MICROPLASTIC*days*

Explore the World of Microplastic Research!

16-18.4.2024

Faculty of Chemistry and Chemical Technology
Ljubljana, Slovenia

Book of abstracts

Ljubljana, 2024

This event is based upon work from COST Action PRIORITY, CA20101, supported by COST (European Cooperation in Science and Technology). COST (European Cooperation in Science and Technology) is a funding agency for research and innovation networks. Our Actions help connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. This boosts their research, career and innovation. www.cost.eu

MICROPLASTIC*days* – Book of abstracts

Published by: Faculty of Chemistry and Chemical Technology, University of Ljubljana

Editors:
Gabriela Kalčíková
Ula Rozman

The publication was published in electronic form and is available at the web address:
https://planterastics.fkkt.uni-lj.si/microplasticdays/

Ljubljana, Slovenia, 2024

This book compiles the abstracts of invited presentations given during MICROPLASTIC*days*, conducted from 16.–18. April 2024 at the Faculty of Chemistry and Chemical Technology, University of Ljubljana (UL FKKT), Slovenia. The abstracts are reproduced as submitted by the authors.

Kataložni zapis o publikaciji (CIP) pripravili v Narodni in univerzitetni knjižnici v Ljubljani
COBISS.SI-ID **201111299**
ISBN 978-961-7078-47-3 (PDF)

MICROPLASTIC*days* are aimed to come together to advance knowledge and collaboration in microplastic research, metrology, and standardization. From seminars and training sessions to presentations and discussions.Each activity offers unique insights and perspectives into the world of microplastics.

MICROPLASTIC*days* are days dedicated to advancing knowledge and expertise in microplastic research, metrology, and standardization organized by various projects, institutions, and researchers.

We bring together experts, professionals, and enthusiasts from across the field to explore innovative solutions and encourage collaboration. MICROPLASTIC*days* are more than just a gathering—it is a collaborative effort to drive positive change and make a difference in tackling microplastic pollution.

MICROPLASTIC*days* are supported by:

PRIORITY

COST EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

Funded by the European Union

UNIVERSITY OF LJUBLJANA | FKKT Faculty of Chemistry and Chemical Technology

PLANT ERASTICS

MEREL

This event is based upon work from COST Action CA20101 PRIORITY, supported by COST (European Cooperation in Science and Technology). COST (European Cooperation in Science and Technology) is a funding agency for research and innovation networks. Our Actions help connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. This boosts their research, career and innovation. www.cost.eu

Table of contents

MICROPLASTIC*day* No. 1

Training day on basic concepts of metrology and standardization organized by COST Action PRIORITY.

The training day aims to introduce basic concepts of metrology and to familiarize participants with the usefulness and simple approaches of the concept and estimation of measurement uncertainty. The production and proper use of reference materials is also an integral part of this training.

As most participants have only a basic knowledge of statistics and metrology, emphasis is placed on theory and concepts. However, this part is broken up by tasks in small groups. One of the tasks are to critically scrutinize publications for their metrological and scientific value.

The second part of the training event deals with the different approaches to measurement uncertainty calculations. Here, measurement uncertainty budgets are applied to practical examples using easily accessible tools.

Meeting of PLASTsensing consortium

The project consortium, consisting of partners from UL FKKT, TU Wien, CEITEC and RECETOX, meets 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 is presented and discussed, and future collaborative research is planned. These regular in-person meetings promote a coherent approach to the further development and success of the project.

MICROPLASTIC*day* No. 2

Seminar for metrology and standardization organized by COST Action PRIORITY.

Scientists and technicians involved in method validation and quality control need reference materials but complain about the lack of reference materials and protocols. As the field of microplastics research expands exponentially, research results often touch on highly sensitive health issues. Indeed, there is a strong call for improved and faster provision of reference materials, quality control materials and harmonized standards and measurement protocols. In order to give the sometimes impatient users a better understanding of how this very important process works "behind the scenes", PRIORITY organizes this seminar, probably the first of its kind, but timely as requests for improvement are increasing.

The aim of this seminar is to give participants who apply metrological standards an insight into the process of standardization and data comparability. The starting point is from the top level, i.e. the ISO view on how ISO standards and guidelines are developed through working groups, technical committees, etc. This follows by the view of a laboratory dealing with MP measurements in a national institution and its relationship with ISO. Another accredited laboratory at university reports on how regulations etc. are implemented through SOP development, method validation etc.

PlasticsFatE, as part of CUSP, provides insights from a large scientific project. Information on ISO standards and guides for reference material production and certification are provided by a liaison member of the TC334 former ISO/REMCO (reference material committee). Efforts to produce method validation and QC material in the laboratory or within a larger project, in the absence of reference materials, may provide useful information for the laboratories involved in microplastic measurement. The final presentation of the seminar addresses how to proceed in situations where protocols, standards, and reference materials are not available.

Program of the ***Seminar for metrology and standardization*** and abstracts of invited lectures

PRIORITY	**8:15 – 8:30**	**Opening**
Impulse lectures and discussion on international, national, regional and laboratory view of standardization of microplastic measurement procedures and terminology	**8:30 – 9:15**	**The ISO view: how an ISO standard is developed & discussion** Ulrike Braun, Umweltbundesamt, Germany
	9:15 – 10:00	**PlasticTrace contribution for standardization of microplastics analysis** Andrea Giovannozzi, INRim, Italy
	10:00 – 10:30	**Coffee break**
	10:30 – 11:15	**The laboratory and user view: how measurement procedures and SOP are developed in the lack of standards & discussion** Aleksandra Tubić, University of Novi Sad, Serbia
	11:15 – 12:00	**PLASTICSFATE contribution to standardization of microplastic research** Damjana Drobne, University of Ljubljana, Slovenia
	12:00 – 13:30	**Refreshment**
Production of reference, quality control and testing materials for microplastic method development, method validation and verification	**13:30 – 14:00**	**Production of reference materials an ISO view** Thomas Meisel, Montanuniversität Leoben, Austria
	14:00 – 14:45	**Production and characterisation of testing materials for health studies and quality control - a laboratory view** Luke Parker, Dutch Organization for Applied Scientific Research, Netherlands
	14:45 – 15:15	**Coffee break**
	15:15 – 15:45	**Good laboratory practice in the absence of ISO and national standards** Thomas Meisel, Montanuniversität Leoben, Austria
	15:45 – 16:30	**Discussion & Recommendations**
Afternoon social program	**16:30 – 18:30**	**Social event at the Faculty of Chemistry and Chemical Technology**

The ISO view: how an ISO standard is developed & discussion?

Ulrike Braun

ulrike.braun@uba.de

German Environment Agency, Berlin, Germany

Plastics and microplastics in environment are issues of public and political concern. There is a large number of activities at international level, e.g. G7, UN, OECD, WHO. It was and is the main motivation to protect the marine environment as the final sink from plastics that are difficult to degrade. Since 2009, scientific publications on this topic have increased significantly. Although so many data are available on the topic, a valid assessment of the situation is not possible due to the non-comparable methodological approaches.

Microplastics are found in aquatic, terrestrial, atmospheric and biological samples as well as in food products or waste materials, such as compost or sewage sludge. Unclear is, which are the main entry paths, how transport pathways contribute to the distribution between the environmental media and what happens to the particles in the final sink. The risks that these particles pose to human health and the environment are still unknown. Nevertheless, this information is a prerequisite for being able to carry out preventive measures at the right place.

Standardized methodological approaches are needed to generate a valid and uniform database. Since unwanted plastics in the environment represent an international challenge, the ISO offers a good working basis for this purpose.

This presentation shows how the joint working group "Microplastics" (JWG 1) of ISO/TC/147 SC 2 managed to bring together experts from different scientific and technical fields on an international level. The JWG consists of members of the Technical Committees (TC) of ISO/TC 61 "Plastics" and ISO/TC 147 "Water quality", who together made a first substantial contribution to harmonising the problem of microplastics analysis in water. The present results in form of a series of ISO standards with various parts, addressing the analysis of microplastic in water with very low or low content of particulate matter, should become an example for further necessary standardisation processes regarding the assessment of microplastics in the environment.

PlasticTrace contribution for standardization of microplastics analysis

Andrea M. Giovannozzi

a.giovannozzi@inrim.it

Quantum Metrology and Nanotechnology Division, Istituto Nazionale di Ricerca Metrologica (INRiM), Strada delle Cacce 91, 10135, Torino (Italy)

Plastic pollution is recognised as a severe anthropogenic issue globally, where complex physico-chemical transformation processes (such as aging, degradation and fragmentation) producing MPs and, subsequently, NPs. These processes occur during production, consumer use, waste processing, as well as through environmental process after vehicles/industrial emissions. Several studies have reported the occurrence, analytical methods and toxicity of larger MPs (>100 µm) in the environment and food matrices. However, MPs (< 100 µm SMPs) and NPs (< 1 µm) in natural systems have been overlooked, primarily due to significant methodological challenges associated with their micro- and nano-specific properties. The present project, PlasticTrace (https://plastictrace.eu/), aims to address the urgent need for the development and harmonisation of methods for the chemical identification, physical characterisation and quantification of released small micro/nanoplastics (SMPs/NPs) in drinking water, food and environmental matrices, as required by the EU's Circular Economy Action Plan (CEAP). In this context, hyphenated and complementary analytical approaches will be developed, optimised, compared and harmonised, leading to the establishment of metrological traceability of measurements through inter-laboratory comparison validation studies. Novel and environmentally relevant SMP/NP reference materials will be developed within the project (e.g. Polyethylene terephthalate (PET), Polyethylene (PE)). International cooperation with key stakeholders such as EURAMET's European Metrology Networks (e.g. "Network for Safe and Sustainable Food" and "Pollution Monitoring"), standards developing organisations (e.g. ISO TC 229, ISO TC 61, and CEN TC 249) and end users (e.g. food and drink producers, environmental monitoring programmes and health experts) will be considered as the basis for a European Metrology network. The need for efficient and reliable measurement infrastructure is required in support of (i) the European Chemicals Agency (ECHA)'s proposed restriction targeting intentionally added MPs in consumer products, (ii) the Marine Strategy Framework Directive (MSFD) which requires specific thresholds for litter types after harmonisation of the methodology, (iii) the new Drinking Water Directive (DWD) that mentions MPs explicitly, and (iv) the new Circular Economy Action Plan (CEAP) adopted in March 2020. In particular, the CEAP promotes circular economy processes, encourages sustainable consumption, and aims to ensure that waste is prevented. However, to support the CEAP and reduce plastic contamination, methods for SMP/NP identification in food and environmental matrices are needed. These methods need to be metrologically validated using appropriate reference materials to establish harmonised and traceable measurements of SMPs and NPs.

The laboratory and user view: how measurement procedures and SOP are developed in the lack of standards & discussion

Aleksandra Tubić

aleksandra.tubic@dh.uns.ac.rs

University of Novi Sad, Faculty of Sciences, Trg Dositeja Obradovića 3, 21000 Novi Sad, R. Serbia

Analysis of different substances in routine laboratory work involves the application of standard operating procedures (SOP) for the analytes for which they have been developed. SOP presents a step-by-step instructions compiled by an organization to help workers carry out routine operations. They are usually based on standard methods issued by relevant organizations. However, there are substances, such as microplastics, for which there are still no widely accepted standard methods. Additionally, reference materials for the analysis of microplastics are still in the development phase. In this situation, laboratories, if they develop their own methods, need to take care of all aspects of quality assurance and quality control (QA/QC). First of all, the laboratory needs to define which polymer types are of interest, taking into account the available analytical equipment, and the type of results that can be obtained (e.g., number of particles, amount of particle color/size/weight, or particle visual coverage). The limitations of the methods used must be taken into consideration, as well as specific regulations and requirements. Matrix-related issues need to be addressed. It is necessary to define which matrix is being analyzed, as well as their properties that can affect the analysis (e.g., organic matter content), as well as whether interfering substances are expected and if they need to be detected and quantified. The laboratory also needs to try to determine the expected concentration levels. QA/QC will require determining at least the following parameters: limit of detection, limit of quantitation, selectivity, working range, analytical sensitivity, trueness, precision, measurement uncertainty, robustness, as well as calibration function/linearity. Thus, laboratories need to be aware of the required quality even if there is no standard method for the analysis of certain parameters. This implies identifying and evaluating relevant performance characteristics and checking them against the analytical requirement.

PLASTICSFATE contribution to standardization of microplastic research

Damjana Drobne

damjana.drobne@bf.uni-lj.si

University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, 1000 Ljubljana

Several regulations and directives are shaping the European landscape on plastics, focusing on reducing plastic waste, enhancing recyclability, minimizing environmental impact and assessing their potential impacts on human health and ecosystems. The key strategy is The EU's plastics strategy, adopted in January 2018, that is part of the broader EU circular economy action plan. This strategy focuses on protecting the environment and reducing marine litter, greenhouse gas emissions, and dependence on imported fossil fuels. But to comply with existing test regulations and strategies, test systems are needed to ensure that all materials, including plastics, meet stringent safety standards, protecting both consumers and the environment. In line with this, the EU has approved new and updated test methods for the regulatory safety testing of chemicals under REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals). These methods are part of the EU's commitment to ensuring that all materials, including plastics, meet stringent safety standards. Our research group has lead preparation ISO/TS 4988:2022 - Nanotechnologies — Toxicity assessment and bioassimilation of manufactured nano-objects in suspension using the unicellular organism *Tetrahymena sp*. In EU-funded PlasticsFatE project, this standard has been successfully upgraded by incorporating of innovative cytotoxicity endpoints to assess hazard of micro- and nanoplastics. By analyzing endpoints at various levels of biological complexity—from subcellular alterations to population-level changes—one could get deeper understanding of the diverse possible modes of action of the tested material. Such data not only enrich the scientific discourse but also contributing for policy-making and regulatory frameworks concerning microplastic pollution.

Production of reference materials - an ISO view

Thomas C. Meisel[1]

thomas.meisel@unileoben.ac.at

General and Analytical Chemistry, Montanuniversität Leoben, Leoben, Austria

Scientists working in the field of microplastic measurement are aware of the need for reference, test and calibration materials for method development, method validation and calibration purposes. However, when searching the literature via WebOfScience using: number of publications with reference material in the abstract (TI=(microplastic*)) AND ((TO=(environment))) AND ((TO=(analysis OR determination OR quantification OR measurement)) AND (AB=(reference material))) and comparing the results with the number of publications containing "microplastic*" with the term "reference material", the ratio is only between 0.01 and 0.012. The low value reflects either a) incorrect terminology used, b) ignorance of the existence of reference materials (RM) or most likely c) the lack of commercially available RM. The ISO committee on reference materials ISO TC334 (formerly ISO/REMCO) is responsible for promoting the international harmonisation and promotion of reference materials, their production and use through the establishment of definitions and the preparation of standards (i.e. written documents, NOT reference materials) for technical committees for making reference to reference materials in ISO documents.

However, there is a paucity of CRMs available for MP research and routine work, and those that are available are certified for very specific properties. As the production of certified RMs is always behind cutting-edge research in providing appropriate materials for method development and method validation, test materials and in-house RM are produced. However, ISO TC334 also provides guidelines on how to produce and characterise them (ISO Guide 80:2014). One crucial aspect of all types of RM is the homogeneity of the materials. In addition, other considerations must be taken into account when procuring or preparing RM or testing materials. For instance, the provided documentation and certification reports (in the case of CRMs) should include comprehensive information about the polymer type (spectra), alterations, morphology, additives, size distribution, and so forth. It is crucial to acknowledge that (C)RM are certified or prepared due to their ease of characterisation. However, it is important to consider that these materials may not be representative of the actual material being studied.

Production and characterisation of testing materials for health studies and quality control - a laboratory view

Luke A. Parker

luke.parker@tno.nl

TNO Environmental Modelling, Sensing and Analysis, Princetonlaan 6, Utrecht 3584 CB, The Netherlands

In order to better understand the impact of micro- and nanoplastics on human health we require more environmentally relevant test materials than the currently used polystyrene microbeads. Particles need to be more diverse in polymer type, shape and size whilst also having a more representative surface chemistry – specifically avoiding the use of surfactants. This is, however, is easier said than done. In this talk, work carried out within the Momentum project on producing more representative micro- and nanoplastic test materials is discussed. Using this method, size reduction is achieved through a two-step (cryogenic) milling process, followed by fractionation using a combination of sieving and sedimentation to achieve several narrow size fractions from <1 µm up to 180 µm. The importance of comprehensive characterisation of test materials through techniques such as light scattering, electron microscopy, x-ray fluorescence and Raman spectroscopy are discussed, as well as some of the challenges that are faced. Finally, the important role that suspension media plays in creating a stable test material matrix is discussed. Examples from the Brigid project of the effect of various liquids on particle stability are presented along with promising routes to predict stable particle suspensions.

This research was supported by funding from ZonMw and Health-Holland (MOMENTUM project, grant agreement No 458001101) and PlasticsEurope (Brigid Project).

Good laboratory practice in the absence of ISO and national standard

Thomas C. Meisel

thomas.meisel@unileoben.ac.at

General and Analytical Chemistry, Montanuniversität Leoben, Leoben, Austria

Scientists in the field of microplastic analysis constantly call for "standardization", "harmonization" and "reference materials". Currently, only 11 ISO standards are available, compared to 37 for nanoparticles. However, even if more were available, would the availability of written standards improve data quality? Is the call for reference materials and standards an excuse?

The situation is not going to change, as standardisation bodies have always lagged behind cutting-edge science. Therefore, scientists need to properly validate their measurement procedures, which helps to understand the limitations of the developed methods. What is also noticeable is the lack of results without associated measurement uncertainties. The latter is particularly important for modelling and toxicological effects studies related to microplastic research.

The lack of fit-for-purpose reference materials, i.e. with properties similar to those of the sample, can be overcome by the production of in-house reference materials, preferably following ISO guides (TC334). Different approaches can be followed, namely bottom-up (synthesis of polymer particles) and top-down (fragmentation of larger particles). The obtained test materials/inhouse reference materials can be characterised either only in-house or better by interlaboratory comparisons.

The in-house materials can be used as 'positive controls' for measurement procedure optimisation and method validation. In this approach, particles with known characteristics are spiked at different steps of the procedure.

A very effective way of validating measurement results is to compare results obtained from independent measurement principles (e.g. vibrational spectroscopy or thermogravimetric methods). Several attempts have been published with mixed results, but it will be essential to bridge the two complementary methods for a better understanding of the measurement results.

Seminar on trends in microplastic research organized by UL FKKT and microplastic researchers from the *PLANTerastics* team.

Microplastic research has significantly advanced in recent years and has led to a better understanding of the sources, distribution, and effects of microplastics. Despite this progress, there is still a growing need for innovative approaches. Standard techniques have provided a solid foundation, but to deepen our understanding it is essential to think outside the box and explore new methods. There are still significant gaps in our knowledge, particularly with regard to long-term environmental and health impacts. Embracing creative and interdisciplinary strategies will be key to gaining new insights, managing the complexity of microplastic pollution more effectively and developing effective mitigation strategies.

In this context, this seminar on the latest trends in microplastic research covers various topics including microplastics preparation and characterization, toxicity testing in relation to human health and the environment, and the use of novel analytical approaches that are not yet routinely applied in microplastic research but have great potential to detect microplastics in complex environmental samples and biota. The event concludes with a focused discussion on potential solutions for mitigating microplastic pollution.

Demonstration of microplastic analysis organized by Merel.

On the same day, a presentation of micro-Raman spectrometry and a demonstration of the characterization of microplastics with a Raman microscope takes place. The presentation and demonstration are carried out by representatives of the companies Merel (Slovenia) and Horiba Scientific (France). During the demonstration, the characterization of microplastic particles on a silicon membrane filter is performed using an automated workflow that includes the steps from sample placement, particle detection and morphological characterization, Raman spectrum measurement and chemical identification to particle classification and reporting.

Program of the ***Seminar on trend in microplastic research*** and abstracts of invited lectures

FKKT	**9:30 - 10:00**	**Welcome**
Preparation & Characterization	**10:00 – 10:20**	**Environmentally relevant particles in microplastic research** Gabriela Kalčikova University of Ljubljana (FKKT), Slovenia
	10:20 – 10:40	**Preparation of microplastics: The effects of preparation on microplastic properties** Stefania Federici University of Brescia, Italy
	10:40 – 11:00	**Size matters: Techniques and challenges in measuring the size of microplastics** Tina Skalar University of Ljubljana (FKKT), Slovenia
	11:00 – 11:20	**Coffee break**
(Eco)toxicity testing	**11:20 – 11:40**	**Effects of microplastics on terrestrial organisms** Anita Jemec Kokalj University of Ljubljana (BF), Slovenia
	11:40 – 12:00	**Investigating the impacts of micro and nanoplastics on humans: experiences from the EU Project PlasticsFatE** Damjana Drobne University of Ljubljana (BF), Slovenia
	12:00 – 12:20	**Impact of microplastics on water flea: what can we learn for future testing?** Dana Kühnel Helmholtz Centre for Environmental Research, Germany
	12:20 – 13:20	**Refreshment**
Analytics	**13:20 – 13:40**	**The use of laser-based spectroscopy for analysis of environmentally relevant microplastics** Pavlina Modlitbova Central European Institute of Technology, Czech Republic
	13:40 – 14:00	**An approach to determine amount of microplastics ingested by zooplankton – Utilization of Differential Scanning Calorimetry (DSC) and Thermogravimetry (TG)** Jiří Kučerík Faculty of AgriSciences, Mendel University in Brno, Brno, Czech Republic
	14:00 – 14:20	**Detection of microplastics by means of X-ray computed tomography** Tomas Zikmund Central European Institute of Technology, Czech Republic
	14:20 – 14:40	**Coffee break**
Solutions	**14:40 – 15:00**	**Green solutions for microplastic pollution: Exploring phytoremediation in the aquatic environment** Ula Rozman University of Ljubljana (FKKT), Slovenia
	15:00 – 15:20	**Innovative strategies for microplastic mitigation in wastewater treatment** Aleksandra Tubić University of Novi Sad, Serbia
	15:20 – 15:30	**Closing**

Environmentally relevant particles in microplastic research

Gabriela Kalčikova[1,2], Ula Rozman[1]

gabriela.kalcikova@fkkt.uni-lj.si

[1]Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia

[2]Faculty of Mechanical Engineering, Brno University of Technology, Brno, Czech Republic

Recently, microplastic pollution has become an urgent environmental issue that requires immediate attention and efforts have been made to understand their impact, behavior and fate in the environment. However, the environmental relevance of microplastic research is constantly being questioned, especially with regard to the relevance of the materials used. Microplastics found in the environment are heterogeneous in size, shape, polymer and additive composition as well as in the degree of biotic and abiotic aging. On the other hand, industrially produced spherical microplastic particles are used in microplastics research very often, but these differ significantly from those found in the natural environment. In addition, the microplastics used in laboratory studies are not always properly characterized. The results of our research have shown that microplastics, which have similar properties and differ, for example, only in shape, have different ecotoxicological properties, they adsorb pollutants to different extents and their biotic aging also varies. Therefore, this underlines the crucial importance of proper characterization of microplastics used in environmental research and to report of minimum information on properties such as size, shape, material, surface morphology and, if possible, also information on surface charge, presence of additives and crystallinity should be provided. Only in this way can we accurately transfer the results from the laboratory to real environmental scenarios.

Preparation of "true-to-life" microplastics: The effects of preparation on microplastic properties

Stefania Federici[1,2], Serena Ducoli[1,2] Laura E. Depero[1,2]

stefania.federici@unibs.it

[1]Department of Mechanical and Industrial Engineering, University of Brescia, via Branze, 38, 20123, Brescia, Italy

[2]INSTM, via Giusti, 9, 50121, Firenze, Italy

Plastic pollution is globally recognized as an environmental challenge affecting ecosystems and human health. The majority of plastic waste flows from land to oceans, significantly impacting marine life and organisms. More recently, there has been growing concern about small fragments at the micro and nanoscale, which originate from the breakdown of larger plastic litter. This concern has driven research efforts to understand the sources, distribution, fate, and impact of these particles. However, their small size poses challenges, including the lack of adequate methods for qualitative and quantitative studies. Within this context, there is a pressing need to create high-quality reference or test materials for laboratory studies, protocol optimization, and database construction. These materials should mimic real particles as closely as possible. This contribution presents a study of micro and nanoplastics through the preparation of more realistic test materials referred to here as "true-to-life" materials. In the case of microscale fragments, "true-to-life" microplastics were generated by mechanically fragmenting commonly used plastic items. These materials were then characterized to reveal specific features of the resulting test materials. These findings indicate that the shape, morphology, and physicochemical characteristics of the fragmented debris vary depending on the type of plastic. This can serve as an indicator for future research into shape analysis of microplastics exposed to the environment and for the development and validation of standardized test procedures to evaluate the biological impacts of plastic particles and chemicals leached from weathered plastic in model organisms. Regarding nanoscale fragments, "true-to-life" nanoplastics separated from larger microscale pieces were used to investigate the nanoscale behavior of interactions with biological systems. Specifically, the formation of a protein corona from human plasma was examined, revealing that "true-to-life" nanoplastics exhibited a different protein corona composition compared to synthetic polystyrene nanobeads. These findings underscore the importance of integrating field and laboratory data under more environmentally relevant conditions. They suggest that "true-to-life" nanoplastics are a more authentic material for studying environmental nanoplastics, opening up the possibility of uncovering new and unexpected results in biological interactions.

Size matters: Techniques and challenges in measuring the size of microplastics

Tina Skalar

tina.skalar@fkkt.uni-lj.si

Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia

The size characterization of microplastic particles is of fundamental importance for their ecological consequences and impact on human health. These parameters are crucial to reliably relate particle size to the dimensions of their environmental impact (degradation pathways, residence time in nature, effects of aging, biofilm formation). Dimensional analysis of microplastics is essential for clarifying their ecological interactions, as only microplastics of certain sizes can be ingested by organisms due to the size of the oral cavity. Larger microplastic particles enter the biota predominantly by bioadhesion, whereas smaller particles are more prone to transcellular migration and systemic dispersal after ingestion, in contrast to larger fragments that are likely to be excreted after gastrointestinal transit.

Particle size and distribution in microplastics can be quantified using a variety of methods of which have their own limitations that must be carefully considered, including cost-effective sieve analysis. Laser particle diffraction is highlighted as a method that can efficiently measure the size distribution of microplastics by analysing the pattern of light scattered from the particles. This technique is particularly valuable as it enables rapid and accurate assessment of a wide range of particle sizes. Furthermore, microscopic techniques (light microscope and/or scanning electron microscope) are discussed as basic tools for the characterization of microplastics. These methods involve direct visual analysis under the microscope, allowing precise measurement of individual microplastic particles and detailed observation of their morphological features. Further image processing based on 2D images is an important but crucial step in the analysis of particle size. These techniques are also illustrated with practical examples, including their limitations due to the parameter determination obtained with the solutions described.

It is crucial for researchers to understand the significance and scope of each parameter measured and to select the most accurate method or combination of methods for assessing the specific characteristics under investigation. These techniques are presented as the most widely used and reliable for the characterization of microplastics, as they provide essential data for environmental science research and policy making. In addition, accurate interpretation of measurement results, especially those related to particle size, is crucial to establish a reliable correlation between particle size and environmental impact.

Effects of microplastics on terrestrial organisms

Anita Jemec Kokalj[1], Andraž Dolar[1], Gabriela Kalčikova[2,3]

anita.jemec@bf.uni-lj.si

[1]University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, 1000 Ljubljana

[2]Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia

[3]Faculty of Mechanical Engineering, Brno University of Technology, Brno, Czech Republic

In recent years, the terrestrial environment has been recognized as highly polluted with microplastics. Agriculture is one of the main inputs through the direct use of plastic in crop production and indirectly by the application of sewage sludge solids as fertilizer. The assessment of the risk to soil biota from microplastics is based on the use of model organisms exposed to microplastics either through ingestion of microplastics or through contact with the body surface. Significant efforts towards testing the hazard of agricultural microplastics on soil invertebrates have been made within the European Union-funded project Papillons (Plastics in agriculture: impacts, lifecycles & long-term sustainability). The focus was on testing multi-generational long-term effects of microplastics on an array of test organisms: Woodlice *Porcellio scaber*, mealworms *Tenebrio molitor*, springtails *Folsomia candida*, enchytraeids *Enchytraeus cripticus* and earthworms *Eisenia andrei.* The organisms were exposed to environmentally relevant microplastic concentrations (0.005-5%) via soil or food. Microplastics was produced from two common mulching films; polyethene and biodegradable polybutylene adipate terephthalate. A number of endpoints were tracked: Survival, reproduction, moulting, growth, energy-related biomarkers and immune response. In general, our results show that the tested microplastics are not lethal to the tested invertebrates. However, exposure to microplastics can have sub-lethal effects, such as changes in reproduction, reduced growth, changes in metabolic activity and triggering an immune response. Microplastics can also change the physico-chemical properties of soil, such as their pH and water retention. This can then indirectly affect the organisms living in soil.

Investigating the impacts of micro and nanoplastics on humans by electron microscopy: experiences from the EU Project PlasticsFatE

Damjana Drobne

damjana.drobne@bf.uni-lj.si

University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, 1000 Ljubljana

The PlasticsFatE project, funded by the European Union's Horizon 2020 Research and Innovation program, aims to enhance our understanding of the impact of micro- and nanoplastics (MNPs) on the human body. Specifically, it focuses on the fate and effects of MNPs, including their potential health impacts. As one of the 28 partners in the PlasticsFatE project, the University of Ljubljana is studying plastic particle uptake, intracellular localization, and effects. For this purpose, we employed transmission electron microscopy (TEM) to explore cellular interactions and ultrastructural changes induced by polystyrene (PS) nanoplastics. Our results demonstrate a dose-dependent uptake of PS nanoplastics by the human alveolar carcinoma cell line (A549). These nanoparticles, with a diameter of 300 nm, were efficiently internalized. Intracellular localization primarily occurred within specific compartments, such as endosomes, suggesting an endocytic pathway for their uptake. Additionally, autophagy-related structures contained PS nanoparticles, indicating their involvement in cellular degradation processes. Lamellar bodies, which play a role in surfactant production, also harbored PS nanoparticles. Importantly, the uptake process did not result in cytotoxicity, as confirmed by cell viability assessments. The study reaffirms the utility of TEM as the gold standard for examining nanomaterial-cell interactions. By providing high-resolution images, TEM enables precise localization and visualization of nanoparticles within cellular compartments. Researchers can leverage TEM to explore subtle changes in cell ultrastructure induced by nanomaterials. The UL team's findings underscore the importance of understanding nanoparticle-cell interactions at the ultrastructural level. Although PS nanoparticles were efficiently taken up by A549 cells without causing cytotoxicity, their presence led to notable cellular changes. Future studies should delve deeper into the molecular mechanisms underlying these effects and explore potential therapeutic applications or safety considerations related to PS nanoparticles.

Study was supported by the European Union's Horizon 2020 research and innovation program via the project PlasticsFatE (grant agreement no 965367).

Impact of microplastics on water flea: what can we learn for future testing?

Dana Kühnel

dana.kuehnel@ufz.de

Helmholtz Centre for Environmental Research, Germany

Water flea represent a group of small planktonic crustaceans that are versatile test organisms and have been used so far in ~ 400 scientific articles (experimental studies and reviews) dealing with the effects of nano- and microplastic particles. Several apical (e.g. immobilisation) and mechanistic (e.g. immune responses) have been assessed. However, it remains difficult to clearly address hazardous effects of nano- and microplastic particles due to contradicting results from many studies. One reason for this is certainly the diversity of plastic materials, which can vary in polymer type, shape, size and additive content, among other properties. Another source of such uncertainties are interferences and artifacts that can occur when testing microplastics. One example the the use of sodium azide as preservative in nanoplastic suspensions. Sodium azide is toxic for daphnids, making it essential to report on its use, to avoid false-positive results. To allow the identification of potential interferences and artefacts, a list of study quality criteria has been developed, covering particle properties, sample preparation and particle behaviour in the test, as well as criteria for the test organisms and the evaluation of study results.

This contribution demonstrates the importance of study quality criteria for the evaluation of toxicological results obtained in water flea for nano- and microplastic particles. The list of criteria is already useful when planning studies, as it covers fundamental aspects that need consideration. In general it helps to identify the reliability of study results. As well, it also fosters the comparability of studies, which is important for meta-analyses of hazard data for nano- and microplastic.

The use of laser-based spectroscopy for analysis of environmentally relevant microplastics

Pavlína Modlitbová[1], Pavel Pořízka[1,2], Jozef Kaiser[1,2]

pavlina.modlitbova@ceitec.vutbr.cz

[1]CEITEC Brno University of Technology, Purkyňova 656/123, 61200 Brno, Czech Republic

[2]Faculty of Mechanical Engineering, Brno University of Technology, Technická 2896/2, 61669 Brno, Czech Republic

The biomonitoring of microplastics (MPs) involves the careful assessment and identification of small plastic particles in association with living organisms and ecosystems. This methodical process aims to understand how widely MPs are distributed, how they might interact with organisms, and how this affects the wider environment. Establishment of robust monitoring tools are important for guiding effective mitigation strategies and ensuring a more sustainable coexistence with this ubiquitous material. The examination of MPs biomonitoring by employing Laser-Induced Breakdown Spectroscopy (LIBS) and Raman Spectroscopy techniques will be present. These methodologies are anticipated to yield a reliable biomonitoring approach. LIBS is a spectroscopic technique that functions by creating a plasma plume through the interaction of a laser with matter. This interaction causes atoms and ions to become excited and emit photons of distinct energy as they transition to lower energy levels. The emitted light is then gathered by collection optics before being transmitted to a spectrometer. Unique spectral lines are allowing the identification of elements present in the sample. This method is employed for differentiating and mapping MPs. Raman spectroscopy employs monochromatic light to interact with a sample, providing insights into molecule vibrations through energy shifts in scattered photons. By analysing the energy shifts in the scattered light, Raman spectroscopy provides insights into molecular vibrations and chemical composition and offers rapid chemical analysis without extensive sample preparation. Both these techniques were used in analysis of environmentally relevant MPs – pristine fragments, aged in freshwater and aged in wastewater. All MPs were analysed directly with biofilm on their surface.

An approach to determine amount of microplastics ingested by zooplankton – Utilization of Differential Scanning Calorimetry (DSC) and Thermogravimetry (TG)

Jiří Kučerík[1], Petra Procházková[2], Eliška Kameníková[2], Martin Brtnický[1], Gabriela Kalčikova[3,4]

kucerik@email.cz

[1]Faculty of AgriSciences, Mendel University in Brno, Brno, Czech Republic

[2]Faculty of Chemistry, Brno University of Technology, Brno, Czech Republic

[3]Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia

[4]Faculty of Mechanical Engineering, Brno University of Technology, Brno, Czech Republic

Microplastics are ubiquitous across all environmental compartments and living systems. Understanding their impact and fate requires analytical methods that are both sensitive and robust. Additionally, in complex substrates, sample pretreatment is sometimes necessary to minimize matrix effects and enhance the sensitivity of the techniques employed. Our group focuses on evaluating the effects of both conventional and biodegradable plastics on soils and living organisms. In some cases, pretreatments such as digestion or extraction are not feasible, prompting us to avoid these labor-intensive procedures. Presented case study involved analyzing biodegradable microplastics made of poly-3-hydroxybutyrate (P3HB) ingested by *Daphnia magna* during ecotoxicological tests. We tested two approaches. The first approach used differential scanning calorimetry (DSC), and relied on the observation that P3HB melts at around 170°C. Determining the melting enthalpy, combined with the measured data on P3HB crystallinity and literature knowledge of the melting enthalpy of 100% crystalline polymer, provided information on the quantity of P3HB ingested by *D. magna*. A shift in the melting temperature suggested some degree of "insulation" of the ingested P3HB, indicating that at lower concentrations, the particles might penetrate deeper into the inner organs. The second approach, thermogravimetry, compared the mass loss in *D. magna* exposed to P3HB with that in specimens not exposed to P3HB. A comparison of both approaches showed that DSC was more sensitive and enabled the analysis of microplastics in a single Daphnia species. Conversely, the thermogravimetry approach can also be applied to plastics with melting temperatures higher than 200°C. This temperature limit is determined by the stability of *D. magna*, whose degradation would compromise DSC measurements above this threshold.

The study was published in Procházková et al. J. Therm. Anal. Calorim. DOI: 10.1007/s10973-024-12985-0

Detection of microplastics by means of X-ray computed tomography

Viktória Parobková[1], Lukáš Maleček[1], Marek Zemek[1], Tomáš Zikmund[1,2], Jozef Kaiser[1,2]

tomas.zikmund@ceitec.vutbr.cz

[1]Central European Institute of Technology, Czech Republic

[2]Faculty of Mechanical Engineering, Brno University of Technology, Brno, Czech Republic

Plastic pollution is a global concern with significant environmental implications. Recent research has shed light on the potential health risks associated with microplastics to human health. Microplastics enter the human body through inhalation and ingestion, accumulating in various tissues. Despite their potential health effects, our understanding of their impacts still needs to be improved. The primary challenge in understanding the correlation between microplastic presence and its effects on humans is achieving reliable detection. Spectroscopic techniques, notably Raman spectroscopy, have effectively detected microplastics in biological samples. However, it's important to note that sample preparation for these techniques involves the irreversible loss of local distribution through tissue digestion and filtration. Local distribution serves as a valuable indicator, as the accumulation of microplastics in specific organ regions can enhance our understanding of their effects on the body. Hence, to preserve information regarding local distribution, we propose the usage of micro computed tomography (microCT) in microplastic research. One major advantage of microCT is its ability to provide 3D imaging of morphology, revealing internal structures non-destructively. Our future objective is to visualize microplastic distribution within specific organs using microCT. However, the low contrast of microplastics and their small size pose challenges to their accurate identification. Therefore, here we assess the limitations of microplastic imaging using microCT through experiments conducted on polyethylene particles, the most prevalent type of microplastics in our environment. Our findings illustrate how various scanning parameters, particularly voxel size, influence the size limitation of analysed particles. Voxel size is closely linked to both the sample size under examination and, consequently, the limited size of detected particles within the organ. Additionally, our study presents a workflow for identifying polyethylene particles in biological samples, emphasizing the importance of selecting an appropriate sample size based on the desired size range of detected microplastics.

Green solutions for microplastic pollution: Exploring phytoremediation in the aquatic environment

Ula Rozman[1], Gabriela Kalčikova[1,2]

ula.rozman@fkkt.uni-lj.si

[1]Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia

[2]Faculty of Mechanical Engineering, Brno University of Technology, Brno, Czech Republic

Microplastic pollution is one of the major environmental problems today. It is therefore necessary to develop efficient methods to limit the entry of microplastics into the environment. As a result, many researchers are currently focusing on limiting the entry of microplastics into the aquatic environment through wastewater. However, microplastics are also generated directly in the environment or enter the aquatic environment via other, uncontrollable pathways. Therefore, there is an urgent need to develop methods to limit the amount of microplastics in the aquatic environment. One of the promising options is in situ phytoremediation, i.e. the removal of microplastics with macrophytes, as our research has shown that microplastics have a high affinity to the surface of floating and submerged aquatic macrophytes. The most promising macrophytes for phytoremediation are floating plants such as *Lemna minor*, as they are widely distributed and can be easily harvested after the process. The interactions between microplastics and aquatic macrophytes are influenced by the properties of the microplastic (shape and chemical composition) as well as by the amount of plant biomass and the exposure time. The phytoremediation experiment carried out under controlled laboratory conditions showed that after 15 cycles (one cycle equaled 24 hours), 80% of the total microplastic particles had been removed and complete removal would have been achieved after 53 cycles. These results indicate the great potential of phytoremediation as a possible in-situ method for removing microplastics from the aquatic environment. However, further studies on large-scale systems and waste management strategies for contaminated biomass are still needed.

Innovative strategies for microplastic mitigation in wastewater treatment

Aleksandra Tubić

aleksandra.tubic@dh.uns.ac.rs

University of Novi Sad, Faculty of Sciences, Trg Dositeja Obradovića 3, 21000 Novi Sad, R. Serbia

One of the significant sources of surface water pollution by microplastics is wastewater and wastewater treatment plants. Sources of primary microplastics include everyday products such as cosmetics, personal hygiene products, cleaning agents, and others. Secondary microplastics in urban wastewater can come from numerous dispersed sources, such as runoff from urban surfaces, atmospheric deposition, degradation of discarded plastic waste in water, synthetic textile fibers from household and commercial wastewater etc. When municipal wastewater is combined with industrial wastewater, the presence of granules used in the production of plastic products, secondary microplastics from process or washing waters, and others can be expected. The presence of microplastics in wastewater can negatively impact treatment processes. It has been found to cause clogging of fine screens, increase the need for reagents in coagulation/flocculation and disinfection processes, and negatively affect the processes of denitrification and filtration on granular activated carbon. Additionally, a significant negative effect of microplastics on bacterial species present in activated sludge has been observed. The significant negative impact of different types of microplastics is also reflected in their high ability to sorb other pollutants, such as metals and various organic compounds, thereby reducing the efficiency of removing these pollutants from wastewater. Due to all the aforementioned reasons, investigations into the possibilities of improving wastewater treatment are the focus of many research projects, aiming for the most efficient removal of microplastics. Coagulation/flocculation, adsorption, advanced oxidation processes, granular activated carbon filtration, as well as electrocoagulation are the most investigated techniques. The efficiency of the treatment is highly variable and depends on the type of microplastics, water characteristics, as well as the previous treatment applied at the wastewater treatment plant. Obtained process efficiencies can't be easily generalized, which is why it is necessary to have a thorough understanding of the system and define process parameters for each individual case to achieve maximum microplastic removal in treatment.

This event is based upon work from COST Action PRIORITY, CA20101, supported by COST (European Cooperation in Science and Technology). COST (European Cooperation in Science and Technology) is a funding agency for research and innovation networks. Our Actions help connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. This boosts their research, career and innovation. www.cost.eu