Temporal variability of microplastic concentration and composition in streams and wastewater treatment plant effluents: mitigation of cross-contamination, long-term mixed sampling, and representativeness assessment

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Temporal Variability of Microplastic Concentration and Composition in Streams and Wastewater Treatment Plant Effluents:

Mitigation of Cross-Contamination, Long-Term Mixed Sampling, and Representativeness Assessment

vorgelegt von

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Abstract

Plastics have many beneficial properties, making them an integral part of everyday life. However, the continuously increasing global production of plastics, as well as their durability and extensive use, entail a serious consequence: The accumulation of plastic fragments of various sizes in the environment. Small plastic fragments, so-called microplastics (MPs), are particularly mobile and have been detected not only in all environmental compartments but also in the most remote parts of the world. Assessing the extent of environmental pollution by MPs is crucial to understanding their impact on ecosystems and, ultimately, on humans, as well as for implementing effective countermeasures. Indeed, the occurrence of MPs in the environment has been intensively researched since the early 2000s, but MP concentrations reported by different studies often deviate by several orders of magnitude, even for the same environmental compartment. Possible reasons for this discrepancy include (i) a high spatial and temporal variability of MP concentrations in the different environmental compartments and (ii) the use of non-uniform methods for sampling, sample processing, and analysis by different studies, as well as insufficient quality assurance and quality control (QA/QC).

This thesis focuses on investigating the temporal variability and representativeness of MP concentration and composition in streams and wastewater treatment plant (WWTP) effluents. The temporal variability of the MP concentration and composition in streams is of great importance in many respects, as streams are the habitat of freshwater biota, a source of drinking water for humans and wildlife, and a transport pathway for MPs into the oceans. In turn, WWTP effluents are a continuous, localized input pathway for MPs into surface waters, and the temporal variability of the MP concentration and composition in WWTP effluents is likely to influence that of receiving water bodies. To obtain not only temporally representative but also reliable MP data, QA/QC, including the avoidance of false positive MP identification, are a fundamental part of this research.

To gain reliable results, MP losses, cross-contamination, and false positive MP findings must be minimized and quantified as far as possible. Two hitherto mostly unknown sources of false positive MP findings are disposable gloves and sodium dodecyl sulfate (SDS). Disposable gloves are commonly worn during sample processing to avoid cross-contamination or for personal protection. SDS is often applied during sample processing to aid the decomposition of animal and plant residues. It was found that SDS and the stearates released from disposable gloves can be falsely identified as polyethylene

(PE) when analyzed using Raman microspectroscopy (μ-Raman), Fourier transform infrared microspectroscopy (μ-FTIR), and pyrolysis-gas chromatography/mass spectrometry (pyr-GC/MS). The extent of the potential PE overestimation due to the use of disposable gloves was experimentally determined for all three analytical methods and their suitability to distinguish between SDS, PE, and stearates was investigated and assessed. Measures to avoid false positives were derived and applied to all subsequent laboratory and field experiments carried out as part of this thesis.

The temporal variability of MP concentration and composition in aquatic matrices has only been researched to a limited extent to date. In particular, MP studies that cover long investigation periods and collect temporally representative mixed samples are rare. This is partly due to a lack of sampling devices specifically designed for long-term sampling of MPs. Therefore, a custom-built, automatic sampling unit for MPs (SAM) was developed within this thesis, which is capable of fully automated, repetitive collection of volume-reduced mixed samples from freshwater matrices over long periods of time. A recovery experiment (positive control) using five different polymer types revealed an overall recovery of 77 $\pm$ 29% for sampling with SAM, subsequent sample processing, and μ-FTIR analysis for particles $\geq$ 11 μm. A low level of cross-contamination was verified based on process blank values, which recorded cross-contamination over the entire workflow from sampling preparation to sample analysis. These process blank values resulted in low, polymer-specific detection limits, demonstrating a high sensitivity of the overall method.

The good suitability of SAM for long-term sampling was proven by two field applications. SAM was applied to collect 24-hour mixed samples over a period of eight days in a stream and over a period of 31 days in a WWTP effluent. By analyzing the corresponding mixed samples with μ-FTIR, the concentration and composition of MPs $\geq$ 11 μm was determined for each sampling day. In both the stream and the WWTP effluent, high day-to-day variability of MP concentration was observed. In the stream, no clear relationship could be identified between the time series of MP concentration and the daily precipitation in the catchment area, the discharge of the stream, the turbidity, or the total particle count in the stream. Similarly, for the WWTP effluent, no correlation could be established between the MP concentration and the discharge from the WWTP effluent, or the daily precipitation sum. However, a high positive correlation was found between the time series of the concentration of many relevant polymer types in the WWTP effluent. Furthermore, a connection was observed between the temporal proximity of sampling days and the similarity in MP concentration and composition of the corresponding samples. The temporal variability of the MP composition by shape, size, and polymer type was much more consistent over the study periods in both the stream and the WWTP effluent than the variability of the MP concentration. Thus, it was concluded that repetitive or continuous collection of 24-hour mixed samples is required to obtain MP concentration data that is representative over long periods of time, while individual mixed samples may be sufficient for making a rudimentary estimate of the MP composition in streams and WWTP effluents.

This thesis has revealed two hitherto mostly unknown causes for overestimation of MP concentrations and has derived effective countermeasures. In addition, an automated device for the repetitive collection of mixed samples over long time periods was developed and validated. Application of this device for sampling from a stream and a WWTP effluent yielded reliable data on the temporal variability of MP concentration and composition and enabled a first assessment of potential variability influencing factors. This work therefore represents a first step towards the collection of reliable, temporally representative MP data from streams and WWTP effluents, and serves as a basis for further research on the temporal variability of MP concentration and composition in water matrices.

Zusammenfassung

Plastik hat viele nützliche Eigenschaften, weshalb es im Alltag omnipräsent geworden ist. Die weltweit kontinuierlich steigende Produktion von Plastik sowie seine hohe Beständigkeit und extensive Verwendung haben jedoch auch eine Schattenseite: Die Anreicherung von Plastikfragmenten unterschiedlicher Größe in der Umwelt. Kleine, als Mikroplastik (MP) bezeichnete Plastikfragmente sind besonders mobil und wurden nicht nur in allen Umweltkompartimenten, sondern auch an den entlegensten Orten der Erde nachgewiesen. Es ist essenziell, das Ausmaß der Umweltverschmutzung durch MP zu erfassen, um die Auswirkungen auf Ökosysteme und letztlich auf den Menschen abschätzen und wirksame Gegenmaßnahmen ergreifen zu können. Zwar wird das Vorkommen von MP in der Umwelt seit Anfang der 2000er Jahre intensiv erforscht, jedoch unterscheiden sich die in den verschiedenen Studien angegebenen MP-Konzentrationen oft um mehrere Größenordnungen, selbst wenn ein und dasselbe Umweltkompartiment untersucht wurde. Mögliche Gründe für diese Diskrepanz sind (i) eine sehr hohe räumliche und zeitliche Variabilität der MP-Konzentration in den verschiedenen Umweltkompartimenten und (ii) die Verwendung uneinheitlicher Methoden für die Probenahme, Probenverarbeitung und Analyse durch verschiedene Studien sowie eine unzureichende Qualitätssicherung und Qualitätskontrolle (QA/QC).

Diese Dissertation befasst sich mit der Untersuchung der zeitlichen Variabilität und Repräsentativität der MP-Konzentration und -Zusammensetzung in Fließgewässern und im Ablauf von Kläranlagen (KAs). Fließgewässer dienen als Lebensraum für Süßwasserbiota, als Trinkwasserressource für Mensch und Tier und als Transportweg für MP in die Ozeane. Dementsprechend ist die zeitliche Variabilität der MP-Konzentration und -Zusammensetzung in Fließgewässern in vielerlei Hinsicht von großer Bedeutung. KA-Abläufe wiederum stellen kontinuierliche, lokale Eintragspfade für MP in Oberflächengewässer dar, und es ist davon auszugehen, dass die Variabilität der MP-Konzentration und -Zusammensetzung im Ablauf von KAs die MP-Konzentration und -Zusammensetzung der entsprechenden Vorfluter beeinflusst. Um nicht nur zeitlich repräsentative, sondern auch belastbare MP-Daten zu erzeugen, bilden QA/QC, einschließlich der Vermeidung falsch-positiver MP-Befunde, einen grundlegenden Bestandteil dieser Forschungsarbeit.

MP-Verluste, Kreuzkontaminationen und falsch-positive Befunde müssen so weit wie möglich vermieden und quantifiziert werden, um belastbare Ergebnisse zu gewinnen. Zwei bisher weitestgehend unbekannte Quellen für falsch-positive MP-Befunde sind Einweghandschuhe und Natriumdodecylsulfat (SDS). Einweghandschuhe werden häufig bei der Probenaufbereitung zur Vermeidung von Kreuzkontaminationen

oder aus Gründen des Arbeitsschutzes getragen. SDS hingegen kommt oft bei der Probenaufbereitung zum Einsatz, um den Abbau von tierischen und pflanzlichen Rückständen in den Proben zu unterstützen. Allerdings können SDS und die von Einweghandschuhen freigesetzten Stearate bei der Analyse mittels Raman-Mikrospektroskopie (µ-Raman), Fourier-Transformations-Infrarot-Mikrospektroskopie (µ-FTIR) und Pyrolyse-Gaschromatographie/Massenspektrometrie (pyr-GC/MS) fälschlicherweise als Polyethylen (PE) identifiziert werden. Das Ausmaß der potenziellen PE-Überschätzung aufgrund der Verwendung von Einweghandschuhen wurde in dieser Arbeit für alle drei Analysemethoden experimentell bestimmt. Zudem wurde untersucht und bewertet, inwieweit die Analysemethoden zur Unterscheidung zwischen SDS, PE und Stearat geeignet sind. Maßnahmen zur Vermeidung von falsch-positiven Befunden wurden abgeleitet und auf alle nachfolgenden Labor- und Feldexperimente angewendet.

Die zeitliche Variabilität der MP-Konzentration und -Zusammensetzung in aquatischen Matrices ist bisher nur in begrenztem Umfang untersucht worden. Insbesondere gibt es nur wenige MP-Studien, die lange Untersuchungszeiträume abdecken und zeitlich repräsentative Mischproben sammeln. Die Ursache hierfür ist unter anderem das Fehlen von Probenahmeapparaturen, die speziell für die Langzeitbeprobung von MP konzipiert sind. Daher wurde im Rahmen dieser Thesis die Probenahmeapparatur "SAM" entwickelt, welche in der Lage ist, vollautomatisch und über lange Zeiträume hinweg volumenreduzierte Mischproben aus Süßwassermatrices zu nehmen. Ein Wiederfindungsversuch (Positivkontrolle) mit fünf verschiedenen Polymerarten ergab eine Gesamtwiederfindung von $77 \pm 29\%$ für die Probenahme mit SAM, die anschließende Probenaufbereitung sowie die µ-FTIR-Analyse für Partikel ≥ 11 µm. Anhand von Prozessblindwerten, die die Kreuzkontamination über den gesamten Arbeitsablauf von der Vorbereitung der Probenahme bis zur Probenanalyse erfassten, wurde ein geringes Maß an Kreuzkontamination nachgewiesen. Zudem ergaben sich aus den Prozessblindwerten niedrige, polymerspezifische Nachweisgrenzen, was eine hohe Empfindlichkeit der Gesamtmethode belegt.

Die gute Eignung von SAM für Langzeitprobenahmen wurde anhand von zwei Feldversuchen unter Beweis gestellt. SAM wurde zur Gewinnung von 24-Stunden-Mischproben über einen Zeitraum von acht Tagen aus einem Bach und über einen Zeitraum von 31 Tagen aus einem KA-Ablauf eingesetzt. Durch Analyse der entsprechenden Mischproben mittels µ-FTIR wurde die Konzentration und Zusammensetzung des MPs ≥ 11 µm für jeden Probentag bestimmt. Sowohl im Bach als auch im KA-Ablauf wurde eine hohe Variabilität der MP-Konzentration über mehrere Tage hinweg festgestellt. Im Bach konnte keine Beziehung zwischen der Zeitreihe der MP-Konzentration und dem täglichen Niederschlag im Einzugsgebiet, den Abflussvolumina, der Trübung oder der Gesamtpartikelzahl festgestellt werden. Auch für den KA-Ablauf konnte kein Zusammenhang zwischen der MP-Konzentration und dem Auslaufvolumen aus der KA oder der Niederschlagssumme im Einzugsgebiet hergestellt werden. Allerdings wurde eine hohe, positive Korrelation zwischen

den Zeitreihen der Konzentration der wesentlichen Polymerarten im KA-Ablauf ermittelt. Außerdem wurde ein Zusammenhang zwischen der zeitlichen Nähe von Probenahmetagen und der Ähnlichkeit der MP-Konzentration und -Zusammensetzung zwischen den entsprechenden Proben festgestellt. Die zeitliche Variabilität der MP-Zusammensetzung nach Form, Größe und Polymertyp war innerhalb der Untersuchungszeiträume im Bach und im KA-Ablauf wesentlich beständiger als die der MP-Konzentration. Daraus wurde geschlussfolgert, dass eine wiederholte oder kontinuierliche Entnahme von 24-Stunden-Mischproben erforderlich ist, um MP-Konzentrationen zu erhalten, die über lange Zeiträume repräsentativ sind, während einzelne Mischproben für eine grobe Abschätzung der MP-Zusammensetzung in Fließgewässern und KA-Abläufen geeignet sein können.

In dieser Arbeit wurden zwei bisher weitestgehend unbekannte Ursachen für die Überschätzung der MP-Konzentration aufgedeckt und wirksame Gegenmaßnahmen abgeleitet. Darüber hinaus wurde eine automatisierte Apparatur zur Sammlung von Mischproben über lange Zeiträume entwickelt und validiert. Der Einsatz dieser Apparatur sowohl in einem Bach als auch im Ablauf einer KA lieferte belastbare Daten zur zeitlichen Variabilität der MP-Konzentration und -Zusammensetzung und ermöglichte eine erste Bewertung potentieller Einflussfaktoren auf die Variabilität. Diese Arbeit stellt somit einen ersten Schritt auf dem Weg zur Erhebung zuverlässiger, zeitlich repräsentativer MP-Daten aus Fließgewässern und KA-Abläufen dar und bietet eine Grundlage für weitere Forschung zur zeitlichen Variabilität der MP-Konzentration und -Zusammensetzung in Wassermatrices.

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Contents

Chapter 1

Introduction

1.1   A Brief History of Plastics

Plastics have become indispensable in medicine, transportation, construction, and the electrical and electronics industry and are firmly embedded in everyday life. This is mainly due to the versatile properties of plastics, such as durability, moldability, flexibility or stiffness, low weight, poor conductivity of electricity and heat as well as the low production costs (Andrady and Neal, 2009; Pilapitiya and Ratnayake, 2024; Thompson et al., 2009). Plastics have helped improve living standards and enabled many technological and medical advances.

The term "plastic" originates from the Greek word "plasticos" (moldable) and is a general expression for synthetic polymers or chemically modified natural polymers. Polymers are macromolecules consisting of a large number of covalently linked basic building blocks, so-called monomers (Koltzenburg et al., 2014). Natural polymers, which do not count as plastics, make up most of the structures of living tissue and include, for instance, proteins, cellulose, deoxyribonucleic acid (DNA), and starch (IUPAC, 2024). The earliest plastic, a human-made chemically modified natural polymer based on cellulose, was patented in 1861 with the product name "Parkesine". The first fully synthetic polymer, called "Bakelite", was developed in 1907 (Thompson et al., 2009). At least 15 new synthetic as well as chemically modified natural polymers were engineered over the subsequent half-century, and industrial mass production of plastics arose in the 1940s and 1950s (Andrady and Neal, 2009; Pilapitiya and Ratnayake, 2024; Thompson et al., 2009). In 1950, the annual global production of plastics was 2 million metric tonnes and has continued to rise ever since (Geyer et al., 2017), exceeding a global annual production of plastics of 400 million tonnes in 2022 (see Figure 1.1a) (Plastics Europe, 2024).

According to Andrady and Neal (2009) there are hundreds of plastic materials today, with a clear dominance of the polymer types polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polyethylene terephtalate (PET), polyurethane (PU) and polystyrene (PS), which account for 74.7% of global plastic production (Plastics Europe, 2024). Most plastics, including those just mentioned, are fossil-based (see Figure 1.2), meaning that they are created by polymerization of monomers derived from petrochemicals, such as oil, coal or combustible gas (Pilapitiya and Ratnayake, 2024). In contrast,

biobased plastics, which are fully or to a significant part derived from renewable bio-
logical material, including raw material and residues (Hatti-Kaul et al., 2020), currently
account for only 0.7% of global plastic production (Plastics Europe, 2024). This is due
to issues such as high production costs compared to conventional fossil-based plastics,
high energy demand during production, competition for arable land for biomass pro-
duction as well as undeveloped recycling and/or disposal routes for biobased plastics
(Ferreira-Filipe et al., 2021; Hatti-Kaul et al., 2020). Nevertheless, research and develop-
ment of biobased plastics is increasing, as they are seen as a way of shifting away from
fossil resources in plastics production and, in the context of global climate change, re-
ducing associated greenhouse gas emissions (Ferreira-Filipe et al., 2021; Hatti-Kaul et
al., 2020; Rosenboom et al., 2022).
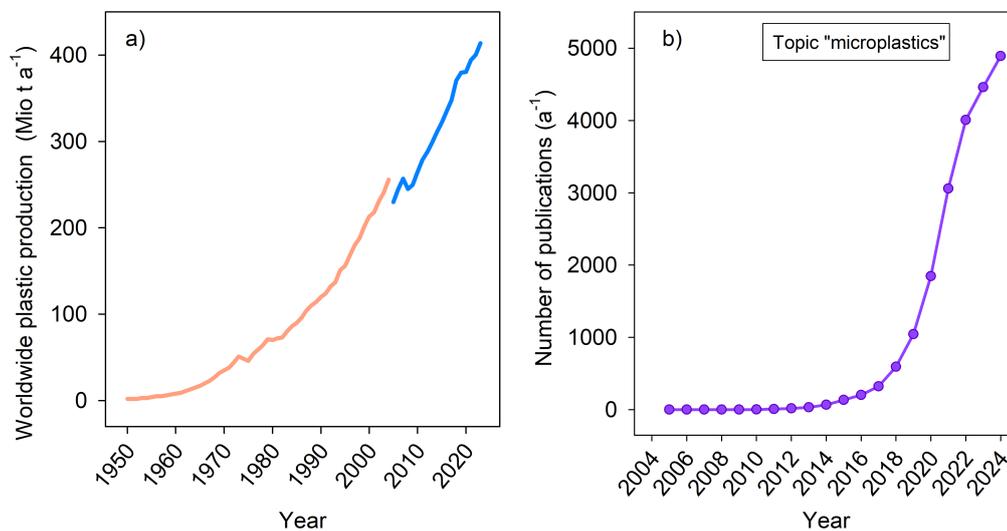
FIGURE 1.1: a) Increase of annual worldwide plastic production since 1950 in million
tonnes. Data displayed in orange (1950–2004) is based on Geyer et al., 2017, OECD, 2022
and Our World in Data, 2025. Data displayed in blue (2005–2023) is based on Plastics
Europe, 2007, 2009, 2010, 2015, 2016, 2017, 2018, 2024. b) Number of research articles
published per year on the topic of "microplastics", according to Web of Science (searched
on 26.01.2025).

Environmental pollution is a major issue associated with the production and use
of plastics. A controversial option for reducing plastic in the environment is the use
of biodegradable plastics. These can be produced from renewable or fossil materials
(see Figure 1.2) and are designed to rapidly degrade (mineralize) under the right condi-
tions, determined by pH, temperature, moisture, oxygen and the available microorgan-
isms (Nizamuddin and Chen, 2024). However, the degradability of biodegradable plas-
tics determined under laboratory conditions or in industrial composting is not directly
transferable to the environment. Degradation in the various environmental matrices is
often much slower and less complete, so that residues (e.g., small fragments) and non-
biodegradable additives remain in the environment (Flury and Narayan, 2021; Qin et

al., 2021). Therefore, biodegradable plastics in general do not represent an environmentally friendly alternative to conventional plastics, although they may offer some benefit over conventional plastics in specific applications where release into the environment is intended or unavoidable (e.g., agricultural mulch films, dolly ropes) and where recovery and reuse or recycling are not feasible for economic or logistical reasons (European Commission, 2021).
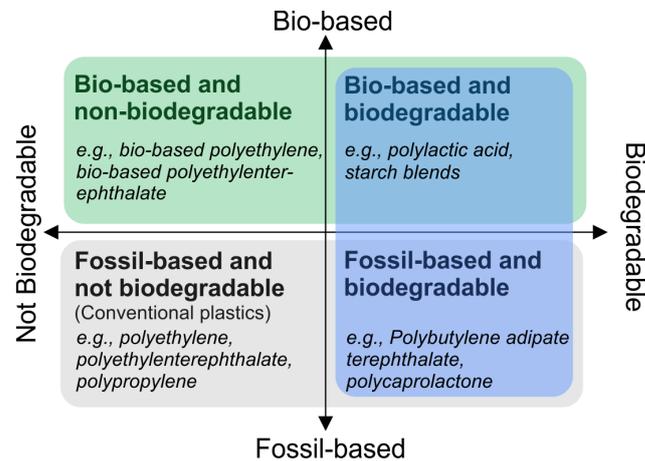
FIGURE 1.2: Categorization of plastics regarding their base material (fossil-based versus biobased) and their design for durability or degradability under certain ambient conditions (non-biodegradable versus biodegradable plastics). Figure modified from Nizamuddin and Chen, 2024.

1.2 Microplastics—a Contaminant of Emerging Concern

1.2.1 Definition, Origin and Pathways

Plastics can enter the natural environment through inappropriate waste management, uncontrolled disposal and littering, but also through their intentional use in environmentally exposed applications (e.g., pond liners, corrugated plastic roofs, polytunnels). The timescales required for plastics to completely degrade (mineralize) under environmental conditions are unknown (Thompson et al., 2024), although ranges from hundreds to thousands of years are assumed (Barnes et al., 2009). Geyer et al. (2017) estimate that 60% of the plastic produced in the world since the 1950s are still accumulating in landfills or in terrestrial and aquatic ecosystems.

For humans, some of the consequences of plastic pollution are clearly visible, such as the aesthetic impairment of nature or the death of wildlife entangled in plastic waste. A much less obvious consequence of plastics introduced into the environment is the breakdown of plastic items into tiny plastic fragments under the influence of solar ultra violet radiation (photo-oxidation) and mechanical stress, for instance friction caused by wind or waves (Andrady et al., 2022). In 2004, the term "microplastics" (MPs) was used for the first time by Thompson et al. (2004) to describe small plastic fragments present in beach sediments, seawater, and plankton samples which had originated from

the breakdown of larger plastic items. In their study, Thompson et al. (2004) stressed that MPs were geographically widespread, that their environmental consequences were unknown and that their abundance was likely to further increase due to the continuously growing plastic production, the longevity of plastics and their disposable nature. This was the kick-off for MP research. In the years that followed, the number of scientific studies on the sources, fate and effects of MPs, but also on the underlying analytical methods, increased substantially (see Figure 1.1b).

Although widely used, the term MP has not yet been standardized. In the context of this thesis, **MPs are referred to as small, solid plastic items, which consist of synthetic or heavily modified natural polymers and, with their largest dimension, are in the size range between 1 μm and 1000 μm** (see Figure 1.3) (Hartmann et al., 2019). This differs from the definition also frequently used in scientific studies, in which plastic particles smaller than 5 mm or in the size range between 1 μm and 5 mm are referred to as MPs (Arthur et al., 2009; Koelmans et al., 2019). However, that definition is not in line with the International System of Units nomenclature and is not scientifically justified (Chae et al., 2023; Hartmann et al., 2019).
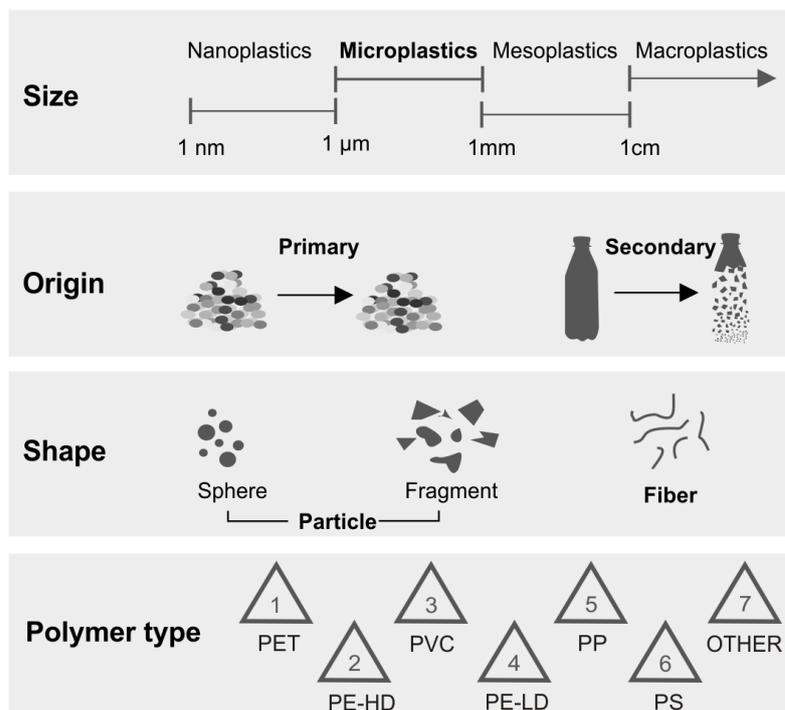
FIGURE 1.3: Characteristics of plastic items by their size, their origin from purposeful production or from the breakdown of larger plastic items, their shape with the main groups of particles and fibers, and by their polymer types, symbolized by Resin Identification Codes according to Geueke (2015) for polyethylene terephthalate (PET), high-density polyethylene (PE-HD), polyvinyl chloride (PVC), low-density polyethylene (PE-LD), polypropylene (PP), and polystyrene (PS).

Based on their origin, a distinction is commonly made between primary and secondary MPs (see Figure 1.3). Primary MPs are intentionally produced MPs (Galafassi et al., 2019; GESAMP, 2016) to be used for instance during abrasive blasting (Coyle et al.,

2020; Gallo et al., 2018), as coating of slow-release fertilizers (Kassem et al., 2024), or as an ingredient in paints (Turner, 2021) and 3D printing (Kühnel et al., 2023). Although the release of primary MPs into the environment is mostly unintentional, primary MPs account for about 15% to 31% of the MPs in the environment (Boucher and Friot, 2017). The remaining 69% to 85% consist of secondary MPs. Secondary MPs are the result of unintentional breakdown of larger plastic items either during the use of plastic products or the weathering of plastic litter (Andrady et al., 2022; Galafassi et al., 2019). Examples include plastic fragments from agricultural mulch films (Khalid et al., 2023), abrasion from road marking paint (Rathnaweera et al., 2023), and fiber fragments created during the washing of synthetic clothing (Galvão et al., 2020). As automotive tires usually consist of 40% to 60% synthetic polymers (e.g., styrene-butadiene rubber or polybutadiene rubber) (Hartmann et al., 2019), tire and road wear particles (TRWP) composed of a mixture of tire and other road-related wear particles, can also be considered as secondary MPs (Galafassi et al., 2019).

There is a wide variety of ways in which MPs can be released into the environment (see Figure 1.4), for example through effluents and stormwater overflows from wastewater treatment plants (WWTP), the application of sewage sludge in agriculture, illegal waste dumping, littering, the loss of industrial pre-production plastic pellets and the use of artificial turf (Galafassi et al., 2019).
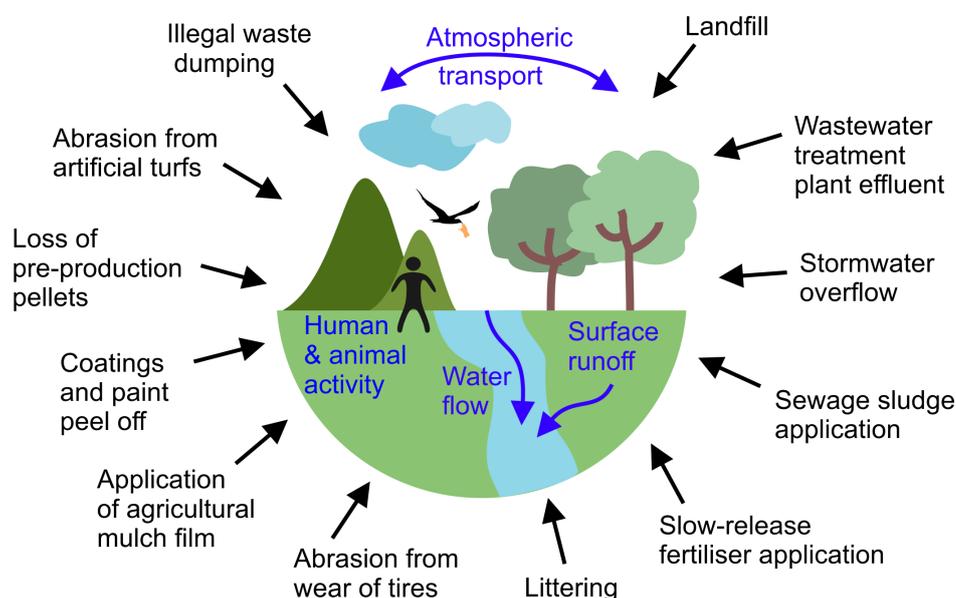
FIGURE 1.4: Major pathways and redistribution of microplastics in the terrestrial environment.

Once in the environment, plastics and MPs are distributed by atmospheric transport (e.g., wind) and water (e.g., surface runoff and river transport), as well as by human and animal activities (see Figure 1.4). Their presence has been reported in all environmental compartments, including soils (Hurley and Nizzetto, 2018; Klaus et al., 2024; Weber et al., 2024), wetlands (Qian et al., 2021), rivers (Gao et al., 2024), lakes (Dusaucy et al.,

2021), marine environments (Coyle et al., 2020; Lorenz et al., 2019) as well as the air (Jenner et al., 2022; Vianello et al., 2019). MPs have even been found in remote areas of the world, such as mountains (Padha et al., 2022) and polar regions (Citterich et al., 2023) and are therefore considered ubiquitous (Rochman, 2018).

1.2.2   Impact on the Environment and Human Health

MPs can be ingested by organisms directly, for example by confusion with food (Ory et al., 2017) or filter feeding (Fernández and Albentosa, 2019; Rendell-Bhatti et al., 2023), or indirectly via trophic transfer (Caldwell et al., 2022; Nelms et al., 2018; Yadav et al., 2024). The review study by Ugwu et al. (2021), focusing on marine biota, found that of the individuals studied, 42% of fish, 59% of marine mammals, 50% of seabirds, and 88% of turtles were contaminated with MPs. The extent to which MPs impact organisms is still unclear, although adverse effects, including neurotoxicity, changes in mortality, growth, behavior, respiration, oxidative metabolism, immune responses, energy reserves, reproduction, as well as cellular and DNA damage, have been reported (Pinheiro et al., 2020; Prata et al., 2021a). These adverse effects may result from physical damage, such as external and/or internal abrasion or blockage of the digestive tract (Wright et al., 2013), but also from the leaching of additives from MPs into the natural habitat of biota or upon ingestion of MPs (Herrera et al., 2022; Yu et al., 2024). Additives (e.g., fillers, plasticizers, pigments, foaming agents and flame retardants) are chemicals typically added during the production process of plastics to induce specific, desired material properties (Andrady and Neal, 2009; Pilapitiya and Ratnayake, 2024) and can, in themselves, negatively impact biota (Yu et al., 2024). Furthermore, MPs have a large specific surface area and can absorb pollutants such as heavy metals, antibiotics and persistent organic pollutants from the environment (Rafa et al., 2024). When ingested, pollutants absorbed by MPs from the environment, as well as plastic additives, can bioaccumulate and cause ecotoxicological effects (Herrera et al., 2022; Rafa et al., 2024). Due to their high specific surface area, MPs also act as a habitat for microorganisms, including potential pathogens, and can transport them over great distances. Whether the increased potential for pathogen transport to hosts leads to an increase in disease outbreaks, remains unknown (Bowley et al., 2021).

Despite numerous ecotoxicological studies (in particular in marine and aquatic environments), the evidence available for conducting a quantitative risk assessment of MPs for biota remains insufficient (de Ruijter et al., 2020; Prata et al., 2021a). This is largely because organisms are often exposed to unrealistic conditions in laboratory tests. Concentrations far exceeding those encountered in the environment are frequently used, which may lead to an overstatement of toxicity. The review study by de Ruijter et al. (2020) on quality criteria for MP effect studies found that only 33% of the studies included at least one environmentally realistic concentration. In addition, MP mixtures used in laboratory tests rarely reflect the heterogeneous polymer types, shapes, and sizes found in the natural environment, so that toxicity effects observed during laboratory tests may not reflect the toxicity effects in the environment (de Ruijter et al., 2020; Phuong et al., 2016).

For instance, Silva et al. (2021) found no effects on the reproduction of *Lumbriculus variegatus* after long-term exposure to irregular-shaped polyethylene (PE) particles, while de Ruijter et al. (2023), using an environmentally realistic MP mixture (naturally aged plastics of varying polymer types, sizes, shapes, colors and concentrations), reported that the species is likely to be affected at the population level. A further issue of ecotoxicological studies is related to the fact that natural particles can have similar properties as MPs and may cause similar toxicological effects. Therefore, it is necessary to assess the toxicological effects of MPs in the presence of and in comparison with natural particles (Koelmans et al., 2022; Schwarzer et al., 2022).

Compared to the effects of MPs on biota, abiotic effects have been much less investigated (Prata et al., 2021a). Nevertheless, MPs have been reported to cause changes in soil properties, such as soil bulk density, water holding capacity, stability of soil water aggregates, pH, conductivity, carbon/nitrogen ratio, and dissolved organic matter, which can further affect the structure and function of the microbial community (Ren et al., 2022). In sediments, MPs can cause changes in permeability and thermal conductivity (Carson et al., 2011; Routier et al., 2024) as well as in compressibility, undrained shear strength and hydraulic conductivity (Routier et al., 2024). Such abiotic changes can potentially have a lasting impact on ecosystems and ultimately also affect humans, beyond the potential effects of direct exposure.

Direct exposure of humans to MPs can occur via ingestion of contaminated foodstuff, such as seafood, sugar, salt, and honey (Cox et al., 2019), as well as through contact of food with plastic materials, such as food packaging (Du et al., 2020; Liu et al., 2022) or plastic cutting boards (Luo et al., 2022). MPs have also been identified in beverages such as drinking water (Koelmans et al., 2019; Mintenig et al., 2019) and bottled water (Koelmans et al., 2019; Oßmann et al., 2018). MPs present in the air can also deposit on food during meal preparation and consumption (Catarino et al., 2018) or can be inhaled directly (Maurizi et al., 2024; Vianello et al., 2019). In contrast, the uptake of MPs via dermal contact is considered unlikely, as particles > 45 nm cannot permeate nor penetrate the skin (Filon et al., 2015).

MPs have been detected in human tissues, organs, and bodily fluids, and their presence in human feces and exhaled air has been reported (Thompson et al., 2024). However, there is currently no evidence of direct adverse effects of MPs on humans (Prata et al., 2021a), although exposure and effect assessments are still incomplete and further research is needed to clarify the extent to which MPs affect human health (Leslie and Depledge, 2020; Thompson et al., 2024). This is due to the fact that most studies on the adverse effects of MPs on human health are performed in vitro or in vivo using model organisms and are compromised by the same limitations described above for ecotoxicological studies (Prata et al., 2021a). Due to steadily increasing plastic production, continued release, and fragmentation of plastics, MPs in the environment are expected to further increase in numbers and mass (Koelmans et al., 2022). According to Koelmans et al. (2022) *"[...] most scientist agree that it is not a question of if, but rather when, the environmental and human health risks of MP particles become apparent."* Until then, the

precautionary principle should be applied, especially in view of the durability of MPs and the fact that it is nearly impossible to remove them from the environment once they have been released (Thompson et al., 2024).

1.3 Sampling, Sample Processing and Analysis

To create realistic testing conditions and achieve conclusive results, ecotoxicological studies require reliable data on the concentrations and composition of MPs in different environmental compartments. However, standardization and harmonization of MP sampling, sample processing, and analysis is limited (Andrade et al., 2020; Cowger et al., 2020; Schymanski et al., 2021). This hampers comparability between MP studies and may be one of the reasons why MP concentrations reported in the environment often differ by several orders of magnitude, even in the same matrix (Koelmans et al., 2019; Lu et al., 2021).

The matrix of particular interest in this thesis is freshwater. Freshwater serves as a relevant pathway for MPs into the environment (e.g., WWTP effluents, stormwater overflows) and for the redistribution of MPs (see Figure 1.4). Furthermore, it is the habitat of freshwater organisms and serves as a source of drinking water for humans and biota. Therefore, in the following, an overview is provided of the most commonly applied methods for sampling MPs from aqueous matrices, as well as for the sample processing and analysis of MPs. The suitability of a sampling and sample processing method generally depends on the analytical technique to be used (Ivleva, 2021).

1.3.1 Sampling

Sampling techniques. Unlike many other pollutants, MPs are not present in water in dissolved form (see Section 1.2.1). Therefore, their distribution is much more heterogeneous, and large sample volumes are generally collected to increase the representativeness of MP samples. However, the required sample volume also depends on the expected MP concentration in the matrix and the limit of detection (LOD) of the analytical technique to be used (Ivleva, 2021; Koelmans et al., 2019).

Volume-reduced sampling is a commonly applied MP sampling strategy, where the water to be sampled is passed through one or several filters or a net, on which the MPs (along with other debris) accumulate (Hidalgo-Ruz et al., 2012; Prata et al., 2019b; Stock et al., 2019). Compared to bulk sampling, where the entire volume of the sample is retained (see Figure 1.5), volume-reduced sampling has the great advantage of keeping only the solid portion of the sample, allowing large volumes to be sampled without increasing the complexity of sample handling and transport (Hidalgo-Ruz et al., 2012). However, this comes with the disadvantage that the minimum mesh width of the net or filter applied determines the smallest size of MPs that can subsequently be quantified.

When using volume-reduced sampling, clogging of filters or nets can force the termination of sampling. To delay clogging and maximize the volume sampled, multiple filters with decreasing mesh sizes are often placed in series along the flow direction.

Some examples of this general setup, refered to as "fractionated filtration" in the following, are the Universal Filtering Objects system (300 μm, 10 μm) by Rist et al. (2020), the filter cascade (100 μm, 20 μm, 5 μm) by Pittroff et al. (2021) and the in-situ filter pump (500 μm, 300 μm, 50 μm) by Schönlau et al. (2020).
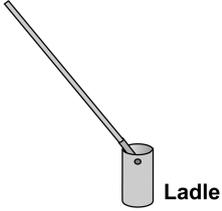

Bulk sampling	Volume-reduced sampling	
Ladle	**Net**	**Filter cascade** — Mesh width
+ All microplastics are preserved **+** Precise volume determination **-** Suited only for small sampling volumes **-** Possibly not a closed system	**+** Sampling of large volumes possible without increased complexity of sample handling & transport **-** Minimum microplastic size determined by mesh width **-** Clogging of filters and nets is possible	
	Nets	**Filters & fractionated filtration**
	+ Sampling at surface and in water column **-** Mesh size between 80 and 500 μm **-** Not a closed system **-** Water flow required (river, towing) **-** Imprecise volume determination	**+** Mesh size ≥ 1 μm **+** Closed system **+** Water supply by pump **+** Precise volume determination **-** Sampling directly at surface not possible

FIGURE 1.5: Comparison of the advantages and disadvantages of bulk sampling and volume-reduced sampling. Within volume-reduced sampling, the advantages and disadvantages of using nets (e.g., plankton net) and fractionated filtration (e.g., filter cascade) are compared.

Nets (e.g., neuston, manta or plankton nets) can be used for sampling at the water surface or in the water column and usually have a mesh size in the range between 80 μm and 500 μm, with 333 μm being the most common mesh size (Prata et al., 2019b; Rios Mendoza and Balcer, 2019; Stock et al., 2019). Compared to the use of filters, nets have three major disadvantages. Firstly, nets must be exposed to the surrounding air when they are deployed and hauled in, which means that there is a risk of cross-contamination. Secondly, nets require a water flow, which, if not naturally present (as for example in a river), must be created by towing (Rist et al., 2020; Schönlau et al., 2020). Thirdly, even when using a built-in flow meter, the amount of water filtered through a net cannot be determined precisely when sampling the water surface, due to the constantly changing immersion depth of the net and the fixed position of the flow meter in the net frame (Montoto-Martínez et al., 2022).

Filters, which are commonly made of stainless steel and available with a variety of mesh widths down to 1 μm, are usually built into a closed system, which is supplied with the sample media via a pump (Mintenig et al., 2017; Pittroff et al., 2021; Rist et al., 2020; Wolff et al., 2019). This enables sampling even when the water body is not big

or deep enough for towing of a net (Bordós et al., 2019) and reduces the risk of cross-contamination from the surrounding air (Pittroff et al., 2021). To prevent the pump from sucking in air, sampling is generally performed from within the water column when using filters (Bordós et al., 2019; Rist et al., 2020; Schönlau et al., 2020).

Sampling in the context of spatial variability. The distribution of MPs in aquatic matrices is not homogeneous but instead depends on external influences such as currents, flow conditions, salinity, wind, and biofilm growth. Furthermore, the varying properties of the MPs themselves (see Figure 1.3), such as shape, size and density, influence their distribution in the water (Prata et al., 2019b; Stock et al., 2019). Therefore, despite the large sample volumes typically collected, the resulting samples may not be spatially (small-scale) representative if taken from a single location in the water matrix. Especially for rivers, there is evidence of high spatial (small-scale) variability of MP concentrations and composition. For instance, Haberstroh et al. (2021) investigated how cross-sectional flow characteristics affect MP distribution in rivers, based on cross-sectional flow profiles and five MP sampling points positioned both vertically and horizontally in a river cross-section. The study revealed a tendency of uneven, large, and dense MPs to be more prevalent in the water column and at the river bottom, while small, smooth, and more circular particles were more abundant at the water surface. Haberstroh et al. (2021) further reported that MP concentrations and fluxes in the cross-section varied by two orders of magnitude, and that the spatial variability was directly influenced by hydrodynamic conditions. Similarly, Pessenlehner et al. (2024), who also used a cross-sectional multi-point approach in a river, reported stratification of MP concentrations at low flow conditions, increased vertical mixing associated with higher discharge, and increased MP concentrations toward the river banks. Based on the observed high (small-scale) spatial variability of MP concentrations and composition, Haberstroh et al. (2021) proposed the adoption of multi-point sampling as a common practice. However, this sampling design, which is also suitable for other aquatic matrices besides rivers, is still the exception rather than the rule, and spatial aspects are often disregarded (Range et al., 2023). This thesis also considers spatial variability only to a limited extent, as its focus is on the temporal variability of the MP concentration and composition. It was not feasible (for technical and sample quantity reasons) to account for both aspects simultaneously.

Sampling in the context of temporal variability. In most cases, MPs in aqueous matrices are sampled at one sampling site at a specific point in time (subsequently referred to as "single-point sampling") without further consideration of the temporal representativeness of the sample. The few studies that do take into account temporal variability, mostly sample the same site on multiple random sampling occasions (Bäuerlein et al., 2023; de Carvalho et al., 2021; Horton et al., 2021; Martín-García et al., 2023; Mintenig et al., 2020) or repeat sampling at fixed intervals, such as daily, weekly, or monthly (Egea-Corbacho et al., 2023; Hitchcock, 2020; Roscher et al., 2022; Xia et al., 2020). Based on

these studies, there is growing evidence that MP concentrations in freshwater matrices, such as lakes and rivers (de Carvalho et al., 2021; Hitchcock, 2020; Mintenig et al., 2020; Skalska et al., 2020; Xia et al., 2020), and WWTP effluents (Bäuerlein et al., 2023; Kukkola et al., 2024; Roscher et al., 2022), can be highly variable over time. For instance, Xia et al. (2020) collected single-point samples at the same time every day over a 41-day period in the surface water of a lake in China and reported that MP abundance varied by a factor of four between the highest and lowest MP concentrations. Hitchcock (2020) reported an over 40-fold increase in MP abundance within two days during a storm event in an Australian river estuary. Roscher et al. (2022) and Akarsu et al. (2020) investigated MP concentrations in the effluents of several WWTPs over a period of one year using monthly single-point sampling and reported that concentrations within the WWTPs differed by factors of 87, 315 (Roscher et al., 2022) 17, six and five (Akarsu et al., 2020) between the highest and lowest MP concentrations in each WWTP.

To increase the temporal representativeness of individual samples, the collection of mixed samples (a mixture of several individual samples taken at different points in time to obtain one temporally representative sample) has been reported (Barkmann-Metaj et al., 2023; Horton et al., 2021). One option for collecting mixed samples is the use of commercially available autosamplers (Barkmann-Metaj et al., 2023; Horton et al., 2021). However, these have the disadvantage that the sample volume taken is usually small and that commercial models may have plastic components that can cause cross-contamination. An alternative is the use of volume-reducing techniques, manually operated to sample defined volumes at timed intervals (Barkmann-Metaj et al., 2023; Kukkola et al., 2024). For instance, Barkmann-Metaj et al. (2023) collected mixed samples with a volume-reducing sampling device from the effluents of industrial park WWTPs by manually sampling for a duration of one minute (max. 20 L) in intervals of 10 min for up to 2 h. However, without automation, mixed sample collection is very time-consuming. This problem is to some extent overcome by the sampling setup presented by Horton et al. (2021), which combines a commercial autosampler with a filter cartridge, allowing the automated collection of volume-reduced mixed samples. However, Horton et al. (2021) reported some quality control and quality assurance issues, such as high procedural blank values and a lack of method validation using a variety of polymer types and MP sizes (see also Section 1.3.4).
To obtain representative data and insights into the temporal variability of the MP concentration and composition in aqueous matrices, the repetitive collection of automated mixed samples (e.g., collecting daily mixed samples over weeks or months) is a promising approach and is therefore pursued in this thesis.

1.3.2 Sample Processing

Environmental samples typically contain a complex particle matrix of which MPs constitute only a small part. The aim of sample preparation is the removal of natural organic and inorganic material from the samples, as it can interfere with subsequent analysis (see Section 1.3.3), while preserving the MPs.

Removal of organic material. Organic material is usually digested or degraded by treating the samples with one or several of the following: oxidizing agents (H_2O_2, Fenton reagent and O_3), acids (HCl and HNO_3), alkali (NaOH and KOH), enzymes (proteinase K, lipase, chitinase, cellulase and protease) and/or the surfactant sodium dodecyl sulfate (Al-Azzawi et al., 2020; Lavoy and Crossman, 2021; Löder et al., 2017; Prata et al., 2019b; Stock et al., 2019). The challenge herein is to achieve an effective removal of organic material while, at the same time, not causing any significant chemical (e.g., changes in spectral signature) or physical (e.g., changes in size, shape, or weight) alteration of the MPs in the sample (Al-Azzawi et al., 2020; Hurley et al., 2018; Lavoy and Crossman, 2021; Stock et al., 2019; Tagg et al., 2017). In particular the treatment of MP samples with Fenton reagent, a mixture of H_2O_2 with a Fe^{2+} catalyst, has proven to be comparatively fast and effective at removing organic material. Furthermore, it has very little to no adverse effects on MPs under controlled temperature conditions (Al-Azzawi et al., 2020; Hurley et al., 2018; Prata et al., 2019a; Tagg et al., 2017). It must be noted, however, that experiments designed to reveal the adverse effects of different sample processing methods on MPs are often carried out using large, virgin plastic pellets, fragments, or particles. Therefore, it remains unclear whether small MP particles and fibers that have undergone environmental aging are equally unaffected by Fenton reagent treatment. Nevertheless, combining findings on the efficiency of organic matter removal with those assessing the impact of sample processing on large MP fibers (Hurley et al., 2018), environmentally aged plastic fragments (Prata et al., 2019a), and small MP particles (Al-Azzawi et al., 2022b) suggests that treatment with Fenton reagent is likely the most suitable of the currently available methods for the removal of organic material from MP samples—even when targeting small, environmentally aged MP particles and fibers.

Removal of inorganic material. Density separation is the most commonly applied method for separating inorganic particles from MPs. It involves the suspension of a MP sample in a separation liquid (e.g., water or a salt solution) with a defined density. Ideally, the density of the separation liquid is higher than the density of the MPs (0.85–2.3 g cm^{-3}; Enders et al., 2015) to be investigated, causing them to rise to the surface, and lower than the density of the inorganic particles (approx. 2.65 g cm^{-3}; Hidalgo-Ruz et al., 2012), causing them to sink to the bottom. Once the particles have settled, the supernatant containing the MPs is isolated (e.g., by decanting, aspiration or draining of the lower part of the liquid) for further processing and analysis.

Commonly applied separation liquids and their densities are: water ($\sim$ 0.99 g cm^{-3}), sodium chloride solution (1.2 g cm^{-3}), calcium chloride solution (1.3–1.35 g cm^{-3}), sodium bromide solution (1.37–1.40 g cm^{-3}), zinc chloride solution (1.5–1.8 g cm^{-3}), zinc bromide solution (1.71 g cm^{-3}), sodium iodide solution (1.8 g cm^{-3}), and sodium polytungstate solution (SPT, 1.4–2.25 g cm^{-3}) (Frias et al., 2018; Halbach et al., 2021; Quinn et al., 2017; Witzig, 2017), as visualized in Figure 1.6. In general, the choice of the separation liquid is based on the desired density, but also on considerations regarding costs (e.g., of salts), availability, human and ecological toxicity as well as reusability

(Frias et al., 2018; Quinn et al., 2017; Stock et al., 2019).

The use of water as a separation liquid is suitable if only the polymer types PE, polypropylene (PP), and ethylene-vinyl acetate, which have a density ≤ 0.98 g cm^{-3}, are of interest. All other polymer types have a greater density than water and therefore sink to the bottom along with the inorganic material (see Figure 1.6). The use of salt solutions with a density ≤ 1.4 g cm^{-3} (sodium chloride, calcium chloride, sodium bromide) can lead to an underestimation of polymer types such as polylactic acid (1.21–1.43 g cm^{-3}), PVC (1.16–1.58 g cm^{-3}), PET (1.37–1.45 g cm^{-3}), polyoxymethylene (POM, 1.41–1.61 g cm^{-3}), polyester (PES, 1.10–2.3 g cm^{-3}), polytetrafluoroethylene (PTFE, 2.1–2.3 g cm^{-3}) as well as TRWP (1.5–2.2 g cm^{-3}) in samples. They may sink to the bottom along with the inorganic particles or, if there is little to no density difference between the polymer and the salt solution, remain suspended in the salt solution. However, even when salt solutions with densities as high as 1.8–1.9 g cm^{-3} are applied (Gomiero et al., 2019; Nuelle et al., 2014; Shaw et al., 2024), PES, PTFE and TRWP may still be underestimated.
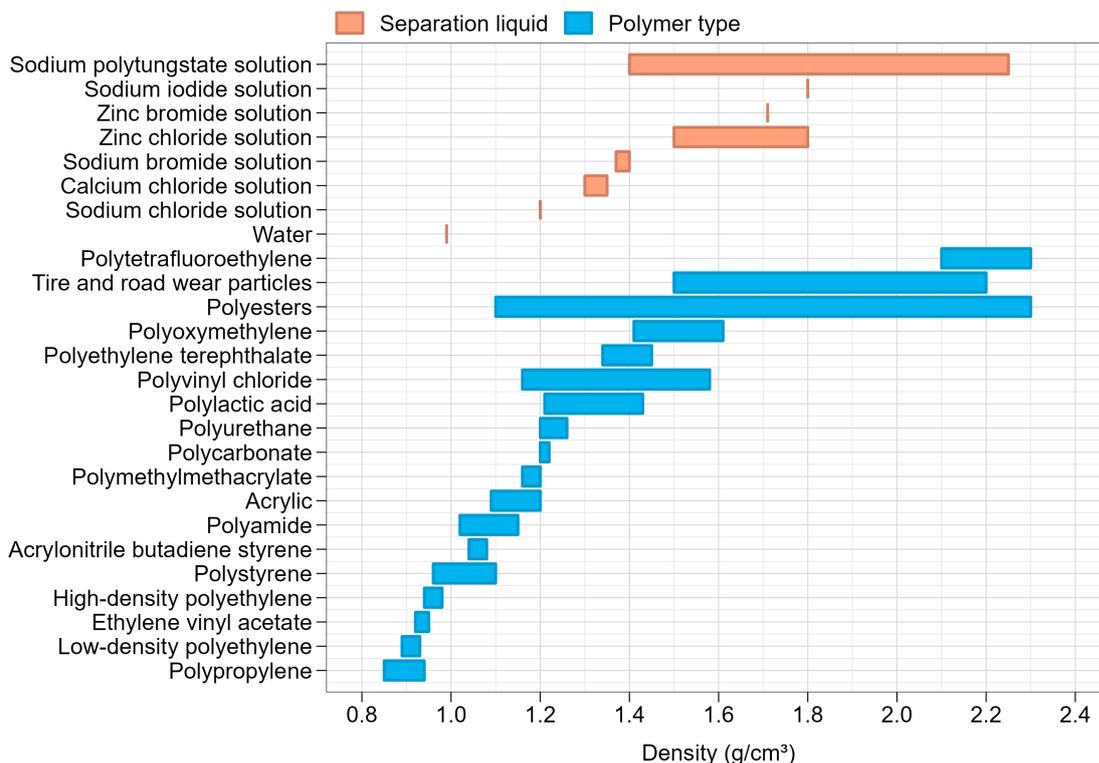
FIGURE 1.6: Density of common polymer types and separation liquids. Data based on Enders et al., 2015, Lambert and Wagner, 2018, Prata et al., 2019b, and Baensch-Baltruschat et al., 2020.

In this thesis, all density separations are performed using a sodium polytungstate solution with an unusually high density of 2.25 g cm^{-3}, as proposed by Witzig (2017). This reduces the risk of underestimating high-density polymers but may cause inorganic particles with particularly low densities, such as halites (2.10–2.20 g cm^{-3}) or organic minerals (2.28 g cm^{-3}), to rise to the surface or remain in suspension, potentially

reducing the effectiveness of density separation. However, this is unlikely, as halites are water-soluble, organic minerals can be removed from the sample by degradation, and most minerals have a density > 2.25 g cm^{-3}.

1.3.3 Analytical Techniques

The simplest method for quantifying MPs is visual identification with the naked eye or using a dissection microscope, whereby optical characteristics such as color, shape and texture can be distinguished (Eriksen et al., 2013; Hidalgo-Ruz et al., 2012; Lusher et al., 2014; Morét-Ferguson et al., 2010; Primpke et al., 2020a). Identification with the naked eye is applicable for particles > 100 µm while the use of a dissection microscope pushes the lower size limit to 3 µm (Primpke et al., 2020a). The advantage of visual identification is that no expensive equipment is required. However, it does not allow chemical identification and differentiation of polymer types.

In the early days of MP research, MP quantification based solely on visual identification was widely used (Hidalgo-Ruz et al., 2012; Sutton et al., 2016), but has since been criticized for its susceptibility to errors. Up to 61% of the particles and fibers visually classified as plastics or MPs are false positives (Di Mauro et al., 2017; Eriksen et al., 2013; Kroon et al., 2018). Verification of chemical particle and fiber identity after visual presorting by means of spectroscopic methods, as proposed by Hidalgo-Ruz et al. (2012), considerably reduces the risk of false positives. However, this does not reduce the probability of MPs being overlooked during visual presorting and thus, the risk of false negatives and underestimation associated with visual identification remains (Primpke et al., 2020a).

Today, many different methods and method combinations for analyzing MPs exist (Primpke et al., 2020a). The three most commonly applied methods are pyrolysis-gas chromatography/mass spectrometry, Fourier transform infrared spectroscopy and Raman spectroscopy (Schymanski et al., 2021). These methods have proven to be particularly suitable for MP analysis due to their reliability in identifying and quantifying MPs and polymer types. All three methods were applied within this thesis and are described below in more detail. Their advantages and disadvantages are summarized in Figure 1.7.

Fourier transform infrared spectroscopy (FTIR) is a non-destructive, spectroscopic method. To determine the chemical identity of a particle, it is radiated with mid-infrared light (∼4000-400 cm^{-1}), causing molecular vibrations. A characteristic vibrational spectrum is recorded, which can be compared with a corresponding database to determine the chemical identity of the particle in question.

Attenuated total reflection (ATR)-FTIR is commonly applied to manually analyze large individual particles (> 200–500 µm), for instance after visual presorting (Ivleva, 2021; Primpke et al., 2020a). A crystal (e.g., diamond, zinc selenide or germanium) is pressed onto the sample surface and the infrared light penetrates a few micrometers into the sample before total reflection takes place. By coupling the ATR-FTIR with a microscope

(µ-ATR-FTIR), small particles and fibers accumulated on a screen or filter can also be analyzed using this method (Käppler et al., 2018). However, especially for great particle numbers or large filter areas, (µ-)ATR-FTIR is very time-consuming (Käppler et al., 2018; Liu et al., 2024; Primpke et al., 2020a).

µ-FTIR is performed using an FTIR spectrometer coupled with an optical microscope. It enables manual analysis of individual particles and fibers as well as automated analysis of entire filters. µ-FTIR is commonly applied to analyze MPs > 10 µm (Gunaalan et al., 2023; Roscher et al., 2021, 2022; Vianello et al., 2019), although theoretically, analysis of particles down to 1.7 µm is possible with this technique (Ivleva, 2021). The information rendered by µ-FTIR comprises the number of MPs in the sample as well as their individual polymer type, size and morphology. By applying a focal plane array (FPA) detector, several thousand spectra can be collected within one measurement, making µ-FTIR very time-efficient (analysis of an entire filter within four to eight hours, depending on the applied settings). Analysis with µ-FTIR can be performed in two different modes: transmission or reflectance. In transmission mode, the infrared radiation penetrating through the sample is detected. Thus, substrates or filters with infrared transparent ranges, such as aluminum oxide or silicon membranes, zinc selenide, barium fluoride, or calcium fluoride windows are required (Käppler et al., 2015; Löder et al., 2015; Simon et al., 2018). The spectra resulting from µ-FTIR in transmission mode are usually high-quality and representative of the entire thickness of the sample (Ivleva, 2021). However, if particles are very thick or dark, total absorption may occur, reducing spectra quality (Ivleva, 2021; Primpke et al., 2020a; Schymanski et al., 2021). In reflectance mode, the infrared radiation reflected by the sample is detected. This requires either a reflective sample surface or, if the sample is sufficiently thin, a reflective substrate (transflection). The spectra recorded using µ-FTIR in reflectance mode can be negatively affected by light scattering.

Regardless of the mode, thorough sample processing prior to µ-FTIR analysis is required to prevent natural organic or inorganic particles from overlapping with MPs, which may lead to an underestimation of MP numbers. FTIR is not suitable for the chemical identification of TRWP, as they contain carbon black, which almost completely absorbs infrared light (Rasmussen et al., 2024). Furthermore, FTIR analysis is only possible for dry samples, as water has strong and broad infrared bands that can partially or completely overlap the spectral signatures of the sample (Ivleva, 2021).

Raman spectroscopy is a non-destructive, spectroscopic method. To determine the chemical identity of a particle, monochromatic light is focused onto the surface of that particle, causing inelastic scattering of photons (Raman effect). A Raman spectrum is recorded, which can be compared with a corresponding database to determine the chemical identity of the particle (Smith and Dent, 2004) as well as of some additives, such as pigments and oxides (Ivleva, 2021). By Raman microspectroscopy (µ-Raman), where a Raman spectroscope is coupled to a confocal optical microscope, particles down to 1 µm (theoretically down to 300 nm) can be analyzed (Anger et al., 2018; Cabernard et al., 2018; Primpke et al., 2020a; Schymanski et al., 2021). As with µ-FTIR, the information rendered by µ-Raman comprises the number of MPs in the sample as well as their

individual polymer type, size and morphology. The measurement can be performed in three different modes, which are (i) manual single particle measurement, (ii) automated particle recognition and measurement and (iii) automated grid-like measurement with fixed distances between measuring points (Primpke et al., 2020a). The time required for the analysis of a sample depends on the measurement mode, the number of particles in the sample, the applied instrument settings and, if applicable, the subset of the sample analyzed (Anger et al., 2018; Schymanski et al., 2021). Especially when particles < 10 μm are investigated and particle numbers in the sample are ≫ 1000, μ-Raman analysis can take days to weeks per sample (Anger et al., 2018; Primpke et al., 2020a). Raman spectroscopy is insensitive to water, allowing analysis in dry, wet and even aqueous samples (Kniggendorf et al., 2019; Schymanski et al., 2021). However, as with FTIR analysis, the carbon black content of TRWP prevents their chemical identification using Raman spectroscopy (Rasmussen et al., 2024; Wagner et al., 2018). To prevent underestimation of MP numbers, μ-Raman analysis of environmental samples requires thorough sample processing, as natural organic and inorganic material in the samples can cause fluorescence (Schymanski et al., 2021) or overlap with MPs (Primpke et al., 2020a).

μ-FTIR

+ Renders particle number, size and morphology
+ Chemical identification
+ Analysis of isolated particles or entire samples
+ Analysis of particles > 10 μm
+ Fast: Analysis of complex samples within one day
+ Non-destructive

- Sensitive to water
- Sample processing required
- No chemical identification of tire wear particles

μ-Raman

+ Renders particle number, size and morphology
+ Chemical identification
+ Identification of some additives (pigments, oxides)
+ Analysis of isolated particles or entire samples
+ Analysis of particles ≥ 1 μm
+ Insensitive to water
+ Non-destructive

- Slow: Analysis of complex samples within days to weeks
- Sample processing required
- No chemical identificaton of tire wear particles

pyr-GC/MS

+ Renders mass concentration
+ Chemical identification
+ Identification of thermo-volatile additives
+ Analysis of isolated particles or entire samples
+ Very fast: Analysis of complex samples within hours
+ Estimaton of tire wear mass concentration

- Limited to a small number of polymer types
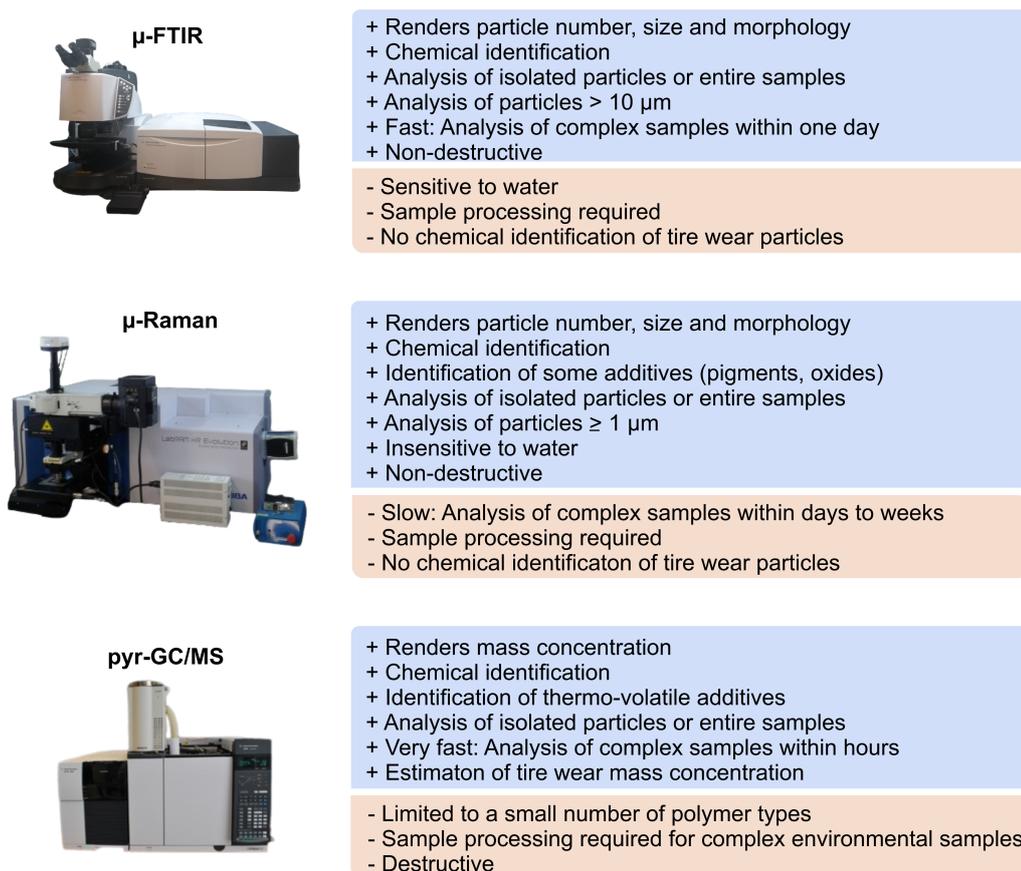- Sample processing required for complex environmental samples
- Destructive

FIGURE 1.7: Comparison of the advantages and disadvantages of Fourier transform infrared microspectroscopy (μ-FTIR), Raman microspectroscopy (μ-Raman), and pyrolysis-gas chromatography/mass spectrometry (pyr-GC/MS).

Pyrolysis-gas chromatography/mass spectrometry (pyr-GC/MS) is a destructive method that is complementary to Raman spectroscopy and FTIR, as it renders polymer mass concentrations instead of information on MP numbers and appearance. A sample to be analyzed with pyr-GC/MS can consist either of a large, isolated MP particle or a few micrograms of a complex particle mixture. During pyr-GC/MS, the sample is degraded by heating it to a defined temperature in an inert atmosphere. The pyrolysis products are separated by gas chromatography and analyzed by mass spectroscopy. The resulting pyrogram is used to identify and quantify the polymer type(s) in the sample based on polymer-specific signature patterns or indicator ions (Primpke et al., 2020a). Pyr-GC/MS measurements are fast, allowing for the analysis of complex samples within hours. However, only a limited number of polymer types can be identified and quantified using pyr-GC/MS to date, including PE, PP, PS, PET, PVC, polymethylmethacrylate, polycarbonate (PC), polyamide-6 and methylene-diphenyldiisocyanate-PU, with polymer-dependent LODs (Primpke et al., 2020a). Furthermore, pyr-GC/MS allows the identification of thermo-volatile additives incorporated in the polymer matrix (Akoueson et al., 2021) and enables estimation of the TRWP concentration in a sample, based on the quantification of styrene-butadiene rubber, butadiene rubber, and natural rubber (Baensch-Baltruschat et al., 2020; Rasmussen et al., 2024). Pyr-GC/MS is less dependent on thorough sample processing than μ-Raman and μ-FTIR. However, organic and inorganic material can interfere with the pyrolysis products of the targeted polymers, so that sample processing is nonetheless advisable for complex environmental samples (Bouzid et al., 2022; Dierkes et al., 2019; Primpke et al., 2020a) and enhances the sensitivity of the method (Ivleva, 2021).

1.3.4 Quality Assurance and Quality Control

MPs have been detected in all environmental compartments and are considered ubiquitous (Rochman, 2018). In order to assess the impact of MPs on the environment, biota and humans, reliable results regarding the concentration, size and morphology of MPs are crucial (de Ruijter et al., 2020). However, there is a lack of standardized methods for sampling, sample processing and analysis, impeding comparability and calling into question the quality of study results (Koelmans et al., 2019; Prata et al., 2021b). The minimization of cross-contamination is essential for data reliability. Cross-contamination can occur through direct contact of samples with plastic, but also indirectly through the air, liquids (e.g., rinsing water, solutions applied during sample processing) or surfaces (vessels, utensils, hands). Effective measures for reducing cross-contamination include wearing cotton lab coats, working in laminar flow hoods, avoiding plastic utensils, using filtered water for cleaning, filtering working solutions and covering samples with glass lids or aluminum foil (Koelmans et al., 2019; Prata et al., 2021b; Schymanski et al., 2021). However, even if all these measures are implemented, cross-contamination cannot be completely prevented (Shruti and Kutralam-Muniasamy, 2023). For this reason, so-called "negative controls" in the form of laboratory blank values and process blank values are indispensable (Koelmans et al., 2019; Prata et al., 2021b; Schymanski

et al., 2021). Laboratory blank values record the cross-contamination introduced during the working steps within the laboratory, while process blank values record the cross-contamination occurring during the entire process of sampling, sample preparation, and analysis. Process and laboratory blank values enable the concentrations determined in samples to be corrected for cross-contamination. However, there is still no consensus on how the correction should be performed (Dawson et al., 2023; Shruti and Kutralam-Muniasamy, 2023). Recently, Dawson et al. (2023) tested 51 correction variants identified from the literature using a dummy dataset and found that variants considering the LOD (mean blank value + 3 × standard deviation) or the limit of quantification (mean blank value + 10 × standard deviation) were particularly suitable for removing cross-contamination from the dummy dataset.

Adulteration of results can also occur through the loss of MPs during various steps of sampling, sample processing, and analysis, for instance if MPs adhere to the surfaces of the sampling equipment, are degraded during sample processing, or roll off the analysis filter. So-called "positive controls", where reference MPs are used to determine recovery rates of individual or multiple steps from sampling to analysis, are crucial for method validation and ensuring a sufficiently high recovery (Cui et al., 2022; Koelmans et al., 2019; Schymanski et al., 2021). Cui et al. (2022) and Koelmans et al. (2019) stress that, in light of the heterogeneity of MPs, reference material with different densities, shapes and a size similar to that of the smallest MP particle to be investigated, should be applied during method validation and recovery experiments. This is, however, complicated by the fact that the production of reference samples containing a defined composition and number of MPs is highly challenging, and so far, certified reference material mixtures for MP recovery experiments are not available (Schymanski et al., 2021). Therefore, recovery experiments are often performed using large MPs of only one or very few shapes and polymer types. This facilitates recovery but undermines the applicability of reported recovery rates to environmental samples. For example, 47% of the publications considered in the review study on recovery experiments by Cui et al. (2022) used large MPs ($\geq$ 500 µm), and almost 80% of the studies used only one shape. Cui et al. (2022) furthermore reported that the polymer types PE, PP and PS—all of which have relatively low densities and are therefore easier to recover during density separation (see Figure 1.6)—were the most commonly used among the studies reviewed.

Prata et al. (2021b) reviewed 50 studies on MPs in biota, air, soil, sediment, freshwater, and saltwater and assessed their compliance with ten basic cross-contamination control parameters. Most studies (median) complied with only four of the ten parameters, and only 60% of the studies collected procedural blanks. In a similar study by Koelmans et al. (2019), which reviewed MP research in drinking water and freshwater, only four out of 50 studies received a positive score regarding all quality criteria proposed by Koelmans et al. (2019). There is clearly a need for more reliable and comparable data on MPs in the environment that has undergone rigorous quality assurance and quality control (QA/QC) and is based on validated methods.

1.4 Motivation and Problem Description

MPs are ubiquitous and continue to accumulate in the environment due to the ever-increasing production and use of plastics, as outlined in Section 1.2.1. Once plastic has fragmented into MPs, it is almost impossible to remove from the environment. A large number of potentially negative effects on humans, biota, and the environment have already been identified, although a conclusive risk assessment is not yet possible (see Section 1.2.2). Among the main reasons why such a conclusive assessment is not yet possible is the fact that ecotoxicological experiments often work with concentrations or MP compositions that are unrealistic or do not reflect the heterogeneity (e.g., polymer types, sizes, shapes) of MPs in the environment (see Section 1.2.2). However, do we even know what concentrations are realistic for MPs in the environment? Even within the same matrix, the results on MP concentrations from different studies often differ by several orders of magnitude (Koelmans et al., 2019; Lu et al., 2021). Due to a lack of standardization, many different methods are used for sampling, sample processing, and analysis of MPs (see Section 1.3), and studies are often deficient in the context of QA/QC (see Section 1.3.4). **Consequently, there is a need for studies on the concentration and composition of MPs in the environment, which implement comprehensive QA/QC measures, uncover potential sources of cross-contamination, and rely on validated methods.**

An important aspect that has so far received very little attention in the context of determining MP concentrations and compositions in surface waters is temporal variability. Most studies use single-point sampling (see Section 1.3.1) and, due to the time-consuming nature of each individual MP sample, do not collect multiple samples at different points in time to account for temporal variability. However, the few studies to date that have investigated the temporal variability of MPs in surface waters show that MP concentrations can be subject to considerable temporal fluctuations (see Section 1.3.1). This calls into question whether snapshot-like single-point sampling, which does not take into account temporal variability, can provide representative MP concentrations and compositions for surface waters at all. **In order to increase our understanding of MP pollution in the environment and to ensure that ecotoxicological studies can base their work on reliable and realistic data in the future, more research is needed on the temporal variability of MP concentrations and composition in surface waters, ideally using continuous or semi-continuous sampling approaches.**

Flowing water courses are the habitat of freshwater organisms, serve as a drinking water source for humans and biota, and are a major pathway for plastics and MPs into the world's oceans (Lebreton et al., 2017). **Therefore, freshwater streams receive particular attention in this thesis. Both the temporal variability of the composition and concentration of MPs in streams and in WWTP effluents, which represent a significant pathway of MPs into streams, are of interest.** Determining the temporal variability of the concentration and composition of MPs in WWTP effluents is crucial to estimate the extent of the MP inputs into streams, to understand the temporal variability of the

MP concentration and composition in streams, and to enable the implementation of targeted, effective measures for the protection of streams and, thus, the oceans.

1.5   Objectives and Structure of the Thesis

1.5.1   Main Research Goal and Objectives

The **main research goal** of this thesis is to reliably determine the temporal variability of the concentration and composition of MPs in streams and WWTP effluents. This main research goal can be broken down into four objectives:

1st objective: Identification and elimination of cross-contamination of samples due to widespread and unquestioned practices in the laboratory (Chapter 2).

2nd objective: Development and validation of a fully automated MP sampling unit, enabling the monitoring of MPs in freshwater samples with high temporal resolution and over extensive periods of time (Chapter 3).

3rd objective: Determination and evaluation of the temporal variability of the concentration and composition of MPs in a stream and in the effluent of a WWTP and investigation of possible variability-influencing factors (Chapters 3 and 4).

4th objective: Assessing the representativeness of single-point and mixed samples in the context of the temporal variability of MP concentration and composition in streams and WWTP effluents (Chapters 3 and 4).

1.5.2   Structure of the Thesis

The main research goal and the four objectives are addressed in three independent research articles that were published in international ISI-listed and peer-reviewed scientific journals. These three research articles form the core of this cumulative thesis, which is structured as visualized in Figure 1.8 and as described in the following.

Chapter 1 provides an introduction to the scientific background and problem description of the three research articles, which are presented in Chapters 2–4 and are briefly outlined below.

Chapter 2 (Article I) examines the potential for cross-contamination of samples by substances associated with disposable laboratory gloves or reagents commonly used during sample processing. Disposable gloves are often worn during sample handling for personal protection or with the good intention of preventing cross-contamination. As a result of this practice, stearates, which are used as mold-release agents in the manufacture of disposable gloves, may be introduced into samples. This can lead to a significant overestimation of PE concentrations, as stearates can be mistaken for PE during chemical identification. The same is true for sodium dodecyl sulfate (SDS), an anionic

surfactant often used during sample processing. Chapter 2 investigates the potential overestimation of PE particles or mass found in MP samples due to the use of powder-free disposable gloves. It also examines the ability of µ-Raman, µ-FTIR and pyr-GC/MS to discriminate between PE, SDS, and stearates. Furthermore, options for overcoming potential bias in PE mass and particle number concentrations and for identifying and eliminating cross-contamination sources in general are discussed.

Misidentification of substances as MPs can significantly undermine the reliability of MP data. Thus, Chapter 2 lays essential groundwork for Chapters 3 and 4, in which the use of disposable gloves and SDS was avoided and extensive QA/QC measures were taken.
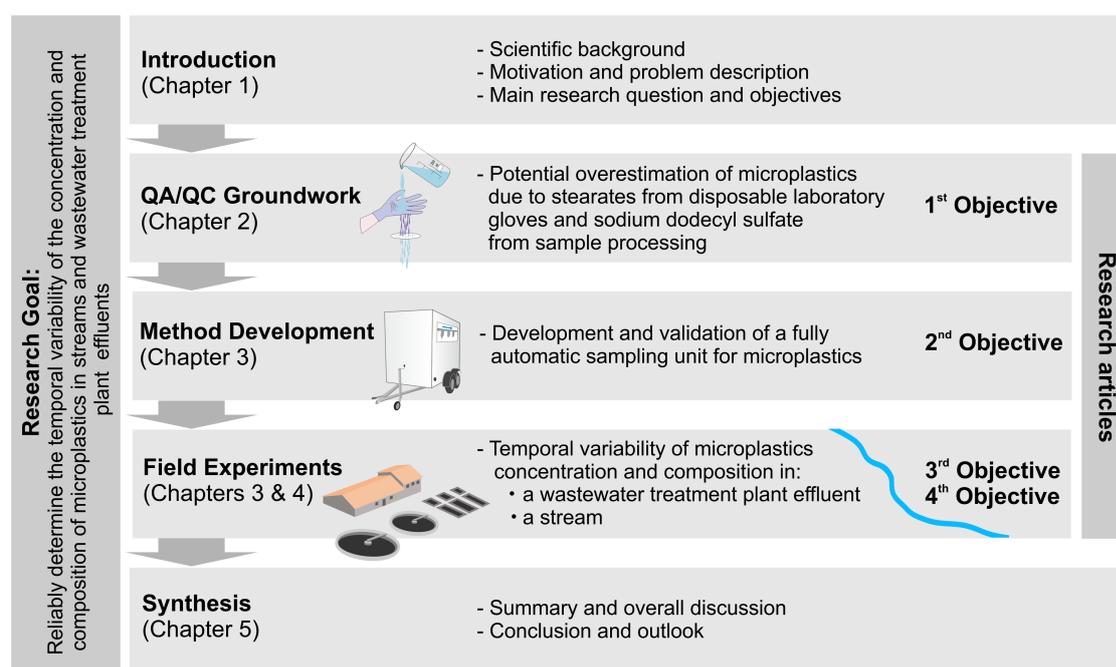

FIGURE 1.8: Structure of the thesis.

In **Chapter 3 (Article II)**, a fully automatic sampling unit for MPs (SAM) is presented, validated, and applied in the field. SAM is custom-built and designed to automatically collect up to seven 24-hour mixed samples by filtration of sample media over filter cascades with three successive stainless steel filters of decreasing mesh widths. In Chapter 3, the functionality of SAM is described and the LOD as well as the recovery rate of the entire process, including sampling with SAM, sample processing, and analysis with µ-FTIR is determined. Furthermore, SAM is applied to collect daily mixed samples of MPs in a stream in southern Germany. The day-to-day variability of the MP concentration and composition over an eight-day period is investigated and compared with total particle count, turbidity, precipitation, as well as discharge in the stream.

In **Chapter 4 (Article III)**, the fully automatic sampling unit for MPs developed and validated in Chapter 3 is applied to investigate the temporal variability of the concentration and composition of MPs in the effluent of a WWTP. To this end, mixed samples are collected daily over a period of one month and analyzed using µ-FTIR. The resulting

time series of MP concentration and composition are evaluated and the representativeness of individual mixed samples is assessed in the context of the observed temporal variability. Furthermore, the MP concentration time series is compared with the time series of precipitation and discharge from the WWTP to uncover possible correlations. Finally, interrelationships between different polymer types are investigated based on their individual time series.

Chapter 5 presents a synthesis comprising the summary and overall discussion of the results of the three research articles, as well as an outlook and conclusion.

Appendix A provides the authors' contributions to the three research articles, while Appendix B lists further publications to which the author contributed during the doctoral phase. Appendices C and D contain the supporting information for Chapters 2 and 3, respectively.

Chapter 2

Article I: When Good Intentions Go Bad—False Positive Microplastic Detection Caused by Disposable Gloves

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Apart from editorial and formatting adjustments, no changes have been made.
The full text of the article can be accessed via this link:
https://pubs.acs.org/articlesonrequest/AOR-RDHAZ4VMSJIC5CEDY26B.

2.1 Abstract

Apart from being considered a potential threat to ecosystems and human health, the ubiquity of microplastics presents analytical challenges. There is a high risk of cross-contamination during sampling, sample processing, and analysis. In this study, the potential of cross-contamination or misinterpretation due to substances associated with disposable laboratory gloves or reagents used during sample processing was investigated. Leachates of ten different types of disposable gloves were analyzed using Raman microspectroscopy (μ-Raman), Fourier transform infrared microspectroscopy (μ-FTIR), and pyrolysis-gas chromatography/mass spectrometry (pyr-GC/MS). There appeared to be polyethylene (PE) in almost all investigated glove leachates and with all applied methods. Closer investigations revealed that the leachates contained long-chain compounds such as stearates or fatty acids, which were falsely identified as PE by the applied analytical methods. Sodium dodecyl sulfate, which is commonly applied in microplastic research during sample processing, may also be mistaken for PE. Therefore, μ-Raman, μ-FTIR, and pyr-GC/MS were further tested for their capability to distinguish among PE, sodium dodecyl sulfate, and stearates. It became clear that stearates and sodium dodecyl sulfates can cause substantial overestimation of PE.
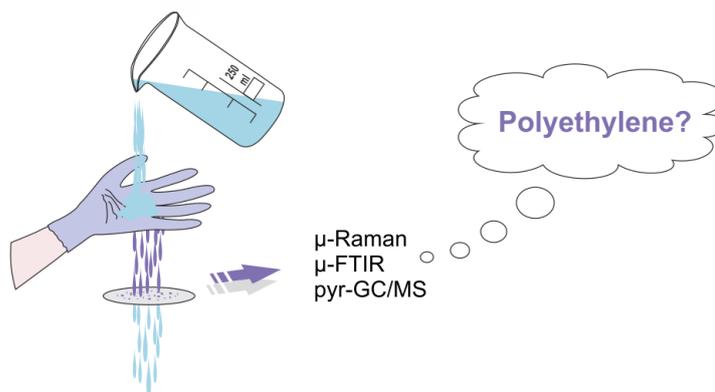
FIGURE 2.1: Graphical abstract of article I.

2.2 Introduction

For the greatest part of the global population, plastics are indispensable materials of daily use (Andrady and Neal, 2009). Due to ever-growing production, as well as high persistence, plastic has become ubiquitous (Rochman, 2018). Since the early 2000s, increasing numbers of studies focus on the toxicological effects and quantification of small plastic particles, so-called "microplastics", in the environment. Microplastics (MPs) are defined as particulate synthetic or heavily modified natural polymers for which the largest dimension is in the size range between 1 and 1000 μm (Braun et al., 2018; Hartmann et al., 2019). So far, methods for sampling, sample processing, and analysis of MPs are still under development. As a result, reported findings of MPs can differ by several

orders of magnitude, even for the same matrix, and must be interpreted with caution (Koelmans et al., 2019).

Raman microspectroscopy (μ-Raman) and Fourier transform infrared microspectroscopy (μ-FTIR), as well as pyrolysis-gas chromatography/mass spectrometry (pyr-GC/MS), are among the most commonly applied analytical methods for identification and quantification of MPs (Cabernard et al., 2018; Müller et al., 2020; Shim et al., 2017). With μ-Raman and μ-FTIR, particles down to 1 μm and 10 μm, respectively, can be detected (Cabernard et al., 2018; Shim et al., 2017). With both methods, the size and number of particles, as well as the chemical identity of the particles, can be determined. Chemical identification is performed for μ-Raman based on inelastic scattering of photons at the sample. These scattered photons reflect the molecular vibrations. For μ-FTIR, the identification is based on molecular vibrations induced by the absorption of infrared light by the sample. For both methods, resulting vibrational spectra are characteristic for individual materials and allow polymer types to be distinguished (Käppler et al., 2016). Mass spectrometric methods, e.g., pyr-GC/MS, are complementary to spectroscopic methods, as they render information on polymer mass concentrations instead of particle numbers. During pyr-GC/MS, samples are thermally decomposed and the decomposition products are separated by gas chromatography and analyzed with mass spectrometry (Unice et al., 2012).

Irrespective of the analytical method, care must be taken to minimize MP cross-contamination during sampling, sample processing, and analysis (Koelmans et al., 2019). Common precautions taken against plastic cross-contamination are the use of laminar flow cabinets, fume hoods, or air treatment devices to prevent plastic input from the air (Liu et al., 2019; Wesch et al., 2017). Further measures against cross-contamination include the avoidance of plastic utensils to prevent direct contact between samples and plastic items, the application of extensive cleaning procedures for all applied utensils, the wearing of disposable gloves and cotton laboratory coats to avoid introduction of plastic particles via hands and clothes, as well as the use of particle-free water for all cleaning and sample processing steps (Liu et al., 2019; O'Connor et al., 2019; Schymanski et al., 2018). Despite precautions, cross-contamination cannot be prevented entirely due to the ubiquitous nature of MP particles (Liu et al., 2019). Therefore, additional examination of blank samples is required in order to assess the level of cross-contamination and to establish a limit of quantification (LOQ).

In this research, cross-contamination induced by the use of sodium dodecyl sulfate (SDS) as well as disposable gloves is investigated. SDS is an anionic surfactant that is commonly applied during sample processing, as it aids the decomposition of animal and plant residues and increases the contact surface for subsequent treatment steps (Löder et al., 2017). Disposable gloves are frequently worn during sample handling for personal protection or in order to prevent cross-contamination. The production of disposable gloves comprises the washing and drying of hand-shaped porcelain formers that are dipped into a coagulant and a compounded latex. The coagulant promotes fast film buildup and is made of polyvalent metal salt, organic acid, or organic acid salt

dissolved in water, methanol, or ethanol (Akabane, 2016).The current state of the art is the addition of stearates to the coagulant as a mold-release agent (Chia and Mohd Sukri, 2019). As described by Akabane (2016), the compounded latex is usually made of natural rubber, polyisoprene rubber, acrylonitrile butadiene rubber, or chloroprene rubber combined with a variety of compounding chemicals (cross-linkers, vulcanization accelerators, vulcanization activators, and antiaging additives). After the dipping of formers into the coagulant and compounded latex, subsequent steps are leaching (residual chemicals and proteins are leached from the glove surface), beading (strengthening/rolling of the cuff), and vulcanization (a curing process where the gloves are heated to gain elasticity and strength). Powdered gloves are additionally treated with talc, silica, or cross-linked starch to prevent adhesion and enable easy donning, while powder-free gloves are either chlorinated or provided with a polymer coating (e.g., acryl, polyurethane) (Akabane, 2016). Although there are already a number of publications on the topic of disposable gloves causing unwanted residues in samples (Castino et al., 2008; Garçon et al., 2017; Sovinski, 2004), the risk and impact of MP samples being contaminated due to the use of disposable gloves has not yet been addressed in MP research, to the best of our knowledge. Preliminary examinations showed that disposable gloves may cause substantial overestimation of polyethylene (PE) in samples. Therefore, the aims of this study are (i) to determine a potential overestimation of PE particles or mass found in MP samples due to the utilization of powder-free disposable gloves; (ii) to analyze the reasons of this overestimation; (iii) to determine the capability of μ-Raman, μ-FTIR, and pyr-GC/MS to distinguish among PE, SDS, and stearates; and (iv) to discuss options for overcoming potential bias in mass and particle number for PE in MP samples.

2.3 Material and Methods

2.3.1 Minimization of Cross-Contamination

Measures for Reducing Cross-Contamination. Three laboratories, each specialized in a different method for MP analysis, were involved in the present study. At all three laboratories, precautions to reduce cross-contamination were taken: 100% cotton lab coats were worn by staff while handling samples, application of plastic tools was omitted, and samples were covered with aluminum foil, glass, or stainless steel lids during storage. To further minimize air-borne cross-contamination, the whole procedure of sample processing and analysis was performed in laminar flow cabinets at the laboratory focused on μ-Raman analysis. The laboratory focused on μ-FTIR analysis conducted sample processing in a laminar flow cabinet and analysis in a room equipped with an air-cleaner including a HEPA filter (for more details on air-cleaning equipment, see Table C.1). At the pyr-GC/MS laboratory, no technical equipment to prevent air-borne cross-contamination was used, because previous long-term investigations had shown no relevant MP input by air (Dierkes et al., 2019).

Protocol for Cleaning of Hands, Glassware, and Utensils. At all three laboratories, a strict and uniform protocol was followed for the washing of hands as well as for the cleaning of glassware and utensils prior to and during sample handling. All glassware and utensils were successively cleaned in a dishwasher, with tap water, with distilled water, and with ultrapure water (for more details on specifications, see Table C.2) and were finally submitted to three-fold ultrasonification with ultrapure water. Hands were washed in a three-step procedure, first using tap water and soap, then using only tap water, and finally using ultrapure water. Between the first two washing steps, hands were dried with paper towels. After the final washing step, hands were either allowed to air-dry within the laminar flow cabinets (µ-FTIR laboratory) or were dried with Kimtech Science Precision Wipes at the µ-Raman and pyr-GC/MS laboratories.

2.3.2 Sample Selection and Distribution

In order to investigate the potential overestimation of PE particles or mass caused by the use of common laboratory powder-free gloves, seven types of nitrile gloves (N1–N7) from different manufacturers, as well as one type of latex gloves (L1), one type of neoprene gloves (Neo1), and one type of vinyl gloves (V1), were acquired in packages of 100 gloves each. Furthermore, three identical looking granular samples were obtained for determining the capability of µ-Raman, µ-FTIR, and pyr-GC/MS to distinguish among sodium stearate (G-S; Alfa Aesar by Thermo Fisher GmbH), PE (G-PE; ultrahigh molecular weight, surface-modified, powder, 40–48 µm particle size, ID: 434272-100G, Sigma-Aldrich by Merck KGaA), and SDS (G-SDS; w > 99%, Fluka). Each laboratory was provided with an identical test package, containing ten pairs of gloves (one pair per glove type) as well as three glass vials with anonymized granulate (one vial per granulate type). For each glove type, duplicates were investigated at each of the three laboratories.

2.3.3 Sample Processing for Glove Leachates

Gloves. The gloves were filled with 50 g of sand as ballast and were immersed in separate glass beakers containing 200 mL of ultrapure water (Figure 2.2). By pulling the opening of each glove over the brim of the beaker, it was ensured that only the outside of each glove came into contact with the water. Gloves were leached for 5 h. Subsequently, each glove was removed from its beaker and was rinsed thoroughly from the outside with ultrapure water. The rinsing water and the leachate of each glove were united for filtration and analysis, which differed between the three participating laboratories (see Section 2.3.4).

Blank Values. In order to assess cross-contamination during sample processing and analysis of the glove leachates, triplicate processing blanks were considered at each laboratory. A 200 mL portion of ultrapure water was poured into each beaker, which was covered with aluminum foil and was left standing for 5 h. Additionally, three blank samples of a washed hand (see Section 2.3.1), hereafter called "hand blanks", were considered at each laboratory. A 200 mL portion of ultrapure water was poured into a beaker
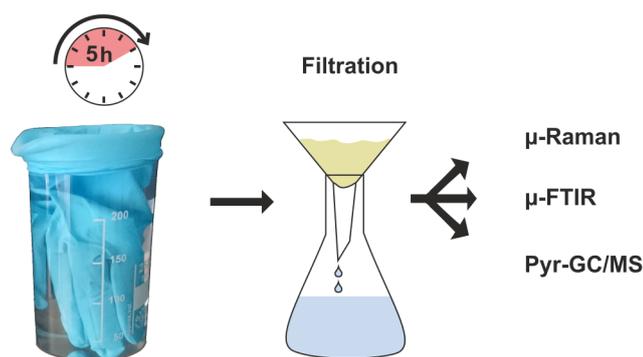
FIGURE 2.2: Schematic visualization of sample processing: Sand is filled into a glove, which is submersed in ultrapure water and leached for 5 h. Leachates are filtered and analyzed.

and laboratory staff brought one hand into contact with the water (either by pouring water over the hand or by immersing the hand in the water). For all blank samples, filtration and analysis were performed as described below for the leachates of the gloves at each laboratory.

2.3.4 Identification and Quantification for Glove Leachates

μ-Raman. Glove leachates were vacuum-filtered through a polytetrafluoroethylene membrane (PTFE; sintered, diameter 25 mm, pore size 1–2 μm, Pieper Filter GmbH) using a stainless steel in-line filter holder (Pall Corp.). The beaker and filter holder were rinsed with 200 mL of ultrapure water. For each glove a separate filter was used and stored in a glass Petri dish prior to analysis. Filters were analyzed using a Horiba XploRa Plus Raman microscope system (Horiba Jobin Yvon) equipped with a Syncerity EMCCD camera and a confocal microscope (Olympus BX51, Olympus). The minimal particle size analyzed was 5 μm. μ-Raman analysis was performed for 6.3% of the filtration area (six square subsections of approximately 2 × 2 mm, distributed to best represent the whole filter; for more details, see Figure C.1) using the ParticleFinder tool of the software LabSpec (ver. 6, Spectroscopy Suite Software, Horiba Jobin Yvon). Spectra were obtained at 20-fold magnification (numerical aperture of 0.45) with an excitation wavelength of 532 nm (air cooled solid-state laser kit) and 7.5 mW laser power on the sample, 600 gr/mm spectral grating, 100 μm slit, 300 μm pinhole, an acquisition time of 2 s, and without accumulations. The examined wavenumber range was 150–3400 cm^{-1}. All acquired spectra were automatically baseline-corrected during acquisition and were compared with a spectra database established at the laboratory using the software True-Match (WITec GmbH). For all particles identified as potential polymers by TrueMatch, spectra attribution was manually verified. By area-weighting, the detected particle number per polymer type was extrapolated to the filtration area. The unit of quantification is particle numbers per filter.

μ-FTIR. Glove leachates were vacuum filtered through an aluminum oxide membrane (Whatman Anopore membrane disk with a polypropylene support ring, pore size 0.2 μm, diameter 25 mm, GE Healthcare) using tailor-made glass filtration equipment with a filtration diameter of 10 mm. Rinsing of the funnel and beaker was performed with 25 mL of ultrapure water, due to very low filtration velocity. For each glove, a separate filter was used. Filters were stored and dried in glass Petri dishes in a desiccator prior to measurement. Filters were analyzed using a Bruker Hyperion 3000 μ-FTIR microscope connected to a Tensor 27 IR-spectrometer (both Bruker Optik GmbH). A visual image of the whole filter area placed on a CaF_2 window (Korth Kristalle GmbH) was acquired with a 4× glass objective. Afterward, IR imaging was performed for the whole sample spot (part of the filter substrate onto which glove leachates were filtrated; average area 76 mm^2) with a 15× IR objective in transmission mode. Spectral data were recorded by a 64×64 elements focal plane array detector (FPA) with six scans for each FPA field and binning of 4×4 pixels. The examined spectral range was 3600–1250 cm^{-1} with a resolution of 8 cm^{-1}. Background measurement was recorded on a sample-free area on the aluminum oxide membrane to overcome self-absorption of the membrane material in the spectroscopic fingerprint area (Löder et al., 2015). Spectra analysis of FPA measurements was carried out with the freeware program siMPle (Liu et al., 2019; Primpke, 2019) and a free FTIR reference database (Primpke et al., 2018). The database results "PE", "chlorinated PE" (Akovali, 2012), and "oxidized PE" are summarized and indicated as findings for "PE" in this research. The reference database was extended with spectra provided by S.T. Japan Inc. for re-evaluation (Section 2.4.1).

Since several glove leachates rendered a film instead of particles on the aluminum oxide membrane, μ-FTIR results are presented as coverage with a substance in percent relative to the sample spot.

pyr-GC/MS. Glove leachates were prepared for pyr-GC/MS analysis by filtration through glass microfiber disks (MGB grade, particle retention 1 μm; Sartorius) using pressurized filtration with stainless steel equipment and rinsing with 200 mL of ultrapure water. For each glove, a separate filter was used. Filters were dried at 40 °C for 24 h and subsequently ground and homogenized in a planet mill (Retsch) using stainless steel grinding tools.

Of each filter, 20 mg (total average weight 0.3 g) was weighed into 80 μL pyrolysis cups (Eco-Cup LF, Frontier Laboratories) and pyrolyzed at 600 °C. The pyrolyzer was operated as "one-shot". Measurements were performed with a Multi-Shot Pyrolyzer EGA/PY-3030D (Frontier Laboratories) equipped with an Auto-Shot Sampler AS-1020E (Frontier Laboratories). Pyrolysis products were injected with a split of 1:20 into an Agilent 7890B gas chromatograph (Agilent) with a deactivated retention gap (5 m length, 0.25 mm inner diameter) and a HP-5 ms Ultra Inert column (Agilent) with the dimensions of 30 m length, 0.25 mm inner diameter, and 0.25 μm film thickness. Chromatographic separation was performed with the following temperature program: hold at 40 °C for 2 min, increase to 320 °C by 20 °C per min, and hold for 13 min. For detection, an Agilent MSD 5977B (Agilent) in scheduled selected ion monitoring mode was used.

To estimate the potential amount of glove-related substances, leading to an over-estimation of PE, appropriate amounts of cryo-milled PE (Sigma-Aldrich) were serially diluted in pulverized glass microfiber disks to achieve a calibration range from 0.005 to 5 mg/g. For quantification, characteristic pyrolysis products of PE were monitored by the abundance of their indicator ion (for more details, see Table C.3). A 10 µL aliquot of a 27 µg/mL solution of polystyrene-d5 (PSd5, Polymer Source) in dichloromethane (Sigma-Aldrich) was added into the pyrolysis cups as an internal standard and monitored at m/z 109.1 and t_r = 6.94 min. The ratio of peak areas of the characteristic pyrolysis product and polystyrene-d5 (relative response) was used for quantification. The unit of quantification was milligram of PE per filter.

2.3.5   Sample Processing and Identification of Granulate Samples

µ-Raman. For the samples G-S, G-PE, and G-SDS, a spatula tip of granulate was placed onto separate sections of a microscope slide and each granulate was analyzed by applying µ-Raman. Single granulate particles were identified manually using the settings described in Section 2.3.4, with exception that the acquisition time was 10 s and the number of accumulations was 2. Raman spectra of each granulate were manually compared with the laboratory internal as well as a commercially available spectra database (Bio-Rad) using the software KnowItAll (Bio-Rad). The correlation between spectra and reference spectra was determined by the software on the basis of mean-centering of all spectra and dot product normalization, as well as calculation of the Euclidean hit quality index (HQI). Note, all HQI information presented in this research (for µ-Raman and µ-FTIR spectra) is scaled to range from zero (no correlation) to one (full consistency).

µ-FTIR. For the samples G-S, G-PE, and G-SDS, a spatula tip of each solid substance was placed on the diamond crystal of an attenuated total reflection (ATR) accessory connected to the IR spectrometer. Spectra were recorded in ATR mode within a range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1} and 16 scans for each sample. The diamond crystal was cleaned before analyzing each sample with 70% isopropyl alcohol. For identification, all obtained spectra were compared with ATR-FTIR Lib complete (462-2 Vol. 1-3, Bruker GmbH) and a free FTIR reference database (Primpke et al., 2018) using the operation "spectrum search" (Opus 7.5.18, Bruker GmbH) with its standard algorithm within the range of 4000–2450 and 2250–400 cm^{-1}. The maximum HQI is assigned "if the position of the absorption band deviates less than the full width at half-maximum (fwhm) and if the compared fwhm as well as the relative intensity deviates less than a factor of two" (Primpke et al., 2017). Further, an aluminum oxide membrane was prepared for identification of granulates G-S, G-PE, and G-SDS by µ-FTIR. Three areas were manually marked on the membrane, and a spatula tip of each substance was strewn on one section each. Measurement and data analysis of an area of 64 mm^2 of the membrane were carried out with the settings and program described above. On the basis of the previous identification of the samples G-S, G-PE, and G-SDS in ATR mode, the free reference database was extended by spectra of stearates and surfactants provided by S.T. Japan Inc. Thresholds, spectral range, and weighting of either raw spectra or

derivatives for spectra correlation were adjusted in order to accomplish identification of granulates G-S, G-PE, and G-SDS on the aluminum oxide filters as PE, stearate, or surfactant. This optimized database was also used for re-evaluation of the leachate samples (Section 2.4.1).

pyr-GC/MS. A microspatula tip of the granulates G-S, G-PE, and G-SDS was weighed into 80 µL pyrolysis cups and pyrolyzed at 600 °C. Measurements were performed as described in Section 2.3.4.

2.4 Results and Discussion

2.4.1 PE Measurements of Glove Leachates.

To determine the potential cross-contamination of samples due to the use of disposable gloves, leachates from all gloves (N1–N7, L1, Neo1, and V1) were analyzed for MPs. The polymers polyamide, polystyrene, polypropylene, and polyethylene terephthalate, as well as indistinguishable acrylates, polyurethane, or varnish, were identified with µ-Raman and µ-FTIR. However, PE was by far the most commonly identified polymer. For this reason, the focus of this research was placed on PE and substances that were falsely identified as PE (false positives).

Within this section, no differentiation is made between PE and substances that are falsely identified as PE, in order to quantify the total potential overestimation of PE in samples due to cross-contamination and misinterpretation. Results for glove leachates are indicated as the mean value from two replicates per glove type. The according range can be derived from Figure 2.3. Process blanks and hand blanks are indicated as the mean value from three replicates with the according standard deviation (Figure 2.3).

µ-Raman. Analysis rendered PE particles neither on process blanks nor on hand blanks. For leachate duplicates per glove type, a mean of eight PE particles per filter (glove N4) to 5897 PE particles per filter (glove N1) was found. High levels of PE particles were identified on gloves L1, Neo1, N1, N2, N3, and N6. The size of PE particles examined across all glove types was in the range between 5 and 20 µm for 82% of the PE particles. Only 18% of the PE particles were in the size range between 20 and 100 µm and none were >100 µm.

µ-FTIR. No PE was identified for process blanks, and the percentage of sample spot covered with PE was at $0.02 \pm 0.02\%$ for hand blanks. Distinct PE films were observed for gloves N1 and N6, where 2.70% and 1.89% of the sample spot were covered with PE, respectively. The highest PE values were determined for glove N3, where 82.0% of the sample spot was covered.

pyr-GC/MS. Using 1-pentadecene, hand blanks and processing blanks were below the limit of detection (LOD, 0.012 mg; mean value of process blank plus three times standard deviation). The same was true for untreated (only ground/ homogenized) filter disks and glove types N4, N5, and N7. Highest concentrations were found for gloves N1, N2, and N6 with a mean of 0.301, 0.324, and 0.161 mg per glove and filter, respectively.

Due to the differing units, results obtained with µ-Raman, µ-FTIR, and pyr-GC/MS can only be compared semiquantitatively. The most evident similarities are the low PE result for gloves V1, N4, N5, and N7 and the comparably high PE results for N1, N3, and N6 with all applied methods. For gloves L1, Neo1, and N2, however, there is no consistent trend between the methods: glove L1 is below the LOQ (0.023 mg, mean value of process blank plus ten times standard deviation) for pyr-GC/MS and results in 0.002% PE sample spot coverage with µ-FTIR, while µ-Raman yields high PE particle numbers (on average 2474 PE particles per filter). Conversely, Neo1 and N2 show substantial PE findings with pyr-GC/MS and µ-Raman, but not with µ-FTIR. These intermethod variations may be explained by two factors: (i) differences in the amount of leachable substances on the gloves due to glove-to-glove variations and (ii) variations caused by differences in the applied methods, e.g., differences in the applied filter substrates (material, pore size) and differences in sensitivity of the methods to certain factors. For instance, µ-FTIR requires a sample thickness (particle or film) in the range where signal intensity is sufficiently different from the substrate background. As the film-building tendency of different leachates (originating from different glove types and manufacturers) is likely to differ, PE coverage identified with µ-FTIR may not only depend on the amount of deposited leachate but also on the lateral distribution and height of the filtration residue.

2.4.2 Distinguishing among PE, SDS, and Stearates.

One of the objectives of this research was to determine the capability of µ-Raman, µ-FTIR, and pyr-GC/MS to distinguish between PE and other substances that may falsely be identified as PE. For this purpose, granulate samples of sodium stearate, PE, and SDS were anonymized and investigated by all three laboratories. Resulting spectra and chromatograms are shown in Figure 2.4.

µ-Raman. With µ-Raman and the applied measurement settings, it was impossible to distinguish between G-S and G-PE. The spectrum of G-PE had distinct peaks at approximately 2850 and 2880 cm^{-1} (CH$_2$ stretching) as well as peaks with lower intensity at approximately 1060 and 1130 cm^{-1} (C–C stretching), 1295 cm^{-1} (CH$_2$ twisting), 1440 cm^{-1} (CH$_2$ deforming), and 2720 cm^{-1} (overtone) (Silva and Wiebeck, 2019; Zavgorodnev et al., 2015). G-S showed the same peaks as G-PE, the only noticeable difference being a less distinct peak at 2850 cm^{-1} for G-S than for G-PE. Both spectra matched very well with reference spectra of PE from the database and were identified as such with µ-Raman. For the samples G-S and G-PE, the HQI for PE was 0.96 and 0.98, respectively. The Raman spectrum of G-SDS was identified as SDS with a HQI of 0.98, although PE was also listed as a match with a HQI of 0.97. The spectrum of G-SDS showed the same peaks as G-PE and G-S, with the main difference being an additional low-intensity peak at 1080 cm^{-1} (C–C stretching) (Zavgorodnev et al., 2015).

µ-FTIR. By µ-FTIR, the unknown samples G-S, G-PE, and G-SDS were first identified using ATR mode. The correlation between the obtained spectra and reference spectra
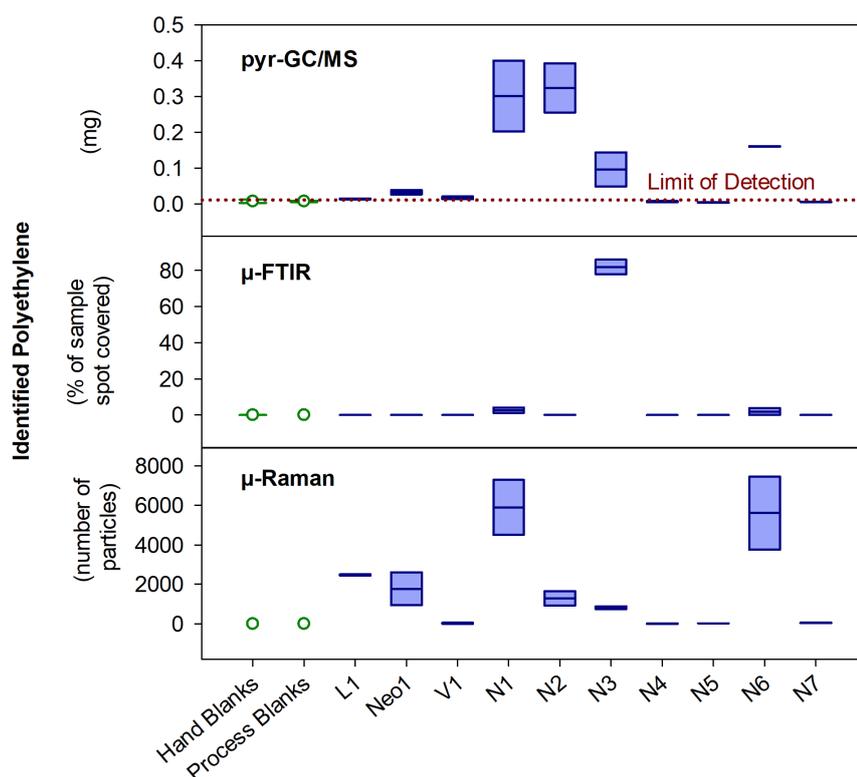
FIGURE 2.3: Amount of substances identified as PE in hand blanks (per filter and hand), process blanks (per filter), and ten different glove types (per filter and glove) using µ-Raman, µ-FTIR, and pyr-GC/MS. For blank values, the mean from three replicates is given (circle) with the standard deviation (error bars). For gloves L1, Neo1, V1, and N1–N7, the mean value from two replicates with the according range (box) is given.

was calculated with the OPUS operations standard search (Primpke et al., 2017). Granulate G-S was identified as sodium stearate with a HQI of 0.79. G-PE matched as PE (high density) with a HQI of 0.99. G-SDS showed a high accordance to sodium dodecyl sulfate with a HQI of 0.96. Hence, ATR-FTIR is suitable to distinguish these substances. However, µ-FTIR imaging of MPs is often carried out in transmission mode with samples immobilized on substrates such as membranes or windows instead of using ATR mode. Commonly applied substrates are for instance aluminum oxide membranes and silicon membranes, as well as zinc selenide, barium fluoride, and calcium fluoride windows (Käppler et al., 2015; Korth Kristalle GmbH, 2025; Löder et al., 2015; Simon et al., 2018). These substrates differ in their IR transparency (aluminum oxide membrane, 3800–1250 cm^{-1} (Löder et al., 2015); silicon membranes, 4000–600 cm^{-1} (Käppler et al., 2015); zinc selenide windows, 3750–950 cm^{-1} (Simon et al., 2018); barium fluoride windows, 71428–714 cm^{-1}; calcium fluoride windows, 80000–952 cm^{-1} (Korth Kristalle GmbH, 2025)). Thus, the choice of substrate is relevant for correct identification of polymers, as the according IR-transparent range determines the wavenumber regions that can be measured and used for sample identification.
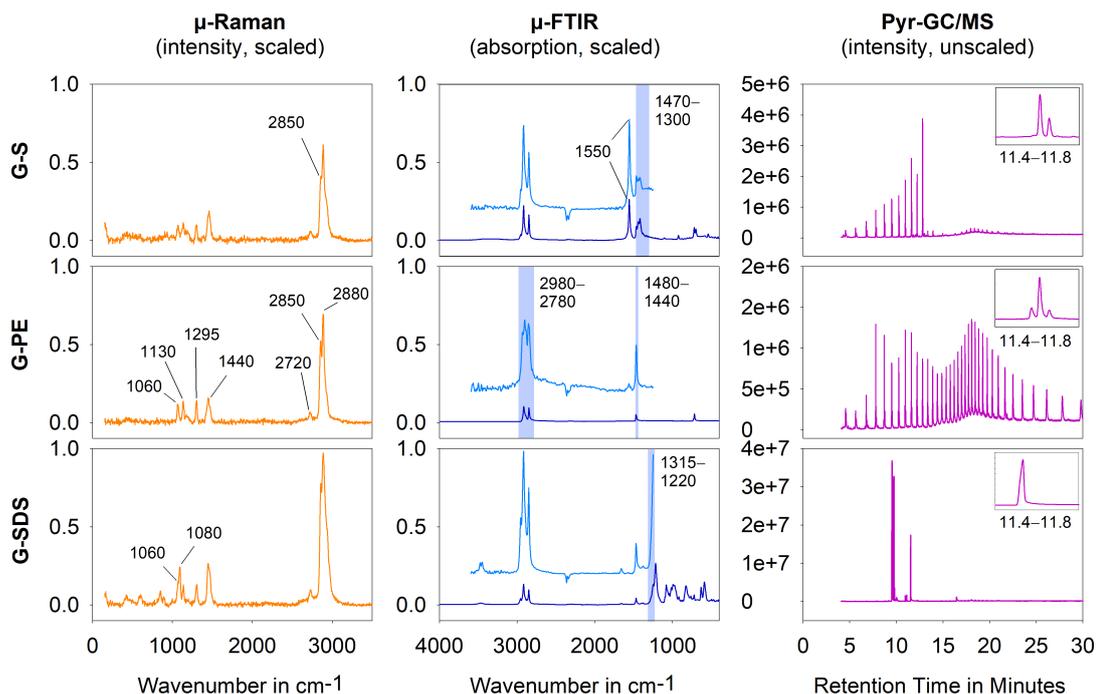
FIGURE 2.4: Comparison of the granulates G-S, G-PE, and G-SDS measured with μ-Raman (intensity, scaled by 31000), μ-FTIR (absorption, scaled by two; dark blue, ATR mode; light blue, transmission mode on aluminum oxide membrane), and pyr-GC/MS (unscaled, a pyrogram cutout for each granulate at t_r = 11.4–11.8 min is displayed enlarged).

Measurements using μ-FTIR in transmission mode and an aluminum oxide substrate were performed with a spectral range from 3600 to 1250 cm^{-1}. Typical spectral regions of interest for polyethylene are the C–H bending at 1480–1440 cm^{-1} and C–H stretching at 2980–2780 cm^{-1} (Löder et al., 2015). Both G-SDS and G-S showed these absorbance bands. Thus, distinction among sodium stearate, PE, and SDS is only possible by considering further absorption bands. G-SDS shows only a weak signal in the region of 1480–1440 cm^{-1} (C–H deformation) but a strong signal from 1315 to 1220 cm^{-1} (SO_2 stretching) in the ATR spectrum (Socrates, 2010). Although spectra acquisition for aluminum oxide membranes was restricted to 1250 cm^{-1}, a section of strong SO_2 stretching vibration band arising at 1300 cm^{-1} was detected. Apart from the C–H vibration bands, G-S has a characteristic strong signal in the region of 1550 cm^{-1} (CO_2 stretching; Hesse et al. (2005)). In the region of 1470–1370 cm^{-1}, the signals 1470–1430 cm^{-1} (C–H vibrations; Hesse et al. (2005)) and 1420–1300 cm^{-1} (carboxylate vibrations; Hesse et al. (2005)) arise next to each other and result in a signal pattern different from those of both PE and SDS. Therefore, a differentiation among stearate, PE, and SDS by μ-FTIR in transmission mode is feasible if the substrate is transparent at least in the abovementioned spectral regions for alkyl, SO_2, and CO_2 vibrations.

pyr-GC/MS. pyr-GC separation of PE and subsequent detection with MS results in a homologous series of peak triplets (Figure 2.4, G-PE). These consist of a weak α,ω-alkadiene peak followed by a dominant 1-alkene peak and a minor *n*-alkane peak, which

can be monitored through the whole pyrogram (t_r = 29 min, maximum observed chain length of 42 C atoms), although the triplets merge to one broad peak at high retention times. Sample G-PE could therefore be clearly identified as PE. However, fatty acids also show similar homologous series with varying repetitions of the triplet peaks as a function of chain length. In each peak triplet, 1-alkene and *n*-alkane are dominant while α,ω-alkadiene is almost negligible, as it can only originate from thermal decomposition of very long chains, such as PE. For G-S, the homologous series was observed up to t_r = 12.72 min (Figure 2.4), which reflects 1-heptadecene resulting from decarboxylation of stearic acid during pyrolysis. The chain length of alkyl compounds such as fatty acids determines the number of triplet repetitions, which is a criterion for identification and distinction. Hence, G-S was identified as a stearate. However, the presence of fatty acids of shorter chain lengths such as palmitic acid could not be excluded, as their signals may be overlapping with the dominating derivatives of stearic acid. The pyrogram for G-SDS showed three dominant peaks at 9.46, 9.56, and 9.64 min, which were identified as isomers of dodecene. Further, a peak at 11.43 min was identified as 1-dodecanol. Therefore, a compound with a dodecyl residue, such as SDS, was assumed. However, a detailed identification for G-SDS was not possible with the applied method. More importantly, the pyrogram of G-SDS showed no relevant peak at t_r = 11.41 min used for PE quantification (for more details see Table C.3) and therefore would not result in overestimation of PE.

2.4.3 Determining the Reason for PE Overestimation in Glove Leachates.

Investigations of the three granulates proved that it is possible to distinguish between PE and stearates with μ-FTIR, if a suited substrate is used and if spectra of stearates are incorporated into the database. As stearates are substances commonly applied in glove manufacturing processes as a mold release agent (Chia and Mohd Sukri, 2019), they were suspected of causing the observed elevated PE results for the glove leachates (Section 2.4.1). Therefore, the μ-FTIR database was expanded to include spectra of stearates, and the μ-FTIR glove leachate data was re-evaluated. This investigation revealed that 89.9 ± 12.6% of the identified PE sample spots of the gloves Neo1, L1, N1, N2, N5, and N6 consisted of stearates. The gloves V1 and N7 contained neither stearates nor PE. For the hand blanks and the process blanks, the identified PE sample spots were composed of 99.9% and 100.0% stearates, respectively. In contrast, gloves N3 and N4 yielded filter coverages of 31.2% and 0.0% stearates, respectively. Whether the remaining percentages of the sample spots are made up of PE (used as stretch modifier or mold-release agent in glove production; Inthasaro (2016) and Lipinski and Tang (2010)) or other substances falsely identified as PE remains unclear. However, additional investigations with pyr-GC/MS (glass microfiber disks soaked with methanol and wiped over gloves) revealed the presence of free fatty acids or corresponding esters/salts (C12, C16, and C18) for L1, N1, N2, N3, N5, and N6.

2.4.4   Preventing False Polymer Classification.

Gloves are a cross-contamination source for MPs, since they are able to release substances that are either actual polymers or misinterpreted as polymers. For instance, stearates resulting from gloves and SDS introduced during sample processing may easily be mistaken for PE, regardless of whether µ-Raman, µ-FTIR, or pyr-GC/MS is used for identification.

µ-Raman. The differences between Raman spectra of stearate, PE, and SDS are minimal. Differentiation is, if at all, possible only if adequate Raman reference spectra are provided in the database and if the risk of confusion between the substances is known. Possibly, the use of higher gratings, longer acquisition times, or multiple spectra accumulations can improve differentiation among PE, SDS, and stearates. This would however increase the already very long measurement time with µ-Raman and is therefore not feasible when dealing with environmental MP samples. Furthermore, interfering substances (e.g., organic and inorganic compounds) are often present in environmental samples and can cause a high level of noise. Thus, minimal differences between spectra—as observed between the Raman spectra of the pure granulates G-S, G-PE, and G-SDS in Figure 2.4—are likely to go unrecognized when dealing with environmental samples. Therefore, special attention must be payed to potential misidentification with µ-Raman, if SDS or disposable gloves are used during sample processing and sample handling. Residues of SDS may be removed prior to analysis by filtration of the sample and thorough rinsing. As washed hands rendered little to no cross-contamination with PE (or false positives) for all three analytical methods (see Section 2.4.1), the application of hand washing procedures may be a suited alternative to using disposable gloves, as long as personal safety precautions permit gloveless handling. In any case, cross-contamination should be quantified by blank value investigations.

µ-FTIR. To avoid misinterpretation or overestimation of PE in IR spectra evaluation, spectra of stearates and surfactants such as SDS should be incorporated into IR databases. Furthermore, only substrates that allow spectra acquisition in the regions of C–H, CO_2, and SO_2 vibration bands (e.g., zinc selenide windows, silicon or aluminum oxide membranes) are recommended for measurements in transmission mode.

pyr-GC/MS. With pyr-GC/MS, misinterpretation of substances like stearates and SDS as PE is less likely than with µ-FTIR or µ-Raman. However, the quantification of PE via 1-pentadecene can lead to an overestimation of PE in the presence of fatty acids. Alternatively, a 1-alkene with a longer chain length (e.g., 1-octadecene) can be chosen to avoid overlapping of pyrolysis products originating from PE and fatty acid, which, however, results in lower sensitivity.

As peak triplets of homologous series of PE tend to merge at higher t_r, it gets harder to differentiate the 1-alkene peak from the weaker peaks of α,ω-alkadiene and *n*-alkane. 1,14-Pentadecadiene can be considered for quantification of PE, as it cannot originate from pyrolysis of fatty acids. Therefore, leachates of studied gloves were additionally evaluated via 1,14-pentadecadiene and 1-octadecene in order to verify the results obtained via 1-pentadecene. 1,14-Pentadecadiene and 1-octadecene rendered significantly

lower PE values: for instance, N1 revealed 0.301 mg via 1-pentadecene but 0.077 and 0.027 mg of PE with 1-octadecene and 1,14-pentadecadiene, respectively. Similar effects were observed for N2, N3, and N6, while all other samples yielded PE values below the LOD and LOQ.

It must be assumed that many more synthetic and natural substances exist in laboratories and the environment that may be misinterpreted as polymers (e.g., proteins, fatty acids). These substances are unknown and unexpected as a potential source of cross-contamination. In order to rule out misinterpretation of synthetic and natural substances as polymers in environmental samples, further research is necessary. For cross-contamination stemming from the lab, the determination of (process) blank values is a reliable method to prevent overestimation of MPs, both by false positive identification of substances as polymers as well as by actual polymers. Furthermore, the identification of conspicuous (process) blank values (e.g., for a specific polymer type, sample, or matrix) is the first step toward uncovering and eliminating a cross-contamination source in the laboratory. Gloves causing sample cross-contamination are just one example of the things that can go wrong during MP investigations. As the pitfalls in MP analysis are manifold and only partially predictable, researchers addressing MP quantification are urged to implement rigorous measures for quality assurance, such as minimization of foreseeable sources of cross-contamination, and to strictly perform quality control by means of blank value investigations and recovery experiments.

2.5 Acknowledgements

Chapter 3

Article II: Temporal Variability of Microplastic Concentrations in Inland Waters: An Automated, Semicontinuous Sampling of Microplastics ≥ 11 µm in a Stream in Southern Germany

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Changes that go beyond editorial and formatting adjustments are indicated as footnotes.
The full text of the article can be accessed via this link:
https://pubs.acs.org/articlesonrequest/AOR-XQMWHMHURV5RNBGXWAEN.

3.1 Abstract

To advance understanding about the temporal variability of microplastic concentrations in inland waters, this study presents a fully automatic sampling unit for microplastics (SAM), which collects daily mixed samples using fractionated filtration. Method validation with five different polymer types revealed an overall recovery of $77 \pm 29\%$ for

sampling, sample preparation, and analysis of particles ≥ 11 µm using Fourier transform infrared microspectroscopy. During an eight-day field test, SAM was applied in a stream in Southern Germany. Microplastic concentrations in the daily mixed samples differed by a factor of 10.8 within the study period, ranging from 1210 to 13052 particles and fibers per m^3. Polypropylene and polymer cluster acrylates/polyurethanes/varnish were the most abundant polymer types observed. The comparison of day-to-day variability of microplastic concentrations with the total particle count, turbidity, precipitation, as well as discharge in the stream did not reveal distinct interrelations. The field application, as well as the good recovery rates of SAM, demonstrate its suitability for future long-term studies focusing on the temporal variability of microplastic concentrations.
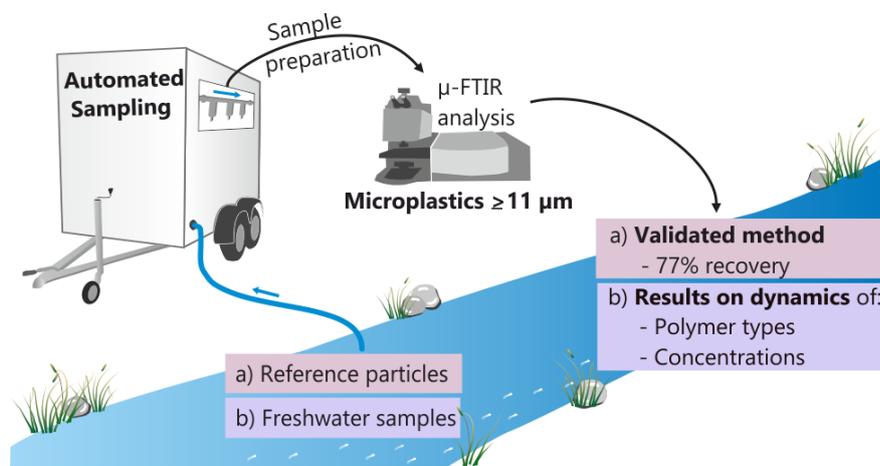
FIGURE 3.1: Graphical abstract of article II.

3.2 Introduction

Over the past decade, there has been a substantial increase in the collection of data concerning the occurrence of microplastics in surface water. Microplastics (MPs) are small, solid plastic items, which consist of synthetic polymers and originate from intentional production or fragmentation of larger plastic objects (Hartmann et al., 2019). Given that there is no standardized size specification for MPs, the range from 1 to < 1000 µm diameter proposed by Hartmann et al. (2019) is used here.

Despite increased research efforts, there are only a few studies that have investigated the temporal variability of MP concentrations in surface water. These studies have predominantly focused on precipitation events or examined monthly to seasonal effects (de Carvalho et al., 2021; Hitchcock, 2020; Mintenig et al., 2020; Skalska et al., 2020; Xia et al., 2020). As a consequence, the assessment of MP concentrations and composition (polymer types) in surface waters mainly relies on the results from single-point samplings. Single-point sampling refers to individual samples taken at a specific point in time, which, especially in rivers, are insufficient to determine representative MP loads.

The limited availability of data related to the temporal variability of MP concentrations and composition is primarily attributed to the lack of automated sampling devices and the immense amount of time required (several days up to weeks) to prepare and analyze MP samples.

MPs are not homogeneously distributed in surface waters, and large sampling volumes are generally required to provide a representative sample (Prata et al., 2019b). A well-established method for sampling larger volumes is fractionated filtration via a filter cascade (Bordós et al., 2021; Funck et al., 2020; Pittroff et al., 2021). The particles in the sample medium are enriched on a sequence of fine stainless-steel sieves, while the sampled water volume is discharged back into the water body. Manual operation or supervision has been necessary for the sampling devices used to date.

The analysis of MP samples is time-consuming and labor-intensive, especially when analyzing particles < 300 µm and using spectroscopic methods such as Fourier transform infrared microspectroscopy (µ-FTIR) and Raman microspectroscopy (µ-Raman). Sophisticated sample preparation techniques are required to remove organic and inorganic interfering substances from surface water samples (Koelmans et al., 2019; Mintenig et al., 2020). However, these methods can reliably determine the number, size, and polymer type of MPs in environmental samples down to 5 µm (µ-Raman; Witzig et al., 2021) and 11 µm (µ-FTIR; Gunaalan et al., 2023; Liu et al., 2023; Roscher et al., 2021).

The lack of standardized procedures has resulted in a large variety of methods being applied to sample, prepare, and analyze MPs in water matrices. This makes comparability between studies difficult and calls data quality into question (Cui et al., 2022). Quality assurance and quality control are therefore particularly important in this regard. The recording of (process) blank values, reporting on measures to avoid cross-contamination, and proof of the suitability of the applied methods (recovery experiments) are increasingly establishing themselves as quality criteria (Cowger et al., 2020; Koelmans et al., 2019; Lu et al., 2021).

This study presents the validation and eight-day field application of a new, fully automated MP sampling unit for semicontinuous monitoring of surface water samples based on daily mixed samples.

3.3 Material and Methods

3.3.1 Study Site

Water samples were collected from the stream Kirchgassgraben, flowing through the city of Bühl, located in Baden-Württemberg, Germany. Kirchgassgraben is a 1 km long side arm of the river Sandbach, originating in the northern Black Forest. The catchment area upstream of the confluence point where Sandbach and Kirchgassgraben merge is approximately 33 km^2. Sample collection was performed under a small pedestrian bridge located at N 48.697927 and E 8.125208.

3.3.2   Sampling

Samples were taken using a custom-built mobile measuring station (M²S). M²S is an enclosed carry-on cargo trailer in which a self-built, automated sampling unit for MPs (SAM) as well as two customary devices for the monitoring of pH, turbidity, conductivity, and total particle count (in the following referred to as accompanying parameters) are installed.

3.3.2.1   Sampling of Microplastics with SAM

The sampling principle of SAM is to collect daily mixed samples of MPs by filtering a constant sample volume over a filter cascade (Pittroff et al., 2021) every hour for 24 h. As SAM is equipped with seven filter cascades (exchangeable via GEKA quick couplings; Karasto Armaturenfabrik Oehler GmbH, Germany), up to seven mixed samples (corresponding to one week of sampling) can be taken in a fully automatic manner. SAM is controlled via a self-written program with LabView (Version 20.0f1, National Instruments Corp., USA), which regulates the peristaltic pump (Verderflex iDura D25 SW US-CLSS VAR1.5 K NR P1 V with a natural rubber tube; Verder Liquids BV, Netherlands) and directs the water flow using motorized valves (stainless-steel, ABVM06S/9AR-MO; ICH B.V., Netherlands). There are three flow paths within SAM (Figure 3.2).
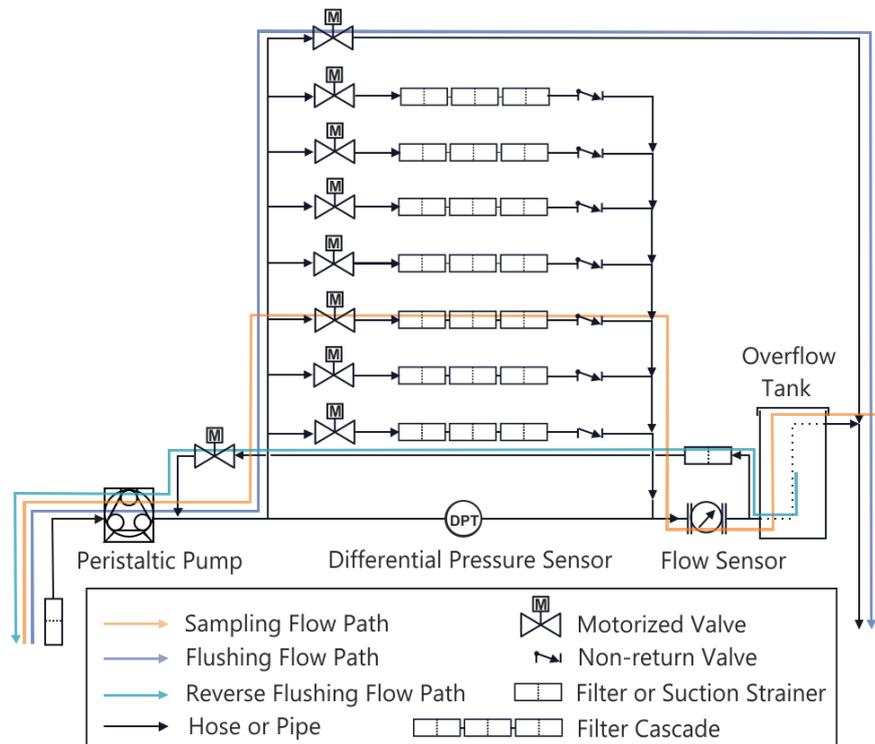
FIGURE 3.2: Setup and functionality of the automated sampling unit for microplastics (SAM).

Sampling Flow Path. Sample media is sucked through a suction strainer (stainless-steel, 1 to 1.8 mm diamond-shaped pores, Esska.de GmbH, Germany) and an approximately 17 m long polyvinyl chloride (PVC) hose (¾ in. diameter, Armoflex, Teguma GmbH, Germany), to the peristaltic pump located in M^{2}S. Following the peristaltic pump, water is pumped over a filter cascade (as presented by Pittroff et al., 2021) and a subsequent non-return valve through stainless-steel pipes (Mapress; Geberit International AG, Switzerland). After passing through a flow sensor (210.906 741G; Huba Control AG, Switzerland) and a 100 L stainless-steel overflow tank, the sampled water is discharged. The sampled volume and differential pressure (DMD 831; DB SENSORS GmbH, Germany) are continuously logged. If the differential pressure (indicator for clogging of the filter cascade currently in use) rises above a predefined maximum value (trigger value), the sampling is automatically paused until the beginning of the next 24-hour cycle, at which point a new cascade is used.

Flushing Flow Path. During the flushing process of the hoses and pipes, the water follows the same flow path as during sampling, but is diverted past the cascades using a bypass and is subsequently discharged.

Reverse Flushing Flow Path. Reverse flushing enables the removal of debris, which may accumulate on the suction strainer at SAM's inlet during sampling and flushing. By reversing the pumping direction, the water is sucked from the overflow tank through a second bypass containing a stainless-steel fine filter (5 μm mesh size; Rolf Körner GmbH, Germany) to the peristaltic pump and is discharged with pressure via the suction strainer, thereby pushing away debris.

3.3.2.2   Monitoring of Accompanying Parameters

A particle counter device with a particle sensor LDS 45/50 (Markus Klotz GmbH, Germany) and a sensor station (s::can GmbH, Austria) with a pH sensor (ph::lyser II eco, E-514-2-075), a turbidity sensor (i::scan, Y05-3-r-075), and a conductivity sensor (condu::lyser, E-511-2-075) are continuously supplied with sample media via a perfluoroalkoxy alkane tube (inner diameter 4 mm, Wolf-Technik eK – VKL, Germany) through a second peristaltic pump (07 528-10 L/S Variable-Speed Digital Drive and 77 253-02 L/S Cytoflow 2- Roller Pump Head; Masterflex SE, Germany). A stainless-steel aquarium filter with a mesh size of 0.5 mm diameter is installed at the tube inlet to prevent clogging of the system.

In addition, data from a gauging station (Landesanstalt für Umwelt Baden-Württemberg, 2023) located 2.3 km upstream and precipitation data from two weather stations (Agrarmeteorologie Baden-Württemberg, n.d.) in the vicinity (weather station 1 located approximately 4.2 km to the Northeast and weather station 2 located approximately 3.3 km to the South) of the sampling point were obtained.

3.3.2.3   Sampling at Kirchgassgraben

Sampling was performed from the 19th until the 27th of September 2022. All samples were taken midstream at 16 cm above the channel bed. The water depth at the sampling

point ranged between approximately 30 and 40 cm during the sampling period.

Conductivity, turbidity, pH, and total particle count were monitored continuously throughout the study period. However, the particle counter experienced two significant downtimes on the 21st of September from 00:14 to 06:59 and on the 23rd/24th of September between 23:09 and 03:25 (see Figure 3.4).

Within the eight-day sampling period, eight mixed samples of MPs were obtained (see Table 3.1) using SAM. Each filter cascade was equipped with three stainless-steel filters of 100, 50, and 10 µm mesh size (Rolf Körner GmbH, Germany). Every hour, before water from Kirchgassgraben was pumped along the sampling flow path (see Section 3.3.2.1), reverse flushing was carried out for 2 min followed by flushing for 2 min. The pumping velocity was approximately 6 L min^{-1} for all three flow paths. On the 19th and 20th of September, the target sampling volume was 100 L d^{-1}. From the 21st of September, it was reduced to 80 L d^{-1}. As presented in Table 3.1, the target volume was never matched exactly due to the closing time of the motorized valves and because the differential pressure trigger value (set to 3.5 bar) was reached on four sampling days. Once sampled, the filter cascades were replaced, sealed with GEKA end couplings, and transported to the laboratory, where they were stored at 8 °C until sample processing.

TABLE 3.1: Microplastics mixed sampling details.

Sampling day	Start of sample collection	End of sample collection	Hours of microplastic sampling	Sampled water volume (L)
D1	19.09.2022 11:45	19.09.2022 22:45	12[a]	52.3
D2	20.09.2022 11:42	21.09.2022 08:44	22[a]	100.4
D3	21.09.2022 11:42	22.09.2022 10:44	24	88.4
D4	22.09.2022 11:42	23.09.2022 10:45	24	89.1
D5	23.09.2022 11:42	24.09.2022 10:45	24	90.2
D6	24.09.2022 11:42	25.09.2022 01:45	15[a]	55.2
D7	25.09.2022 11:42	26.09.2022 10:45	24	89.2
D8	26.09.2022 11:58	27.09.2022 03:01	16[a]	57.5

[a]Differential pressure trigger point was reached.

3.3.3 Sample Processing

The three size fractions per filter cascade (referred to as one sample) were extracted separately using an ultrasonic bath (Elmasonic P 180 H, 100% intensity, 37 kHz; Elma Schmidbauer GmbH, Germany). To counteract the effect of filter cake formation during sampling, each sample was refractionated in the lab using a filtration tower, equipped with stainless-steel filter discs of 100, 50, and 10 µm mesh width (48 mm diameter; Rolf Körner GmbH, Germany). The sample was treated with 300 mL of ultrapure water (18.2 MΩ cm, Arium 611 UF; Sartorius AG, Germany) enriched with ozone (approximately 3.2 mg L^{-1}), sealed to be airtight, and left overnight at room temperature. The following day, samples were treated with Fenton reagent. For this, 100 mL of ultrapure

water, 10 mL of iron sulfate solution ($0.1\ \mathrm{mol\ L^{-1}}$; Thermo Fisher Scientific Inc., USA), 15 mL of sodium hydroxide solution ($0.1\ \mathrm{mol\ L^{-1}}$; Merck KGaA, Germany), and 85.5 mL of hydrogen peroxide (50%; Carl Roth GmbH & Co. KG, Germany) were added to each size fraction. The sample was maintained at a controlled temperature of $\leq 30\ ^\circ$C for a duration of 4 h, using an ice bath to regulate the temperature. Subsequently, the sample was left in a water bath overnight. The size fraction $\geq 100\ \mu$m was split into the size fractions $\geq 250\ \mu$m and < 250 to $\geq 100\ \mu$m by filtration over a 250 μm stainless-steel filter disc (48 mm diameter; Rolf Körner GmbH, Germany). The filter disc with size fraction $\geq 250\ \mu$m was transferred to a glass Petri dish and not subjected to any further sample processing. All size fractions $< 250\ \mu$m were density separated using sodium polytungstate solution ($2.25\ \mathrm{g\ cm^{-3}}$; TC-Tungsten Compounds GmbH, Germany) and a centrifuge (Sigma 8KS, SIGMA Laborzentrifugen GmbH, Germany) with a centrifugal acceleration of 742g. The size fractions < 250 to $\geq 100\ \mu$m and < 100 to $\geq 50\ \mu$m were filtered onto silicon filters (10 μm pore diameter, 10×10 mm square membranes, Smart-Membranes GmbH, Germany). Up to four silicon filters per size fraction were used to minimize the superposition of particles on top of each other. Due to the high number of particles and fibers, aliquots were taken for the smallest size fraction. Up to four aliquots in the size fraction < 50 to $\geq 10\ \mu$m per sample were filtered onto separate silicone filters after enrichment in a small amount of ultrapure water and while the particles were well mixed. Per sample, 6.8–37.7% of the size fraction < 50 to $\geq 10\ \mu$m was analyzed.

3.3.4 Chemical Identification

MP samples were measured using μ-FTIR. The applied system from Agilent Technologies Inc. (USA) consists of a Cary 670 IR spectroscope coupled with a Cary 620 FTIR microscope and is equipped with a 128×128 mercury cadmium telluride focal plane array detector. All measurements were performed with triangular apodization, the spectral range of 3800–900 $\mathrm{cm^{-1}}$ at 4 $\mathrm{cm^{-1}}$ resolution, and using a Cassegrain objective with 15× magnification. Pixel resolution was 5.5 μm. Spectra comparison was accomplished with software siMPle (version 1.1.ß; Primpke et al., 2020b) and the adapted database presented by Roscher et al. (2022).

Particles and fibers $\geq 250\ \mu$m were manually transferred from the stainless-steel filter disc to a gold-coated microscope slide. The measurement of each particle or fiber was performed in reflection mode using 30 co-added scans and was immediately followed by taking 30 co-added background scans on an empty and clean spot on the gold-coated microscope slide. Particles and fibers $\geq 250\ \mu$m were identified as polymers, if the single spectra match resulted in a hit quality $\geq 60\%$ and the assigned polymer type was manually confirmed.

The silicon filters containing particles and fibers in the size ranges < 250 to ≥ 100, < 100 to ≥ 50, and < 50 to $\geq 10\ \mu$m were measured in full (grid of 15×15 measurement fields), using transmission mode and 30 co-added scans. Prior to the measurement of each silicon filter, 120 co-added background scans were taken on an empty and clean silicon filter. Image analysis rendering MP numbers, classification as particle or fiber and

polymer assignment was performed using the MPAPP pipeline (MPAPPv1.1.1; Primpke et al., 2019). Since the smallest mesh size used during sampling and sample preparation was 10 µm, and because the pixel resolution of the µ-FTIR analysis is at 5.5 µm, the smallest MPs reported in this study have a size of 11 µm, corresponding to the edge length of two pixels.

3.3.5   Quality Assurance and Quality Control

3.3.5.1   Cleaning of SAM

Before and after the sampling at Kirchgassgraben, SAM was cleaned using pre-filtered tap water. For this purpose, a single filter cartridge (MiniPlus-FF06–3/ 4AAM, Honeywell, Germany) containing a stainless-steel fine filter (5 µm mesh size) was attached to a drinking water pipe on one side and a PVC hose on the other side. The PVC hose was rinsed for 10 min, and then its free end was connected to the inlet side of SAM's peristaltic pump (see Figure 3.2). Instead of filter cascades, short pieces of PVC hose were installed in SAM as bridging. All flow paths, including the overflow tank, were thoroughly rinsed with pre-filtered tap water. All filter cascades were prepared in the laboratory under high cleanliness conditions (see Section 3.3.5.2).

3.3.5.2   Reducing Cross-Contamination and Sample Loss

Airborne cross-contamination was minimized by using a closed system setup for sampling (see Section 3.3.2), by processing and measuring samples under laminar flow cabinets (HEPA 14; Thulab GmbH, Germany) and by covering samples during storage. The application of plastic tools was omitted as far as possible, 100% cotton lab coats were worn, and a strict protocol for washing hands (see Chapter 2, Section 2.3.1) was followed. All utensils were cleaned by scrubbing them in tap water with an animal hairbrush, rinsing with distilled water, and cleaning them in an ultrasonic bath with ultrapure water three times. All temperature-resistant utensils (e.g., stainless-steel filters, glassware) were previously pyrolyzed in a heating cabinet for one hour at 500 °C. To reduce the loss of MPs, the number of utensils brought into contact with a sample was kept to a minimum by reusing filter discs, containers, and filtration devices until the processing of that sample was completed.

3.3.5.3   Process Blank Samples

On the 30th of September, three process blank samples were taken. For this purpose, a single filter cartridge containing a stainless-steel fine filter (5 µm mesh size) was attached to a drinking water pipe and connected to the peristaltic pump of SAM via a PVC hose (24 m long). Three filter cascades, equipped in the same way as for sampling at Kirchgassgraben, were installed in SAM. 100 L of pre-filtered tap water was sampled with each filter cascade. The samples (hereafter referred to as process blank samples) were subsequently processed and analyzed as described in Sections 3.3.3 and 3.3.4.

3.3.5.4 Laboratory Blank Samples

To determine cross-contamination from sample processing, a random filter cascade, which was installed in SAM during the sampling campaign at Kirchgassgraben, was replaced before stream water was filtered through it. The filter cascade (hereafter referred to as laboratory blank sample) was processed and analyzed as described in Sections 3.3.3 and 3.3.4. The resulting laboratory blank value is volume independent (since no water was filtered over the filter cascade) and was used to assess unforeseen laboratory cross-contamination arising during the processing of the sample batch from Kirchgassgraben.

3.3.5.5 Recovery Experiment

Fifteen samples with (theoretically) identical composition as well as three blank samples were prepared (see Appendix D, Page 97). Each sample contained MP reference particles in the size range between 100 and 10 μm of the polymer types polyethylene (PE), polypropylene (PP), PVC, polyethylene terephthalate (PET), and polystyrene (PS). To determine their initial composition, a subset of nine reference particle samples was filtered directly onto silicon filters. These nine samples are hereafter referred to as untreated spiked samples (U-SS). A further six reference particle samples were enriched on filter cascades using SAM. For this purpose, a single filter cartridge with a stainless-steel fine filter (5 μm mesh size) was connected to a drinking water pipe to pre-filter the water. The pre-filtered drinking water was passed into a funnel. The funnel outlet was connected to a 10 m long PVC hose, which led to the inlet side of SAM's peristaltic pump. Each blank sample and each one of the six reference particle samples was poured into the funnel and pumped onto a separate filter cascade using 150.1 ± 0.4 L of pre-filtered water. Between (blank) samples, flushing via the bypass was carried out for 5 min. The cascades were processed as described in Section 3.3.3, and the corresponding samples and blank samples are henceforth referred to as treated spiked samples (T-SS) and treated blank samples (T-BS), respectively. All samples from the recovery experiment (U-SS, T-SS and T-BS) were analyzed as described in Section 3.3.4. However, because the recovery experiments focused on particles and five specific polymer types, the standard database for automated IR analysis (version 1.0.1; Primpke, 2019) and APA pipeline (APAv1.1.1; Primpke et al., 2017) were used. Because the database identifies PET particles (as used in the recovery experiments) as polyester (PES), the corresponding results are reported as PET/PES.

3.3.5.6 Image Analysis in MPAPP

To ensure adequate image analysis, data subsets were subjected to manual spectra evaluation (as proposed by Primpke et al. (2020b), Moses et al. (2023) and Lorenz et al. (2019)) and the thresholds for automated image analysis were adapted (see Appendix D, Page 98).

3.4　Results and Discussion

The results presented in this study do not comprise all polymers from the standard (Primpke, 2019) and adapted database (Roscher et al., 2022). Polyoxymethylene and rubber are constituents of SAM and were excluded from all results due to observed cross-contamination. Polysulfone, polyether ether ketone, polychloroprene, polyisoprene-chlorinated, polycaprolactone, polyimide, polybutadiene, and acrylonitrile-butadiene were analyzed but could not be detected in any of the samples. For the sake of simplicity, the following presentation and discussion of results refer only to the remaining polymer types, PE, PP, PS, polycarbonate (PC), polyamide (PA), PVC, polyester (PES), cellulose artificially modified (CAM), polymer cluster acrylates/polyurethanes/varnish (A/PU/V), polylactic acid (PLA), and ethylene-vinyl-acetate (EVA).

3.4.1　Recovery Experiment

Reference particles made of the five most relevant polymer types for freshwater (Koelmans et al., 2019) were used, which also cover a broad range of material densities (approximately 0.90–1.45 g cm^{-3}). The smallest diameter of the reference particles (10 µm) applied during the recovery experiment corresponds to the diameter of the smallest MP particles (11 µm) reported in this study. Based on the total number of MP particles in each U-SS (1834 $\pm$ 248 particles) and the volume of water applied to accumulate the particles on the filter cascades (150.3 $\pm$ 0.2 L), a concentration of approximately 1.22×10^4 MP particles per m^3 was sampled during the recovery experiment, which is a realistic MP concentration for riverine surface water (Koelmans et al., 2019; Lu et al., 2021).

The results of the recovery experiment are summarized in Figure 3.3. The overall recovery is 77 $\pm$ 29% (T-SS relative to U-SS). The overall cross-contamination level was very low at 3 $\pm$ 2% (T-BS relative to U-SS). While the recovery for the polymer types PE, PS, and PVC can be rated as good, there were substantial losses for PP and PET/PES. This is not surprising, as PP and PET were among the polymers with the lowest recovery rates in other studies (Bordós et al., 2021; Mári et al., 2021). A possible reason for this is the high surface hydrophobicity of pristine PP and PET particles, which may lead to reduced transport efficiency (particle loss) during sampling and processing (Hildebrandt et al., 2019). However, Hildebrandt et al. (2019) observed a significant increase in transport efficiency and recovery for PP and PET particles, which had been exposed to river water for 40 days. Thus, even though an underestimation of PP and PET/PES in environmental samples cannot be ruled out, losses are likely to be less pronounced than indicated by the recovery of pristine PP and PET/PES particles in Figure 3.3.
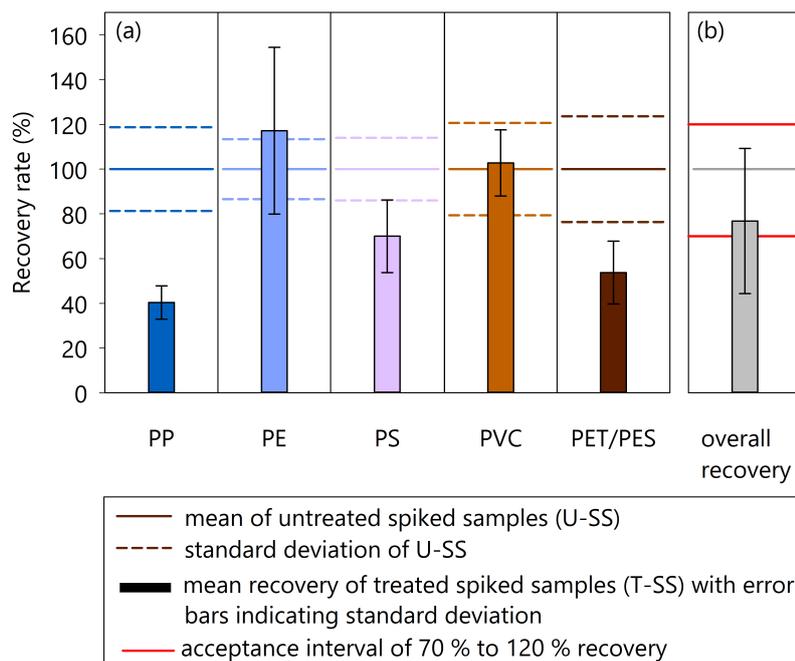
FIGURE 3.3: (a) Recovery for treated spiked samples (T-SS; n = 6) relative to untreated spiked samples (U-SS; n = 9), shown with mean and standard deviations for the polymer types PE, PP, PS, PVC, and PET/PES. (b) Mean and standard deviation for overall recovery and recovery acceptance interval.

3.4.2 Laboratory and Process Blank Samples

The laboratory blank sample revealed a low level of cross-contamination of in total 14 particles and three fibers. In the three process blank samples, a mean of 263 ± 129 particles per m^3 and 80 ± 16 fibers per m^3 were detected, summed over all polymer types. For each polymer type, the limit of detection (mean process blank value $+ 3 \times$ standard deviation) was calculated, distinguishing between particles and fibers (Table 3.2). Since the laboratory blank sample, which was processed in parallel to the samples from Kirchgassgraben, only contained MPs below the limit of detection, there is no indication of any unforeseen cross-contamination from the laboratory during the processing of the sample batch from Kirchgassgraben.

TABLE 3.2: Limit of detection calculated based on the three process blank samples.

Parameter (m^{-3})	PE	PP	PS	PC	PA	PVC	PES	A/PU/V	CAM	PLA	EVA
Particles	363	132	0	0	94	0	62	108	30	0	54
Fibers	34	21	0	0	21	0	64	80	17	0	17

3.4.3 Microplastics and Accompanying Parameters in Kirchgassgraben

For the samples from Kirchgassgraben, only MP concentrations above the corresponding limit of detection (Table 3.2), considering the shape and polymer type, are reported. MP numbers and data of the accompanying parameters (total particle count, precipitation, turbidity, and discharge) from the eight-day sampling at Kirchgassgraben are shown in Figure 3.4.
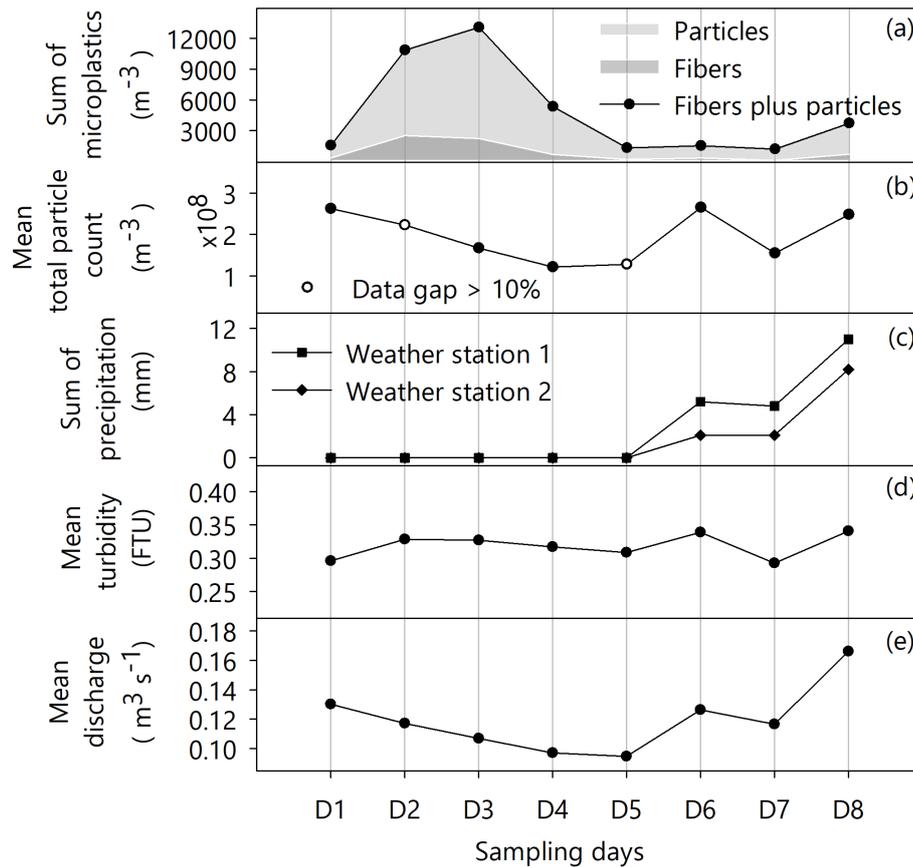
FIGURE 3.4: Results from the eight-day sampling at Kirchgassgraben, with (a) number of microplastics, distinguishing between particles and fibers, (b) mean total particle count, (c) sum of precipitation at the two weather stations in the vicinity of the sampling site, (d) mean turbidity, and (e) mean discharge at the gauging station upstream of the sampling site. Only data collected simultaneously to microplastic sampling was used to calculate the means.

3.4.3.1 Microplastic Shapes

The sum of MPs given in Figure 3.4a consists of particles and fibers, whereby particles make up the largest share (82.2 ± 4.7% as mean for the sampling period). Although less abundant (17.8 ± 4.7% as mean for the sampling period), fibers are present on every sampling day, absolute numbers ranging between 112 fibers per m^3 (D7) and 2529 fibers per m^3 (D2). The particle to fiber partitioning agrees with the observation

of Mintenig et al. (2020), who found a median of 87.1% particles and 12.9% fibers in the surface water of two Dutch rivers when investigating MPs > 20 µm.

3.4.3.2 Microplastic Concentrations

While D1, D5, D6, and D7 show remarkably uniform MP concentrations (mean of 1409 ± 149 MPs per m^3), increased MP concentrations occurred during D2 to D4 and on D8. The observed span of MP concentration at Kirchgassgraben, from a minimum of 1210 MPs per m^3 (D7) to a maximum of 13052 MPs per m^3 (D3), is within the range expected based on literature, although tending toward the upper end. In the review article by Lu et al. (2021), MP concentrations with a maximum of 5.42×10^5 MPs per m^3 are reported for freshwater environments while Mintenig et al. (2020) and Koelmans et al. (2019) report a more conservative range between 0 and 1.3×10^4 MPs per m^3 for global riverine surface waters. The comparably high MP concentrations in Kirchgassgraben are related to the very small particles and fibers investigated (≥ 11 µm). An increase in particle numbers with decreasing particle size is a commonly reported trend (Hidalgo-Ruz et al., 2012; Lu et al., 2021).

3.4.3.3 Microplastic Sizes

Following the abovementioned trend, 95 ± 3% of the MPs at Kirchgassgraben were <100 µm. Further subdivided, most MPs were in the size class ≥ 11 to < 20 µm ($45 \pm 7\%$) followed by the size class ≥ 20 to <50 µm ($36 \pm 12\%$) and the size class ≥ 50 to < 100 µm ($14 \pm 8\%$).

3.4.3.4 Accompanying Parameters

The accompanying parameters shown in Figure 3.4b to e were recorded to determine possible relationships between processes in the water body and the observed MP concentrations. Therefore, the mean total particle count and the mean turbidity, as shown in Figure 3.4b and d, respectively, only include data collected per minute and simultaneously with the MP sampling. Similarly, when determining the sum of precipitation for each sampling day (Figure 3.4c) and the mean discharge per sampling day (Figure 3.4e), based on hourly precipitation and mean discharge, respectively, only data from the hours in which MPs were sampled (see Table 3.1) were taken into account.

Mean total particle count, mean turbidity, and mean discharge show a very similar trend over the sampling period. In general, the values decrease continuously from D1 to D5. Exceptions to this are the increase in the mean turbidity from D1 to D2 and the increase in the mean total particle count from D4 to D5. No precipitation was recorded in the period between D1 to D5.

The precipitation on D6 (5.2 mm at weather station 1; 2.1 mm at the weather station 2) is reflected in increased mean total particle count and mean turbidity, presumably due to the resuspension of sediment and input via runoff from sealed surfaces. On D7, precipitation sums are as the day before (4.8 and 2.1 mm, respectively), but the mean

total particle count, turbidity, and discharge decreased compared to D6. The highest precipitation sum is reached on D8 (11 and 8.2 mm, respectively) and is accompanied by increased values for mean total particle count, mean turbidity, and mean discharge. As the comparison of Figure 3.4a and c shows, precipitation on D6 and D7 does not influence the MP concentration in Kirchgassgraben. However, on D8, the MP concentration increases to 3725 MPs per m^3. The substantially higher MP concentrations in the first half of the week (D2 to D4) cannot be explained by any of the accompanying parameters from Figure 3.4b to e.

3.4.3.5 Microplastic Polymer Types

The absolute and relative contributions of the polymer types PP, PE, EVA, PS, PA, A/PU/V, CAM, PES as well as PC, PVC, and PLA to the total number of MPs on each sampling day are shown in Figure 3.5. On each sampling day, polymer types with a density below and above the density of water were found. This indicates a good mixing of the water column. Good mixing of the water column is important for the representativeness of the results from Kirchgassgraben, as sampling was only possible at one point in the stream cross-section and only at one water depth. The narrow course of the stream, the shallow water depth, and the presence of several rapids upstream of the sampling point were therefore important factors taken into account when selecting the sampling point at Kirchgassgraben. Isokinetic sampling was also considered during the planning phase of SAM, but was not technically practicable in combination with the volume-reducing sampling technique.
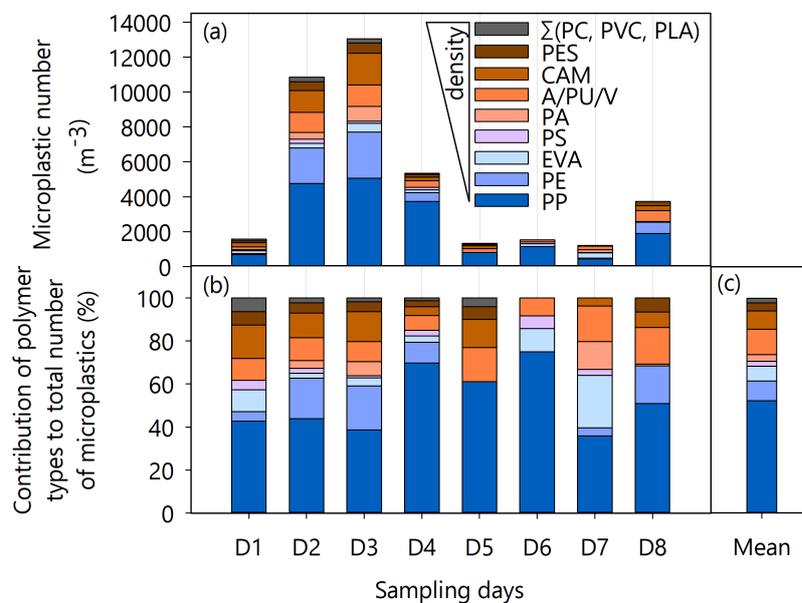
FIGURE 3.5: Contribution of different polymer types to the total number of microplastics on each sampling day at Kirchgassgraben (a) in absolute numbers per m^3 and (b) in percent. (c) Percentage share of the polymer types as mean over the eight sampling days.

PP and the polymer cluster A/PU/V are the only polymer types present on every sampling day and make up the largest share over the eight days with 52 and 12%, respectively, followed by PE and CAM (9% each), EVA (7%), PES (4%), and PA (3%), as well as PC, PVC, and PLA (together 2%). PE and PP are commonly reported as the most abundant polymer types in freshwater, reflecting global plastic demands (Lu et al., 2021). In agreement with this, PP and PE are two of the three most abundant polymer types detected in Kirchgassgraben.

High levels of A/PU/V have previously been reported by studies using the reference database designed by Primpke et al. (2018). In the comparative study by Moses et al. (2023) in which µ-FTIR sample sets are compared after evaluation with siMPle (siMPle_database_Version 1.0.1) and Bayreuth Particle Finder (Hufnagl et al., 2019), siMPle yields significantly higher findings for A/PU/V. The authors of the comparative study neither rule out an underestimation of the A/PU/V findings by Bayreuth Particle Finder nor an overestimation by siMPle due to "missing awareness of specific natural materials causing systematic false positive assignments" (Moses et al., 2023).

To reduce the risk of false positive assignments, the adapted database presented by Roscher et al. (2022), which contains waxy plant cuticles, was used in the evaluation of the samples from Kirchgassgraben.

During the sampling period, PE has a particularly high contribution (16 $\pm$ 4%) on the four days with the highest MP concentrations (D2 to D4 and D8). On the remaining sampling days, PE is not present at all (D5 and D6) or is only present to a small extent (4% on D1 and D7). However, PE alone is not responsible for the high MP concentrations of D2 to D4 and D8, and no pattern can be discerned in the contributions of the other polymers.

3.5 Conclusion

Intensive testing of SAM showed very good results in the form of high recovery rates (overall 77 $\pm$ 29%[1]), low limits of detection[2] (0–397 MPs per m^3 for different polymer types), and plausible MP concentrations (between 1210 and 13052 MPs per m^3) for a riverine surface water body. The eight-day sampling at Kirchgassgraben revealed large variations in MP concentrations by a factor of up to 10.8 between daily mixed samples. These variations could not be explained based on total particle count, precipitation, turbidity, and river discharge data. This confirms two things:

(i) Processes and factors influencing temporal variability of MP concentrations in rivers, for example, catchment hydrology, precipitation events, diffuse and point sources, as well as sedimentation and resuspension require more research (Skalska et al., 2020).

(ii) Samples taken at one point in time (without repetition or continuous sampling) are not suitable for studying MP concentrations and composition in riverine surface

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[1]An incorrect number given in the publication was corrected here.
[2]The publication incorrectly states "limits of quantification".

waters. This is because they disregard temporal variability and are likely to lead to incorrect conclusions about MP fluxes.

In the past, taking several single-point samples at different times on one day (Mintenig et al., 2020), repeatedly collecting manual mixed samples (Barkmann-Metaj et al., 2023) or repeating a single-point sampling daily at the same time over several days (Xia et al., 2020) were the only approaches available to study the variability of MP concentrations and composition. Now, SAM provides a validated, more efficient, and representative alternative by automatically collecting daily mixed samples.

3.6   Acknowledgements

Chapter 4

Article III: Long-term Investigation on the Daily Variability of Microplastic Concentration and Composition—Monitoring in the Effluent of a Wastewater Treatment Plant

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4.1 Abstract

The discharge of treated wastewater into receiving waters by wastewater treatment plants (WWTPs) is considered as one of the main pathways for microplastics (MPs) to enter the environment. To gain a better understanding of the temporal variability of MPs in WWTP effluents, this study investigated the concentration and composition (size, shape and polymer type) of MPs in the effluent of a German WWTP over the course of one month. 24-hour mixed samples were collected daily by a custom-built automated sampling unit for MPs. Particles and fibers ≥ 11 µm were analyzed using Fourier transform infrared microspectroscopy. The MP concentration showed large daily fluctuations

and ranged between 9.64×10^3 m^{-3} and 8.44×10^4 m^{-3} MPs over the study period. However, there was no significant correlation between the MP concentration and the precipitation or discharge from the WWTP. In contrast to the MP concentration, the MP composition in terms of size and shape was consistent over the study period. There were strong correlations between the time series of the polymer types polypropylene, polyethylene, polystyrene, polyester, ethylene-vinyl-acetate, cellulose artificially modified as well as the polymer cluster acrylates/polyurethanes/varnish. The time series of polyamide showed no significant correlation with the time series of any other polymer type. This study established a one month long high-resolution time series of MP concentration and composition, and thus provides a valuable basis for future research on the temporal variability of MP inputs into the environment from WWTP effluents.
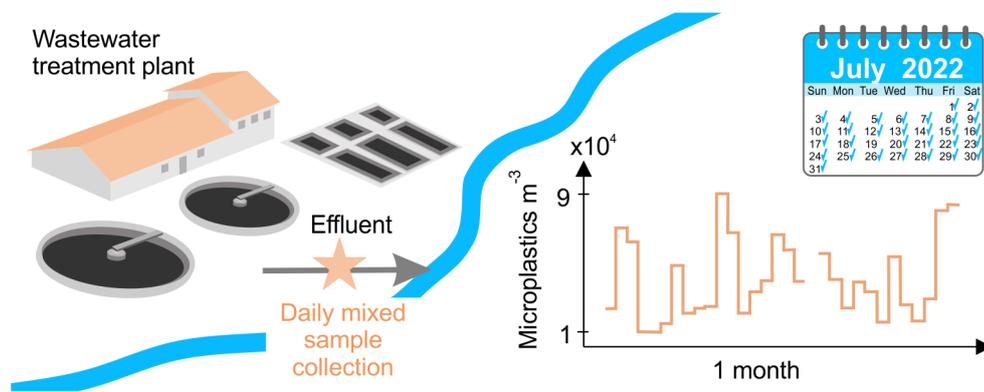
FIGURE 4.1: Graphical abstract of article III.

4.2 Introduction

In the Eurobarometer public opinion survey on the environment, 88% of the participants declared themselves concerned about the environmental impact of microplastics (European Commission, 2022). Microplastics (MPs) are synthetic polymers or highly modified natural polymers in the size range of 1 µm to 1000 µm (Chae et al., 2023; Hartmann et al., 2019). Their impact on ecosystems has not yet been clarified conclusively, although ecotoxicological effects related to the ingestion of MPs, the release of additives by MPs and their accumulation and transport of pollutants are being investigated (Catarino et al., 2021; Pinheiro et al., 2020; Rafa et al., 2024; Yu et al., 2024). MPs can enter the environment through the loss of purposefully produced MPs or by fragmentation of or abrasion from plastic objects that have been introduced into or used in the environment, e.g., plastic litter, road paints, artificial turfs (Galafassi et al., 2019) and agricultural mulch film (Khalid et al., 2023). In the case of inland waters, one of the most relevant pathways for MPs is associated with effluents of wastewater treatment plants (WWTPs).

Wastewater from households, industry and surface runoff entering WWTPs can contain MP concentrations of up to 1.8×10^7 MPs m^{-3} (Gao et al., 2023; Simon et al., 2018). However, due to the generally high removal efficiencies of WWTPs (up to 99% depending on the treatment stages; Monira et al., 2023), MP concentrations in the treated waste

water, which is discharged to receiving waters, are much lower than the concentrations in the WWTP influents. Nevertheless, WWTPs release significant amounts of MPs into the environment because of the large volumes of treated wastewater that are continuously discharged. For instance, Simon et al. (2018) calculated that the total load of MPs released from all Danish WWTP effluents is approximately 3 t per year. Horton et al. (2021) estimated that 7.8×10^{10} MP items are discharged daily from WWTPs in the whole of England and Wales. For the effluent of a single German WWTP, a flux of approximately 4×10^{11} MP items per year, corresponding to annual 91 kg MPs, can be derived from Roscher et al. (2022).

While MP masses are very useful for establishing mass balances and tracing material flows (Rasmussen et al., 2021, 2024), MP item concentrations in combination with information on MP size and polymer type are important for ecotoxicological studies (Triebskorn et al., 2019), for instance to set up environmentally realistic experimental conditions. One of the most common techniques to reliably determine polymer type, number, size, and morphology of MPs down to a size of 10 µm is Fourier transform infrared microspectroscopy (µ-FTIR) (Schymanski et al., 2021). The sample preparation required prior to the application of this and other methods for MP analysis is very labor-intensive and time-consuming (Gao et al., 2023; Okoffo et al., 2019). In addition, until recently, there were no means of automatically collecting samples of MPs over extended periods of time. For these reasons, to the best of our knowledge, no time series of MP concentrations in (treated) wastewater with daily or even higher temporal resolution exceeding a one-week examination period exist to date (Gündoğdu et al., 2018; Kukkola et al., 2024). Instead, MP concentrations in wastewater treatment plant effluents were mostly investigated at individual points in time (single-point sampling) (Magni et al., 2019; Simon et al., 2018), compared between different sampling occasions (Bäuerlein et al., 2023; Egea-Corbacho et al., 2023; Horton et al., 2021; Martín-García et al., 2023; Wolff et al., 2019) or examined on the basis of samples taken in fixed intervals of weeks or months (Akarsu et al., 2020; Ben-David et al., 2021; Egea-Corbacho et al., 2023; Roscher et al., 2022). These previous studies have shown that MP concentrations in WWTP effluents are not constant over time but can fluctuate by up to two orders of magnitude (Roscher et al., 2022). The study by Kukkola et al. (2024), which compared the temporal variability of MP concentrations and loads between hourly, weekly and monthly samplings in a stream fed solely by a WWTP, emphasizes the risk of misinterpretation when generalizing MP data from single-point, weekly or monthly sampling. Kukkola et al. (2024) as well as Bäuerlein et al. (2023) stress the need for future long-term and high-frequency studies on MPs.

The automated sampling unit for MPs recently described by Witzig et al. (2024) makes it possible to investigate the temporal variability of MPs over long periods of time with a high temporal resolution. In the study presented here, the automated sampling unit was applied to establish a continuous MP concentration time series, based on daily 24-hour mixed samples collected over a month from the effluent of a WWTP. The data was used to gain a better understanding of the temporal variability of MP

concentration and composition (size, shape, polymer types) in WWTP effluents. This study also investigated and evaluated i) correlations between the precipitation in the catchment area, the WWTP discharge volume and the MP concentration in the WWTP effluent, ii) correlations between the time series of the individual polymer types and iii) the representativeness of individual mixed samples in WWTP effluents with regard to the concentration and composition of the MPs.

4.3 Material and Methods

4.3.1 Study Site

Samples were collected from the effluent of a municipal WWTP located in the south of Germany, Baden-Württemberg. The WWTP is designed for a population equivalent of 15,000 and receives domestic wastewater, industrial wastewater, and runoff from sealed surfaces. It has a mechanical treatment step (bar screen, grit and sludge removal) followed by phosphorus elimination, intermittent aerobic biological treatment and secondary clarification (Figure 4.2). In 2022, the year of the study, a total of approximately 94,000 m^3 of wastewater passed through the WWTP.
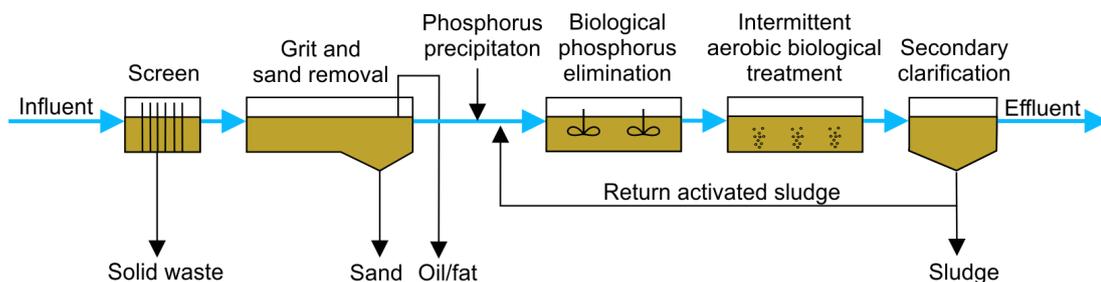
FIGURE 4.2: Schematic of the WWTP and its treatment stages.

4.3.2 Sampling

4.3.2.1 Sampling of Microplastics

For the purpose of monitoring the WWTP effluent, a carry-on cargo trailer converted into a mobile measuring station (M^{2}S), recently presented by Witzig et al. (2024), was installed next to the WWTP outlet shaft, which had a filling level of approximately 1.75 m. The automated sampling unit for MPs (SAM), which is part of M^{2}S, was used to automatically collect daily mixed samples from 85 cm above the bottom of the outlet shaft from the 1st of July to the 1st of August 2022. A brief summary of the general sampling principle of SAM is given below (for a detailed description see Witzig et al., 2024), followed by specifics related to the sampling performed at the WWTP.

At the heart of SAM, there are seven filter cascades (Pittroff et al., 2021) arranged in parallel, separated by stainless-steel electric valves. Each filter cascade corresponds to a daily mixed sample which is collected, by passing a defined, constant volume of sample

medium (e.g., tap water, surface water, WWTP effluent water) through one filter cascade every hour for 24 h, using a peristaltic pump. Every hour, before sample medium is passed through a filter cascade, two rinsing steps are automatically performed. First, the stainless steel suction strainer at the sampling inlet (to retain debris; 1 to 1.8 mm diamond-shaped pores) is flushed for two minutes using a reverse flow of pre-filtered water. Secondly, the polyvinyl chloride (PVC) sampling hose and the stainless-steel tubing are rinsed for two minutes with the sample medium. The volume of water flowing through a filter cascade and the pressure building up in the filter cascade during sampling are continuously monitored. If filter cake formation causes the differential pressure to reach 3.5 bar (trigger value), the sampling of the current mixed sample is prematurely terminated and sampling is resumed with a clean filter cascade (next mixed sample) at the start of the following sampling day.

During the sampling at the WWTP, filter cascades equipped with three successive stainless-steel filters with the mesh sizes 100 µm, 50 µm and 10 µm (Rolf Körner GmbH, Germany) were used. The filter cascades filled with a mixed sample were regularly exchanged for sample-free ones via GEKA quick couplings (Karasto Armaturenfabrik Oehler GmbH, Germany). Each filter cascade containing a mixed sample was sealed with GEKA end couplings, transported to the laboratory and stored at 8 °C until further processing.

For practical reasons, each sampling day at the WWTP ran from 11 AM on one day to 11 AM on the next day. In the following, all mixed samples are referred to by the date on which the respective sampling was started. On eleven sampling days, the sampling was prematurely terminated because the pressure trigger value was reached. In addition, technical problems caused an early termination of sampling on one day, a delayed start of sampling on two[1] sampling days, and no sampling on one day. The sampling duration of the mixed samples can be derived from Figure 4.3a. The targeted sampling volume for each mixed sample was set to 1.4 L each hour for 24 h. However, due to the closing time of the motorized valves, the hourly target volumes were generally slightly exceeded. The mean daily volume sampled was 41 L, not counting the day without sampling (Figure 4.3b).

4.3.2.2 Acquisition of Accompanying Parameters

The operators of the WWTP provided two-hourly means of conductivity, pH, temperature, discharge velocity and discharged volume, monitored in the WWTP effluent. The daily mean conductivity in the WWTP effluent ranged between 0.7 and 1.5 µS cm^{-1} while the mean daily pH was between 7.2 and 7.6. The mean daily water temperature was in the range between 20.8 °C and 23.7 °C. All three parameters showed a rising trend over the month July.

Total particle number and turbidity were monitored in the treated waste water using a particle sensor (LDS 45/50, Markus Klotz GmbH, Germany) and a turbidity sensor (i::scan Y05-3-r-075, s::can GmbH, Austria). Unfortunately, the data could not be

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[1]The publication incorrectly states three sampling days with delayed start of sampling.

compared with the MP concentration monitored in the WWTP effluent, as was initially planned, due to a sensor error (jumps in the measured turbidity values) and massive data gaps (total particle count).

Hourly precipitation data was acquired from a weather station (Agrarmeteorologie Baden-Württemberg, n.d.) located approximately 1.4 km north of the WWTP.
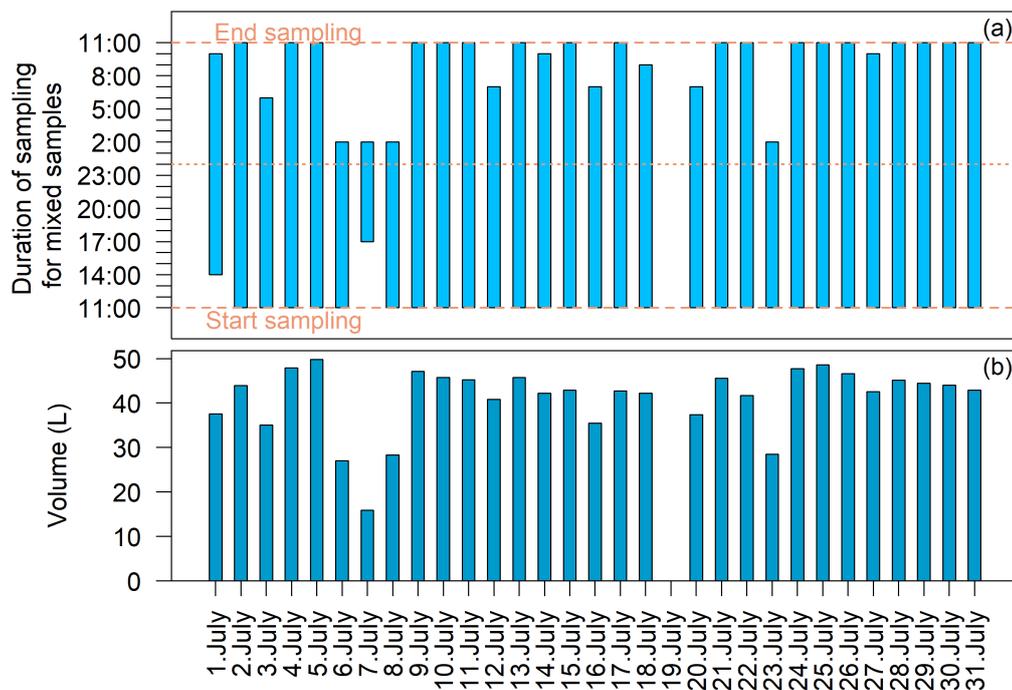

FIGURE 4.3: (a) Timespan represented by the mixed samples on the different sampling days and (b) water volume sampled from the effluent of the WWTP.

4.3.3 Sample Processing

Sample processing was performed in accordance with the validated method described by Witzig et al. (2024) and is briefly summarized here: The particles accumulated on the three stainless-steel filters of each filter cascade were extracted (Elmasonic P 180 H, 100% intensity, 37 kHz; Elma Schmidbauer GmbH, Germany) and re-fractionated, using a filtration tower equipped with three stainless-steel filter discs (48 mm diameter; Rolf Körner GmbH, Germany) matching the mesh sizes used in the filter cascade. The three size fractions per mixed sample were then treated with ozone-rich water (approx. 3.2 mg L^{-1}) for one night at 21 °C. In contrast to the description by Witzig et al. (2024), the mixed samples were not immediately processed further after the ozone treatment, but were instead stored at 8 °C until the end of the sampling campaign at the WWTP. This was necessary to enable rapid extraction of the samples and preparation of the filter cascades (cleaning, drying, equipping with new stainless-steel filters) in time for the next filter cascade exchange in SAM.

After completion of the sampling campaign at the WWTP, the stored samples (each consisting of three size fractions) were treated with Fenton reagent under controlled temperature conditions (for details see Witzig et al., 2024). Subsequently, particles and fibers $\geq$ 250 μm were separated from the size fraction $\geq$ 100 μm by filtration onto a 250 μm stainless-steel filter disc (48 mm diameter; Rolf Körner GmbH, Germany), which was then analyzed.

The size fractions $<$ 250 to $\geq$ 100 μm, $<$ 100 to $\geq$ 50 μm and $<$ 50 to $\geq$ 10 μm were subjected to density separation, using sodium polytungstate solution (2.25 g cm^{-3}; TC-Tungsten Compounds GmbH, Germany) as the separatory medium and a centrifuge (742g centrifugal acceleration, Sigma 8KS, SIGMA Laborzentrifugen GmbH, Germany) to speed up the separation process. The size fractions were each accumulated on up to four silicon filters with a pore diameter of 10 μm (square membranes with 1 cm edge length, SmartMembranes GmbH, Germany) for subsequent analysis. It was not possible to filter the entire corresponding sample volume through silicon filters for the smallest size fraction ($<$ 50 to $\geq$ 10 μm). Instead, up to four aliquots were taken, which together on average accounted for 36% of the sample volume of the smallest size fraction per sample.

4.3.4 Chemical Identification of Microplastics

Analysis of particles and fibers was performed using a μ-FTIR system (Agilent Technologies Inc., USA) equipped with a Cary 670 IR spectroscope, a Cary 620 FTIR microscope and a 128 × 128 mercury cadmium telluride focal plane array detector. Spectra were acquired in the range 3800–900 cm^{-1} at 4 cm^{-1} resolution with triangular apodization and 15× magnification (Cassegrain objective). Subsequent spectra comparison was carried out using the software siMPle (version 1.1.ß) by Primpke et al. (2020b) and the spectra database by Roscher et al. (2022).

4.3.4.1 Microplastics $<$ 250 μm

All silicon filters containing particles and fibers $<$ 250 μm were measured in imaging transmission mode with 30 co-added scans. Prior to each measurement, background collection was performed on a clean silicon filter with 120 co-added background scans. Pixel resolution was at 5.5 μm. For subsequent image analysis, MPAPP pipeline (MPAPP v1.1.1) by Primpke et al. (2019) was applied. The thresholds applied during the automated image analysis were previously determined based on the manual evaluation of spectra from a subset of the data.

4.3.4.2 Microplastics $\geq$ 250 μm

Particles and fibers $\geq$ 250 μm were placed on a gold-coated microscope slide. Measurement was performed in single point reflection mode with 30 co-added scans immediately followed by 30 co-added background scans on an empty spot of the gold-coated microscope slide. In general, all particles and fibers $\geq$ 250 μm were measured with the

exception of black particles and fibers. Experience had shown that black particles and fibers ≥ 250 µm collected with SAM are mostly abrasion from the natural rubber tube of the peristaltic pump. For practical reasons, only 10% of the black particles and fibers (and where possible not less than three black items) were randomly selected and measured.

During the single spectra database comparison, the following two acceptance criteria were applied. Firstly, a hit quality index of $\geq 60\%$ had to be achieved and secondly, the match had to be manually verified.

4.3.5 Quality Assurance and Quality Control

4.3.5.1 Mitigation of Cross-Contamination

To reduce cross-contamination of samples, the same protocol as presented by Witzig et al. (2024) was followed. This includes working in laminar flow cabinets (HEPA 14; Thulab GmbH, Germany), using ultrapure water (18.2 MΩ cm, Arium 611 UF; Sartorius AG, Germany), avoiding plastic tools, wearing 100% cotton laboratory coats and adhering to strict rules for cleaning of hands and utensils (including filter cascades). All liquids applied during sample processing were previously filtered through a 1 µm glass microfiber filter. Furthermore, to prevent cross-contamination, SAM was thoroughly cleaned prior to the sampling at the WWTP (for details see Chapter 3, Section 3.3.5.1).

Polyoxymethylene and rubber were excluded from all results as they are installed in SAM and are known to cause cross-contamination within this setup (Witzig et al., 2024). Cross-contamination of the samples with PVC due to the use of a PVC hose during sampling with SAM is very unlikely, as the according process blank samples investigated by Witzig et al. (2024) did not show any PVC cross-contamination (see also Section 4.3.5.3).

4.3.5.2 Laboratory Blank Samples

Each week during the sampling campaign at the WWTP (n = 5; including one partial week), one of the seven filter cascades installed in SAM was replaced prior to sampling. This sample-free filter cascade underwent the same sample processing and analysis as the corresponding filter cascades containing mixed samples.

The five sample-free filter cascades served as laboratory blank samples, which allowed to monitor the cross-contamination originating from processes in the laboratory and from the installation and dismantling of filter cascades in the field.

The level of cross-contamination in the five laboratory blank samples was very low, with a mean of 30 ± 2 MPs per laboratory blank sample and a mean of < 10 MPs per polymer type. As the MP concentrations determined in the mixed samples of the WWTP effluent are much higher than the mean laboratory blank (factor > 300), cross-contamination from the laboratory is considered negligible.

4.3.5.3 Validation and Limit of Detection

The method validation and limit of detection (LOD = mean process blank value $+ 3 \times$ standard deviation) for particles and fibers presented by Witzig et al. (2024), is applicable to the samples of this study, because the same sampling apparatus as well as method for sampling, sample preparation and analysis was used. Polymer numbers $<$ LOD were not included in the results presented in Section 4.4.

4.4 Results and Discussion

4.4.1 Microplastic Concentration Range

The mean MP concentration across all 30 mixed samples was $3.85{\times}10^4$ $\pm$ $2.12{\times}10^4$ MP m^{-3}. The lowest and highest MP concentrations determined were $9.64{\times}10^3$ MP m^{-3} (5th of July) and $8.44{\times}10^4$ MP m^{-3} (11th of July), respectively (Figures 4.4 and 4.5c). The size, shape and polymer type of the MPs is discussed in Section 4.4.3.

Figure 4.4 compares the range of MP concentrations observed in this study to other previous studies that have investigated European WWTP effluents using spectroscopic methods for polymer detection and an equivalent lower cut-off size, meaning 10 µm or 11 µm was the smallest MPs size investigated. The concentration range spanned by the studies in Figure 4.4 extends from a minimum concentration of 88 MP m^{-3} (Roscher et al., 2022) to a maximum concentration of $1.1{\times}10^6$ MP m^{-3} (Al-Azzawi et al., 2022a). The highest concentration reported by Al-Azzawi et al. (2022a) was associated with a disturbance in WWTP operation concerning the flow feeding of the sand filters and therefore may not be representative of WWTPs under normal conditions. However, disturbances in WWTP operation are among the many factors driving the temporal variability of MP concentrations in WWTP effluents, which is why the corresponding concentration was not excluded from the overview in Figure 4.4. Even if the study by Al-Azzawi et al. (2022a) is disregarded, the MP concentrations observed in the study here are clearly within the concentration range to be expected for European WWTP effluents when considering MPs down to a size of approximately 10 µm (see Figure 4.4).

Although the studies in Figure 4.4 were chosen for their high comparability, they differ from each other in detail. For example, due to limited standardization, the methods used for sampling and sample preparation, as well as the settings applied during analysis, vary between the studies. Furthermore, the studies all investigated different WWTPs (one or more per study), which inherently introduces variables, for example in relation to the treatment processes and the origin of the wastewater. In this regard, it is important to note that the concentration of MPs in WWTP effluent is not constant over time within one WWTP, but is subject to fluctuations. For example, Roscher et al. (2022) and Akarsu et al. (2020) each investigated the MP concentration in the effluents of multiple WWTPs over a one-year period through a monthly sampling regime. The highest and lowest measured concentrations within each WWTP differed by factors of

approximately 87 and 315, respectively, for the two WWTP investigated by Roscher et al. (2022) and by factors of 17, six, and five, respectively, for the three WWTPs studied by Akarsu et al. (2020). Likewise, in this study, where mixed samples were taken daily from a WWTP effluent over a period of one month, the variation between the lowest and highest measured MP concentration is a factor of nine and high day-to-day variability is evident (see Figure 4.5c). Indeed, based on the temporal variability of the MP concentrations in the WWTP effluent observed in this study, at least eleven daily mixed samples are required in order to obtain a mean MP concentration that lies within the standard deviation of the "actual" mean MP concentration (derived from all 30 mixed samples) with a probability of > 50%.

This result demonstrates, that even when performing mixed sample collection, individual samples or low sample numbers are not suitable to determine representative MP concentrations in WWTP effluents. Single-point samples (individual samples taken at a specific point in time) are even less representative than individual mixed samples (Witzig et al., 2024). However, only few studies focus on the temporal variability of MPs and due to the high time expenditure associated with each MP sample, low samples numbers are common (e.g., Al-Azzawi et al. (2022a), Simon et al. (2018), Leslie et al. (2017), Simon et al. (2019) and Wolff et al. (2021)). It seems that the differences in MP concentration ranges shown in Figure 4.4 are therefore most likely influenced by the timing of sampling—or rather the temporal variability of MP concentrations.
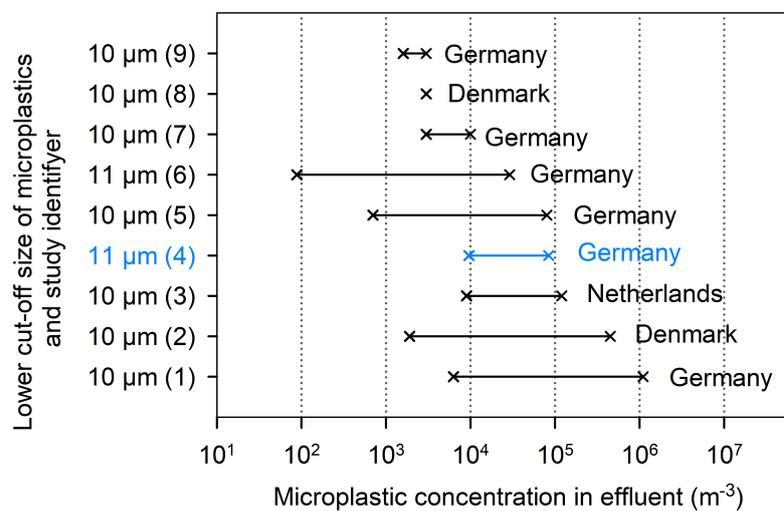
FIGURE 4.4: Microplastic concentrations determined in the effluents of European wastewater treatment plants by studies investigating a similar size range as this study (highlighted in blue) and applying spectroscopic methods. In the following, the studies are indicated by the identifier in brackets, the reference and the analytical method: (1) Al-Azzawi et al. (2022a), μ-FTIR/μ-Raman; (2) Simon et al. (2018), μ-FTIR; (3) Leslie et al. (2017), microscopy/μ-FTIR; (4) this study, μ-FTIR; (5) Barkmann-Metaj et al. (2023), μ-Raman; (6) Roscher et al. (2022), μ-FTIR; (7) Wolff et al. (2019), μ-Raman; (8) Simon et al. (2019), μ-FTIR; (9) Wolff et al. (2021), μ-Raman.

4.4.2 Relationship Between Precipitation, Discharge and Microplastic Concentration

Precipitation data from the weather station in the vicinity of the WWTP, the WWTP's discharge volume as well as the MP concentration determined in the WWTP effluent are shown in Figure 4.5 for the MP sampling period. As it rained on the day prior to the start of MP sampling, precipitation data and discharge volume are additionally shown for the 29th and 30th of June. To allow direct comparison of MP concentrations with precipitation sums and discharge volumes, daily data in Figure 4.5 uniformly refers to the timespan between 11 AM on one day to 11 AM on the next day (see Section 4.3.2.1).
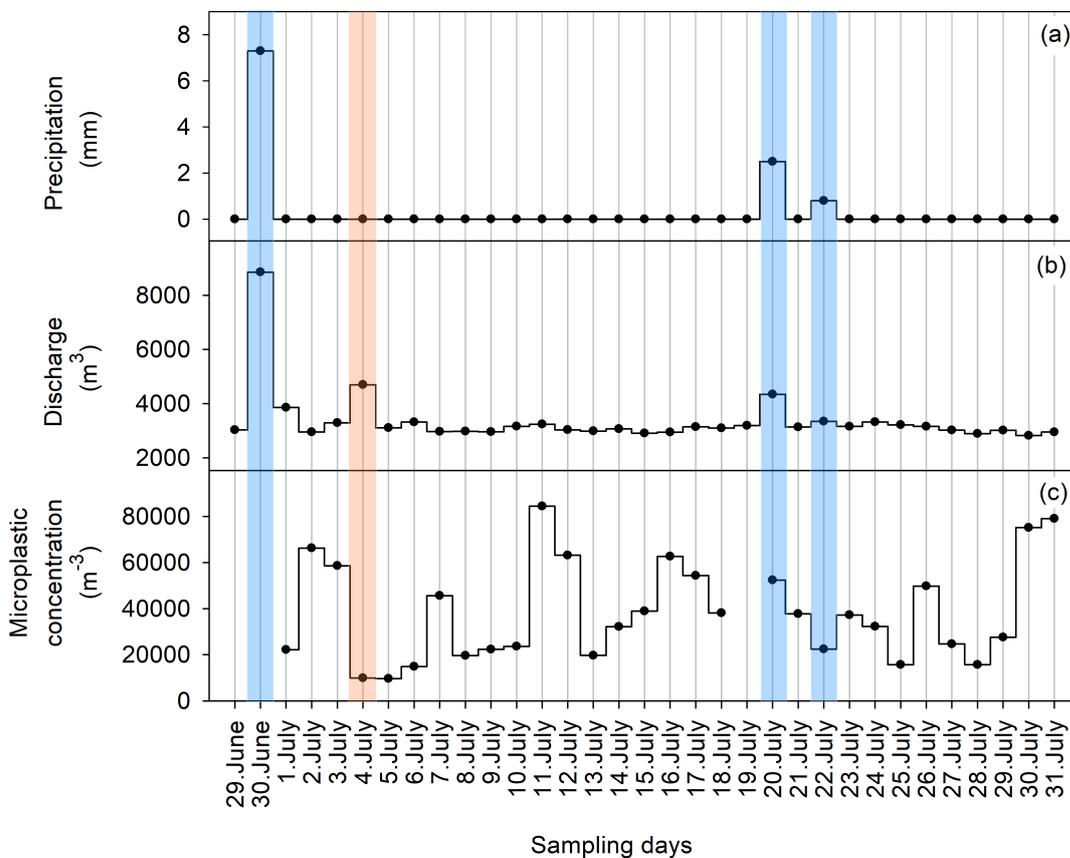
FIGURE 4.5: Daily precipitation sums (a), daily discharge volume from the WWTP (b) as well as microplastic concentration in daily mixed samples from the WWTP effluent (c). Blue areas highlight days with precipitation. Orange areas highlight days without precipitation but an elevated discharge volume.

As evident from Figure 4.5a, the month of July 2022 was very dry in the region of the WWTP and there were only two days with light rain, the 20th of July and the 22nd of July (2.5 mm and 0.8 mm, respectively). On the 30th of June, the day before sampling started, there was 7.4 mm of rainfall.

The discharge volume showed little variability in July 2022 and a mean of 3204 m^3 per day (corresponding to a mean discharge rate of 37.1 L s^{-1}) were discharged via the WWTP effluent. It is noteworthy that higher discharge volumes were recorded on all

days with precipitation (e.g., 30th of June and 20th of July). The exception being the 22nd of July, on which there was very little rain and no clear increase in the discharge volume was observed.

To investigate the dependency between precipitation and discharge volume, discharge data on a two-hourly basis provided by the WWTP was cross-correlated with two-hourly precipitation sums from the weather station, testing different lag times between rainfall and effluent discharge. Due to the low number of precipitation events in July 2022, this was performed for an extended time period from the 1st of June to the 31st of August (data not shown). A positive correlation between precipitation and discharge volume was determined and it was the highest for a lag of two to four hours (correlation coefficient 0.46, $p < 0.001$). This is surprising, as the residence time of the water in the WWTP was indicated to be between 3.5 and six days by the WWTP's operators. However, the determined time lag of only a few hours between precipitation and an increase in the discharged volume is in accordance with the uniform behavior of daily precipitation and discharge volumes in Figure 4.5a and b.

On the 4th of July there was an increased discharge, although no precipitation was recorded. Upon enquiry, the WWTP operators stated that the rainwater overflow basin may have been cleaned on that day.

The MP concentrations in Figure 4.5c show a high degree of temporal variability. A significant correlation between the MP concentration and the precipitation or the WWTP discharge volume could not be determined (Spearman's rank correlation; $p > 0.05$, correlation coefficient 0.02 and -0.24, respectively), although either a dilution effect or a concentrating effect were expected. In principle, a dilution effect can occur, if the MP concentration of the surface runoff is lower than that of the sewage water. This effect has been observed, for example, by Martín-García et al. (2023), who reported low MP concentrations in the influent (but not in the effluent) of a WWTP in context with heavy rainfall. By contrast, a concentrating effect may occur if the surface runoff has a higher MP concentration than that of the sewage water. Elevated MP concentrations in WWTPs (influent or effluent) related to rainfall events or the rainy season have been reported by e.g., Akarsu et al. (2020), Roscher et al. (2022) and Ben-David et al. (2021). Roscher et al. (2022) furthermore theorized that during heavy precipitation, the clearance rate of WWTPs could be reduced due to shorter pre-clearance, higher turbulence and shorter residence times, resulting in the discharge of higher MP concentrations from the WWTP.

The concentrating effect, the dilution effect and the reduced clearance effect could not be observed in the effluent of the WWTP monitored in this study on the days with precipitation. These effects are probably only apparent during events with high precipitation intensity or prolonged precipitation, both of which were absent during the study period (see Figure 4.5a). Interestingly, however, the second-lowest MP concentration of the entire study period was recorded on the 4th of July, the day on which, according to the WWTP operator, the stormwater overflow basins may have been flushed and

elevated discharge volumes were observed (see Figure 4.5b–c). The lowest MP concentration was recorded on the following day. Thus, it is possible that the low MP concentrations on the 4th and 5th of July in the WWTP effluent are the result of the dilution effect caused by cleaning of the stormwater overflow basins with tap water, which has very low MP concentrations (Adediran et al., 2024; Kirstein et al., 2021; Pittroff et al., 2021; Weber et al., 2021).

As the study period only covered one month, no general statement can be made about how much MP is discharged from the WWTP into the environment. However, based on the mean daily water volume discharged from the WWTP (3204 m^3 d^{-1}) and the mean daily MP concentration detected in the WWTP effluent (see Section 4.4.1), approximately 1.2×10^8 MPs were released from the WWTP every day during the study period (equivalent to 8×10^3 MPs cap^{-1} d^{-1}). This is in accordance with other studies that have concluded that WWTPs are a constant point source of MPs. Despite mostly high retention efficiencies of WWTPs (depending on the treatment stages), the large volumes of treated wastewater discharged from WWTPs daily result in high numbers of MPs being released into surface waters (Gao et al., 2023; Komorowska-Kaufman and Marciniak, 2024; Kukkola et al., 2024; Roscher et al., 2022).

4.4.3 Composition of Microplastics

4.4.3.1 Composition by Size

The MP samples from the WWTP effluent are very homogeneous in terms of size. 95 $\pm$ 2% of the MPs are in the size range < 100 μm and 5 $\pm$ 2% are in the size range ≥ 100 μm (see Figure 4.6a). Similar size distributions have been found in the effluents of other German WWTPs. For example, Roscher et al. (2022) reported that 93 $\pm$ 15% and 93 $\pm$ 24% of the MPs in two German WWTPs were in the size range ≥ 11 to < 100 μm, respectively, and Wolff et al. (2019) presented data for another German WWTP where 76 $\pm$ 7% of the MPs were in the size range ≥ 10 to < 100 μm. Wolff et al. (2019) further differentiated between particles and fibers and reported that approximately 95% of the MPs particles and 10% of the MP fibers were in the size fraction ≥ 10 to < 100 μm. While the findings of the study presented here match well with those by Wolff et al. (2019) for MP particles (98 $\pm$ 1% < 100 μm), a much higher proportion of MP fibers in the size range < 100 μm (83 $\pm$ 4%) was observed in this study. However, as larger MPs are more efficiently removed during the treatment process than smaller MPs (Okoffo et al., 2019; Talvitie et al., 2017), the results presented here, where both fibers and particles in the WWTP effluent are mostly in the size range < 100 μm, are plausible. More small fibers may have been detected in this study than by Wolff et al. (2019) because the automated particle/fiber analysis pipeline MPAPP (Primpke et al., 2019) was applied in this study.

4.4.3.2 Composition by Shape

As visualized in Figure 4.6b, the majority of the MPs detected in the WWTP effluent in July 2022 were particles (mean of 82 $\pm$ 2%), while fibers were much less abundant (mean

of 18 ± 2%). This is, for example, in accordance with Wolff et al. (2021) who observed more particles than fibers in the effluents of two municipal WWTPs and one industrial WWTP. In the study by Kukkola et al. (2024), where MPs were investigated in a stream fed solely by a WWTP, roughly ³/₄ of all MPs were particles. Roscher et al. (2022) also reported a dominance of particles over fibers in the effluent of two WWTPs but stated that this was in contrast with previous studies, which reported a dominance of fibers over particles in WWTP effluents. According to Roscher et al. (2022), this discrepancy between studies may be due to differing treatment processes in WWTPs or to the use of methodologically different approaches.
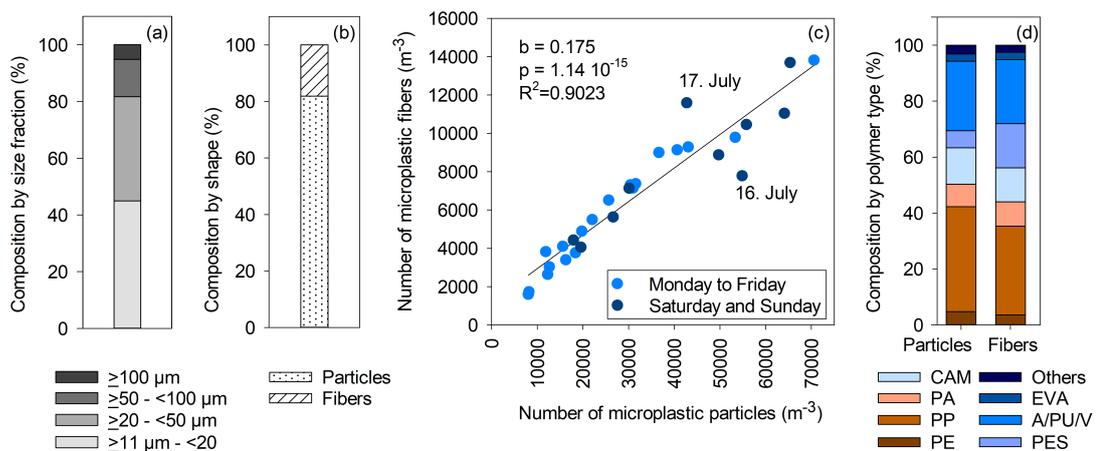
FIGURE 4.6: Composition of the microplastics in the effluent of the WWTP. Mean composition of the microplastics (a) by size fraction and (b) with distinction between particles and fibers. (c) Relationship between the number of microplastic fibers and the number of microplastic particles and (d) mean composition of the microplastic particles and the microplastic fibers by polymer type.

If differences in methodological approaches were a main reason for discrepancies between studies, similar methodological approaches should render similar results. Indeed, the results presented by Roscher et al. (2022) match strikingly well with those presented in this study, not only in terms of the ratio of particles to fibers but also in terms of the size distribution of the MPs (Section 4.4.3.1) and the most dominant polymer types present in the samples (Section 4.4.3.3). The study by Roscher et al. (2022) and this study investigated different WWTPs but used the same sampling principle (closed setup, water is pumped through stainless-steel filters), the same analytical method (μ-FTIR and application of siMPle and MPAPP Pipeline) and both investigated MPs down to 11 μm. Thus, the good agreement between the two studies may partially be due to methodological similarities.

Okoffo et al. (2019) suggested that more fibers could enter WWTPs at weekends than on weekdays because more laundry is washed at weekends. However, as shown in Figure 4.6c, there was no clear increase in MP fibers compared to MP particles in the WWTP effluent at weekends. Taking into account the retention time of 3.5 to six days at the WWTP (see Section 4.4.2), the shift in the ratio between fibers and particles would

be expected to occur with a corresponding delay. However, no systematic shift could be detected for the other days of the week either. This could be due to the fact that human behavior in the WWTP catchment area on weekends during the study period did not lead to an increased fiber input relative to particle input, or that the effect was offset by the treatment of the wastewater, so that it was no longer detectable in the WWTP effluent.

The largest shift in the ratio between fibers and particles visible in Figure 4.6c was observed on the 16th of July (towards more particles) and on the 17th of July (towards more fibers). The cause of these shifts is not apparent, although comparatively high MP concentrations were detected on both days (Figure 4.5c). Indeed, Figure 4.6c reveals that high concentrations of MPs (both particles and fibers) in the WWTP effluent occurred disproportionately often at weekends. If the residence time of the wastewater in the WWTP is in the range of hours rather than days, as indicated by the cross-correlation between precipitation and discharge (see Section 4.4.2), then the elevated concentrations in the WWTP effluent at weekends may be due to increased household and recreational activities.

4.4.3.3 Composition by Polymer Type

Assessing particles and fibers together, the most abundant polymer types detected in the treated wastewater during the study period were polypropylene (PP, 36%), the polymer cluster acrylates/polyurethanes/varnish (A/PU/V, 27%) and cellulose artificially modified (CAM, 12%), followed by polyester (PES, 8%), polyamide (PA, 7%), polyethylene (PE, 5%) and ethylene-vinyl-acetate (EVA, 3%).

While the composition of wastewater by polymer type can vary greatly between WWTPs, PP, PES, PA and PE have commonly been reported by wastewater studies (Gao et al., 2023; Sun et al., 2019) and their presence in the WWTP effluent of this study meets expectations. A/PU/V (or individual components from this polymer cluster) and CAM (e.g., cellulose acetate), have also previously been reported in wastewater (Akarsu et al., 2020; Becucci et al., 2022; Egea-Corbacho et al., 2023; Roscher et al., 2022; Simon et al., 2018). However, previous studies have found CAM to account for only a small proportion of MPs, usually < 10%. The slightly higher percentage of CAM in the WWTP effluent of this study may be due to the use of CAM by regional industry or to the rapidly increasing worldwide production (and use) of bio-based and bioattributed plastics over the past few years, which is particularly pronounced in Europe (Plastics Europe, 2023).

As shown in Figure 4.6d, there are only small differences (mostly two or fewer percent) between the composition of MP particles and fibers by polymer type, with the exception of the polymers PP and PES. There were 10% more fibers consisting of PES than particles (Figure 4.6d). This may be related to the fact that PES is commonly used in synthetic clothing and that high numbers of PES fibers may enter the sewage system in consequence of the washing of clothes (Gao et al., 2023; Napper and Thompson, 2016). In contrast, there were 6% more particles consisting of PP than fibers (Figure 4.6d), indicating the predominant use of PP as film or ridged material (which fragment into particles)

rather than as fabric within the catchment area of the WWTP (Figure 4.6d). However, drawing conclusions about the sources of MPs based on data from the WWTP effluent is hampered by the fact that wastewater treatment generally modifies both the abundance and composition (in terms of shape, size, and/or polymer type) of the MPs (Akarsu et al., 2020; Egea-Corbacho et al., 2023; Magni et al., 2019; Talvitie et al., 2017). For this reason, the concentration and composition of MPs in the WWTP effluent is more suitable for obtaining an overview of the MP inputs into surface waters than for drawing conclusions about the sources of MPs.

4.4.4 Analysis of Polymer Concentration Time Series

In order to identify the relationships between the concentration time series of the main polymer types, which were present in the WWTP effluent on $\geq 90\%$ of all sampling days, a principal component analysis (PCA) was performed. 77% of the variance in the polymer concentration time series is explained by the first two principal components (PC 1 and PC 2). The according score plot and loading plot are presented in Figure 4.7. PC 1 is positively correlated with all polymer types (see Figure 4.7a) and represents the total MP concentration while PC 2 has a high, positive correlation (0.8) with the polymer type PA and a negative correlation (-0.5) with the polymer type CAM (see Figure 4.7a).
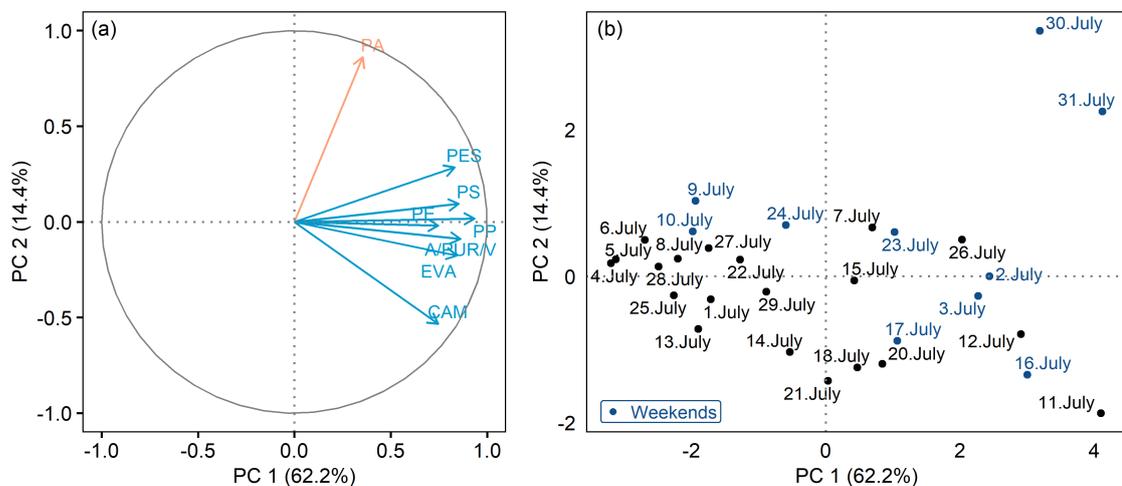
FIGURE 4.7: Results of principal component analysis performed on the concentration time series of the different polymer types in the wastewater treatment plant effluent in July 2022 displayed as (a) score plot and (b) loading plot for the first two principal components.

The PCA indicates, that there is a high correlation between the MP concentrations of the polymer types PP, PE, PS, PES, A/PU/V, EVA and CAM (Figure 4.7a). This was confirmed by Spearman's Rank correlation ($p < 0.05$). Only PA appears to behave differently and is negatively correlated with CAM. This negative correlation is, however, not significant (Spearman's Rank correlation, $p > 0.05$, correlation coefficient -0.12) and

may therefore be incidental. In fact, the concentration time series of PA is not significantly correlated with that of any other main polymer type. This raises two questions: i) why do the concentration time series of the main polymer types behave so uniformly, and ii) why does the concentration time series of PA deviate?

The former is presumably related to the fact that MPs enter wastewater through numerous, unrelated inputs (so-called diffuse inputs) resulting from various human activities (e.g., personal and household cleaning and washing, industry, construction, etc.). On consecutive days, similar general conditions (air temperature, hours of daylight, etc.) and similar activities can be expected in the catchment area of the WWTP, resulting in the input of similar polymer types into the wastewater. This, together with the applied sampling method, which averages short-term variations in polymer composition over up to 24 h (see Section 4.3.2.1), may explain the observed uniform behavior of the time series of the main polymer types.

This does not contradict the observed high temporal variability of the MP concentration. If the changes in MP concentration are due to increased or decreased (and not entirely different) activities in the catchment, they do not necessarily have to be accompanied by significant changes in polymer composition. Kukkola et al. (2024), who used a multiple-time scale sampling approach in a solely WWTP-fed stream, also observed a high temporal variability of MP concentration coupled with a temporally consistent MP composition (polymer type, shape and size).

The differing behavior of PA can have various reasons. One possibility would be the accidental, systematic cross-contamination of the WWTP effluent samples with PA during sampling or sample processing. However, cross-contamination during sampling with SAM is unlikely, as it is a closed system and the LOD, which is based on process blank values including sampling, was taken into account (see Section 4.3.5.3). Cross-contamination during sample processing is also unlikely, as the weekly laboratory blank samples, which were processed together with the samples from the WWTP effluent, are very low for all polymer types including PA (see Section 4.3.5.2). Another possibility is the entry of PA in the context of WWTP operation. Upon enquiry, the operators checked the processes and substances used (e.g., precipitants) in the WWTP, but were unable to identify any source of PA. Interference from natural PA cannot be completely ruled out, even though, to the best of our knowledge, there have been no reports to date of misidentification of natural PA as synthetic PA when using µ-FTIR and the software siMPle. An alternative explanation is that PA may have a dominant source in the catchment area of the wastewater treatment plant in addition to diffuse sources. For example, local industry could contribute a correspondingly high PA input with its own temporal variability, which differs from that of the other polymers. Further research would be needed to reach a final conclusion.

In the loading plot[2] (Figure 4.7b), sampling days with similar MP concentration and

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[2]In this paragraph, the publication incorrectly refers to the "score plot", although it is actually the "loading plot".

composition are clustered together. Sampling days with comparably high MP concentrations of PP, PE, PS, PES, A/PU/V, EVA and CAM are located on the far right of Figure 4.7b, while sampling days with comparably low MP concentrations of PP, PE, PS, PES, A/PU/V, EVA and CAM are located on the far left (compare Figures 4.5c and 4.7b). Similarly, sampling days with comparably high PA and/or low CAM concentrations are located in the upper part of the loading plot (Figure 4.7b), while sampling days with comparably low PA and/or high CAM concentrations are located in the lower part of the loading plot. As can be deduced from Figure 4.7, the polymer composition on weekends does not differ greatly from that on other days of the week, despite the observed tendency towards higher MP concentrations on weekends (Section 4.4.3.2). Only the 30th and 31st of July are situated some distance away from all the other sampling days in the loading plot. The MP concentration on these two days is high, but of the same order of magnitude as on other sampling days, such as the 2nd, 11th, 12th, and 16th of July (see Figures 4.5c and 4.7b). However, the PA concentration on the 30th and 31st of July is comparatively high, while the CAM concentration is low, which is why these two days are isolated in the loading plot. The remaining sampling days form a cluster, with sampling days that are temporally close to each other showing a tendency to appear close to each other in the loading plot. This is consistent with the expectation that, despite the observed high temporal variability, similar concentrations and compositions of MPs are more likely to be found on consecutive sampling days than on sampling days that are far apart in time.

4.5   Conclusions

SAM has proven to be suitable for sampling MPs in the effluent of a WWTP. Samples were collected semi-continuously over a period of one month on a daily basis using mixed samples. Despite fairly constant general conditions (e.g., dry weather), the concentration of MPs showed strong day-to-day fluctuations and ranged between 9.64×10^3 m^{-3} and 8.44×10^4 m^{-3} MPs over the study period. There was no significant correlation between the MP concentration and the precipitation or discharge from the WWTP. The observed high temporal variability of the MP concentration between the daily mixed samples allows the conclusion, that individual or low numbers of mixed samples (as well as single-point samples) taken in WWTP effluents are unsuited for the determination of representative MP concentrations. Instead, continuous or a regular collection of mixed samples is required to provide representative MP concentrations, but this is a significant gap within MP research.

   The MP composition, in terms of size, shape and polymer type, was consistent across the investigated 30 mixed samples. It appears that the MP composition in the WWTP effluent is much less dynamic over time than the MP concentration. Therefore, individual mixed samples may be appropriate for making a rudimentary estimate on the MP composition in a WWTP effluent.

Analysis of the concentration time series of the polymer types PP, PE, PS, PES, EVA, the polymer cluster A/PU/V, CAM and PA showed a high correlation between the time series of all these polymer types, except for PA. It also revealed, that despite high temporal variability, the concentration and composition of MPs in the WWTP effluent tends to be more alike between sampling days that are temporally close to each other (e.g., consecutive sampling days) than between sampling days that are temporally further apart. These finding provide a good basis for further in-depth research into the underlying relationships.

By applying the automated sampling device SAM, this study has proven that it is possible to collect a high-resolution MP time series over a significant duration and that such time series can reveal relationships that remain hidden when only investigating individual, temporally unrelated MP data. MP time series have great potential for expanding our current knowledge on MPs and this study is a first step towards this goal.

4.6 Acknowledgements

Chapter 5

Synthesis

5.1 Summary and Overall Discussion

Due to the continuous production and use of plastics, MPs accumulate in the environment. Numerous studies have already been carried out to determine the quantity and composition of MPs in various environmental compartments and it has become apparent, that the concentrations determined by different studies often differ by several orders of magnitude, even for the same matrix (Koelmans et al., 2019; Lu et al., 2021). These differences may be caused by individual or a combination of the following factors:

- spatial variability

- temporal variability

- limited standardization of methods for sampling, sample processing and analysis

- inadequate QA/QC

For a realistic evaluation of the global pollution of the environment with MPs and for a meaningful assessment of the ecotoxicological risks associated with MPs, reliable and representative data on MP concentrations and compositions is required for all environmental compartments. Insufficient QA/QC can lead to over- or underestimation of MPs in environmental samples and the concentration and composition of MPs obtained based on single-point samples from aqueous matrices may not be representative over long periods of time. Therefore, the focus of this thesis is on the factors of "insufficient QA/QC" and "temporal variability" of the concentration and composition of MPs, considering in particular the matrices streams and WWTP effluents.

The following paragraphs summarize the main contents and findings of the research work and describe how the four objectives defined in Chapter 1.5.1 were achieved.

5.1.1 1st Objective: Identification and Elimination of Cross-Contamination of Samples due to Widespread and Unquestioned Practices in the Laboratory

In Chapter 2, cross-contamination and false positive MP identification in consequence to the use of SDS and disposable gloves in the laboratory were investigated. SDS is an anionic surfactant that is commonly applied during sample processing, as it aids the

decomposition of animal and plant residues and increases the contact surface for subsequent treatment steps (Löder et al., 2017). Disposable gloves, in turn, are frequently worn during sample handling for personal protection or in order to prevent cross-contamination. However, as presented in Chapter 2, SDS and stearates, which are commonly applied as mold-release agent during the production of disposable gloves (Chia and Mohd Sukri, 2019), may false positively be identified as PE.

Firstly, in order to determine the potential overestimation of PE due to the use of disposable gloves, leachates of ten different types of disposable gloves (made of nitrile, latex, neoprene and vinyl) were prepared, filtered and analyzed using the three most commonly applied analytical methods in MP research, namely μ-Raman, μ-FTIR and pyr-GC/MS. As control, ultrapure water blank samples and "hand blanks", where the washed hands of laboratory staff instead of gloves were brought in contact with a portion of ultrapure water, were processed and analyzed in the same way as the glove leachates. The detected amount of alleged PE per glove type was semiquantitatively compared between the three analytical methods. The results of this experiment proved that disposable gloves can cause very high levels of cross-contamination and that all three considered analytical methods are susceptible to falsely identify stearates as PE. Up to 5897 alleged PE particles, up to 82% filtration area coverage with alleged PE and up to 0.324 mg alleged PE were detected per disposable glove using μ-Raman, μ-FTIR and pyr-GC/MS, respectively. In contrast, PE was absent or below the limit of detection in the blank samples and little to no cross-contamination with PE (or false positives) was found in the "hand blanks" with all three analytical methods.

Secondly, in order to determine the capability of μ-Raman, μ-FTIR, and pyr-GC/MS to distinguish among PE, SDS, and stearates, three identically looking granular samples of sodium stearate, PE and SDS were anonymized and analyzed with all three analytical methods. A comparison between the according μ-Raman spectra did not allow differentiation between the sodium stearate granulate and the PE granulate. The SDS granulate was successfully identified, although its spectrum differed only minimally from those of the PE and sodium stearate granulates. For μ-Raman, it was concluded that even when adequate reference spectra of all three substances are provided in the spectra database, confusion between SDS, stearates, and PE is likely to occur due to the great similarity between the Raman spectra of these substances and because of the higher level of noise expected in natural samples.

Using ATR-FTIR and μ-FTIR, the granulates of PE, SDS and sodium stearate were successfully distinguished from each other. The main differences between the spectra of the three substances were found in the regions of the C–H, CO_2, and SO_2 vibration bands. This means that, when μ-FTIR is applied in transmission mode, the correct distinction between SDS, PE, and stearates depends greatly on the filter substrate used. Differentiation between PE, SDS and stearates in transmission mode is only possible if the filter substrate is IR-transparent in the ranges of the C–H, CO_2, and SO_2 vibration bands (e.g., zinc selenide windows, silicone or aluminum oxide membranes).

Pyr-GC/MS allowed the three granulates to be distinguished from each other. However, it was discovered that in the presence of fatty acids, the quantification of PE via 1-pentadecene may lead to overestimation of PE due to the overlapping of the pyrolysis products originating from PE by those originating from fatty acids. To avoid this, quantification of PE via a 1-alkene with a longer chain length than 1-pentadecene (e.g., 1-octadecene) or via 1,14-pentadecadiene, which cannot originate from pyrolysis of fatty acids, was suggested.

In short, the research in Chapter 2 revealed that the use of SDS and disposable gloves in the laboratory may cause the quantity of PE, and thus MPs, to be overestimated. It showed that both μ-Raman and μ-FTIR are susceptible to false positive identification of stearates and SDS as PE, and that overestimation of PE in the presence of fatty acids is possible with pyr-GC/MS. Several measures to eliminate or reduce the risk of cross-contamination and overestimation of MP concentrations were identified and discussed, such as omitting the use of SDS and disposable gloves in the laboratory as well as removing SDS residues by thorough rinsing of filters prior to analysis. Furthermore, it was demonstrated that PE, SDS and stearates can be differentiated by adapting practices related to μ-FTIR and pyr-GC/MS analysis. For cross-contamination stemming from the laboratory, regular investigation of blank samples was advised as a reliable method to prevent overestimation of MPs, both by false positive identification of substances as polymers as well as by actual polymers.

5.1.2 2nd Objective: Development and Validation of a Fully Automated Microplastic Sampling Unit, Enabling the Monitoring of Microplastics in Freshwater Samples with High Temporal Resolution and over Extensive Periods of Time

Despite increased research efforts, there are only a few studies that have investigated the temporal variability of MP concentrations in surface waters. Most of these studies have focused on precipitation events or on monthly to seasonal effects and applied single-point sampling (de Carvalho et al., 2021; Hitchcock, 2020; Mintenig et al., 2020; Skalska et al., 2020; Xia et al., 2020). However, when high temporal variability is expected to occur in the medium (e.g., in streams), single-point samples may not render representative results for the concentration and composition of MPs. A promising approach to gain representative data on the temporal variability of MP concentrations and composition in such matrices is the collection of mixed samples in close succession. However, to enable this form of sampling over long periods of time, an automated sampling apparatus that requires minimum manual handling and maintenance and renders reliable samples (validated by positive and negative controls) is needed. Therefore, a custom-built, automatic sampling unit for MPs called "SAM", which is designed for the repetitive collection of mixed samples from freshwater matrices over long periods of time, was presented in Chapter 3. The main functionalities of SAM were described and a thorough validation by means of positive and negative controls was performed.

Essentially, SAM consists of seven filter cascades. Each filter cascade contains three stainless-steel filters with decreasing mesh sizes in the direction of flow. The sample medium is drawn in by a peristaltic pump and directed either through a filter cascade or through a bypass using electric valves. Both the pump and the electric valves are controlled by a self-developed computer program, which also documents the data obtained from a differential pressure sensor for detecting filter clogging and a flow sensor for monitoring the sample volume. The computer program automatically initiates regular hose rinsing, switches between filter cascades (which corresponds to switching from one mixed sample to the next), and controls the mixed sampling, based on the user settings (e.g., timing and volume). SAM also has a function for automatically ending a mixed sampling process prematurely, when a user-defined maximum differential pressure is reached. SAM can also be monitored and controlled remotely via remote access to the control computer. Human intervention is only required after every seventh mixed sample collection, when all sampled filter cascades have to be manually replaced by clean, unused filter cascades.

The validation of SAM comprised a negative and a positive control. The negative control, which has the purpose of uncovering cross-contamination, was performed by collecting three process blank samples with SAM. For this purpose, pre-filtered tap water was pumped through filter cascades installed in SAM. The according samples were subsequently processed and analyzed in the same way as performed for the environmental samples investigated in Chapters 3 and 4 and thus reflect the cross-contamination occurring during the entire process of sampling, sample processing and analysis. In this context, it has to be mentioned that extensive measures were taken to avoid cross-contamination during the handling of all environmental and (process) blank samples described in Chapters 3 and 4. These measures include, for example, avoiding the use of plastic utensils as far as possible, wearing 100% cotton laboratory coats, working under laminar flow benches, and, based on the findings in Chapter 2, not using SDS and avoiding the use of disposable gloves unless absolutely necessary for personal protection (e.g., when handling hydrogen peroxide to produce Fenton reagent).

In the three process blank samples, a mean of 263 ± 129 particles per m^3 and 80 ± 16 fibers per m^3 was detected, summed over all polymer types. Compared with other studies that considered process blank values, this level of cross-contamination is low (Horton et al., 2021; Roscher et al., 2022). Furthermore, the LOD was calculated separately for particles and fibers of each polymer type. This allows cross-contamination to be accounted for or corrected in environmental samples collected with SAM.

The positive control was performed by means of a recovery experiment. For this purpose, several vials were filled with a MP reference particle mixture. The aim was for each vial to contain approximately the same number of MPs and the approximately same polymer type composition, which posed a major challenge. The reference particle mixture consisted of five different polymer types (PP, PE, PS, PVC and PET/PES) with densities ranging from approximately 0.90 to 1.45 g/cm^{-3}. The particles in the reference mixture had a size ≥ 10 µm, which corresponds to the MP particle size that SAM

is designed to sample. In addition to the vials filled with the reference particle mixture, several vials filled with ultrapure water for cross-contamination control were prepared. Some of the vials containing the reference particle mixture (n = 9) were filtered directly onto analytical filters and were analyzed in order to determine the number and composition of the MPs in the original reference particle mixture (U-SS). This revealed that the vials containing the reference particle mixture exhibited good, albeit not perfect, consistency. The remaining vials containing the reference particle mixture (n = 6) and the vials filled with ultrapure water (n = 3) were used to spike pre-filtered drinking water, which was sampled with a different filter cascade per vial using SAM. The volume of tap water was chosen so that a realistic concentration of MPs for surface water was achieved during sampling of the tap water spiked with the reference particle mixture. The blank samples (T-BS) and samples with reference particles (T-SS) obtained with SAM were processed and analyzed in the same way as the environmental samples investigated in Chapter 3. Cross-contamination and recovery were determined by comparison of T-BS and T-SS with U-SS.

The overall cross-contamination level of the recovery experiment was very low at 3% (T-BS relative to U-SS). The overall recovery rate determined was 77 $\pm$ 29% (T-SS relative to U-SS). This can be rated as good, especially when considering that recovery experiments in MP research have rarely been carried out where, as in this case, different types of polymers with different density ranges are applied, where realistic environmental concentrations are used, and where the particle size employed is based on the particle size to be investigated in the environment (Bordós et al., 2021; Cui et al., 2022). However, it should be taken into account that there were notable differences in the recovery rates of the individual polymer types, and that underestimation for some polymer types, such as PP and PET/PES (see Figure 3.3), cannot be ruled out. Furthermore, the recovery experiment was only carried out for MP particles and not for MP fibers, although these were also subject of the study. This was due to the much poorer availability of MP reference material in fiber form than in particle form, especially in the lower micrometer size range. As MP fibers may behave differently, the good overall recovery rate obtained for MP particles cannot be extrapolated to MP fibers but remains to be determined separately.

The low LOD of the individual polymer types and the high overall recovery rate achieved for MP particles indicate that SAM is very well suited for collecting reliable MP samples. This has also been confirmed during practical applications: As presented in Chapters 3 and 4, SAM was used to collect temporally representative 24-hour mixed samples from a stream and a WWTP effluent over a period of eight days and 31 days, respectively. During these two sampling periods, there was only one day on which sampling was not possible due to a technical defect of SAM. On three further days, technical problems with SAM occurred, which were, however, permanently resolved by adjustments to the control code. This confirms that SAM requires little manual handling and

maintenance and is suitable for collecting MP mixed samples with high temporal resolution (e.g., one mixed sample per day) over long periods of time. However, one shortcoming of SAM was identified during application in the field: During sampling in the stream and WWTP effluent (see Chapters 3 and 4), only a mean of 78 L and 41 L, respectively, could be collected per mixed sample. Moreover, even with these small sample volumes, the filter sieves tended to clog, causing the sampling to be automatically terminated prematurely for several mixed samples. Ideally, a volume of $\geq$ 500 L should be collected from WWTP effluents and streams (Koelmans et al., 2019), although sampling volumes of $\leq$ 50 L are very common for WWTP effluents (Sheriff et al., 2024). Due to the low LOD determined and the high MP concentration $\geq$ 11 µm observed in both the stream and WWTP effluent in question, the volumes collected in this thesis can, nevertheless, be considered adequate. However, for future applications, SAM should be equipped with filters with a larger surface area to prevent clogging and enable the sampling of large volumes, even at high concentrations of suspended solids in the medium of interest. SAM should also be further optimized to enable not only temporally but also spatially (small-scale) representative sampling in future. This is advisable because, in contrast to dissolved substances, MPs are much less homogeneously distributed in water. The different densities of MPs can lead to an uneven distribution of polymer types in the water column and, especially in streams, factors such as flow velocity, turbulence, distance from the bank, stream geometry, bed friction, etc. can influence the spatial (lateral and vertical) distribution of MP concentration and composition (Haberstroh et al., 2021). During the sampling campaigns described in Chapters 3 and 4, samples were collected from one location in the stream and from one location in the WWTP effluent, respectively, using a peristaltic pump. Good mixing of the water was assumed in the WWTP effluent shaft, and a narrow cross-section downstream of several rapids was deliberately selected for sampling from the stream. Nevertheless, small-scale spatial variability of MP concentration and composition, that was not accounted for, cannot be ruled out. For future investigations, simultaneous sample collection with numerous, spatially distributed mini-submersible pumps could be an approach to improve the small-scale spatial representativeness of sampling with SAM.

5.1.3 3rd Objective: Determination and Evaluation of the Temporal Variability of the Concentration and Composition of Microplastics in a Stream and in the Effluent of a WWTP and Investigation of Possible Variability-influencing Factors

The temporal variability of the concentrations and composition of MPs in flowing surface waters and WWTP effluents is as yet poorly understood. Therefore, SAM was applied to collect 24-hour mixed samples over a period of eight days in a stream in southern Germany (see Chapter 3) and over a period of 31 days in the effluent of a WWTP (see Chapter 4). By analyzing the corresponding mixed samples with µ-FTIR, the concentration and composition of MPs $\geq$ 11 µm was determined for each of the sampling days. Based on this data, the day-to-day variability of the MP concentration and composition

as well as the influence of various factors on this variability were investigated. For this purpose, the daily precipitation sums from the nearest weather stations, the total particle count and turbidity in the stream and WWTP effluent as well as the discharge in the stream and the discharge from the WWTP effluent were obtained. Unfortunately, the data on the total particle count and turbidity in the WWTP effluent was not suitable for interpretation due to a sensor error and data jumps.

It is important to note that the WWTP effluent investigated in Chapter 4 does not discharge into the stream considered in Chapter 3. Consequently, there is no systemic link between the stream data and the WWTP effluent data. However, Chapters 3 and 4 contribute to the same research objective and almost the same methodology was applied to gain both datasets.

The mean MP concentration determined from the eight mixed samples collected in the stream is $4.83 \times 10^3 \pm 4.66 \times 10^3$ MPs per m^3. The lowest and highest MP concentrations observed in the stream are 1.21×10^3 MPs per m^3 and 1.31×10^4 MPs per m^3, respectively. In the WWTP effluent, the mean MP concentration determined across 30 mixed samples is one order of magnitude higher than in the stream, with $3.85 \times 10^4 \pm 2.12 \times 10^4$ MPs per m^3. The corresponding lowest and highest MP concentrations are 9.64×10^3 MPs per m^3 and 8.44×10^4 MPs per m^3, respectively. As is evident from the high standard deviations, a high day-to-day variability of the MP concentrations was observed in both the stream and the WWTP effluent for the periods investigated.

In the stream, no clear relationship could be observed between the MP concentration time series and the course of the precipitation sum, the discharge of the stream, the turbidity or the total particle count in the stream. Similarly, for the WWTP effluent investigated, no correlation could be established between the time series of MP concentration and the course of the discharge from the WWTP effluent or the precipitation sum. However, precipitation was very light (daily precipitation sums ≤ 4.8 mm) and occurred only on two days each during the sampling in the stream and the WWTP effluent. Therefore, a positive or negative correlation between the precipitation sum and the MP concentration in the stream and/or the WWTP effluent is still considered likely in the case of heavy precipitation events. For instance, heavy rain may cause an increase of MP inputs (high surface runoff), a reduced clearance rate of WWTPs, resuspension of MPs or the dilution of MPs (Ben-David et al., 2021; Hitchcock, 2020; Martín-García et al., 2023; Roscher et al., 2022; Xia et al., 2020).

In contrast to the high temporal variability of the MP concentration observed in the WWTP effluent and in the stream, the composition of MPs by size and shape (fibers and particles) in both these matrices was very consistent over the periods studied. $95 \pm 3\%$ and $95 \pm 2\%$ of the MPs were < 100 μm in the stream and in the WWTP effluent across all sampling days, respectively. Furthermore, $82 \pm 5\%$ and $82 \pm 2\%$ of the MPs were present as particles in the stream and in the WWTP effluent, respectively, over the periods studied. This direct comparison of the composition of MPs by size and shape between the wastewater effluent and the stream reveals a very good agreement. Similar particle size

distributions and particle contributions are also reported in the literature for other surface waters and WWTP effluents (e.g., Mintenig et al., 2020; Roscher et al., 2022; Wolff et al., 2019, 2021). Therefore, neither a (non-existent) systemic link between the stream and the WWTP effluent nor the largely identical methodology used in Chapters 3 and 4 is decisive for the observed similarity of the MP composition (size and shape) between the stream and the WWTP effluent.

The composition of MPs by polymer type varied to some extent from day to day, but the polymers PP and A/PU/V were clearly dominant across all sampling days in both the stream ($52 \pm 15\%$ PP and $12 \pm 4\%$ A/PU/V) and the WWTP effluent ($36 \pm 6\%$ PP and $27 \pm 7\%$ A/PU/V). It can be concluded that the composition of MPs by size, shape, and polymer type in both the stream and the WWTP effluent was much less subject to temporal variability over the respective study periods than the MP concentration.

In order to be able to recognize relationships within a dynamic system with many influencing factors, long time series are of advantage. For this reason, the exceptionally long period of 31 days was chosen for the investigation of the WWTP effluent. This made it possible to analyze the concentration time series of the individual, main polymer types (present in $\geq 90\%$ of the mixed samples) in the WWTP effluent by means of PCA. A high correlation between the time series of the polymer types PP, PE, PS, PES, A/PU/V, EVA and CAM was uncovered. Only the time series of the polymer PA did not correlate with that of any of the other main polymer types. Possible reasons for the high correlation between the time series of all main polymer types except PA and the deviating behavior of PA are discussed in detail in Chapter 4. The PCA also revealed that the MP composition by polymer type on weekends does not differ greatly from that on other days of the week, even though slightly higher concentrations in the WWTP effluent were observed on weekends. Furthermore, PCA showed that the MP concentration and the composition by polymer type were more similar between samples collected on days close to each other (e.g., subsequent sampling days) than between samples collected on days further apart.

It remains to be investigated, to what extent the observations presented in Chapters 3 and 4 on the temporal variability of the concentration and composition of MPs also apply to other sampling periods (other weeks, months, seasons) and can be transferred to other streams and WWTP effluents.

5.1.4   4th Objective: Assessing the Representativeness of Single-Point and Mixed Samples in the Context of the Temporal Variability of Microplastic Concentration and Composition in Streams and WWTP Effluents

In general, measurement data obtained from a mixed sample is temporally more representative than data from a single-point sample, because each mixed sample represents the mean over the according sampling period. Based on the 24-hour mixed samples collected over a period of eight days in a stream and over a period of 31 days in the effluent

of a WWTP, a high temporal variability of MP concentrations was observed in both matrices (see Chapters 3 and 4). It can be assumed that the real-time variability of the MP concentration in the stream and in the WWTP effluent is even higher than determined on the basis of the 24-h mixed samples. Assuming that the high temporal variability of the MP concentration observed in the stream and WWTP effluent can be transferred to other watercourses and other WWTP effluents, it can be concluded that single-point samples (without regular repetition) are not suitable for representing the MP concentration in watercourses and WWTP effluents. Single-point samples do not take into account temporal variability and therefore likely lead to false conclusions about MP concentrations and MP fluxes.

Based on the day-to-day variability of the MP concentration determined over the 31-day period in the effluent of the WWTP, it was calculated in Chapter 4 that at least eleven daily mixed samples were required in order to obtain a mean MP concentration that lies within the standard deviation of the "actual" mean MP concentration (derived from all mixed samples) with a probability of > 50%. This demonstrates that individual or low numbers of mixed samples may also not be sufficient to determine a representative MP concentration in a medium with high temporal variability. Instead, continuous or regular collection of mixed samples is required for this purpose. In contrast to this, however, individual mixed samples may well be suited for roughly estimating the composition (size, shape, polymer type) of MPs in watercourses and WWTP effluents. This was concluded from the observation that the MP composition in the WWTP effluent and in the stream, investigated in Chapters 3 and 4, was temporally much more consistent than the MP concentration.

A follow-up question arising from these findings is how temporal variability interacts with small-scale spatial variability. It must be assumed that the variability of the MP concentration and composition has a temporal as well as a spatial component. In future, both should be taken into account in order to obtain spatiotemporally representative data. Furthermore, accounting for small-scale spatial variability may be the key to identifying the drivers of the temporal variability of MP concentration and composition.

5.2 Scientific Contribution

The main innovations of this thesis and its contributions to the scientific community are summarized in the following:

- Attention was drawn to the fact that the use of stearates (in the form of disposable gloves) and SDS in the laboratory can lead to false positive PE results when using µ-Raman, µ-FTIR and pyr-GC/MS analysis, and that MP concentrations may be overestimated as a result. Options for avoiding this false positive identification of SDS and stearates were identified and the importance of blank value determination for the detection and elimination of cross-contamination in the laboratory was stressed. These findings are important for assessing the reliability of reported MP

concentrations from other studies and for ensuring the reliability of researchers' own MP results.

- A MP sampling device enabling the automated collection of mixed samples with high temporal resolution over long periods of time and requiring minimal manual handling and maintenance was developed, validated and successfully applied in the field. This device represents a first step towards investigating the temporal variability of MP concentration and composition over long time periods using mixed samples, thus contributing to obtaining a representative estimate of the MP pollution in different surface water systems.

- Based on one case study each and by collecting representative 24-hour mixed samples, it was shown that under normal conditions (no heavy rainfall), MP concentrations in watercourses and WWTP effluents are highly variable from day to day. This led to the conclusion that single-point samples and individual, non-repetitive mixed samples collected from a matrix with high temporal variability of the MP concentration are not suitable for determining a MP concentration that is representative over a longer period of time. Instead, continuous or repetitive mixed samples are required for this purpose. It was also discovered, that the discharge volume from the WWTP effluent, the stream discharge, and the precipitation sum of light rain, as well as turbidity and total particle number, do not reflect the MP concentration and its temporal variability. These findings are important for evaluating the temporal representativeness of samples but also for the planning of further investigations and the development of a deeper understanding of the factors influencing the temporal variability of MP concentrations in flowing surface waters and WWTP effluents.

5.3 Outlook

As stated at the beginning of Section 5.1, MP concentrations determined by different studies often differ by several orders of magnitude, even in the same matrix. The following factors were listed as possible causes: limited standardization of methods for sampling, sample processing, and analysis, inadequate QA/QC, spatial variability and temporal variability. To obtain reliable and comparable results, a thorough understanding of the spatial and temporal variability of MP concentration and composition in various environmental matrices as well as extensive QA/QC measures are required. Furthermore, the use of standardized methods—to the extent that this is compatible with answering the research question—may enhance comparability between studies. For the standardization of methods, a good basis is provided by the already available research results from literature. Minimum requirements for analyzing MPs in the environment have already been defined in *DIN EN ISO 24187:2024-04* (2024), while more specific MP standards that also consider different case constellations are being developed by various committees and standardization bodies. This, however, is a lengthy process and it

may take years before these standards are finalized and put into practice. Nevertheless, there are already a number of publications containing guidelines, minimum requirements, and best practice suggestions for different aspects of MP research (Andrade et al., 2020; Cowger et al., 2020; Cui et al., 2022; Schymanski et al., 2021). Taking into account these publications and carrying out extensive QA/QC in the form of positive and negative controls can improve comparability between studies. Positive controls in particular are not widely performed, and when they are, they often involve only one or very few types of polymers and/or large MPs, typically pellets, while shapes such as fibers and fragments are rarely used (Cui et al., 2022). This is mainly due to the fact that it is very challenging to produce reference particle mixture samples containing (almost) identical MP numbers and compositions in the lower MP size range. The development and commercialization of corresponding MP reference suspensions is a necessary step to make recovery experiments for method validation easier, more common and reliable, and thus to promote inter-laboratory comparability.

Similar to the temporal variability, the spatial variability of MP composition and concentration in aquatic matrices has not yet been studied in sufficient detail, either. However, it has already been shown that differences in MP concentration and/or composition can exist in the same system both at the closest range (Haberstroh et al., 2021; Lenaker et al., 2019; Pessenlehner et al., 2024; Range et al., 2023) and over greater distances (Mintenig et al., 2020; Pakhomova et al., 2024; Range et al., 2023). It can make a difference whether samples are taken at the surface or subsurface of a flowing water body, if a sampling point is located in the middle or close to the bank of a river (Haberstroh et al., 2021; Pessenlehner et al., 2024), whether sampling is performed close to the source or the mouth of a river, and whether the region of sampling is rural or urban (Range et al., 2023). Further research is required to gain a deeper understanding of the spatial variability of the MP concentration and composition as well as of the underlying factors. In this context, it should be noted that the spatial variability and the temporal variability of the concentration and composition of MPs are most likely interlinked. Therefore, spatial variability should be investigated in conjunction with temporal variability. This is important for preventing over-simplification, for gaining both spatially and temporally representative data, and for calculating meaningful concentrations and fluxes.

An open question arising from this work is whether the observed high temporal variability in MP concentration would also be found in other WWTP effluents and streams using the same experimental set-up. The results of other studies suggest that this would be the case (see Chapters 3 and 4), but a direct comparison using the same methodology would be more meaningful in order to assess the transferability of the results of this work to other watercourses and WWTP effluents. Furthermore, the causes of the temporal variability of the MP concentration and composition could be further researched as part of these investigations. Based on the example of a stream and a WWTP effluent, some factors (e.g., light precipitation and stream as well as WWTP effluent discharge) could already be excluded as main drivers of the temporal variability of the

MP concentration and some relationships (e.g., between the concentration time series of several individual polymers) could be established. Nevertheless, the main drivers of the high temporal variability of MP concentration in both the stream and the WWTP effluent remain unclear. Further research is needed to identify these drivers.

For example, a study at the catchment area level would be revealing, in which temporally and spatially (e.g., vertical and cross-sectional multi-points) representative mixed samples are taken from a river over several days under normal weather conditions, while at the same time all point source inputs (e.g., WWTP effluents, tributaries, industrial discharges) into the river are also sampled. The data obtained would improve the understanding of the influence of both point and diffuse MP inputs on the variability of MP concentration and composition in the river, also taking into account processes at the water-sediment interface, (e.g., sedimentation and resuspension of MPs). However, the personnel and material expenditure of such a study would be immense, calling its feasibility into question.

It would likewise be interesting to use SAM to investigate the variability of MP concentration and composition at higher temporal resolution. A mode for automated, hourly composite sampling is already included in the SAM program code. This would make it possible to determine how much higher the temporal variability of MP concentrations is from hour to hour compared to from day to day and whether there are diurnal, recurring patterns in the concentration profile, e.g., in a WWTP effluent in relation to the peak times of wastewater production.

Investigations with SAM in a stream or WWTP effluent based on 24-h mixed samples covering a period of several months would also be of interest. This would allow not only the investigation of the temporal variability of the MP concentration and composition under normal conditions, as in this thesis, but also during heavy rainfall events, dry periods, and possibly even in different seasons. Consequently, it would be possible to determine the conditions under which maximum and minimum values of MP concentrations are reached, how high these maximum and minimum values are, and whether MP composition is subject to greater variability under extreme weather conditions than under normal conditions. In addition, the variability of the MP concentration and composition between representative monthly averages and between different seasons could be determined. This would provide important insights for estimating MP loads and could serve as a basis for ecotoxicological studies. However, for such long-term investigations to become feasible, sample processing must be accelerated considerably or automated. For example, with the processing method used in this work, a maximum of two samples per week can be processed by one person. However, the collection of 24-hour mixed samples yields seven samples per week, not counting blank values. The objective would therefore be a sample processing procedure that is fast enough for at least as many samples to be processed per week as would be collected in the same time during a long-term mixed sampling campaign. An approach to solving this issue can be the use of thermoanalytical methods such as pyr-GC/MS for analysis, as these, in contrast to spectroscopic methods (e.g., μ-Raman, μ-FTIR), are less dependent on thorough

sample processing (see Chapter 1). Using thermoanalytical methods would also have the advantage that TRWP, which are emitted in great amounts (e.g., $\sim$1.3 million t a^{-1} in Europe, Baensch-Baltruschat et al. (2020)) could be taken into account. In this thesis, TRWP could not be quantified in the investigated WWTP effluent and stream, as the analytical method used (μ-FTIR) is not suitable for the chemical identification of TRWP. However, thermoanalytical methods are complimentary to spectroscopic methods, as they determine the mass rather than the number of MPs in a sample. While mass data can be useful for calculating representative MP loads, e.g., of a river, ecotoxicological studies require representative MP concentrations based on the number of MP particles, determined for various environmental compartments. Accordingly, further investigations of the temporal and spatial variability of the MP concentration in relation to the particle number and mass are needed, along with the development of faster or even automated sample processing methods.

5.4 Conclusion

MPs are ubiquitous and accumulate in the environment due to the worldwide ever increasing production and use of plastics as well as their longevity. Despite the rapid increase in the number of publications in the field of MP research since the beginning of the 2000s, many open questions remain. This includes the determination of reliable, temporally and spatially representative MP concentrations for various environmental compartments, which is required as a basis for sound ecotoxicological studies and the still-pending, comprehensive risk assessment of MP pollution. This thesis is one step towards this overarching goal and contributes by:

- drawing attention to the fact that the use of stearates and SDS in the laboratory can lead to relevant overestimation of MP number and mass concentrations in environmental samples.

- developing and validating a sampling device that enables the repetitive collection of temporally representative mixed samples over extensive periods of time and requires minimal manual handling and maintenance. This provides a basis for future long-term investigations of the temporal variability of the MP concentration and composition in freshwater bodies and for collecting temporally representative MP data.

- investigating the temporal variability of the concentration and composition of MPs in a stream and in a WWTP effluent over eight and 31 days, respectively, assessing the observed variability and identifying several variability (non-)influencing factors.

Aside from further research on the temporal variability of the MP concentration and composition, e.g., in different environmental compartments and over longer periods of time including extreme weather events, spatial variability must receive more attention

with regard to the representativeness of samples. Furthermore, the standardization of methods and a more widely implemented extensive QA/QC are needed to promote comparability between studies, collaboration, and cumulative gain of knowledge.

Appendix A

Author Contributions

Article I: When Good Intentions Go Bad—False Positive Microplastic Detection Caused by Disposable Gloves

Witzig, C. S., Földi, C., Wörle, K., Habermehl, P., Pittroff, M., Müller, Y. K., Lauschke, T., Fiener, P., Dierkes, G., Freier, K. P., and Zumbülte, N. (2020). When good intentions go bad—False positive microplastic detection caused by disposable gloves. In: *Environmental Science & Technology* 54 (19), pp. 12164–12172. DOI: 10.1021/acs.est.0c03742.

C.S.W. conceived and wrote the manuscript with text contributions by C.F. and K.W., who contributed equally. Presented investigations were mainly carried out by C.S.W. at TZW: DVGW-Technologiezentrum Wasser, by C.F. and T.L. at the Bundesanstalt für Gewässerkunde, and by K.W. at the Bayerisches Landesamt für Umwelt. Figures were created by C.S.W. with inspiration from N.Z. The person to first consider MP cross-contamination by disposable gloves was P.H., while M.P. subsequently recognized the connection among disposable gloves, PE cross-contamination, and stearates. Y.K.M. identified the risk of confusion between SDS and PE with μ-Raman during previous investigations and helped incorporate her insights into this research. All co-authors critically discussed, commented on, and reviewed the manuscript.

Article II: Temporal Variability of Microplastic Concentrations in Inland Waters: An Automated, Semicontinuous Sampling of Microplastics ≥ 11 µm in a Stream in Southern Germany

Witzig, C. S., Fiener, P., König, M., and Zumbülte, N. (2024). Temporal variability of microplastic concentrations in inland waters: An automated, semicontinuous sampling of microplastics ≥ 11 µm in a stream in southern Germany. In: *ACS ES&T Water* 4 (4), pp. 1443–1450. DOI: 10.1021/acsestwater.3c00591.

C.S.W. devised the first construction plan of SAM, which was subsequently optimized with input by M.K. With joint effort, M.K. and C.S.W. built and programmed SAM. C.S.W. planned and conducted all field and laboratory experiments and conceived and wrote the manuscript. N.Z. supervised the research work and arranged access to the

sampling site at Kirchgassgraben. N.Z. and P.F. both provided valuable input and feedback throughout the different stages of the study and critically reviewed the manuscript.

Article III: Long-Term Investigation on the Daily Variability of Microplastic Concentration and Composition—Monitoring in the Effluent of a Wastewater Treatment Plant

C.S.W. planned and carried out the sampling campaign at the WWTP and performed the laboratory work as well as the data analysis. C.S.W. furthermore conceived and wrote the manuscript including all visualization. N.Z. and P.F. both provided valuable input and feedback throughout the different stages of the study and critically reviewed the manuscript. N.Z. supervised the research work.

Appendix B

Further Publications by the Author

List of additional publications in peer-reviewed journals to which the author contributed during the doctoral phase:

Müller, Y. K., Wernicke, T., Pittroff, M., Witzig, C. S., Storck, F. R., Klinger, J., and Zumbülte, N. (2020). Microplastic analysis—are we measuring the same? Results on the first global comparative study for microplastic analysis in a water sample. In: *Analytical and Bioanalytical Chemistry* 412, pp. 555–560. DOI: 10.1007/s00216-019-02311-1.

Pittroff, M., Müller, Y. K., Witzig, C. S., Scheurer, M., Storck, F. R., and Zumbülte, N. (2021). Microplastic analysis in drinking water based on fractionated filtration sampling and Raman microspectroscopy. In: *Environmental Science and Pollution Research* 28, pp. 59439–59451. DOI: 10.1007/s11356-021-12467-y.

Schymanski, D., Oßmann, B. E., Benismail, N., Boukerma, K., Dallmann, G., von der Esch, E., Fischer, D., Fischer, F., Gilliland, D., Glas, K., Hofmann, T., Käppler, A., Lacorte, S., Marco, J., El Rakwe, M., Weisser, J., Witzig, C. S., Zumbülte, N., and Ivleva, N. P. (2021). Analysis of microplastics in drinking water and other clean water samples with micro-Raman and micro-infrared spectroscopy: Minimum requirements and best practice guidelines. In: *Analytical and Bioanalytical Chemistry* 413, pp. 5969–5994. DOI: 10.1007/s00216-021-03498-y.

Appendix C

Supporting Information of Chapter 2

When Good Intentions Go Bad—False Positive Microplastic Detection Caused by Disposable Gloves

Details on Air-Cleaning Systems and Ultrapure Water

Information on the systems applied at µ-Raman and µ-FTIR laboratory to minimize airborne cross-contamination during various steps of sample handling is given in Table C.1. At the three laboratories, different ultrapure water systems with subsequent filter capsules (additional filter at the water outlet of the ultrapure water system) were applied. Information on the type and manufacturer but also conductivity and pore size for the ultrapure water systems and the filter capsules are given in Table C.2.

TABLE C.1: Air-cleaning systems applied at µ-Raman and µ-FTIR laboratory during sample processing and sample analysis.

Laboratory	Type of System	Manufacturer	Steps Applied for
µ-Raman	Laminar flow cabinet, HEPA 14	Thulab, Germany	Sample processing and sample analysis
µ-FTIR	Laminar flow cabinet, UVF 6.12 S	BDK Luft- und Reinraumtechnik GmbH, Germany	Sample processing
	Dustbox DB 1000, HEPA 14	Moecklinghoff Lufttechnik GmbH, Germany	Sample analysis

TABLE C.2: Ultrapure water systems and filter capsules applied at μ-Raman, μ-FTIR and pyr-GC/MS laboratory.

| Laboratory | Ultrapure Water System | | Filter Capsules | |
	Type and Manufacturer	Conductivity	Type and Manufacturer	Pore Size
μ-Raman	Arium 611 UF, Sartorius AG, Germany	18.2 MΩ cm	Satopore 2 Sartorius AG, Germany	0.2 μm
μ-FTIR	Ultra Clear TP UV UF TM, Evoqua Water Technologies GmbH, Germany	18.2 MΩ cm	Capsule Filter Evoqua, Evoqua Water Technologies LLC, USA	0.2 μm
pyr-GC/MS	Milli-Q, Integral, Merck, Germany	18.0 MΩ cm	Milipak, Merck, Germany	0.22 μm

Details on μ-Raman Evaluation

As μ-Raman analysis is very time consuming, only 6.3% of the filtration area of the filter was analyzed. Six square sub-sections with the size of approximately 2 mm × 2 mm (4.5 mm^2 each) were measured using the Particle Finder tool of the software LabSpec (Version 6, Spectroscopy Suite Software, Horiba Jobin Yvon, Japan). As experience had previously shown that particles are often distributed inhomogeneously across the filter (less particles at the filter center and more particles at the filter edge), three of the measured sections were chosen to lie close to the edge of the filter and three sections were chosen to lie close to the filter center. The position of the six sections was identical for all analyzed filters as fixed coordinates were used for their positioning (Figure C.1).
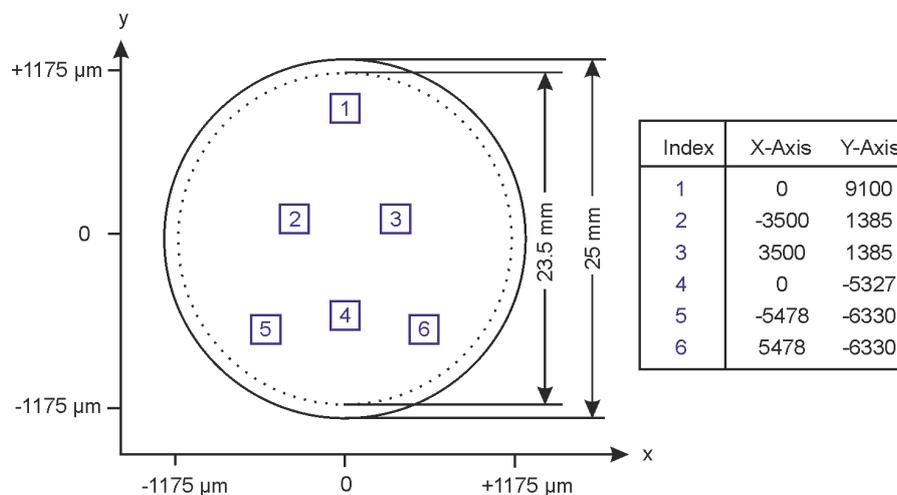
FIGURE C.1: Coordinates and visualization of the six 2 mm × 2 mm sections within the filtration area (dotted line) of the filter.

After data base comparison, the particle number identified for each polymer type was extrapolated from the total analysis area of the filter (six sections excluding poorly focused areas) to the entire filtration area (433.7 mm^2). The upscaling factor (entire filtration area divided by the well-focused areas) was between 15.9 and 25.5.

Details on Characteristic Pyrolysis Products of PE—Pyr-GC/MS

For quantification, characteristic pyrolysis products of PE were monitored by the abundance of their indicator ion, as summarized in Table C.3.

TABLE C.3: Characteristic pyrolysis products of PE, their indicator ions and calibration curve data.

Pyrolysis product	Indicator ion (m/z)	t_r (min)	Calibration curve	R^2
1-pentadecene	97.1	11.41	y = 0.487 x + 0.006	0.994
1,14-pentadecadiene	81.1	11.36	y = 0.215 x + 0.001	0.992
1-octadecene	97.1	13.12	y = 0.499 x + 0.004	0.995

Appendix D

Supporting Information of Chapter 3

Temporal Variability of Microplastic Concentrations in Inland Waters: An Automated, Semicontinuous Sampling of Microplastics $\geq$ 11 µm in a Stream in Southern Germany

Details on Preparation of Reference Particle Samples

Reference particles of the polymer types PVC, PET, and PS (Goodfellow GmbH, Germany) as well as PE (Sigma-Aldrich, USA), and PP (PolyGroup Inc, USA) were acquired and 0.35 to 0.55 g (see Table D.1) were weighed for further use. Other than the reference particles of the polymer types PE and PP, the reference particles made of PVC, PET, and PS were mostly larger than 100 µm. Therefore, the latter were fractionated separately into smaller secondary particles using ultrasound treatment (Elmasonic P 180 H for eight hours with 100% intensity and 37 kHz; Elma Schmidbauer GmbH, Singen) under hydrolytic conditions (suspension of particles in 0.25 mol L^{-1} potassium hydroxide (KOH) solution; von der Esch et al., 2020). Subsequently, all particles with a diameter > 100 µm and < 10 µm were removed by filtering the particle suspension (KOH solution for PVC, PET, PS and ultrapure water for PE and PP) of each polymer type over a 100 µm and subsequently a 10 µm stainless steel filter disc (48 mm diameter; Rolf Körner GmbH, Germany) separately.

TABLE D.1: Information on the mass of reference particles, density of stock solutions as well as volume of stock solution used in the process of preparing reference particle samples of five different polymer types.

Polymer type	Weighed mass of reference particles (g)	Density of stock solution (g cm^{-3})	Volume of stock solution (µL), used to prepare reference particle samples
PVC	0.362	1.40	800
PET	0.351	1.35	75
PS	0.550	1.05	200
PP	0.350	0.90	10
PE	0.350	0.94	10

In parallel, five solutions (see Table D.1) were prepared that corresponded to the

density of the five types of reference particles, using mixtures of ultrapure water with sodium polytungstate solution or ethanol. The reference particles in the size range < 100 µm and ≥ 10 µm were transferred into the corresponding solutions, resulting in a 200 mL stock solution for each polymer type. The particle size distribution for each stock solution (created by filtration of each stock solution onto a silicon filter, subsequent microscope imaging and particle size evaluation using software ImageJ, version 2.35) is shown in Figure D.1. Settling processes take place at a very slow rate within these stock solutions due to the approximately equal density of the solution and the particles. This facilitates the collection of uniform aliquots. In total, 15 samples, each containing reference particles of all five polymer types, were prepared by pipetting a fixed volume (see Table D.1) of each stock solution into 15 glass jars. Analogously, ultrapure water was pipetted into three glass jars, which were to be used as blank samples.
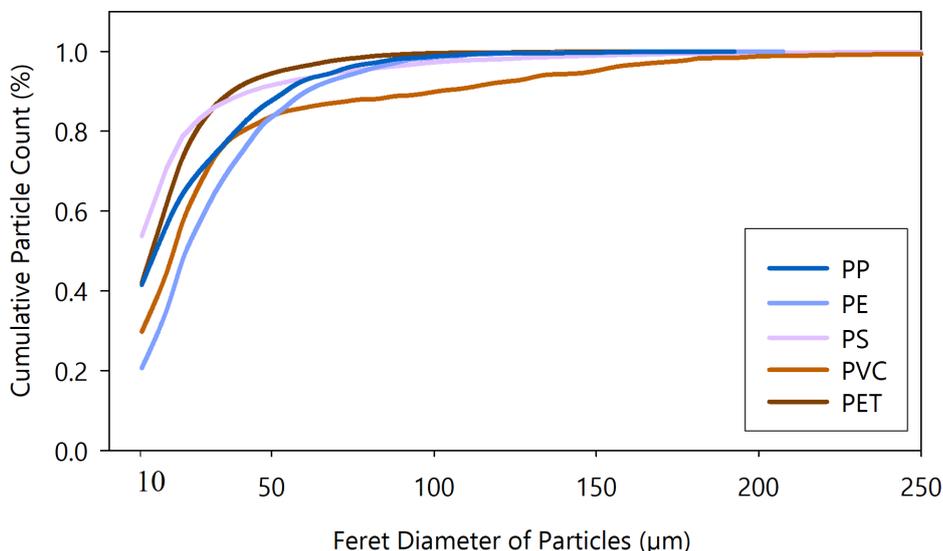
FIGURE D.1: Cumulative distribution function of the particle ferret diameter in the five stock solutions containing PP, PE, PS, PVC, and PET, respectively. Data is presented for the diameter range from 10 to 250 µm.

Information on Adapted Thresholds for Automated Image Analysis

When using MPAPP pipeline for automated image analysis with siMPle, manual evaluation of a data subset with subsequent adaption of thresholds is a necessary part of QA/QC (Lorenz et al., 2019; Moses et al., 2023). The subset of the environmental samples from Kirchgassgraben comprised nine filters from random sampling days, with three filters of each of the size fractions < 250 to ≥ 100 µm, < 100 to ≥ 50 µm and < 50 to ≥ 11 µm. The adapted thresholds are shown in Table D.2.

TABLE D.2: Thresholds of hit quality (max. 2000) defined by manual spectra evaluation for Kirchgassgraben samples and process blank samples.

	Hit quality threshold	
Material type	**Kirchgassgraben samples**	**Process blank samples**
polyethylene	840	750
polyethylene oxidized	600	600
polyethylene, chlorinated	970	929
polypropylene	850	790
polystyrene	630	600
polycarbonate	600	600
polyamide	1000	800
polyvinylchloride	815	956
cellulose artificially modified	850	739
nitrile rubber	600	600
polyester	850	600
acrylates, polyurethanes, varnish	1060	960
animal furs, natural polyamides	960	600
cellulose, plant fibers	1000	922
quartz	850	690
polysulfone	600	600
polyether ether ketone	600	600
polychloroprene	600	600
chitin	600	600
polyisoprene-chlorinated	600	600
polylactic acid	600	600
polycaprolactone	600	600
ethylene-vinyl-acetate	1000	955
polyimide	600	600
polyoxymethylene	700	600
polybutadiene	600	600
acrylonitrile-butadiene	600	600
rubber type 1	945	962
rubber type 2	980	1140
charcoal	600	600
coal	600	600
rubber type 3	1200	1000
cuticula	1200	1090
acrylamides	1100	600

For the process blank samples, all filters of the size fractions < 250 to ≥ 100 µm, < 100 to ≥ 50 µm and < 50 to ≥ 11 µm were manually evaluated to determine adequate thresholds, which are shown in Table D.2.

The evaluation of a subset of filters from the recovery experiment was discontinued due to the very high spectra quality and the corresponding good agreement with the reference spectra. Standard threshold values of 600 were used.

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List of Abbreviations

A/PU/V	polymer cluster acrylates/polyurethanes/varnish
ATR	attenuated total reflection
CAM	cellulose artificially modified
DNA	deoxyribonucleic acid
EVA	ethylene-vinyl-acetate
FPA	focal plane array
FTIR	Fourier transform infrared spectroscopy
fwhm	full width at half-maximum
G-PE	granulate made of PE
G-S	granulate made of sodium stearate
G-SDS	granulate made of SDS
HQI	hit quality index
L1	one type of latex gloves
LOD	limit of detection
LOQ	limit of quantification
M^2S	mobile measuring station
MP	microplastics
N1–N7	seven types of nitrile gloves
Neo 1	one type of neoprene gloves
PA	polyamide
PC	polycarbonate
PC 1	principal component 1
PC 2	principal component 2
PCA	principal component analysis
PE	polyethylene
PE-HD	high-density polyethylene
PE-LD	low density polyethylene
PES	polyester
PET	polyethylene terephthalate
PLA	polylactic acid
POM	polyoxymethylene
PP	polypropylene
PS	polystyrene
PTFE	polytetrafluoroethylene
PU	polyurethane
PVC	polyvinyl chloride
pyr-GC/MS	pyrolysis-gas chromatography/mass spectrometry
QA/QC	quality assurance and quality control
SAM	automated sampling unit for microplastics

SDS   sodium dodecyl sulfate
SI   supporting information
T-BS   treated blank samples
TRWP   Tire and road wear particles
T-SS   treated spiked samples
TZW   TZW: DVGW-Technologiezentrum Wasser
U-SS   untreated spiked samples
V1   one type of vinyl gloves
WWTP   wastewater treatment plant
µ-FTIR   Fourier transform infrared microspectroscopy
µ-Raman   Raman microspectroscopy