Sources, fate, and impact of microplastics

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SOURCES, FATE, AND IMPACT OF MICROPLASTICS

BY
MÁRTA SIMON

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By

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2019
SETAC Europe 29th Annual Meeting
µFT-IR imaging to analyse the effect of antifouling paint leachates on a freshwater microalga (Poster)

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Removal of 10-500 µm microplastics from wastewater effluent by disc filter (Poster)

ENGLISH SUMMARY

The recognition of microplastic particles (MPs) in the 2000s has led to increasing scientific and public concern. The interest has been driven by the MPs' suspected adverse ecological impact on organisms at all trophic levels related to the MPs' small size. Numerous knowledge gaps regarding the abundance, potential sources, fate, and ecotoxicological effect of MPs hamper risk assessment despite the growing number of scientific studies on these topics. The discrepancy originates from the lack of standard operating procedures that gives rise to incomparable results, and study designs often irrelevant to natural conditions. Nevertheless, what is the representative or environmentally realistic concentration, physical and chemical properties of MPs? Answering the question by defining these parameters is the crucial step to infer the impact of MPs on the ecosystem as well as to compare the contribution of various sources to microplastic pollution.

The concentration of a pollutant is not only a key factor regarding its toxic effect, but its determination at various sources possibly leads to appropriate discharge mitigation actions. MPs in the environment continuously undergo fragmentation, which creates smaller particles, simultaneously increasing their quantity. Sample preparation procedures can similarly cause further particle fragmentation, which likely affects MP quantification by particle number. In contrast, particle mass is a measure that is conserved throughout sample handling and analysis that yield accurate determination of MP abundance and comparable results. However, measuring MP mass is no easy task. While MPs larger than a few hundred micrometres can be weighed on analytical scales, determining the mass of particles of few microns, let alone in the nanometre range, is impossible with this method. Thermoanalytical techniques could provide the necessary sensitivity in such cases, yet only a handful of studies have quantified MP mass this way. The present PhD aimed to improve MP mass quantification through the development of a method, which combines particle detection and mass estimation by infrared spectroscopy. The approximation of MP masses allowed to assess the role of wastewater treatment plants in MP pollution in Denmark. The study concluded that MPs comprise a minor fraction of solids in wastewater, of which over 98 % is retained in the treatment facilities. Additionally, wastewater discharge was deemed a minor contributor to MP pollution compared to other MP sources, e.g., transportation.

Nevertheless, exact MP concentrations in environmental samples cannot be ascertained without the accurate detection of MPs. The quality of identification by a frequently applied technique, infrared spectroscopy, depends on the extent of the employed spectral libraries that contain spectra of known materials and on the skills of the analyst. Although automatic algorithms have been developed to eliminate the bias rooted in the latter one, the coverage of materials by the applied library limits MP detection. Furthermore, reference spectra used for the identification of the unknown ones are mostly collected of pristine materials. However, the spectra of particles in environmental samples might differ from those of standard materials as particles are exposed to environmental weathering impacts that can induce chemical modifications. This PhD examined the effect of weathering on the identification of paint materials by their infrared spectra collected by attenuated total reflection infrared spectroscopy and concluded that weathering could alter the chemical makeup of the material to the extent that impedes identification. Consequently, the findings propose the inclusion of spectra of weathered material in spectral libraries. Furthermore, laboratory experiments revealed that the chemical modifications increased the mobility of heavy metals from weathered paint microparticles, indicating that weathering can alter the particles' impact.

Therefore, the PhD also assessed the effect of weathering on MP leachate toxicity in laboratory growth inhibition tests with a freshwater microalga, *R. subcapitata.* Such organisms are essential for freshwater ecosystems as they comprise the base of the food chain. The study showed that weathering significantly increased the toxicity of the leachates, thereby emphasised the importance of applying weathered MPs in impact studies.

The findings of the present PhD advanced MP quantification, identification, and ecotoxicity testing. It contributed to determining MP concentrations more accurately while ensuring the comparability of results. It has, additionally, given insight into the changes that MPs in the environment possibly undergo and how these can influence the particles' impact and quantification. The contributions of this PhD are small but necessary steps towards finding the sought environmentally realistic conditions that enable us to understand the threat that MP pollution may pose on the ecosystem.

DANSK RESUME

Erkendelse af, at der findes mikroplastik partikler (MPs) i miljøet, har i 2000'erne ledt til øget videnskabelig og offentlig bekymring. Interessen er drevet af MPs formodede negative økologiske indvirkninger på organismer på alle trofiske niveauer relateret til specielt de mindre MPs. Talrige videnshuller med hensyn til forekomst, potentielle kilder, skæbne, og økotoksikologiske indvirkninger af MPs hæmmer dog risikovurderinger til trods for et voksende antal videnskabelige studier i disse emner. Uoverensstemmelsen stammer fra mangel på standard protokoller for prøvetagning og analyse, som giver anledning til usammenlignelige resultater, og undersøgelsesdesign som ofte er irrelevant for naturlige forhold. Derudover: Hvad er repræsentative og miljømæssigt realistiske koncentrationer, samt fysiske og kemiske egenskaber af MPs? Svaret på spørgsmålet kræver, at disse parametre defineres, og er et afgørende skridt til at forstå, hvordan udledning af MPs indvirker på økosystemet, samt for at sammenligne bidraget af forskellige mikroplastik forureningskilder.

Koncentrationen af et forurenende stof er ikke kun en afgørende faktor angående dets toksiske virkning, men også for design og dimensionering af renseforanstaltninger ved diverse kilder. I miljøet fragmenterer MPs konstant, som kreerer mindre partikler, samt forhøjer deres kvantitet målt som antal partikler. På samme måde kan prøvebehandling før analyse fragmenterer partikler, hvilket formentlig påvirker MP kvantificeringen på basis af partikel antal. Partikelmasse er derimod en parameter, der er bevaret helt igennem prøvebehandling og analyse, og giver et præcist mål for forekomst af MP og herved grundlag for at kunne sammenlignelige resultater. Dog er bestemmelse af MP-masse ikke en nem opgave. Mens MPs større end få hundrede mikrometre kan vejes på analytiske vægte, er determination af masse af partikler på få mikrometer størrelse, endsige på nanometre niveau, umuligt med denne metode. Termoanalytiske teknikker kunne give den nødvendige følsomhed og præcision, men der findes kun få studier, der har kvantificeret MP masse på den måde. Denne PhD forsøgte at forbedre MP masse kvantificering ved at udvikle en metode, der kombinerer partikel detektion af antal og masse estimering ved brug af infrarød spektroskopi. Approksimationen af MP masserne gav mulighed for at vurdere rollen af spildevandsanlæg i MP forurening i Danmark. Studiet konkluderede, at MPs fylder en mindre fraktion af det suspenderede stof i spildevand, og mere end 98% af MP er tilbageholdt i renseanlæg. Derudover, blev udledning af renset

spildevand vurderet som en mindre bidragyder af MP forurening sammenlignet med andre MP kilder, f. eks. transport.

MP koncentrationer i miljøprøver kan ikke bestemmes uden præcis detektion af MPs. Kvaliteten af identificering ved infrarød spektroskopi, en ofte anvendt teknologi på området, er afhængelig af størrelsen af det brugte spektrumbibliotek, der indeholder spektre af kendte materialer, samt af analytikerens evner. Selvom automatiske algoritmer har været udviklet for at eliminere subjektivitet hos analytikeren, begrænses MP detektion af hvilke materialer, der er indeholdt i det brugte bibliotek. Desuden er referencespektre brugt i identifikation af ukendte spektre ofte lavet fra materialer upåvirket af miljøforhold. Sidstnævnte kan være et problem, idet spektre af partikler i miljøprøver kan afvige fra standardspektre, da materialerne bliver påvirket af miljøet i form af forvitring, som kan give kemiske ændringer. Denne PhD har undersøgt indflydelse af forvitring på identificering af malingsmaterialer ved infrarød spektroskopi. Herfra konkluderedes, at forvitring kan ændre den kemiske sammensætning af materialet i en grad, der hæmmer identificering. Følgeligt blev en udvidelse af spektrumbiblioteker med spektre af forvitrede materiale foreslået. Herudover, viste laboratorieeksperimenter, at kemiske ændringer forøgede mobiliteten af tungmetaller fra forvitrede malingsmikropartikler, hvilket indikerer, at forvitring af malingspartikler kan ændre deres miljøpåvirkning.

Nærværende PhD undersøgte hvordan forvitring af MP af maling påvirker toksiciteten af hvad der vaskes ud af partiklerne. Ferskvandsmikroalgen, *R. subcapitata* blev brugt vækshæmmer testene. Denne type organisme er essentiel i ferskvandsøkosystemer, da de former bunden af fødekæden. Studiet viste, at forvitring forøger toksiciteten fra det der udvaskes signifikant, og dermed understreger vigtigheden af at inkludere forvitrede MPs i indvirkningsstudier.

Denne PhD har ført til forbedret MP kvantificering, identificering, og økotoksikologiske test. Den har bidraget til mere præcis bestemmelse af MP koncentrationer, og dermed bedre sammenlignelighed af resultater. Derudover fremmede den indsigt i potentielle kemiske ændringer som MPs kan undergå i miljøet og hvordan disse kan påvirke partiklernes miljøindvirkning og kvantificering. Bidraget fra denne PhD er et lille men vigtig skridt i jagten efter at forstå risici fra MP på miljøet under miljørealistiske forhold.

PREFACE

This thesis has been submitted for assessment in partial fulfilment of the PhD degree. As part of the assessment, co-author statements have been made available to the assessment committee and are also available at the Faculty.

The PhD was carried out in the period from January 2018 to November 2020 at the Department of the Built Environment, Aalborg University. The PhD included a collaboration with the Department of Environmental Engineering, Technical University of Denmark. The joint study resulted in a manuscript (Paper-III), which is ready for submission.

This thesis is based on one published scientific paper (Paper-I), and two scientific manuscripts (Paper-II,-II). Contents of the papers are briefly discussed in the extended summary of the thesis, while additional details are given in the paper and manuscripts. Furthermore, the thesis presents the current state of microplastic research in a nutshell by reviewing the latest key findings of the scientific area.

ACKNOWLEDGEMENTS

Let's build a passive house on Antarctica! Using sticks and stones. And it needs to be completed in ten months. This is how our very first microplastic project felt to me: challenging, exciting, and impossible. Our tiny group was quite lonesome with the task. The available knowledge about microplastics was limited at that time, and we could not expect external help from other research groups. Nevertheless, we somehow managed to build, well, not a passive house, rather a little hut. The project was delivered on time, thereby, we set our base in this research field. Since then, the continuous addition of bright minds to the group enabled us to build a huge and comfortable house that accommodates all of us with our needs.

I am grateful for being one of the pioneers and witnessing such growth. It would not have been possible without my supervisor, Jes, who trusted me with the task of laying down the groundwork. He also allowed me to work on my pet project during my PhD and provided the necessary funding, which was a privilege. I have learned much from him from setting up a project to communicate its results. He was always there to point me in the right direction when I could not find it. I could not have wished for a better mentor. I am also thankful for Asbjørn, Diana, Jytte and Henrik who have helped to manage the group and the projects with great scientific expertise and efficiency. Their efforts have provided the framework for a safe work environment ensuring state-of-the-art solutions and high-quality results.

I thank Greta for her support as a co-supervisor for my master thesis. She introduced me to algae research which turned out to be one of my great interests. I always admired her infinite positivism and commitment to work. I also acknowledge my fellow colleagues who contributed to my PhD in any way, such as starting a scan, assisting with sampling, or lifting the mood during lunch break. I am likewise grateful to Nanna and Susanne from DTU for giving me a warm welcome during my external stay and for their assistance in my algae experiments.

I would particularly like to thank Alvise for his guidance throughout my PhD. He was a kind of 'supervisor on ground', who regularly advised on experiments, helped to understand data, explained techniques, just to name a few. I always enjoyed working with him as it was not only educational but much fun at the same time. I could turn to him with any issue, and he was

always there to lend a hand. I sincerely appreciate his commitment to this research group and the enormous effort he has put into evolving it both scientifically and socially.

I am especially grateful for the support of Nikki, Kristina, and Elena. These smart, kind, skilful, and fun ladies have been not only terrific friends but also inspirational colleagues. Elena always made my day with her stone-cold sarcasm. I respect her persistence in her PhD despite all the obstacles she had had to overcome. I deeply value Kristina for being disciplined, detail-oriented and having high working standards. I appreciate her encouragement when I faced difficulty in my private life. I always admired Nikki's unmatched practicality, analytical and language skills. She has always been supportive as a fellow student, a colleague, and a friend. She never hesitated to give a helping hand from discussing an idea to moving from one apartment to another. I hold the kindness she has shown to me in high esteem.

I owe my deepest gratitude to my family, who pushed me through 15 years or so of education, supported me in moving to another country, which has ultimately led to this PhD. I profoundly value all their effort and encouragement. And last but not least, I would not be writing these lines without having my husband, my true companion, a partner in crime, my best friend by my side for all these years. He has always shown genuine interest in my work, provided tangible help, cheered for my success, and encouraged me during more challenging times. I am deeply grateful for his unconditional support.

Clicking on the submit button will close a significant chapter and open a new for me. The coming years are full of uncertainties and challenges like the first years of my life in Denmark. It all had turned out well for the first time, and I hope that this will be the case in the future as well. I stay curious and look forward to tackling them.

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II. M. Simon, A. Vianello, Y. Shashoua, J. Vollertsen, Accelerated weathering affects the chemical and physical properties of marine antifouling paint microplastics and their identification by ATR-FTIR spectroscopy (Submitted to Chemical Engineering Journal, under review)

III. M. Simon, N.B. Hartmann, J. Vollertsen, Accelerated weathering increases the release of toxic leachates from microplastic particles as demonstrated through altered toxicity to the green algae *Raphidocelis subcapitata* (Manuscipt)

OTHER SCIENTIFIC PAPERS AND REPORTS

(not included in the thesis)

I. M. Simon, A. Vianello, J. Vollertsen, Removal of >10 µm Microplastic Particles from Treated Wastewater by a Disc Filter. *Water* 2019, *11*, 193. https://doi.org/10.3390/w11091935

II. S. Primpke, R.K. Cross, S.M. Mintenig, M. Simon, A. Vianello, G. Gerdts, J. Vollertsen, EXPRESS: Toward the Systematic Identification of Microplastics in the Environment: Evaluation of a New Independent Software Tool (siMPle) for Spectroscopic Analysis, 2020. https://doi.org/10.1177/0003702820917760.

III. U.E. Bollmann, **M. Simon**, J. Vollertsen, K. Bester, Assessment of input of organic micropollutants and microplastics into the Baltic Sea by urban waters, Mar. Pollut. Bull. 148 (2019) 149–155. https://doi.org/10.1016/j.marpolbul.2019.07.014

IV. K.B. Olesen, N.V. Alst, **M. Simon**, A. Vianello, F. Liu, J. Vollertsen, Analysis of microplastics using FTIR imaging — Identifying and quantifying microplastics in wastewater, sediment and fauna. Application Note, Agilent Technologies, pp. 6

V. G. Minelgaite, D.A. Stephansen, **M. Simon**, M.L. Fejerskov, J. Vollertsen, No Clear Response in Stormwater Phytoplankton Community to Biocide Contamination (Submitted to Water, under review)

CHAPTER 1. INTRODUCTION

The dramatic images of sea turtles entangled in discarded fishing nets, nestlings fed with plastic litter, divers swimming in the turquoise ocean surrounded by plastic bottles and bags have drawn the spotlight on plastic pollution since its recognition in the 1970s (1). Such pictures show that the material once associated with modernisms, growth, and future has become a major planetary burden that requires urgent actions to put an end to its detrimental effects on the ecosystem (2). The properties that made plastic materials essential to modern life, such as durability, cheap production cost, excellent chemical resistance, and versatility, have led to the mass production of a myriad of different plastic materials summing up to 8300 million tonnes in 2015 (3,4).

An estimated 60 % of the total mass has been discarded and served as a source of plastic litter in the environment through mismanaged land-based plastic waste as well as cargo-loss or fishing activities (1,3,5,6). Since these materials are rather resistant to biodegradation, plastic litter accumulates in the ecosphere (7). The build-up and continuous influx of plastic litter possibly result in that plastic will outweigh fish in the world's oceans by 2050 (8). Furthermore, the recent recognition of plastic particles smaller than a few millimetres, called microplastic particles (MPs), have raised new concerns about plastic pollution (9–11).

MPs are defined by particle size, though a scientific unanimity on their classification is yet to be attained (12,13). A broad definition of MPs includes plastic particles and fragments ranging from 1 nm to 5 mm (14). Particles of synthetic as well as chemically modified natural polymers falling into this size-range are considered MPs (13). Primary and secondary MPs can be distinguished depending on the origin of the particle (4). Primary MPs are manufactured small particles serving as raw materials for plastic production or additives in personal care products (PCPs). In contrast, the fragmentation of larger plastic items and abrasion of plastic surfaces by various environmental impacts (Figure 1) through the weathering of the plastic material generates secondary MPs (4,15,16).

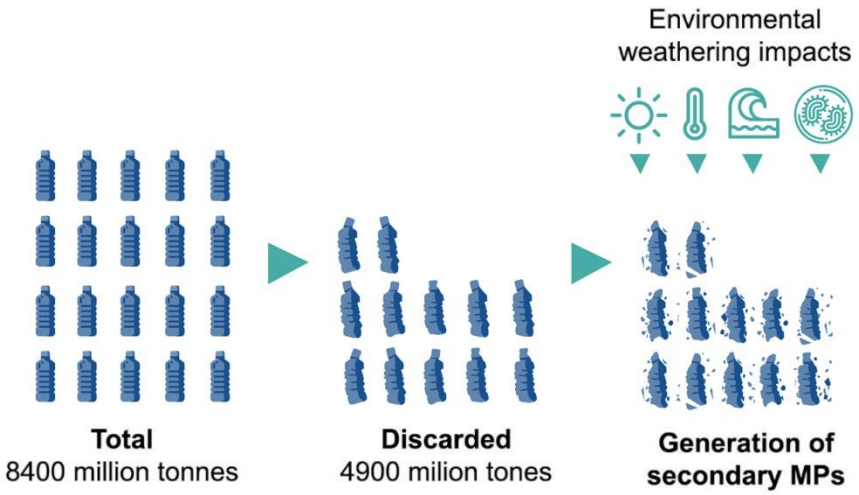

Figure 1. Generation of plastic waste and secondary microplastic particles from the total mass of plastic produced between 1950 and 2015 based on Geyer et al. (2017) (3) and Andrady (2011) (1).

1.1. ANALYTICAL METHODS OF MPS

The MP research field is currently lacking universally accepted standardised analytical procedures due to the novelty of the subject (13,17), which has led to divergent investigative practices. Numerous techniques are currently in use for the identification of the constituting plastic material and quantification of particle size and mass. Such inconsistency encumbers the comparison of studies and gives rise to data of varying quality (11).

MP analysis is preceded by sample purification to remove interfering natural material from the sample matrix (18). The methods vary from gently removing attached natural material from the surface of larger MPs to the elaborate process of extracting small MPs in the micrometre range from complex environmental matrices. Although a standard procedure awaits to date (11,15,18,19), Löder et al. (18) proposed an extraction method that applies enzymatic digestion to organic matter and density separation for the removal of inorganic particles. The method is applicable for various matrices with a high recovery rate of MPs and has been widely adopted by the scientific community.

The most suitable analytical method depends on the addressed size range of particles, the composition of the sample, and the parameters of interest. Visual inspection is a commonly applied technique for the identification of collected MPs (11,20). The method can be supplemented with a hot needle test or selective dying of particles to facilitate and increase the accuracy of distinguishing particles of natural and plastic origin (21,22). Nevertheless, as the uncertainty of visual inspection increases with decreasing particle size, techniques that examine the molecular composition of the particles should be consulted to identify MPs smaller than 500 µm (11,23).

Molecular spectroscopic techniques, e.g., Fourier-transform infrared (FTIR) spectroscopy, is widely used in MP analysis that allows for the identification of the constituting molecules in a specimen (11,24,25). While MPs larger than a few hundred micrometres can be characterised by Attenuated Total Reflection FTIR (ATR-FTIR), µFTIR imaging is suitable for the analysis of smaller particles (19,23,25). Since millions of spectra comprise the yielded infrared map of the sample, automatic algorithms have been developed to handle the generated vast data and to prevent bias stemming from subsampling and human error (17,26). The algorithms compare all collected spectra with reference spectra of known materials for identification and measure particle size as well as estimate particle mass. Particle numbers are often reported as a measure of MP concentration, albeit it is associated with uncertainty as MPs in environmental samples are prone to fragmentation during the extraction process, resulting in their potential overestimation (1,27). Consequently, the mass of MPs is of great importance to generate accurate and comparable data as this measure is independent of the incidental fragmentation of particles (28). Thermoanalytical techniques, such as pyrolysis-gas chromatography-mass spectrometry, may provide the required accuracy of MP masses (29). This method, moreover, can determine plastic additives and detect tire wear particles which is infeasible with molecular spectroscopic techniques as the tires' black carbon content absorbs the illuminating light resulting in uninterpretable spectra (6,28,30,31). Nevertheless, this destructive method prevents further investigation, for instance, particle size analysis (6,31). Consequently, the imperfection of each analytical method necessitates the combined application of several techniques to cover the analysis of MPs of various materials and to report all required parameters accurately (17,20,32).

1.2. SOURCES OF MPS

Plastic litter can be a significant source of secondary MPs, as up to 12.7 million tonnes of plastic waste was estimated to enter the environment in 2010 alone (5). Other sources of MPs include transportation, e.g., through the abrasion of tire, deteriorating plastic surfaces and exterior coatings, as well as household activities that can generate textile fibre fragments and release primary MPs from PCPs (33). As a result, MPs can either be formed in the environment by the weathering of plastic litter or enter via stormwater runoff from urban surfaces and wastewater effluent (6). The role of wastewater treatment plants in MP pollution has been under scrutiny as these facilities receive and discharge a substantial volume of wastewater that can contain MPs of various origins (34,35). Nevertheless, research has shown that the wastewater treatment process efficiently retains more than 98 % of the incoming MPs, which become enriched in the removed grease and sewage sludge (36–40). These findings suggest that sewage sludge applied as fertiliser on farmlands might be a more prominent source of MPs compared to wastewater effluent despite the latter's considerable discharge flux (39,40).

1.3. FATE OF MPS IN THE ENVIRONMENT

MPs are omnipresent contaminants in the environment polluting even remote areas (41–44). Their easy dispersion by wind, streams, and currents, owing to their small size and low weight, has led to 250 000 tonnes of plastic fragments floating on ocean surfaces (45). However, the disparity between the estimated mass of plastic waste entering the oceans and floating MPs, as well as the recent discovery of the substantial mass of MPs in the interior of the Atlantic Ocean, indicate that environmental processes remove floating MPs from water surfaces (9,45,46).

MPs in the environment are exposed to several weathering factors, e.g., UV-radiation, microbial activity, fluctuating temperature and humidity, and mechanical friction (2,47–49). These impacts alter the physical properties or the chemical composition of the particle (Figure 2), thereby influencing its fate and interaction with the environment (2,48). The principal weathering factor is UV-radiation that causes the photooxidation of the material at the exposed surface of the particle (47,48,50). UV-exposure changes the chemical makeup of the material, which is expressed in altered polarity and disintegrated polymeric structure (2,48,51). The changed polarity may influence the adsorption of compounds to the particle's surface from the surrounding

environment and attachment of microbes. The latter process, called biofouling, affects the vertical distribution of MPs as it increases particle density and promotes hetero-aggregate formation with natural particulate matter that enhances the sinking rate of MPs, possibly contributing to the loss of MPs from water surfaces (52–54).

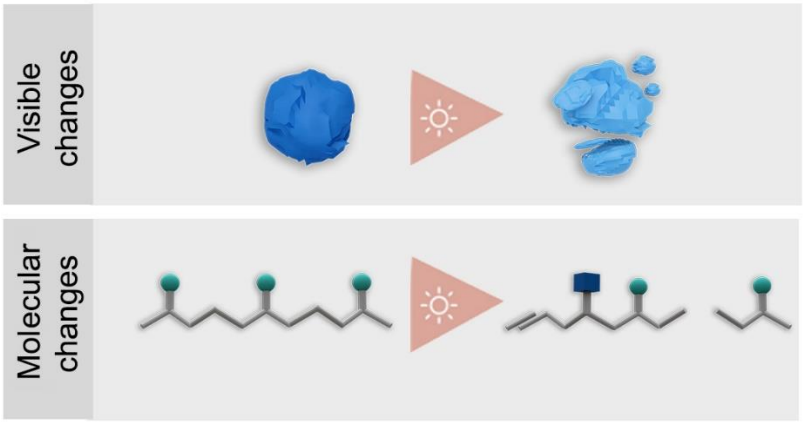

Figure 2. UV-exposure induces changes in the physical properties of MPs, such as colour loss and fragmentation, while it generates new functional groups and breaks chemical bonds in the constituting polymer (47,48).

1.4. IMPACT OF MPS IN THE ENVIRONMENT

MP pollution has become an emerging concern because the small size of MPs extends the bioavailability of plastic litter to a wide range of organisms and may introduce new harmful modes of action compared to macro-sized plastic litter (1,4,55). Ingestion of particles is the primary risk frequently associated with MPs (56). Intake of MPs potentially causes the blockage of the gastrointestinal tract (Figure 3) and malnutrition because plastic is an inaccessible carbon source for most organisms (8,55). Although clear evidence shows that various organisms ingest MPs, particles were found in small numbers in the guts of fish, invertebrates, and birds (39,56,57). Low internal concentration can be an indication of eliminating mechanisms or low abundance of MPs entailing minor probability of organism encounter with MPs (16,55,56,58,59). That is, MPs take up a negligible fraction, for example, in the Atlantic Ocean despite their mass of a staggering 210 million tonnes therein (46). Assuming an average density of 920 kg m^{-3} (27) of the most

abundant plastic types, polyethylene, and polypropylene (46), the approximated volume of the reported MP mass contributes to $6x10^{-8}$ % of the total volume (60) of the Atlantic Ocean. In other words, one cubic kilometre ocean water contains 0.6 m^3 of MPs. Such small fraction implies a low average abundance of MPs in the Atlantic basin. Nevertheless, a higher concentration of MPs is expected in accumulation zones, such as ocean gyres and areas of increased human activity (55,61,62).

An additional cause for concern regarding the adverse impact of MPs is the potential release of toxic compounds adsorbed to the particle's surface, similar to the possible leaching of harmful plastic additives (4,63–65) in the organism upon ingestion (Figure 3). However, the exhaustive literature review of Burns and Boxall (2018) (39) found no unequivocal evidence of the harmful effect of the release of adsorbed pollutants. Several authors (6,32,39) claim that inadequate laboratory study designs applying compounds and MPs in unrealistically high concentrations, as well as the use of pristine MPs, not representing particles occurring in the environment, hinder concluding the ecotoxicological impact of MPs.

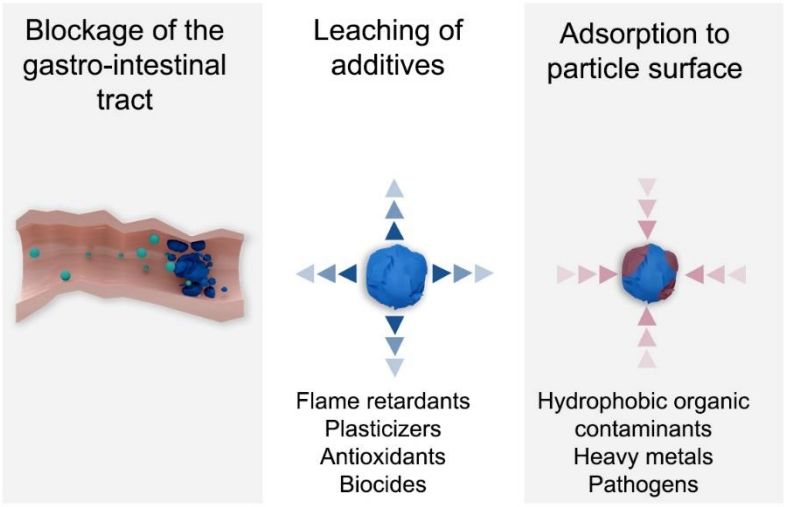

Figure 3. Possible adverse impacts of microplastic particles on biota (4,55).

The novelty of the research area and the lack of standard methods for effect studies explain that divergent studies often investigate unrealistic scenarios,

while the consequent knowledge gaps impede a sound risk assessment of MPs (32,39,66,67). A pivotal step towards carrying out effect studies representing natural conditions may be the use of weathered MPs. This practice could account for weathering-induced changes in particle shape, size, surface characteristics, and composition of released substances that can significantly affect the particle's impact (2,68–71). Degradation products and additives can be released from weathered MPs that may result in a mixture of compounds that possibly differ from the leachates of pristine MPs (68,70). Consequently, the altered chemical composition and concentration of substances in the leachates derived from weathered MPs conceivably affects organisms at all trophic levels differently from pristine MPs (54,72,73).

1.4.1. IMPACT OF MPS ON FRESHWATER PHYTOPLANKTON

Phytoplankton are autotrophic primary producers that convert inorganic substances, carbon dioxide, and water, to biomass with the simultaneous release of molecular oxygen, using the energy of solar radiation. As such, these organisms provide an energy source and contribute to a breathable atmosphere and a good ecological status of this group is a prerequisite of life on Earth.

The freshwater environment is underrepresented in MP research, though it serves as a gateway for MP pollution from land to oceans (6). The knowledge about the effect of MPs on freshwater primary producers is, hence, also incomplete despite their vital role in freshwater ecosystems (74–76). Several studies have reported adverse effects of MPs on freshwater phytoplankton, but the ecological relevance of the laboratory experiments is uncertain (74,77). Though MPs may exert toxic effects dependent on their size and the type of the constituting polymer, the observed changes in phytoplankton biochemistry could only be observed at unrealistically high MP concentrations (77). Moreover, several studies were restricted to investigate the effects of MPs consisting of the pristine form of a few polymer types that fail to cover the diversity of plastic materials (74,77). Therefore, the application of weathered MPs derived from commercial plastic products is essential to explore the potential harmful impact of plastic additives as well as weathering-induced changes that can exacerbate the toxicity of MPs (65,68,70,78–80). Such studies could contribute to a realistic risk assessment of the effects of MPs on the freshwater phytoplankton community.

CHAPTER 2. STUDY AIMS AND OBJECTIVE

The lack of harmonised MP analytical methods gives rise to incomparable data that hampers the identification of the most significant MP sources and delays the understanding of these particles' fate in the environment. This PhD study aimed to improve MP analytical practices by the development of a method for MP mass estimation and by the assessment of the effect of weathering-induced chemical changes on the identification of paint materials. Furthermore, it evaluated the role of wastewater treatment plants as one of the MP sources. The PhD also intended to improve the representativeness of MP impact studies by the comparison of the toxic effect of pristine and weathered MPs on freshwater phytoplankton.

The study was divided into several research questions, and each was answered to complete an objective to address these aims.

Research question 1: How can the quantification of environmentally realistic MP be improved?

This research question was addressed by improving the quantification and analytical practices of MPs. On the one hand, a novel method for estimating the mass of MPs based on their infrared image was developed to provide a concentration measure that is unaffected by particle fragmentation and which enables the comparison of results. On the other hand, the effect of weathering on the automatic identification of paint materials was assessed. Chemical changes induced by UV-exposure in paint microparticles were evaluated by ATR-FTIR spectroscopy and were quantified by a similarity index used by automatic identification algorithms.

Research question 2: Are wastewater treatment plants significant sources of MP pollution to the environment in Denmark?

This objective was addressed by evaluating the MP removal rate in ten of the largest wastewater treatment plants in Denmark by measuring MP concentrations in the influent and effluent wastewater. The developed method was applied to estimate MP masses in raw and treated wastewater. The MP mass in treated wastewater samples was related to other emission sources to assess the significance of wastewater discharge in MP pollution.

Research question 3: *How does weathering affect the fate and impact of MPs on freshwater phytoplankton?*

This aim was addressed by assessing heavy metal mobility from non-weathered and weathered antifouling paint microparticles and toxicity tests carried out with selected MP leachates in laboratory studies. Heavy metal release from pristine and UV-exposed paint microparticles was investigated in leaching experiments and related to the UV-induced changes in the paint materials evaluated by ATR-FTIR spectroscopy. The toxicity tests were conducted with *Raphidocelis subcapitata*, a freshwater phytoplankton species. Growth inhibition tests were performed with the organisms and the leachates of UV-weathered and non-weathered paint, unplasticized polyvinyl chloride, and tire microparticles. The tests were followed by the chemical analysis of the leachates to identify the inorganic substances contributing to toxicity.

CHAPTER 3. METHODOLOGY

The PhD study was carried out focusing on three subtopics, one per each research question:

- Quantification and identification of MPs
- The occurrence of MPs in wastewater
- The effect of UV-exposure on heavy metal release and toxicity of microplastic leachates on freshwater phytoplankton

The first and third research focus was addressed in laboratory studies, while the second one by field sampling.

3.1. QUANTIFICATION AND IDENTIFICATION OF MPS

Method development for the quantification and identification of MPs was carried out, and the research topic was divided into two work packages.

3.1.1. ESTIMATION OF MP MASS

Rapid chemical mapping of entire filters or slides containing the sample of interest is feasible by μFTIR imaging spectroscopy (23,25,81) (Figure 4). The resulting infrared image of complete particles in the analysed area allows for measuring the particles' size. The method developed for particle mass estimation employed the estimated volume of the MP calculated from its dimensions measured on the resultant infrared map and the density of the identified material. The details of the method are found in Paper-I.

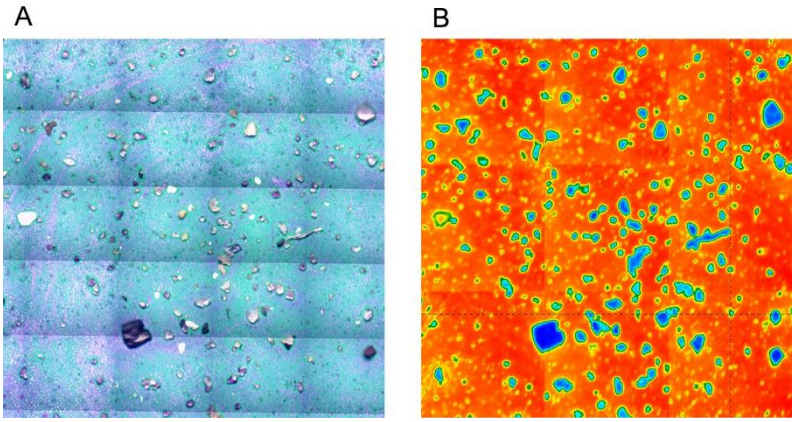

Figure 4. Visible image (A) of a segment of the slide containing particles extracted from influent wastewater and its infrared image (B) generated by µFTIR imaging.

3.1.2. CHEMICAL CHANGES INDUCED IN PAINT MATERIALS BY EXPOSURE TO UV-C RADIATION

A laboratory study was carried out to investigate the chemical changes induced in antifouling paints upon exposure to UV-C radiation and their consequent impact on the identification of paint materials. Paint flakes were generated from commercial marine antifouling paints and were exposed to accelerated weathering through UV-C exposure for between seven and 28 days (Figure 5). Infrared spectra of the flakes were collected by ATR-FTIR spectroscopy, and the changes were manually evaluated by peak-by-peak comparison of non-weathered and weathered spectra. The differences and the impact of weathering on the identification of paint materials were quantified by calculating a similarity index, the Score, used for the automatic identification of MPs described in Primpke et al. (2020) (17). Paper-II provides further details about the study design, the evaluation of chemical changes, and the identification of weathered paint materials.

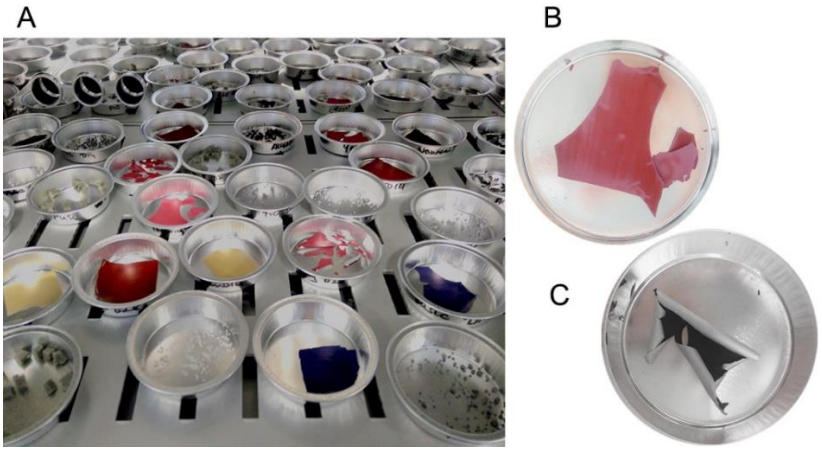

Figure 5. Paint flakes and microplastic particles in the UV-weathering chamber (A) and weathered paint flakes after 14 days of UV-C exposure (B and C).

3.2. OCCURRENCE OF MPS IN WASTEWATER

The MP removal efficiency of Danish wastewater treatment plants was assessed to evaluate the role of these facilities in MP pollution. Untreated wastewater, as well as wastewater effluent, was collected at several wastewater treatment plants around Denmark (Figure 6). The differing suspended solid (SS) content and chemical oxygen demand (COD) of the two sample types required different sampling strategies. Flow-proportional samples of untreated wastewater were collected from the autosamplers at the wastewater treatment plants. In contrast, larger volumes of treated wastewater were filtered on-site onto stainless steel filter meshes. Both sampling methods accounted for the significant variability of wastewater and enabled representative samples.

Figure 6. The locations of the studied wastewater treatment plants in Denmark are denoted by white dots.

The subsequent sample purification and MP extraction included enzymatic digestion of cellulose fibres, the removal of the remaining organic matter by advanced oxidation process, and particle separation into two size classes by wet sieving. The particles of each size class that remained after the procedure were concentrated in ethanol, and a subsample of the particle suspension was analysed by µFTIR imaging spectroscopy. A fraction of the resulting infrared maps was analysed manually owing to the lack of automatic identification methods at the time of the study. The unknown spectrum of each particle was compared to spectra of known materials from infrared spectral libraries encompassing natural organic, inorganic, and various synthetic polymer materials. Subsequently, the mass of each identified MP was estimated. MP concentrations by particle number and mass determined in raw and treated wastewater in the respective treatment facility were considered to ascertain the removal efficiency of each wastewater treatment plant. Furthermore, the contribution of wastewater discharge to MP pollution was evaluated by relating the combined MP discharge from the studied treatment plants to the total MP emission in Denmark estimated by Lassen et al. (2015) (82). Details about the MP extraction and analytical methods, as well as the calculations, are found in Paper-I.

3.3. THE EFFECT OF UV-EXPOSURE ON HEAVY METAL RELEASE AND TOXICITY OF MICROPLASTIC LEACHATES ON FRESHWATER PHYTOPLANKTON

The effect of UV-C exposure on the fate and impact of MPs was evaluated in two work packages.

3.3.1. THE IMPACT OF UV-EXPOSURE ON HEAVY METAL RELEASE FROM ANTIFOULING PAINT MICROPARTICLES

The impact of UV-induced chemical changes on heavy metal release from paint microparticles was investigated in a laboratory study. Microparticles of marine antifouling paints measuring 20-500 µm were exposed to UV-C radiation for seven and 14 days based on the results of a weathering experiment conducted with paint flakes. The heavy metal release from non-weathered and weathered particles was evaluated by leaching the particles for between 1 and 168 hours in ISO standard algae growth medium to simulate freshwater conditions (Figure 7). Heavy metal concentrations in the leachates were measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES). Further details of the leaching experiment can be reviewed in Paper-II.

Figure 7. 72-hour leachate of weathered paint microparticles (left-hand side) and the leachate of non-weathered paint microparticles of the same material (right-hand side).

3.3.2. THE EFFECT OF UV-EXPOSURE ON THE TOXICITY OF MP LEACHATES ON FRESHWATER PHYTOPLANKTON

The impact of UV-exposure on MP leachate toxicity on a freshwater microalga species was evaluated in laboratory growth inhibition tests. MPs derived from marine antifouling paints, tire granulates of end-of-life tires, and unplasticized polyvinyl chloride (PVC) were exposed to UV-C radiation for between seven and 28 days depending on the material. The weathered and non-weathered particles were leached in ISO standard algae growth medium for 72 hours. The leachates were used in growth inhibition tests carried out with *Raphidocelis subcapitata,* according to Hartmann et al. (2010) (83), to evaluate leachate toxicity (Figure 8). The inorganic components of the leachates were determined by ICP-OES. The details of the method and the results of the toxicity tests are found in Paper-III.

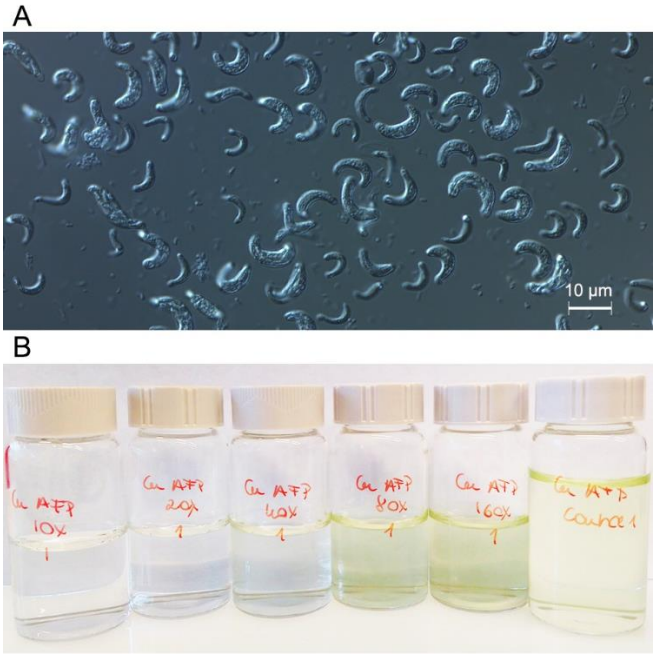

Figure 8. Microscope picture (630x, oil immersion) of R. subcapitata, the model organism used in the growth inhibition test (A), and the results of the test with antifouling paint particle leachates (B). The most concentrated leachate is on the left-hand side, and the deepening green colour towards the right indicates a gradual increase in the phytoplankton concentration.

CHAPTER 4. RESEARCH OUTCOMES

The PhD study resulted in three scientific papers that addressed three research topics to answer the initially raised questions:

- MP analytical procedures- *How can the quantification of environmentally realistic MP be improved?*
- The occurrence of MPs in wastewater- *Are wastewater treatment plants significant sources of MP pollution to the environment?*
- The effect of weathering on heavy metals release and toxicity of selected microplastic leachates- *How does weathering affect the fate and impact of microplastics on freshwater phytoplankton?*

4.1. MP ANALYTICAL PROCEDURES

MP analytical procedures had been in dire need for a simple MP mass quantification method that could yield comparable data across studies. While the accurate determination of this parameter is feasible by thermoanalytical techniques, their application is uncommon at the current stage of MP research. In contrast, the widespread use of FTIR spectroscopy for MP identification called for a method that is suitable for determining MP masses based on the resulting infrared data. Paper-I presented such a procedure that yielded estimated MP masses by μFTIR imaging spectroscopy. This measure, subsequently, enabled to compare the significance of MP sources.

Nevertheless, the correct identification of MPs is essential for the reliable determination of particle concentrations. Infrared spectra of MPs are commonly compared to known spectra of the pristine form of various polymers collected in spectral libraries to identify their material. However, MPs are exposed to weathering factors in the environment that can alter their chemical composition. The study presented in Paper-II revealed that weathering could influence the automatic identification of paint materials as the UV-induced chemical changes decreased the similarity index, the Score, of the infrared spectra of non-exposed and UV-exposed paint materials. Score values below a certain threshold may result in the misidentification of weathered paint materials.

4.2. THE SIGNIFICANCE OF WASTEWATER TREATMENT PLANTS IN MP POLLUTION

Paper-I assessed the role of wastewater treatment plants in MP pollution in Denmark, as wastewater effluent had been a presumed significant source of MPs. Nevertheless, the study presented in Paper-I demonstrated that Danish wastewater treatment plants efficiently retain MPs from raw wastewater as less than 2 % of the incoming MPs were detected in wastewater effluent. The results also showed that MPs accounted for a minor fraction of suspended solids and COD in raw wastewater. Furthermore, MP mass estimation enabled to compare the discharged MP mass from wastewater treatment plants to the total MP emission in Denmark. The calculations indicated that wastewater treatment plants are not major contributors to MP pollution in Denmark.

4.3. THE EFFECT OF WEATHERING ON HEAVY METAL RELEASE AND TOXICITY OF SELECTED MICROPLASTIC LEACHATES

The results of Paper-II showed that UV-exposure of paint microparticles increased the mobility of heavy metals that gave rise to their higher aqueous concentration in the leachates of the exposed particles than of the non-exposed ones. The observed difference was related to the UV-induced chemical changes in the materials, such as a weakened structure and modified polarity. Such altered particle characteristics imply differing impact of weathered and pristine paint microparticles.

Paper-III followed up on the observation of greater metal release from weathered paint microparticles and investigated the impact of non-weathered and weathered paint, tire, and PVC microparticles on biota. The study was carried out with a freshwater phytoplankton species as toxicity studies with such organisms have mainly applied MPs of pristine polymers. The results demonstrated that weathering significantly increased the toxicity of the leachates of the studied six marine antifouling paint, tire, and unplasticized PVC microparticles. The increased toxicity was attributed to higher heavy metal concentrations measured in the leachates of the weathered MPs than of the non-weathered MPs.

CHAPTER 5. CONCLUSIONS

The PhD study was divided into three main research questions, and the following conclusion was drawn for each question.

Research question 1: How can the quantification of environmentally realistic MP be improved?

The PhD study provided tools described in Paper-I and Paper-II that may facilitate to answer the research question. The development of an MP mass quantification method (Paper-I) enables to estimate unbiased MP concentrations in various environmental samples. Although the method was based on µFTIR imaging spectroscopy, it is not restricted to this technique and is applicable for other procedures that can determine the size and material of the investigated particle. The study presented in Paper-II concluded that UV-induced chemical changes could impede the identification of weathered paint materials. This finding suggests the inclusion of spectra of weathered materials to spectral libraries to improve the quality and certainty of MP identification and their related quantification.

Research question 2: Are wastewater treatment plants significant sources of MP pollution to the environment in Denmark?

Paper-I reported MP masses in wastewater for the first time that enabled to relate MP concentrations to their estimated total emission in Denmark. The comparison revealed that MPs comprise a small fraction of organic particulate matter in raw wastewater and that wastewater treatment plants are minor sources of MP pollution in Denmark that contribute less than 0.5 % to the estimated total emission.

Research question 3: How does weathering affect the fate and impact of MPs on freshwater phytoplankton?

MPs in the environment are continuously exposed to various weathering factors; hence their chemical makeup and physical properties are unlikely identical to that of manufactured pristine materials often applied in toxicity studies. The results of Paper-II demonstrated that UV-exposure increased the mobility of heavy metals through the induced chemical changes in marine antifouling paints. Therefore, the behaviour of weathered MPs could

markedly differ from non-weathered materials that potentially influence the MPs' fate and impact on biota as illustrated by the findings of Paper-III. The results emphasised the importance of applying MPs of weathered material in impact studies as weathering can significantly increase the toxicity of MP leachates to freshwater phytoplankton. Therefore, the relevance of toxicity tests employing only pristine MPs is questionable.

5.1. MAIN CONTRIBUTION TO SCIENCE

The MP research field lacks standard protocols for sampling, MP extraction, identification, and impact studies that impedes the comparability and limits the representativeness of measurements and experimental results (1,19,81). The objective of this PhD was to advance MP analytical methods regarding quantification and identification of MPs as well as the improvement of ecotoxicity testing through the investigation of an environmental impact that can significantly alter the chemical makeup of MPs.

MPs are typically quantified by the number of particles which measure can be affected by the incidental fragmentation of MPs during sampling and MP extraction. Paper-I presented a novel method for MP mass estimation based on µFTIR-imaging spectroscopy, which measure is unaffected by sample handling. The study underlined the importance of MP mass quantification that enable to compare results across studies and to assess the potency of different MP sources.

Infrared spectra of pristine materials are often used as references for MP identification despite that these particles are continuously exposed to environmental impacts that can induce chemical alterations in their material. Paper-II aimed to improve MP identification by suggesting the addition of spectra of weathered materials to infrared spectra libraries when applying automatic identification algorithms. Such an extension of the libraries may enable the detection of MPs that would be overlooked by the current identification practices, thus could improve the accuracy of MP measurements.

Impact studies are often carried out with MPs of pristine polymer materials, albeit organisms are more likely to encounter chemically and physically modified MPs due to weathering processes. The findings of Paper-II and Paper-III contributed to the advance of MP toxicity testing by suggesting the application of weathered MPs in impact studies. The experimental results

demonstrated that weathering-induced alterations possibly influence the ecological impact of MPs and identified a probable underlying cause of the observed dissimilar toxic effect of MPs of pristine and weathered materials.

LITERATURE LIST

1. Andrady AL. Microplastics in the marine environment. Mar Pollut Bull. 2011;62(8):1596–605. http://dx.doi.org/10.1016/j.marpolbul.2011.05.030

2. Jahnke A, Arp HPH, Escher BI, Gewert B, Gorokhova E, Kühnel D, et al. Reducing Uncertainty and Confronting Ignorance about the Possible Impacts of Weathering Plastic in the Marine Environment. Environ Sci Technol Lett. 2017 Mar 14;4(3):85–90. https://pubs.acs.org/doi/10.1021/acs.estlett.7b00008

3. Geyer R, Jambeck JR, Law KL. Production, use, and fate of all plastics ever made. Sci Adv. 2017;3(7):e1700782. http://doi.org/10.1126/sciadv.1700782

4. Cole M, Lindeque P, Halsband C, Galloway TS. Microplastics as contaminants in the marine environment: A review. Mar Pollut Bull. 2011;62(12):2588–97. http://dx.doi.org/10.1016/j.marpolbul.2011.09.025

5. Jambeck JR, Geyer R, Wilcox C, Siegler TR, Perryman M, Andrady A, et al. Plastic waste inputs from land into the ocean. Science (80-). 2015;347(6223):768–71. http://doi.org/10.1126/science.1260352

6. Lambert S, Wagner M. Freshwater Microplastics. Vol. 58, Freshwater Microplastics The Handbook of Environmental Chemistry. 2018. 1–23 p. http://doi.org/10.1007/978-3-319-61615-5

7. Villarrubia-Gómez P, Cornell SE, Fabres J. Marine plastic pollution as a planetary boundary threat – The drifting piece in the sustainability puzzle. Mar Policy. 2017. http://doi.org/10.1016/j.marpol.2017.11.035

8. Crawford CB, Quinn B. Microplastic pollutants. 2017. 1–17 p. Elsevier Science, ISBN 9780128094068 http://dx.doi.org/10.1016/B978-0-12-809406-8.00001-3

9. Thompson RC, Olsen Y, Mitchell RP, Davis A, Rowland SJ, John AWG, et al. Lost at Sea: Where does all the plastic go? Science. 2004;304:838. http://doi.org/10.1126/science.1094559

10. Barnes DKA, Galgani F, Thompson RC, Barlaz M. Accumulation and fragmentation of plastic debris in global environments. Philos Trans R Soc B Biol Sci. 2009;364(1526):1985–98. http://doi.org/10.1098/rstb.2008.0205

11. Hidalgo-Ruz V, Gutow L, Thompson RC, Thiel M. Microplastics in the marine

environment: A review of the methods used for identification and quantification. Environ Sci Technol. 2013;46:3060–75. http://doi.org/10.1021/es2031505

12. Frias JPGL, Nash R. Microplastics: Finding a consensus on the definition. Mar Pollut Bull. 2019;138:145–7. http://doi.org/10.1016/j.marpolbul.2018.11.022

13. Hartmann NB, Hüffer T, Thompson RC, Hassellöv M, Verschoor A, Daugaard AE, et al. Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris. Environ Sci Technol. 2019;53(3):1039–47. http://doi.org/10.1021/acs.est.8b05297

14. GESAMP. Sources, Fate and Effects of Microplastics in the Marine Environment – Part Two of a Global Assessment. IMO/FAO/UNESCOIOC/UNIDO/WO/IAEA/UN/UNEP/UNDP Jt Gr Expert Sci Asp Mar Environ Prot (ed P J Kershaw C M Rochman). 2015;220.

15. Andrady AL. Microplastics in the marine environment. Mar Pollut Bull. 2011;62(8):1596–605.http://dx.doi.org/10.1016/j.marpolbul.2011.05.030

16. Browne MA, Galloway TS, Thompson RC. MICROPLASTIC—AN EMERGING CONTAMINANT OF POTENTIAL CONCERN? Integr Environ Assess Manag. 2007;preprint(2008):1. http://doi.org/10.1897/ieam_2008-022.1

17. Primpke S, Cross RK, Mintenig SM, Simon M, Vianello A, Gerdts G, et al. EXPRESS: Toward the Systematic Identification of Microplastics in the Environment: Evaluation of a New Independent Software Tool (siMPle) for Spectroscopic Analysis. Applied Spectroscopy. 2020. http://doi.org/10.1897/ieam_2008-022.1

18. Löder MGJ, Imhof HK, Ladehoff M, Löschel LA, Lorenz C, Mintenig S, et al. Enzymatic Purification of Microplastics in Environmental Samples. Environ Sci Technol. 2017;51(24):14283–92. http://doi.org/10.1021/acs.est.7b03055

19. Löder MGJ, Gerdts G. Methodology Used for the Detection and Identification of Microplastics—A Critical Appraisal. In: Marine Anthropogenic Litter. Berlin: Springer; 2015. p. 1–447. http://doi.org/10.1007/978-3-319-16510-3

20. Ivleva NP, Wiesheu AC, Niessner R. Microplastic in Aquatic Ecosystems. Angew Chemie - Int Ed. 2017;56(7):1720–39. htpp://doi.org/10.1002/anie.201606957

21. Maes T, Jessop R, Wellner N, Haupt K, Mayes AG. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. Sci Rep. 2017;7:1–10. http://dx.doi.org/10.1038/srep44501

22. Lusher A, Hollman P, Mandoza-Hill J. J. Microplastics in fisheries and aquaculture. Vol. 615, FAO Fisheries and Aquaculture Technical Paper. 2017. 127 p. ISBN 978-92-5-109882-0

23. Löder MGJ, Kuczera M, Mintenig S, Lorenz C, Gerdts G. Focal plane array detector-based micro-Fourier-transform infrared imaging for the analysis of microplastics in environmental samples. Environ Chem. 2015;12(5):563. http://doi.org/10.1071/EN14205

24. Smith BC. Infrared spectral interpretation: a systemic approach. CRC Press LLC; 1999. ISBN 0-8493-2463-7

25. Veerasingam S, Ranjani M, Venkatachalapathy R, Bagaev A, Mukhanov V, Litvinyuk D, et al. Contributions of Fourier transform infrared spectroscopy in microplastic pollution research: A review. Crit Rev Environ Sci Technol. 2020;0(0):1–63. https://doi.org/10.1080/10643389.2020.1807450

26. Primpke S, Lorenz C, Rascher-Friesenhausen R, Gerdts G. An automated approach for microplastics analysis using focal plane array (FPA) FTIR microscopy and image analysis. Anal Methods. 2017;9(9):1499–511. http://dx.doi.org/10.1039/C6AY02476A

27. Simon M, van Alst N, Vollertsen J. Quantification of microplastic mass and removal rates at wastewater treatment plants applying Focal Plane Array (FPA)-based Fourier Transform Infrared (FT-IR) imaging. Water Res. 2018;142:1–9. http://doi.org/10.1016/j.watres.2018.05.019

28. Fischer M, Scholz-Böttcher BM. Microplastics analysis in environmental samples-recent pyrolysis-gas chromatography-mass spectrometry method improvements to increase the reliability of mass-related data. Anal Methods. 2019;11(18):2489–97. http://doi.org/10.1039/c9ay00600a

29. Fischer M, Scholz-Böttcher BM. Simultaneous Trace Identification and Quantification of Common Types of Microplastics in Environmental Samples by Pyrolysis-Gas Chromatography-Mass Spectrometry. Environ Sci Technol. 2017;51(9):5052–60. http://doi.org/10.1021/acs.est.6b06362

30. Sarkissian G, Keegan J, Du Pasquier E, Depriester JP, Rousselot P. The analysis of; tires and tire traces using FTIR and PY-GC/MS. J Can Soc Forensic Sci. 2004;37(1):19–37. http://doi.org/10.1080/00085030.2004.10757566

31. Käppler A, Fischer M, Scholz-Böttcher BM, Oberbeckmann S, Labrenz M, Fischer D, et al. Comparison of μ-ATR-FTIR spectroscopy and py-GCMS as identification tools for microplastic particles and fibers isolated from river sediments. Anal Bioanal Chem. 2018;410(21):5313–27. http://doi.org/10.1007/s00216-018-1185-5

32. Koelmans AA, Besseling E, Foekema E, Kooi M, Mintenig S, Ossendorp BC, et al. Risks of Plastic Debris: Unravelling Fact, Opinion, Perception, and Belief. Environ Sci Technol. 2017;51(20):11513–9. http://dx.doi.org/10.1021/acs.est.7b02219

33. Boucher J, Friot D. Primary microplastics in the oceans: A Global Evaluation of Sources. Gland, Switzerland: IUCN.; 2017. 43 p. ISBN 9782831718279 http://dx.doi.org/10.2305/IUCN.CH.2017.01.en

34. Ziajahromi S, Neale PA, Leusch FDL. Wastewater treatment plant effluent as a source of microplastics: review of the fate, chemical interactions and potential risks to aquatic organisms. Water Sci Technol. 2016. http://doi.org/10.2166/wst.2016.414

35. Talvitie J, Heinonen M, Pääkkönen JP, Vahtera E, Mikola A, Setälä O, et al. Do wastewater treatment plants act as a potential point source of microplastics? Preliminary study in the coastal Gulf of Finland, Baltic Sea. Water Sci Technol. 2015;72(9):1495–504. http://doi.org/10.2166/wst.2015.360

36. Murphy F, Ewins C, Carbonnier F, Quinn B. Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environment. Environ Sci Technol. 2016;50(11):5800–8. http://doi.org/10.1021/acs.est.5b05416

37. Talvitie J, Mikola A, Setälä O, Heinonen M, Koistinen A. How well is microlitter purified from wastewater? - A detailed study on the stepwise removal of microlitter in a tertiary level wastewater treatment plant. Water Res. 2017;109:164–72. http://doi.org/10.1016/j.watres.2016.11.046

38. Lares M, Ncibi MC, Sillanpää M, Sillanpää M. Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology. Water Res. 2018;133:236–46. http://doi.org/10.1016/j.watres.2018.01.049

39. Burns EE, Boxall ABA. Microplastics in the aquatic environment: Evidence for or against adverse impacts and major knowledge gaps. Environ Toxicol Chem. 2018;37(11):2776–96. http://dx.doi.org/10.1002/etc.4268

40. Sun J, Dai X, Wang Q, van Loosdrecht MCM, Ni BJ. Microplastics in wastewater treatment plants: Detection, occurrence and removal. Water Res. 2019;152:21–37. https://doi.org/10.1016/j.watres.2018.12.050

41. Bergmann M, Wirzberger V, Krumpen T, Lorenz C, Primpke S, Tekman MB, et al. High Quantities of Microplastic in Arctic Deep-Sea Sediments from the HAUSGARTEN Observatory. Environ Sci Technol. 2017;51(19):11000–10. http://doi.org/10.1021/acs.est.7b03331

42. Kelly A, Lannuzel D, Rodemann T, Meiners KM, Auman HJ. Microplastic contamination in east Antarctic sea ice. Mar Pollut Bull. 2020;154:111130. https://doi.org/10.1016/j.marpolbul.2020.111130

43. Lusher AL, Tirelli V, O'Connor I, Officer R. Microplastics in Arctic polar waters: The first reported values of particles in surface and sub-surface samples. Sci Rep. 2015;5(June):1–9. http://doi.org/10.1038/srep14947

44. Cincinelli A, Scopetani C, Chelazzi D, Lombardini E, Martellini T, Katsoyiannis A, et al. Microplastic in the surface waters of the Ross Sea (Antarctica): Occurrence, distribution and characterization by FTIR. Chemosphere. 2017;175:391–400. http://dx.doi.org/10.1016/j.chemosphere.2017.02.024

45. Eriksen M, Lebreton LCM, Carson HS, Thiel M, Moore CJ, Borerro JC, et al. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. PLoS One. 2014;9(12):1–15. http://doi.org/10.1371/journal.pone.0111913

46. Pabortsava K, Lampitt RS. High concentrations of plastic hidden beneath the surface of the Atlantic Ocean. Nat Commun. 2020;11:4073:1–11. http://dx.doi.org/10.1038/s41467-020-17932-9

47. Andrady AL. The plastic in microplastics: A review. Mar Pollut Bull. 2017;119(1):12–22. http://dx.doi.org/10.1016/j.marpolbul.2017.01.082

48. Rabek JF. Photostabilization of polymers: Principles and application. Elsevier Science Publishers Ltd; 1990. ISBN 978-94-010-6821-5

49. Kämpf G, Sommer K, Zirngiebl E. Studies in accelerated weathering. Part I. Determination of the activation spectrum of photodegradation in polymers. Prog Org Coatings. 1991;19(1):69–77. http://doi.org/10.1016/0033-0655(91)80011-7

50. Hammer J, Kraak MHS, Parsons JR. Plastics in the Marine Environment: The Dark Side of a Modern Gift. Vol. 220, Reviews of Environmental

Contamination and Toxicology. 2012.
http://doi.org/10.1007/978-1-4614-3414-6

51. Wypych G. Principles of Uv Degradation. PVC Degrad Stab. 2015;167–203.
http://dx.doi.org/10.1016/b978-1-895198-85-0.50007-8

52. Oberbeckmann S, Labrenz M. Marine Microbial Assemblages on Microplastics: Diversity, Adaptation, and Role in Degradation. Ann Rev Mar Sci. 2020;12:209–32.
http://doi.org/10.1146/annurev-marine-010419-010633

53. Lagarde F, Olivier O, Zanella M, Daniel P, Hiard S, Caruso A. Microplastic interactions with freshwater microalgae: Hetero-aggregation and changes in plastic density appear strongly dependent on polymer type. Environ Pollut. 2016;215:331–9. http://doi.org/10.1016/j.envpol.2016.05.006

54. Rummel CD, Jahnke A, Gorokhova E, Kühnel D, Schmitt-Jansen M. Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. Environ Sci Technol Lett. 2017;4(7):258–67.
http://doi.org/10.1021/acs.estlett.7b00164

55. Wright SL, Thompson RC, Galloway TS. The physical impacts of microplastics on marine organisms_A review. Environ Pollut. 2013;178:483–92.
http://doi.org/10.1016/j.envpol.2013.02.031

56. Bergmann M, Gutow L, Klages M. Marine anthropogenic litter. 2015. 1–447 p. ISBN 9783319165097. http://doi.org/10.1021/acs.est.9b01360

57. Lusher AL, McHugh M, Thompson RC. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar Pollut Bull. 2013;67(1–2):94–9.
http://dx.doi.org/10.1016/j.marpolbul.2012.11.028

58. Steer M, Cole M, Thompson RC, Lindeque PK. Microplastic ingestion in fish larvae in the western English Channel. Environ Pollut. 2017;226:250–9.
http://dx.doi.org/10.1016/j.envpol.2017.03.062

59. Pazos RS, Maiztegui T, Colautti DC, Paracampo AH, Gómez N. Microplastics in gut contents of coastal freshwater fish from Río de la Plata estuary. Mar Pollut Bull. 2017;122(1–2):85–90.
http://dx.doi.org/10.1016/j.marpolbul.2017.06.007

60. National Oceanic and Atmospheric Administration. ETOPO1 Global Relief Model. http://doi.org/10.7289/V5C8276M

61. Browne MA, Crump P, Niven SJ, Teuten E, Tonkin A, Galloway T, et al. Accumulation of microplastic on shorelines woldwide: Sources and sinks. Environ Sci Technol. 2011;45(21):9175–9. http://doi.org/10.1021/es201811s

62. Cozar A, Echevarria F, Gonzalez-Gordillo JI, Irigoien X, Ubeda B, Hernandez-Leon S, et al. Plastic debris in the open ocean. Proc Natl Acad Sci. 2014;111(28):10239–44. http://www.pnas.org/cgi/doi/10.1073/pnas.1314705111

63. Hahladakis JN, Velis CA, Weber R, Iacovidou E, Purnell P. An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. J Hazard Mater. 2018;344:179–99.http://dx.doi.org/10.1016/j.jhazmat.2017.10.014

64. Lithner D, Larsson A, Dave G. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Sci Total Environ. 2011;409(18):3309–24. http://dx.doi.org/10.1016/j.scitotenv.2011.04.038

65. Lithner D, Nordensvan I, Dave G. Comparative acute toxicity of leachates from plastic products made of polypropylene, polyethylene, PVC, acrylonitrile-butadiene-styrene, and epoxy to Daphnia magna. Environ Sci Pollut Res. 2011;19(5):1763–72. http://doi.org/10.1007/s11356-011-0663-5

66. Horton AA, Centre NO, Spurgeon DJ, Lahive E, Svendsen C. Microplastics in freshwater and terrestrial environments : Evaluating the current understanding to identify the knowledge gaps and future research priorities. 2017. http://doi.org/10.1007/s11356-011-0663-5

67. Eerkes-Medrano D, Thompson RC, Aldridge DC. Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Res. 2015;75:63–82. http://dx.doi.org/10.1016/j.watres.2015.02.012

68. Luo H, Li Y, Zhao Y, Xiang Y, He D, Pan X. Effects of accelerated aging on characteristics , leaching , and toxicity of commercial lead chromate pigmented microplastics. Environ Pollut. 2020;257. https://doi.org/10.1016/j.envpol.2019.113475

69. Bråte ILN, Blázquez M, Brooks SJ, Thomas K V. Weathering impacts the uptake of polyethylene microparticles from toothpaste in Mediterranean mussels (M. galloprovincialis). Sci Total Environ. 2018;626:1310–8. https://doi.org/10.1016/j.scitotenv.2018.01.141

70. Bejgarn S, MacLeod M, Bogdal C, Breitholtz M. Toxicity of leachate from weathering plastics: An exploratory screening study with Nitocra spinipes. Chemosphere. 2015;132:114–9. http://dx.doi.org/10.1016/j.chemosphere.2015.03.010

71. Rummel CD, Escher BI, Sandblom O, Plassmann MM, Arp HPH, Macleod M, et al. Effects of Leachates from UV-Weathered Microplastic in Cell-Based Bioassays. Environ Sci Technol. 2019;53(15):9214–23. http://doi.org/10.1021/acs.est.9b02400

72. Zhu L, Zhao S, Bittar TB, Stubbins A, Li D. Photochemical dissolution of buoyant microplastics to dissolved organic carbon: Rates and microbial impacts. J Hazard Mater. 2020;383:121065. https://doi.org/10.1016/j.jhazmat.2019.121065

73. Romera-Castillo C, Pinto M, Langer TM, Álvarez-Salgado XA, Herndl GJ. Dissolved organic carbon leaching from plastics stimulates microbial activity in the ocean. Nat Commun. 2018;9(1). http://dx.doi.org/10.1038/s41467-018-03798-5

74. Wang W, Gao H, Jin S, Li R, Na G. The ecotoxicological effects of microplastics on aquatic food web, from primary producer to human: A review. Ecotoxicol Environ Saf. 2019;173:110–7. http://doi.org/10.1016/j.ecoenv.2019.01.113

75. Anbumani S, Kakkar P. Ecotoxicological effects of microplastics on biota: a review. Environ Sci Pollut Res. 2018;25(15):14373–96. 1http://doi.org/0.1007/s11356-018-1999-x

76. Ma P, Wei Wang M, Liu H, Feng Chen Y, Xia J. Research on ecotoxicology of microplastics on freshwater aquatic organisms. Environ Pollut Bioavailab. 2019;31(1):131–7. https://doi.org/10.1080/26395940.2019.1580151

77. Prata JC, da Costa JP, Lopes I, Duarte AC, Rocha-Santos T. Effects of microplastics on microalgae populations: A critical review. Sci Total Environ. 2019;665:400–5. https://doi.org/10.1016/j.scitotenv.2019.02.132

78. Gu S, Zheng H, Xu Q, Sun C, Shi M, Wang Z, et al. Comparative toxicity of the plasticizer dibutyl phthalate to two freshwater algae. Aquat Toxicol. 2017;191:122–30. http://doi.org/10.1016/j.aquatox.2017.08.007

79. Wik A, Nilsson E, Källqvist T, Tobiesen A, Dave G. Toxicity assessment of sequential leachates of tire powder using a battery of toxicity tests and toxicity identification evaluations. Chemosphere. 2009;77(7):922–7.

http://dx.doi.org/10.1016/j.chemosphere.2009.08.034

80. Adams WJ, Biddinger GR, Robillard KA, Gorsuch JW. A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environ Toxicol Chem. 1995;14(9):1569–74. http://doi.org/10.1002/etc.5620140916

81. Hidalgo-Ruz V, Gutow L, Thompson RC, Thiel M. Microplastics in the marine environment: A review of the methods used for identification and quantification. Env Sci Technol. 2013;46:3060–75. http://doi.org/10.1021/es2031505

82. Lassen C, Hansen SF, Magnusson K, Hartmann NB, Rehne Jensen P, Nielsen TG, et al. Microplastics: Occurrence, effects and sources of releases to the environment in Denmark. Copenhangen K: Danish Environmental Protection Agency; 2015. ISBN 9788793352803

83. Hartmann NB, Von der Kammer F, Hofmann T, Baalousha M, Ottofuelling S, Baun A. Algal testing of titanium dioxide nanoparticles-Testing considerations, inhibitory effects and modification of cadmium bioavailability. Toxicology. 2010;269(2–3):190–7. http://dx.doi.org/10.1016/j.tox.2009.08.008

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