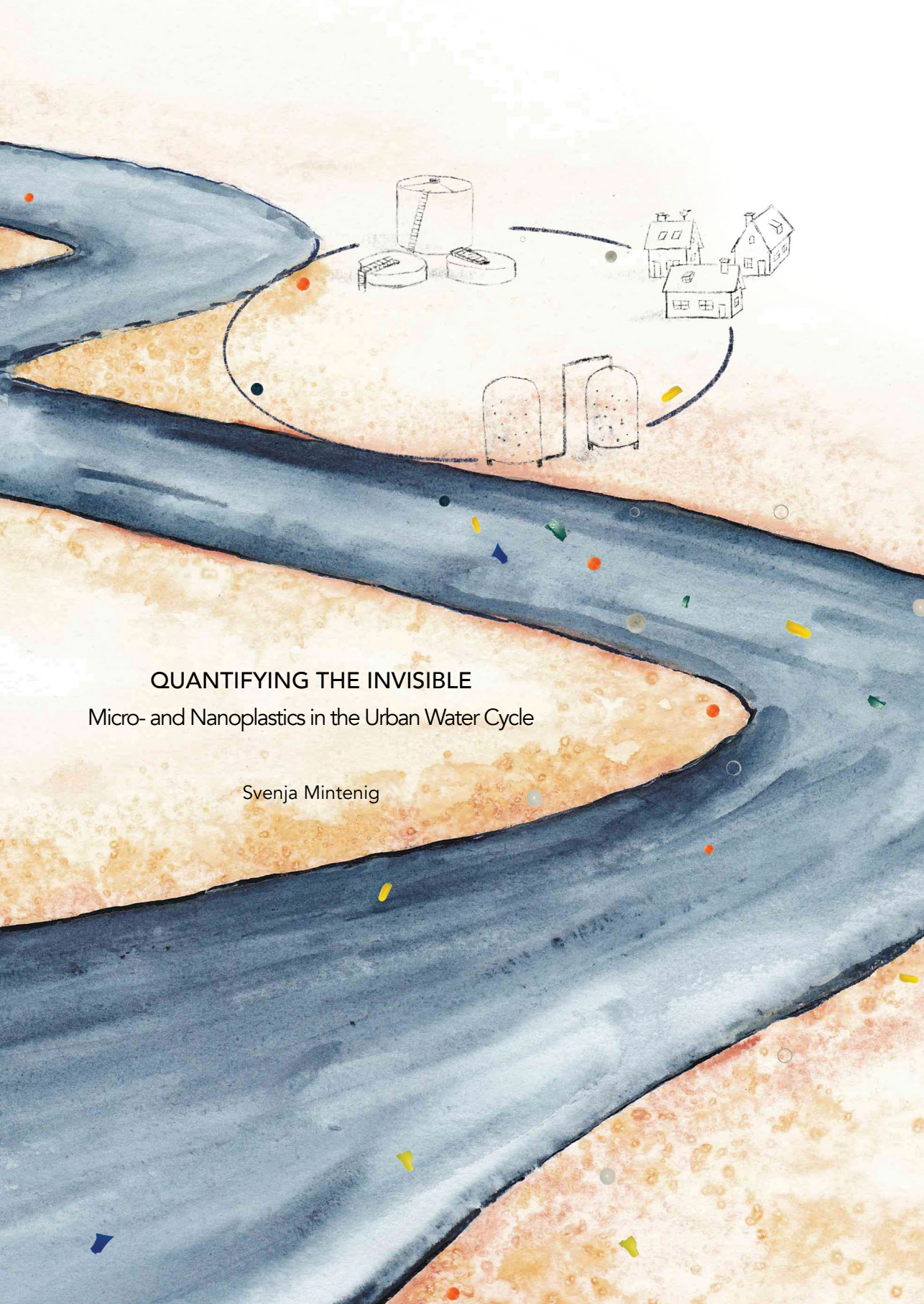

QUANTIFYING THE INVISIBLE

Micro- and Nanoplastics in the Urban Water Cycle

Svenja Mintenig

QUANTIFYING THE INVISIBLE -

MICRO- AND NANOPLASTICS IN THE URBAN WATER CYCLE

SVENJA MARIA MINTENIG

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Quantifying the Invisible –
Micro- and Nanoplastics in the Urban Water Cycle

Het onzichtbare zichtbaar maken –
Micro- en nanoplastic in de urbane watercyclus
(met een samenvatting in het Nederlands)

Das Unsichtbare sichtbar machen –
Mikro- und Nanoplastik im urbanen Wasserkreislauf
(mit einer Zusammenfassung in Deutsch)

Proefschrift

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CHAPTER 1

INTRODUCTION

1.1 Plastic pollution

The term plastic is a collective name for synthetic or semi-synthetic organic polymers. Depending on its designated usage, a plastic item can be made of various polymer types. Its chemical and physical properties can further be modified by variations in the production process itself and by the addition of numerous additives and plasticisers. As a result, thousands of different types of plastic are on the market, all different from each other. Most polymer types can be modified to such a degree that they can be applied in various sectors, including packaging, building and construction, automotive, electrics, households, and agriculture (PlasticsEurope 2019).

What the various plastics have in common, is that they are lightweight, durable, and cheap. These properties increased the global demand and production to 359 million tonnes in 2018 (PlasticsEurope 2019). After generally growing production rates in Europe, a declining growth could be registered in the last two years and is also expected for the year 2020 (-5% compared to the production in 2015) (PlasticsEurope 2019). From the globally produced plastic in 2015, 12% were incinerated, 9% were recycled, and 79% were collected in landfills or, potentially, released into the environment (Geyer et al. 2017). The European plastic manufactures have agreed to reduce the latter to avoid plastic leakage to the environment, therefore they aim in an increased circularity, i.e. a higher recycling or energy recovery (EEA 2021, PlasticsEurope 2018). During their lifecycle plastics, however, can be emitted into the environment, relevant for this are the improper management or littering with, for example, single-use plastics. Geyer et al. (2017) estimated that 12,000 million tons of plastic waste can be expected in the environment by 2050 if the current plastic production trend continues to rise and no preventive measures are taken to reduce plastic emissions. Once in the environment, accumulating plastic could eventually cause ecological harm to species and habitats, and socio-economic harm due to potential human health risks, reduced recreational and aesthetic attractiveness, as well as income losses in the tourism and fishery sector (Galgani et al. 2013). Plastic items reported in seafood attracted attention not only of the scientific community and regulatory authorities, but also of the general public. When entering the environment durability, one of plastics' greatest assets becomes its curse. In contrast to natural materials plastic cannot be degraded easily (Amobonye et al. 2020, Yuan et al. 2020), still, its appearance will change. Exposure to UV radiation causes the plastic to get brittle, and physical abrasion due to wind and wave actions will cause the plastic to fragment into smaller pieces (Andrady 2011). These plastic items can be categorized by their size, shape, source, chemical composition, and density (SAPEA 2019). Most prominently is the definition based on size: all plastic items larger than 5 mm are defined as macroplastics, all items between 1 µm to 5 mm are microplastics (MP), and with a size smaller 0.1 µm plastics are defined as nanoplastics (NP) (Hartmann et al. 2019).

Special attention has been given to MP that can nowadays be found in any type and shape in, probably almost all, natural ecosystems (Arthur et al. 2009, Hurley et al. 2018). A first, semi- quantitative proof for NP in the environment has been given by Ter Halle et al. (2017) and its formation was proven experimentally by Lambert and Wagner (2016) and Gigault et al. (2016). Further, it has not yet been possible to quantify NP in environmental samples, which leads to a high degree of uncertainty about the extent of this problem.

Next to the size definition, MP are characterized into primary or secondary MP based on their source. Primary MP are small items of plastic that are purposefully manufactured, such as pre-production pellets and spheres used in cosmetics or in abrasive blasting (Lambert and Wagner 2018). Secondary MP, in contrast, are formed through the fragmentation of larger plastic items. As a result, these MP can have almost any shape or size. This class of MP are found predominantly in environmental samples (Hidalgo-Ruz et al. 2012). The different polymer types produced cause MP being further highly diverse.

1.2 Microplastic in the (aquatic) environment

The first studies reporting MP in marine surface waters date back to the early 1970s (Carpenter and Smith 1972, Colton et al. 1974). Thirty years later this topic gained again greater attention when several studies confirmed the presence of (micro)plastic in different marine ecosystems (Galgani et al. 2000, Moore et al. 2001, Thompson et al. 2004). The following years MP were identified in marine surface waters (Eriksen et al. 2013b, Law et al. 2010, Moret-Ferguson et al. 2010), coastal (Browne et al. 2011, Claessens et al. 2011) and deep sea sediments (Van Cauwenberghe et al. 2013), the polar region (Bergmann et al. 2017, Peeken et al. 2018) and in the stomachs of several marine species (Avery-Gomm et al. 2013, Markic et al. 2020, Murray and Cowie 2011, Rummel et al. 2016).

Assessing how plastics find their way into the environment, Derraik (2002) already indicated that "land-based sources provide major inputs of plastic debris into the oceans". Later it was estimated that even up to 80% of the marine (micro)plastics actually derives from terrestrial sources (Andrady 2011, GESAMP 2010, Rochman 2018). Although freshwaters, and rivers in particular, were expected to play an important role for the MP transport, it took relatively long before MP were confirmed in freshwater systems (Eriksen et al. 2013a, Free et al. 2014, Morritt et al. 2013). Regarding the presence of MP in riverine systems it is important to answer several questions, among others, what are the actual sources of MP, where in the river are MP found, and in which concentrations?

Within the hydrologic, natural water cycle, another one exists- this one can be referred to as the urban water cycle (Van Dijk et al. 2006). This smaller water cycle describes how humans get, use and treat water before it is released back into the environment (Figure 1-1). Traditionally waste water treatment plants (WWTPs) were built to remove organic carbon and nutrients from waste waters, but they can further reduce levels of harmful pollutants, e.g. MP.

The same applies to drinking water treatment plants (DWTPs) that remove pollutants to produce clean and safe drinking water. Assessing the possible entry points of MP contamination into the cycle, and the removal potentials of current water treatment technologies are main challenges before aiming to reduce this type of pollution.

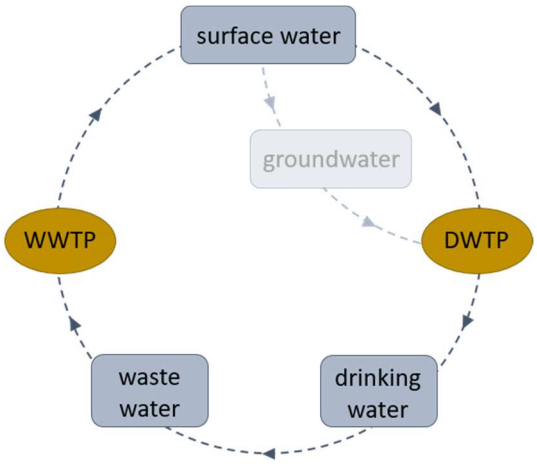

Figure 1-1 | The urban water cycle illustrates how water is used and treated by humans, via surface and groundwaters this cycle is connected to the natural hydrological water cycle (from: Van Dijk et al. (2006)).

Sources or pathways of MP into the aquatic environment, are expected to include MP particles or fibres released by WWTPs, runoff from urban areas and agricultural land, storm water overflows, the exhaust from industrial plants, and atmospheric deposition (Hurley and Nizzetto 2018, Lambert and Wagner 2018). The relative importance of these sources remains, however, unclear. In sediments and surface waters of large and small scaled rivers the presence of MP was confirmed by several field studies (Castaneda et al. 2014, Hurley et al. 2018, Kataoka et al. 2019, Klein et al. 2015, Mani et al. 2019a, Mani et al. 2019b, Morritt et al. 2013, Rodrigues et al. 2018, Vermaire et al. 2017, Watkins et al. 2019).

Reviewing studies on MP in aqueous samples from the urban water cycle, Koelmans et al. (2019) illustrated how widely reported concentrations can vary: in riverine surface waters reported number concentrations varied by five orders of magnitude. Regional differences might explain some of these differences. But also the different approaches to sample and analyse MP are expected to cause at least some degree of variation in the reported results. The subsequent section gives an overview of the most common approaches to determine MP in environmental samples.

1.3 Microplastic determination

Several reviews have illustrated the various methods applied to sample, extract and analyse MP in environmental samples (Figure 1-2) (Elkhatib and Oyanedel-Craver 2020, Hanvey et al. 2017, Hermsen et al. 2018, Hidalgo-Ruz et al. 2012, Klein et al. 2018, Koelmans et al. 2019, Li et al. 2018, Rocha-Santos and Duarte 2015, Shim et al. 2017).

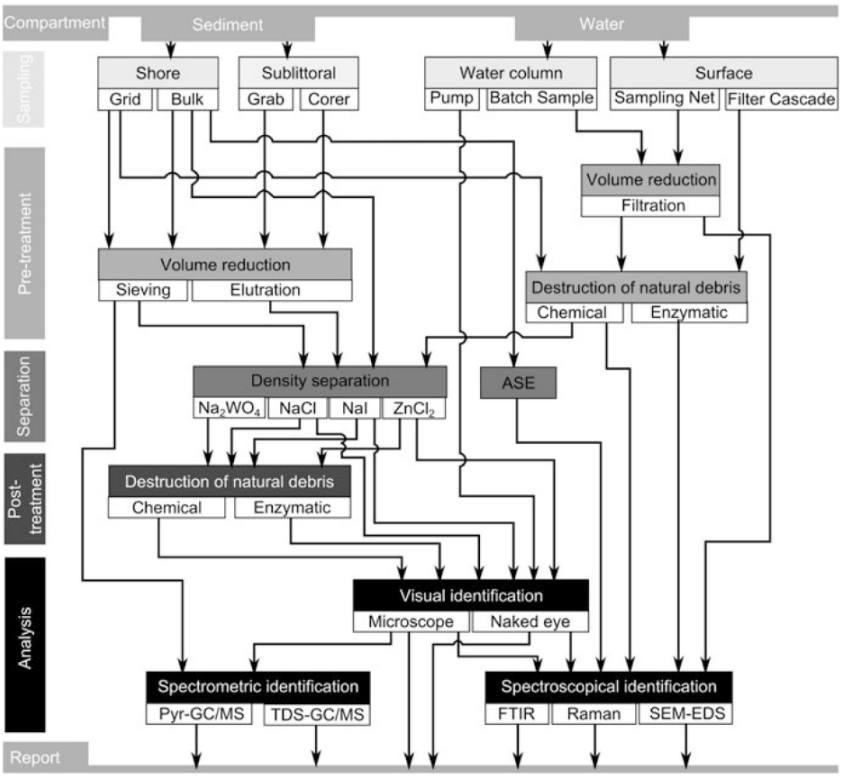

Figure 1-2 | Possible strategies described in literature to sample, extract and analyse MP in sediment and water samples, summarized by Klein et al. (2018).

Almost ten years ago Hidalgo-Ruz et al. (2012) already concluded that methods need to be standardized to be able to compare results of different studies. Instead, the number of methods increased in the past eight years; a trend not uncommon in a relatively young research field (Cowger et al. 2020). This analytical development is needed to increase the quality and accuracy of generated data. Significant analytical advances were made, for example, by the implementations of Focal Plane Array (FPA) based Fourier Transform Infrared (FTIR) microscopy to identify MP down to 20 µm in a relatively short time (Löder et al. 2015); by pyrolysis GC-MS to detect polymer mixtures (Fischer and Scholz-Böttcher 2017); by Raman spectroscopy opening the possibility to detect MP < 10 µm (Oßmann et al. 2018, Pivokonsky et al. 2018); or by the use of automated software tool to analyse results from FTIR microscopy

fast, reliable and accurate (Primpke et al. 2020, Primpke et al. 2017b). The next section discusses common approaches to sample, extract and identify MP, and how findings should be reported.

MP sampling Despite the methodological developments, the accurate identification of MP in environmental samples remains challenging: before the actual analysis and characterisation of MP can take place, a suitable sampling is needed that adequately represents the heterogeneously distributed MP. The sampling approach obviously depends mostly on the environmental matrices studied, and on the targeted MP sizes. Water samples are frequently taken by using surface nets, like manta trawls (Eriksen et al. 2013a, Lorenz et al. 2019, Mani et al. 2015). These nets cannot retain particles smaller than 300 µm, but they allow the sampling of large water volumes (>10 m^3) which specifically are needed when low MP concentrations are expected (Koelmans et al. 2019, Löder and Gerdts 2015). With decreasing size MP are expected to be more diverse and also more hazardous (Haave et al. 2019, Koelmans et al. 2015a), thus more and more studies are aiming in detecting smaller MP. Particles down to 5 to 20 µm can be retained when filtering water using filter cascades, stacked sieves (Carr et al. 2016, Dyachenko et al. 2017, Mintenig et al. 2020, Talvitie et al. 2015), or cartridge filters (Mintenig et al. 2017, Mintenig et al. 2019, Wolff et al. 2019). To sample NP, ultrafiltration (Ter Halle et al. 2017) and crossflow ultrafiltration (Mintenig et al. 2018) have been proposed.

MP extraction Samples typically contain relatively low numbers of MP and high loads of natural materials, such as organic detritus, plants, cellulose, sand, or clay. An appropriate sample preparation is thus essential to extract MP (Klein et al. 2018). Organic materials are removed by the addition of chemicals, such as acids (Claessens et al. 2013, Van Cauwenberghe and Janssen 2014), bases (Claessens et al. 2013, Rochman et al. 2015), hydrogen peroxide (Anderson et al. 2017, Mani et al. 2019b, Simon et al. 2018), or by the addition of enzymes (Cable et al. 2017, Löder et al. 2017, Mintenig et al. 2017). It is of high importance that this step does not affect the MP weights, counts and shapes (Koelmans et al. 2019). Inorganic particles are removed using their higher density by applying saturated sodium chloride (NaCl) (Fischer et al. 2016, Hoellein et al. 2017, Thompson et al. 2004), zinc chloride ($ZnCl_2$) (Lahens et al. 2018, Lorenz et al. 2019, Mintenig et al. 2020) or sodium iodide (NaI) (Karami et al. 2017, Ziajahromi et al. 2017). While NaCl is most cost efficient, the denser $ZnCl_2$ and NaI solutions enable higher extraction efficiencies.

MP identification Numerous studies relied on a purely visual determination of MP (Baldwin et al. 2016, Estahbanati and Fahrenfeld 2016, Kosuth et al. 2018). The misidentification rate, however, can be high and no information on the polymer types can be provided (Kroon et al. 2018, Löder and Gerdts 2015). The visual determination of particles > 300 µm is still feasible, but it should be completed by a subsequent polymer identification, using e.g. attenuated total reflectance (ATR)- FTIR (Koelmans et al. 2019, Mintenig et al. 2017). Any visual pre-selection for smaller particles should be avoided as error rates can be unacceptably

high (Shim et al. 2017). Instead the accurate identification for such small MP can be achieved using FTIR or Raman microscopy (Cabernard et al. 2018, Käppler et al. 2016, Löder et al. 2015, Mintenig et al. 2020, Wolff et al. 2019), or using spectrometric approaches (Dümichen et al. 2017, Fischer and Scholz-Böttcher 2017). In any case MP should be characterized in regard to their size, shape and polymer identity.

MP reporting Describing in detail the MP sizes, shapes and polymer identities enables a broad usability of the data. As an example, providing shape and polymer types of MP is required to pin-point possible sources (Haave et al. 2019). Knowledge on the MP sizes is required when assessing potential health or ecological effects (Koelmans et al. 2017a, Redondo-Hasselerharm et al. 2018). Although not yet clear, the latter might also depend on polymer type and shape (Kögel et al. 2020). The same could hold true for the association of bacterial assemblages (Amaral-Zettler et al. 2020, Frère et al. 2018) or the sorption of different chemicals (Hüffer and Hofmann 2016, Tourinho et al. 2019). Finally, providing such detailed information will also be beneficial when studying the environmental fate of MP (Kooi and Koelmans 2019). But not only the MP characteristics need to be described well. Due to the diverse methodologies applied to sample, extract and identify MP generated results lack reproducibility and comparability (Cowger et al. 2020, Klein et al. 2018). Hermsen et al. (2018) and Koelmans et al. (2019) provided a set of QA/QC criteria that need to be fulfilled and clearly documented to produce reliable data when examining MP in biota and water samples. Recently Cowger et al. (2020) proposed a detailed set of reporting guidelines, spanning from field work, to the reporting of raw data, and to toxicological considerations, to produce data enabling to answer large scale questions.

Only such a detailed reporting of MP findings in various ecosystems will increase our understanding on MP, by which it will get easier to assess the MP related risks accurately. Our current knowledge on these risks is summarized in the subsequent section.

1.4 Microplastic and its risks

The environmental pollution with plastic items is of growing societal concern, not only due to their persistency and accumulation in the environment, but also due to their potential negative effects on the ecological health (Kögel et al. 2020). The size of a plastic item determines if plastics can be hazardous to (aquatic) biota and to what extend bioaccumulation could take place. While macroplastics can cause entanglement or suffocation (Koelmans et al. 2017a, Rodriguez et al. 2013), MP, due to their small size, can be ingested by almost all levels of the trophic chain (Adam et al. 2019). Ingested MP might be excreted easily (Mazurais et al. 2015, Redondo-Hasselerharm et al. 2018), stay in the gastro-intestinal tract where it might accumulate and cause a reduced nutritional value of food (Au et al. 2015) or alter the exposure to plastic-associated chemicals (Bucci et al. 2020). Furthermore, the smallest plastics might even reach the circulatory system. Once ingested,

laboratory studies indicate that MP effects can be diverse and depend mainly on the exposure time, and the MP's concentration, size, and polymer types. Several studies reported an induced reduction of survival, growth and activity, as well as a higher physiological stress or an altered lipid metabolism (Kögel et al. 2020). Potential effects are also visible beyond the individual species level: in a long-term exposure study Redondo-Hasselerharm et al. (2020) demonstrated that MP and NP altered the composition of a macroinvertebrate community.

To assess if MP pose a risk to environmental communities one needs to compare data on actual MP exposure and data on MP ecotoxicity that stem from laboratory or mesocosm experiments. Until now, four studies provided a provisional risk assessment with estimates for the Predicted No Effect Concentration (PNEC), which is the threshold concentration at which no adverse effects for aquatic biota are expected to occur (Adam et al. 2019, Besseling et al. 2019, Burns and Boxall 2018, Everaert et al. 2018). Although the calculated PNECs vary by four orders of magnitude, all four studies conclude that these are higher than, so far, measured environmental MP concentrations. Burns and Boxall (2018) and Kögel et al. (2020), however, highlight that laboratory experiments often used smaller MP and in higher concentrations than found in the environment. At the same time experiments are based on much shorter exposure times (Bucci et al. 2020). Measured concentrations of MP < 20 µm, however, could possibly be higher because current analytical techniques cannot detect them. To tackle these, Koelmans et al. (2020) proposed an approach to align methods to improve our understanding of MP related risks in the environment. Still, more accurate data is needed to assess these hazards correctly.

The same holds true when investigating if MP (and NP) could have negative effects on the human health. Several studies indicate that humans are exposed to MP via different routes, including the inhalation of atmospheric MP (Dris et al. 2016, Gasperi et al. 2018, Vianello et al. 2019), and the consumption of MP in seafood (Barboza et al. 2018, Rochman et al. 2015, Van Cauwenberghe and Janssen 2014), salt (Fischer et al. 2019b, Yang et al. 2015), and also drinking water (Kosuth et al. 2018, Schymanski et al. 2018). As for all species, it is expected that NP are the most hazardous (Koelmans et al. 2015a). The consumption of MP might cause local immune responses or gut inflammation, NP in contrast, might also reach and penetrate organs, including placenta and brain (Bouwmeester et al. 2015). To assess the effects of MP and NP on the human health more accurate knowledge on exposure levels, via ingestion and inhalation, is thus needed (Prata et al. 2020, Rahman et al. 2020, Wright and Kelly 2017).

1.5 Thesis

1.5.1 Thesis objective

To be able to assess the impacts MP (and NP) could pose within the urban water cycle, they first need to be determined accurately. More information on where, how and in which concentrations MP are released, and how this relates to characteristics of WWTPs is needed. It also needs to be understood what types of MP and how they are transported once being in a river, and lastly, if the production of drinking water could be affected by MP and NP present in surface waters. Although numbers of studies on this topic are steeply increasing, existing information is still limited. Among others, this can be explained by sampling and analytical tools still being under development. Thus, the first part of this thesis addresses the requirements to reliably identify especially the small MP. Applying these criteria, the second part provides accurate data on MP in waste waters and riverine surface waters. In a third part, we assessed experimentally if current purification techniques could retain NP present in surface waters when producing drinking water. In the subsequent section the three parts of this thesis are motivated individually and concrete research questions are formed.

Requirements to reliably identify small MP

At the start of this thesis, sampling and analytical methodologies were still in the early development phase. Frequently, MP were determined purely visually, missing out information on polymer identities and on MP smaller than approximately 300 µm. Only two studies had applied FTIR microscopy to examine MP in WWTPs (Tagg et al. 2015) and in the sediments of the Lagoon of Venice (Vianello et al. 2013). Being especially interested in small MP, the main research questions are: Which analytical techniques can be applied to determine the size, and polymer types of individual MP and NP? Given the wide range of applied analytical methodologies; what are the key criteria that need to be fulfilled and reported to represent environmental MP reliably?

Occurrence and variability of MP in waste waters and riverine surface waters

Being still scarce today, data on MP in freshwater ecosystems were hardly available at the time this thesis started. It was widely assumed that the riverine transport of MP to the sea was significant. However, only a handful of studies had confirmed MP in large water bodies, while no data were published on MP in smaller streams. Further, suggestions on potential sources were made, but were not yet confirmed (Wagner et al. 2014). WWTPs were named a significant source or pathway for MP to the freshwater environment (Cole et al. 2011). However, their absolute contribution to a river had not been assessed properly. Even less knowledge was available on the relative contribution of WWTPs in comparison to other sources or in relation to MP already present in a river. The main research questions here are: Which exact types of MP can be detected in different WWTP effluents and in riverine surface waters? What are the absolute concentrations released by WWTPs, and what is their relative contribution to the MP load already present in a river? How does the riverine MP transport vary over time and over a river's length?

NP removal during drinking water production from surface waters

The third part addresses what impacts NP could have on the drinking water production. Few studies assessed the removal of MP when producing drinking water from ground and surface waters (Shen et al. 2020). It is highly likely that bigger MP are retained by the different purification techniques applied. To date NP has not yet been identified in freshwater systems, their presence in surface waters is, however, expected. Drinking water purification techniques are designed to remove bacteria and viruses from the water, it is thus likely that also NP are removed. This, however, has not yet been examined. The research question here: Could NP, potentially present in surface waters, be retained by the commonly applied drinking water purification techniques?

1.5.2 Thesis outline

As outlined above, the aim of this thesis is to improve our knowledge on MP present in the urban water cycle of the freshwater environment. The focus of this thesis is on riverine surface waters, treated waste waters and drinking water, the potential contamination of groundwater with MP is not considered (Figure 1-1). For this we will look specifically into following research question grouped into the three previously discussed fields of interest (Figure 1-3):

1. Which analytical techniques can be applied to determine the size, and polymer types of individual MP and NP? Given the wide range of applied analytical methodologies; what are the key criteria that need to be fulfilled and reported to represent environmental MP reliably?
2. Which exact types of MP can be detected in different WWTP effluents and in riverine surface waters? What are the absolute concentrations released by WWTPs, and what is their relative contribution to the MP load already present in a river? How does the riverine MP transport vary over time and over a river's length?
3. Could NP, potentially present in surface waters be retained by the commonly applied drinking water purification techniques?

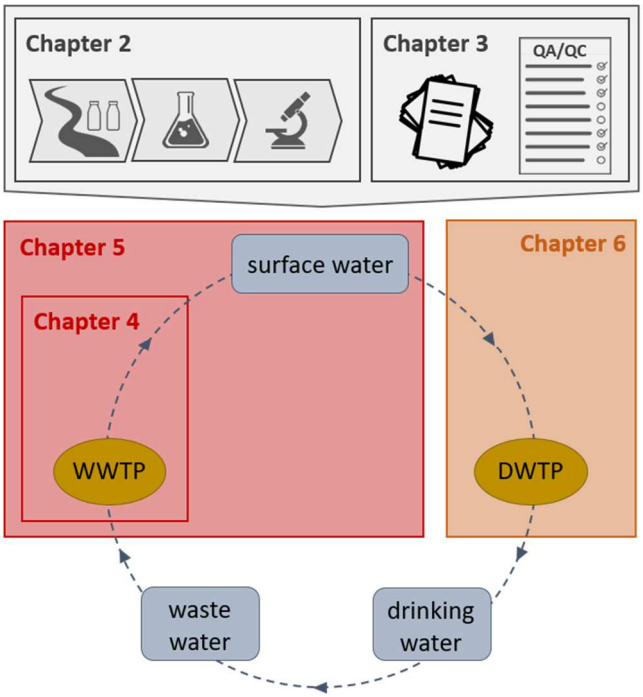

Figure 1-3 | Thesis outline illustrating how individual chapters correspond to the three substantive parts described in section 1.5.1.

After this general introduction (**Chapter 1**) this thesis is structured in three parts, consistent with the thesis' objectives (Figure 1-3). The first question, covering analytical aspects and requirements that need to be taken into account to reliably identify and report MP in environmental samples, is addressed in **Chapter 2** and **Chapter 3**. Applying these techniques and criteria, the second question is the starting point for two field studies examining MP in the effluents of WWTPs (**Chapter 4**) and in one Dutch river basin (**Chapter 5**). In **Chapter 6** the last question is addressed: By performing an experimental study we try to assess if the presence of NP in freshwater systems could pose a problem for the drinking water sector.

Chapter 2, titled 'Closing the gap between small and smaller: towards a framework to analyse nano- and microplastics in aqueous environmental samples', presents a framework able to consistently determine a broad spectrum of plastic particle sizes in aqueous environmental samples. Independently of the targeted MP size it is required to conduct (i) an appropriate sampling, and a subsequent identification of the actual MP (ii) sizes and (iii) polymer types. To achieve these three goals, different methods need to be applied for MP and NP. For MP down to about 20 µm we follow the most used approach that combines conventional filtration and FTIR microscopy. Further we show how this can be extended for NP by using crossflow ultrafiltration, followed by asymmetrical flow field flow fractionation (AF4) and Pyrolysis-GC-MS.

Chapter 3, titled *'Quality Criteria for the Analysis of Microplastic in Biota Samples',* presents a scoring system that was developed based on ten critical criteria to reliably determine MP in marine biota samples. This was done by critically reviewing and evaluating MP ingestion studies in aquatic biota, after which we propose a quality assessment method based on clearly defined criteria, and also apply this assessment method to the reviewed studies. Alongside, a standardized protocol is provided with the quality criteria incorporated to accurately detect MP in biota samples.

Chapter 4, titled 'Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging', contains the results of the first field study examining MP in the effluents of 12 WWTPs. Being aware of the quality requirements from Chapter 3, this was the first study applying an enzymatic-oxidative purification approach in combination with FPA-based micro-FTIR imaging to identify MP down to a size of 20 µm. Additionally, we provide data on the presence of MP in sewage sludge and on the removal capacity of an installed post-filtration unit.

Chapter 5, titled *'A systems approach to understand microplastic occurrence and variability in Dutch riverine surface waters',* pursues this work. Strictly following the defined quality criteria from Chapter 3, MP was identified in the effluents of WWTPs and in the surface waters of two Dutch river systems. This way the study addresses, firstly, the WWTPs' MP release relative to the amounts and types present in riverine surface waters and, secondly, how the presence of MP in a river is altered by spatial and temporal variations. It is the first study examining MP in riverine surface waters with FTIR microscopy and an automated image analysis, thus providing new, detailed insights.

Chapter 6, titled *'Nanoplastics removal during drinking water purification',* follows up the urban water cycle. The previous chapters have proven the presence of MP in riverine surface waters which are also used for the production of drinking water. Although not yet proven, it is widely assumed that also NP will be present in this water. Conducting bench-scale experiments we assessed the NP removal potential for three commonly used purification techniques, namely coagulation-flocculation-sedimentation (CFS), rapid sand (RS) filtration and granular activated carbon (GAC) filtration. Due to the relevance of drinking water these results provide important information when assessing exposure routes and potential human health impacts.

Chapter 7 is a summary and synthesis of all previous chapters. It includes a discussion on the current status of knowledge, the methodologies used to examine MP (and NP) in environmental samples, the reported concentrations of MP in freshwater systems and the potential of current water purification techniques to remove MP and NP. Based on the findings of this thesis recommendations for future research are provided at the end of this chapter.

CHAPTER 2

CLOSING THE GAP BETWEEN SMALL AND SMALLER: TOWARDS A FRAMEWORK TO ANALYSE NANO- AND MICROPLASTICS IN AQUEOUS ENVIRONMENTAL SAMPLES

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2018. Environmental Science: Nano 5, 1640-1649.

Abstract

Detecting nanoplastics and measuring concentrations and sizes of plastics in the environment are essential to assess the risks plastic particles could pose. Microplastics have been detected globally in a variety of aquatic ecosystems. The determination of nanoplastics, however, is lagging behind due to higher methodological challenges. Here, we propose a framework that can consistently determine a broad spectrum of plastic particle sizes in aquatic environmental samples. Analytical evidence is provided as proof of principle. FTIR microscopy is applied to detect microplastics. Nanoplastics are studied using field-flow-fractionation and pyrolysis GC-MS that gives information on the particle sizes and polymer types. Pyrolysis GC-MS is shown to be promising for the detection of nanoplastics in environmental samples as a mass of approximately 100 ng is required to identify polystyrene. Pre-concentrating nanoplastics by crossflow ultrafiltration enables polystyrene to be identified when the original concentration in an aqueous sample is > 20 µg L^{-1}. Finally, we present an approach for estimating polymer masses based on the two-dimensional microplastic shapes recorded during the analysis with FTIR microscopy. Our suite of techniques demonstrates that analysis of the entire size spectrum of plastic debris is feasible.

2.1 Introduction

A growing body of literature is documenting the widespread occurrence of plastic litter in various ecosystems (Cozar et al. 2014, Law et al. 2014, Mani et al. 2015) and its ecological consequences (Jeong et al. 2016, Kühn et al. 2015). Considerable attention has been given to microplastics (MP): plastics smaller than 5 mm (Arthur et al. 2009, Verschoor 2015). MP and the much smaller particles usually referred to as 'nanoplastics' (NP) can be released to the environment directly (Hernandez et al. 2017, Koelmans et al. 2015a) or can be formed when larger plastic items degrade and fragment under the impact of various environmental stressors (Gewert et al. 2015, Gigault et al. 2016, Lambert and Wagner 2016). The actual fragmentation processes are unknown and currently under research (Song et al. 2017, Weinstein et al. 2016). However, it is widely assumed that the fragmentation into small MP and eventually into NP is one of the explanations for the 'missing plastic' budget, a term defined by Cozar et al. (2014), who detected lower MP concentrations in the open ocean surfaces than predicted by their model. Recent experimental, modelling and field studies further support this hypothesis (Gigault et al. 2016, Koelmans et al. 2017b, Lambert and Wagner 2016, Song et al. 2017, Ter Halle et al. 2017).

MP has been studied and detected globally in almost all natural habitats, but no lower size limitations or sub-classes have been officially defined. Yet the term 'nanoplastic' is widely used, but interpreted differently. Here, we primarily acknowledge the formal definition of a nanomaterial by the EU (2011/696/EU) (Koelmans et al. 2015a, Mattsson et al. 2015), according to which at least 50% of the particles must have at least one dimension smaller than 100 nm. Other studies define NP as plastic particles < 1µm (da Costa et al. 2016, Lambert and Wagner 2016, Ter Halle et al. 2017) or even < 20 µm (Wagner et al. 2014).

There are currently several protocols for detecting MP (Filella 2015), but they lack consistency in sampling, sample pre-treatment, analysing and reporting of results. The analysis of NP is more elaborate, and protocols are currently under development (Ter Halle et al. 2017). One of the major challenges is the pre-concentration of samples required to match the detection limits of currently available instrumentation. The aim of the present paper is twofold. First, we aim to provide a framework for quantitatively analysing NP and MP that is based on three criteria: (a) a sampling strategy to reproducible concentrate plastic particles of targeted sizes, (b) the determination of particle sizes and (c) the identification of polymer types. Second, we aim to provide empirical data on the applicability of novel steps in the proposed framework.

2.2 A framework for the analysis of nano- and microplastics in aqueous environmental samples

In order to concentrate MP and NP for a representative analysis, starting with an appropriate sampling strategy is of high importance. The protocol used most widely today entails filtering surface water through nets with a mesh size of 333 µm (Eriksen et al. 2013a, Hidalgo-Ruz et al. 2012, Law et al. 2014, Mani et al. 2015). The size of smaller particles retained is 25 to 45 µm when water is filtered through a stack of sieves (Carr et al. 2016, Ziajahromi et al. 2017), and 10 µm when stainless steel cartridge filters are used (Mintenig et al. 2017). Sampling NP is more challenging as conventional filtering is not applicable in these low size ranges. Ter Halle et al. (2017) used ultrafiltration to concentrate the colloidal fraction (< 1.2 µm) of a 1 L seawater sample. Another concentration technique is crossflow ultrafiltration, which uses a filter originally made as dialysis equipment (Hemoflow, Fresenius Medical Care, Germany). This crossflow ultrafiltration setup has been applied successfully to concentrate microorganisms in drinking and surface waters by factors of 4000 and 1000, respectively (Veenendaal and Brouwer-Hanzens 2007).

To date, a variety of analytical techniques has been applied to determine MP in environmental samples. Numerous studies have relied on visual sorting of MP of a few hundred µm micrometres in size (Mani et al. 2015, Nuelle et al. 2014). In recent years, the scientific focus has shifted from determining visible plastic particles to determining microscopic plastic particles, usually using spectroscopic (Imhof et al. 2016, Käppler et al. 2015, Löder et al. 2015) or thermal degradation analyses (Dümichen et al. 2017, Fischer and Scholz-Böttcher 2017, Majewsky et al. 2016). When coupled to a microscope, Fourier transform infrared (FTIR) or Raman spectroscopy reveals the chemical identity of particles and allows the estimation of individual particle sizes and shapes. However, both techniques are limited by particle size: 500 nm for Raman microscopy (Käppler et al. 2015) and 20 µm for FTIR microscopy (Löder et al. 2015). In contrast, thermal degradation analyses are not limited by size when analysing mixed environmental samples, but also, they do not provide information on particle sizes. Recent studies have used thermal degradation to identify polymer mixtures in surface water (Ter Halle et al. 2017), soil (Dümichen et al. 2015, Dümichen et al. 2017), fish (Fischer and Scholz-Böttcher 2017) and wastewater treatment plant effluents (Majewsky et al. 2016).

A major problem arising from using such different techniques is the incomparability of data (Cannon et al. 2016, Filella 2015, Song et al. 2015, Twiss 2016). Manual particle sorting or spectroscopic analyses yield numbers of MP particles or fibres, whereas water volumes (Carr et al. 2016, McCormick et al. 2014), surface areas (Collignon et al. 2014, Law et al. 2014), sediment weight (Claessens et al. 2011, Vianello et al. 2013) and suspended particulate matter weight (Leslie et al. 2017) are presented in metric units. A bigger problem occurs when comparing these data with data from thermal degradation procedures that aim to

simultaneously identify and quantify polymers (Dümichen et al. 2017, Fischer and Scholz-Böttcher 2017) per sample volume or weight. Eventually, exposure data are needed that can be linked to results generated during effect studies. And as the hazards posed by MP and NP are likely to depend on the concentration, size (Bouwmeester et al. 2015, Jeong et al. 2016) and potentially on polymer types, these data are of high interest (Koelmans et al. 2015b, Redondo-Hasselerharm et al. 2018). Information on polymer masses will be required to enable mass-balance models that link production and emission data to environmental occurrence data (Koelmans et al. 2017a, Koelmans et al. 2017b).

Given that plastic debris comes in a broad spectrum of sizes, its identification requires a combination of different sampling techniques (criterion a) and analytical techniques to determine sizes (criterion b) and polymer types (criterion c) (Figure 2-1). The sequence of the techniques, and their relationships, are shown also in a flow scheme (Figure 2-S1). In addition to conventional filtration to concentrate MP, we introduce crossflow ultrafiltration to concentrate NMP (nano- and microplastics <20 µm) prior to analysis. For NMP analysis two techniques are needed: Asymmetrical Flow Field-Flow Fractionation (AF4), which is a versatile tool for sample fractionation based on particle sizes (Gigault et al. 2017), in combination with pyrolysis gas chromatography–mass spectrometry (GC-MS), to identify polymers in size fractions collected individually. Here, a filtration step is essential since the particle size separation of the AF4 occurs in two modes: in the 'normal' mode, increasing particle sizes lead to an increased retention, whereas this is reversed for bigger particles in the so-called 'steric' mode. The sizes and polymer types of MP particles exceeding 20 µm are identified with micro-FTIR (Figure 2-1). Manual sorting and subsequent identification of MP becomes feasible for plastics bigger than 300 µm; thus this common procedure (Filella 2015, Law et al. 2014, Mani et al. 2015) completes the proposed protocol.

The framework has several components new to this field of research that we have tested individually and in combination. These tests are presented below and comprised (a) sampling surface and drinking water by concentrating them using crossflow ultrafiltration, including the determination of recovery rates, (b) NMP size determination using AF4 and (c) polymer identification of NMP using pyrolysis GC-MS.

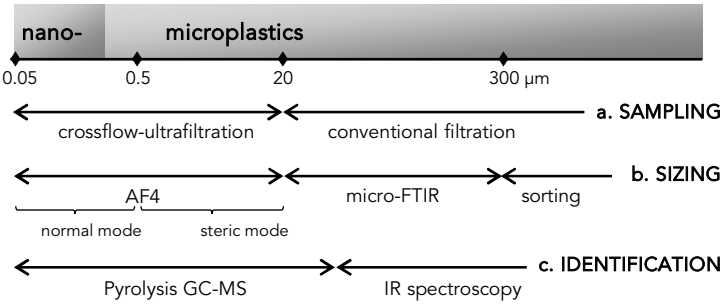


Figure 2-1 | Protocol applied to: (a) sample; and detect sizes (b) and identify polymer types (c) of nano- and microplastics in an environmental aqueous sample.

2.3 Materials and Methods

2.3.1 Materials and instrumental setup

Chemicals Monodispersed NP suspensions of polystyrene (PS) spheres with specified diameters (50, 100, 200, 500 and 1000 nm) and uncharged surfaces were purchased from Polyscience Inc. (Illinois, USA). Monodispersed gold and silver nanoparticles (50 nm) in solutions with a citrate- based agent were purchased from NanoComposix (California, USA). Green fluorescent MP polyethylene (PE) beads in sizes ranging from 90 to 106 µm were purchased from Cospheric (California, USA). To facilitate dosing, these PE beads were suspended in ultrapure water containing a surfactant (0.01% sodium dodecyl sulphate (SDS), Sigma Aldrich), which yielded a final concentration of 260 mg (5 x 10^5 particles) L^{-1}. To determine MP number concentrations, the solutions were filtered through cellulose nitrate filters (0.45 µm Whatman, Germany) and PE beads were counted using a dissecting microscope (Zeiss STEMISV8, Germany). Further, transparent PS pellets were cooled with liquid nitrogen, ground and sieved over an installed 100 µm mesh (Retsch Centrifugal Grinding Mill ZM1000, Germany).

Crossflow ultrafiltration To increase MP and NMP concentrations we used a crossflow ultrafilter (Hemoflow filter HF80S, Fresenius, Medical Care) consisting of bundled hollow fibre membranes made of polysulfone that had an inner diameter of approximately 200 µm. The exact pore sizes were not specified, but the cut-off was defined for proteins sized between 40 and 60 kDa. Samples were pumped (Masterflex, Cole Parmer, USA) through the crossflow ultrafilter at a constant flow rate of 4 L min^{-1} and an overpressure of 0.4 bar. Thereby the permeate was pressed through the filter while the concentrate was retained and rinsed back into the tank, raising particle concentrations (see Figure 2-S2).

AF4 An AF4 system was used (Postnova Analytics GmbH, AF2000, Landsberg, Germany), coupled online to a UV detector (Shimadzu) and a multi-angle light-scattering (MALS) detector (Postnova, Landsberg, Germany). The trapezoidal channel was 27.5 cm long and 250 µm thick. There were two membranes, 10 kDa regenerated cellulose (RC) and 10 kDa polyethersulfone (PES) (Postnova, Landsberg, Germany), and three carrier liquids: ultrapure water (> 18 MΩ), a solution containing an anionic surfactant (0.01% SDS, Sigma Aldrich) and a solution containing a non-ionic surfactant (0.01% TWEEN, Sigma Aldrich) surfactant (see Table 2-S1). The fractionation and presence of particles were recorded by the MALS detector. Plotting the detection signal against the fractionation time, the area under the curve (AUC), proportional to the particle concentrations injected, was determined using GraphPad Prism (5.01, GraphPad Software, San Diego California USA). Further information on the general ability and limitations of the AF4 to separate particles can be found elsewhere (Gimbert et al. 2003, Messaud et al. 2009).

Pyrolysis GC-MS Polymers in environmental samples were analysed using pyrolysis GC-MS. The samples were pyrolysed at 560 ºC (Pyromat, GSG Mess- und Analysegeräte, Germany) in a tubular pyrolysis wire with a capacity of approximately 15 µL. The instrumental details for pyrolysing a sample are provided as Supplementary Information (Table 2-S2). The degradation gases were separated using a GC (Trace GC, ThermoFisher Scientific, Madison, USA) and identified using an MS system (Trace MS Plus, ThermoFisher Scientific, Madison, USA). The settings of the GC-MS system are shown in Table 2-S3. Generated pyrograms, peak intensities and polymer characteristic mass-to-charge (m/z) ratios were analysed using the software XCalibur (Thermo XCalibur 2.2 SP1.48, ThermoFisher Scientific, Madison, USA). Individual compounds were searched within a library of organic compounds (NIST/EPA/NIH MS Library (NIST 11), USA) and an in-house generated library.

Micro-FTIR An FTIR microscope equipped with an ultra-fast motorized stage and a single mercury cadmium telluride (MCT) detector (Nicolet iN10, ThermoFisher Scientific, Madison, USA) was used to identify MP, using chemical mapping. This entailed enrichment of the samples on aluminium oxide filters (Anodisc 25 mm, Whatman, UK) placed on a calcium fluoride (CaF_2) crystal (EdmundOptics, Germany) to prevent filter bending. All measurements were taken in transmission mode (Löder et al. 2015). Polymers were identified with the aid of the "Hummel Polymer and Additives FTIR Spectral Library" (ThermoFisher Scientific, Madison, USA). The spectra and chemical maps generated were analysed using Picta software (1.5.120, ThermoFisher Scientific, Madison, USA).

Samples To test the individual techniques that make up the framework, samples of drinking and surface water were spiked with different monodispersed plastic particles. The drinking water was tapwater from Nieuwegein; the ultrapure water was obtained by purifying demineralized water in a Milli-Q system (Millipore, MA, USA). The surface water samples were from two freshwater systems in the Netherlands: the Lek canal and Lake IJssel. The Lek canal was sampled in April 2016 using a stainless steel bucket. Surface water of Lake IJssel was sampled using crossflow ultrafiltration (Figure 2-S2) in January 2016. Using a water standpipe at an official sampling point, we obtained surface water pumped from a depth of 0.5 m by placing a small stainless steel cask with a volume of approximately 20 L under the open tap and allowing it to fill with water. The volume of water was maintained at a constant level by means of a float valve that allowed more water to be pumped into the cask automatically when the level fell. This allowed the concentration process to proceed unsupervised for 24h. During this time, 635 L surface water were filtered and concentrated into a volume of 0.4L. Contamination with plastic particles was minimized by using tubes rinsed with ultrapure water and by covering the tank with aluminium foil. Subsequently, the Lake IJssel sample was filtered through a 20 µm stainless steel sieve, the retentate was treated with 1 M sodium hydroxide (NaOH, 3 days, 50 °C, similar to (Dehaut et al. 2016). During sample handling cotton lab coats were worn at all times and the sample was kept covered whenever possible. Further controls and blanks could be omitted because spiked particles were used.

2.3.2 Testing the analytical framework using spiked environmental samples

A) Sampling Crossflow ultrafiltration was further validated by adding NP (PS 50 and 200 nm) or MP (PE 90-120 µm) to drinking water samples. The drinking water came directly from the tap and was not filtered before usage. For both plastic types, three 100 L samples were concentrated into final volumes of 0.5 L. Further, one sample of pure drinking water was filtered and used as a blank. For MP, the starting concentration was 2.6 µg (5 particles) L^{-1}. For NP, 0.4 mg L-1 PS (50 nm) and 0.585 mg L^{-1} (200 nm) PS were added. Standard suspensions with particle concentrations 200 times higher than indicated concentrations were produced and used to determine NP recovery rates. Pre-concentration was done as follows: The 100 L samples were distributed among five jerry cans (20 L, HDPE) and pumped through the crossflow ultrafilter. Each jerry can was thoroughly rinsed with ultrapure water and ethanol (30%). After two hours the concentrate was collected in a glass jar, and the tubes and filters were rinsed twice by pumping 150 mL of collected permeate through the filter. The MP beads were counted using a dissecting microscope and the numbers compared to the originally admixed concentrations. The NP samples and standard suspension were analysed in quadruplicate using AF4-MALS, and the AUCs were determined. Because this AUC is proportional to a NP concentration range of 0.1 to 140 mg L^{-1} ($R^2 > 0.99$), it was used to evaluate the NP recovery. In addition to AF4-MALS measurements, all NP samples were re-analysed using spectrophotometry (UNICAM UV 500, ThermoSpectronic). The UV absorbance was measured at 229 nm wavelength, at which PS in ultrapure water shows the highest absorption. The system was calibrated for PS concentrations between 4 to 23.6 mg L^{-1}, resulting in a linear increase of measured absorbance ($R^2 > 0.99$). The UV absorbance of the concentrated crossflow samples was measured after samples had been diluted with ultrapure water (1:10) and ultrasonicated for five minutes to prevent erroneous measurements arising from aggregation.

B) Size determination To detect the sizes of plastics accurately, different techniques were used. For MP, the two-dimensional shape (maximum and minimum diameters) of individual particles can be assessed during chemical mapping by using micro-FTIR, as will be explained in the following section. More challenging is the size determination for NMP; although AF4 is a powerful technique for separating a variety of nanoparticles, it needs to be adapted for the particles of interest (Gigault et al. 2017). First, two membranes, RC and PES, were tested in combination with different carrier liquids: ultrapure water, or a solution containing an anionic (SDS) or a non-ionic (TWEEN) surfactant. These surfactants were added to reduce particle–membrane interactions that could cause erroneous results. Each combination was evaluated using the data recorded by the MALS detector. We tested for distinct signals by injecting monodispersed NMP suspension (50 and 500 nm, 50 mg L^{-1}, injection volume of 30 µL). To test for complete size separation we injected a mixture of 50, 100, 200, and 500 nm spheres (each 200 mg L^{-1}, 20 µL). The settings to run the AF4 system are presented in Table 2-S1; using these, the elution times of the various NMP sizes were recorded. In a second

step, a monodispersed suspension of 1000 nm spheres (200 mg L^{-1}, 10 µL) was injected to determine elution time and signal intensity recorded by the MALS detector. A new mixture of all five NMP sizes was analysed under different crossflow conditions (0.5, 1, 2, 3, 4 mL min^{-1}, Table 2-S1) to test if a simultaneous separation might be feasible or if there had been a transition from the "normal mode" to the "steric mode". This was done because previous studies have shown that this transition occurs for particle sizes of about 1 µm (Dou et al. 2013, Gigault et al. 2017). The MALS detector provides data on the particles' radii. For a concentration range for particles of 50 and 200 nm (100–0.1 mg L^{-1}, 50 µL) it was determined when discernible peaks were detected compared to the baseline and when the particle sizes given by the MALS detector matched the supplier's specifications. NPs are made of polymers with different densities. To test the effect of different densities on the elution times of particles, we injected monodispersions of 50 nm PS, gold and silver nanoparticles.

C) Polymer identification The final polymer characterization was also conducted using two techniques, Pyrolysis GC-MS for NMPs and micro-FTIR for MP. Pyrolysis GC-MS was used to determine the presence of polymers in size fractions previously separated by AF4. Lek canal and Lake IJssel surface waters were examined using pyrolysis GC-MS. To do so, pyrolysis tubes were filled with 12.5 µL sampled water, and the water evaporated at 60ºC. This step was repeated resulting in a total sample volume of 25 µL. The sample from the Lek canal was tested solely for PS (200 nm) that had been added at concentrations of 0.6 mg L^{-1} (mimicking the status before crossflow ultrafiltration), 117 mg L^{-1} (after crossflow ultrafiltration) and 1200 mg L^{-1}, resulting in PS masses of 15 ng, 3 µg and 30 µg within the sample volumes of 25 µl. These tubes were pyrolysed several times (Table 2-S2) to ascertain whether full material pyrolysis occurred and, if so, when. The analysis focussed on characteristic PS degradation products: styrene (mass 104) and tristyrene (mass 312) (Fischer and Scholz-Böttcher 2017). Fischer and Scholz-Böttcher (2017) showed that the more abundant styrene is non-specific, since it is also produced when chitin is pyrolysed. In contrast, the tristyrene is less abundant, but specific for the presence of PS. Finally, PS was added to organic rich Lake Ijssel sample yielding in PS concentrations of 1 to 20 mg L^{-1}. Pyrolysis tubes were filled with 25 µl of these solutions, and thus contained 25 to 500 ng PS. The limit of detection (LOD) was determined based on an S/N ratio of 3; the limit of quantification (LOQ) was assessed considering an S/N ratio of 10.The second technique used was micro-FTIR to identify MP. In order to measure MP down to 20 µm in a feasible time frame, when using micro-FTIR equipped with a single MCT detector, we tested filter surface chemical mapping at two spectral and spatial resolutions. For all measurements, the aperture size was set at 50 x 50 µm. The spatial resolution, i.e. the step sizes between measurement points, was set at 20 or 35 µm. In combination with the changed step sizes, we tested a spectral resolution of 8 cm^{-1} with four scans per point and of 16 cm^{-1} with one scan per point (ultra-fast mapping option). To do so, PS fragments (9 to 90 µm) were spread on an Anodisc filter. The area of the mapped filter area covered with PS as well as the particle numbers were determined using Picta software.

2.4 Results

2.4.1 NMP recovery using crossflow ultrafiltration

The recovery rates of NMP and MP particles were evaluated after concentrating drinking water samples by crossflow ultrafiltration. The three samples revealed an MP recovery of 50.2% (± 11.9). The NMP samples were analysed using AF4-MALS and spectrophotometry and both methods yielded a reproducible NMP recovery (Figure 2-2, Table 2-1). Spectrophotometry yielded a total NMP (50 and 200 nm PS) recovery of 54.0% (± 2, n=3).

Table 2-1 | Recovery of NMP (measured with AF4-MALS and UV-Vis spectrophotometry) and MP after concentrating 100 L of drinking water with crossflow ultrafiltration.

		Initially added plastics	Recovery (%)	SD
NMP	AF4	50nm (0.4 mg L^{-1})	12.7	1.3
		200nm (0.585 mg L^{-1})	49.3	3.7
		50 + 200nm	48.6	3.6
	UV	50 + 200nm	54.0	2.0
MP		(2.6 µg L^{-1})	50.2	11.9

During AF4 separation the MALS detector revealed that the peak of the 200 nm spheres was less intense, broader and lagged behind after crossflow concentration. The 50 nm NP hardly peaked (Figure 2-2). The recovery rates calculated using the AUCs were 49.3% (± 3.7, n=3) for the 200 nm particles and 12.7% (± 1.3, n=3) for the 50 nm spheres, which together makes a total NMP recovery of 48.6% (± 3.6, n=3) (Table 2-1). The values are within the error ranges of the measurements of the total recovery determined earlier. Further, the MALS detector specified average radii of 115 nm (± 1.5, n=125) and 53 nm (± 2.4, n=28) for the concentrated samples and of 111 nm (± 0.5, n=73) and 67 nm (± 1.9, n=19) for the standard suspension analysed (Figure 2-2). The variations might be attributable to matrix effects yet suggest that homo-aggregation during the concentration was not relevant.

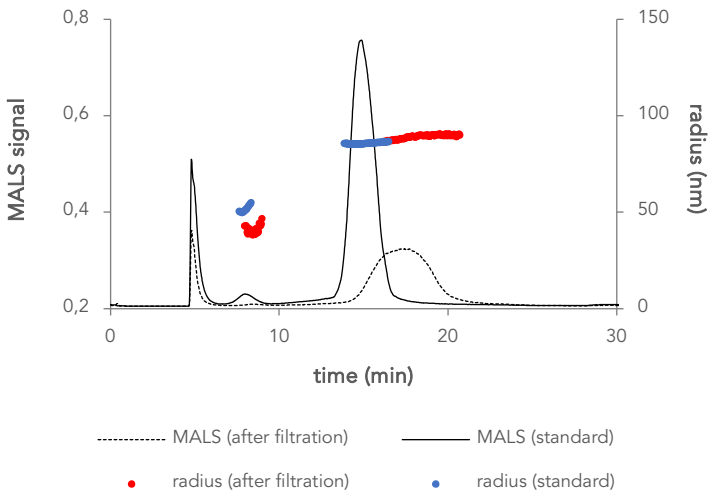


Figure 2-2 | MALS signal and NMP radii in drinking water after crossflow ultrafiltration and in the standard suspension containing calculated target concentrations.

2.4.2 Size determination of NMP using asymmetrical Flow Field-Flow Fractionation (AF4)

First, two membranes, RC and PES, were tested in combination with different carrier liquids. As only the RC membrane and the 0.01% SDS solution led to distinct peaks and a satisfactory size separation (Table 2-S4), this combination was chosen for further tests. A complete size separation of PS spheres in a polydispersion (50, 100, 200 and 500 nm) was possible. Although the 200 and 500 nm peaks were close, they were still distinguishable (Figure 2-3A).

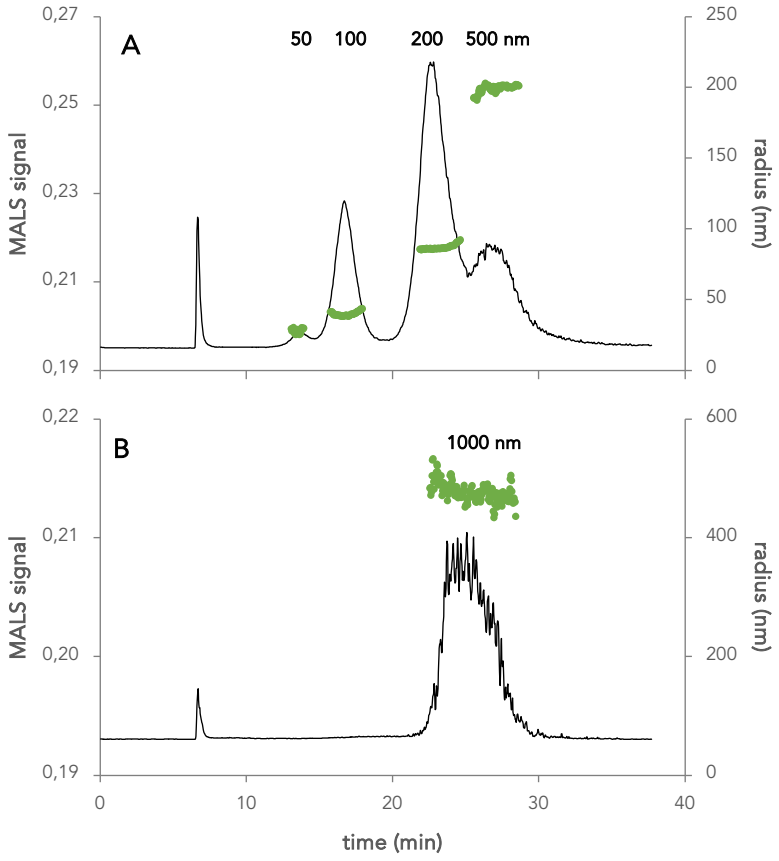


Figure 2-3 | MALS signal (black line) and NMP radii (green dots) when analysing (A) a polydispersion of 50, 100, 200 and 500 nm spheres and (B) a monodispersion of 1000nm sphere.

In a second step, a monodisperse suspension of 1000 nm spheres (200 mg L^{-1}, 10 µL) was injected. These particles had a similar elution time as the 200 and 500 nm spheres under crossflow conditions of 2 mL min^{-1} (Figure 2-3B). A new mixture of these five NMP sizes was analysed under different crossflow conditions (0.5, 1, 2, 3, 4 mL min^{-1}, Table 2-S1) but none of these could fractionate particles of 1000 nm successfully, which implies that transition from the "normal mode" to the "steric mode" occurred, and that prior to analysis, particles larger than 500 nm need to be removed by filtration. The scope of the present study did not allow a further, detailed evaluation of particle fractionation in the steric mode.

The MALS detector indicated average particle sizes of 72 nm (± 4.3, n=23), 103 nm (± 3.8, n=60), 225 nm (± 4.5, n=76) and 514 nm (± 7.1, n=85) (Figure 2-3A), and further 1233 nm (± 41.7, n=161) (Figure 2-3B), which fairly matches the characteristics of originally injected spheres. For particles of 50 and 200 nm (100 – 0.1 mg L^{-1}, 50 µL) the concentration range was determined where distinguishable peaks were detected and where particle sizes were in accordance with the supplier's specifications. The particles of 200 nm were still detected correctly at a PS concentration of 1 mg L^{-1}, but not at a concentration of 0.5 mg L^{-1}. For particles of 50 nm the detection limit was between 5 and 10 mg L^{-1}. In combination with pre-concentration using crossflow ultrafiltration, these LODs would further decrease by 200 times, resulting in values between 5 and 50 µg L^{-1}.

Lastly, to test the effect of different particle densities on the elution times of particles, monodispersions of 50 nm PS, gold and silver nanoparticles were injected. Using the same settings, the particles eluted at the same time (Figure 2-4), indicating that different polymer densities will not hinder a satisfactory size fractionation of NMP.

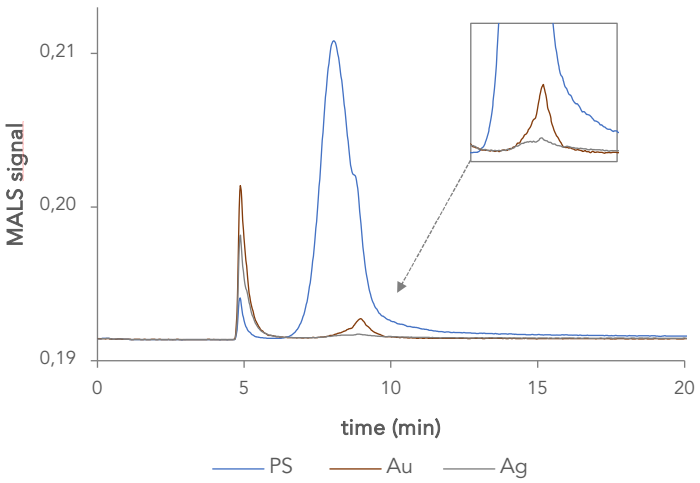


Figure 2-4 | MALS signal revealed similar elution times for nanoparticles (50 nm) made of PS, gold and silver.

2.4.3 Identification of NMP using Pyrolysis GC-MS

First, samples from the Lek canal with added PS masses of 15 ng, 3 µg and 30 µg were analysed and examined for the presence of the styrene (mass 104) and tristyrene (mass 312) (Figure 2-S3). Compared with the values detected for 3 µg PS, the styrene intensity for 30 µg PS was ten times higher but the tristyrene intensity was only twice as high, indicating that pyrolysis of the material was incomplete. Although this does not hamper polymer identification, it might hamper a quantification with one run.

Secondly, pyrolysis tubes containing masses of 25 to 500 ng PS in organic-rich surface water were analysed to ascertain the LOD and LOQ of this method. The styrene was detected in all pyrolysis tubes with lower PS concentrations (Figure 2-S3). The tristyrene was identified for PS of at least 100 ng (S/N ratio of 7). As tristyrene is specific for PS, the analysis should focus on this compound, which will result in an LOD between 50 and 100 ng and an LOQ between 100 and 250 ng for environmental samples. Under the given settings and pyrolysed volumes of 25 µL, an LOD of 4 mg L^{-1} and an LOQ of 4–10 mg L-1 were assessed.

2.4.4 Identification of MP using Micro-FTIR

Using different spectral and spatial resolutions during chemical mapping yielded slightly varying PS-covered areas and particle counts between the step sizes of 20 µm (29 particles and 9.4%; 32 particles and 9.2%) and 35 µm (25 particles and 9.3%; 28 particles and 10.6%). Step sizes of 20 µm were preferred since we aimed to detect small MP for which information would be lost if step sizes were bigger. Further, the smaller step sizes allow a more precise determination of sizes and numbers for particles that lie close to each other. Both spectral resolutions yielded spectra of sufficient quality to identify polymer types. We used the lower spectral resolution for further measurements, since it required shorter measuring times.

Based on data generated during micro-FTIR analysis we estimated polymer masses. This is based on the length (l), width (w) and depth (d) of the particles and their density. While the two-dimensional shape of each particle (l x w) can be assessed from the micro-FTIR data, the third dimension (d) cannot be measured. However, we can assume that the particles will prefer a 'flat' position on the filter, implying that the unknown third dimension (d) will be the smallest of the three. Consequently, it can be assumed that particles on average have a third dimension which is half of the second dimension. This assumption will become accurate when the number of particles is sufficiently large.

2.4.5 Evaluation of the proposed framework

Several techniques are needed in order to determine a wide size range of plastics. The framework we present makes it possible to concentrate NMP and MP, and to identify and quantify the sizes and polymer types of various NMPs and MPs in an aqueous environmental matrix. During this study, individual techniques were tested that proved to be promising for application in this field of research (Figure 2-5). The approach is in parts comparable to the one presented recently by Ter Halle et al. (2017) who sampled plastic of various sizes in the North Atlantic. They applied micro-FTIR for MP detection > 25µm and a combination of dynamic light scattering (DLS) and pyrolysis GC-MS to identify plastics <1.2 µm.

Compared to the techniques' theoretical size constraints as presented in Figure 2-1, only slight adaptions needed to be made (Figure 2-5). Using a micro-FTIR that is not equipped with an advanced focal plane array detector which can measure several pixels at the same time (Löder et al. 2015, Tagg et al. 2015) we suggest mapping the surface of a filter in steps of 20 µm at a reduced spectral resolution. This enables MP down to 28 µm to be determined. To assess polymer masses from the generated results, we propose a particle shape analysis. Although based on an assumption about the particles' third dimension, this approach offers a solution for combining MP and NMP data not only within the framework presented, but also in studies in general.

NMP particles are examined using a combination of AF4-MALS and pyrolysis GC-MS. The AF4-MALS was tested and the settings optimized to allow NMP between 50 and 500 nm to be separated. Based on these settings and depending on the particle sizes, the coupled MALS detector detected particle sizes for PS concentrations of 1–10 mg L^{-1}. In our approach, the AF4-MALS sample fractionation is based on previously determined elution times and thus, is not concentration-dependent. Subsequently, individual fractions are analysed using pyrolysis GC-MS. Although there is no size limitation, a minimum of approximately 100 ng is required to guarantee the detection of PS in an environmental matrix. Based on the analysed sample volume of 25 µL, a concentration of 4 mg L^{-1} PS would be required.

To decrease the LODs, particles need to be concentrated during sampling. To do so, we introduced crossflow ultrafiltration and determined that NMP were recovered reproducibly for sample volumes of 100 L. At a concentration factor of 200, the LOD for originally present particles would decrease to 20 µg L^{-1}. Recommendations for addressing the remaining "gaps" in the NMP – MP size continuum (dashed lines, Figure 2-5) of the proposed framework are discussed below.

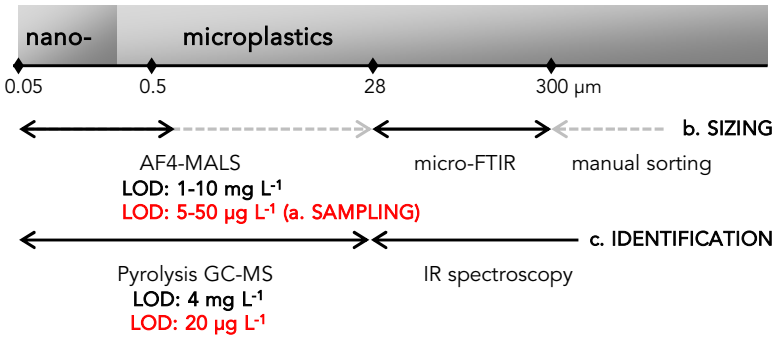


Figure 2-5 | Overview of techniques applied, showing respective size and concentration limitations. Using a crossflow filter, particles were concentrated by a factor of 200, which further decreased the LODs.

2.5 Discussion

2.5.1 Closing the gap between small and smaller

The field of micro- and nanoplastic research is relatively young, implying that methods are still under development. So far, the use of FTIR or Raman microscopy has been favoured for the examination of MP at micrometre sizes. Although these techniques enable sizes, shapes and polymer types to be detected simultaneously, they have shortcomings regarding detectable particle sizes, their semi-quantification and their long measurement and data analysis times. As previous studies (Löder et al. 2015, Ter Halle et al. 2017) have noted, it is preferable to analyse whole samples, especially if they are very heterogeneous. However, this is laborious and time-consuming. Of great benefit is an automatic approach to handle data generated by micro-FTIR, reducing the workload and increasing objectivity and comparability of the data generated (Primpke et al. 2017b). In addition, the particle shape analysis we propose enables the relationship between data derived from spectroscopic and thermal degradation methods to be ascertained. Recently, polymer mixtures in environmental matrices have been determined using thermogravimetric analysis (TGA) (Dümichen et al. 2017) or pyrolysis (Fischer and Scholz-Böttcher 2017, Ter Halle et al. 2017) coupled to a GC-MS system. A TGA system offers controlled continuous heating with a simultaneous weight loss determination and sample volumes of 20 mg soil (Dümichen et al. 2017). Using pyrolysis GC-MS and sample volumes of 1 mg, Fischer and Scholz-Böttcher (2017) evaluated the LOD and LOQ for various polymer types in fish samples and were constrained only by the scale used (repeatability of 0.25 µg). They therefore expect these limits to lie in the range of nanograms, which makes thermal degradation methods appealing for detecting NMP.

As already mentioned, micro- and nanoplastic sizes should be routinely provided, due to size-related effects (Redondo-Hasselerharm et al. 2018) and to enable comparisons with other studies. Using AF4 and analysing individual size fractions generates broad and valuable results.

This could complement the protocol proposed by Ter Halle et al. (2017) using DLS and pyrolysis GC-MS. In comparison with DLS, the size determination using AF4-MALS is not concentration-dependent but is based on a priori determined elution times of injected NMP standards (Figure 2-3A). Applying DLS for heterogeneous samples might cause misinterpretation of particle sizes and an underestimation of very small particles (Gigault et al. 2017). The different polymer densities will not hinder a satisfactory separation, as separation is dependent on particle sizes, not densities. We did not elaborate on the particle fractionation in the steric mode, but after Dou et al. (2013) separated PS spheres from 1 µm up to 40 µm satisfactorily, we conclude the AF4 being appropriate to fill the remaining gap in the proposed protocol (Figure 2-5).

2.5.2 Sampling and sample preparation

Adequate sampling of NMP to reach methodological detection limits of further analyses is especially challenging. We propose using crossflow ultrafiltration to concentrate NMP. To evaluate this technique, we tested NMP recovery and potential aggregation processes. Although reproducible, the recovery of the 50 nm spheres was not yet at its full potential (Table 2-1). The crossflow ultrafilters are used as dialysis equipment and are made to retain proteins of 60 kDa. SEM microscopy might be used to test if damaged membranes were limiting the recovery of 50 nm spheres, or if the current limitation could be attributed to attachments on the inner walls of the equipment used. Doses of a surfactant in low concentrations might reduce particle–membrane and attachment interactions and subsequently increase the recovery rates. Further, we demonstrate the potential of this crossflow ultrafiltration setup for sampling surface waters: 635 L were filtered and the particles concentrated into a volume of 0.4 L, which corresponds to a concentration factor of 1580. This might be increased by a subsequent ultrafiltration (Ter Halle et al. 2017). Ter Halle et al. (2017) concentrated surface water samples of 1 L using ultrafiltration in a polysulfone-based cell. The filtration had to be repeated several times because the cell volume was 180 ml, but they succeeded in reducing the sample volume to 10 ml – a concentration factor of 200.

Although we tested the fibrous crossflow ultrafiltration membranes with an inner diameter of approximately 200 µm for filtering MP of 100 µm size, we suggest to combine conventional filtration, e.g. with stacked sieves, with crossflow ultrafiltration (Figure 2-S1). Using sieves of e.g. 20 µm, 300 µm and 1 mm allows for large volumes of water to be filtered, as larger particles would no longer clog the membrane used during crossflow ultrafiltration.

A further point to consider is sample preparation. This is already laborious for MP, but will be even more challenging for NMP. Several approaches have been presented for MP, but studies are now focusing on an enzymatic (Cole et al. 2014, Courtene-Jones et al. 2017, Fischer and Scholz-Böttcher 2017, Löder et al. 2017, Mintenig et al. 2017) or alkaline (Dehaut et al. 2016, Hermsen et al. 2017, Kühn et al. 2017) treatment to reduce the organic sample matrix while inorganic particles are removed conducting a density separation. As our study aim was to test the handling and applicability of individual techniques, we did not include contamination controls. Though often neglected, these tests are needed when analysing environmental samples to determine a method's representability and reliability. Positive controls need to assess if and how much NMP adsorbs to filter or filter equipment when filtering NMP prior to AF4 analysis. The negative controls are particularly important given the broad usage of plastic materials and the frequently discussed contamination with synthetic fibres (Filella 2015, Wesch et al. 2017).

2.6 Conclusion and outlook

The presented analytical framework contributes to a more consistent determination of a broad size spectrum of plastic particles, including nanoplastics, in aqueous environmental samples. We have shown empirical data on the applicability of the techniques used to sample, to determine plastic sizes and to identify polymer types. The sampling is especially challenging for NMP, but crossflow ultrafiltration proved to reproducibly concentrate these. By doing so, it completes conventional filtration methods.

The data this framework generates will help elucidate environmental fate (including fragmentation processes), will allow a system-based mass balance to be achieved and, ultimately, will allow assessing environmental risks of micro- and nanoplastics.

Acknowledgements

This study was funded by the Dutch Technology Foundation TTW (project number 13940). We acknowledge additional support from and discussions with representatives from KWR, IMARES, NVWA, RIKILT, the Dutch Ministry of Infrastructure and the Environment, The Dutch Ministry of Health, Welfare and Sport, Wageningen Food & Biobased Research, STOWA, RIWA and the Dutch water boards (BTO Joint Research Program). Thanks to J. Burrough for advising on the English.

2.7 Supplementary Information

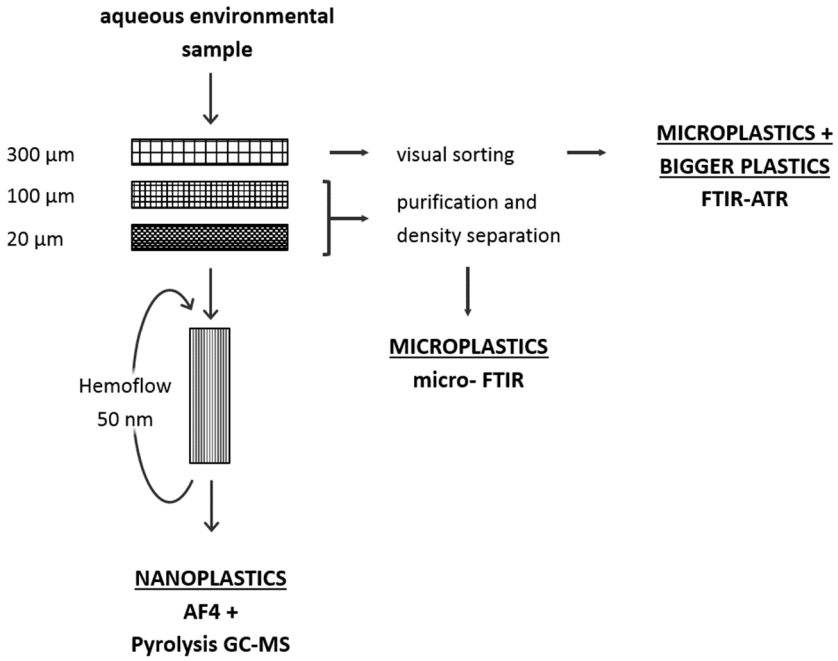


Figure 2-S1 | Scheme of combined techniques to sample and analyse nano- and microplastics.

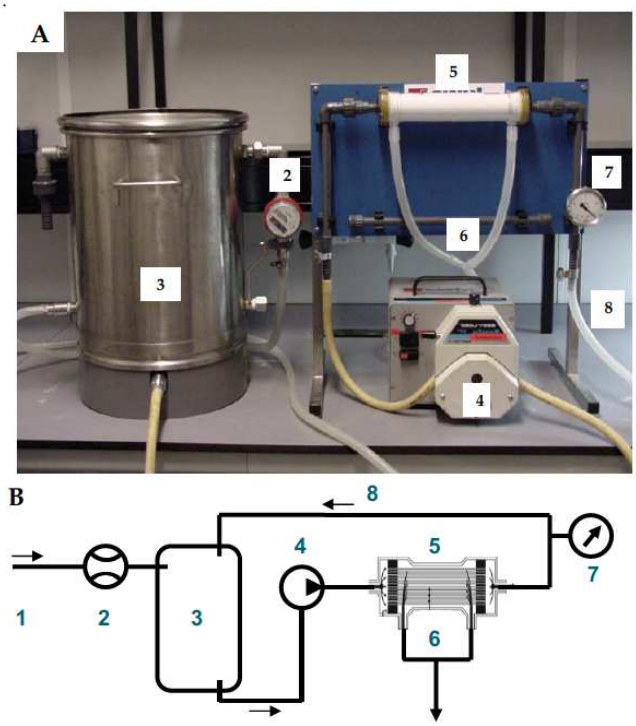


Figure 2-S2 | Schematic presentation of the Hemoflow crossflow ultrafiltration. 1 = water inflow, 2 = water meter, 3 = tank with float valve, 4 = pump, 5 = Hemoflow filter, 6 = permeate, 7 = pressure gauge, 8 = concentrate, circulation back into (3) tank (Source: Veenendaal and Brouwer-Hanzens (2007)).

Table 2-S1 | Settings used at the AF4. For the separation of a NP mixture different crossflows were tested.

	Tested Settings (membrane & carrier liquid)	Applied Settings
Membrane	Reg. cellulose (RC) 10 kDa Polyethersulfone (PES) 10 kDa	RC
Carrier liquid	Milli-Q 0.01% SDS 0.01% TWEEN	0.01% SDS
Spacer thickness	250 µm	250 µm
Detector flow	1.0 ml min^{-1}	1.0 ml min^{-1}
Split flow	0 ml min^{-1}	0 ml min^{-1}
Cross flow	1.5 ml min^{-1} (0-11 min) 1.5-0 ml min^{-1} (11-50 min, exp. 0.2) 0 ml ml min^{-1} (50-65 min)	1 ml min^{-1} (0-8 min) 1-0 ml min^{-1} (8-28 min, exp. 0.2) 0 ml min^{-1} (28-33 min)
Focusing flow	2.3 ml min^{-1}	1.8 ml min^{-1}
Injection flow	0.2 ml min^{-1}	0.2 ml min^{-1}
Injection time	6 min	4 min
Injection volume	30 µl (monodispersed) (10 µl PS polydispersion)	50 µl

Table 2-S2 | Settings to run the pyrolysis of the samples.

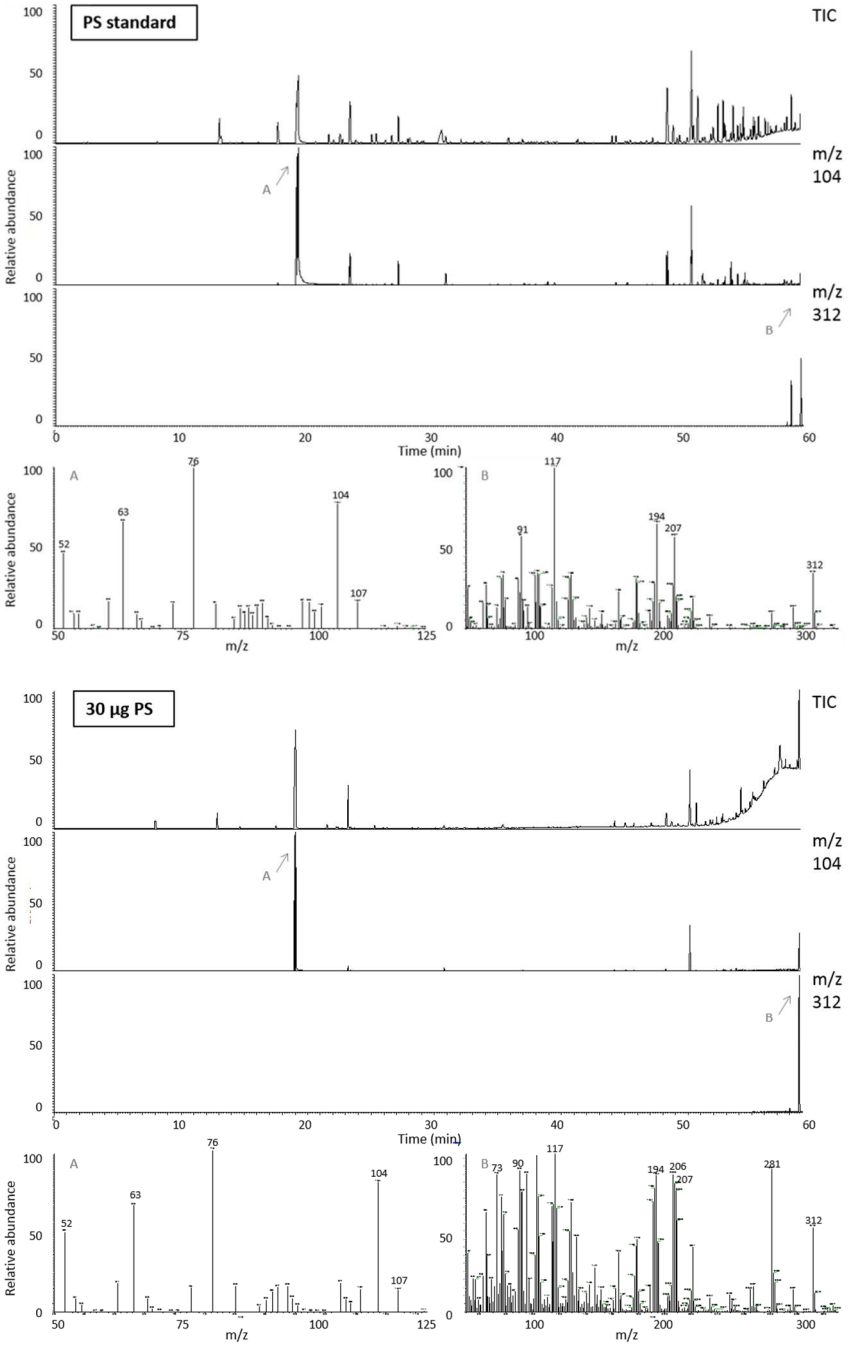
General timing	
Clean time	20.0 s
Clean time #2	60.0 s
Delay time	0.0 min
Equilibration time	20.0 s
Standby	
Temperature	
Head temperature	150.0 °C
Offset AS	50.0 °C
Default Parameters	
Temperature	150.0 °C
Pyro Time	10.0 s
Table	single
Pyrolysis Cup	560 °C

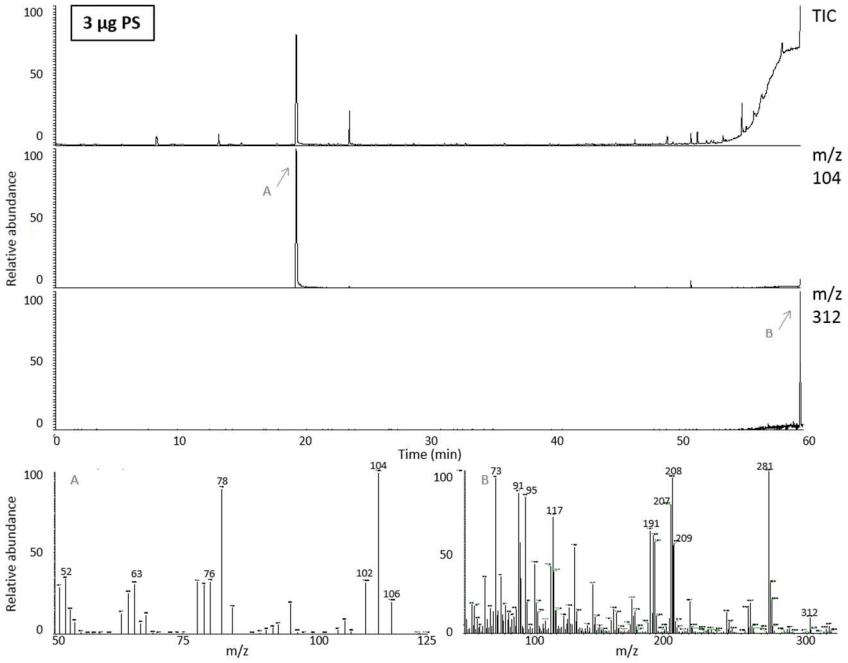
Table 2-S3 | Settings to run the GC-MS.

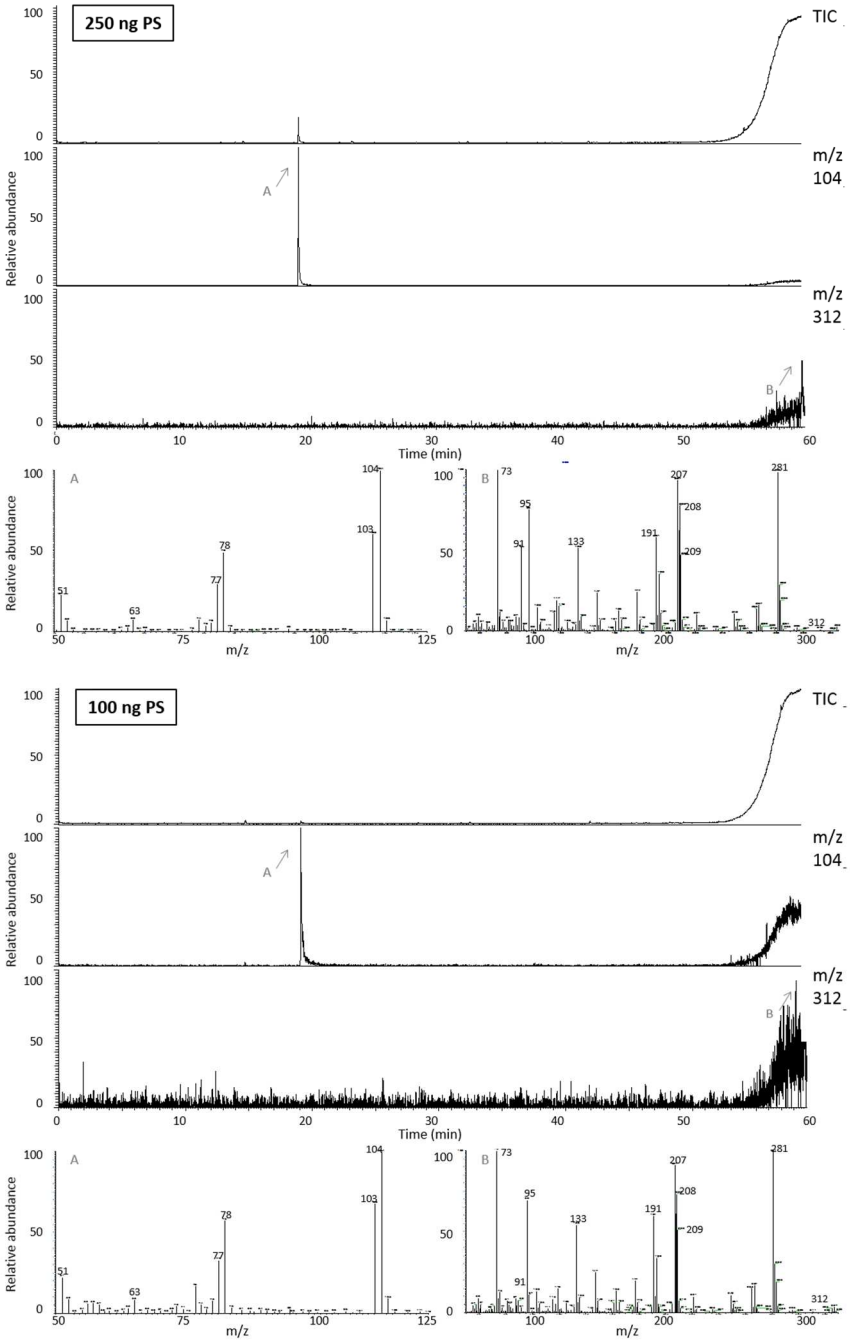
Oven	
Initial Temperature	40 °C
Initial Time	4.00 min
Number of Ramps	2
Rate #1	4.0 °C min^{-1}
Final Temperature #1	230 °C
Hold Time #1	0.00 min
Rate #2	20.0 °C min^{-1}
Final Temperature #2	325 °C
Hold Time #2	5.00 min
Maximum Temperature	350 °C
Prep Run Timeout	10.00 min
Equilibration Time	0.50 min
Inlet	
Mode	split
Base Temperature	200 °C
Split Flow	40 ml min^{-1}
Split ratio	10
Carrier	
Mode	Constant flow
Initial Value	4.00 ml min^{-1}
Detector	
Mode	Full scan
Mass Range	50 – 1000 amu
Time Range	0 – 59 min
Peak Format	Centriod
Scan Time	0.40 s
Multiplier	600 V
Ionisation Mode	EI+
Source Temperature	200 °C
Interface Temperature	280 °C

Table 2-S4 | Separation efficiency for various AF4 membrane/ carrier liquid combinations. The fractionation of mono- and polydispersed solutions was concerned successful (marked with an "Y") when resulting in clear distinct peaks.

	50 nm	500 nm	fractionation of mixture
PES & Milli-Q	y	y	-
PES & SDS	-	-	-
PES & TWEEN	-	-	-
RC & Milli-Q	(y)	y	-
RC & SDS	y	y	y
RC & TWEEN	-	-	-


PS standard
TIC
m/z
104
m/z
312
Relative abundance
Time (min)
A
B
30 µg PS
m/z


3 µg PS
TIC
m/z
104
m/z
312
Relative abundance
Time (min)
A
B
m/z
500 ng PS
TIC
m/z
104
m/z
312
Relative abundance
Time (min)
A
B
m/z


250 ng PS
TIC
m/z 104
m/z 312
Relative abundance
Time (min)
A
B
m/z
100 ng PS
TIC
m/z 104
m/z 312
Relative abundance
Time (min)
A
B
m/z

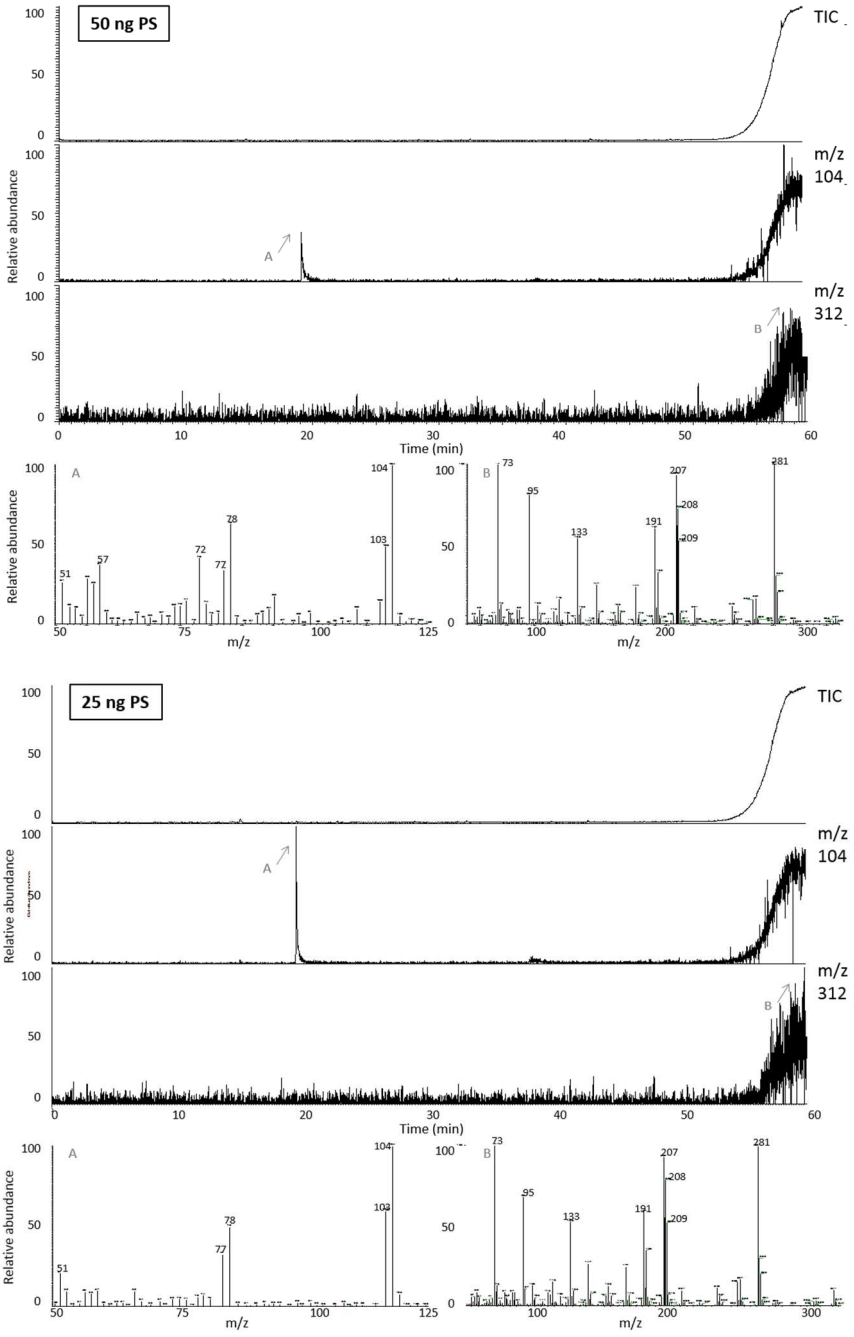


Figure 2-S3 | The pyrograms of a PS standard, and of PS (30 µg to 25 ng) that was added to surface water samples after analysis with Pyrolysis GC-MS. Each showing the total ion current (TIC), the chromatogram of selected masses (styrene m/z 104; tri-styrene m/z 312) and the mass spectra of selected peaks (A, B)

CHAPTER 3

QUALITY CRITERIA FOR THE ANALYSIS OF MICROPLASTIC IN BIOTA SAMPLES

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Abstract

Data on ingestion of microplastics by marine biota are quintessential for monitoring and risk assessment of microplastics in the environment. Current studies, however, portray a wide spread in results on the occurrence of microplastic ingestion, highlighting a lack of comparability of results which might be attributed to a lack of standardisation of methods. We critically review and evaluate recent microplastic ingestion studies in aquatic biota, propose a quality assessment method for such studies, and apply the assessment method to the reviewed studies. The quality assessment method uses ten criteria: Sampling method and strategy, Sample size, Sample processing and storage, Laboratory preparation, Clean air conditions, Negative controls, Positive controls, Target component, Sample (pre-)treatment, and Polymer identification. The results of this quality assessment show a dire need for stricter quality assurance in microplastic ingestion studies. On average studies score 7.8 out of 20 points for 'completeness of information', and 'zero' for 'reliability'. Alongside the assessment method, a standardised protocol for detecting microplastic in biota samples incorporating these criteria is provided.

3.1 Introduction

The ubiquity of microplastic (plastic particles < 5 mm (GESAMP 2016)), combined with associated effects, has raised concerns regarding marine species, ecosystems, and the impact it may have on human health. Microplastic have been detected in a wide variety of habitats in the ocean, from shallow coasts to the deep sea (Browne et al. 2007, Chen et al. 2018, Wright et al. 2013). Increasing numbers of studies report the ingestion of microplastic by marine biota across multiple trophic levels, including animals often targeted by fisheries (Table 3-1) (Foekema et al. 2013, Lusher et al. 2013, Mathalon and Hill 2014, Neves et al. 2015, Romeo et al. 2015). The ingestion of microplastics seemingly concerns a wider range of species than the ingestion of meso- and macroplastics; indeed, it is considered the most frequent interaction between plastic debris and marine organisms (Lusher 2015).

Ingested microplastic particles are thought able to evoke a biological response through both physical and chemical mechanisms, although many of these effects have yet to be studied. Ingestion of microplastics is thought to cause physical damage in small organisms (Wright et al. 2013), and has been speculated to provide a pathway for some associated chemicals to enter and spread in the food web all the way up to humans, with microplastic particles as vectors (Diepens and Koelmans 2018, Mato et al. 2001, Teuten et al. 2009). Additionally, ingestion by biota is considered a possible sink for microplastics (Cozar et al. 2014). Therefore, measuring quantities of ingested plastic is of high priority in order to properly assess the risk of such hazards.

Physical impacts for small organisms like internal abrasions and blockages have been reported (Wright et al. 2013). Moreover, microplastic particles were shown to cause damage leading to cellular necrosis, inflammation, and lacerations of tissues in gastrointestinal tracts according to a review of plastic impact on biota (Rochman et al. 2016a). In bigger organisms, ingestion of larger objects (i.e. macroplastics) has been demonstrated too (Besseling et al. 2015, Lusher et al. 2018).

In addition to the impact of ingested microplastics proper, persistent organic pollutants (POPs) may concentrate on the particles. It is suggested this could pose a possible new route for POPs to enter the food chain (Mato et al. 2001, Teuten et al. 2009); however, it has not been irrefutably shown that this actually happens (Herzke et al. 2016, Koelmans 2015, Koelmans et al. 2013). Contrarily, evidence in Northern Fulmars (*Fulmarus glacialis*) suggests a transfer of POPs from the lipids in the animal to the plastic, rather than the other way around (Herzke et al. 2016).

The concerns for the impacts of microplastic are reinforced by the hypothesis that microplastics may be able to spread through the food web by means of trophic transfer, a phenomenon that has been observed in a few instances (Farrell and Nelson 2013, Setälä et al. 2014). This is cause for concern especially in commercially valuable species, as it possibly

poses a threat to human food-safety (Wright and Kelly 2017). To what extent this transfer occurs in the food web remains to be studied further.

Despite these worries concerning microplastic ingestion, the effects in the natural environment and the implications for the food web remain poorly understood. Due to the absence of suitable standardized methods, data are too often incomparable, are not representative, and lack quality assurance (Connors et al. 2017, Filella 2015, Hanvey et al. 2017, Vandermeersch et al. 2015, Wesch et al. 2016b). Hence, our knowledge on the fate and impacts of microplastics remains incomplete. The microplastic research field is young, and as research performed now lays down the foundations for later studies, there is a dire need for a standardised protocol for carrying out studies on the ingestion of microplastics by marine biota in order to mitigate this issue (Vandermeersch et al. 2015). Although first steps towards standardisation of methodologies in environmental samples are being made (Vandermeersch et al. 2015, Wesch et al. 2016b), the comparability of current data is being impeded by the wide variety of methodologies, which has led to data of different quality (Filella 2015, Löder and Gerdts 2015). In order to deal with the wide spread in quality of the data produced by studies, an example can be taken from the field of toxicology. In toxicology, it is common practise to assess the reliability of studies with consensus criteria, like the so-called Klimisch score (Klimisch et al. 1997), or the recently proposed CRED (Criteria for Reporting and Evaluating Ecotoxicity Data) (Kase et al. 2016). These methods both offer scoring systems with different reliability categories, generating standardised documentation of validity evaluation. They were developed to guide risk assessors in performing unbiased, transparent, and detailed evaluations, while guiding researchers in performing and reporting studies in a manner deemed appropriate (Kase et al. 2016). We argue that research and risk assessment with respect to the impacts of plastic debris are in urgent need for the development and use of such criteria (Koelmans et al. 2017a).

The aim of the present study is to critically review the literature on ingestion of microplastic by marine biota. Based on this review, we develop a scoring method for ecological studies and the analytical methodologies employed to detect plastic debris in aquatic biota samples. The scoring method is subsequently applied retrospectively to the reviewed studies. This assessment does not result in an absolute judgement, but is an indicator of the usefulness of these studies for risk assessment and monitoring purposes of microplastic ingestion in natural populations. We also provide average scores per evaluation criterion, illustrating which methodological aspects need improvements most. Finally, our synthesis provides the basis for a quality assurance protocol for the analysis of microplastic debris in biota samples.

3.2 Materials and Methods

An extensive literature review was undertaken by accessing the Web of Science, ScienceDirect, and Scopus databases for studies of microplastic ingestion in marine biota in natural populations, including studies from all years up until those published in June 2017. Queries included the following search terms: "microplastic AND ingestion AND marine", "microplastic AND uptake AND marine", "microplastic AND marine biota", "microplastic AND biota AND monitor*". Reference lists of the found articles, reviews, and 'reversed searches' were consulted as well, resulting in a representative collection of 37 currently available studies. Laboratory exposure experiments were excluded from the collection. Furthermore, studies were only included if they provided data on the ingestion of microplastic. For these studies, the ingestion incidence was calculated as the fraction of sampled individuals containing microplastic. The 95% confidence intervals for these binominal proportions were assessed using the Wilson method (Brown et al. 2001). Subsequently, studies were scored according to method quality criteria discussed in the next section. All studies were assessed by two separate authors independently, after which differences in scoring were discussed, and tuned until the assessment was done consistently across all studies. To maximize transparency and traceability, the scoring explanations, scoring criteria and scorings for all papers are provided as Supplementary Information (Table 3-S1, Table 3-S2 and Table 3-S3, respectively). The eventual assessments do not express the value of studies. They only reflect the compliance of studies to reliability criteria as perceived by the authors of the present paper, in hindsight. Although we maximized our effort to be complete and thorough in this process, misinterpretations or misjudgements cannot be completely excluded.

The here presented scoring method was designed to assess current studies on reliability of their data on microplastic ingestion in marine field biota, and is based on several aspects that define a reproducible and controlled study. The method evaluates the inherent adequacy of the employed methods for monitoring and risk assessment purposes, relating to a standardised methodology, and the description of the procedure and results. By scoring high in all categories, a study can be defined as "reliable", providing reproducibility, clarity, and plausibility of its findings.

3.3 Quality Assessment System

Previous scoring systems that have been proposed for assessing the reliability of ecotoxicology studies are the Klimisch (Klimisch et al. 1997), and the more recent CRED scoring systems (Kase et al. 2016). The Klimisch criteria have received critique for being unspecific and for lacking essential criteria and guidance, leaving too much room for interpretation (Kase et al. 2016). The CRED evaluation method gives extensive guidance on how to use the set criteria, and gives recommendations for reporting (Kase et al. 2016). Following the example set by the CRED method, the present evaluation method for microplastic ingestion studies provides several criteria which must be assessed, including guidance on how to assess each criterion. The quality assessment method is made up of ten criteria: (1) Sampling method and strategy, (2) Sample size, (3) Sample processing and storage, (4) Laboratory preparation, (5) Clean air conditions, (6) Negative controls, (7) Positive controls, (8) Target component, (9) Sample (pre-)treatment, and (10) Polymer identification (Table 3-1). For each criterion, a score of 0, 1, or 2 can be assigned to the publication under review. Scores signify the following: 2= reliable without restrictions, 1= somewhat reliable, but with restrictions, 0= not reliable. If information is lacking on certain aspects in the publication this is considered unreliable, leading to a lower score. After each criterion is scored, an overall reliability score is calculated by taking the product of all criteria scores, resulting in a maximum attainable overall theoretical reliability score of 1024 points, indicating a high reliability of a publication. This contrasts with both the CRED and Klimisch method: these methods assign a category of reliability to each criterion, but do not quantify it with a score (Kase et al. 2016, Klimisch et al. 1997). In the evaluation method presented here, the quantification through scoring is deemed important, because each criterion is considered crucial and equally important to the reliability of the results of a study. This means when a study scores zero points on a criterion, too much uncertainty still surrounds the results of the study, marking the results unreliable. This also means that when only one criterion is evaluated as "not reliable" (zero points) the overall reliability score of the study will be zero. Besides this overall reliability score, we provide an accumulated score; calculated as the sum of the individual scores. This score has a maximum of 20 points and can be seen as a combination of the reliability and the completeness of information in a publication.

In the following ten paragraphs, argumentation is provided on each of the ten scoring categories, including explanation based on the currently reviewed studies, and specification of scoring criteria. A supporting, more detailed overview of the scoring criteria is provided as Supplementary Information (Table 3-S1, Table 3-S2).

Table 3-1 | The scoring of the reviewed articles in the current quality assessment [a]

Study	Criterion										
	1 Sampling methods	2 Sample size	3 Sample processing and storage	4 Laboratory preparation	5 Clean air conditions	6 Negative control	7 Positive control	8 Target component	9 Sample treatment	10 Polymer identification	Accumulated Score
Lusher et al. (2016)	2	2	2	2	1	2	0	2	2	0	15
Tanaka and Takada (2016)	2	2	2	0	0	1	0	2	2	2	13
Davidson and Dudas (2016)	1	1	2	2	0	2	2	2	0	0	12
Rummel et al. (2016)	2	2	0	2	1	2	0	0	1	2	12
Courtene-Jones et al. (2017)	0	0	2	2	0	1	0	2	2	2	11
Devriese et al. (2015)	2	1	2	0	2	2	0	2	0	0	11
Mathalon and Hill (2014)	1	1	2	1	1	2	1	2	0	0	11
Wesch et al. (2016a)	0	2	2	0	2	0	0	2	2	1	11
Cannon et al. (2016)	0	2	2	0	2	0	0	2	0	2	10
Desforges et al. (2015)	2	2	2	0	0	2	0	2	0	0	10
Li et al. (2015)	2	2	0	0	0	1	1	2	0	2	10
Murphy et al. (2017)	2	1	0	2	0	1	0	2	0	2	10
Vandermeersch et al. (2015)	1	1	2	0	2	2	0	2	0	0	10
Davison and Asch (2011)	2	2	2	0	0	1	2	0	0	0	9
Foekema et al. (2013)[b]	2	2	1	0	0	0	0	2	2	0	9
Karlsson et al. (2017)	1	1	2	1	0	2	0	2	0	0	9
Nadal et al. (2016)	2	2	2	2	0	1	0	0	0	0	9
Torre et al. (2016)	0	2	2	2	1	2	0	0	0	0	9
Bellas et al. (2016)	2	1	2	1	0	1	0	0	1	0	8
Jabeen et al. (2017)	0	2	0	1	0	1	0	2	0	2	8
Lusher et al. (2013)	2	2	2	0	0	0	0	0	0	2	8
Van Cauwenberghe and Janssen (2014)	1	0	1	0	2	2	0	2	0	0	8
Bråte et al. (2016)	0	2	0	2	0	1	0	0	0	2	7
Anastasopoulou et al. (2013)	0	2	2	0	0	0	0	2	0	0	6
Besseling et al. (2015)[b]	2	0	0	0	0	0	0	0	2	2	6
Jantz et al. (2013)	1	2	2	0	0	0	0	0	1	0	6

Murray and Cowie (2011)	2	2	2	0	0	0	0	0	0	0	6
Peters et al. (2017)	1	2	2	0	0	1	0	0	0	0	6
Vendel et al. (2017)	2	2	1	0	0	0	0	0	0	0	5
Boerger et al. (2010)	2	2	0	0	0	0	0	0	0	0	4
Liboiron et al. (2016)	0	2	0	0	0	0	0	2	0	0	4
Neves et al. (2015)	0	0	1	0	0	0	0	0	0	2	3
Wójcik-Fudalewska et al. (2016)	0	1	2	0	0	0	0	0	0	0	3
Romeo et al. (2015)	1	1	0	0	0	0	0	0	0	0	2
Miranda and de Carvalho-Souza (2016)	0	0	0	0	0	0	0	0	0	0	0
Average all-study score (n=35)	1.14	1.46	1.31	0.57	0.40	0.86	0.17	1.03	0.43	0.66	8.0

[a] Scores of 0-2 were assigned to each publication in each of the 10 categories. The publications are sorted from high to low based on the 'Accumulated score'. The overall reliability score was zero for all studies and is not indicated.

[b] Studies with involvement of 1 or more of the authors of the present paper.

Sampling methods and strategy - Several factors related to sampling method and strategy affect the results of microplastic detection in biota samples. For instance, due to differences in density, and sinking as a result of biofouling, plastic is found at different depths of the water column (Kooi et al. 2017, Lusher 2015). Microplastics are also known to accumulate in the sediment (Claessens et al. 2011, Gall and Thompson 2015, Wesch et al. 2016b), with deep sea bottoms likely to make up a sink for the particles (Kooi et al. 2017, Van Cauwenberghe et al. 2013, Woodall et al. 2015). It is plausible that feeding strategy has an influence on the type and amount of microplastic ingested (Redondo-Hasselerharm et al. 2018, Setälä et al. 2016), with planktivorous and filter feeders expected to be more susceptible to ingestion of low density particles floating in the top layers of the water column, while demersal and bottom dwelling species are more likely to encounter high density microplastics. Additionally, some species are known for diurnal vertical migration and are subjected to a wide variety of microplastic encountered, possibly affecting their ingestion rates. Non-ecological factors such as mesh size will have influence on life stage of the caught individuals in the sample, whereas a small mesh size could lead to cod-end feeding (Davison and Asch 2011). Sampling methods can greatly influence the outcome of a study; therefore, it is important that such characteristics of the sampling are recorded, in order to create a reproducible study (ICES 2015, Wesch et al. 2016b). Also, by reporting such details it could be easier to interpret the outcome, and account for possible contamination in the results.

In this section, studies are scored on reportage, and therefore reproducibility, of the sampling, but also on choice of sampling methods itself. Studies scoring high in this section

reported extensively on their methods (e.g. type of gear, sampling location and depth) and controlled their own sampling, or were fully aware of what had happened to the specimens during sampling. Articles with low scores either failed to report on (parts of) their sampling (Table 3-2), or used, for instance, store bought individuals when making inferences on natural populations (Cannon et al. 2016, Jabeen et al. 2017). The use of store or market bought individuals is not inherently wrong, as long as the interest of the study lies on contamination of sea food, not on natural populations. Scores of 1 indicate that for part of the sample, sampling was not performed correctly, while for another part of the sample it was: the aim of the study should be correctly matched to the sampling method. For example, Vandermeersch et al. (2015) partially used store bought individuals, while using self-sampled ones for a different part of the study. The microplastic uptake in mussels from different estuaries was compared with the uptake by commercial mussels. The commercial mussels were bought in stores, leading to uncertainty about the treatment of these mussels prior to the analysis: microplastic found in these mussels could have originated from contamination during handling in the production chain, rather than from microplastic ingestion by the mussels themselves. Would the aim of this study have been to check microplastic content in store bought individuals (i.e. checking on general contamination, not ingestion) this would not have been an issue. This study scored 1 in this section, since part of the study can be considered reliable with sampling method correctly matched to the aim of the specific part of the study.

Sample size - Both the International Council for the Exploration of the Sea ICES (2015) (ICES 2015) and the European Strategy Framework Directive's Technical Subgroup on Marine Litter (MSFD-TSGML) (2013) (MSFD (Technical Subgroup on Marine Litter) 2013) recommend a sample size of at least 50 individuals. This sample size of 50 is arbitrarily chosen, since, due to the wide variety in microplastic ingestion reported by different studies, no clear indication of the true ingestion incidence of microplastic by biota can be estimated. When more clarity can be given in the future, this recommended sample size should be adjusted accordingly. If ingestion incidence appears to be low, higher sample sizes will be needed to give reliable results; if populations show high incidence of microplastic ingestion, lower sample sizes will suffice.

The scoring in this category is fairly straightforward, using the recommended 50 individuals as a threshold until it is possible to perform a reliable power analysis in order to calculate a more appropriate sample size for ingestion studies. Too low a sample size may provide interesting data, but no conclusions should be drawn, as the statistical power of such a study simply would be too low to infer any trends. A larger sample size is always advisable, since it will lead to more reliable results, i.e. narrower confidence intervals (Figure 3-1). Studies with a sample size over 50 specimens taken from a food web or ecoregion scored 2. A score of 0 was ascribed to studies using less than 50 specimens. Studies with > 50 specimens in total and > 25 specimens per research unit (e.g. a species, a food web or an ecoregion) received

a score of 1. For now, we also applied these criteria to a study that reported the presence of microplastic in a single stranded whale (Besseling et al. 2015) leading to a very wide confidence interval (Figure 3-1). However, for whales or for rare and protected species the n=50 criterion is difficult or even unethical to achieve in a sampling effort meant to assess trends in microplastic ingestion. For such big or protected organisms, retrospective data obtained from stranded animals and from bycatch through different reports need to be combined in order to reach a sample size with sufficient rigour (Lusher et al. 2018). This would require harmonisation of protocols in order to increase comparability of studies, for which guidance is beyond scope of the current review.

We further advise provision of the confidence interval in the reported count (Hermsen et al. 2017, Lusher et al. 2013); however, this was not yet included as criterion in the current scoring. Based on the total number of animals and the number of animals that ingested microplastics, we calculated the confidence intervals and provide an overview in Figure 3-1.

Sample processing and storage - After sampling, samples need to be stored until examination in the laboratory. Samples are often frozen (Bellas et al. 2016, Lusher et al. 2013, Lusher et al. 2016, Romeo et al. 2015), or whole specimens of smaller species are preserved in fixatives such as formalin, ethanol, or formaldehyde (Boerger et al. 2010, Courtene-Jones et al. 2017, Desforges et al. 2015, Karlsson et al. 2017, Murray and Cowie 2011). ICES (2015) recommends to store biota samples on board using aluminium foil for freezing at -20°C or preservation in ethanol in glass containers. In the present study it was not considered necessary to wrap each individual in aluminium foil, as long as specimens were quickly frozen after capture at -20°C, and stored in a closed container. If this is combined with a pre-examination rinse of the specimens (see "Laboratory preparation"), it should suffice in mediating contamination of the exterior of the specimen. Under no circumstance should the specimen be opened on board. This is considered as a high, and difficult to assess risk, for contamination due to unregulated conditions on board. We further recommend to avoid dissecting individuals outside clean air conditions at all times (see "Clean air conditions").

High scores were assigned to studies freezing their samples shortly after capture at -20°C or storing them on ice, leaving any further handling till the laboratory. Alternative methods storing the samples in closed off containers with a fixative were also given highest scores in case potential effects of these chemicals on different plastics were studied before application. Recently, the resistance of microplastics to formaldehyde/ethanol has been confirmed (Courtene-Jones et al. 2017). Studies scoring low in this section performed dissections, or otherwise opened the specimens, on board. Middle scores again indicate some aspects of the study do not comply, but do partially meet the standards (e.g. different processing for different subsamples).

Laboratory preparation - Contamination is a prevalent issue in microplastic research, creating uncertainty around the results of many studies (Torre et al. 2016, Vandermeersch et al. 2015, Wesch et al. 2016b). This risk and uncertainty have been dealt with in different ways. Different forms of prevention have been applied, in varying degrees of success. Foekema et al. (2013) decided to exclude small fibres from analyses after finding a sharply decreased abundance when working under clean air conditions. ICES (2015) proposed in their preliminary protocol to exclude all fibres smaller than 5 mm in length from results. Although this may provide a way to reduce the issue of contamination in results, it is less than ideal; by excluding all small fibres from results, truly ingested fibres will be excluded from the results too. This could lead to an underestimation of ingestion rates and a potential knowledge gap in the ingestion of microplastic. Therefore, proper prevention is needed. In the laboratory, contaminations with synthetic polymers should be avoided as they may influence ingestion results (Foekema et al. 2013, Vandermeersch et al. 2015). Equipment, tools and work surfaces should be free of particles, in order to avoid easy contamination. To this end all materials used should be washed and rinsed thoroughly with high quality water (e.g. Milli-Q water) before use, and preferably kept in a clean air cabinet.

Factors such as clothing should be considered. Often, contamination arises in the form of microfibers (Vandermeersch et al. 2015, Wesch et al. 2016b). Additional contamination originating from researchers' clothing can easily be avoided by solely wearing 100% natural fibre clothing, such as cotton. Only wearing a 100% cotton lab coat may not suffice; if one was to wear a polyester shirt underneath, it would not be unimaginable that some fibres could end up in the samples. For the current scoring in this study, if all other precautions were met, a 100% cotton lab coat was considered sufficient.

In some studies, precautions were made by wiping surfaces and tools using alcohol (Liboiron et al. 2016). This method is probably not thorough enough to deal with contamination; merely wiping surfaces, be it with alcohol or water, could still leave particles. They could be missed, detach from the wipe during wiping, or the wipe itself could even prove to be a source of contamination (i.e. the material, or dust already collected on the wipe before use). Rigorously washing and rinsing of the equipment are considered to be the only proper option here.

Additional to the preparation of surfaces and tools, the sample specimens itself require some preparation. The exterior of the animal should be rinsed (Foekema et al. 2013, Hermsen et al. 2017), and checked for contamination. In case of small specimens such as zooplankton, this is not an easy feat. In the study performed by Desforges et al. (2015) this issue was overcome by individually checking each specimen under a microscope, and picking off any external contamination with a pair of tweezers.

In summary, a score of 2 was assigned when non-synthetic clothing and a lab coat were used, and equipment and organism exterior were rinsed. A score of 1 was assigned for solely wiping laboratory surfaces and equipment or not wearing a lab coat, as long as negative

control samples were run in parallel and examined for contamination. A score of zero was assigned when no precautions were met.

Clean air conditions - Problems with airborne contamination are unavoidable, unless work is performed in clean air conditions (Foekema et al. 2013, Vandermeersch et al. 2015, Wesch et al. 2016b). To this end, sample handling should be done in a laminar flow cabinet (Devriese et al. 2015, Hermsen et al. 2017, ICES 2015) or in a "clean room", which is designed to minimise airborne contamination during sample handling and analysis (Wesch et al. 2016b, Wesch et al. 2017). The use of such facilities is a necessity in microplastic research; any handling of samples outside clean air conditions creates a high risk of airborne contamination (Wesch et al. 2017).

Other studies placed their samples in a fume hood in order to minimise the risk of contamination (Devriese et al. 2015). However, since a fume hood draws air from the room into the hood (contrarily to a positive pressure laminar flow cabinet, which blows filtered air through the cabinet into the room), the risk of airborne contamination remains (Wesch et al. 2017).

A few studies were seen that mitigated contamination by closing off samples as much as possible, and handling them as fast as possible (Jabeen et al. 2017, Karlsson et al. 2017). These methods are not fool proof, and should not be relied upon without further indication on results of negative samples treated in parallel to actual samples.

The proper use of clean air conditions was given a score of 2. A score of 0 was assigned to studies taking no regard for airborne contamination. Studies mitigating contamination by carefully keeping samples in a closed off situation as much possible scored 1 in this category, provided that negative controls were run in parallel and examined for contamination.

Negative controls - Although increasing in recent studies, the use of controls in microplastic research is not standard practise. During sample handling the chances of contamination by microplastic particles and fibres are high, thus, the use of controls, treated and analysed in parallel to actual samples, is crucial.

For a study to score 2, proper blanks should be included for each batch of samples, with at least three replicate blanks per batch. These controls should be performed without tissue, or with tissue that was confirmed to be devoid of microplastic, in parallel with samples containing the target component (ICES 2015, Rummel et al. 2016). By doing so, the controls are given the same full treatment as the studied specimens. Controls should be run regularly and with special attention to moments of high risk of contamination, such as moving specimens in and out of the laminar flow cabinet (Löder and Gerdts 2015). Also the visual examination of samples forms a moment of high risk, which is why additionally placed and examined petri dishes next to the sample might be advisable (Hermsen et al. 2017).

Scores of 1 indicate a blank analysis of some form, nevertheless deemed insufficient here. This includes, for instance, solely open petri dishes or soaked paper that were placed next to the work surface and checked for contamination (Hermsen et al. 2017, Lusher et al. 2016) or the filtration of air. These do not account for contamination deriving from used chemicals or equipment. Studies scored 0 when no form of negative control was included in the study.

Positive controls - It is generally difficult to assess whether all microplastics present in a sample are effectively recovered from that sample. Especially small particles may be overlooked or missed, and losses may occur during all steps of sample preparation, processing and analysis. Therefore, it is considered crucial to include controls (triplicate) with added microplastic particles that are treated in parallel to the samples, in order to determine the recovery rate (score of 2 points). Ideally, positive controls should be included also for the smallest targeted size class, and the limit in the detected size should be reported. We are aware of only three studies that included reliable positive controls (Davidson and Dudas 2016, Davison and Asch 2011, Hermsen et al. 2017). Davison and Asch (2011), for instance, blindly added random numbers of spherical beads from two size classes into fish stomach contents, so that the researcher would not know this number, and were able to trace back all added particles to achieve 100% recovery. A score of 1 was assigned to studies with some form of a positive control (e.g. testing only a part of the protocol) and a score of zero was assigned when no positive controls were included.

Target component - Among the reviewed studies, different target components were described, that are mainly (parts of) the digestive tracts for larger biota like fish (Boerger et al. 2010, Foekema et al. 2013, Hermsen et al. 2017, Lusher et al. 2013, Romeo et al. 2015), or whole specimens for smaller species, like bivalves (Desforges et al. 2015, Van Cauwenberghe and Janssen 2014, Vandermeersch et al. 2015). Choosing a suitable target component is an important part of the study setup. For accurate estimation of microplastic ingestion, it is important to examine the entire gastrointestinal tract (GIT) (oesophagus to vent). By only examining the stomach, particles in the gut would be missed, leading to an underestimation of ingestion rate. When small animals such as bivalves and zooplankton are being studied, the entire specimen should be used.

Studies examining full specimens or entire GITs received the highest score. Examination of parts of the GIT were scored lowest. In case a study examined a part of the GIT for a subsample, yet full GITs for the rest of the sample, it was scored 1.

Sample (pre-)treatment - To extract and characterise microplastics in biological samples, a digestion step is a crucial component, namely dissolving organic matter without degrading plastic polymers. Detection of microplastic in a biological sample without getting rid of the organic matter makes for an unreliable method; the chance of missing particles is high, especially small particles that are not visually detectable (Vandermeersch et al. 2015). Therefore, it is advised to make use of a digestion pre-treatment (ICES 2015, Löder et al. 2017).

Dehaut et al. (2016) performed a study testing six existing methods (including enzymatic, alkaline, and acidic digestion), comparing their effects on 15 different plastic polymers, as well as their efficiency in biological samples. Their tests showed that out of the six protocols, an adapted protocol of Foekema et al. (2013) was most successful. The original protocol involves the samples being left for digestion in 10% KOH solution and kept at room temperature for 3 weeks. The adapted protocol used 10% KOH solution, with 24 hours of incubation at 60°C (Dehaut et al. 2016). This adaptation was made in order to shorten the incubation time. The heating of samples during digestion pre-treatments to speed up the process is fairly common, and especially with acidic digestion methods this is often part of the protocol. However, this practise may be ill advised, since the heating of the samples could cause some microplastic particles to deform, or clump together (Munno et al. 2018). Therefore, it is advised to apply the original protocol of Foekema et al. (2013). The adequacy of the 10% KOH protocol has recently been confirmed by Kühn et al. (2017) and Munno et al. (2018). However, for smaller organisms, like the soft tissue of mussels or plankton species, also enzymatic methods have been shown to provide high digestion rates with no damage to microplastic (Catarino et al. 2016, Cole et al. 2014).

Based on these findings, studies using a 10% KOH solution-based digestion, or an enzymatic digestion received the highest score of 2. Studies not incorporating a digestion step received no points. Studies using other digestion methods were scored 1. A score of 1 was also assigned to studies that did not need a digestion step because the size of particles was large enough, which can be achieved by sieving the samples over 300 µm. This mesh size allows adequate particle sorting as it is done frequently for e.g. water samples (Eriksen et al. 2013a, Imhof et al. 2016, Law et al. 2014).

Polymer identification - Accurate identification of polymer types in environmental samples can be laborious. Hence, two aspects are relevant when assessing the polymer identities of a microplastic sample: (1) the quality of the method used for the identification (efficiency, sensitivity, accuracy, reproducibility) and (2) the quality of the selection of the subsample (representativeness).

Polymer identity - Visual inspection (i.e. characterising microplastic by eye under a dissection or stereomicroscope) was found to be a frequently used identification method (Bellas et al. 2016, Desforges et al. 2015, Devriese et al. 2015, Mathalon and Hill 2014, Peters et al. 2017, Romeo et al. 2015, Rummel et al. 2016). However, visual examination cannot be used to identify the (polymer) identity of a particle. Without formal evidence of polymer identity, a particle cannot be reported as being a microplastic particle. The quality of visual examination is influenced by the observer, properties of the plastic, the targeted microplastic size, the magnification of the microscope, and the sample type (Wesch et al. 2016b). In a case study on microplastics in North Sea sediments, the usage of focal plane array (FPA) micro-Fourier transform infrared (micro-FTIR) spectroscopy revealed that only 1.4% of the particles visually sorted as microplastic actually were synthetic polymers (Löder and Gerdts 2015). Fibres with a size over 500µm were found to be of natural origin after an initial selection as microplastic (Remy et al. 2015, Wesch et al. 2016b). This uncertainty of visual identification further increases as particle size decreases which illustrates the importance of verifying the chemical origin of potential microplastics.

To date, potential microplastics are identified mostly using spectroscopic (Imhof et al. 2016, Käppler et al. 2015, Löder and Gerdts 2015) or thermal degradation analyses (Dümichen et al. 2017, Fischer and Scholz-Böttcher 2017, Fries et al. 2013). Particles sorted manually are mostly analysed using attenuated total reflectance (ATR) Fourier transform infrared (FTIR) spectroscopy (Courtene-Jones et al. 2017, Rummel et al. 2016) but also pyrolysis GC-MS is applied (Fries et al. 2013). Both techniques result in a clear identification, but are restricted to bigger particles due to the manual particle handling. When aiming for microscopic particle determination, the coupling of a microscope to FTIR or Raman spectroscopy reveals the chemical identity of particles and allows particle sizes to be estimated. Both techniques are limited by a certain minimum particle size (Käppler et al. 2015, Löder et al. 2015, Mintenig et al. 2018). Alternatively, unsorted samples, i.e. where a polymer mixture might be present, can be analysed using thermal degradation techniques (Dümichen et al. 2017, Fischer and Scholz-Böttcher 2017). Since particles are not sorted manually, these techniques are not limited by a minimum particle size required, however they do not provide information on microplastic size either. Furthermore, they do provide information on ingested polymer masses instead of presenting the numbers of ingested microplastic particles. One of these techniques should be applied, and should always be favoured over the so-called 'hot point-test' applied by several studies (Devriese et al. 2015, ICES 2015, Vandermeersch et al. 2015). Plastic particles are 'identified' when a particle shows a sticky dark mark when touched with a hot needle. However, this test does not allow polymer identification, is less suitable for thermoset and smaller plastics, and should therefore only be seen as a facilitation for visual sorting.

Representative subsample of particles – Many studies report polymer identities for a small subset of sorted particles only (Foekema et al. 2013, Karlsson et al. 2017). This leaves considerable uncertainty with respect to the actual distribution of polymer types among samples. Based on practical experience using ATR-FTIR to determine polymer identities (Besseling et al. 2015, Foekema et al. 2013, Hermsen et al. 2017), we advise that when numbers of pre-sorted particles are < 100, all particles should be analysed. For particle numbers > 100, analysis becomes more laborious but > 50 % should be identified for a representative subsample, with a minimum of 100 particles being analysed. The information given in the results section should contain the following: particle counts with confidence intervals, detection limits for the count and for minimum particle size, the polymer types determined, their percentages with regard to other polymer types and natural particles, and the microplastic size (classes).

If a study identified polymer identities and applied the latter criteria, 2 points were assigned. For insufficient numbers of identified particles that could result in an unrepresentative subsample, 1 point was assigned. Zero points were given if no polymer identification (i.e. purely visual sorting) was conducted.

3.4 Protocol for microplastic ingestion studies in biota

Here, as synthesis of our review and method assessment, we propose a standardised protocol for the detection of ingested microplastic in (marine) biota, alongside the quality assessment method (Table 3-2). The protocol is adaptable for both vertebrates and invertebrates, as long as the components of the quality assessment system are upheld. The protocol was developed taking the recommended protocol by ICES (2015) into account and amending with knowledge and evaluation of currently existing methodologies as outlined above. The protocol and the quality assessment system are such that, when following the protocol successfully, high reliability scores can be acquired. This protocol relies on the same literature analysis and argumentation as the assessment method, and follows the categories step-by-step.

Table 3-2. Standardised protocol for the detection of ingested microplastic in (marine) biota

1. Sampling methods	Sampling characteristics that should be recorded: - Gear - Mesh size and mesh size at cod-end (if applicable) - Material - Location - Depth - Date and time of day - Presence of plastic materials
2. Sample size	A suitable sample size of 50 individuals per research unit (species, food web, ecoregion, feeding type, etc.) is required (ICES 2015, MSFD (Technical Subgroup on Marine Litter) 2013). The confidence interval of the ingestion incidences should be reported (Figure 3-1).
3. Sample processing and storage	Between the moment of capture and the examination in the lab the biota samples should be stored on ice or frozen at -20°C. Smaller organisms can also be preserved in a glass container with ethanol or formaldehyde. Any sample handling, such as dissections, should be left for the lab.
4. Laboratory preparation	All materials, equipment, and laboratory surfaces need to be thoroughly washed and rinsed, afterwards all materials should be kept in clean air conditions. Used solutions and filters, should be checked before usage, the same applies for the outside of the sample specimens (Foekema et al. 2013).
5. Clean air conditions	The handling of samples should be performed in clean air facilities (Wesch et al. 2016b). Samples should not be taken out of the clean air facilities without being sealed off. If sampling processing and analysis cannot fully be conducted under clean air conditions, the implementation of negative controls (see criterion 6) will get even more important.
6. Negative control	A replicate of 3 negative controls is advised that are included for each batch of samples and treated in parallel to the sample treatment (ICES 2015). Additionally, if the samples have to be analysed outside of the clean air facilities, clean Petri dishes should be placed next to the sample, and checked for any occurred air- borne contamination.
7. Positive controls	A replicate of 3 is advised in which microplastics of known polymer identity and of targeted sizes are added to 'clean' samples which are then treated and analysed the same way the actual samples are. The particle recoveries are calculated by tallying the numbers of retrieved particles to the amounts added.

8. Target component	To ensure monitoring all ingested microplastic, the full gastrointestinal tract (oesophagus to vent) of fish and the entire body of smaller species, e.g. bivalves, should be examined.
9. Sample treatment	A digestion step must be included to dissolve organic matter in the sample when aiming in the detection of small microplastics (< 300 µm). The digestion method described by Foekema et al. (2013) using a 10% KOH-solution and enzymatic digestion methods (yet only for small organisms) are most suitable (Cole et al. 2014, Courtene-Jones et al. 2017, Löder et al. 2017). In any case, heating or drying of the samples at high temperatures should be avoided.
10. Polymer identification	Until now, most common methods in the field of microplastic research are FTIR or Raman spectroscopy, pyrolysis or TGA- GC-MS. The polymer identification is required for all, or at least a subsample of particles: When numbers of pre- sorted particles are <100, all particles should be analysed. For particle numbers >100, >50 % should be identified, with a minimum of 100 particles. Particle counts with confidence intervals, detection limits for the count and for minimum particle size, polymer types and percentages (of different polymer types, of synthetic vs natural material) and particle sizes should be reported.

3.5 General Discussion

Considerable uncertainty with respect to methodology was observed and quantified via the scoring system. Accumulated reliability scores ranged from 0 to 15, out of a maximum of 20, with an average of 8.0 (Table 3-1). As mentioned before, the results of such an assessment are not an absolute judgement, and the results should not be used as a ranking list of the value of studies. The scores are an indicator of the usefulness of these studies for risk assessment and monitoring purposes with respect to natural populations. The assessment evaluates common characteristics of a variety of studies. Not all decisions in a study can be captured in the scoring system; therefore, it is still important to critically look at a study and reflect upon its plausibility and comparability to other studies, not just upon its results.

Often studies could not be assigned a high score due to missing information on certain characteristics, such as details of the sampling or analytical procedures. Average scores (n=35) per evaluation criterion were especially low (<1) for the criterion 'positive controls' (0.17), 'clean air conditions' (0.40), 'sample treatment' (0.43), 'laboratory preparation' (0.57), 'polymer identification' (0.66) and 'negative controls' (0.86) (Table 3-1). By leaving out such essential information, a study immediately becomes irreproducible, and thus less reliable. One reason for initiating the present review was to systematically define this crucial information, such that future studies can avoid this by using standardised consensus methods.

Based on the assessment of reviewed papers (considered representable for currently available knowledge, Table 3-S3), we conclude that all reviewed studies are not fully reliable. All studies scored zero in at least one category, indicating an uncertainty around at least one of its aspects. Therefore, the overall reliability score, calculated as the products of individual scores, were all zero and thus were not included in Table 3-1. Each category of the assessment was defined by the consideration that if its set criteria were not up to par, the possibility of contamination could not be excluded. This is problematic, and for future studies the use of the proposed protocol is strongly recommended, in order to obtain reliable and reproducible results. Following the proposed protocol we conducted a study focussing on microplastic detection in North Sea fish, while giving special attention to quality assurance and full reportage (Hermsen et al. 2017).

Our meta-analysis of microplastic ingestion data shows a wide variability among studies, which may be due to methodological, ecological, and/or spatial differences. Ingestion incidence ranges from zero to 100% with confidence intervals that are narrower for higher sample sizes (Figure 3-1). Based on pooled data from all studies an overall biota ingestion incidence of 16.6% (15.9 – 17.2 95% CI) was calculated. This 'whole ocean' value can be interpreted as the percentage of the 13722 biota individuals sampled across all oceans, in which microplastic was detected in the period of 2010 to 2017. The data underlying Figure 3-1 further reveal that with sample sizes lower than 50, the confidence intervals can become as wide as 35-80% (Figure 3-1).

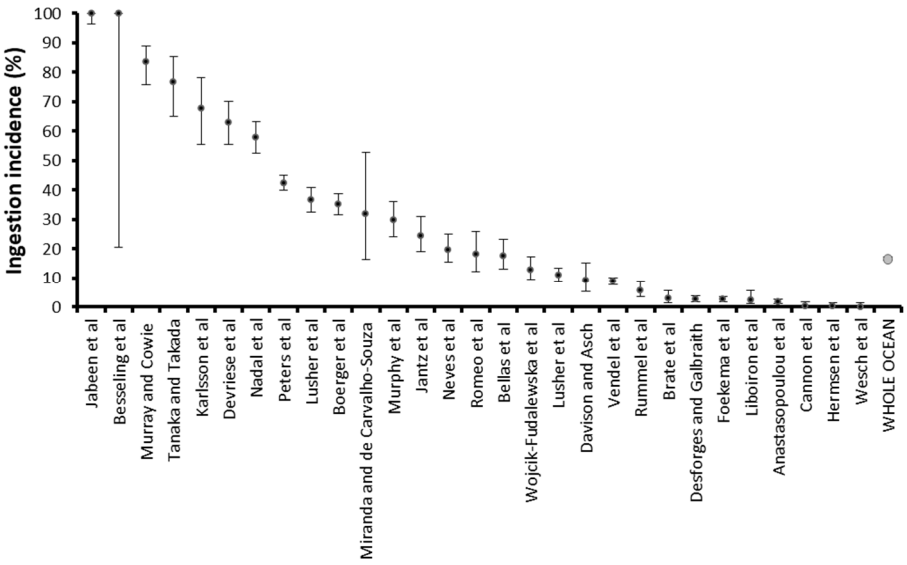

Figure 3-1. Ingestion incidence and 95% confidence intervals recalculated from data provided in microplastic ingestion studies. Data are combined to obtain a 'Whole ocean' biota ingestion incidence value (°).

3.6 Perspective and Outlook

We provided an evaluation method for the quality of studies reporting microplastic ingestion by biota. The applied quality criteria were defined based on a critical review of the literature available. Current studies are not of such a level of reliability that they could be used confidently for risk assessment or monitoring of microplastic by biota in the natural environment. Reliable ingestion rate studies are needed in order to define whether there is a risk posed by microplastic ingestion to the natural environment, and to human food-safety. The proposed protocol can be used to perform these studies; the quality assessment system can be applied to control the quality of these data and enable an easier comparison of studies, in order to move towards standardisation and reliability. The quality assessment system may provide a tool and set an example that will help regulators and policy makers in their activities to mitigate contamination with plastic debris. Until now the majority of studies focussed on visually sortable microplastics. Our present scoring system is tuned to this research aim and used today's best available information. However, we foresee that our recommendations may need adaptations when the focus is on much smaller microplastic, which are more difficult to detect. It is also conceivable that our proposed scoring system needs modification if the research field evolves, for instance when new analytical technologies become available, just like the aforementioned CRED criteria (Kase et al. 2016) can be seen as evolving from the original Klimisch criteria (Klimisch et al. 1997) for

ecotoxicology studies. For now, all criteria were weighed equally as we considered all of them to be crucial for generating reliable results. Future research however, may provide a rationale for using unequal weights, which thus would lead to another outcome of the scoring. Finally, we emphasise that a protocol and scoring system for microplastic analytical studies should be seen as a product of the scientific community, rather than a product of a limited set of authors. In this sense we see the present paper as a starting point in assessing quality assurance criteria for microplastic analytical studies rather than the final stage.

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3.7 Supplementary Information

Table 3-S1. Explanation of scores - Quality assessment, MP in (marine) biota

Criterion		Explanation
1	Sampling methods	Several sampling characteristics should be recorded: this includes the exact sampling gear and information on the net type, material and its mesh sizes. Furthermore, the sampling location and depth ("upper 10 m", "bottom trawling," are sufficient) need to be recorded, as well as the date and time of the day sampled. This will enable identifying any potential contamination from the gear, or occurred during the sampling. This information also enables the replication of the sampling, and provides insight in comparability with other studies.
2	Sample size	50 or more individuals per research unit are defined as a suitable sample size (ICES 2015, MSFD (Technical Subgroup on Marine Litter) 2013). The confidence interval of the ingestion incidences should be reported (Figure 1). In larger animals, e.g. marine mammals, this criterion is difficult to achieve but samples should be as diverse and large as possible.
3	Sample processing and storage	Biota samples should be stored between the moment of capture and the examination in the lab. At best, the samples should be frozen at -20°C (ICES 2015). For small species the preservation in a glass container using a fixative is an option. However, the effects of the fixative on different types of plastic should be evaluated before application. Recently, the usage of formaldehyde/ ethanol were found to have no effects on different microplastics (Courtene-Jones et al. 2017). If any other fixative is used, an application test will be required. Additionally, any sample handling, such as dissections, should be left for the lab, never on board.
4	Laboratory preparation	All materials, equipment, and laboratory surfaces need to be thoroughly washed and rinsed. After rinsing, all materials should be kept in clean air conditions. All other materials, such as solutions and filters, should be checked before usage and covered afterwards. If possible, the sample specimens should be rinsed and checked for external contamination (Foekema et al. 2013). Sample contamination originating from researchers' clothing should be avoided by solely wearing 100% natural fibre clothing and a cotton lab coat. The coat alone may not be sufficient; wearing a polyester shirt underneath, it is imaginable that some fibres could end up in the samples. However, for the current scoring, a 100% cotton lab coat was considered sufficient when all other precautions were met.

5	Clean air conditions	The handling of samples should be performed in clean air facilities, such as a (positive pressure) laminar flow cabinet or a "clean room". Samples should not be taken out of the clean air facilities without being sealed off. Since the analysis often cannot be conducted under clean air conditions the implementation of negative controls becomes an even higher necessity.
6	Negative control	Controls (in triplicate) should be included for each batch of samples and should be performed in parallel to the sample treatment (ICES 2015). The controls should be conducted using filtered water, or biota tissue that is free of plastic. Only then a contamination deriving through air, clothes, added chemicals or used equipment can be discovered. Additionally (not instead!), controls might be taken again at "high risk moments" that are moving materials or samples in-/ outside the clean air facilities, or during analysis outside the clean air facilities (e.g. visual inspection, or polymer identification). Here, clean petri dishes or soaked paper can be placed next to the sample, and checked for any occurred contamination.
7	Positive control	Positive controls (triplicates) should be included to determine the microplastic detection efficiency. This is a necessary quality assurance, providing information on the effectiveness of the purification and analysis methods applied. Positive controls should be performed in parallel to the sample treatment using samples with an added number of microplastic particles of known polymer identity. Then, the numbers of retrieved microplastic particles are tallied to the amounts added.
8	Target component	To ensure monitoring of all ingested microplastic, a suitable target component for larger species, such as fish, is the full gastrointestinal tract (GIT). For smaller species, such as bivalves, the entire organism should be used.
9	Sample treatment	A digestion step must be included to dissolve organic sample matter so that especially small microplastics are not overlooked. The digestion method described by Foekema et al. (2013) using a 10% KOH-solution is considered suitable for fish (Dehaut et al. 2016). However, heating of the samples during digestion should be omitted. For smaller organisms, applying an enzymatic digestion is considered adequate as well (Cole et al. 2014, Courtene-Jones et al. 2017, Löder et al. 2017). The digestion of organic material can be circumvented when focussing solely on the ingestion of bigger microplastics. A lower size limit needs to be defined by e.g sieving the sample over 300 µm (Rummel et al. 2016).

10	**Polymer identification and reporting**	For all microplastics a polymer identification is required. The choice of the analytical method depends on the targeted microplastic sizes. The most common methods are FTIR or Raman (micro)spectroscopy, and pyrolysis- or TGA GC-MS. Any of these can be applied. For pre-sorted particles and when these numbers are < 100, all particles should be analysed. For particle numbers > 100, >50 % should be identified, with a minimum of 100 particles. The reporting should include: particle counts with confidence intervals, detection limits for the count and for minimum particle size, detected microplastic sizes, polymer types and percentages.

Table 3-S2. Definition of scores - Quality assessment, MP in (marine) biota

Scores	
2	Reliable without restrictions
1	Somewhat reliable, with restrictions
0	Not reliable

		Definition of scores		
Criterion		2	1	0
1	**Sampling methods**	Extensive reportage of applied sampling methods (gear, mesh size, material), as well as locations and times sampled.	Minor part of the sampling was not performed or reported correctly.	No/ insufficient reportage of sampling methods. **OR** The sampling was done by a third party (e.g. fishermen) without a special instruction on handling of organisms. **OR** The organisms were bought in stores (this is not inherently wrong, provided that the interest of the study lies in contamination of sea food, not in natural populations).
2	**Sample size**	> 50 individuals per research unit (that can be: species, food web, ecoregion, or feeding type).	> 50 individuals in total and > 25 individuals per research unit.	< 50 individuals.
3	**Sample processing and storage**	Sample freezing or storing on ice shortly after capture, and any sample handling was avoided before being in the laboratory. **OR** sample storage in a fixative when effects of the fixative on different polymers were tested before application.	Standards only partially met.	Dissection of organisms performed on board.
4	**Laboratory preparation**	Fulfilled precautions that are: wearing non-synthetic clothes and a	Solely wiping laboratory surfaces and equipment or not	No precautions.

		lab coat. All equipment and lab surfaces, as well as the exterior of organisms were rinsed and checked for contamination.	wearing a lab coat **IF** negative samples were run in parallel and examined for contamination.	
5	**Clean air conditions**	Usage of clean room conditions for sample handling in the laboratory.	Mitigation of airborne contamination by carefully keeping samples closed as much as possible **IF** negative samples were run in parallel and examined for occurring contamination.	No regard of airborne contamination.
6	**Negative control**	Controls (in triplicate) treated and analysed in parallel to actual samples.	Insufficient form of a control, e.g. the filtration of air, or the sole examination of petri dishes/ soaked papers placed next to the samples.	No negative controls.
7	**Positive control**	Controls (triplicate) with an added amount of microplastic particles treated the same way the samples are, and for which the particle recovery rates are determined.	Insufficient form of a positive control (e.g. if only a part of the protocol is tested).	No positive controls.
8	**Target component**	Examination of complete organisms or entire GITs.	Examination of parts of the GITs, while complete GITs where analysed for a subsample.	Examination of stomach contents only (missing information on the gut/ throat).
9	**Sample treatment**	Digestion of complete GIT/ organisms using a protocol with KOH or enzymes. If another chemical was used, effects on different polymers have to be tested before application.	Digestion of parts of the GIT **OR** if proof is missing that polymers are not affected by protocol (e.g. heated KOH) **OR** in case studies are aware of the need for a purification step when going for smaller MP,	No digestion of GIT.

			but exclusively focus on the bigger microplastics by sieving the samples (mesh size > 300µm).	
10	**Polymer identification**	Analysis of all particles when numbers of pre-sorted particles are <100. For particle numbers >100, 50% should be identified, with a minimum of 100 particles.	Insufficient polymer identification, potentially resulting in an unrepresentative subsample.	No polymer identification.

Table 3-S3 | Scoring of individual papers. (Table not printed in this thesis, for this, the reader is referred to the online version of the published article).

CHAPTER 4

IDENTIFICATION OF MICROPLASTIC IN EFFLUENTS OF WASTE WATER TREATMENT PLANTS USING FOCAL PLANE ARRAY-BASED MICRO-FOURIER-TRANSFORM INFRARED IMAGING

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Abstract

The global presence of microplastic (MP) in aquatic ecosystems has been shown by various studies. However, neither MP concentrations nor their sources or sinks are completely known. Waste water treatment plants (WWTPs) are considered as significant point sources discharging MP to the environment.

This study investigated MP in the effluents of 12 WWTPs in Lower Saxony, Germany. Samples were purified by a plastic-preserving enzymatic-oxidative procedure and subsequent density separation using a zinc chloride solution. For analysis, attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FT-IR) and focal plane array (FPA)-based transmission micro-FT-IR imaging were applied. This allowed the identification of polymers of all MP down to a size of 20 µm. In all effluents MP was found with quantities ranging from 0 to 5×10^{1} m^{-3} MP > 500 µm and 1×10^{1} to 9×10^{3} m^{-3} MP < 500 µm. By far, polyethylene was the most frequent polymer type in both size classes. Quantities of synthetic fibres ranged from 9×10^{1} to 1×10^{3} m^{-3} and were predominantly made of polyester. Considering the annual effluxes of tested WWTPs, total discharges of 9×10^{7} to 4×10^{9} MP particles and fibres per WWTP could be expected. Interestingly, one tertiary WWTP had an additionally installed post-filtration that reduced the total MP discharge by 97%. Furthermore, the sewage sludge of six WWTPs was examined and the existence of MP, predominantly polyethylene, revealed. Our findings suggest that WWTPs could be a sink but also a source of MP and thus can be considered to play an important role for environmental MP pollution.

4.1 Introduction

Plastic litter contamination is considered one of the most serious manmade threats for the natural environment and hence a topic of emerging concern (Eerkes-Medrano et al. 2015). Polymer particles < 5 mm are defined as microplastic (MP). Primary MP is intentionally produced in small sizes and used as industrial pellets or scrubbers added to personal care products. For the latter category, Chang (2015) determined a size range of 60 to 800 µm when testing MP in various cosmetics. Being exposed to environmental abiotic and biotic processes, plastic undergoes degradation and fragmentation into smaller particles (Cole et al. 2011). Thereby, so called secondary MP is formed. In environmental samples both, primary and secondary MP, is found (Eerkes-Medrano et al. 2015, Phuong et al. 2016). So far, the occurrence of MP was mostly determined in marine water, sediment or biota samples. Here, studies showed a global presence of MP in all, even remote habitats (Andrady 2011, Browne et al. 2011, Eriksen et al. 2013b, Watters et al. 2010), or revealed ingestion by several species (Kühn et al. 2015). MP amounts and classification of potential sources vary considerably among studies. However, it is widely assumed that with up to 80% the largest share of marine plastic derives from terrestrial sources (Andrady 2011, Wagner et al. 2014), including inadequately disposed plastic and (micro)plastic introduced via riverine transport. Nevertheless, only recently studies started quantifying MP in major rivers e.g. the Thames (Morritt et al. 2013), the Danube (Lechner et al. 2014) or the Rhine (Mani et al. 2015), determined abundances vary considerably. Similar to results from the marine environment, detailed information on sources for MP in limnic habitats are scarce (Jambeck et al. 2015). While acknowledging substantial fluxes coming from industrial plants (Lechner et al. 2014, Mani et al. 2015) or littering (Morritt et al. 2013), the effluents of waste water treatment plants (WWTPs) are often seen as a major source for MP (Cole et al. 2011, Mani et al. 2015). Considering the high likelihood of municipal and industrial effluents as well as urban surface runoffs containing macro- and microplastic, it is a plausible assumption. While large plastics are likely being removed in water processing, used technologies are not specifically designed to retain small MP. So far, the effluents of WWTPs are studied rarely. Quantitative data are available from few non-peer-reviewed reports (Leslie et al. 2013, Magnusson and Norén 2014, Talvitie and Heinonen 2014) or peer-reviewed studies from Australia (Browne et al. 2011), the UK (Murphy et al. 2016), France (Dris et al. 2015), Finland (Talvitie et al. 2015) and the United States (Carr et al. 2016). In the study by van Wezel et al. (2016) emissions of primary MP by Dutch WWTPs were estimated. Scarce data on MP in the influents and removal efficiencies during water purification complicate this approach. While McCormick et al. (2014) detected significantly more MP in the surface water of a highly urbanized river in Chicago after the introduction of treated waste water (TWW), Klein et al. (2015) could not link MP data of Rhine sediments to WWTPs. Further, Carr et al. (2016), Talvitie et al. (2015) and Murphy et al. (2016) showed high retention potentials of WWTPs by examining influent and effluent. Nevertheless, comparability of data is limited since mentioned studies apply different methods for MP sampling and analysis.

Methods used for MP determination range from visual sorting via a dissection microscope over gas chromatography-mass spectrometry (GC-MS) (Fries et al. 2013) to Fourier- transform infrared spectroscopy (FT-IR) and RAMAN microscopy (Fischer et al. 2015, Löder et al. 2015). Without any further chemical analysis, particles determined via visual analyses show error values up to 70% (Hidalgo-Ruz et al. 2012). For a distinct determination the sample must be either targeted with a destructive method, e.g. GC-MS analysis, or measured via spectroscopic methods, e.g. FT-IR or RAMAN spectroscopy. In the present work FT-IR analysis was the method of choice. Through coupling to a microscope even small transparent particles with a size down to 20 µm can be measured as manual sorting becomes unfeasible. Different detectors can be attached. While extremely long measurement times are required for complete filter analyses when using a single element detector (Harrison et al. 2012), time consumption can be reduced when applying modern focal plane array (FPA) detectors. At the same time a maximum in resolution can be kept (Löder et al. 2015, Tagg et al. 2015). In general, a FPA detector consists of a n x n field of single detector elements. During data collection each element is read out individually resulting in n^2 spectra within one measurement. Within the FT-IR microscope these fields can be arranged as arrays, allowing the measurement of wide fields. For transmission measurements, optimized parameters concerning time consumption and data quality are already available (Löder et al. 2015).

Targeting at the exact determination of MP contamination in TWW, the effluents of 12 German WWTPs were sampled. For the first time FPA-based transmission micro-FT-IR imaging was applied to detect MP in large volumes of organic-rich TWW. Samples were purified by a plastic-preserving enzymatic-oxidative procedure which enabled the identification of all MP down to a size of 20 µm. Since this is the first study providing this detailed information, selected WWTPs varied in waste water derivation and applied water purification technologies to further evaluate results. To explore the potential of sewage sludge to be a sink for MP, drained sewage sludge from six WWTPs was purified according to the alkaline protocol of Cole et al. (2014) and analysed for MP.

4.2 Materials and methods

4.2.1 Information about sampled WWTPs

The Oldenburg-East-Frisian water board supplies the drinking water for an area of 7500 km² and manages the sewage disposal in an area of 3700 km² in the north-western part of Germany. Overall, 46 WWTPs are responsible for treating 32.5 million m³ of waste water each year. Targeting at the determination of MP amounts and sizes in TWW, 12 WWTPs were selected which vary in capacity, waste water derivation and composition (Table 4-S1). With an annual efflux of approximately 13 million m³, the WWTP of Oldenburg has the largest capacity. Another four WWTPs discharge between 1 to 2.6 million m³ TWW yearly. The smallest facility records an annual efflux of 0.185 million m³. In general the WWTPs receive municipal and industrial effluents, whereby industrial sources are combined, accounting for approximately 20% of the inputs. In three facilities a higher industrial share can be attributed directly to an abattoir, a dairy and a textile processing plant. In contrast, five WWTPs purify almost exclusively municipal waste waters. All WWTPs receive waste water through a combined sewer system and require two to three days for purifying waste water under normal weather conditions.

Individual WWTPs handle waste water by different treatment technologies (Table 4-S1). Six WWTPs have primary skimming tanks where lighter, floating materials are removed from the water surface and further processed as primary sludge. All WWTPs apply secondary treatment reducing organic materials, nitrogen and phosphate compounds. Additionally, four plants provide tertiary treatment to further reduce suspended matter. In three WWTPs this is facilitated by settling processes in maturation ponds. In Oldenburg the daily flux of 3.6×10^4 m³ is finally filtered over pile fabrics (MECANA, Switzerland). Comparable to a fur, these fabrics are made of very fine polyamide (PA) fibres, attached to a netting made of polyester (PEST) with a small amount polyethylene (PE). The nominal pore size of 10 to 15 µm can filter even smaller materials due to the crosslinked fibres. The fabrics are backwashed several times per day. The retained material is combined with the sewage sludge and treated equivalently. To reduce the large water proportion in the sewage sludge polymeric flocculants can be added. Further, the sludge can be drained through centrifugation and compression. Nowadays more than half of the sludge produced by the 46 WWTPs is burnt for energy generation, while agricultural usage is decreasing.

4.2.2 Sampling

The sampling at the 12 WWTPs took place between April 22th and 29th 2014. It was intended to sample purified water from the effluent. Depending on the spatial circumstances, the exact sampling point had to be adapted for each WWTP. Samples were taken from the effluent, at the overflow of clarifying tanks or at the intake of maturation ponds. At the WWTP of Oldenburg two samples were taken, directly before and after the installed post-filtration.

The TWW samples were taken by a custom made mobile pumping device (Figure 4-S1,A). It consisted of a flexible polyvinylchloride (PVC) hose with a weighted end-piece connected to a membrane pump (Jabsco EMG 590-8023, Xylem, Germany), a filter housing (polycarbonate (PC) with polypropylene (PP) lid) containing a 10 µm stainless steel cartridge filter (4 7/8", Wolftechnik, Germany) and a flowmeter (Gardena, Germany). At each sampling station a separate filter unit (filter housing with stainless steel cartridge filter), previously rinsed with analytical grade water (Milli-Q), was used. Prior to sampling the pumping system was primed for five minutes with TWW. For sampling the weighted end-piece was located 10 cm below the water surface. Generally a sample volume of 1 m^3 was intended, yet sampling had to be stopped in case of a significant reduction of flow rate. Six out of 13 sampling procedures were stopped prematurely, with a minimum of 390 litres at WWTP of Holdorf (Table 4-S1). After completing each filtration the units were kept sealed and stored refrigerated at 4°C. The pumping system was rinsed thoroughly with tap water before subsequent sampling.

Additionally, we sampled drained sewage sludge at six WWTPs which apply a primary skimming treatment (Table 4-S1). Samples of 500 g wet weight were taken by shovel, stored dark and refrigerated (4 °C) in PVC sample containers that were previously rinsed with Milli-Q.

4.2.3 Purification of MP

4.2.3.1 Contamination mitigation

To reduce the risk of contaminating TWW samples several steps were taken. During sample purification only clothes made of natural fabric and clean lab coats were worn. Before usage all lab materials were rinsed with Milli-Q and ethanol (30%, Carl Roth GmbH & Co. KG, Germany, filtered over 0.2 µm) and covered with aluminium foil. We limited the usage of plastic materials, but could not avoid it. Therefore three negative controls were implemented in the purification process of TWW to examine a potential secondary contamination. Negative controls consisting of 150 litres filtered tap water (3 µm stainless steel cartridge filters) were treated and analysed simultaneously to TWW samples.

4.2.3.2 Purification of MP in TWW

To remove natural organic and inorganic material prior to FT-IR analyses, TWW samples were treated with a multi-step, plastic-preserving enzymatic maceration according to (Löder and Imhof unpubl. data). All applied enzymes (ASA Spezialenzyme GmbH, Germany) were of technical grade and sterile filtered (0.2 µm) before application to remove organic residues from the production process. Enzyme activities were not checked before usage and are specified according to the manufacturer. For simplicity solely Phosphate Buffered Saline (PBS, Thermo Fisher Scientific Inc., USA) was used as a buffering solution but adjusted to the pH optima of respective enzymes according to the manufacturer's recommendations. All purification solutions, with exception of hydrogen peroxide (H_2O_2) and chitinase, were pumped directly into the filter units. After each treatment the liquid was removed by pressure (0.2 µm filtered compressed air) and samples were flushed with 5 litres of 3 µm filtered tap water.

Purification started with the addition of sodium dodecyl sulphate (5% w/vol). Samples were incubated at 70°C for 24 h. Next, protease (Protease A-01, ~1800 U L^{-1} in PBS pH 9) was added and samples were incubated at 50°C for 48 h. Then lipase (Lipase FE-01, ~2320 U L^{-1} in PBS pH 10.5) and cellulase (Cellulase TXL, ~44 U L^{-1} in PBS pH 4.5) were added. Samples were incubated at 40°C for 96 h and at 50°C for 6 d, respectively. Finally, cartridge filters were removed from housings and rinsed with Milli-Q and ethanol (30%). If necessary, attached material was scraped off from the cartridge filters using a metal brush. The cartridge filters were placed in Milli-Q containing beakers, sonicated (Sonorex RK514, Bandelin, Germany) for 3 minutes and rinsed again. Subsequently, samples were fractionated by filtration over a 500 µm PA net. Retained material was stored in glass petri dishes for photographic documentation and ATR-FT-IR (see 2.4.1) analyses.

Sample fractions < 500 µm were filtered onto 10 µm stainless steel screens. These screens were placed in 100 ml glass bottles, covered with 30 ml H_2O_2 (35%, Carl Roth GmbH & Co. KG, Germany) and incubated at 50°C for 24 h. After removal of H_2O_2 by filtration on the rinsed 10 µm screens, they were placed back in the glass bottles and covered with 30 ml of a chitinase solution (Chitodextrinase, ~96 U L^{-1} in PBS pH 5.6). Samples were incubated at 37°C for 48h. Finally, the application of H_2O_2 was repeated as described above.

Inorganic compounds (e.g. sand, rust) were removed by performing density separation using a zinc chloride ($ZnCl_2$, Carl Roth GmbH & Co. KG, Germany) solution with a density of 1.6 g cm^{-3}. After a settling time of 24 h in 100 ml separation funnels, the settled material was purged. For later micro-FT-IR analyses the residing fluid was enriched onto 0.2 µm aluminium oxide filters (Anodisc 25 mm, Whatman, U.K.) by using an in-house fabricated filter-funnel having an inner diameter of 11 mm (Figure 4-S1,B). To prevent sample loss, the funnels were thoroughly rinsed with Milli-Q and ethanol. In case of large residues of material, aliquots were distributed on additional filters. The filters were placed in individual, partly opened petri dishes and dried at 40°C.

Residues of the 500 µm net were visually inspected using a stereo light microscope (Olympus SZX16, Olympus K.K., Japan) with an attached camera (Olympus DP26, Olympus K.K., Japan). Screening for potential plastic particles was facilitated by using a Bogorov chamber and by applying following criteria. Particles that showed a bright and homogenous colour, no cellular structures and certain bending properties were isolated. All these particles were photographed and measured at their longest dimensions. Afterwards, the inspected samples were filtered onto 0.2 µm aluminium oxide filters (Anodisc 47 mm, Whatman, UK) to count fibres that remained unaffected by visual inspection. The fibres were categorized in terms of colour (black/blue, red, transparent and other). Likewise, fibres in the fraction < 500 µm were counted and categorized during micro-FT-IR analyses. To determine the proportion of synthetic fibres, 60 fibres per sample were identified individually by using micro-FT-IR (see 2.4.2).

4.2.3.3 Purification of MP in sewage sludge

To extract MP from sewage sludge an alkaline treatment according to Cole et al (2014) was applied. Therefore, 125 g drained sewage sludge was diluted in 825 ml Milli-Q, admixed with 400 g solid sodium hydroxide (Sigma Aldrich Chemie GmbH, Germany) and kept stirred for 24 hours at 60°C. After neutralizing with hydrochloric acid (37 %, Carl Roth GmbH & Co. KG, Germany) the NaCl solution had a density of 1.14 g cm^{-3}. A settling time of 96 hours allowed separating the most common polymers, PE, PP and PS (Hidalgo-Ruz et al 2012). Therefore, supernatants were rinsed over a 500 µm PA net. Residues of the 500 µm net were visually inspected using a stereo light microscope and all particles potentially made of plastic were identified by ATR-FT-IR (see 2.4.1). Aliquots (20 % of original samples) of the smaller fraction were filtered onto 0.2 µm aluminium oxide filters for micro-FT-IR analysis.

4.2.4 FT-IR analyses

FT-IR analyses were performed on a Tensor 27 FT-IR spectrometer (Bruker Optik GmbH, Ettlingen, Germany) further equipped with a Platinum-ATR-unit (Bruker Optik GmbH), a Hyperion 3000 FT-IR microscope with a 15× cassegrain objective and a 64×64 FPA detector (Bruker Optik GmbH).

4.2.4.1 ATR-FT-IR

Previously sorted material (> 500 µm) was photographed and identified by using ATR-FT-IR. Therefore, individual particles were placed onto the ATR crystal. To analyse recorded IR spectra the software OPUS 7.2 (Bruker Optik GmbH) was used and identified by a spectrum search in a customized polymer library. The library contained spectra of all common polymers, natural materials and lab materials used during sampling and purification.

4.2.4.2 Micro-FT-IR

For microscopic measurements with the FPA detector the filters with the enriched samples were placed on calcium fluoride windows on a customized sample holder. All measurements were performed with the optimized measurement settings published by Löder et al. (2015) with a binning factor of 4 and a spectral resolution of 8 cm^{-1} with 6 co-added scans.

To screen all filters in a reasonable amount of time, it was decided to analyse 25% of each filter (see 4.3 for a detailed discussion). This approach used two crossed 7x65 FPA arrays, considering the overlapping area in the centre of the cross only once.

The data were analysed using the software OPUS 7.2. False colour images were produced using two polymer specific regions, firstly between 1480-1430 cm^{-1} (C-H bending, aromatic ring stretching) and secondly between 1790-1700 cm^{-1} (C=O stretching). The images derived from this integration have a colour scheme proportional to the area above or below the baseline (that is the straight line between the data points at the upper and lower wavenumber of the targeted region). Based on these information potential MP was marked and the spectra identified via a library search (Löder et al. 2015). Same time, for each particle colour and size at the longest dimension were recorded.

To target fibres in a suitable manner, a different approach needed to be used. Fibres from the fraction > 500 µm were concentrated on 0.2 µm aluminium oxide filters (Anodisc 47 mm, Whatman,UK) and measured individually using micro-FT-IR. From each colour a representing amount of 15 fibres per sample was chosen and identified. Compared to settings of the wide imaging, the settings for fibre analysis were modified. After focusing a single fibre, a grid of 2 FPA fields (each resulting in 64 x 64 measured pixel) was placed and measured without active binning, resulting in a lateral pixel resolution of 2.7 µm. The number of scans was increased to 32 (instead of previously chosen six) to gain an optimal signal to noise ratio. With a process time of ca. ten hours per sample this method was highly time demanding but enabled to estimate amounts of synthetic fibres by projecting determined proportions to total fibre counts.

4.2.5 Statistical analyses

Spearman rank correlation was performed between polymer specific counts of MP < 500 µm and population equivalents of respective WWTPs (Table 4-S1). Yearly effluents of the WWTPs were not included since these were highly correlated with population equivalents. The spearman rank correlations were calculated by using Statistica 11 (Statsoft, Germany).

4.3 Results

4.3.1.1 Contamination

Negative controls were found to be slightly contaminated with particulate (av. number 21) and fibrous (av. number 130) MP representing five different polymers: PP, PE, PA, styrene acrylonitrile (SAN) and PEST. Contamination predominantly consisted of PP particles (av. 81%), whereas remaining polymers were detected in comparably low numbers. The averaged polymer specific counts from the three negative controls were subtracted from the counts of all analysed TWW samples.

4.3.1.2 MP > 500 µm in TWW

In ten of the 12 WWTPs effluents MP > 500 µm was detected (Figure 4-1,A). The effluents in Schillig and Oldenburg after post-filtration contained no MP > 500 µm. MP of the remaining WWTPs (including Oldenburg before post-filtration) comprised of eight synthetic polymers: PE, PP, PA, PVC, polystyrene (PS), polyurethane (PUR), silicone and PUR-based coatings ("paint"). The majority of MP was identified as PE (av. 59 %), followed by PP (av. 16 %). Other polymers occurred sporadically (Figure 4-S2,A). In total one to five polymers in a size range of 500 to 7200 µm were detected per sample (Figure 4-S3,A and S4,A). Discharges of MP > 500 µm ranged from 0×10^{1} m^{-3} in Schillig to 4×10^{1} m^{-3} in Holdorf (Figure 4-1,C). The sample taken after the installed post-filtration in Oldenburg did not contain MP > 500 µm (5×10^{1} m^{-3} before post-filtration). Annual discharges of MP > 500 µm were extrapolated by taking the annual effluxes of the respective WWTP into account and ranged from 1×10^{6} y^{-1} in Lohne to 5×10^{7} y^{-1} in Varel (Figure 4-1,B).

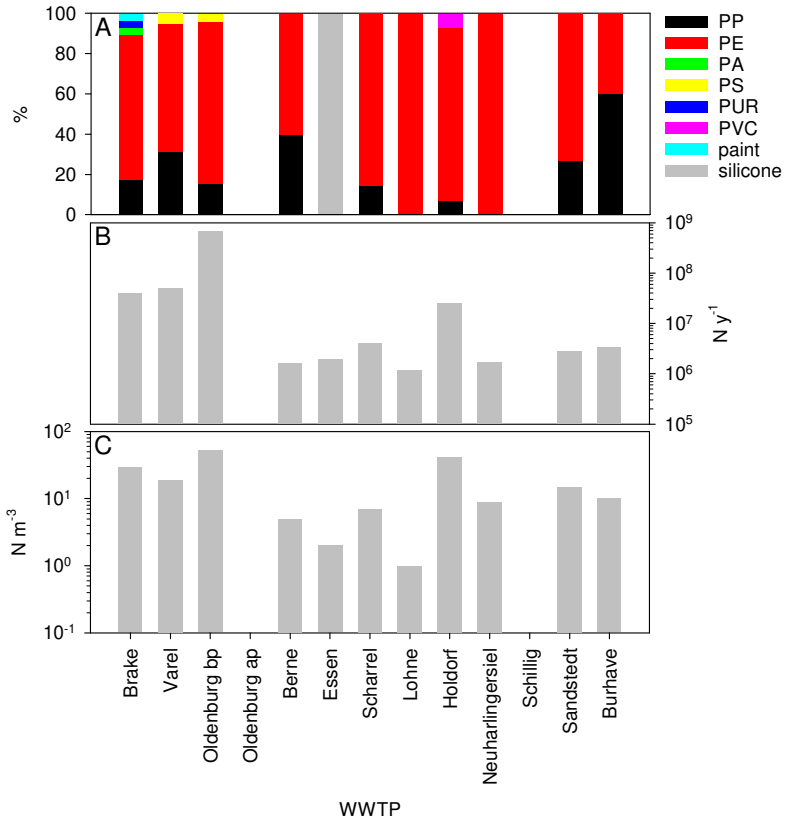

Figure 4-1 | Microplastics (MP) > 500 µm in treated waste water (TWW) of 12 waste water treatment plants (WWTP) in Lower Saxony (Germany). At the WWTP Oldenburg a sample was taken before (bp) and after (ap) post-filtration. A: Percentage composition of synthetic polymers; B: Annual load of MP in the effluent (based on yearly effluent); C: MP numbers per cubic meter.

4.3.1.3 MP < 500 µm in TWW

All samples contained MP < 500 µm including Oldenburg after post-filtration and Schillig (Figure 4-2,A). Detected MP comprised of 14 polymers: PE, PP, PS, PA, SAN, PEST, PVC, PUR, polyethylene terephthalate (PET), ethylene vinyl acetate (EVA), polyvinyl alcohol (PVAL), acrylonitrile butadiene styrene (ABS), polylactide (PLA) and paint. PE clearly dominated all samples (av. 40 %), followed by PVAL (av. 16 %), PA and PS (each 8 %, Figure 4-S2,B). Three to 12 polymers were detected per TWW sample. In Oldenburg six different polymers (PE, PVAL, PP, SAN, PET and paint) were detected before post-filtration, while only PE, PA, SAN were detected afterwards. In all samples most particles were of a size between 50 to 100 µm (av. 59 %), only 4 % were determined bigger than 250 µm (Figure 4-S3,B and S4,B).

The discharges of MP < 500 µm varied considerably between 8 × 10^1 m^{-3} in Neuharlingersiel, 7×10^2 m^{-3} in Essen and 9×10^3 m^{-3} in Holdorf (Figure 4-2,C). The post-filtration in Oldenburg decreased the amount of MP < 500 µm from 2×10^2 m^{-3} to 1×10^1 m^{-3}. Extrapolated annual discharges of MP < 500 µm ranged from 1×10^7 y^{-1} in Neuharlingersiel to 5×10^9 y^{-1} in Holdorf (Figure 4-2,B).

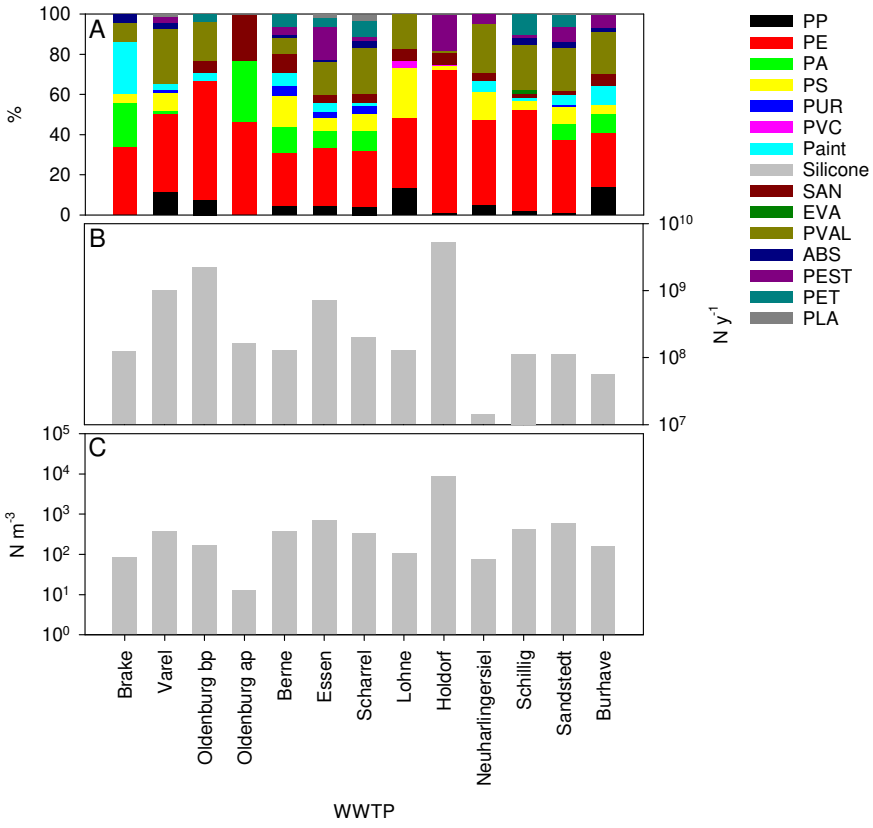

Figure 4-2 | Microplastics (MP) < 500 µm in treated waste water (TWW) of 12 waste water treatment plants (WWTP) in Lower Saxony (Germany). At the WWTP Oldenburg a sample was taken before (bp) and after (ap) post-filtration. A: Percentage composition of synthetic polymers; B: Annual load of MP in the effluent (based on yearly effluent); C: MP numbers per cubic meter.

4.3.1.4 Synthetic fibres in TWW

Both sample fractions contained fibres of comparable sizes. Thus, it was assumed that fibres were able to pass the 500 µm net in an upright orientation and micro-FT-IR analyses were only conducted for fibres of the fraction > 500 µm. The results were extrapolated to both fractions. In total 2×10^4 fibres (of synthetic and natural materials) were detected in the TWW samples. Most fibres were categorized as transparent (av. 61 %). For each TWW sample and colour group 15 fibres were analysed by micro-FT-IR (see 2.3.2). All samples contained synthetic fibres comprising of three different polymers (PA, PP, PEST, Figure 4-3,A). PEST was found predominantly (av. 74 %) followed by PA (av.17 %) and PP (av. 9 %, Figure 4-S2,C). Individual samples contained fibres of one to three different polymers. After post-filtration in Oldenburg only PEST fibres were detected. Discharges of synthetic fibres ranged from 1×10^2 m^{-3} in Burhave to 5×10^3 m^{-3} in Holdorf (Figure 4-3,C). Post-filtration installed in Oldenburg reduced the load of synthetic fibres from 9×10^2 m^{-3} to 2×10^1 m^{-3}. Annual discharges of synthetic fibres were extrapolated and ranged from 3×10^7 y^{-1} in Burhave to 3×10^9 y^{-1} in Holdorf (Figure 4-3,B).

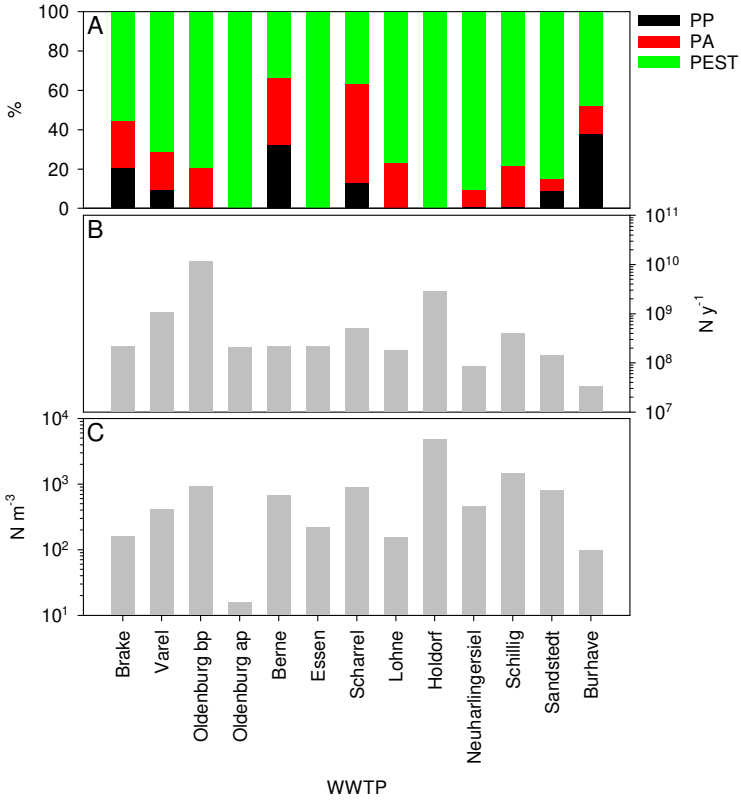

Figure 4-3 | Synthetic fibers in treated waste water (TWW) of 12 waste water treatment plants (WWTP) in Lower Saxony (Germany). At the WWTP Oldenburg a sample was taken before (bp) and after (ap) post-filtration. A: Percentage composition of synthetic polymers; B: Annual load of synthetic fibers in the effluent (based on yearly effluent); C: Numbers of synthetic fibers per cubic meter.

4.3.2 Statistical Analyses

Spearman rank correlation was performed between polymer specific counts concerning MP < 500 µm and population equivalents of the respective WWTP. No significant correlation ($p < 0.05$) was observed.

4.3.3 MP in sewage sludge

While none of the samples contained MP > 500 µm, MP < 500 µm was detected in all sewage sludge samples (Figure 4-S5,A) made of PE, PP, PA and PS (Figure 4-S6). Fibres were not considered in the analyses since we did not estimate fibre contamination during purification. The samples displayed strong variations in estimated MP concentration, ranging from $1x10^3$ kg^{-1} (dry weight, dw) in Oldenburg to $2.4x10^4$ kg^{-1} dw in Scharrel (Figure 4-S5,C). This results in strong variations of the estimated amounts of MP in the yearly produced sewage sludge (Table 4-S1). Estimations vary between the WWTPs from 1.24×10^9 y^{-1} in Schillig to 5.67×10^9 y^{-1} in Scharrel (Figure 4-S5,B).

4.4 Discussion

4.4.1 Sampling

While previous studies examined MP in rather small volumes (< 50 litres) of TWW (Browne et al. 2011, Dris et al. 2015, Murphy et al. 2016), the custom made pumping and sampling device allowed the filtration of large volumes (0.39- 1 m^3) of TWW over cartridge filters with a pore size of 10 µm. Carr et al. (2016) also examined large sample volumes of TWW, but different pore sizes of 45 and 180 µm hinder the comparison of results. This emphasizes again the necessity of standardized and harmonized approaches (Hidalgo-Ruz et al. 2012).

4.4.2 Sample purification

The enzymatic-oxidative purification is a new approach for MP extraction from environmental samples (Cole et al. 2014, Löder and Imhof unpubl. data). For the first time we applied it successfully to extract MP from large volumes of TWW. It prevents the risk of partially disintegration or even loss of certain polymers that can occur through aggressive chemicals used for the removal of organic matter (Cole et al. 2014). This can also be seen as a precautionary step since the effects on very small or weathered polymers were not determined yet. However, enzymatic purification required a considerable amount of time and effort. The risk of contamination or sample loss increased due to several filtration steps, necessary for sample incubation at individual enzymatic pH-optima. To minimize these risks, first purification steps were performed in the individual, sealed filter units. H_2O_2 was used to oxidize organic matter (Imhof et al. 2012, Nuelle et al. 2014, Tagg et al. 2015). It was the only applied chemical having a potential impact on MP. Tagg et al. (2015) and Nuelle et al. (2014)

found no significant changes for different polymers after a weeklong exposure, so an exposure time of 48 h seemed reasonable. The finally applied density separation led to a considerable reduction of contained inorganic material. With a density of 1.6 g cm^{-3} the used $ZnCl_2$ solution ensured the separation of all common polymers. This cannot be guaranteed by the often used, cheaper and non-hazardous saturated sodium chloride solution with a density of 1.2 g cm^{-3} (Klein et al. 2015, Thompson et al. 2004). Hereby, the common polymers PE, PP, PS and PA are kept, while PVC or PEST are discounted (Hidalgo-Ruz et al. 2012), both types were identified in TWW samples. Acquisition and disposal costs were reduced by reusing the environmentally harmful $ZnCl_2$ solution after filtration over 3 µm stainless steel filters. Generally, the application of the enzymatic-oxidative purification for TWW samples led to a successful reduction of natural materials and the detection of various synthetic polymers. However, it should be noted, that the protocol was originally developed for plankton samples. Further adaptations could optimize future purification results.

To extract MP from sewage sludge we decided to follow the advice of Cole et al. (2014) and used heated 10 M NaOH. However, an insufficient purification prevented us from filtering whole samples onto aluminium oxide filters for micro-FT-IR analyses.

4.4.3 Contamination

The widespread usage of plastic results in a high risk of contaminating environmental samples and is widely discussed in this research field (Filella 2015, Imhof et al. 2012, Murphy et al. 2016). To estimate the contamination we implemented three negative controls into the process of TWW purification. Although care was taken (e.g. clothes made of natural fabrics and lab materials that were previously cleaned and covered directly) the three negative controls revealed a contamination of samples with plastic particles and fibres. Our aim was to present an applicable approach that can be repeated easily at different WWTPs and so we did not fully avoid the usage of plastic materials. However, this should be reconsidered. The high contamination with PP is probably caused by filter housings. A replacement by stainless steel housings would be possible but needs to be assessed against higher costs and weight. Further, techniques limiting fibres contamination should be discussed and implemented. So far the handling of fibrous contamination varies between studies. While Dris et al. (2015) and Murphy et al. (2016) determined fibre contamination directly, Leslie et al. (2013) displayed general numbers for particulate and fibrous MP. Carr et al. (2016) as well as Magnusson and Norén (2014) did not present negative controls. This hampers the comparison of results and underlines the need to implement negative controls when examining MP in environmental samples.

4.4.4 FT-IR analyses

As already stated by Hidalgo-Ruz et al. (2012) as well as Löder and Gerdts (2015) the analyses of MP solely based on visually inspection is insufficient and harbours the risk of MP overestimation by misidentification but also -vice versa- underestimation due to barely visible or transparent MP particles and fibres. For the presented results we consequently relied on FT-IR techniques for material identification.

For small, not sortable MP particles micro-FT-IR techniques have been applied successfully. Thereby, the optical resolution of a microscope is combined with the analytical strength of a FT-IR spectrometer to identify particles that are enriched on filters or plates. While Ng and Obbard (2006) and Murphy et al. (2016) conducted a visual pre-selection of suspicious particles, Harrison et al. (2012) as well as Vianello et al. (2013) mapped complete, randomly selected filter sub-areas. With spatial resolutions of 150 µm and 50 µm, respectively, around 5% of total filter areas were analysed. In contrast to these single point measurements the coupling of an FPA detector (n x n single detector elements) enables the imaging of whole filter areas, resulting in the avoidance of any pre-selection (Löder et al. 2015). The usage of FPA-based micro-FT-IR analysis for detecting MP in TWW was recently published by Tagg et al. (2015) as a "proof of principle" study. Mentioned studies applied reflectance micro-FT-IR analysis (Harrison et al. 2012, Murphy et al. 2016, Ng and Obbard 2006, Tagg et al. 2015, Vianello et al. 2013). However, we measured IR- transmission since Löder et al. (2015) showed better imaging results. Their settings enabled the analysis of entire filters (11 mm in diameter) with a spatial resolution of 20 µm. Initially, the same approach was intended for the analysis of TWW samples. It became unfeasible since high sample loads had to be split on up to nine filters, resulting in 38 filters for the 13 TWW samples. As a consequence, two cross-shaped areas were analysed which allowed an automatic analysis of 25 % of all filters while maintaining the spatial resolution of 20 µm. For the first time MP in TWW was determined by an unbiased approach due to micro-FT-IR imaging.

In contrast, MP > 500 µm had to be visually sorted. Therefore, presented results bear the risk of underestimation. To minimize this risk as good as possible, all suspicious particles were sorted since ATR-FT-IR analysis prevented from overestimation. Such an approach is commonly used for bigger MP in environmental samples (Dris et al. 2015, Filella 2015, Mani et al. 2015, Talvitie et al. 2015).

The greatest challenge faced by this study was the identification of fibres. Due to several reasons it was not possible to determine fibres simultaneously during imaging of MP < 500 µm. Next to a fineness of 10 to 20 µm and an often occurring slight protrusion from the focal plane of the analysed filter cake, the spherical shape led to a non-ideal interaction (e.g. diffraction at the surface, lens effects) with the IR beam reducing the accuracy of the measurement. An additional difficulty arose through the high loads of fibres which prohibited individual handling and identification by ATR-FT-IR. Therefore, the technique of imaging was stretched to its limits to identify fibres. Individual fibres were focused and analysed by two

high resolution FPA fields. The differentiation between fibres of synthetic or natural materials required about ten hours per sample and resulted in the analysis of subsamples. Thus, presented results documenting the discharge of synthetic fibres and of MP > 500 µm should be seen as estimates and handled with care.

4.4.5 MP in TWW and sewage sludge

In this study we present for the first time reliable data on the presence of MP and synthetic fibres in the effluents of selected German WWTPs. Both types of MP were detected in all TWW samples. The results have to be discussed and compared to other monitoring data in terms of amounts, sizes and polymers. As mentioned previously, this is hindered by considerably varying sampling, sorting and identification methods. Further, these results should be validated and repeated by replicates taken at different times and seasons. Only then reliable daily and yearly MP discharges of individual WWTPs can be presented.

In the TWW samples MP < 500 µm was quantified in a wide range between 1×10^{1} m^{-3} (Oldenburg after post-filtration) to 9×10^{3} m^{-3} (Holdorf, Figure 4-2,C). Previously reported MP discharges in TWW vary stronger from 2×10^{4} MP m^{-3} (Leslie et al. 2013, Talvitie et al. 2015), to 2.5×10^{2} MP m^{-3} (Murphy et al. 2016) and 4 MP m^{-3} (Magnusson and Norén 2014). Carr et al. (2016) found even less with 1 MP m^{-3} in secondary plants and the effluents of tertiary plants seemed free of any MP. While earlier studies examined MP in TWW visually using microscopes, Carr et al. (2016), Magnusson and Norén (2014), Talvitie et al. (2015) and Murphy et al. (2016) used FT-IR to analyse selected particles. Here, for the first time, polymer origin of MP in TWW was identified intensively. Determined polymers were mostly made from PE (av. 40%, Figure 4-S2,B), which is in accordance with earlier studies. In total 14 different polymers were identified, from which some occurred only rarely (e.g. PVC, PUR, PLA, EVA). The majority of MP was transparent and had a size between 50 to 100 µm (av. 59.2%), only 8.5% of MP was bigger than 200 µm (Figure 4-S4,B). Since no other data on MP sizes in TWW exist and no correlation with sewage characteristics was determined, size classes are presented (Figure 4-S3 and S4) but not further discussed. It should be noted that recording sizes and polymers of MP is important when concentrations should be stated on weight basis or compared to such data (van Wezel et al. 2016). While it is apparent that synthetic fibres seem to be a main contaminant of TWW (Browne et al. 2011), no fibres were documented by Carr et al. (2016). Here, we showed high loads of fibres in the TWW samples. But on average, only half of the fibres were identified being made from synthetic polymers. In 11 of the 13 TWW samples, synthetic fibres exceeded MP counts. Finding PEST predominately could be a reflection of the frequent use of synthetic clothing (e.g. fleece garments). Discharges of synthetic fibres were estimated ranging from 2×10^{1} m^{-3} (Oldenburg after post-filtration) to 5×10^{3} m^{-3} (Holdorf). Results of our negative samples confirm that despite all precautions fibre contamination occurred. Thus, results have to be handled with care. Additionally, despite

high difficulties of distinguishing synthetic from natural fibres, chemical identification of fibres should be included to prevent from overestimation.

Finally, assertions about shares of primary and secondary MP were difficult. Primary spherical plastic items as used in peelings (Chang 2015) were observed only rarely. And also Murphy et al. (2016) documented them mostly in the sampled grease fraction. Carr et al. (2016) documented mostly irregular shaped primary MP made of PE in toothpaste, whereby the discrimination from secondary material becomes impossible. It could be that the documented high PE loads derived from cosmetics. But also abrasion of household items could result in these high numbers.

We also addressed potential differences between WWTPs. No correlations between MP numbers, sizes or polymers and population equivalents of respective WWTPs were found.

In all effluents clearly more MP < 500 µm was found compared to the larger fraction (Figure 4-1,C and 2,C). While it can be assumed, that larger plastic items are removed from sewage water during purification, the fate of MP is hardly studied. So far, Carr et al. (2016), Talvitie et al. (2015) and Murphy et al. (2016) pointed towards a high potential of WWTPs retaining MP from incoming water. Primary skimming and settling processes had the biggest effect leading to high amounts of MP in sewage sludge. Here, we did not examine the WWTP influx, but aimed in confirming a potential retention by taking samples from the light fraction skimmed during primary treatment and the sewage sludge of six WWTPs. A sufficient purification to quantify MP in the former could not be conducted. While visible plastic promotes the retention during primary treatment, we did not find differences in MP numbers comparing WWTP with and without primary skimming tanks (Table 4-S1). Same limited conclusions can be drawn for the sewage sludge samples that consisted of highly persistent organic material. The treatment with heated, strong alkaline leach potentially attacked polymers like PEST and PA (Cole et al. 2014). Due to the uncertainties caused by small sample volumes and the untested effects of heated 10 M NaOH on different polymers, we decided to not further analyse the results of MP in sewage sludge, but to present our results as estimates (Figure 4-S5 and S6). Linking results of TWW and sewage sludge would have been highly interesting, since backwashed material from the post-filtration in Oldenburg (MP retention of 97%, Figure 4-1 to 4-3) is added to the sewage sludge. But instead of determining higher MP loads, MP contamination was estimated the lowest ($1x10^3$ MP kg^{-1} dry weight sewage sludge, Figure 4-S5,C) which indicates that results of the sewage sludge should be handled with care.

For tertiary water treatments, the impact for MP retention by gravity filters (Carr et al. 2016) or by a membrane reactor (Leslie et al. 2013) were rated as low. In contrast, we found a high removal rate for post-filtration at WWTP of Oldenburg which was installed to diminish the discharge of suspended matter. The system of 12 rolling filters of pile fabric removed MP > 500 µm completely, MP < 500 µm was reduced by 93% and synthetic fibres by 98%. Higher amounts of PA detected in the TWW after post-filtration compared to the previously taken sample derived most likely from the pile fabric itself. Still, they are minor compared to retained MP. Further studies should be conducted that examine the fate and removal of MP during waste water purification and that link MP counts from TWW and sewage sludge samples.

4.5 Conclusions

- The presence of MP particles and fibres was confirmed in effluents of 12 German WWTPs.
- The applied enzymatic-oxidative purification approach in combination with FPA-based micro-FT-IR imaging allowed the identification of MP down to a size of 20µm.
- MP particles were made of 14 different polymers, with the majority determined as PE.
- Amounts of synthetic fibres were on average higher than MP counts and predominantly made of PEST.
- The installed post-filtration unit (WWTP of Oldenburg) reduced the load of MP and synthetic fibres in TWW substantially.
- Standardization and harmonization of sampling, purification and analysis approaches is urgently needed to compare future studies.

Acknowledgements

This study was funded by the Oldenburg-East-Frisian water board (OOWV) and the Lower Saxony Water Management, Coastal Defence and Nature Conservation Agency (NLWKN).

4.6 Supplementary Information

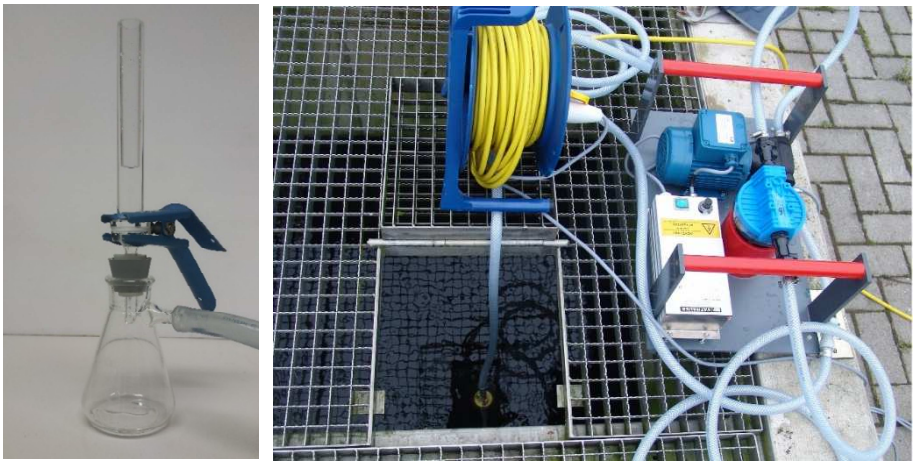

Figure 4-S1 | Treated waste water (TWW) samples were taken by a custom made mobile pumping device. It consisted of a flexible polyvinylchloride hose with a weighted end-piece connected to a membrane pump (Jabsco EMG 590-8023, Xylem, Germany), a filter housing (polycarbonate) with polypropylene lid containing a 10 µm stainless steel cartridge filter (4 7/8", Wolftechnik, Germany) and a flowmeter (Gardena, Germany) here at the WWTP Scharrel (picture left).To filter the purified TWW samples onto 0.2 µm aluminium oxide filters (Anodisc 25 mm, Whatman, U.K.) we used an in-house fabricated filter-funnel with an inner diameter of 11 mm (Polymethylmethacrylate, right picture).

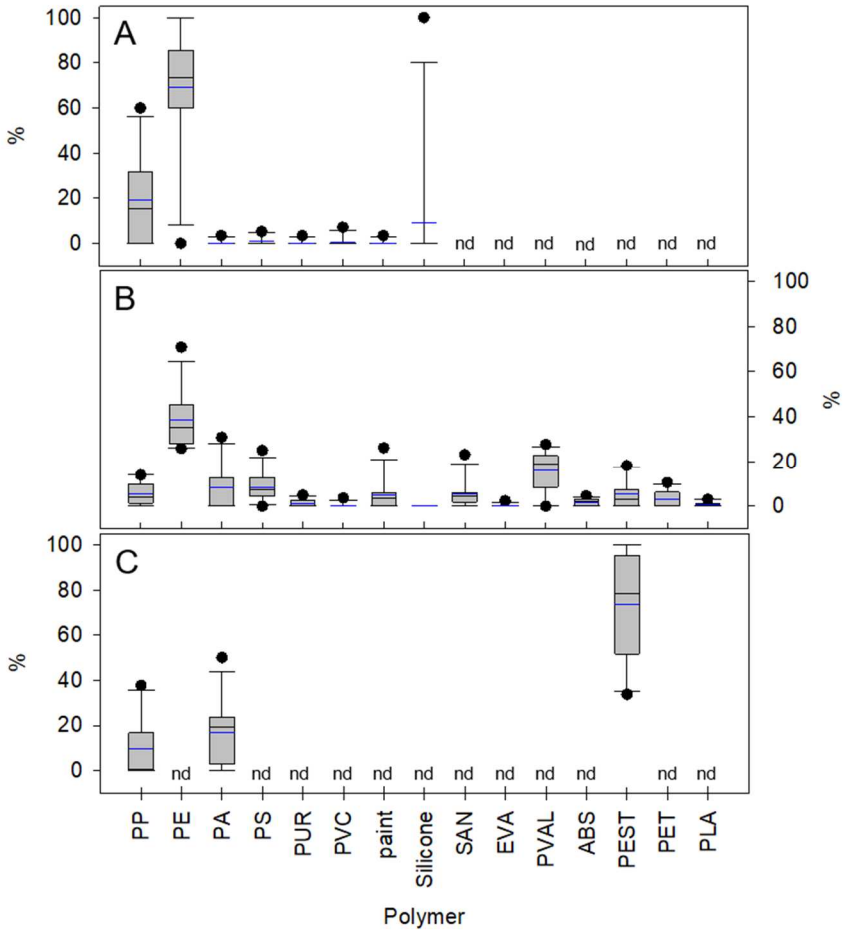

Figure 4-S2 | Microplastic (MP) > 500 µm (A), MP < 500 µm (B) and synthetic fibers (C) in treated waste water (TWW) of 12 waste water treatment plants (WWTP) in Lower Saxony (Germany) with n=13. Polymers that were not determined in one of the groups are marked with nd (not determined). Box- and Whiskers plots of percentage shares of synthetic polymers detected in all WWTPs. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. Blue lines are indicating the mean and black bullets outliers.

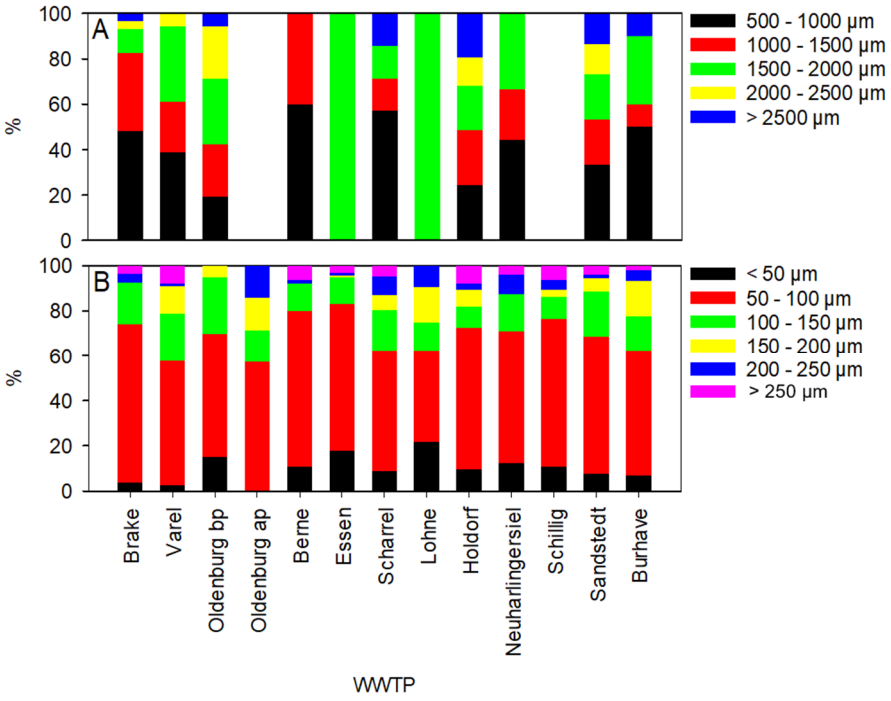

Figure 4-S3 | Percentage composition of size classes of MP > 500 µm (A) and MP < 500 µm (B) in TWW of 12 WWTPs in Lower Saxony (Germany).

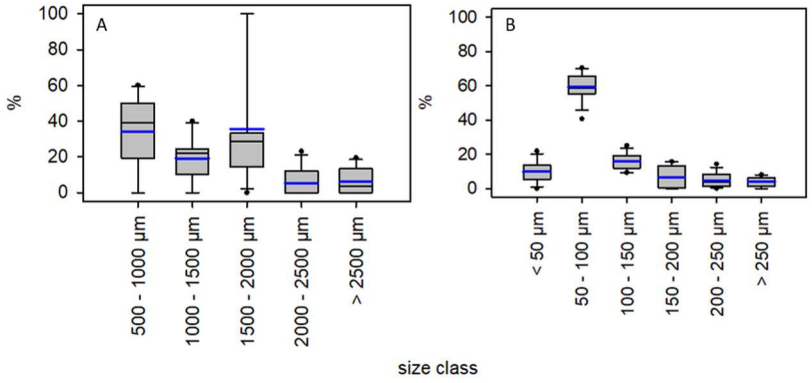

Figure 4-S4 | Size classes of MP > 500 µm (A) and MP < 500 µm (B) in TWW of 12 WWTPs in Lower Saxony (Germany) with n=13. Box- and Whiskers plots of percentage shares of synthetic polymers detected in all WWTPs. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. Blue lines are indicating the mean and black bullets outliers.

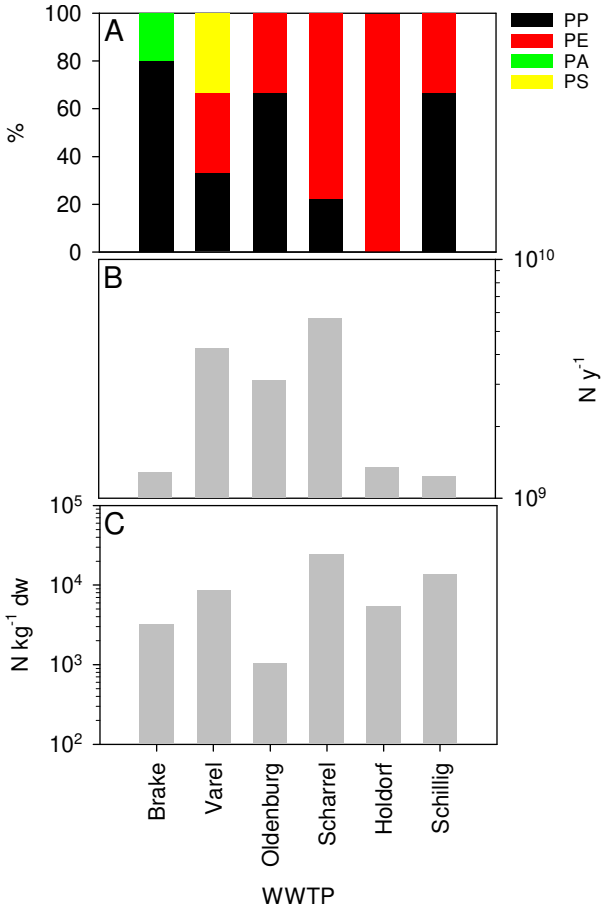

Figure 4-S5 | MP < 500 µm in the sewage sludge of 6 WWTPs in Lower Saxony (Germany). A: Percentage composition of synthetic polymers; B: Annual load of MP in sewage sludge (based on data Table 4-S1); C: MP numbers per kg dry weight

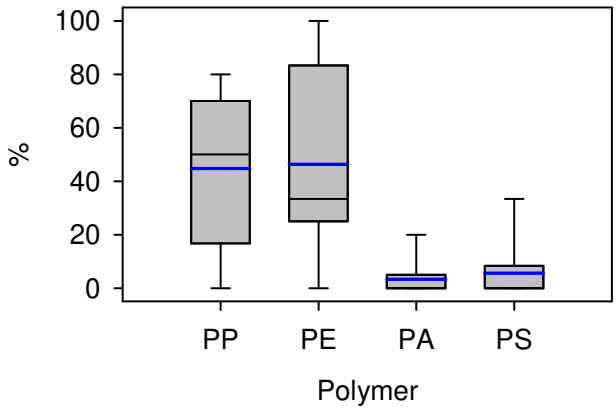

Figure 4-S6 | MP < 500 µm in sewage sludge of 6 WWTPs in Lower Saxony (Germany) with n=6. Box and Whiskers plots of percentage shares of synthetic polymers detected in all WWTPs (The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. Blue lines are indicating the mean).

Table 4-S1 | Sampling sites, sample sizes and characteristics of the investigated WWTPs in Lower Saxony (Germany). At the WWTP of Oldenburg samples were taken before and after an installed postfiltration (PF).

WWTP	Population equivalent	Annual efflux (m^3)	Annual sewage sludge (t)	Influx composition	Water processing				Sampling			
					1st TM	2nd TM	3rd TM		Precipitation, 5 d prior sampling (mm)	Date (2014)	Sampling position	Sample (L)
							PF	Maturation pond				
Oldenburg	2.1×10^5	1.3×10^7	3.0×10^3	municipal/ industrial	Y	Y	Y	N	1.6	23.4.	Before PF	600
										23.4.	After PF	1000
Varel	5.6×10^4	2.6×10^6	5.0×10^2	municipal/ industrial	Y	Y	N	Y	2.4	22.4.	Secondary clarifier overflow	685
Brake	3.4×10^4	1.4×10^6	4.0×10^2	municipal/ industrial	Y	Y	N	N	2.0	22.4.	Facility efflux	1000
Lohne-Rießel	4.3×10^4	1.2×10^6	1.6×10^2	municipal/ industrial	N	Y	N	Y	1.2	25.4.	Facility efflux	1000
Essen	4.6×10^4	1.0×10^6	3.6×10^2	70 % abattoir, municipal/ industrial	N	Y	N	N	2.0	24.4.	Secondary clarifier overflow	500
Holdorf	2.6×10^4	6.0×10^5	2.5×10^2	40 % dairy, municipal/ industrial	Y	Y	N	N	2.4	25.4.	Secondary clarifier overflow	390
Scharrel	1.4×10^4	5.8×10^5	2.4×10^2	10 % textile industry, municipal/ industrial	Y	Y	N	N	4.4	24.4.	Facility efflux	1000
Burhave	1.4×10^4	3.4×10^5	9.0×10^1	municipal, (touristic)	N	Y	N	N	1.8	29.4.	Secondary clarifier overflow	1000
Berne	8.0×10^3	3.3×10^5	9.5×10^1	municipal	N	Y	N	N	1.8	23.4.	Secondary clarifier overflow	600
Schillig	1.1×10^4	2.7×10^5	9.0×10^1	municipal, (touristic)	Y	Y	N	Y	1.2	28.4.	Influx maturation pond	800
Neuharlingersiel	1.0×10^4	1.9×10^5	4.5×10^1	municipal, (touristic)	N	Y	N	N	0.8	28.4.	In secondary clarifier	1000
Sandstedt	7.0×10^3	1.9×10^5	7.2×10^1	municipal	N	Y	N	N	9.4	29.4.	Facility efflux	1000

CHAPTER 5

A SYSTEMS APPROACH TO UNDERSTAND MICRO-PLASTIC OCCURRENCE AND VARIABILITY IN DUTCH RIVERINE SURFACE WATERS

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Abstract

Assessment methods on data quality and environmental variability are lacking for microplastics (MP). Here we assess occurrence and variability of MP number concentrations in two Dutch rivers. Strict QA/QC procedures were applied to identify MP using Fourier-transform infrared (FTIR) microscopy followed by state of the art automated image analysis. For a series of randomly selected, yet ever smaller subareas of filters, we assessed how accurately MP numbers and polymer types are represented during partial filter analysis. Levels of uncertainty were acceptable when analysing 50% of a filter during chemical mapping, and when identifying at least a subset of 50 individual particles with attenuated total reflection (ATR)-FTIR. Applying these guidelines, MP number concentrations between 67 and 11532 MP m^{-3} were detected in Dutch riverine surface waters. Spatial differences caused MP number concentrations to vary by two orders of magnitude. Temporal differences were lower and induced a maximum variation of one order of magnitude. In total, 26 polymer types were identified, the most common were polyethylene (23%), polypropylene (19.7%) and ethylene propylene diene monomer rubber (18.3%). The highest diversity of polymer types was found for small MPs, whereas MP larger than 1 mm was scarce and almost exclusively made of polyethylene or polypropylene. Virtually all sampling locations revealed MP number concentrations that are considerably below known effect thresholds for anticipated adverse ecological effects.

5.1 Introduction

Microplastics (MP) have been detected globally across all major environmental compartments. It is expected that most of the plastic litter originates from land based sources and is relocated by e.g. urban runoff or riverine transport towards the marine environment (Hurley and Nizzetto 2018, Rochman 2018). The latter has been confirmed recently by Lorenz et al. (2019) who examined the MP distribution in the southern North Sea and found highest concentrations in surface waters where riverine input of the Thames and Rhine occurs. Although awareness of the relevance of this transport route is rising, knowledge on MP in rivers is still scattered. MP have been reported in various river systems, in sediment (Hurley et al. 2018, Mani et al. 2019b), water (Cheung et al. 2018, Kataoka et al. 2019, Koelmans et al. 2019) or biota samples (Roch et al. 2019), with reported concentrations varying by several orders of magnitude (Adam et al. 2019, Koelmans et al. 2019).

Various large and small scale processes affect MP concentrations and its distribution within a river system. Higher MP concentrations in surface waters have been linked to the vicinity to urbanized areas (Kataoka et al. 2019, Mani et al. 2015), or higher flow velocities during rain events (Cheung et al. 2018) that cause settled MP to be released from sediments. Flow conditions can thus substantially change the spatial distribution of MP in a river (Eo et al. 2019, Hurley et al. 2018, Kooi et al. 2018) and can strongly vary over the seasons. Yet only a few studies considered this and determined MP over a longer time period. Watkins et al. (2019) identified higher MP concentrations in surface waters during low flow conditions in summer, and Eo et al. (2019) found highest MP abundances during the dry and wet season while concentrations were significantly lower during a moderate season. Further insights on seasonal variations are needed.

Discharges of waste water treatment plants (WWTPs) are expected to be an important point source for MP, thereby affecting the distribution of MP in a river (Boucher et al. 2019). The presence of MP in WWTP effluents has been confirmed by multiple studies (Mintenig et al. 2017, Simon et al. 2018, Talvitie et al. 2017), though reported concentrations vary considerably (Koelmans et al. 2019).

These variations in observed MP concentrations may exist due to when and where samples were taken and thus reflect the variability on a WWTP or system's level. These variations, however, can also depend on how the samples were taken or how MP were extracted and analysed (Connors et al. 2017, Filella 2015). To be able to distinguish between system level variability and procedural uncertainty it is required to follow strict quality assurance/ quality control (QA/QC) procedures. Recently, Hermsen et al. (2018) and Koelmans et al. (2019) assessed the reliability of studies by evaluating a set of defined QA/QC criteria. Only four out of 50 reviewed studies that examined MP in aqueous samples were confirmed to fulfil all proposed quality criteria (Koelmans et al. 2019), which indicates limitations with respect to the reliability of studies, and increases the uncertainty around generated data. One of the proposed criteria describes the need to identify the particles' chemical nature for a sufficient

amount of particles. Numerous studies did not include any polymer identification step, but purely relied on a visual selection of particles. Confirming the presence of specific polymer types is essential to be able to see patterns, to point out potential sources and to properly link results of exposure and effect studies (Kooi and Koelmans 2019, Potthoff et al. 2017). The more recent studies do identify the particles' chemical nature (Lorenz et al. 2019, Mani et al. 2019b). Due to long analysis times this is frequently done for a part of a sample only (Lorenz et al. 2019, Mintenig et al. 2017, Simon et al. 2018). Peeken et al. (2018) applied chemical imaging on three subareas of a filter by which they found that MP were distributed unevenly on a filter. Analysing small subareas of filters with Fourier- transform Infrared (FTIR) or Raman spectroscopy might thus considerably under- or overestimate actual MP abundances. Still, the uncertainty introduced by partial filter analysis or by identifying a subset of particles using attenuated total reflectance (ATR)-FTIR has not yet been quantified. We argue that it is needed to systematically assess the trade-off between shortening the analysis time, and the loss of information and accuracy on MP numbers and polymer types. As sample handling and MP analysis are laborious, finding the balance between a doable analysis time and an acceptable level of analytically introduced uncertainty constitutes a major step forward.

This study aimed to assess the occurrence of MP and to explore its variability in surface waters of two Dutch river systems. Strict QA/QC procedures (Hermsen et al. 2018, Koelmans et al. 2019) were followed to identify MP down to 20 µm using FTIR microscopy followed by an automated image analysis (Primpke et al. 2019). Sampling locations were chosen (1) with a high spatial resolution and at different flow velocities in the river Dommel to assess the spatial variability of MP within one river, (2) with two locations where the sampling was repeated to assess the seasonal and daily variation of MP occurrences, and (3) included WWTP effluents, as well as an up- and downstream sampling location of their discharging points to assess the relative contribution of WWTPs to the total riverine MP load. A secondary aim was to assess the introduced uncertainty through partial filter analysis and the analysis of a subset of particles by comparing generated MP data for a series of randomly selected, yet ever smaller fractions on their accurate representation of MP numbers and polymer types. Finally, generated MP data are discussed in the context of previously reported concentrations and of anticipated risks for aquatic biota.

5.2 Material and Methods

5.2.1 Study area

MP were identified and quantified in surface waters of the Dutch part of the Meuse river basin. One of its tributaries is the Dommel that originates in Belgium and flows over a length of 80 km through the Netherlands. Flow velocities of the Dommel vary over the river length and over the seasons (0.001- 0.98 m s^{-1}, mean 0.28 m s^{-1} with a mean discharge of 3.1 m^3 s^{-1}). The Dommel is fed by the discharges of three WWTPs, as well as by several smaller tributaries and combined sewer overflows (de Klein et al. 2016). The Dommel is well studied and described in a temporal and spatial explicit model (NanoDUFLOW), which is based on hydrological data of the Dommel and has been applied to study transport of metal- based nanoparticles (de Klein et al. 2016) and nano- and microplastics (Besseling et al. 2017).

The Meuse has an average width of about 100 m and an average discharge of 350 m^3 s^{-1}. The Meuse is mostly rain fed, resulting in strong differences between summer and winter flow regimes. The Dutch part of the river basin is characterized by a high number of inhabitants, intensive agriculture and industry. At the same time the Meuse is used as a source for drinking water production. Three subsequent basins, built in the 1970s in the national park 'De Biesbosch', enable water storage and sedimentation processes to improve water quality.

Surface water samples were taken in the Dutch part and over the lengths of both rivers, the Meuse (N= 12) and the Dommel (N= 20) (Figure 5-2). Locations included the sedimentation basins and the effluents of five WWTPs discharging directly or indirectly into the two rivers were sampled. The majority of samples was taken in autumn 2017. The sampling was repeated at two sites during different seasons in 2018 (Table 5-S1).

5.2.2 Sampling

A centrifugal water pump (Leo 4xCm 120C, China) was used to filter surface water over stacked stainless steel sieves with mesh sizes of 300 µm, 100 µm and 20 µm (ø 20 cm, ThermoFisher Scientific, USA). The inlet tube (polyvinyl chloride, PVC) was equipped with a metal cap (opening 2 cm) and mounted on a wooden pallet. This enabled sampling the upper 5 cm of the water column, and an upstream orientation of the tube. Before sampling, tubes and pump were primed for 5 minutes, and the sieves rinsed with filtered surface water. The sample volume was determined by a connected water meter. Between 1.3 and 8 m^3 were filtered with a flow rate of approximately 2 m^3 h^{-1} over the two bigger sieves. The sample volume depended on the amount of suspended matter. Regularly the flow was lowered and the 20 µm sieve placed underneath the other sieves. By doing so 0.03 to 2.25 m^3 water were filtered over the 20 µm sieve, which on average represented 15 % of the total sample volume (Table 5-S1). The residues were rinsed into individual glass bottles that were closed with aluminium foil and stored at 4˚C until further processing in the laboratory. Materials retained on the sieves, or MP enclosed in aggregates, resulted in capturing also MP smaller than the

respective mesh sizes. During sampling care was taken that the outlet tube discharged the filtered water downstream of the sampling location. Airborne contamination was abated by covering the upper sieve with a metal lid in which the inlet tube was hung.

5.2.3 Sample preparation

Sample preparation to extract MP retained on the 20 and 100 µm sampling sieves was done at KWR Watercycle Research Institute (The Netherlands). The sorting of MP > 300 µm and all FTIR analyses were conducted in the laboratories of Wageningen University and Research (The Netherlands). During sample preparation quality criteria as presented by Koelmans et al. (2019) were followed. Before starting to handle environmental samples, the susceptibility to contamination and the achievability of a good MP recovery were tested for three working places used in literature, namely a normal lab bench (Dris et al. 2015, Mintenig et al. 2017), a glove box (Torre et al. 2016) and a laminar flow hood (Lorenz et al. 2019, Peeken et al. 2018). To do so samples of 1 L of Milli-Q water underwent the same steps as environmental samples. For further details on these pre- tests, contamination mitigation and quality assurance we refer to the Supplementary Information (Paragraph S1).

MP > 300 µm The residues of the 300 µm sampling sieve were visually inspected using a stereomicroscope (Nikon Stereo SMZ2T, Japan). The sorting of potential MP particles was facilitated using a Bogorov chamber (Polymethyl-methacrylate, PMMA 70ml, HydroBios Germany). All particles with a bright or transparent colour, no cellular structures and certain bending properties were isolated, photographed (Euromex CMEX 5 MP, The Netherlands) and measured at their longest and shortest dimension. At the same time their shape and colour were noted.

MP < 300 µm Multiple steps were taken to reduce natural organic and inorganic sample components when extracting MP retained on the 20 and 100 µm sampling sieves (Figure 5-S1). Purification started with the addition of sodium dodecylsulphate (SDS, 5 %, Serva Electrophoresis GmbH, Germany), after which potassium hydroxide (KOH, 10 %, Carl Roth GmbH, Germany) and hydrogen peroxide (H_2O_2, 32 %, Carl Roth GmbH, Germany) were added. Before adding the subsequent chemical, samples were filtered over a stainless steel 20 µm mesh placed in a stainless steel filter capsule. Further required were a vacuum pump (ME1C, Dijkstra Vereenigde, The Netherlands) and a Teflon tube attached to the filter capsule. The sample residues on the 20 µm meshes were transferred into beakers and the subsequent chemical was added. During all steps an incubation temperature of 35°C was kept for which samples were placed in an oven. Inorganic particles were removed by performing a density separation using a zinc chloride ($ZnCl_2$, Carl Roth GmbH, Germany) solution with a density of 1.6 g cm^{-3}. From the 20 µm mesh, residues were rinsed with the $ZnCl_2$ solution into separation funnels and were left to settle for 24 h. Materials with a density above 1.6 g cm^{-3} settled to the bottom and were removed by regularly and slowly turning the

outlet valve. All lighter materials were filtered one more time over the 20 µm mesh, then onto 0.2 µm aluminium oxide filters (anodisc 25 mm, Whatman, U.K.) for which a filtration funnel with an inner diameter of 15 mm was used. These filters were placed into slightly opened glass petri dishes and dried at 35°C for five days.

During sample preparation, cross- contamination was minimized by always using the same 20 µm steel mesh and glass beaker for individual samples. In parallel to actual samples, procedural blanks were treated and analysed and their results were considered when analysing MP in environmental samples (Paragraph S1).

5.2.4 MP identification and quantification

MP > 300 µm – ATR-FTIR Sorted, potentially synthetic, particles larger than 300 µm were identified using ATR-FTIR (Varian 1000 FT-IR, Agilent USA). Particles were placed individually onto the ATR crystal, polymer types were identified based on the recorded spectra (600-4000 cm^{-1}) with the aid of the 'Hummel Polymer and FTIR Spectral Library' (ThermoFisher Scientific, USA). If the number of sorted particles was < 50, all particles were analysed. A subset of 50 randomly chosen particles (32- 76 %) was identified for eight samples with numbers of sorted particles > 50 (Koelmans et al. 2019). To assess the loss of accuracy when analysing only a subset of pre-sorted particles all particles (73 to 123) were analysed for further three samples (section 2.6).

MP < 300 µm – Micro-FTIR To identify MP < 300 µm, an FTIR microscope equipped with an ultrafast motorized stage and a single mercury cadmium telluride (MCT) detector (Nicolet iN10, ThermoFisher Scientific, USA) was used. The anodisc filters with sample residues were placed on a calcium fluoride (CaF_2) crystal (EdmundOptics, Germany) to avoid the filter from bending. Chemical mapping of these samples was conducted in transmission mode for pre-defined filter areas (Löder et al. 2015, Mintenig et al. 2018, Mintenig et al. 2017). MP from the 100 µm sampling sieve was analysed on a filter area of approximately 12 x 16 mm (66 % of the total filter area), IR spectra were recorded with a spatial pixel resolution of 30 µm and in a wavenumber range of 1250 to 3200 cm^{-1}. The aperature size was set as 50 x 50 µm controlling the energy amount presented to the sample, the spectral resolution was set as 16 cm^{-1}, and 1 scan per pixel was conducted. For MP analysis retained on the 20 µm sampling sieve two areas (both approximately 88 mm^2, and together 62 % of the filter area) were mapped with a spatial pixel resolution of 20 µm, with remaining settings kept unchanged. The loss of accuracy by partial analysis of the filter area was quantified separately (section 2.6).

The generated FTIR data were automatically analysed using two software tools, MPhunter (Liu et al. 2019) and MPAPP (Primpke et al. 2019), in combination with the reference database presented by Primpke et al. (2018). These software tools were later transferred into siMPle, a freeware which can be downloaded via https://simple-plastics.eu/. Within the software all recorded spectra are compared against the spectra of a reference library, this is done for the raw spectrum and for its first derivative. The resulting hits are afterwards evaluated as described by Primpke et al. (2017b) followed by an image analysis using MPAPP. This analysis uses first a pixel hole closing mechanism prior to a particle/fibre recognition with set parameters (Primpke et al. 2019) and yields in numbers and polymer types for MP particles and fibres, including the longest and shortest dimension for individual MP. Threshold values for individual polymers were adapted after evaluating the spectra of five samples manually (Table 5-S2).

Based on the MP' two- dimensional shapes the mass per MP, and subsequently per sample, was estimated. To do so, we followed the approach by Simon et al. (2018) and calculated the ratio of the shortest and longest dimension of all identified particles, which on average was 0.56 with a standard deviation (SD) of 0.19. For particles it was assumed that this ratio would be the same for a particle's height and its shorter dimension. For fibres the individual lengths were given while a fixed diameter of 15 µm was assumed (Napper and Thompson 2016, Pirc et al. 2016). The mass was calculated from the MP volume and the density of its material. As exact particle densities cannot be determined during analysis, the mean polymer densities indicated in literature were used (Table 5-S2).

5.2.5 Statistical analysis

The Shapiro-Wilk normality test was used to test if MP number concentrations were normally distributed amongst WWTPs and river systems. A non-parametric Kruskal-Wallis rank sum test was used to compare MP in grouped locations. The Dunn test resulted in an adjusted p-value based on the Benjamini- Hochberg method which was used to compare differences between individual groups. All tests were performed using the software RStudio (v.1.1.463).

5.2.6 Assessment of data reliability

Based on generated MP data we assessed how randomly selected, yet ever smaller sample fractions being analysed can impact final results and when levels of introduced uncertainties become unacceptable. This was done for the partial analysis of a filter during chemical mapping, and for the identification of only a subset of pre-sorted particles > 300 µm using ATR-FTIR. For both analyses five samples with varying MP abundances were selected. These were assessed in regard to an accurate representation of (i) MP number concentrations and polymer types during chemical mapping and (ii) polymer types for ATR-FTIR analysis. MP abundances varied for the environmental samples from 157 to 2928 per filter during chemical mapping (Figure 5-1) that were detected when analysing 66% of the filter. For ATR-FTIR analysis the MP abundances varied between 18 to 123 per sample (Figure 5-S3). Using Microsoft Excel, randomly 10 filter areas, subsets of particles respectively, were generated representing 75, 50, 25, 10, 5, 1 and 0.5 % of the total sample. Within these sample fractions the representation of polymer types and MP number concentrations was assessed and their coefficient of variation calculated. A coefficient of variation ≤ 1 indicates an acceptable variance and was set as threshold to provide sufficiently robust data on MP numbers and respective polymer types.

5.3 Results and Discussion

5.3.1 Assessment of data reliability

This study was conducted by following the QA/QC recommendations by Koelmans et al. (2019) which have also been adopted in recent reports (UKWIR 2019, WHO 2019). Based on the provided quality criteria this study would score 17 out of 18 points and data would thus be assessed as 'reliable'. One point was subtracted as sample preparation was not done under clean air conditions. The overall score is higher than the average scores from studies on surface waters (4- 15, mean 7.9) and on WWTP effluents (3- 13, mean 7.3) (Koelmans et al. 2019).

Generated data will still inhere a certain degree of uncertainty introduced during sampling, sample handling and analysis of parts of a sample only. The latter was assessed for an accurate representation of MP number concentrations (Figure 5-1A) and polymer types after micro-FTIR analysis (Figure 5-1B, Figure 5-S2) as well as polymer types after ATR-FTIR analysis (Figure 5-S3).

Independently of varying MP abundances, MP number concentrations were represented with an acceptable coefficient of variance (≤ 1) when mapping 50 and 75 % of the filter area. The same applied for 25 % if several thousand MP were concentrated on a filter. Lower MP abundances and especially a partial analysis of 10 % or less of a filter considerably increased uncertainties (Figure 5-1A). The five samples contained 9 to 15 polymer types, all were presented with an acceptable level of uncertainty (coefficient of variance 0.02- 0.53) when analysing 75 % of a filter (Figure 5-1B, Figure 5-S2). The same applied for the most common polymer types, e.g. polyethylene (PE), polypropylene (PP) or rubber type 3, when analysing at least 5 % of the filter. The correct representation of more rare polymer types by analysing ever smaller filter areas depends on original MP abundances (Figure 5-1B, Figure 5-S2). However, these rare polymer types are likely overlooked when analysing ≤ 5 % of a filter, and represented inaccurately for a partial filter analysis of 10 to 50 % depending on MP abundances.

Combining these two aspects, it is thus recommended to map at least 50 % of a filter using micro-FTIR. If a filter contains several thousand MP it might also be sufficient to analyse 10 to 25 % of a filter. Analysing 5 % or less of a filter should certainly be avoided, otherwise data on MP numbers and polymer types will entail high uncertainty levels. This becomes even more important considering that MP are not distributed evenly on a filter (Peeken et al. 2018). However, there are further restrictions during chemical mapping as the correct identification of MP can be hampered by a thick filter cake (Löder et al. 2015, Lorenz et al. 2019). To generate accurate results high sample volumes and a good sample preparation with a high MP extraction efficiency should be strived for (Lorenz et al. 2019).

We also assessed the accurate representation of polymer types when analysing ever smaller subsets of pre- sorted particles with ATR-FTIR. Independently of the samples' total number of sorted particles, the presence of polymer types was depicted accurately when analysing 75 % of all particles (Figure 5-S3). Rare polymers were overlooked and uncertainties around presented polymer distributions became unacceptably high when analysing 10 to 25 % of the particles, which corresponds to approximately 10 to 20 particles. In agreement with the recommendations by Koelmans et al. (2019), analysing all, or at least 50 of the pre- sorted, potential synthetic particles, will depict abundances of different polymer types with an acceptable level of uncertainty.

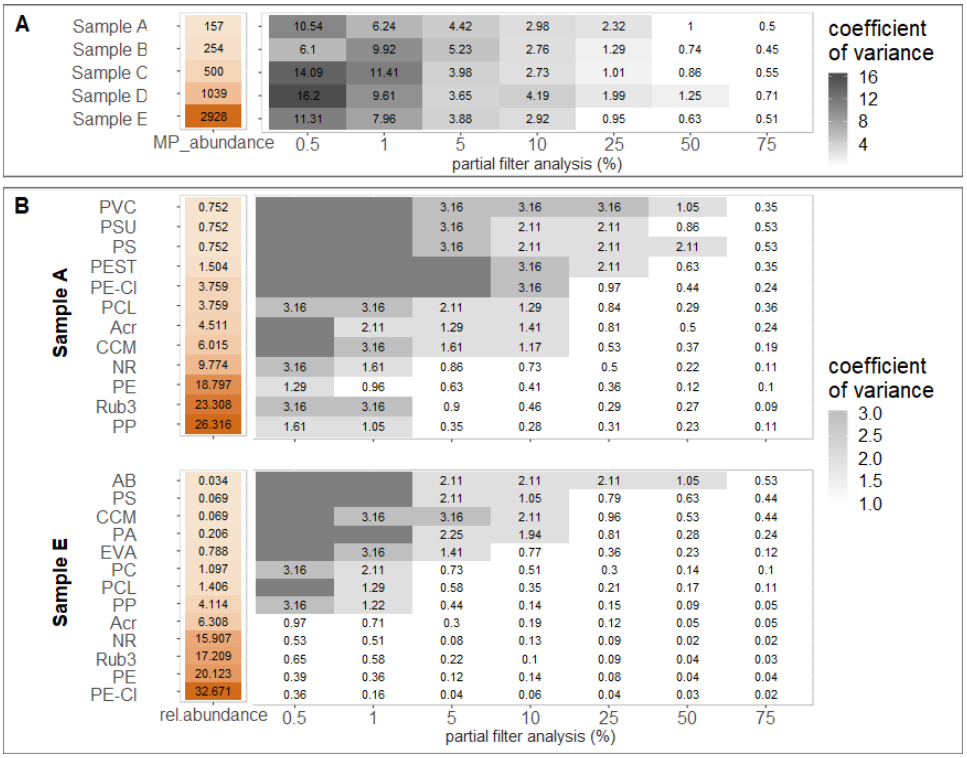

Figure 5-1 | Uncertainties expressed as coefficient of variance (CV) of identified (A) total MP number concentrations, and (B) polymer types during partial filter analysis with micro-FTIR. In orange (A) total MP abundances per filter are given for five individual samples, while in (B) relative abundances of polymer types are indicated for the samples with the lowest and highest MP abundances (see Figure 5-S2 for the remaining three samples). The CV was calculated for individual samples based on 10 randomly selected filter areas of specified size (0.5- 75%). Areas that miss a CV and are coloured in dark grey indicate that this polymer type was not identified in any of the generated 10 filter areas.

5.3.2 MP in riverine surface waters

MP number concentrations MP particles and fibres down to a detection limit of 20 µm were detected in all samples. Number concentrations varied between 67 and 11532 (median 862) MP m^{-3} (Figure 5-2, Figure 5-4). MP in surface waters of the Meuse have been assessed by one earlier study, the results, however, are not comparable due to different methodologies and reportage of results (Leslie et al. 2017). Globally, between 0 and 1.3 x 10^4 MP m^{-3} (median 2.75 MP m^{-3}) were reported in riverine surface waters (Koelmans et al. 2019). The here presented numbers are at the higher ends of this range. This could be explained by studies targeting different MP sizes. The current size limit is lower compared to studies that sampled with a 333 µm neuston net and reported lower number concentrations (Baldwin et al. 2016, Hoellein et al. 2017). Further, the majority of studies reported on a few polymer types only (Koelmans et al. 2019), while in the present study 26 different polymer types were identified (Figure 5-S4). The here applied automated image analysis of FTIR data is a major step forward as it circumvents human bias and automatically compares spectra against a standardized database of common polymer types (Primpke et al. 2018). In this way rare polymer types, or very small MP are not overseen but identified correctly (Mani et al. 2019b). Remarkably, several types of rubbers were highly abundant, which has not yet been reported for riverine surface waters in earlier studies.

Data on MP in environmental samples constitute merely a snapshot of the environmental situation and the processes taking place. Until now only a few studies examined individual rivers over their length in detail (Ding et al. 2019, Hurley et al. 2018, Mani et al. 2015). To better understand the MP distribution on a systems' level the sampling was restricted to two river systems, reducing the number of variables that need to be taken into account when comparing generated data. The snapshot character of data was reduced by choosing sampling locations at a high spatial resolution, and by assessing temporal variations.

Slight differences in regard to MP number concentrations were found for the two river systems. In the Meuse, the concentrations ranged from 177 to 1381 (median 867) MP m^{-3} (Figure 5-2A), while concentrations in the Dommel and its tributaries were in the range of 160 to 11,532 (median 654) MP m^{-3} (Figure 5-2B). Although the discharges of Dommel and Meuse differ by a factor of 100, MP concentrations are rather similar. In both rivers MP abundances did not increase continuously over the river length.

Highest concentrations were detected in the downstream part of the Dommel close to the cities Eindhoven (8450 MP m^{-3}) and StOedenrode (11532 MP m^{-3}). This is in line with the findings of Kataoka et al. (2019) and Mani et al. (2015) who could link higher MP abundances to the vicinity to urban areas. In contrast, the more rural part of the Dommel, including all locations that lie upstream of Eindhoven, revealed much lower MP concentrations (median 309 MP m^{-3}) (Figure 5-2B). The Shapiro- Wilk normality tests indicated that MP number concentrations were non-normally distributed amongst WWTP effluents and river systems. Thus a non-parametric Kruskal- Wallis rank sum test was conducted revealing significant

differences between the upstream and downstream locations of the Dommel (p-value= 0.0032) which was also confirmed by the Dunn test (adjusted p-value of 0.0024). No significant differences were found between these locations and the samples from the Meuse or the WWTP effluents.

To reduce levels of suspended matter the Dommel passes the Klotputten, a wide basin with low flow velocities (0.002 m s^{-1} on average) that facilitates sedimentation processes. Surface water was sampled at the upstream and downstream end of this basin. Contrary to expectations, MP concentrations did not decrease, and were even slightly higher at the downstream location (587 compared to 460 MP m^{-3}) (Figure 5-2B). In contrast, the sampled sedimentation basins of 'De Biesbosch' revealed a considerable decrease of MP from surface waters due to low flow velocities. This might be explained by the much longer residence times. At Drimmelen, water from the Meuse is pumped into the basins to facilitate the settling of natural suspended matter. MP also settled resulting in decreasing MP concentrations from 789 MP m^{-3} (Drimmelen) to 607 MP m^{-3} (Biesbosch1) and 67 MP m^{-3} (Biesbosch2) (Figure 5-2A). Similar polymer types were present in the three samples, however their numbers and proportions changed. Compared to the inlet water, surface water in the first sedimentation pond contained less particles made from PP and the Acrylates/ Polyurethanes/ Varnish cluster. The lower MP numbers in the second pond can be explained by the strong decline of PE and Rubber type 3 particles in comparison to the first pond (Figure 5-2A).

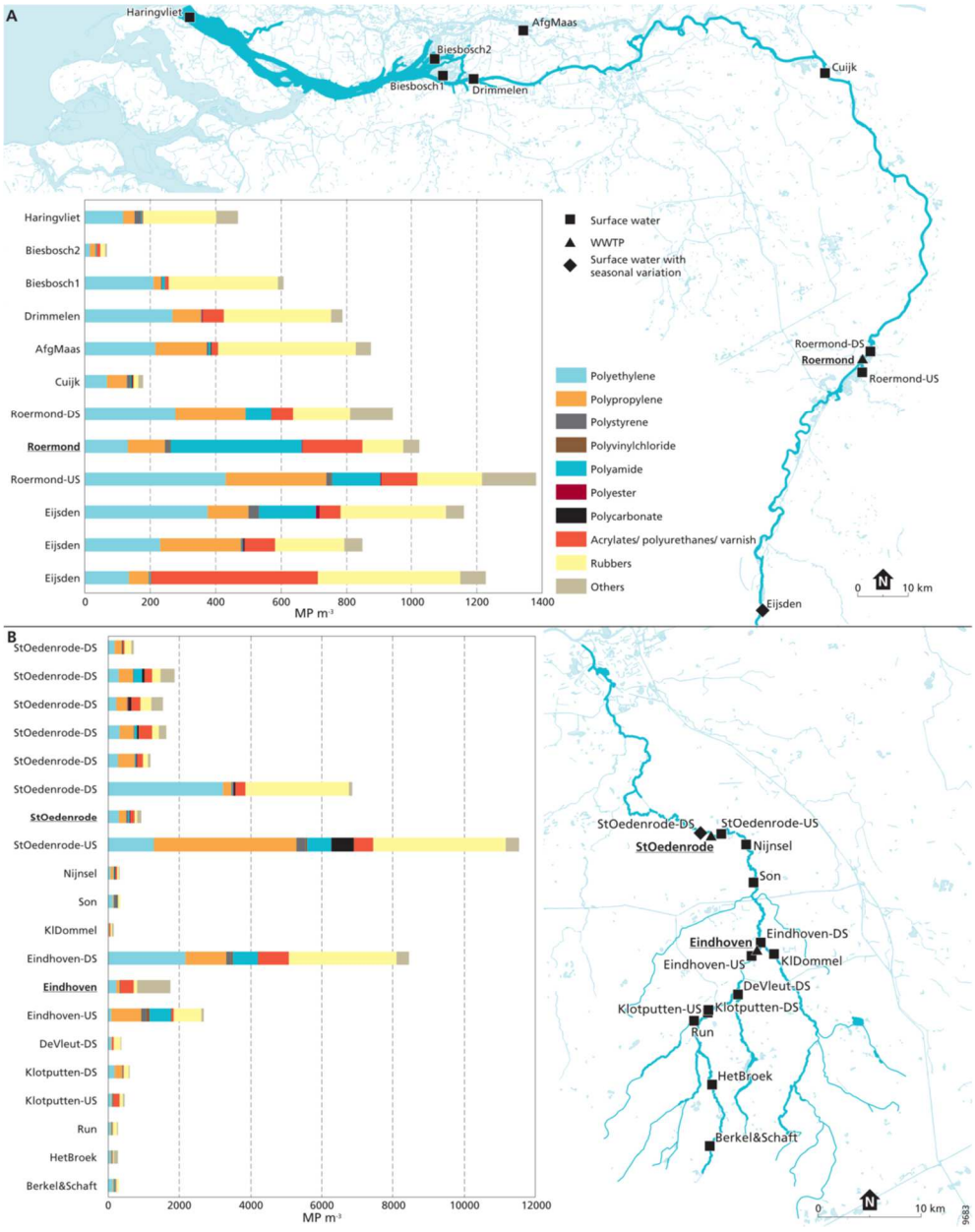

Figure 5-2 | Sampling locations in the river catchments of the Meuse (A) and the Dommel (B). Sampled WWTP effluents are written in bold, surface water was examined upstream (US) and downstream (DS) of the WWTPs' discharging points. The detected MP concentrations (MP m^{-3}) are indicated with respect to the different polymer types identified.

The discharges of WWTPs are expected to influence the distribution of MP in a river. MP concentrations in the effluents ranged from 941 to 1741 (median 1024) MP m^{-3} (Figure 5-2A&B). They were thus higher than MP detected in the upstream regions of the Dommel, but comparable to the ones reported for the downstream locations. Riverine surface water was sampled up- and downstream of these effluents' discharging points. The upstream locations in StOedenrode and Roermond revealed a higher contamination than the effluents or the downstream locations. The MP concentration in the effluent of the WWTP Eindhoven was lower than the one identified in the upstream location and can thus not explain the concentration increase in the Dommel from 2678 to 8450 MP m^{-3} (Figure 5-2B). Although WWTPs continuously add MP to the river systems we could not see a general increase in MP abundances at the sampled downstream locations. Higher MP concentrations might rather be linked to diffuse sources (Hurley and Nizzetto 2018) or other riverine dynamics and processes.

One of these processes could also be weather induced variations. These were assessed at WWTP effluents when examining the effluents of two smaller WWTPs (Maasbommel and Oijen) for MP > 300µm for which concentrations of 46 and 1494 MP m^{-3} were observed (Figure 5-S5). The latter was recorded at the WWTP Oijen when sampling the effluent during a strong rain event with an effluent discharge of 10560 m^3 h^{-1} which was considerably higher than the monthly average of 1,550 m^3 h^{-1}. Two weeks later, the same effluent was sampled again three subsequent times under normal weather conditions (Table 5-S1). Lower concentrations of 211, 279 and 711 MP m^{-3} were detected. Still, MP abundances were much higher than determined in the effluents of the other four WWTPs where a mean concentration of 77 (SD 44) MP m^{-3} was retained on the 300 µm sieve. These data confirm findings that MP number concentrations can vary between WWTP effluents (Mintenig et al. 2017, Simon et al. 2018), and that this strongly depends on weather and rain conditions (Primpke et al. 2017a, Wolff et al. 2019). This not just holds true for WWTP samples, but environmental samples in general where an increase of MP in surface water has been linked to higher flow regimes (Cheung et al. 2018, Hurley et al. 2018, Watkins et al. 2019). MP concentrations in the Dommel were linked to respective flow velocities (Table 5-S1, Figure 5-S6). The critical shear stress equation (Waldschläger and Schüttrumpf 2019) indicates a critical flow velocity of 0.275 m s^{-1} that led to an increase of MP in surface waters. At flow velocities higher than that concentrations above 6800 MP m^{-3} were detected for MP > 20 µm around Eindhoven and StOedenrode. These concentrations are considerably higher than at lower flow velocities, where we found a mean concentration of 924 (SD 722) MP m^{-3}.

One of the study aims was to interpret data in respect to the system scale behaviour. Thus, we assessed if the variability of detected MP concentrations would be influenced more by spatial than by temporal aspects. Within three weeks in autumn 2017, 13 locations in the Dommel were sampled under very similar weather conditions. The sampling was repeated at StOedenrode for another five times in 2018 (Table 5-S1, Figure 5-3).

The Kruskal- Wallis rank sum test revealed no significant differences (p-value= 0.1346) between spatial, seasonal or daily variability (Figure 5-3). Differences within the Dommel only become significant if differentiating again between the rural and urban areas (p-value= 0.0157). While spatial differences caused MP number concentrations to vary by two orders of magnitude, temporal variations were lower and induced a maximum variation of one order of magnitude. This confirms that spatial differences, like geographical and demographic differences or the inputs of WWTPs, induce larger variations in a system compared to the temporal variations at one location due to e.g. differences in flow or wind conditions. Also within a day the determined MP concentrations varied, however, differences were comparably low and varied by a factor of 1.4 in StOedenrode (Figure 5-3, Table 5-S3), and 3.4 in the effluents of the WWTP Oijen where MP > 300 µm was identified (Figure 5-S5, Table 5-S3).

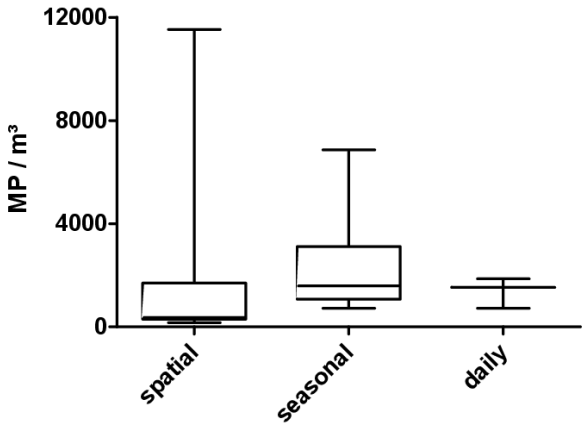

Figure 5-3 | Variation of MP m^{-3} detected in the surface water of the Dommel, whiskers indicate the 95 % confidence interval. Spatial: 13 samples taken over the whole length of the Dommel in October 2017; Seasonal: 6 samples taken at StOedenrode (downstream the WWTP) on 4 time points in 2017 and 2018; Daily: variation of 3 samples taken within 4 hours at StOedenrode.

MP sizes and shapes In all samples more particles than fibres were detected. Fibres accounted for 1.4 to 34 % (median 12.9 %) of the total MP numbers in surface water, and for 12.5 to 22.9 % (median 12.4 %) in WWTP effluent samples. On average, fibres were 300 µm long. Their width was approximately 15 µm which is in accordance to other studies (Napper and Thompson 2016, Pirc et al. 2016).

At all locations increasing abundances with decreasing MP sizes were detected. In total, 67.1 % of all MP was smaller than 100 µm (Figure 5-4A), with 26.3 % being smaller than 25 µm, and 18.5 % of the MP having their longest dimension between 25 to 50 µm. Only 6.7 % were longer than 300 µm, and 1.1 % longer than 1 mm respectively. The fitted power law resulted in an exponent $\alpha = 2.79$ with an R^2 of 0.93 (Figure 5-S7) which is comparable to the ones found in literature (Kooi and Koelmans 2019). The mesh size during sampling and sample handling was 20 µm. Smaller MP could have been retained when filters started clogging. However, small MP particles or fibres might also have passed sieves vertically or might not have been detected during micro-FTIR analysis. As such, number concentrations of MP of approximately 20 µm are likely to even be higher.

Polymer types In total 26 different polymer types were identified (Figure 5-S4). The samples from the Meuse contained on average 13 (SD 2) different polymer types, while 12 (SD 3) were detected in the Dommel, and 15 (SD 2) in the WWTP effluents respectively. Most abundant polymer types were PE, PP, rubber type 3, nitrile rubber and acrylates/ polyurethanes/ varnish which together represented 81 % of all identified MP particles and fibres (Figure 5-2, Figure 5-S4). PE (23 %) and PP (19.7 %) were detected most frequently. Both polymer types have been reported in high concentrations earlier. Rubber type 3 (18.3 %), which is ethylene propylene diene monomer (EPDM) rubber, was the third most abundant polymer type identified. EPDM is commonly used as sealing material, for tubes, car doors, but also in building and roof constructions. Until now it was rarely detected in the environment. This might be explained by the identification methods used, as recent studies that also applied micro-FTIR with automated image analysis were able to identify EPDM and other types of rubber in considerable concentrations (Haave et al. 2019, Mani et al. 2019b). As knowledge on rubber particles in the environment is very limited, these data are of particular interest. This especially holds true for the abrasion of car tyres which are considered a major source of MP to the environment (Boucher et al. 2019, Hurley and Nizzetto 2018), however no data are yet available to verify emission statistics (Kole et al. 2017). As already indicated by Haave et al. (2019) it is difficult to assess car tyre abrasives. High contents of Carbon Black hampers the identification by micro-FTIR of one of its main components, styrene butadiene rubber (SBR). Further, its density is higher than the commonly used $ZnCl_2$ solution (1.6 g cm^{-3}). Therefore, other sample preparation methods than used in the current study are required to accurately assess abrasions of car tyres in the environment. Also remaining rubber types, such as Nitrile rubber, cannot solely be linked to traffic activities as their fields of application are so diverse.

The different MP sizes also reflect differences in relative abundances of polymer types: MP larger than 1 mm were almost exclusively made from PE or PP. Smaller MP had a much higher polymer diversity (Figure 5-4A). The same conclusions were drawn by recent studies which also applied micro-FTIR with automated image analysis (Haave et al. 2019, Lorenz et al. 2019, Mani et al. 2019b). This emphasizes the importance of examining these small MP to correctly depict occurrences and distributions of polymer types in the environment.

MP mass concentrations For all samples taken in the Dommel and in the Meuse, the total MP mass concentration was estimated as described in section 2.4. The mass based concentration varied between 51 and 7270 (median 670) µg m^{-3}. In general, samples with low MP number concentrations also revealed low mass concentrations. Samples taken in the Haringvliet and StOedenrode (first replicate taken on 21.8.2018) revealed comparably low mass concentrations which, however, could not be explained by particularly high numbers of small particles (*Table 5-S3*). MP smaller than 100 µm were most frequent by numbers, however, their individual weight was so low that they contributed only for approximately 2 % to the total MP mass concentration (Figure 5-4B). Total mass was largely determined by the presence of MP > 2 mm. As these MP are almost exclusively made of PE and PP, it is not surprising that the two polymer types constitute the largest share in terms of MP mass concentration (Figure 5-4B, Table 5-S3).

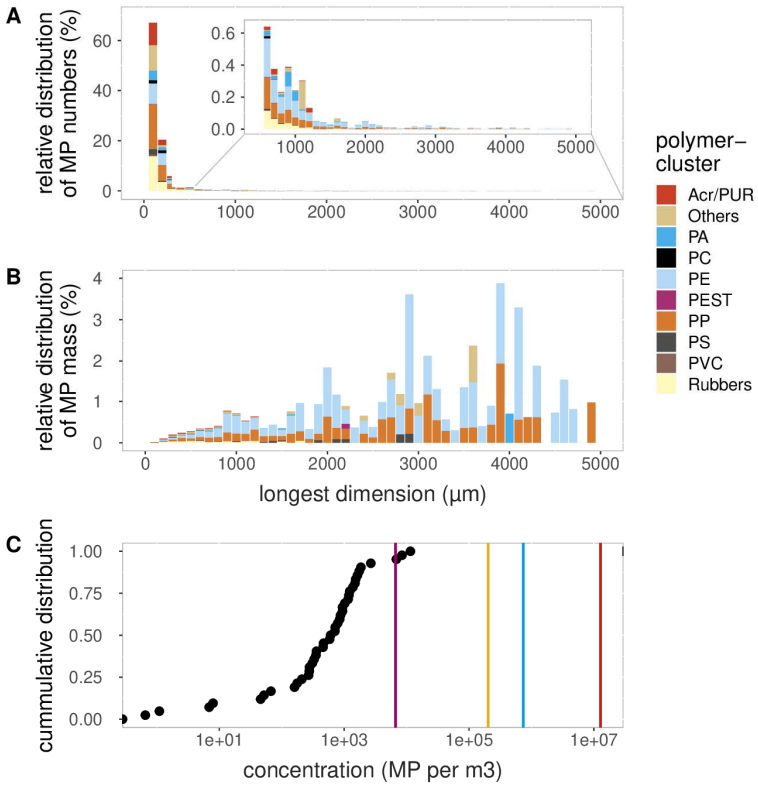

Figure 5-4 | Relative distribution of (A) MP numbers and (B) mass concentrations in relation to MP sizes, and (C) the cumulative frequency distribution of total MP number concentrations identified in the Dommel and the Dutch part of the Meuse, vertical lines represent predicted no effect concentrations (PNEC by Everaert et al. (2018): 6.7 x 10^3 MP m^{-3} (purple), Besseling et al. (2019): 2.0 x 10^5 MP m^{-3} (yellow), Adam et al. (2019): 7.4 x 10^5 MP m^{-3} (blue), Burns and Boxall (2018): 1.3 x 10^7 MP m^{-3} (red)). Acr/PUR: acrylates/polyurethanes/varnish cluster, PA: polyamide, PC: polycarbonate, PE: polyethylene, PEST: polyester, PP: polypropylene, PS: polystyrene, PVC: polyvinyl chloride.

5.3.3 General discussion

Until now only a few studies have focused on examining the spatial distribution of MP in individual rivers in detail (Ding et al. 2019, Mani et al. 2015). Only five studies identified MP with a detection limit of approximately 50 µm, but all selected particles for further identification and did not include chemical mapping (Koelmans et al. 2019). This is the first study assessing the spatial and temporal variability of MP down to a size of 20 µm using FTIR microscopy followed by an automated image analysis (Primpke et al. 2019). As such, the results provide a valuable insight into the presence and distribution of various polymer types in riverine surface waters and WWTP effluents.

Several studies have discussed the need to implement standardized QA/QC criteria to generate reliable and comparable data (Filella 2015, Hermsen et al. 2018). Koelmans et al. (2019) provided guidelines for nine criteria for the analysis of MP in aqueous samples, one of them recommends that at least 25 % of a filter should be analysed during chemical mapping. Based on here presented data we favour that at least 50 % of a filter should be mapped to accurately assess MP number concentrations and polymer types. This also reduces the risk of data misinterpretation due to the uneven distribution of MP on a filter (Peeken et al. 2018). Further aspects that should be considered are high original sample volumes and efficient MP extraction steps to increase actual numbers of identified particles. Further, the analysis of at least 50 pre-sorted particles with ATR-FTIR showed that different polymer types were presented with an acceptable level of uncertainty. In this study, care was taken that the complete sample after MP extraction was concentrated on one or more anodisc filter, and that of each filter at least 60 % were chemically mapped. For MP > 300 µm a visual pre-selection of potentially synthetic particles was unavoidable from which a minimum of 50 particles (32 to 76% of the sorted particles) was analysed with ATR-FTIR. As such, we consider our data to reflect actual MP numbers and polymer types relatively accurate. However, we might have underestimated concentrations of MP of around 20 µm which would imply that levels of MP pollution are higher than concluded here.

The cumulative frequency distribution of detected MP number concentrations covers a range from 67 to 11532 MP m^{-3} (Figure 5-4C). Samples were taken from different representative water system types that are typical for the Netherlands, including small ditches, the rural and urban parts of an intermediate river and a big international waterway. Therefore, we argue that this distribution of MP concentrations represents that of all Dutch riverine surface waters reasonably well, enabling a generic assessment of risks for aquatic biota. To do so, one needs to combine exposure and effect concentrations (Koelmans et al. 2017a). Until now, four studies presented an environmental risk assessment of MP with estimates for the Predicted No Effect Concentration (PNEC), which is the threshold concentration at which no adverse effects are expected to occur (Adam et al. 2019, Besseling et al. 2019, Burns and Boxall 2018, Everaert et al. 2018). The PNEC estimates do not differentiate between MP sizes or polymer types, therefore neither does the frequency distribution of MP number concentrations from

Dommel and Meuse. The majority of locations revealed MP concentrations that are below, or even considerably below, the determined PNEC concentrations (Figure 5-4C). Only in three surface water samples, all taken in the region of Eindhoven and StOedenrode, MP concentrations were higher than the PNEC as derived by Everaert (6.7×10^3 MP m^{-3}). In comparison, the PNECs of the other three studies are two to four orders of magnitude higher than the PNEC defined by Everaert et al. (2018).

Adam et al. (2019) found no significant differences of expected effects for different polymer types or shapes. It is, however, assumed that effects vary for the various MP sizes and that smaller sizes will be more hazardous (Koelmans et al. 2017a, Redondo-Hasselerharm et al. 2018). It should also be mentioned that MP used in toxicity studies underlying the PNEC calculations might not represent the various forms, sizes and polymer types of environmental MP well enough (Kooi and Koelmans 2019). Further, it is assumed that concentrations of today are not what can be expected in the future because of increasing plastic production and use, the plastics' persistance and continous fragmentation in the environment which imply that future MP concentrations will be higher than the ones currently measured (Everaert et al. 2018, Koelmans et al. 2017a).

5.4 Conclusions

In this study we assessed the distribution of MP in riverine surface waters of two Dutch river systems. Samples were taken at a high spatial resolution, and repeated over time at selected locations. We further attached particular value to the implementation of high QA/QC criteria (Koelmans et al. 2019) to identify MP with a detection limit of 20 µm using FTIR microscopy followed by an automated image analysis (Primpke et al. 2019). The latter circumvents any human bias during data analysis by which it is more likely that rare polymer types, and very small MP are not overseen but identified correctly. This way 26 different polymer types, including partly highly abundant rubbers, were identified of which several have not yet been reported in riverine surface waters.

Frequently only parts of a sample are analysed for MP by partial filter analysis or subsampling. It is, however, unclear how much analytical uncertainty can be introduced by doing so. We estimated that during chemical mapping at least 50 % of a filter should be analysed to guarantee an accurate representation of MP number concentrations and polymer types.

In two Dutch river systems, between 67 to 11532 MP m^{-3} were identified. Virtually all of these concentrations are considerably below known effect thresholds. Thus, based on the current knowledge, MP associated risks for aquatic biota are not likely to be anticipated in Dutch riverine surface waters. The three locations with MP number concentrations above one of the PNECs were in the vicinity to big cities, if risks are to be expected, they will most likely be highest in highly urbanized and polluted areas.

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5.5 Supplementary Information

Paragraph S1 | Contamination mitigation and quality assurance

Before starting to handle environmental samples three working places used in literature were tested for their susceptibility to contamination and their easiness to handle which influences the recovery of MP. Tests were conducted on a normal lab bench (Dris et al. 2015, Mintenig et al. 2017), in a glove box (Torre et al. 2016) and in a laminar flow hood (Lorenz et al. 2019, Peeken et al. 2018).

Positive controls: The protocol of described sample purification (see 2.3. MP < 300 µm) was tested and the handling optimized to enable a high MP recovery during the sample purification. For this, green fluorescent PE beads (90- 106 µm, Cospheric, USA) were added to 5 samples (1 L of Milli-Q water) per working place and counted before and after purification steps. The final batches of positive controls yielded in a recovery rate of 93.1 ± 1.2 % on the lab bench, of 91.1 ± 5.8 % in a laminar flow hood and of 79.0 ± 9.6 % in a glove box.

Negative controls: Prior usage all lab equipment was thoroughly rinsed, and the lab surfaces cleaned with ethanol (30 %, Carl Roth GmbH & Co. KG, Germany) and Milli-Q water. Then, the level of contamination was determined for five negative samples (1 L of Milli-Q water) per working place, also these samples underwent described sample purification (see 2.3.) Particles and fibres were counted under a stereomicroscope after the samples had been filtered onto gridded cellulose nitrate filters (Whatman, U.K.). The samples on the lab bench contained on average 9.3 (SD 1.8) fibres and 1.8 (SD 1.4) particles, in the laminar flow hood 3.7 (SD 1.2) fibres and 2.3 (SD 1.1) particles were detected, and in the glove box 5.6 (SD 3.6) fibres and 4 (SD 0.9) particles respectively.

The samples from the glove box showed a medium level of contamination, but especially a low and strongly varying recovery rate of MP (79.0 ± 9.6 %). This can be explained by the restricted freedom to operate which complicated handling and rinsing steps and resulted in the methods rejection. Samples from the laminar flow hood showed the lowest contamination levels while enabling a high recovery of MP (91.1 %). The samples on the lab bench contained the highest amount of fibres, but also had a high and continuable recovery of MP (93.1 ± 1.2 %). Ideally, all sample handling should thus be made in a laminar flow hood. However, as the access to the laminar flow hood was very limited, the recovery rates were high and reproducible on the lab bench and the levels of contamination on the lab bench were not that much higher than the ones in the laminar flow hood it was decided to conduct all work on a previously cleaned lab bench. Because of this, and because not all laboratory equipment used could be made from glass, procedural negative samples (1 L of Milli-Q water) were treated and analysed in parallel to the environmental samples which were corrected for the level of identified contamination (see below). Per sample batch (five to seven environmental samples) one negative sample was treated in parallel. At the end, environmental samples

were corrected for the contamination assessed through eight negative samples. Listed are the detected MP and their respective polymer types. The samples were corrected for these MP in respective size classes.

Table: Polymer specific MP counts in eight individual negative samples

Polymercluster	B1	B2	B3	B4	B5	B6	B7	B8	mean	SD
Polyethylene	0	26	68	6	0	10	3	16	16	23
Polyethylene-chlorinated	0	0	26	13	3	0	0	5	6	9
Polypropylene	0	7	0	0	0	0	3	0	1	2
Polyamide	0	16	3	3	0	0	0	0	3	6
Nitrile rubber	0	0	0	0	0	3	0	0	0	1
Polyester	0	7	0	0	0	0	0	0	1	2
Acrylates/polyurethanes/varnish	0	3	0	3	3	0	0	0	1	2
Rubber type 1	0	0	3	0	0	0	0	0	0	1
Rubber type 2	0	3	0	0	0	0	0	0	0	1
Rubber type 3	7	39	334	22	0	19	0	26	56	113

To further mitigate sample contamination when working with actual environmental samples several steps were taken: During sample handling a lab coat and clothes, both made from natural fabric, were worn. Prior to any working step the lab surfaces were wiped off with ethanol (30 %, Carl Roth GmbH & Co. KG, Germany) and paper towels. Prior usage, all chemicals were filtered over 20 µm, all lab materials were rinsed with ethanol and Milli-Q water and covered with aluminium foil. Also the samples were covered immediately with aluminium foil after finishing working with them.

Table 5-S1 | Sampling dates and locations in the Dommel (D), Meuse (M), and their tributaries/related locations (+). Riverine surface waters and WWTP effluents were sampled by filtering water over stacked sieves (mesh sizes of 20, 100 and 300 µm), sample volumes vary for the different sieves and are indicated below (m^3). When sampling the WWTP effluents the tube inlet was hung directly in the effluent (15 cm below surface). The WWTPs varied in size and capacity, below their maximum hydraulic capacities are listed. When sampling riverine surface waters the tube inlet was mounted horizontally on a wooden pallet (see section 2.2) which was hung in the water to sample the upper 5cm of the water, in case of turbulent water (due to wind or waves) the tube was mounted vertically (*, with specific depth indications). In general, this was done from small bridges in the Dommel, and from the riverside (~5m from shore) in the Meuse (it is indicated if done differently).

River	Location	Latitude, Longitude	Date	Weather condition	Flow (m/s)	Start time	Sample volume (m^3)			Vertically placed inlet tube/ varying sampling location
							20µm sieve	100µm sieve	300µm sieve	
D	Berkel & Schaft	51.291384, 5.438432	18.10.2017	dry, no wind	0.29	15:30	0.213	2.151	2.183	
D	Eindhoven (WWTP, 35,000 $m^3\ h^{-1}$)	51.462441, 5.504710	09.10.2017	dry, no wind	-	10:30	0.061	1.515	2.965	
D	Eindhoven DS	51.468845, 5.509891	09.10.2017	dry, no wind	0.29	17:00	0.030	1.703	1.703	
D	Eindhoven US	51.460012, 5.501451	09.10.2017	dry, no wind	0.24	13:30	0.045	1.898	1.995	
D	Het Broek	51.344994, 5.441739	18.10.2017	dry, no wind	0.28	12:00	0.198	2.084	2.160	
D	Klotputten DS	51.410085, 5.436638	26.10.2017	little wind, little rain	0.002	10:30	0.500	2.000	2.000	small boat
D	Klotputten US	51.407783, 5.435758	26.10.2017	dry, little wind	0.002	13:00	0.409	2.419	2.419	riverside
D	Nijnsel	51.554369, 5.489739	13.10.2017	dry, little wind	0.22	15:00	0.115	3.080	3.080	
D	Son	51.521215, 5.499958	13.10.2017	dry, no wind	0.23	11:30	0.102	3.370	3.370	
D	St Oedenrode (WWTP, 1,930 $m^3\ h^{-1}$)	51.561801, 5.444830	10.10.2017	dry, little wind	-	09:00	0.100	2.389	3.829	
D	St Oedenrode US	51.561768, 5.446611	10.10.2017	little wind, little rain	0.28	12:00	0.100	1.751	2.650	

D	St Oedenrode DS	51.564389, 5.425714	10.10.2017	dry, little wind	0.27	15:00	0.100	3.529	2.999	
D			13.02.2018	dry, little wind	0.35	15:30	0.200	2.020	2.020	
D			06.06.2018	dry, no wind	0.17	15:00	0.351	2.000	2.000	
D			21.08.2018	dry, no wind	0.1	09:50	0.300	2.000	2.000	
D			21.08.2018	dry, no wind	0.09	11:50	0.300	2.000	2.000	
D			21.08.2018	dry, no wind	0.09	13:05	0.300	2.000	2.000	
D+	De Vleut	51.423584, 5.477953	13.11.2017	dry, little wind	0.27	11:30	0.510	2.159	2.159	
D+	Kleine Dommel	51.458808, 5.528436	13.10.2017	dry, no wind	0.265	08:30	0.150	3.000	3.000	
D+	Run	51.400646, 5.416487	18.10.2017	dry, no wind	0.23	09:10	0.300	2.565	2.565	
M	Afgedamde Maas	51.806161, 5.025366	12.10.2017	dry, little wind	-	13:00	0.105	3.525	3.700	
M	Cuijk	51.730406, 5.883969	19.10.2017	dry, no wind	-	13:00	0.995	3.000	3.000	* 10-30 cm depth, moving pond
M	Drimmelen	51.720030, 4.884695	14.11.2017	little wind, little rain	-	10:30	0.510	2.600	2.600	small boat
M	Eijsden	50.779497, 5.699936	19.10.2017	dry, no wind	-	09:00	0.980	3.000	3.000	
M			13.02.2018	dry, no wind	-	09:30	0.500	3.243	3.243	* 15 cm depth
M			06.06.2018	dry, no wind	-	10:30	0.650	2.000	2.000	
M	Haringvliet	51.822818, 4.074128	15.02.2018	dry, little wind	-	11:00	2.250	7.990	7.990	* 5- 50 cm depth, boat

M	Roermond (WWTP, 7,000 $m^3 h^{-1}$)	51.223334, 5.984140	04.10.2017	dry, little wind	-	10:00	0.200	2.825	2.825	
M	Roermond DS	51.238896, 6.006217	04.10.2017	dry, little wind	-	13:00	0.200	3.431	3.431	* 10-30 cm depth, moving pond
M	Roermond US	51.199460, 5.981864	04.10.2017	dry, little wind	-	16:30	0.400	3.603	3.603	
M+	Biesbosch1 (De Gijster)	51.725490, 4.797129	12.04.2018	dry, little wind	0	11:00	0.980	2.960	2.960	small boat
M+	Biesbosch2 (Petrusplaat)	51.754803, 4.773473	07.06.2018	dry, no wind	0	10:00	2.200	4.000	4.000	small boat
M+	Maasbommel (WWTP, 150 $m^3 h^{-1}$)	51.829682, 5.522084	06.10.2017	dry, little wind	-	09:00	n.a.	1.335	2.371	
M+	Maasbommel DS	51.828649, 5.519383	06.10.2017	dry, little wind	-	11:30	n.a.	3.058	3.058	
M+	Maasbommel US	51.830167, 5.519385	06.10.2017	dry, little wind	-	16:00	n.a.	2.033	2.033	almost no flow, wind blowing in US direction
M+	Oijen DS	51.797160, 5.512459	05.10.2017	wind, little rain	-	17:00	0.070	2.768	2.768	
M+	Oijen US	51.801022, 5.487776	05.10.2017	wind, little rain	-	14:00	0.450	3.643	3.643	
M+	Oijen (WWTP, 12,250 $m^3 h^{-1}$)	51.810053, 5.488648	05.10.2017	wind, strong rain	-	10:30	0.045	0.767	0.767	
M+			20.10.2017	little wind, little rain	-	08:50	0.647	2.000	2.000	
M+			20.10.2017	dry, little wind	-	10:25	0.500	2.050	2.050	
M+			20.10.2017	dry, little wind	-	11:40	0.520	1.995	1.995	

Table 5-S2 | Thresholds of spectral hit quality (max. 2000) defined by manually evaluation of spectra of 3 surface water samples and 2 WWTP effluent samples based on criteria presented by Primpke et al. (2018). This was done to determine polymer specific thresholds for a safe MP identification (confidence interval of 95%). These thresholds were used during final image analysis using MPAPP (Primpke et al. 2019). (* After a subsequent spectra evaluation of the sample 'Eindhoven WWTP' the threshold for this sample for polymer #12 was increased to 1050). Also indicated are polymer densities applied to calculate the MP mass (see section 2.4), these densities are the mean of reported varying densities for individual polymer types.

no.	Polymer cluster	Hit quality threshold	Density (g cm^{-3})
1	Polyethylene	600	0.91
2	Polyethylene oxidized	600	0.91
3	Polyethylene-chlorinated	1000	0.91
4	Polypropylene	600	0.92
5	Polystyrene	600	1.04
6	Polycarbonate	800	1.21
7	Polyamide	1100	1.22
8	Polyvinylchloride	900	1.38
9	Cellulose chemical modified	900	1.20
10	Nitrile rubber	900	1.00
11	Polyester	1000	1.35
12	Acrylates/polyurethanes/varnish	850*	1.20
13	Animal fur	800	-
14	Plant fibers	800	-
15	Sand	600	-
16	Polysulfone	600	1.24
17	Polyetheretherketone	600	1.32
18	Polychloroprene	800	1.23
19	Chitin	800	-
20	Polyisoprene chlorinated	600	0.91
21	Polylactic acid	600	1.30
22	Polycaprolactone	1000	1.15
23	Ethylene-vinyl-acetate	1100	0.94
24	Polyimide	600	1.60
25	Polyoxymethylene	600	1.41
26	Polybutadiene	600	0.90
27	Acrylonitrile-butadiene	600	1.22
28	Rubber type 1	600	1.03
29	Rubber type 2	600	1.03
30	Charcoal	600	-
31	Coal	600	-
32	Rubber type 3	900	1.10

Table 5-S3 | MP detected in surface water and WWTP effluent samples taken in the Dommel (D), Meuse (M), or their tributaries/ related locations (+). Analysis using ATR-FTIR and µ-FTIR provided information on the individual MPs' 2 dimensional shapes and polymer types, based on this, total mass concentrations were estimated (see section 3.2). * For these samples concentrations are indicated for MP > 300 µm, the remaining locations summarize findings on MP down to a size of 20µm.

	Location	Acr/PUR		Polyethylene		Polypropylene		Polystyrene		Rubbers		Others		**Total**	
		# m^{-3}	µg m^{-3}	# m^{-3}	µg m^{-3}	# m^{-3}	µg m^{-3}	# m^{-3}	µg m^{-3}	# m^{-3}	µg m^{-3}	# m^{-3}	µg m^{-3}	**# m^{-3}**	**µg m^{-3}**
D	Berkel & Schaft	33	2	129	401	31	10	17	6	75	11	24	0.01	**309**	**430**
D	Eindhoven (WWTP)	379	52	224	2677	109	289	3	13	93	7	932	29	**1741**	**3068**
D	Eindhoven DS	862	446	2180	5422	1148	982	127	50	3019	300	1114	69	**8450**	**7270**
D	Eindhoven US	71	64	95	234	826	176	146	21	781	106	760	107	**2678**	**708**
D	Het Broek	17	0	63	716	35	26	0.5	11	35	3	126	6	**277**	**761**
D	Klotputten DS	19	2	182	715	221	210	9	13	151	18	5	25	**587**	**981**
D	Klotputten US	179	205	84	173	28	20	2	10	101	16	68	12	**460**	**435**
D	Nijnsel	49	20	72	668	80	107	0	0	46	2	84	26	**331**	**824**
D	Son	2	2	129	1411	42	134	105	9	73	4	3	0	**354**	**1561**
D	St Oedenrode (WWTP)	132	126	302	1533	207	135	40	26	75	18	185	47	**941**	**1884**
D	St Oedenrode DS	53	3	182	1109	204	187	0	0	188	10	94	29	**721**	**1339**
D	St Oedenrode US	544	95	1282	834	3995	518	259	48	3726	611	1725	269	**11532**	**2375**
D	St Oedenrode 13-2	279	54	3232	2178	227	211	22	1	2906	591	193	64	**6859**	**3099**
D	St Oedenrode 6-6	191	51	293	284	396	158	41	29	247	90	691	138	**1859**	**750**
D	St Oedenrode 21-8-R1	156	8	281	136	454	78	28	1	144	4	129	11	**1192**	**237**
D	St Oedenrode 21-8-R2	372	57	326	180	388	60	32	1	192	4	327	338	**1636**	**641**
D	St Oedenrode 21-8-R3	266	28	228	250	312	39	7	1	296	8	422	35	**1531**	**360**
D+	De Vleut	35	1	79	69	36	32	8	1	182	13	21	3	**360**	**119**
D+	Kleine Dommel	26	1	19	179	23	43	1	0	34	2	57	36	**160**	**261**
D+	Run	12	0	76	12	11	5	12	19	129	3	29	12	**270**	**51**
M	Afgedamde Maas	21	5	215	348	157	119	7	17	423	68	52	12	**876**	**569**
M	Cuijk	2	0	67	131	62	59	10	25	15	2	21	5	**177**	**223**
M	Drimmelen	64	13	269	82	87	46	3	1	330	13	36	23	**789**	**178**

M	Eijsden 13-2	512	29	134	72	63	9	2	1	437	285	81	21	**1228**	**418**
M	Eijsden 19-10	96	10	230	1354	247	314	3	16	212	28	62	17	**849**	**1738**
M	Eijsden 6-6	64	18	377	169	124	98	31	56	322	281	241	92	**1160**	**713**
M	Haringvliet	4	2	116	64	36	17	20	6	224	57	67	11	**468**	**156**
M	Roermond (WWTP)	183	84	132	857	112	76	15	26	124	26	458	182	**1024**	**1250**
M	Roermond DS	66	36	277	207	215	130	1	14	175	21	207	40	**942**	**447**
M	Roermond US	110	9	431	728	309	275	13	6	200	42	317	38	**1381**	**1098**
M+	Biesbosch1 (De Gijster)	12	2	209	128	24	10	2	1	333	287	26	6	**607**	**435**
M+	Biesbosch2 (Petrusplaat)	12	3	16	37	17	12	1	0.01	16	2	6	7	**67**	**62**
M+	Maasbommel *	0	0	45	915	1	17	0	0	0	0	0	0	**46**	**932**
M+	Maasbommel-DS *	0	0	1	13	0	0	0	0	0	0	0	0	**1**	**13**
M+	Maasbommel-US *	0	0	5	110	1	30	0	0	0	0	0	0	**7**	**140**
M+	Oijen *	1	35	1468	29761	20	400	1	30	0	0	4	106	**1494**	**30332**
M+	Oijen-DS *	0	10	7	139	1	15	0	0	0	0	0	0	**8**	**164**
M+	Oijen-US *	0	0	1	22	0	0	0	0	0	0	0	0	**1**	**22**
M+	Oijen-R1 *	0	0	203	4105	7	143	0	0	0	0	1	27	**211**	**4276**
M+	Oijen-R2 *	1	26	263	5330	12	239	0	0	0	0	3	81	**279**	**5677**
M+	Oijen-R3 *	0	0	692	14033	15	308	2	46	0	0	2	50	**711**	**14437**

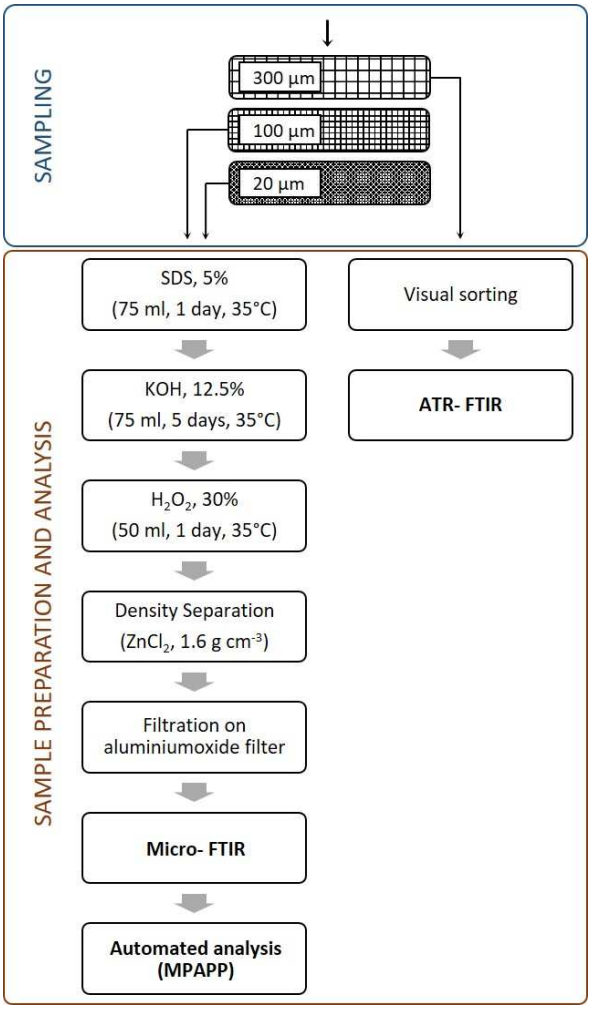

Figure 5-S1 | Schematic flowchart of steps taken to extract and analyse MP from aqueous samples. All sample preparation to extract MP retained on the 20 and 100 µm sampling sieves was done at KWR Watercycle Research Institute (The Netherlands), the sorting of MP > 300 µm and all FTIR analyses were conducted in the laboratories of Wageningen University and Research (The Netherlands).

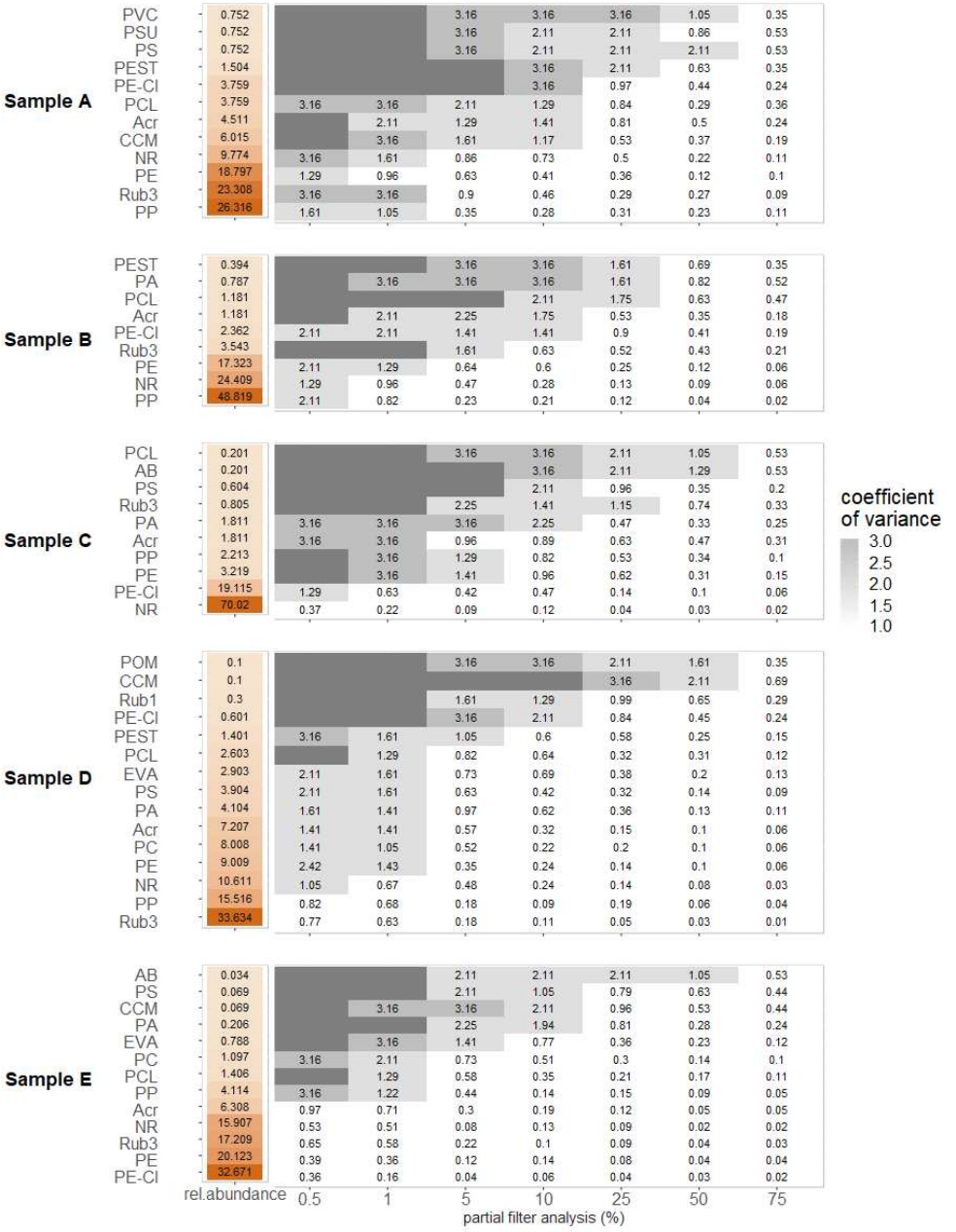

Figure 5-S2 | Uncertainties expressed as the coefficient of variance (CV) of identified polymer types during partial filter analysis with micro-FTIR. In orange the relative abundances of polymer types are indicated, total numbers of MP were Sample A: 157; Sample B: 254; Sample C: 500; Sample D: 1039; Sample E: 2928 (Figure 5-1). The CV was calculated for the individual samples based on 10 randomly selected filter areas of specified size (0.5- 75%). Areas that miss a CV are coloured in dark grey indicating that this polymer type was not identified in any of the generated 10 areas.

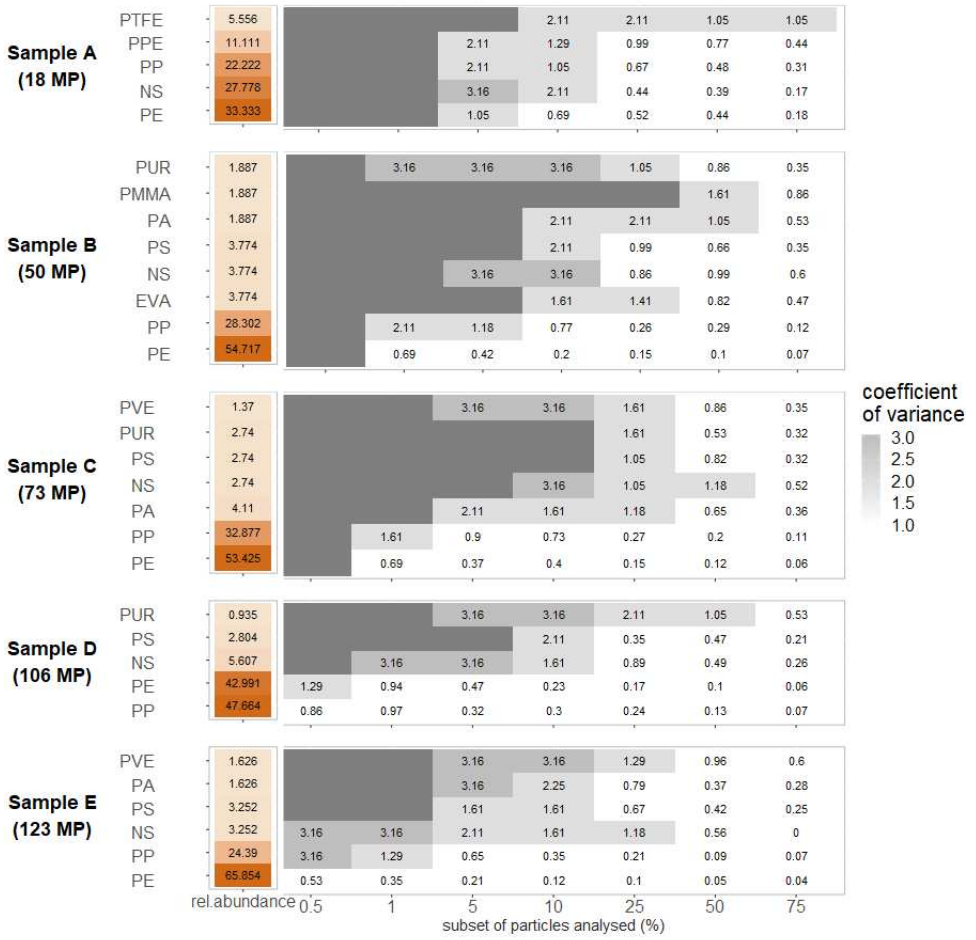

Figure 5-S3 | Uncertainties expressed as the coefficient of variance (CV) of identified polymer types when analysing a subset of particles with ATR-FTIR. In orange the relative abundances of polymer types are indicated for each sample. The CV was calculated for the individual samples based on 10 randomly selected subsamples of specified size (0.5- 75%). Areas that miss a CV are coloured in dark grey indicating that this polymer type was not identified in any of the generated 10 subsamples.

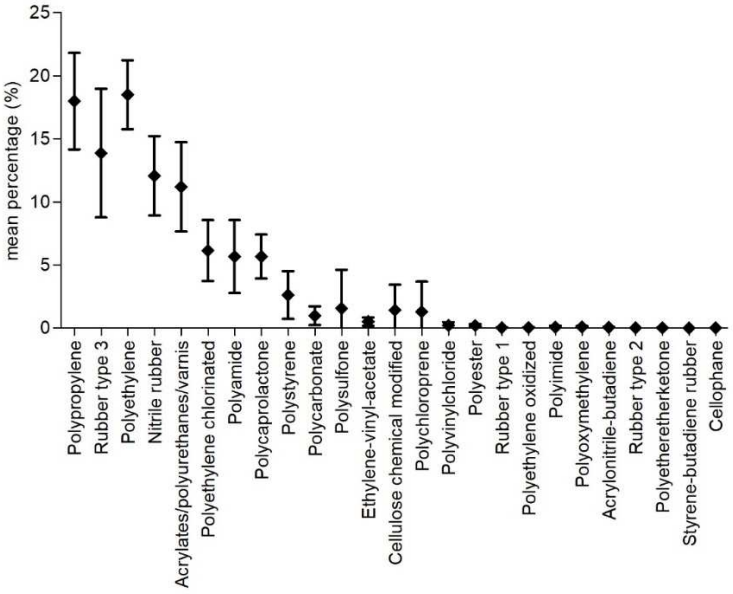

Figure 5-S4 | Mean percentages of detected polymers in riverine surface water samples. Whiskers show the 95 % confidence interval. Polymers are ordered on the x axis based on the total amount of detected MP.

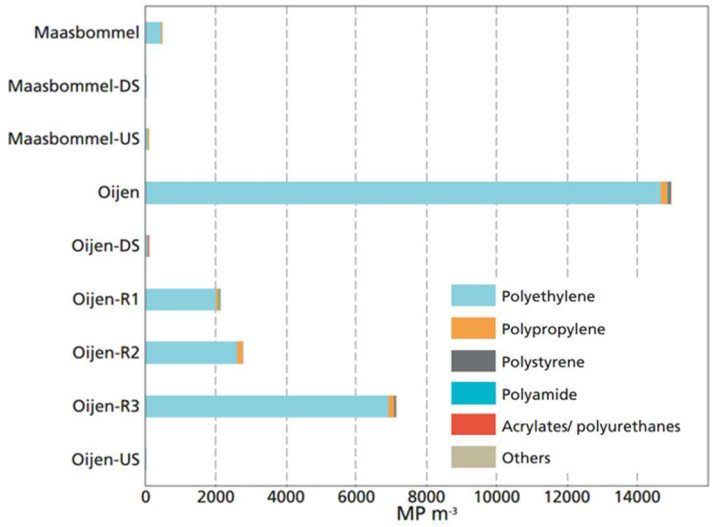

Figure 5-S5 | MP number concentrations (MP > 300µm m^{-3}) with respect to the different polymer types identified in the effluents of the WWTPs Maasbommel and Oijen, as well as in riverine surface water upstream (US) and downstream (DS) of the discharging point.

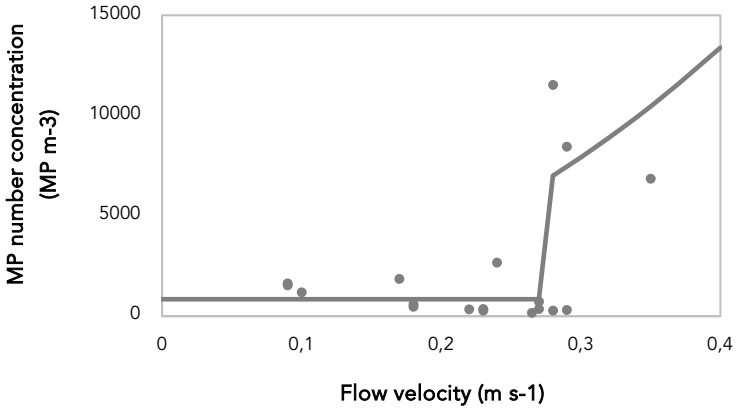

Figure 5-S6 | MP concentrations detected in the Dommel in dependency of flow velocities. The critical shear stress equation indicated a critical flow velocity of 0.275 m/s that will result in increased MP concentrations in riverine surface water.

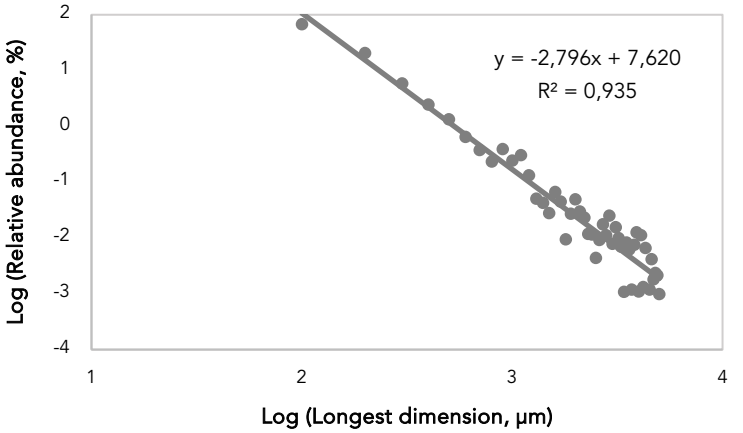

Figure 5-S7 | Relative abundance of MP numbers in relation to MP sizes.

CHAPTER 6

REMOVAL OF NANOPLASTIC PARTICLES DURING DRINKING WATER PURIFICATION

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Abstract

To date, the presence of microplastics in freshwater ecosystems has been confirmed by numerous studies. Subsequently, questions on human health effects were raised after microplastics were determined in raw and treated drinking water. This concern also applies for nanoplastics, potentially the most hazardous size range. Here we assessed experimentally if three commonly applied drinking water purification techniques have the potential to remove nanoplastic particles. This was done simulating coagulation- flocculation-sedimentation (CFS), rapid sand filtration and granular activated carbon (GAC) filtration. Nanoplastics varying in size and surface charge were partly removed by all tested techniques. Rapid sand filtration revealed the lowest removal rates. CFS was most efficient for bigger nanoplastics (200 nm), while smaller nanoplastics (50 nm) were removed better by GAC filtration. Total removal rates were found to be considerable. As drinking water treatment plants in the Netherlands consist of a set of different treatment processes, it can be expected that a large part of nanoplastics present in surface waters will thus be removed. However, it cannot be excluded that some of these plastics will remain in the drinking water. More research is thus needed to specify removal rates, and to strive for an improved removal to reduce the human exposure to nanoplastics via drinking water.

6.1 Introduction

The widespread occurrence of microplastics (MP) in freshwater ecosystems has been confirmed by numerous studies (Koelmans et al. 2019, Mintenig et al. 2020). However, with decreasing size, knowledge on MP becomes more scarce. Until now, MP down to 1 µm have been identified by a few studies only in (bottled) drinking water (Oßmann et al. 2018, Pivokonsky et al. 2018, Pivokonský et al. 2020, Schymanski et al. 2018, Wang et al. 2020). Particles smaller than 1 µm are defined as nanoplastics (NP) (Hartmann et al. 2019, SAPEA 2019). Experimental studies have revealed their presence in cosmetics (Hernandez et al. 2017) and their formation as a consequence of UV exposure (Gigault et al. 2016, Lambert and Wagner 2016). Although confirmed to exist in the environment (Ter Halle et al. 2017, Wahl et al. 2021), it has not yet been possible to quantify environmental NP concentrations.

The detection of MP in raw and treated drinking water (Kosuth et al. 2018, Mintenig et al. 2019, Pivokonsky et al. 2018, Wang et al. 2020) has raised the question if human health could be affected by MP present in drinking water (WHO 2019). This concern also applies for NP. Freshwater sources, contaminated with MP and most likely NP, are relevant for producing drinking water (Wang et al. 2020), and knowledge on removal processes is still fragmentary.

Almost all MP detected in treated drinking water were of a size smaller than 100 µm, suggesting that larger plastics are removed effectively (Mintenig et al. 2019, Pivokonsky et al. 2018, Wang et al. 2020). Pivokonsky et al. (2018) determined that full-scale drinking water treatment plants (DWTP) applying different techniques removed, on average, 73% of the MP present in surface water. Wang et al. (2020) quantified the removal of MP down to 1 µm between treatment steps in a DWTP. MP removal was highest for coagulation- flocculation-sedimentation (CFS), and the subsequently applied rapid sand filtration removed all MP > 10 µm, but only 30% of the smaller MP. This is in accordance to laboratory experiments studying transport mechanisms in porous media. While Hou et al. (2020) reported that 35% of 45 µm sized MP migrated through a sand column, this increased to approximately 80% for smaller MP and NP (Chu et al. 2019, Pradel et al. 2020, Zhao et al. 2020).

Because of their small size, NP are likely the most hazardous plastic items (Koelmans et al. 2015a, Lehner et al. 2019). Reducing NP emissions into the environment, and lowering the human exposure to NP is thus of utmost importance. An efficient removal of NP during drinking water purification would contribute to the latter. DWTPs are designed to remove particles from nanoscale viruses to micrometre sized bacteria (Westerhoff et al. 2018). A retention of NP is thus likely, but fairly unknown due to the high analytical challenges.

Our study aims to assess the removal efficiencies of purification techniques commonly applied in DWTPs, namely CFS, rapid sand filtration (subsequently abbreviated 'sand filtration') and granular activated carbon (GAC) filtration. CFS reduces the water turbidity by adding a coagulant to initiate the aggregation of particles smaller than 1 µm into flocs and that way enabling them to settle (Van Dijk et al. 2006). In surface water treatment, rapid sand

filtration is typically applied after CFS to remove remaining flocs and pathogens. GAC filtration often aims specifically to remove organic micro-pollutants that can sorb to the porous structure (Troester et al. 2016).

NP removal efficiencies of these three purification techniques were assessed in laboratory bench-scale experiments. Therefore, four different types of polystyrene NP were added to surface water relevant for drinking water production. The added NP varied in surface charge but also in size, enabling to further study their transport and deposition processes during sand and GAC filtration applying colloid filtration theory (CFT).

6.2 Material and Methods

6.2.1 Materials

For the CFS experiments, surface water from the Lek canal (Nieuwegein, The Netherlands) was used.-The sampling location was at the inlet point to a DWTP where surface water is pre-treated with CFS, for which on average 3 mg L^{-1} Fe^{3+} is added (Van Dijk et al. 2006) and rapid sand filtration. This pre-treated surface water was used to conduct filtration experiments which were performed within four days after sampling. All experiments were performed using four different types of NP, i.e. monodispersed polystyrene spheres (Polyscience Inc., US) that differed in size (50 and 200 nm) and functionalized groups. The surfaces of the NP were either plain (uncharged) or modified with carboxyl groups (COOH) which commonly occur on weathered plastics (Pradel et al. 2020) and by which the latter were more negatively charged. For the CFS, iron chloride ($FeCl_3$) was used as coagulant. For the sand filtration virgin sand with a median grain size (d_{50}) of 1.016 mm (AcquaSilica®, Kremer zand en grind BV, Nederland) was employed. Before usage, the sand was thoroughly rinsed with tap water. The GAC (Norit Row 08 supra, 0.93 mm) was obtained from a full-scale DWTP where it had been used for several months.

6.2.2 Experimental setup

For CFS, all experiments were conducted after adjusting the pH of the surface water to 8 using 0.1 M NaOH. Two different concentrations of the coagulant $FeCl_3$ (12 and 18 mg L^{-1}) were tested, which are similar to concentrations applied in full-scale DWTPs (Shen et al. 2020, Van Dijk et al. 2006, Zhang et al. 2020a). In pre-tests it was confirmed that these sufficiently reduce the water turbidity (2100Q PorTable 6-Turbidimeter, HACH, US) and TOC (LCK380, HACH, US).

The process of CFS was simulated in jars (1.8 L) that were each equipped with two dosing units and a stirring paddle. After adding NP to the surface water (10 mg L^{-1}, admixed for 30 minutes at 60 rpm) the actual CFS started by injecting NaOH followed by a rapid mixing phase (400 rpm, 10 s). Then, coagulation was initiated by adding $FeCl_3$ (12 and 18 mg L^{-1}) followed by another rapid mixing phase (400 rpm, 10 s). The flocculation step consisted of a slow stirring phase (70 rpm, 15 minutes), followed by a subsequent sedimentation phase. Settling times of 10 minutes (Volk et al. 2000) to 30 minutes (Floris 2017) seem to represent full-scale DWTPs the best. Using a syringe the supernatant (2 cm below the water surface) was sampled at 0, 20, 60 and 120 minutes. The discussion, however, will mainly focus on the removal after 20 minutes.

The sand and GAC filtration were simulated using a glass column, through which a continuous water flow from top to bottom was maintained (see SI, Table 6-S1). Before starting the experiment a conservative tracer (sodium chloride, 1 g L^{-1}) was injected to determine pore velocity and porosity.

The experiments started with continuously injecting treated surface water spiked with NP (2 mg L^{-1}). Subsequently, columns were eluted using the same water without NP added. The column effluent was sampled regularly to determine the NP concentration. Starting with every few minutes, the sampling frequency of the column's effluent declined towards the end of the experiments. NP specific breakthrough curves were generated by plotting the effluent NP concentration normalized by the inlet concentration (C/C_0).

For all experiments, the NP concentration was determined using UV-VIS spectrophotometry (Thermo Spectronic Unicam UV-500, US) as the concentration of polystyrene was proportional to the UV absorption at 229 nm (Figure 6-S2). Samples from the CFS tests were subjected to ultrasonic treatment prior to measurements to break up aggregates, and samples taken during the filtration experiments were analysed within two hours after finishing the experiments.

6.2.3 Colloidal filtration theory

Colloidal filtration theory (CFT) has been applied frequently to study the transport and deposition of nanoparticles or microbes while propagating in saturated or unsaturated porous media (Molnar et al. 2015, Schijven and Hassanizadeh 2000, Schrick et al. 2004). Within CFT the deposition of particles depends on their size and density, but also on the characteristics of the stationary and mobile phase. The approach by Tufenkji and Elimelech (2004) was followed to predict the single- collector contact efficiency (η_0), the attachment efficiency (α), and the single- collector removal efficiency (η) for the experimental filtration data. The equations to calculate these parameters for saturated porous media are summarized here (eq. 6-1 to 6-3), further calculations can be found in the original study (Tufenkji and Elimelech 2004).

The first parameter, η_0 (-), describes the efficiency of NP delivery to the filter surfaces (Molnar et al. 2015). Together with the term α (-), indicating how efficiently NP stick to the filter material after a collision, the removal efficiency η (-) can be calculated. These parameters enable comparing the retention of different NP and different filter materials.

The contact efficiency η_0 is calculated by summing individual transport mechanisms, Eq. 6-1:

$$\eta_0 = \eta_D + \eta_I + \eta_G \quad (6\text{-}1)$$

in which:

η_D	=	the transport by diffusion	[-]
η_I	=	the transport by interception	[-]
η_G	=	the transport due to gravity	[-]

Larger colloids typically have a larger η_I and η_G, while Brownian motion, and thus η_D, is larger for smaller colloids (Molnar et al. 2015). Using η_0, the attachment efficiency (α) was determined which indicates the fraction of contacts needed for a colloid to attach to the filter material, Eq. 6-2:

$$\alpha = -\frac{2}{3} * \frac{d_{50}}{(1-f)L\eta_0} * \ln\frac{C}{C_0} \quad (6\text{-}2)$$

in which:

d_{50}	=	median diameter of the used filter material	[m]
f	=	porosity	[-]
L	=	length of the filter bed	[m]
C	=	effluent NP concentration	[mg L^{-1}]
C_0	=	dosing NP concentration	[mg L^{-1}]

Lastly, the single-collector removal efficiency (η) is calculated which expresses the capacity of the filter material to trap NP. It is the product of the attachment efficiency (α) and the single-collector contact efficiency (η_0), Eq. 6-3.

$$\eta = \alpha * \eta_0 \quad (6\text{-}3)$$

6.3 Results and Discussion

6.3.1 CFS

In pre-tests, the reduction of turbidity and of TOC were determined for the two different coagulant doses. The turbidity was reduced by approximately 80% (30 min) to 95% (120 min), and TOC by 25% to 60% respectively (Figure 6-S1). Requirements for the production of drinking water were thus reached within 30 min (WHO 2017).

During the actual experiments, both coagulant dosages resulted in a similar NP removal, increasing considerably with longer sedimentation times to an almost complete removal for both, the plain and carboxylated, larger NP after two hours (Figure 6-1). The process of coagulation with the addition of $FeCl_3$ is tailored for negatively charged particles (Enfrin et al. 2019, Matilainen et al. 2010, Novotna et al. 2019). But, contrary to our expectations, the negatively charged carboxylated NP were not systematically removed better than the plain NP. Instead, a considerable effect of NP size could be determined.

The settling of the bigger NP started immediately and in the supernatant a reduced NP concentration was determined already at the start of the experiments. After a settling time of 20 min, the bigger NP were removed by 83% (79-86%), while this was much lower for the smaller NP (6-47%). Also Zhang et al. (2020a) reported very low removal rates (2-13%) for plastic particles between 0.18-125 µm. The authors hypothesized that this was caused by the MPs' generally low settleability, and a relatively large size by which they were not enmeshed in the formed flocs. This floc formation also depends strongly on other factors (e.g. water pH, surface charges or coagulant concentration). Such differences could thus be an explanation for the contradictory results (Enfrin et al. 2019). Nevertheless, the here presented results are similar to removal efficiencies determined in laboratory-scale experiments for other nano-sized particles. Removal efficiencies of approximately 80% for nano-silver, of more than 90% for TiO_2 particles (Chalew et al. 2013b) and of more than 80% for nC_{60} fullerenes (Floris 2017) were reported. Similarly, Lapointe et al. (2020) determined removal rates of around 80% for smaller MP (15 and 140 µm) for which settling started instantly and for which removal was highest for weathered MP. The authors attributed this to the irregular MP shape, or to the presence of hydroxyl and carboxylic groups. It might have rather been the shape, as the current findings could not support an effect of the modified NP surfaces with carboxylic groups. However, further research is required to test this.

Lastly, it has to be considered that the initial NP concentration of 10 mg L^{-1} is likely too high to depict the environmental situation accurately, but had to be chosen due to analytical restrictions. Previous studies demonstrated a negligible effect of the initial concentration of nano-sized particles on their removal rates (Chalew et al. 2013b, Honda et al. 2014), however, further research might be needed to confirm this for NP specifically.

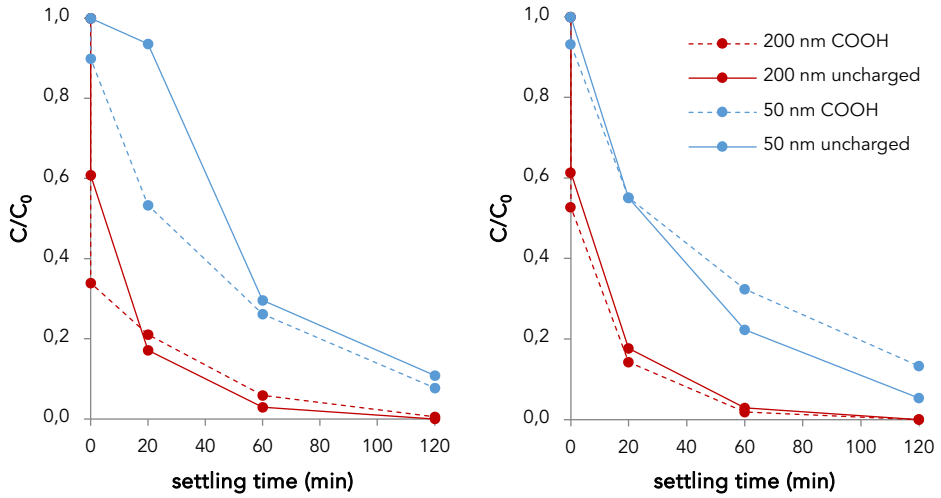

Figure 6-1 | NP residual concentrations, determined in jar tests simulating coagulation- flocculation-sedimentation (CFS) for two iron chloride concentrations (left: 12 mg/L and right: 18 mg/L). Measured NP concentrations in the aqueous phase were normalized to initial NP concentrations.

6.3.2 Sand and GAC filtration

Neither sand, nor GAC filtration fully removed the injected NP (Figure 6-2). The breakthrough curves of most of the NP types reached a plateau almost instantly which is in accordance to several other studies (Chu et al. 2019, Molnar et al. 2015, Pradel et al. 2020). Exemptions are the 200-COOH NP during GAC filtration and the 50-PL (plain) NP during both experiments. For these particles the retention became less efficient over time.

During sand filtration the 50-COOH NP were removed by 63%, while lower NP removal rates (4- 22%) were determined for the remaining three NP types. These results are comparable to the ones from Chu et al. (2019) and Zhao et al. (2020) who reported that 20% of the smaller MP and NP were retained in a sand column. No explanation could be found for the higher removal of the 50-COOH NP. Further, no clear trend was observed between NP removal and the NP size or charge, which was expected as the sand grains (d_{50} = 1016 µm) formed pores considerably bigger than the used NP. Using finer sand, Pradel et al. (2020) concluded that larger NP (460 nm) deposited more easily than smaller NP (200 nm). More importantly, the authors also demonstrated that removal rates increased by one order of magnitude when injecting same- sized, but irregular shaped NP. Since environmental NP are mostly a result of fragmentation and thus of irregular shape, higher removal efficiencies in full-scale DWTPs could be expected. In addition, Bertelkamp et al. (2018) reported that approximately 40% of nano-silver and -gold were removed using virgin sand, while the retention increased to approximately 65% using sand covered with a biofilm. This could also indicate towards a

higher NP retention in a full-scale DWTP. The present study could therefore be considered as a worse-case estimate.

As expected, NP removal during GAC filtration was higher than during sand filtration. Approximately 60-70% of three types of initially added NP were retained in the column, except for the 200-COOH from which only 20% were removed. Smaller NP (50 nm) were removed to a larger extent than the bigger (200 nm) NP. In addition, for both sizes, the uncharged NP showed a higher removal than the carboxylated NP. In contrast to current findings, an almost complete removal was determined experimentally for nano-silver (Gicheva and Yordanov 2013), and for nC_{60} fullerenes (Floris 2017). In full-scale DWTPs an almost complete removal of MP with a size of approximately 1 µm was found when applying GAC filtration (Pivokonsky et al. 2018, Wang et al. 2020).

For both filter materials, the elution phase was accompanied by a sharp decline of NP detected in the effluent. The NP were thus well attached and retained by the column (Chu et al. 2019, Molnar et al. 2015). This is in accordance to the findings by Pradel et al. (2020) studying NP transport in a sand column. An exception to this is given by the smaller carboxylated NP during GAC filtration which got mobilized again during elution phase leading to increasing NP concentrations in the effluent (Figure 6-2B).

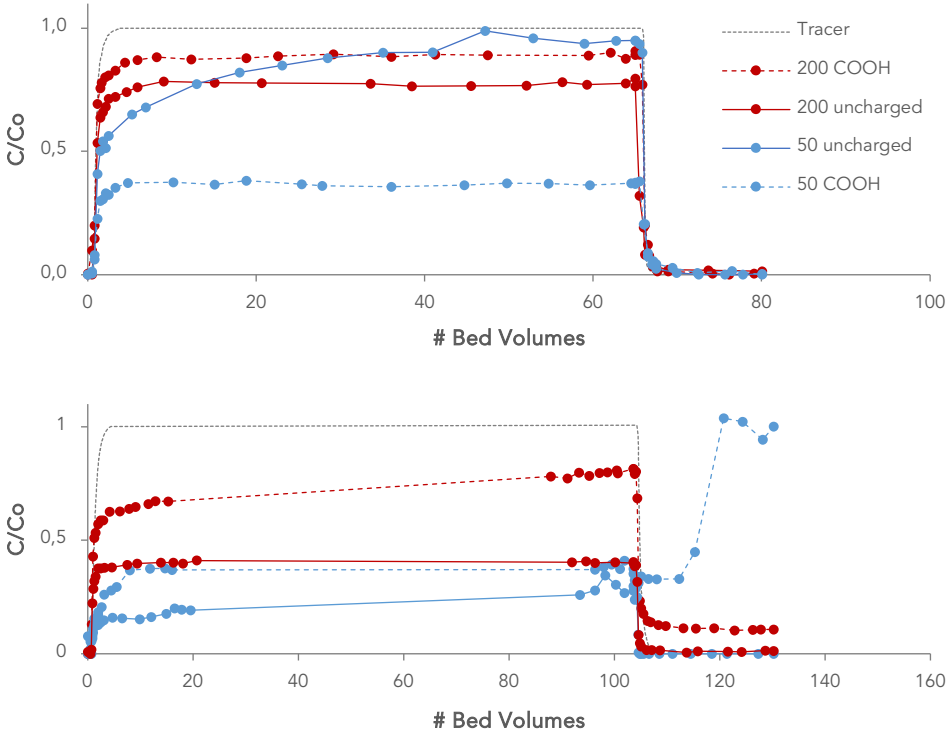

Figure 6-2 | NP breakthrough curves during sand filtration (upper plot) and during granular activated carbon (GAC) filtration (lower plot). NP in the effluent are presented in relation to the constant inlet concentration (C_0 2 mg L^{-1}).

6.3.3 Quantifying the NP attachment efficiency

Applying CFT and thus calculating the attachment efficiency (α) enables a quantitative comparison of NP transport in different filter materials (Pelley and Tufenkji 2008).

During sand filtration an increasing NP breakthrough, and thus a lower single-collector removal efficiency (η) also resulted in a lower α (Table 6-1). This term α describes how efficiently NP stick to the filter material after a collision with the filter grains. Attachment efficiencies were generally lower for GAC than for sand filtration; however the smaller, and more porous GAC grains have a higher surface area making a NP collision more likely. Consequently, the attachment efficiencies for the 50-COOH NP for both column materials varied considerably despite similar removal rates (Table 6-1). Apparently, more collisions were needed for NP to be removed during GAC filtration. Similarly, during GAC filtration the 200-PL and the 50-COOH showed an almost identical removal, and thus η, while alpha was almost three times larger for the larger NP. While the injected NP mass concentration was the same, the injected NP number concentration was obviously higher for the smaller NP.

The lower alpha indicates that the smaller NP attached less good to the GAC than the bigger NP.

Recent studies on the transport of small MP and NP in saturated sand media also applied CFT to elaborate on experimental results. Attachment efficiencies were considerably different to the here presented results from the sand filtration which can be attributed to different plastic sizes (Chu et al. 2019, Zhao et al. 2020), and much smaller sand grains (Chu et al. 2019, Pradel et al. 2020).

For the current study, the attachment to sand seemed more efficient than to the irregular shaped GAC. These results, however, should be interpreted with care as we applied CFT for polydisperse, irregular shaped GAC grains, for which it is not originally made (Pradel et al. 2020). More research is needed to test the applicability of CFT for such experiments. Similarly, and although frequently applied, it is not yet clear how well alpha can be extrapolated to the conditions in a full-scale DWTP (Bertelkamp et al. 2018).

Table 6-1 | Experimentally determined NP breakthrough (C/C_0) during rapid sand and GAC filtration, calculated attachment efficiencies (α) and single-collector removal efficiencies (η) following Tufenkji and Elimelech (2004). Indications of individual transport mechanisms (diffusion, interception and gravity), and on the single-collector contact efficiency (η_0) can be found in Table 6-S2.

	Sand filtration			GAC filtration		
	C/C_0	α (-)	η (-)	C/C_0	α (-)	η (-)
200 COOH	88.9	0.22	1.9E-04	80.2	0.07	2.2E-04
200 uncharged	77.7	0.47	4.1E-04	40.2	0.28	9.0E-04
50 COOH	36.8	0.62	1.6E-03	38.6	0.10	9.4E-04
50 uncharged	95.7	0.03	7.1E-05	29.5	0.12	1.2E-03

Applying CFT could further give an indication of the deposition processes taking place. Because smaller nanoparticles exhibit larger Brownian motion, their deposition due to diffusion is higher compared to bigger particles (Molnar et al. 2015). This can clearly be seen during sand filtration where the transport by diffusion was one order of magnitude bigger for the 50 nm compared to the 200 nm NP. This, however, was not the case during GAC filtration where the transport by diffusion was within the same order of magnitude for both NP sizes. For both filtration types, the NP deposition due to interception and gravity was one to two orders of magnitude higher for the bigger NP (Table 6-S3).

In their study, Zhao et al. (2020) reported that increasing the flow velocity or the ionic strength led to a higher MP deposition, and a thus higher attachment efficiency respectively. Pradel et al. (2020) documented that the attachment efficiency largely depended on the sand grains. Further research on these aspects is recommended when aiming in an increased NP removal during drinking water purification.

6.3.4 Transferability to DWTPs

Most of the DWTPs in the Netherlands operate at least these three purification techniques in sequence. In theory total NP removal rates between approximately 80–95% could then be expected (Table 6-2). This is comparable to experimental findings by Donovan et al. (2016) reporting an almost complete removal of nano-gold, -silver and -TiO_2 particles during drinking water production. The here presented removal rates, however, were determined under specific laboratory conditions and should thus be used as a first indication only.

Table 6-2 | Removal rates (%) determined experimentally, and under specific conditions, for individual water purification techniques coagulation- flocculation- sedimentation (CFS), rapid sand filtration and GAC (granular activated carbon) filtration, which were subsequently combined to estimate a total removal efficiency for different types of NP. The retention rates for CFS correspond to an experimental settling time of 20 min and are an average of the two $FeCl_3$ dosages, and, for sand and GAC the total removal efficiency at the end of the experiments are indicated.

NP type	CFS (%)	Sand filtration (%)	GAC filtration (%)	Total (%)
200 COOH	82 (SD 5)	11 (SD 1)	20 (SD 1)	87 (SD 4)
200 uncharged	83 (SD 1)	22 (SD 1)	60 (SD 1)	95 (SD 1)
50 COOH	46 (SD 1)	63 (SD 1)	61 (SD 2)	92 (SD 1)
50 uncharged	26 (SD 27)	4 (SD 2)	71 (SD 3)	79 (SD 10)

Several aspects should further be considered for actual DWTPs. For example, Bertelkamp et al. (2018) reported an increased deposition of nano-silver and nano-gold on biofouled sand grains compared to virgin sand grains. Similarly, Lapointe et al. (2020) and Pradel et al. (2020) suggested that irregular shaped and weathered NP will be removed better. Also the NP surface charge and the water matrix will have an effect (Fischer et al. 2019a, Pelley and Tufenkji 2008) and thus influence the actual removal of NP in a full-scale DWTP.

6.4 Conclusions

Three different purification techniques were assessed individually. All techniques partially removed the injected NP. During CFS, larger NP (200 nm) were removed more efficiently than the smaller NP (50 nm), however, no effect of NP surface charge was observed. GAC filtration revealed a higher capacity to remove NP compared to rapid sand filtration. Especially the smaller NP were removed well during GAC filtration, and uncharged NP were removed to a larger extent than carboxylated NP.

Future research is be required to elucidate to what extent these experimental findings can be transferred to actual drinking water production, and what removal rates can be achieved by advanced treatment such as oxidative or size-exclusion techniques. To date, analytical techniques are not yet available to use plain NP for this. Mitrano et al. (2019), however, have proven that metal-doped NP have a high potential to be applied for such tests by which environmentally realistic NP concentrations and irregular NP shapes can be realized.

Acknowledgements

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6.5 Supplementary Information

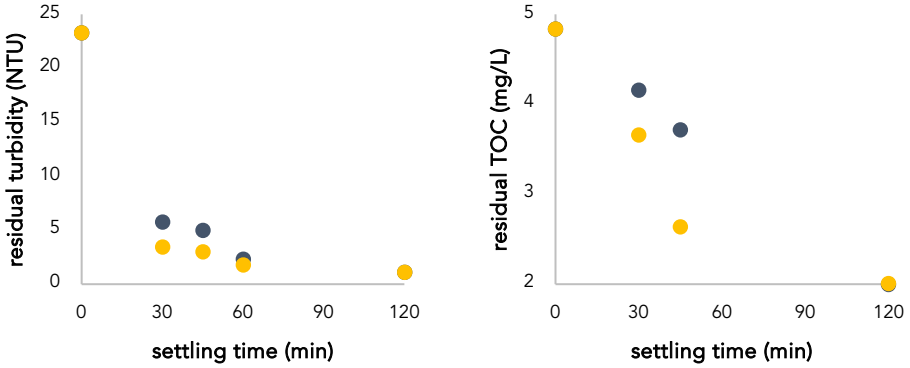

Figure 6-S1 | Pre-tests conducted to determine the reduction of residual turbidity and TOC when simulating coagulation- flocculation- sedimentation (CFS) using two concentrations of iron chloride as coagulant (12 (blue) and 18 (yellow) mg L^{-1}), and different settling times.

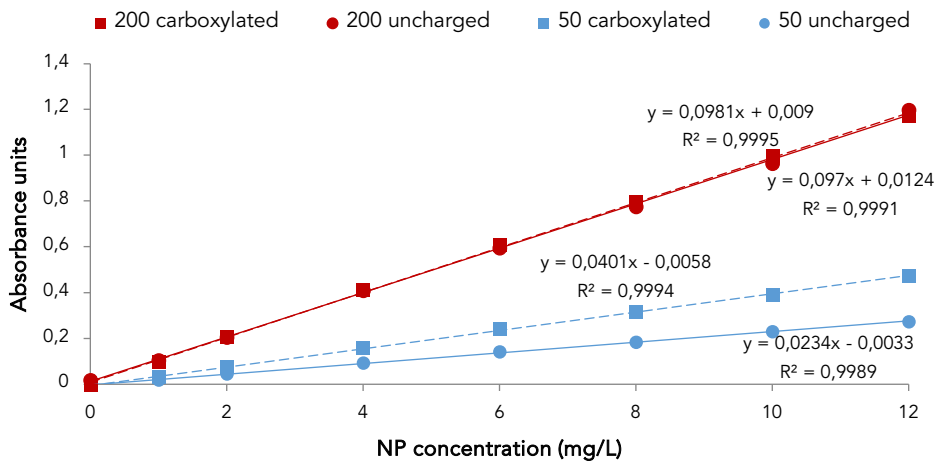

Figure 6-S2 | Calibration curve produced using UV-VIS spectrophotometry to quantify different NP (polystyrene spheres) spiked into riverine surface water based on the UV absorption at 229 nm.

Table 6-S1 | Characteristics of used columns to assess the removal capacity for NP during rapid sand and granular activated carbon (GAC) filtration. Further water parameters can be found in Van Dijk et al. (2006).

		Sand filtration	GAC filtration
Column characteristics	porosity (n) [b]	0.405	0.22
	median grain size filter - d_{50} (µm)	1016	925
	bed height (m)	0.70	0.80
	column diameter (m)	0.035	0.035
	bed volume (ml)	673.48	769.69
	contact time (h)	0.102	0.169
	discharge Q (m^3 s^{-1})	1.84E-06	1.27E-06
	column area A (m^2)	9.62E-04	9.62E-04
	A, corrected for porosity (m^2)	3.90E-04	4.31E-04
	flow through pores (m s^{-1})	4.72E-03	2.94E-03
Water matrix	Influent:		
	initial NP concentration (mg L^{-1})	2.0	
	duration NP injection phase (pore volumes)	65	104
	duration elution phase (pore volumes)	15	27
	temperature (°C)	20.1 (SD 0.2)	
	pH [a]	8.06 (SD 0.08); 8.13 (SD 0.16)	7.99 (SD 0.2); 7.77 (SD 0.2)
	conductivity (µS cm^{-1}) [a]	611 (SD 14); 608 (SD 14)	588 (SD 6); 591 (SD 20)
	UV 254 nm [a]	0.61 (SD 0.1); 0.59 (SD 0.09)	0.56 (SD 0.08); 0.37 (SD 0.04)
	Tracer 1 (C_{eff}/C_{inf}=50%, in s)	357	765
	Tracer 2 (C_{eff}/C_{inf}=50%, in s)	366	757

[a] The first value corresponds to the influent measured, the second to the effluent respectively.

[b] The porosity was determined experimentally for the sand filter by adding water to a packed column. The porosity (n) was calculated by dividing the volume of water in the pores by the volume of the filter material. This approach was not applicable to the GAC. Therefore the porosity of the GAC filter was determined by mercury intrusion porosimetry, helium pycnometry and nitrogen adsorption. These calculations were done by Delft Solids Solutions, and subsequently by KWR Water Research Institute. The first three techniques applied differentiated the total porosity (V_{tot} = 1.50 cm^3 g^{-1}) and the internal porosity (V_{tot} = 1.06 cm^3 g^{-1}) of the GAC. The pores accessible for the NP can thus be estimated as: V_{inter} = 0.49 cm^3 g^{-1}. Using also the density of the GAC itself (2.2 cm^3 g^{-1}), the porosity of the GAC was calculated: 0.49 cm^3 g^{-1} x 2.2 cm^3 g^{-1} = 0.22.

Table 6-S2 | Experimentally determined NP breakthrough during column rapid sand and granular activated carbon (GAC) filtration. For these experiments the individual transport mechanisms (diffusion, interception and gravity) were assessed following Tufenkji and Elimelech (2004). Respective attachment efficiency (α) and single-collector contact efficiency (η) can be found in Table 6-1.

	Sand filtration			GAC filtration		
	η_D	η_I	η_G	η_D	η_I	η_G
200 COOH	8.7E-04	6.6E-06	1.5E-07	3.2E-03	3.6E-05	5.0E-07
200 uncharged	8.7E-04	6.6E-06	1.5E-07	3.2E-03	3.6E-05	5.0E-07
50 COOH	2.6E-03	9.1E-07	9.9E-09	9.7E-03	5.0E-06	3.2E-08
50 uncharged	2.6E-03	9.1E-07	9.9E-09	9.7E-03	5.0E-06	3.2E-08

CHAPTER 7

SYNTHESIS

7.1 Introduction

Plastic is the common name given to a wide range of synthetic or semi-synthetic organic polymers. Typically, items made of plastic are lightweight, durable and cheap, which increased their global production to 359 million tonnes in 2018 (PlasticsEurope 2019). After usage, plastic can be incinerated, recycled, collected in landfills or, potentially, released into the environment (Geyer et al. 2017). Once in the environment and apart from ethical and aesthetical concerns, plastic litter can cause ecological, and socio-economic harm (Galgani et al. 2013, SAPEA 2019). In contrast to natural materials, plastic items are not degraded. The exposure to UV radiation and physical abrasion, however, will cause the formation of smaller plastic fragments (Andrady 2011).

Plastic items larger than 5 mm are usually referred to as macroplastics; all items between 1 µm and 5 mm are defined as microplastics (MP); and plastics smaller than 1 µm are defined as nanoplastics (NP) (SAPEA 2019). Special attention, not only in science, but also in media and policy, has been given to MP which to date can be found in almost all natural habitats (Hurley et al. 2018).

First studies on MP almost exclusively focussed on the marine environment. From there, the focus shifted towards coastal zones, and then to terrestrial, freshwater and atmospheric environments (Rochman 2018, Zhang et al. 2020b). This implies that the scientific focus is getting closer to examining the actual sources, which are largely terrestrial (Andrady 2011, Rochman 2018). In this thesis I will focus only on the freshwater environment.

Jambeck et al. (2015) estimated that 4.8 to 12.7 million tons of plastic were released in 2010 by coastal countries into the sea. But not only coastal countries discharge (micro)plastics. Within the natural water cycle of evaporation, precipitation and runoff, the urban water cycle is embedded (Figure 1-1) (Van Dijk et al. 2006). This cycle describes how humans get, use and re-use water. MP can enter rivers via waste waters, littering, runoff from urban and agricultural areas, or tyre abrasion (Boucher et al. 2019, Hurley and Nizzetto 2018). Riverine surface waters are again used to produce drinking water. MP have been traced throughout this urban water cycle, in (treated) waste waters, surface waters and, lastly, also drinking waters (Koelmans et al. 2019). Their ubiquity has caused considerable scientific attention (Figure 7-1), but also media attention. Although it is not yet feasible to fully assess the risks of MPs, policy makers from local to global scale started implementing regulations to prohibit the release of (micro)plastics into the environment (Rochman et al. 2016b).

Several studies have determined MP within various components of the urban water cycle. Reported concentrations, however, vary by several orders of magnitude (Koelmans et al. 2019, Lambert and Wagner 2018). Regional differences might explain some of these concentration differences, but the various approaches to sample and analyse MP also contribute to this variability (Koelmans et al. 2020). To be able to compare results of different studies, strict quality assurance and quality control (QA/QC) procedures have to be followed

and reported extensively (Cowger et al. 2020, Koelmans et al. 2019). Although a recent progress in the quality of applied methods can be seen, the majority of published studies miss on crucial aspects of quality assurance (Koelmans et al. 2019). As a result, and despite the yearly numbers of MP studies have increased considerably (Figure 7-1), our knowledge on MP is still fragmented. The lack of knowledge becomes even more apparent for NP due to the even higher analytical challenges, and the delayed start of NP research in comparison to the work on MP (Figure 7-1).

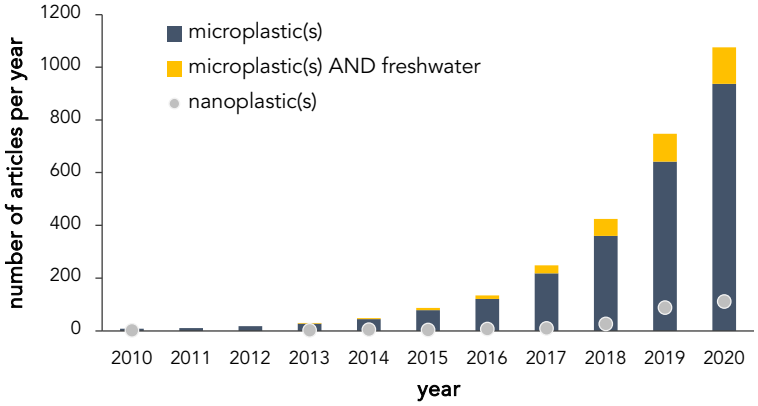

Figure 7-1 | Number of articles found using the Web of Science database on 19th November 2020, including all articles on microplastic(s) (total bar), with articles focussing on the freshwater environment highlighted in yellow. The grey dots indicate number of articles on nanoplastic(s).

Accurate data on MP types and concentrations in the environment are needed to assess their related risks, to trace back their emission sources, and to mitigate these sources. Although the numbers of studies are increasing, a limited amount of studies have and is focused on the freshwater environment (Figure 7-1). For this thesis three fields of interest were further defined (Figure 1-3), for which the following research questions were formulated:

1. Which analytical techniques can be applied to determine the size, and polymer types of individual MP and NP? Given the wide range of applied analytical methodologies; what are the key criteria that need to be fulfilled and reported to represent environmental MP reliably?
2. Which exact types of MP can be detected in different WWTP effluents and in riverine surface waters? What are the absolute concentrations released by WWTPs, and what is their relative contribution to the MP load already present in a river? How does the riverine MP transport vary over time and over a river's length?
3. Could NP, potentially present in surface waters, be retained by the commonly applied drinking water purification techniques?

In this final synthesis chapter (**Chapter 7**), based on the findings of previous chapters and recent literature, I provide and reflect on answers to the posed research questions. The synthesis is structured in accordance to the three fields of interest. The first set of research questions covered analytical requirements to accurately identify MP, and was addressed in **Chapter 2** and **Chapter 3**. Applying herein defined criteria, the second set of research questions was the starting point for two field studies examining MP in and around the effluents of WWTPs (**Chapter 4** and **Chapter 5**). Thirdly, the last research question was addressed experimentally in **Chapter 6**. At the end of the chapter a short prospect section is provided, where some implications and key recommendations for further research are presented.

7.2 Advancing analytical methods to assess MP and NP behaviour in the urban water cycle

7.2.1 Requirements to reliably identify small MP and NP

The field of MP research is still relatively young, implying that methods to detect MP in environmental samples are still under development. Methods to identify NP are even more in their infancy. Therefore, the first research question was: *Which analytical techniques can be applied to determine the size, and polymer types of individual MP and NP?*

Only a detailed characterization of the plastics' sizes, shapes and polymer identities assures that generated data can be used broadly (Haave et al. 2019). We thus developed, in **Chapter 2**, a framework able to consistently determine these parameters for a broad particle size spectrum (Figure 2-5). While building on analytical techniques commonly applied to detect MP, we provided data on a new set of techniques to detect NP.

Plastic particles are neither distributed nor transported homogeneously in the environment. This has to be considered during sampling. For the aquatic environment volume-reduced or bulk sampling can be conducted (Hidalgo-Ruz et al. 2012). Reducing the sample volume is achieved by filtering water, thus concentrating the MP, which increases the representativeness of a sample (Hidalgo-Ruz et al. 2012, Koelmans et al. 2019). However, this type of sampling might affect the MP' size distribution. MP smaller than the filter's grid size can be retained when filters start clogging, while MP just bigger than that can pass the filter vertically (Lorenz et al. 2019, Mintenig et al. 2020). This problem is avoided applying bulk sampling. Frequently, MP concentrations are not high enough to accurately capture the environmental situation when applying bulk sampling. We thus recommend (**Chapter 2 and 3**) and also applied volume-reduced sampling using cartridge filters (**Chapter 4**) or stacked sieves (**Chapter 5**). Sampling becomes more challenging when targeted MP sizes decrease. Completing the rather conventional filtration steps we applied crossflow ultrafiltration to sample particles smaller than 20 µm (**Chapter 2**). We demonstrated its applicability by concentrating particulate matter from 635 L surface water into a volume of 0.4 L (factor of

1600) and by determining a reproducible recovery (Table 2-1). With the same aim, Ter Halle et al. (2017) applied ultrafiltration and Hildebrandt et al. (2020) applied continuous flow centrifugation. Considerably lower sampling volumes of 1 L, and 5 L respectively, were concentrated. However, especially the latter seems of high potential as high and reproducible recovery rates were achieved when concentrating NP (160 nm) in filtered (92%) and unfiltered (75%) river water.

Due to the diversity of polymers, but also to differentiate between synthetic and natural materials, the particles' nature needs to be identified applying spectroscopic or spectrometric techniques.

To date, most studies sort potential MP larger than 300 to 500 µm visually. This alone could lead to an incorrect estimate of MP, but error rates seem negligible if subsequently conducting ATR- FTIR analysis (Kroon et al. 2018). Using field data from **Chapter 5** we quantified that, independently of the samples' total number of sorted particles, polymer distributions were depicted accurately when analysing 75% of all sorted particles (Figure 5-S3).

Analysing MP smaller 300 µm is even more error-prone, and should thus avoid any manual sorting. Here, mainly spectroscopic techniques, namely Raman and FTIR microscopy, have been applied that are able to identify polymer types, and to estimate the size and shape of MP down to a few micrometre (Cabernard et al. 2018). In contrast, spectrometric approaches (Dümichen et al. 2017, Fischer and Scholz-Böttcher 2017) are not limited by MP sizes, but also do not provide any size estimation. Such analyses result in polymer specific MP mass concentrations, while applying spectroscopy typically results in MP number concentrations.

FTIR microscopy is able to analyse MP down to several micrometres, and its usability is proven by a wide range of studies (Liu et al. 2019, Lorenz et al. 2019). We thus built on its usage developing the protocol from **Chapter 2** (Figure 2-1), and applied it to identify MP in **Chapter 4** and **Chapter 5**. For smaller MP, Pyrolysis GC-MS seems most promising (Fischer and Scholz-Böttcher (2017), **Chapter 2**). Natural sample materials can hinder tracing the polymer specific degradation products during GC-MS analysis. Considering this, we used organic rich surface water and identified a limit of detection for polystyrene between 50 and 100 ng. Pyrolysis GC-MS thus is a likely appropriate technique to detect NP, especially when applying volume-reduced sampling (Hildebrandt et al. (2020), **Chapter 2**).

Next to analytical demands, further considerations need to be taken into account to produce accurate data. We thus formulated the second research question: *Given the wide range of applied analytical methodologies, what are the key criteria that need to be fulfilled and reported to represent environmental MP reliably?*

Almost ten years ago Hidalgo-Ruz et al. (2012) already concluded that methods to sample and analyse MP need to be standardized to be able to compare results of different studies. Instead, the numbers of methods increased, while the necessity of standardization has been emphasized continuously (Cowger et al. 2020, Filella 2015, Frias et al. 2019, Provencher et al. 2020, Twiss 2016). In **Chapter 3** we showed how variable applied methodologies, and how widely spread QA/QC implementations of published studies that focus on MP ingestion by marine biota are. This compromises data quality and data comparability. In order to deal with this problem, we defined ten quality criteria to be considered after reviewing 37 studies. Based on these criteria we further developed a scoring system to assess a studies' data quality. Inspired by approaches to QA/QC in the field of toxicology, this approach can guide risk assessors in performing unbiased, transparent, and detailed evaluations, while guiding researchers in performing and reporting studies in a manner deemed appropriate (Kase et al. 2016, Klimisch et al. 1997).

Due to its clarity, this work was received well and caused follow-up work. Together with the World Health Organization (WHO) this scoring system was extended to aqueous samples relevant for the production of drinking water (Koelmans et al. 2019), and more recently to atmospheric samples (Wright et al. 2021) and to toxicological studies (de Ruijter et al. 2020).

These scoring systems should not be seen as static. Instead, they ask for incorporation of new knowledge or analytical developments once these become available. As an example, while general recommendation were kept unchanged, they were, for example, adapted in regard to targeted MP sizes as these are typically lower in water samples compared to biota samples. For the future, I expect, for example, adaptations in regard to data handling and interpretation, as automated image analysis has proven to provide more accurate information on MP numbers, sizes, shapes, and polymer types (Primpke et al. 2020, Primpke et al. 2017b). Further, implementation and reporting guidelines defined in **Chapter 3** and Koelmans et al. (2019) are in line with the newest ones from Cowger et al. (2020) and Provencher et al. (2020), who additionally demand reviewers and editors to stronger control the implementation of sufficient QA/QC procedures.

7.2.2 Occurrence and variability of MP in waste waters and riverine surface waters

After discussing quality assurance criteria in the previous section, here I will discuss the results of two field studies conducted for this thesis. Four research question were posed in this section, starting with: *Which exact types of MP can be detected in different WWTP effluents and in riverine surface waters?,* and *What are the absolute concentrations released by WWTPs?*

In **Chapter 4**, MP particles and fibres were identified in all of the 12 examined WWTP effluents. Total MP number concentrations varied around a mean of 717 MP m^{-3} by three orders of magnitude. In total, 14 different polymer types were identified. While the majority of particles was made of polyethylene (PE) (Figure 4-1, Figure 4-2, and Figure 4-S2), polyester (PEST) was predominately found for fibres (Figure 4-3, and Figure 4-S2). Furthermore, in almost all effluents numbers of MP fibres outweighed MP particles. Building on this study, we determined MP in WWTP effluents (**Chapter 5**), and riverine surface waters up- and downstream of the WWTPs' discharging points. The MP number concentrations in the three WWTP effluents varied from 1050 to 1950 MP m^{-3} (Figure 5-2), with 18 different polymer types identified. Including also the results from riverine surface water samples, we found 26 different polymer types. We mostly detected MP particles, which is contrary to the previous chapter with MP fibres outweighing MP particles.

In the course of this thesis methods became available and were thus adapted, these differences might explain some of the mentioned deviations within generated results. For example, both studies applied conventional filtration, but sampling with a mesh size of 10 µm (**Chapter 4**) instead of 20 µm (**Chapter 5**) might explain the higher proportions of fibres and thus avoids drawing conclusions on the environmental situation. As these fibres typically have a diameter of 15 µm (Napper and Thompson 2016, Pirc et al. 2016), they could have passed the bigger mesh sizes vertically. Further, in both studies we applied FTIR microscopy. In **Chapter 4** an advanced focal plane array (FPA) detector enabled a higher spatial resolution during measurements, by which MP down to a size of 11 µm could be detected. In comparison, the single- point mercury- cadmium- telluride (MCT) detector from **Chapter 5** had a size limit of 20 µm. Neglecting environmental differences, and considering that a smaller mesh size during sampling and a more advanced FTIR system were used in the first study, higher MP concentrations could have been expected here. That this was not the case can be explained by the analysis of generated FTIR data. For the second study an automated image analysis (Primpke et al. 2019) was applied which circumvents human bias and automatically compares spectra against a standardized database of common polymer types (Primpke et al. 2018). In this way rare polymer types and very small MP are identified correctly, which are likely to be overseen when analysing FTIR data manually, as was done in **Chapter 4** (Primpke et al. 2017b). Until now, only a few studies have applied automated image analysis: Two of them in riverine sediments (Mani et al. 2019b, Pan 2021), and two in marine sediments and surface waters (Haave et al. 2019, Lorenz et al. 2019). This way several types

of rubbers have been identified in high abundances, which have not yet been reported in the environment in earlier studies (Haave et al. 2019, Mani et al. 2019b, Mintenig et al. 2020).

Both studies of this thesis were conducted strictly following QA/QC implementations. This can be supported by the scoring system of Koelmans et al. (2019) which is based on the developments in **Chapter 3**. The first study was scored with 13 from a maximum of 18 points (Koelmans et al. 2019). Improving on several points, the subsequent study (**Chapter 5**) scored 17 out of 18 points, thus producing even more reliable data.

After quantifying MP in all WWTP effluents examined in **Chapter 4** and **Chapter 5**, we focussed on the third research question in this section: *What is the WWTPs' relative contribution to the MP load already present in a river?*

Despite the continuous MP release we could not see a general increase of MP abundances in riverine surface water samples downstream the discharge points (Figure 5-2) as suggested by earlier studies (Estahbanati and Fahrenfeld 2016, Kay et al. 2018). We could thus not explain peaks in MP concentrations to WWTP discharge, but rather to urban areas in general (Kataoka et al. 2019, Mani et al. 2015). This might be caused by diffuse sources such as urban and road runoff (Hurley and Nizzetto 2018). Varying MP concentrations, however, also depend on other riverine dynamics and processes (Kooi et al. 2018). As such we formulated our last research question in this section as: *How does the riverine MP transport vary over time and over a river's length?*

As indicated, MP concentrations can vary considerably between urban and rural areas. To assess the MP load in a river requires enough sampling points that depict the diverse environmental situation and the processes taking place. If this is not considered, results merely constitute snapshots of the actual situation. In **Chapter 5** we tried to reduce this snapshot character to some extent by choosing sampling locations at a high spatial resolution, and by assessing temporal variations. In the small, yet diverse river Dommel we found that spatial differences induced a high variation of MP number concentrations, i.e. by two orders of magnitude (Figure 5-2B). In contrast, temporal differences caused MP number concentrations to vary less; by only one order of magnitude. This is similar to the findings of Watkins et al. (2019) and Hurley et al. (2018) focussing on changes due to flooding events. Examining more samples further improves our understanding of the microplastic diversity, in regard to concentrations and properties (Kooi and Koelmans 2019). This improved understanding is not only useful when designing sampling campaigns, but can also form the basis for transport models. Based on our findings, it is evident that models, e.g. exploring hotspots, need detailed spatial information of the river systems, while temporal variability in inputs and boundary conditions might be less essential.

7.2.3 NP removal during drinking water production from surface waters

After determining MP in riverine surface waters used for the production of drinking water, and assuming the presence of NP, this section follows up the urban water cycle and discusses the findings of **Chapter 6** with following research question: *Could NP, potentially present in surface waters, be retained by the commonly applied drinking water purification techniques?*

The removal efficiency for MP can be assessed in two ways, by (1) measuring MP at the inlet and outlet of a drinking water treatment plant (DWTP) or, (2) evaluating the removal rate by individual techniques in laboratory experiments (Novotna et al. 2019). Following the first approach, Pivokonsky et al. (2018) and Wang et al. (2020) demonstrated high removal rates for MP down to 1 µm for DWTPs. However, considerably less is known for the removal of NP. Due to high analytical challenges the first approach has not yet been applied to trace NP. Therefore we chose the second approach based on experimental work.

In **Chapter 6** we tested three purification techniques commonly applied on DWTPs, namely coagulation- flocculation- sedimentation (CFS), rapid sand filtration and granular activated carbon (GAC) filtration. Overall NP removal seems promising, but the efficiencies to retain NP varied strongly between the three techniques. Rapid sand filtration was least efficient in removing NP. CFS was especially successful in removing the bigger NP (200 nm), while GAC filtration more efficiently removed the smaller ones (50 nm) (Table 6-2). Results of previous studies suggest that the removal on an actual DWTP might be higher because subsequent techniques can benefit from the flocs formed during CFS (Troester et al. 2016) and because a biofilm on the sand grains can increase the entrapment of NP (Bertelkamp et al. 2018). Further, environmental NP might be retained better than the regular spheres used in our experiments due to formed hydroxyl and carboxylic acid groups (Lapointe et al. 2020) and irregular shapes (Pradel et al. 2020).

It can be concluded that common drinking water purification can partly remove NP present in surface water. Still, at least some of the NP present in surface waters will likely pass the DWTP. Beside an incomplete removal, the deterioration of plastic materials used during water purification or distribution could again contaminate the drinking water (Mintenig et al. 2019). To date, studies are investigating if and how the removal potential of DWTPs could be increased. Most promising should be advanced purification techniques, such as membrane filtration or reverse osmosis (Enfrin et al. 2019, Troester et al. 2016). By applying, for example, membrane filtration; > 99% of C_{60} fullerenes were removed when the membrane's pores was smaller or similar to the fullerenes' size (Floris et al. 2016). Also Chalew et al. (2013a) determined high, however not complete, removal rates for metallic nanoparticles.

All these results seem promising and form a good starting point. Most of them, however, remain restricted to experimental studies tracking one type of added NP (or of another material) throughout specific techniques. It has not yet been possible to detect what different types and in which concentration NP pass a DWTP. This might become possible rather quickly, after Mitrano et al. (2019) found a way to produce NP with a metal core that is used as a tracer.

7.3 Outlook

Focussing on the detection of MP in the urban water cycle, this thesis provided answers to some of the most pressing questions on the identification of MP in and around WWTPs. Despite that numbers of studies are rapidly increasing (Figure 7-1), important questions remain or have evolved more recently. In this section I will discuss *'What do we know, and what should we (not) examine more?'.* This question can be answered from various perspectives. Finally, I will provide suggestions for several fields of interest that are relevant within the frame of this thesis.

Monitoring Our knowledge is still too fragmentary to point out MP sources and pathways into and through the environment. Thus, more studies are needed to monitor these, to assess spatial and temporal trends and to estimate related risks. But where should a monitoring strategy focus on? The field studies in this thesis (**Chapter 4** and **Chapter 5**), and numerous other studies, have proven that number concentrations and polymer diversity are highest for small MP. This is also the size fraction considered most hazardous, which could imply that future studies should focus on examining those smaller group of MP, and NP. A growing number of studies, among others **Chapter 3**, have shown that insufficient QA/QC implementations considerably increase the uncertainty around generated data. In particular, this applies to the smaller plastics. For monitoring purposes I would thus argue that good data restrained to bigger MP sizes are more valuable than less reliable data on smaller plastics. Here, robust data are of main interest, while scientific studies should elaborate if and to what extent data on larger plastics can be used as a proxy for smaller ones. For the moment, this could be the solution for an effective monitoring strategy. Here, the interests are high in a simplified approach that is less expensive by requiring less handling and less advanced analytical equipment. One idea, namely assessing plastics purely based on mass concentrations, could fulfil these requirements, however, too much information would be missed. In contrast, laser-based microscopy is a new technique that might be more applicable for monitoring purposes as it provides required information (MP numbers, sizes, and polymer types) for small MP within a relatively short time. Acquisition costs are still high, avoiding such systems to become standard laboratory equipment. However, decreasing costs and achievements towards an easier sample preparation might simplify monitoring of smaller MP sizes in the future.

Standardization of analytical methodologies Assessing the distribution of MP in the environment would certainly benefit from standardized procedures. Ongoing standardization efforts, however, illustrate accompanying difficulties (Bessa et al. 2019, Koelmans et al. 2020, Rochman et al. 2017, Ryan et al. 2019) as individual procedures already lack comparability. Apart from this, aspects related to competition, network building and funding opportunities should not be neglected. In a competitive field with limited funding resources, each laboratory attempts to stand-out in some specific technique that they use to gain funding, publish results, buy equipment, or build a professional network to support this competitive advantage. These dynamics cause standardization to be a rather slow process. However, promising developments were made, and it can be expected that the most efficient ones, eventually, will establish themselves. For example, Primpke et al. (2018) compiled a reference database of various polymeric and natural materials which, nowadays, is used widely to analyse FTIR data and which considerably increases the comparability of study results.

Next to asking if standardization is realistic to be achieved in the near future, it is further arguable if and how much it is wanted at this point in time in this still developing field. In the long term best practices will be defined, either as a result of a pure scientific evolution, or accelerated by legislatory bodies. But for now Rist et al. (2020) asked pointedly 'How much should we harmonize, and, how much freedom do we need?'. This second question should not be neglected, we should guarantee flexibility to incorporate new developments. These analytical advancements are of scientific value, and also drive scientific advancements. They are ongoing and because of this, we are able to identify ever smaller MP or a higher number of polymer types, for example tyre abrasives. Analytical developments are further urgently needed to tackle the, so far, biggest remaining gap of knowledge: nanoplastics.

Nanoplastics This smallest size fraction is studied the least, resulting in high uncertainties when assessing NP concentrations and fate. Analytical techniques are only evolving slowly. While extracting and analysing MP in environmental samples is already challenging, this seems rather easy compared to the challenges related to NP. Still, similar requirements have to fulfilled. At best an analysis should provide information on the polymer types, and on NP size estimates. A combination of Pyrolysis GC-MS and NP size fractionation, like the AF4 used in **Chapter 2**, or the usage of Nano-FTIR (Meyns et al. 2019) seem most promising. These methodologies still need to be developed further, until then, controlled laboratory studies could already benefit from the usage of metal doped NP, enabling, for example, studying the fate or bioaccumulation of NP (Mitrano et al. 2019, Redondo Hasselerharm 2020).

Data alignment Arguing that methodological advancements are needed, and that agreeing on standardized methods is a slow process we, in the meantime, need to find ways to increase the comparability of results from different studies. Koelmans et al. (2020) proposed a rescaling method by which MP number concentrations within any size range can be translated into MP concentrations within a default size range, for instance 1- 5000 µm. Such workarounds are needed to use data which are already generated, and to align effect and

exposure data when assessing the risks of (micro)plastics in the environment. This task is seen as one of today's major challenges (SAPEA 2019) and has gained broad attention, for example, also through the initiative of the European Chemicals Agency (ECHA 2019).

One aim of this thesis was thus to provide accurate exposure data on MP types and number concentrations in Dutch riverine surface waters. In **Chapter 5** we concluded that MP number concentrations from three locations only exceeded the predicted no effect concentration (PNEC) from Everaert et al. (2018), but were still considerably lower than the PNECs from three other studies (Figure 5-4). Here, we aligned our data to represent the size range from 1- 5000 µm following the approach by Koelmans et al. (2020). Further, we used their likewise aligned effect data revealing a hazardous MP threshold concentration for 5% of the species (HC_5) of 75.6 MP L^{-1} (with a 95% confidence interval of 11- 521 MP L^{-1}). The majority of sampled locations revealed MP number concentrations considerably below this HC_5 concentration. However, it was approximately met at one location, and MP number concentrations of several locations are within or close when considering the 95% confidence interval (Figure 7-2). Following the data alignment and considering this HC_5 threshold concentration protects 95% of the species, earlier drawn conclusions are still valid. However, exposure and effect threshold concentrations are now closer to each other. This trend could increase as a high plastic production, the plastics' persistance and continous fragmentation could imply that future MP concentrations will be higher than the ones currently measured (Everaert et al. 2018, Koelmans et al. 2017a).

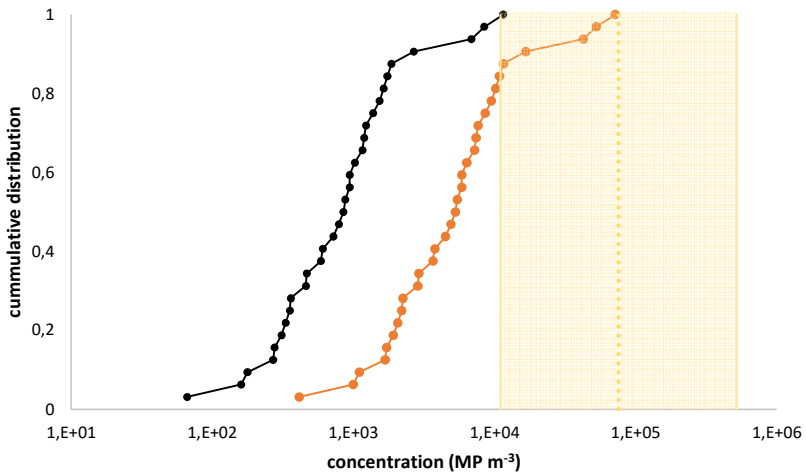

Figure 7-2 | Cumulative frequency distribution of MP number concentrations that were identified in riverine surface waters in Chapter 5 (20- 5000 µm, black line) and that were subsequently rescaled to a size range of 1- 5000 µm following the approach by Koelmans et al. (2020) (orange line). Based on likewise aligned effect data, that also consider the bioavailability of MP, a HC_5 threshold concentration of 75.6 MP L^{-1} (yellow dotted line), with an indicated 95% confidence interval of 11- 521 MP L^{-1}, could be expected.

Mitigation strategies Recalling again the opening question of this section, we know already that WWTPs efficiently remove MP from the incoming sewage water. So we rather not need future studies generally stating that large shares of MP are retained on a WWTP after examining the water at the plant's inlet and outlet. Instead, specific insights are needed into individual purification techniques, and especially into the retention of small MP and NP. The agricultural usage of sludge, as a side note, might thus also need some reconsiderations. As previously discussed, the methodologies to detect NP in environmental samples are yet to be developed further and applied. Until then, at least experimental studies could benefit greatly from the usage of metal doped NP. For example, such NP could be used to repeat the work from Chapter 6, preferably on a full-scale DWTP, and with more environmentally realistic NP concentrations.

Increasing the potential of specific techniques to remove MP and NP is of high interest for WWTPs, as well as for DWTPs. The consumption of drinking water is, among others, seen as a pathway for the human exposure to MP and NP (Wright and Kelly 2017). In (bottled) drinking water the contamination with MP was partly traced back to the abrasion of plastic materials used during production, transportation or packaging. The formation of NP is thus also likely. Although that does not change the exposure route, it has to be considered that it might not be the drinking water itself that poses the highest contamination risk. This implies that using a plastic bottle might revert the achievements of a DWTP in removing MP and NP. In case the biggest source of pollution can actually be related directly to our daily usage of plastic materials, water companies and engineers, as well as citizens themselves are responsible to reduce the human exposure to plastics when drinking water. Monitoring plastics in the environment and implementing mitigation strategies will be a task for authorities, e.g. the water managers, but also for plastic producers and citizens.

SUMMARY

SUMMARY

Plastic is the common name given to a wide range of synthetic or semi-synthetic organic polymers. These polymers are lightweight, durable, and cheap which explains a global production of 359 million tonnes in 2018 (PlasticsEurope 2019). However, due to several reasons plastic items can end up in the environment where its durability eventually can cause ecological, and socio-economic harm (Galgani et al. 2013, SAPEA 2019). Special attention by science, media and policy, has been given to plastic items smaller than 5 mm, so called microplastics (MP) which, to date, can be found in almost all natural habitats (Hurley et al. 2018). It has been estimated that about 80% of the environmental plastic is released by terrestrial sources (Andrady 2011, Rochman 2018), and that riverine transport plays an important role in distributing the plastic. Over the last years, numbers of studies on (micro)plastics have increased exponentially. Still, our knowledge on occurrences and types of MP in the freshwater environment remains fragmentary. Due to the even higher analytical challenges it is yet to be determined if, how and where even smaller plastics, so called nanoplastics (NP), occur and behave in the environment.

This thesis focussed on assessing the presence of MP in the urban water cycle which describes how humans get, use and re-use freshwater. Accurate data on MP types and concentrations are needed to assess their related risks, to trace back their emission sources, and to mitigate these sources. For this thesis three fields of interest were defined. After a general introduction (**Chapter 1**), we (i) addressed analytical requirements to accurately identify MP and NP (**Chapter 2** and **Chapter 3)**. Applying herein defined criteria, (ii) **Chapter 4** and **Chapter 5** contain the results of two field studies examining MP in and around the effluents of WWTPs. And (iii) in **Chapter 6** the removal of NP during drinking water purification was assessed experimentally. Finally, **Chapter 7** provides answers to the posed research questions, and ends with an outlook section discussing which research topics should (not) be studied in the future. This was done based on the findings of previous chapters, of which main conclusions are summarized below.

In **Chapter 2**, we presented a framework able to consistently determine a broad spectrum of plastic particle sizes in aqueous environmental samples. To accurately depict the environmental situation and to adequately assess its risks, it is, independently of actual targeted plastic sizes, required to conduct (i) an appropriate sampling, and a subsequent identification of individual MP (ii) sizes and (iii) polymer types. For MP down to about 20 µm we followed the most common approach that combines conventional filtration and chemical mapping using FTIR microscopy. We further showed how this could be extended for NP using crossflow ultrafiltration, followed by asymmetrical flow field flow fractionation and pyrolysis-GC-MS analysis. The latter seems most promising to detect NP in environmental samples. Together with pre-concentrating NP with crossflow ultrafiltration the identification of polystyrene with an original concentration > 20 µg L^{-1} was enabled. Lastly, an approach to

estimate polymer masses based on the two-dimensional MP shapes recorded during chemical mapping was provided. Thereby this suite of techniques demonstrated being feasible to analyse the entire size spectrum of small plastic debris.

Chapter 3 was motivated by MP ingestion studies which portray a wide spread assessment of ingestion incidences. These varying results might be attributed to a lack of standardisation of methods. In this chapter we critically reviewed and evaluated studies assessing the ingestion of MP by marine biota, we then proposed a quality assessment method for such studies and applied this method retrospectively to the reviewed studies. The quality assessment method was based on scoring ten quality criteria. On average, studies scored only 7.8 out of 20 points', revealing a dire need for stricter quality assurance in MP ingestion studies. Alongside the assessment method, a standardised protocol incorporating these criteria was provided.

This work was received well and caused follow-up work in which this scoring system was extended to aqueous samples relevant for the production of drinking water (Koelmans et al. 2019), to atmospheric samples (Wright et al. 2021) and to toxicological studies (de Ruijter et al. 2020).

Implementing previous findings, Chapter 4, was the first study applying an enzymatic-oxidative purification approach in combination with FPA-based micro-FTIR imaging to identify MP down to a size of 20 µm in the effluents of 12 waste water treatment plants (WWTPs). In all effluents MP was found with quantities ranging from 0 to 5×10^{1} m^{-3} MP > 500 µm, and 1×10^{1} to 9×10^{3} m^{-3} MP < 500 µm. By far, polyethylene was the most frequent polymer type in both size classes. Quantities of synthetic fibres ranged from 9×10^{1} to 1×10^{3} m^{-3} and were predominantly made of polyester. Considering the annual effluxes of tested WWTPs, total discharges of 9×10^{7} to 4×10^{9} MP particles and fibres per WWTP could be expected. Interestingly, one tertiary WWTP had an additionally installed post-filtration that reduced the total MP discharge by 97%. Further, the sewage sludge of six WWTPs was examined proofing the presence of MP. Our findings suggested that WWTPs could be a sink, but also a source of MP and should thus be considered playing an important role for the environmental MP pollution.

Then, in Chapter 5, we pursued this work focussing on two Dutch river systems. Following strict QA/QC implementations, we firstly, determined the WWTPs' MP release relative to MP amounts and types present in riverine surface waters and, secondly, assessed how spatial and temporal variations altered MP occurrences. For the first time, FTIR microscopy and an automated image analysis were applied to study MP in riverine surface waters. We identified MP number concentrations between 67 and 1×10^{4} MP m^{-3}, which were comprised of 26 different polymer types. Polyethylene (23%), polypropylene (19.7%) and ethylene propylene diene monomer rubber (18.3%) were the most common ones. The highest diversity of polymer types was found for small MP, whereas MP larger than 1 mm was scarce and almost exclusively made of polyethylene or polypropylene. Determined MP number concentrations

were affected stronger by spatial, than by temporal differences. Further, virtually all sampling locations revealed MP number concentrations that were considerably below known effect thresholds for anticipated adverse ecological effects.

Based on these environmental samples we assessed how accurately MP numbers and polymer types are represented during partial filter analysis. We found that an acceptable level of uncertainty was only achieved when analysing 50% of a filter during chemical mapping, and when identifying at least a subset of 50 individual particles with attenuated total reflection (ATR)-FTIR.

In **Chapter 6** we assessed if NP present in surface waters could be removed by current drinking water purification techniques. This was done simulating coagulation- flocculation-sedimentation (CFS), rapid sand filtration and granular activated carbon (GAC) filtration in individual bench-scale experiments. NP varying in size, 50 and 200 nm, and surface charge were removed partly by all tested techniques. Rapid sand filtration revealed the lowest removal rates. CFS was most efficient for bigger nanoplastics (200 nm), while smaller nanoplastics (50 nm) were removed better by GAC filtration. Total removal rates were found to be considerable. Drinking water treatment plants in the Netherlands consist of a set of different treatment processes, it could thus be expected that a large part of NP present in surface waters will be removed. However, it cannot be excluded that some of these plastics will remain in the drinking water. More research is thus needed to specify removal rates, and to improve removal rates to reduce the human exposure to nanoplastics via drinking water.

Finally, **Chapter 7** provides a summary and synthesis of all previous chapters. Three fields of interest were defined for this thesis. Answers to the therein posed research questions are provided based on the findings of earlier chapters and of recent literature. At first, analytical requirements to analyse MP and NP are discussed, followed by the results of two field studies examining MP in and around the effluents of WWTPs, and experimental results on NP removal during drinking water purification. Finally, recommendations are provided on research topics that should (not) receive future attention.

SAMENVATTING

Plastic is de algemene naam voor een breed scala van synthetische en semi-synthetische organische polymeren. Deze polymeren zijn licht, duurzaam en goedkoop, wat een wereldwijde productie verklaart van 359 miljoen ton in 2018 (PlasticsEurope 2019). Via verschillende routes kan plastic in het milieu terechtkomen en, uiteindelijk, ecologische en sociaaleconomische schade veroorzaken (Galgani et al.2013, SAPEA 2019). Wetenschap, media en beleid hebben veel aandacht besteed aan plastic voorwerpen kleiner dan 5 mm, zogenaamde microplastics (MP) die tot op heden in bijna alle natuurlijke habitatten voorkomen (Hurley et al. 2018). Geschat wordt dat ongeveer 80% van deze plasticdeeltjes via terrestrische bronnen in het milieu terecht komen (Andrady 2011, Rochman 2018), en dat riviertransport een belangrijke rol speelt bij de distributie van het plastic. Het aantal onderzoeken naar (micro)plastics is de laatste jaren exponentieel toegenomen. Toch blijft onze kennis over hoeveelheden en soorten MP in het zoetwatermilieu fragmentarisch. Ook liggen er nog analytische uitdagingen om de zogeheten nanoplastics (NP) te kunnen bepalen.

Dit proefschrift was gericht op het beoordelen van de aanwezigheid van MP in de urbane watercyclus. Deze cyclus beschrijft hoe mensen zoetwater aangevoerd krijgen, hoe ze zoetwater gebruiken en uiteindelijk hergebruiken. Nauwkeurige gegevens over MP typen en concentraties zijn nodig om hun risico's te beoordelen, te traceren waar ze vandaan komen en natuurlijk om deze bronnen te beperken. Voor dit proefschrift zijn drie interessegebieden gedefinieerd. Na een algemene inleiding (**Hoofdstuk 1**), bespreken wij (i) de analytische vereisten die nodig zijn om MP (**Hoofdstuk 2**) en NP (**Hoofdstuk 3**) te identificeren. De hierin gedefinieerde criteria zijn toegepast in (ii) **Hoofdstuk 4** en **Hoofdstuk 5** waar de resultaten van twee veldstudies de aanwezigheid van MP in en rond het effluent van rioolwaterzuiveringsinstallaties (RWZI's) laten zien. In (iii) **Hoofdstuk 6** is de verwijdering van NP tijdens drinkwaterzuivering experimenteel onderzocht. **Hoofdstuk 7** geeft tenslotte antwoorden op de gestelde onderzoeksvragen en eindigt met een verkenningsparagraaf waarin wordt besproken welke onderzoeksthema's in de toekomst (niet) bestudeerd dienen te worden. Dit is gedaan op basis van de bevindingen uit eerdere hoofdstukken, waarvan de belangrijkste conclusies hieronder worden samengevat.

In **Hoofdstuk 2** presenteerden wij een raamwerk dat in staat is plasticdeeltjes van verschillende groottes in waterige milieumonsters te bepalen. Om de milieusituatie nauwkeurig weer te geven en de risico's ervan adequaat in te schatten is het, onafhankelijk van de deeltjes grootte, vereist om (i) een passende steekproef uit te voeren, (ii) de grootte en (iii) het polymeertype van de MP te bepalen. Voor MP tot 20 µm gebruikten wij een conventionele filtratie combineert met FTIR-microscopie. Wij lieten verder zien hoe dit kon worden uitgebreid naar NP met behulp van een crossflow-ultrafiltratie techniek voor de bemonstering, gevolgd door asymmetrische flow-veldstroomfractionering (AF4) en pyrolyse-

GC-MS-analyse. Pyrolyse GC-MS lijkt het meest veelbelovend om NP in milieumonsters te detecteren. Samen met die crossflow-ultrafiltratie, die er voor zorgt dat NP wordt voorgeconcentreerd, werd de identificatie van polystyreen met een oorspronkelijke concentratie > 20 µg L^{-1} mogelijk. Ten slotte werd een benadering gepresenteerd om polymeermassa's te kunnen schatten op basis van de tweedimensionale MP vormen, detecteert tijdens de analyse met FTIR. Deze technieken samen zijn in staat om het hele spectrum, van nano- tot microplastic, te detecteren.

MP ingestieonderzoeken, studies die de opname van MP door maritieme dieren bestuderen, laten een breed spectrum aan resultaten zien. Die wijd uiteenlopende resultaten kunnen gedeeltelijk worden verklaard door een gebrek aan standaardisatie van meetmethoden. Dit was de motivatie voor **hoofdstuk 3.** Hier hebben wij deze ingestieonderzoeken kritisch bekeken en geëvalueerd. Vervolgens hebben wij een kwaliteits-beoordelingsmethode opgesteld en deze methode retrospectief toegepast op de meegenomen studies. De kwaliteitsbeoordelingsmethode was gebaseerd op het scoren van tien kwaliteitscriteria. Gemiddeld scoorden onderzoeken 7,8 van de 20 punten, wat de behoefte aan strengere kwaliteitsborging bij MP innameonderzoeken benadrukt. Naast de beoordelingsmethode is een gestandaardiseerd protocol opgesteld waarin deze criteria zijn opgenomen. Dit werk is goed ontvangen en leidde tot vervolgwerk waarbij het scoresysteem werd uitgebreid tot (i) watermonsters die relevant zijn voor de productie van drinkwater (Koelmans et al. 2019), (ii) tot atmosferische monsters (Wright et al. 2021) en (iii) tot toxicologische studies (de Ruijter et al. 2020).

Hoofdstuk 4 implementeert alle bovengenoemde bevindingen. Verder was het de eerste studie waarbij een enzymatisch-oxidatieve zuiveringsbenadering werd toegepast in combinatie met FPA-gebaseerde micro-FTIR-analyse. Deze combinatie van methodes werd gebruikt om MP tot een grootte van 20 µm te identificeren in het effluent van 12 RWZI's. In alle effluenten werd MP gevonden met hoeveelheden variërend van 0 tot 5×10^{1} m^{-3} MP > 500 µm, en 1×10^{1} tot 9×10^{3} m^{-3} MP < 500 µm. Polyethyleen was het meest voorkomende polymeertype in beide afmetingsklassen. De hoeveelheden synthetische vezels varieerden van 9×10^{1} tot 1×10^{3} m^{-3} en waren voornamelijk gemaakt van polyester. Gezien de jaarlijkse effluent van de geteste RWZI's, kunnen totale lozingen van 9×10^{7} tot 4×10^{9} MP deeltjes en vezels per RWZI en jaar worden verwacht. Eén RWZI had een extra geïnstalleerde tertiaire postfiltratie die de totale afvoer van MP met 97% verminderde. Verder werd het zuiveringsslib van zes RWZI's onderzocht om ook hier de aanwezigheid van MP te bekijken. Onze bevindingen suggereerden dat RWZI's een reservoir, maar ook een bron voor MP kunnen zijn. RWZI's spelen hoe dan ook een belangrijke rol bij de milieuverontreiniging met en de verdeling van MP MP.

Vervolgens, in **hoofdstuk 5**, hebben wij dit werk voortgezet door te concentreren op twee Nederlandse rivieren: de Maas en de Dommel. Door de kwaliteitscriteria uit hoofdstuk 3 te volgen hebben we, ten eerste, bepaald welke MP hoeveelheden en typen worden geloosd bij verschillende RWZI's in de rivieren. Ten tweede hebben wij beoordeeld hoe ruimtelijke en temporele variaties MP concentraties veranderden. Voor het eerst, werd in deze studie FTIR-microscopie en een geautomatiseerde beeldanalyse toegepast om MP in oppervlaktewater van rivieren te bestuderen. Op deze manier hebben wij MP concentraties tussen 67 en 1 × 10^4 MP m^{-3} kunnen bepalen. Ook hebben we 26 verschillende polymeertypes kunnen identificeren: polyethyleen (23%), polypropyleen (19,7%) en ethyleen-propyleen-dieen-monomeerrubber (18,3%) waren de meest voorkomende polymeertypes. De grootste diversiteit aan polymeertypes werd vooral gevonden in de kleinste grootteklasse MP. MP groter dan 1 mm waren schaars en vrijwel uitsluitend gemaakt van polyethyleen of polypropyleen. De MP concentraties werden sterker beïnvloed door ruimtelijke verschillen dan door temporele verschillen. Verder lieten vrijwel alle bemonsteringslocaties MP concentraties zien die aanzienlijk lager waren dan de drempelwaardes waarbij nadelige ecologische effecten worden verwacht.
Op basis van die hier genereerde data konden wij verder beoordelen hoe nauwkeurig de MP aantallen en MP polymeertypes kunnen worden bepaald met een partiële filteranalyse. Een acceptabele kwaliteit werd alleen bereikt wanneer de micro-FTIR analyses ten minste 50% van de filter meeneemt. En bij de ATR-FTIR analysetechniek moeten ten minste 50 individuele deeltjes worden geanalyseerd.

In **Hoofdstuk 6** hebben wij onderzocht of NP verwijderd zou kunnen worden uit oppervlaktewater met de huidige drinkwaterzuiveringstechnieken. Dit werd gedaan door de volgende technieken experimenteel te simuleren: (i) coagulatie-flocculatie-sedimentatie (CFS), (ii) snelle zandfiltratie en (ii) granulaire actieve kool (GAC) filtratie. NP variërend in grootte, 50 en 200 nm, en variërend in oppervlaktelading werden gedeeltelijk verwijderd door alle geteste technieken. Snelle zandfiltratie was het minst effectief in het verwijderen van plasticdeeltjes. CFS was het meest efficiënt voor de grotere NP (200 nm), terwijl kleinere NP (50 nm) beter werden verwijderd door GAC-filtratie. Het totale verwijderingspercentage was aanzienlijk. Drinkwaterzuiveringsinstallaties in Nederland bevatten verschillende zuiveringsprocessen, het is dus te verwachten dat een groot deel van het NP aanwezig in oppervlaktewater zal worden verwijderd tijdens de productie van drinkwater. Het kan echter niet worden uitgesloten dat een deel van deze plastic deeltjes in het drinkwater achterblijft. Meer onderzoek is dus nodig om verwijderingspercentages duidelijker te maken en deze te verbeteren om de menselijke blootstelling aan NP zo laag mogelijk te houden.

Hoofdstuk 7 geeft ten slotte een samenvatting en synthese van alle voorgaande hoofdstukken. Voor dit proefschrift zijn drie interessegebieden gedefinieerd. Antwoorden op de daarin gestelde onderzoeksvragen worden gegeven op basis van de bevindingen uit eerdere hoofdstukken en recente literatuur. Ten eerste worden de analytische vereisten voor het analyseren van MP en NP besproken, ten tweede worden de resultaten van twee veldstudies weergegeven waarin MP in en rond het effluent van RWZI's zijn onderzocht, en ten derde worden de experimentele resultaten over de verwijdering van NP tijdens drinkwaterzuivering besproken. Ten slotte worden aanbevelingen gedaan over onderzoeksthema's die in de toekomst (geen) aandacht zouden moeten krijgen.

ZUSAMMENFASSUNG

Plastik ist die gebräuchliche Bezeichnung für eine Vielzahl von synthetischen oder halbsynthetischen, organischen Polymeren. Diese Polymere sind leicht, langlebig und billig, was die weltweite Produktion von 359 Millionen Tonnen im Jahr 2018 erklärt (PlasticsEurope 2019). Plastik kann aus verschiedenen Gründen in die Umwelt gelangen und dort ökologische und sozioökonomische Schäden verursachen (Galgani et al. 2013, SAPEA 2019). Große Aufmerksamkeit, sowohl in Wissenschaft, als auch in Medien und Politik, haben Plastikteilchen mit einer Größe von weniger als 5 mm, sogenanntes Mikroplastik (MP), erlangt. Diese Teilchen können heutzutage in fast allen natürlichen Lebensräumen nachgewiesen werden (Hurley et al. 2018). Es wird geschätzt, dass etwa 80% dieser Plastikpartikel über terrestrische Quellen in die Umwelt gelangt sind (Andrady 2011, Rochman 2018), und dass Flüsse eine wichtige Rolle bei ihrer Verteilung spielen. In den letzten Jahren hat die Zahl der Studien über (Mikro) Plastik exponentiell zugenommen. Dennoch ist unser Wissen über deren Mengen und Polymertypen in verschiedenen Süßwassergebieten fragmentarisch. Aufgrund der großen analytischen Herausforderungen stehen die Untersuchungen ob, wie und wo noch kleineres Nanoplastik (NP) auftritt, noch aus.

Ziel dieser Arbeit war es, MP im urbanen Wasserkreislauf zu charakterisieren und mehr über das Vorhandensein von NP zu lernen. Dieser Kreislauf beschreibt, wie Menschen Süßwasser erhalten, nutzen und wiederverwenden. Hier sind detaillierte Informationen zu MP Konzentrationen und den einzelnen Polymertypen erforderlich, um die damit verbundenen Risiken einschätzen zu können, sowie ihre Emissionsquellen verfolgen und reduzieren zu können. Für diese Arbeit wurden drei Interessensgebiete definiert: Nach einer allgemeinen Einführung (**Kapitel 1**) werden (i) die analytischen Anforderungen diskutiert, die zur genauen Identifizierung von MP und NP (**Kapitel 2** und **Kapitel 3**) erforderlich sind. Unter Anwendung der hier definierten Kriterien werden (ii) in **Kapitel 4** und **Kapitel 5** Ergebnisse zweier Feldstudien wiedergeben, in welchen MP in verschiedenen Klärwerksausflüssen sowie in deren Umgebung nachgewiesen wurde. In **Kapitel 6** wurde (iii) abschließend die Entfernung von NP während der Trinkwasseraufbereitung experimentell untersucht. **Kapitel 7** beinhaltet Antworten auf die gestellten Forschungsfragen und endet mit einer Diskussion welche Forschungsthemen in Zukunft (nicht) weiter fokussiert werden sollten. Dafür wurden die Ergebnisse der einzelnen Kapitel berücksichtig, deren wichtigste Schlussfolgerungen nachstehend noch einmal zusammengefasst sind.

In **Kapitel 2** haben wir ein analytisches Protokoll vorgestellt, welches die Analyse eines breiten Größenspektrums an Plastikpartikeln in wässrigen Umweltproben ermöglicht. Um die Umweltsituation genau darstellen und Risiken angemessen bewerten zu können, ist es dabei erforderlich (i) eine geeignete Probemenge zu untersuchen sowie (ii) die Größe und (iii) den Polymertyp der einzelnen MP zu bestimmen. Für MP bis zu einer Größe von etwa 20 µm

wurde die Beprobung mittels konventioneller Filtrationstechniken mit der Analyse mittels FTIR-Mikroskopie kombiniert. Im Weiteren konnten wir aufzeigen, dass eine Beprobung mittels Cross-Flow- Ultrafiltration, kombiniert mit asymmetrischer Feldflußfraktionierung (AF4) und anschließender Pyrolyse GC-MS Analyse NP erfolgreich nachweisen kann. Der Einsatz von Pyrolyse GC-MS scheint für den Nachweis von NP in Umweltproben am vielversprechendsten zu sein. Zusammen mit der Beprobung mittels Cross-Flow-Ultrafiltration, bei der NP vorkonzentriert wird, ist der Nachweis von Polystyrol mit einer Ausgangskonzentration von > 20 µg L^{-1} möglich. Abschließend war es notwendig einen Ansatz zu finden, um die ermittelten Teilchenkonzentrationen der FTIR- Analyse mit den Massenkonzentrationen der Pyrolyse GC-MS vergleichen zu können. Die Analyse mittels FTIR- Mikroskopie liefert Angaben über die zweidimensionalen MP Formen, welche genutzt wurden, um die Polymermassen abschätzen zu können. Die Kombination vorgestellter Techniken hat sich somit als erfolgreich erwiesen, um ein breites Größenspektrum an Plastikpartikeln in wässrigen Umweltproben nachweisen zu können.

Die Ergebnisse von Studien, welche die MP Aufnahme durch marine Organismen untersuchen und bestimmen, variieren erheblich. Diese unterschiedlichen Ergebnisse könnten jedoch auch auf eine mangelnde Standardisierung der Methoden zurückgeführt werden. Aus diesem Grund haben wir, in **Kapitel 3**, diese Studien zunächst kritisch bewertet und anschließend eine Methode entwickelt, um die Qualität der jeweils angewendeten Methodik objektiv beurteilen zu können. Dies ist möglich anhand der Bewertung von zehn ausführlich beschriebenen Qualitätskriterien. Im Durchschnitt erzielten Studien nur 7,8 von 20 Punkten, was einen großen Bedarf an einer strengeren Qualitätssicherung in MP Aufnahmestudien zeigt. Zusätzlich zu dieser Bewertungsmethode haben wir ein standardisiertes Protokoll bereitgestellt, in welchem diese Kriterien berücksichtigt sind und welches daher einen guten Ausgangspunkt für zukünftigen Arbeiten bietet.

Diese Arbeit wurde sehr positiv aufgenommen und führte zu Folgearbeiten, die das Bewertungssystem ausgeweitet und für Wasserproben, die für die Trinkwasserproduktion relevant sind (Koelmans et al. 2019), für atmosphärische Proben (Wright et al. 2021) und für toxikologische Studien (de Ruijter et al. al. 2020) angepasst haben.

Kapitel 4 setzt die Erkenntnisse der ersten beiden Studien konsequent um. Auch wurde in dieser Studie zum ersten Mal eine enzymatisch-oxidative Probenaufbereitung in Kombination mit einer FPA-basierten Mikro-FTIR-Analyse angewendet. Somit konnte MP bis zu einer Größe von 20 µm im geklärten Abwasser von 12 Kläranlagen identifiziert werden. In allen Abwasserproben konnte MP nachgewiesen werden. Für MP > 500 µm variierten Konzentrationen zwischen 0 bis 5×10^{1} MP m^{-3} und für MP < 500 µm zwischen 1×10^{1} bis 9×10^{3} MP m^{-3}. Polyethylen war der am häufigsten gefundene Polymertyp in beiden Größenklassen. Die Mengen an synthetischen Fasern lagen zwischen 9×10^{1} bis 1×10^{3} m^{-3} und waren hauptsächlich aus Polyester. Angesichts der jährlichen Abwassermengen der getesteten Kläranlagen können die Freisetzung von 9×10^{7} bis zu 4×10^{9} MP Partikeln und

Fasern pro Kläranlage und Jahr erwarten werden. In der Kläranlage Oldenburg war eine zusätzliche tertiäre Filtrationsanlage installiert, diese konnte die totale Menge an MP um 97% reduzieren. Darüber hinaus wurde der Klärschlamm von sechs Kläranlagen untersucht, und auch hier konnte MP nachgewiesen werden. Unsere Ergebnisse legen daher nahe, dass Kläranlagen sowohl eine Senke aber auch eine Quelle für MP sein können. Sie spielen daher eine wichtige Rolle bei der Freisetzung und Verteilung von MP in der Umwelt.

Diese Arbeit wurde in **Kapitel 5** fortgesetzt wobei wir uns auf zwei niederländische Flüsse, Maas und Dommel, konzentriert haben. Den strengen Qualitätskriterien aus Kapitel 3 folgend, haben wir untersucht, welche MP Konzentrationen und Polymertypen durch Kläranlagen freigesetzt wurden und welche bereits im Oberflächenwasser der beiden Flüsse vorhanden waren. In dieser Studie wurde zum ersten Mal FTIR-Mikroskopie mit einer automatisierten Bildanalyse kombiniert, um MP im Süßwasser Bereich nachweisen zu können. Auf diese Weise konnten zwischen 67 und 1×10^4 MP m^{-3} und insgesamt 26 verschiedene Polymertypen identifiziert werden. Polyethylen (23,0%), Polypropylen (19,7%) und Ethylen-Propylen-Dien-Monomer- Kautschuk (18,3%) wurden am häufigsten detektiert. Die größte Vielfalt an Polymertypen wurde für die kleinste MP Fraktion gefunden. MP größer als 1 mm wurde dagegen nur selten nachgewiesen, und war fast ausschließlich aus Polyethylen oder Polypropylen hergestellt. Im Oberflächenwasser von Flüssen beeinflussen räumliche Gegebenheiten die bestimmten MP Konzentrationen deutlich stärker als zeitliche Variationen. Darüber hinaus lagen die MP Konzentrationen von fast allen untersuchten Proben deutlich unter den bisher angenommenen Schwellenwerten, ab welchen mit nachteiligen ökologischen Auswirkungen gerechnet werden kann.

Die hier generierten Daten wurden im Weiteren dazu genutzt, um die Menge an MP zu bestimmen, welche untersucht werden muss, um Konzentrationen und Polymertypen akkurat abschätzen zu können. Bei der MP Analyse mittels FTIR- Mikroskopie ist es gängige Praxis nur einen Teil des Filters zu messen, und auch bei vorsortierten, größeren MP Partikeln wird häufig nur eine Unterprobe mittels ATR-FTIR identifiziert. Ein akzeptables Maß an Unsicherheit kann jedoch nur dann erreicht werden, wenn 50% oder mehr eines Filters, und wenn mindestens 50 einzelne Partikel analysiert werden.

Es wird angenommen, dass neben MP auch NP in Oberflächengewässern vorhanden sind. In **Kapitel 6** haben wir untersucht, ob NP während der Trinkwasseraufbereitung entfernt werden kann. Dafür wurden drei gängige Techniken, Koagulation- Flokkulation- Sedimentation (KFS), schnelle Sandfiltration und Aktivkohlefiltration, in Experimenten simuliert. NP unterschiedlicher Größe (50 und 200 nm) und unterschiedlicher Oberflächenladung wurden durch alle drei Techniken zumindest teilweise entfernt. Die Aufbereitung mittels schneller Sandfiltration ergab die niedrigsten Entfernungsraten. Größeres NP (200 nm) wurde am effektivsten durch KFS, und kleineres NP (50 nm) durch Aktivkohlefiltration zurückgehalten. In den Niederlanden sowie zahlreichen anderen Ländern werden verschiedene Aufbereitungsverfahren kombiniert, um Trinkwasser aufbereiten zu können. Aus diesem

Grund kann erwartet werden, dass ein großer Teil des im Oberflächenwasser vorhandenen NP während der Trinkwassererzeugung entfernt wird. Es kann jedoch nicht ausgeschlossen werden, dass einige dieser Plastikteilchen im Trinkwasser verbleiben. Weitere Untersuchungen sind daher notwendig um Entfernungsraten spezifizieren und verbessern zu können.

Kapitel 7 ist eine abschließende Zusammenfassung und Synthese aller vorherigen Kapitel. Für diese Arbeit wurden drei Interessensgebiete definiert. Basierend auf den Kapiteln dieser Arbeit und den Ergebnissen neuerer Literatur beinhaltet das letzte Kapitel Antworten auf die einzelnen Forschungsfragen. So werden zunächst (i) die analytischen Anforderungen für die Analyse von MP und NP diskutiert, gefolgt von den (ii) Ergebnissen zweier Feldstudien welche MP in und um Kläranlagen untersucht haben sowie einer (iii) experimentelle Studie zur Entfernung von NP während der Trinkwasseraufbereitung. Das Kapitel endet mit Empfehlungen zu Forschungsthemen, die in Zukunft (keine) Aufmerksamkeit erhalten sollten.

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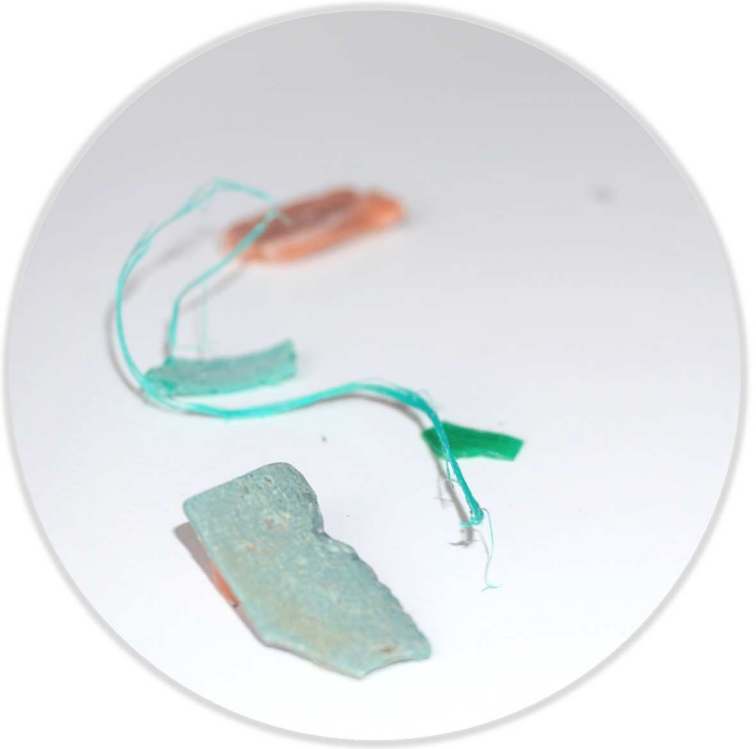

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CURRICULUM VITAE

SCIENTIFIC PUBLICATIONS

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CURRICULUM VITAE

Svenja Mintenig was born on the 20th of January 1988 in Neuwied, Germany. She grew up in Mendig where she also went to primary school. At secondary school she chose biology as a major subject. Enjoying the course of Limnology the most she decided to deepen this interest. After graduating from secondary school she thus moved to Oldenburg. Here she attended University and finished the bachelor in Environmental Science, and the master in Marine Environmental Science. For her master thesis she moved to Helgoland where she worked on a method to isolate microplastics from marine surface water samples.

In 2015 she moved to Utrecht where she started her PhD at the Copernicus Institute of Sustainable Development within the project 'Technologies for the Risk Assessment of Microplastics' which was funded by the Dutch Technology Foundation NWO-TTW. Specialised on the analysis of microplastic she was working towards this goal with two other PhDs involved, Merel Kooi and Paula Redondo Hasselerharm. After her PhD research she started working at the Aquatic Ecology and Water Quality Management Group (AEW) at Wageningen University where she continues working in the field of microplastic research.

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