202), were conducted. Microtox results demonstrated effects on the bioluminescence produced by A. fischeri in all samples, with the final product, diafiltrated liposomes loaded with diclofenac, being the one with the highest effect, with an EC50=3.5mg/L. In agreement with this, the results obtained for D. magna demonstrated lower survival rates from the diafiltrated sample with an EC50=26.2mg/L. On the other hand, the commercial diclofenac solution induced the lowest effects in both organisms, with an EC50=15.1mg/L and EC50=144.3mg/L, for A. fischeri and D. magna, respectively. The results from the YES assay suggested that the final product and the placebo liposomes may have potentially induced endocrine disruption, as they were able to bind to the estrogen receptor in the yeast. Even though this new method may solve a current problem, in this case, liposomal encapsulation of diclofenac showed higher toxicity than the commercial formulation, suggesting that the process may affect the targeted and non-targeted delivery of the compound.

P15 | **Impact of Antimicrobial Substances in Bottom Up regulated Food Webs**

Frederik Meyer, Institute of Environmental Science; RPTU Kaiserslautern Landau; Verena Schreiner, One Health Ruhr, University of Duisburg-Essen; Eric Bollinger, iES Rptu Kaiserslautern Landau; Mirco Bundschuh, iES Rptu Kaiserslautern Landau

Microbial decomposers play a crucial role in detritus-based freshwater food webs, yet their disruption by antimicrobial contaminants and the subsequent bottom-up effects on aquatic food webs remain poorly understood. This study investigates how Azoxystrobin (fungicide) and Ciprofloxacin (antibiotic) affect microbial leaf conditioning, indirectly macroinvertebrate community structure and thus functional feeding groups in stream mesocosms. Artificial stream channels were used with established macroinvertebrate communities, providing those communities with leaf litter conditioned in presence of increasing levels of Azoxystrobin and Ciprofloxacin, respectively. Azoxystrobin significantly reduced fungal biomass on leaf litter, likely leading to lower detritus quality, impaired conditioning, and cascading shifts in macroinvertebrate communities. Gammarus fossarum as a dominant species increased in abundance most likely through compensatory feeding and dietary flexibility. Chironomidae, in contrast, declined, likely due to their reliance on well-conditioned detritus. Shredders, predators, and scrapers contributed most to community differentiation, indicating trophic propagation beyond primary consumers. In contrast to the fungicide, the antibiotic did not induce significant changes, suggesting fungal decomposers is the primary driver of detritivore-mediated energy flow. These findings emphasize the critical role of microbial-mediated leaf decomposition in structuring freshwater food webs and highlight potential ecological risks associated with fungicide contamination.

P16 | **The Impact of Caffeine on Freshwater Ecosystems: Behavioral and Physiological Effects on the Gastropod Physella acuta**

Ahlam Mohamed-Benhammou, National University of Distance Education (UNED), Spain

Caffeine, one of the most widely consumed psychoactive substances globally, has emerged as a prevalent environmental pollutant, particularly in aquatic ecosystems. Research has demonstrated that caffeine contamination in freshwater habitats can adversely affect the physiology and behavior of various aquatic organisms. Studies indicate that exposure to caffeine can disrupt feeding patterns, reproductive success, and overall health in freshwater invertebrates, raising concerns about its potential impacts on biodiversity and ecosystem functionality. This study aimed to investigate the effects of caffeine on the behavior of adult freshwater snails and its physiological impact by assessing heart rate responses in juvenile Physella acuta. Caffeine was tested at different concentrations to evaluate its effects on locomotion, activity levels, feeding behavior, and cardiac function in these snails. Additionally, the study sought to identify age-related variations in how both adult and juvenile snails respond to caffeine exposure. Snails were exposed to environmentally relevant caffeine concentrations (0, 5, 30, and 50 µg/L) for either a short-term period of 24 hours or a prolonged duration of 7 days. Behavioral changes were analyzed through video recordings using specialized software capable of tracking velocity, trajectory, and other movement patterns essential for assessing organismal behavior, while heart rates were measured non-invasively by counting beats per minute under a microscope. The results revealed that caffeine exposure decreased locomotor activity in adults, whereas juveniles exhibited a dose-dependent increase in heart rate. Notably, juvenile snails demonstrated a heightened sensitivity to caffeine compared to adults, underscoring differences in vulnerability based on developmental stage. These findings provide insight into age-dependent physiological responses to caffeine pollution in freshwater ecosystems. The study offers essential data for ecological risk assessments concerning this widespread contaminant. This work has been funded by Ministerio de Ciencia e Innovación (PID2022-136669OB-I00). A.M-B is a recipient of a fellowship from Ministerio de Ciencia e Innovación (PRE2022-000781)

P17 | **Identification and Mapping of Priority Freshwater Ecosystem Services (ES) Across England's River Catchments to Inform Chemical Risk Assessment**

Judith Mugambi, University of Exeter; Ross Brown, University of Exeter; Charles Tyler, University of Exeter

Freshwater ecosystems are rich in biodiversity with 9.5% of known animal species on Earth, including around one third of the vertebrates (mainly fishes), all the crocodilians, most of the amphibians and turtles. Biodiversity forms the basis of ecosystem services that are critical to human well-being and any changes in biodiversity can influence the supply of ecosystem services. Ecosystem services (ES) are the benefits that humans derive from ecosystems also referred to as nature's contributions to people. Biodiversity and subsequently ES faces several threats such as chemical pollution, climate change, habitat degradation, land use change, etc. Riverine ecosystem services vary along a river course, indicating that ES delivery varies spatially too. Mapping ES enables visualization of the effect of different management strategies on the supply of the services. It is therefore important for spatial variation to be considered in chemical risk assessment to ES delivery. Mapping can therefore be a starting point for developing strategies for freshwater resources management and conservation. It is therefore important for spatial variation to be considered in risk assessment i.e., of chemicals to ES delivery. Environmental Quality Standards (EQSs) used to protect freshwater ecosystems from chemical pollution are currently based on toxicity data of the most sensitive species 'lowest toxicity threshold' and don't consider the ecosystems functionality as a whole and the services it provides. This poster highlights the identification and spatial distribution of selected freshwater ecosystem services (ES) across England's River catchments. The information mapped is obtained through stakeholder engagement and secondary data sources. Stakeholder engagement was through questionnaires with some of the key persons in the management of river basin catchments in England. While the secondary data is from freshwater ES related mapping exercises in England, publications, verifiable data from ES related recreational or economic activities. These findings will be presented using GIS maps and graphs. This is part of the findings of the 2nd chapter of a PhD research project that seeks to develop a chemical risk assessment approach for freshwater rivers that considers impacts at the ecosystem level and provides a framework for ecosystem service-based Environmental Quality Standards (EQS).

P18 | **Indirect Effects in Mudsnails Through Dietary Uptake?**

Sophie Oster, Rhineland-Palatinate Technical University Kaiserslautern-Landau (RPTU); Patrick Fink, Helmholtz Centre for Environmental Research (UFZ); Mirco Bundschuh, Rhineland-Palatinate Technical University Kaiserslautern-Landau (RPTU)

Aquatic ecosystems are characterized by multiple connections within and among trophic levels. Chemical stress can disrupt these interactions and lead to shifts in aquatic ecosystems' structure and function. Pesticides or pharmaceuticals can enter surface waters via different pathways such as spray drift, agricultural runoff, or sewage treatment plants. Direct effects of these chemicals towards aquatic primary producers such as river biofilms have been widely studied. Biofilm communities not only contribute to primary production, but also represent an important food source for grazing animals and host a large biodiversity including bacteria and algae. Studies of potential indirect effects towards higher trophic levels (primary consumers) are scarcer. We therefore hypothesized that species turnover in the biofilm community composition (horizontal interaction) could in turn indirectly affect grazers (vertical interaction) feeding on biofilm. We studied river biofilms as food source the biofilm grazing New Zea

P19 | **Investigating Aquatic Chemical Exposure Across the Galapagos Archipelago, Using Rapid Assessment Techniques**

Georgie Savage, University of Exeter; Katie Deakin, University of Exeter; Stephanie Andrews, University of Exeter; Ricardo Zambrano, Galapagos National Park; Alfonso Venlasteguí, Galapagos National Park; Patrick Moldowan, Charles Darwin Foundation; Courtney Pike, Charles Darwin Foundation; Ildiko Kriston, Greenpeace Research Laboratories; David Santillo, Greenpeace Research Laboratories; Beatriz Callejo, Greenpeace Research Laboratories; Alexandra Richardson, Imperial College London; Leon Barron, Imperial College London; Jen Jones, Galapagos Conservation Trust; Ceri Lewis, University of Exeter; Tamara Galloway, University of Exeter;

As a developing island province, the Galapagos Archipelago is at the forefront of the Anthropocene, facing intensifying pressures from its growing human footprint, accelerated global connectivity and unique environmental factors, which are cumulatively driving chemical contaminant across the islands' aquatic environments. Despite their geographical isolation, the islands are at risk of exposure from oil, plastics, pesticides, emerging contaminants, persistent organic pollutants and heavy metals; such contaminants threaten to overwhelm the adaptive capacity and resilience of these fragile aquatic ecosystems. Here we characterise pesticides and emerging contaminant exposure levels in water from thirteen coastal sites and nine highland ponds across the three main inhabited Islands (San Cristobal, Santa Cruz and Isabela. Alongside standardised 'grab' sampling and in-field solid-phase extraction techniques, novel passive samplers including 'HiSorb' probes (stainless steel probes coated with polydimethylsiloxane solid phase) and miniaturised 3D-printed discs (methacrylate housing holding five hydrophilic-lipophilic balance membranes), were deployed for 1-2 weeks to examine temporal variability in contaminant exposure and evaluate their potential as rapid assessment monitoring tools. HPLC-MS analysis revealed carbendazim and diuron to be the most frequently detected pesticides found at the highest concentrations in seawater (43.75% and 37.5% of samples; 24.6 ng/L and 24.9 ng/L, respectively), and trace levels of fungicide griseofulvin (<LOQ) found in pond water. Proximity to urbanisation and point-sources influenced coastal pesticide contamination, with enclosed brackish waterbodies exhibiting the highest exposure concentrations. Forensic GC-MS screening of HiSorb

probes identified medium-to-long chain hydrocarbons, chemical ingredients associated with sunscreen products and residues of the widely used insect repellent DEET in seawater. Analysis of emerging contaminant on 3D-printed passive sampling discs is still ongoing. This investigation provides valuable baseline data on chemical exposure across the Galapagos Archipelago aquatic environments to inform future management and mitigation strategies by the Galapagos National Park, and help navigate this evolving chemical landscape.

P20 | **In Vitro Toxicity of Road Runoff From Different Road Types Using Reporter-Gene Assays**
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Rapid urbanization and the rising number of motor vehicles over the past few decades have resulted in dramatic environmental changes. In particular, tire and road wear particles (TRWPs) in road runoff are of increasing concern. Nonetheless, the environmental fate and ecotoxicological effects of road runoff are yet to be understood. Furthermore, the possibilities of interactions between TRWPs and other road runoff pollutants are rarely known. The presented study will be carried out as part of the interdisciplinary RoadTox project, which aims to conduct an ecotoxicological risk assessment of road runoff and further prioritize measures for input mitigation.
Runoff samples were collected over the course of two years (2022 to 2023) at three different model road sites (motorway, rural and urban road) in Aachen, Germany, covering seasonal and meteorological variability (e.g., dry periods, freezing conditions, heavy rain events). Subsequently, the water samples were filtered and extracted via Solid Phase Extraction (SPE). In addition, organic extracts were prepared from suspended particulate matter (SPM), which were isolated from corresponding runoff samples and freeze dried prior to Ultrasound-Assisted Extraction (UAE). For use in in vitro assays, aliquots of the extracts were transferred to DMSO. An in vitro biotest battery with several reporter gene assays (dioxin-like activity, (anti-)estrogenic, and anti-androgenic activity) was applied to assess the mechanism-specific toxicity. Prior to the measurement of the mechanism-specific endpoints, the neutral red assay was performed for each used cell line to determine the cytotoxicity.
The occurrence of endocrine activity and dioxin-like effects was detected in a great number of samples. Moreover, extensive chemical data are available for the samples analyzed in the current study. Hence, the aim of the presented study is to provide corresponding mechanism-specific in vitro data focusing on the comparison of different road types. The results will contribute to a better understanding of road runoff ecotoxicity and to identify possible toxicity drivers.
The project is funded by the Ministry for the Environment, Nature Conservation and Transport of the State of North Rhine-Westphalia (MUNV) and the RobustNature network, Germany.

P21 | **Toxicity of Tire Rubber Leachate to Daphnia magna and Temporal Trends of Additive Leaching**
Onni Sirkiä, University of Eastern Finland; Pius Kairigo, University of Jyväskylä; Elina Kalenius, University of Jyväskylä; Jussi Kukkonen, University of Eastern Finland; Victor Carrasco Navarro, University of Eastern Finland

Tire rubber (TR) is a semisynthetic polymer with varying physical and chemical properties. It consists not only of the polymer chains but also of many different kinds of additives that are used to modify the properties of rubber mass depending on the intended use. These additives are not chemically bonded to the TR and can leach out of the rubber mass over time, therefore causing potentially harm to the organisms in the surrounding environment. Still, the majority of TR-related research focuses on physical particles rather than the released additives.
In our study, we wanted to assess the toxicity of tire rubber leachate to Daphnia magna in the absence of physical particles. D. magna is a common freshwater crustacean and a vital part of the aquatic food web. In addition, we wanted to evaluate temporal trends of additive leaching and attempt to identify the additives released from the TR particles.
Tire rubber leachate was prepared in medium-high concentration (10 g/L) over six subsequent leaching periods (20 d, room temperature, darkness, 160 rpm). After each leaching, TR particles were removed via filtration (0.22 μm pore size) and the toxicity of obtained leachate was tested on D. magna neonates (< 24h old) in different concentrations (5, 20, 50 and 75%) by monitoring their mobility at 24 h and 48 h according to the OECD guidelines. We are currently analyzing the leachate characterization data from untargeted high-resolution LC–MS and will also include ICP–MS analyses for the metal content determination.
Our results indicate that early TR leachates can induce acute mortality on D. magna already at 5% concentration, thus making the determination of any ECxx values impossible. However, the observed toxicity begins to diminish on the third leaching cycle and practically disappears by the fifth leaching. Ideally, MS analyses will provide us with the identity of leached additives as well as possible candidates for causing the observed toxicity. Preliminary results indicate high levels of 1,3-diphenylguanidine, a common tire rubber vulcanization agent, together with some of its degradation products. However, the metal analyses and additional toxicity testing using the potentially hazardous additives must be performed before drawing any conclusions.
Overall, our study demonstrates the important role of TR-derived chemicals in ecotoxicology and the hazards they may pose to the environment already at relatively low concentrations.

P22 | **Integrated Assessment of Groundwater Ecosystems: Acute and Mechanism-specific Effects of Groundwater Samples from the Urban Area of Hanover, Germany**
Sarah Wohlmann, Goethe University Frankfurt; Carolin Bertold, Goethe-University Frankfurt; Christian Forberg, Goethe-University Frankfurt; Sven Berkhoff, Institute for Groundwater Ecology, IGÖ GmbH; Hans Jürgen Hahn, Institute for Groundwater Ecology, IGÖ GmbH; Heide

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Groundwater represents the biggest freshwater compartment in the global hydrological cycle. It provides water for half of the world's population, used as drinking water and for irrigation. However, this vital resource is under great pressure, both quantitatively due to increased water extraction and reduced recharge, and qualitatively due to pollutant infiltration from surface. Sources for pollutants are mainly industry, wastewater discharge or agriculture, resulting in complex chemical mixtures occurring in groundwater consisting of nitrate, pesticides, pharmaceutical residues and perfluorinated substances (PFAS). Public and institutional awareness of groundwater quality and its impact on groundwater as a habitat is increasing. However, effect-based ecotoxicological data, using groundwater samples or stygobionts as testspecies are still very scarce. This study focuses on the acute toxicity towards a stygophilic species and the endocrine disrupting potential of groundwater samples on cellular level. Nine samples were taken from aquifers in the urban area of Hanover, Germany and extracted via large-volume solid phase extraction (LV-SPE) and solid-phase extraction (SPE). Following, a series of chemically activated luciferase expression assays (CALUX®) to detect (anti-) estrogenic or (anti-) androgenic effects were conducted. Three native samples were further tested for acute toxicity with the stygophilic copepod species Eucyclops serrulatus. To investigate developmental effects on the organismic level, a 21-day developmental toxicity test with < 24 h old nauplii was conducted. Regarding endocrine disrupting effects detected with the CALUX® assays, all nine sampling sites showed effects, mostly anti-androgenic and partly estrogenic potential. This correlates with the detection of a variety of pharmaceuticals, such as 2-hydroxyibuprofen. First data regarding the acute toxicity of the three selected native groundwater samples suggest no lethal effects of nauplii or adult copepods. Further ecotoxicological relevant endpoints will be tested with the same groundwater samples and corelated to chemical and faunistic data. This study is part of the joint project "gwTriade", which aims to develop a holistic assessment concept of groundwater ecosystems based on a triad approach, combining chemical analysis, standardised ecotoxicological bioassays and fauna diversity.The project is funded by the BMBF (funding line: "Nachhaltiges Grundwassermanagement" (LURCH)) as part of the federal program "Wasser: N".

2. Terrestrial Ecotoxicology

17 | **Evaluating the Impact of Cassava Mill Effluent Discharge on Soil Quality in Selected Agricultural Areas of Abia State, South Eastern Nigeria**
Precious Emole, Abia State University; Victoria Kelechi Emmanuel, Abia State University; Ifeoma Uzoamaka Nwabekee, Abia State University; Prince Onyedinma Ukaogo, Abia State University

Cassava is a major source of carbohydrate intake in Nigeria. However, cassava processing releases huge amount of effluent called cassava mill effluent (CME). CME is harmful to the environment and has the potential to evade traditional wastewater treatment, It is categorized as a contaminant of emerging concern that can seep into soil and water bodies. In this study, the impact of CME discharge on agricultural soils' physicochemical and elemental properties using standard analytical methods in three locations (Umudike, Ibeku and Oboro) in Abia State, Nigeria were comprehensively evaluated. Soil samples (topsoil: 0-15 cm and subsoil: 15-30 cm) were taken randomly from CME-receiving areas as well as control sites to determine changes in selected soil properties such as pH, moisture content, organic matter, nutrients, and heavy metal concentrations. The findings showed that CME significantly altered the soil's physicochemical and elemental properties compared to the control. The significant increase in moisture content and the reduced bulk density suggested potential implications for soil structure and water dynamics that can lead to waterlogging and nutrient loss if not properly managed. The low pH of the CME-affected soil indicated a higher acidity and thus could negatively affect the availability of nutrients to plants. Though the polluted soils had more organic matter and nitrogen, HCN (Hydrogen Cyanide) was present at levels that exceeded permissible limits. The elevated HCN level poses adverse effect on soil biota and crop performance. The elemental analysis showed enhanced levels of calcium, magnesium, potassium, and phosphorus in the CME-impacted soils, suggesting potential benefits for plant nutrition. However, the disproportionate increase in certain elements could disrupt nutrient balance and lead to an imbalance in the soil-plant system. This research provides insight into the environmental fate and effects of CME discharge on the quality and productivity of agricultural soils. The findings highlight the need for the development of effective strategies to regulate and manage the disposal of this effluent, ensuring the sustainability of soil resources and food production in cassava-growing regions thus advancing the scientific understanding of the environmental implications of improper waste management practices in the agricultural sector.
Keywords: Soil, Cassava mill effluent, Hydrogen Cyanide, Physicochemical properties, Toxicity

18 | **Plants on Prescription: Long Term Effects on Fertility of Diclofenac and 17B-EstradiolPlants on Prescription: Long Term Effects on Fertility of Diclofenac and 17B-Estradiol**
Andrea Garduno Jimenez, University of Leeds; Narmin Garazade, University of Leeds; Sarah Hunt, University of Leeds, Leeds; Laura J. Carter, University of Leeds

The use of wastewater and treated sludges to meet agricultural nutrient and irrigation demand results in the inadvertent release of many emerging contaminants including active pharmaceutical ingredients and naturally excreted hormones. It has been shown that these chemicals can be taken up by plants from soils and they can elicit an effect in these non-target organisms. Two contrasting examples are the demonstrated growth hindering effect of diclofenac and growth promotion from 17B-estradiol. However, we know very little about longer term effects at environmentally relevant concentrations, in particular around plant reproduction (flowering, seeding and hereditary effects). To address this knowledge gap we carried out a study to i) identify whether diclofenac and 17B-estradiol influenced Raphanus sativus reproduction (Phase 1) and ii) whether the seeds produced by the first generation of plants reacted differently under repeated exposure to diclofenac and 17B-estradiol in comparison to controls (Phase 2). Phase 1 results revealed that flowering was delayed by 23- and 12-days following exposure to environmentally relevant concentrations of diclofenac and 17B-estradiol, respectively, compared to the controls. Total plant biomass was not significantly different ($p < 0.01$) depending on the presence of either pollutant. However, seed yield from the diclofenac treated plants was only about 10% that of the controls (0.152 and 1.5 g dw seeds per plant for diclofenac and control, respectively). For 17B-estradiol, seed yield was not significantly ($p < 0.01$) different to that of the control. Seeds collected in Phase 1 were planted to follow up on hereditary effects, by exposing them to either chemical or to neither. Plants grown from seeds produced by plants exposed to a pollutant in Phase 1 presented a flowering delay in Phase 2, even when not exposed to the pollutant again during the second phase. In the case of diclofenac exposure, the delay was of 47 days and for 17B-estradiol of 35 days . For diclofenac the delay was greater when plants were exposed to the pollutant again in Phase 2, with a 56-day delay. However, for 17B-estradiol, the flowering delay was reduced to 26 days when the plants were exposed again in Phase 2. Preliminary results also indicate a significantly lower ($p<0.01$) seed yield from plants exposed to diclofenac during Phase 1 only and during Phase 1 and 2. Transcriptome analysis on first- and second-generation plants exposed to both pollutants is underway to begin to elucidate the molecular mode of action of the observed effects. Effects on flowering delay and seed yield have significant implications for reproductive success and survival of plant species, which in turn impacts ecosystems, agriculture, and biodiversity. Hereditary effects passed on through seeds have very relevant implications in terms of long-term population development, which highlights the importance of these findings.

19 | **The Impacts of Gaseous and Particulate Air Pollution on the Moth Species Spodoptera littoralis**
Rachael Haw, University of Sheffield; Karl L. Evans, University of Sheffield; Laurence Jones, United Kingdom Centre for Ecology and Hydrology (UKCEH); Stuart Campbell, University of Sheffield

Tropospheric air pollution is a major public health concern, contributing to approximately seven million deaths annually. While extensive research has focused on the human health impact of air pollution, far fewer studies have addressed its ecological consequences. Insects may be especially sensitive to air pollution due to their foraging behaviours, which increase their exposure to particulate matter (PM) and oxidising pollutants such as nitrogen dioxide (NO_2) and ground-level ozone (O_3 These pollutants could negatively impact insect fitness in several ways. Particulate deposition on foliar surfaces could act as a non-nutritive barrier to herbivores, discouraging feeding behaviour and physically blocking the digestive tract when ingested. The increased production of reactive oxygen species (ROS) and subsequent oxidative stress from NO_2 could demonstrate deleterious impacts on growth and development, causing dysfunction or death. Most studies addressing this have pursued a field-based approach, yet there is a notable lack of empirical evidence on how such pollutants directly affect insect fitness. In two experiments we tested the individual effects of NO_2 and PM on the growth and fitness of a generalist moth species Spodoptera littoralis (Lepidoptera: Noctuidae). Lifetime exposure of larvae to elevated NO_2 levels (~100 ug/m3) via a specialised fumigation chamber system elicited weak effects on growth and fitness with indications of stage-specific mortality. For the PM experiment, elemental carbon was deposited onto the surface of artificial insect diet at several densities reflecting current PM deposition levels. Counterintuitively, larvae feeding on PM experienced up to an average 475% mass gain compared to control larvae. The unexpected results of this study challenge existing assumptions about the ecological impacts of air pollution, and further study into the mechanisms underpinning these responses is critical for understanding the broader implications of air pollutants on insect populations and ecosystem health.

20 | **Prochloraz Affects the Toxicokinetics of Azoxystrobin in Enchytraeus crypticus (Annelida)**
Kevin Noort, UK Centre for Ecology & Hydrology; Elma Lahive, UK Centre for Ecology & Hydrology; Emily Eagles, UK Centre for Ecology & Hydrology; Alexander Robinson, UK Centre for Ecology & Hydrology; Stephen Short, UK Centre for Ecology & Hydrology; Nele Horemans, SCK CEN; Henriette Selck, Roskilde University; Dave Spurgeon, UK Centre for Ecology & Hydrology

Chemical mixtures can exhibit additive, synergistic, or antagonistic effects. Synergistic interactions are particularly concerning as they amplify toxicity beyond predicted levels. Among known contributors to synergy, azole fungicides play a significant role due to their inhibition of cytochrome P450 monooxygenases (CYPs), essential enzymes in the phase I metabolism of lipophilic pesticides. However, the few attempts at making a mechanistic link between CYP inhibition and synergistic interactions had to conclude that inhibition does not fully explain synergy, insinuating that other mechanisms might also be effected.
This study aims to assess how PCZ affects the uptake and elimination kinetics of AZX, contributing to a better understanding of mixture toxicity and biotransformation in soil invertebrates, and arriving at a mechanistic attribution of synergistic interactions observed in mixtures with azole fungicides.
This study examines the toxicokinetics of azoxystrobin (AZX) in Enchytraeus crypticus (Annelida) in the presence of prochloraz (PCZ), a known cytochrome P450 inhibitor. It does so by using 14C-radiolabelled AZX. The experiment includes two phases: an Uptake phase and a Depuration phase, both lasting 2 weeks. During Uptake, worms are exposed to 14C-AZX (1.35 MBq total activity, ~1 kBq/g); during Depuration, they are transferred to clean soil. The experiment is conducted using LUFA 2.2 soil and uses a full factorial design: two AZX concentrations (high/low) and two PCZ treatments (present/absent). Sampling occurs at 3h, 16h, 3d, 7d, and 14d in both phases, and worms are allowed to purge gut contents for 4 hours before being frozen for radioactivity measurements.
Preliminary results confirm differences in uptake and elimination processes of AZX, indicating that PCZ affects the toxicokinetic parameters of AZX through other mechanisms. The presenter will further discuss results and explore how these findings contribute to mechanistic insights into the role of biotransformation in mixture toxicity. Special attention will be given to how these insights may inform future risk assessment frameworks for pesticide mixtures in soil ecosystems.

21 | **Unearthing Plastic: A Global Review of Microplastic Contamination**
Kelly O'Shea, University of Leeds; Paul Kay, University of Leeds; Bob Barnes, Environment Agency; Laura Carter, University of Leeds

Plastics are ubiquitous in modern life, with applications ranging from packaging and construction to electronics and agriculture. Global plastic production continues to rise, with the generation of virgin plastics far outpacing recycling efforts. While the environmental impact of plastic pollution has largely focused on marine systems, recent research suggests that soils may harbour even greater concentrations of plastic debris. Microplastics—plastic particles ranging from 1 µm to 5 mm—enter terrestrial environments through diverse pathways, including irrigation with wastewater, compost application, plastic mulching, landfill leachate, road runoff, and direct littering.
This review of 512 articles examines the global distribution of microplastics in soils, focusing on reported concentrations, sources, polymer types, and particle sizes. Reported concentrations varied widely, from as low as 1 microplastic particle per kilogram of soil to as high as 69,407 MP/kg. The most common sources of soil contamination were linked to agricultural practices, particularly plastic mulching (up to 38,649 MP/kg) and the use of wastewater sludge (up to 16,164 MP/kg). Microplastics are found to influence soil properties such as structure, water retention, and cation exchange capacity, with potential implications for soil health and ecosystem function.

A significant challenge identified across the literature is the lack of standardised methodologies for microplastic extraction and quantification in soils. This absence of consistency limits the comparability of studies and may lead to over- or underestimations of contamination levels. Country specific data is also inconsistent, with China publishing 120 studies and Brazil publishing only 1 study from the period of 2008 to 2024. Despite extensive inclusion criteria, not all countries are represented as their research did not return any results. Overall, the review highlights both the widespread nature of microplastic pollution in soils and the urgent need for harmonised analytical protocols to accurately assess and address this emerging environmental threat.

22 | **Pharmaceutical Pollution in Terrestrial Habitat: Investigating Effects of Antibiotic Pollution on Microbial Functioning and Modelling Scenarios for Antibiotic Pollution in S/Western Nigeria**
Oluyemi Ojo, University of York; Alistair Boxall

Pharmaceuticals and personal care products (PPCPs) are regarded as pollutants of rising concern due to their capacity to elicit physiological alterations at low levels in non-target species within the environment. . This study focused on bridging this gap in the terrestrial ecosystem. This research seeks to examine the impact of antibiotics on microbial function through the use of standardised ecotoxicological testing protocols. The subsequent objectives were formulated to achieve the goal: A systematic review of existing publications to identify gaps, The development and validation of analytical methods and Toxicity testing of target antibiotics on soil microbial functioning.A systematic review of previous publications and ecotoxicological databases revealed that PPCPs have an inhibitory or lethal effect on the biochemistry, physiology, population, and yield of some plants, soil microbiota, and soil invertebrates. A total of 2678 publications were identified exploring the antimicrobial fate, occurrence, and effects in the soil environment. A total of 857 soil entries were found in the Umweltbundesamt database (UBA 2021). The most highly monitored antimicrobials in soils were tetracycline, oxytetracycline, and chlortetracycline, and the antimicrobials detected at the highest concentrations were enrofloxacin, ciprofloxacin, and chlortetracycline. The highest measured environmental concentration of antimicrobials was found in South America, Asia, and Europe. Effects data were found for 11 of the top 15 antimicrobials monitored. Of these, only 2 had data on all key ecological components of the soil environment (microbes, invertebrates, and plants).
Based on the ubiquity of these PPCPs, a risk assessment was carried out using the risk quotient (RQ) approach. Risk characterization of these chemicals indicates that sulfonamides, tetracyclines, and fluoroquinolones are of environmental concern as they have risk quotients greater than 1, i.e., RQ > 1. Furthermore, several knowledge gaps have been identified. This includes toxicity data for chemicals and the degree to which the ecotoxicological effects of PPCPs could disrupt ecosystem service delivery is yet to be well elucidated. The impacts of PPCPs on vital microbial functions at the soil-rhizosphere are also yet to be constrained.
Studies on persistence, effects, and adsorption were undertaken on five target active pharmaceutical ingredients (APIs). The findings indicate potential hazardous impacts of these antibiotics on terrestrial microorganisms. These will enhance the corpus of information regarding the behaviour and possible hazards associated with the studied substances.

23 | **Dual Impact of Organochlorine Pesticides on the Nutritional Integrity of Plantains and Bananas**
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This ground-breaking study investigates the interplay between organochlorine pesticide (OCP) contamination and the nutritional quality of two globally vital tropical staples—plantain (Musa paradisiaca) and banana (Musa acuminata). Leveraging AOAC protocols for proximate analysis, we reveal stark contrasts in their nutritional profiles: plantains exhibited higher crude fiber (5.05–7.20%) and ash (2.21–4.29%) but lower carbohydrates (10.19–16.69%) compared to bananas, which showed elevated moisture (71.34–71.46%) and carbohydrate content (21.18–21.90%). Concurrently, a robust QuEChERS-based extraction paired with GC-MS analysis detected trace OCP residues at quantification limits of 0.1–10 ng/g, with recoveries of 70–120%, underscoring method reliability. While pesticide levels fell below regulatory thresholds, their pervasive presence raises critical concerns about chronic ecotoxicological risks to fruit quality and consumer health. This dual-focused research bridges a crucial gap in food safety, highlighting how pesticide residues—even at sub-toxic levels—may subtly alter nutrient dynamics, potentially compromising dietary value. Our findings provide actionable data for nutritional policymakers and agronomists and sound an urgent call for enhanced monitoring of agrochemical use in tropical fruit cultivation. By linking contamination risks to nutritional degradation, this study pioneers a holistic framework for safeguarding the purity and health benefits of these dietary cornerstones in an era of escalating food safety challenges.Keywords: Organochlorine pesticides, Nutritional profiling, Food safety, Musa spp., QuEChERS, GC-MS, Ecotoxicology.

P23 | **Herbicide Atrazine Act as a Long-Term Endocrine Disruptor in Drosophila Melanogaster**
Estefania Arroyo Jilote, National Autonomous University of Mexico (UNAM); Patricia Ramos-Morales, National Autonomous University of Mexico (UNAM)

Atrazine is an herbicide widely used control broadleaf weed growth in the fields. While its endocrine-disrupting activity has been debated, it has shown several adverse on development and reproductive effects in mammals, fish, and amphibians. However, information on the long-term disruptive activity of atrazine is scarce. The ability of atrazine to alter development and reproductive traits in four subsequent generations after treated female Drosophila melanogaster was evaluated. Wild type, 72 h age larvae were fed with medium enriched with atrazine (GESAPRIM®Calibre90) [7.45E-

08 at 80 ppm] or distilled water as negative control. From recovered adults, 5 treated females were crossed with untreated wild type males and put by pairs in fresh medium. From the F1 progeny, five couples were put into vials with fresh food without Atrazine to get the next generation (F2). This mating system was repeated until the fourth generation (F4) was obtained. From each concentration and generation, flies recovered were counted to estimate the average number of progeny and sorter by sex to obtain the sex ratio. Flies were also reviewed under microscope to score possible morphological alterations. In addition, 10 females were randomly selected to dissect the ovaries and measure their size. Progeny average, sex ratio and size of the ovaries was affected in some concentrations of F1 to F4 progeny. Atrazine increased the frequency of alterations in all subsequent untreated generations. The frequency of alterations was higher in females that in males. More than 40 morphological alterations were observed, including reduction in size, changes in different structures of the head, thorax, abdomen and genital plate. Our results confirm that Atrazine can act as disruptor endocrine in long term because induce change's reproductive function and malformations transgenerational mode in different way in female and male. This effect was detected even at concentrations below the permitted limits in water, thus posing a risk to exposed wildlife organisms and their descendants. It is therefore essential to carry out more studies on the long-term effect of atrazine and other pesticides in other biological models. Acknowledgments: Adriana Muñoz-Hernández and Hugo Rivas-Martínez by technical support. Sciences Graduate Program, UNAM. CONACYT-CVU-630455, PAPIIT-UNAM IN226923, Drosophila Stock Center Mexico, UNAM.

P24 | **The Soil Behind the Oil: Heavy Metal Contamination of an Oil Palm Plantation**

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The high yield potential of oil palm, alongside ever-increasing demand for palm oil derivatives, drives frequent agrochemical use to maximise productivity. Agrochemicals, such as fertilisers, can contain impurities such as heavy metals. These unintentional releases of heavy metals can cause well documents toxic effects in organisms and pose a threat to health of the wider ecosystem, yet this has been largely overlooked in oil palm production. With over 30 million hectares planted with oil palm globally, and ongoing expansion in many areas, understanding the ecological risk of such pollutants is critical knowledge that will underpin future management strategies to promote sustainable production. In this study, a reconnaissance survey for heavy metals in the soils of an oil palm estate in Sabah, Malaysia was performed. A total of 140 plantation soils, and 25 background soils, from a nearby forest were analysed for total heavy metal concentrations by ICP-MS. Heavy metal contamination of plantation soils was assessed through standard indices such as enrichment factor, geoaccumulation index and pollution load index. Further, the ecological risk of heavy metals was estimated though comparison to ecotoxicologically derived thresholds such as Predicted No Effect Concentrations (PNECs). Total metal concentrations of were then compared to PNECs to assess risk using a risk quotient approach, whereby a risk quotient >1 indicates that soil organisms may be at risk of toxic effects. This study presents an initial investigation into the pollution load and ecological risk of heavy metals in soils of an oil palm plantation, providing a basis for improved chemical risk assessment in this agricultural setting. Future work should investigate how metal translocation, and risk varies with climatic factors such as drought, flooding, and rainfall. Furthermore, understanding the direct contribution of fertiliser application rates to soil metal concentrations is needed. Finally, the PNEC values used here are for temperate species, therefore, the relative sensitivity of analogous native species is needed to account for sensitivity differences and determine real world risk in these ecosystems.

P25 | **Insect Frass as a Sustainable Alternative to Conventional Fertilizers: Enhancing Soil Health and Agricultural Productivity**

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The increasing need for sustainable food production has driven research into novel organic fertilizers, such as insect frass, an entomofertilizer resultant of insect farming. This study explores the use of frass from Hermetia illucens (black soldier fly) larvae as an alternative to conventional synthetic mineral fertilizers, NPK (nitrogen, phosphorus, potassium), associated with nutrient hotspots and eutrophication events. Two different frasses were used: 1) frass obtained from insect digestion of cereal waste and 2) olive-pomace frass, an entomofertilizer resulting from the bioconversion of olive pomace, a residue of the olive oil industry. The aim of this study was to assess the potential of both entomofertilizers to replace or complement mineral fertilizers in agriculture at a laboratory scale. In addition to the control group, which consisted of natural LUFA 2.2. soil with no added fertilizer, the treatment groups included both mineral fertilizer and each type of frass at relevant rates, and different rations between NPK and frass application. Plant performance endpoints were assessed (germination, biomass, photosynthetic activity and productivity) in two full life cycle plant pot tests with Brassica rapa, following the ISO 22030 guideline. In the first cycle, all fertilization regimes were applied to the soil. For the second full life cycle plant test, the soil that remained after harvesting was used without further amendment. Soil function was assessed by measuring

soil enzyme activities (urease, β-glucosidase, aryl sulfatase, dehydrogenase, and phosphatases) at the end of each cycle. Additionally, to evaluate the safety of all used fertilization regimes, ecotoxicological assays were performed using two soil model invertebrates, Enchytraeus crypticus and Folsomia candida, following the OECD-220 and OECD-232 guidelines, respectively. Results showed that all fertilization regimes increased significantly plant performance endpoints. Frass induced soil enzymatic activity, especially at the end of the second plant cycle. This reveals a long-term positive influence on soil biochemical processes and nutrient cycling. Survival and reproduction of F. candida were not affected, while reproduction of E. crypticus increased significantly with organic fertilization. This study highlights the efficacy and safety of insect frass as a promising solution for sustainable agricultural practices.

P26 | **Heavy Metals Bioaccumulation in Urban and Rural Conspecific Ants**

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Heavy metals are a major pollutant of urban ecosystems, where they have accumulated over centuries of intense industrial and transportation activities. Although many species thrive in cities, our understanding of their response to heavy metal exposure is still limited. Strikingly, it remains unclear whether conspecifics inhabiting urban and rural habitats exhibit divergent physiological responses to heavy metal pollution. To address this gap, we measured the concentrations and bioaccumulation of six heavy metals (Cd, Cr, Cu, Fe, Pb, Zn) in 22 colonies of the urban-tolerant ant Lasius niger, sampled from two urban and two rural populations. We tested whether ants from urban habitats exhibit higher metal bioaccumulation and stronger antioxidant responses. We found that most metals were indeed more concentrated in urban soil and urban ants compared to their rural counterparts. Zn and Cd bioaccumulated in ants at levels 2 to 9 times greater than those found in the surrounding soil. Despite the overall trend of urban ants being exposed to higher heavy metal concentrations than their rural counterparts, Zn showed the opposite pattern, bioaccumulating 1.6 times less in urban ants. Total antioxidant capacity showed a positive correlation with heavy metal concentrations in the soil, and to a lesser degree, in the ants. Finally, increased oxidative stress defences in highly polluted sites suggest that metal pollution has a physiological cost and might therefore induce selective pressure on urban ants. These findings should stimulate further research on urban ecotoxicology and the physiological responses of urban wildlife to heavy metal pollution.

3. One Health

24 | **Geospatial Analysis for Monitoring and Assessing Oil Spills in Nigeria**
Nguamo Jessica Angula, University of Strathcylde; Christine Switzer, University of Strathclyde

The frequency of crude oil and refined product spills in Nigeria during exploration, extraction, and distribution yields devastating impacts on human health and the environment. Oil spills contaminate surrounding soils and water bodies, resulting in losses of agricultural lands, forest ecosystems, mangroves, and biodiversity within oil-rich territories. This research, using the National Oil Spill and Detection Response Agency (NOSDRA) database, applies a quantitative approach with geospatial analysis to identify patterns in spill locations, assess spill volumes and examine trends in regulatory reporting over period. A total of 16,425 spill incidents were recorded by NOSDRA, with data primarily self-reported by petroleum industry operators between 1990 and 2023. Approximately 49.6% (833,000 barrels) spill volume was recorded and 50.6% had no records. Filling in the missing spill volumes with maximum estimates reveals an additional estimate of 226 million barrels of oil which could have been lost in the Niger Delta environment, where oil exploration is in proximity with humanity and wildlife. Moreso, primary cause of oil spill incidents is sabotage, which accounts for 65% of total spill reported and 73% spill volume, mainly, in Rivers (4,685), Bayelsa (3,185), and Delta (1,631) states predominantly onshore. However, the polluters pay principal exempts sabotage-related spills from compensation claims. Combined with unclassified reported spill causes 15% with 4% spill volume, this policy framework effectively excludes about 88% of spill incidents from liability, leaving affected communities to bear environmental and economic damages without support. States such as Imo with low spill incident cases are marked as a hotspot area due to the strategic location of boundaries to communities with high spill incidents. Onshore areas especially swampy areas are more affected by oil related pollution due to weaker regulatory presence of oil spill management. Issues with discrepancies in underreporting shows significant gaps in transparency and accountability in oil spill data management framework. Therefore, there is an urgent need for improved spill detection system, better classification of causes of spills, and development of improved enforcement strategies. Additionally, local communities should be compensated even though spills are attributed to sabotage because of damages incurred during the spill affecting their livelihood, and well-being.

25 | **Non-Steroidal Anti-Inflammatory Drug (NSAID) Use and Avian Scavenger Contamination Risk in South America: A Survey of Livestock Veterinarians in Argentina**
Kane Colston, University of Bristol; Irene Bueno, University of Bristol; Juan Manuel Grande, Universidad Nacional de La Pampa; Nicola J. Rooney, University of Bristol

The significant declines of Old World vultures across the Indian subcontinent have highlighted the substantial ecotoxicological risks faced by avian scavengers. These declines, driven by the ingestion of livestock carcasses contaminated with the non-steroidal anti-inflammatory drug (NSAID) diclofenac, led to significant public health costs, with a number of species still listed as critically endangered today, despite ongoing conservation. However, the potential impact of NSAIDs on scavenger species beyond Asia and Europe remains poorly understood, particularly in South America, where an expanding livestock sector is combined with a unique array of avian scavengers, including the IUCN Vulnerable Andean condor (Vultur gryphus). We conducted an online questionnaire of livestock veterinarians from Argentina, to investigate for the first time the potential threat of NSAIDs to avian scavengers in the Americas. We aimed to establish the identities and frequencies of NSAID used, the contexts and temporal trends in NSAID usage and the livestock management practices of farmers, including carcass disposal methods, to understand the potential risk of avian scavenger NSAID exposure. We obtained 81 responses from 17 of the 23 provinces in Argentina, from veterinarians in 68 different towns and cities nationwide. Our survey revealed that multiple known Old World vulture-toxic NSAIDs including flunixin, ketoprofen, and diclofenac are commonly used in the treatment of several livestock species such as cattle, pigs and sheep, and for a variety of purposes. With increasing attention paid to livestock welfare on the continent, respondents perceived and increasing trend in NSAID use, which may continue to elevate exposure risks to scavenging species. A large majority of respondents indicated that potentially contaminated livestock carcasses are routinely left accessible to avian scavengers in both extensive and intensive farming systems, and that withdrawal periods are typically not observed when carcasses are left for consumption by wildlife. These findings suggest that New World avian scavengers in Argentina, including vultures, caracaras and other raptors, are likely exposed to potentially harmful NSAID residues via livestock carcasses consumption. Further research is needed to quantify the level of exposure through contaminated carcasses, and to better characterize the sensitivity of these species to acute and chronic NSAID exposure.

26 | **Tropical Mesopredator Ecology And The Impacts Of Heavy Metals In The Environment**
Tyler Cuddy, Cardiff University; Pablo Orozco Ter-Wengel, Cardiff University; Benoit Goossens, Danau Girang Field Centre, Cardiff University; Claus Svendsen, UKCEH

Heavy metals, as inorganic pollutants, are an increasing global concern. In Southeast Asia, the oil palm industry has caused mass deforestation, with the extensive use of fertilizers, pesticides, and herbicides contributing to potential heavy metal pollution in biodiversity hotspots with high endemism. Despite these changes, many generalist species have adapted to plantations thanks to their behavioural plasticity and dietary flexibility – facilitated by the different resources that the plantation can provide. However, their persistence in agricultural areas may increase exposure to inorganic pollutants from agricultural products, leading to potentially adverse accumulations. This PhD research, conducted in the Lower Kinabatangan Wildlife Sanctuary, Sabah, Borneo, aims to use generalist mesopredators inhabiting both

plantations and surrounding forests as indicators of heavy metal pollution. We hypothesize that trace metals in herbicides, pesticides, and fertilizers will be found in higher concentrations in mesopredators close to plantations compared to those in the forest. Additionally, we predict that species with higher protein percentages in their diets will exhibit greater concentrations of non-essential heavy metals due to bioaccumulation and biomagnification.
By conducting extensive fieldwork and ecotoxicological assessments using inductively coupled plasma optical emission spectroscopy (ICP-OES), we seek to determine whether bioaccumulation and biomagnification of certain heavy metals pose a threat to the area, and to identify which environmental systems are most at risk. We also plan to use dietary metabarcoding to analyse dietary composition in relation to heavy metal burdens. This combination of ecotoxicological and dietary data will hopefully ultimately illuminate the invisible impacts of intensive agriculture on tropical forest mesopredator communities along the Kinabatangan and help identify key risk areas and aid the establishment of mitigation strategies through working with the plantations towards more sustainable practices.

27 | **Novel Sorbents for Remediation of Crude Oil and Refined Product Spills in Aquatic Systems**
Alamin Khamis, University of Strathclyde; Christine Switzer, University of Strathclyde

Oil spills to surface water bodies cause significant human health and environmental damage. Oil spill response is often a race against time to mobilise response teams and equipment while natural forces disperse the oil and increase the complexity of the necessary response. In oil-rich regions like Nigeria, spill response is further complicated by the relative remoteness of spill locations and potential clandestine activities that may have contributed to their occurrences. With the increasing incidence of oil spills, cost-effective, sustainable remediation strategies are crucial to mitigate the adverse effects of these spills on local communities and ecosystems. This research explores the efficacy of sorbents derived from readily available agricultural waste and biochar in the remediation of oil pollution.
Biosorbents derived from agricultural wastes were evaluated for their sorption capabilities. Agricultural wastes included chaff from wheat, barley, and rapeseed crops as well as potato peelings, carrot peelings, and banana peels. These materials were air- and oven-dried and ground to particle sizes <5mm. A subset of each waste was converted to biochar under slow pyrolysis conditions adapted for each material. Crude oil from the Tern oilfield near Shetland, UK, with published API of 34° was used for this work. Sorption studies were carried out comparing oil recovery capabilities of each dried waste and biochar. Accelerated Solvent Extraction methods were devised for each type of material followed by Total Extractable Petroleum Hydrocarbons (TPH) quantification using GC-FID. Further work is underway to assess the resilience of these materials in various environmental conditions, simulating real-world scenarios of oil spill contamination.
Both raw and biochar agricultural wastes exhibit significant potential for sorption, influenced by differences in their physical properties and chemical composition. Generally, the biochars outperformed the raw waste materials and the food wastes outperformed the chaffs, but some uptake was observed by all materials. Some water uptake was also observed. Further work is underway to examine the effects of biochar production on uptake and potential to optimise production characteristics for oil recovery. These findings will contribute to a comprehensive understanding of how different biosorbents can be utilised to address crude oil pollution, emphasising the concurrent benefits of waste management and environmental protection.

P27 | **Addressing the Global Data Imbalance of Contaminants of Emerging Concern in the Context of One Health**
Andrea Garduno Jimenez, University of Leeds; Yolanda López-Maldonado, Indigenous Science. Merida; Rachel L. Gomes, Food Water Waste Research Group. University of Nottingham, University Park; Laura Carter, University of Leeds

Contaminants of emerging concern pose a significant global threat due to their link with the development of antimicrobial resistance and the wider ecotoxicological risk they pose. Therefore, it is urgent to effectively address this pollution challenge. However, tackling this global issue is hindered by the fact that there is considerably more contaminant of emerging concern data available for the Global North than South. Utilising research on Global North situated pollutants and impacts may lead to strategies that are inappropriate and even detrimental to the Global South, with differing pollution profile and/or environmental risk. In addition, to effectively address pollution, efforts must be truly global and equitable, given that pollution does not respect political boarders. Therefore, it is paramount that principles from One Health, which view the planet as a whole and with a clear interdependency between human and environmental health, are applied to address this global data imbalance. To achieve this, it is key to include all stakeholders and acknowledge and address the role that global resource inequalities has on this data imbalance. This work examines the critical importance of including Indigenous Peoples and Local Communities in contaminants of emerging concern research and provides specific recommendations to achieve this. This inclusion is not only a matter of social justice but a necessity for acquiring representative global data. Globally comprehensive data will in turn inform more equitable global policy to address contaminants of emerging concern environmental pollution and safeguard the health of our planet as whole. Specific recommendations to achieve this aim are made in four key areas for scientists and policy makers working on contaminants of emerging concern: 1) Understanding the context and adapting sampling processing and analysis accordingly; 2) Respectful and equitable collaborations, ensuring Indigenous Peoples and Local Communities views are respected; 3) Funding and mechanisms for fair and equitable collaborations, recognition and transparency; 4) Sensitive language and narrative use. We believe that if those working at the science-policy interphase understand and apply the recommendations made in these areas, it is possible that small actions within their fields will pave the way towards a more equitable and

effective tackling of global environmental pollution in the context of planetary health.

P28 | **Human Health Risk Assessment of Heavy Metals in Locally Grown Vegetables**
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Because of the increasing risk of contamination of food by pesticides, heavy metals, and/or toxins, the food safety issues have attracted the attention worldwide. The present study was carried out to assess the essential metals and toxic metals As, Ca, Cd, Co, Cr, Cu, Fe, Hg, Mn, K, Mg, Na, Ni, Se, Pb and Zn levels in locally grown vegetables by inductively coupled plasma mass spectrometry (ICP-MS) analysis and determined the potential human risks to consumers. The vegetables samples were collected from the local farmer market. The overall all studied metal accumulated levels in different vegetables showed followed decreasing trend: spinach > tomato > sugar beet > white eggplant > kale > cucumber > green chili > green bean > dill soya > potato > capsicum > onion > corn. Overall, potassium and magnesium were in higher amount while Pb and Hg were found in lower amount in studied vegetables. On average basis in all vegetables, metals showed the following decreasing trend: K > Mg > Ca > Na > Fe > Zn > Mn > Cu > Ni > Cd > Se > As > Co > Cr > Pb > Hg. In micro elements (Ni, Mn, Cu, Se and Co), Mn and Cu are the dominants while in toxic elements (As, Cd, Cr, Hg and Pd), Cd and As were the higher. Highest Cd concentrations (0.99 µg/g) was noted in spinach while highest As concentration (0.44 µg/g) was found in cucumber followed by white eggplant. The highest level of K, Mg, Fe, Mn, Zn, Cr, Pb, and Hg was noted in spinach. The highest level of Cu, Na, Se, Co and Ni were found in tomato, sugar beet, dill soya, green chili, and green bean, respectively. Most of the metals were found to be lower than maximum allowable concentrations set by international agencies among the analyzed vegetables. Health risks associated with the intake of these metals were evaluated in terms of estimated daily intake (EDI), and non-carcinogenic risks by target hazard quotient (HQ) and hazard index (HI). EDI values of all the metals were found to be below the maximum tolerable daily intake. The HQs of Cd were noted to be higher > 1 in most of the analyzed vegetables, followed by As, suggesting health hazards for population. HQs value for Zn and Mn were also higher than 1 in spinach. HI of Cd, As and Mn was comparatively very high as compared to other metals in studied vegetables. The findings of this study reveal the health risks associated with Cd and As through the intake of selected vegetables in consumer. Overall, Cd and As was noted to be concern in future. In conclusion, the levels of these metals should be regularly monitored in vegetables especially leafy vegetables like spinach for pollution control and human health.

P29 | **Polycyclic Aromatic Hydrocarbons in the Breast Milk of Selected Nigerian Lactating Mothers and Implications for Carcinogenic Risk in Newborns**
Oluwafemi Sarumi, Rhineland-Palatinate Technical University Kaiserslautern-Landau (RPTU); Olukayode Bamgbose, Federal University of Agriculture Abeokuta; Adetola Adebowale, Rheinland-Pfälzische Technische Un aiversität Kaiserslautern-Landau (RPTU); Abraham O. James, São Paulo State University (UNESP)

Breast milk is a crucial source of essential nutrients for infant growth, immune system development, and overall health. However, rapid industrialization and continued reliance on fossil fuels in developing countries has led to increased levels of polycyclic aromatic hydrocarbons (PAHs), raising concerns about heightened exposure and toxicity within affected populations. This baseline study evaluated the concentrations and carcinogenic risks of PAHs in the breast milk of selected lactating mothers during their first postpartum week. Twenty-eight breast milk samples were collected with written informed consent, and lifestyle information was gathered using a structured questionnaire. Eight PAH compounds were analyzed using Gas Chromatography equipped with Flame Ionization Detector. All samples showed PAH concentrations significantly exceeding the European Union's maximum permissible limit for human exposure (0.001 mg/kg). Benzo[a]pyrene (BaP) had the highest concentration (1.07 mg/kg), while benzo[b]naphtho[2,1-d]thiophene (BNT) had the lowest (0.07 mg/kg); both values were higher than global averages reported in most studies. These findings suggest a considerable risk of both acute and chronic health effects for vulnerable groups such as lactating mothers and infants. Carcinogenic risk assessment indicated that 60% of infants could face carcinogenic health risks from ingesting PAH-contaminated breast milk. Additionally, participating mothers reported experiences of low birth weight, preterm birth, and fetal loss. The results highlight the increased vulnerability of early life stages to environmental pollutants and underscore the urgent need for interventions to reduce exposure.

4. Computational Ecotoxicology & Environmental Modelling

28 | **Modelling the Role of Antarctic Krill in the Vertical Transport of Microplastics in the Southern Ocean**

Elizabeth Candish, University of Cambridge; Nan Wu, British Antarctic Survey; Clara Manno, British Antarctic Survey

Microplastics (plastics <5mm) have been frequently detected in the deep ocean and pose a potential ecological risk to this vulnerable environment. Observations of these buoyant microplastics in the deep sea suggest they are likely being transported vertically altering their distribution. Recent findings argue that the settling of microplastics with marine particles such as marine snow aggregates and zooplankton faeces are influencing the vertical transport and fate of microplastics in the ocean.
Antarctic Krill contribute to a large export of carbon in the Southern Ocean because the fast-sinking faeces can escape various attenuation processes to reach the deep ocean. It has also been shown that they consume, but do not bioaccumulate, microplastics. Therefore, krill have the potential to contribute to this vertical transport.
In this study, a one-dimensional, mechanistic model is developed to explore the interaction between Antarctic krill and microplastics in the Southern Ocean. This model describes the ingestion of microplastics by krill and the subsequent egestion. These faecal pellets are then tracked as they sink, undergoing both remineralization and fragmentation along their descent. Fragmentation may lead to the microplastics being re-released into the environment where they will rise back to the surface. Results show that krill have the capacity to transport a significant amount of microplastic particles to the deep sea. This model contributes to a greater understanding of the transport and subsequent fate of microplastics in the Southern Ocean.

29 | **Risk Assessment of Pesticides in French Surface Waters Within Protected Areas**

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Water pollution from synthetic chemicals is a leading cause of freshwater habitat degradation. As such, studying their presence and effects on aquatic ecosystems can help develop effective protection strategies. This study addresses the lack of large-scale, long-term analyses on the effectiveness of Protected Areas (PAs) in mitigating exposure to synthetic chemicals and reducing associated risks in freshwater ecosystems. To this end, a dataset comprising 179,288,140 entries recorded between 2005 and 2023 was analysed, covering surface water concentrations of 1,688 synthetic chemicals across 10,151 monitoring stations in France. The goal of this study was to assess the spatial and temporal trends of chemicals in freshwater across France and to compare these trends between PAs and Non-Protected Areas (NPAs). An environmental risk assessment was conducted for three organism groups (aquatic invertebrates, aquatic plants, and fish) by comparing Measured Environmental Concentrations with EU Regulatory Acceptable Concentrations, yielding Toxic Units (TU). A cumulative TU by sample (sumTU) and its yearly maximum by monitoring station (sumTUmax) were calculated. A sumTUmax > 1 was used as an indicator of high ecological risks for aquatic plants, invertebrates, and fish. Results show that chemical pollution of freshwater systems is widespread across France, with 55.5% of the stations measuring high-risk concentrations to at least one species group at least once (sumTUmax >1). Overall, aquatic plants and invertebrates faced high risks (sumTUmax) more often than fish (30.21%, 19.57% and 5.25%, respectively). Significant differences were found between PAs and NPAs regarding the quantification of chemicals (2.72% NPA, 1.92% PA, $R2= 0.93$, $p < 0.001$, $\delta = -0.63$) which also translated to significant differences in environmental risks (58.3% NPA, 48.3% PA, $R2 = 0.97$, $p < 0.005$, $\delta = -0.53$). These findings suggest that while PAs contribute to reducing chemical exposure and risk, they do not fully eliminate it. Lastly, gammarid body burden patterns were also examined. A principal component analysis shows that pesticide exposure and agricultural land use are negatively correlated to gammarid survival, highlighting a clear, direct link between those variables, as well as broader ecological consequences for freshwater food webs. These findings emphasise the persistent threat posed by synthetic chemicals, even in protected areas.

30 | **Evaluation of Metal Bioavailability in Aqueous Media by Mechanistic Analysis of the Time-Response of Luminescent Metal Biosensors: Theoretical Concepts and Case Study**

Lorenzo Maffei, University of Lorraine (UL); Christophe Pagnout, Université de Lorraine; Marie Zaffino, Université de Lorraine; Elise Rotureau, Université de Lorraine; Jérôme F.L. Duval, Université de Lorraine

Whole-cell bacterial sensors have been used for decades to assess metal bioavailability in aquatic environments. Some of these sensors involve the lux-reporter gene and are thus capable of producing a bioluminescence signal that is proportional to the concentration of the targeted bioactive (i.e. internalizable) metal species. However, the exploitation of the metal-mediated response of such biosensors is still currently limited to empirical observations and/or to the consideration of the only peak signal intensity. In turn, such signal processing makes it difficult to derive robust quantitative information on metal speciation and metal bioavailability properties in solution, especially in situations when standard Biotic Ligand Model (BLM) framework does not apply. Based on recent theoretical and experimental work from our group, the current contribution details how the full time-response of metal-detecting luminescent bacterial sensors reflect the speciation of metals in solution and their bioavailability beyond the conventional BLM picture. The derived theoretical framework defines the conditions in line with scenarios where free metals are the only bioactive metal species (as assumed in BLM), and the conditions where metal complexes can contribute to the biouptake depending on their lability. The formalism further accounts for the depletion of bioactive metal

species from bulk solution due to metal bioaccumulation over time. These concepts are integrated in a theory for the time-response of luminescent bacterial metal-sensors, which makes the mechanistic connection between metal speciation/bioavailability and bioluminescence production kinetics. Predictions by theory are successfully supported by bioluminescence data measured over time on Cadmium-inducible luxCDABE-E. coli in controlled media where we varied cell concentration, type and concentration of chelators. In particular, the formalism successfully explains how the time-position and amplitude of the bioluminescence maxima depend on the bioluminescence assay medium composition and on the interplay between the kinetics of cell photoactivity and bulk metal depletion. Overall, our work paves the way for the development of original strategies to decipher the time-response of luminescent biosensors for the assessment of metal speciation/bioavailability in aquatic media.

31 | **Extrapolating the Effects of Chlorpyrifos from Lab to Field Using Time-Variable Mean Species Abundance Relationships**

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Laboratory toxicity tests are mandatory for bringing a new plant protection product onto the market, but how well these tests predict field effects is still an important question. Specifically, it is challenging to translate short-term laboratory results into long-term outcomes that are relevant to field conditions. This study examines the applicability of lab-to-field extrapolation by comparing the impacts of the insecticide chlorpyrifos on biodiversity in both laboratory tests and a mesocosm study by adjusting the lab data for time-dependency.
To assess the chemical impact on biodiversity, we use mean species abundance relationships (MSAR), increasingly used for multistress assessment, describing the mean species abundance dependent on chemical concentration. This method has an advantage over the commonly used species sensitivity distributions because it can be derived directly from field or mesocosm abundance samples. The laboratory MSARs were calculated using species-specific median effect concentration (EC50), median lethal concentration (LC50), and slope values from acute toxicity data.
The time-dependency of the lab MSAR was achieved by predicting the time dependence of the EC50 and LC50 values, using an equation based on the critical body residue concept. To compare the laboratory and mesocosm MSARs, the laboratory data was extrapolated to the time point at which the hazardous concentration 50 (HC50) of the laboratory-calculated MSAR equaled the HC50 of the mesocosm MSAR. This approach allowed us to determine the day on which the predicted impact from the laboratory data matched that observed in mesocosm studies.
Results show that, the predicted day at which lab HC50 equals mesocosm HC50 is similar, suggesting that the model can predict field effects from lab data. When all macroinvertebrates were included, the mesocosm MSAR slopes were less steep than those from the laboratory. However, when focusing solely on arthropods—the taxa tested in the lab—the slopes became steeper and more comparable to the lab curves. As arthropods are usually the most sensitive invertebrates to chlorpyrifos the selection of lab species among this phylum is reasonable. In conclusion, MSARs are a promising tool for integrating lab and field data into ecological risk assessments. By showing how laboratory toxicity data can reflect field conditions, this study offers a more ecologically realistic framework for environmental regulation and chemical risk evaluation.

P30 | **Modeling Chemical Bioaccumulation in Black-Tailed Godwits: Analyzing the Extra Risk for Migratory Birds and Identifying High-Risk Sites Along Migration Routes**

Eva Alexandri, University of Osnabrueck; Florian Schunck, University of Osnabrueck; Andreas Focks, University of Osnabrueck

Migratory birds are increasingly exposed to chemical contaminants introduced into the environment through agricultural and industrial activities. These chemicals may accumulate in wildlife, especially in large predators and migratory species with high food intake demands. The Black-tailed Godwit (Limosa limosa), a prominent migratory shorebird, forages across diverse habitats such as estuaries, rice fields, and grasslands as it travels between its breeding grounds in Europe and wintering sites in North Africa. To assess the chemical exposure risks faced by this species, we are developing a mechanistic bioaccumulation model that integrates body weight dynamics, feeding behavior, and chemical kinetics. The model tracks the internal chemical concentrations in godwits by combining ingestion-based uptake with internal redistribution driven by energy use, particularly during energetically demanding periods such as migration. By expressing all state variables as mass concentrations and reformulating uptake and elimination processes using concentration-based (Q x C) terms, we ensure a consistent mass balance framework, especially for blood. This model enables spatial and temporal analysis of contaminant accumulation and allows us to identify critical exposure windows and high-risk sites along the migratory route. Ultimately, the approach aims to quantify the additional chemical exposure risks specific to migratory life histories and to provide a scientific basis for targeted conservation strategies. Our findings contribute to linking environmental quality with individual health status in migratory bird species.

P31 | **Does Cytochrome P450 Inhibition Lead to Synergy? A Mechanistic Study of Azole-Pesticide Mixtures in Enchytraeus crypticus (Annelida)**

Kevin Noort, UK Centre for Ecology & Hydrology; Alexander Robinson, UK Centre for Ecology & Hydrology; Stephen Short, UK Centre for Ecology & Hydrology; Henriette Selck, Roskilde University; Dave Spurgeon, UK Centre for Ecology & Hydrology

Chemical mixtures can exhibit additive, synergistic, or antagonistic effects. Synergistic interactions are particularly concerning as they amplify toxicity beyond predicted levels. Among known contributors to synergy, azole fungicides play a significant role due to their inhibition of cytochrome P450 monooxygenases (CYPs), essential enzymes in the phase I metabolism of lipophilic

pesticides. However, a direct quantitative link between azole CYP inhibition and synergy has not been fully established.
This study explores whether in vivo CYP inhibition can predict synergy in binary mixtures of azole fungicides and pesticides (azoxystrobin or cypermethrin). Using Enchytraeus crypticus as a model soil organism, CYP inhibition was quantified with an ECOD assay during azole exposure. Dose-response curves for three imidazoles (prochloraz, imazalil, miconazole) and three triazoles (propiconazole, prothioconazole-desthio, epoxiconazole) were constructed. Concurrently, survival dose-response curves were developed for azoles, pesticides, and their mixtures in a 96h survival study. Mixtures were tested using a full factorial design, combining three lethality levels of pesticides (estimated LC10, 25, and 50) with four inhibition levels of azoles (IC10, IC25, IC50, IC75). Mixture effects were modelled using concentration addition (CA) and observed deviations from model predictions were quantified in model deviation ratios (MDRs). MDRs, indicating synergy (≥2) or antagonism (≤0.5), were correlated with CYP inhibition to establish predictive relationships. Preliminary results confirm robust, reproducible CYP inhibition by azoles in E. crypticus. Initial survival tests provide accurate LCxx values for pesticides, enabling further synergy assessments. Data will be presented and discussed, linking CYP inhibition and synergistic toxicity.

P32 | **Are French Surface Waters at Risk From Chronic Pesticide Exposure?**
Veronica Rodriguez Careaga, Rhineland-Palatinate Technical University Kaiserslautern-Landau (RPTU); Larissa Z. Hermann, Rhineland-Palatinate Technical University Kaiserslautern-Landau (RPTU)

Pesticides are widely used to secure agricultural productivity, leading to their frequent and large-scale application. As a result of overspray, drift, and agricultural runoff, freshwater systems are consistently exposed to pesticide residues, raising concerns about chronic ecotoxicity risks. This study aims to assess the chronic ecotoxicological risk of pesticide exposure in French surface waters and investigates how such risk varies spatially, according to factors such as land use, pesticide sales, and geo-hydrological conditions. Furthermore, since chronic toxicity is inherently linked to sustained exposure over time, the role of sampling frequency is considered to evaluate how monitoring intensity affects the detection and assessment of long-term pesticide risk.
The study is conducted by performing a national-scale risk assessment based on measured pesticide concentrations, employing statistical analyses and machine learning techniques to identify spatial patterns of risk and their key environmental drivers. The assessment uses a dataset spanning 10 years, from 2013 to 2023, and covering over 500 pesticide substances. The study is expected to identify influential factors contributing to and patterns within chronic pesticide risks in French surface waters, e.g., specific surface water sites and substances. Additionally, this study aims to explore how sampling frequency influences the ability to detect chronic pesticide exposure. The outcomes of this research are relevant for informing pesticide monitoring strategies and regulatory efforts, as identifying the drivers of higher risk can guide the development of targeted monitoring guidelines.

P33 | **To Which Extend Can the Fungicide Azoxystrobin Affect the Uptake and Elimination of Copper in Daphnia Magna?**
Marius Schmitt, Ghent University; Karel De Schamphelaere, Ghent University

Copper (Cu) is an essential metal for aquatic organisms and crucial for a well-functioning metabolism. However, when internal metal concentrations exceed essential levels, metals can cause toxicity. With active uptake and elimination processes, organisms can balance and regulate their internal metal concentrations to avoid toxicity and ensure homeostasis.
However, in the environment metals often cooccur with organic micropollutants such as pesticides. In such mixtures, metal uptake and elimination processes could potentially be negativly impacted. Even though metal uptake and elimination have been extensively studied in aquatic organisms, little is known about how organic substances can affect metal uptake and elimination processes. Using the stable isotope 65Cu, we investigated to which extent the presence of the fungicide azoxystrobin affects the uptake and elimination of Cu in Daphnia magna. Cu and azoxystrobin are both used as fungicides in agriculture. Azoxystrobin inhibits mitochondrial respiration and interferes with organisms energy metabolism. Due to its MoA, we hypothesise that azoxystrobin can negatively impact uptake and elimination processes in D. magna, disrupting its ability to regulate its internal metal concentrations.
Uptake and elimination studies were performed for 72h with a 48h uptake and 24h elimination phase. Uptake and elimination rate constants were determined using the standard one-compartment model. Daphnia were sampled for 12h every 2h, after 24h and 48h to study effects on metal uptake. During the elimination phase, the daphnia were sampled for 10h every 2h and after 24 h. At each time point, three replicates of 10 Daphnia (5-6 days of age) were sampled. Internal 65Cu concentrations were measured in organisms. Effects were studied at effect concentrations of 5 and 50% effect, derived from previously performed chronic reproduction tests. Tested treatments contained only 65Cu, only azoxystrobin, a binary mixture of 65Cu-azoxystrobin and a control treatment. Adverse effects on the organism's Cu uptake- and elimination processes can have serious implications, not only due to enhanced toxicity if elimination is impaired but also because organisms could suffer copper deficiency in the long term when azoxystrobin is found to negatively affect metal uptake.

P34 | **Addressing Data Gaps for Safe and Sustainable by Design: A Substance-Function Dataset to Inform on Functional Alternatives for PMT/vPvM Chemicals**
Bianca Stadelmann, University of Amsterdam; Milo L. de Baat, University of Amsterdam; Antonia Praetorius, University of Amsterdam; Agnes Oomen, University of Amsterdam/ National Institute for Public Health and the Environment (RIVM); Annemarie van Wezel, University of Amsterdam

Persistent, mobile and toxic (PMT) as well as very persistent and very mobile (vPvM) pose a threat to drinking water quality and ecosystem health. PMT/vPvM substances do not readily degrade in the environment, can travel large distances and are difficult to remove with current water treatment technologies. PMT chemicals also exhibit toxicity to humans and biota. Hence, there is an urgent need to phase out these hazardous substances. An efficient process to identify functional, safe and sustainable by design (SSbD) alternatives is critical to achieving this. However, the scattered and rather generic nature of knowledge of the specific substance-use combinations for PMT/vPvM chemicals is a major barrier to assessing the suitability of potential alternative substances.
The research presented in this poster aims to address this challenge by manually compiling a high-quality dataset of substance-use combinations for a defined group of high-volume PMT/vPvM substances registered under REACH. Use categories, key functions and properties of each chemical fundamental to the uses are extracted from databases, such as the substances in preparations in Nordic countries (SPIN), as well as patent literature and systematically catalogued. This allows for additional evaluation of the necessity of P, M and T properties for individual uses and informs future SSbD assessments of potential functional alternatives. This approach, targeting specifically the high-volume PMT/vPvM chemicals, allows for an in-depth assessment of all relevant substance-use combinations, enabling better evaluations of alternatives at early data-poor stages of the innovation process.
This substance-use dataset provides a critical foundation for developing and applying quantitative structure-use relationships (QSURs) to support targeted design workflows. Additionally, this dataset can facilitate the development of machine learning and artificial intelligence approaches, assisting in the knowledge collection of additional substance-use combinations. Ultimately, the compiled dataset provides a basis for allowing innovators to rapidly identify high-potential functional SSbD alternatives for further evaluation, accelerating the transition away from hazardous chemicals.

P35 | **Mechanistic Modelling for Aquatic Mesocosms with Endectocide Treatments**
Chuxinyao Wang, Swiss Federal Institute of Aquatic Science and Technology (Eawag); Andreas Scheidegger, Swiss Federal Institute of Aquatic Science and Technology (Eawag); Lukas Kruckenfellner, Mesocosm GmbH; Louis Sollinger, Mesocosm GmbH; Peter Ebke, Mesocosm GmbH; Nele Schuwirth, Swiss Federal Institute of Aquatic Science and Technology (Eawag)

Mesocosms are controlled aquatic outdoor model ecosystems that are capable of investigating ecological responses to toxicants at the community and ecosystem levels. They provide an insight into the ecological interactions under ecotoxicological influences. Natural variability is represented, as well as the toxic effects of exposure to pesticides and their direct and indirect effects. In such condition, the collected data must be presented transparently and comprehensibly so that meaningful interpretations and conclusions can be made, e.g. for the risk assessment of chemicals.This research aims to develop mechanistic ecological models that support the interpretation of aquatic mesocosm observations by disentangling direct and indirect effects while providing uncertainty quantification. The study is structured into four phases: (1) constructing a baseline mechanistic model capturing key ecological processes and species interactions from pesticide-free (control) mesocosm replicates using Bayesian inference for calibration and uncertainty quantification; (2) expanding the model to incorporate pesticide treatments to evaluate both direct and indirect toxicant effects; (3) assessing model transferability by applying it to datasets across different years of mesocosm experiments; and (4) exploring the potential for improving model performance through iterative refinement based on findings from previous phases. We are currently working on the first phase. So far, a prototype model and the overall modelling framework have been developed. The next steps involve grouping species into functional categories and converting observational data into units suitable for model input. This foundational work will enable effective model calibration and serve as a basis for subsequent phases.
We will present the current state of work in the first phase. This includes a prototype model and the implementation of the overall modelling framework. The next steps involve testing different groupings of species into functional categories, collecting prior information for model parameters from literature, and developing probabilistic observation models for the calibration data. This foundational work will enable us to perform Bayesian model calibration and uncertainty quantification. Eventually, this study seeks to establish a feedback loop between mesocosm experiments and modeling. By enhancing our understanding of system dynamics and variability, we aim to enhance interpretation of experimental outcomes and refine experimental designs. Future applications will explore the sensitivity of various functional groups to a range of toxicant exposures, contributing to improved ecological risk assessment and guiding ecosystem management and regulatory decision-making.

P36 | **Predicting the Presence of Pyrethroids: Spatio-Temporal Distribution in Agricultural Environments in Germany**
Katharina Wifling, iES Landau; Jakob Wolfram, iES Landau

Pyrethroids are synthetic insecticides derived from natural pyrethrins that are now among the most commonly used pesticides in agriculture. Despite their relatively low application rates and fast degradation through hydrolysis, they are highly toxic to non-target organisms, particularly aquatic species. Their widespread use and hydrophobicity lead to pseudo-persistent behavior in the environment, especially in areas with frequent applications. Concerns regarding their neurotoxic, immunosuppressive, and potentially endocrine-disrupting effects have been raised, alongside evidence of bioaccumulation in both aquatic organisms and mammals. While pyrethroids are often assumed to be environmentally benign due to their low persistence, recent assessments of total applied pesticide toxicity in Germany indicate a significant increase in their ecological risk towards aquatic and soil organisms.

This study aims to develop a forecasting tool to predict when and where pyrethroids are likely to occur in the environment based on agricultural application patterns. Using data from the German Federal Office of Consumer Protection and Food Safety (BVL), we compiled information on authorized pyrethroid products, crop associations, and application periods. These data were integrated with high-resolution crop distribution maps to predict the expected spatio-temporal distribution of pyrethroids in agricultural landscapes in Germany. The resulting map identifies regions and timeframes with high likelihoods of pyrethroid presence, providing a valuable tool for targeted environmental sampling (e.g., soil, vegetation, insect). This approach can inform monitoring strategies and contribute to the refinement of pesticide risk assessments by aligning sampling events with periods of peak exposure potential.

5. New Approach Methodologies: Advancing Chemical Safety Assessment and Reducing Animal Harm

32 | **Proteomics-Based Evaluation of the Zebrafish PAC2 Cell Line as a Model to Study Molecular Mechanisms of Chemical Toxicity in Fish**

Mihai-Ovidiu Degeratu, Swiss Federal Institute of Aquatic Science and Technology (Eawag); Nikolai Huwa, Swiss Federal Institute of Aquatic Science and Technology (Eawag); Jessica Bertoli, Swiss Federal Institute of Aquatic Science and Technology (Eawag)/ETH Zürich; Marion Revel, Swiss Federal Institute of Aquatic Science and Technology (Eawag); René Schönenberger, Swiss Federal Institute of Aquatic Science and Technology (Eawag); Kristin Schirmer, Swiss Federal Institute of Aquatic Science and Technology (Eawag)/ETH Zürich; Colette vom Berg, Swiss Federal Institute of Aquatic Science and Technology (Eawag); Ksenia Groh, Swiss Federal Institute of Aquatic Science and Technology (Eawag)

Fish cell lines represent promising alternatives to traditional animal models for environmental risk assessment of chemicals, but their uptake into regulatory frameworks remains limited. Insufficient functional characterization of these in vitro models limits the confidence in their reliability and applicability for the toxicity assessment of chemicals. Here, molecular expression data could be used to deduce functional capacity and suitability for studying certain types of toxicity. Another key issue is the “black box” nature of the current tests. We argue that gaining a better understanding of chemical-induced molecular alterations could help improve the in vitro tests’ capacity to predict chronic toxicity effects.
My project aims to perform protein-focused studies seeking to address these research needs in selected zebrafish and rainbow trout cell lines. Here, I present the work done so far in the zebrafish (Danio rerio) embryonic cell line PAC2.
First, we characterized protein expression across the cell culture growth cycle, including early lag-phase, exponential and stationary phases. Global proteomics allowed measuring over 7200 proteins. Time-dependent differences in expression profiles revealed cellular changes across growth phases. Gene ontology analysis demonstrated a broad coverage of cellular processes, including toxicologically relevant protein groups, such as those involved in stress responses, or energy and lipid metabolism. Comparison with transcriptomics data obtained at two selected timepoints is currently ongoing and will be used to assess the added value of protein-level characterization.
Second, we set out to develop a zebrafish protein marker panel that could allow rapid insights into selected mechanisms of toxicity as well as early and late toxicity responses in the exposed cells, using targeted proteomics. For this, over 200 zebrafish proteins were selected for inclusion on the tentative panel based on a literature review. Global proteomics data showed that 140 candidate markers are expressed in unexposed PAC2 cells. This model is thus well-suited for testing the candidate panel using a set of reference chemicals, which is now ongoing.
In conclusion, our work demonstrates that proteomics can enable comprehensive exploration of molecular profiles and that the PAC2 cell line could become a valuable model for performing proteomics-enhanced chemical toxicity testing.

33 | **Towards Streamlined Environmental Persistence Assays for Trace Organic Contaminants: Findings from High-Throughput Method Optimization and Biodegradation Testing**

Chiel Kaal, University of Zürich/ Swiss Federal Institute of Aquatic Science and Technology; Sarah Partanen, Swiss Federal Institute of Aquatic Science and Technology/University of Zürich; Nicolas Mueller, Swiss Federal Institute of Aquatic Science and Technology; Kathrin Fenner, Swiss Federal Institute of Aquatic Science and Technology/University of Zürich

The 2022 European Commission framework entitled “Safe and Sustainable by Design Chemicals and Materials” emphasizes the need for hazard assessment of chemicals at an early stage of their design process. One such chemical hazard is environmental persistence, which is currently evaluated using time-intensive and resource-heavy methods. These methods are unsuitable for integration into in early stages of the chemical design process as they do not allow for the assessment of a wide range of chemical alternatives.
To overcome this issue, we developed a high-throughput method for experimentally evaluating chemical persistence using activated sludge as a microbial inoculum source. Performing the assay in activated sludge provides enough diverse and dense biomass to rapidly screen for pollutant biodegradation within 48 hours. In addition, results can be extrapolated to other relevant environmental compartments such as soil. Our approach combines a 24-well plate format with an eight-minute runtime liquid chromatography-based separation and high-resolution mass spectrometry for the high-throughput analysis of chemical degradation kinetics. Our experiments in 24-well plates yielded half-lives comparable to the outcomes of traditional large-volume biotransformation experiments. To further assess the method's throughput and applicability domain, we applied it to a mixture of 200 compounds—including pharmaceuticals and agrochemicals—and analysed their degradation and adsorption behaviour in the miniaturized setup compared to large volume reference data. The presented results will include comparisons of persistence between the large-volume and miniaturized formats for the 200 compounds, as well as an investigation into the impact of chemical mixtures and dissolved organic carbon (DOC) content on degradation behaviour. We hypothesize that minimal mixture effects will be observed due to the environmentally relevant spike concentrations and based on preliminary experiments with a smaller test set of 40 compounds. However, the DOC introduced by the solvent used to dissolve the standards may influence biotransformation rates, as these spikes significantly elevate the carbon content in the samples and provide an easily accessible carbon source for microorganisms. By developing a high-throughput screening method for measuring primary biotransformation, a first-tier assessment on the persistence of chemicals is made possible, which is

especially useful for early-stage chemical discovery pipelines.

34 | **Immune Cell-Based Bioassay Approaches for Examining the Immunotoxic Effects of Oil Sands Process Waters and Naphthenic Acids**
Sunanda Paul, University of Alberta; James L. Stafford, University of Alberta; Isaac Sánchez-Montes, University of Alberta; Mohamed Gamal El-Din, University of Alberta

Surface mining in the oil industry produce large amounts of wastewater called oil sands process water (OSPW). This water contains complex mixtures of organic and inorganic components that can cause acute and chronic toxicity to aquatic organisms and affect the environment. One of the main toxic components in OSPW is a group of organic compounds called naphthenic acids (NAs), which naturally occur in oil sands. Due to their complex chemical properties, NAs are difficult to remove from OSPW, which makes them a major environmental concern. To address this, recent research has focused on ways to remove NAs from OSPW, such as using advanced oxidation processes (AOPs). Additionally, studies have aimed to develop alternatives to animal models that are sensitive and cost-effective for evaluating water samples after treatment. In this study, we used mouse (RAW 264.7) and human (THP-1) macrophage cells, to investigate the immunotoxic potential of untreated and treated OSPW samples. Since NAs are a key contributor to OSPW toxicity, we also tested both commercially available NAs and NAs extracted from OSPW to evaluate their impact on immune cell responses. After 24 hours of exposure to OSPW or NAs, we measured the release of inflammatory cytokines from the cells and used synchronous fluorescence spectroscopy (SFS) and ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UPLC-QTof-MS) analyses to measure NAs in OSPW samples before and after treatment. Our findings show that untreated OSPW induced significantly higher levels of inflammatory cytokines compared to treated samples, aligning with the removal of NAs during treatment. We also observed that both commercial and extracted NAs independently triggered cytokine release, confirming their inflammatory potential. Notably, our research show that the innate sensing protein toll-like receptor 4 (TLR4) may act as a key sensor in the recognition of NAs, as we observed that cells specifically require TLR4 to respond to OSPW and NAs using both pharmacological blockers and TLR4-based reporter cell lines. In summary, this study highlights the potential of immune cell-based bioassays as sensitive tools for detecting inflammatory components in OSPW (such as NAs) and for evaluating the effectiveness of remediation strategies.

35 | **The Rainbow Trout Gill at Single-Cell Resolution: Cellular Diversity Underlying Toxicological Response**
Owen Trimming, Cardiff University; Peter Kille, Cardiff University

The surface area of the gill is 90% epithelial pavement cells, apparently simple for such a complex and multifunctional organ. Not only is it the site of gas exchange and waste excretion, but it also acts as the main point of xenobiotic absorption. Therefore, the minority of gill cells support a wide range of physiological processes essential to understanding ecotoxicology in fish. These cell populations include the ionocytes that control osmoregulation and chemical uptake, the chemoreceptors that sense and respond to environmental chemical changes, and the progenitors maintaining this vital barrier. In bulk RNA sequencing, the dominance of epithelial cell transcripts can obscure the contributions of rarer cell populations. Utilizing single cell RNA sequencing, we have painted a much more complete picture of the Rainbow Trout (Oncorhynchus mykiss) gill. Twenty separate cell populations were identified in the gill and blood. This highlights that even within well-characterized cell types, there exists a spectrum of functional states and specialized subpopulations. Genes involved in the response to a chemical can be disproportionately represented in specific cell populations. To address this challenge, single cell sequencing can be utilised to reveal otherwise overlooked Rainbow Trout vulnerability to pollutants. Furthermore, where single cell sequencing is not financially viable in larger studies, we have outlined gene markers that can be used when analysing RNAseq data to assess the presence of the different cell types in the sample. Given that Rainbow Trout are a commonly used model species and of great importance to aquaculture, the gill cell atlas provided could have a wide range of uses in ecotoxicology and fish health. For example, the variety of gill cells revealed in this work brings in to question the validity of transcriptomic analysis of in vitro gill models, particularly the commonly used RTgill-w1 cells which only represent epithelial cells. We propose that the markers identified here can be used as a quality control step when establishing multicellular primary cultures to examine the types of gill cells present. In doing so we hope to demonstrate what these cell cultures can and cannot represent when studying how the genome responds to xenobiotic exposure.

P37 | **Assessment of the In Vitro Test Guideline OECD 455 for Detecting Estrogenic Activity using Animal Free Conditions and Animal-Free Metabolization Systems**
Denise Horte, Goethe University Frankfurt; Inska Reichstein, Goethe-Universität Frankfurt; Carolin Bertold, Goethe-Universität Frankfurt; Christian Forberg, Goethe-Universität Frankfurt; Sarah Wohlmann, Goethe-Universität Frankfurt; Henner Hollert, Goethe-Universität Frankfurt/Fraunhofer Institute for Molecular Biology and Applied Ecology (FhG-IME)

Regarding cell-based in vitro assays, controversial animal-derived components like the fetal bovine serum (FBS) collected from non-anesthetized calf fetuses and the phenobarbital/ß naphtoflavone induced liver homogenate fraction (S9) collected from rats are frequently used. These components, used for promoting cell growth and for simulating biotransformation processes respectively, are known for interacting with test substances, being potentially contaminated by microorganisms and causing increased variability within the assays due to their undefined composition. To decrease animal cruelty and provide stable comparable

assay results efficient alternatives such as chemically defined matrices should be considered.
Contributing to this, the aim of this study is to develop the in vitro assay ERα CALUX® for detecting estrogenic activity towards animal-free conditions and the incorporation of animal-free metabolization systems. This will be established by evaluating the lowest amount of FBS needed for a valid ERα CALUX® test result applying the manufacturers validity criteria established for the standard, FBS containing procedure. After evaluating the outcome of this approach, the sensitivity of the optimized assay will be tested by using model substances and groundwater samples from the project "gwTriade", a project aiming to establish an integrated assessment of groundwater systems. Therefore, different scenarios will be compared regarding their performance and comparability based on the in vitro test guideline OECD 455: (1) The currently standardized FBS-containing medium (2) The chemically defined medium without FBS and (3) The chemically defined medium with the optimized amount of FBS from the improvement approach. Additionally, those approaches will be tested with the supplementation of animal-derived S9 and biotechnological, animal-free S9 (ewoS9).
At present, the addition of 0.1 % FBS to chemically defined medium for conducting a valid in vitro ERα CALUX® seems promising. If evaluated as reproducible in further experiments, this would result in the use of 98 % less FBS per assay plate contributing to a successful reduction in animal components following the 3R's principle of replacing, reducing and refining the use of animals in scientific research. Consequently, this could also contribute in achieving less variability within the assays, providing better reproducible and comparable results on estrogenic activity in environmental samples.

P38 | **Fish Cell Lines in Chemical Risk Assessment: Temperature and Species Sensitivity Influence**
Kehinde Olajide, Swiss Federal Institute of Aquatic Science and Technology (Eawag); Marco Franco, Swiss Federal Institute of Aquatic Science and Technology (Eawag); René Schönenberger, Swiss Federal Institute of Aquatic Science and Technology (Eawag); Ksenia Groh, Swiss Federal Institute of Aquatic Science and Technology (Eawag); Kristin Schirmer, Swiss Federal Institute of Aquatic Science and Technology (Eawag)/ETH Zurich

Environmental legislation mandates a risk assessment of chemicals before their authorization into the market to protect the environment from harmful effects. In aquatic ecosystems, short term (acute) and long term (chronic) toxicity tests are fundamental components in evaluating a chemical impact on survival and growth, and its potential to bioaccumulate in organisms. However, these studies are resource intensive and heavily rely on the sacrifice of live animals. More recently, alternative approaches have been developed for the reduction and replacement of animal experimentation including the use of fish cell lines. The RTgill-W1 cell line is an example of a fish cell line derived from rainbow trout (Oncorhynchus mykiss) that is gaining global relevance following its adoption by the Organization for Economic Cooperation and Development for determining acute fish toxicity (OECD TG 249). Fish cell line assays are also used to derive relevant information on bioaccumulation, and impact on fish growth.
To further advance the use of fish cell lines in environmental risk assessment, this research aims to explore the influence of non-chemical factors, like temperature, and inter-specific differences on chemical risk assessments. For this purpose, acute toxicity of three pesticides namely trifloxystrobin, tebuconazole and tembotrione are conducted according to the OECD TG 249 and at different temperature regime. In addition, a cell proliferation assay will be used to predict reduced fish growth, while the distribution of chemicals will be monitored within the cell culture system to assess chemical uptake, biotransformation and bioaccumulation using permanent rainbow trout cell lines from the gills (RTgill-W1) and liver (RTL-W1). Cell line-based assays will also be established using cell lines from other fish species to inform inter-species sensitivity. The generated cell line-based data will then be introduced into the framework of the Dynamic Energy Budget (DEB) model to infer the impact of chemicals on energy allocation in fish. Altogether, this study aims to improve the relevance and applicability of fish cell lines in chemical risk assessment.

P39 | **The Regulation of Essential Metal Toxicity: Assessing Mechanisms Driving Metal-Nutrient Interaction Responses**
Eleanor Phillips, University of Sheffield

Trace metals, such as copper, are essential for life but can become toxic in excess. Traditionally, ecotoxicology has focused on the dose-response of individual metals, often lacking mechanistic insights and overlooking mediating factors like nutrient availability. Using Drosophila melanogaster, a powerful genetic model, I investigate how essential metals interact with nutrients to alter toxicity thresholds. Preliminary findings suggest that dietary composition can impact an organism's ability to cope with copper toxicity during development. Additionally, manipulating a key metal-regulating transcription factor alters the response to copper toxicity under different protein diets, suggesting nutritional dependencies in metal regulation. By incorporating functional genetics into ecotoxicology, we can better understand how organisms handle metal exposure, predict species sensitivity to complex scenarios, and link molecular mechanisms to environmental impacts.

P40 | **Leveraging Genomics and Artificial Intelligence to Develop Predictive Pesticide Risk Assessment Frameworks for Wild Pollinators**
Chung Tsui, University of Exeter; Chris Bass, University of Exeter; Richard ffrench-Constant, University of Exeter; Angie Hayward, University of Exeter; Ralf Nauen, Bayer AG; Nicola Nadeau, University of Sheffield

Cytochrome P450s are a huge group of enzymes that exist in virtually all organisms. P450s are involved in both endogenous functions such as the regulation of hormones, and exogenous functions including xenobiotic detoxification. In bees, previous work has revealed that the CYP9Q-type subfamily of P450s in the CYP3 clan is involved in insecticide detoxification. In other bee

species, closely related P450s, collectively referred to as CYP9Q-type, play a similar role in insecticide detoxification. However, some bee species, such as Megachile rotundata, lacks these P450s and is more sensitive to insecticides, even if the insecticide is considered bee safe.

The current risk assessments rely heavily on data collected from a handful of model species; however, given that different insects have different susceptibility to insecticides, the robustness of this approach is highly questionable. This project aims to develop a pesticide risk assessment framework that exploits genomic information for beneficial insects to predict their sensitivity to pesticides. Not only can this framework screen for multiple species at a time but also reduce the use of insects in risk assessments by focusing on molecular methods. I am focusing on parasitoid wasps as recent advances in understanding of insecticide detoxification by P450s in bees serves as an excellent entry point. Parasitoid wasps branched off from the Aculeata, a subclade of Hymenoptera containing ants, bees, and stinging wasps, therefore, it is likely that the P450s in parasitoid wasps evolved from similar or ancestral P450s to those of bees.

Using the genomic data from the National Center for Biotechnology Information database, CYP9 P450s in parasitoid wasps were identified and extracted. The CYP9s of 212 Hymenopteran species were compiled into a dataset in Geneious including 5 species of bees as reference. A Maximum Likelihood phylogeny tree was run to locate the CYP9Q-type P450s in parasitoid wasps. None of the CYP9s in parasitoid wasps clade with CYP9Q-type P450s in bees. Instead, they clade within their own superfamily/family, except for CYP9P P450s, which is not known for detoxification. It is possible that CYP9Q-type P450s in parasitoid wasps were lost due to their life histories as parasitoid or that they have evolved their own CYP9 P450s that can detoxify insecticide that are very distantly related to CYP9Q-type P450s in bees. Protein expression is needed to explore the function of CYP9s in parasitoid wasps.

6. Applied Ecotoxicology, Including Life Cycle Assessment, Science to Policy & Regulation

P41 | **The Role of Spices in Modulating Polycyclic Aromatic Hydrocarbon Formation in Suya**
Favour Agboola, Kwara State University Malete

Polycyclic aromatic hydrocarbons (PAHs) in grilled meat (suya) are toxic compounds generated during the high temperature grilling of meat, particularly under traditional open-flame conditions. Suya, a widely consumed grilled meat in Nigeria, is often prepared using various spice blends that may influence the formation of PAHs. This study aims to investigate the effect of spices on PAH profiles by comparing three sample types: meat spiced before grilling, suya spiced after grilling, and unspiced suya. The goal is to determine whether the timing of spice application alters the occurrence or concentration of PAHs, especially high-molecular-weight variants associated with carcinogenic risks. To address this, suya samples will be collected from vendors in Malete, Kwara State, Nigeria. Standardized preparation and preservation techniques will be applied to ensure sample integrity. Analytical methods will include Soxhlet extraction and gas chromatography-mass spectrometry for PAH identification and quantification. The study will assess whether spices function as physical barriers, chemical modulators, or potential sources of alternative PAHs when exposed to heat. The preliminary expectations suggest that spicing before grilling may reduce harmful PAH formation, while spicing after grilling may not provide the same benefit. This research is expected to inform safe food practices and contribute to ongoing discussions on reducing dietary exposure to carcinogens from traditionally processed meat.

36 | **Reevaluating New and Existing Challenges That Early Career Researchers Face Across Regulatory Ecotoxicology**
Dylan Asbury, University of Sheffield; Lowenna Jones, University of Sheffield; Susanna Mölkänen, University of Sheffield; Rana Al-Jaibachi, University of Sheffield; Thomas Malpas, University of Sheffield; Niamh O'Connor, University of Sheffield; Hana Mayall, University of Sheffield; Harry Bond-Taylor, University of Sheffield; Grzegorz Sowa, University of Sheffield

A SETAC Pellston Workshop® on “Improving the Usability of Ecotoxicology in Regulatory Decision-Making” was held in August 2015. One outcome of the workshop was a publication, which highlighted the challenges ecotoxicologists of the future face in applying academic research to regulatory decision making. The authors propose that training students on issues of relevance and reliability will lead to improvements in the applicability of academic research for regulatory decision making. Whilst also set out the importance of cross-sector partnerships across industry, academia and government. As nearly a decade has passed since the initial workshop, we believe it is important to reevaluate previously identified challenges faced in applying academic research to regulatory decision-making (OBJ1), highlight challenges not previously identified (OBJ2), and assess the uptake of proposed training and skills (OBJ3). To obtain the necessary data a survey was developed and deployed online to gain understanding on the challenges early career researchers (ECRs) face in applying academic research to regulatory decision making, and the training and skills received throughout their career. In total, 52 ECRs responded to the survey. Just over a quarter of respondents (27%) consider themself to be part of a training network, however approximately half of all respondents state that they have received training to understand the regulatory context of their research (52%); ecotoxicology methodologies or guidelines (44%); and principles of quality assurance and good laboratory practice (54%). We explore why ECRs may or may not receive training and assess the extent to which they agree that it has improved the applicability of their research for regulatory decision making. We conclude that whilst some areas of training for ECRs have developed, some areas remain a challenge. These include aspects of interdisciplinary research, cross-sector partnerships, and government or industry participation.

37 | **Potential Indoor Sources of Aromatic Amines in Dust: Implications for Indoor and Environmental Exposure**
Özge Edebali, Masaryk University; Lisa Melymuk, Masaryk University; Marek Stiborek, Masaryk University; Anna Goellner, UFZ Helmholtz Centre for Environmental Research; Melis Muz, UFZ Helmholtz Centre for Environmental Research; Zdeněk Šimek, Masaryk University; Branislav Vrana, Masaryk University

Aromatic amines (AAs), such as aniline and N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD), are increasingly recognized as emerging contaminants with potential toxicological significance. Although AAs are known to be used in products such as dyes, pharmaceuticals, pesticides, and rubber materials, their presence and behavior in indoor environments are still not well understood. While some studies have reported their occurrence indoors, comprehensive data on sources, pathways, and exposure risks remain limited. This study contributes new data on the occurrence of AAs in five indoor environments by quantifying their concentrations in dust samples and discussing their relevance within existing regulatory frameworks.
A total of 42 AAs were detected across all indoor environments. Among the different environments, the smoking pub had the highest number of detected compounds (n = 35), followed closely by the smoking home (n = 34) and the restaurant kitchen (n = 32). In comparison, the hairdresser showed 20 detected AAs, while non-smoking homes had the lowest number, with 18. These findings suggest a trend of increased AA diversity in environments with tobacco smoke or cooking activities. In addition to direct exposure indoors, AAs can accumulate in textiles through contact with contaminated dust or air, and can subsequently be released to wastewater during laundering, potentially making their way to surface water bodies. This highlights the importance of understanding indoor sources not only from a human health perspective but also in terms of their broader environmental impact.
Identifying which indoor environments are associated with AAs can inform both exposure assessments and targeted mitigation strategies. Current regulations on

AAs primarily address workplace exposure and industrial emissions. There is a lack of source-based studies and specific guidelines for the general population, particularly for non-industrial indoor environments. Expanding research efforts to better understand indoor sources and transport mechanisms is essential for evaluating potential public health impacts and developing more comprehensive environmental policies.

38 | **Non-Standard Toxicity Endpoints: Regulatory Challenges for Integration in the Environmental Risk Assessment Framework for Plant Protection Products**
Sofia Sangiorgi, Health and Safety Executive (HSE) - Chemicals Regulation Division

Plant Protection Products (PPPs) are regulated according to assimilated Regulation (EU) 1107/2009 which includes specific data requirements for a range of non-target organisms. The regulation requires environmental risk assessment to protect non-target organisms from potentially harmful chemicals released into the environment and to ensure consistent regulatory decisions. The current framework relies on the assessment of toxicity data in line with standardised and validated study guidelines. This includes observations of effects on mortality, reproductive output, growth and development of model organisms following exposure to the chemical under assessment. In addition, investigation of other non-standard sub-lethal toxicity effects (e.g. behavioural, phytotoxic, and other morphological effects) has been attracting attention amongst stakeholders due to their sensitivity and potential ecological relevance in the context of environmental protection.Guidance documents driving the assessment in Great Britain typically do not contain methods for addressing the risk to non-target organisms based on non-standard sub-lethal effects, meaning that to date the use of such data in environmental hazard and risk assessments has been limited and its relevance questioned.In order to ensure the best possible risk assessments, barriers to integration of these non-standard endpoints in the current environmental risk assessment for plant protection products have been considered further. The barriers identified include linking sub-lethal effects observed in toxicity studies to specific protection goals, the suitability of currently available environmental risk assessment methodologies and the diversity in endpoints derived and study designs used. Common issues experienced with the inclusion of non-standard toxicity endpoints in regulatory submissions have been investigated as case studies. These include ensuring that the risk assessment for plants is sufficiently protective of phytotoxic effects observed in toxicity studies, and exploring challenges in linking effects observed in honeybee brood colony feeding studies to exposure quantified under realistic conditions of use. These case studies provide a regulatory perspective on opportunities to develop approaches for an integrated environmental risk assessment that includes the use of non-standard yet potentially ecotoxicologically relevant sub-lethal endpoints.

39 | **Challenges in Using Scientific Results in Regulatory Risk Assessment of Plant Protection Products**
Sonja Schaufelberger, RWTH Aachen University

As former employee at a national competent authority working with authorisation of PPP in the area of ecotoxicology, I would like to share some insights about the everyday work and what stands in the way of using current scientific knowledge in applied regulatory risk assessment (RA). Conference sessions like this one often focus on how to transfer new insights into regulations, but it is rarely talked about how the practical execution could look like. This is not a research project but rather a report from personal experience backed up with quotes from relevant sections of EU regulations and guidances. Some issues: 1) Legislation usually limits teh RA to the data submitted by the applicant with limited options to include other data. Companies are obliged to report new data which may affect the authorisation of their products, but there is little guidance on what constitutes "adverse data" and how to handle it, and no obligation for them to e.g. screen open literature for lower endpoints (EP). 2) Experiments with non-standard test protocols can bring new insights tailered to specific characteristics of a substance, but can't be compared to other data. Validity criteria are sometimes not reported, e.g. only nominal but no measured concentration. 3) Datasets on the a.s. are usually already more complete than for the PPP, but scientific experiments often performed with the a.s. Regulatory gaps where additional information would be interesting are mostly related to PPPs. Example situation: A product is more toxic for soil-living organisms than the a.s., which accumulates in soil over the years. PECsoil,accu is only modelled for the a.s. The product EP can only be used for RA in the first year because the environmental fate of co-formulants is unknown. However, information on co-formulants is confidential. 4) Tight deadlines mean that we usually don't have the time to look at anything else apart from the submitted dossier. Long queues of product applications in many countries, increasing the time pressure.
Suggestions for improving the situation: 1) Researchers: Design experiments as close as possible to OECD guidelines (when it makes sense). Consider testing formulated products instead of or in addition to pure active substances. 2) Industry: Take the opportunity of pre-submission meetings to improve the completeness and quality of your application, and reduce processing time at authorities. 3) More career switches between academia and authorities to exchange expertise.

7. Multiple Stressors in a Changing World

40 | **Insights Into the Toxicity and Subcellular Partitioning of Platinum (Pt) and Palladium (Pd) in Chironomus Riparius Under Mixture Exposures**
Alice Carle, Université du Québec à Montréal (UQAM); Marc Amyot, Université de Montréal; Maikel Rosabal, Université du Québec à Montréal

Most ecotoxicological studies are focused on the effects of individual metals and in water exposure, but only few are conducted for sediments considering mixture exposure. This is the case of platinum (Pt) and palladium (Pd) that are not very soluble, so they accumulate mainly in sediments, often found in mixtures. Classified as strategic minerals in Quebec, the use and release of these elements into the environment are increasing, but their effects on organisms remain little explored. No studies have assessed their impact on benthic organisms. To fill this gap, our research determines the effects and the subcellular distribution of the mixtures of Pd and Pt in Chironomus riparius, usually used in ecotoxicology to assess sediment quality. Our objectives are: (i) to assess the interaction and the resulting effect of mixture exposures of Pd and Pt at different concentrations, and (ii) to determine the subcellular distribution of these two elements applying a validated subcellular partitioning protocol. In the exposure experimental design, we fixed the concentration (µg.gsed-1 d.w.) of one metal (i.e., Pd LC20 = 47 ± 29 or Pt LC20 = 124 ± 19) and for the other, increasing concentrations were tested (including its LC10, LC20, LC50, LC60, LC70 values). Responses measured include survival and relative growth. The subcellular distribution of both metals is analyzed for exposure condition where metals are alone (at their LC20 values) as well as in mixtures. Metal quantification was performed using ICP-QQQ in each subcellular fraction. Our data show significant ($P < 0.05$) effects on survival measurements when Pt is fixed (LC20) and Pd concentration increases from LC20 to LC70. Similar results were found for the relative growth rate ($P < 0.05$) as Pt is stable at LC20 and Pd levels are exposed at LC50 as well as at LC70 values. The resulting effects are more than additive or less than additive, indicating a synergistic or antagonistic effect of Pt on Pd depending on the conditions tested. Subcellular analyses are in progress and will enable us to understand the metal-handling strategies used by Chironomus riparius to cope with both metals. We expect these two B metals will be found mainly in the detoxified fractions, mainly associated with metallothioneins. Furthermore, significant accumulation will be observed in sensitive compartments (e.g., mitochondria, other organelles) leading to toxic effects. This study will contribute to our understanding of the impact of these strategic elements, in particular their intracellular behaviour and their interaction in mixtures.

41 | **Assessing Impact of Pesticides on Benthic Diatoms in the Context of Multiple Stressors: A Mesocosm Approach**
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Protecting aquatic ecosystems from pesticide pollution is a critical regulatory task. Benthic diatoms, commonly used to indicate nutrient pollution, are known to exhibit variable species sensitivities to pesticides. Therefore, we assessed if they could also serve as bioindicators for pesticide pollution.
We conducted a laboratory study using flow-through chambers for biofilm colonisation in a closed, controlled system. We continuously exposed the biofilm to 44 treatments, consisting of combinations of 36 environmentally relevant pesticide levels and three environmental factors (i.e., light intensity, temperature, and nutrient levels) over a 23-day colonisation period. Following colonisation and exposure, we examined the diatom community composition and structure using both morphological and molecular methods.
The results showed that pesticides had no significant effect on the overall diatom community composition, as revealed by ordination techniques, while light and temperature had a significant impact. At the species level, the results indicated patterns of sensitivity or tolerance to pesticides, as well as to the other factors, as revealed by beta regression analysis. Community-level biofilm parameters (i.e., effective photosynthetic activity, chlorophyll-a content, ash-free dry weight, bacterial abundance) were not significantly affected, except by light, where an increase in light intensity had a significant positive impact on bacterial abundance. Both identification methods revealed discrepancies, with molecular approaches detecting morphologically challenging taxa and methodological biases affecting species abundances. Lastly, the Swiss Diatom Index (DI-CH), calculated from diatom samples using both identification techniques, showed weak correlations between the two methods.
Overall, our study highlights that while community-level responses to pesticides may be masked by confounding environmental drivers, species-level analyses can reveal differential sensitivities and demonstrate the complementarity of the two identification methods. These findings emphasize the need for multifactorial experimental designs that better represent realistic scenarios and help disentangle the combined effects of pesticides from other environmental stressors, ultimately supporting more ecologically relevant risk assessments.

42 | **Temperature Effects on Species Sensitivity Distributions Across Aquatic Taxa**
Lea Grenc, Radboud University Nijmegen; A. Jan Hendriks, Radboud University; Paul J. van den Brink, Wageningen University and Research; Leo Posthuma, Dutch National Institute for Public Health and the Environment

Rising global temperatures are changing aquatic ecosystems and altering the toxicity of contaminants, challenging the robustness of current environmental risk assessment frameworks. Species sensitivity distributions

(SSDs), used to derive protective concentration thresholds, are typically based on standard temperature conditions and may not account for thermally induced changes in species responses.
We compiled toxicity data for a broad range of chemicals tested across multiple temperatures and aquatic species. From this dataset, 22 chemicals with sufficient data were selected for further analysis. To assess within-species sensitivity patterns, we first examined the LC50–temperature relationships for individual species–chemical combinations. We then investigated the influence of temperature on SSDs for the selected chemicals across three aquatic taxonomic groups: algae, crustaceans, and fish.
For species–chemical combinations with multiple temperature data points, LC50 values showed no consistent temperature trends. Some species exhibited decreasing LC50s with increasing temperature within the observed temperature ranges, while others demonstrated little to no change.
The analysis shows that HC50 most often increases as temperature increases, suggesting reduced sensitivity under warmer conditions. However, several chemicals exhibit different trends, with the lowest or highest sensitivity occurring at intermediate temperatures. The differences can also be observed when it comes to different taxa. Taxonomic differences in SSD shifts appear to be primarily driven by the mode of action of a chemical. All data were based on short-term exposures between 24 and 96 hours, all classified as acute, and showed minimal variation in SSD shape or position related to exposure time. Life stage was rarely reported, limiting comparisons; in cases where it was, observed differences likely reflect taxon-specific characteristics rather than developmental stage.These results emphasise the importance of considering temperature as a modulator of chemical sensitivity in aquatic organisms. Our study contributes to ongoing efforts to integrate climate-relevant parameters into chemical risk assessments and underscores the potential value of temperature-adjusted SSDs in future regulatory frameworks.

43 | **Temporal Dynamics of Sorption/Desorption Interactions: Microplastics Modulate Phenanthrene Bioavailability and Toxicity in Parhyale hawaiensis**
Ibrahim Lawan, Heriot-Watt University; Mariana A. Dias; Cassiana C. Montagner; Lyndon, A. Robert; Theodore, B. Henry

Understanding the interactions between microplastics (MPs) and polycyclic aromatic hydrocarbons (PAHs) in aquatic environments is crucial due to their potential role in modulating pollutant bioavailability and consequent ecological impacts. However, the temporal dynamics of these interactions, particularly the mechanisms by which MPs influence the toxicity and bioavailability of PAHs like phenanthrene (Phe) over varying exposure periods, remain underexplored. This study used adult Parhyale hawaiensis (2-3 months old) as a model organism to investigate these dynamics through short-term (96 h) and long-term (21 d) exposure experiments with three MP types—polyethylene terephthalate (PET), polyethylene (PE), and polyamide (PA). Short-term exposure revealed significant mitigation of Phe-induced mortality by MPs, as indicated by higher LC50 values and 69-75% sorption rates. However, long-term exposure showed no significant differences in mortality, growth, or foraging behaviour between Phe-only and Phe-MP treatments, suggesting possible desorption of Phe from MPs. A confirmatory 24-hour test, conducted after ageing the exposure media for 21 days, revealed that MPs continued to mitigate Phe toxicity, in contrast to the earlier long-term findings. This suggests that MP-sorbed Phe likely settled in the initial 21-day study, thereby increasing its accessibility to the bottom-dwelling P. hawaiensis. These findings emphasise the dynamic nature of MP-Phe interactions and highlight the importance of exposure duration and organism behaviour in assessing the environmental impact of MP-bound contaminants. Comprehensive assessments considering both short- and long-term scenarios are essential for understanding the fate and effects of these pollutants in aquatic ecosystems.

44 | **Investigating the Allergenic Potential of Different Pollen Species and Their Chemical Modifications on Exposure to Air Pollutants**
Dimple Pathania, Deakin University/Coventry University; Svetlana Stevanovic, Deakin University; Ivan Kourtchev, Centre for Agroecology, Water and Resilience (CAWR)/Coventry University; Matthew McKenzie, Deakin University; Anna Bogush, Centre for Agroecology, Water and Resilience (CAWR)/Coventry University

Airborne pollen may lead to severe allergies such as asthma, pollinosis, rhinitis, and lung inflammation. Climate change induced extreme weather occurrences, fluctuations in temperature and relative humidity, can influence the transport, dispersion and interaction of pollen allergens and air pollutants (Tran et al. 2023). In an urban setting, presence of pollutants can influence the allergenic potential of pollens via chemical modifications of allergenic proteins and thereby altering their immune response. The overall aims of this work are to (1) to assess the allergenic potential of grass, pine and acacia pollen species by assessing cell viability and oxidative stress in lung cells (2) to investigate the protein modifications in intact and ruptured grass and birch pollen mixture upon exposure to air pollutants.
In this work, cell viability was determined by MTT assay and reactive oxygen species (ROS) measurements were conducted using 2′,7′- dichlorodihydrofluorescein diacetate (H2DCFDA) probe. Pollen are assessed using liquid chromatography mass spectrometry (LC-MS) to determine the chemical protein modifications occurring in ‘exposed’ and ‘non-exposed’ pollens to anthropogenic pollutants. This will be achieved using a non-targeted proteomics workflow, allowing identification of the pollen proteome and any modifications following exposure to pollutants.
Cell viability results showed a varied concentration dependent response among all species with lowest viability for pine pollen followed by grass pollen. Rate of ROS production was highest for grass pollen and increased in a concentration dependant manner. Allergenicity analysis of grass pollen extract suggested increasing group 5 grass allergen Ph1 p 5 allergen concentration with increasing pollen extract concentration. Additionally, the impact of anthropogenic pollutants on the protein modifications in intact and ruptured pollen will be discussed. It is hypothesised that

with rising levels of atmospheric oxidants, pollen proteins can undergo modifications (oxidation, nitration, cross linking) and the formation of resulting complex could be the biomarker for oxidative stress.
References
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45 | **Bioaccumulation Studies With Aquatic Invertebrates – Exploring the Role of Receptor Binding and Temperature**
Johannes Raths, Swiss Federal Institute of Aquatic Science and Technology (Eawag); Linda Schinz, Swiss Federal Institute of Aquatic Science and Technology (Eawag); Juliane Hollender, Swiss Federal Institute of Aquatic Science and Technology (Eawag)

In order to promote animal welfare in bioaccumulation science, aquatic invertebrates provide a promising alternative to animal studies using fish. For this purpose, the OECD Test Guideline 321, which uses Hyalella azteca for bioaccumulation studies, has recently been adopted. Beyond animal welfare, these experiments also reduce the time and resources required, allowing for higher throughput. However, some difficulties arise in these test systems with compounds that do not follow simple one-compartment kinetics, such as neonicotinoids, which bind with very high affinity to the nicotinic acetylcholine receptor (nAChR).
Toxicokinetic experiments with the neonicotinoid thiacloprid were conducted using two amphipod model species, Hyalella azteca and Gammarus pulex, under different temperature scenarios (6–21°C) or exposure concentrations (ranging from ng/L to mg/L; only for G. pulex). Samples were extracted and analyzed using an online-SPE system coupled with LC-HRMS/MS. A recently developed multicompartment toxicokinetic model for G. pulex was applied to determine toxicokinetic parameters. This model includes a structure compartment with free thiacloprid and a second compartment accounting for the receptor-bound fraction. The resulting parameters were compared with those obtained using the conventional one-compartment approach.
Applying the conventional one-compartment model resulted in poor fits for the temperature-dependent experiments. In contrast, the new two-compartment approach provided good fits and even allowed for the application of basic physical chemistry theory (Arrhenius Equation) to generalize the temperature effect on toxicokinetic parameters. Furthermore, the one-compartment model showed an increase of the bioconcentration factor (BCF) with decreasing exposure concentrations - even exceeding regulatory thresholds at 50 ng/L. The receptor-model demonstrated that this concentration dependence was an artifact of the simplified approach and revealed a first compartment with a very low BCF, but a second, elimination-resistant receptor-bound compartment.
These studies demonstrate that an approach accounting for receptor binding can improve the interpretation of toxicokinetic data. Furthermore, this approach may be more relevant for evaluating the toxicological significance of tissue concentrations. It remains to be discussed how regulatory studies can benefit from these new insights and how they could be implemented into future regulatory frameworks.

P42 | **Urban Forests as Hotspots for Novel Insect-Host Interactions: Alder Leaf Beetle Expansion onto Birch**
Safia El-Amiri, University of Sheffield; Holly Croft, University of Sheffield; Jill Edmondson, University of Sheffield; Stuart Campbell, University of Sheffield

Urban forests (UF) provide multiple ecosystem services, shaping the health of cities by improving air quality, regulating temperature, supporting biodiversity, and enhancing human well-being. However, these trees are increasingly exposed to multiple biotic and abiotic stressors, including air and soil pollution, rising temperatures associated with the urban island heat effect, drought, and pathogen and pest attack. Trees subjected to multiple stressors may have reduced capacity to allocate resources to defence, increasing their vulnerability to herbivory, and facilitating the emergence of new pest-tree associations that could threaten UF. Here, we report an apparent host range expansion by the alder leaf beetle (ALB) in urban environments, and assess the role of urban environmental conditions and plant traits on host use by ALB. Surveys of four commonly-planted young urban street tree species, including Quercus robur (English oak), Tilia cordata (small-leaved lime), Betula ermanii (Erman's birch), and Liquidambar styraciflua (American sweetgum) were coupled with no-choice feeding bioassays of adult and larval ALB to evaluate their potential suitability as alternative hosts for ALB. Preliminary results show that larvae feed on a wider range of hosts than adults, with significant differences in herbivory among tree species. Betula ermanii sustained the highest damage, indicating its potential as an alternative host. Assessment of foliar traits suggested that specific leaf area (SLA), a key leaf phenotype linked to abiotic stress tolerance, was positively correlated with larval feeding on birch leaves, suggesting an indirect link between urban tree abiotic stress and attack by a novel insect herbivore. Future work will investigate how abundance of Alnus spp. (the primary host of ALB) and urban stressors, such as NO2 exposure, affect the distribution and feeding intensity of ALB on birch. Findings highlight the role of urban environments in shaping novel insect-host interactions. Understanding these dynamics is key to managing pest pressures and supporting resilient UF in the face of environmental change.

P43 | **Beyond the Individual: Modelling the Effects of Contamination on Food Web Structure and Invasibility**
Laura Landon Blake, University of Sheffield

Global ecosystems face increasing threats from chemical contaminants and the spread of invasive species, with their consequences spanning multiple ecological scales - from individual physiology to broad community-level changes that can disrupt fundamental ecosystem functions. While the individual impacts of these stressors

are well recognised, their combined effects remain critically under-explored, hindering our ability to forecast and manage their pervasive effects on our ecosystems.To date, research has largely examined contaminant effects at the organismal level, revealing disruptions across sensory, cognitive, behavioural, reproductive, and metabolic pathways. These individual-level effects can propagate through food webs, altering species interactions and community dynamics. Conversely, the study of biological invasions has predominantly focused on community-level impacts, where their introduction can alter the flow of energy by establishing novel interactions, modifying resource availability, and mediating competitive dynamics. Despite this, the mechanisms through which contaminants modulate invasion dynamics, particularly by altering the structure of the recipient community, represent a significant gap in our understanding. To address this knowledge gap, my research investigates the role of chemical contamination and biological invasion in altering community composition, network structure, and metabolic function through models of food web biomass dynamics. Using a bio-energetic framework, this approach bridges the traditional divide between individual-focused contaminant studies and community-level invasion biology by examining how chemical contamination mediates food web structure and subsequently alters its susceptibility to invasions (i.e., invasibility). The study focuses on developing a predictive model that incorporates the effects of contamination on fundamental metabolic processes, such as growth and species trophic interactions (such as feeding rates and handling times), to determine how these contaminant-driven alterations to the recipient community ultimately influence food web invasibility. Utilising these network studies will allow us to predict emergent community effects and facilitate the development of comprehensive models to inform effective management strategies for ecosystems facing multiple anthropogenic stressors, contributing to the preservation of biodiversity and ecosystem function.

P44 | **Integrating Transcriptomic Points of Departure (tPODs) with Bio- and Chemical Analyses for Hazard Assessment of Road Runoff in Zebrafish**

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Recent studies have demonstrated severe ecological impacts of road runoff and tire wear particles, such as the Urban Runoff Mortality Syndrome in coho salmon along the Washington coast. Despite such findings, little is known about the environmental impacts in other regions, particularly in Europe. The present study, part of the RoadTox project, addresses this gap by integrating bioanalytical, chemical, and molecular methods to investigate the effects of road runoff on zebrafish larvae and derive transcriptomic Points of Departure (tPODs): Three consecutive runoff samples were collected from the BAB4 motorway in winter 2022, and zebrafish embryos were then exposed to total and particle-free runoff fractions. Our analyses included acute toxicity, enzymatic biomarkers, neurotoxic endpoints, and whole transcriptome analysis using Illumina sequencing. tPODs were then calculated of the obtained transcript profiles based on benchmark concentrations of individual genes for which concentration-response models could be fitted. Acute toxicity varied highly among the three samples, with EC10 thresholds ranging from 2.3% to 28% for total samples and 9.8% to 45% for filtered samples, confirming a strong contribution of particulate matter. Additional effects were observed on spontaneous tail coiling, light-dark transition behaviour, and EROD activity differing in intensity between samples and fractions. On the transcriptome level, an increasing number of transcripts were dysregulated with increasing concentrations of all three runoff samples. Preliminary tPOD results showed effect thresholds ranging from one-quarter of the EC10 concentration to nearly the EC10 value, within the single-digit percentage range of road runoff. The upregulation of cyp1A, linked to dioxin-like activity and oxidative stress, was consistent across all samples, matching previous observations of cardiovascular teratogenic effects and enzyme biomarker data. Further regulated genes (e.g., cyb5a, alox5b.2, cbr1l, sult6b1) were associated with oxidative stress and xenobiotic metabolism, linking to measured PAHs and benzothiazoles as likely toxicity drivers. The results of this study, funded by the Ministry for Environment, Agriculture, Conservation, and Consumer Protection of North Rhine-Westphalia (MULNV), Germany, demonstrate that road runoff affects gene expression at low concentrations and provide a foundation for identifying toxicity drivers through molecular pathway analyses.

P45 | **Evaluating Osmia bicornis as a Model Organism in Ecological Risk Assessment: A Comparison to Other Pollinator Species**

Dominika Twaróg, Institute of Nature Conservation Polish Academy of Sciences; Grzegorz S. Sowa, Institute of Environmental Sciences, Jagiellonian University; Agnieszka J. Bednarska, Institute of Nature Conservation Polish Academy of Sciences

The global decline in arthropod abundance, diversity, and biomass observed in recent years is largely driven by various human activities such as agricultural intensification, in particular widespread use of pesticides. Among pesticides, especially insecticides should draw our attention, as by targeting the nervous systems, they act not only on pest insects but are dangerous to many beneficial insects, including pollinators.

Current regulations concerning plant protection products consider only the well-being of the honeybee (Apis mellifera), but different pollinator species exhibit varying sensitivities to different substances. Thus, there is an urgent need for including mandatory testing on other pollinators, such as solitary bees, bumblebees, butterflies or hoverflies. In this study we assessed sensitivity of the red mason bee Osmia bicornis towards two insecticides with different modes of action (dimethoate and flupyradifurone) by determining the acute LD_{50} value (lethal dose for 50% of individuals) after both topical and oral exposure of bees.
The LD_{50} values for dimethoate and flupyradifurone, determined 48 hours after exposure to a single dose of each insecticide, were 0,62 and 78,8 µg/individual for topical application, and 0,459 and 1,93 µg/individual for oral exposure, respectively. Dimethoate was found to be much more toxic than flupyradifurone via both exposure routes and both insecticides exhibited greater toxicity in oral exposure. The data for Osmia bicornis will be compared with other pollinator species for their sensitivity toward studied insecticides, to predict intrinsic sensitivity across species and construct species sensitivity distributions (SSDs) for pollinators in the future.
This research was conducted as part of the EU project PollinERA (HORIZON-RIA No. 101135005)

P46 | **Toxic Heatwave: Chemical Pre-Exposure Alters Springtail Survival in Rising Extreme Temperatures**
Micha Wehlri, Swiss Federal Institute of Aquatic Science and Technology (Eawag); Jian Ge, Aarhus University; Stine Slotsbo, Aarhus University; Martin Holmstrup, Aarhus University

Global climate change is increasing the frequency and intensity of heat waves, posing a significant threat to ectothermic organisms. Concurrently, chemical pollution, including heavy metals and pesticides, remains a pervasive environmental stressor. This study investigates the effects of sublethal copper and fluazinam exposure on the thermal tolerance of the soil22 dwelling springtail, Folsomia candida. Using a Thermal Death Time (TDT) framework, we assessed how pre-exposure to these toxicants at two acclimation temperatures (20 °C and 24 °C) influenced survival under heat stress. Our findings indicate that toxicant exposure reduced heat tolerance at moderately high temperatures (32.5 °C) but had negligible effects at extreme temperatures (37 °C). Acclimation at 24 °C mitigated the negative effects of both toxicants, suggesting an enhanced capacity for cellular homeostasis under warm conditions. Additionally, soil type influenced thermal tolerance, highlighting the importance of environmental context in multiple stressor interactions. These findings highlight the need to integrate realistic thermal exposure scenarios in ecotoxicological assessments to improve predictions of organismal vulnerability under climate change.

8. Exploring the Role of Microplastics as Environmental Contaminants

46 | **Multifaceted Effects of Microplastics on Soil-Plant Systems: Exploring the Role of Particle Type and Plant Species**

Zhangling Chen, University of Leeds; Laura J. Carter, University of Leeds; Steven A. Banwart, University of Leeds; Devlina Das Pramanik, University of Leeds/Amity Institute of Biotechnology, Amity University; Paul Kay, University of Leeds

Microplastics (MPs) have emerged as a global environmental concern, yet their impact on terrestrial environments, particularly agricultural soils, remains underexplored. Due to intensive farming, agricultural soils may serve as significant sinks for MPs. This study investigated the effects of different types of MPs—polyester (PES) microfibers, polyethylene terephthalate (PET) microfragments, and polystyrene (PS) microspheres—on soil properties and radish growth. At the same time, a complementary experiment examined the impact of PES microfibers on the growth of lettuce and Chinese cabbage. Through both horizontal and vertical comparisons, it comprehensively evaluated the interactions between plastic particles and plant species in soil-plant systems. The results showed that PES microfibers significantly affected soil bulk density, with effects varying based on planting conditions ($p < 0.01$). PET microfragments and PS microspheres reduced the proportion of small soil macroaggregates under radish cultivation ($p < 0.01$). Additionally, PS microspheres significantly altered the total organic carbon stock in radish-growing soil, potentially affecting the microclimate ($p < 0.01$). Interestingly, PES microfibers promoted lettuce seed germination and significantly enhanced the root biomass of Chinese cabbage ($p < 0.05$). Overall, the environmental effects of MP exposure varied depending on particle type and plant species, indicating that MPs may not always exert negative impacts on soil–plant systems and could potentially exhibit context-dependent promoting effects in certain scenarios. Given the important role of soil-plant systems in terrestrial ecosystems and their direct connection to food safety, human health, and global change, further research should explore both the positive and negative impacts of MPs on agricultural practices.

47 | **Sustainable Cellulase Biosynthesis Valorizing Post-consumer Textile Waste**

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Textile waste has posed a serious environmental challenge globally. This created a need to explore the valorization of textile waste into value-added products. Therefore, in this study, different compositions of textile waste i.e. 100% cotton, 80% cotton + 20% PET-polyethylene terephthalate, 60% cotton + 40% PET, 50% cotton + 50% PET and 100 % PET were analyzed for their potential as substrate in submerged fermentation to produce cellulase using Trichoderma reesei. The experiment was conducted in 250 ml conical flask with 50 ml of fermentation media in each, having 0.5g of textile waste as substrate. Each flask was inoculated with 0.1 ml of T. reesei spore suspension and incubated for 72 days in a shaking incubator at 30°C and 180 rpm. After 72 hours of the fermentation process, the maximum cellulase activity of (107.79/91.34 filter paper unit per gram – FPU/g) was observed using 100% cotton and 100% PET respectively and the least cellulase activity of (59.53 FPU/g) was observed in 50% cotton + 50% PET. The remaining textile waste substrate in the flasks were hydrolyzed and separated into cotton and PET fibers. The use of textile waste to produce cellulase for the separation and recycling of cotton and PET into fibres will help in achieving the concept of integrating biorefinery and bioeconomy for a clean environment.KEY WORDS: Clean environment, Valorizing, Submerged Fermentation, Biorefinery, Bioeconomy.

48 | **Breakdown of Plastic Waste Into Microplastics During an Industrial Composting: A Case Study From a Biowaste Facility**

Noora Risku, University of Jyväskylä; Olli Dahl, Aalto University; Hanna Vanhanen, Aalto University; Katja Pulkkinen, University of Jyväskylä; Jaakko Litmanen, University of Jyväskylä; Cyril Rigaud, University of Jyväskylä; Sami Taipale, University of Jyväskylä

Microplastic pollution is an increasing environmental concern, and further research into its sources is urgently needed. One potential pathway for microplastics to enter agricultural lands is the use of compost-based soil amendments or recycled organic fertilizer. While techniques exist to remove visible plastics from biowaste, microplastics present a hidden challenge. EU fertilizer regulations only account for particles ≥ 2 mm, excluding smaller ones. Research on plastics in biowaste management systems is limited. Our study investigated plastic fragmentation into microplastics and the role of microbial communities in plastic degradation. Samples were collected before sanitation, after tunnel composting, after outdoor maturation, and from a compost-based fertilizer, focusing on particles > 20 µm using Raman spectroscopy. Microbial community analysis was conducted using 16S rRNA sequencing and phospholipid fatty acid analysis. We observed a significant increase in microplastic particle counts and a decrease in size throughout composting. Mature compost contained an average of 944 ± 586 particles/g of dry weight (<0.25 mm), primarily polyethylene terephthalate, with plastics accounting for up to 0.25 % of dry weight. Only 7 ± 2 bigger plastic particles (>0.5 mm) were found from all samples. Subtle changes were observed in microbial communities during the composting process, predominantly among fungal communities, while Firmicutes remained the most abundant bacterial phylum in all samples. Our results suggest that plastics are fragmented into smaller particles during the industrial composting process and are not efficiently decomposed by microbes during the process.

49 | **Understanding the Impacts of UV-Weathering and Plastic Additives on Microplastic Toxicity to Ammonia-Oxidizing Bacteria**
Mara Walters, Virginia Institute of Marine Science; Bongkeun Song, Virginia Institute of Marine Science; Meredith Seeley, Virginia Institute of Marine Science; Hyo-Bang Moon, Hanyang University at Ansan; Sori Mok, Hanyang University

Nitrification is a key nitrogen cycle process in various environments, essential for nutrient bioavailability and denitrification. Recent studies have shown that some microplastics can affect the nitrification activities of sediment- and wastewater-associated microbial communities. However, it remains unclear to what degree variable chemical factors of microplastics in the environment, including plastic additive composition and degree of UV photooxidation, drive these effects. To investigate these questions, we conducted an exposure experiment with the model ammonia-oxidizing bacterium Nitrosospira sp. AV. Untreated or UV-weathered microplastics composed of polyethylene (PE), polylactic acid (PLA), polyurethane foam (PUF), or polyvinyl chloride (PVC) were added to cultures at a concentration of 1 mg/mL. Among polymer types, only unweathered PVC inhibited the nitrification activity of Nitrosospira sp. AV after four days. Interestingly, no inhibition was observed with UV-weathered PVC. To identify possible chemical differences driving these effects, we utilized pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) as well as nonpolar solvent extraction followed by gas chromatography coupled to quadrupole time-of-flight mass spectrometry (GC-QTOF-MS) to characterize the additive composition of the microplastics before and after UV-weathering. Both mass spectrometry analyses revealed changes in the concentrations of several plastic additive chemicals. Furthermore, we investigated the toxicity of one additive of interest, which was detected in the unweathered PVC microplastics but absent from the UV-weathered PVC microplastics. Our results indicated that this additive may have driven the nitrification inhibition. Overall, this work provides clear evidence of the potential for microplastics and their additives to negatively impact nitrogen-cycling microbes. Furthermore, this study provides an example of how strategic analyses can pinpoint drivers of effect—a success story towards identifying practical mitigation strategies for impacts of plastics on the environment.

P47 | **Assessing the Microplastic Contribution From Sewage Sludge to Agricultural Soils With Regular Sludge Application**
Thilakshani Atugoda, University of Exeter; Ceri Lewis, University of Exeter; Mark E. Hodson, University of York; Vinuela Ruth, South West Water; Bob Barnes, Environment Agency; Richard Brazier, University of Exeter

The transition to sustainable agriculture emphasises the adoption of organic alternatives, such as biosolids, in place of inorganic fertilisers that contribute to the circular economy. Biosolids are widely utilised in the United Kingdom as a fertiliser for replenishing organic matter in agricultural soils. However, their application has been identified as a potentially significant pathway for microplastic (MP) contamination in terrestrial environments. This study aims to evaluate the presence and concentration of microplastics in soil obtained from farmland soils in Southwest England, which have records of biosolid application history. Soil from agricultural land that has not had biosolid for a prolonged time, but has an otherwise similar land use history was used as controls for identifying non-sludge-derived microplastics. The collected soil samples were digested using Fenton's reagent, and a series of density separations using saturated ZnCl2 were carried out to extract microplastics from the soil matrix. The biosolid amended soil accounted for 80-95% of the MP fibres and 6-20% of the MP fragments recovered. The average concentration of microplastics was 4,465 particles per kilogram of dry weight of soil, although there was significant variability across different fields, with values ranging from 1,200 to 11,400 particles per kilogram of dry weight. This variation highlights the complexity of microplastic contamination in soils affected by biosolid application. Additionally, soil samples were analysed for other properties such as water-holding capacity, pH, organic matter, NH3, NO-3, NO-2, and PO43-. Organic matter was measured using the loss on ignition method, and nutrient analysis was performed using a Skalar Bluvision discrete analyser. The average organic matter, NH3, NO-2 and NO-3 content for biosolid applied fields were 10.38 ± 1.18 %, 1.11 ± 0.48 mg/kg, 0.05 ± 0.02 mg/kg, and 48.57 ± 22.30 mg/kg and for control fields were 11.83 ± 1.32 %, 1.45 ± 0.06 mg/kg, 0.08 ± 0.07 mg/kg, and 36.60 ± 15.47 mg/kg respectively. The higher concentrations of fibres in soil primarily come from synthetic textiles in sewage, and their tendency to entangle in soil aggregates contributes to their persistence. Aside from biosolid application, soil properties varied across the fields due to factors such as soil type, complexity of the matrix, geographical distribution, and land use.

P48 | **Tracking Microplastics in the Marine Environment: Methodological Obstacles and Advances**
Janika Reineccius, Leibniz Institute for Baltic Sea Research Warnemünde; Joanna J. Waniek, Leibniz Institute for Baltic Sea Research

Widespread microplastic pollution in marine environments remains a global concern, primarily due to persistent challenges in identifying their sources, fates, and quantifying their overall distribution. These difficulties arise from the high versatility and ubiquitous use of plastics, combined with the limited comparability between studies due to the absence of standardized methodologies.
The lack of standardized detection techniques for marine microplastics often results in inconsistent inclusion of microplastic particle size ranges, differing types of included polymer types, or untraceable false-positive or negative microplastic detections, e.g., due to divergent evaluations of manually screened polymer spectra. Recalculations of reported microplastic abundances, accounting for some of these inconsistencies, indicate that previous studies may have significantly underestimated concentrations by up to a factor of 15, in which untraceable inaccuracies could not even be considered.

One of the most problematic error assessments is the subjectivity, especially arising from visual identification methods, strongly dependent on the sample processor. Therefore, the micro-Fourier-transform infrared spectroscopy (μFTIR) is largely used for microplastic detection, which enables rapid and accurate identification of various polymer types. While several software solutions offer automated data analysis, discrepancies between algorithms and their limitations in providing consistent results often necessitate manual verification of spectral data. However, not all researchers possess the expertise to reliably assess polymer spectra.
To address this gap and support the development of standardized protocols for MP detection, this work proposes the first systematic guideline for the evaluation of microplastic spectra by highlighting essential and polymer characteristic peaks vs. less important or environmental-related variable peaks with the potential to mislead standardly used evaluation algorithms.

P49 | **Toward Standardized Microplastic Uptake Assessments in Bivalves: Experimental Trials in Exposure System Design and Particle Quantification**
Venecia van Balla, Cape Peninsula University of Technology (CPUT); Jaime Leigh Johnson, Cape Peninsula University of Technology; Conrad Sparks, Centre for Sustainable Oceans, Cape Peninsula University of Technology

In the face of growing global concerns over the ecological impacts of microplastics (MPs), the development of reliable and standardized laboratory exposure methods have become increasingly essential. This preliminary study aimed to optimize experimental conditions for MPs exposure by assessing quantification methods of lab-made MP particles, its uptake pathways in bivalves, and system retention efficiency. The experiments assessed different aeration systems to ensure adequate particle suspension and to maintain overall water quality. Aeration was tested by comparing the flow rate between air stones and pipettes, while water quality parameters were monitored over several days using a portable testing kit and a bench meter. Key parameters measured included temperature, salinity, pH, turbidity, nitrates, nitrites, ammonia, copper, and phosphates. These measurements were taken to safeguard the health and survival of the test organisms. Two MP quantification methods were investigated, namely, mass-based (g) and a volume-based method using Nile Red staining with Sedgewick Rafter chamber analysis. To investigate the dynamics of uptake, the exposure of MPs was examined in dead mussels to understand passive deposition. Additionally, the system retention of the MPs particles was monitored over time to account for the efficiency of exposure and to determine potential losses due to settling, adhesion, or clumping. The pipette method resulted in a higher retention rate of 93.33% as opposed to 84.17% of fragments from the air stone experiment. Retention experiments suggest that low flow bubbling via the pipette method maintained homogenous distribution of particles and system stability, while vigorous bubbling from the air stone disrupted stability and water quality. Comparison of quantification methods indicated that the mass-based method produced inconsistent weight measurements, while the volume-based analysis demonstrated greater sensitivity and consistency for detecting low plastic particle concentrations. Uptake pathways experiments using dead mussels revealed that dead mussels cannot actively uptake MP particles, but they may act as negative controls for assessing passive deposition. These findings will support protocol refinement for live mussel exposure experiments, enhance standardization across studies, and improve understanding of MP interactions with bivalves, supporting the development of environmental policy and regulation.

9. Analytical Challenges in Environmental Sciences

50 | **Assessment of Physico-Chemical and Microbial Variability in Poultry Litter and the Impact on Veterinary Medicine and Feed Additive Degradation**
Bethany Adams, University of Leeds; Paul Kay, University of Leeds; Chris Sinclair, Bayer; Edward Haynes, Fera Science Ltd; Philip Rooney, Smithers; Victoria Pratt, Fera Science Ltd; Laura Carter, University of Leeds

The potential environmental impact of veterinary medicines and feed additives is assessed through an environmental risk assessment as part of the regulatory process. Simulating manure degradation can be used as an approach to refine environmental exposure and currently the guidance provided by the European Medicines Agency in 2016 only requires testing in one source of manure per animal type. Most of the research exploring degradation of veterinary medicines has been conducted on pig slurry or cattle manure, leading to an OECD guideline published in 2022 specific to these 'liquid' manures. In comparison, poultry litter is relatively understudied. The variability of poultry litter properties and the potential effect of this variability on the rate of degradation of veterinary medicines is relatively unknown. Subsequently, the current guidance on the number and type of litters to be used may not accurately evaluate the true environmental risk posed by these compounds. A series of experiments have been designed to explore the impact of poultry litter collected from different farms on the degradation of five veterinary antibiotics: tiamulin, trimethoprim, sulfadiazine, monensin and salinomycin. Three litter types that differed in the type of chickens reared and the type of bedding material were collected from three separate farms and used in simulated degradation studies performed according to the harmonised experimental guidance published by the European Medicines Agency in 2011. Results revealed that the rates of degradation for salinomycin differed significantly ($p = 2.0 \times 10\text{-}16$) between the poultry litters , in the litter collected from one farm, salinomycin was persistent (DT50 >1000 days) and in the litters from other farms it degraded rapidly (DT50 <1). Our results suggest that we need to better understand how the variability in the chemical and biological properties of poultry litters can influence degradation processes. Based on these initial findings, a number of litters from different farms may be needed to perform manure degradation studies to deliver a comprehensive, and realistic environmental risk assessment.

51 | **Advancing Analytical Tools for High-Throughput Assessment of Polysaccharide Biodegradation**
Prabodhi Preethika Dehiwalage Dona, Newcastle University; Russell Davenport, Newcastle University; Jennifer Menzies, Procter & Gamble; Kathleen Mcdonough, Procter & Gamble

Polysaccharide-based polymers are increasingly investigated as sustainable alternatives to synthetic polymers; however, their structural complexity presents considerable analytical challenges for environmental fate assessment. Standardized biodegradation tests are widely used for regulatory purposes but often suffer from low throughput, extended testing durations, and limited resolution when applied to high-molecular-weight or structurally diverse biopolymers. These limitations underscore the need for complementary analytical approaches that are sensitive, scalable, reproducible, and rapid. This study focuses on the development and evaluation of quantitative analytical workflows to assess how structural modifications of polymer backbones affect degradation under controlled conditions. Our example polymers were unmodified and chemically modified water-soluble polysaccharides, with an initial focus on pullulan. Polymers were degraded using specific enzyme assays, followed by screening with different methods; initially, thin-layer chromatography and colorimetric monomer detection in a 96-well plate format. These methods enabled the preliminary detection and semi-quantification of degradation products, including mono- and oligosaccharides. To enhance analytical resolution, Ultra-High Performance Liquid Chromatography with Charged Aerosol Detection was employed, achieving a limit of detection of 15 µg/mL and a limit of quantification of 20 µg/mL for selected saccharides. Additional techniques, including derivatization-based UHPLC with Diode Array Detection and Liquid Chromatography–Mass Spectrometry, are under optimization to improve sensitivity and compatibility with both complex degradation matrices and existing screening methods. The resulting method framework offers a scalable and transferable platform for characterising the environmental transformation of polysaccharide-based polymers. Future work will extend the approach to materials such as dextran and carboxymethyl cellulose to further investigate how chemical modifications influence biodegradation performance and support enhanced analytical capacity for the environmental assessment of emerging sustainable polymers.

52 | **Development of an Ultrahigh Resolution Mass Spectrometry-Based Method for Nontarget and Suspect-Screening of Nitroaromatic Compounds in Atmospheric Particulate Matter (PM2.5)**
Sergi Grebenyuk, Goethe University Frankfurt; Sergi Grebenyuk, Goethe University Frankfurt; Jonathan Martin, Stockholm University; Ioannis Sadiktsis, Stockholm University

Air pollution is an escalating challenge in modern societies and a major contributor to disease and premature mortality worldwide. Its impacts are particularly severe in developing countries, where exposure is often highest. Nitroaromatic compounds, like particulate matter (PM), are primarily emitted from combustion processes and are thus ubiquitously present in the atmosphere. These compounds are highly toxic and significantly contribute to climate forcing due to their light-absorbing characteristics.Fine particulate matter (PM2.5) can penetrate deeply into the human respiratory system. Organic compounds sorbed onto these particles may dissolve in alveolar fluid and become bioavailable, potentially entering systemic circulation. Consequently, a combination of physical damage from particle intrusion and chemical toxicity may be responsible for adverse

health outcomes.To address this, a sensitive non-targeted analytical method has been developed using ultrahigh-resolution mass spectrometry and diagnostic neutral loss scanning. After initial validation using model compounds—including mono- and poly-nitroaromatic species (e.g., nitro-PAHs)—the method was applied to real PM2.5 samples collected on quartz fiber filters during the SAPOEX18 campaign in the Maldives (January–April 2018). Air mass back trajectories for each sampling period were computed using the HYSPLIT model. Three sample sets, each representing distinct geographic origins (Bangladesh, India, Arabian Sea), were selected for detailed analysis. The resulting nitroaromatic profiles were statistically evaluated and correlated with meteorological data.

53 | **Pharmaceuticals and Personal Care Products in Estuarine Systems Across a Gradient of Effluent Contributions to Base Flow: Influences of Grab and Time Weighted Composite Sampling**

Adam Wronski, Baylor University; Joseph Choi, Baylor University; Sara Kamanmalek, Howard University; Jacelyn Rice-Boayue, NC State University; Alistair B.A. Boxall, University of York; Kenneth M.Y. Leung, City University of Hong Kong; Bryan W. Brooks, Baylor University

Billions of humans live within a few hundred kilometers from coastlines, but aquatic exposure information for pharmaceuticals and personal care products (PPCPs) are limited in coastal regions. This represents a key research need because PPCPs can present water quality risks, and further development and utilization of these systems for aquaculture and coastal fisheries will be required to meet future global food demand. To better understand the influence of PPCPs in effluent discharges on estuarine systems, we used a hydrological model to identify 14 estuarine systems with varying proportions of wastewater effluent contributing to base flows across the Gulf Coast of Texas, USA. In both sampling campaigns (wet and dry season), we specifically utilized traditional grab sampling methods on study day 1 and study day 2 while also employing time-weighted composite sampling over a 24-hour period, and then determined surface water concentrations of 30 PPCPs by isotope dilution LC-MS/MS. When sufficient data was available, we developed probabilistic environmental exposure distributions to examine influences of sampling approaches on potential exceedances of water quality thresholds. We quantified 18 target analytes in these coastal systems, including several benzodiazepines and glucocorticoids. Frequently detected compounds were identified at the highest concentrations in Houston, TX or Corpus Christi, TX, the two largest cities included in our sampling effort. Among the sampling methods utilized, we observed higher likelihoods of encountering temazepam, sulfamethoxazole, and trimethoprim, based on 10th centile values, using grab sampling, while for time-weighted autosamplers, alprazolam, caffeine, and diphenhydramine were more likely to be observed. These findings can inform future surface water quality monitoring efforts of PPCPs in coastal systems across gradients of urbanization.

P50 | **Developing Innovative Techniques to Monitor Chemical and Microbial Pollution**

Charley Clayton, University of York; Harriet Sleight, University of York; John Wilkinson, University of York

Surface waters across the UK are increasingly threatened by a complex mixture of chemical pollutants and microbial contaminants. Critically, none of the 982 regional water bodies in Yorkshire achieved a high ecological or chemical status in their last assessment. There is a need for increased environmental monitoring and management, however, current monitoring capacity is limited by the high cost and technical demands of analytical methods.
This project addresses the challenge of high monitoring costs and limited data coverage by developing an accessible, standardised toolkit for environmental monitoring. The primary aim is to adapt advanced analytical techniques, which are traditionally confined to laboratories, into simple, harmonised methods suitable for use by citizen scientists.
The toolkit will advance the boundaries of current citizen science methods by including validated protocols for detecting pharmaceutical and personal care products and E. coli, with robust quality control measures ensuring data integrity across different individual users and locations. This toolkit will equip citizen scientists across Yorkshire to collect and analyse water samples, significantly expanding the spatial and temporal scale of current monitoring.
Preliminary implementation of this toolkit has demonstrated its feasibility in generating high-quality, comparable data across multiple locations. These large-scale datasets will provide valuable insights into the chemical and ecological health of surface water bodies in Yorkshire. These datasets will support targeted efforts to protect and restore surface waters, in collaboration with key stakeholders, including Yorkshire Water, the Environment Agency and the Yorkshire Wildlife Trust. This toolkit will also engage and empower citizen scientists and community groups with growing interest and concern among the public and media regarding the condition of surface waters.

P51 | **Towards Quantitative Method Development Using Pyrolysis–Gas Chromatography–High-Resolution Mass Spectrometry: Estimating Phthalate Ester Emissions From E-waste Pyrolysis**

Amoluck Eluri, Coventry University; Will P. Gates, Deakin University; Damien L. Callahan, Deakin University; Susanne Charlesworth, Coventry University; Ivan Kourtchev, Coventry University

The increasing consumption of electronic products is driving a surge in e-waste generation, posing serious environmental and human health risks. A major concern is the use of phthalates (PAEs) as plasticisers in electronics manufacturing, as they are typically retained during preliminary stages of e-waste recycling and can potentially volatalise during the pyrometallurgical metal recovery process. Current analytical methods for estimating PAE emissions during e-waste pyrolysis are unsuitable because they rely on offline sampling and preparation, which are highly susceptible to contamination from ubiquitous environmental PAEs. While online pyrolysis (Py) – gas chromatographic (GC)-

mass spectrometric (MS) based methods facilitate direct analysis of samples and avoid contamination concerns, existing methods are only semi-quantitative. In this study, we advanced the existing Py-GC-MS-based technique by developing a fully quantitative analytical method for determining airborne PAEs during e-waste pyrolysis. Six PAEs, namely, dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), di-2-ethylhexyl phthalate (DEHP), and dioctyl phthalate (DOP), were selected for method development. The method was evaluated across various pyrolysis conditions (e.g., pyrolysis temperature and time), and analytical parameters, including accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ), and matrix effects. The chromatographic responses of PAEs demonstrated good linearity ($r2 > 0.990$) over the 5-1000 pg µL-1 range, with limits of detection (LODs) ranging from 28-77 pg µL-1. The method showed acceptable accuracy and precision (% CV and RE < 20%) at five tested concentrations for most of the targeted analytes, with matrix effects of up to 50% observed for DEHP and DOP. The validated method was successfully applied to three electronic matrices, observing DEHP and DOP emissions ranging from 3-30 mg kg^{-1}, marking the first Py-GC-HRMS study on the quantitative determination of DEHP and DOP during e-waste pyrolysis, to the best of our knowledge.

P52 | **Next Generation Passive Samplers for Monitoring of Organic Contaminants in Water at Increased Time Resolution**

Kai Wilschnack, University of Portsmouth; Xiangning Zeng, University of Southampton; Adrian Nightingale, University of Southampton; Gregory Slavik, University of Southampton; Gary Fones, University of Portsmouth

Anthropogenic organic contaminants such as pharmaceuticals, plant protection products, and tire compounds, are found in various water sources worldwide in the ng to µg L−1 range. Typical monitoring approaches use spot sampling and targeted liquid chromatography mass spectrometry methods, allowing the determination of pre-selected compounds at one point in time. However, the number and frequency of sampling events is limited by cost and time required for sampling and analysis. Alternatively, passive samplers, e.g., Chemcatcher, are left in the water for several weeks allowing the determination of time-weighted average concentrations at relatively low costs without further preconcentration. However, the temporal variation within the deployment period cannot be assessed, making it difficult to identify pathways and point source events. Hence, a next generation passive sampling for monitoring organic contaminants on a shorter time scale was developed. By autonomously exposing the sampling discs, organic contaminants can be measured at increased time resolution (days instead of weeks) for the same operational costs. The newly developed passive sampler will be used to analyse organic contaminants in the River Test using a non-target screening. This allows an increased understanding of pathways and temporal trends of a large variety of organic contaminants in the environment.

P53 | **Global Inter-regional Depositions of Benzo [α] Pyrene**

Priyanka Yadav, Indian Institute of Technology ; Asif Qureshi, Indian Institute of Technology Hyderabad

Benzo[α]pyrene (BaP), a polycyclic aromatic hydrocarbon (PAHs), is a ubiquitous environmental contaminant. Since 1998, PAHs have been listed in the Convention on Long Range Transboundary Air Pollution (CLRTAP) Protocol on Persistent Organic Pollutants. This pollutant is of great public concern because of its toxicity and potential carcinogenicity. Emissions of BaP occurred as early in 1970s, increased till 1990, then decreased before spiking again from 2000 onwards. This shift in emissions from developed to developing countries is largely attributed due to shifting of BaP emitting industries, with China and India being the largest emitters of PAHs. In the light of environmental significance, it is important to know the emission scenario of BaP. Results indicated that Asia has the highest regional emissions (1.73 x 108 kg), while Australia (1.03 x 106 kg) has the lowest. In the present study, we have used the BETR-Global model to understand the BaP scenario at global scale. The model dynamically integrates air, vegetation, freshwater, ocean, soil, and sediment compartments at a 3.75° × 3.75° resolution. This study investigates the long-term (1970–2018) global distribution with emphasis on spatial and seasonal variability. Here, we will highlight the long-term trends (1970 – 2018) of BaP transboundary seasonal depositions across Asia, Europe, Africa, North America, South America, Australia, Arctic and, Antarctica. Overall, seasonal depositions were highest during DJF (Dec, Jan, Feb). Contributions from its own domain were large, among all these regions, Asia had the highest seasonal depositions (3.36 x 107 kg). BETR model results were also evaluated through the comparison between ground-based observation data and the BaP simulations of the model. This research underscores the importance of understanding the shifting dynamics of BaP emissions for effective environmental management and policy development.

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