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Spatiotemporal characteristics of microplastics in a university wastewater treatment plant: Influence of sudden on-campus population swings

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ABSTRACT

We studied the occurrence and characteristics (sizes, shapes, and polymer compositions) of microplastics (MPs) in a secondary wastewater treatment plant (WWTP) at the University of Mississippi both spatially and temporally. Putative MPs were isolated by sieving, matrix digestion, and density separation, and quantified using stereomicroscopy, with a subset of samples analyzed by Focal Plane Array (FPA) μ-FTIR imaging. In the influent, the highest MP concentration (particles/L ± 1 SD, n = 3) occurred after a football game on campus (62.3 ± 7.6) and the lowest (19.7 ± 2.1) during the summer with little activity on campus. Over 90% of the MPs were removed in the primary treatment. Downstream, MPs were most abundant in the closed loop reactor with concentrations as high as 1962 particles/L. Concentrations in secondary clarifier and final treated effluent were both < 4 particles/L during both normal flow (~2000 m^3/d) and high-flow (>2500 m^3/d) periods, and between 16 and 39 particles/L during a low-flow (<1500 m^3/d) period. This difference likely stems from changes in plant operations during the low-flow period to support the activated sludge, including longer process times. MPs were mainly composed of polyester, polyethylene, acrylates, polypropylene, polyurethane, polyvinyl chloride, and polystyrene. Fibers were most abundant throughout the system, averaging 61%, followed by fragments (21%), films (13%), and beads and foams (~5%). Overall, we show that flow rates and treatment times can profoundly influence MPs concentrations in the treated effluent, and that the optimal wastewater treatment conditions also yield the best MP removal efficiency.

1. Introduction

Plastic pollution has been exacerbated with increased global demand for plastics and with the COVID-19 pandemic, which has amplified reliance on plastics for safety and hygiene [1]. Microplastics (MPs) are a diverse suite of contaminants consisting of synthetic polymers ranging in size from 1 μm to 5 mm, which have polluted waters globally, raising serious concerns about their impact on aquatic life and their incorporation into food webs [2,3]. Most MPs are generated by the breakdown of larger items such as clothing, tires, and mismanaged plastic waste, but intentionally manufactured MPs, including those used in abrasives and personal care products, are also a significant source [4]. Most of the MPs in the marine environment originate from land-based sources [5]. One study modeled the export of MPs from land to sea, estimating 42% from tire and road wear particles, 29% from textiles abrasion during laundry, 19% from household dust, and 10% from personal care products [6].

Wastewater treatment plants (WWTPs) are considered one of the last opportunities to capture significant amounts of MPs before they are released into the natural environment. Fortunately, many WWTPs effectively remove MPs regardless of their concentration in the influent [7-9]. MP removal efficiencies have been reported to range from 53.6 to > 99.9% for primary and secondary WWTPs [10,11], with MPs concentrations remaining in the effluent ranging from 0.004 to 447 particles/L [11,12]. Nevertheless, as WWTP effluent typically contains higher MP concentrations than the recipient waters and with large volumes of effluent discharged, WWTPs still account for a significant source of MPs to downstream waterways [13,14].

MP types and concentrations entering WWTPs are influenced by a number of factors, including the local population, level of urbanization, the surrounding geographic environment, and industrial activity in the wastewater catchment area [8,15]. In the treatment plant itself, the level of organic matter in the wastewater can affect MP removal efficiency since the quantity and quality of activated sludge, which accumulates MPs, greatly depending on nutrient concentration [16]. Whereas the

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majority of municipal WWTPs have sufficient incoming organic matter to continually support activated sludge microorganisms, others may need to alter treatment processes during periods with low influent flows. This can be accomplished by, for example, changing process times or returning more sludge from the secondary clarifier back to the biological unit.

Numerous studies have examined the distribution and characteristics of MPs in municipal WWTPs, but there is nothing in the literature about a WWTP that strictly serves a university community. Such WWTPs are unique considering their highly variable flows dependent on the on-campus population, which itself varies depending on the University calendar and sporting events, and because the wastewater stems from teaching and research laboratories in addition to student dormitories and cafeterias. Thus, our study aimed to characterize MPs from a WWTP in University of Mississippi both spatially (in the influent, grit chamber, closed loop reactor, secondary clarifier, and treated effluent) and temporally (during summer and remote learning with most students off campus due to the COVID-19 pandemic and during normal in-person learning and sporting events with high populations on campus). Herein, we provide empirical data on MP abundances and characteristics within treatment compartments in a university WWTP, as well as address MP removal efficiencies under considerably different inflows and treatment conditions. The environmental significance being a better understanding of the conditions that maximize MP retention and minimize MP discharge into water masses.

2. Materials and methods

2.1. Study site and sample collection

The WWTP at the University of Mississippi (UM) consists of grit chambers, closed loop reactors, secondary clarifiers, and UV disinfection where wastewater flows in continuous and closed process (Fig. S1). This WWTP serves the university community through a separate sewer system collecting and treating University wastewater only. The system does not include stormwater runoff. The influent flow is highly dependent on the on-campus population, ranging from < 1000 m^3/d during campus closures to ~2000 m^3/d for regular operation during semesters to $>$ 3000 m^3/d during major sporting events, especially football games that can attract crowds above 65,000 spectators.

We collected wastewater samples from multiple WWTP compartments on four separate occasions (Table 1). These sampling campaigns include August 5 2020, during a low-flow period (1270 m^3/d) with a low student population due to summer break; September 2 2020, during a relatively low-flow period (1410 m^3/d) with low student population due to remote learning during the pandemic; September 7 2021, during a moderate (more typical) flow period (1670 m^3/d) with students fully back on campus; and September 12, 2021, during a high-flow period (2500 m^3/d) with a relatively high population on campus due to a football game. We collected triplicate grab samples from both the influent and closed loop reactor with 1 L glass mason jars that were previously washed with 0.2 μm filtered Milli-Q water (Milli-Q, Millipore, Burlington, MA, USA), hereafter termed ultrapure water. We collected triplicate 50 L samples from the grit chamber effluent, secondary clarifier, and final treated effluent using a stack of stainless-steel sieves (20.32 cm diameter) with mesh sizes of 45, 125, and 1000 μm. Wastewater was pumped to fill a 50 L polypropylene carboy container which was subsequently allowed to drain through sieves assembled from coarse to fine. Particles retained in the sieves during sampling were rinsed to 1 L glass mason jars using a PTFE squeeze bottle containing ultrapure water. Note that we only sampled the grit chamber effluent in 2021, as we were initially focused on other compartments and were unaware of the access point during earlier sampling.

Table 1
Sample information for the wastewater treatment plant at University of Mississippi.

Date	Flow (m^3/d)	Campus population	Compartment sampled
Aug. 5, 2020	1270 "low-flow"	Low / Summer break (<1000)	Influent, closed loop reactor, secondary clarifier, treated effluent
Sept. 2, 2020	1410 "low-flow"	Low / No in-person classes due to COVID-19 (~1000–2000)	
Sept. 7, 2021	1670 "normal or moderate-flow"	Normal operation (~20,000)	Influent, primary effluent, closed loop reactor, secondary clarifier, treated effluent
Sept. 12, 2021	2500 "high-flow"	Sport event (~50,000)	

2.2. Sample extraction and quality control

Wastewater samples were dried at < 40 °C for 24 h to increase the digestion efficiency. Labile organic matter was digested using Fenton's reagent following a modified version of our one pot method described elsewhere [17]. Briefly, 30% H_2O_2 (97% purity, Fisher Scientific, Hampton, NH, USA) and 0.05 M $FeSO_4$ were used to oxidize organic matter for 24 h at room temperature. Following the digestion step, inorganic particles were removed by density separation (three times per sample) using a solution of $ZnCl_2$ (>99% purity, Fisher Scientific, Hampton, NH, USA) with a density of 1.6 g/mL. The top layer of the solution was collected and filtered through a 45 μm sieve. Retained particles were rinsed with 5 mL 1% HCl (to remove any floc due to the dilution of $ZnCl_2$ solution) and ultrapure water before being filtered through 47 mm polycarbonate track-etched filters (Sterlitech Corp., Kent, WA, USA) for visual inspection.

We validated the sampling method by spiking fluorescent polyethylene microbeads (Cospheric LLC, Santa Barbara, CA) into a 50 L container filled with wastewater from the secondary clarifier and treated effluent. Twenty microbeads, each from three different size classes, were used (75–90, 150–180, and 300–355 μm). The spiked microbeads were counted under UV light once the sample glass mason jars were brought back to the lab. We also evaluated our MP extraction method by spiking three samples of the wastewater effluent and three blanks (consisting of reversed osmosis water) with both weathered and virgin MPs. The spiked MPs included ten weathered low density polyethylene films (1–3 mm), ten polyethylene microbeads (300–330 μm), ten weathered polyvinyl chloride fragments (500–1000 μm), and ten polyester fibers (1–3 mm). Films and fragments were cryogenically ground, and fibers were cut with a scissor. Spiked MPs, except microbeads, were stained with 2 μg/mL Nile red at 70 °C for 3 h for easier identification. The density of the spiked MPs ranged from ~0.92 to ~1.4 g/cm^3.

To minimize MP contamination, we cleaned all materials with soap and water and rinsed them three times with ultrapure water. Additionally, we heated glass components (Mason jars and filtering apparatus) to 450 °C. We wore bright orange-dyed cotton laboratory coats and covered samples and associated assemblies with aluminum foil when not being actively processed. The entire MP extraction process was conducted in a clean laminar flow hood. To assess contamination, we evaluated six blanks consisting of 50 L of reversed osmosis (RO) water passed through the mesh sieves and processed along with the samples.

2.3. Microplastic quantification and characterization

Each filter containing the extract was visually examined at 40 × magnification under a stereomicroscope (SteREO Discovery V12; Carl Zeiss Jena GmbH, Germany) equipped with an Axiocam 105 color digital camera and an X-Cite 120Q fluorescence lamp illuminators. Morphology and number of putative MPs were documented. Stereomicroscopy images of representative MPs were shown in Fig. S2. Daily discharges of putative MPs were determined from the flow rate and MP

abundance. Microbeads used for sampling method validation were enumerated under UV light. Spiked fluorescent MPs used for evaluating MP extraction were counted under green fluorescence range (excitation at 47/22 nm, emission at 525/50 nm), except PE microbeads which used a simple UV (black) light. The surface structures of selected particles were examined by a field-emission scanning electron microscopy (SEM) equipped with energy dispersive X-ray spectroscopy (EDX) (JSM-7200FLV, JEOL Ltd., Japan).

To identify the MPs in selected samples, we carried out μ-FTIR imaging spectroscopy using an Agilent Cary 620 FTIR microscope equipped with a 128 × 128 pixel FPA detector coupled to an Agilent Cary 670 FTIR spectrometer. FPA-μ-FTIR imaging was chosen because it is considered one of the most suitable techniques for analyzing small microplastics (<500 μm) without presorting [18-20]. First, the filters used for stereomicroscopy were briefly sonicated in 50% ethanol in 25 mL glass vials to dislodge the MPs. The filter was removed from the vial and rinsed again with the ethanol capturing the liquid in the vial. The solution was then evaporated using a stream of filtered air at room temperature. The vials were sealed and shipped to Aalborg University, Denmark, for analysis. Five milliliters of 50% ethanol were added to the vial and mixed to put the MPs into a solution. A subsample of known volume was deposited onto two zinc selenide (ZnSe) windows held in compression cells (PIKE Technologies, Fitchburg, WI, USA) using a capillary glass pipette and dried at 55 °C on a heating plate overnight. The analysis was performed by scanning the entire active area of the windows (~78.5 mm^2). This was achieved by analyzing 14 × 14 tiles, each composed of 16384 spatially resolved spectra simultaneously collected over an area of 704 × 704 μm/tile using 15 × IR Cassegrain objective-condenser system, which provides a pixel size of 5.5 μm. The full chemical map obtained included more than 3.2 million infrared spectra. The instrument operated in transmission mode with an active spectral range from 850 to 3750 cm^{-1}, collecting 120 co-added scans for the background and 30 co-added scans for the samples at a spectral resolution of 8 cm^{-1} and a beam attenuation of 50%. The scan time was ~4 h.

Data analysis was carried out using siMPle, a software developed by Aalborg University and the Alfred Wegener Institute [21]. The software automatically detects particles on the scanned surface, correlating the raw spectra, and the first derivative of all sample spectra to a custom--built database containing 461 reference spectra (polymers, paints and resins, and non-synthetic materials). Moreover, siMPle automatically measures the size of the particles and can also provide a mass estimation using the area, the density, and an estimated thickness of the identified particles [21].

Samples from all WWTP compartments were analyzed as described above, except for the primary treatment effluent. These samples were characterized by μ-FTIR using a Bruker LUMOS II microscope with a liquid nitrogen cooled FPA detector at the University of Mississippi. Prior to μ-FTIR analysis, the polycarbonate filters (previously analyzed by optical microscopy) were sonicated for 5 min in ethanol to dislodge the particles, and the solution was filtered through a 25-mm gold-coated polycarbonate filter (5 μm pore size; Sterlitech Corp.). μ-FTIR measurements were conducted from 750 to 4000 cm^{-1} in reflectance mode using a resolution of 4 cm^{-1}. Two different regions of the filter were analyzed, amounting to ~1/2 of the entire filter area. The data was treated with siMPle as before.

3. Results and discussion

3.1. Validation of microplastic sampling and extraction methods

As detailed in Section 2.2, we conducted recovery rate studies for spiked MPs in both the field (to assess sampling) and laboratory (to assess sample preparation). In the field, we spiked 50 L of water collected from the secondary clarifier and the treated effluent each with three size classes of fluorescent polyethylene microbeads, then passed the sample through a stack of sieves (45, 125, and 1000 μm) on-site at the WWTP. Recoveries ranged from 85% for the 75–90 μm fraction to 95% for the 300–355 μm fraction (Table S1). There was no statistical difference ($p < 0.05$) between recovery rates for the secondary clarifier samples and the treated effluent samples.

We also spiked samples with weathered and virgin MPs, including polyethylene beads, polyethylene films, polyvinyl chloride fragments, and polyester fibers, each made to fluoresce brightly to aid in detection, and carried them through the entire sample preparation procedure, including matrix digestion and density separation. Average recovery rates for the different types of MPs are given in Table S1. For the microbeads recoveries were 100%. Several microbeads were randomly chosen to observe their surfaces under the stereomicroscope. We found no apparent changes (damage) from the chemical treatment (Fig. S3a). The other three types of polymers were counted under green fluorescent light (Fig. S3b-d). The low-density polyethylene film was successfully extracted, giving 100% recovery rates. The high-density polyvinyl chloride fragments also had excellent recovery rates (97%). Fibers had slightly lower recovery rates, ranging from 80% to 100%, with an average rate of 88.3%. Because both the sampling and sample preparation recoveries were deemed acceptable and because the MPs used in these experiments are not representative of all plastics (e.g., types, shapes, sizes) we do not correct for recoveries herein.

Using optical analysis, putative MPs ranged from 5 to 9 particles in our procedural blanks (50 L of RO water), of which 82.9% were fibers and 17.1% were fragments. At < 1% of sample concentrations, these levels were deemed acceptable. Nevertheless, the following sample data were blank subtracted: each individual particle type (morphology) in the blank sample(s) was subtracted from the samples' data to obtain more accurate blank-corrected results.

3.2. Occurrence of MPs in wastewater compartments

3.2.1. Microplastic abundance

3.2.1.1. Influent. On August 5, 2020 prior to the start of the semester, the average abundance of MPs in the influent was 19.7 ± 2.1 particles/L. A few weeks later, on September 2, 2020, with more people on campus, but still relatively few due to remote learning during the pandemic, the MP abundance increased slightly to 26.0 ± 4.0 particles/L. The following year on September 7, 2021, when students returned to campus for in-person learning, MP levels increased to 46.3 ± 9.5 particles/L. Several days later (on September 12, 2021), the day after a football game that drew a large crowd on campus, the abundance increased to 62.3 ± 7.6 particles/L. Still, these influent concentrations are within the range found in previous WWTP studies (0.28–18285 particles/L) [11,22]. This despite differences in sampling, extraction, and analysis methods between studies. Overall, we found a significant correlation between influent flow rates and MP abundances ($r = 0.904$, $p < 0.01$).

3.2.1.2. Primary treatment effluent. Larger-sized MPs are prone to be removed in the grit chamber (primary treatment). Here we found the MP abundance ranged from 3 to 6.1 particles/L, with 91.8–92.9% of MPs being removed from the raw sewage. This is consistent with prior reports that primary treatment removes 50–98% of MPs [23]. MP removal at this stage was mainly achieved through surface skimming as well as settling according to the MP densities [24,25].

3.2.1.3. Closed loop reactor. The highest MP abundance was extracted from wastewater in the closed loop reactor, ranging from 1125 to 1962 particles/L in a low-flow period and from 153 to 409 particles/L during a high-flow period (Fig. 1). This difference may be due to several factors, including the longer process times during the low-flow period resulting in greater accumulation of MPs. Another factor may be related to the condition and quality of the activated sludge itself. With a low

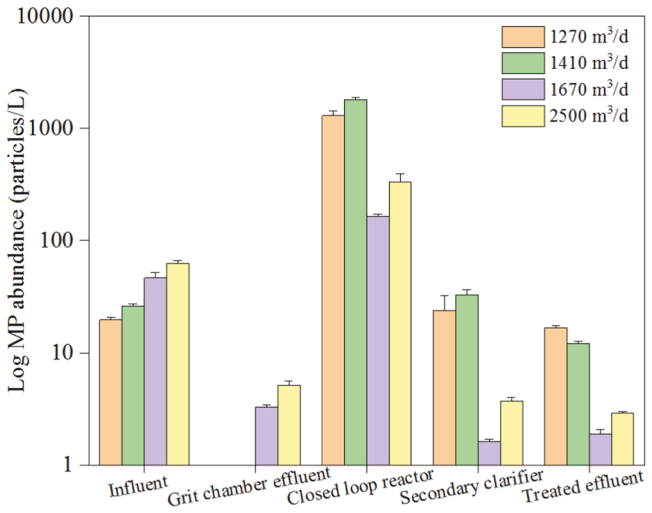

Fig. 1. Microplastic abundance in WWTP compartments at the University of Mississippi with little campus activity (1270 m^3/d); with increased activity but relatively low student population due to remote learning (1410 m^3/d); with return to full in-person learning (1670 m^3/d); and following a football game which drew a large crowd in excess of 50,000 (2500 m^3/d).

population on campus and low organic matter inputs the wastewater can become nutrient deficient for the activated sludge. Smaller and weaker flocs form under such starvation conditions, and these flocs are more easily sheared and subject to hydraulic surge flotation in the secondary clarifiers, which can lead to microscopic flocs in the effluent [26]. This was corroborated by the relatively high concentration of MPs in the secondary clarifier during the low-flow period. Further, during this period flocs were typically < 50 μm in diameter and consisted of floc-forming microorganisms without a filament, which may affect the trapping of suspended particles [26]. As the on-campus population returned to normal, flows increased and more typical conditions returned for the WWTP. Subsequently, more of the MPs were captured by activated sludge and/or were removed through flocculation due to the addition of ferric or aluminum salts, as has been the case elsewhere [14].

3.2.1.4. Secondary clarifier. Extracellular polysaccharides produced by activated sludge are partly responsible for floc formation. Overproduction of this polysaccharide occurs with nutrient deficiency which can lead to poor sludge settling. Moreover, enhancing the sludge return ratio (from the secondary clarifier to the biological unit) effectively increases the age of the activated sludge, which can result in denitrification, with tiny bubbles further affecting the settlement of sludge in the secondary clarifiers [26]. All this could have a great effect on the MP abundance both in the secondary clarifier as well as downstream in the effluent. In our study, with a low on-campus population and the changes in WWTP operation described above, we measured 16.6–38.9 putative MPs/L in the secondary clarifier, with an average abundance of 28.5 ± 7.7 particles/L. In contrast, only 1.5–4.5 particles/L were found in the secondary clarifier during normal and high-flow periods, with the corresponding improvement of sludge activity and settling in the secondary clarifier. Overall, the MP removal efficiency by closed loop reactor alone ranged from 27.5% to 50.6%, but when combined with the primary treatment this range increases to 94.0–96.5%.

3.2.1.5. Treated effluent. As WWTP effluent is directly discharged into the natural environment, it is critical to assess the concentration of pollutants in the treated effluent. Here, we found on average 14.4 ± 2.9 putative MPs/L during the low-flow period, which is slightly higher than the most municipal WWTPs, and < 4 MPs/L during normal and high-flow periods, which is similar to other WWTPs [27-29,14,30]. Overall, MP removal efficiencies ranged from 14.8% to 53.4% during the low-flow period (with minimal on-campus population), where changes to the plant operation were required (as described above), but increased to a high of 95.6% ± 0.41% during normal operation and high-flow days. MP abundance in the effluent was 1.9 ± 0.3 particles/L on September 7 2021 under normal/moderate flow and 2.9 ± 0.2 particles/L after the football game. The daily discharges of putative MPs were 1.7–2.1 × 10^7 particles/day during low-flow period and 3.2–7.3 × 10^6 particles/day during high-flow days from the UM WWTP. Similarly, municipal WWTPs from other studies discharged 2.7 × 10^3 to 8.9 × 10^9 MP particles per day [7,25,29,31].

3.2.2. Microplastic morphological and size characteristics

Fibers were the predominant shape (37–76%), followed by fragments (10–30%) and films (6–24%) during all sampling campaigns (Fig. 2). The predominance of fibers was consistent with previous wastewater studies [27,32,25,9,33]. Fibers typically stem from laundering of synthetic fabrics and shedding of textiles. New garments were found to release a significantly greater number of fibers than used ones ([15,34,35]; [36]; [37]). A significant increase in the percentage of fibers during high-flow period was not observed ($p = 0.104$). Although fibers were prone to be removed via surface skimming and captured by activated sludge due to their relatively large surface area, they were still dominant in the treated effluent because of their large quantity.

Fragments and films were also present throughout all treatment stages during the four sampling dates. We did not find significant percentage changes of films in different compartments, although they were more brittle than other morphological MPs. Potential sources for these could be personal care products and the fragmentation of plastic waste. A dull and irregularly shaped blue polyethylene fragment (Fig. S2F) observed in the treated effluent was similar to the MPs extracted from toothpaste by another study [24]. While microbeads have been banned in the US in some personal care or cosmetic products under the Microbead-Free Waters Act of 2015 (H.R.1321), they were universally found in all wastewater samples of this study, accounting for 2–14% of the total MPs. Glitter were also sporadically found in the closed loop reactor, secondary clarifier and treated effluent, though they only composed 0.26%−2.6% of the total MPs. Glitter have not often been found in wastewater and are rarely reported in the sludge, likely due to the ban on MPs in personal care and cosmetic products in some countries. However, glitter was detected in this WWTP, and was primarily composed of polyethylene terephthalate (1.30–1.40 g/cm^3) [38,30,9]. Compared to microbeads added into personal care products designed for adults, glitters have a wider application and target audience. They are commonly used in products marketed to children. Foams were also observed in the wastewater but only accounted for 1.5%−9.4% of the MPs identified. This may be associated with the much lighter density of foams which keeps them floating at the surface. As such, they tend to evade sampling efforts.

Each particle analyzed by FTIR microscopy was also measured using its major dimension. Among the particle size classes sampled with sieves (<45 μm, 45–125 μm, 125–500 μm, 500–1000 μm, >1 mm), 45–125 μm was predominate (43–59%) during the low-flow period, followed by 125–500 μm (23–39%) (Fig. 3a). However, the particle size distribution shifted to larger sizes during moderate- and high-flow periods, with 33–49% of the particles between 125 and 500 μm and 12–27% between 45 and 125 μm (Fig. 3b-c). The long retention time of activated sludge could increase either the mechanical or biological fragmentation of MPs trapped by activated sludge in the biological unit, resulting in more small sizes of MPs than normal- and high-flow periods. Additionally, larger-sized MPs (>1 mm), mostly fibers, were also frequently observed, especially during moderate and high flow (10–25%). Yang et al. [39] demonstrated that the average size of fibers in the treated effluent was 1110.72 ± 862.95 μm, but other shapes of MPs were 681.46 ± 528.73 μm, with most particles concentrated at around 300 μm. For the smallest size fraction (<45 μm), only 0–4.8% were observed during

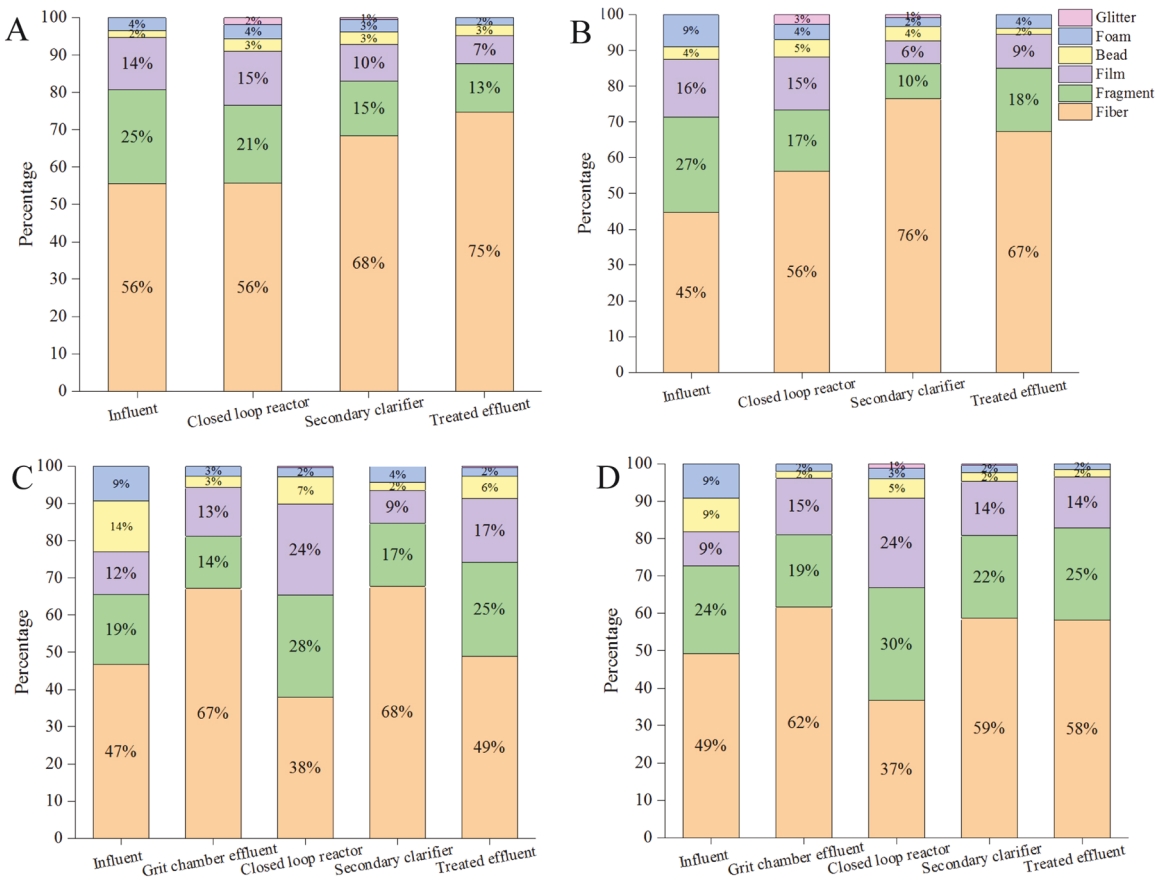

Fig. 2. MP morphology distributions during (A) 8–5–2020 (low-flow period), (B) 9–2–2020 (low-flow period), (C) 9–7–2021 (normal-/moderate-flow period), and (D) 9–12–2021 (high-flow period).

moderate- and high-flow periods, but a greater percentage (12–17%) was observed for this size class during the low flow period. Because our smallest sieve pore size was 45 μm, the presence of this fraction suggests that some smaller MPs were either retained on the sieve, perhaps agglomerated with other particles, or were generated during sample preparation. Others have also reported significant abundances of this smaller-sized fraction of MPs in WWTPs [9,12]. We did not find significant changes in the percentage of small MPs across the wastewater treatment stages ($p = 0.890$).

3.2.3. *Surface structure characterization*

Several particles were examined by SEM-EDX, which can provide both surface texture and elemental composition information. A particle mainly composed of oxygen and silicon is considered inorganic, while those composed of carbon are considered organic (Fig. S4). Due to their similar elemental compositions, it is challenging to distinguish plastics from organic matter depending only on EDX information. One exception is tire wear particles, as they have a unique morphology and texture and often have elevated concentrations of heavy metals like zinc. Particle 1 and particle 2 (Fig. 4) isolated from the influent and treated effluent are considered to be putative MPs, since they were both mainly composed of carbon and oxygen. These two particles showed brittle, hackly, wrinkled, and fractured surface morphologies, which are prone to break down and increase surface area for potential sorption and concentration of contaminants. A recent study demonstrated that the fragmentation of MPs into nano-particles by WWTPs processes increased the number of MPs/nano-particles in water by one order of magnitude [40].

3.2.4. *Polymer types characterization*

Imaging an entire filter was deemed to be too laborious and time-consuming as a full spectrum is collected for each pixel in the analysis area regardless of the presence or absence of a particle. Here, we measured 28–40% of the processed samples, and siMPle software was employed to facilitate the processing of data provided by the FTIR imaging system. Various polymers were detected in wastewater samples (Fig. 5). During the whole treatment stages, polyester (PEST - including polyethylene terephthalate and other polyesters) was the predominant polymer, accounting for 29.9–48.7% of all MPs. This is consistent with previous studies of MPs in WWTPs. For example, Lares et al. [28] reported PEST as the most abundant MPs in the influent, grit chamber effluent, and treated effluent. In other studies, PEST was frequently detected even after tertiary treatment processes [28,33]. As only domestic wastewater was collected in this study, the most likely potential source for PEST would be the laundering of synthetic textiles. A high percentage of acrylates were also found from raw sewage to treated effluent, ranging from 14.5% to 23.7%. Some other common polymers, such as polyethylene, polypropylene, and polyurethane were universally extracted. Together these polymers represent a large portion of the plastics manufactured globally [41]. Additionally, the sampling method may also have contributed to the increased frequency of low-density MPs present in the wastewater samples. Despite having a higher density than wastewater, PVC accounted for 0.9–6.1% of total identified MPs in wastewater samples. These heavy particles could be covered by biofilms or attach to other large light materials, enabling them to float. Biodegradable polymers (e.g., polylactic acid, polyglycolic acid, polycaprolactone, poly(vinyl alcohol)) were not detected in any of our samples. In general, the main polymers detected in all wastewater treatment stages were similar, including polyester, acrylates, polyethylene, polypropylene, polyvinyl chloride, and polyurethane.

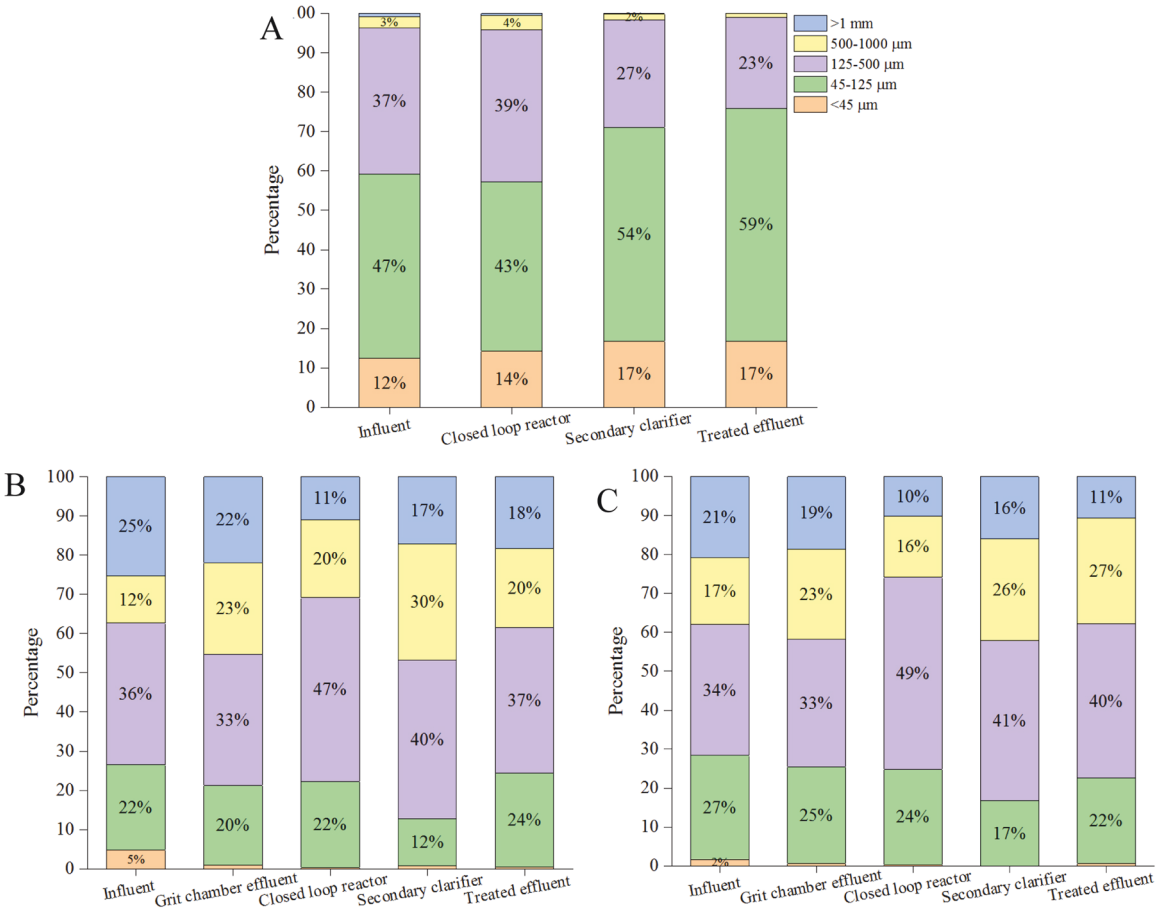

Fig. 3. MP size distributions during (A) low flow periods (8–5–2020 and 9–2–2020), (B) normal-/moderate-flow period (9–7–2021), and (C) high-flow period (9–12–2021).

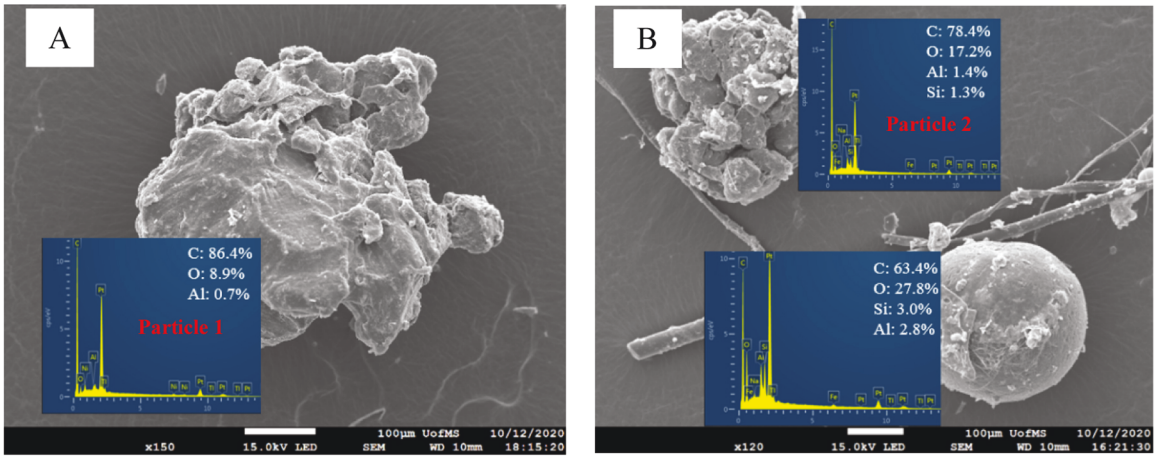

Fig. 4. Scanning electron microscopy images (top) and elemental composition determined by Energy Dispersive (X-Ray) Spectroscopy (bottom) of putative microplastics isolated from (A) influent and (B) treated effluent.

4. Conclusions

We assessed the occurrence and characteristics of MPs in major treatment compartments within a university WWTP during periods with different inflows due to drastic population changes on-campus. Concentrations of putative MPs in the influent increased after a football game, suggesting that the event affected MP loadings. We also found a higher abundance of MPs in the treated effluent during a low-flow period compared to normal- and high-flow days, even though the latter had higher inflow concentrations. We attribute this difference with the increased retention time of activated sludge during low-flow periods and effects of that on both the accumulation of MPs in the compartment and the removal efficiency of the secondary clarifier. Morphology affects MP removal in WWTPs. Fibers were predominant in all selected compartments, including treated effluent, with PEST the most abundant polymer. Therefore, more attention is needed to reduce

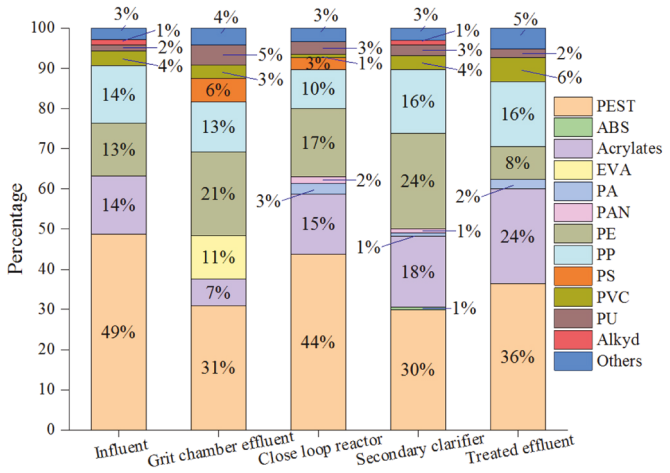

Fig. 5. Microplastic polymer compositions detected at the University of Mississippi wastewater treatment plant. ABS= acrylonitrile butadiene styrene, PA= polyamide, PAN= polyacrylonitrile, PEST= polyester, PE= polyethylene, PP= polypropylene, EVA= ethylene-vinyl acetate, PS= polystyrene, PU= polyurethane, PVC= polyvinyl chloride.

the occurrence of PEST fibers entering to natural environment via WWTPs. We observed larger MPs more frequently during normal- and high-flow periods than a low-flow period. Differences in plastic sources as well as sludge retention time likely alter the size distribution of MPs in the sludge. Thus, the impacts of changing the parameters of WWTP operation on the transport and fate of MPs in system deserve further scrutiny. Overall, we determined that WWTPs play a significant role in reducing MPs from wastewater, but can still discharge significant quantities of MPs into the natural environment, particularly when they are not operating optimally.

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CRediT authorship contribution statement

Z.G. and J.V.C. led the sampling campaign. J.V.C. and Z.G. conceived the project; Z.G. and A.V. analyzed the samples; K.W. helped with data interpretation; J.V.C. supervised and administered the project. All authors were involved in the Writing – review & editing, and data interpretation, and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: James Cizdziel reports financial support was provided by US Geological Survey. James Cizdziel reports equipment, drugs, or supplies was provided by National Science Foundation.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2022.108834.

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