

## Microplastics in urban wastewater and estuarine water: Importance of street runoff

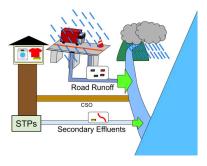
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[Received October 27, 2020; Accepted February 13, 2021]

## **ABSTRACT**

We measured microplastics [(MPs):  $10 \mu m-5 mm$ ] in untreated and secondary-treated sewage, highway runoff, and estuarine water in Tokyo, Japan, during both dry and wet weather. MPs smaller than 200  $\mu$ m were predominant, making up > 60% of the total number, in all our water samples. The abundance of the MPs was 420 pieces / L in the influent and 8.7 pieces / L in the secondary effluent, indicating a removal efficiency of 98% during primary + secondary treatment. Of the measured polymers—polyethylene (PE), polypropylene, polystyrene, polyethyleneterephthalate (PET), polyethylene polypropylene copolymer, polyethylene polypropylene diene (PEPD), polyethylene vinyl acetate, and acrylonitrile styrene, PET was predominant in the sewage influent (88%) and effluent (49%) and its form was mostly fibrous,



indicating contribution from laundry effluents. Highway runoff water samples contained high concentrations of MP (81-292 pieces/L). The highway runoff contained more PE (25%-49%) and PEPD (13%-30%) than PET (3%-12%) indicating contribution from debris from automobile tire wear and plastics weathered on the ground. MP concentrations in estuarine water samples were lower (1.4-2.3 pieces/L in dry weather), and PET was predominant, at 52%-77%, indicating a dominant contribution from sewage effluent. Following rainfall, MP concentrations in the estuarine water increased to 1.8-4.3 pieces/L, and PEPD, in particular, increased significantly, with a proportion of 43%-52%, indicating the contribution of MPs derived from street runoff. Although analyses of molecular markers of sewage, i.e., linear alkylbenzenes and coprostanol, confirmed the input of combined sewer overflow (CSO) to the estuarine water, we did not observe any significant increase in PET fiber MPs, indicating that there was no significant increase in the contribution of MPs from sewage including CSO to the estuarine waters after a rain event. This study highlights the importance of street runoff as a source of MPs in urban aquatic environments. Further investigation should be performed to quantitatively access the individual sources and processes.

**Key words:** sewage influent; sewage effluent; highway runoff; combined sewer overflow (CSO); linear alkylbenzenes; coprostanol

## INTRODUCTION

Studies on microplastics [(MPs) < 5 mm] began with those in marine environments (Bowmer and Kershaw, 2010). Recent studies have identified various sources of MPs in terrestrial environments; these include MP fibers from textiles, microbeads in cosmetics, and particles from tire wear. Sources of MPs can be categorized into two groups: primary and secondary MPs. Primary MPs are plastic particles that are originally manufactured to be of a size such as that of resin pellets (Ogata et al., 2009; Antunes et al., 2013) and of microbeads in cosmetics and cleaners (Eriksen et al., 2013; Tanaka and Takada, 2016). Secondary MPs are produced by the fragmentation and weathering of larger plastic items and litter. Globally, more than 400 million tons of plastics are produced each year (Geyer et al., 2017). Approximately 40% of this amount consists of single-use plastics such as packaging and food containers that are discarded as plastic waste just after being used (Geyer et al., 2017). Although plastic waste is subject to waste management, some of it ends up being emitted to the environment and is washed out by streams and rivers to the ocean. Approximately eight million tons of plastic waste is discharged into the oceans each year (Jambeck et al., 2015). Plastic waste floating on the surface of the water and strewn on beaches is fragmented and weathered into smaller pieces, i.e., MPs, by physical impacts and UV radiation (Andrady, 2011). Weathering and fragmenta-

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tion of consumer plastic products is an important process in creating MPs in aquatic environments.

Weathering of plastics occurs even during their use in terrestrial environments. A typical example is MP fibers derived from clothes of chemical textiles such as polyesters. MP fibers have been reported in laundry wastewater (Browne et al., 2011; Napper and Thompson, 2016). In addition, MPs from the wear of plastic products, such as plastic sponges, used in the home are introduced to sewage together with primary MPs, such as microbeads in facial cleaners (Eriksen et al., 2013). MPs used and generated in the home are carried to sewage treatment plants (STPs), where they are subject to treatment (Karapanagioti, 2017). Primary and secondary treatment removes MPs with an efficiency of 95%-99% (Carr and Thompson, 2019). However, small but significant amounts of MPs are discharged from STPs as secondary effluents. It is important to understand their contribution to MP pollution in riverine and coastal environments.

Furthermore, in areas that have a combined sewer system, untreated sewage is discharged directly to rivers and coastal zones as CSO during a rainfall event. CSO can introduce MPs to rivers and coastal zones (Katsanou et al., 2019). However, there has been insufficient study of its contribution to MP pollution. This study has compared the abundance and composition of MPs in estuarine water in Tokyo, Japan, immediately after rainfall with the same parameters in dry weather to explore the contribution of CSO to MP pollution in urban waters. To detect the contribution of CSO to estuarine water, we utilized two types of molecular markers: linear alkylbenzenes (LABs) and coprostanol. LABs are feedstock of linear alkylbenzenesulfonates (LAS), and small but significant amounts of unreacted LABs are contained in LAS-type synthetic detergents and sewage (Eganhouse et al., 1983; Takada and Ishiwatari, 1987). Coprostanol is generated from cholesterol via enzyme activity in the intestines of mammals, including humans, and is contained specifically in human feces and sewage (Murtaugh and Bunch, 1967; Takada and Eganhouse, 1998). For the past few decades, both LABs and coprostanol have been utilized as markers of sewage inputs to aquatic environments (e.g., Takada and Eganhouse, 1998; Isobe et al., 2004; Ni et al., 2009; Wang et al., 2010; Wang et al., 2012; Harwood, 2014; Albuquerque de Assis Costa et al., 2018). Because of their hydrophobicity, both are sorbed to sewage particles and, thus, are removed efficiently by the settling of sewage particles during sewage treatment. Consequently, untreated sewage contains relatively high concentrations of LABs and coprostanol, whereas secondary effluents contain minimal concentrations of both compounds (Morimoto et al., 2011). Therefore, higher concentrations of LABs and coprostanol are indicative of CSO inputs.

Street runoff is another route whereby MPs from terrestrial sources enter aquatic systems. Weathering of plastics occurs not only in aquatic environments but also on the surface of the land. Plastic waste discarded on roads can be fragmented by physical impacts, such as being trodden on by walkers or run over by automobiles, and weathered by UV radiation. In fact, MPs have been detected in street dust (Abbasi et al., 2017; Yukioka et al., 2020). Street dust also contains tire-wear particles, which are considered as being MPs (Kole et al., 2017).

Street runoff can wash MPs in street dust to rivers and coastal waters. However, there have been few studies of the contribution of MPs in street runoff to MP pollution in riverine and coastal waters (Müller et al., 2020). Consequently, this study has measured MPs in highway runoff and compared the abundance and compositions of the MPs with those in estuarine water in both dry and wet weather.

To elucidate the contribution of STP effluents, CSO, and road runoff to MP pollution in estuarine water in Tokyo, Japan, we measured MPs in sewage influent, secondary effluent, highway runoff, and estuarine water in both dry and wet weather. The target polymers were polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethyleneterephthalate (PET), polyethylene polypropylene copolymer (PEP), polyethylene polypropylene diene (PEPD), polyethylene vinyl acetate, and acrylonitrile styrene (AS).

# MATERIALS AND METHODS SAMPLE COLLECTION

We collected sewage influent and secondary effluent from a municipal STP in the Tokyo Metropolitan area in November 2019. This STP was designed to treat sewage from  $\sim 113 \times 10^3$  people. It employs secondary treatment using activated sludge (reaction time  $\sim 6$  hours; settling time  $\sim 5$  hours) after primary treatment with a settling pond (settling time  $\sim 2$  hours). The final effluent is discharged into Tokyo Bay. We collected 24-h composite samples of influent (1 L) and secondary effluent (20 L) in a glass bottle and a stainless steel container, respectively, and transported them to our laboratory in the university for sample treatment.

We collected highway surface runoff (first flush and transitional and steady state runoff) from Chuo Expressway at a discharge pipe in Chofu city (N 35°39'48", E 139°32'52"; Fig. S1), where the runoff is carried to Nogawa River, a tributary of the Tamagawa River, which flows into Tokyo Bay. The highway has five lanes and a daily traffic density of ~80 × 10<sup>3</sup> vehicles, and the pipe receives runoff from a road surface area of 600 m<sup>2</sup> (Kumata et al., 2000a). The sample collection was performed during the continuous rainfall event that lasted for 48 h between November 22 and November 24, 2019. At the beginning of the rainfall event (11:10 on November 22), we collected the first flush of runoff in a 10 L stainless steel container, as shown in Fig. S1. At 16:30 on the same day, when the accumulative rainfall was 18 mm, we collected another sample in a 20 L stainless steel container; in this study it is called a "transitional and steady state sample." We transported both runoff samples to our laboratory.

We collected estuarine water samples from two locations (st.MR and st.SB, Fig. 1) in the estuary of Sumidagawa River as it flows into Tokyo Bay. Sumidagawa River receives secondary effluents of ~27 m³/s from ~3 × 106 people via nine municipal STPs in dry weather (Bureau of Sewerage of Tokyo, 2019). The secondary effluent represents ~70% of the river water in the lower reaches (NILIM Public Investment Project Team, 2005). In wet weather, the river receives a CSO via 29 pumping stations and numerous chambers (Poopipattana et al., 2020) together with surface runoff. St.MR is located at the river mouth of Meguro River, which receives a large volume of CSO via the pumping stations and chambers. St.SB is located near

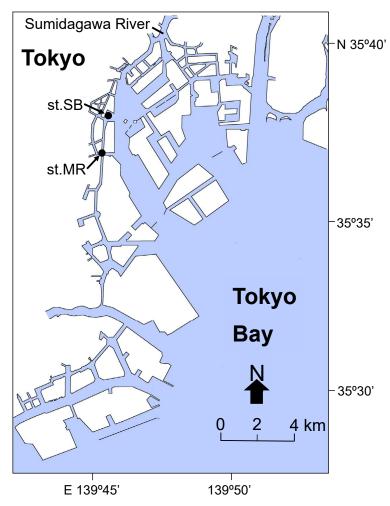


Fig. 1 Sampling locations of the Sumidagawa Estuary

to a large pumping station. Our previous study suggested that both locations are influenced by CSO following rainfall events (Poopipattana et al., 2018). We collected surface water samples during dry weather on December 16, 2019 and during a rain event on November 23, 2019. The rainfall event survey was conducted when there was 86 mm rainfall in the 24 h preceding the sample collection; this was sufficient to induce CSO. Basic water quality data (electric conductivity or salinity, temperature, pH, and suspended solids) are listed in Table S1. We used a stainless steel bucket to collect 20 L of surface water for MP analysis and another 3 L for sewage marker analysis, and we stored these samples in a stainless steel container and glass amber bottle, respectively, for transportation to our laboratory.

#### SPECIAL INSTRUMENTS AND REAGENTS

We used a stainless steel pressurized large volume (20 L) closed filtration system (Fig. S3) that was custom-made by Advantec Toyo Kaisha, Ltd. (Tokyo, Japan). We purchased a cellulose nitrate filter (A500A090C; pore size:  $5.0~\mu m$ , diameter 90 mm) from Advantec Toyo Kaisha, Ltd. (Tokyo, Japan). We used a stainless steel mesh filter (mesh size:  $10~\mu m$ , diameters 13 mm and 25 mm) that was custom-made by Nippon Donaldson. We purchased polyethylene microbeads (Cospheric; several sizes ranging from 27  $\mu m$  to  $150~\mu m$ , and several densities ranging from 1.0 to 1.5; Table 1) from Corefront Corporation

Table 1 Spiked microplastics and their recovery

Density (g/cm³)	Size (µm)	Polymer type	Recovery (%)
Beads			
1.0	27- 45	PE*	63
1.0	63- 75	PE	80
1.0	125-150	PE	93
0.96	63- 75	PE	92
1.25	63- 75	$PE + TiO_2$	72
1.5	63- 75	$PE + BaSO_4$	67
Fiber			
~1.2	$3.3 \text{ T} \times 0.5 \text{ mm}$	PET**	70

\*PE: polyethylene, \*\*PET: polyethyleneterphthalate

(Tokyo, Japan). Reference PET fiber (thickness 3.3 T, length 0.5 mm) was kindly supplied by the Japan Chemical Fibers Association (Tokyo, Japan). We purchased sodium iodide, hydrogen peroxide, ferrous sulfate heptahydrate, sodium hydroxide, hydrochloric acid, and methanol, all of reagent grade, from FUJIFILM Wako Pure Chemical Corporation (Tokyo, Japan). Glass or stainless steel items of apparatus were rinsed using detergents, tap water, and methanol. Sodium iodide solution

and ferrous sulfate solution were pre-filtered just before use to remove interfering solid materials.

#### SAMPLE TREATMENT FOR MP ANALYSIS

Using the large volume stainless steel filtration system pressurized by nitrogen, we filtered secondary effluents, highway runoff, and estuarine water samples (0.6 L-20 L) through the cellulose nitrate filter (Roch and Brinker, 2017) to collect suspended solids larger than five micrometers (Fig. S2). We used a glass vacuum filtration system to filter the STP influent sample (100 mL) through the cellulose nitrate filter. The cellulose nitrate filter containing MPs was transferred to a 500 mL glass beaker, to which we added 10 mL of NaOH aqueous solution (1 mol/L) to dissolve the filter at 40°C. Following neutralization through the addition of 10 mL of HCl (1 mol/L), we added 50 mL of 30% H<sub>2</sub>O<sub>2</sub> aqueous solution containing 5 mL of FeSO<sub>4</sub> (50 mmol/L) to degrade natural organic matter through the Fenton reaction at room temperature under fluorescent light for three days. After three days, we added a few drops of NaI solution (6.7 mol/L) to deactivate residual H<sub>2</sub>O<sub>2</sub> and transferred the beaker's contents to a 1 L glass funnel. We added 250 mL of the NaI aqueous solution to the glass funnel and hand shook the resultant solution (d = 1.6) for one minute. We allowed the solution to stand still overnight and removed deposits by opening the cock. We then repeated the density separation, i.e., removal of heavier particles. The resultant supernatant was filtered through the stainless steel mesh filter (mesh size  $10 \mu m$ , diameter 25 mm), on which the MPs were collected. We dried the particles on the mesh filter overnight in a desiccator with silica gel.

#### **MICRO FTIR ANALYSIS**

We measured the MPs on the mesh filter using a  $\mu$ FT-IR Nicolet iN10 infrared microscope (Thermo Fisher Scientific, Madison, USA). Measurement was conducted in reflectance mode, with scanning from 675 cm<sup>-1</sup> to 4,000 cm<sup>-1</sup> and a resolution of 16 cm<sup>-1</sup>. FTIR imaging was conducted with a 100  $\mu$ m × 100  $\mu$ m aperture and 50  $\mu$ m step size. As a reference library, we used HR Hummel Polymer and Additives, HR Rubber Compounding Materials, and Spouse Polymer by ATR. We obtained FTIR profiles of PE, PP, PS, and PET (Fig. S3). For each profile, we identified polymers with the highest hit quality, with a hit quality of 60% as the threshold.

Following FTIR imaging analysis of MPs, we observed individual particles in a microscopic image obtained using the same instrument (Nicolet iN10) to ascertain their shape (fiber or flake) (Fig. S4). Some fibers were identified as multiple disconnected flakes only by imaging FTIR. Through observational confirmation from a microscopic image, such disconnected flakes were determined as being single fiber. In some cases, we could not see particles identified by FTIR imaging in the microscopic image due to creeping of the particles on the stage. This paper reports and discusses values obtained by imaging with visual confirmation, although these values could be underestimated by 38% (at most) compared with values obtained only by FTIR imaging.

Based on the measurement of sizes of MPs (major axis and minor axis) and polymer type, we estimated the weights of individual MP particles by using the following equation:

Weight of MP particle = major axis × minor axis × thickness × density — (1)

As the particle thickness was assumed to be the same as the minor axis, the estimated weight would be an overestimation. We assumed the density of individual MP particles to be  $1~\rm g/cm^3$  for all the polymer types, apart from PET, which we assumed to have a density of  $1.3~\rm g/cm^3$ . As sizes were measured on FTIR images with  $100~\mu m$  aperture, they, especially those of the minor axis (diameter) of fibers, were overestimated (Fig. S4). After calculating the weight of individual MP particles, we calculated the size distribution of the weight of MPs and total weight of MPs in individual samples. As these calculations contain assumptions leading to uncertainty, particularly overestimation, we have not used them for the main discussion in this study but rather for additional discussion.

## QUALITY ASSURANCE AND QUALITY CONTROL FOR MP ANALYSIS

There have been several reports of MPs in the atmosphere (Allen et al., 2019; Chen et al., 2020) that could contaminate samples during collection and treatment. We carried out several measures to minimize MP contamination from ambient air, as follows: We introduced a stainless steel pressurized closed filtration system; we filtered FeSO<sub>4</sub> and NaI aqueous solutions through the stainless steel mesh filter just before use; we carried out vacuum filtration and laboratory sample treatment in a bench-top aluminum foil chamber; no operators wore clothing made of synthetic fibers; to access airborne contamination, we ran a travel blank with all the samples apart from runoff samples; i.e., tap water filtered through stainless steel mesh was placed in either glass bottles or stainless steel containers of the same volume as those used for the samples, brought to the field, and subjected to all the analytical processes. In the case of the runoff samples, we ran laboratory procedural blanks with the samples.

The reproducibility of sample treatment was assessed by triplicate analyses of a secondary effluent sample. We examined recoveries by spiking 20 pieces of each of seven kinds of PE microbeads of various sizes and densities and PET fibers to 5 L of the secondary effluents, as listed in Table 1. We measured the beads and fibers spiked to the samples by FTIR following the entire analytical procedure, including filtration,  $\rm H_2O_2$  oxidation, and density separation. We performed the recovery experiments in triplicate.

## **ANALYSIS OF SEWAGE MARKERS**

We filtered the estuarine water samples (two locations in both dry and wet weather conditions; 2 L each) by using a prebaked glass fiber filter (Whatman GF/F, 90 mm diameter). The filter containing suspended particles was freeze-dried. As surrogate standards, 1-C<sub>n</sub> LABs (n: carbon number of alkylchain; 8–14) and cholesterol- $d_6$  were spiked to the filter. The filters were extracted successively with methanol, dichloromethane (DCM)/methanol (1:1, v/v), and DCM by ultra-sonication for 15 minutes each. We combined the extracts and roto-evaporated the solvent to dryness. We re-dissolved the extracts in 1 mL of hexane and placed them onto a 10% H<sub>2</sub>O-de-activated silica gel column (1 cm × 5 cm) with activated copper

 $(1~\rm cm \times 1~\rm cm)$  on top. We obtained three fractions by using 15 mL of hexane/DCM (3:1,v/v), 15 mL of DCM/MeOH (99:1,v/v), and 15 mL of DCM/acetone (7:3,v/v). We used the first fraction for LABs measurement using a gas chromatograph equipped with a mass spectrometer (GC-MS; Agilent 6890 with 5973). Hartmann et al. (2000) describe the detailed conditions of gas chromatography-mass spectrometry (GC-MS). The sum of 26 LAB congeners with  $C_{10}$ - $C_{14}$  alkylcarbons is expressed as total LABs. We recovery-corrected their concentrations by using the surrogates  $(1-C_n$  LABs) with the same carbon number. We combined the second and third fractions and acetylated them for the measurement of coprostanol. Isobe et al. (2002) describe details of acetylation and their GC-MS analysis. We recovery-corrected the coprostanol concentration by using cholesterol- $d_6$ .

To study contamination during all the processes, including sample collection, filtrations, and the analytical procedure, we brought distilled water to the field in a glass bottle and subjected it to all the analytical processes as a travel blank. The amounts of total LABs and coprostanol found in the travel blank were 36 ng and < 0.3 ng, respectively, corresponding to the limit of quantification (three times that of the travel blank value) of 50 ng/L for total LABs and 0.5 ng/L of coprostanol in case 2 L of water was used for the analysis. We assessed reproducibility via replicate analyses (n = 4) of sediment from Tokyo Bay. The relative standard deviations of total LABs and coprostanol were 8% and 4%, respectively. We checked recovery by spiking native standards to the sediment. The recoveries of total LABs and coprostanol were 74 ± 2 and 115% ± 3%, respectively.

## **RESULTS AND DISCUSSION**

## ANALYTICAL RELIABILITY OF MP MEASUREMENT

The recoveries of PE microbeads spiked to the secondary effluent are listed in Table 1. Smaller MPs showed lower recovery; e.g., 125 μm-150 μm PE beads showed 93% recovery, whereas 27 μm-45 μm PE beads showed 63% recovery. As smaller particles have a larger specific surface area, there is an increased chance of either adsorption loss on the surface of analytical apparatus such as a separatory funnel or incorporation into heavier particles that were settle-removed during density separation. In this study, the number of MPs smaller than 45  $\mu$ m might be underestimated by 40%. In terms of density, heavier plastics showed lower recovery; i.e., 67% for microbeads with a density of 1.5 g/cm<sup>3</sup>. This can also be explained by loss during density separation. PET fibers were recovered over 70%. Our findings suggested that light (i.e., density < 1) MPs measuring more than 50  $\mu$ m had satisfactory recovery (>70%).

Contamination from the atmosphere is a critical source of MPs in blank. Of the analytical steps, filtration was the greatest contributor to contamination. For example, we found 18 pieces of MP when 10 L of distilled water was vacuum-filtered followed by  $\rm H_2O_2$  oxidation and density separation, whereas we found three pieces of MP during  $\rm H_2O_2$  oxidation and density separation. The polymers we found in the blank procedure were mainly PET fibers and were consistent with the MP composition in the air (Chen et al., 2020). To eliminate the possible contact of the water sample with the air, we had a pressurized,

stainless steel, closed filtration system custom-made and applied it to samples of large volume. This reduced MPs in the blank to 0–5 pieces/10 L. In the collection and analysis of environmental water samples, travel blanks were run together with the samples to monitor contamination. The number of MPs we found in the travel blanks was insignificant (2%–5% of those in the samples) for most of the samples, whereas they were 13%–43% of those in the estuarine water samples. In tables showing the numbers of MPs in the samples, MPs found in the corresponding travel blank are indicated.

## MPs IN SEWAGE AND REMOVAL EFFICIENCY OF MPs DURING SEWAGE TREATMENT

In sewage, both STP influent and secondary effluent, PET was the predominant polymer (Fig. 2). PET made up 88% of polymers, with PEP and PE as significant but minor components of the influent. The PET was mainly fibers derived from the wear of fabrics (e.g., clothes) made of PET. Previous studies have reported generation of PET fibers from laundering polyester clothes (Browne et al., 2011; Napper and Thompson, 2016). According to Browne et al. (2011), laundering clothes and blankets generated large numbers of polyester fibers, resulting in concentrations of hundreds of fibers per liter of laundry effluents. The predominance of PET fibers in sewage (Fig. 2 and Fig. 3) is consistent with previous reports (Talvitie et al., 2017; Lares et al., 2018). In the present study, the total number of MPs in the sewage influent was 420 pieces/L (Table 2). This is orders of magnitude higher than the numbers reported in some previous studies (Mason et al., 2016; Murphy et al., 2016; Liu et al., 2019b; Bayo et al., 2020), but it is comparable to the numbers reported in other studies (Talvitie et al., 2017; Edo et al., 2020). The greater abundance is mainly a result of the smaller size range, down to 10  $\mu$ m, included in our study. The size distribution of MPs in this study showed that there

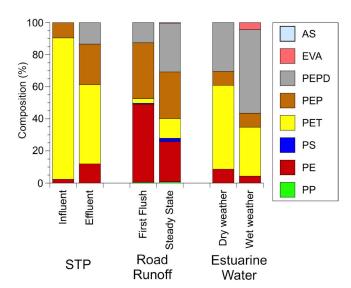


Fig. 2 Polymer type compositions of microplastics in STP influent and effluent, highway runoff, and estuarine water. Based on number of microplastics; STP: sewage treatment plant; AS: acrylonitrile styrene, EVA: polyethylene vinyl acetate, PEPD: polyethylene polypropylene diene, PEP: polyethylene-polypropylene copolymer, PET: polyethyleneterphthalate, PS: polystyrene, PE: polyethylene, and PP: polypropylene.

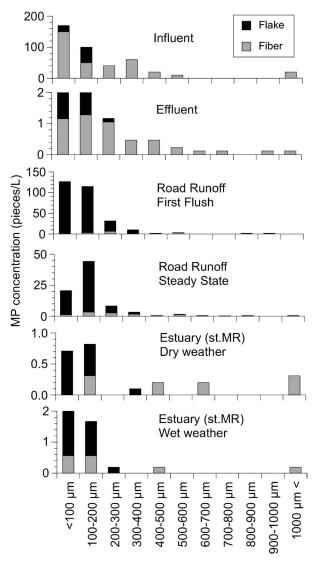


Fig. 3 Size distribution of microplastic samples in sewage treatment plant influent and effluent, highway runoff, and estuarine water.

Based on number of microplastics.

was a greater abundance of smaller plastics (Fig. 3). MPs  $<200~\mu m$  were predominant—i.e., >60%—with MPs  $<100~\mu m$  making up 40% of the total number of MPs.

The abundance of MPs in secondary effluent was 8.7 pieces/L. Once more, this is orders of magnitude higher than in some previous reports (Mason et al., 2016; Murphy et al., 2016), whereas it is comparable to the abundances reported in other studies (Talvitie et al., 2017; Edo et al., 2020). In the present study, the higher abundance was due to our measurement of smaller MPs. Based on MP concentrations in the influent and secondary effluent, we calculated removal efficiency during primary treatment followed by activated sludge treatment to be 98%. This is comparable to the previous studies: i.e., 98.4% (Murphy et al., 2016), 93.7% (Edo et al., 2020), 97%-99% (Gies et al., 2018), and 98.3% (Lares et al., 2018). The efficient removal of MPs was because they were incorporated in sewage sludge. High concentrations of MPs have been observed in sewage sludge (Lares et al., 2018; Edo et al., 2020). Lares et al. (2018) studied MP removal in individual steps during sewage treatment and found that most MPs were removed during primary treatment. Once more, MPs of smaller sizes were predominant in secondary effluent (Fig. 3), with MPs  $< 200 \mu m$ making up more than 60%. PET fiber predominated the secondary effluent (Fig. 2 and Fig. 3), whereas the proportion of the other polymers-PE, PEP, and PEPD-was higher in the effluent than in the influent. As PE, PEP, and PEPD are lighter than PET, they may be sedimented and removed less efficiently in a primary settling tank.

## **MPs IN HIGHWAY RUNOFF**

We found large numbers of MPs (81–292 pieces/L) in the highway runoff samples (Table 3). To the best of our knowledge, there have been no studies of MPs in highway runoff water samples during rainfall events. Water samples collected from the retention ponds of highway storm water have been analyzed, and much lower concentrations of MPs (0.494 pieces of MP/L) have been reported (Liu et al., 2019a). This lower concentration was because of sedimentation of MPs for seven

Table 2 Microp	lastic concentrations	in STP* inf.	luent and second	lary effluent

Polymer type**	Influent		Secondary Effluent		
	Sample	Blank	Sample	Blank	
	(pieces/L)	(pieces/L) (pieces/L)		(pieces/L)	
AS	0	0	0.0	0.0	
<b>EVA</b>	0	0	0.0	0.0	
PEPD	0	0	1.2	0.0	
PEP	40	0	2.2	0.0	
PET	370	20	4.3	0.3	
PS	0	0	0.0	0.0	
PE	10	0	1.0	0.0	
PP	0	0	0.0	0.0	
Total	420	20	8.7	0.3	

<sup>\*</sup>STP: sewage treatment plant

<sup>\*\*</sup>AS: acrylonitrile styrene, EVA: polyethylene vinyl acetate, PEPD: polyethylene polypropylene diene, PEP: polyethylene-polypropylene copolymer, PET: polyethyleneterphthalate, PS: polystyrene, PE: polyethylene, and PP: polypropylene.

Polymer type*	First	Flush	Steady State		
	Sample (pieces/L)	1		Blank (pieces/L)	
AS	0	0	0	0.0	
EVA	0	0	0	0.0	
PEPD	37	2	25	0.0	
PEP	102	0	24	0.3	
PET	8	10	10	2.0	
PS	2	0	2	0.0	
PE	142	0	20	0.0	
PP	2	3	1	0.0	
Total	292	15	81	2.3	

Table 3 Microplastic concentrations in highway runoff

days or more following rainfall events. Concentrations of MPs (66–191 pieces/L) similar to those of our highway runoff samples have been reported for stormwater runoff samples (Piñon-Colin et al., 2020). As MP concentrations in the highway runoff were orders of magnitude higher than those in secondary effluents, road runoff may represent an important source of MPs in urban aquatic environments.

The composition and shape of polymers in the highway runoff samples were quite different from those in sewage. Their shapes were mostly flakes while fibers were a minor component (Fig. 3). They were dominated by PE (25%-49%) and PEP (29%-35%), whereas PET was a minor component (3%-12%), as shown in Fig. 2. We detected considerable amounts (13%-30%) of PEPD or ethylene propylene diene rubber (EPDM), components of natural and synthetic rubber, in the highway runoff samples. This finding is reasonable, as street runoff contains significant amounts of particles from tire wear (Kumata et al., 2000b). EPDM is a significant component of MPs in street dust (Yukioka et al., 2020). Our previous studies (Kumata et al., 2000b; Kumata et al., 2002) have revealed the significant contribution of tire-wear particles to suspended solids in highway runoff by measuring benzothiazole-type molecular markers-2-(4-morpholinyl) benzothiazole and N-cyclohexyl-2-benzothiazolamine—both of which are impurities of vulcanization accelerators and specific to automobile tires. PEP may be derived from the ethylene propylene rubber that is used for anti-vibration materials for road and wire coating (Debek et al., 2015). Road construction and maintenance materials, such as triangular cones, sheets, and anti-vibration materials may contribute to the PE MPs in the highway runoff. Further characterization, such as color and additives, may help identify the sources. Once again, small MPs predominated the highway runoff samples, with MPs < 200 μm constituting more than 80% of the total number of MPs. There were fewer MPs in the transitional and steady state stage sample (81 pieces/L) than in the first flush (292 pieces/L), and there was a slight shift to larger particles. These findings could be explained by washing out of MPs, particularly smaller MPs, on the road surface during the early stage of rainfall. However, we obtained only two samples in one rainfall event, and more observations are required for further discussion.

#### MPs IN ESTUARINE WATER

The concentrations of MPs in the estuarine water in dry weather was 2.3 pieces / L and 1.4 pieces / L at st.MR and st.SB (Table 4), respectively, and were lower than that in the secondary effluent (8.7 pieces/L). Once again, smaller MPs were predominant (Fig. 3). In the estuarine water, MP compositions were dominated by PET (52% and 77% of the total numbers of MPs) and were similar to those in sewage effluents (Fig. 2), implying that MPs in the estuarine water are derived from sewage effluent. Secondary effluents make up ~70% of the water volume of Sumidagawa River (NILIM Public Investment Project Team, 2005). MPs in the estuarine water are likely supplied mainly from sewage effluent and are diluted with river water and seawater. The numbers and polymer compositions of MPs are consistent with this idea. However, the shape of MPs is not consistent, i.e., flakes are dominant in the estuarine water and fibers are dominant in the secondary effluent. Further observation is required.

Kataoka et al. (2019) carried out a nationwide survey of MPs in Japanese rivers. According to their findings, the concentration of MPs in 29 Japanese rivers ranged from 0 to 0.012 pieces/L, with an average of 0.0016 pieces/L and 0.00023-0.0049 pieces/L for rivers in the Tokyo Metropolitan area, where the present study was conducted. Moreover, Isobe (2016) has reported MP concentrations ranging from 0.00036 to 0.011 pieces/L for seawater in Tokyo Bay. In the present study, we have found numbers of MPs in the estuarine water that are two to three orders of magnitude higher than those both for the river water and Tokyo Bay in the two aforementioned studies. This finding is reasonable as both studies measured MPs larger than 300 µm and disregarded smaller MPs. We measured smaller MPs down to 10  $\mu$ m, and the size distribution revealed that a greater number of MPs is present in a smaller range than 300  $\mu$ m (65%–91% of the total number of

<sup>\*</sup>AS: acrylonitrile styrene, EVA: polyethylene vinyl acetate, PEPD: polyethylene polypropylene diene, PEP: polyethylene-polypropylene copolymer, PET: polyethyleneterphthalate, PS: polystyrene, PE: polyethylene, and PP: polypropylene.

Table 4 Microplastic and molecular marker concentrations in estuarine water

	Dry Weather survey			Rain Event survey				
Polymer type*	st.MR		st.SB		st.MR		st.SB	
	Sample (pieces/L)	Blank (pieces/L)	Sample (pieces/L)	Blank (pieces/L)	Sample (pieces/L)	Blank (pieces/L)	Sample (pieces/L)	Blank (pieces/L)
AS	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
EVA	0.0	0.0	0.0	0.0	0.2	0.0	0.5	0.0
PEPD	0.7	0.0	0.2	0.0	2.2	0.0	0.8	0.0
PEP	0.2	0.1	0.1	0.1	0.4	0.2	0.3	0.3
PET	1.2	0.3	1.1	0.3	1.3	0.4	0.3	0.5
PS	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PE	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0
PP	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	2.3	0.4	1.4	0.4	4.3	0.6	1.8	0.8
Total LABs** (ng/L)	38	18	21	18	353	18	498	18
coprostanol (μg/L)	0.44	< 0.0003	0.18	< 0.0003	4.0	< 0.0003	8.3	< 0.0003

<sup>\*</sup>AS: acrylonitrile styrene, EVA: polyethylene vinyl acetate, PEPD: polyethylene polypropylene diene, PEP: polyethylene-polypropylene copolymer, PET: polyethyleneterphthalate, PS: polystyrene, PE: polyethylene, and PP: polypropylene.

## MPs (Fig. 3).

The concentrations of MPs in estuarine water, at 1.4-4.3 pieces/L, are close to the threshold concentration of MPs (6.65 pieces/L) below which adverse effects are not likely to occur (Everaert et al., 2018). As we measured smaller MPs directly in the present study, we measured a greater number of smaller MPs and assessed the risk they pose more appropriately than previous studies (e.g., Isobe et al., 2015; Kataoka et al., 2019). Measurement of smaller MPs is important to assess the particle toxicity associated with MPs. Furthermore, we found large numbers of MPs in the smallest size range, between  $10-100 \mu m$  (Fig. 3), indicating the presence of MPs much smaller than 10  $\mu$ m in the river water and the estuarine water. If we were to include such smaller MPs, particle toxicity would be more critical. Future studies should measure smaller MPs. To assess the risk associated with chemicals, one should calculate the weight of MPs and incorporate the findings into the discussion, as the risk posed by hazardous chemicals is evaluated in terms of the amount of chemicals, which can be estimated from the weight of MPs and the concentrations of chemicals they contain. The size distribution of the weight of MPs in the water samples (sewage, road runoff, and estuarine water) suggests that larger particles of > 100  $\mu$ m are more important than smaller particles of  $< 100 \mu m$  (Fig. S5). However, there was considerable uncertainty involved in the estimation of the weight of MPs, and more precise measurement of the weight of MPs, especially in the range of hundreds of micrometers, is vital to assess the risk associated with chemicals to plastics. Therefore, it is essential to monitor MPs in a wide range of sizes, from nanometers to millimeters, to ascertain the total risk posed by plastics, i.e., exposure of toxic chemicals and particle toxicity, in aquatic environments.

#### MP DISCHARGE DURING RAINFALL EVENTS

During rainfall events, greater amounts of contaminants are supplied to urban aquatic environments through CSO and surface runoff. We surveyed MPs during a rainfall event with a preceding rainfall of 86 mm, which was sufficiently intense to induce CSO. The concentrations of sewage markers in the rainfall event survey were one to two orders of magnitude higher than those in the dry weather survey (Table 4). Total LABs in the rainfall event survey were 353 ng/L at St.MR and 498 ng/L at St.SB, both of which were one order of magnitude higher than were those in dry weather, at 38 and 21 ng/L, respectively. In the rainfall event survey, the concentrations of coprostanol were 4.0 and 8.3  $\mu$ g/L, respectively, whereas the concentrations in dry weather were 0.44 and 0.18  $\mu$ g/L, respectively. Both markers indicate clearly the inputs of untreated sewage, i.e., CSO, to the estuary in the rainfall event survey. Conversely, we found no indication of sewage-derived MP inputs. PET MP concentrations (1.3 and 0.3 pieces/L) in the rainfall event survey were either similar to or lower than those in the dry weather survey (1.2 and 1.1 pieces/L), although there was a slight increase in the numbers of all the polymers (2.3 and 1.4 to 4.2 and 1.8 pieces/L). PET was abundant in sewage influent, and the concentrations would increase if the CSO-derived MPs were to contribute to the estuarine water. However, we found no significant increase in PET, indicating that CSO did not contribute to the MPs in the estuarine water in this rainfall event. Using the ratios of marker concentrations in the estuarine water to those in sewage influent [9,500 ng/L for LABs and 170 μg/L for coprostanol (Morimoto et al., 2011)], sewage (CSO) is estimated to represent 2%-5% of the estuarine water on a volume basis. This would increase MP concentrations in the estuarine water up to either 10 pieces/L or 20 pieces / L. However, we observed no such increase in the present study (Table 4). The difference may be explained by

<sup>\*\*</sup>LABs: Linear alkylbenzenes

differential transport between MPs and the sewage markers. Future observations under various rainfall conditions in various seasons would provide a clearer evaluation.

Surface runoff is another potential contributor of MPs to the urban water body during rainfall events. In this study, the numbers of pieces of PEPD in the rainfall event survey (2.2 and 0.79 pieces/L) were higher than were those in the dry weather survey (0.7 and 0.2 pieces/L, respectively), unlike the case with PET. These increases in the numbers of PEPD imply the contribution of street runoff during a rainfall event, because PEPD is a characteristic component in highway runoff (Fig. 2). This shifting of polymer composition toward runoff, together with higher MP concentrations in the highway runoff, indicates that road runoff is one of the major contributors of MPs to estuarine and coastal environments during wet weather.

To more quantitatively discuss the contribution of road runoff to MP pollution, we conducted a first-order mass input calculation by using MP numbers in the present study and some socio-statistical data. We multiplied the total area of roads in Tokyo prefecture—188 km<sup>2</sup> (Bureau of Construction of Tokyo, 2017) —by the total rainfall (1,870 mm/year in 2019) and estimated that  $3.52 \times 10^8$  m<sup>3</sup> of street runoff is generated annually. When we multiplied the concentrations of MPs in the highway runoff (transitional and steady state: 81 pieces/L) by the estimated volume of street runoff  $(3.52 \times 10^8 \text{ m}^3 \text{ per year})$ we estimated the total number of MPs generated from street runoff to be  $28 \times 10^{12}$  pieces per year. This is comparable to the figure from sewage effluent (17 × 10<sup>12</sup> pieces per year), which is estimated by multiplying the volume of secondary effluent discharged annually in Tokyo (2 × 10<sup>9</sup> m<sup>3</sup> per year; Bureau of Sewerage of Tokyo, 2019) by the concentration of MPs in sewage effluent (8.7 pieces/L). However, in the present study, the observations of MPs in sewage effluent and street runoff were very limited and there is considerable uncertainty with the estimation. In particular, it depends on measurement of MPs in the highway runoff. Previous studies of MPs in street dust (Abbasi et al., 2017; Yukioka et al., 2020) have indicated contribution from both traffic activity and non-traffic activities such as unintentional emissions of container/packaging-derived plastic wastes that subsequently undergo weathering on the streets to become MPs. Highway runoff may contain fewer MPs, and more MPs may be found in street runoff from general roads. In Tokyo, highways represent only 0.5% of the total road area (Bureau of Construction of Tokyo, 2017). Consequently, the estimation based on the data on highway runoff could be low. To obtain more accurate and realistic mass calculation, it is necessary to collect more runoff samples from general roads and highways and analyze them for MPs.

## **CONCLUSION**

MPs smaller than 200  $\mu$ m were predominant in all the water samples. PET was predominant both in sewage and secondary effluents, whereas PE, PEP, and PEPD were predominant in street runoff samples. The detection of PEPD indicates contribution from automobile tires. PET predominance was observed in estuarine water during dry weather, indicating a contribution from sewage effluent. During rainfall events, the proportion of PEPD in estuarine water increased, indicating a

contribution from street runoff. However, the present study is preliminary, depending on a limited number of samples, larger number and other types of road runoff, and estuarine water should be collected and analyzed for more a robust discussion.

## **ACKNOWLEDGMENTS**

We thank Japan Chemical Fibers Association for their kind supply of Reference PET fiber. Students and staffs of our laboratories provided welcome assistance on the field and laboratory works. The present study was financially supported by Grant-in-Aid from the Ministry of Education and Culture of Japan (Projects No. K219H00783 and K120H02283) and The University of Tokyo FSI–Nippon Foundation Research Project on Marine Plastics.

## **SUPPLEMENTARY MATERIAL**

Fig. S1, Sampling location of highway runoff from Chuo Expressway at N 35°39'48", E 139°32'52" and photo of the location and the runoff sample; Fig. S2, Image and schematic view of stainless-steel pressurized closed filtration system; Fig. S3, Imaging map of a secondary effluent sample. Red portions are substances profiled for polyethyleneterphthalate (PET); Fig. S4-1, Zoomed imaging map of individual microplastics (left), microscope image (middle), and IR spectrum (right); Fig. S4-2, Zoomed imaging map of individual microplastics (left), microscope image (middle), and IR spectrum (right); Fig. S5, Size distribution of microplastic samples in sewage treatment plant influent and effluent, highway runoff, and estuarine water. Based on weight of microplastics; Table S1, Basic water quality of the sewage, highway runoff, and estuarine water samples; Table S2, Volume-base number of microplastics in sewage treatment plant influent (pieces/L); Table S3, Volume-base number of microplastics in sewage treatment plant secondary effluent (pieces/L); Table S4, Volume-base number of microplastics in Highway Runoff: First Flush (pieces / L); Table S5, Volume-base number of microplastics in Highway Runoff: Transitional and Steady State (pieces/L); Table S6, Volume-base number of microplastics in estuarine water: st.MR on dry weather (pieces/L); Table S7, Volume-base number of microplastics in estuarine water: st.MR on rain event survey (pieces/L); Table S8, Volume-base number of microplastics in estuarine water: st.SB on dry weather (pieces/L); Table S9, Volume-base number of microplastics in estuarine water: st.SB on rain event survey (pieces/L); Table S10, Estimated concentration of microplastics in the water samples (ug/L).

This material is available on the Website at https://doi.org/10.5985/emcr.20200006.

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