Microplastic Analysis in NJ Wastewater Treatment Systems and Receiving Water Bodies

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Contents

1. Introduction

Over 12.7 million metric tons of mismanaged plastic enter the ocean on an annual basis (Jambeck et al., 2015). There is growing concern about the ecotoxicity of microplastics (MPs), many of which enter the ocean through freshwater systems. Wastewater treatment plants (WWTPs) are pathways of entry for MPs to water bodies. Studies on MP contamination in municipal wastewater have shown that although WWTPs can remove a significant portion of MPs from wastewater influent (Fahrenfeld et al., 2019), treated effluent is a likely vector for freshwater contamination (Murphy et al., 2016; van Wezel et al., 2016). However, removal rates can vary by unit process.

Cross-study comparison for MP studies is challenging due to the lack of consistent methods applied by different researchers for MP sampling, extraction, and analysis. Our recent meta-analysis of MP studies in freshwater including wastewater indicated that several factors impacted the MP concentrations and polymer profiles reported including location and sampling volume (Antoine et al., in review). Most researchers have collected volumes less than recommended by ASTM 8332 of 5,450 L for wastewater (ASTM, 2020), potentially biasing the concentration and polymer profiles reported. Extraction is required prior to analysis of MPs by vibrational spectroscopy with many researchers applying a peroxide or wet peroxide oxidation with or without additional steps such as cellulose and enzymatic digestion or density separation with a variety of salts (Masura et al., 2015; ASTM, 2021). Recent reviews indicate that most researchers studying wastewater (59-67% of studies) applied a density separation (Zoccali et al., 2025; Antoine et al., in review). These separations can introduce bias in polymer profiles and inaccuracies in polymer concentrations given the potential for losses of dense polymer types such as polyvinyl chloride (PVC) and acrylonitrile butadiene styrene (ABS) (Nabi et al., 2022). With respect to analysis, researchers have reported a wide range of subsampling strategies to address the analytical challenges posed by the number of post extraction particles (see Table 1). However, there is concern for methods that include visual sorting due to the potential significant biases towards white, black, or clear particles (Ziajahromi et al., 2017).

Several researchers have explored MP removal at wastewater treatment plants using filters including rapid sand (Talvitie et al., 2017; Wolff et al., 2021), disk (Talvitie et al., 2017; Simon et al., 2019; Salmi et al., 2021), and membrane biofilters (Talvitie et al., 2017). Removal efficiencies across these studies ranged from 40 to >99% with researchers applying a range of sampling, extraction, and analysis strategies and had different minimum particle size targets (Table 1). [Note: only studies that applied chemical confirmation are included in Table 1.] Among these studies, only Talvite et al. (2017) sampled multiple filter types. This team found similar removal rates for MP across filter types and reported chemical confirmation particles (N=752) from effluent samples. Thus, studies that allow for comparison of multiple filter types are desirable, particularly those that can provide robust MP analysis including polymer profiles and particle sizes. MPs have been detected in New Jersey water bodies (Ravit et al., 2017; Bailey et al., 2021; Boni et al., 2022; Parmar et al., 2023; Ochoa et al., 2024) and zooplankton (Sipps et al., 2022).

Further data from NJ WWTPs would be useful to identify and propose future potential mitigation strategies. A recent literature review focused on the removal efficiencies of MP from full-scale WWTPs over ten years (2014 to 2024) revealed that only 30 out of the 147 papers analyzed were from US WWTPs [excluding reviews, laboratory-scale, and pilot plants studies] (Zoccali et al., 2025). Our team previously demonstrated higher concentrations of MP 500-2000 µm in wastewater influent (1300 ± 790 MP/m^3) compared to effluent (63 ± 125 MP/m^3) (Bailey et al., 2021). Both of these observations were orders of magnitude greater than concentrations observed at the mouth of Raritan River and in the Hudson-Raritan estuary (0.30 ± 0.63 MP/m^3). And, to the authors knowledge, no NJ-specific MP data are available in smaller particle size ranges. These sizes are of particular interest, given that these particles are more abundant and able to translocate (Mehinto et al., 2022) and thus potentially more adverse health impacts.

The aims of this research were to (1) understand the removal and/or generation rates of MPs during wastewater treatment and (2) compare MP concentrations and polymer profiles upstream and downstream of outfalls in receiving surface water bodies. To achieve (1), paired wastewater influent, pre-filtration, and effluent samples were collected from four WWTPs with varying average daily flows and filtration types (i.e., disk, sand, or membrane). For (2), paired surface water samples were collected in the receiving surface water bodies up and downstream of the wastewater outfalls. MP concentrations and polymer profiles were compared for surface water upstream and downstream of WWTP outfalls. Comparisons were also made between the upstream and downstream surface water samples from the different utilities.

Table 1. Summary of studies evaluating MP removal in wastewater treatment plants with filters

Location (Reference)	Sampling Volume	MP Reported Size Ranges	MP Analysis	Filter Type	MP Concentration Before (MP/L)	MP Concentration After (MP/L)	Removal Rate
Finland (Talvitie et al., 2017)	Grab samples for different pore sizes: 20 µm 0.5-140 L, 100 µm 1-1000 L, 300 µm 6-1000 L 24-hr composite samples: 0.4-27.4 L	20–100 µm 100–300 µm >300 µm	FTIR on selected effluent samples, visual inspection for others	Rapid sand	0.7±0.1	0.02±0.007	97.1%
				Disk	0.5±0.2, 2.0±1.3	0.3±0.1, 0.03±0.01	40-98.5%
				Membrane	6.9±1.0	0.005 ±0.004	99.9%
Denmark (Simon et al., 2019)	Before DF: 200 L After DF: 1600 L	>10 µm	FPA-FTIR microscopy	Disk	29	3	89.7%
Germany (Wolff et al., 2021)	WWTP 1 Prefiltration: 26 L WWTP 1 Effluent: 2255 L WWTP 2 Prefiltration: 134 L WWTP 2 Effluent: 2503 L	10-50 µm 50-100 µm 100-500 µm 500-1000 µm 1000-5000 µm	Raman, analysis of 50% of filter	Sand filter WWTP 1	0.88± 0.27* (0.018 ± 0.2 fibers/L)	0.007 ± 0.0022* (0 fibers/L)	WWTP 1: 99.2 ± 0.29% WWTP 2: 99.4 ± 0.15%
				Sand filter WWTP 2	1.9 ± 0.28 (0.27 ± 0.083 fibers/L)	0.011 ± 0.0042 (0.003± 0.005 fibers/L)	
Finland (Salmi et al., 2021)	Influent: 1 L Prefiltration: 1-50 L Effluent: 10-1000 L	20-300 µm >300 µm	Raman, at least ¼ of filter or 10 polymers measured	Disk	(Influent 61± 26) 13	0.8±0.4	Influent to prefiltration: 79% Prefiltration to DF: 94% WWTP overall: 99%
Murcia, Spain (Bayo et al., 2020)	Influent: 4.08 ± 0.15 L MBF (Prefiltration):4.43 ± 0.33 L RSF (Effluent): 4.04 ± 0.14 L	200-400 µm 400-600 µm 600-800 µm 800-1000 µm 1-2 mm, 2-3 mm, 3-4 mm, 4-5 mm	FTIR	Membrane	4.40 ± 1.01	0.92 ± 0.21	MBF: 79.01% RSF: 75.49%
				Rapid sand		1.08 ± 0.28	

*Researchers reported MP and microfibers separately

MP – Microplastics, DF – Disc Filtration, WWTP – Wastewater Treatment Plant, MBF – Membrane biofilter, RSF – Rapid Sand Filter
FTIR – Fourier Transform Infrared Spectroscopy, FPA-FTIR – Focal Plane Array Fourier Transform Infrared Spectroscopy

2. Materials & Methods

2.1 Sample Collection

A total of 36 samples were collected from four WWTPs located in New Jersey by NJ DEP from September 28th, 2023 to November 21st, 2023. Wastewater influent, before filtration, and treated effluent samples were collected from each treatment plant on three separate occasions. Average sampling volumes varied depending on the stage sampled (influent = 6.2±1.9 L, before filtration = 6.8±1.3 L and effluent = 21.7±0.2 L, Table 2).

Additionally, a total of 11 surface water samples were collected from April 9th to 10th, 2024. Surface water samples were collected from the receiving water bodies upstream and downstream of each treatment plant outfall (N=8, Table 3). During the surface water sampling campaign, one wastewater influent sample from WWTP 4 was collected on the same date as the surface water sampling was performed. This sample was collected to allow for a paired comparison of the wastewater influent to the surface water given that the main wastewater sampling campaign occurred in Fall 2023. Two additional surface water samples were collected from WWTP 1 to serve as field replicates.

For QA/QC, five field blanks (1 L) were collected during wastewater sampling, and one field blank was collected during surface water sampling (0.51 L).

2.2 MP Extraction

Wastewater samples (including field blanks, one per utility and one for surface water sampling for N=6) were wet-sieved using a 20 µm sieve and transferred to glass beakers after being rinsed with deionized (DI) water, as modified from ASTM D882 (ASTM, 2020). The beakers were covered with aluminum foil to prevent contamination before being dried at 60°C. Matrix spikes consisted of polystyrene (PS) beads: 10-20 beads per wastewater sample (White, 355–425 µm, PolySciences, Inc., Warrington, PA) and 20 beads for every 10 L of surface water (Red, polystyrene divinylbenzene, 202 µm ChromoSphere). Matrix spikes were counted after extraction to facilitate the calculation of matrix spike recovery.

MPs were extracted from the residual solids following ASTM D8333 with minor modifications (ASTM, 2021). All residual solids were subjected to extraction except for pre-filtration samples from WWTP 2, for which 20% of the solids mass were extracted. First, peroxide oxidation was performed to remove labile organic material. Briefly, 25 mL of 30% hydrogen peroxide was added to solids in 50mL Falcon tubes, shaken for 60 minutes (100 rpm), then centrifuged at 5000 rpm for 3 min to create a pellet. To prevent particle loss, the supernatant was filter-concentrated on a 20 µm steel mesh. Peroxide oxidation was repeated until addition of hydrogen peroxide did not result in a further reduction of sample turbidity, which was required for influent and some pre-filtration samples.

Table 2. Wastewater treatment plant (and filter type), sampling dates, and volumes collected.

WWTP	**Sampling Date**	**Sampling Volume (L)**			**Average Daily Flow (MGD**)**
		Influent	Pre-filtration	Effluent	
1 (disk filter)	9/28/2023	5.1	9.0	21.6	3
	10/04/2023	8.0	8.6	21.7	
	10/06/2023	11.5	8.9	22	
2 (sand)	10/12/2023	4.7	6.4	21.6	13
	10/18/2023	6.4	6	21.2	
	10/20/2023	6.3	6.1	21.8	
3 (membrane)	10/25/2023	5.4	5.8*	21.8	1
	11/01/2023	5.7	6.0	22.0	
	11/02/2023	5.2	5.5	21.7*	
4 (disk filter)	11/15/2023	5.7	6.9	21.8	3
	11/16/2023	5.7	5.7	21.8	
	11/21/2023	4.9	6.4	21.8*	

**Indicates samples where volumes were not measured and therefore estimated by averaging volumes of samples of the same type.*

** Millions Gallons per Day

Table 3. Sampling dates, volumes, and locations for the surface water study. NA indicates this sample type was not collected for the given WWTP.

WWTP	Sampling Dates m/d/y	Sampling Volume (L)		
		Upstream	Influent	Downstream
1	04/09 - 04/10/2024	11.7, 20.5	NA	17.0, 19.3
2	04/09 - 04/10/2024 (upstream), 04/06/2024 (downstream)	39.1	NA	37.4
3	04/09 - 04/10/2023	53.4	NA	29.0
4	04/09/2024 (up- and downstream); 4/10/2024 (influent)	21.6	21.9	42.1

Next, cellulose digestion was performed on the pellet and filter-concentrated particles by adding 40 mL of a modified Schweizer's reagent (2.5 g copper (II) hydroxide for every 100 mL of 30% ammonium hydroxide). The reaction was performed in Falcon tubes that were shaken for 5 min, then centrifuged at 5000 rpm for 3 min. Disposable glass pipettes were used to remove the supernatant without disturbing the pellet. An additional 25 mL of 30% ammonium hydroxide was added to the Falcon tubes to solvate any remaining modified Schweizer's reagent. After repeating the process of shaking, centrifuging, and removing the supernatant, the remaining particles were concentrated on 20 µm steel mesh.

Wastewater influent samples underwent additional processing through enzymatic digestion to remove organic material (Appendix Figure A1). Particles were resuspended from the wire mesh and transferred to the Falcon. Particles were rinsed with 20 mL of 1M Tris HCl buffer (pH 8), shaken for 5 min, then centrifuged for 3 min at 5000 rpm before removing the Tris HCl buffer solution. Next, 15 mL of protease solution, 20 mL of Tris HCl, pH 8 buffer solution, and 5 mL of lipase were added to the tube before shaking for 20 hours at 20 rpm and 45°C. After centrifuging the tubes again for 3 min at 5000 rpm, the remaining particles were filtered onto a 20 µm steel mesh. Then, the samples were sonicated in methanol for 15 min before being rinsed onto a 63 µm steel mesh for analysis. The methanol filtrate was then filtered onto a 20 µm mesh, to allow for future potential analysis of smaller particles.

2.3 Polymer Identification

Particles were analyzed via Fourier transform infrared spectroscopy (FTIR). Attenuated Total Reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was performed on a spectrometer equipped with a single-bounce diamond internal reflection element (IRE; Bruker Alpha, Bruker, Billerica, Massachusetts or Nicolet iS50, ThermoFisher, Waltham, MA). Particles were transferred to the IRE using tweezers. Spectral data were collected by conducting 32 scans per particle at a resolution of 4 cm^{-1} within a wavenumber range of 4000 to 400 cm^{-1}. Background scans of air were performed before and periodically during analysis using the same number of scans, resolution, and wave number range. Selected particles were analyzed in transmittance mode on a FTIR microscope with an electronically cooled TE-MCT detector (Bruker Lumos II, Bruker Optics, Billerica, MA) on a CaF_2 window (Harrick Scientific Products, Pleasantville, NY). These spectra were also collected by conducting 32 scans per particle at a resolution of 4 cm^{-1}, but with a wavenumber range of 4000 to 1000 cm^{-1} due to spectral artifacts below 1000 cm^{-1} from the CaF_2 window.

Spectral analysis was conducted using OpenSpecy for R and the derivative library, an open-source polymer library that reports Pearson correlations between reference and uploaded spectra (Cowger et al., 2020). The library was filtered so that only FTIR spectra would be compared to the uploaded spectra, and the recommended pre-processing was conducted including conforming and smoothing with the following settings: polynomial = 3, windows = 11, and derivative order = 1. Generally, particles with over a 70% match (i.e., Pearson correlation coefficient) (California State Water Resources Control Board, 2021) with consistent polymer identification over the top five matches were classified as that polymer type. Selected particles identified as MP had additional physical characteristics documented such as color, morphology (fiber, fragment, sphere, and film), and size (as described below).

Total particles per sample > ~140 µm were counted after collecting filter images (either via the LUMOS microscope or cellphone camera) using ImageJ software. In this study, all particles were analyzed if the total number of particles in the sample after extraction was less than ~50 particles. If the total number of particles was greater, then subsampling was performed. For subsampling, the largest 20 particles were analyzed first, as needed, to prevent the obstruction of smaller particles. Then, a second twenty particles were randomly analyzed by overlaying a numbered, boxed grid over a petri dish. The centermost particle was then analyzed when the box was randomly selected using a number generator. The percentage of MPs found within the randomly selected 20 particles would then be multiplied by the total number of particles in the sample to estimate the number of MPs. A total of 1483 spectra were collected across the wastewater sampling campaign and 259 for the surface water samples, plus nine spectra for blanks.

2.4 Particle Size

For 459 of the wastewater MPs, the longest particle dimension (Feret's diameter maximum, FDM in μm) and longest perpendicular dimension (Feret's diameter horizontal, FDH in μm) were recorded. MP included in the particle size analysis were from WWTP 2 samples before sand filter and effluent samples. Photos were taken using either a Lumos II microscope and measured using the Bruker Opus software or particles were placed on a scale grid (¼ in) and photographed using a cell phone camera. Using Webplot Digitizer to overlay a XY coordinate plane over the scale grid, X and Y coordinates were placed to mark the FDM and FDH of each particle. FDM and FDH were then calculated using the distance formula adjusted as a ratio to the length and width of the scale grid.

2.5 Data Analysis

All statistical tests were performed in R and data visualized using the ggplot2 package. The number of polymer types observed (1) in wastewater by sampling site and WWTP was compared via PERMANOVA and (2) in the surface water campaign in up- and downstream samples across all WWTP were compared via a Wilcoxon Rank sum test. The same tests were used to compare MP concentrations (and for WWTP, removal rates) by these factors. Matrix spike recoveries were compared by WWTP and sampling location using a Kruskal-Wallis test.

To compare polymer profiles between utilities and across sampling sites for each sampling campaign, polymer counts were square root-transformed and a Bray–Curtis dissimilarity matrix was created for use in ordination and represented via non-metric multidimensional scaling (nMDS, vegan package). An analysis of similarities (i.e., ANOSIM) test was performed on the dissimilarity matrix to understand if WWTP and sampling site were factors associated with the polymer profiles observed for each sampling campaign. Correlations were tested between total post-extraction particle concentrations and observed MP concentrations (excluding any scaled samples) using Spearman Rank correlation tests. MP dimensions (FDM and FDH) were compared between WWTPs, sampling sites, and polymer types using PERMANOVA (adonis2 function in vegan package). Kolmogorov-Smirnov tests were applied to compare MP size distributions between sampling sites, as well as for the two most prevalent plastic types: polypropylene (PP) and polyester (PES). An Anderson-Darling test was applied to compare MP size distributions across WWTPs.

3. Results

3.1 WWTP Microplastic Observations

Of the 1483 particles analyzed from the wastewater influent, pre-filtration, and effluent sampling campaign, 548 were identified as manufactured polymers. These manufactured polymers were categorized into 17 polymer classes. Sampling site was significantly associated with the number of unique polymer types (i.e., polymer richness) observed per volume (R^2=0.30, p=0.002, PERMANOVA): influent samples had 0.65±0.36 unique polymer types/L (average ± standard deviation), pre-filtration samples had 0.51±0.19 unique polymer types/L, and effluent samples had 0.30±0.07 unique polymer types/L. This corresponds to 3.8±1.9, 3.5±1.5, and 6.3±1.6 unique polymer types per sample, for the influent, pre-filtration, and effluent samples, respectively. Example FTIR spectra, microscope images, and library spectral matches are shown in Fig. 1.

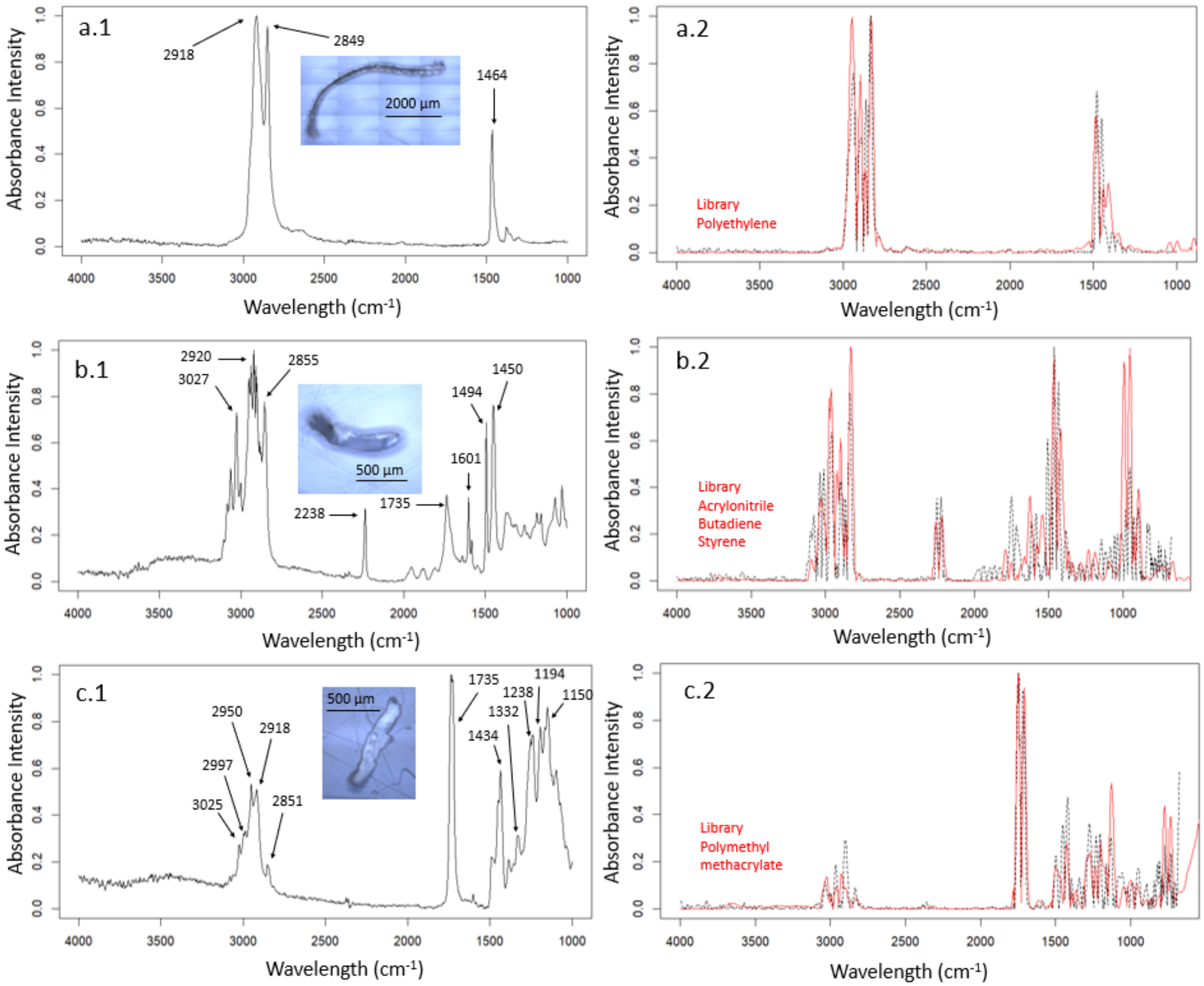


Fig. 1. Example spectra and corresponding particle images for a. polyethylene (PE), b. acrylonitrile butadiene styrene (ABS) fragment, and c. Poly(methyl methacrylate) (PMMA) fragment, (1) shows raw spectrum. (2) shows processed spectrum (black dotted lines) and reference spectrum (red) for the matched polymer. (continued on next page)

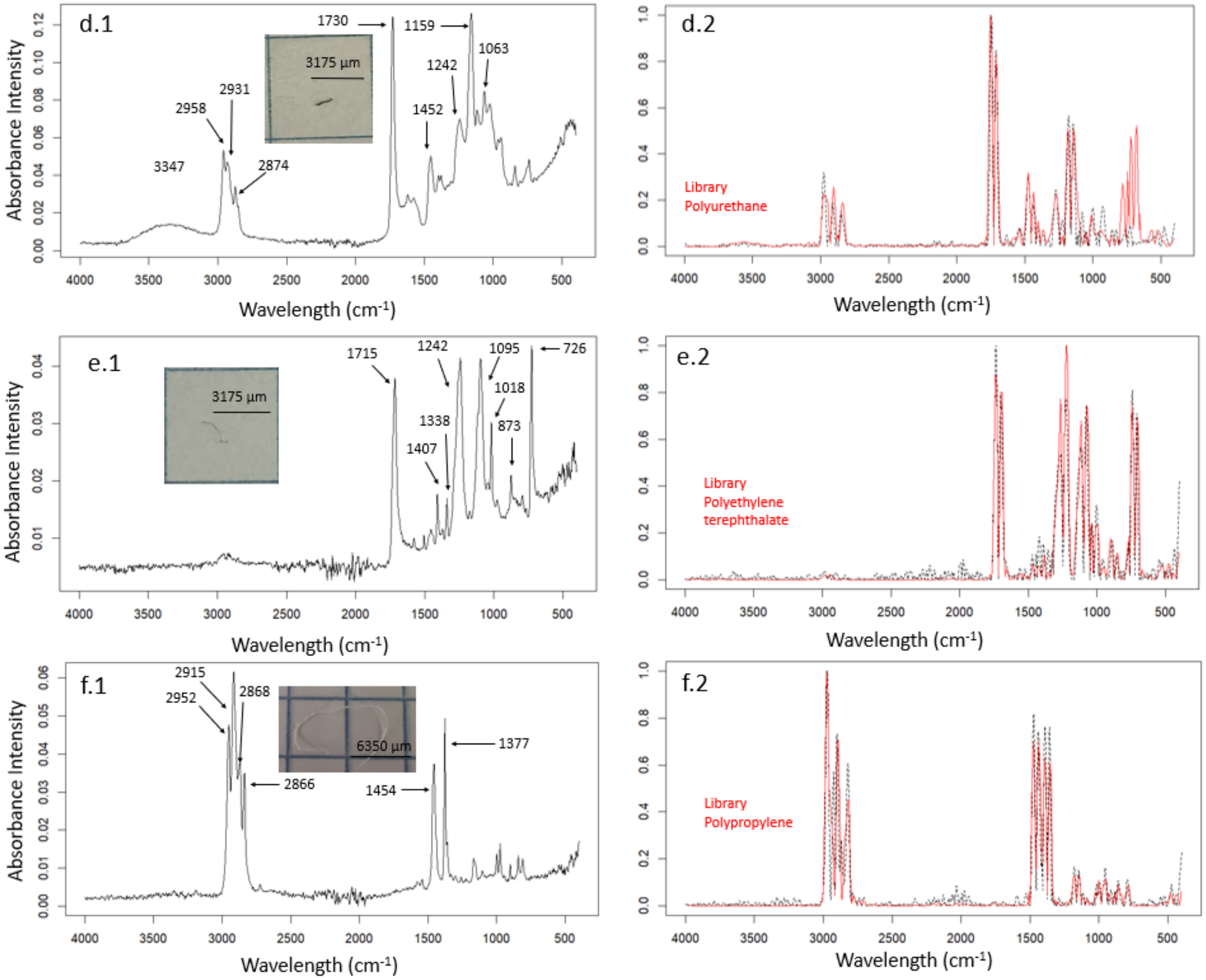
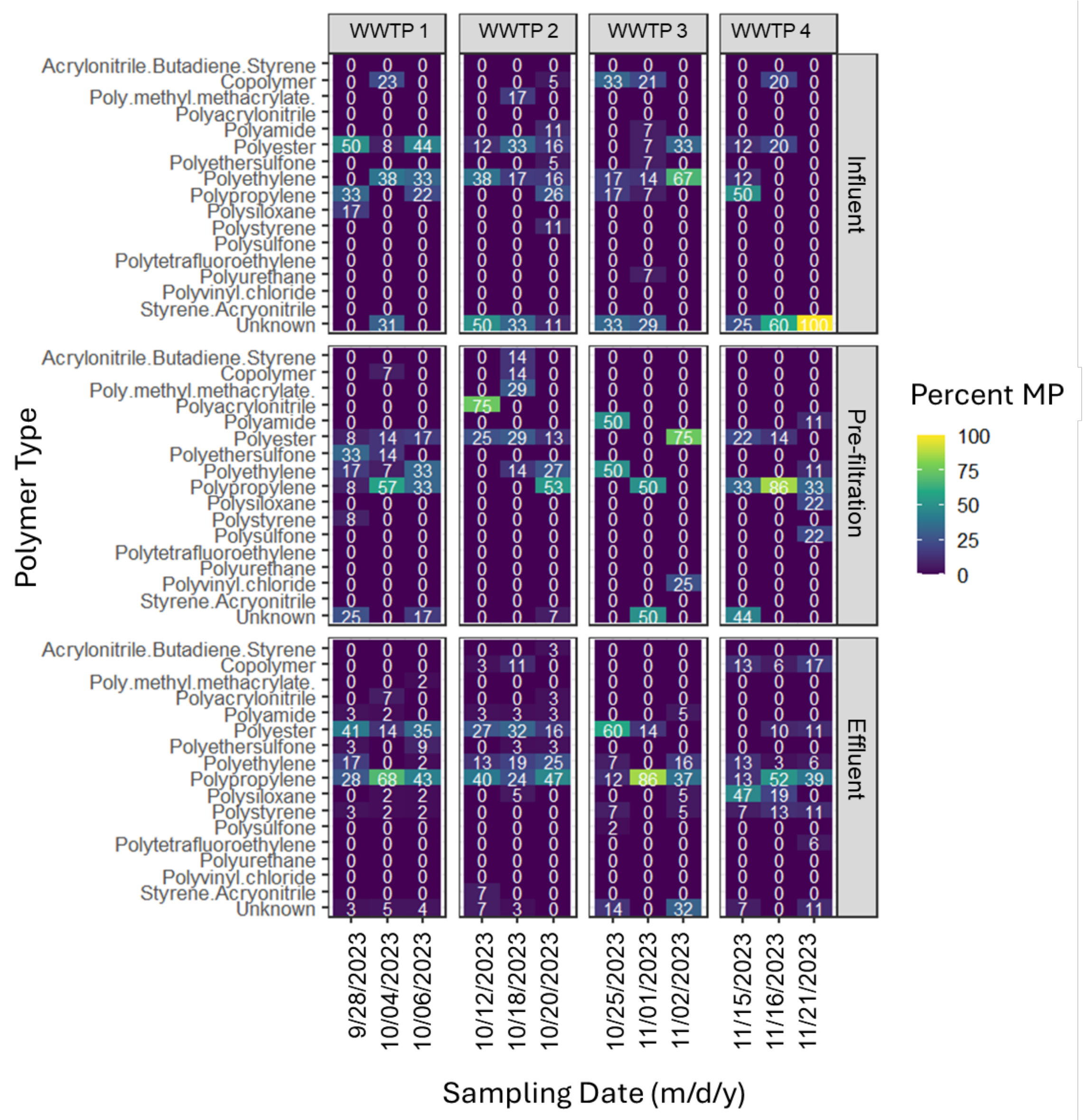


Fig. 1. (Continued) Example spectra and corresponding particle images for d. polyurethane (PU), e. polyethylene terephthalate (polyester - PES) fiber, and f. polypropylene (PP) fragment. (1) shows raw spectrum. (2) shows processed spectrum (black dotted lines) and reference spectrum (red) for the matched polymer.

The most prevalent polymer types across samples were polypropylene (35% of MPs), polyester (23% of MPs), and polyethylene (12% of MPs, Fig. 2). Polymer profiles were associated weakly with sampling stage (p=0.001, R=0.22) but not WWTP (p=0.32, ANOSIM). This is reflected in the nMDS showing clustering by sampling stage of the wastewater treatment process, but not by the WWTPs themselves or sampling dates (Fig. 3). (Note, the comparisons in polymer profiles discussed here were made using the polymer concentrations observed without scaling the samples that required subsampling.) Across all blanks, no MPs were observed. All particles in the blanks either resulted in spectra classified as non-plastics or produced poor quality spectra.



	Polymer Type	WWTP 1			WWTP 2			WWTP 3			WWTP 4		
		9/28/2023	10/04/2023	10/06/2023	10/12/2023	10/18/2023	10/20/2023	10/25/2023	11/01/2023	11/02/2023	11/15/2023	11/16/2023	11/21/2023
Influent	Acrylonitrile.Butadiene.Styrene	0	0	0	0	0	0	0	0	0	0	0	0
	Copolymer	0	23	0	0	0	5	33	21	0	0	20	0
	Poly.methyl.methacrylate.	0	0	0	0	17	0	0	0	0	0	0	0
	Polyacrylonitrile	0	0	0	0	0	0	0	0	0	0	0	0
	Polyamide	0	0	0	0	0	11	0	7	0	0	0	0
	Polyester	50	8	44	12	33	16	0	7	33	12	20	0
	Polyethersulfone	0	0	0	0	0	5	0	7	0	0	0	0
	Polyethylene	0	38	33	38	17	16	17	14	67	12	0	0
	Polypropylene	33	0	22	0	0	26	17	7	0	50	0	0
	Polysiloxane	17	0	0	0	0	0	0	0	0	0	0	0
	Polystyrene	0	0	0	0	0	11	0	0	0	0	0	0
	Polysulfone	0	0	0	0	0	0	0	0	0	0	0	0
	Polytetrafluoroethylene	0	0	0	0	0	0	0	0	0	0	0	0
	Polyurethane	0	0	0	0	0	0	0	7	0	0	0	0
	Polyvinyl.chloride	0	0	0	0	0	0	0	0	0	0	0	0
	Styrene.Acryonitrile	0	0	0	0	0	0	0	0	0	0	0	0
	Unknown	0	31	0	50	33	11	33	29	0	25	60	100
Pre-filtration	Acrylonitrile.Butadiene.Styrene	0	0	0	0	14	0	0	0	0	0	0	0
	Copolymer	0	7	0	0	14	0	0	0	0	0	0	0
	Poly.methyl.methacrylate.	0	0	0	0	29	0	0	0	0	0	0	0
	Polyacrylonitrile	0	0	0	75	0	0	0	0	0	0	0	0
	Polyamide	0	0	0	0	0	0	50	0	0	0	0	11
	Polyester	8	14	17	25	29	13	0	0	75	22	14	0
	Polyethersulfone	33	14	0	0	0	0	0	0	0	0	0	0
	Polyethylene	17	7	33	0	14	27	50	0	0	0	0	11
	Polypropylene	8	57	33	0	0	53	0	50	0	33	86	33
	Polysiloxane	0	0	0	0	0	0	0	0	0	0	0	22
	Polystyrene	8	0	0	0	0	0	0	0	0	0	0	0
	Polysulfone	0	0	0	0	0	0	0	0	0	0	0	22
	Polytetrafluoroethylene	0	0	0	0	0	0	0	0	0	0	0	0
	Polyurethane	0	0	0	0	0	0	0	0	0	0	0	0
	Polyvinyl.chloride	0	0	0	0	0	0	0	0	25	0	0	0
	Styrene.Acryonitrile	0	0	0	0	0	0	0	0	0	0	0	0
	Unknown	25	0	17	0	0	7	0	50	0	44	0	0
Effluent	Acrylonitrile.Butadiene.Styrene	0	0	0	0	0	3	0	0	0	0	0	0
	Copolymer	0	0	0	3	11	0	0	0	0	13	6	17
	Poly.methyl.methacrylate.	0	0	2	0	0	0	0	0	0	0	0	0
	Polyacrylonitrile	0	7	0	0	0	3	0	0	0	0	0	0
	Polyamide	3	2	0	3	3	3	0	0	5	0	0	0
	Polyester	41	14	35	27	32	16	60	14	0	0	10	11
	Polyethersulfone	3	0	9	0	3	3	0	0	0	0	0	0
	Polyethylene	17	0	2	13	19	25	7	0	16	13	3	6
	Polypropylene	28	68	43	40	24	47	12	86	37	13	52	39
	Polysiloxane	0	2	2	0	5	0	0	0	5	47	19	0
	Polystyrene	3	2	2	0	0	0	7	0	5	7	13	11
	Polysulfone	0	0	0	0	0	0	2	0	0	0	0	0
	Polytetrafluoroethylene	0	0	0	0	0	0	0	0	0	0	0	6
	Polyurethane	0	0	0	0	0	0	0	0	0	0	0	0
	Polyvinyl.chloride	0	0	0	0	0	0	0	0	0	0	0	0
	Styrene.Acryonitrile	0	0	0	7	0	0	0	0	0	0	0	0
	Unknown	3	5	4	7	3	0	14	0	32	7	0	11

Fig. 2. *Heatmap showing percentage of polymer type by sampling event (x-axis), WWTP (columns), and sampling stage (rows). Overlaid text is rounded percentage of MP.*

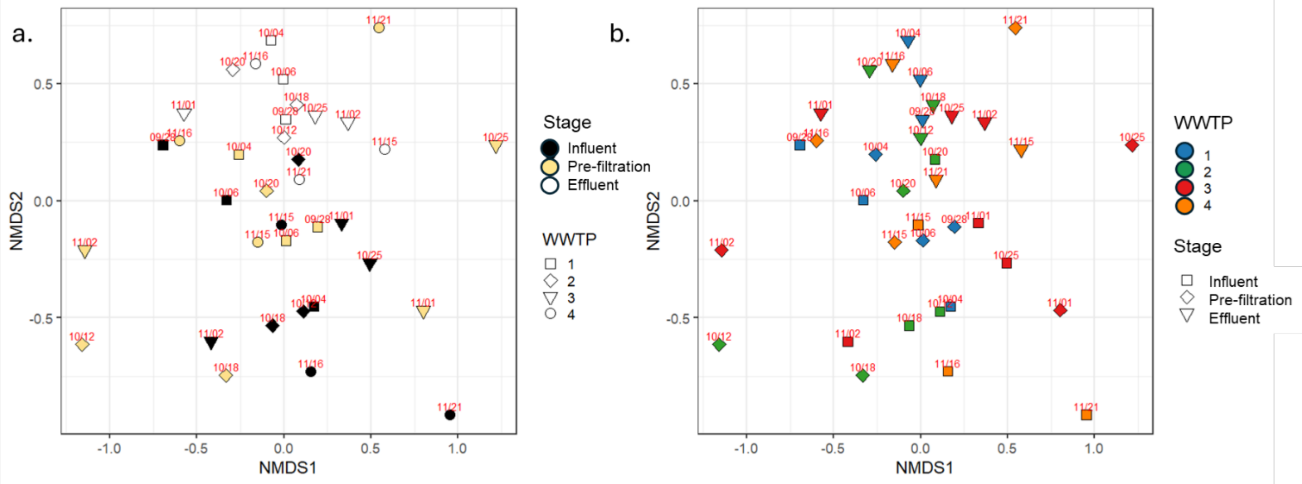


Fig. 3. nMDS of polymer profiles illustrated with a. color corresponding to sampling stage and shapes corresponding to WWTP and b. color corresponding to WWTP and shape corresponding to sampling location. Stress = 0.19.

The non-plastic particles observed in the wastewater samples were primarily classified as other organic materials (66% of non-plastics) or minerals (10% of non-plastics) along with several particles which produced poor quality spectra (6% of non-plastics). The non-plastic organics included top library matches to cotton and other natural fibers, cellulose, and "tomato skin" among others. Several white fragments in wastewater influent (N=1-30 particles/sample) had inconsistent spectral matches to polymers in OpenSpecy and were manually identified as closer matches to calcium stearate and/or fat, oil, and grease (FOG) spectra (Fig. 4). Calcium stearate is an additive to plastics and several other materials including soaps but is not a plastic itself. Fat, oil, and grease can form solid white globules at room temperature in wastewater and sewers and FTIR spectra for lab-generated and sewer FOG samples are available in the literature (He et al., 2011). The rest were unknown materials. The presence of these non-plastic materials post-extraction, which represented 63% of total particles analyzed, underscores the need for improved extraction protocols to help reduce analytical efforts for analyzing non-target materials using count-based methods.

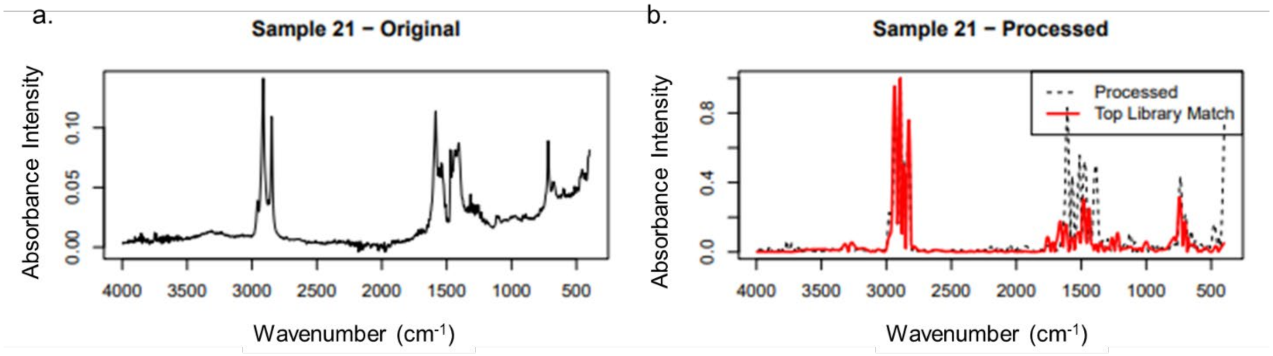


Fig. 4. FTIR spectrum from a white particle in WWTP 4 influent 11/21/2023 (a) and after spectral processing (dashed black) compared to the top OpenSpecy library match (red) (b).

3.1.1 MP concentrations and removal across treatment

Total particle counts were determined after extractions with 2.8±2.0 particles/L in the pre-filtration samples (for the disk and sand filters) and 1.8±0.8 particles/L in the effluent samples. Subsampling was required for all influent samples and the pre-filter sample from WWTP 3 (i.e., pre-membrane filtration). The influent samples had an estimated 294±347 particles/L, and the membrane samples had 105±47 particles/L after extraction, following the additional lipase and proteinase treatment described in Section 2.2. For these samples, 6.2±3.8% of the total estimated particles were analyzed and scaled to estimate total MP concentrations.

Wastewater influent concentrations averaged 33±45 MP/L, ranging from 2.6 to 153 MP/L (Fig.5). Average pre-filtration concentrations were 14±24 MP/L and effluent concentrations were 1.4±0.52 MP/L. Thus, MP concentrations varied moderately by sampling site within the treatment facilities (R^2=0.42, p=0.001), but not by utility or the interaction of sampling site and utility (both p>0.30, PERMANOVA).

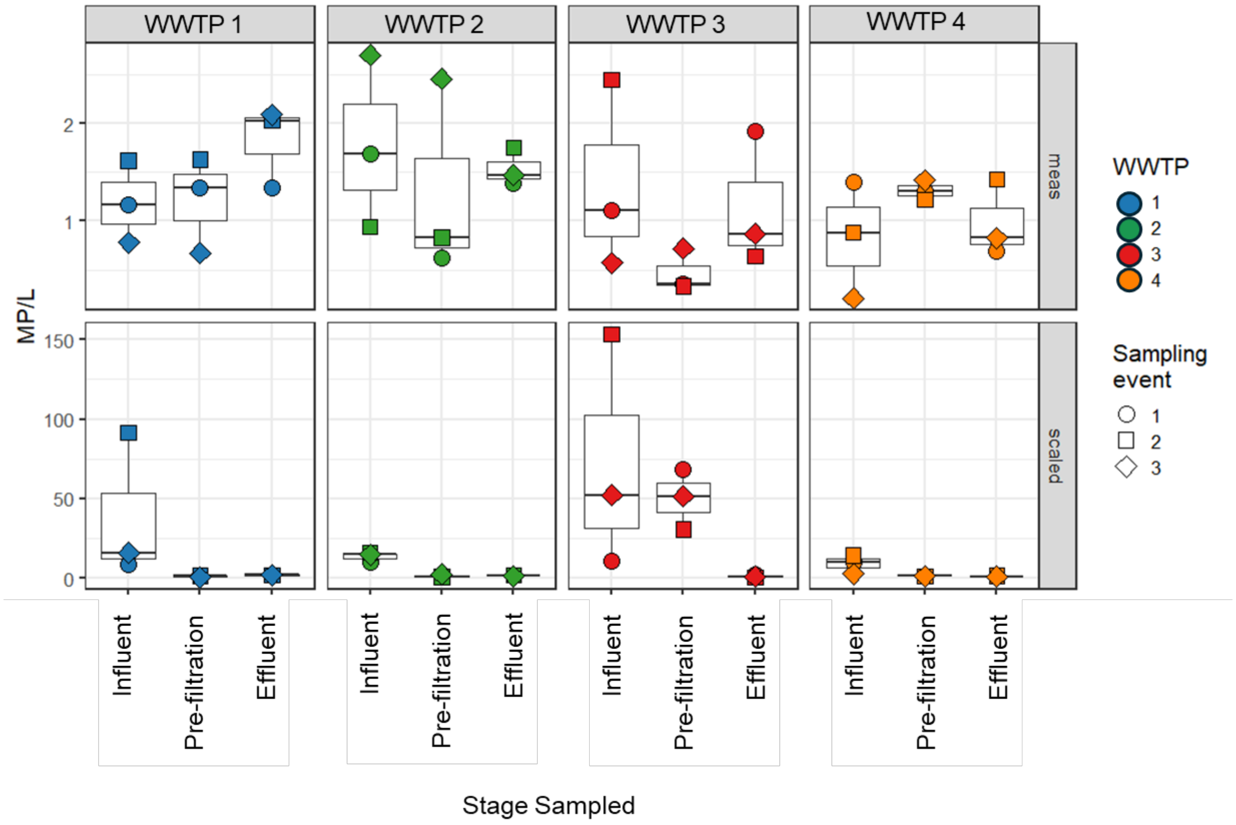


Fig. 5. *Box plots of total MP concentration (MP/L) versus sampling site. Overlaid jitter for individual sampling events (shape) at each sampling site. Colors correspond to WWTP. Data shown in "meas" panels (top) represent the MP concentration without scaling for subsampling, "scaled" panels (bottom) includes scaling for subsampled samples.*

Of particular interest was estimation of MP removal/generation rates during treatment for the particle size range studied (Table 4). Between influent and pre-filtration samples, there was on average 93±7%, 90±6%, and 75±25% removal for WWTP 1, 2, and 4, respectively. WWTP 3 had one sampling date with higher MP concentrations at pre-membrane filtration (68 MP/L) compared to influent (11 MP/L), representing a 544% increase in concentration. On the other two sampling dates, there was 1-80% (40±56%) MP removal between the influent and pre-filtration samples. This corresponds to an average overall -154 ± 340% removal across the three sampling events, influenced heavily by the date with the higher pre-filtration MP concentration. Between influent and effluent samples, there was 90±7% removal for WWTP 1, 88±2% removal for WWTP 2, 93±10% removal for WWTP 3, and 84±14% removal for WWTP 4. Removal rates varied moderately by treatment type (i.e., before and after each filter type, R^2=0.54, p=0.036) but not by utility or their interaction (both p>0.26, PERMANOVA). These removal rates likely reflect the pattern of the utilities without membrane treatment having similar MP concentrations in pre-filtration samples as in the effluent. Meanwhile, WWTP 3 with membrane treatment had most removal observed at the membrane filtration step. These decreases in MP concentrations with treatment could correspond with accumulation of MP in the solid phase (which was not measured here) and/or losses through other mechanisms (e.g., fragmentation below the particle size range investigated here).

Table 4. MP removal/generation rates for each utility by sampling stage. Average values are shown ± standard deviation of triplicate samples.

WWTP (filter type)	MP concentration average percent difference (%) from	
	Influent to pre-filtration	Influent to effluent
1 (disk filter)	93±7	90±7
2 (sand filter)	90±6	88±2
3 (membrane filter)	-154 ± 340	93±10
4 (disk filter)	75±25	84±14

Strong correlations were observed between total post-extraction particle concentrations and total MP concentrations for pre-filtration samples (p=0.026, rho=0.75, Spearman Rank correlation) and very strong correlations for the effluent samples ($p<2.2\times10^{-6}$, rho=0.92, Spearman Rank correlation, Fig. 6). These correlations were tested using only samples for which analysis of all post-extraction particles was performed given that scaled sample concentrations were estimated using the total post-extraction particle counts and would bias these tests. Overall, 57±23% of the total post-extraction particles from the pre-filtration samples (for the two disk- and one sand filtration WWTP) and 77±11% of the total post-extraction particles from the effluent samples were identified as MP.

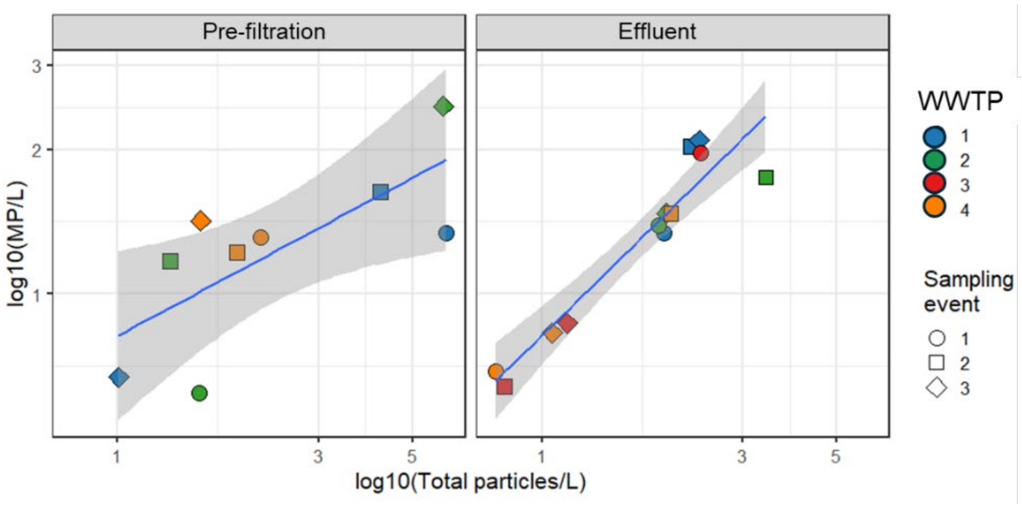


Fig. 6. *MP/L versus total post-extraction particles/L with color corresponding to WWTP, shape corresponding to sampling event. Linear regression shown as blue line and gray shading showing 95% confidence interval.*

Particle size analysis was performed on 459 MP out of the 548 identified from wastewater samples and shown as a function of MP polymer type (Fig. 7). Influent, pre-filtration, and effluent samples showed right-skewed distribution when plotted on a linear histogram but are displayed on a logarithmic histogram to show the wide distribution of sizes. Influent, prefiltration, and effluent samples showed an average FDM of 1657±1739 μm, 924±978 μm, and 828±303 μm, and median values of 1326 μm, 571 μm, and 491 μm, respectively. Nine macroplastic particles were found in the samples (effluent = 5, pre-filtration = 1, and influent = 3). Both the smallest (90.65 μm) and largest MP (15744 μm) were found in 10/18 effluent samples from WWTP 2.

FDM had an association with sampling stage and polymer class (both $R^2 = 0.10$ and $p = 0.001$, PERMANOVA) and a weaker but significant association with WWTP ($R^2 = 0.02$ and $p = 0.007$, PERMANOVA). FDH similarly showed an association with sampling stage ($R^2 = 0.15$, $p = 0.001$, PERMANOVA) and weak associations with WWTP and no association with polymer class ($R^2 = 0.02$, $p = 0.010$ and $p=0.44$, respectively, PERMANOVA). FDH also had a weak association for the interaction between sampling stage and WWTP ($R^2 = 0.03$ and $p = 0.008$, PERMANOVA).

The size distributions between sampling stages were also analyzed. Influent and pre-filtration MP size distributions were different ($D = 0.37$, p-value = 0.001, Kolmogorov-Smirnov). No differences were observed between the size distributions of samples collected pre-filtration and from the effluent ($D=0.13$, $p = 0.187$, Kolmogorov-Smirnov). MP size distributions varied by WWTPs ($A^2 = 5.86$, $p <0.001$, Anderson-Darlington). Comparing the two most prevalent polymer types (polypropylene and polyester), these polymers had different size distributions ($D = 0.45$, $p<0.001$, Kolmogorov-Smirnov). This is likely explained by the morphology of these MP: the most common morphology for polyester was fiber, whereas polypropylene fragments and fibers were both prevalent.

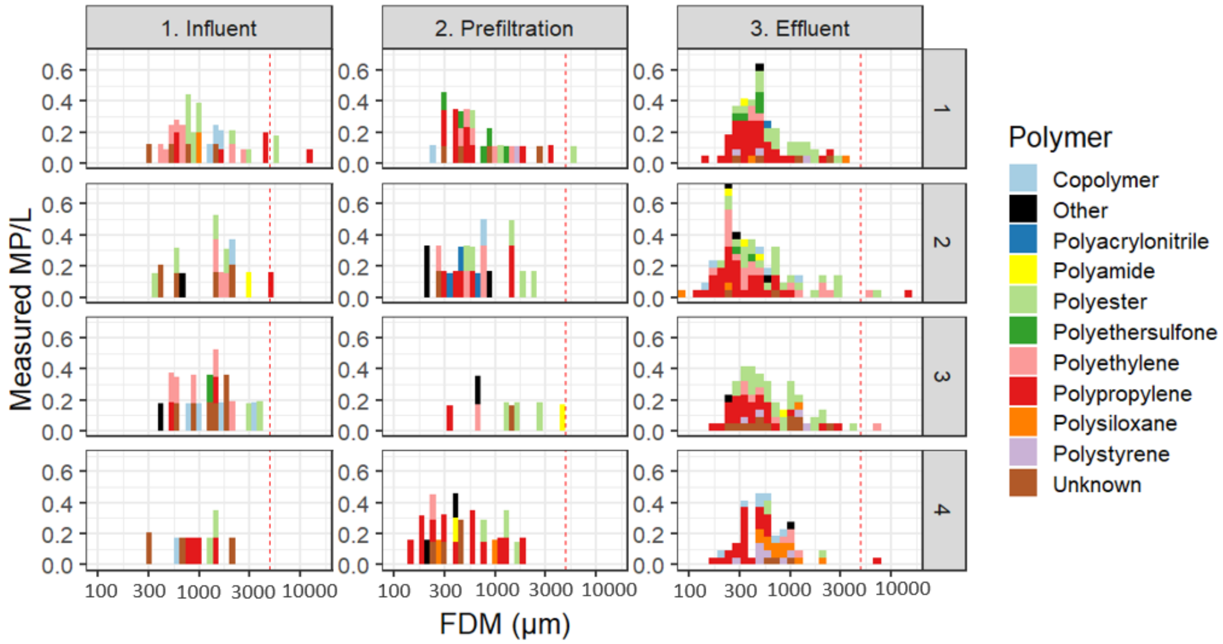


Fig. 7. *Histogram of maximum particle length (FDM, in microns) of 459 MP from wastewater samples. Colors correspond to polymer type and samples are sorted by WWTP (rows) and sampling stage (columns). Vertical red lines are at 5000 μm to distinguish particles that are considered macroplastics. Y-axis values for influent and prefiltration differ from effluent samples to account for difference in MP count.*

3.2 Surface Water Microplastic Observations

Of the 259 particles analyzed from the surface water sampling campaign, 95 were identified as manufactured polymers (three of which were identified in the paired wastewater influent sample collected at WWTP 4). All post-extraction particles >140 μm were analyzed in six of the ten surface water samples (average of 24.7±11.5 particles/sample) and the paired influent sample from WWTP 4 (18 particles). The other four surface water samples had 73-302 particles remaining after extraction and therefore required subsampling. For these samples, 7-27% of the total estimated particles were analyzed (Table 5).

Table 5. *Total post extraction particle counts and percent of particles analyzed for each WWTP and sampling location. NA indicates this sample type was not collected for the given WWTP.*

WWTP	Total Post-Extraction Particle Counts (% analyzed if <100%)		
	Upstream	Influent	Downstream
1	16 or 145 (14%)	NA	24-28
2	257 (13%)	NA	23
3	73 (27%)	NA	12
4	302 (7%)	18	45

Materials were categorized into 11 polymer classes. The most prevalent classes across the surface water samples were unknown (N=21), copolymers (N=21), polystyrene (N=13), and polypropylene (N=11) (Fig. 8a). Upstream samples had an average of 3.8±1.8 polymer types observed compared to 4.4±1.5 polymer types in downstream samples, thus there were a similar number of polymer types observed in the downstream samples (p=0.23, Wilcoxon Rank Sum) across the receiving water bodies sampled. The receiving river for WWTP 3 had all polymer types observed in the upstream (i.e., polytetrafluorethylene, polystyrene, and polypropylene for WWTP 3) also observed in the downstream samples, the latter which also contained two types not observed upstream (i.e., polyether sulfone, co-polymer, and polysiloxane). The receiving water bodies for the other WWTPs had some polymers observed upstream that were not detected in the downstream samples. This observation is reinforced with the nMDS plots (Fig. 8b) that demonstrated clustering for WWTP 3 but otherwise overlapping profiles for the other WWTPs. (Note, the comparisons in polymer profiles discussed here were made using the polymer concentrations observed without scaling the samples that required subsampling.) Interestingly, there was not clustering by sampling location nor consistent trajectories across the sampling locations (ANOSIM, all p>0.13). The polymer profiles for the upstream and downstream varied the most for WWTP 2. These samples were collected three days apart rather than one day apart as done for the other sampling locations, which could potentially explain the greater variation in profiles observed. The profiles for the upstream and downstream samples for WWTP 4 were about equidistant to one another as the influent sample collected for that utility. For WWTP 4, the up and downstream samples were collected on the same day while the influent was collected the day after.

The non-plastic particles were classified as inorganic or minerals (66.5% of the non-plastics), polyamides (3.7%), and natural organic materials including cotton and cellulose (14%). Several other particles produced poor quality spectra (7.9%). The rest were unknown materials. The presence of these non-plastic materials post-extraction, which represented 63% of total particles analyzed, underscores the need for improved extraction protocols to help reduce analytical efforts for analyzing non-target materials using count-based methods.

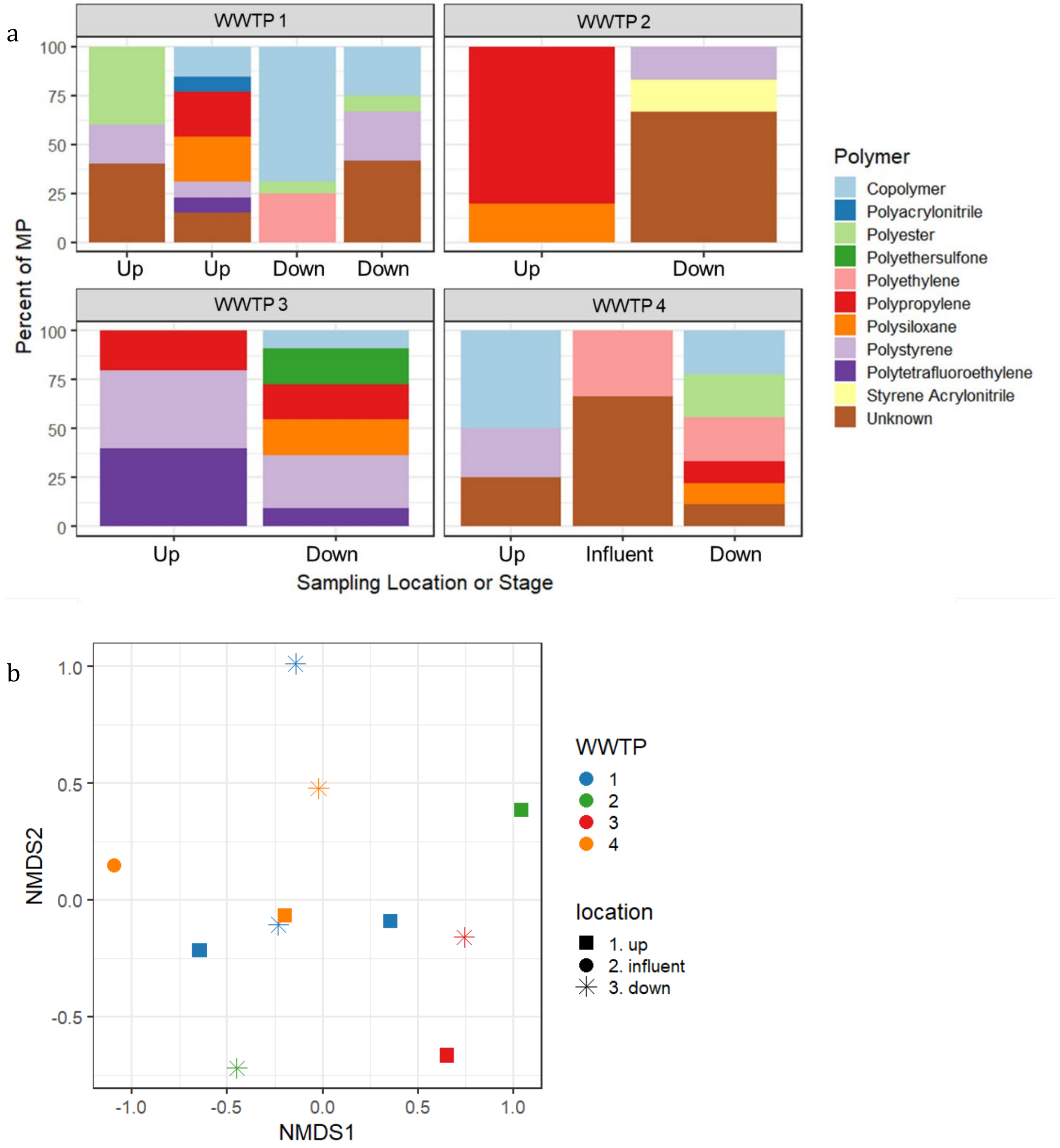


Fig. 8. *Stacked bar charts of MP observed with colors corresponding to polymer types by sampling site. Upstream samples are labeled Up downstream samples "Down." Influent refers to the influent from the WWTP sampled (a). nMDS of polymer profiles with color corresponding to utility number and symbols corresponding to sampling location. Stress=0.13* (b).

Given that most researchers report the total MP concentrations rather than by polymer type, measured and estimated MP concentrations (the latter after scaling as described in Section 2.3) by sampling location are shown in Fig. 9. The estimated MP concentrations in the surface water ranged from 0.21 downstream of WWTP 4 to 6.0 MP/L downstream of WWTP 1. Matrix spike recoveries for the surface water sampling campaign were 66±16% (N=11) and did not vary significantly by WWTP or by sampling location ($p>0.19$, Kruskal Wallis). While this value is lower than desired in the QAPP, it is well within the range reported for environmental samples that we found was as low as 30% (Antoine et al., in review).

The average MP concentrations in upstream samples was 1.0±1.0 MP/L and downstream was 1.5±2.5 MP/L. There were not significant differences by sampling locations across the receiving water bodies ($p=0.28$, Wilcoxon Rank Sum). This reflects the observation of higher estimated total upstream concentrations for WWTP 4 and 2 and the opposite pattern for WWTP 1 and 3. The WWTP 2 downstream surface water sample was collected the day after a 4.6 mm rainfall event that could have impacted the concentrations observed at that site where no rainfall was reported on the day of sampling or day prior for the upstream samples. The paired influent concentration for WWTP 4 was 0.14 MP/L, compared to 0.21 MP/L observed in downstream surface water (Appendix Table A1/A2).

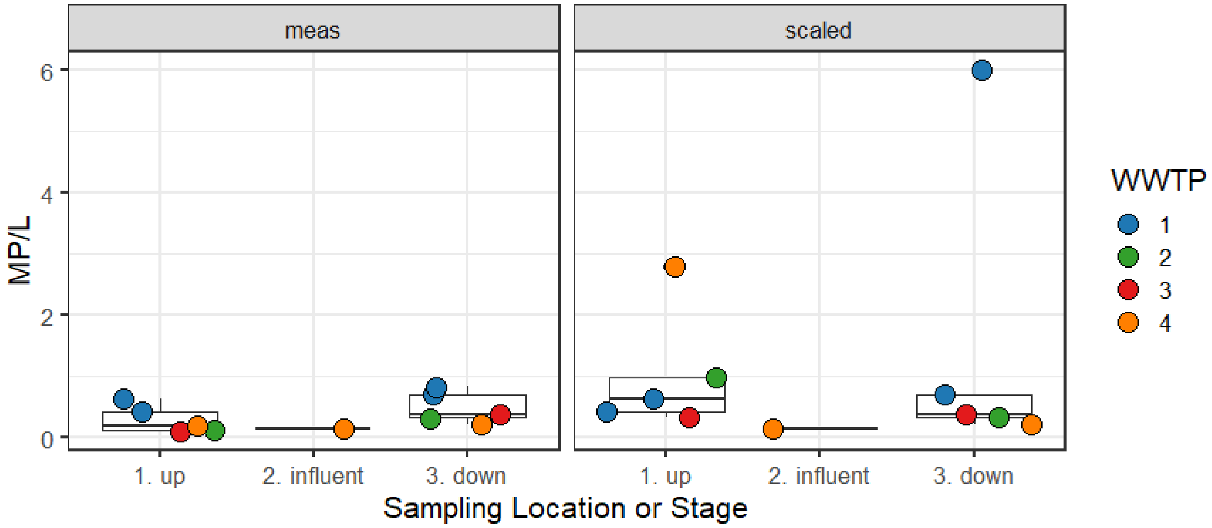


Fig. 9. *Boxplots of MP concentrations directly measured in samples ("meas") or estimated after scaling the samples that requires subsampling by sampling location. The colors for the overlaid jitter points correspond to the different WWTP. Box represents lower and upper quartiles with the line inside the box showing the median, whiskers are 1.5 times the interquartile range.*

3.3 Comparison of WWTP and Surface Water Samples

Given that the two sampling campaigns were not performed on paired dates, comparisons between the wastewater and surface water observations should be performed with caution. This is reinforced by the low WWTP influent concentration observed during the surface water sampling campaign in comparison to those observed during the wastewater sampling campaign (comparing influent concentrations from Fig. 5 of 2.6-14.3 MP/L to that shown in Fig. 9 of 0.14 MP/L). Nonetheless, total MP concentrations in the WWTP influent ranged from 2.6 to 153 MP/L and effluent from 0.6 to 2.0 MP/L in wastewater effluent, compared to 0.21 to 6.0 MP/L in the surface water samples. Thus, downstream surface water had up to three orders of magnitude lower MP concentrations compared to WW influent.

4. Discussion

4.1 Microplastic Polymer Types in Wastewater

The polymer richness per sample was generally in the lower range for wastewater influent and upper for wastewater effluent in comparison to those recently reviewed by Antoine et al. (in review). This could be due to the smaller sampling volumes and subsampling utilized in the influent samples or due to other spatiotemporal differences between this and other studies available. Comparing to the other studies of wastewater filters, Simon et al. (2019) reported 32 unique polymer types compared to the 17 observed here. That study analyzed smaller MP than was feasible here, sampled volumes 1-2 orders of magnitude greater, and had greater diversity of polymer types is generally expected when looking at smaller particles. The other studies of wastewater filtration reported fewer polymer types: 6 from samples collected before and after filtration (Wolff et al., 2021), 9-14 across treatment (influent, prefiltration, effluent) (Bayo et al., 2021; Salmi et al., 2021), and 13 in wastewater filter effluent (Talvitie et al., 2017).

The most prevalent polymer types found in this study are consistent with the high and medium relatively abundant polymer types in WWTPs reviewed by Talukdar et al. (Talukdar et al., 2024): polyethylene, polypropylene, polyamide, polyester, polystyrene, and acrylics (such as polymethylmethacrylate (PMMA) and polyacrylonitrile). The WWTP samples here also had some polymer types others have reported in lower relative abundance (e.g., ABS polysulfone, polytetrafluoroethylene (PTFE), polyvinyl chlorine, styrene acrylonitrile, and polysiloxane). Others have reported moderate relative abundances of polymer types not observed here such as polylactide and polyvinyl alcohol. With respect to polylactide, this polymer is biodegradable and bio-based thermoplastic. In a recent study, we found that biopolymers polylactide and polybutylene adipate terephthalate were all classified as other polyesters in the database we used for classification (Ochoa et al., (in review)). A summary of all plastic detection types by sampling location and frequency is provided in Appendix Figure A2.

While many studies report polymer profiles of samples, few studies compare them between sampling site or treatment type. Wang et al. (2020b) found that the distribution of type, shape and size of MPs in effluent samples were not significantly different than influent samples for WWTPs with a maximum of secondary treatment. In contrast, we found that both size and polymer type varied by sampling stage (i.e. influent, prefiltration, and effluent). This could be due to differences in extraction methodology (i.e., the use of density separation by Wang et al.), sampling volumes (i.e., 1 L by Wang et al.), and study location. A summary of MP detection by events and wastewater treatment location is provided in Appendix Figure A3.

Other researchers also reported challenges in processing wastewater influent samples due to the high amounts of natural organic material. Several also sampled smaller volumes of influent compared to effluent to account for this [e.g., (Murphy et al., 2016; Alavian Petroody et al., 2021)].   Some reported that collection of grab samples was required to avoid wastewater influent samples clogging the filters used for collecting samples (Rasmussen et al., 2021; Salmi et al., 2021). Others modified their extraction methods for the high organic matter samples including drying samples (Salmi et al., 2021), using iron-catalyzed wet peroxide oxidation (Tagg et al., 2020; Rasmussen et al., 2021), or subsampling after extraction (Murphy et al., 2016).

4.1.1 Microplastic Concentration and Removal

MP influent concentrations observed here had a wide variance, particularly for WWTP 1 and 3, with a standard deviation exceeding the mean concentration. More research on these utilities' catchments could provide insight as to why. However, such large variations in wastewater influent are not uncommon with Salmi et al. (Salmi et al., 2021) reporting average influent concentrations of 61± 26 MP/L (for a larger particle size range, Table 1). Focusing instead on the two utilities with lower MP influent variation (WWTP 2 and 4), an average of 11±5 MP/L was observed, closer to the average influent concentrations reported by Bayo et al. (2021), who targeted a similar size range (4.40 ± 1.01 MP/L).

Again, including all pre-filtration observations results in large variance in concentration of 14±24 MP/L for all utilities, mostly driven by WWTP 3 with the membrane biofilter, whereas average concentrations of 1.3±0.5 MP/L were observed for the other utilities in this study. The latter is similar to Talvitie et al. (2017) who reported 0.5±0.2 MP/L and 2.0±1.3 MP/L for two WWTPs that used disk filters, 0.7±0.1 MP/L before rapid sand filtration, for a larger particle size range (Table 1). Salmi et al reported higher pre-disk filter concentrations of 29 MP/L for a larger particle size range (Table 1).

The higher pre-filtration concentrations for WWTP 3 with the membrane bioreactor was not consistent with (Bayo et al., 2021) who targeted a similar MP size range finding 0.92 ± 0.21 MP/L. However, that team, as noted above, reported lower average influent concentrations compared to the utility sampled here using a membrane bioreactor (4.40 ± 1.01 MP/L versus 72 ± 73 MP/L for WWTP 3).

The effluent MP concentrations observed here of 1.4±0.52 MP/L were reasonably consistent with others studying wastewater filters studying similar size ranges [1.08 ± 0.28 MP/L (Bayo et al., 2021)] and within an order of magnitude for those who included smaller MP [0.02-0.3 MP/L (Talvitie et al., 2017), 3 MP/L (Simon et al., 2019) 0.8±0.4 MP/L (Salmi et al., 2021)].

With respect to removal rates, the values presented here were in comparison to wastewater influents with 84-93% removal, lower than the 99% removal reported by Salmi et al. (2021) who compared wastewater influent to effluent for a utility with disk filters. Salmi et al. (2021) also reported removal of MP pre-disk filtration of 79%, within the ranges for the utilities sampled here with disk filters that had 75-93% removal between influent and pre-disk filtration.

Most researchers also compared the pre-filtration and post-filtration MP concentrations (Table 1). Meanwhile, this study only collected pre-filtration and WWTP effluent samples. Comparing the pre-filtration and effluent samples resulted in only WWTP 3 having consistent removal via membrane filtration of 98±1% of MPs. This removal percentage is within the range reported by Talvite et al. (2017) of 99.9% (for a larger particle size range) and Bayo et al. (2021) of 79% (for a similar particle size range). Between the pre-filtration samples for the disk and sand filters and final effluent, N=3/15 samples had removal of MPs of 40-47% resulting in a final effluent concentration of 0.69-1.47 MP/L. Other samples from these utilities had no change (N=1/15) or a 21-212% increase (N=11/15) in MP concentrations between the pre-filtration and effluent stages resulting in final effluent concentrations of 1.34-2.09 MP/L. Others have reported 75-99.9% removal of MPs by sand filters (Talvitie et al., 2017; Bayo et al., 2021; Wolff et al., 2021) and 40-99.4% removal for disk filtration (Talvitie et al., 2017; Simon et al., 2019; Salmi et al., 2021) when sampling pre- and post-filtration.

With respect to particle size distribution, two other studies of WWTP filters reported information about particle sizes (Simon et al., 2019; Bayo et al., 2021). Here, the average and median FDM pre-filtration MPs were larger than in effluent in contrast to Simon et al. (2019) who reported an increase in median FDM post disk filtration. Bayo et al. (2021) also reported that MP sizes significantly increased from influent to prefiltration to effluent, hypothesizing that was due to fibers dominating MP morphology within WWTPs and slipping through pores in disk and sand filters. While fibers had the longest lengths among MPs found in this study, including in the treated effluent, there was a mix of fragments and fibers as opposed to the dominance of fibers reported by Bayo et al. (2021).

A similar strong correlation between total particle post-extraction concentrations and total MP concentrations has been reported in other studies of surface water (Nitzberg et al., 2024) and surface water, wastewater influent, and stormwater samples (Bailey et al., 2021). Both of these studies used a wet peroxide oxidation followed by sodium chloride density separation (Masura et al., 2015) while this study used a peroxide oxidation and for some samples additional cellulose and enzymatic digestions (ASTM, 2020, example Figure

A1). This observation indicates that the number of post-extraction particles may be used as an estimate of MP concentrations and could be considered as a screening technique.

4.2 Surface Water Observations

The number of polymer types observed in the present study (11 types) was within the range reported by others. In studies that investigated NJ waters, six polymer types were observed for particles 500-5000 µm (i.e., a smaller size range) in our study of the DE Bay Estuary (Nitzberg et al., 2024) and 12 polymer types for particles 250-5000µm from the Raritan-Hudson Estuary (Bailey et al., 2021). These observations are also comparable to studies from other rivers near WWTPs reporting five to fourteen polymer types [e.g., (Kazour et al., 2019; Wang et al., 2020a; Xia et al., 2022)].

PE typically dominates MP freshwater studies (Jolaosho et al., 2025) as seen in previously compared papers (Kazour et al., 2019; Wang et al., 2020a; Bailey et al., 2021; Xia et al., 2022; Nitzberg et al., 2024). In this study, PE was detected in 3 out of our 11 surface water samples while PS, unknown plastics, and copolymers were predominant. PS is a common plastic found in MP freshwater studies (Jolaosho et al., 2025), and copolymers were similarly abundant in Wang et al.'s (2020a) study.

Comparing the surface water MP concentrations to other studies in our region, higher concentrations were observed here (1-1.5 MP/L on average) than by either Bailey et al. (2021) or Nitzberg et al. (2024) who reported in MP/m^3. However, both of those studies involved sampling with towed nets from river mouths into estuaries, as opposed to the smaller creeks sampled here. Towed nets can allow for much larger water volumes, which is known to result in lower MP concentrations (Watkins et al., 2021). Studies from other regions reported comparable or greater MP concentrations (Wang et al., 2020a; Xia et al., 2022).

4.3 Comparison of Wastewater and Surface water

The apparent impact of WWTP effluent on surface water MP concentrations varied here by WWTP, similar to the literature where the relative importance of different sources varied by catchment specific factors (Rochman et al., 2022). For example, some studies observed elevated MP concentrations near WWTP outfalls (Kazour et al., 2019) whereas others reported relative low MP abundances (Wang et al., 2020a). With respect to polymer profiles, as seen here, some profiles were consistent with wastewater effluent serving as a source (Wang et al., 2020a). This is despite the general observation here and by others that wastewater effluent has orders of magnitude greater concentrations of MPs than the receiving surface waters [e.g., (Bailey et al., 2021; Xia et al., 2022), among others].

4.4 Recommendations for Future Work

Improved extraction methods are desirable in future studies to improve removal of natural organic matter from high suspended solids water samples. Despite following ASTM D833, the amount of natural organic matter left over in samples made it necessary for subsampling of influent and pre-membrane filtration samples. More information about the filters studied here (e.g., pore size, materials) and the utilities sampled (e.g., industrial inputs, catchment, sewer pipeline materials, flowrates on sampling days, etc.) could aid in the interpretation of the results presented here. Since there was limited surface water sampling in this study, future work with higher replication and greater volumes could improve the reliability of the results in this lower solids matrix.

Appendix

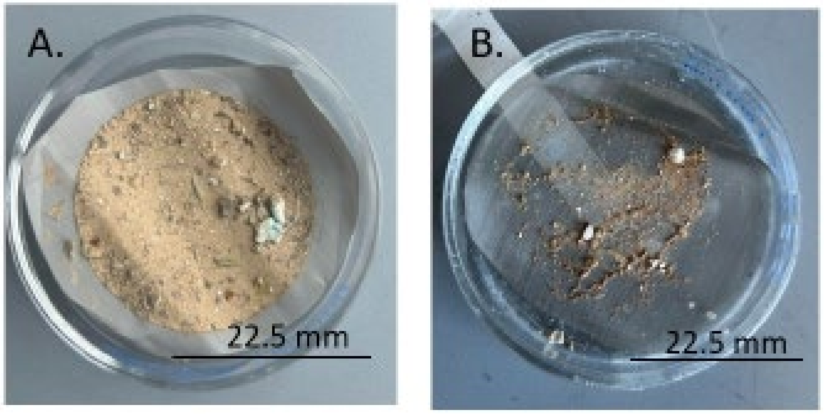


Fig. A1. *Images of particles from wastewater influent (WWTP 2, collected 10/12/2023) post a. peroxide oxidation and cellulose digestion concentrated on a 20 µm wire mesh and b. after enzymatic digestion concentrated on a 63 µm wire mesh.*

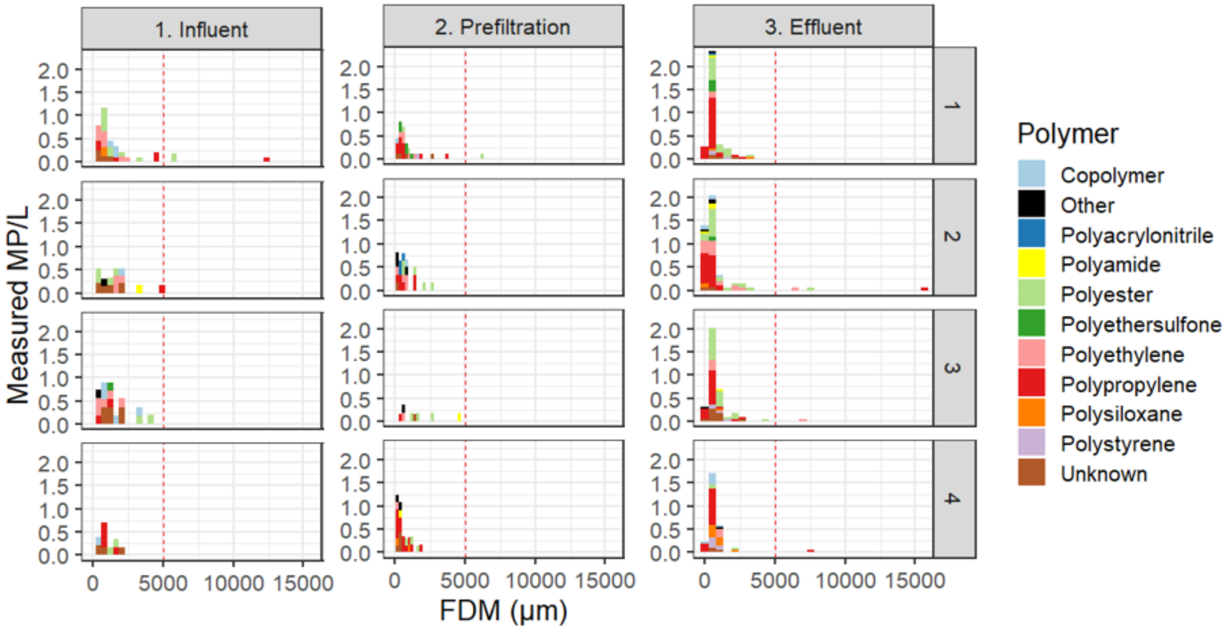


Fig. A2. *Histogram of maximum particle length (FDM, in microns) of 459 MP from wastewater samples (non-logarithmic). Colors correspond to polymer type and samples are sorted by WWTP (rows) and sampling stage (columns). Vertical red lines are at 5000 µm to distinguish particles that are considered macroplastics.*

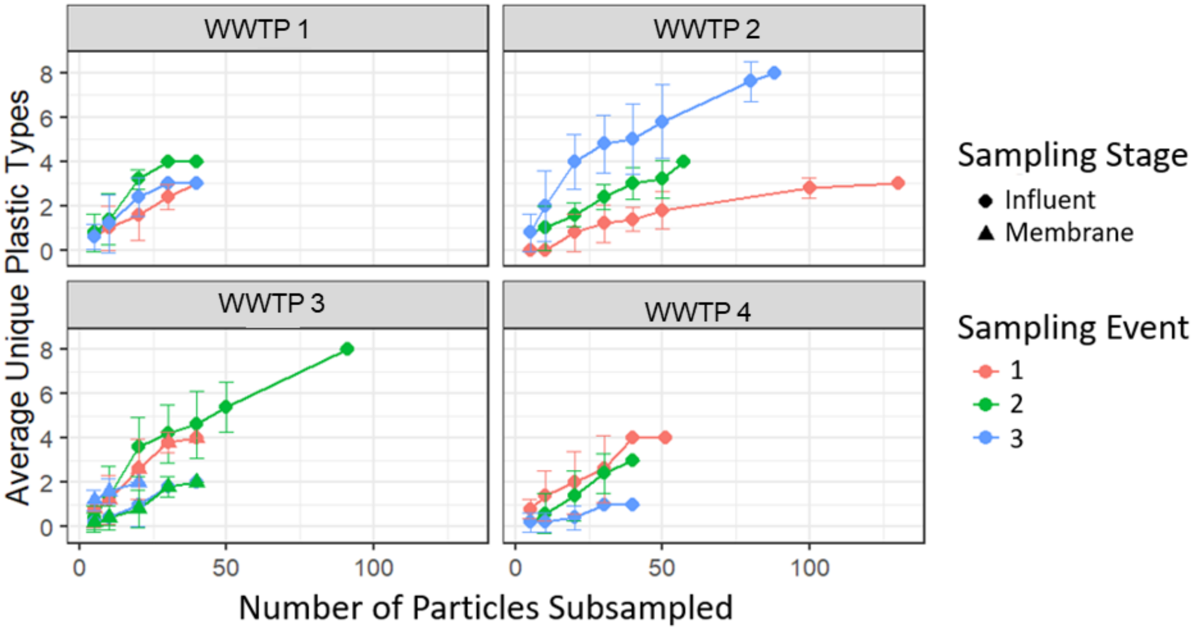


Fig. A3. *Polymer rarefaction curves, grouped by WWTP, showing the average number of unique plastic polymers versus the number of particles subsampled. Error bars represent standard deviation (replication N = 5). The colors correspond to different sampling events (dates) and shape to sampling stage (circles for influent and triangles for membrane, only found in WWTP 3).*

Table A1. *Rainfall data obtained through National Oceanic Atmospheric Administration (NOAA) Climate Data Online on wastewater sampling days.*

WWTP	Date	Rainfall (mm)		Station
		Day Before	Day Of	
1	9/28/2023	2.5	0.0	USC00286964
1	10/4/2023	0.0	0.0	USC00286965
1	10/6/2023	0.0	0.0	USC00286966
2	10/12/2023	0.0	0.0	US1NJMC0057
2	10/18/2023	1.0	0.0	US1NJMC0057
2	10/20/2023	0.0	2.3	US1NJMC0058
3	10/25/2023	0.0	0.0	US1NJSM0065
3	11/1/2023	0.3	1.5	US1NJSM0066
3	11/2/2023	1.5	0.0	US1NJSM0067
4	11/15/2023	0.0	0.0	US1NJBT0090
4	11/16/2023	0.0	0.0	US1NJBT0091
4	11/21/2023	0.0	0.0	US1NJBT0092

Table A2. *Rainfall data obtained through National Oceanic Atmospheric Administration (NOAA) Climate Data Online for surface water sampling days.*

		Rainfall (mm)		
WWTP	Date	Day Before	Day Of	Station
1	4/9/2024	0	0	USC00286964
1	4/10/2024	0	0	USC00286965
2	4/6/2024	4.65	0	US1NJMC0057
2	4/9/2024	0	0	US1NJMC0057
2	4/10/2024	0	0	US1NJMC0058
3	4/9/2024	0	0	US1NJSM0065
3	4/10/2024	0	0	US1NJSM0066
4	4/9/2024	0	0	US1NJBT0090
4	4/10/2024	0	0	US1NJBT0091

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