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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.

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 60–800 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., 2013; Watters et al., 2010), or revealed ingestion by several species (Kuhn 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

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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 $n \times n$ 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.

2. Materials and methods

2.1. Information about sampled WWTPs

The Oldenburg-East-Frisian water board supplies the drinking water for an area of 7500 km^2 and manages the sewage disposal in an area of 3700 km^2 in the north-western part of Germany. Overall, 46 WWTPs are responsible f for treating 32.5 million m^3 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 S1). With an annual efflux of approximately 13 million m^3, the WWTP of Oldenburg has the largest capacity. Another four WWTPs discharge between 1 and 2.6 million m^3 TWW yearly. The smallest facility records an annual efflux of 0.185 million m^3. 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 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 m^3 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 10–15 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.

2.2. Sampling

The sampling at the 12 WWTPs took place between April 22nd 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 (Fig. S1A). 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 5 min 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 L at WWTP of Holdorf (Table 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 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.

2.3. Purification of MP

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 L filtered tap water (3 μm stainless steel cartridge filters) were treated and analysed simultaneously to TWW samples.

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 (Imhof & Löder 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 L 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 min 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 48 h. 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 (Fig. S1B). 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).

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 h 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 h 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.

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).

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.

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 7 × 65 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 and 1430 cm^{-1} (C–H bending, aromatic ring stretching) and secondly between 1790 and 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 × 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.

2.5. Statistical analyses

Spearman rank correlation was performed between polymer specific counts of MP < 500 μm and population equivalents of respective WWTPs (Table 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).

3. Results

3.1. 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.

3.2. MP > 500 μm in TWW

In ten of the 12 WWTPs effluents MP > 500 μm was detected (Fig. 1A). 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 (Fig. S2A). In total one to five polymers in a size range of 500–7200 μm were detected per sample (Figs. S3A and S4A). Discharges of MP > 500 μm ranged from 0 × 10^1 m^{-3} in Schillig to 4 × 10^1 m^{-3} in Holdorf (Fig. 1C). 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 (Fig. 1B).

3.3. MP < 500 μm in TWW

All samples contained MP < 500 μm including Oldenburg after post-filtration and Schillig (Fig. 2A). 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%, Fig. S2B). Three to 12 polymers were

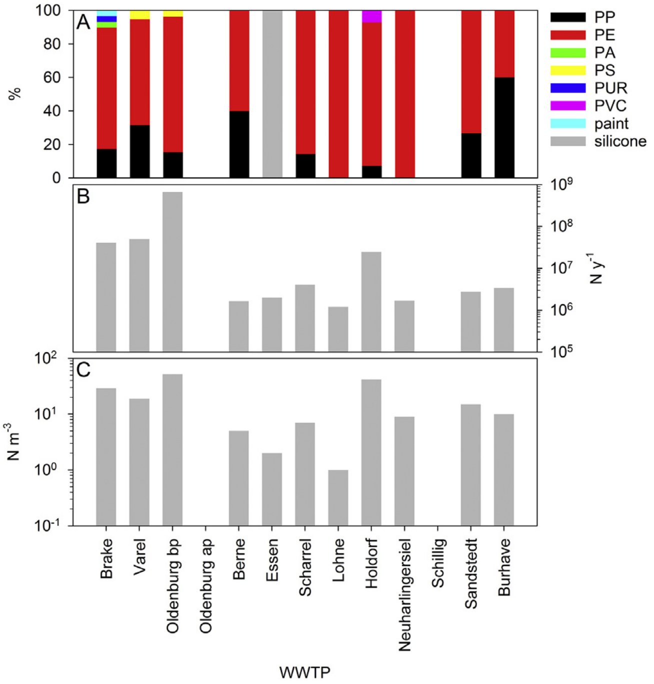

Fig. 1. Microplastics (MP) >500 μm in treated waste water (TWW) of 12 waste water treatment plants (WWTP) in Lower Saxony (Germany). At the WWTP Oldenburg a sample was taken before (bp) and after (ap) post-filtration. A: Percentage composition of synthetic polymers; B: Annual load of MP in the effluent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

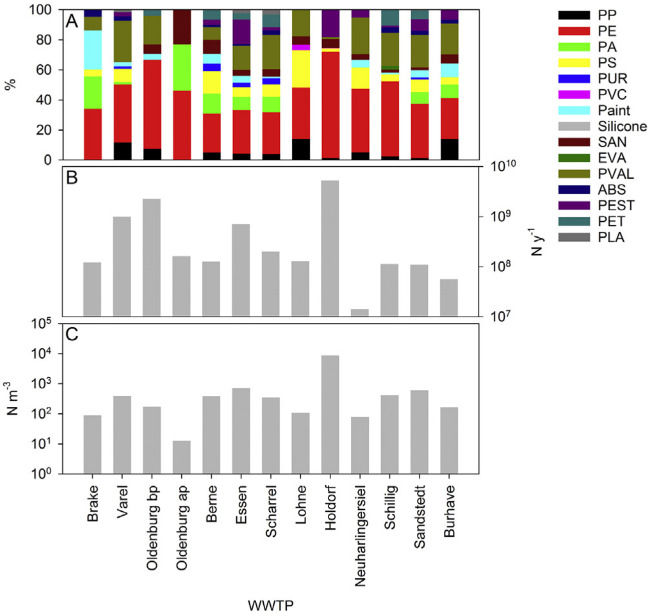

Fig. 2. Microplastics (MP) <500 μm in treated waste water (TWW) of 12 waste water treatment plants (WWTP) in Lower Saxony (Germany). At the WWTP Oldenburg a sample was taken before (bp) and after (ap) post-filtration. A: Percentage composition of synthetic polymers; B: Annual load of MP in the effluent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

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 and 100 μm (av. 59%), only 4% were determined bigger than 250 μm (Figs. S3B and S4B).

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 (Fig. 2C). 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 (Fig. 2B).

3.4. *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, Fig. 3A). PEST was found predominantly (av. 74%) followed by PA (av.17%) and PP (av. 9%, Fig. S2C). 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 (Fig. 3C). 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 (Fig. 3B).

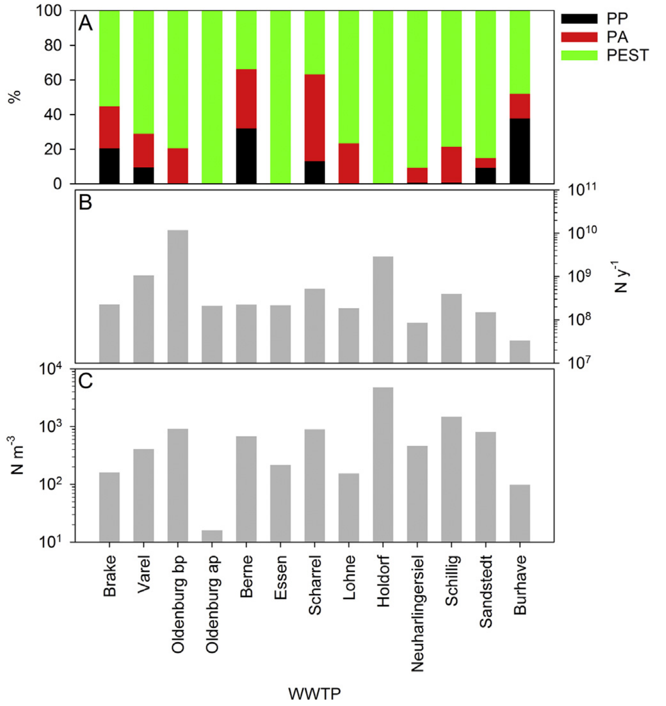

Fig. 3. Synthetic fibers in treated waste water (TWW) of 12 waste water treatment plants (WWTP) in Lower Saxony (Germany). At the WWTP Oldenburg a sample was taken before (bp) and after (ap) post-filtration. A: Percentage composition of synthetic polymers; B: Annual load of synthetic fibers in the effluent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

3.5. *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.

3.6. *MP in sewage sludge*

While none of the samples contained MP $>$ 500 μm, MP $<$ 500 μm was detected in all sewage sludge samples (Fig. S5A) made of PE, PP, PA and PS (Fig. 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 1×10^3 kg^{-1} (dry weight, dw) in Oldenburg to 2.4×10^4 kg^{-1} dw in Scharrel (Fig. S5C). This results in strong variations of the estimated amounts of MP in the yearly produced sewage sludge (Table S1). Estimations vary between the WWTPs from 1.24×10^9 y^{-1} in Schillig to 5.67×10^9 y^{-1} in Scharrel (Fig. S5B).

4. Discussion

4.1. *Sampling*

While previous studies examined MP in rather small volumes (<50 L) 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).

4.2. Sample purification

The enzymatic-oxidative purification is a new approach for MP extraction from environmental samples (Cole et al., 2014; Imhof & Löder 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.

4.3. 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. 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 ($n \times n$ 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 10–20 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 10 h 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.

4.5. 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, Fig. 2C). 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%, Fig. S2B), 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 and 100 μm (av. 59.2%), only 8.5% of MP was bigger than 200 μm (Fig. S4B). Since no other data on MP sizes in TWW exist and no correlation with sewage characteristics was determined, size classes are presented (Figs. 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 (Figs. 1C and 2C). 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 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 (Figs. 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%, Figs. 1–3) is added to the sewage sludge. But instead of determining higher MP loads, MP contamination was estimated the lowest (1×10^3 MP kg^{-1} dry weight sewage sludge, Fig. S5C) 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.

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 20 μm.
- 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.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2016.11.015.

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