MICROPLASTIC*days*

Shaping the future of microplastic research

25-27.3.2025

Faculty of Chemistry and Chemical Technology
Ljubljana, Slovenia

Book of abstracts

Ljubljana, 2025

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MICROPLASTIC*days* – Book of abstracts

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MICROPLASTIC*days* are dedicated to fostering collaboration and advancing knowledge in the field of microplastic research, metrology, and standardization. Through seminars, training sessions, presentations, and discussions, participants gain diverse insights into the complex world of microplastics.

Organized by a network of research projects, institutions, and experts, MICROPLASTIC*days* serve as a dynamic platform for scientists, professionals, and engaged stakeholders to exchange ideas, showcase innovations, and explore collaborative solutions.

More than just a conference, MICROPLASTIC*days* represent a collective effort to drive meaningful progress in understanding and mitigating microplastic pollution.

MICROPLASTIC*days* are supported by:

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MICROPLASTIC*day* No. 1

Current and future metrology and chemometrics in microplastic research
organized by COST Action PRIORITY.

The seminar aims to introduce participants to the concepts of metrology and chemometrics in microplastic research, highlighting the practical applications and straightforward methods for estimating measurement uncertainty. Additionally, the effective production and use of test and reference materials was a key component of this training.

The first part of the workshop featured a series of lectures focused on the materials utilized in microplastic research, as well as guidance on how to prepare test and reference materials for environmentally relevant studies. The second part was specifically designed for students and early-career researchers, providing them with the skills to effectively manage and analyse data in their microplastic research.

Program of the seminar ***Current and future metrology and chemometrics in microplastic research***

8:30 – 9:00	**Welcome & coffee**
9:00 – 9:30	**Microplastic research: viewpoint of producers** Camila Carteny Plastics Europe, Belgium
9:30 – 10:00	**Small micro- and nanoplastic test and reference materials for research: Current status, ongoing efforts and future needs** Andy Booth SINTEF, Norway
10:00 – 10:30	**Small micro- and nanoplastic reference materials supporting harmonization and standardization** Korinna Altmann BAM, Germany
10:30 – 11:00	**Coffee break**
11:00 – 11:30	**Standardization and harmonization effort on microplastics analysis by spectroscopic methods** Andrea Giovannozzi INRim, Italy
11:30 – 12:00	**Progress towards standardized microplastic test materials in Europe** Luke Parker Dutch Organization for Applied Scientific Research (TNO), Netherlands

Practical workshop for young researchers

Moderators: Thomas Meisel, Montanuniversität Leoben, Austria; Stefania Federici, University of Brescia, Italy; Aleksandra Tubić, University of Novi Sad, Serbia

13:30 – 15:15 Introductory lecture on chemometrics

Lecturer: Enmanuel Cruz Muñoz, University of Milano-Bicocca, Italy

15:15 – 15:30 Coffee break

15:30 – 17:30 Practical exercise and discussion

Lecturer: Benedikt Hufnagl, Hufnagl Chemometrics, Austria

17:30 – 20:00 City tour & networking

Abstracts of invited lectures from the seminar ***Current and future metrology and chemometrics in microplastic research***

Microplastics research: Viewpoint of producers

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Over the past five years, the plastics industry has significantly increased its engagement in microplastics research, recognising its responsibility to contribute to science-based, policy-relevant solutions. This presentation outlines key industry-led and collaborative initiatives designed to address critical knowledge gaps in microplastic emissions, fate, and impacts. It highlights strategic programmes such as Brigid and the MARII platform, which support robust human health risk assessments, improved analytical methodologies, and real-world exposure modelling. The presentation also provides a comprehensive mapping of European research activities in the field, reflecting a surge in publicly funded projects driven by the EU Green Deal and the 2030 microplastics reduction target. These include initiatives under Horizon Europe, national and regional programmes, and coordinated research consortia. Projects span a range of topics, from microplastics in freshwater, soil, and marine environments to human health impacts, metrology, and remediation strategies. In parallel with this growing research landscape, the industry's focus is shifting from hazard identification to actionable mitigation. Key priorities include the minimisation of pellet losses and the reduction of emissions across the product lifecycle. Emphasis is placed on the development of harmonised methods, representative testing materials, and lifecycle-based product design. The presentation underscores the importance of cross-sector collaboration in translating scientific insights into effective regulatory and environmental outcomes.

Small micro-and nanoplastic test and reference materials for research: Current status, ongoing efforts and future needs

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Micro- and nanoplastic pollution in the natural environment comprises partially degraded particles representing a continuum of sizes, shapes, polymer types and chemical compositions. Small microplastic particles (sMP; <100 µm) and nanoplastics (NPs) are considered to be a potential risk to organisms due to their potential for biological uptake and accumulation. To quantify sMP and NP exposures, assess hazards and understand the associated risks, it is critical to understand how these particles behave in the environment and how environmental matrices affect their detection. To achieve this, high quality, well-characterised and environmentally relevant test and reference materials are crucial. A current lack of widely available environmentally relevant sMP and NP reference materials, as well as methods for their production, has resulted in many studies utilising commercially available spherical, homogenous and monodisperse particles. Such particles are typically produced for specific purposes and lack both environmental relevance and the detailed physicochemical characterization necessary for interpreting fate and effects data. As such, there is a need for sMP and NP test/reference materials for fate and effects assessments and analytical protocol validation that more accurately represent the sMP and NP present in the environment. Feasible methods for producing relevant sMP and NP test materials in sufficient quantities for environmental fate and effects studies remain lacking. This presentation provides and overview and laboratory comparison of a suite of reported sMP and NP methods. We highlight those methods that show the most promise for producing environmentally relevant sMP and NP with further development and optimisation.

Small micro- and nanoplastic reference materials supporting harmonization and standardization microplastic research

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Microplastics occurrence, fate, transportation, and possible risks are not related to research only anymore since regulators started to formulate directives like the Drinking Water Directive (Directive (EU) 2020/2184) and the Urban Wastewater Treatment Directive (Directive (EU) 2024/3019). Microplastics monitoring will become mandatory in EU the next years. Microplastics are defined as solid particles mainly consisting of polymers and a size between 1 and 1000 µm. Nanoplastics are smaller than 1 µm (ISO/TR 21960:2020). Basic requirements to fulfill the criteria of the directives are harmonized analytical approaches for sampling, sample preparation and detection. This includes standard operation procedures as well es international standards and reference materials to guarantee true and accurate results that are comparable all over the world. The talk provides information about reference materials in general and possible production routes for microplastics and nanoplastics. It shows the broad diversity in needs according to the user's perspective and the intended use, e.g. polymer type, size, shape and surface functionality and tries to sort, what is possible so far and what remains as potential research for the future. All provided case studies have in common that the final products are homogeneity and stability tested to really earn their names as reference materials. This talk focuses on top-down particle production methods by cutting large microplastics with cryogenic milling or Ultra-Turrax to smaller fragments together with further sieving or filtration for size separation. It also highlights the need of very small concentrations for particles masses and numbers and present tableting and stable suspensions as possible solutions.

Standardization and harmonization effort on microplastics analysis by spectroscopic methods

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Plastic pollution is widely recognised as a major global anthropogenic issue, driven by complex physicochemical transformation processes such as aging, degradation, and fragmentation. These processes lead to the formation of microplastics (MPs, 1–5000 µm) and subsequently nanoplastics (NPs, <1 µm). Plastic debris exists in a broad spectrum of sizes, polymer types, and degradation levels, often carrying various associated chemicals. Therefore, a combination of diverse sampling, processing, and analytical techniques is required. Numerous analytical methods have been developed to assess samples containing different polymer types and particle size ranges. However, validated and standardised methodologies are still lacking. The most commonly used techniques to reliably identify MPs include micro-spectroscopy (e.g., FTIR, Raman) and thermo-analytical methods. Thermo-analytical approaches provide mass-based quantification of polymers (with limits of detection in the µg range), while spectroscopic techniques offer detailed particle-level data on polymer type, count, size, and distribution—even down to the sub-micron scale. Despite some progress toward harmonisation, there is a critical need for collaborative method development, certified reference materials, and inter-laboratory comparison (ILC) studies to validate and align methods and reporting protocols. This contribution provides an overview of current efforts toward method harmonisation, including:

i) European projects (e.g. EUROqCHARM, 21GRD07 PlasticTrace) aiming to critically review the state of the art and develop reference materials for QA/QC;

ii) ILC studies assessing the performance of spectroscopy (µFTIR, µRaman) and thermo-analytical techniques (e.g. VAMAS TWA45);

iii) International initiatives at ISO level (e.g. ISO/TC 147/SC 2/JWG 1), focused on defining key principles for analysing microplastics in drinking water and other matrices, with emphasis on sample preparation, measurement, data processing, and method validation.

Linking microplastic size with material properties and applying this to reference material production

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Microplastics formed through wear, tear and/or degradation of larger plastics, so-called secondary microplastics, are by far the most common type of microplastics in the environment. These particles are characterized by a heterogeneous size distribution and fragmented morphology which is desirable to replicate in the production of reference materials. This is often achieved by processes such as ball- or centrifugal milling, however producing the smallest micro- and nanoplastics by these methods remains a challenge. By gaining a greater understanding of the formation mechanisms of microplastics we can hopeful optimise methods for the production of the smallest micro- and nanoplastics. The Microplastics Index (MPI) is a mathematical model derived from fracture mechanics and relates 12 material properties (such as Young's modulus and yield strength) to the amount of microplastics a material will form under a specific stress and the smallest particle size that will be generated. The MPI has been calculated for both impact and wear stresses. Literature data was used to calculate the MPI for 15 different plastics and it was seen that polyethylene has the lowest tendency to form microplastics and the formed particles are large. Polystyrene, on the other hand, was found to have the highest tendency to form microplastics and these will be much smaller. We found that microplastics formed through wear (abrasion) are in general around an order of magnitude smaller than those formed through impact forces. UV-ageing of plastic was also found to increase the amount of microplastics formed and decrease their size. These findings are of use for reference material production as they show that using a production process that incorporates abrasion may facilitate the production of small particles, as will pre-ageing the material through means of UV.

MICROPLASTIC*day* No. 2

Advances in microplastics research by UL FKKT and researchers from the PLANTerastics team.

This seminar brought together leading experts to explore the latest developments in microplastics research. We focused on state-of-the-art approaches to the preparation and characterization of microplastics, the development of reliable reference materials, and the application of chemometrics in data interpretation. Beyond the materials and methods, we looked at the wider impacts, highlighting not only the effects of the microplastic itself, but also the importance of additives and their potential health risks. Particular attention was paid to how microplastics change as they age in the environment, affecting their behaviour and toxicity.

The seminar also highlighted new trends in analytical techniques, including novel monitoring strategies and the use of advanced tools such as computed tomography (CT) and laser-based methods. Importantly, we expanded the discussion beyond technology and recognized the critical role of education, public awareness and social science in shaping effective responses to microplastic pollution. Finally, space was given to young minds to present their research through dynamic pitch talks, showcasing emerging talent and fresh perspectives in the field.

By bridging scientific innovation and interdisciplinary dialog, the seminar underscored the urgent need for integrated solutions to one of the most complex environmental problems of our time.

Program of the seminar ***Advances in microplastics research***

	9:30 - 10:00	**Welcome & coffee**
Preparation & Characterization	**10:00 – 10:20**	**Comparison of fragmentation techniques for the production of test microplastics and their effects on physicochemical characteristics** Stefania Federici, University of Brescia, Italy
	10:20 – 10:40	**Linking microplastic size with material properties and applying this to reference material production** Luke Parker, Dutch Organization for Applied Scientific Research (TNO), Netherlands
	10:40 – 11:00	**On data analysis of μ(FT)IR imaging data using different spectrometers, filter types and measurement geometries - a chemometric perspective on the problem of creating data-agnostic software for microplastics analysis** Benedikt Hufnagl, Hufnagl Chemometrics, Austria
	11:00 – 11:20	**Coffee break**
Impacts	**11:20 – 11:40**	**Chemical additives in plastic materials and their role in plastic degradation processes** Andy Booth, SINTEF, Norway
	11:40 – 12:00	**Microplastic health risk assessment framework in allergic immune disorders** Tanja Ćirković Veličković, University of Belgrade, Republic of Serbia
	12:00 – 12:20	**The implication of biofilm development on interaction of microplastics with their surroundings** Ula Putar, University of Ljubljana, Slovenia
	12:20 – 13:20	**Refreshment**
Analytics	**13:20 – 13:40**	**Monitoring of microplastics in water samples: standardization, legislation and field experiences in the Danube-region** Gabor Bordós, Eurofins, Hungary
	13:40 – 14:00	**Combined laser induced breakdown spectroscopy (LIBS) and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) in microplastic analysis** Lukas Brunnbauer, TU Wien, Austria
	14:00 – 14:20	**Detection of microplastics in fish by X-ray computed tomography** Tomas Zikmund, Central European Institute of Technology, Czech Republic
	14:20 – 14:40	**Coffee break**
Solutions	**14:40 – 15:00**	**Harmonized protocols for surveying and monitoring litter, plastics and microplastics - UPSTREAM project approach** Aleksandra Tubić, University of Novi Sad, Republic of Serbia
	15:00 – 15:20	**Prevention and solutions: social science perspectives on microplastics** Nina Vaupotič, University of Vienna, Austria
	15:20 – 15:40	**The role of formal education in raising awareness and action regarding microplastics and other environmental issues** Iztok Tomažič, University of Ljubljana, Slovenia
Young minds	**15:40 – 17:00**	**Innovations and insights: Perspectives of early-career scientists** 3 min pitch of early-career scientists to introduce their research
	17:00	**Closing & Social event at FKKT**

Abstracts of invited lectures from the seminar ***Advances in microplastics research***

Comparison of fragmentation techniques for the production of test microplastics and their effects on physicochemical characteristics

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Plastic pollution remains a major environmental concern, with micro- and nanoplastics emerging as key contributors due to their pervasive presence and potential risks to ecosystems and human health. These small plastic fragments often result from the degradation of larger plastic waste, and understanding their properties and behavior is critical for assessing their environmental impact. However, progress in this field is hindered by the lack of representative test materials that accurately reflect those found in the environment. This contribution focuses on the generation of “true-to-life” micro- and nanoplastics through different fragmentation techniques applied to common plastic items. By mechanically fragmenting everyday plastics, we produced microscale particles whose shape, morphology, and physicochemical features were closely analyzed. The results highlight how fragmentation methods and polymer types influence the characteristics of the resulting particles, insights that are essential for refining protocols and interpreting environmental samples. At the nanoscale, particles separated from microscale fragments were used to explore interactions with biological systems. Notably, “true-to-life” nanoplastics formed a protein corona in human plasma that differed from that of synthetic nanobeads, emphasizing the relevance of using environmentally realistic materials in such studies. Altogether, the findings reinforce the need for realistic test materials in micro- and nanoplastics research. They also demonstrate how fragmentation technique choice directly affects the properties of test plastics, with implications for toxicity testing, environmental modeling, and regulatory development.

Progress towards standardized microplastic reference materials in Europe

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Recently, great progress has been made by various national and international research projects Europe in the production of environmentally relevant micro- and nanoplastic testing materials and steps have been made towards producing standardized reference materials. This talk identifies four crucial criteria in the definition of certified tests materials and summarises the progress made towards each. These include: particle properties, characterisation, homogeneity and stability, and certification. When discussing particle properties the influence of the production method on particle size and morphology is investigated along with the use of different tracers (such as fluorescent probes, metals and radioisotopes) and influence of UV-ageing. An overview of characterisation techniques is given and the requirement of a complementary suite of techniques is presented. This includes the techniques that are often used to measure common particle properties (size, morphology, chemical identity) and also shines a light on particle properties, such as endotoxin contaminants, that are recently gaining more attention. The importance of dispersion media is examined with regards to particle stability along with new routes for predicting suspension stability. Finally, progress towards traceability and certification is gathered, including the important role FAIR data storage and open-science platforms play in hosting SOPs and particle characterisation.

On data analysis of µ(FT)IR imaging data using different spectrometers, filter types and measurement geometries - a chemometric perspective on the problem of creating data-agnostic software for microplastics analysis

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While the comparability of microplastics data is a well-recognized problem, it is still unclear how the use of different spectrometers and different analysis software contributes to the observed differences. Both within the domain of µ(FT)IR and µRaman spectroscopy we can use instruments from various manufacturers and perform the classification of spectra using different libraries as well as different matching criteria. More recently, machine learning is gaining more and more momentum as an alternative to spectral library search. While this new methodology opens new possibilities, it also adds to the complexity of choosing the right methods for an optimal analysis workflow. This circumstance raises the question of how to compare automatic analysis solutions. Are there measures that allow algorithms to be compared in an objective way? In this presentation we demonstrate software tools that implement the recommendations in ISO 24187:2023 for algorithm comparison. By comparing analysis results obtained with Artificial Neural Networks, Random Forests and Spectral Library Search, we show the impact of different algorithms on error rates such as true-positive and false-negative rates.

Chemical additives in plastic materials and their role in plastic degradation processes

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The abundant plastic pollution in all environmental matrices globally has led to an increasing number of studies investigating the degradation processes, mechanisms and products acting upon these materials. The goal of many studies is to gain a clearer understanding of how plastic materials degrade in the environment and how this is linked to their various physicochemical properties. Despite the widespread usage of additive chemicals in the majority of consumer products, the influence of such chemicals on plastic degradation is typically not considered. Given that most additive chemicals are incorporated into the polymer matrix of consumer products to enhance durability and protect against degradation, this appears to be a significant oversight in many reported studies. In this presentation, we take a look at why additive chemicals are added to plastic materials and what implications this might have on their degradation in the natural environment. We start by taking a closer looking into the many infographics available that claim to provide information on the expected lifetimes of many consumer products and highlight that they are all based upon non-peer-reviewed data. Next, we look at the primary degradation mechanisms influencing plastic in the environment, namely UV degradation, mechanical degradation and microbial degradation. This includes looking into the fragmentation and ablation of plastic materials over time. We consider these degradation mechanisms in the context of additive chemicals that are specifically used to prevent UV degradation or increase the flexibility and durability of plastic materials (i.e. reducing mechanical degradation and abrasion), and the implications this has on laboratory and field studies that do not characterize the presence of such chemicals. Finally, we take a look at microbial degradation and how the use of antimicrobial additives may influence this degradation mechanism.

Microplastics health risk assessment framework in allergic immune disorders

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Micro- and nanoplastics pollution has become a serious environmental concern. MNP omnipresence, particularly in biological fluids, has raised concern of its potential health effects. Allergy is a chronic disorder, characterized by hypersensitivity to otherwise harmless environmental and food (glyco)proteins. It shares exposure routes with MNP. The MNP health outcome assessment framework in allergic immune disorders integrates risk assessment data to scientifically identify and quantify hazards that contribute to allergy-related health outcomes. This integration relies on several essential components: MNP characterization, MNP as a vector for allergen and effect assessment, exposure and allergen/MNP co-exposure assessment, and health effect assessment. Each of these components is critical for building a comprehensive understanding of how micro- and nanoplastics may contribute to allergenic risks. The MNP characterization is fundamental for identifying properties of various MNP types. By investigating particle size, surface charge, and agglomeration behavior, we gained insights into the mechanisms by which MNPs interact with the allergenic proteins and immune system. These properties are central to assessing potential allergenic risks, as they impact how MNPs are absorbed and distributed within biological systems. Laboratory studies conducted in both *in vitro* cell cultures and *in vivo* animal models are invaluable for examining cellular responses to MNPs alone and in combination with allergens. Our work provided deep insights into how MNPs through interactions with allergens and immune cells might affect immune modulation or allergic response, suggesting hazard for allergic diseases. To provide a reliable health impact assessment of MNP in allergic disorders, several major gaps should be filled in future studies: comprehensive exposure assessment, particularly to nanoplastics, which depends on harmonized and standardized analytical methods and their development, improved *in vitro* and *in vivo* models to reflect chronic exposure, more environmentally relevant MNP and their mixture available for testing in environmentally relevant concentrations.

This study was supported by the IMPTOX European Union's Horizon 2020 research and innovation program (grant number 965173).

The implication of biofilm development on interaction of microplastics with their surroundings

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Biofilm development, also known as biofouling, plays a significant role in the environmental fate of microplastics. This complex process involves the attachment of diverse microorganisms to microplastic surfaces, followed by the secretion of extracellular polymeric substances (EPS) that help stabilize the biofilm community. As a result, the physical and chemical properties of microplastics can undergo substantial changes, influencing their interactions with surrounding environments. The formation of biofilm is influenced by different properties of microplastics. Studies have shown that biofilm attachment is shape-dependent: microorganisms preferentially colonize irregularly shaped particles (e.g., fragments and films), while smoother and/or spherical particles tend to activate greater EPS production. Chemical composition, including polymer type and additives, also plays a critical role in biofilm formation. Subsequent to biofilm development, the physico-chemical characteristics of microplastics are altered. For instance, microplastics made of low-density polymers may experience increased density and sink, while density of microplastics made of high-density polymers may decrease. Surface morphology and chemical composition also change through aging. These changes affect microplastic interactions with biota and pollutants. While aged microplastics may exhibit reduced direct toxicity to organisms, they can act as vectors for pollutant transfer, accumulating higher concentrations of contaminants. Moreover, aged microplastics can transport microorganisms between ecosystems, potentially altering microbial diversity. Understanding the role of biofilm development on microplastics is essential for predicting their environmental behaviour, ecological risks, and long-term impacts.

Monitoring of microplastics in water samples: standardization, legislation and field experiences in the Danube-region

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Recently, microplastics (MPs) were found along the whole Danube River Basin (DRB). In contrast to well researched seawater, occurrence and effects of MP in surface water and groundwater, especially those used for drinking water supply of the DRB region remain largely unexplored. Because of the lack of integrated MP management in water environment, effective monitoring tools, and improved policies to mitigate MP emission and reduce pollution are urgently needed. As a first step, EU Directive 2020/2184 on the quality of water intended for human consumption (EU DWD) identified MPs as potentially hazardous substances and included it on watch list mechanism monitoring from 2024, in order to carry out a complete risk assessment by 2029. Sampling and analysis methodology for this was released in Commission Delegated Decision C(2024) 1459. To tackle the above-mentioned issues and start collecting EU DWD harmonized data, further to our previous experiences in the region gained on surface water sampling and analysis, during MicroDrink project microplastics will be monitored in 9 designated transboundary pilot sites equally distributed in 3 clusters (karst, intergranular, surface/river bank filtration) representing the vast majority of DRB drinking water resource types. Sampling knowledge will be transferred to the partners during a joint sampling event via sharing and demonstrating an EU DWD harmonized methodology (1000 L sample on 20 µm sieves). Samples from the following one year-long monitoring campaign will be analyzed harmonized to the directive's strict quality assurance and quality control protocols (blanks, recoveries, etc.) both in local and in a central lab to compare results accuracy. As an outcome of the project, a comprehensive online MicroDrink knowledge base, meanwhile relevant international stakeholders will be engaged via targeted meetings, workshops and events.

MicroDrink (DRP0200442) project was supported as part of the Interreg Danube Region Programme, co-funded by the European Union, with the financial contribution of the partner states and institutions.

Combined LIBS and LA-ICP-MS in microplastic analysis

Lukas Brunnbauer[1]*, Laura Kronlachner[1], Ula Putar[2], Mark Starin[2], Pavel Porizka[3], Jozef Kaiser[3]
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In the last years, efforts to study and investigate the effects of microplastics (MPs) have greatly increased. To fully understand the impact of MPs, comprehensive characterization is required. Besides established techniques such as FTIR/Raman Spectroscopy useful for the identification of the polymer type of MPs, recently the application of LA-ICP-MS for MPs analysis was proposed. Due to its high sensitivity, LA-ICP-MS can offer insights into the metal content of individual MPs which can provide additional information about the presence or absence of potentially harmful species. Additionally, recent works showed the capabilities of directly detecting MPs based on the 13C+ signal. In this work, recent results of applying LA-ICP-MS for MPs analysis are presented. Two different approaches for measuring MPs via LA-ICP-MS are presented and their advantages and limitations are discussed. Mounting of individual particles in a resin and preparing cross-sections: With this approach, conventional imaging experiments can be carried out revealing the distribution of the elements of interest. This approach is applied to study the uptake of heavy metals (Pb, Cr, Cd) of three different MP types (PVC, PE, PS). Two different ageing experiments were conducted. The first is based on exposing the particles to river water spiked with heavy metals. In the second experiment, MPs are previously exposed to UV radiation for artificial ageing before exposure to a solution spiked with heavy metals. Quantitative LA-ICP-MS analysis using matrix-matched standards revealed differences in the uptake of the heavy metals in relation to the polymer type and ageing procedure providing insights into the environmental fate of MPs. Direct sampling of individual MPs based on LA-single particle-ICP-MS. With this approach individual MPs particles are non-destructively sampled from a substrate and transported to the ICP-MS. The ICP-MS is operated in single-event mode and each particle is detected as a spike in the transient 13C+ signal. To enable the determination of the particle mass and therefore also the particle size based on the carbon signal, we developed a calibration approach based on the ablation of polymer thin films. With this approach, the size of spherical PS, PVC, and PMMA particles in the low µm range are correctly determined.

Detection of microplastics in fish by X-ray computed tomography

Tomáš Zikmund[1,2]*, Viktória Parobková[1], Gabriela Kalčíková[2,3], Ondřej Adamovský[4], Jozef Kaiser[1,2]

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The ubiquitous presence of microplastics (MPs) in aquatic environments poses a significant threat to ecosystems and human health. While various analytical techniques exist to detect MPs, many require destructive sample preparation and lack the capacity for spatial visualization within biological tissues. This study presents a novel, non-destructive approach using micro-computed tomography (microCT) for detecting and 3D visualization of microplastics in zebrafish (*Danio rerio*), offering new insights into MP localization and behavior in biological systems. Our methodology focuses on the targeted detection of spherical polyethylene (PE) microplastics chosen to align with the detection limits of the CT setup. Zebrafish samples were prepared ex vivo, including staining of soft tissue to enhance contrast. The analysis concentrated on the abdominal region to assess MP accumulation in the digestive tract. Results demonstrate the successful detection of spherical PE particles as small as 30 µm, with sufficient contrast for detailed morphological and quantitative analysis. MPs were tracked through the digestive system, providing insights into their distribution and retention. However, as microCT is a density-based technique, challenges remain in detecting MPs of varying compositions, shapes, and sizes, particularly non-spherical types or materials like PVC, PP, and PET. This proof-of-concept study highlights the potential of microCT for MP research in biological specimens. Future work will focus on expanding the range of detectable MP types and improving segmentation algorithms. The findings contribute to advancing non-invasive analytical tools for environmental toxicology and microplastic impact assessments.

Harmonized protocols for surveying and monitoring litter, plastics and microplastics - UPSTREAM project approach

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Plastic pollution is a diverse contaminant that represents a continuum of properties, from macro scale litter, down to micro and nanoplastic particles, as well as the leachable compounds associated with such plastics. As such no single analytical technique can monitor “plastics” in the environment, and the region of this continuum targeted for monitoring in the Horizon Europe project UPSTREAM is dependent on the technology under development at each of the demonstration sites in the project and the analytical techniques available across the partners.

A total of five demonstration sites connecting seven rivers, feeding into five sea basins are to be investigated in the UPSTREAM project. Of these demonstration sites, four technologies associated with wastewater treatment works are investigated whilst the fifth site is the Danube River itself in Serbia. At each demonstration site, either litter, microplastics, leachable compounds associated with plastics, or a combination of these are the target of monitoring. Given the diversity of analytes, locations and institutions required to achieve the monitoring goals in the project, it was essential that standards are used where available, and harmonisation of methods is encouraged where such standards do not exist.

There are four critical areas for harmonisation when considering monitoring in the environment: sample collection, sample preparation, sample analysis, as well as data analysis and interpretation. Taking each of the major analytes for monitoring, these aspects of harmonisation are considered, standard operating procedures collected, and agreement reached for recommendations for harmonisation across methodologies. The ambition is to allow evaluation of demo sites themselves but also between sites.

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Prevention and solutions: social science perspectives on microplastics

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Microplastic pollution is a consequence of individuals' choices, attitudes, and behaviors. Conversely, whether individuals are concerned about microplastics can influence their consumer behavior, political action, and support for regulation. These actions are shaped by political and communication contexts, but different stakeholders are also sensitive to the public's perceptions and decision-making. Our research focuses on risk perception of microplastics, support for mitigation measures, and the socio-psychological drivers behind these attitudes among the public and other stakeholders. Public survey results from several European countries show that awareness and risk perception of microplastics are relatively high, especially compared to other pollutants such as PFAS, excess nutrients, and underwater noise. Based on surveys with other stakeholders, risk perception is highest among NGO representatives, followed by decision-makers, scientific experts, and lowest among industry representatives. Of the mitigation measures, subsidizing pro-environmental technologies is most supported, followed by labeling eco-friendly products, while penalties for non-eco-friendly behavior are least supported across all stakeholders. Moreover, pro-environmental norms (perceived social rules for environmental protection), biospheric values (environmentally focused moral values), and low trust in industry are associated with higher risk perception of microplastics and support for mitigation measures. Considering scientific uncertainties regarding the health effects of microplastics, and recognizing that public perceptions are shaped by science communication, we investigated how various approaches to communicating uncertainty influence both risk perception and support for mitigation measures. We found that communicating a lack of knowledge does not significantly affect risk perception or policy support. However, communicating a lack of scientific consensus may reduce both risk perception and support for action. These findings provide important input for prevention and potential solutions to microplastic pollution, as effectively addressing the issue will depend not only on technological advances and the feasibility of measures, but also on how they are communicated and adopted by the public.

The role of formal education in raising awareness and action regarding microplastics and other environmental issues

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Formal education plays a crucial role in raising awareness and fostering action regarding a plethora of environmental issues. With increasing concerns about pollution, climate change, and biodiversity loss, integrating environmental education into curricula at various levels is essential for equipping students with knowledge and informing them about the possible adaptation and mitigation strategies that lead to pro-environmental action (behaviour). There are several dimensions at which professional community can introduce environmental topics in formal education environment: (1) cooperation with or within the universities in pre-service teacher education, (2) cooperation in in-service teacher education (i.e. conferences/seminars), (3) preparation of teacher manuals with the proposed school activities (i.e. LIFE projects or national research projects), (4) writing or coauthoring textbooks (i.e. science, biology, chemistry, physics), (5) participation in introduction of systemic changes (i.e. curricular changes and legislation), and (6) educational research in the field of interest (i.e. microplastics). With the ongoing renewal of school curricula, there were five common themes of learning goals assigned to all school curricula, one of them being sustainability. With the latter and with the subject specific goals, environmental topics can be meaningfully integrated into formal education, especially within the natural science curricula. Within our presentation, mentioned possibilities will be explained in more detail and will be linked to one of the studies conducted in Slovenia regarding students' knowledge and attitudes regarding conventional plastics, bioplastics and biodegradable plastics. Furthermore, the presentation will also draw from the experiences gained from other Slovenian research and research projects that had an effect on all above-mentioned dimensions and link them to the topics of microplastics.

Young Minds: Abstracts of pitch talks

Microplastic and microfiber pollution in the Classical Karst Region. Springs and caves preliminary assessment

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Research on microplastic (MP) and microfiber (MF) pollution in karst areas is still at the early stages. The Classical Karst Region hosts different protected habitats and species, including numerous stygobionts, such as the *Proteus anguinus.* However, existing regulations often fail to consider the ecological connections between different habitats. In this preliminary study, we collected and investigated several submerged sediment and water samples from both surface (springs) and subterranean (caves) aquatic environments of this Region. Detected MPs and MFs (5-0.1 mm) were quantified and characterized by size, shape, and color via visual identification under a microscope, with and without UV light. Spectroscopic analyses were conducted on selected particles to determine their chemical composition. All examined samples contained significant amounts of MPs and MFs. Most MPs and MFs were smaller than 1 mm and abundances increased with the decrease of the considered size. Over 70% of MPs and MFs exhibited fluorescence under UV light, predominantly with a blue hue. Fluorescent MPs and MFs were mainly transparent, while non-fluorescent ones were especially dark. Samples contained especially polyesters and copolymers. Of the analyzed MFs only 10-15% were synthetic. Of the natural and regenerated MFs, the major part was cotton. MPs and MFs pose a threat to ecosystems and water resources. Vulnerable species hosted in these habitats could assimilate them with potentially negative consequences for subterranean water safety at all the levels, such as ecological functionality, biodiversity distribution, ecosystem services and human health. Our findings confirm the presence of MPs and MFs in all examined aquatic environments, contributing to a better understanding of micro-pollutant contamination, and providing valuable insights for future research. MPs and MFs pollution monitoring in karst areas must be prioritized to support habitat conservation, species protection and water resources management, taking into account the ecological interconnections between surface and subterranean habitats.

Advancing microplastic analysis in biological systems with LA-ICP-MS

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The pervasive disposal of plastic waste into the environment presents a substantial challenge for the coming decades, particularly concerning the monitoring and quantification of microplastics (MPs) across various environmental matrices and within living organisms. Microplastic contamination originates from multiple sources, including seafood, food additives, packaging materials, and industrial/agricultural products. Despite the recent detection of MPs in a broad spectrum of environmental media and biota, their dispersion mechanisms and toxicological impacts on humans remain inadequately understood. Emerging evidence suggests that microplastics, which can enter the human body through contaminated food or packaging materials, may pose significant risks to both human health and the environment. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) holds significant promise for identifying polymer alterations and metal markers. However, its direct application in detecting MPs has been limited to tracking trace metals adsorbed on MPs. By enhancing the detectability and imaging of MPs in biological tissues, this research aims to open new pathways for monitoring MPs throughout the human body and assessing potential health effects. Additionally, integrating element-specific imaging from LA-ICP-MS could establish the "golden analytical standard" for µCT volumetry to precisely characterize the distribution of MPs within the organ systems of living organisms. Understanding how microplastics interact with biological systems is crucial to mitigating potential health risks. This short presentation focuses on imaging two sizes of polyethylene terephthalate (PET) MPs, artificially introduced into homogenized rat liver tissue, whole heart, and brain to simulate MPs accumulation. MPs were directly imaged by analyzing 121Sb and 59Co as metal markers in the form of MPs additives.

Evaluation of toxicity of model micro- and nanoplastics in human umbilical vein endothelial cells

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Plastic pollution, particularly in the form of micro- and nanoplastics (MNPs), has become a significant concern for both environmental and human health. MNPs have been detected in human placental &ssue, raising serious concerns about poten&al maternal-fetal exposure and its implications for fetal development. We used model-MNPs made of these polymers labeled with fluorescent-quantum dots (qDs) to investigate the effects of these potentially circulating polymers at a fetal level, particularly on Human Umbilical Vein Endothelial Cells (HUVECs) as representative of the structural and biological barrier. Thanks to the red fluorescent signal of qDs, the combined use of confocal microscopy and flow cytometry allows us to demonstrate the different behavior of two polymer models (PVC and PP). In particular, flow cytometry and confocal microscopy analyses further uncovered significant morphological changes, characterized by increased complexity and granularity, alongside elevated colocalization of MNPs with cellular membranes. Cytotoxicity assays revealed a concentration- and &me-dependent decline in cell viability, with PP exerting a more pronounced effect, particularly at higher concentrations and extended exposure. These findings suggest that the physicochemical properties of PVC and PP drive their differential interaction patterns. While both MNP types penetrate cells, PVC exhibits stronger affinity and uptake, potentially posing greater risks to endothelial integrity and vascular health. This study claims the need for further investigation, taking account of the specific plastic composition to address the biological effects and mechanisms of nanoparticle interactions with vascular cells to unravel their risk in maternal and fetal contexts.

Why do polyolefin microplastics sink?

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Microplastics (MPs) in the ocean are a serious concern and a potential threat to marine ecology. The abundance of MPs leaked and observed in the oceans reveals a massive imbalance. Buoyant polyolefin MPs such as polyethylene (PE) and polypropylene (PP) are expected to be floating on the ocean surface, but a large amount is found in the deep ocean and sediments. In the present study, we examined the accelerated weathering of low-density PE, PP films, as well as high-density PE (HDPE), PP fragment MPs that mimic environmental conditions. We found that weathering increases the density of polyolefin MPs, which is one of the main reasons for its sinking. We assessed the effect of accelerated weathering on their physical, chemical, thermal and surface properties and correlated it with the change in density. The density of pristine HDPE and PP fragments are 0.94 g/cm^3 and 0.89 g/cm^3, which increase to 1.00 g/cm^3 and 1.02 g/cm^3 after 1000 h of weathering, respectively. The weathering time was found to be proportional to the increase in density of polyolefin MPs. Thus, it is critical to consider MP degradation to accurately predict its fate and transport in the marine environment.

Research approach to study the occurrence and transport of microplastics in groundwater

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Microplastics (MPs) are emerging pollutants of growing environmental concern, yet their presence and behavior in groundwater remain largely unexplored. Given that 98 % of Slovenia's population depends on groundwater for drinking water, understanding the occurrence and transport mechanisms of MPs in aquifers is crucial. Despite the increasing recognition of groundwater vulnerability to MPs, research on their transport dynamics, influencing factors, and sampling methodologies is still in its early stages. This presentation will describe the methodology and approach that will be used in my PhD thesis and the steps and techniques to investigate the occurrence and transport of microplastics in groundwater. The research aims to assess the presence and transport of MPs in Slovenia's intergranular and karst aquifers while refining monitoring techniques. A comprehensive study will be conducted to evaluate MP occurrence in groundwater across different hydrogeological settings, considering the influence of land use, recharge conditions, and human activities. Additionally, a novel tracing experiment using lysimeters under atmospheric conditions will be implemented to investigate MP migration through the unsaturated gravel zone. The study will explore how MP characteristics (e.g., type, size) and aquifer media properties affect their transport behavior. By developing improved sampling and analytical approaches and advancing knowledge on MP mobility in groundwater, this research will better understand MPs as a potential contaminant in aquifers. The findings will support future risk assessments and groundwater protection strategies.

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Detection and analysis of microplastics by LIBS

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Plastics have become a pillar of modern society, influencing a wide range of industries such as transport, urban development, agriculture, manufacturing and even textiles. However, the widespread use of plastics has given rise to a growing environmental problem – microplastics. These tiny plastic particles, ranging in size from 1 to 1000 µm, are of particular concern due to their durability, widespread distribution and potential to cause long-term damage to ecosystems and human health. To address this issue, our research group focuses on the detection and analysis of microplastics using laser-induced breakdown spectroscopy (LIBS). LIBS is a powerful spectroscopic technique that works by creating a plasma plume when a high energy laser interacts with a material. This process excites the atoms and ions in the sample, causing them to emit photons of different energies as they return to lower energy levels. The emitted light is then collected by an optical system and analysed by a spectrometer. By studying the unique spectral lines, we can identify the elements present in the sample. Our group has already gained valuable experience in using LIBS to analyse aged microplastics. However, as the application of LIBS in microplastic research is still in its early stages, there is still considerable room for growth and exploration in this area. In this study, we aim to demonstrate the advantages of LIBS for microplastic analysis and explore its potential to improve our understanding of microplastic pollution. Through this work, we hope to highlight the capabilities of LIBS and its promising future in addressing this environmental challenge.

PET microplastics in concrete reused as natural aggregate replacement to reduce environmental impact

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Polymers or plastics are widely used materials since their properties are easily adjustable by adding various additives according to different needs and preferences. Nowadays, polyethylene terephthalate (PET) plastic is used in large quantities, especially in the food and packaging industry. Since most PET plastic materials are made for singular use and therefore, relatively quickly become waste, it poses a significant burden on the environment. At the same time, the demand for concrete is also increasing, due to population growth, urbanization, and industrialization. The dependence of concrete production on natural resources (natural aggregate and cement) and the large amount of CO_2 emissions from cement production raise more and more concern. All things considered, the possibility of plastic waste use in concrete as partial natural aggregate replacement has recently been presented as one of the most promising ways. The actual use of such concrete could address the CO_2 emissions, excessive use of natural resources, and PET plastic waste problem at the same time. However, the PET content in concrete raises a concern about its ecotoxicity, if such material is exposed to different conditions. Therefore, our research aimed to determine the ecotoxicity of PET microplastic–containing concrete and compare it with the ecotoxicity of conventional concrete. The ecotoxicological tests using test organism duckweed (*Lemna minor*) and concrete leachates were performed according to standards. The first results of ecotoxicological tests indicate that the ecotoxicity of concrete with PET microplastics is similar to that of conventional concrete. It was found that the presence of PET microplastics in concrete showed no increase regarding the inhibition of duckweed growth and the content of chlorophyll a and b in its fronds. These confirm and add to the potential of the actual use of PET microplastic in building materials, such as concrete.

Detection of microplastics in sea salt by SWIR-HIS: pollution control in the Mediterranean Sea as a case study

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Microplastics represent 80 % of the marine waste, becoming one of the main problems worldwide today, one of the reasons they have been categorised as the 10th greatest threat in the World Economic Forum's Global Risks Report 2024. To address this issue, many recognised organisations have developed action plans for monitorization, mitigation and prevention of microplastic contamination. Aligned with these initiatives, the presented study shows an innovative analytical methodology based on the application of hyperspectral imaging (HSI) together to machine learning methods to monitor the presence of these pollutants in the Mediterranean Sea, specifically in the region of Almeria (Spain). For this purpose, salts sampled from solar saltworks instead of Mediterranean seawater were analysed, since solar saltworks act as a high-scale natural "pre-concentrators" of solid pollutants in water, including microplastics, thus salts can be considered as an indicator of their presence in marine environment. All of them were crushed to reach microplastics size and to homogenise particle size. Natural salt samples and microplastics (PE, PET, PS, PP, PVC) materials were analysed and as a result, a hyperspectral image of each one was obtained. Then, the region of interest (ROI) of each sample was selected. As exploratory analysis, hierarchical clustering analysis (HCA) was performed using the ROI average spectrum of sea salt samples and microplastics standard materials. In addition, partial least square discriminant analysis (PLS-DA) was applied to build a classification model capable of discriminate between ROI pixel-spectral which contain salt or any kind of microplastic. Once the model was trained and validated it was applied to detection of microplastics in salts from coastal saltworks.

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Reuse of PVC plastic waste to reduce microplastics occurrence in the environment

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Today, plastic plays an important role in modern society, with global production surpassing 400 million tonnes in 2023. 9% of plastic is recycled, with the rest usually incinerated, landfilled or mismanaged. Plastic incineration can present a source of harmful pollutants (dioxins, furans), while improper disposal and mismanagement are some of the main reasons why plastic ends up in the environment. Plastic particles can be exposed to different conditions, becoming brittle and breaking down into smaller particles – microplastic and nanoplastic – which can contaminate water, soil, and air. Nanoplastic appears to be even more harmful than microplastic since it can be ingested, inhaled or toxic additives (plasticisers) can leach out from the surface of the plastic particles. Polyvinyl chloride (PVC) plastic accounts for approximately 13% (53 million tonnes) of global plastic production. It is often used for pipes, window frames, packaging, and cable insulation. It is known that plasticisers (such as Bisphenol A) leaching from PVC microplastic present a significant environmental stressor and are an ongoing environmental concern. One of the most promising approaches to reducing the amount of PVC microplastic is its reuse in construction materials, i.e. concrete. Adding PVC microplastic to concrete as an additive or partial substitute for natural aggregate (sand, gravel) would at the same time contribute to PVC plastic waste reduction in the environment and natural resources consumption reduction. However, the mechanical properties and ecotoxicity of PVC-containing concretes must be assessed, to ensure that they comply with construction requirements and to avoid inadvertently causing more damage to the environment. The aim of our research is to evaluate PVC-containing concrete compared to conventional concrete in terms of ecotoxicological aspects. Therefore, the assessment of the ecotoxicity of PVC microplastic-containing concrete leachates using standard ecotoxicity tests with different test organisms will provide an insight into the feasibility of such a material for the future.

The use of magnetically modified microplastics in environmental studies

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In the past decade, microplastics (MPs) have received attention due to their widespread presence and their potential effects on the environment and health. Conducting various environmental research on MPs remains difficult due to the small size of the particles. A potential solution for making laboratory MPs experiments easier is to use modified MPs that are easy to retrieve and track. The main objective of our research was to investigate stability, potential use, and recovery of magnetically modified MPs. We conducted experiments on PE, PP, PET, and PVC particles that were magnetically modified using magnetic iron oxide particles. First, we determined the iron (Fe) content on MPs (1.85–2.82 wt.%) and evaluated potential leaching of Fe from particles at different pH levels. Leaching was minimal in the neutral pH range. In addition, no statistically significant impact was observed when evaluating potential toxicity on specific growth rate, root length, and the content of photosynthetic pigments of duckweed *Lemna minor*. In the next step, the recovery efficiency of capturing the magnetic MP particles from aqueous solution (by magnet) was tested. The recovery rate of MPs remained very high (above 90 %) even after 10 repeated experiments with the same particles. Recovery potential was further tested in sediment matrix of two different size ranges (2.5 mm and 4-8 mm). The recovery rate from sediment with bigger size range was slightly higher, but recovery rates from both sediment matrixes were in all tries above 80 %. These findings suggest that the use of magnetically modified MPs represent a promising approach in various environmental studies, which will provide valuable insights for future environmental studies and remediation strategies.

Microplastics and poultry

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Microplastics are found in various animals, e.g. poultry, and can thus enter the food chain. Poultry meat is an important source of protein and also a potential route for microplastics to enter the human body. Microplastics are found in all ecosystems, including agricultural systems, where they can contaminate feed, water and the environment in which chickens are raised. This raises questions about their impact on animal growth, metabolism and overall health. In addition, the potential transfer of microplastics in poultry meat poses a risk to the production of safe food and potential transfer to humans, reducing the economic efficiency of chicken farming. The aim of the study was to sample feed, water and parts of the digestive tract of chickens and to search for polymer particles in the samples obtained. For this purpose, methods for tissue degradation, transfer of microplastics to filters and characterization of microplastics based on chemical analysis, size and color were used. The study of microplastics in the food chain is important for the development of sustainable agricultural practices with less impact of microplastics on the food chain and the assessment of the potential risk of microplastic transfer to humans.

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Effects of BP-3 additive on the formation of biofilm on polyethylene microplastics in a freshwater system

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Benzophenone-3 (BP-3) is commonly used additive in plastics to protect against UV radiation. It can leach from plastics into freshwater ecosystems, where it persists and poses a risk to aquatic life and human health. The aim of this study was to investigate whether the presence of BP-3 in polyethylene microplastics affects biofilm formation in a simulated freshwater environment. Therefore, two types of microplastics were prepared: PE microplastics (MP) and PE microplastics with 3% BP-3 (MP/BP-3), as described in previous research. Environmental aging was simulated in natural stream water. The incubation was carried out for 4 weeks under controlled laboratory conditions. The biofilm formed on MP and MP/BP-3 was analysed in terms of the amount of biofilm, chlorophyll *a* content, extracellular polymeric substance (EPS) content. The change in the density of microplastics after ageing was also evaluated. The results showed that the biofilm parameters on aged MP/BP-3 differed significantly from those on aged MP. Namely, 38 % less biofilm, 63 % less chlorophyll *a* and 91 % less EPS content were measured on MP/BP-3 compared to MP. Consequently, the density of microplastics after aging also differed significantly: the density of aged MP/BP-3 was for 11 % lower than that of aged MP. In conclusion, BP-3 negatively affects biofilm formation on microplastics in freshwater. This can affect the properties, behaviour and fate of microplastics in the aquatic environment. Given the persistence and toxicity of BP-3 in the environment, further research is needed to assess the long-term ecological impacts and potential risks to the aquatic environment and human health.

A SAXS and MD study of fluorinated alcohols inspired by environmental concerns regarding PFAS

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Per- and polyfluoroalkyl substances (PFAS) are a highly useful group of chemicals in technical and industrial applications that exhibit characteristic physicochemical properties due to the presence of fluorine atoms in the hydrocarbon chain but unfortunately pose significant environmental risks. Our primary focus is on nanoplastics in aqueous environments, specifically examining their structure, migration, transport and remediation. In this context, we also investigate the transport of PFAS mediated by the migration of nanoplastics. This process is strongly influenced by the interactions between nanoplastics and PFAS. One of the first critical steps in these studies is to gain a better understanding of the structure and dynamics of PFAS in bulk and various solvents at the molecular level. Therefore, we perform a structural study of simple pure liquid fluorinated alcohols as simplified models for PFAS. Here, we use molecular dynamics (MD) simulations and further theoretical calculations of small- and wide-angle X-ray scattering (SWAXS) intensities using the complemented system approach method to interpret the experimental SWAXS data. The MD simulations are performed with the AMBER, CHARMM, GROMOS-UA, GROMOS-AA, OPLS and TraPPE force fields. The comparison between the calculated and the experimental SWAXS data serves firstly as a robust structural test of the model used and secondly provides insights into the structure of the investigated liquid model system at the molecular level.

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Using TED-GC/MS for the analysis of microplastics and tyre wear particles in environmental matrices

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Microplastics have been detected in various environmental compartments across the globe and are discussed as emerging pollutants due to their resistance to biodegradation and their bioaccumulative properties. However, to investigate which pathways microplastics take in the environment and to determine future environmental limit values, reliable analytical methods are required. TED-GC/MS (thermal extraction desorption gas chromatography mass spectrometry) is a new analytical method that allows the qualification and quantification of plastics. A method for the simultaneous determination of tyre wear particles (styrene-butadiene rubber (SBR), and natural rubber (NR)), polyethylene (PE), polypropylene (PP), polystyrene (PS) and polyethylene terephthalate (PET) in aqueous and solid environmental samples was developed and validated. This included the development of a suitable sample preparation strategy, as well as the preparation of cryomilled mixed standard to ensure a more efficient calibration of multiple polymers.

Novel process design for quantifying secondary microplastics formed during accelerated weathering of plastic-based materials

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In recent years, significant progress has been made in understanding the formation, properties, distribution, and impacts of microplastics (MPs), but many questions remain. Secondary MPs are known to be more abundant in the environment, but less controllable. They could be reduced by introducing regulatory restrictions and limits, similar to those for primary MPs. However, methods and techniques to quantify the formation of secondary MPs from plastic-based materials during their service life are still lacking. The objective of this study was to develop a process design that would allow the release of MPs during accelerated weathering of plastic-based materials to be collected and quantified. The process design was developed by integrating a sieve system (smallest mesh size 20 µm) into the QUV accelerated weathering tester. Two plastic-based materials were tested according to the developed process design: recycled polypropylene (rPP) and wood plastic composite (WPC) consisting of 60 % rPP and 40 % wood particles. The results showed that the mass of MPs collected in the sieves was relatively small for rPP, but significant for WPC, reaching a cumulative MPs release of 9.4 g/m^2 after 2 months of accelerated weathering. The gravimetric and surface analysis of the weathered rPP suggested that the MPs could be smaller than 20 µm, therefore, most of them passed through the sieves and ended up in the effluent. The developed process design can be used to evaluate the release of MPs, but further analysis of the effluent is required to quantify the exact amount. The process design has the potential to be used as a tool to quantify the release of MPs during weathering, facilitating the introduction of regulatory limits in the future.

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The influence of polystyrene nanoplastics on the uptake and distribution of elements in tomato plants

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Climate change and population growth escalate agricultural challenges, increasing water scarcity. Reusing treated wastewater for plant irrigation presents a sustainable solution, but the risks associated with contaminant uptake in edible plants remain unknown. Nanoplastics (NPs), ubiquitous in all environmental compartments, pose a significant concern due to their small size, allowing them to penetrate plant tissues. Additionally, NPs can influence the mobility and bioavailability of other pollutants, such as heavy metals. To fully assess the risks posed by NPs along with other potentially toxic elements (PTEs) in the human food chain, quantitative research on their uptake and translocation is essential. In this study, hydroponically grown tomato plants were exposed to polystyrene NPs doped with europium (PS-Eu NPs, 200 nm, 1 mg/L), either alone or in combination with isotopically enriched elements: 53Cr, 70Zn, and 204Pb (0.1 mg/L), and 106Cd (0.05 mg/L). After five weeks of exposure, harvested plant samples (roots, stems, leaves, fruits) were analysed using ICP-MS. By assessing the stability of PS-Eu NPs under exposure conditions, we established a correlation between the concentrations of Eu and PS-NPs, allowing Eu to be used as a proxy for NPs concentration. The results showed that PS-Eu NPs were present in all parts of the tomato plant, with the highest accumulation in the roots, followed by translocation to the leaves, stems, and fruits. The presence of PS-Eu NPs influenced the uptake and distribution of PTEs, with no impact on Cd and Zn concentrations, but a decrease in Cr and Pb levels, likely due to steric effects, their adsorption on NPs, and/or precipitation. Changes in the uptake of other elements present in the nutrient solution were also observed. Consuming tomatoes grown under the studied conditions could lead to excessive intake of toxic Cd, posing potential health risks, while the unknown effects of NPs remain a concern.

Selective labelling of small microplastics with SERS-tags and application in environmental samples

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Microplastics pollution is being unanimously recognized as a global concern in all environments. Routine analysis protocols foresee that samples, which are supposed to contain up to hundreds of microplastics, are eventually collected on nanoporous filters and inspected by micro-spectroscopy techniques like micro-FTIR or micro-Raman. All particles, whether made of plastic or not, must be inspected one by one to detect and count microplastics. This makes it extremely time-consuming, especially when Raman is adopted, and indeed mandatory for the small microplastic fraction. Inspired by the principles of cell labelling, the present study represents the first report in which gold nanostars (AuNS) are functionalized to act as SERS-tags and used to selectively couple to microplastics. The intrinsic bright signals provided by the SERS-tags are used to run a quick scan over a wide filter area with roughly 2 orders of magnitude shorter analysis time in respect of state-of-the-art in micro- and nanoplastics detection by μ-Raman. The applicability of the present protocol has been validated at the proof-of-concept level on both fabricated and real offshore marine samples. It is indeed worth mentioning that a SERS-based approach is herein successfully applied on filters and protocols routinely adopted in environmental microplastics monitoring, paving the way for future implementations and applications.

Assessing the toxic effects of polypropylene and pyrene on mussels at environmentally relevant concentrations: a multi-biomarker approach

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Presented study investigates the effects of aged polypropylene (PP) microplastic and pyrene on the *Mytilus galloprovincialis* focusing on the early toxic effects after exposure to environmentally relevant concentrations. Polypropylene and pyrene were selected as both are common pollutants found in the coastal waters, and PP microplastics can adsorb PAHs in the seawater. MPs affect mussels via inflammatory response, bioenergetic disruption, chemical leaching and transfer of pollutants, while pyrene causes oxidative stress and DNA damages. The effects of a 14-days of exposure to virgin and laboratory-aged PP particles (~ 40 µm, 1 mg L^{-1}) with the addition of pyrene (50 µg L^{-1}) at 20 °C and defined feeding regime were evaluated through various endpoints including biomarkers of oxidative stress, antioxidative capacity, neurotoxicity and DNA damage after 7 and 14 days of exposure. Digestive gland and gills were used as indicator organs. Additionally, susceptibility of mussels to PP and/or pyrene was assessed by measuring respiration and heart rates. Among the most sensitive outcomes were the respiration rate of mussels towards aged PP, and changes in heart rate to pyrene and pyrene adsorbed to aged PP. Biochemical biomarkers showed variable trends between different exposures. These findings emphasize the complexity of responses towards simultaneous effects of MPs and adsorbed pollutants on biota. Moreover, studies simulating environmental conditions are needed due to unprecedented decline of mussels from their natural beds.

Spatiotemporal dynamics and trophic transfer of microplastics in some endemic fish species of Lake Victoria

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Microplastics (MPs) are a complex suite of environmental contaminants which may enter aquatic food chains by inadvertent ingestion, branchial intrusion, prey misidentification by a predator or trophic transfer. However, little is known about the predictive traits and the extent to which fish biomagnify or transfer MPs in food webs of freshwater ecosystems. We investigated the spatiotemporal variations, possible biomagnification and trophic transfer of MPs in three fish species (*Rastrineobola argentea*, *Protopterus aethiopicus* and *Synodontis victoriae;* n = 54) from Ripon Falls, Katosi and Port Bell fish landing beaches of Lake Victoria, Uganda. No MPs could be detected in whole samples of the agastric *R. argentea*, edible muscles and gills of *P. aethiopicus* and *S. victoriae*. The gastrointestinal tracts (GIT) of *P. aethiopicus* and *S. victoriae* had 145 and 169 MPs, with the highest mean numbers (16.0±7.8 and 14.7±6.7 items/fish taxon) recorded in dry season samples from Ripon Falls, respectively. Two Way-ANOVA revealed that sampling location had a significant effect on the number of MPs recovered in *S. victoriae* when the data were standardized to items/g of GIT ($P<0.05$). Blue and brown-colored 0.3–0.9 mm fragments and filaments made of nylon 11, polyamide and polypropylene were the most dominant MPs according to µATR-FTIR results. Pyrolysis-gas chromatography-mass spectrometry quantified polypropylene and nylon 66 as the most abundant polymers. While we cannot completely rule out biomagnification and trophic transfer (especially for MPs <0.25 mm), no direct evidence supporting that they occur were found in the present study. These results support the hypothesis that a multispecies approach, trophic position and individual fish traits are important aspects for a comprehensive understanding of the complex microplastic dynamics in freshwater ecosystems.

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Advancing microplastic detection: non-destructive 3D imaging of microplastics in zebrafish using micro x-ray computed tomography

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Plastic pollution is a global environmental issue, and microplastics (MPs) pose increasing concerns due to their potential impact on ecosystems and human health. MPs enter organisms through ingestion and inhalation and accumulate in various tissues. Understanding their distribution within biological systems is crucial for assessing their effects, yet current detection methods face significant limitations. Conventional spectroscopic and pyrolytic techniques provide chemical composition data but require extensive sample preparation, leading to a loss of spatial distribution information and increased contamination risks. To address these challenges, this study explores the application of X-ray micro-computed tomography (microCT) as a non-destructive method for detecting MPs in biological tissues. Using zebrafish as a model organism, we demonstrate how microCT enables precise 3D visualization of MP localization without altering tissue integrity. By optimizing scanning parameters and employing contrast-enhancing techniques, we successfully detected polyethylene MPs within fish intestines. Our findings highlight the potential of microCT for tracking MP accumulation in biological samples, offering a complementary approach to conventional methods. MicroCT's ability to retain spatial information presents new opportunities for studying MP behavior in biological systems. This technique could be further developed for environmental monitoring and biomedical research, providing insights into the potential health risks of MP exposure. Future work will focus on refining detection capabilities for MPs of different sizes, densities, and shapes, expanding the applicability of microCT in microplastic research.

Impact of sampling techniques on microplastic concentration: A comparative study of manta trawl and grab sampling in the north-east Atlantic ocean

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A lack of standardization in monitoring protocols has hindered the accurate evaluation of microplastic (MP) pollution in the open sea and its potential impacts. As sampling techniques significantly influence the amounts of MPs contained in the sample, the aim of this study was to compare two sampling methods: Manta trawl (size selective approach) and grab sampling (volume selective approach). Both approaches were applied in the open sea surface waters of the North-east Atlantic Ocean. Onshore sample processing was carried out using the innovative tape lifting technique, which affords a series of advantages, including prevention of airborne contamination during analysis, without compromising integrity of the results. The results obtained indicated an MP concentration over four orders of magnitude higher using grab sampling compared to the Manta net approach (mean values equal to 0.24 and 4050 items/m3, respectively). Consequently, the sole quantification of MPs using results obtained with the Manta trawl resulted in a marked underestimation of abundance. Nevertheless, the grab sampling technique is intricately linked to a risk of collecting non-representative water volumes, consequently leading to an overestimation of MPs abundance and a significant inter-sample variability. Moreover, the latter method is unsuitable for use in sampling larger MPs or in areas with low concentrations of MP pollution. The optimal sampling method therefore is dependent on the specific objectives of the study, often resulting in a combination of size and volume selective methods. The results of this study have the potential to contribute to the standardization of monitoring protocols for microplastics, both during the sampling phase and sample processing.

Detection of natural and synthetic fibers in the core of giant hailstones, using eSEM-EDS and Raman spectroscopy

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Hail storm is a known weather hazard that forms high in the troposphere, in the highest parts of the cumulonimbus cloud. Under conditions of prolonged duration and intensified updraft, giant hailstones can form, causing destruction on impact with the ground. In a recent study, we delve into the intricate influence of microscopic fragments, both biotic and abiotic in nature, which influenced the growth of unusually large specimen. By meticulously examining the distribution of various fragments found within the hailstones, we offer a fresh insight into its formation. Our research involved melting and examining concentric layers of giant hailstones that fell in a small region of Slovenia in 2019. We analyzed the biotic and abiotic constituents that may have contributed to their formation, among other techniques also environmental scanning electron microscopy (eSEM) combined with energy-dispersive X-ray spectroscopy (EDS) and Raman spectroscopy were used. Notably, our findings revealed the presence of natural and synthetic fibers concentrated at the cores of the hailstones. The results indicate that variations in hailstone composition and the diversity of embedded materials are critical factors for future research. Moreover, evidence suggests that anthropogenic microfiber pollutants played a significant role in the formation of the giant hailstones analyzed in this study.

Fragmentation techniques and their impact on the biotic aging of microplastics

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When microplastics (MPs) enter the environment, they undergo different biotic and abiotic aging processes that can change their chemical and physical properties and as a result alter their interaction with the environment. For laboratory studies of biotic aging processes, it is important to use test materials that mimic those found in the environment. Top-down fragmentation methods appear to produce environmentally relevant MPs. The chemical and physical properties of the produced MP may vary depending on the fragmentation method used. The aim of this study was to investigate the influence of two different fragmentation techniques on the biotic aging of polystyrene (PS) and polypropylene (PP) MPs. PS and PP MPs were produced with two different mills (ultracentrifugal and mixer mill) and were biotically aged in natural stream water under controlled laboratory conditions for 16 weeks. The differences between MPs produced by two different milling techniques were evaluated by analysing the amount of biofilm, chlorophyll *a* content, content of extracellular polymeric substance (EPS), morphology, changes in particle density and crystallinity. The results showed that the biofilm parameters differed between used mills. The amount of biofilm, the concentrations of EPS and chlorophyll *a*, were higher in PS MPs prepared with the ultracentrifugal mill. For the PP MPs, the amount of biofilm was lower in those prepared with ultracentrifugal mill, whereas both the EPS and chlorophyll *a* contents were higher. Morphological analysis revelled clear differences between the used mills. The difference in densities were observed only between aged samples prepared with ultracentrifugal and mixer mill. In the case of crystallinity, a significant difference was only observed in aged PP samples, as all tested PS MPs were amorphous. The results of this study showed clear influences of MPs preparation on the biotic aging processes and provided valuable insights for development of test MPs materials.

Application of respirometry to monitor the biodegradation of polymer materials

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In an effort to reduce plastic pollution, the European Union has been steadily refining its regulatory frameworks and standards to promote the bioeconomy and circular economy. The umbrella standard governing the field of materials that are considered biodegradable or compostable is EN 13432. Bioplastics can be naturally biodegradable in various ecological environments such as compost, sewage wastewater, sea or algae environments of sweet or salt waters. For this reason, it is very important to have suitable laboratory methods and equipment to simulate different degradation conditions. Measuring the biodegradation of natural and synthetic polymers by respirometry relies on microorganisms in the inoculum media, which act as natural sensors to detect and determine the degradation process. Advanced analytical methods following standardized measurement protocols increase the efficiency of biodegradation analysis, reduce complexity and eliminate time-consuming titrations typical of classical respirometric approaches. ECHO Instruments multi-channel respirometry systems enable the rapid and efficient analysis of multiple samples simultaneously. These systems allow for the laboratory-scale simulation of various natural environments, enabling the monitoring of complete mineralization of biodegradable polymers through CO_2 and gas measurements. As well, the degradation of microplastic particles made from biodegradable materials is possible to determine. In this study we have analyzed the Symbiotic Culture of Bacteria and Yeast (SCOBY), natural biofilm polymer produced by the symbiotic relationship between bacteria and yeast. SCOBY primarily consists of bacterial cellulose, a natural polymer formed during the fermentation of kombucha as a byproduct of microbial activity. Scoby has excellent physicochemical and mechanical properties and has found numerous applications in packaging materials, textiles, food, biomedical applications, metal ions removal, electronics. In the respirometer, SCOBY was found to produce a similar amount of CO_2 as micro-cellulose in twice the time.

MICROPLASTIC*day* No. 3

Open laboratory day at UL FKKT organized by UL FKKT and microplastic researchers from the *PLANTerastics* team.

During the open lab day different methods used for microplastic characterization in laboratory research were demonstrated:

- size analysis by laser diffraction analyser,
- chemical analysis by µFTIR, and
- analysis of thermal properties by thermal analysis.

The laser diffraction analyser Microtrac Bluewave (Microtrac, Germany) is a laser-based particle size analyser developed for the precise and fast measurement of particle size distribution. During the demonstration, the particle size distribution of a microplastic sample is measured using the dry dispersion unit. The process of the analysis was demonstrated, and the observed results were discussed, focussing on the differences between number-based and volume-based distributions.

Chemical analyses are in our laboratory performed by FTIR microscope Lumos II (Bruker, Germany) that enables us measuring of samples in transmission, reflection or ATR mode. During the demonstration, we showed single particle measurement in ATR mode and chemical image analysis (used for identification of microplastics in environmental samples) in transmission mode.

The thermal properties of microplastics were analysed using the differential scanning calorimeter DSC1 from Mettler Toledo (Schwerzenbach, CH). It measures the heat flow that occurs in a sample during a controlled temperature programme and is used to identify and characterise materials by determining their glass transition, melting and crystallisation temperature, enthalpy, degree of crystallinity, heat capacity and cold crystallisation parameters. We use thermogravimetric analysis (TGA), often coupled with MS or FTIR spectrometry and gas chromatography (GC), such as the Mettler Toledo TGA/DSC3+–IST16–GC/MS system to analyse the gases produced, to determine thermal and oxidative stability, decomposition patterns and filler content. The operation of the robotised devices, the sample preparation by encapsulation and the evaluation of the measured curves were demonstrated.

Meeting of PLASTsensing consortium

The project consortium, consisting of partners from UL FKKT, TU Wien, CEITEC and RECETOX, met annually during MICROPLASTIC*days*.

The project is focused on bridging the gap between technical and biological sciences and to implement novel methodologies in conventional microplastic research. More specifically to employ state-of-the-art techniques to detect microplastics in aquatic organisms. At the meeting, the progress of each partner was presented and discussed, and future collaborative research was planned. These regular in-person meetings promote a coherent approach to the further development and success of the project.

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