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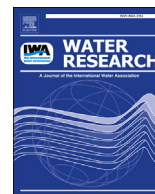
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Quantification of microplastic mass and removal rates at wastewater treatment plants applying Focal Plane Array (FPA)-based Fourier Transform Infrared (FT-IR) imaging

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ABSTRACT

This paper presents a method for microplastic (MP) mass quantification using a Focal Plane Array-based Fourier Transform Infrared imaging technique. It discusses the issue that particle number is not a conserved base quantity and hence less suited than mass to compare independent studies on MP in the environment. It concludes that MP mass should be included when quantifying MP pollution in the environment, supplementing the conventional approach of reporting particle numbers. Applying mass as the unit of MP measurement, the paper presents data showing that Danish wastewater treatment plants discharge around 3 t/year of MP in the size range 10–500 μm . This value corresponds to an annual per capita emission from these plants of 0.56 g MP/(capita year). The distribution of polymer types by mass and particle number differed because the size of MP particles of the different material types varied.

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1. Introduction

Plastic is a versatile material which can be manufactured to suit multifarious applications, making its usage unavoidable in modern life (Andrady, 2011). The ever-growing use of these artificial polymers, however, entails the increase of plastic litter due to the persistent nature of the material (Thompson et al., 2004). The improper disposal of plastic waste has led to its widespread presence in the environment (Van Cauwenberghe et al., 2013; Villarrubia-Gómez et al., 2017), where the long-term ubiquity of plastic litter poses various threats to different ecosystems and organisms at all trophic levels (Barnes et al., 2009; Browne et al., 2011). The environmental impact and fate of plastic particles smaller than 5 mm, called microplastics (MP), in particular, has been the focus of interest for more than a decade (Hidalgo-Ruz et al., 2013).

Based on the origin of the particles, two types of MP are commonly distinguished. Primary MP is used as raw material in the production of plastic items and additives in cosmetic products, whereas secondary MP is formed by the physical or photo-oxidative breakdown of larger plastic items (Andrady, 2011). Both

primary and secondary MP has been found in the marine environment as well as in freshwater systems. MP particles are considered potentially harmful environmental contaminants by acting as vectors for organic pollutants and heavy metals or by causing physical harm to organisms upon ingestion (Cole et al., 2011). Although the exact and most significant sources of MP are yet to be discovered, wastewater treatment plants (WWTPs) have been found to be one of the urban sources releasing MP. Studies have shown that despite the high retention of MP in WWTPs, a considerable number of these particles enters the environment due to the high flux of wastewater (Murphy et al., 2016).

The MP research field has so far not established standardized analytical methods, which is a significant obstacle in studying the occurrence of MP pollution in the environment. Although some studies have proposed techniques as standard procedures (Andrady, 2011; Hidalgo-Ruz et al., 2013; Löder et al., 2015), a scientific consensus is yet to be reached in all aspects of MP research from sampling to data interpretation. Owing to the different methodologies the results of some studies can be difficult to compare, creating uncertainties over the fate and impact of MP in the environment (Löder et al., 2015).

Quantification of MP has focused on identifying particle numbers and sizes as impacts and behavior of MP is strongly linked to these parameters (Andrady, 2011). However, particle number data cannot be adequately compared with estimates of MP mass

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loads, such as, for example, the one by Lassen et al. (2015) who estimated the Danish MP discharge based on information on plastic use and wear. Owing to the brittle nature of MP particles aged in the environment (Andrady, 2011), the incidental fragmentation of MP particles can affect particle number and result in a potential over-estimation of their concentrations to varying degrees. Furthermore, particle number cannot alone describe the extent of MP pollution as the size of these particles spans a range three orders of magnitudes wide. For example, a cubical particle of 1 mm side length takes up equal space in the environmental matrix as one billion cubical particles with 1 μm side length, as particle volume comes in the third power of particle dimensions. The fact that particles continuously break up in the environment (Andrady, 2011), and can fragment during MP analysis, entails that particle number cannot be viewed as a conserved base quantity. Mass opposed to particle number is a conserved base quantity and as such a consistent measure unaffected by the physical and chemical processes to which the MP is exposed. Supplementing determination of MP numbers by the mass of MP, therefore, would allow for the experimental quantification of MP loads to the environment and a direct comparison of the contribution from different sources (Rocha-Santos and Duarte, 2015).

Despite the apparent advantages of including mass as a measure of MP concentration, it has, to our best knowledge, not been systematically estimated and reported in peer-reviewed MP studies applying FT-IR (Fourier Transform Infrared Spectroscopy) or similar quantification techniques. Plastic mass has been measured applying pyrolysis techniques, for example with the purpose of quantifying car tire rubber (Unice et al., 2013). Additionally, several studies have been conducted to assess the mass load to various environments based on theoretical considerations and information on plastic use and wear (for example Lassen et al. (2015); sundt et al. (2014)). One objective of our study was to present a procedure to estimate the mass of MP in wastewater applying an FT-IR based technique, allowing both mass and particle number to be determined on the same samples. We further aimed to gather information about the overall MP loads in typical Danish municipal wastewater and discharges from the associated WWTPs. We sampled inlet and outlet wastewater at ten of the largest WWTPs in Denmark, and assessed the mean MP load and mean removal efficiency of the WWTPs. We extracted particles in the size range of 10–500 μm from wastewater samples, analyzed their material composition with a Focal Plane Array (FPA)-based FT-IR imaging technique, and estimated the mass of the identified MP particles.

2. Materials and methods

2.1. Sampling

Raw and treated wastewater was collected flow-proportionally from 10 of the largest WWTPs in Denmark, managing 26% of all Danish wastewater (MFVM, 2017). Nine of the examined WWTPs manage wastewater mainly from households, while one receives wastewater of 75% industrial origin (WWTP #4, supplementary material, Table S1). All WWTPs do biological N and P removal based on activated sludge technologies. One of the plants, WWTP #8, had rapid sand filtration as an additional polishing step. Samples were taken during dry weather, defined as a maximum of 3 mm of precipitation for 48 h before and during sampling. Raw wastewater was collected from the inlet as flow-proportional samples covering 24 h of inflow, applying the auto-samplers of the WWTPs. The samplers were all permanently installed after the screens and accredited for sampling of raw wastewater for monitoring and compliance purposes. It was chosen to use the accredited sampling devices at the WWTPs as these are certified to yield representative

samples of the raw wastewater inflow. While it is not ideal to use samplers containing plastic parts, it was deemed a better alternative than taking grab samples at locations where representativeness of samples could not be ensured. Possible contamination by the auto-samplers was not assessed as this was not practically feasible with the already installed samplers.

One liter of raw wastewater was subsampled at each WWTP and shipped for MP analysis. Treated wastewater was collected in glass bottles from the outlet flow and filtered on-site through 10 μm Ø47 mm stainless steel meshes until three filters had clogged. The filtered effluent varied for each location depending on water quality (from 4.1 to 81.5 L per site, supplementary material, Table S1). Except for the auto-samplers at the treatment plants, all equipment used for sampling was either metal or glass.

The WWTPs are anonymized as only one inlet and outlet sample was taken from each plant. The variability between sampling days is hence not quantified and the data not necessarily representative of that plant. Furthermore, it is assumed that the two times ten wastewater samples could be viewed as random samples drawn from the same set – raw and treated wastewater – and that it hence is valid to combine them for further calculations regarding MP removal efficiency and material composition.

2.2. Extraction of microplastics

2.2.1. Raw wastewater

1 L raw wastewater was wet-sieved with a Retsch AS 200 vibratory sieve shaker through 2 mm, 1 mm and 500 μm sieve meshes to eliminate particles and fibers larger than 500 μm . Sodium dodecyl sulfate (SDS) as a surfactant was added to a final concentration of 0.15 g/L before sieving to detach adhered MP particles from the larger solids (Enders et al., 2015). Afterwards, 200 mL of the pre-sieved wastewater was incubated with cellulase enzyme (*Aspergillus* sp., Sigma-Aldrich, CAS no. 9012-54-8) for 48 h at 40 °C to degrade cellulose fibers deriving mainly from toilet paper. Subsequently, organic material was oxidized with hydrogen peroxide where iron(II) was added to catalyze the reaction (Fenton reaction). Peroxide was added to a final concentration of 250 g/L and iron(II) sulfate to 2.5 g/L (Masura et al., 2015).

Temperature, pH, and the ratio of peroxide to ferrous ions are key factors to ensure maximum removal of organic matter (Babuponnusami and Muthukumar, 2014; Gulkaya et al., 2006). The pH of the mixture was adjusted to approximately 3 with sodium hydroxide, as the formation of complex species of ferric or ferrous ions reduce the amount of free iron(II) ions and decelerate the reaction at pH-values below 2 and above 4 (Babuponnusami and Muthukumar, 2014). The reactor was kept in an ice-bath to keep the temperature between 15 and 30 °C as the oxidation reaction is exothermic and the mixture can boil violently. Keeping the designated temperature interval was critical as a yellow-colored substance, probably a ferric compound tended to precipitate at temperatures below 15 °C. Subsequently, the oxidized sample was wet-sieved (demineralized water with 0.15 g/L SDS) into two size fractions through an 80 μm sieve mesh. The effluent containing particles <80 μm was collected into a glass beaker. Particles >80 μm were removed from the sieve mesh into filtered demineralized water containing 0.15 g/L SDS by treatment in an Elma S50R ultrasonic bath. Particles from this liquid and the collected effluent were gathered on separate 10 μm steel meshes. Particles were removed from the filters into 25 mL HPLC grade ethanol by ultrasonic treatment. The resulting particle-ethanol suspensions of the two size fractions were transferred into glass vials where their final volume was set to 5 mL by evaporation with nitrogen gas.

2.2.2. Treated wastewater

The collected solids were removed by ultrasonic treatment from the 10 μm steel filters used for sampling in the WWTP and collected in filtered demineralized water containing 0.15 g/L SDS. The resulting suspension was incubated in a serum flask for 48 h at 40 °C with the same cellulase enzyme used for raw wastewater. Samples were oxidized in 180 g/L hydrogen peroxide catalyzed by 1.8 g/L iron(II) sulfate and pH adjusted to approximately three by sodium hydroxide. The rest of the sample preparation was identical to that of raw wastewater, i.e., size fractionation by wet-sieving and transferring the particle-ethanol suspension into glass vials.

2.3. Analysis

The chemical composition of the extracted particles was determined with an FPA-based FT-IR imaging technique. The equipment was a Cary 620 FT-IR microscope from Agilent Technologies coupled with a Cary 670 IR spectroscope. The microscope was equipped with 15x Cassegrain objective and a 128×128 Mercury Cadmium Telluride (MCT) FPA detector of 5.5 μm pixel resolution. The 10–80 μm particles were analyzed in transmission mode on a \emptyset 13 mm \times 2 mm zinc selenide transmission window, whereas 80–500 μm particles were scanned in reflection mode on a MirrIR low-e microscope reflection slide from Kevley Technologies. Samples were scanned in the spectral range of 3750–950 cm^{-1} at 8 cm^{-1} resolution applying 30 co-added scans in both transmission and reflection mode. The sample was homogenized with a vortexer, and an aliquot, corresponding to 2–6% of the sample was transferred onto either the transmission window or the reflection slide using disposable glass capillary pipettes. Samples were covered with a glass beaker and dried at 40 °C on a heating plate, and the area of the entire slide was scanned. While representing a significant sub-sampling (corresponding to actual sample volumes between 0.14 and 4.05 L of treated wastewater), the aliquots allowed quantification of 10–32 MP particles per sample in both treated and untreated wastewaters.

Upon acquiring the infrared map – which consisted of more than 3 million individual spectra – organic particles were denoted by highlighting the aliphatic CH stretch (Fig. 1). Afterwards, spectra of the highlighted organic particles were analyzed by comparing them to polymer spectra from commercial libraries using the KnowItAll software from Bio-Rad and Omnic 8.3 from Thermo Scientific. At least 52 tiles in the arrangement of a double cross

were examined for plastic particles (approx. 26% of the window or slide) and a maximum of 98 tiles, corresponding to half of the area of the window or slide. Identification was stopped when ten plastic particles were encountered or until the 98th tile was reached.

2.4. Mass calculation

The major and minor dimensions of the identified MP particle were measured on the infrared image. The major dimension was defined as the longest continuous axis in the center of the particle, and the minor was the longest axis perpendicular to the major axis. The ratio of the minor and major dimension of all the identified MP ($n = 398$) particles was calculated and yielded a median value of 0.67 ± 0.22 . It was assumed that the ratio of the thickness and the minor dimension of the particle was the same; hence the thickness was estimated as 67% of the minor dimension. The mass of the particle was calculated from the volume of the particle assuming an ellipsoid shape and the density of its material. The volume of fibers was calculated presuming a cylindrical shape with 40% void fraction. The density values of the identified polymers applied in the calculation are found in the supplementary material, Table S2.

2.5. Method validation

2.5.1. Recovery

Recovery tests were performed with raw wastewater spiked with a known amount of red 100 μm PS beads (Sigma-Aldrich, product no. 56969) and with 80–150 μm green high-density polyethylene (HDPE) particles of random shape (supplementary material, Figure S1). The latter was produced by cryo-milling a large piece of an HDPE water bottle and sieved to size fractions. Triplicate spiked samples were processed, and the particles were extracted as described for raw wastewater. Extracted particles were transferred onto a glass microscope slide, and since these particles had a distinct appearance, they were identified and counted visually using a Meiji Techno MT4310 trinocular phase microscope.

2.5.2. Contamination

Precautions such as meticulous flushing of glassware before usage, minimizing the application of plastic tools for sampling and analysis, muffling of steel filters, and covering glassware with aluminum foil, were implemented to mitigate the contamination of samples. Nevertheless, airborne contamination and contamination

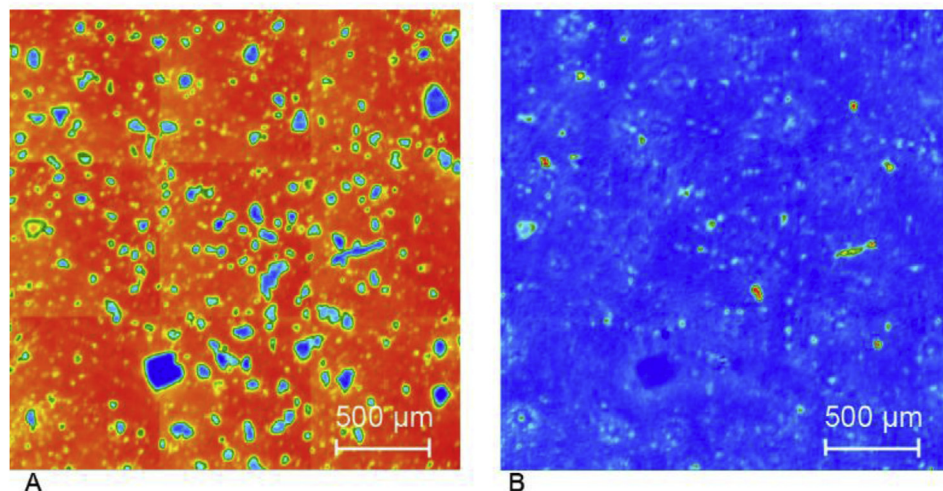


Fig. 1. The acquired infrared map of the scanned area (A) and the filtered IR map (B) of the same area, where organic particles are highlighted.

from tools will have occurred. Blanks were analyzed to assess the extent of contamination throughout the sample preparation procedure. One liter of demineralized water filtered through 0.8 µm mixed cellulose ester filters was used as a model medium for this, and triplicate samples were processed and analyzed as described for raw wastewater.

2.5.3. Statistical analysis

Normality of datasets was tested by a Shapiro-Wilk normality test. A non-parametric ANOVA Kruskal-Wallis test by ranks was used to compare univariate groups. Both test types were performed in the software Past (v.3.05).

3. Results and discussion

3.1. Method validation

Blank samples showed that each sample preparation gave rise to 2110 MP particles (84 µg) of contamination per liter sample. Assuming that the demineralized water used for the contamination test was free of MP, this contamination must have come from the equipment and the surrounding air during sample preparation. For the raw wastewater, this means that 16.3% of all the particles in raw wastewater regarding particle number and 28.4% regarding particle mass were associated with contamination. These contamination levels are comparable to those of Talvitie et al., (2017) who reported 30% contamination of microlitter. They might be above the contamination rates in Mintenig et al., (2017), reporting 21 particles and 130 fibers in 150 L tap water processed simultaneously to wastewater samples. The limit of detection of MP in raw wastewater we determined from the blanks by the 3-Sigma method to 89 µg/L in terms of particle mass and 3093 particles/L in terms of particle number. MP concentration of raw wastewater consequently was below detection limit in the sample from WWTP 10 (Table 1). We did not explicitly quantify the contamination of the sample preparation for the treated wastewater as the preparation steps in the laboratory were nearly identical to those of the raw wastewater. The concentration and polymer type of the identified MPs in the three subsamples of blanks are shown in Table S3 and the distribution of particle size in Figure S3 in the supplementary material.

We performed recovery tests to evaluate the efficiency of the extraction method with triplicate raw wastewater samples spiked with PS microspheres and HDPE particles of irregular shape. We recovered $77.7 \pm 11.6\%$ of the PS microbeads and $57.6 \pm 25.1\%$ of HDPE particles from the samples, which we deemed acceptable. The tendency to aggregation and overlapping of the HDPE particles could lead to their miscount which could explain the lower recovery and larger standard deviation compared to microbeads, where aggregation and overlapping would not have affected the particle counting. However, the recovery rates are only indicative of the efficiency of the extraction method as the features of the MP particles used for spiking did not cover all the diversity of MP particles in the sample regarding their size, material, and shape. Therefore, to avoid introducing an unknown bias, data is not

corrected for recovery.

3.2. Quantification of MPs

The concentration of MP regarding particle number and particle mass in the ten raw and treated wastewater samples along with the general removal efficiency of Danish WWTPs is presented in Table 1. Shapiro-Wilk normality tests showed that MP in units of number as well as mass were non-normal distributed amongst WWTPs (MP numbers: raw wastewater, $p = 0.071$, treat wastewater $p = 0.004$; MP mass: raw wastewater, $p = 0.004$, treat wastewater $p = 0.029$); thus, median values were considered to describe the overall concentrations of MP particles.

The median MP concentration of 7216 particles/L and 54 particles/L in influent and effluent wastewater, respectively, was high compared to previous studies. Magnusson and Norén (2014) and Murphy et al. (2016) have reported approximately 15 particles/L in influent wastewater. The reported MP concentrations in effluent wastewater range from $6 \cdot 10^{-3}$ particles/L to 9 particles/L in other studies (Magnusson and Norén, 2014; Murphy et al., 2016; Mintenig et al., 2017; Talvitie et al., 2017). The differences can be related to random natural variations among the studied treatment plants (Luo et al., 2014). However, this seems an unlikely explanation as our study covered ten individual WWTPs, and the previous studies also covered a significant number of WWTPs. More probable is that the variations in the number of detected particles were due to differences in size ranges addressed, sampling, sample preparation, and in analytical methods (Ziajahromi et al., 2016). Our study focused on the smaller particle sizes and hence bound to find more particles per volume than studies that have addressed larger particle sizes. It also applied an FPA detector for quantification, which is likely to yield better estimates of especially the smallest particles than would single point FT-IR (Tagg et al., 2015). However, the particle numbers of our study are also large compared to previous studies that applied FPAs. Those studies did though use a somewhat higher pixel resolution and also a different substrate for the scanning. Whether or not these differences in quantification technique gave our study a better resolution of MP particles is, however, difficult to assess. Differences in sample preparation can also affect particle numbers. For instance, sonication steps during the sample preparation process could cause some particles to break up as especially aged particles can be brittle (Andrady, 2011) and break due to ultrasonic treatment, owing to the asserted mechanical stress on the particles (Löder and Gerds, 2015). However, the sample preparation method of our study has most likely been no more and no less harsh than that of other studies. All in all we are of the opinion that the cause of the discrepancy between our and previous studies needs further investigation, for example through inter-laboratory comparison on identical samples and development of automated approaches for data analyses as suggested by Primpke et al. (2017).

The removal efficiency of the WWTPs did, however, correspond well with other studies, and we also conclude that WWTPs are efficient in retaining MPs. Regarding particle number, the determined efficiency was 99.3%. Previous studies showed over 98%

Table 1
The concentration of MP at each WWTP (marked with numbers 1–10) in the inlet and outlet wastewater in mass concentration (µg/L) and particle numbers (item/L). *Removal efficiency calculated from the median particle concentration. **Removal efficiency calculated from the median mass concentrations.

WWTP#		1	2	3	4	5	6	7	8	9	10	Median	Removal efficiency [%]
Raw wastewater	item/L	10044	8762	6830	6021	18285	4994	2223	8149	7601	5362	7216	99.3*
	µg/L	181	407	268	193	482	1189	212	407	118	61	250	98.3**
Treated wastewater	item/L	127	447	42	29	214	182	35	19	43	65	54	
	µg/L	3.6	11.9	0.6	0.5	5.4	11.6	0.7	1.4	4.8	3.8	4.2	

removal efficiency regarding particle number (Carr et al., 2016; Magnusson and Norén, 2014; Murphy et al., 2016; Talvitie et al., 2017). With regards to particle mass, 98.3% of the MP was removed in the WWTPs. The mass of MP particles in wastewater, being it raw or treated, has to our best knowledge not previously been reported and these values can hence not be compared to previous findings. Nevertheless, high particle numbers do not necessarily mean that MP is a significant contributor to the total amount of suspended solids in raw wastewater as a high particle number is essentially not associated with high particle mass, and vice versa. Comparing WWTP #5 and #6, raw wastewater (Table 1), illustrates this phenomenon well. Relating the particle numbers and masses to typical raw wastewater chemical oxygen demand (COD) and typical suspended solids (SS) (320–740 mg COD/L and 190–450 mg SS/L (Henze et al., 1997) results in MP mass concentrations in raw wastewater of $7.80 \cdot 10^{-4}$ – $3.37 \cdot 10^{-4}$ mg MP/mg COD and $1.31 \cdot 10^{-3}$ – $5.55 \cdot 10^{-4}$ mg MP/mg SS, respectively. In other words, MP accounts for roughly one thousandth of the raw wastewater constituent mass.

Based on the total amount of wastewater in Denmark ($7.67 \cdot 10^8$ m³/year for 2015, MFVM, 2017), and the median MP mass concentrations estimated in this study, the sum of all Danish WWTPs receive approx. 191 t MP over the course of one year. The estimated MP load on the aquatic environment via the treated wastewater is slightly more than 3 t/year. To put it into context, Lassen et al. (2015) estimated 600–3100 t/year total MP emission in Denmark, indicating that WWTPs are minor contributors to MP discharge. Relating the MP emission to the Danish population (approx. 5.7 million) yields an annual per capita emission from Danish WWTPs of 0.56 g MP/(capita year). Assuming that MP is not degraded in the WWTPs, the 188 t/year MP removed from wastewater, must have gathered in the sewage sludge, of which in Denmark roughly 70% is applied as agricultural fertilizer and 30% for energy recovery.

3.3. Polymer type of MPs in raw and treated wastewater

Our research focused on the overall MP retention in the size range of 10–500 µm by WWTPs in Denmark. Since determining the material of such small particles with visual inspection is highly uncertain (Löder et al., 2015; Song et al., 2015), an FT-IR imaging technique was applied to identify the chemical composition of the examined particles. However, MP is a heterogeneous group of particles varying considerably in chemical composition, size, and shape, and identification is not always straightforward even with the powerful tool of FT-IR spectroscopy coupled with FPA imaging. The diversity of particles was narrowed down by dividing the samples for FT-IR imaging into suitable size ranges and applying scanning techniques optimized for each size range to improve the accuracy of identification. However, the quality of the acquired spectra, regarding the resolution and intensity of the peaks, varied. That is because the variable shape and rough surface of the particles can result in light scattering causing baseline drift and noise interfering with spectral peaks (see supplementary material). Furthermore, residues of organic material attached to the particle surfaces or altered chemical composition of polymers caused by environmental impacts can hinder matching unknown spectra to those of commercial libraries and hence, impede material identification (Andradý, 2011; Lenz et al., 2015; Vianello et al., 2013). Nevertheless, as the sample preparation procedure described in this paper was efficient to remove the vast majority of the organic material in both raw and treated wastewater samples, interference of organic matter with the particle's spectra was not a considerable obstacle in spectral identification.

With these caveats, we could identify 176 particles as MPs in the

analyzed samples of raw wastewater and 222 of treated wastewater. Of these 393 were particles and 5 fibers. The latter all were of polyester. The number of particles of each identified polymer type in raw and treated wastewater is shown in Table 2.

Fig. 2 shows the distribution of polymers based on particle number and particle mass in raw and treated wastewater. In raw wastewater based on particle number, the most abundant type was acrylates (27%, 47 particles), yet they contributed only 12% to the mass while PP contributed the most with 39%. In treated wastewater, PEst (25%) and PE (27%) were the most abundant based on particle number, while PEst and acrylates contributed 34% and 20%, respectively to the total mass of the identified particles, and PE only 9%. The reason for the different proportion of a polymer regarding particle number and mass can be related partly to the different density of polymers. For instance, PE particles were present in the highest number in treated wastewater, but due to the lower density of PE (Table S2) compared to acrylates and PEst, the total mass of PE particles was lower than of the other two polymers. Another aspect is that the mass of a polymer type in the sample depends on both particle number and size. In the case of the raw wastewaters, PP particles contributed more to the mass than acrylates even though it consisted of fewer particles than the acrylates and the density of PP is lower than that of acrylates.

3.4. Size and mass of MPs

In Fig. 3 the major dimension of each particle from raw wastewater is shown against the particles' minor dimension. The size of the data points represents the calculated mass of the particles. The figure reveals that the presence of one 200×300 µm sized PP particle with a considerable mass of 4.44 µg increased the mass fraction of this polymer type. Despite PP particles not being the most abundant, they contributed the most to the total mass of MPs, which highlights the importance of describing MP concentrations not only by particle number but by plastic mass as well.

The method for calculating particle mass we present in this paper allows for estimating the MP mass in the sample rather than determining its absolute value. For example, identifying the margins of a particle on the IR image can be challenging as the quality of the IR spectra deteriorates gradually towards the edge of the particle without outlining a clear-cut border. Further assumptions such as the extent of the particles' third dimension, their presumed ellipsoid shape for volume calculations, and the applied density values are also associated with uncertainties of unknown degree. While IR imaging techniques are suitable to estimate the mass of

Table 2

The identified polymer types in raw and treated wastewater and the number of particles of the given material. The type named "Other" includes poly(phthalimide), polycarbonate, terpene resin, Plexar resin and poly(oxymethylene) particles which were sparse throughout the samples.

Polymer type	Raw wastewater	Treated wastewater
Acrylate	47	27
SAN	11	1
VAC-acrylic copolymer	6	3
PE	18	60
PP	21	26
PE-PP copolymer	22	31
PEst	24	56
PS	3	3
PUR	11	1
PVC	4	0
EVA	3	1
PA	4	7
PVA	0	2
Other	2	4

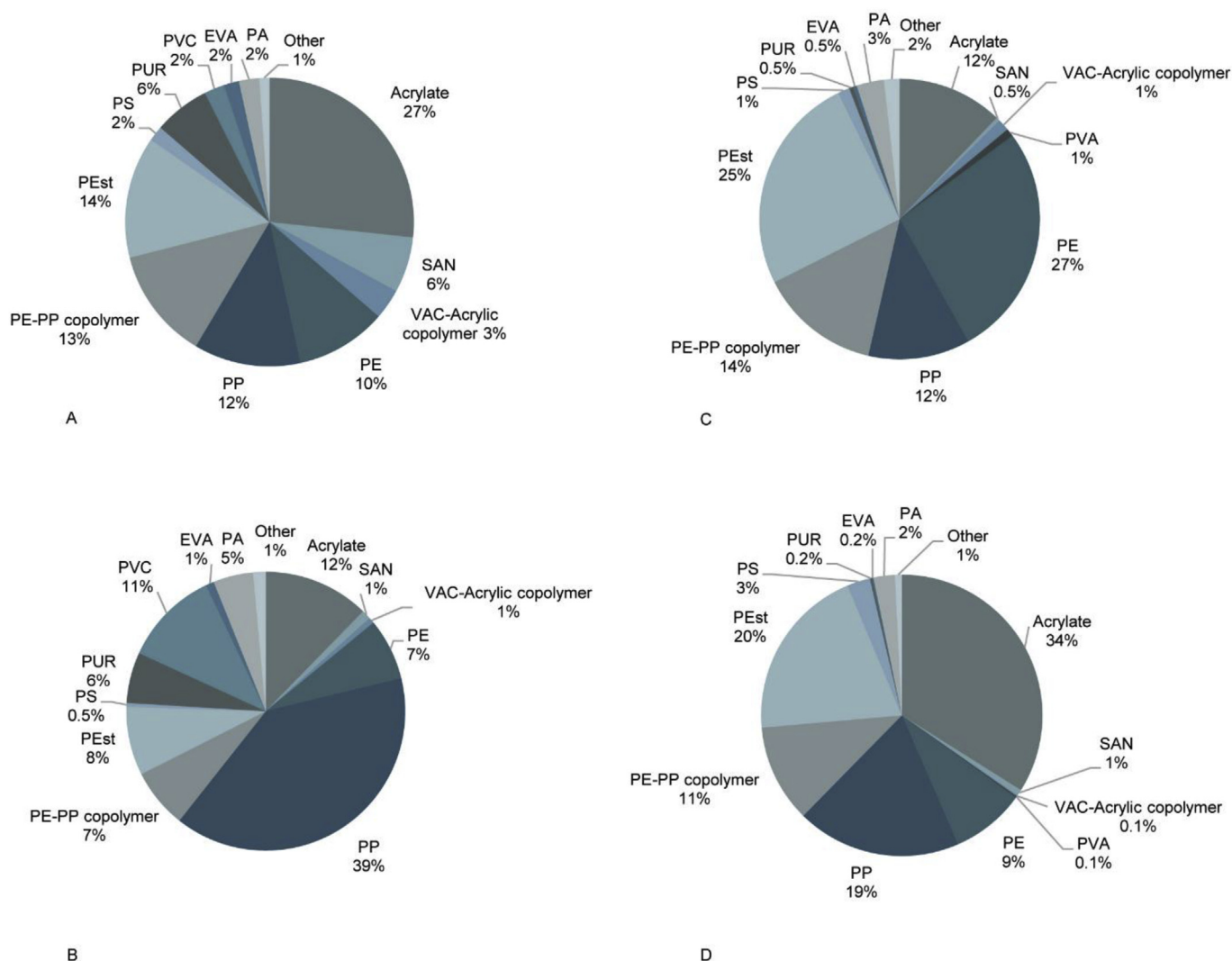


Fig. 2. The distribution of polymers based on particle number (A) and particle mass (B) in raw wastewater and particle number (C) and particle mass (D) in treated wastewater.

MP particles, thermoanalytical techniques might have the potential to increase the accuracy of the plastic mass quantification (Dümichen et al., 2015; Fischer and Scholz-Böttcher, 2017).

Although particle size plays a fundamental role in MP research, reporting this parameter alone seems inadequate and only gives an illustrative characterization of identified particles (Filella, 2015). MPs have been defined as plastic particles typically smaller than 5 mm in their diameter (GESAMP, 2015). However, MP particles are irregularly shaped meaning that describing their size with only one dimension can be insufficient and opens up data interpretation to bias. For instance, in the case of fibers which typically are rather long (often several 100 of μm) with small diameters ($<50 \mu\text{m}$), one dimension apparently cannot define size appropriately or provide sufficient information about its contribution to the total amount of MPs in a sample. Therefore, standard parameters already applied in colloid science could be used to obtain reliable and comparable data about the actual size of MP particles in a normalized manner (Filella, 2015; Merkus, 2009). Accordingly, Fig. 4A presents the particle size distribution based on the measured major dimension of the identified MP particles in raw wastewater. The data for treated wastewater and raw wastewater based on the measured minor dimension can be found in the supplementary material (Figure S4). Based on Cozar et al. (2014) we chose the increment of

the bins to 0.1 on a logarithmic scale. The figure also shows the abundance of particles normalized by the width of the size classes in microns. The relative cumulative frequency of particles in raw and treated wastewater samples concerning major dimension is illustrated in Fig. 4B and Figure S5 in the supplementary material regarding the minor dimension. The figures show that the median minor dimension was $26 \mu\text{m}$ in raw and $30 \mu\text{m}$ in treated wastewater samples, while the median major dimension was $48 \mu\text{m}$ and $45 \mu\text{m}$ in raw and treated wastewater samples, respectively. The D_{90} particle size in raw wastewater was $100 \mu\text{m}$ and $91 \mu\text{m}$ in treated wastewater regarding the major measured dimension, meaning that smaller particles were more abundant in the samples. A Kruskal-Wallis test comparing the sizes of particles in inlet and outlet showed that the samples were not significantly different ($p = 0.563$). Since the majority of MP particles originate from the breakdown of plastic fragments or larger plastic particles (Lassen et al., 2015) higher abundance of smaller particles was expected. Another aspect is that larger volumes of wastewater than the volumes sampled in our study need to be collected to achieve a higher abundance of the larger particles. Mintenig et al. (2017) reported that 59% of the MP particles in treated wastewater measured in their longest dimension ranged between 50 and $100 \mu\text{m}$. Talvitie et al. (2017) found 70% of particles in the range of 20– $100 \mu\text{m}$ in

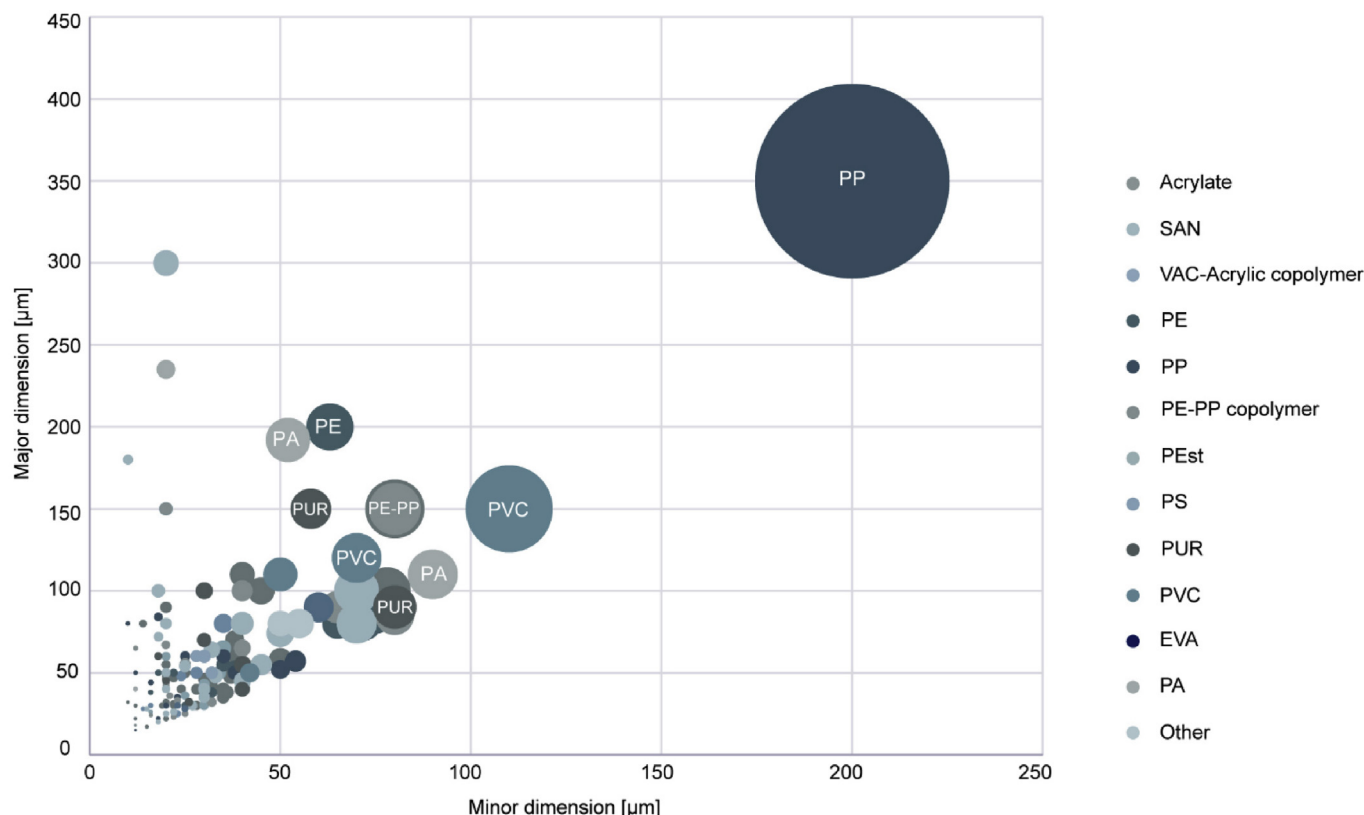


Fig. 3. The major dimension of particles of each polymer type from raw wastewater plotted against their minor measured dimension. The size of the data points represents the calculated mass of the particles. The polymer type named “Other” includes poly(phthalimide), polycarbonate, terpene resin, Plexar resin and poly(oxymethylene) particles which were sparse throughout the samples.

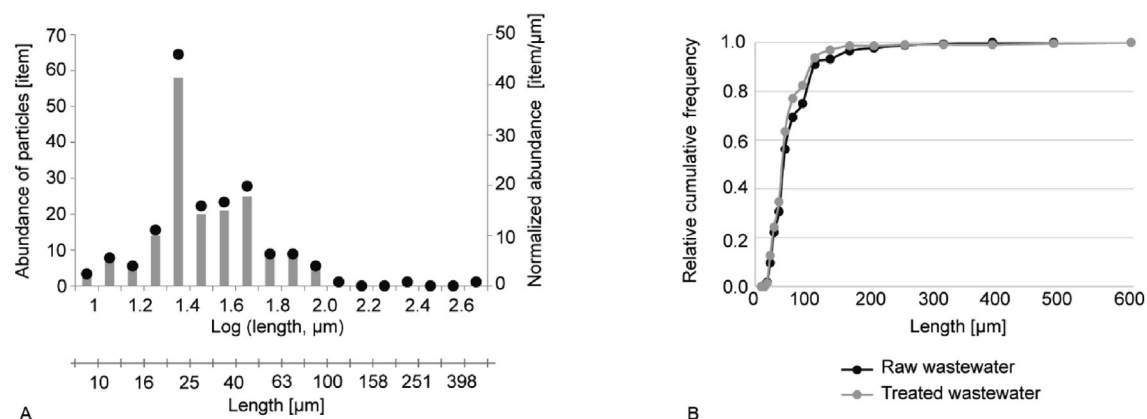


Fig. 4. (A) Particle size distribution in raw wastewater samples based on the measured major particle dimensions in abundance and normalized by the width of the size classes in microns. The increment of the bins was chosen 0.1 on a logarithmic scale. (B) The relative cumulative frequency of the major measured dimension of particles both in raw and treated wastewater samples.

effluent while approximately 40% of the same size range in influent wastewater. Unfortunately, owing to the different size classes reported in the studies, direct comparison of data is infeasible (Filella, 2015).

There was no significant difference between the mass of the identified particles in raw and treated wastewater samples (Kruskal-Wallis test, $p = 0.778$). The median particle mass was 13.45 ng and 13.52 ng of raw and treated wastewater samples, respectively. The non-significant differences between inlet and outlet wastewater concerning particle size and mass indicate that there was no

preferential removal of particles in the WWTPs related to the size of the particles.

4. Conclusions

Our study has shown over 98% removal of 10–500 μm sized MP in Danish WWTPs. The total annual MP discharge in this size range from the studied plants corresponded to approx. 3 t/year. The MP discharge via WWTPs related to the population of Denmark was 0.56 g/(capita year) and accounted for less than 0.5% of the total MP

emission estimated for Denmark. This comparison to total emissions is, though, highly uncertain as the absence of harmonized sample treatment methods, as well as data reporting, currently impedes relating the significance of WWTPs to other sources of MP. Our study is the first providing an estimate of the mass concentration of MP in wastewater and has pointed out the importance of describing MP by mass in addition to particle number. We find mass concentrations to be a more robust measure, less affected by differences in analytical procedures and targeted size ranges. Although FPA-based FT-IR imaging is a suitable technique to identify the material of particles in the micrometer size range, it only allows for the rough estimation of the MP mass. Determination of microplastics would benefit from being supplemented by other methods, e.g., thermoanalytical techniques, to increase the certainty of measuring MP mass. Applying MP mass as the conserved base of MP quantification along with standardized sample treatment and analytical methods would enable to apprehend the extent of MP pollution and consistently compare sources and occurrence in the environment. Such knowledge allows, for example, the comparison of source strengths so that appropriate measures to decrease MP emission can be assessed and implemented.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.watres.2018.05.019>.

References

- Andrady, A.L., 2011. Microplastics in the marine environment. *Mar. Pollut. Bull.* 62, 1596–1605. <https://doi.org/10.1016/j.marpolbul.2011.05.030>.
- Babuponnusami, A., Muthukumar, K., 2014. A review on Fenton and improvements to the Fenton process for wastewater treatment. *J. Chem. Environ. Eng.* 2, 557–572. <https://doi.org/10.1016/j.jeece.2013.10.011>.
- Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364, 1985–1998. <https://doi.org/10.1098/rstb.2008.0205>.
- Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines worldwide: sources and sinks. *Environ. Sci. Technol.* 45, 9175–9179. <https://doi.org/10.1021/es201811s>.
- Carr, S.A., Liu, J., Tesoro, A.G., 2016. Transport and fate of microplastic particles in wastewater treatment plants. *Water Res.* 91, 174–182. <https://doi.org/10.1016/j.watres.2016.01.002>.
- Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. *Mar. Pollut. Bull.* 62, 2588–2597. <https://doi.org/10.1016/j.marpolbul.2011.09.025>.
- Cozar, A., Echevarria, F., Gonzalez-Gordillo, J.L., Irigoien, X., Ubeda, B., Hernandez-Leon, S., Palma, A.T., Navarro, S., Garcia-de-Lomas, J., Ruiz, A., Fernandez-de-Puelles, M.L., Duarte, C.M., 2014. Plastic debris in the open ocean. *Proc. Natl. Acad. Sci. Unit. States Am.* 111, 10239–10244. <https://doi.org/10.1073/pnas.1314705111>.
- Dümichen, E., Barthel, A.K., Braun, U., Bannick, C.G., Brand, K., Jekel, M., Senz, R., 2015. Analysis of polyethylene microplastics in environmental samples, using a thermal decomposition method. *Water Res.* 85, 451–457. <https://doi.org/10.1016/j.watres.2015.09.002>.
- Enders, K., Lenz, R., Stedmon, C.A., Nielsen, T.G., 2015. Abundance, size and polymer composition of marine microplastics $\geq 10 \mu\text{m}$ in the Atlantic Ocean and their modelled vertical distribution. *Mar. Pollut. Bull.* 100, 70–81. <https://doi.org/10.1016/j.marpolbul.2015.09.027>.
- Filella, M., 2015. Questions of size and numbers in environmental research on microplastics: methodological and conceptual aspects. *Environ. Chem.* 12, 527–538. <https://doi.org/10.1071/EN15012>.
- Fischer, M., Scholz-Böttcher, B.M., 2017. Simultaneous trace identification and quantification of common types of microplastics in environmental samples by pyrolysis-gas chromatography-mass spectrometry. *Environ. Sci. Technol.* 51, 5052–5060. <https://doi.org/10.1021/acs.est.6b06362>.
- GESAMP, 2015. In: Kershaw, P.J., Rochman, C.M. (Eds.), *Sources, Fate and Effects of Microplastics in the Marine Environment – Part Two of a Global Assessment*. IMO/FAO/UNESCOIOC/UNIDO/WO/IAEA/UN/UNEP/UNDP Jt. Gr. Expert. Sci. Asp. Mar. Environ. Prot. vol. 220.
- Gulkaya, I., Surucu, G.A., Dilek, F.B., 2006. Importance of H₂O₂/Fe²⁺ ratio in Fenton's treatment of a carpet dyeing wastewater. *J. Hazard Mater.* 136, 763–769. <https://doi.org/10.1016/j.jhazmat.2006.01.006>.
- Henze, M., Herramóes, P., La Cour Jansen, J., Arvin, E., 1997. *Wastewater Treatment: Biological and Chemical Processes*. Springer pages 28 and 30.
- Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2013. Microplastics in the marine environment: a review of the methods used for identification and quantification. *Environ. Sci. Technol.* 46, 3060–3075. <https://doi.org/10.1021/es2031505>.
- Lassen, C., Hansen, S.F., Magnusson, K., Hartmann, N.B., Rehne Jensen, P., Nielsen, T.G., Brinch, A., 2015. *Microplastics Occurrence, Effects and Sources of Releases*.
- Lenz, R., Enders, K., Stedmon, C.A., MacKenzie, D.M.A., Nielsen, T.G., 2015. A critical assessment of visual identification of marine microplastic using Raman spectroscopy for analysis improvement. *Mar. Pollut. Bull.* 100, 82–91. <https://doi.org/10.1016/j.marpolbul.2015.09.026>.
- Löder, M.G.J., Gerdts, G., 2015. Marine anthropogenic litter. *Mar. Anthropog. Litter* 1–447. <https://doi.org/10.1007/978-3-319-16510-3>.
- Löder, M.G.J., Kuczer, M., Mintenig, S., Lorenz, C., Gerdts, G., 2015. Focal plane array detector-based micro-Fourier-transform infrared imaging for the analysis of microplastics in environmental samples. *Environ. Chem.* 12 (563). <https://doi.org/10.1071/EN14205>.
- Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S., Wang, X.C., 2014. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Sci. Total Environ.* 473–474, 619–641. <https://doi.org/10.1016/j.scitotenv.2013.12.065>.
- Magnusson, K., Norén, F., 2014. Screening of Microplastic Particles in and Downstream a Wastewater Treatment Plant, pp. 1–22. <https://doi.org/naturvardsverket-2226>.
- Masura, J., Baker, J., Foster, G., Arthur, C., 2015. *Laboratory Methods for the Analysis of Microplastics in the Marine Environment: Recommendations for Quantifying Synthetic Particles in Watersand Sediments*, Vol. 39.
- Merkus, H.G., 2009. *Particle Size Measurements; Fundamentals, Practice, Quality*. Springer, pp. 14–19.
- MFVM, 2017. *Punktkilder 2015*.
- Mintenig, S.M., Int-Veen, I., Löder, M.G.J., Primpke, S., Gerdts, G., 2017. Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. *Water Res.* 108, 365–372. <https://doi.org/10.1016/j.watres.2016.11.015>.
- Murphy, F., Ewins, C., Carbonnier, F., Quinn, B., 2016. Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. *Environ. Sci. Technol.* 50, 5800–5808. <https://doi.org/10.1021/acs.est.5b05416>.
- Primpke, S., Lorenz, C., Rascher-Friesenhausen, R., Gerdts, G., 2017. An automated approach for microplastics analysis using focal plane array (FPA) FTIR microscopy and image analysis. *Anal. Meth.* 9, 1499–1511. <https://doi.org/10.1039/C6AY02476A>.
- Rocha-Santos, T., Duarte, A.C., 2015. A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. *TrAC Trends Anal. Chem. (Reference Ed.)* 65, 47–53. <https://doi.org/10.1016/j.trac.2014.10.011>.
- Song, Y.K., Hong, S.H., Jang, M., Han, G.M., Rani, M., Lee, J., Shim, W.J., 2015. A comparison of microscopic and spectroscopic identification methods for analysis of microplastics in environmental samples. *Mar. Pollut. Bull.* 93, 202–209. <https://doi.org/10.1016/j.marpolbul.2015.01.015>.
- Sundt, Peter, Schultze, Per-Erik, Syversen, Frode, 2014. *Sources of microplastic-pollution to the marine environment*. Mepex. Nor. Environ. Agency 1–108. <https://doi.org/M-321/2015>.
- Tagg, A.S., Sapp, M., Harrison, J.P., Ojeda, J.J., 2015. Identification and quantification of microplastics in wastewater using focal plane array-based reflectance micro-FT-IR imaging. *Anal. Chem.* 87, 6032–6040. <https://doi.org/10.1021/acs.analchem.5b00495>.
- Talvitie, J., Mikola, A., Setälä, O., Heinonen, M., Koistinen, A., 2017. How well is microlitter purified from wastewater? - A detailed study on the stepwise removal of microlitter in a tertiary level wastewater treatment plant. *Water Res.* 109, 164–172. <https://doi.org/10.1016/j.watres.2016.11.046>.
- Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at Sea: where does all the plastic go? *Science* 304, 838.
- Unice, K.M., Kreider, M.L., Panko, J.M., 2013. Comparison of tire and road wear particle concentrations in sediment for watersheds in France, Japan, and the United States by quantitative pyrolysis GC/MS analysis. *Environ. Sci. Technol.* 47, 8138–8147. <https://doi.org/10.1021/es400871j>.
- Van Cauwenbergh, L., Vanreusel, A., Mees, J., Janssen, C.R., 2013. Microplastic pollution in deep-sea sediments. *Environ. Pollut.* 182, 495–499. <https://doi.org/10.1016/j.envpol.2013.08.013>.
- Vianello, A., Boldrin, A., Guerriero, P., Moschino, V., Rella, R., Sturaro, A., Da, Ros L., 2013. Author æTM s personal copy Estuarine, Coastal and Shelf Science Microplastic particles in sediments of Lagoon of Venice, Italy: first observations on occurrence, spatial patterns and identification. *Estuar. Coast Shelf Sci.* 130, 54–61.
- Villarrubia-Gómez, P., Cornell, S.E., Fabres, J., 2017. Marine plastic pollution as a

planetary boundary threat – the drifting piece in the sustainability puzzle. *Mar. Pol.* in press <https://doi.org/10.1016/j.marpol.2017.11.035>.
Ziajahromi, S., Neale, P.A., Leusch, F.D.L., 2016. Wastewater treatment plant effluent

as a source of microplastics: review of the fate, chemical interactions and potential risks to aquatic organisms. *Water Sci. Technol.* 74 (10), 2253–2269 wst2016414. <https://doi.org/10.2166/wst.2016.414>.