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Particle balance and return loops for microplastics in a tertiary-level wastewater treatment plant

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ABSTRACT

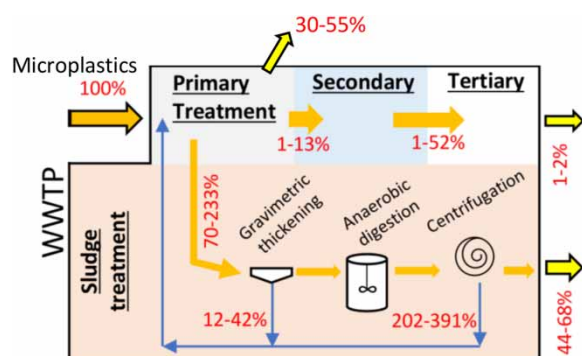
Microplastics (MPs) from households, stormwater, and various industries are transported to wastewater treatment plants (WWTPs), where a high proportion of them are captured before discharging their residuals to watersheds. Although recent studies have indicated that the removed MPs are mainly retained in wastewater sludge, sludge treatment processes have gained less attention in MP research than water streams at primary, secondary, and tertiary treatments. In this study, we sampled twelve different process steps in a tertiary-level municipal WWTP in central Finland. Our results showed that, compared to the plant influent load, three times more MPs circulated via reject water from the sludge centrifugation back to the beginning of the treatment process. Especially fibrous MPs were abundant in the dewatered sludge, whereas fragment-like MPs were observed in an aqueous stream. We concluded that, compared to the tertiary effluent, sludge treatment is the major exit route for MPs into the environment, but sludge treatment is also a return loop to the beginning of the process. Our sampling campaign also demonstrated that WWTPs with varying hydraulic conditions (such as the one studied here) benefit from disc filter-based tertiary treatments in MP removal.

Key words: enzymatic purification, microplastics, reject water, return loop, wastewater sludge

HIGHLIGHTS

- Dewatering by centrifugation was a step that removed a high number of MPs from the sludge.
- Sludge retained especially the fibrous microplastics.
- Reject water transported microplastics inside a wastewater treatment plant.
- Disc filter based tertiary treatment ensured removal of 99% of microplastics in wastewater.

GRAPHICAL ABSTRACT



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INTRODUCTION

Microplastics (MPs) are small (<5 mm), synthetic or partly synthetic polymer particles that have either been manufactured small (primary MPs) or have formed by the weathering of larger plastic items (secondary MPs, [Frias & Nash 2019](#); [Hartmann et al. 2019](#)). The fate of MPs in wastewater treatment plants (WWTPs) is of central importance due to high MP concentrations in untreated raw wastewater originating from households, stormwater, and various industries. Previous studies have indicated that despite MPs being trapped from wastewater rather efficiently with the existing treatment methods, WWTPs represent point sources of MPs into the environment ([Gatidou et al. 2019](#); [Sun et al. 2019](#)). MPs are released to the receiving watersheds in treated effluents and to terrestrial environment in wastewater sludge that is used on land applications and construction sites ([Mahon et al. 2017](#); [Talvitie et al. 2017a](#); [Li et al. 2018](#); [Gatidou et al. 2019](#)). [Zhang & Chen \(2020\)](#) suggested that, in general, wastewater sludge could be at the forefront of all MP sources.

In WWTPs, plant influent (raw wastewater) undergoes treatments typically categorized as primary, secondary, and tertiary treatments and sludge treatments. In the beginning of the primary treatment, coarse and fine screening decreases the size of the plastics entering the following treatments but does not effectively remove MPs ([Gatidou et al. 2019](#); [Sun et al. 2019](#)). [Talvitie et al. \(2017a\)](#) have reported that grit removal and primary sedimentation treatments removed 97% of the microlitter, including MPs, in a municipal WWTP in Helsinki, Finland. Secondary treatments, comprised of the activated sludge process and secondary sedimentation, increased the removal rate further by 7–20%, leading to good removal rates ([Talvitie et al. 2017a](#)). However, the daily discharge of microlitter was 1.97×10^8 via plant effluent ([Talvitie et al. 2017a](#)). Similarly, [Gies et al. \(2018\)](#) reported that a secondary-level WWTP in Vancouver, Canada, removed up to 99% of incoming MPs, but the WWTP still released billions of MPs annually. [Sun et al. \(2019\)](#) calculated a synthesis based on concentrations from several published studies and summarized that WWTPs without tertiary treatments removed approximately 88% of the MPs in untreated raw wastewater. With tertiary treatments – mechanical, biological, or chemical technologies situated at the end of the wastewater treatment process to complete the removal of dissolved or solid materials – efficiency increased to 97%, according to [Sun et al. \(2019\)](#). [Hidayaturrahman & Lee \(2019\)](#) studied various tertiary treatments in four WWTPs in South Korea and found that ozone, a membrane disc filter, and rapid sand filtration decreased MP concentrations by 98.9–99.2%, compared to concentrations in the influent. Decreases due to only primary treatments were 56.8–64.4%, and decreases due to primary plus secondary treatments were 42.1–77.3% ([Hidayaturrahman & Lee 2019](#)). [Carr et al. \(2016\)](#) reported that tertiary treatments were effective in MP removal in WWTPs in Southern California. However, primary and secondary processes already reached rates above 99%. In line with these results, [Zhang & Chen \(2020\)](#) summarized that up to 99% of the MPs in the wastewater inflow end up in the treated wastewater sludge.

The variation of the abundance of MPs in different sludge processing steps is a knowledge gap in MP research in WWTPs. Unlike the effects of primary, secondary, and tertiary treatments in the aqueous phase in the WWTPs, there has been less detailed research on how MPs are transported and transformed within the sludge treatment processes, despite demonstrations of a large amount of microplastics in the final dewatered sludge ([Lares et al. 2018](#); [Gatidou et al. 2019](#)). [Mahon et al. \(2017\)](#) studied MPs in treated sludge in seven WWTPs in Ireland and reported concentrations from thousands to tens of thousands of MPs per kilogram of dry weight sludge. [Talvitie et al. \(2017a\)](#) reported in their particle balance study for the WWTP in Helsinki that 80% of the microlitter in plant influent is transported out from the WWTP via the treated sludge. This corresponded to $>10^{11}$ microlitter particles per day ([Talvitie et al. 2017a](#)).

The study by [Talvitie et al. \(2017a\)](#) indicated that a notable amount of microlitter circulated inside the WWTP within the reject water that are returned to the primary and secondary treatments. However, the process where the circulating litter originated was not specified. Previous studies have also indicated that especially fiber-like MPs were retained in treated sludge more easily than fragment-like particles ([Mahon et al. 2017](#); [Talvitie et al. 2017a](#); [Lares et al. 2018](#); [Edo et al. 2020](#)) and the possibly selective processes are a focus of interest, too. On their route from primary to treated sludge, MPs have been proposed to have negative impacts on microbial populations in the WWTP process ([Zhang & Chen 2020](#)) and membrane-based treatments ([Enfrin et al. 2019](#)). On the other hand, microbes have been proposed to degrade MPs during sludge treatment ([Mahon et al. 2017](#)). MPs have also been suggested to shred during treatments, and more studies on that have been called for ([Enfrin et al. 2019](#)). A comprehensive investigation of MPs at different subprocesses of sludge treatment is therefore of central importance in understanding the contribution of these processes to a WWTP's MP balance.

The purpose of this study was to scrutinize the fate of MPs (20–5,000 μm) in a municipal wastewater treatment plant in central Finland that utilizes a conventional activated sludge process followed by a disc filter as a tertiary-level treatment and anaerobic digestion for sludge treatment. Samples were purified enzymatically to isolate MPs, according to Löder *et al.* (2017), with small practical modifications targeted for sludge samples, and MPs were characterized with Raman microscopy. To scrutinize the route of microplastics in more detail and to tackle the knowledge gap of the fate of MPs in various sludge treatment processes, we collected samples from twelve different phases of the WWTP treatment process.

METHODS

Sampling site

This study was done at the Nenäinniemi wastewater treatment plant (hereafter the ‘Nenäinniemi WWTP’), located in Jyväskylä, central Finland. The Nenäinniemi WWTP treats municipal wastewater for a population of 160,000. Primary treatments include coarse and fine screening (25 and 6 mm, respectively), grit removal, and primary sedimentation (Figure 1). Secondary treatment is based on a conventional activated sludge process that includes aeration and sedimentation. Excess sludge from secondary sedimentation is recirculated to the primary clarifier, where all the sludge is removed. The Nenäinniemi WWTP installed disc filtration in the tertiary treatment in 2018, prior to our sampling occasion. The disc filter (Veolia Hydrotech HSF2628/27-2F) sieve material is polyester with a pore size of 10 μm . A total of 5 units were installed, each containing 27 filter discs, which corresponds to a total net filtration area of $5 \times 133.4 \text{ m}^2$. The treated wastewater (hereafter ‘tertiary effluent’) discharges into Lake Päijänne. The sludge treatment consists of two gravity thickeners and a screw thickener. The thickened sludge is digested in a mesophilic digester and dewatered using centrifuges. Reject water from the sludge treatment returns to the beginning of the process. Wash water from the screenings’ wash press and grit washer returns between grit removal and fine screening. Wash water from disc filtration returns to a spot prior to primary sedimentation. Key parameters for the process at the Nenäinniemi WWTP are given in Table S1 (Supplementary material).

Sample collection

All samples were taken on September 7, 2018, between 9 AM and 8 PM. At the time of our sampling, the flow rate for the plant influent was $30,180 \text{ m}^3 \text{ d}^{-1}$, the flow rate for effluent was $29,371 \text{ m}^3 \text{ d}^{-1}$, and the amount of outgoing

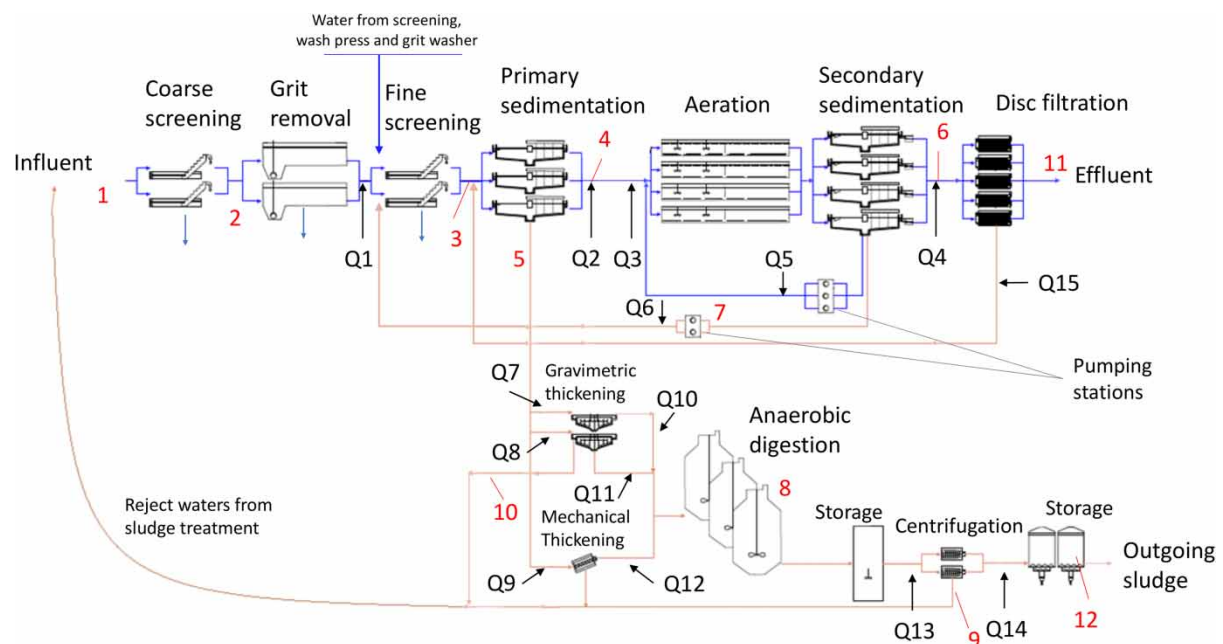


Figure 1 | Flow chart illustrating the sampling points (red numbers 1–12) and flow rate monitoring stations (black numbers and letters Q1–Q15) within the main processes at the Nenäinniemi WWTP. The chart is simplified from the original provided by the Nenäinniemi WWTP. Gas diversions from digesters, chemical additions, UV disinfection treatments, and security bypasses are omitted from the original flow chart.

dried sludge was $9.6 \text{ tons dw d}^{-1}$ (Table 1). Three replicate samples were taken from each sampling point (Figure 1). Samples of plant influent (sampling point 1 in Figure 1) and water after coarse screening and fine screening (sampling points 2 and 3, Figure 1) were taken by lifting the samples from the wastewater stream with a zinc bucket attached to a rope (a method also called ‘grab sampling’, Talvitie *et al.* 2017a). Samples were poured into 1 L plastic bottles using a polypropylene measuring jug. All bottles were closed with aluminum foil between the cap and the bottles.

Samples from the primary sludge (sampling point 5, Figure 1), excess sludge (sampling point 7, Figure 1), digested sludge (sampling point 8, Figure 1), centrifuge reject (sampling point 9, Figure 1), and thickening reject (sampling point 10, Figure 1) were taken into the measuring jug and poured into 1 L plastic bottles from the maintenance and monitoring hatches and taps. Samples of the dried sludge (three replicates, point 12, Figure 1) were taken from a storage chamber with a spatula, encapsulated with aluminum foil, and enclosed in 1 L plastic boxes. Due to varying retention and delay times in each treatment, samples taken from each point do not represent plant influent at the same point in time. However, as the samples were taken on the same day, proceeding in logical order from influent to effluent, we consider that the balance calculation is justifiable, although we recognize that temporal variation in MP concentration can be notable.

Samples from water streams after primary, secondary, and tertiary treatments (effluents) were taken from the water stream using a hollow PVC pylon (inner diameter 45 mm) equipped with nylon filters in three sizes (20, 100, and 300 μm). The sampling system was developed by Talvitie *et al.* (2017a) for the purpose of sampling MPs from wastewater streams. Wastewater was pumped until the filters were almost clogged, and the filters were stored in Petri dishes until the analysis. The filter housing was flushed with ultrapure water between replicates. Pumped volumes were determined using a Gardena Smart Water Flow Meter. (See Table 1 for sample volumes.) Three replicate samples were filtered with each filter size.

To control the variation caused by microplastic contamination during sampling due to textile fibers and fragments present in the ambient air and surfaces, negative control samples were prepared by filling similar 1 L plastic bottles that were used for sampling with ultrapure water and opening them at the sampling sites at the

Table 1 | Sampling point of each subprocess and the measured total daily flows that were used to calculate daily loads of microplastics

Treatments	Sampling point (Figure 1)	Description	Sampled volume (L)	Flow rate monitoring station (see Figure 1)	Flow rate ($\text{m}^3 \text{d}^{-1}$)
Primary treatment	1	Influent	1	=Q1 (sludge rejects, $1,440 \text{ m}^3 \text{d}^{-1}$, subtracted)	30,180
	2	Coarse screening	1	=Q1	31,620
	3	Fine Screening	1		
	4	Primary effluent	20 μm : 1 100 μm : 17 300 μm : 120	=Q1-sum(Q7,Q8,Q9)	30,024
	5	Sludge to thickening	1	=sum(Q7,Q8,Q9)	1,596
Secondary treatment	6	Secondary effluent	20 μm : 1 100 μm : 7 300 μm : 50	=Q1-Q6	30,470
Sludge treatment	7	Excess sludge	1	=Q6	1,150
	8	Digested sludge	1	=Q13	578
	9	Centrifuge reject	1	=Q13*	578
	10	Thickening reject	1	=(Q7 + Q8) - (Q10 + Q11)	1,152
	12	Dewatered sludge	100–200 g	=Q14	9.6 (tons dw d^{-1})
Tertiary treatment	11	Final effluent	20 μm : 10 100 μm : 200 300 μm : 1,000	=Q4-Q15	29,371

Sample volumes are given in liters (L), except for dried sludge in grams (g). Three replicates of the given volumes were taken. Centrifuge reject (*) was considered to have a similar flow rate as the flow to centrifugation because the dry matter content of dewatered sludge was high (average 32%, SD = 4).

same time as the actual samples. Replicates of negative controls were opened at coarse screening, centrifuge reject, and excess sludge sampling points (Figure 1). For the pumped samples, negative controls were prepared by holding clean filters on the filter holders for a few seconds and checking their purity with a stereomicroscope. All samples were stored in the dark at +4 °C until processed further.

Sample processing in the laboratory

As particles of the largest size fraction were relatively easy to hand-pick with tweezers, the >300 µm pumped samples on nylon filters were screened on a stereomicroscope, and particles were hand-picked for Raman microscopy. The smaller size fractions of the pumped samples (20–300 µm) and all other samples underwent the enzymatic purification protocol and density separation (Löder *et al.* 2017).

Enzymatic purification and density separation

To count and characterize the MPs, they were isolated from each sample matrix with an enzymatic purification protocol developed and validated by Löder *et al.* (2017). The three replicates were purified and analyzed, except from the sample point after coarse screening where one sample was lost. The protocol is based on hydrogen peroxide and technical grade enzymes that decompose and remove materials other than plastics from the samples. We followed the sequence of reagents (Table S2) and incubation temperatures and durations as described by Löder *et al.* (2017), except that in the beginning, the first sodium lauryl sulfate (SDS) step was replaced by a hydrogen peroxide treatment because the foaming of SDS appeared to us to be problematic for filtration. Therefore, the reagents used were 30% (v/v) hydrogen peroxide (WVR International), cellulase, protease, lipase, and chitinase (ASA Spezialenzyme GmbH; see S2 for a list of reagents and incubation times). Additionally, instead of incubating H₂O₂ at 50 °C, we incubated at 60 °C to intensify the effect on more recalcitrant organic litter. After the enzymatic purification, MPs were separated from inorganic solids by density separation. Samples were poured into 100 mL glass separation funnels, and a ZnCl₂ (WVR International) solution prepared at a density of 1.6–1.8 g cm⁻³ was added and allowed to settle for at least 24 h (Löder *et al.* 2017). The upper phase was filtered into a 20 µm stainless steel filter, and microplastics were rinsed using a squirt bottle from the filter with 96% ethanol into glass bottles. Subsamples (V_d, Equation (1)) from ethanol were filtered through aluminum oxide filters (Anodisc, Whatman, pore size 0.2 µm, diameter 25 mm). Filters were stored in glass Petri dishes for the Raman microscope analysis that followed immediately or within one month.

Modifications for samples with high dry matter content

Before the enzymatic purification, water was evaporated from samples taken from points 7, 8, and 12 by keeping them in a thermal cabin at 50 °C for 1–3 days. The dry matter content of samples taken at point 12 (dried sludge) was 28–35% (assessed according to the standard SFS-3008, 1981), which made filtration of representative volumes impossible. Therefore, samples of dried sludge were divided into 50 mL centrifuge tubes (VWR International) covered with aluminum foil and centrifuged at 3,000 RCF for 2 min. The supernatant was collected into a beaker, and solids were suspended in ultrapure water and centrifuged again in a similar way. After the second centrifugation, the supernatants were combined and filtered through 20 µm steel filters. This centrifugation and filtration procedure was done between each step of the protocol by Löder *et al.* (2017) for the dried sludge.

Quality controls

All samples were handled in a laminar flow cabin using metal and glass equipment. However, polyethylene squirt bottles (Nalgene, Thermo Scientific) were used in rinsing the equipment with ultrapure water between each replicate sample. Sample processing was done wearing cotton lab coats and nitrile gloves. Reagents were filtered through GF/A (Whatmann) to remove possible MP contaminants, except for ZnCl₂, which was filtered through a 20 µm stainless steel filter.

Negative control samples were treated as influent samples, except for the incubation time, which is considered to have a negligible effect on MPs themselves (Löder *et al.* 2017). To estimate the recovery rate of the enzymatic purification process, ten pink polypropylene particles shredded from a larger plastic item, 0.52 mm in size, were added to three influent wastewater samples taken only for the recovery rate test. Positive control samples were treated as influent samples. Recovered pink MPs were counted with a stereomicroscope, and the recovery rate was calculated as a percentage of found particles from added particles.

Characterization with Raman microscopy

MPs were characterized with Raman microscopes so that 16 samples were counted with the Thermo DXR2 microscope and 40 samples with the DXR2xi microscope. The microscope was changed after we noticed a weakening of the Raman signal in the former equipment due to a technical problem. Filters were imaged with the microscopes' cameras, and Raman spectra were acquired by visually selecting measurement spots from every particle on the examined area. Because part of the samples contained a very large amount of particles, known sub-areas (k) were analyzed from filters until at least one of the following conditions were met: At least a quarter of the filter area was analyzed, or at least 10 polymer particles had been measured (see Table S3 for the subsampling ratios).

Spectra were measured with the Thermo DXR2 using a 785 nm laser, 10X objective, 400 lines/mm grating resulting in approximately 5 cm^{-1} spectral resolution, $3,250\text{--}50 \text{ cm}^{-1}$ spectral range, 20 mW laser power, $50 \mu\text{m}$ slit aperture, preferred signal-to-noise ratio (S/N) 100, and max 1 min exposure time. For the Thermo DXR2xi, the measurement parameters were otherwise similar, but the spectral range was $3,300\text{--}50 \text{ cm}^{-1}$; and instead of an automatic S/An -based measurement time, the exposure time was 0.1 s, and the number of scans was 20.

The data was analyzed with Thermo Omnic 9/Omnic xi software by comparing the Raman spectra of particles to commercial spectral libraries consisting of reference spectra of plastics. If the correlation between the sample spectrum and the plastic was $>70\%$, the particle was counted as a reliably recognized plastic. MPs in the environment are often weathered and do not necessarily display complete matches to the reference libraries by automatic comparison (Lenz *et al.* 2015). For this reason, spectra with lower correlations were manually interpreted based on peak positions. In addition to plastic types, MPs were also coarsely categorized based on their shape and size into fragments, defined as thick 'three-dimensional' particles, and fibers that had the largest dimension at least ten times longer than the smallest dimension.

Calculation of particle balance

MP concentration (C) of each sample was calculated from the Raman microscopy results as

$$C \text{ (MP L}^{-1}\text{)} = \frac{N \times k}{v} \times V_d \quad (1)$$

where N is the number of MPs, k is the subsampling ratio for Raman analysis ($\text{Area}_{\text{filtration}}/\text{Area}_{\text{examined}}$), v is the volume of the processed sample, and V_d is the subsampling ratio for filtration through Anodiscs (volume of ethanol-preserved sample divided by volume of sample filtered on Anodisc) if the whole sample was not filtered for Raman analysis.

Concentrations were converted to daily loads (f) with the following equation:

$$f \text{ (MP d}^{-1}\text{)} = C \times Q \quad (2)$$

where C is the concentration of MPs (in MPs m^{-3}), and Q is the flow rate in the subprocess (Table 1).

RESULTS AND DISCUSSION

We first present the results from the primary treatment processes, followed by the sludge treatment and secondary treatment. Finally, we present results from the disc filter-based tertiary treatment and establish an MP removal rate for the Nenäinniemi WWTP. When flows are given as $\times 10^9 \text{ MP d}^{-1}$, the standard deviations are also $\times 10^9 \text{ MP d}^{-1}$, but for readability, the units were omitted from standard deviations.

The negative control samples, which represented background concentrations for actual samples, contained on average 4 MP L^{-1} ($\text{SD} = 6$, $N = 3$). The concentration in the negative control samples can be considered as a limit of detection (LOD) only for the bottled samples, not for the pumped samples where the sampling volumes were up to several orders of magnitudes larger (Table 1). The negative controls for the pumped samples that were formed by holding a clean filter on the tubing in the field showed no attachment of contaminating MPs. Positive controls showed that the recovery rate of MPs was 97% ($\text{SD} = 6$, $N = 3$). The results were not corrected based on negative controls or recovery, but the results for the control samples were interpreted as sufficiently successful sampling and laboratory work.

Primary treatment

The average MP concentration in the three replicate samples of the wastewater influent was 61 MP_s L⁻¹ (SD = 26). After the coarse screening with a 25 mm screen, the concentration decreased to 27 MP_s L⁻¹ (SD = 23). However, after the fine screening (6 mm), the concentration increased again to 171 MP_s L⁻¹ (SD = 149). This observation can be explained by the fact that, although a significant amounts of MPs (30–55% as estimated based on the balance) was removed with sand and screenings, the wash water from the screening and grit washers and the reject water from sludge thickening and dewatering were pumped back to the process irregularly, all creating a return loop for the MPs back to the process (Figures 1 and 2). Sludge dewatering reject recirculated a large amount of MPs back into the process. The total concentration of MPs in the aqueous phase after primary sedimentation was on average 3 MP_s L⁻¹. Aqueous samples taken by pumping were size-fractionated, which revealed that the highest MP concentration was in the smallest (20–100 μm) fraction (average 4 MP_s L⁻¹, SD = 6). Average concentrations in the midmost (100–300 μm) and largest size fraction (>300 μm) were 0.4 MP_s L⁻¹ (SD = 0.1) and 0.3 MP_s L⁻¹ (SD = 0.0), respectively.

When the concentrations presented above were converted to daily loads of MPs using the flow rates monitored by the Nenäinniemi WWTP (Table 1), the conversion yielded almost two billion MPs arriving daily (1.8×10^9 MP_s d⁻¹, Figure 2). The amounts after coarse and fine screening were 0.8×10^9 MP_s d⁻¹ and 5.4×10^9 MP_s d⁻¹, respectively. The load from primary sedimentation effluent to secondary treatment was 0.1×10^9 MP_s d⁻¹.

MP concentration in the Nenäinniemi WWTP influent was an order of magnitude lower than in the study of microlitter >20 μm by Talvitie *et al.* (2017a) in a WWTP with a population equivalent of 800,000 inhabitants. In their study, the microlitter also included the litter of natural material, such as cotton textile fibers. Lares *et al.* (2018) studied MPs >38 μm in a WWTP with a 10,000 m³ daily inflow and a reported average concentration of 57.6 (SE = 12.4) MP L⁻¹. The amount of incoming water in the Nenäinniemi WWTP was three times higher, but the total concentration in the influent was like the plant influent in eastern Finland studied by Lares *et al.* (2018). This congruence was likely due to the similar origins of the wastewater, but these studies also utilized similar sampling, sample processing, and characterization methods.

Sludge treatment

The sludge that was pumped from the primary sedimentation included raw sludge and excess sludge from secondary sedimentation contained on average 1,560 MP_s L⁻¹ (SD = 990). The excess sludge from the secondary

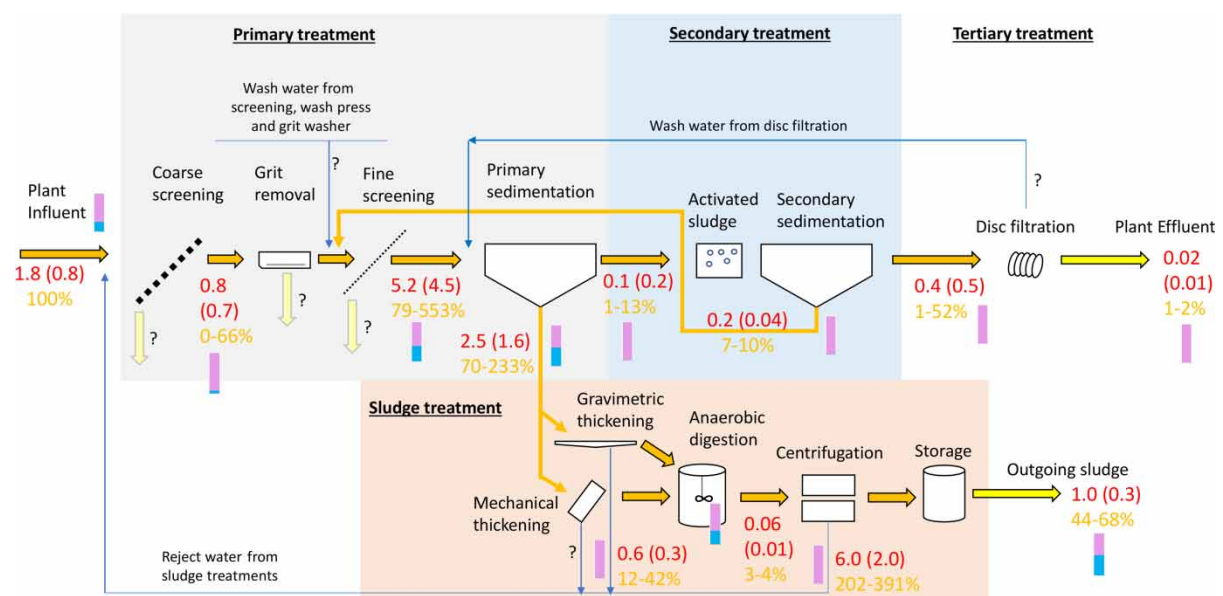


Figure 2 | Microplastic particle loads on the Nenäinniemi WWTP with all the sampled process phases at the plant. Red numbers indicate $\times 10^9$ microplastics d⁻¹ (standard deviation based on three replicates), and orange numbers indicate the average flow normalized to the average MP_s d⁻¹ entering the WWTP. For size-fractionated samples, the highest observed standard deviations are shown. Bars indicate the average proportion (%) of fibers (blue) and fragments (pink).

sedimentation was directed back to the primary sedimentation (Figure 1). This excess sludge contained on average 142 MPs L^{-1} ($\text{SD} = 31$).

The concentration in the reject of gravimetric thickening was on average 475 MPs L^{-1} ($\text{SD} = 55$), which was returned to the stream of plant influent. Digested sludge contained 102 MPs L^{-1} ($\text{SD} = 20$). The centrifuge reject that similarly returned from the later sludge treatment processes back to the beginning of the process had a high mean concentration of MPs, $10,400 \text{ MPs L}^{-1}$ ($\text{SD} = 3,464$). Finally, the dried sludge contained on average $9,379 \text{ MPs g}^{-1} \text{ DW}$ ($\text{SD} = 4,474$).

The load from the raw sludge including excess sludge from secondary sedimentation was $2.5 \times 10^9 \text{ MPs d}^{-1}$ ($\text{SD} = 2$, Figure 2). Excess sludge transported $0.2 \times 10^9 \text{ MPs d}^{-1}$ ($\text{SD} = 0.04$) to primary treatment. Gravimetric thickening reject conveyed $6.0 \times 10^9 \text{ MPs d}^{-1}$ ($\text{SD} = 0.3$) to the reject water returning to the plant influent. The load via digestion towards centrifugation was $0.06 \times 10^9 \text{ MPs d}^{-1}$ ($\text{SD} = 0.01$). The returning centrifuge reject brought a high amount MPs: $6.0 \times 10^9 \text{ MPs d}^{-1}$ ($\text{SD} = 2.0$), which also entrained reject water returning to the plant inflow. The dried sludge conveyed $1.0 \times 10^9 \text{ MPs d}^{-1}$ ($\text{SD} = 1.0$, Figure 2) out from the Nenäinniemi WWTP.

In agreement with Talvitie *et al.* (2017a), Lares *et al.* (2018) and Edo *et al.* (2020) the proportions of fibrous MPs were higher in sludge than in water (Figure 2). In this study, fibers were captured in the raw sludge during primary sedimentation. A negligible amount of clearly fibrous particles was observed in the gravimetric thickening and centrifuge rejects, indicating that fibers stayed in the sludge during these treatments and anaerobic digestion (Figure 2).

Mahon *et al.* (2017) examined the impact of different sludge treatments on MP abundance in sewage sludge. Mesophilic anaerobic digestion (MAD) resulted in a significantly lower abundance of MPs compared to two other examined treatments – thermal drying (TD) and lime stabilization (LM). Mahon *et al.* (2017) raised an interesting question on the possible role of MAD in the microbial degradation of MPs during the treatment. In this study, we also observed relatively low MP loads from digestion to centrifugation. However, the load via centrifugation reject back to the process influent was up to two orders of magnitude higher. The possible reasons for the observed variation between the MP loads from anaerobic digestion, centrifugation, and outgoing dewatered sludge might include the varying distribution of microplastics on the temporal and spatial scales and, despite our best efforts, relatively small sample volumes representing a rather negligible fraction of the treatment volumes. However, the observed MP loads that increased dramatically in the sampling points after anaerobic digestion suggest that MPs were not mineralized by bacteria, but this topic undoubtedly requires further studies.

Sludge samples with a high dry weight content are typically difficult to study because a sample volume that is practical to purify for MP characterization often remains small. Fourier Transform Infrared Microscopy (FTIR) is one of the most widely used methods to characterize MP. However, Raman microscopy that generally yields higher spatial resolution is also increasingly used (Sun *et al.* 2019). Both methods require a high level of purity for samples from materials other than MPs; and the prevailing purification method is time-consuming, utilizing a sequence of strong oxidizers and enzymes. This is especially important for sludge samples representing complex and variable matrixes and is likely a major reason why sludge is scrutinized less often in MP studies (Gatidou *et al.* 2019). In this study, we used a gentle bench-top centrifugation procedure for sludge before the enzymatic purification protocol. This increased the sample volume, and we were able to process and analyze 11–13 g DW (>40 g WW) of dewatered sludge.

Secondary treatment

Concentration of MPs was higher in secondary effluent compared to primary effluent. After aeration and secondary sedimentation, the MP concentrations in the water stream was 13 MPs L^{-1} . Similarly to primary effluent, the highest concentration was observed in the smallest size fraction (12 MPs L^{-1} , $\text{SD} = 17$). The midmost and largest size fraction contained 0.4 MPs L^{-1} ($\text{SD} = 0.2$) and 0.2 MPs L^{-1} ($\text{SD} = 0.1$), respectively.

Similarly to the concentrations, the daily loads of MPs consistently showed that the number of MPs increased in the secondary sedimentation compared to the primary sedimentation. The load after the secondary sedimentation basin to the tertiary treatment was $0.4 \times 10^9 \text{ MPs d}^{-1}$ (Figure 2). There was a high concentration of solids during the filtration of samples, and the filters where samples were collected from the secondary sedimentation effluent tended to clog much earlier than the filters did after the primary settling basin. Monitoring data from the Nenäinniemi WWTP also showed relatively high concentrations of suspended solids in the secondary sedimentation effluent (Table S4). Resuspension of solid material in the secondary settling basin could explain the increased amount of MPs in the secondary effluent as the concentration of MPs in the excess sludge (sludge

in the secondary settling pool) was relatively high 142 MPs L^{-1} ($\text{SD} = 31$). Lares *et al.* (2018) also reported slightly higher MP concentrations in the final effluent in a secondary-level WWTP compared to the effluent after primary treatment. They discussed that the increase might have been caused by turbulent water flow after primary treatments. They also noted that the particles observed were mainly positively buoyant polyethylene. In the Nenäinniemi WWTP, the observed MPs after secondary sedimentation were mainly polyethylene and polypropylene, which are typically positively buoyant (Sun *et al.* 2019) and for this reason likely susceptible to upwelling turbulent flows.

Tertiary treatment and MPs removal rate

Concentration in the plant effluent was 0.8 MPs L^{-1} ($\text{SD} = 0.4$). Similarly to primary and secondary effluent, the highest average concentration in the effluent was observed in the smallest size fraction (20–100 μm , 0.8 MPs L^{-1} ($\text{SD} = 0.4$)). In the other size fractions, the average concentrations were $\leq 0.01 \text{ MPs L}^{-1}$. Converted to load, on average $0.02 \times 10^9 \text{ MPs d}^{-1}$ was released into the receiving lake (Figure 2). Like Simon *et al.* (2018) in Denmark, we also observed particles larger than the nominal 10 μm mesh size of the disc filter in the tertiary effluent. Likely, this observation was facilitated by our high sampling volume of plant effluent (1,000 L per replicate, see Figure S1 for a micrograph of the particles).

After primary treatment, the MPs removal rate of the Nenäinniemi WWTP from the water stream was 95%. However, due to the increase of MPs concentration in secondary treatment, the removal rate of primary and secondary treatments was 79% of the MPs in the influent water (Figure 2). The disc filter retained 94% of the MPs coming from the secondary treatment. This meant that the total removal rate of the Nenäinniemi WWTP was 99%. In agreement with the previous studies, we observed that sludge was the main exit route for WWTP MPs. The average MP concentration in the dried sludge (1×10^5 per kg DW) in the Nenäinniemi WWTP was of the same order as the microlitter concentrations in Talvitie *et al.* (2017a) and the microplastic concentration in Lares *et al.* (2018).

The Nenäinniemi WWTP reached a high MP removal percentage (99%), with the disc filter playing a crucial role in the high removal rate, as expected from previous studies on disc filters (Talvitie *et al.* 2017b; Simon *et al.* 2018; Hidayaturrehman & Lee 2019). In the Nenäinniemi WWTP, the disc filter as a tertiary treatment completed the removal of MPs from secondary effluent by which the removal rate was 80%. At the time of this study, the suspended solids content in secondary effluent was two times higher than the yearly average (see the water quality table from Table S4). This was likely due to operational problems in the process at the time of sampling, causing poor settling in the secondary sedimentation (Nenäinniemi WWTP, Personal communication). For this reason, the disc filter was demonstrated to be effective for capturing not only MPs but also other suspended solids that otherwise would have entered Lake Päijänne.

Plastic morphology and types

Most of the counted MPs were characterized as fragments (Figure 2). The rest, having a clearly thin and long shape, were characterized as fibers. Fibers were especially present in the influent (26%), after coarse and fine screening (8 and 41%, respectively), primary sludge (45%), digested sludge (35%), and dried sludge (49%). In other sampling points, the average proportion of fibers stayed at 2% or below (Figure 2). The most common types characterized with Raman microscopy were polypropylene (PP) and polyethylene (PE), which were observed in all the sampling points (Figure 3). Polyethylene terephthalate (PET) was also rather common (Figure 3). Together, these three plastics covered >90% of the daily sum loads of MPs. Other observed plastic types were polystyrene (PS), polyoxymethylene (POM), ethylene-vinyl acetate (EVA), polydimethylsiloxane (PDMS), polytetrafluoroethylene (PTFE), and polymethyl methacrylate (PMMA).

The most abundant polymer types found from the Nenäinniemi WWTP – PP, PE, and PET – are used broadly for various purposes, such as beverage containers, plastic bags, bottles, and caps (Andrady 2011; Webb *et al.* 2013; Chubarenko *et al.* 2016). Polyethylene is also typical in personal care products (Leslie 2014) and PET in textile fibers (Webb *et al.* 2013). Lares *et al.* (2018) reported polyester (PES) and polyethylene (PE) to be the most abundant MP types with PES fibers being dominant in all the treatments.

Sources of variation

Variation in our results – as MP studies in general – was introduced by retention times and variation in the surface loading within the sampling day (Table S1), and despite our quality control measures, also no doubt challenges

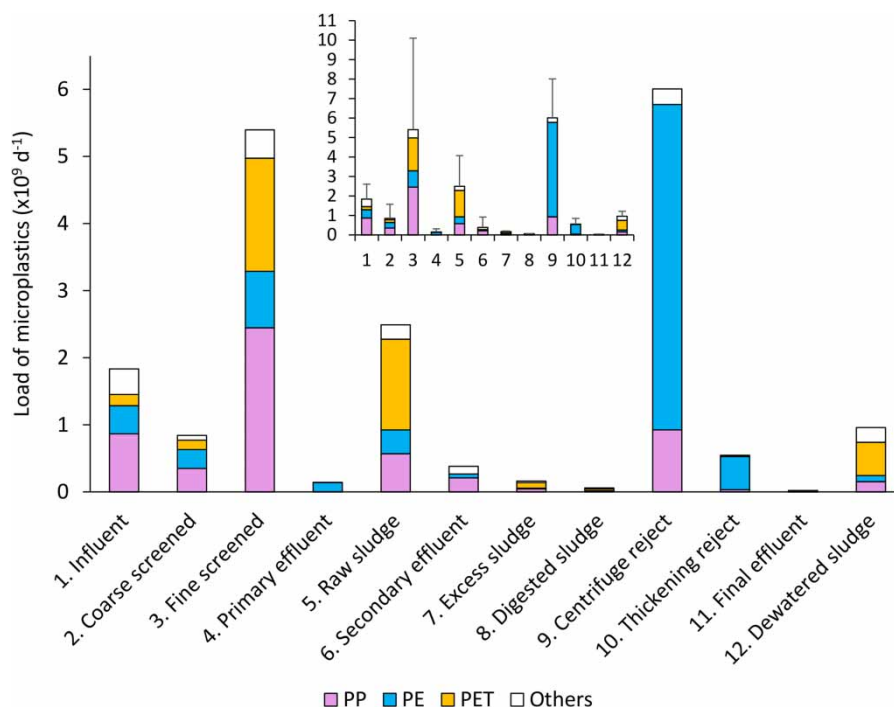


Figure 3 | Microplastics substance distribution in the Nenäinniemi WWTP. The insert shows standard deviations of replicate samples (\pm SD, $n = 3$). PP, polypropylene; PE, polyethylene; PET, polyethylene terephthalate. For size-fractionated samples, the highest observed fraction-specific standard deviations are shown.

the sampling, sample processing, and MP characterization (Gatidou *et al.* 2019; Koelmans *et al.* 2019). Also, we used two microscopes, which could have caused a variation in the results. However, we aimed to set the measurement parameters as similar as possible in both instruments. In the future, the analysis equipment between different studies would also benefit from intercalibration. Ideally, the amount of microplastics in the tertiary effluent, outgoing sludge and in the outgoing load leaving after the screenings and grit removal processes equals the inflow of microplastics to the WWTP. In this study, we did not sample the amount of microplastics leaving the WWTP after the screenings and grit removal. Instead, that part of MP particle balance was estimated as a residue from tertiary effluent and dried sludge, which automatically led to an equilibrium in the balance. However, as the total mass removal after the screenings and grit removal is small (0.7 tons d^{-1} , Table S1), the conclusion of dried sludge (mass removal 30 tons d^{-1} , wet weight) being a major exit for MPs is justifiable. In the future studies, the MP exits at the primary treatment could also be considered.

The internal circulation of MPs in the reject water could also affect balance calculations because of the difficulty to estimate the duration of time that those particles have spent in the WWTP. We did not sample reject water from mechanical thickening (Figure 2) so the total amount of MPs in the return loop could be even higher. Most importantly, this study lacks information of temporal variation.

In the case of the disc filter, the wash water removed the solids collected on the filter cloths, and thus it can be estimated that this wash water stream caused a recycle load back to the primary sedimentation corresponding to the removed MP load, too. However, compared to the return loop from the sludge treatment, this is likely a minor internal circulation of MPs.

Variation in MP polymers types was high between the sampling points that could also be of importance to study where that variation accumulates from. We anticipate that if the sample sizes and frequency could be further increased, this variation might be reduced.

CONCLUSIONS

Overall, our study indicated that wastewater sludge represents a notable source of MPs to the environment in areas where the treated sludge is land-applied. Although this study emphasized sludge treatment, we also demonstrated that disc filter –based tertiary treatments enhance MP removal at WWTPs due to the typical variations in the performance of the secondary treatment. The internal circulation of MPs that we observed from sludge

thickening and centrifugation to the beginning of the primary treatment is important because that affects the particle balance calculations. A large amount of internally circulating microplastics might also be shredded and modified during transportation and process, especially during dewatering using centrifuges. In our study, despite the sometimes even large standard deviations between sample replicates, the large differences between sampling points indicated that especially dewatering by centrifugation was a step that removed a high amount of MPs from the sludge, possibly selectively leaving the fibrous particles attached. In future studies at WWTPs, the calculation of MP mass balances in addition to particle balances could reveal more about the behavior of MPs in these processes. The observed accumulation of MPs in the dewatering reject is also of interest for future development of MP removal processes and it opens up the possibility of targeting MPs in this in this concentrated internal flow.

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DATA

Data generated and processed during this study is available at the University of Jyväskylä's JYX repository (DOI: 10.17011/jyx/dataset/71624; URI: <http://urn.fi/URN:NBN:fi:ju-202009035741>).

DATA AVAILABILITY STATEMENT

All relevant data are available from an online repository or repositories.

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