Long-term assessment of nanoplastic particle and microplastic fiber flux through a pilot wastewater treatment plant using metal-doped plastics

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In recent years, several studies have investigated the flux of particulate plastic through municipal wastewater treatment plants (WWTP). Challenges related to time consuming analytical methods have limited the number of sampling points and detection limits have hampered quantification of nanoplastic and microplastic fiber fluxes through WWTPs. By synthesizing nanoplastic particles and microplastic fibers labeled with a rare metal (Pd and In, respectively) which can be measured as a proxy for the plastic itself, we have circumvented major analytical pitfalls associated with (micro)plastic measurements. In this study, we spiked the labeled materials to a pilot WWTP mimicking the activated sludge process (nitrification, de-nitrification and secondary Clarification). Using a mass flow model for WWTP sludge, we assessed the behavior of particulate plastic in relation to the removal of organic matter. Triplet samples were collected from the mixed liquor and from the effluent at least twice weekly over the entire experimental run time of 40 d. Our findings show that in discrete grab samples during steady state conditions, at least 98% of particulate plastics were associated with the biosolids. A positive correlation between total suspended solids (TSS) and plastic concentrations was observed in the sludge as well as in the effluent. Because of the strong association between particulate plastic and TSS, TSS removal is likely a good indicator of plastic removal in a full scale WWTP. Therefore, additional process steps in a full-scale WWTP which further reduce the TSS load will likely retain nanoplastic particles and microplastic fibers effectively and consequently increase the removal rates.

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1. Introduction

Reports on the occurrence of particulate plastic (nano- and microplastic particles and fibers) in the environment emerge on a weekly basis. However, analytical challenges and difficulties with sampling collection strategies interfere with a reliable quantification of the fluxes of particulate plastic through wastewater treatment plants (WWTP) (Horton et al., 2017; Rocha-Santos and Duarte, 2015; Shim et al., 2017). This is particularly true for nanoplastic particles (due to their small size and low concentration) and microplastic fibers (due to contamination during sampling and analysis) (Gigault et al., 2016; Schwaerts et al., 2019). Nevertheless, a sound understanding of transport processes is key for an assessment of the environmental fate of particulate plastics (Kawecki and Nowack, 2019; Kawecki et al., 2018). WWTP are at the interface between technical and natural systems (Dris et al., 2015), where potentially large quantities of particulate plastic resulting from either domestic sources (e.g. textile washing (Carr, 2017; Hernandez et al., 2017), personal care products (Napper et al., 2015)) or from mismanaged fragmented macroplastic waste and tire wear in road runoff may reach surface waters (Kole et al., 2017; Vogelsang et al., 2018; Wagner et al., 2018) or agricultural fields from sludge application (Kelessidis and Stasinakis, 2012; Keller et al., 2020; Zubris and Richards, 2005). Therefore, WWTP have been considered key hubs regarding the flux of particulate plastic into natural environments (Conley et al., 2019; Mason et al., 2016), and the presence of particulate plastic in effluent streams has led to speculation as to whether (particulate) plastic may pose a threat to environmental ecosystems (Enfrin et al., 2019). A number of studies

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investigating the removal of microplastics along the WWTP treatment chain have indicated that over 90% of the incoming plastics are effectively sequestered in the wastewater biomass and thus removed from the effluent stream (Carr et al., 2016; Lares et al., 2018; Talviite et al., 2017b). Nevertheless, the concentration of microplastics downstream of WWTP have been reported to be higher than those upstream of the plants, suggesting that cumulatively a measurable release of particulate plastic into receiving waters is conceivable. As the majority of particulate plastic is retained in the sludge, the burden of plastic pollution would shift with sewage sludge application to soil, or effectively be eliminated if sludge were incinerated. However, assessing particulate plastic fluxes through the WWTP have so far been based on limited surveys of individual WWTP at single time points, with few exceptions (Blair et al., 2019; Conley et al., 2019), and for the most part have excluded microplastic fibers and have not assessed nanoplastic particles at all.

To better understand the extent and distribution of particulate plastic in various technical and environmental compartments, including WWTP, more effective comparisons between various studies must be drawn (Chae and An, 2018). For this, standardization of analytical workflows and sampling protocols would greatly improve comparability and potentially allow for more-robust results for each study individually (Talviite et al., 2017a). Currently, a range of approaches have been adopted, though lower particle size cutoffs are often determined by the sampling setup (filtration using mesh sizes between 10 and 300 μm is most commonly applied) which can subsequently exclude smaller (e.g. nanoplastic) particle analysis. Extraction of microplastics from complex media, degradation of organic material and chemical analysis by an array of techniques (micro FT-IR, micro RAMAN, etc.) have considerably improved (Schwaferts et al., 2019; Shim et al., 2017), which has allowed for more reliable quantification of microplastic concentrations in various media, albeit with time consuming methods. However, the detection limits for commonly applied techniques that allow assessment of the particle size on a semi-automated basis are in the micrometer size range, which is already close to the diffraction limitation of the respective techniques. Although further advances in detector technologies will enable faster measurements in the future, the detection of individual nanoplastic particles will not be possible with these methods. Currently, the most pressing issues in assessing particulate plastic distribution in any media are 1) ensuring accurate sampling protocols to reliably assess the diversity of an inherently heterogeneous particulate plastic population, both in terms of particle size and particle composition, 2) measuring plastic particles in the sub-micrometer size range and 3) capturing time resolved data for particulate plastic fluxes in various systems, which will require reducing the time and labor of current analytical workflows.

To date, the time and resource intensive nature of particulate plastic analysis has hindered researchers from performing in-depth studies to understand the fate, transport and biological interactions of these materials. While progress is ongoing to develop analytical methods to measure particulate plastic in field studies and at the submicron scale (Mintenig et al., 2018), researchers who study these processes in bench top or pilot scale studies can take advantage of an entirely different approach. In the last years, we have synthesized a variety of particulate plastics, including nanoplastic particles (Mitrano et al., 2019) and microplastic fibers (Schmiedgruber et al., 2019), with a rare element embedded in the plastic which can be used to trace plastic by common analytical techniques for metals analysis, such as inductively coupled plasma mass spectrometry (ICP-MS). In this study, we calculated the flux of spiked particulate plastic through a pilot WWTP, based on measurements of the metal tracer. By using these Pd- and In-doped plastics, it was possible to quantify nanoplastic and microplastic fibers in a complex and heterogeneous wastewater matrix using ICP-MS. Besides circumventing the inherent difficulties of measuring nanoplastic and microplastic fibers specifically, the ease and speed of the procedure allowed us to measure up to daily samples over the course of several weeks. We, therefore, were able to 1) investigate replicate samples at each time point, 2) monitor plastic concentrations at several points, thereby allowing us to capture temporal fluctuations in the system and 3) follow plastic release patterns over an extended period of time and correlate them to standard properties commonly measured in WWTP, such as TSS content. We assessed the association between particulate plastic and biosolids over time and determined to what extent nanoplastic particles and microplastic fibers passed through the WWTP. Based on these results, we extrapolated the expected performance of full-scale municipal WWTP and anticipated the likely loads of particulate plastic to receiving waters using current water treatment technologies.

2. Materials and methods

2.1. Chemicals for plastic synthesis and feed stock suspensions

For all experiments, deionized water from a NANOpure Diamond purification system (Thermo Fisher Scientific, 18.2 M-Ω cm) was used for nanoplastic synthesis, dilutions, and preparing feed stock suspensions for the pilot WWTP. Chemicals used for nanoplastic synthesis included a selection of monomers (Acrylonitrile (>99%, Aldrich Chemistry), styrene (>99%, Sigma Aldrich) and DVB (>60%, Sigma Aldrich)), and initiator (water-soluble potassium persulfate (KPS) (Merck, ACS, Reag. Ph. Eur.), surfactants (potassium poly(ethylene glycol) 4-nonphenyl 3-sulfopropylether (KPE) (>99% Sigma Aldrich), sodium dodecyl sulfate (SDS) (>99%, Sigma Aldrich)) and a water soluble Pd precursor (K3PdCl4, 99%, ABCR). For fiber production, post-condensed PET in granular form was obtained from Terssuise Multils SA (PET #5997 with a density of 1.4 g/cm³ and viscosity index of VI 158). Indium (III) oxide nanoparticles (In2O3) were acquired from Nanographi (16–68 nm, 99.996% purity, as reported by the manufacturer). The feed suspension of microplastic fibers into the pilot WWTP was made in 0.001% surfactant (FL-70, Fisher Scientific).

2.2. Nanoplastic synthesis and characterization

Emulsion polymerization of nanoplastic spheres containing entrapped Pd were made in house and characterized following the protocol described in Mitrano et al. (Mitrano et al., 2019). Briefly, the procedure consisted of a two-step emulsion polymerization in which first the particle core was synthesized (which contained the metal), after which a further shell of polystyrene was grown through feeding a second monomer-containing solution over time to augment the surface chemistry and morphology of the original particle. For the nanoplastic core, the surfactant (SDS) and initiator were charged inside the reactor. When the initial nucleation point was achieved, the dissolved metal precursor and KPE were added slowly (over the course of 5 min) to the reaction vessel. The evolution of the polymer conversion and particle size were followed through dynamic light scattering (DLS) (Malvern Zetasizer Nano Z, backscatter angle 173° at 25 °C) and thermogravimetric analysis (TGA) at T = 120 °C (Mettler Toledo), respectively. After a conversion rate of approximately 90% was achieved, the shell feed was directly plumbed into the reaction vessel along with an additional shot of initiator (KPS) and water. The shell feed, a mixture of water, styrene, DVB and SDS, was dosed for 4 h. The particle growth and extent of conversion was again closely monitored with samples collected every half hour to follow the reaction progress. The
nanoplastics were stored at room temperature. To produce enough material for the entirety of the experiments, 0.8 L of nanoplastic stock was synthesized in one large batch. The solids content of the stock dispersion content was assessed by TGA (approximately 10% dry weight), total metal content was acquired by microwave (ultraCLAVE, MLS GmbH, Leutkirch, Germany) assisted acid digestion (see details below) followed by ICP-MS analysis (255 mg Pd/L) and the particle size and electrophoretic mobility was measured with the Malvern Zetasizer (z-average: 187 nm, polydispersity index: 0.04, zeta-potential (derived from the electrophoretic mobility): −43 mV). Scanning electron microscopy and transmission electron microscopy images along with the operational parameters of the microscopes can be found in the supplemental information (Figs. S1 and S2).

2.3. Microplastic fiber production and characterization

The main steps for microplastic fiber production included compounding, fiber melt-spinning and cutting the endless filaments into microplastic fibers. A PET compound containing indium oxide was prepared by melt extrusion in a co-rotating 36 L/D twin screw extruder (Dr. Collin GmbH, Germany) in two steps. First, 5 wt % of In₂O₃ was melt-mixed with 95% PET, followed by a second compounding with 95% PET, resulting in the final In nominal concentration of approximately 0.25 wt%. Corresponding extrusion temperature and output were 300 °C and 0.9 g/h, respectively. Prior to compounding, the polymer was dried at 140 °C for 8 h. The extruded polymer was quenched on a conveyor belt down to room temperature and subsequently pelletized (Fig. S3, upper panel). Fiber melt-spinning was carried out on a customized pilot melt-spinning plant originally built by Fourné Polymertechnik (Alfter-Impekoven, Germany). This pilot plant enables prototype production of fibers with a throughput of 0.1–5 kg/h. Further details about the pilot melt spinning pant can be found elsewhere (Hufenus et al., 2012). In this work, the pilot melt-spinning line was assembled with a single screw extruder with 14 mm diameter and length to diameter ratio of 25, which was used to melt and feed the polymer to a melt pump which in turn supplied the polymer melt at a fixed throughput to the spin pack (Fig. S3, lower panel). A static mixer was installed between extruder and melt pump to improve the distributive mixing and temperature uniformity of the melt stream. Extruder pressures needed for an effective operation of the melt pump was set to 40 bar, extruder and spin pack temperatures were graduated between 270 and 300 °C, and nominal throughput of the gear pump was 10.5 cm³/min. The draw ratio, namely the ratio between the winder speed and that of the take-up godet, was 3.5, applying a final winding speed of 700 m/min.

The fully drawn PET filaments proved difficult to cut in a reproducible fashion due to their flexibility and high tensile strength. To aid fiber cuttability, the filaments were annealed for 30 min at 200 °C to increase their crystallinity and thus render them more brittle. The load-strain behavior of the filaments before and after annealing was assessed using the Statimat ME+ tensile tester (Textechno, Germany) with 10 N load cell, following ASTM D2256 (Table S1).

Due to the large mass of (cut) microplastic fibers which were needed for the experiments, a device was built in-house to quickly and reproducibly cut the endless filaments than cutting by hand (e.g. with scissors or a knife) or using a cryotome (Fig. S4). Briefly, the microplastic fiber cutting device consisted of an assembly housing two stainless steel wheels, which counter-rotate when turning a crank lever. This further propelled and guided fibers between the wheels as the crank was spun. The wheels were equipped with teeth at an angle of 60°, where the main reason for the fibers breaking was the force between the wheels. The length of the resulting microplastic fibers was adjusted to approximately 500 μm, corresponding to the length of the fibers released by textiles when laundering (Hernandez et al., 2017), by selecting the angle of the teeth on the wheel and increasing the pressure on the filaments through tightening screws on the external housing of the device. Once cut, the fibers were collected via a vacuum hose into a water bath to securely capture them (Fig. S5). Finally, fibers were collected on a filter and allowed to air dry before being used in further experiments.

During processing, a subsample of cut microplastic fibers was observed under a stereo microscope (Olympus SZX 10) to ensure complete scissions were made with the cutting device. Fiber length (distribution) and diameter were measured using an Olympus DP 72 camera and the Olympus CellSens software (Fig. S6). Average fiber length was 500 μm ± 12.2 μm and 30 μm in diameter. The Indium content of randomly selected fibers from each spool averaged 0.213 ± 0.005 wt% (Table S2).

2.4. Pilot wastewater treatment plant (WWTP) setup and operational procedures

The pilot WWTP located at Eawag was fed with municipal wastewater and simulates the activated sludge system, consisting of a non-aerated tank (92 L, denitrification stage) and aerated tank (147 L, nitrification stage) as well as secondary clarifier (150 L) with an internal sludge recirculation. A schematic layout of the activated sludge system (our experimental boundaries) and associated treatment facilities is given in Fig. 1.

Two months prior to spiking the pilot WWTP with particulate plastic, the tanks were filled with activated sludge from a full-scale WWTP (ARA, Neugut, Duebendorf, Switzerland) and operated under the conditions listed in Table 1 to establish the activated sludge process. The activated sludge process was monitored using both automated sensors and manual performance tests for chemical oxygen demand (COD), NH₄, NO₃, temperature, pH and total suspended solids (TSS) (Table 1). In the influent, the average COD was 485 mg/L ± 1.2 mg/L and NO₃ was 1.4 mg/L ± 0.2 mg/L. The background Pd and In concentrations in the influent were both below the ICP-MS detection limit (approximately 0.07 μg Pd or In/L). The activated sludge was internally recirculated at a rate of 47 L/h (ratio of recirculated sludge to wastewater inflow = 2:1). The WWTP was operated at a hydraulic retention time (HRT) of 17 h and sludge age (SA) of 15 d. The oxygen concentration in the nitrification reactor was kept between 2 and 3 mg/L by periodically aerating the reactor. The pH in both the nitrification and the denitrification reactors averaged 7.5 across the experiment with a temperature between 19 and 22 °C. Excess sludge was withdrawn from the nitrification reactor at a rate of 17.5 L/d into a thickener, where it was mixed with primary sludge (50/50). After thickening, the sludge mixture was pumped into an anaerobic digester. The flow rate of the effluent of the secondary was between 22 and 23 L/h, with average values for COD 31.2 mg/L ± 0.9 mg/L, NO₃ 10.9 ± 0.3 mg/L and NH₄ 0.06 mg/L + - 0.04 mg/L.

2.5. Particulate plastic stock suspensions and feeding into the pilot WWTP

Three stages of particulate plastic feeding were performed including 1) a loading phase of 72 h, to increase concentration of the particulate plastic in the sludge to a selected value, 2) a continuous feeding phase of 598 h, to maintain the particulate plastic concentration in the sludge at a constant value, corresponding to a metal concentration of 150 μg metal/L through steady addition and 3) a clearing phase of 330 h, where no particulate plastic was added to investigate removal of plastic from the system. The plastic particles
were assumed to immediately attach to the biosolids and the WWTP was treated as a continuously stirred tank reactor as described in Kaegi et al. (2011). Spiked particulate plastic concentrations were chosen based on the known analytical detection limits of the metal-doped plastics in activated sludge from our previous work.

Nanoplastic particles were dispersed in DI H₂O and remained stable for extended periods of time, as assessed through bi-monthly DLS and ICP-MS measurements of the stock suspension (data not shown). Depending on the feeding schedule (loading phase or continuous phase), nanoplastic concentrations of 13.78 mg plastic/L and 2.5 mg plastic/L (equivalent to 6.89 mg Pd/L and 1.25 mg Pd/L) were prepared in 5 L glass Schott bottles. The suspension was continuously stirred at 300 rpm using a magnetic stirrer. Across the experiment, 24.2 g of nanoplastic, equating to approximately \( \frac{1}{10^{16}} \) particles, were spiked into the pilot WWTP.

Due to the increased surface tension and higher density of the microplastic fibers, additional measures needed to be taken to ensure fibers were evenly dispersed in the stock suspension for feeding. A 5 L glass Schott bottle was filled with a 0.01% FL-70 surfactant solution made up in DI H₂O and spun with a magnetic stir bar at 300 rpm. Fibers were slowly added while continuously stirring with a metal spatula to disperse and separate fiber aggregates until the final desired mass loading was reached (27 mg fiber/L for the loading phase and 5.04 mg fiber/L for continuous feeding phase, equivalent to 6.89 mg In/L and 1.25 mg In/L). Once fibers were visibly dispersed, the suspension was transferred to the feeding platform of the WWTP, where the magnetic spin bar was replaced with a mechanical stainless steel mixer which provided greater mixing force. When continuously stirred, the microplastic fibers remained evenly dispersed but would quickly settle if/when stirring was interrupted. In total, 30.3 g PET fiber, equating to approximately \( \frac{2.2}{10^{7}} \) individual microplastic fibers, were fed into the pilot WWTP.

To feed the particulate plastic suspensions into the pilot WWTP, a programmable peristaltic pump (Ismatec, MPC-Z gear driven pump) equipped with two double mounted pump heads (Masterflex, Easy-load PPS/SS) and 3.2 mm diameter tubing (Masterflex) was used (Fig. S7). Additional information on pumping rates can be found in the supplementary information (Fig. S8). Although there were physical differences (particle size, shape, polymer) between the nanoplastic particles and microplastic fibers, the same feeding system was used for both materials. The stock feeding solutions were replenished every 2–4 days. See supplemental information for further assessment of the testing and reliability of the feeding protocol. Despite prior testing, the fiber suspension still clogged in the peristaltic pump tubing over extended time periods and fewer fibers were delivered to the pilot WWTP than initially envisioned. Therefore, after the dosing of each feed stock aliquot, fibers remaining in the feeding flask were carefully collected, dried, and weighed to adjust the expected feeding concentration to the effective feeding concentration. This effective feeding concentration was used for calculating the recovery of fibers over the course of the experiment and for the evaluation of the mass balance.

**2.6. Sampling strategy**

Sampling frequency was adjusted based on the phase of

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**Table 1**

Operational parameters for and characteristics of the pilot WWTP. BDL indicates below detection limit.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (average)</th>
<th>Unit</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pilot Scale WWTP Physical Set-up</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Non-aerated tank</td>
<td>92</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td>Aerated tank</td>
<td>147</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td>Secondary clarifier</td>
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<td>L</td>
<td></td>
</tr>
<tr>
<td>Wastewater inflow</td>
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<tr>
<td>Excess sludge removal</td>
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<td>L/d</td>
<td></td>
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<tr>
<td>Hydraulic retention time</td>
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<td>h</td>
<td></td>
</tr>
<tr>
<td>Sludge age</td>
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<td>d</td>
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<tr>
<td><strong>Chemical Parameters Sludge</strong></td>
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<td></td>
</tr>
<tr>
<td>Chemical oxygen demand</td>
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<td>mg/L</td>
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</tr>
<tr>
<td>NO₃</td>
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<td>mg/L</td>
<td>0.2</td>
</tr>
<tr>
<td>Oxygen concentration</td>
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<td>pH</td>
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<td>BDL</td>
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</tr>
<tr>
<td>Background In</td>
<td>BDL</td>
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<td></td>
</tr>
<tr>
<td><strong>Chemical Parameters Effluent</strong></td>
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<tr>
<td>Chemical oxygen demand</td>
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<td>mg/L</td>
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</tr>
<tr>
<td>NH₄</td>
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<td>mg/L</td>
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<td>NO₃</td>
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<tr>
<td>Background Pd</td>
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<tr>
<td>Background In</td>
<td>BDL</td>
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</tbody>
</table>

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Fig. 1. A simplified schematic of a municipal WWTP. The activated sludge system, the operational boundaries for our experiment, is indicated by the orange dashed line. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
particulate plastic addition. During the loading phase \((t = 0–72 \text{ h})\), samples were collected three times per day from the activated sludge (nitrification tank) and effluent (supernatant of the secondary clarifier tank). During the continuous feeding phase \((t = 72–672 \text{ h})\), samples were taken from the same locations approximately every 48 h. At each time point, triplicate samples were collected, where an aliquot \((10 \text{ mL})\) was immediately digested and analyzed for the metal content and another aliquot \((40 \text{ mL})\) was stored at \(-20 \degree \text{C}\) as a backup.

2.7. Assessment of total suspend solid (TSS) in the activated sludge and effluent

TSS in the mixed liquor and effluent were measured weekly before initiating the spiking experiments and at each measurement point during the plastic dosing phase of the experiment. After pre-drying a filter paper (Whatman, Glass Microfilter Filter, 1.5 \(\mu\)m pore diameter) in an oven at 105 \(\degree\)C for 60 min, the dry-weight was obtained using a laboratory balance (Mettler Toledo, AX 205) and subsequently placed into the filtration funnel of a vacuum filtration unit. After wetting the filter paper, 150 mL of mixed liquor or 500 mL of effluent were filtered to retain the biosolids. The filter paper was placed in an oven at 105 \(\degree\)C for 60 min, allowed to cool and subsequently re-weighed to assess TSS.

2.8. Digestion protocols

For sample digestions, hydrogen peroxide \((\text{H}_2\text{O}_2, >30\%, \text{Sigma Aldrich})\), stored in the refrigerator at 5 \(\degree\)C wrapped in aluminum foil, nitric acid \((\text{HNO}_3, 65\%, \text{Merck})\), and sulfuric acid \((\text{H}_2\text{SO}_4, 95–98\%, \text{Sigma Aldrich})\) were used. Sample digestion was performed in a microwave system (ultra-CLAVE 4, MLS GmbH), operated at a pressure of 120 bar and temperature of 250 \(\degree\)C for 10 min. Digestion tubes and caps were made of Teflon, with sample volumes of 10 mL. The digestion protocol was optimized to digest both particulate plastic and organic matter in the samples. First, 0.4 mL of \(\text{H}_2\text{O}_2\) was added to the sample directly in the digestion tube and allowed to stand for 30 min. Second, 4 mL of \(\text{HNO}_3\) was added and again the sample was left to rest for 30 min. Finally, 0.2 mL of \(\text{H}_2\text{SO}_4\) was added and the samples were loaded into the microwave system. After digestion, the samples were quantitatively transferred to polypropylene FacIon tubes with DI \(\text{H}_2\text{O}\) with a final volume of 50 mL.

2.9. ICP-MS analysis

Elemental analysis was performed by ICP-MS (Agilent Technologies, QQQ 8900) featuring an integrated sample introduction system (ISIS), microMIST spray chamber and nickel cones. A standard curve was performed on each day of ICP-MS analysis. Ionic palladium (Pd) and Indium (In) standard solutions \((10,000 \text{ mg/L and 1000 mg/L, respectively, Sigma Aldrich})\) were diluted and used for calibration solutions, made fresh daily, at concentration of 0, 0.5, 1, 2.5 and 5 \(\mu\)g/L. An ionic Rhodium standard \((10,000 \text{ mg/L, Sigma Aldrich})\) was diluted to approximately 100 \(\mu\)g/L as an internal standard during all sample measurements.

3. Results and discussion

3.1. Mass balance model and particulate plastic concentrations assessed in the activated sludge

The expected Pd and In concentrations, reflecting the spiked nanoplastic and microplastic fiber concentrations in the excess sludge and in the effluent, were calculated by treating the WWTP as a continuously stirred reactor assuming that the plastic particles immediately attach to the biosolids, as previously described by Kaegi et al., 2011. If the particulate plastic was removed proportionally to the sludge (TSS) in the secondary clarifier, we can argue that 1) particulate plastic is associated with the wastewater biosolids and thus is removed in the same proportion as the biosolids and/or 2) that the particulate plastic does not functionally differ from other solids in the WWTP and thus similar removal of particulate plastic can be expected as other solids in the system. For the mass balance calculations, we assumed instantaneous, uniform and complete mixing of the particulate plastic within the mixed liquor and identical particulate plastic attachment to the biosolids in the recirculated sludge, the excess sludge, and the effluent. The (time resolved) mass fluxes of particulate plastic were calculated by scaling the Pd and In concentrations measured in the excess sludge and in the effluent to their respective concentrations in the labeled plastic and multiplying these plastic concentrations with the volumetric flow rates of the excess sludge removal and of the effluent. Further details and assumptions included in the model can be found in the supplemental information.

By assuming a constant TSS concentration in the mixed liquor/ excess sludge and in the effluent over time \((3 \text{ g/L in the mixed li-}

quent and 5 \text{ mg/L in the effluent})\), the expected concentrations of plastic, derived from the associated metal concentrations, were calculated and the spiking concentrations of the particulate plastic were estimated. The TSS measured over the course of the experiment fluctuated both in the sludge and in the effluent \((\text{Fig. 2A})\). In the model calculations, we therefore adjusted the TSS contents in the mixed liquor and effluent correspondingly at each phase of the experiment. For both nanoplastic and microplastic fibers, the measured concentrations of Pd and In remained constant during the steady state feeding of the pilot WWTP \((\text{Fig. 2, panels B and C})\) and generally followed the predicted trends of the concentrations derived from the mass balance calculations. A higher metal-doped particulate plastic concentration than expected was observed in the effluent during the loading phase \((t < 72 \text{ h})\) \((6.3\% \text{ for nanoplastic particles, 10.64\% for microplastic fibers})\), potentially due to limited attachment of the spiked plastic particles with the biosolids during the high (pulse) influx of particulate plastic into the system. Due to the logarithmic scale of \(\text{Fig. 2},\) this effect can only be seen in the effluent and not in the mixed liquor phase. However, throughout the continuous feeding phase, the concentration of the plastic in the effluent decreased to approximately 1% of the plastic in the mixed liquor for the remainder of the experiment, in agreement with the expected proportions from the sludge model corrected for actual TSS values. After ceasing to add particulate plastic into the pilot WWTP at \(t = 744 \text{ h}(\text{i.e. the clearing phase})\), the plastic concentration in the mixed liquor decreased, although only one data point was collected during this time.

The high microplastic fiber concentrations in the stock suspension during the initial loading phase caused clogging and blockages in the peristaltic pump tubing during the initial loading phase, disrupting delivery of microplastic fibers to the pilot WWTP. The feeding system was monitored frequently \((\text{several times daily})\), and fibers were additionally spiked when needed. Adjustments to the fiber feeding strategy and clumping of fibers in the more concentrated stock suspension may have resulted in an uneven loading and distribution of microplastic fibers in the mixed liquor \((\text{i.e. small entanglements of fibers})\). In combination with the decreased delivery of microplastic fiber mass into the pilot WWTP, this explains the perpetually lower In \((\text{microplastic fiber})\) concentration measured in the mixed liquor than predicted based on the model calculations. Fewer challenges with feeding microplastic fibers were evidenced in the continuous feeding phase, when the fiber concentration in the stock suspension was six times lower.
equivalent volumes, particularly for effluent samples. This is likely due to the higher number of individual nanoplastics in the system compared to the microplastic fibers in the same sample volume. Given the metal concentration in the fibers and their average dimensions, approximately 200 fibers per 10 mL of mixed liquor and 3 fibers per 10 mL of effluent were calculated for the continuous feeding phase. Particularly for the effluent samples, a change in the sampling strategy to collect fibers from an increased sample volume through e.g. filtration may have improved the precision and, thus, decreased the standard deviation between these measurements.

Diurnal and temporal variations of pollutants in wastewater flows have to be considered by assessing annual loads of pollutants of interest (Georgantzopoulou et al., 2018; Ort et al., 2010). Caused by the limited sample throughput of currently established analytical techniques for microplastic analyses, reported removal efficiencies of microplastic particles during wastewater treatment are based on a limited amount of samples collected from a few treatment steps. Assessing the temporal fluctuations of microplastic in- and outputs through time-resolved sampling or through collecting multiple samples for triplicate analyses is thus unrealistic using currently available microplastic analyses schemes. Our method for particulate plastic measurement by way of measuring metals enabled us to take many samples over an extended period of time and allowed us to accurately construct a mass balance of particulate plastic over the course of the experiment. Based on the calculated plastic particle fluxes and in combination with the fraction of TSS which is lost via the WWTP effluent, allowed us to better describe the behavior of microplastics during the activated sludge treatment. Because the inflow of plastics into the pilot WWTP was well controlled, any fluctuations observed in the plastic concentrations can be related to operational parameters of the pilot WWTP. At steady state conditions (continuous phase) we observed only negligible fluctuations in the concentrations of our metal-doped plastics, and thus operational variability of the pilot WWTP was of minor importance during this phase. In contrast, lower than expected removal rates were found during the dynamic loading phase, when the spiking concentrations were high and the system was not yet at equilibrium. Our results, thus, suggest that high pulses of plastic into the activated sludge system may result in a higher transmission of the plastic particles through the WWTP than observed during steady state conditions.

To date there has been little investigation as to if and to what extent plastic may degrade within the WWTP. In laboratory testing conditions, water sheer forces have been shown to induce nanoplastic formation from microplastic fragments (Enfrin et al., 2020). However, these principals may not be directly applicable in the context of water treatment depending on differences in the strength of sheer forces, the relatively short residence time in the WWTP, and the unlikelihood of chemical or biological degradation of the polystyrene and polyester studied here under our test conditions. Therefore, in this context we hypothesize that the plastic particles which were introduced into the WWTP do not significantly change in size, shape or chemistry after passing through the WWTP. However, we cannot completely exclude the possibility of fragmentation. Nevertheless, even in the unlikely case of fragmentation, we anticipate that these fragments would ultimately have the same fate as the larger particulate plastics which were introduced to the system: that they would still largely be associated with the sludge flocs and be retained in the WWTP sludge.

### 3.2. Particulate plastic association with total suspended solids

During the steady state conditions (continuous feeding phase), the proportions of Pd and In, reflecting nanoplastic particles and compared to the fiber concentrations in the stock suspension used during the loading phase. Therefore, it is recommended to feed a more dilute concentration of fibers at higher flow rates or to increase the time for the initial loading phase to reach comparable plastic concentrations in the mixed liquor in similar experiments in the future. When the initial spiking concentration of In in the model was matched to the measured concentration of In at the end of the loading phase (i.e. adjusted to the anticipated effective dosing concentrations), the measured tracer concentrations during the steady state operation of the WWTP were in line with the model predictions (Fig. 2).

At each sampling instance, a low variability (Sludge: 4.7%; Effluent: 7%) in Pd (nanoplastic) concentrations between triplicate samples suggested that the plastic was well mixed within the activated sludge for a 10 mL sample. Notably, In (microplastic fibers) concentrations showed a higher variability (Sludge: 13.32%; Effluent: 58.79%) than nanoplastic particles when sampling

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**Fig. 2.** The total suspended solids (TSS) in both the mixed liquor and effluent (panel A), and the corresponding measured nanoplastic concentrations (as assessed by Pd concentrations) (panel B) and microplastic fiber concentrations (as assessed by In concentrations) (panel C). The experiment comprised of three distinct phases of particulate plastic feeding: (I) initial dosing of a high concentration of plastic (shaded red), (II) continuous dosing of particulate plastic, corresponding to the steady state concentrations under the respective operational conditions (shaded blue) and (III) no dosing of additional particulate plastic, where plastic already in the system was cleared/eliminated (shaded green). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
The mass fraction of nanoplastic particles to TSS (Pearson correlation coefficient $r = 0.94$) was obtained implying that the relationship between the nanoplastic and TSS is independent of time. This suggests that nanoplastics are dominantly associated with the biosolids and removed during secondary clarification along with the biosolids, as has been documented for other nanoscale particles (Dris et al., 2018; Murphy et al., 2016; Wang et al., 2012; Westerhoff et al., 2011). Generally, a similar trend was observed for microplastic fibers and TSS in the effluent (Fig. 3C), but the trend was not as clear as with the nanoplastics and TSS (Pearsons correlation coefficient $r = 0.61$, Fig. 3D). This difference likely stems from hotspots of microplastic fibers in suspension, which are not adequately assessed due to small sample sizes, opposed to a decreased association between microplastic fibers and sludge flocs compared to nanoplastics. Thus, this positive correlation between particulate plastic and TSS suggests that reducing TSS loads in the effluent would also result in a proportional reduction of plastics being released to receiving waters.

### 3.3. Mass balance of particulate plastic through the WWTP

In total, 99.7% and 82.1% of nanoplastic particles and microplastic fibers, respectively, were recovered (i.e. measured via their respective metal content to assess mass closure) over the course of the experiment (Fig. S9). As the experiments were conducted in parallel using the same reactors, temporal fluctuation of the TSS cannot explain the lower recovery obtained for the microplastic fibers compared to the nanoplastic particles. The poorer recovery of the microplastic fibers could have originated from a number of sources including 1) a consequence of the small sampling sizes which did not fully capture the microplastic fiber concentration at each time point and/or 2) an inadequate feeding strategy which led to incomplete dispersion of fibers in the mixed liquor. In the latter case, the fiber entanglements could potentially be removed to a greater extent than individual fibers attached to sludge flocs since they would more likely settle in the secondary clarifier. Their increased size and density would mean they would have a reduced likelihood of exiting the pilot WWTP via the effluent. The initial pitfalls with the microplastic fiber feeding strategy ultimately led to a decreased concentration of microplastic fibers in the WWTP than initially aimed for, resulting in incomplete recovery.

Over the course of the entire experiment, we determined the flux of both plastics and biosolids through the pilot WWTP to assess retention of particulate matter in system. 66.5% of the nanoplastic particles were associated with the excess sludge and 31.1% were recovered from the effluent. For microplastic fibers, a similar proportion was calculated in the effluent (29.4%), yet only 49.3% was recovered in the activated sludge. Our study exclusively targeted the activated sludge process and thus, lower removal efficiencies of TSS as compared to full-scale WWTP (which also include primary clarification) are conceivable. For example, the primary clarifier

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**Fig. 3.** The fluctuation of nanoplastic particle concentration recovered in the effluent tracked well with the proportion of total suspended solids (TSS) concentration in the effluent (A). The mass fraction of nanoplastic particles to TSS (B) were positively correlated, with a Pearson correlation coefficient of $r = 0.9415$. The microplastic fiber concentration did not appear to track the TSS content as well (C), which is reflected in a lower Pearson correlation coefficient of $r = 0.6133$ (D). Total TSS and total metal are the sum of either TSS or metal in both the activated sludge and effluent.
Likewise, most WWTP systems would have a higher removal efficiency of TSS, e.g. through additional treatment steps such as sand filtration and tertiary filtration, compared to our pilot system, which is only equipped with a secondary clarifier. Our results support our hypothesis that particulate plastics aggregate with TSS and that this aggregation and settling of plastic/TSS aggregates is responsible for the retention of plastics in the sludge. This is why plastics of different shape, size and density are effectively retained in the system. We anticipate that the strong association between particulate plastic and TSS would remain the same in full-scale WWTP as we have seen in our pilot WWTP. Therefore, actions which reduce the TSS load of the effluent would consequently reduce particulate plastic concentrations to receiving waters.

4. Conclusions

The influx of particulate plastic into the urban wastewater system can come from a variety of sources, resulting in a poly-disperse population in terms of size, shape, polymer and arrival time to the WWTP. In full-scale WWTP, elevated loads of particulate plastic may result from industrial discharges (e.g. holding basins which are released). This discontinuous discharge from industrial sites would translate to temporally increased plastic loads in the influent of WWTP, with possibly reduced retention rates of particulate plastic. However, in principal, these industrial sources would not affect municipal WWTP in most locations and thus a more continuous influx of particulate plastic is anticipated under most conditions. In our study, a constant feed of particulate plastic was spiked over time to correlate with realistic input parameters with removal rates.

Currently, it is not possible to measure nanoplastic particles in full-scale WWTP and, while feasible, it is lengthy and cumbersome to assess microplastic fibers, fragments and films because of analytical challenges. While analytical capabilities are constantly being refined and improved, a robust and reliable monitoring strategy for nanoplastic particles and microplastic fibers seems still far away and is unlikely to be developed in the near future. However, if the processes which control the removal of plastics in WWTP (or engineered systems in general) are understood, then it will be possible to extrapolate results on the fate and behavior of plastic particles obtained from simplified model systems, such as pilot WWTP to full-scale systems. In this study, the use of our metal-doped particulate plastics has enabled us to investigate the behavior of nanoplastic particles and microplastic fibers during the activated sludge process, a key element in modern wastewater treatment plants. Our finding, based on the analyses of a large set of samples collected over several weeks, allowed us to estimate the degree of nanoplastic particle and microplastic fiber removal in full-scale WWTP. A strong correlation between particulate plastics and TSS was observed in this study, in agreement with the results from our previous batch experiments suggesting an efficient (hetero)aggregation between particulate plastic and biosolids in less than half an hour (Mitrano et al., 2019; Schmiedgruber et al., 2019). Considering the relevant time scales of the path of particulate plastic to the WWTP (e.g. through sewers and all treatment stages), it is highly likely that in larger scale systems nanoplastic particles and microplastic fibers will be largely associated with TSS, and a large fraction of which would already be removed by settling in a primary clarifier in a full-scale WWTP.

Given the strong correlation between particulate plastic and TSS in this study and as well as the high retention rate of particulate plastic observed during wastewater treatment processes in other published literature, there may be little added value in continuing to perform removal efficiency studies for municipal WWTP. While investigating the flux of particulate plastic may not provide any additional information, understanding the particle population in terms of number, size, and polymer may still be of interest. However, because of the high temporal fluctuations in the influent and effluent, the particle population moving through the WWTP is not well characterized by current studies. As most particulate plastic has been assessed to remain with the biosolids, it would be most beneficial (i.e. more efficient) to characterize the plastic particles residing in the digested sludge. The sludge age of the digested sludge is typically in the order of 30 days and thus, temporal fluctuations in type and concentration of plastic particles in the inflow will be well-balanced. By focusing on sampling strategies and analysis of particulate plastic in the digested sludge, this would save time (opposed to sampling every stage of wastewater treatment) and provide increased understanding for the material that may enter the environment in those locations where sludge is still used as an amendment to agricultural soils. Based on the strong correlation between particulate plastics and TSS, the known TSS removal efficiencies and flow rates of a given full-scale WWTP, the plastic particle concentrations in the inflow and in the outflow of full-scale WWTP may be estimated based on the concentrations observed in the sludge. The strong association between particulate plastic and TSS likely makes the TSS removal a good and easy way to access indicator of plastic removal rates in a full scale WWTP. Therefore, additional process steps in a full-scale WWTP which further reduce the TSS load will likely retain nanoplastic particles and microplastic fibers effectively and consequently increase the removal rates.

5. Associated content

Additional information is provided in the supplemental information including SEM (Fig. S1), TEM images and EDX of the nanoplastic particles (Fig. S2), a schematic of filament compounding and spinning line (Fig. S3), physical properties of filaments (Table S1), schematic of microplastic fiber cutting device and workflow (Figs. S4 and S5), images and length distribution of microplastic fibers (Fig. S6), assessment of indium content in microplastic fibers (Table S2), assessment of particulate plastic feeding strategy (Figs. S7 and S8, additional discussion), further mass balance model explanation calculations and measured versus sludge model calculations of plastic in the experiment (Fig. S9).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watres.2020.115860.

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