A Study on the Proper Sampling Method for Microplastic Distributions in the Surface Freshwater:

From Case Studies in Japan and Indonesia

河川の表層水におけるマイクロプラスチックの分布のための適切なサンプリング方法に関する研究:

日本とインドネシアにおけるケーススタディから

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ABSTRACT

Microplastics (MPs) are recently considered anthropogenic pollutants. However, studies on MPs have been primarily conducted in the marine environment. Many pollutants materials, including MPs, from land, are released through the point/nonpoint sources and adversely affect the marine environment. Therefore, the freshwater environment plays an essential role in the fate of MPs.

One of the arguments to describe the MPs distributions is which sampling method should be employed. Naturally generated MPs quantity differences, which are caused by different net sizes, have been reported by many researchers, but how much qualitative MPs distribution is overlooked in large mesh size is still insufficient. Notably, verifying the validity of the sampling method in the freshwater environment is more limited than the marine environment. Therefore, the present study carefully determined the validities of the samplings with 100, 355 μm meshes and the Bulk water sampling method in five perspectives: MPs sources, abundances, features, river characteristics, and weather conditions. The final goal is to provide support in the selection of a proper mesh size to establish future study plans, which evaluate MPs pollution in the freshwater environment.

Samplings with 100, 355 μm meshes were conducted in the T River, Japan, 2020, which is the pristine freshwater characteristic that represents Japanese basic freshwater without specific pollution sources such as industries and Wastewater treatment plants (WWTPs). In the Bulk water sampling method, the surface water was grabbed in Citarum River, West Java, Indonesia, which is an optimized sampling area to evaluate the validity of the sampling method that reveals fewer MPs losses. The 100 μm mesh and the Bulk water sampling method samples were analyzed by the modified Sugiura & Takada (2019) *Symposium of JEC*, **28**, 125. coupled with an FT-IR microscope (ultrafast mapping method) and the 355 μm mesh sample was analyzed by Kudo et al. (2018) *J. JSCE Ser. B1,* **74**(4), 529-534. using FT-IR ATR and Micro Raman spectrometer.

The median values of numerical and mass abundances were 13.9 particles/m^3 and 6.0 μg/m^3, respectively in the 100 μm mesh (n=9), and 0.4 particles/m^3 and 1.0 μg/m^3, respectively in 355 μm mesh (n=9). A difference in the mass abundances was

approximately six times between both meshes, but for pristine river characteristics, the difference was ignored in this present study. A gradual increase in the numerical fragment abundance toward a smaller size was observed in 100 μm mesh, whereas the 355 μm mesh had no specific size distribution. The cumulative probabilities relating to the minimum Feret diameter of fragment and film were divided into three parts. Three parts implied respective 96% and 67% potential MPs underestimation in the 355 and 100 μm meshes. The 85% fragment, 8% film, 7% fiber with seven polymers (40% PE, 39% PP, 10% PS, 7% PA, 2% PU, 1% PVC, 1% PET) were found in 100 μm mesh, but only 99% fiber and 1% fragment shapes and three polymers (71% PET, 28% polyester, 1% PA) types were revealed in 355 μm mesh. In this respect, 355 μm mesh was challenging to represent tracing microplastic origins and presuming bioaccumulation potentialities. Whereas the distribution tendency along the flow direction by the 355 μm mesh was affected by accidental irregularities discovered in the sampling analysis step, the 100 μm mesh showed the highest abundances in the lower sampling station reflecting the adjacent urban and tributaries. Therefore, 100 μm mesh revealed completely different MPs distributions collected by 355 μm mesh and was recommended in the present study in microplastic abundances, sources, features perspectives.

In the Bulk water sampling method, the MPs were collected in the wet season (St.1-6 in Mar. 2018, St.3-5, 3-a in Jan. 2020). The results showed that the MPs numbers decreased in 2020 due to rainy events, the mean of 4.2±2.6 (n=6), and 2.8±5.5 (n=4) particles/20mL in 2018 and 2020. Notably, the tributary recorded the highest MPs abundance in 2020 with unknown pollutant sources. The upper area (Wagisagara, St.1) was anticipated pristine water, but the MPs pollution was shown, and the alkyd resin and PET were found in this area, unlike other stations in 2018. The Koyod (St.2), which had intensive textile industries, revealed high MPs pollution with one fiber in the 1000-5000 μm group. Additionally, the central part (Cisirung, St.3) of vast Bandung city showed heavy MPs pollution in 2018. The bioaccumulation of fishes inhabiting this river due to dominant PP (61%) and PE (17%) polymers was concerned. Comparison with other studies indicated inadequate wastewater treatment systems affected the MPs abundance in this basin. Therefore, the Bulk water sampling method was valid in microplastic abundances, sources, features perspectives.

Notably, the Bulk water sampling method effectively represented the MPs distribution in the 2020 tributary (St.3-a) station, which was different river characteristics compared with the mainstream (inadequate water exchange and numerous plastic debris). Therefore, it was regarded as a powerful tool to present MPs distribution in stagnant water flow and heavily polluted freshwater environments. Conversely, it also indicated that the Bulk water sampling method is more vulnerable to fluid river characteristics than the Volume-reduced method.

This viewpoint was more highlighted in the weather condition perspective. Except for the tributary (St.3-a), the Bulk water sampling method did not collect the MPs in the 2020 sampling stations in which sampling was implemented with precipitation. Based on previous studies and the maximum sizes (90 μm) of randomly selected suspended particles, which may clog the mesh, the relatively larger mesh than 100 μm was recommended to avoid clogging the mesh due to suspended matter. It indicated that the 355 μm mesh is likely to collect valid MPs distribution in rainfall events and numerous suspended matters. Although it was unsuitable in the present study, several previous studies found that approximately 355 μm mesh collected acceptable MPs distribution in the MPs features (shape and polymer types).

In this present study, the 100 μm mesh and the Bulk water sampling revealed more specific distributions than 355 μm, but a suitable sampling method should be employed to describe MPs distributions depending on the river characteristics and study purposes.

Keyword: Microplastics, Freshwater, Surface water, Volume-reduced sampling method, Bulk water sampling method, Numerical and mass abundances, Microplastics sources, Microplastics Features, River characteristics, Weather conditions

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CHAPTER 1

Backgrounds

1 Backgrounds for Study on the Microplastics

Plastics are essential materials for our life in this contemporary era and are anthropogenic pollutants that cause a threat to the aquatic environment. From 15 million metric tons in 1964, global plastic production reached 311 million metric tons in 2014, a twenty-fold increase within the past 50 years (WEF, 2016). In 2010, Jambeck et al. (2015) estimated that at least 4.8 to 12.7 million metric tons of plastic wastes from the land were released into the marine environment. Emitted plastics are gradually broken down in size by photolysis and are circulated by sea currents in the oceans (Andrady, 2011).

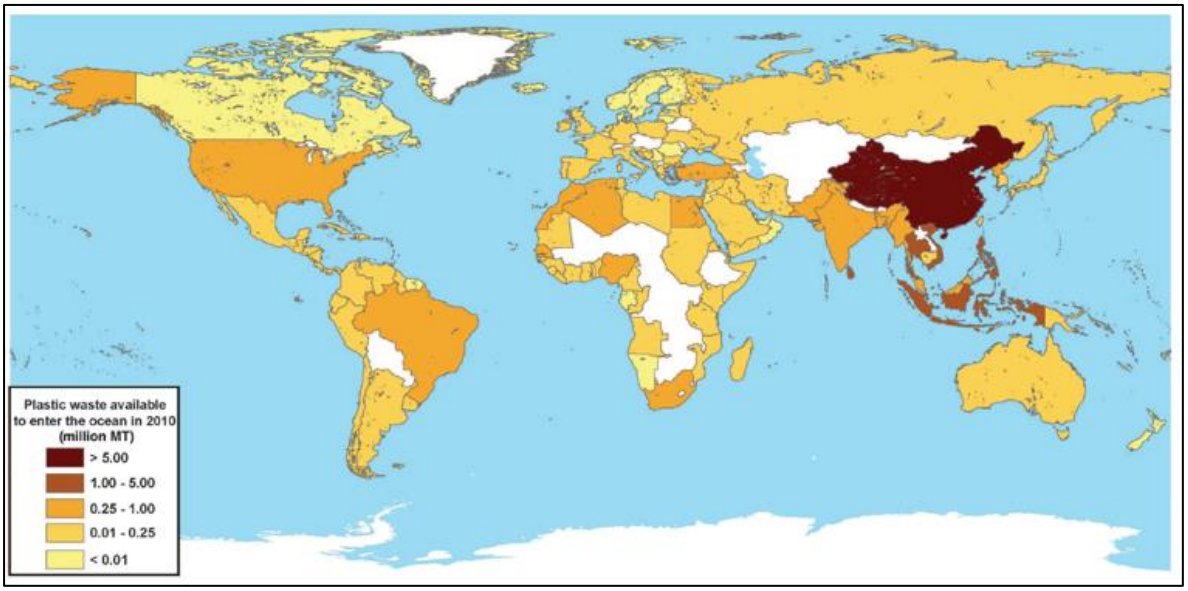

Figure 1. Global map with each country shaded according to the estimated mass of mismanaged plastic waste (millions of metric tons (MT)) generated in 2010 by populations living within 50 km of the coast (192 countries, Countries not included in the study are shaded) (Jambeck et al., 2015).

Emitted plastic particles are categorized according to the diameter, less than 25 mm (meso), 5 mm (micro), and 1 μm (nano-plastics) (GESAMP, 2019). Microplastics (MPs) have a similar size as the zooplankton, have broad surface areas per unit volume, and are distributed from the pelagic to the benthic ecosystem. These characteristics pose a threat to aquatic organisms in the following ways: accidental ingestion by the sea animals, leaching of harmful chemicals (nonylphenol, bisphenol-a, PBDE) from the MPs, absorbed hydrophobic compounds (PAHs, PCBs) to the MPs (Cole et al., 2011). Therefore, MPs were recently pointed out as the new pollutant matrix, different from the water, suspended matters, and sediments (Yamashita et al., 2016).

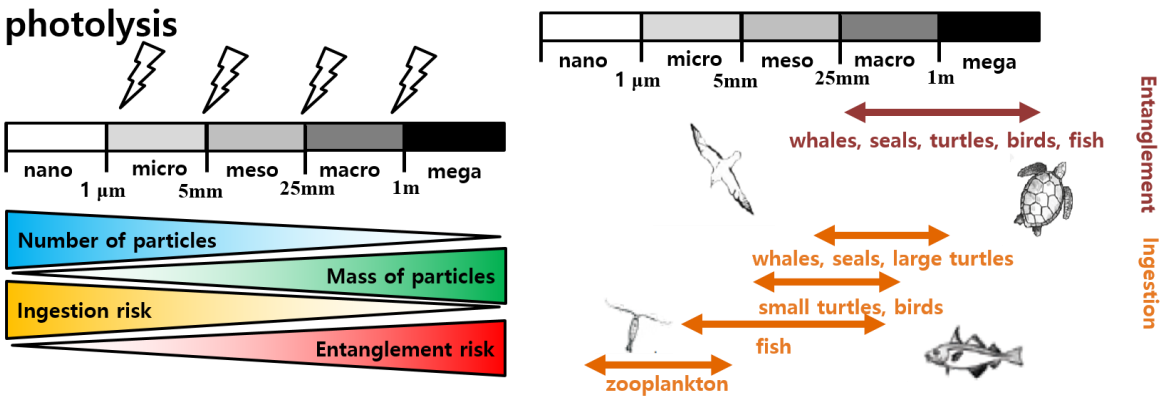

Figure 2. Schematic representation of the impacts of different sized plastics on marine biota including entanglement, ingestion and habitat associated risk. The figure was extracted from (GESAMP, 2019) and re-illustrated.

These MPs are categorized into two groups by origins. 1) Primary MPs, which are manufactured by the plastic industry for having some purposes, such as cosmetics, personal care products, cleaning agents, 2) Secondary MPs, which are irregularly derived from the large plastic debris through weathering (GESAMP, 2019; Fabres et al., 2016).

On the other hand, *in vivo* exposure tests have shown decreased reproductive output in copepods (*C.helgolandicus*) (Cole et al., 2015), depleted energy reserves in marine worms (*A.marina*) (Wright et al., 2013), decreased growth rates and changes of feeding preference in European perch (*P.fluviatilis*) larvae (Lönnstedt & Eklöv, 2016), and intestinal alterations in European sea bass (*D.labrax*) (Pedà et al., 2016). Indeed, many researchers have discovered these MPs on the zooplankton (Desforges et al., 2015), bivalve, gastropod, crustacean (Danopoulos et al., 2020), riverine, estuarine, and oceanic fishes (Barboza et al., 2020; Bessa et al., 2018; T. J. Park et al., 2020), including human feces (Zhang et al., 2021).

In this perspective, recent studies on MPs have been implemented in various study fields, such as the technical methods of microplastic analysis, the fate of MPs in the basin area, the current distribution of MPs in the land, freshwater, and sea (Otsuka et al., 2021). However, studies on MPs have primarily focused on the marine environment. According to Lembert & Wagner (2018), a literature search on Thomson Reuters's ISI Web of Science returns 1,228 manuscripts containing the term ‘microplastic’, of which only 3.7% of publications contain the term ‘freshwater’. It indirectly indicated that research on MPs in freshwater has a little attention.

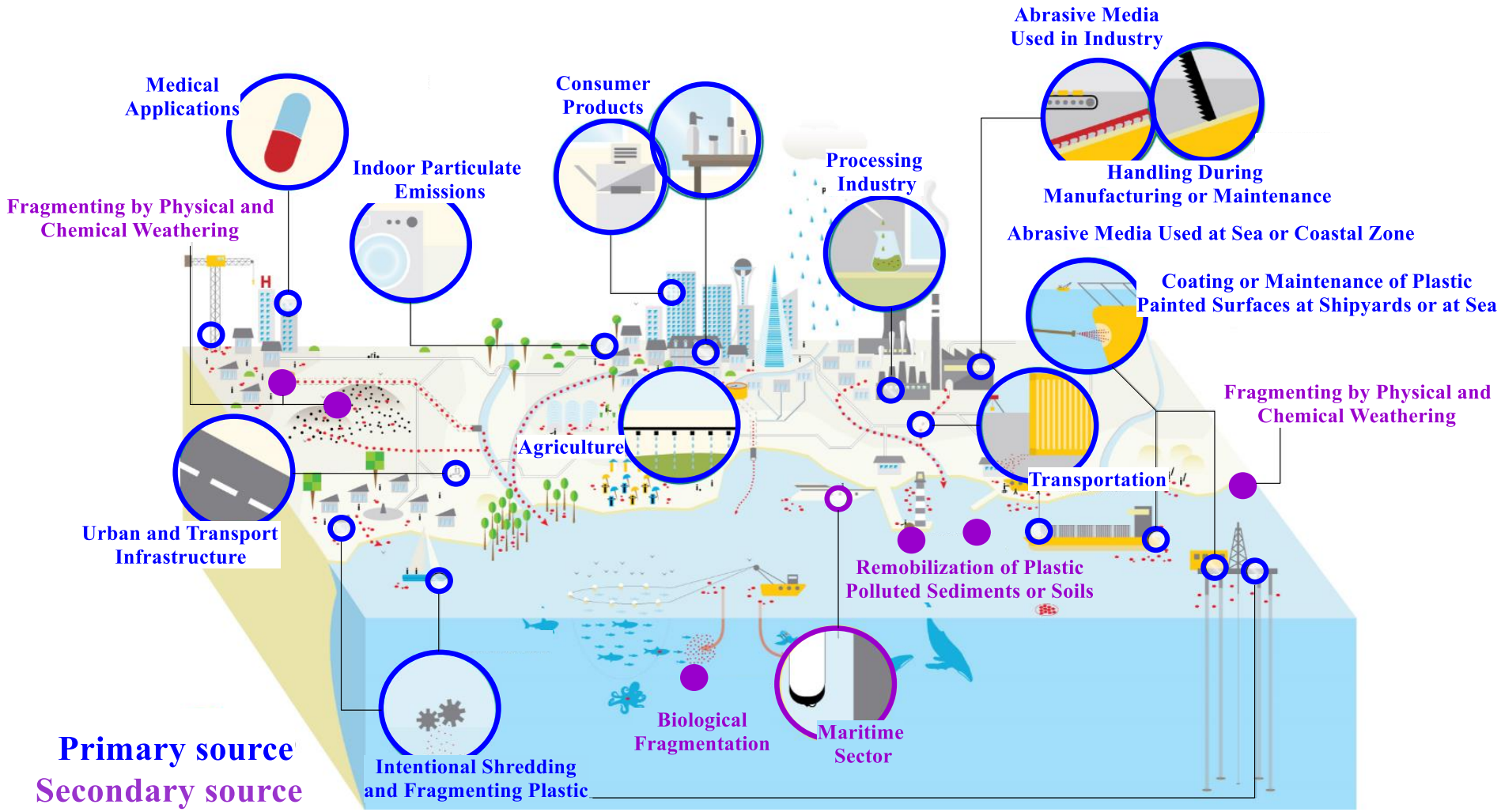

Figure 3. How microplastics are generated. The figure was extracted from Fabres et al. (2016) and re-illustrated.

CHAPTER 2

Recognizing Importance and Problems

in the Study on MPs in the Freshwater Environment

Citation:
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2.1 Pollution Materials From the Land to the Marine Environment

The general background for the study on MPs in the aquatic environment was stated in Chapter 1. A recent research trend of the study on MPs has been focused on the maritime sector. Therefore, before entering the main idea, it is essential to understand why studying MPs in freshwater is necessary and its problems. In Chapter 2, the importance and problems in the study on MPs in the freshwater environment are emphasized. Firstly, we determined the fate of general pollution materials from land to the marine environment in this perspective.

The Gamak Bay, Yeosu City, Korea, was designated as the environmental preservation sea area to conserve fishery resources such as bivalves farming (*C.gigas*) by the Korean government (Jeong et al., 2017). However, northwestern Gamak bay was affected by eutrophication in the marine environment every year (Jeong et al., 2019; J.-B. Kim et al., 2006; J.-S. Lee et al., 2003; T. Lee, 2015). Therefore, Yeosu City carried out various purification & restoration programs to improve the polluted sediments (Jeong et al., 2019; S. D. Kang, 2018). Nevertheless, adverse effects such as hypoxia and hydrogen sulfide formation reoccur recently (Jeong et al., 2019; J.-B. Kim et al., 2006). The primary cause of reoccurred adverse effects was the previous purification & restoration programs, which did not consider the pollutant sources (Jeong et al., 2019; S. D. Kang, 2018). These characteristics had an advantage in determining how many terrigenous origin pollutant materials affect the aquatic environment, and therefore surface sediments in Gamak Bay were investigated. The surface sediments are the primary environmental medium denoting the long-term adverse impacts of pollutant materials effectively, unlike the water medium that condition constantly changes depending on various parameters such as tide, precipitation, solar radiation, evaporation, oxidized-reduced environment, and fishery farming.

Thus, the heavily eutrophicated organic matter on the surface sediment in Gamak Bay was determined origins (terrigenous or oceanic) in this Chapter. The surface sediments were grabbed using a Van Veen grab sampler on ten sampling stations in northwestern Gamak bay on 26th May, 26th June, 12 and 19th July, 8 and 16th August, 13th September, and 13th October, and additionally on six sampling stations in Sunso area on 26th June 2017 (Figure 4). Target parameters, which represent the eutrophication on sediments, were Ignition loss (IL), Chemical Oxygen Demand (COD), Acid Volatile Sulfur (AVS), Total Organic Carbon (TOC), Total Organic Nitrogen (TON). Analytical methods were employed the ignition method with 550℃ for IL, the Alkaline potassium permanganate method for COD, the Sulfur dioxide detector tube (GASTEC Corp., Kanagawa, Japan) for AVS, the Organic elemental analyzer (vario MACRO cube, Elementar Analysensysteme GmbH, Langenselbold, Germany) after removing the calcium carbonate using a 1N hydrochloric acid for TOC and TON.

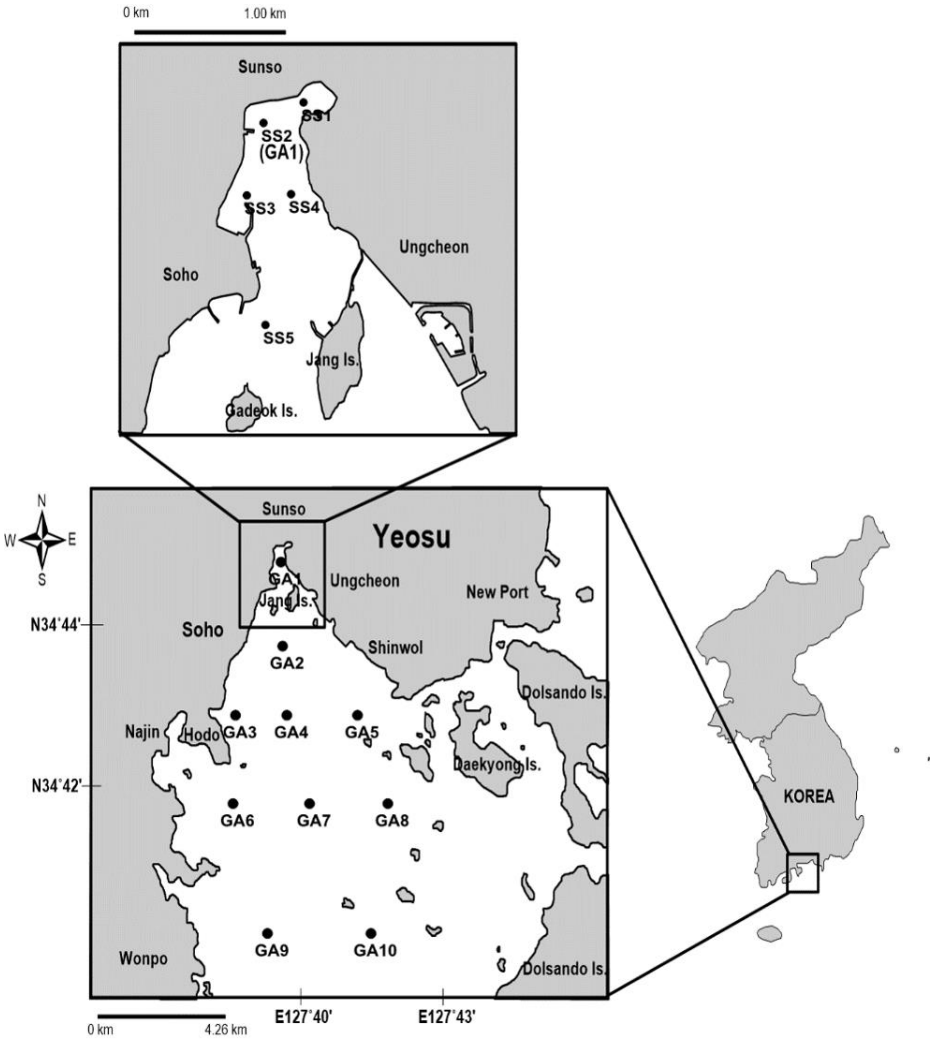

Figure 4. Map showing surface sediment sampling stations (Sunso: n=5, Gamak bay: n=10).

The results of IL, COD, AVS, TOC, TON on the surface sediment in the sampling areas are shown in Table 1. Besides, the C/N ratios to determine the origin of organic matters in the whole sampling date are illustrated in Figure 5. The C/N ratio above twelve indicates the terrigenous, a range of 6-9 is phytoplankton origin, and 5-12 implies the oceanic organic matter (Holligan et al., 1984; P.-J. Kim et al., 2012; Kukal, 1971; Müller, 1977; Stein, 1991).

In Figure 5, the whole sampling date and station in northwestern Gamak Bay indicated the oceanic organic matter. However, SS1, SS2 sampling stations in the Sunso sea areas showed the terrigenous origin. These areas are located adjacent to three sewers (Figure 6). The sewers are played a role in the 'Point source,' inputting the heavily eutrophicated organic matter to the marine environment constantly (S. D. Kang, 2018). There is one wastewater treatment plant: Yeosu WWTP. However, it is pointed out that the WWTP could not cover the whole wastewater in the surrounding city (S. D. Kang, 2018). Significantly, Yeosu City employs the Combined Sewer System (CSS), which collects rainwater runoff, domestic sewage, and industrial wastewater into one pipe (S.

D. Kang, 2018; US EPA, 2021a). Due to this characteristic, the three sewers also act as a 'Nonpoint source' outputting numerous organic matter during rain events to the bay (S. D. Kang, 2018).

The point/nonpoint sources resulted in a necessity to purify the heavily eutrophicated organic matters on the surface sediments exceeding the global sediment criteria: COD 20 mgO_2/g-dw., AVS 0.2 mgS/g-dw. (JFRCA, 2013), and TOC 1.0% (Canada Ontario, 2019). The input of organic matter through the sewers also resulted in significantly poor water quality in COD, DO in SS1 and SS2, Sunso sea area (Jeong et al., 2019; S. D. Kang, 2018). In particular, the sources in Gamak Bay are pointed out as the primary route of inputting other pollution chemicals and heavy metals, such as PAHs, Dibutyltin, Zinc, Lead, Copper, and Mercury (Jeong et al., 2017, 2019; S. D. Kang, 2018). Consequently, the corresponding study (Jeong et al., 2019) indicated that the freshwater environment is vital as the primary route of pollution materials via pollution sources.

Table 1. The result of MIN-MAX (MEAN±SD) concentrations for IL, COD, AVS, TOC, TON on surface sediments in northwestern Gamak bay and Sunso area, Yeosu, Korea, 2017

Sea area	Sampling date	n	IL	COD	AVS	TOC	TON
			%	mgO_2/g-dw.	mgS/g-dw.	%	%
Northwestern Gamakbay, Yeosu City	26th May	10	1.3-3.3 (2.2±0.7)	7.2-27.4 (14.9±6.7)	0.008-0.796 (0.320±0.269)	0.7-2.4 (1.4±0.5)	0.11-0.34 (0.20±0.07)
	26th June	10	6.9-11.9 (9.2±2.0)	7.9-39.3 (21.5±10.6)	0.010-0.825 (0.262±0.245)	0.6-1.9 (1.2±0.5)	0.08-0.28 (0.17±0.07)
Sunso, Yeosu	26th June	5	1.8-8.8 (4.0±3.0)	9.5-36.8 (23.6±10.9)	0.240-4.362 (2.085±1.950)	1.4-6.2 (3.1±2.0)	0.2-0.4 (0.3±0.1)
Northwestern Gamakbay, Yeosu City	12th July	10	11.6-21.0 (16.8±3.1)	10.4-47.1 (25.5±22.1)	0.008-0.908 (0.304±0.295)	0.7-2.6 (1.4±0.6)	0.11-0.40 (0.22±0.09)
	19th July	10	6.1-11.3 (8.3±1.8)	9.2-45.0 (22.1±12.2)	0.002-1.114 (0.317±0.352)	0.6-2.4 (1.4±0.6)	0.11-0.37 (0.21±0.08)
	8th August	10	5.9-13.6 (8.6±2.3)	5.2-41.7 (20.0±10.9)	0.011-1.125 (0.347±0.346)	0.7-2.6 (1.4±0.6)	0.12-0.40 (0.21±0.08)
	16th August	10	4.8-13.0 (9.8±2.5)	4.1-33.9 (14.6±9.2)	0.010-0.660 (0.267±0.238)	0.7-2.3 (1.4±0.5)	0.10-0.29 (0.18±0.06)
	13th September	10	7.1-15.0 (10.8±2.6)	8.5-33.2 (21.1±8.6)	0.029-0.792 (0.328±0.316)	0.8-2.6 (1.6±0.6)	0.12-0.34 (0.20±0.08)
	13th October	10	5.5-10.6 (7.9±1.5)	6.1-31.6 (20.3±9.4)	0.016-0.749 (0.287±0.241)	0.6-2.0 (1.4±0.5)	0.07-0.28 (0.18±0.07)

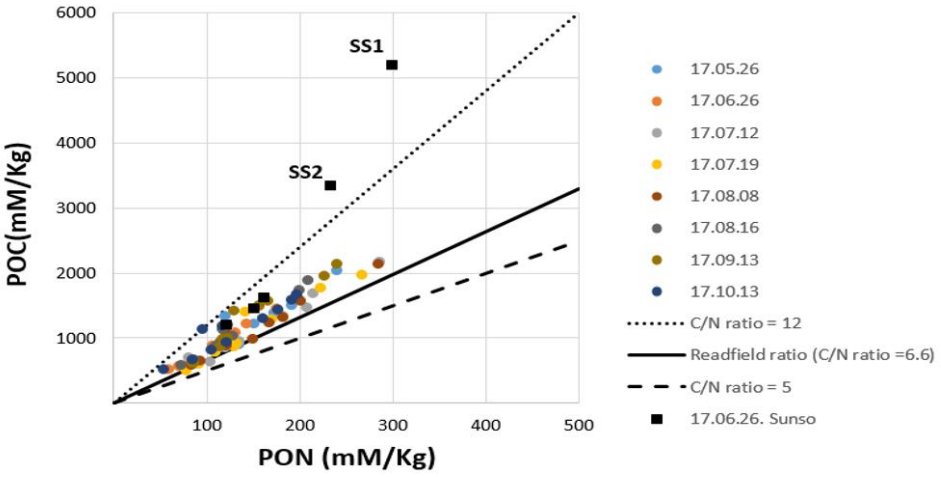

Figure 5. Distributions of Carbon/Nitrogen ratio on surface sediment in Sunso and Gamak bay.

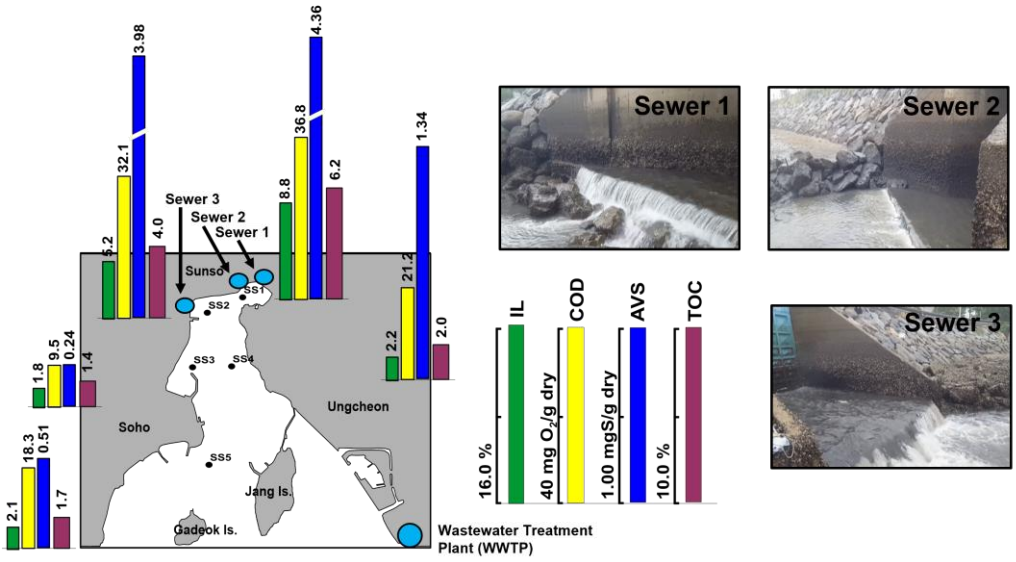

Figure 6. Spatial distribution of IL, COD, AVS, and TOC on June 26th on surface sediment and three sewers in Sunso sea area, Yeosu City, Korea.

2.2 Microplastics From the Land to the Marine Environment

As mentioned above, the freshwater environment plays an essential role as the primary route for pollution materials via point/nonpoint sources. In this perspective, we need to discuss whether the MPs in the surface water are also followed this trend to understand the importance of the study on MPs in the freshwater environment.

Firstly, the point sources are representative processes increasing the MPs abundance in surface freshwater. According to the US EPA (2021b), the term 'Point source' means any discernible, confined, and discrete conveyance, including but not limited to any pipe, ditch, channel, tunnel, conduit, well, discrete fissure, container, rolling stock, concentrated animal feeding operation, or vessel or other floating craft, from which pollutants are or may be discharged.

In the studies on MPs, point sources representatively include the wastewater derived from indoor emissions, industrial plants, effluents of WWTPs. Many researchers mention the existence of MPs in personal care products, such as hand and facial cleansers, toothpaste, body scrubs, shower gel, shampoo, shaving foam (Guerranti et al., 2019; Sharma et al., 2021; Siegfried et al., 2017). Notably, laundry is pointed out as the crucial microfiber emission source in indoor activities (Corami et al., 2020; Siegfried et al., 2017). Another indoor emission, the MPs based on household dust, which is generated by cleaning air conditioner filters, floors, or dusty surfaces, end up in the drain and make their way to sewage (Siegfried et al., 2017; Webster et al., 2009).

Meanwhile, several researchers estimated that point sources of tire wear emissions account for 13% (Verschoor et al., 2016) or 15% (Kole & Ragas, 2015) of the total tire wear inputs to rivers (Siegfried et al., 2017). MPs in industrial wastewater are reported in chemical, electroplating (F. Wang et al., 2020), spinning, textiles (Alam et al., 2019), polishing eyeglass (J. Lee et al., 2021) industries. Although these wastewaters reach to WWTPs, the effectiveness of reducing MPs in WWPTs is reported at 64-99% globally (Edo et al., 2020; H. J. Park et al., 2020; Sugiura et al., 2021). It is indicated that the WWTPs may constantly release tiny MPs, which could not be removed in the WWTPs (Edo et al., 2020; Kataoka et al., 2019; H. J. Park et al., 2020; Sugiura et al., 2021).

Secondly, the definition of point sources does not include agricultural stormwater discharges and the return flows from irrigated agriculture, and the term 'Nonpoint source' is defined to mean any source of water pollution that does not meet the definition of 'Point source' (US EPA, 2021b).

Notably, precipitation is an essential factor in the nonpoint source. The rainy events facilitate MPs to be emitted to the aquatic environment from the road (Verschoor et al., 2016), urban area (Piñon-Colin et al., 2020), agriculture (Chijiwa & Agusa, 2021; Katsumi et al., 2021). Additionally, the CSS allows pollutant materials to be released

into the aquatic environment with the precipitation, despite the wastewater being connected to the sewage pipe, as shown earlier in Yeosu City, Korea (Jeong et al., 2019). These rainy events generate the seasonal MPs distributions resulting in the increased (Cheung, Fok, et al., 2018; Eo et al., 2019; Kataoka et al., 2013; Piñon-Colin et al., 2020) or decreased (Lin et al., 2018; Moore et al., 2011; Yan et al., 2019) numerical abundance.

On the other hand, other nonpoint sources are the MPs fallen from the atmosphere (Dris et al., 2015) and the re-floated MPs from the riverbeds (Hurley et al., 2018). Additionally, plastic pollution also occurs in the maritime itself, such as derivatives of fishery behavior (Eo et al., 2018) and shipment (Chae et al., 2015).

However, Morales-Caselles et al. (2021) stated that approximately 80% of plastic litter originated from land floats into the seawater. Moreover, Kataoka et al. (2019) determined the correlations between MPs and freshwater quality parameters, indicating the effects on WWTPs. These results emphasized the significance of studies on MPs in the freshwater environment (Figure 7).

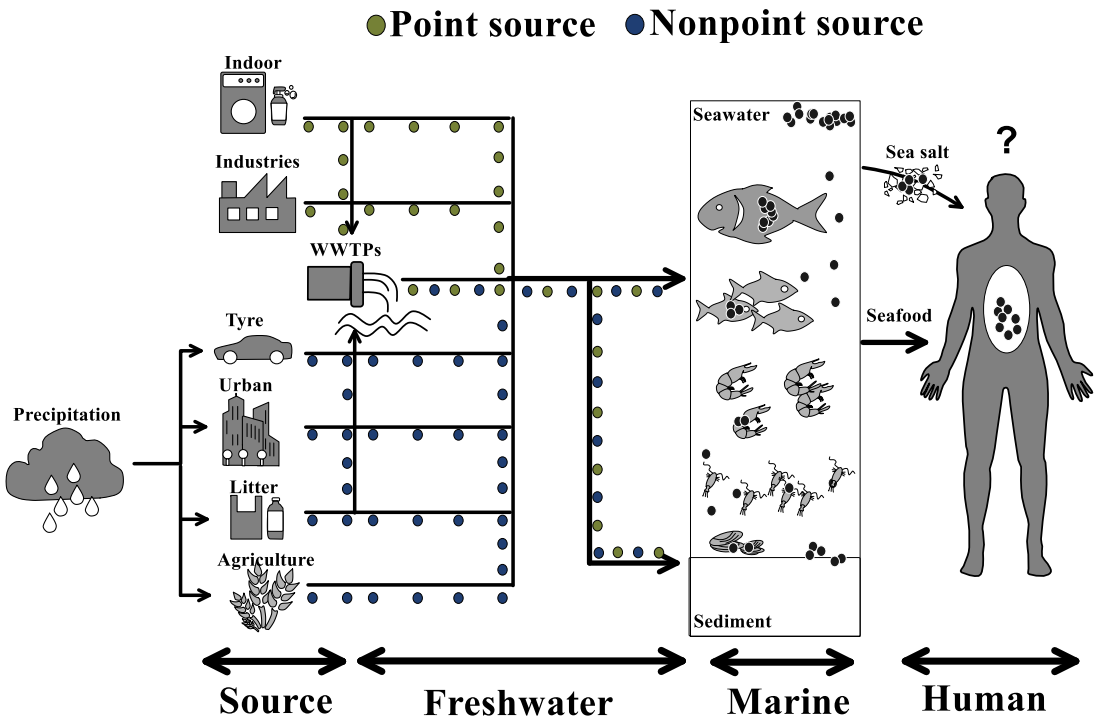

Figure 7. Representative fate of Microplastics in aquatic environments.

2.3 Selecting Mesh Size for Microplastics

Hidalgo-Ruz et al. (2012) reviewed three methods of sampling MPs in aquatic environments: selective, bulk, and volume-reduced. Of which the selective sampling method is adequate in situations where different MPs of similar morphology and size greater than 1 mm are present, but the less obvious heterogeneous items can be often overlooked (Hidalgo-Ruz et al., 2012). The Volume-reduced method is commonly employed to describe MPs in the surface water from past to date because it has the advantage that large areas or quantities of water can be sampled using mesh screens (Chae et al., 2015), plankton nets (Cheung, Fok, et al., 2018; Cheung, Hung, et al., 2018; Eo et al., 2019; Faure et al., 2015; Kataoka et al., 2019; Rodrigues et al., 2018), handle nets (Moore et al., 2011). In contrast, though bulk sampling limits the sampled amount of water, all MPs in the specimens can be collected, regardless of their size or visibility, in theory (Crwaford & Quinn, 2017).

When researchers discussed MPs pollution, one of the crucial topics is the mesh size to collect the MPs. The Guidelines for Harmonizing Ocean Surface Microplastic Monitoring Methods recommended a 350 μm mesh because of its ability to filter vast amounts of seawater (Michida et al., 2019). Kataoka et al. (2019) indicated that suspended matters disturbed sampling and clogged 100 μm mesh in the freshwater. Contrary to this, the number of MPs <300 μm reached 74% and 81% of the total numbers in the river and sand beaches, respectively (Eo et al., 2018, 2019). This implied that many particles are not collected when a 300 μm mesh is employed (Eo et al., 2018, 2019). However, 74% and 81% were considered only for maximum particle size, and it was insufficient for considering the potentiality in which the particles pass the mesh in a minimum size via particle rotation (Abeynayaka et al., 2020).

Significantly, approximately 80% of MPs investigations focus MPs debris collection >300 μm in the aquatic environments (Conkle et al., 2018; Lindeque et al., 2020), but many studies have pointed out undervalued MPs distributions in large mesh sizes (Hidalgo-Ruz et al., 2012; Prata et al., 2019): 100, 333, 500 μm meshes (Lindeque et al., 2020), 333 and 1000 μm meshes (Tokai et al., 2021) in the surface seawater.

On the other hand, underestimating tiny MPs is indispensable in the volume-reduced method based on mesh sizes (Hidalgo-Ruz et al., 2012). The grabbing water may descript MPs validly in a situation in which MPs abundances should be carefully illustrated. There are also reported numerically different MPs between grabbing water and the Volume-reduced sampling method in the marine environment: 50 and 330 μm meshes (J. H. Kang et al., 2015), 0.45 and 333 μm meshes (Barrows et al., 2017).

However, while difference that naturally arises in terms of quantities has been enumerated, there are still insufficient qualitative distributions, particularly in the freshwater environment. Therefore, the present study determined to evaluate two

different size meshes (100 and 355 μm) adequacy in Chapter 3 and the Bulk water sampling method in Chapter 4. Subsequently, comprehensive validities for 100, 355 μm meshes and the Bulk water sampling methods were demonstrated in Chapter 5 based on five perspectives: MPs sources, abundances, features, river characteristics, and weather conditions.

The present study qualitatively and quantitatively demonstrated the differences of MPs distributions in two different meshes and bulk water sampling. The final goal is to provide support in the selection of a proper mesh size to establish future study plans, which evaluate MPs pollution in the Japanese freshwater environment.

CHAPTER 3 (CASE1)

The Microplastics Distributions in the Surface Freshwater Collected by 100 and 355 μm meshes in the First-Grade Freshwater, Japan

Citation:
JEONG Huiho, KUSANO Teruhiko, ADDAI-ARHIN Sylvester, NUGRAHA Willy Cahya, NOVIRSA Randy, PHAN DINH Quang, SHIROSAKI Tomohiro, FUJITA Emiko, KAMEDA Yutaka, CHO Hyeon Seo, ISHIBASHI Yasuhiro, ARIZONO Koji.
A Study on the Differences of Microplastics Distributions in the Surface Freshwater Collected by 100 and 355 μm nets
Environment Monitoring Contaminant & Research, [revised]

3.1 Introduction

The importance and problems in the study on MPs in the freshwater environment were emphasized in Chapter 2. In addition, the previous chapter highlighted that studies are insufficient to evaluate the MPs distributions collected by the small and large meshes in the surface freshwater in qualitative aspects.

Therefore, the present chapter aimed to evaluate the adequacy of two different mesh sizes based on collected microplastic features. Specifically, the results between 100 and 355 μm mesh samples were compared by the size, shape, polymer types, and the potentially overlooked particles were estimated by the minimum Feret diameter.

Both mesh sizes were selected based on the mesh sizes that have been generally employed to collect MPs in aquatic environments; 80, 100, 125 μm (Abeynayaka et al., 2020; Dris et al., 2015; Michida et al., 2019; H. J. Park et al., 2020; T. J. Park et al., 2020; Sembiring et al., 2020; Syakti et al., 2018) and 300, 330, 333, 335, 350 μm (Abeynayaka et al., 2020; Faure et al., 2015; Kataoka et al., 2019; Kudo et al., 2018; Lima et al., 2014; Mani et al., 2015; Michida et al., 2019; Moore et al., 2011; Naidoo et al., 2015; Nihei et al., 2020).

On the other hand, the T River, Japan, was designated as the 'First-Grade Freshwater,' a vital water system for national land conservation and the national economy in Japan (MIC, 2020). This pristine freshwater characteristic is the representative Japanese basic freshwater without specific pollution sources such as industries and WWTPs and. Chapter 3 qualitatively and quantitatively demonstrated the differences in MPs distributions in two different meshes (100 and 355 μm).

3.2 Materials and Methods

3.2.1 Sample Collection

Microplastics on the surface water were collected from three sampling stations in the T River, Japan, on 26th August 2020 (Figure 8). The samples were collected at positions where the investigator could easily access the river edge. The 100 µm (the mouth diameter 30 cm, length 75 cm, Simple plankton net, Rigo Co. Ltd., Tokyo, Japan) and 355 µm (the mouth diameter 30 cm, length 75 cm, an order made net, Tanaka Sanjiro Co. Ltd., Fukuoka, Japan) meshes were employed for 5-10 minutes with three replications at each sampling station.

In the front of the mesh net mouths, a flowmeter (Digital Flowmeter 2030R, General Oceans Inc., Miami, FL, USA) was installed to estimate filtered water volumes, and the volumes were 3.2±1.1 and 7.8±1.7 m^3 (n=9) for the 100 and 355 µm meshes, respectively. Collected samples were stored in glass bottles and transported to the laboratory. Depending on the sampling nets, two different methods were employed to optimize the microplastic distributions because of the detection size limitations of the spectroscopy.

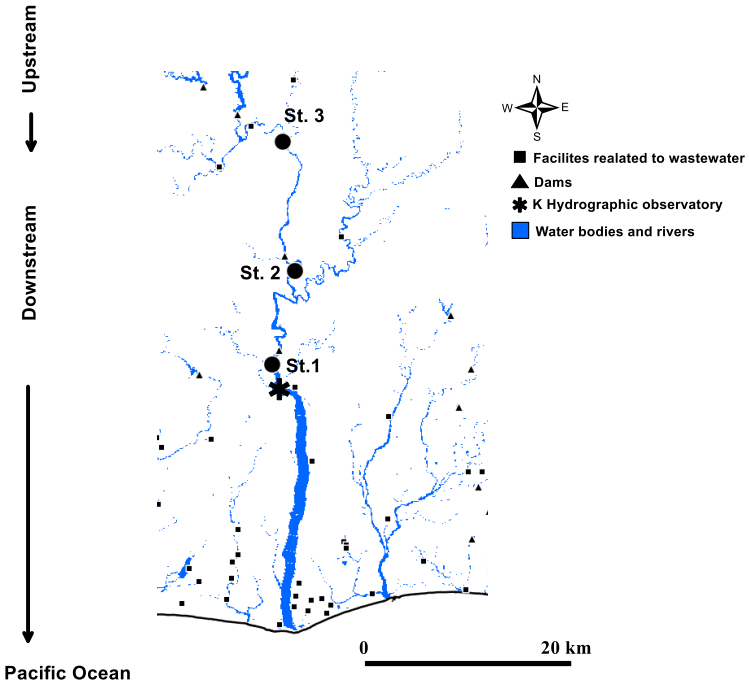

Figure 8. Schemed the microplastic sampling stations collected by 100 and 355 µm nets and K Hydrographic observatory in the First-Grade River, T River, Japan.

3.2.2 Sample Treatment

1) 100 μm mesh sample (Figure 9)

The 100 μm mesh sample was pre-treated by the modified Sugiura & Takada (2019) method. In brief, the specimen was filtered by the Cellulose Nitrate filter paper (CN filter paper, pore size 8 μm, diameter 47 mm, Whatman PLC., Maidstone, UK). The CN filter paper was dissolved at 40℃ using 25 mL of 1 M NaOH (CAS#1310-73-2, Sodium hydroxide) solution. The 1 M NaOH solution was neutralized using 25 mL of 1 M HCl (CAS#7647-01-0, Hydrochloric acid) solution.

In sequence, 50 mL of 30% H_2O_2 was added to digest organic matters in the solution, followed by the addition of 0.070 mg $FeSO_4 \cdot 7H_2O$ (CAS#7782-63-0, Ferrous sulfate heptahydrate). The solution was kept under florescent light for one week with an aluminum foil cover to prevent contamination.

The resultant solution was transferred into a glass separatory funnel for the density separation using 350 mL of 6.7 M NaI (CAS#7681-82-5, Sodium iodide) solution (1.6 g/cm^3). The funnel was shaken by hand for one minute and kept stable for 24 hours. The lower part of the solution was collected into a beaker, re-separated, and the supernatant was filtered using a stainless filter (pore size 100 μm, diameter 47 mm). This step was repeated three times, and each stainless filter was kept in the desiccator until the final repetition.

Total particles on the three filters were transferred by ultrasonication (AU-166C, Aiwa Medical Industry Co. Ltd., Tokyo, Japan) into 200 mL of the ultrapure water. Finally, the ultrapure waters were filtered on a polytetrafluoroethylene (PTFE) OMNIPORE membrane filter (pore size 5 μm, diameter 47 mm, Merck Millipore Ltd., Tullagreen, Ireland) using a filtration set, which had 17 mm of a diameter of filtered circle area (SIBATA SCIENTIFIC TECHNOLOGY Ltd., Saitama, Japan), and the filter was dried in the desiccator for one day, ensuring no contaminations.

The Micro FT-IR was utilized for the plastic polymer identification on this filter. The detected MPs were sized and photographed by a Stereoscopic Zoom Microscope, and the particle weights were calculated using Equations (1)-(4). Every chemical reagent in this process was purchased from FUJIFILM Wako Pure Chemical Corp., Osaka, Japan.

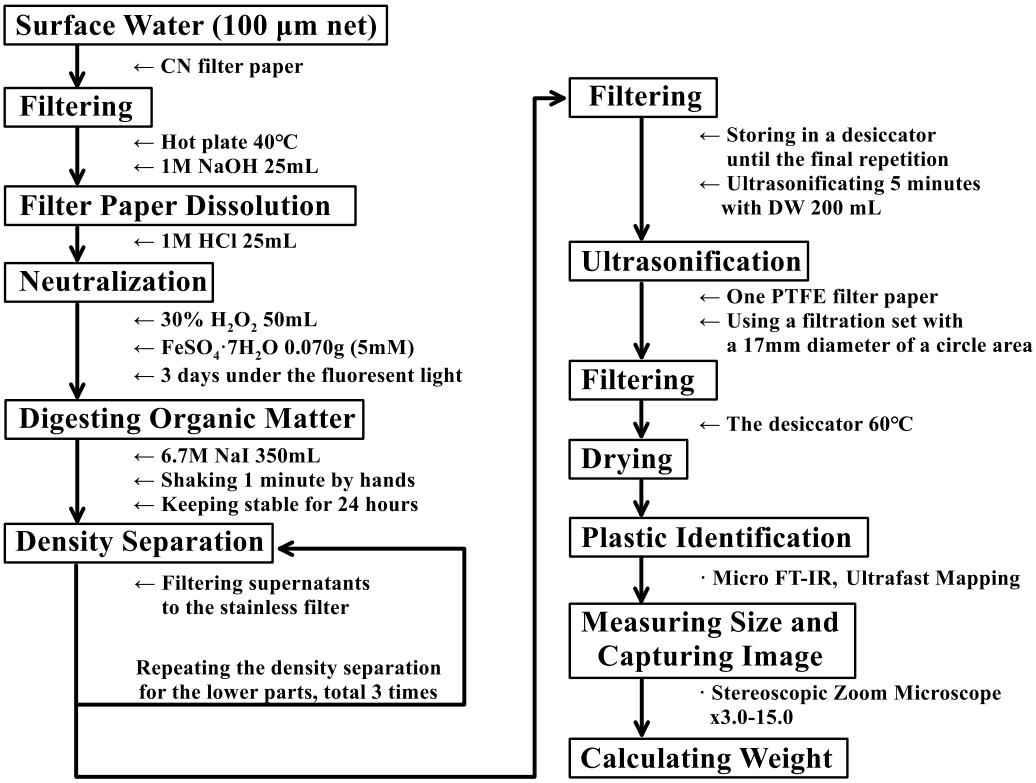

Figure 9. Flowchart of laboratory analysis for microplastics collected by the 100 µm net. The Sugiura & Takada (2018) method was modified and employed.

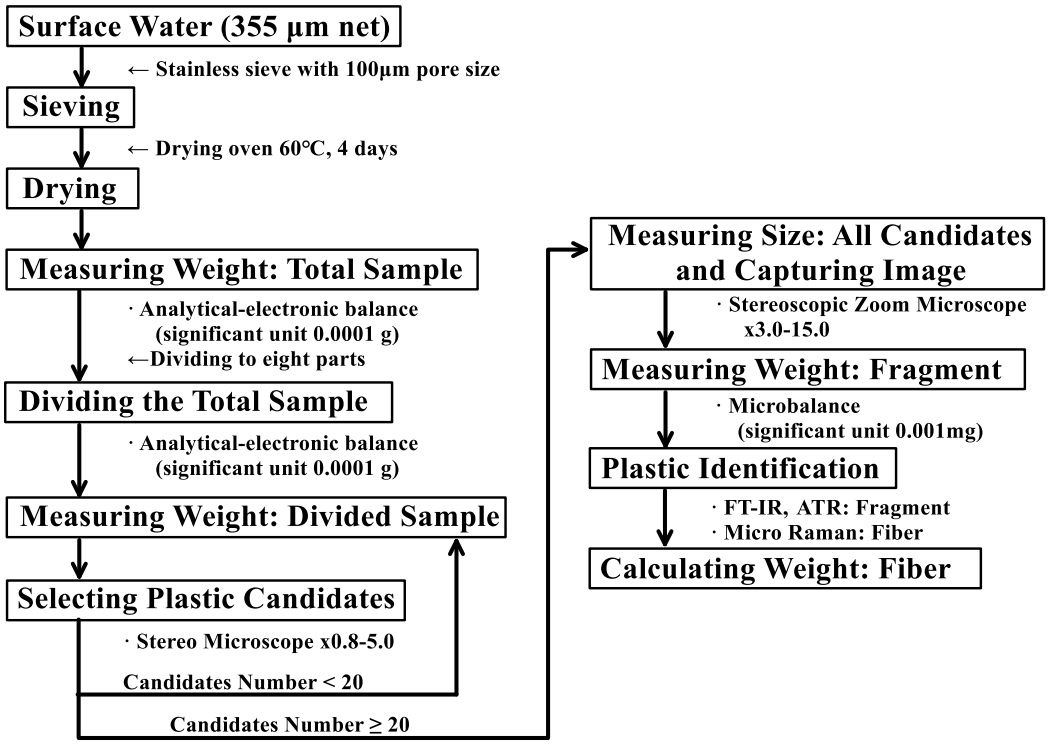

Figure 10. Flowchart of laboratory analysis for microplastics collected by the 355 µm net. The Kudo et al. (2018) method was modified and employed.

2) 355 μm mesh sample (Figure 10)

For the identification analysis of the 355 μm mesh samples, the Kudo et al. (2018) method was modified. Briefly, the sample was filtered by a 100 μm stainless sieve and transferred into a glass Petri dish containing a minimum amount of ultrapure water.

The dish with the sample was dried in a drying oven (DO-450A, AS ONE Corp., Osaka, Japan) at 60℃ for four days, after which the weight was taken using an analytical-electronic balance (significant unit 0.0001 g, AS ONE Corp., Osaka, Japan). The dried dish and sample weight was subtracted from an already known weight of the Petri dish to obtain the weight of the filtered sample. Only 1/8 of the dried sample was measured again for the first stereo microscopic observation (0.8-5.0 magnifications, SMZ1000, Nikon Corp., Tokyo, Japan).

If the plastic candidates picked were over the twenty particles in the first observation, the sizes of the candidate particles were measured using the Stereoscopic Zoom Microscope. If the candidates were found below twenty, another 1/8 of the sample was used repeatedly until over twenty candidates were obtained. The weights of fragment candidates were taken using a microbalance (significant unit 0.001 mg, MT5, Mettler-Toledo International Inc., Columbus, OH, USA). The weights of fibrous candidates were difficult to measure using the balance; therefore, they were calculated using Equations (1) and (2).

The plastic polymer was identified by the Fourier transform infrared - attenuated total reflectance (FT-IR - ATR) and Micro Raman spectrometer in fragments and fibers, respectively.

3.2.3 Plastic Polymer Identification

1) 100 μm mesh sample

Particles on the PTFE filter paper of 100 μm mesh samples were identified by the Micro FT-IR (Nicolet iN 10MX, Thermo Fisher Scientific Inc., Waltham, MA, USA) ultrafast mapping method in the transmission mode using the 4000-715 cm^{-1} infrared spectrum range, 0.1 sec collection time, 16 cm^{-1} spectral resolution, and one scan at each measurement (T. J. Park et al., 2020). The selected step size was 50 x 50 μm (Sugiura et al., 2021), and three arbitrary 5 mm square areas were analyzed in different positions on the filter paper. The plastic polymers were identified by the OMNIC Picta version 9.8.286 software (Thermo Fisher Scientific Inc., Waltham, MA, USA) using twenty-one reference spectra library and nine commercial plastic spectra, such as polypropylene (PP), polyethylene (PE), polystyrene (PS), polyamide (PA), polyurethane (PU), polyethylene terephthalate (PET), polyvinylchloride (PVC) (Figure 11a). Only spectra with >70% accuracy were regarded as detected microplastic.

2) 355 μm mesh sample

The FT-IR (Frontier, Perkin Elmer Inc., Norwalk, CT, USA) with a universal diamond ATR method was used to identify the fragment candidates in the 355 μm net samples (Scott et al., 2019). A total of 32 scans were collected across a mid-infrared (MIR) region using a wavenumber range from 4000 to 400 cm^{-1} and a spectral resolution of 4 cm^{-1}. The background line of the spectra was corrected using the Perkin Elmer Spectrum version 10.03.09.0139 software (Perkin Elmer Inc., Norwalk, CT, USA). The candidate spectra were compared with the reference spectra and commercial plastic spectra manually (Figure 11b).

The fibrous candidates in 355 μm mesh were analyzed by the Micro Raman spectroscopy (NRS-5100, JASCO Inc., Easton, MD, USA) (J. Lee et al., 2021). The fibrous candidates were put on a slide-glass and focused by objective lenses with 20 or 100 magnifications. The excitation laser was a green laser having a 532 nm wavelength, and other operation details were 500-4000 cm^{-1} detected range, 5 seconds of excitation time, and a resolution of 1.08 cm^{-1}. The fluorescence and baselines on the sample spectra were corrected by the Spectra Manager version 2.10.01 [Build 1] software (JASCO Inc., Easton, MD, USA). The sample Raman spectra were compared with commercial product spectra (Figure 11b), another study (Cho, 2007), and an online public database (https://publicspectra.com/SpectralSearch).

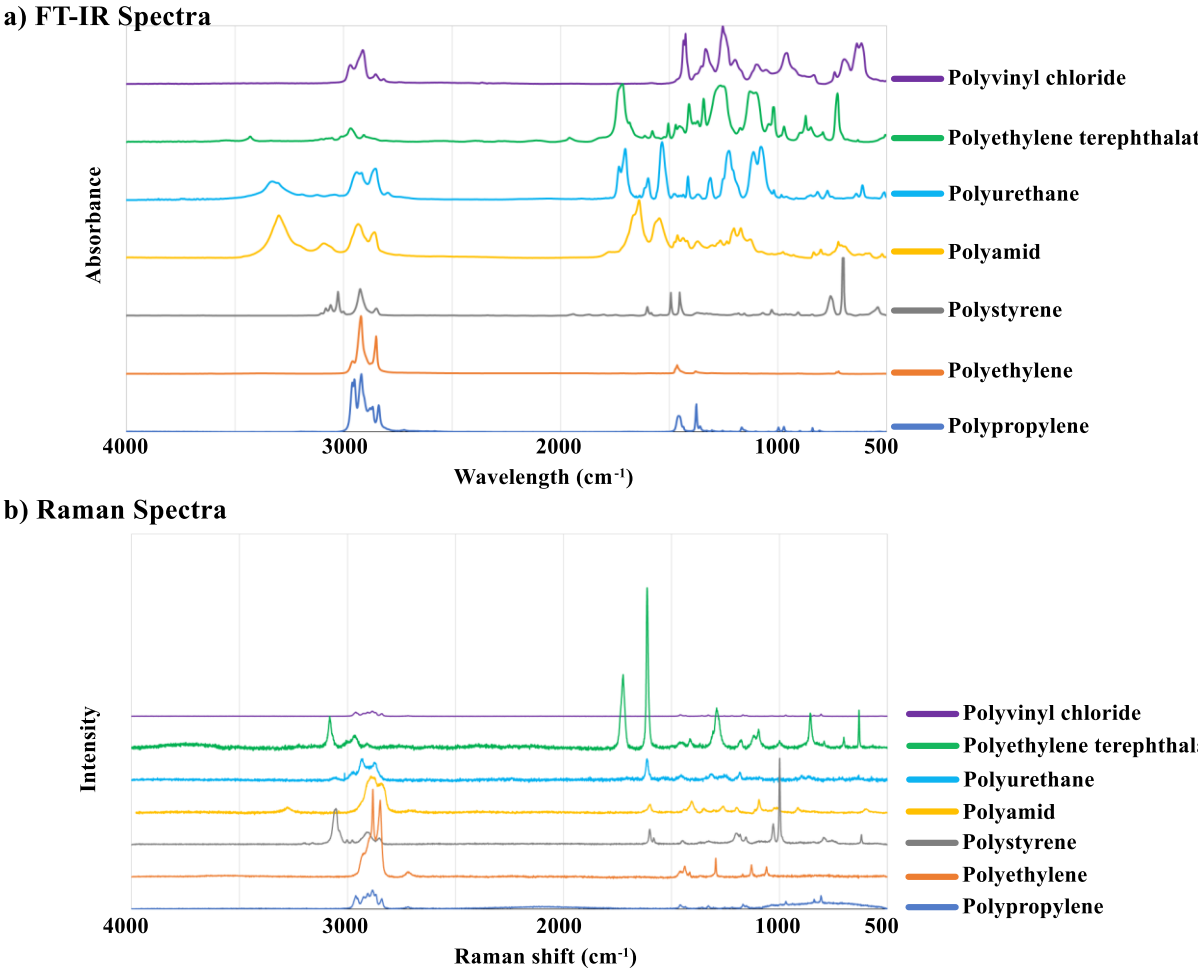

Figure 11. The spectra of seven polymer types found in the present study. a) FT-IR and b) Raman spectroscopy. a) The reference spectra in the FT-IR library, b) Commercial product spectra.

3.2.4 Size Measurement and Weight Determination

The maximum Feret diameters (FL), which are the perpendicular distances between parallel tangents touching opposite sides of profile (Walton, 1948), the minimum Feret diameters, the thickness and the surface areas of the particles (films and fragments) were measured using the Stereoscopic Zoom Microscope (3.0-15.0 magnifications, SMZ25, Nikon Corp., Tokyo, Japan) with the NIS-Elements BR version 5.30.00 [Bild1531] software (Nikon Corp., Tokyo, Japan) (Figure 12).

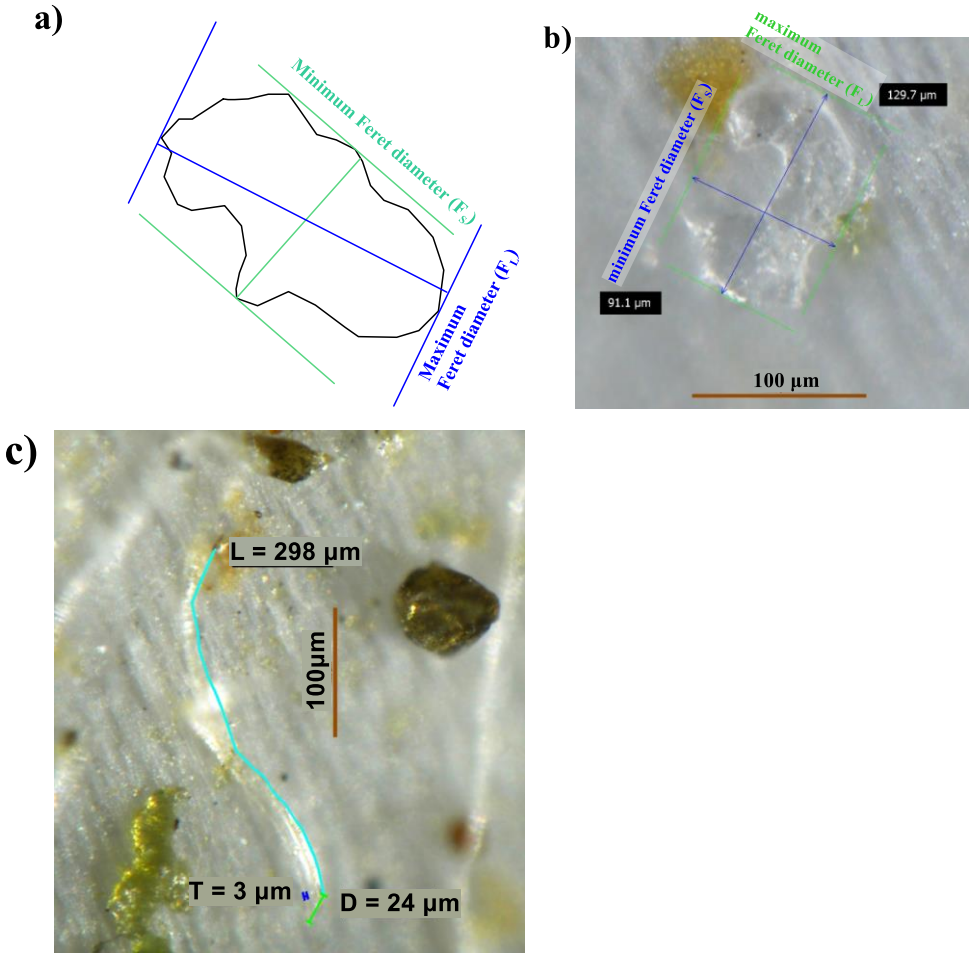

Figure 12. Employed a maximum Feret diameter (F_L) and a minimum Feret diameter (F_S) to measure particle sizes in the present study. a) was extracted from Yap et al. (2013) re-illustrated, and b) is an arbitrary sample in this study, (c) is measured the length (L), diameter (D), thickness (T) in an arbitrary rectangular microfiber using the Stereoscopic Zoom Microscope (3.0-15.0 magnifications, SMZ25, Nikon Corp., Tokyo, Japan) with the NIS-Elements BR version 5.30.00 [Bild1531] software (Nikon Corp., Tokyo, Japan). The microfiber length was measured by a continuous line drawn from end-to-end of the fiber outline using the Stereoscopic Zoom Microscope, the diameter was the shortest line between one end of the fiber, the thickness was the edge line between surface and bottom areas.

The microplastic fibers categorized into two groups: cylindrical ($W_{C\text{-}fiber}$) and rectangular ($W_{R\text{-}fiber}$) in the 100 and 355 μm specimens were estimated by Equations (1)-(2).

$$W_{C\text{-}fiber}\ (\mu g) = L \times 1/4 \times \pi \times D^2 \times \rho \times c \quad (1)$$

$$W_{R\text{-}fiber}\ (\mu g) = L \times D \times T \times \rho \times c \quad (2)$$

where L and D are the length and diameter (μm) respectively (Figure 12), T is the thickness (μm) (Figure 12), c is a constant to convert the units (10^{-6} μg/g · $cm^3/\mu m^3$), and ρ is a polymer density (g/cm^3). The density (ρ) was obtained by taking the average of the maximum and minimum values reported in previous studies; 0.91 PP, 0.94 PE, 1.07 PS, 1.04 PA, 1.20 PU, 1.41 PET, 1.37 PVC, and 1.77 g/cm^3 polyester (Hidalgo-Ruz et al., 2012; Prata et al., 2019). The weight of the fragment ($W_{Fragment}$) and film (W_{Film}) shapes in the 100 μm were determined using Equation (3), and the total weight of microplastic (W_{100}, W_{355}) was calculated using Equation (4).

$$W_{Fragment} \text{ or } W_{Film}\ (\mu g) = S \times H \times \rho \times c \quad (3)$$

$$W_{100} \text{ or } W_{355}\ (\mu g) = W_{Fragment} + W_{Film} + W_{C\text{-}fiber} + W_{R\text{-}fiber} \quad (4)$$

where S is the measured particle surface area (μm^2) by the Stereoscopic Zoom Microscope.

3.2.5 Calculation of MPs Abundances

Microplastic numbers (N_{100}, N_{355}) were counted from some parts of the total sample in the laboratory analysis processes. Therefore, microplastic numerical (C_{N100}, C_{N355}) and mass (C_{W100}, C_{W355}) abundances were corrected by Equations (5) and (6) (Corami et al., 2020; Kudo et al., 2018).

$$C_{N100}\ (particles/m^3) \text{ or } C_{W100}\ (\mu g/m^3) = N_{100} \text{ or } W_{100} \times A_{total} / A_{analyzed} \times 1/ V_{net} \quad (5)$$

$$C_{N355}\ (particles/m^3) \text{ or } C_{W355}\ (\mu g/m^3) = N_{355} \text{ or } W_{355} \times W_{total} / W_{analyzed} \times 1/V_{net} \quad (6)$$

where A_{total} and $A_{analyzed}$ are the total filtered area (227 mm^2) and the analyzed areas (75 mm^2) respectively on the PTFE filter paper in the 100 μm net sample, W_{total} and $W_{analyzed}$ are the total filtered sample weight (g-dw.) and the analyzed sample weight (g-dw.) respectively in the 355 μm net sample, and V_{net} is the filtered volume by nets (m^3).

3.2.6 Quality Assurance (QA) / Quality Control (QC)

The ultrapure water, which was slightly conductive at 18.2 Mohm-cm (Direct-Q 3UV, Merck Millipore Ltd., Bedford, MA, USA), was used for rinsing the entire experimental apparatus. Additionally, every experiment was conducted in a closed area which is separated from the general laboratory, and a 100% of cotton laboratory coat, nitrile gloves, a mask were worn.

Blank tests were repeated for the two pretreatment methods using ultrapure water. Utmost care was ensured to prevent contamination. No plastic was found in the blank experiment in the modified Kudo et al. (2018) method. However, two polyethylene fragments with sizes of 157 and 178 μm were found in the modified Sugiura & Takada (2019) method, but these numbers were fewer than the whole replicated samples. The experimental contaminations were ignored in the present chapter.

3.2.7 Literature Survey

A map of land use in the sampling area, including dams and wastewater treatment facilities, was obtained from databases on the National Land Numerical Information (NLNI) service of Japan (https://nlftp.mlit.go.jp/ksj/) and was illustrated by QGIS version 3.10.1-A Coruña (https://www.qgis.org/en/site/) (Kabir et al., 2021; Nihei et al., 2020).

Water quality parameters from 1990-2018 in the sampling area were obtained from the Water Information System database (http://www1.river.go.jp/) in the K Hydrographic observatory located near St. 1 in T River, Japan. The parameters such as pH, biological oxygen demand (BOD), suspended solids (SS), dissolved oxygen (DO), the total coliform were assessed by the Environmental Quality Standards for Conservation of the Living Environment (Rivers), Japan (MOEJ, 2021).

3.2.8 Statistical Analysis

The Shapiro-Walk normality test and Wilcoxon Signed-Rank test were employed to compare the median values, and statistical analysis was implemented by the R version 4.0.2. (2020-06-22) (https://www.r-project.org/).

3.3 Results and Discussion

3.3.1 Microplastics Collected by 100 and 355 μm Meshes

The numerical abundances (n=9) (C_{N100}, C_{N355}) in St.1-3 ranged from 3.2-38.8 (mean 14.1±10.7, median 13.9) in C_{N100}, 0.0-12.4 (mean 1.9±4.0, median 0.4) particles/m^3 in C_{N355}, and mass abundances (C_{W100}, C_{W355}) ranged from 0.2-101.6 (mean 25.1±41.5, median 6.0) in C_{W100}, 0.0-26.7 (mean 3.8±8.7, median 1.0) μg/m^3 in C_{W355} (Table 1). The sum of numerical MPs abundances (n=9), *i.e.*, regardless of sampling stations, was 127.3 and 16.8 particles/m^3, respectively in C_{N100} and C_{N355}.

Table 2. The result of numerical and mass microplastics abundances collected by 100 and 355 μm meshes in T River, Japan

Sampling station	Replication	100 μm mesh				355 μm mesh			
		Filtering time	Filtered water	Numerical	Mass	Filtering time	Filtered water	Numerical	Mass
		seconds	m^3	particles/m^3	μg/m^3	seconds	m^3	particles/m^3	μg/m^3
St.1	1st	301	1.8	14.9	11.1	601	7.7	1.1	1.5
	2nd	301	2.8	4.3	0.9	588	8.9	0.0	0.0
	3rd	301	2.3	38.8	94.5	421	6.9	0.0	0.0
	Median	-	-	14.9	11.1	-	-	0.0	0.0
	Mean±SD	301±0	2.3±0.5	19.4±17.7	35.5±51.3	537±100	7.8±1.0	0.4±0.6	0.5±0.9
St.2	1st	302	3.2	5.6	0.5	601	5.5	1.2	1.4
	2nd	301	2.4	13.9	5.3	601	5.4	12.4	26.7
	3rd	301	2.8	18.1	101.6	601	7.5	1.7	3.8
	Median	-	-	13.9	5.3	-	-	1.7	3.8
	Mean±SD	301±1	2.8±0.4	12.5±6.4	35.8±57.0	601±0	6.1±1.2	5.1±6.3	10.6±14.0
St.3	1st	422	4.2	15.9	6.2	602	10.3	0.0	0.0
	2nd	602	4.9	12.5	6.0	601	8.1	0.4	1.0
	3rd	601	4.7	3.2	0.2	601	8.8	0.0	0.0
	Median	-	-	12.5	6.0	-	-	0.0	0.0
	Mean±SD	542±104	4.6±0.3	10.5±6.5	4.2±3.4	601±1	9.0±1.1	0.1±0.3	0.3±0.6
St.1-St.3	Median	-	-	13.9	6.0	-	-	0.4	1.0
	Mean±SD	381±131	3.2±1.2	14.1±10.7	25.1±41.5	580±60	7.7±1.5	1.9±4.0	3.8±8.7

3.3.2 Microplastics Numerical and Mass Abundances

Statistically significant differences were found in the numerical (p=0.001<0.05) and mass (p=0.062<0.1) abundances (Figure 13). The median values of both abundances in the 100 µm mesh (C_{N100}, C_{W100}) were approximately 35 and 6 times higher than C_{N355} and C_{W355}, respectively. Notably, Poulain et al. (2019) demonstrated that small MPs (25-1000 µm) was the main contributor to plastic mass balance in the ocean. This indicated that several studies utilized an approximately 355 µm size of the mesh were underestimated the significant microplastic mass balances (Figure 13b).

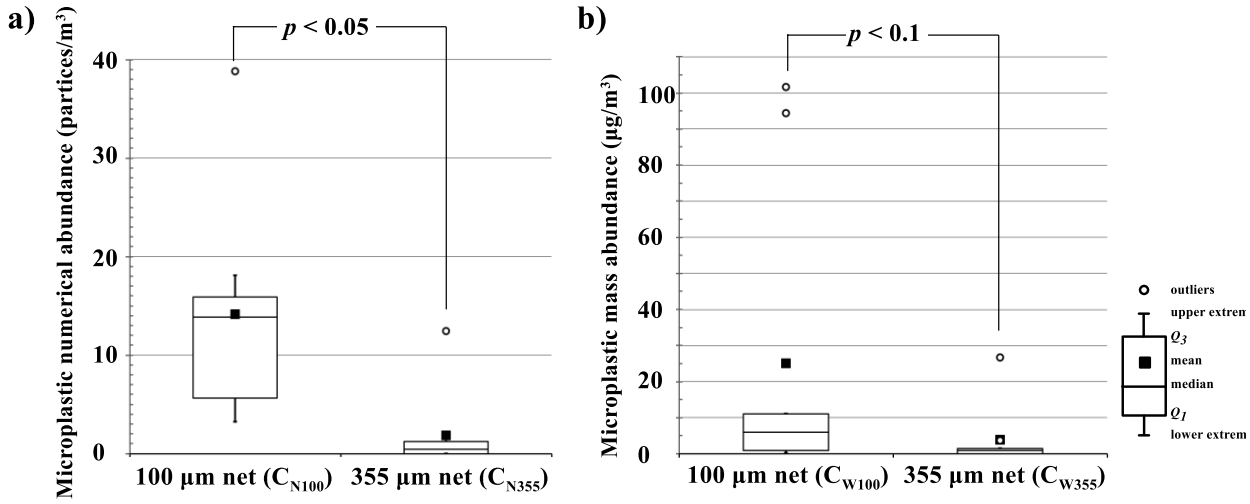

Figure 13. The boxplot compared microplastics; a) numerical and b) mass abundances between the 100 (C_{N100}, C_{W100}) and 355 µm (C_{W355}, C_{W355}) meshes (n=9).

3.3.3 Microplastics Sizes

Although the size distribution in the microfibers was widely dispersive, the film shape showed a relatively smaller size (*i.e.*, below 500 µm) in 100 µm mesh (Figure 14). Contrary to this, fragment abundance was increased toward smaller sizes, and the 100-150 µm group was the predominant size (Figure 14a). However, there was no film shape in the 355 µm mesh, and the microfibers were dominant with 800-5000 µm size. The most frequent size group of fiber was 2000-3000 µm, but the specific distribution was not shown (Figure 14b).

Generally, large plastics degrade into smaller particles, and the number of small plastic fragments increase as their size decreases (Andrady, 2011; GESAMP, 2019; Isobe et al., 2015). The 100 µm mesh revealed the specific fragment size distribution reflecting the tendency, unlike the 355 µm mesh (Figure 13a). However, it was shown that, though film and fragment plastics above 350 µm were collected in the 100 µm mesh, only one fragment was found in the 355 µm mesh.

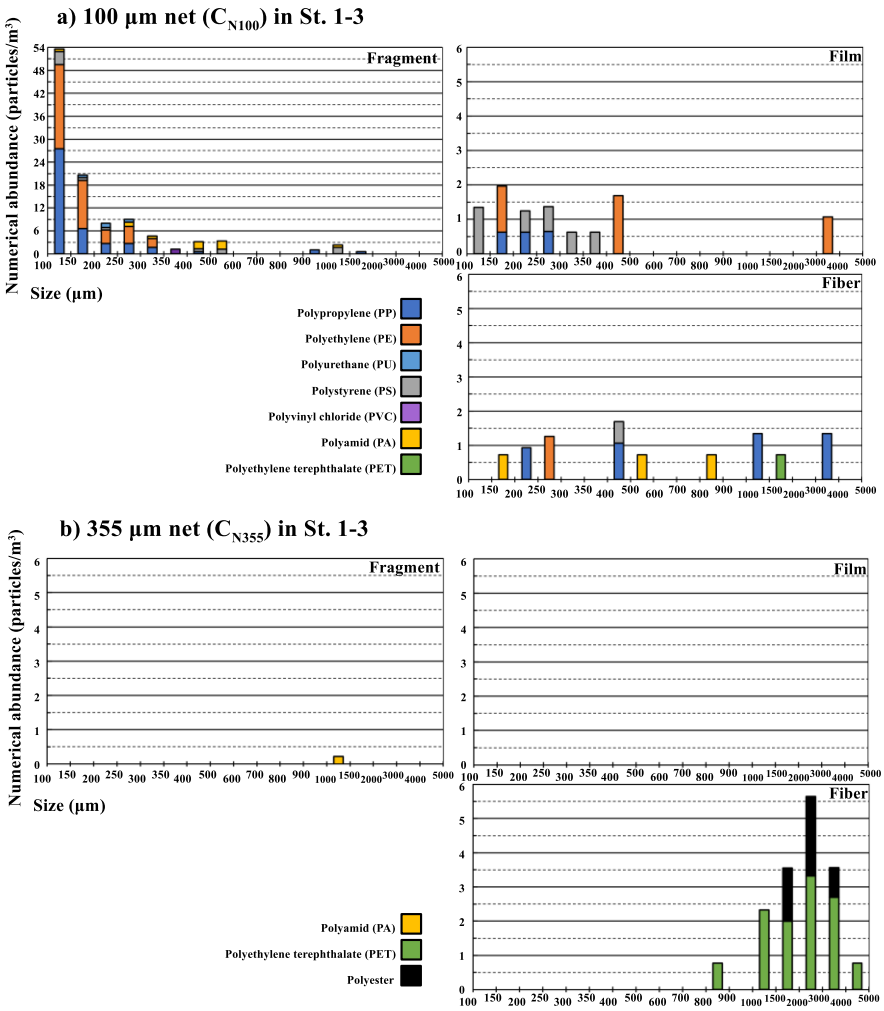

Figure 14. The shape, size, and the polymer type distributions of numerical abundances (C_{N100}, C_{N355}) collected by; a) 100 and b) 355 µm nets in entire sampling stations.

Several researchers have suggested that the particles above the mesh size may pass the mesh due to changed orientation by rotating in the water column (Abeynayaka et al., 2020; Michida et al., 2019). In the 355 μm mesh, the maximum length in which particles can pass is extended to approximately 502 μm by the Pythagorean Theorem (Figure 15).

Additionally, Tokai et al. (2021) emphasized that the minimum Feret diameter (F_S) may be a principal factor for expressing the probability that a plastic fragment can pass through the mesh. If the F_S is shorter than the 502 μm, the particles were likely to pass the net on the cater-corner orientation via rotation (Figure 15 Case 4) (Abeynayaka et al., 2020; Michida et al., 2019; Tokai et al., 2021).

This possibility can also apply to the 100 μm mesh, and the extended mesh is approximately 141 μm (Figure 15). Thus, considering the underestimated MPs in mesh sizes, two factors (particles rotation and F_S) should be conferred simultaneously (Abeynayaka et al., 2020; Michida et al., 2019; Tokai et al., 2021).

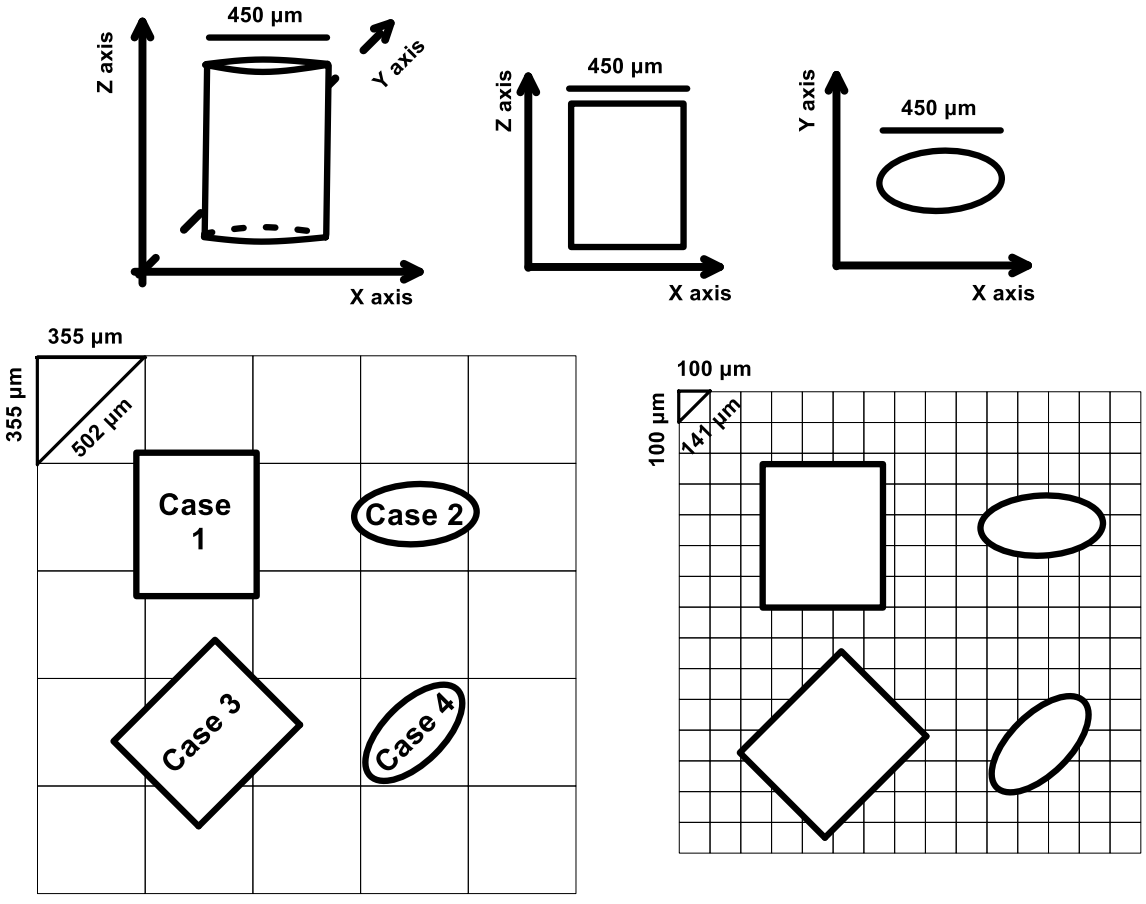

Figure 15. The maximum length on the diagonal that appeared in 100 and 355 μm nets. A virtual oval cylinder particle can be collected on the 355 μm net (Case 1-3) and where it is likely to pass through the 355 μm net (Case 4). The figure was extracted from Abeynayaka et al. (2020), supplemented and re-illustrated.

Cumulative probabilities of F_L and F_S for the fragment and film in 100 μm mesh are enumerated to presume how many MPs were underestimated in both meshes (Figure 16). The fibers are not illustrated in Figure 16 because they are selectively collected in the two meshes (Barrows et al., 2017; Tokai et al., 2021). When the extended maximum lengths were considered, *i.e.*, the diagonal lengths, the probabilities in F_S were respectively 67.3% and 96.7% in 141 and 502 μm. These probabilities significantly increased from F_L, which showed 8.5% and 86.7% in 100 and 355 μm, respectively.

Notably, it was suggested that Figure 16 was divided into three parts based on the extended length (141 and 502 μm) and Fs (Abeynayaka et al., 2020; Michida et al., 2019; Tokai et al., 2021). Theoretically, Part 1 and Part 2 indicated the collectable fragment and film using 355 and 100 μm meshes, respectively. Part 3 implied the potential fragment and film that are likely to be overlooked in 100 μm mesh in theory. These three parts suggest the following: 1) 96.7% of the fragment and film were underestimated in the 355 μm mesh than 100 μm mesh, 2) 67.3% of the fragment and film, which were likely to pass the 100 μm mesh, might be potentially overlooked.

These arguments reflect the microplastic underestimation in a large mesh (Lindeque et al., 2020; Tokai et al., 2021) and the unavoidable overlooking of MPs in the Volume-reduced method (Barrows et al., 2017; Hidalgo-Ruz et al., 2012; J. H. Kang et al., 2015).

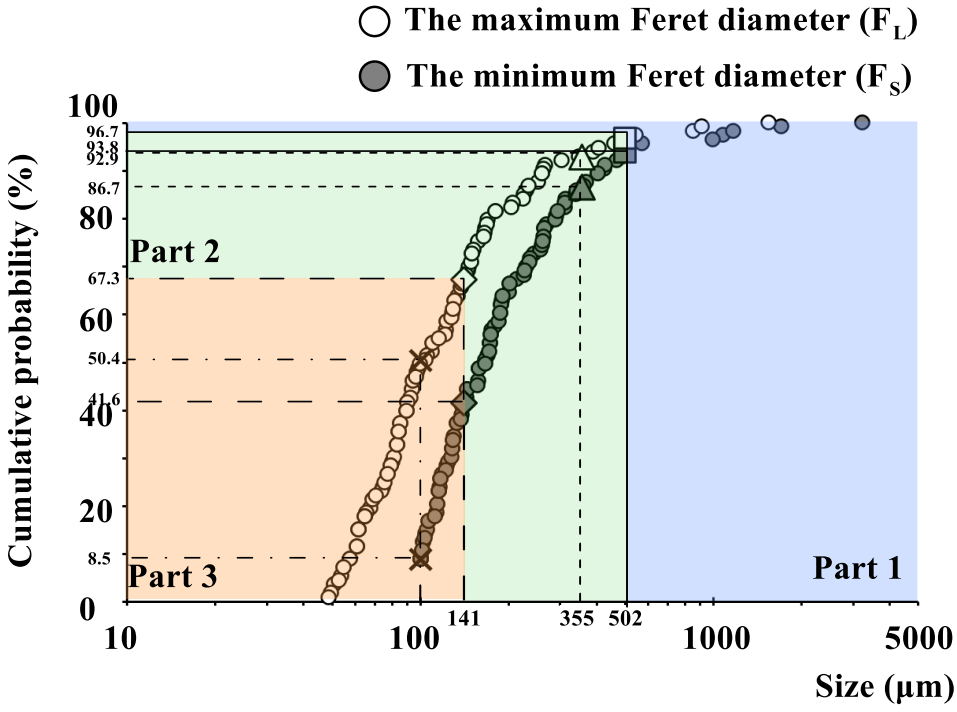

Figure 16. The cumulative probability plot of particles (film and fragment) size using the maximum (F_L) and minimum (F_S) Feret diameter in the 100 μm net. The x-symbols (×) refer to the cumulative probability at 100, the diamonds (◇) at 141, the triangles (△) at 355, and the squares (□) at 502 μm.

3.3.4 Microplastics Shape Types

The shape compositions were 84.8% fragment, 7.8% film, 7.4% fiber in the 100 μm mesh, 98.7% fiber, 1.3% fragment in the 355 μm mesh (Figure 17). The represented MPs in this present chapter are photographed in Figure 18.

The primary shapes were the fragment in the 100 μm mesh, the fiber in the 355 μm mesh. The film (Figures 18a, b), fiber (Figures 18c-e), and fragment (Figures 18f-o) types above the 100 μm mesh were collected, but pellet and foam shape were not discovered in both meshes. The Figure 18j particle could be considered as a pellet in a 2-dimensional image, but it was shaped like a concave bowl in the Stereoscopic Zoom Microscope observation. This tendency in which the pellet type was not found is consistent with what was previously reported in the Japanese inland bay, located in the same prefecture with T River in this study (Isobe, 2016).

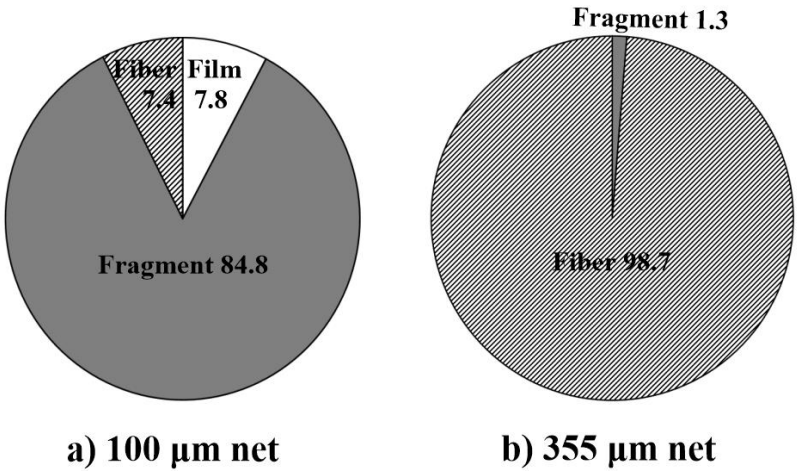

Figure 17. Microplastic shape compositions collected by; a) 100 and b) 355 μm meshes.

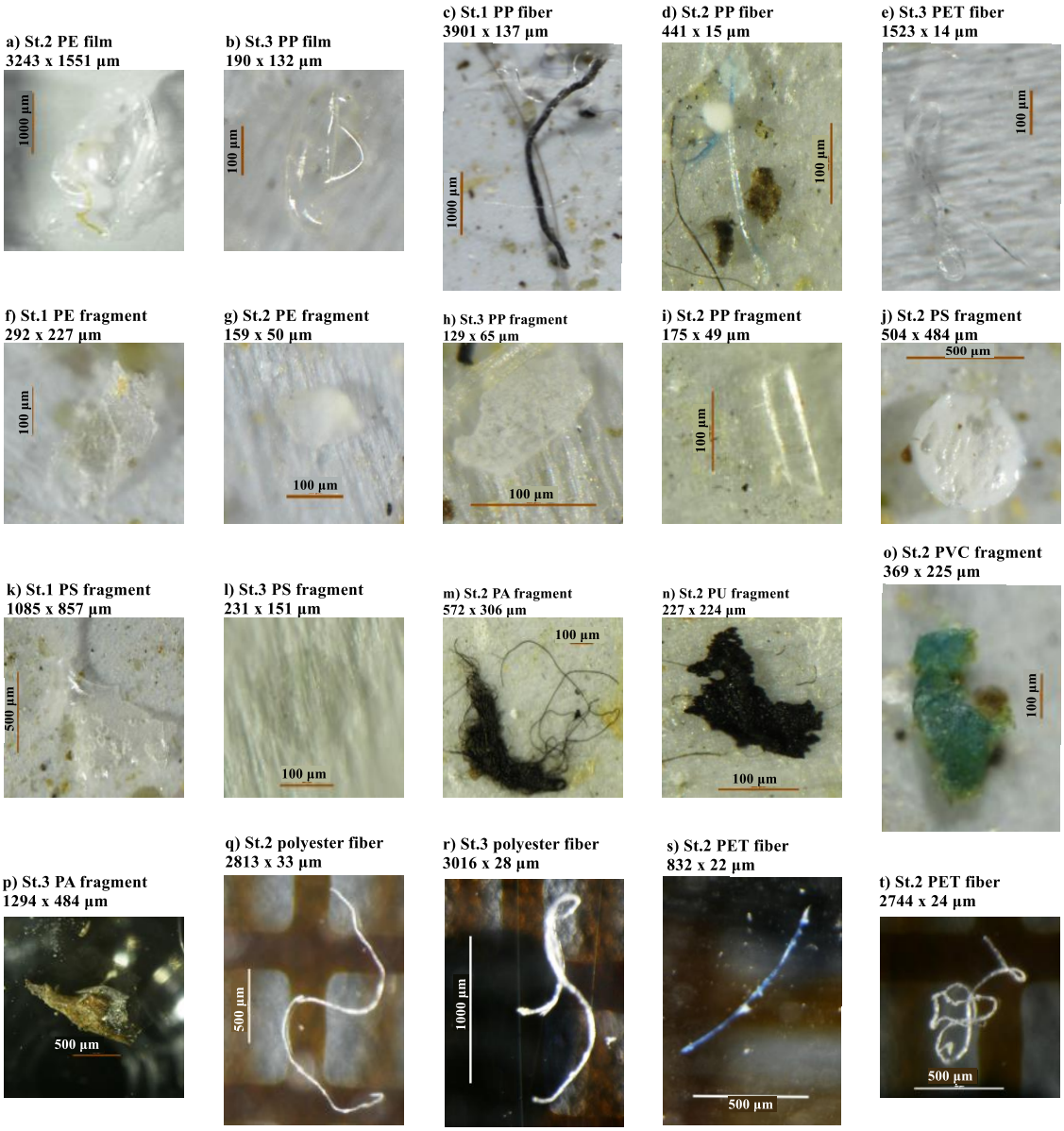

Figure 18. The photographed representative microplastics by the Stereoscopic Zoom Microscope (3.0-15.0x) in the 100 (a-o) and 355 µm (p-t) meshes.

The absence of fragment and film in the 355 μm mesh was stated above, but the microfiber proportions were remarkably different between both meshes. However, the numerical microfiber abundances (Figure 19a) ranged from 0.0-2.9 (mean 1.1±1.1, median 0.9) and 0.0-12.4 (mean 1.8±4.0, median 0.2) particles/m^3 in 100 μm and 355 μm meshes, respectively, and there was no statistically significant difference (p=0.716). This tendency was consistent with mass microfiber abundances (p=0.649), which were 0.0-85.6 (mean 9.6±28.5, median 0.1) in 100 μm mesh and 0.0-26.7 (mean 3.8±8.7, median 0.6) μg/m^3 in 355 μm mesh (Figure 19b).

Three orders of magnitude higher numerical microfiber abundance (>2 mm) in the Bulk water method (50 μm) than 330 μm mesh was reported (J. H. Kang et al., 2015), contrary to this, there was a report that remarkably few string-like fragment numbers were collected in 333 μm (29 particles) and 1000 μm (32 particles) meshes (Tokai et al., 2021). Notably, Tokai et al. (2021) suggested that the string-like fragment have different mesh selectivity from other shaped fragments. This may result from the

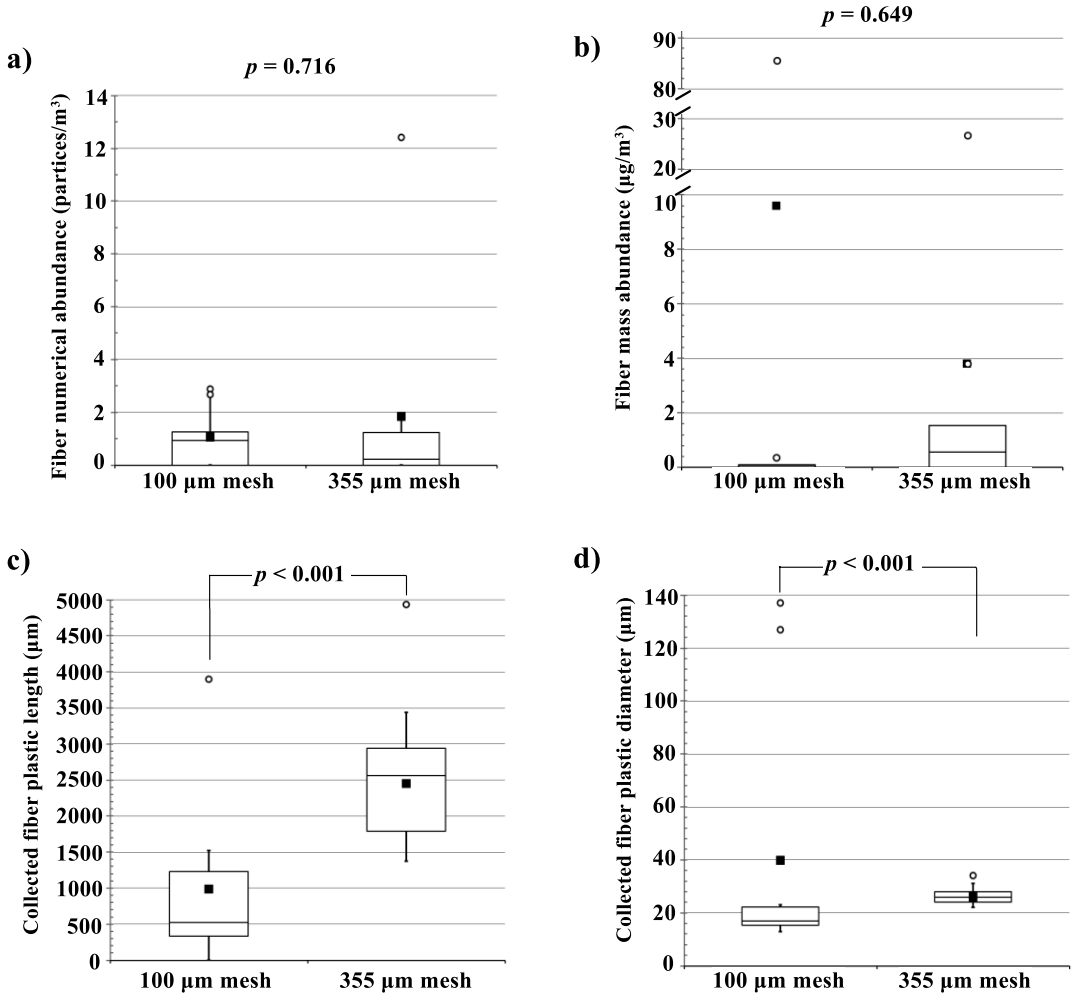

Figure 19. Numerical (a) and mass (b) microfibers abundances in 100 and 355 μm meshes and the lengths (c) and diameters (d) of the microfibers.

morphological characteristics, which can become more easily entangled or bent than other plastic shapes (Barrows et al., 2017).

On the other hand, Lindeque et al. (2020) presented mutually statistical significances on fiber length and diameter in 100, 333, 500 μm meshes. In the present study, fiber length (median 523 and 2562 μm, p=0.0004<0.001) and diameter (median 17 and 26 μm, p=0.03<0.05) in 100 μm mesh were statistically significantly shorter than 355 μm mesh (Figures 19c, d). Dris et al. (2018) stated that the small volume (compared with large mesh) and clogging problem might induce fewer microfiber numbers in the short mesh. The filtered water volumes were 3.2±1.1 and 7.8±1.7 m^3 in 100 and 355 μm meshes, respectively, and the times, which filter the one cubic meter (m^3) of water, were 120±22 and 78±20 seconds in 100 and 355 μm meshes, respectively. Indeed, numerous particles, which were shorter than the mesh size, were found in both meshes, indicating that clogging occurred in both meshes (Treilles et al., 2021). Notably, clogging is a critical factor emphasized in many studies (Dris et al., 2018; Kataoka et al., 2019; Michida et al., 2019; Ryan et al., 2020; Tokai et al., 2021).

Discussions of the significantly different fiber lengths in both meshes are limited even in the previous reports (Barrows et al., 2017; Lindeque et al., 2020), but we assume the one hypothesis in which fiber lengths are affected by clogging. This hypothesis is based on the possibility of capturing small particles when there adhere to the mesh itself and fail to pass through the holes (Covernton et al., 2019). When moderate clogging that did not generate un-filtering water occurs, the relatively large MPs that do not adhere to the mesh may be overflowed by turbulence in the mesh net. However, this hypothesis should be inquired the validity in the future.

Meanwhile, Lindeque et al. (2020) stated for the statistically significant difference that sampling with a smaller mesh effectively provides an indication of the microplastic quantity and estimating MPs abundances in which small marine organisms are bioavailable. From this perspective (Barrows et al., 2017; Lindeque et al., 2020), though fibers were selectively collected in both meshes (Barrows et al., 2017; Tokai et al., 2021), the 100 μm mesh was regarded as a more valid mesh to represent the MPs shapes, including fragment and film distributions mentioned earlier.

3.3.5 Microplastics Polymer Types

Seven polymers were detected in 100 µm mesh, and major polymers were 39.5% PE, 39.4% PP, 10.3% PS, followed by 7.3% PA, 2.0% PU, 1.0% PVC, 0.6% PET (Figure 20a). However, fewer polymer types were collected by 355 µm mesh than 100 µm mesh; 70.6% PET, 28.2 polyester, and 1.3% PA (Figure 20b). Some Raman sample spectra in 355 µm mesh samples were insufficient to identify the obvious PET spectrum due to the fluorescence and were categorized to the polyester.

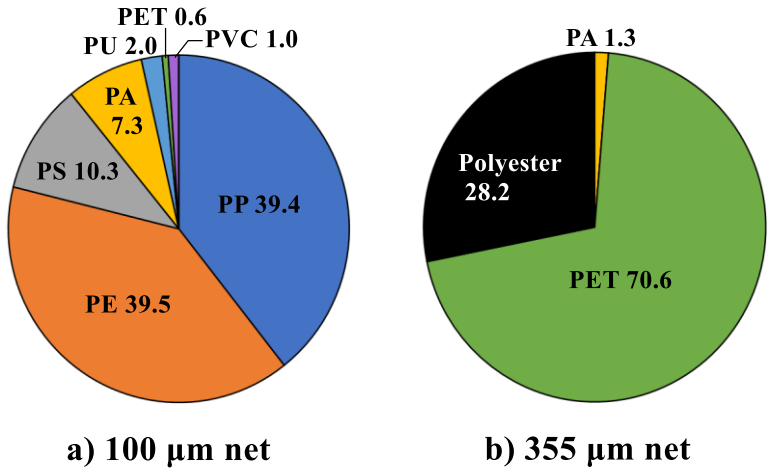

Figure 20. Microplastic polymer compositions collected by; a) 100 and b) 355 µm nets.

The 39.5 % PE was the major polymer in the 100 µm net with film (Figure 18a), fragment (Figures 18f, g) followed by 39.4% PP with film (Figure 18b), fiber (Figures 18c, d), fragment (Figures 18h, i). The two polymers are the most frequently used polymers in everyday life and are known to have a light density (Hidalgo-Ruz et al., 2012; Prata et al., 2019), generally accounting for the highest percentage of total polymer composition on the surface water in the aquatic environment (Eo et al., 2019; Rodrigues et al., 2018). The next highest composition was 10.3% PS (Figures 18j-l), widely used as food contact materials (Gelbke et al., 2019). The PA (Figure 18m) is well known for textile products worldwide. In Japan, PU (Figure 18n) is mainly used in construction, vehicles, and electronics (Furukawa, 2018), and PVC (Figure 18o) is also employed in pipes, cable sheaths, and in construction (Mitsumata & Hashimoto, 2019).

New virgin PET products can be produced from used PET products by re-polymerizing the monomer derived from chemical recycling in Japan (Semba et al., 2020). Globally, the PET and polyester fibers, which are primary polymers and

accounted for 70.6% and 28.2%, respectively in 355 µm mesh, have been used frequently in the production of polyester textile products to date (Semba et al., 2020) and can be originated from lifestyle microplastics, particularly washing machines (Corami et al., 2020; Sillanpaa & Sainio, 2017).

On the other hand, this river plays a vital role as a spawning ground for Ayu (*P. altivelis*). The fish is an important species in this river basin for food resources, leisure behaviors, and tourism accompanying economic benefits. However, the primary polymer PP and PE types are very susceptible to photo-oxidation resulting in physical and chemical changes (Rodrigues et al., 2018). The photolysis will degrade these polymers until becoming nano plastics which has high ingestion potential (GESAMP, 2019). Therefore, understanding the MPs distribution with the potentiality for bioaccumulation in the fish should be supplemented. However, the potentiality was not represented in the 355 µm mesh, which covered only PA, PET, and polyester.

Apart from the fiber, polymer composition with the fragment and film collected by 100 µm mesh is illustrated into three parts based on F_S (Figure 21). Part 1 and Part 2 accounted for 89-100% of the total composition in entire polymer types. It implied that 355 µm mesh might be insufficient to capture the six polymer types of the fragment and film in the particle size aspect. Polymer type is a crucial parameter to trace microplastic origins and estimate potentials for bioaccumulation, and 355 µm mesh was challenging to determine the origins and the potentials of microplastics bioaccumulation (Lindeque et al., 2020).

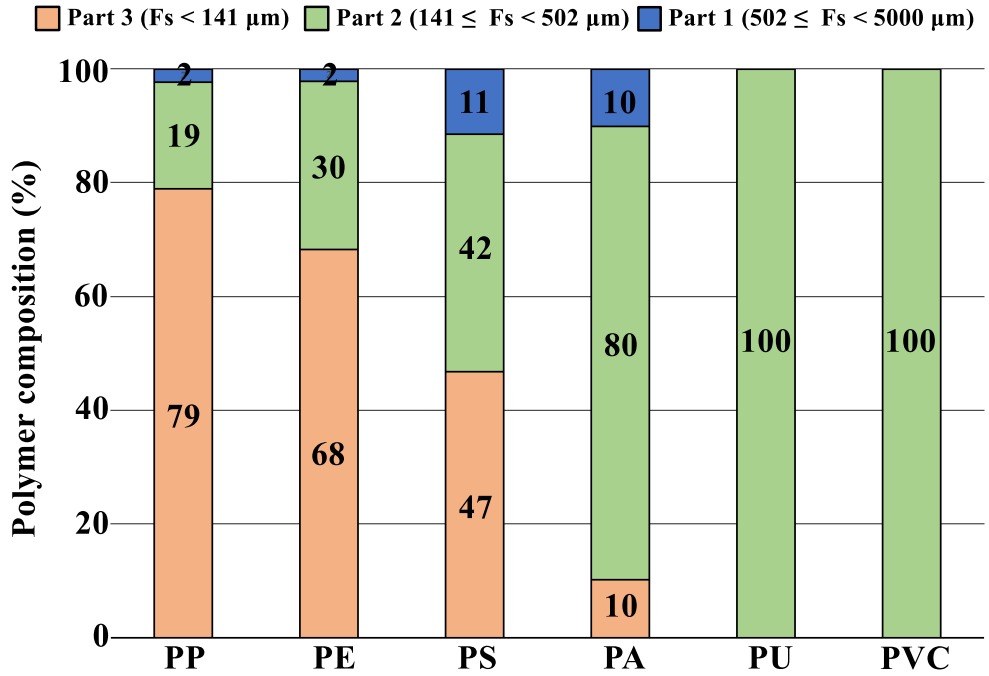

Figure 21. Six polymer compositions, which were detected from fragment and shape types in 100 µm mesh, were categorized by three Parts based on the minimum Feret diameter (F_S).

3.4 Summary

The present chapter determined the differences of detailed MPs distributions in the surface freshwater collected by 100 and 355 μm meshes in numerical/mass abundances and microplastic features (size, shape, polymer type). Apart from the numerical abundance that is naturally increased, the median value of mass abundance in 100 μm mesh was six times higher than 355 μm mesh. The cumulative probabilities for the minimum Feret diameter of particles were divided into three parts, indicating 96% and 67.3% of potential underestimation in the 355 and 100 μm meshes, respectively. Additionally, 355 μm mesh was challenging to trace microplastic origins and estimate bioaccumulation potentialities in the size, shape, and polymer types. In this chapter, 100 μm net revealed more specific distributions than 355 μm in the pristine Japanese freshwater environment.

CHAPTER 4 (CASE2)

The Microplastic Distributions in the Surface Freshwater Collected by the Bulk Water Sampling Method in the Citarum River Basin, West Java, Indonesia

Citation:
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4.1 Introduction

The importance and problems in the Study on MPs in the freshwater environment were emphasized in Chapter 2, and Chapter 3 evaluated the validity of the 100 and 355 μm mesh sampling MPs in the pristine freshwater environment.

However, the Fine Microplastics (FMPs) >20 μm in the freshwater are emphasized recently (Kameda et al., 2021), and this indicates that previous studies using large mesh to estimate the MPs emission through freshwater are likely to remarkably underestimate the emission (Kabir et al., 2021; Kataoka et al., 2019; Nihei et al., 2020).

Moreover, underestimating tiny MPs is indispensable in the Volume-reduced Method (Hidalgo-Ruz et al., 2012). The grabbing water may descript MPs validly in a situation in which microplastic abundances should be carefully illustrated, such as the effect of WWTPs (Kameda et al., 2021; Lahens et al., 2018).

On the other hand, the numerous mismanaged plastic waste emissions from rivers in developing countries were estimated by Lebreton et al. (2017). Notably, four rivers (Brantas, Solo, Serayu, and Progo) in Central Java, Indonesia, were ranked among the top twenty polluting rivers by the global river plastic inputs model (Lebreton et al., 2017).

This result reveals that the research on plastics pollution in freshwater is at issue in Indonesia. In Indonesia, the various research realms in the marine environment have been studied for MPs pollutions, such as seawater (Cordova & Nurhati, 2019; Syakti et al., 2018), sediments (Falahudin et al., 2019), beaches (Syakti et al., 2017). However, the studies on MPs pollutions in freshwater are limited compared to that of the marine environment (Alam et al., 2019; Lodo Pe et al., 2020; Sembiring et al., 2020).

The Citarum River Basin (CRB) is located in the western part of Java Island, Indonesia. It covers about 13,000 square kilometers through three inter-connected river basins (Boer et al., 2019). The CRB is a primary water source because it provides 80% of Jakarta surface water through the West Tarum Canal (Asian Development Bank, 2019). Additionally, millions of people rely on the CRB to support their economic activities and livelihood (Boer et al., 2019).

Nevertheless, over the past two decades, the water resources CRB have come under increased pressure due to urbanization and industrial growth, and severe water pollution (Asian Development Bank, 2019; Boer et al., 2019; Pamungkas et al., 2021). This river is evaluated as one of the most polluted rivers globally because of poor wastewater treatment systems (Honingh et al., 2020; Izza Indah Afkarina et al., 2020). Finally, environmental aggravation has reached levels that endanger public health and livelihoods (Asian Development Bank, 2019).

Researchers have reported in this perspective the water and surface sediment quality for the river environment, including humans (Chazanah et al., 2017; Fulazzaky, 2010; Sudarningsih et al., 2019). However, the studies on the MPs pollution in this river were targeted only in the partial river such as the industrial sector (Alam et al., 2019), downstream (Sembiring et al., 2020), reservoir (Ramadan & Sembiring, 2020).

These CRB characteristics, such as heavy microplastic pollution, specific pollutant sources, and less studied area, indicate optimized sampling areas to evaluate the validity of the Bulk water sampling method that reveals fewer MPs losses. Therefore, in Chapter 4, the distributions of MPs by shape, size, and polymer type in the surface water from the upper to the downstream of the entire Citarum River Basin were first determined using the FT-IR microscope.

This corresponding study (Jeong et al., *in press*) was the first study that determined the details (shape, size, polymer type) of MPs pollutions using the FT-IR microscope in entire Citarum River Basin. The results can contribute to the plastics reduction management proposal focused on the pollutant sources along the local area in Citarum River Basin, West Java, Indonesia, in the future.

4.2 Materials and method

4.2.1 Sample Collection

The sampling area and the land use of CRB, West Java, Indonesia, are shown in Figure 22a (LAPAN, 2019). Six sampling stations from the upstream; Wangisagara (St.1), Koyod (intensive industry sector, St.2), Cisirung (the central part of the Bandung city, St.3), Nanjung (the vicinity of the city, St.4) in the upper basin, Jatiluhur (the reservoir for drinking water resource, St.5), the downstream Walahar (St.6) in the lower basin, and one other sampling station, a local tributary near the vast city, Bandung (St.3-a) were used for the study. On 15-16th March 2018, sampling was conducted during the wet season for the six sampling locations (St.1-6). The Cisirung (St.3), Nanjung (St.4), and Jatiluhur (St.5) samples were collected again with the tributary (St.3-a) on 22nd-23rd January 2020. The freshwater specimens were carefully grabbed on the one sampling position using a basket at each station for only surface water. The collected samples were transported to the laboratory and were pre-treated for MPs analysis.

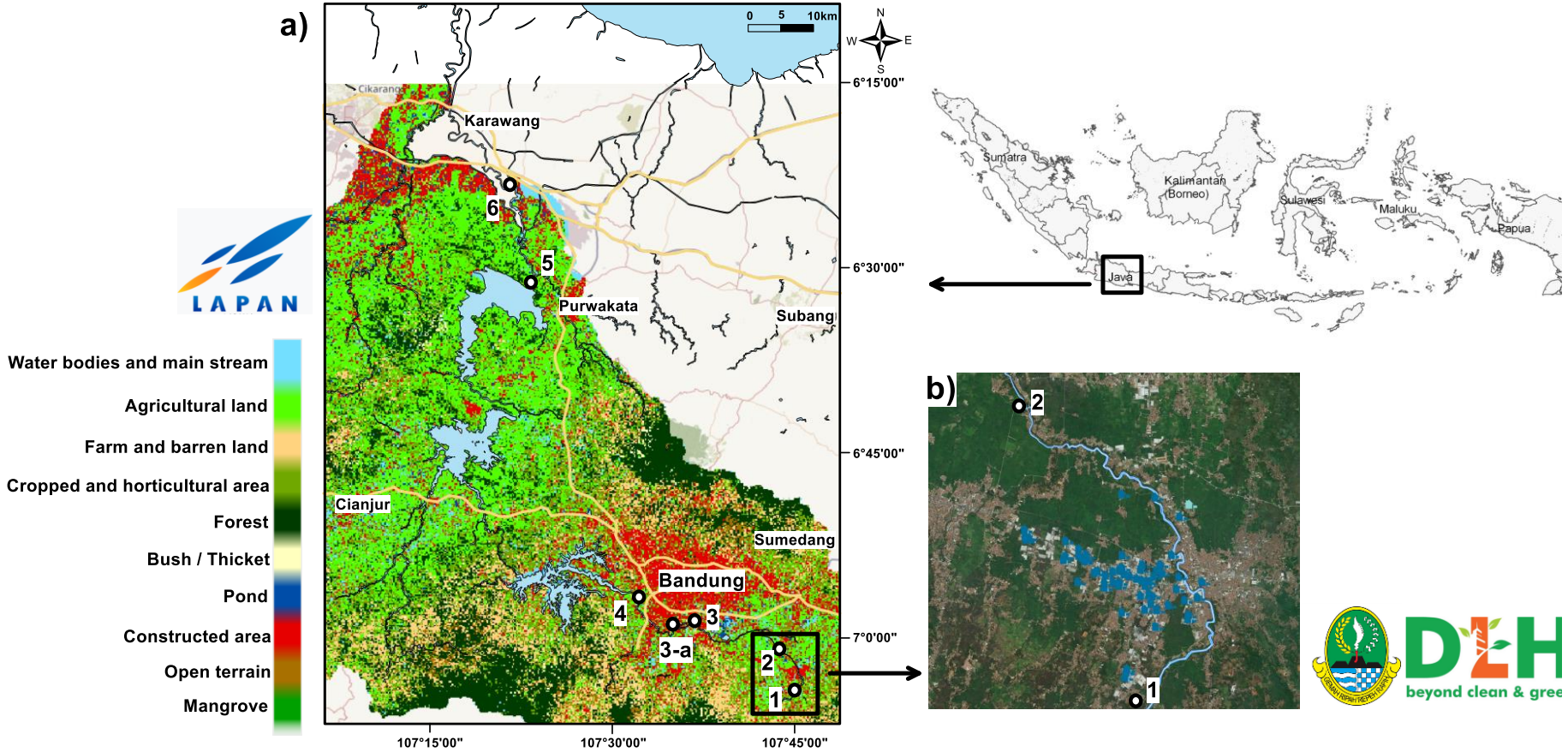

Figure 22. a) A map showing the land use of the Citraum River Basin (CRB) and the sampling stations from upstream Wangisagara (St.1) to the downstream Walahar (St.6) in the Citraum River, West Java, Indonesia, and b) the precise locations of industries as described by the blue icons between Wangisagara (St.1) and Koyod (St.2).

4.2.2 Sample Treatment

The surface water sample was pretreated to identify MPs distribution by the (Sugiura & Takada, 2019) modified partially. The freshwater samples were filtered on a Cellulose nitrate (CN) membrane filter (diameter 47 mm, pore size 8 μm, Whatman, UK) by a vacuum pump.

The filtered water volumes were 20 mL for whole sampling stations in 2018, while the amount was increased to 1000 mL in 2020. However, the tributary (St.3-a) sample in 2020 had remarkably many plastics in the surface water; hence, only 20 mL of filtrate was obtained.

The filter paper was dissolved in 1 M NaOH by stirring at 40℃, and the resultant solution was neutralized using 1 M HCl. 30% H_2O_2 was added to digest organic matters in the solution, followed by the addition of $FeSO_4 \cdot 7H_2O$ as a catalyst.

The entire solution was kept under the fluorescent for light three days until the organic digestion was completed. Subsequently, 6.7 M NaI solution (1.6 g/cm^3) was used for density separation using the glass separatory funnel.

The funnel was sufficiently shaken by hand and kept stable for more than 24 hours. The lower part of the solution was discarded, and the only surface solution was filtered using a polytetrafluoroethylene (PTFE) OMNIPORE™ membrane filter (diameter 47 mm, pore size 5 μm, Merck Millipore, Ireland). A filtration set (SIBATA SCIENTIFIC TECHNOLOGY, Japan) with a 17 mm diameter of the filtered circle area was used in this step; the total filtered area was 227 mm^2.

The filter was dried in the desiccator for one day, ensuring no atmospheric contamination. Blank was prepared by repeating the same operations using pure deionized water; the water was slightly electrically conductive 18.2 Mohm-cm (Barnstead™ SMART2PURE™, Thermo Scientific, USA).

4.2.3 Plastic Polymer Identification, Size Measurement and Calculation of MPs Abundance

The sizes and polymer type of whole particles available on the filter paper were identified by the FT-IR microscope (Nicolet™ iN 10MX™, Thermo Fisher Scientific, USA). Images via infrared mapping were detected using ultrafast mapping for more than 100 μm particles in the transmission mode: the apertures were width 100 μm, height 100 μm and the step size was 50 x 50 μm (Sugiura et al., 2021). The detailed setting for ultrafast mapping was the 4000-715 cm^{-1} of infrared spectrum range, 0.1 sec of collection time, and one scan at each measurement (T. J. Park et al., 2020).

In each specimen, a square that imaged a 5 x 5 mm area was measured three-times (75 mm^2) in arbitrarily different positions on the filter paper, and the numbers of MPs in the total inspected area (75 mm^2) were utilized to the MPs abundance (Equation 7).

$$\text{MPs abundance (particles/mL)} = N \text{ (particles)} / V \text{ (mL)} \quad (7)$$

where N is the numbers of MPs in three measured areas (75 mm^2), V is filtered water volume.

The Relative Standard Deviation (RSD%) between inspected areas in each sampling station ranged from 50-173%, except for one sample with 20%. It implied that the MPs distribution on the filter paper was biased in partial areas and that there was a possibility the three inspected areas (75 mm^2) in the present study were inspected only in the biased MPs areas. Therefore, converting from local inspected areas (75 mm^2) to the total filtered area (227 mm^2) was not employed in this study because of uncertainty due to biased MPs distribution on the filtered paper (Chae et al., 2014).

The spectra of particles were compared with the reference spectra databases to identify the polymers using OMNIC Picta version 9.8.286 software (Thermo Fisher Scientific, USA). Additionally, the twenty-one reference spectra included nine spectra collected by generally well-used commercial plastic products: polypropylene (PP), polyethylene (PE), polystyrene (PS), polyamide (PA), polyethylene terephthalate (PET), polymethyl methacrylate (PMMA), polyvinyl chloride (PVC), polyurethane resin (PU), and zein (Figure 11a). The zein is non-plastic, but it has a similar FT-IR spectrum with PA. The spectra, which had >50% of accuracy, were considered to be target substances.

4.2.4 Quality Assurance (QA) / Quality Control (QC)

In this study, only 20 mL of surface water was filtered for MPs analysis. A multiplication factor of 50 was used to obtain the final unit (particles per liter). In this case, the final MPs numbers were likely to be overestimated due to atmospheric contaminations during experiments. In one hypothesis in which the same atmospheric contaminations were repeated in the samples in each experiment, the sample results were subtracted by the blank experiment.

Firstly, whole MPs were distinguished by fiber and fragment. The fiber was defined as a minimum of a 10:1 length: diameter aspect ratio. Secondly, the divided MPs were classified into two groups by size: a) 100 (a minimum detection size)-1000 μm (the boundary between 'micrometer' and 'millimeter') and b) 1000-5000 μm (a maximum value of MPs definition). Finally, the four groups were re-categorized into several groups by polymer types of the plastics. In each group, if three classes (*i.e.*, shape, size, and polymer type) were the same, the data were subtracted by the blank. The averaged blank result and the Method Detection Limit (MDL) in this method were shown in Table 3.

For a hypothetical example, in a fictional sample, there is raw data with one PP fragment, one PE fragment in the 100-1000 μm group, and one PS fragment in the 1000-5000 μm. An imaginary blank has one PP fragment in the 100-1000 μm, three PE fragments, and five PS fibers in the 1000-5000 μm. Although the imaginary blank has MPs more than the fictional sample, the last modified data in the hypothetical example shows one PE fragment in the 100-1000 μm and one PS fragment in the 1000-5000 μm.

Table 3. The averaged blank results (n=5) and the Method Detection Limit (MDL) in this study

	Averaged Blank ± STD Fragment (particles)		**Averaged Blank ± STD Fiber (particles)**		**MDL*** **Fragment (particles)**		**MDL*** **Fiber (particles)**	
Size	**Small****	**Large*****	**Small**	**Large**	**Small**	**Large**	**Small**	**Large**
PP	1.2±0.4	0.0±0.0	0.0±0.0	0.0±0.0	1.7	0.0	0.0	0.0
PE	1.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0	0.0	0.0	0.0
Alkyd	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0	0.0	0.0	0.0
PA	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0	0.0	0.0	0.0
PS	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0	0.0	0.0	0.0
PET	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0	0.0	0.0	0.0

*MDL = blank standard deviation × 3.747 (t (4, 0.99)) **Small MPs: 100 μm ≤ x < 1000 μm

***Large MPs: 1000 μm ≤ x ≤ 5000 μm

4.2.5 Literature Survey

Literature was searched for the information on industries in the CRB via the Environmental Agency (DLH), West Java province, Indonesia (http://dlh.jabarprov.go.id/index.php/data-gis), the daily precipitation in the Bandung City through the Meteorology, Climatology, and Geophysical Agency in Indonesia (http://dataonline.bmkg.go.id/home), and the populations of the Sukabumi city and Bandung city by the World Population Review (https://worldpopulationreview.com/).

Additionally, water quality parameters of the 18 sampling stations located along the mainstream from Wangisagara to Nanjung, were obtained from the Summary of Water Quality Data in Citarum River and Its Tributaries in 2018 reported by the Environmental Agency (DLH), Bandung district, Indonesia (2018): Dissolved Oxygen (DO), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), and total coliform.

4.3 Results and Discussion

4.3.1 Microplastics Collected by the Bulk Water Sampling Method

The numbers of MPs (particles/20mL) shown in Figures 23a and 23b were six for Wangisagara (St.1), Koyod (St.2), seven for Cisirung (St.3), three for Nanjung (St.4), zero for Jatiluhur (St.5), three for Walahar (St.6) in 2018, and zero for Cisirung (St.3), Nanjung (St.4), Jatiluhur (St.5), eleven for tributary (St.3-a) in 2020. The ranges (particles/20mL) were 0-7 (mean 4.2±2.6) in 2018, 0-11 (mean 2.8±5.5) in 2020.

The figure represented differences in which the MPs numbers were depleted in Cisirung (St.3), Nanjung (St.4), in 2020 compared with that of 2018. There were no MPs in Cisirung (St.3), Nanjung (St.4) in 2020. However, the significantly highest MPs abundance of 11 particles/20mL (550 particles/L) were found in the tributary (St.3-a). No MPs were detected in Jatiluhur (St.5), while other sampling locations were exposed to MPs in the surface water in 2018.

The distribution tendency along the Citarum River in the upper basin showed that the MPs number increased from Wangisagara (St.1) to Cisirung (the center of the vast city, St.3) and became a low level at Nanjung (St.4) in 2018. In the downstream sector, the MPs quantity was increased from Jatiluhur (St.5) to Walahar (St.6).

As results of the ratio for fragments: fibers (Figure 23a), fragments were dominant in the entire sampling stations in both years; Wangisagara (St.1, 100:0), Koyod (St.2, 83:17), Cisirung (St.3, 100:0), Nanjung (St.4, 100:0), Walahar (St.6, 100:0) in 2018, the tributary (St.3-a, 91:9) in 2020. The small MPs with 100-1000 μm size were more frequent than the large ones in all sampling stations (Figure 23b). The 1000-5000 μm group was found in only Koyod (St.2) for a fiber in 2018, the tributary (St.3-a) for a fragment in 2020.

Meanwhile, the most frequently detected polymers were PP (61%), PE (17%) in both years. The proportions of polymer type were 52% PP, 16% PE, 12% PA, 8% PET, 8% PS, 4% Alkyd in 2018, 82% PP, 18% PE in 2020 (Figure 23c). Only Wangisagara (St.1) had distinct PET and alkyd resin compared with the other stations in 2018. Other notable polymer distribution patterns were the continuous presence of PA from the industrial zone (Koyod, St.2) toward Nanjung (St.4) and the PS around Bandung City; Cisirung (St.3), Nanjung (St.4) in 2018 (Figure 23c).

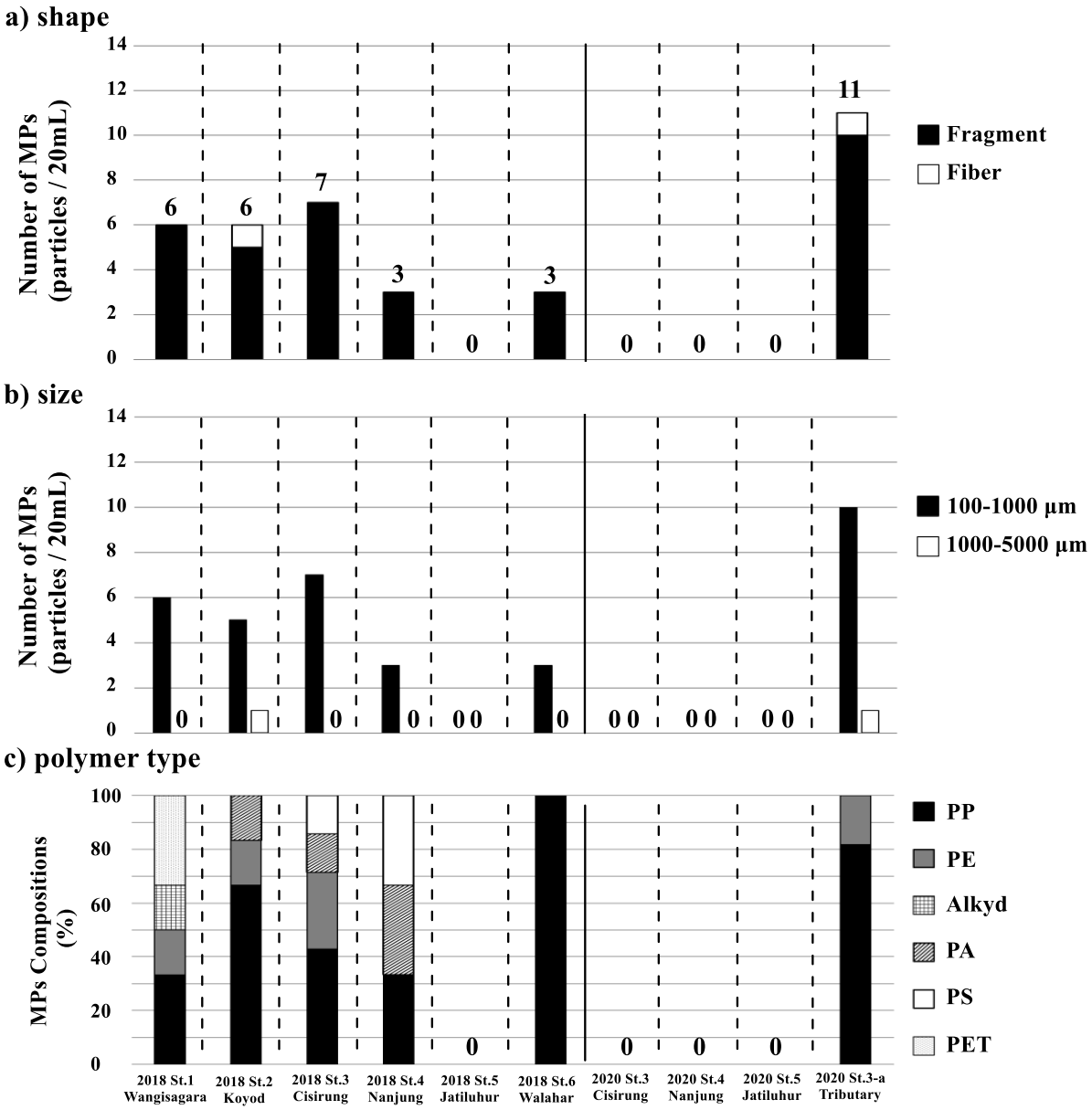

Figure 23. The numbers of microplastics (MPs) by a) shape, b) size, and c) the compositions of MPs polymer type in surface water in the Citarum River, West Java, Indonesia in 2018 and 2020.

4.3.2 The Compared Microplastics Distribution in the Citarum River Between 2018 and 2020

The MPs quantity decreased in Cisirung (St.3) and Nanjung (St.4) in 2020 compared with 2018 (Figure 23). Additionally, the exceptionally high numbers of MPs in the tributary (St.3-a) were distinguished from other locations in 2020 (Figure 23). In the tributary, much debris was discovered in the visual at sampling field (St.3-a). This implied that unknown plastic sources have been flowing continuously into the tributary (St.3-a). Notably, the numerous fragment shapes (91%) of MPs in this tributary (St.3-a) were likely derived from this accumulated debris (Figure 23a). It is urgent to identify specific pollutant sources and the water quality management in this tributary (St.3-a).

Moreover, at the same sampling station, the time-related alternations in MPs numbers have been frequently stated as the seasonal variability. For example, Eo et al. (2019) reported the seasonal variabilities of MPs in the Nakdong River Korea, 2017, and the MPs were significantly higher in the wet season than in the dry season; the wet season meant from July to September in the manuscript. Nevertheless, in this present study, both samplings were implemented in the same wet season. Thus, we focused on daily rain events, not seasonal precipitation, and a difference occurred during sampling between both years (Figure 24). The second sampling was carried out with 33.7 mm of rain on 22nd-23rd January 2020, whereas the sampling in 2018 was implemented with no precipitation.

This variability implied that an intense response to rainfall might vary the MPs numbers. The decreased MPs abundance due to rainfall is also shown in other studies. Lin et al. (2018) recorded a mean 2.7 particles/L of MPs in the Pearl Rivers China in June 2017, while Yan et al. (2019) reported approximately seven-time higher abundances in December 2017 at almost similar sampling stations as Lin et al. (2018). Thus, Yan et al. (2019) discussed the reason for extreme rain events in the summer. As for previous results, reduced MPs abundances on the mainstream (St.3, 4) in 2020 implied that MPs were diluted or were discharged to the sea by rainfall (Lin et al., 2018; Moore et al., 2011; Yan et al., 2019).

Contrary to this, the temporary rainfall rapidly increased the MPs abundance in a semiarid region (Piñon-Colin et al., 2020). Although the tributary (St.3-a) was connected to the mainstream, it showed extremely poor water exchanges with the mainstream in the sampling field. This characteristic implied that the precipitation in the sampling area might affect the accumulation of the numerous MPs abundance without discharging to the mainstream.

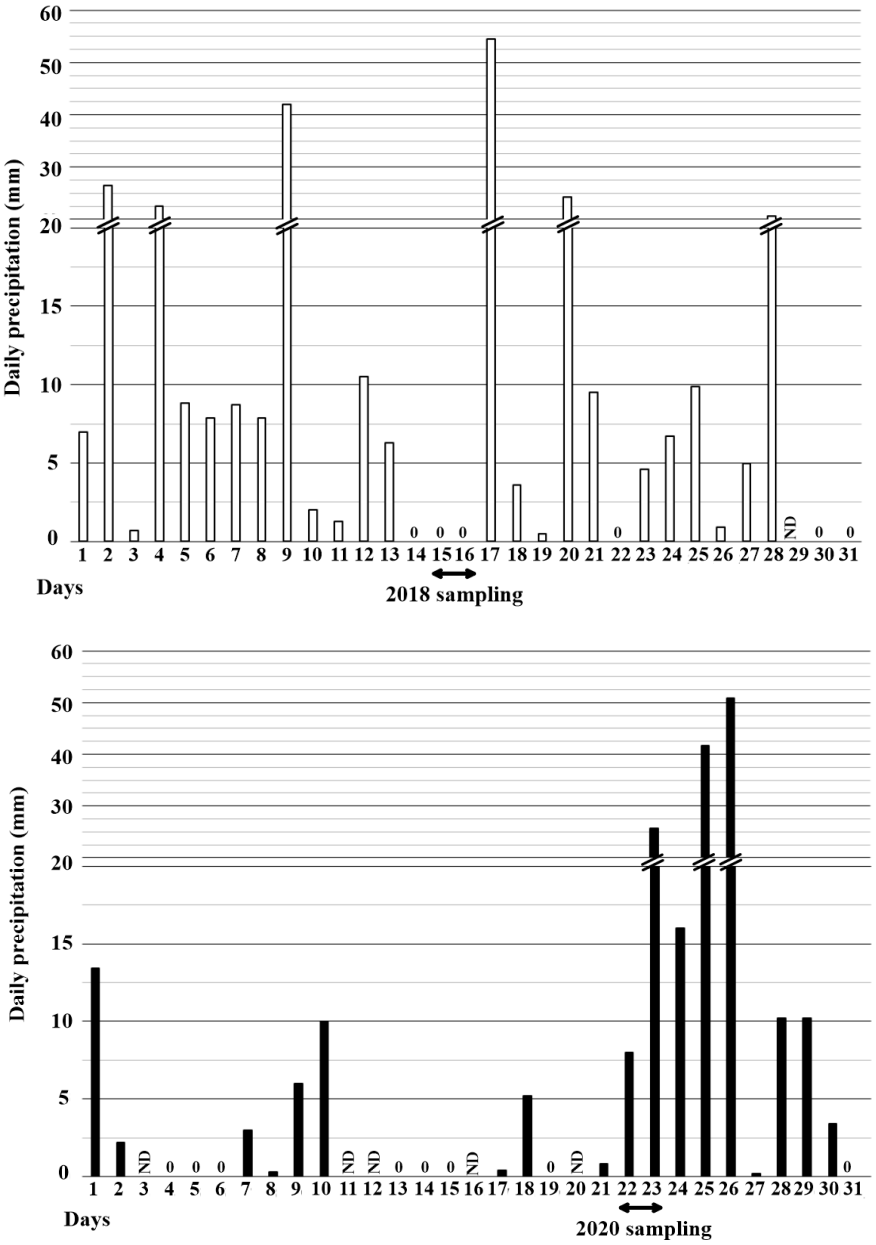

Figure 24. The daily precipitation in Bandung city, West Java, Indonesia, in March 2018 and January 2020. ND means no data on daily precipitation from the Meteorology, Climatology, and Geophysical Agency in Indonesia and the zero is no precipitation.

4.3.3 The Features of Microplastics in the Citarum River

The most common polymers detected in both years were PP, PE (Figure 23c). These polymers are well known for having a lower density than the freshwater (Prata et al., 2019), and this specific feature allows a widespread distribution in the surface water (Eo et al., 2019; Kataoka et al., 2019; Lin et al., 2018). This feature was also demonstrated in the Indonesian studies in which these two polymers predominated (Sembiring et al., 2020; Syakti et al., 2017, 2018).

Notably, the polymers are very susceptible to photo-oxidation resulting in physical and chemical changes (Rodrigues et al., 2018). Photolysis is the most efficient degradation process in the aqueous system, which continues until MPs become nano plastics, increasing potential ingestion, and bioaccumulation (Andrady, 2011). Edible fish species such as catfish (*C.gariepinus*), tilapia (*O.mossambicus*) inhabit the upper Citarum River in the wet season (Sunardi et al., 2012), and the bioaccumulation of MPs via the fishes is concerned. Several researchers already reported the small MPs existence in the digestive tract of commercial fishes in Indonesia (Hastuti et al., 2019; Priscilla & Patria, 2019; Sembiring et al., 2020).

In the Citraum River, this study covered that the 100-1000 μm groups of PP and PE were predominant. This finding demonstrated the potential threat of these small polymers via bioaccumulation (GESAMP, 2019; Rodrigues et al., 2018) (Figure 23b). Additionally, this study established the bulk water sampling, and many particles below 100 μm were found. Nevertheless, the group was excluded because it was regarded as the atmospheric contaminations during the experiment. This process implied that MPs pollutions were likely to exceed the results of this study and that analyzing the tiny plastics is necessary with the experiment that the contaminations are utterly excluded.

The studies in Indonesia on MPs pollutions in the river are initial stage, and the studies are expected to be gradually active in the foreseeable future (Alam et al., 2019; Cordova & Nurhati, 2019; Honingh et al., 2020; Izza Indah Afkarina et al., 2020; Lodo Pe et al., 2020; Pamungkas et al., 2021; Purba et al., 2019; Sembiring et al., 2020). Additionally, CRB plays a vital role in supplying water to the capital city, economic activities, and livelihood to riparian inhabitants in West Java, Indonesia (Asian Development Bank, 2019; Boer et al., 2019). This study is the first study that determined the details (shape, size, polymer type) of MPs pollutions using the FT-IR microscope in entire Citarum River Basin. However, it was indicated that this study was insufficient to express statistical differences for the specific amount of MPs due to a shortage of sample numbers in each sampling station. It is necessary to supplement the limitations by establishing a systematic study plan.

4.4 Summary

In the present study, the MPs distribution were examined in the entire Citarum River, West Java, Indonesia, in 2018 and 2020. The MPs numbers showed a decreasing pattern in 2020 compared with 2018 due to daily precipitation. Notably, in the tributary in 2020, the considerable numbers of MPs were revealed by unknown pollutant sources. The bioaccumulation via fish was concerned due to the frequently detected PP, PE in 100-1000 μm. It was concluded that the daily rain events and the different pollutant sources in each location resulted in various MPs abundance in this river.

CHAPTER 5

Proper Sampling Method Selection

Citation:
JEONG Huiho, KUSANO Teruhiko, ADDAI-ARHIN Sylvester, NUGRAHA Willy Cahya, NOVIRSA Randy, PHAN DINH Quang, SHIROSAKI Tomohiro, FUJITA Emiko, KAMEDA Yutaka, CHO Hyeon Seo, ISHIBASHI Yasuhiro, ARIZONO Koji
A Study on the Differences of Microplastics Distributions in the Surface Freshwater Collected by 100 and 355 μm nets
Environment Monitoring Contaminant & Research [revised]
JEONG Huiho, NOVIRSA Randy, NUGRAHA Willy Cahya, ADDAI-ARHIN Sylvester, PHAN DINH Quang, FUJITA Emiko, BAMBANG Wispriyono, KAMEDA Yutaka, ISHIBASHI Yasuhiro, ARIZONO Koji
The Distributions of Microplastics (MPs) in the Citarum River Basin, West Java, Indonesia
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5.1 Introduction

Chapter 5 evaluated comprehensive validities on 100, 355 μm meshes and the Bulk water sampling in five perspectives based on results in Chapters 3 and 4. The perspectives are MPs sources, abundances, features, river characteristics, and weather conditions.

Concretely, MPs abundances in the three methods were compared with other studies. This helps to understand the meaning of MPs abundance differences in macro-perspective with river water quality parameters (Kameda et al., 2021; Kataoka et al., 2019).

Moreover, verifying that the sampling methods reflect the pollution sources in the adjacent rivers (Alam et al., 2019; Kameda et al., 2021; Kataoka et al., 2019) and the general MPs distributions (particularly polymer types in the surface water) (Eo et al., 2019; Rodrigues et al., 2018) is an essential factor to evaluate the validities of the sampling methods.

On the other hand, the Citarum River had a specific sampling field that showed stagnant water flow in the tributary (St.3-a) and two samplings with different weather conditions (*i.e.*, 2018 and 2020). These river characteristics simultaneously suggest the specific advantages and disadvantages of using the Bulk water sampling method (Hidalgo-Ruz et al., 2012; Prata et al., 2019). In this regard, we employed two perspectives to discuss the advantages and disadvantages of the sampling methods.

Apart from the five perspectives, many factors affect MPs distributions, such as sampling positions in the river (Dris et al., 2018), seasonal differences (Eo et al., 2019). However, the samplings were carried out at the edge of the river where the analyzer could access the position quickly and safely in three methods, and one season analysis was conducted. Therefore, these viewpoints were not discussed in the present study.

5.2 Five Perspectives

5.2.1 Perspective 1: Microplastics Sources

1) Volume-reduced sampling methods (100 and 355 μm meshes)

The median values of microplastic abundances in each sampling station (n=3) along the river flow is presented in Figure 25. The median C_{N100} value gradually increased tendency from the upper (12.5 particles/m^3) to the lower (14.9 particles/m^3). However, the C_{N355} showed the highest in the middle area (1.7 particles/m^3), and the other stations were below 0.1 particles/m^3.

Urban and population ratios significantly affect microplastic abundances (Kataoka et al., 2019; W. Wang et al., 2017; Yan et al., 2019). The St. 2 and 3 are mainly the mountains and the forests, but St.1 is located close to the urban area (Figure 25). Moreover, tributaries between St. 3 and St. 1 combines with the mainstream and flows into the estuary through St.1. These land use and topographical features are likely to cause gradually increased microplastic abundances in 100 μm mesh.

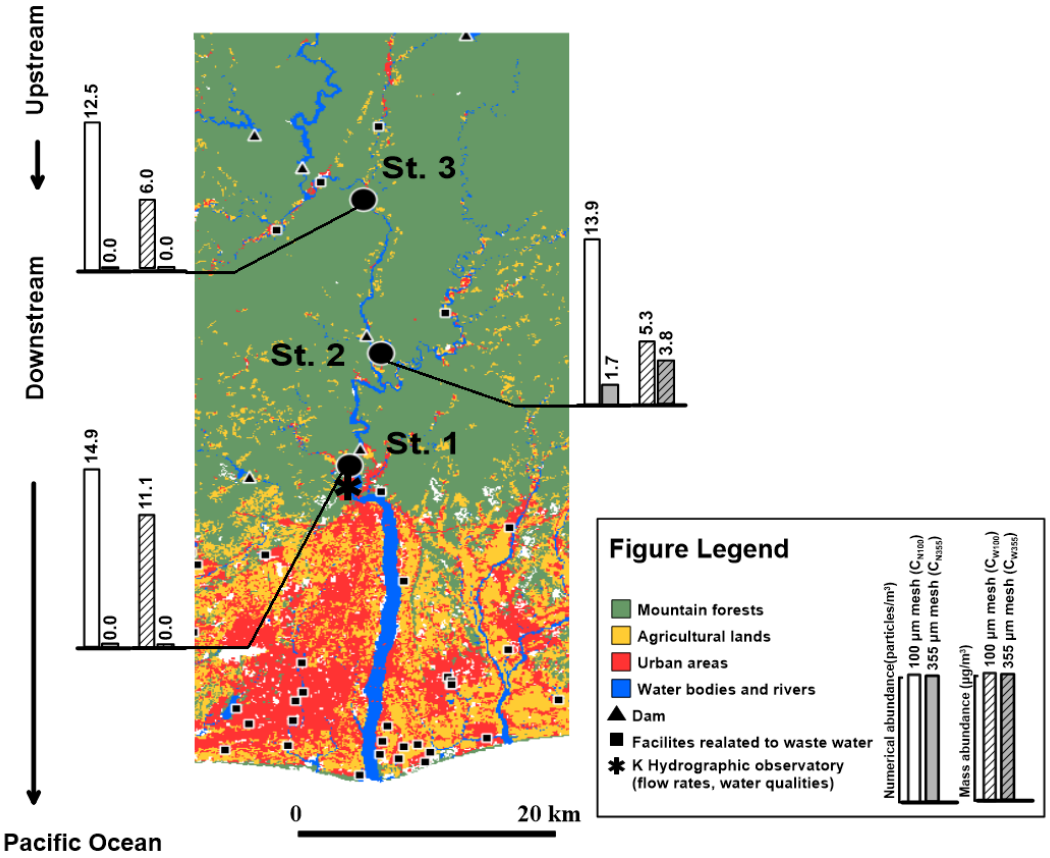

Figure 25. The microplastic numerical (CN100, CN355) and mass (CW100, CW355) abundances distributions (n=3) following the river direction.

On the other hand, the C_{N355} and C_{W355} were the highest abundances in St.2. One sampling in the three replicated samplings in St.2 had 16 microfibers and resulted in the highest abundances. In picking the plastic candidates phase with the stereomicroscope (Figure 10), a small fiber cluster was taken and separated into individuals, and the individual fibers were identified as the PET. This cluster might be derivatives of 'fabric pilling,' which commonly occurs in lifestyle textile products (Corami et al., 2020; Sillanpaa & Sainio, 2017). A PET cluster morphologically similar to this study was found in crabs (*C. dehaani*) in Osaka Bay, Japan (Nakao et al., 2019).

In Chapter 3, the two analytical methods (Kudo et al., 2018; Sugiura et al., 2021) were employed to optimize MPs distributions by two size meshes. Therefore, accidental irregulars were regarded as derivatives of the difference between two meshes. Accidentally discovered irregulars in 355 μm mesh might generate uncertain distribution characteristics.

2) Bulk water sampling method

In Citarum River, Indonesia, during this study planning phase, Wangisagara (St.1) was designated the pristine area due to the upstream sector. In the Wangisagara (St.1), the 6 particles/20mL (300 particles/L) of MPs were recorded in 2018. Although Wangisagara (St.1) is located around the forest area, many villages are situated in the upper parts of this area. In terms of MPs polymer compositions (Figure 23c), the polymer patterns of MPs in Wangisagara (St.1) were distinguished from the others in 2018; polyesters consisting of alkyd resin and PET were identified. This finding was consistent with the results of a previous study by (Alam et al., 2019).

This composition indicated that different MPs sources with other stations existed in the Wangisagara (St.1) area. The previous result on the water quality for drinking water use was improper below the industry zone (Fulazzaky, 2010), *i.e.*, from Koyod (St.2) to Nanjung (St.4) area of this study. However, the water quality was moderately suitable in the upstream area (Wangisagara, St.1), where industries are not located (Fulazzaky, 2010). Additionally, Chazanah et al. (2017) studied the surface water quality in the upper Citarum River, which involved the Wangisagara area (St.1). According to the results, concentrations of COD, Total Nitrogen (TN), and Total Phosphorous (TP) in the Wangisagara (St.1) area were not lower than other sampling stations, indicating an inflow of adjacent human activities (Chazanah et al., 2017).

The tendency, which was anticipated heavy microplastic pollution in the high population densities (Kataoka et al., 2019; W. Wang et al., 2017; Yan et al., 2019), was revealed in the Bandung city (Cisirung, St.3). However, the high MPs numbers with fibers were also shown in Koyod (St.2) (Figure 23). Sixty-six industries related to

textiles and spinning were situated near this area, and the fiber might be driven from the industries (Alam et al., 2019; Izza Indah Afkarina et al., 2020) (Figure 23b). Additionally, there is also a possibility of the cottage industry, which is difficult to specify the amount and pollutant sources.

Alam et al. (2019) examined MPs pollutions in the Ciwalengke River around this industrial area using Wanisagara station as a control, in October and November 2017. Previous results showed that MPs main shape was fiber (65%) in the surface water, but the MPs had a mean of 5.85±3.28 particles/L in the surface water (Alam et al., 2019). This was enormously lower than our study in which 6 particles/20mL (300 particles/L) were recorded in Koyod (St.2). The MPs in the water can be variably affected by various factors, particularly rainfall (Eo et al., 2019; Lin et al., 2018; Moore et al., 2011; Piñon-Colin et al., 2020; Yan et al., 2019). Referred to daily rainfall in Bandung city, there is a possibility that the three samplings (12^{th}, 26^{th} October, and 16^{th} November 2017) in the previous study (Alam et al., 2019) were implemented with rainfall events; 8-12^{th} 68 mm, 17-26^{th} 154 mm in October, and 4-16^{th} 304 mm in November 2017. These massive rainfall events might result in the difference of MPs abundances in both studies (Moore et al., 2011; Yan et al., 2019).

Additionally, heavy MPs pollutions were shown not only in the represented industry zone (Koyod, St.2) but also in prosperous urban central (Cisirung, St.3) and the vicinity of the urban (Nanjung, St.4) in 2018. This tendency also was demonstrated by past studies on the water quality in the Citarum River. The water quality in these areas was unsuitable for drinking water use, the leisure/aquatic sports use, and the aquaculture use (Fulazzaky, 2010). Besides general water quality, heavy metals in the water were also higher concentrations than other stations; Zn, As, Hg, Cu, Pb, Ni, Co, Cd (Sudarningsih et al., 2019).

In the Jatiluhur (St.5), which supplies surface water to the capital city, MPs pollution was not found in both samplings. However, in this reservoir, Ramadan & Sembiring (2020) reported a mean of 2.58×10^5 particles/km^2 of MPs due to the human and fisheries activities. Although the direct comparison is difficult due to different units, supposing the surface water depth is 0.3 meter, the MPs numbers were estimated to 0.774×10^{-4} particle/L. This number was close to zero and was significantly lower than other sampling stations in this study.

The study on the MPs presence at the downstream area of Citrarum River was reported by Sembiring et al. (2020) in the three sampling stations located in nearby Jakarta Bay, far below the Walahar (St.6) station in this study. In the previous research (Sembiring et al., 2020), MPs were considerably lower than 3 particles/20mL (150 particles/L), in Walahar (St.6) of this study. It implied that the MPs distribution along downstream, where no industrial zone or a large city was located, is expected.

Microplastic distribution collected by the Bulk water sampling method reflected the regional characteristics (Figure 26), such as human activities (Wangisagara (St.1)), the industrial sector (Koyod (St.2)), the populous city (Cisirung (St.3) and Nanjung (St.4)), and the relatively clean reservoir (Jatiluhur (St.5)). Therefore, the method was evaluated to represent pollutant sources validly in the Citarum River, Indonesia.

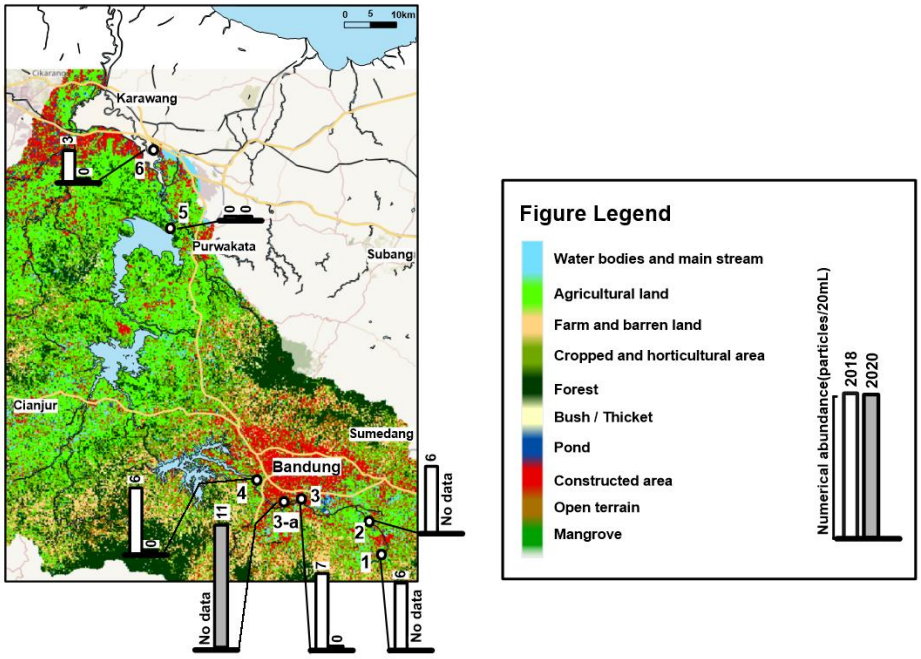

Figure 26. The numerical microplastic abundances distributions following the river direction in Citarum River, Indonesia 2018, 2020.

5.2.2 Perspective 2: Microplastic abundances

1) Volume-reduced sampling methods (100 and 355 μm meshes)

When we compared the median values, C_{N100} and C_{W100} were approximately 35 and 6 times higher than C_{N355} and C_{W355}, respectively (Figure 13). This tendency apparently reflected the natural increase due to the different mesh sizes and concurred with previous studies that reported the discriminations between the large and small meshes (Barrows et al., 2017; J. H. Kang et al., 2015; Lindeque et al., 2020; Tokai et al., 2021). However, we need to understand the extent of discriminations from a macro-perspective via comparing with other studies on MPs abundances.

Nihei et al. (2020) collected the MPs using 335 μm mesh in the seventy rivers, ninety sites in Japan to compute the annual emissions from land to the ocean. The MPs numerical (C_{N100}, C_{N355}) and mass (C_{W100}, C_{W355}) abundances in the present study are compared with the Japanese rivers in Figure 27.

When MPs abundance rankings were listed from the lower order, the numerical and mass abundances in this river were ranked 88th (C_{N100}), 10th (C_{W100}), 19th (C_{N355}), and

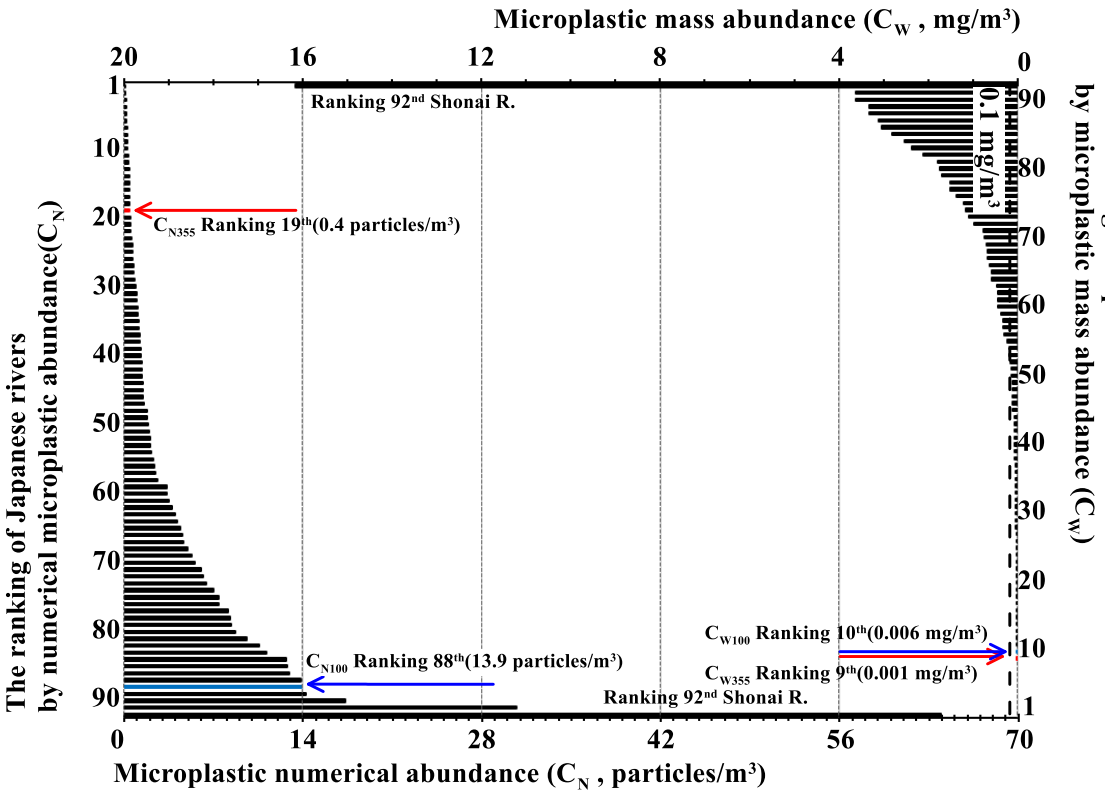

Figure 27. The ranking of microplastics numerical (C_N) and mass (C_W) abundances in this study compared with seventy rivers, ninety sites in Japan by Nihei et al. (2020). The median values in the T River were used for comparison because the mean values showed variable standard deviations in the C_{N355} and C_{W355}.

9th (C_{W355}). Apart from a natural difference in numerical abundances, the rankings of mass abundances did not represent a relatively remarkable difference (10th in C_{W100} and 9th in C_{W355}). Additionally, both mass abundances were less than 0.1 mg/m^3. The 0.1 mg/m^3 figure was the median value of the mass abundance in the previous study and was considerably lower than the top 20 rivers which had above 1.0 mg/m^3 mass abundance (Nihei et al., 2020).

The MPs numerical and mass abundances were correlated with freshwater quality factors in BOD, DO (Kataoka et al., 2019). In this regard, the water quality parameters for the past 28 years in the T River were evaluated to determine the clean river characteristics (Figure 28). As a result, the entire parameters reached the AA grade for three decades except for the total coliform. Based on the Environmental Quality Standards for Conservation of the Living Environment (Rivers), the AA grade means a first-class water supply, and the conservation of the natural environment is required (MOEJ, 2021). Although the median mass abundance values differed seventy-one times in Chapter 3 (Figure 13), Figures 27 and 28 implied that the clean river characteristics might neglect the difference.

On the other hand, Poulain et al. (2019) stated the same order of magnitude for the weight in small MPs (25-1000 μm) compared with large MPs (1000-5000 μm). Neglecting the mass difference between both mesh nets will underestimate microplastic distribution significantly in some freshwaters, which are not pristine characteristics, because of numerous mathematical magnifications.

For example, when the seventy-one times were multiplied directly, the Shonai River, which was ranked 92nd (Figure 27) as the most polluted by MPs according to Nihei et al. (2020), reached 1,146 mg/m^3 comparable with Yamaguchi prefectural four small-

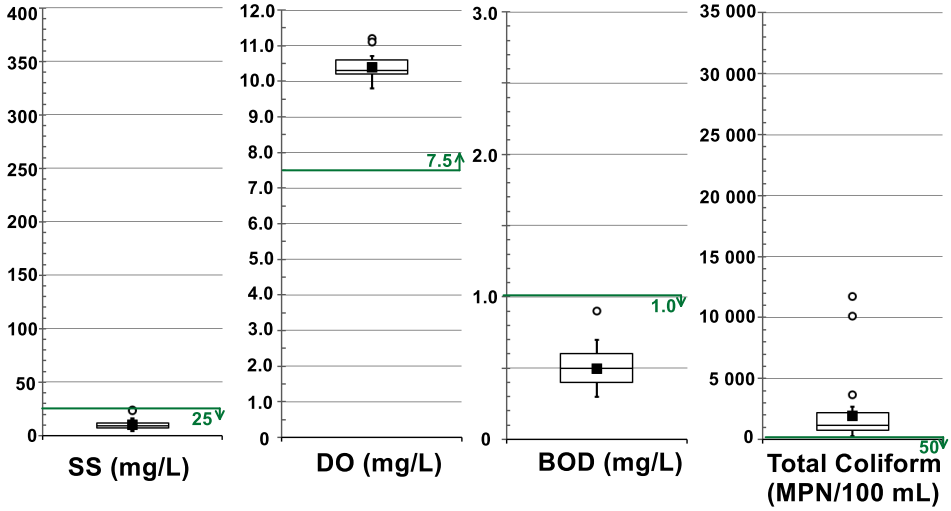

Figure 28. The figure assessed water quality parameters on the K Hydrographic observatory, T River, during the past three decades (1990-2018) by the Environmental Quality Standards for Conservation of the Living Environment (Rivers), Japan (MOEJ, 2021).

scale rivers that showed heavy MPs (50-5000 μm) pollution range from 50-25,850 mg/m^3 (Kabir et al., 2021). Nihei et al. (2020) concluded that the regional differences probably occurred due to the local status of sewage development, but it was insufficient to considering a small mesh size. It may be suggested to correct the MPs numerical and mass abundances collected by the 335 μm mesh in the entire Japanese rivers through a linear regression model between 100 and 335 μm meshes in several rivers, where the 100 μm mesh is applicable.

There are reports on the magnifications of the numerical abundance in the marine environment: two and three orders of magnitude between the bulk water sampling and 330, 335 μm mesh nets (Barrows et al., 2017; J. H. Kang et al., 2015), the 2.5-fold difference between 100 and 335 μm mesh nets (Lindeque et al., 2020). Therefore, whereas 355 μm mesh showed significantly underestimated MPs abundances, the 100 μm mesh net is recommended from the numerical and mass abundances perspective.

2) Bulk water sampling method

Microplastics abundance in Chapter 4 recorded considerably higher abundances than other studies such as Nakdong River Korea (Eo et al., 2019), Pearl River China (Lin et al., 2018; Yan et al., 2019), twenty-nine rivers Japan (Kataoka et al., 2019). This tendency was similar to the other freshwater media, Wuhan Lake (Wang et al., 2017) and Yangtze Estuary (Zhao et al., 2014) China (Table 4).

In this study (Table 4), the MPs abundance reached the influent water levels of the WWTPs in Korea (H. J. Park et al., 2020) and the Sewage Treatment Plant (STP) in Japan (Sugiura et al., 2021). Kataoka et al. (2019) reported the positive correlations between microplastic pollution and poor water quality in BOD and DO.

The mean water quality parameters of the 18 sampling stations located along the mainstream from Wangisagara (St.1) to Nanjung (St.4) as reported by the DLH, Bandung District, Indonesia (2018) were 2.9±1.5 mg/L DO, 31±38 mg/L BOD, 72±74 mg/L COD, 5460±8816 MPN/100 mL total coliform. These parameters (Figure 29) showed the poor water quality due to inadequate wastewater treatment systems (Honingh et al., 2020; Izza Indah Afkarina et al., 2020) in this area compared with the Freshwater Quality Standard, Indonesia (Indonesia Government, 2021), and the Environmental Quality Standards for Conservation of the Living Environment (Rivers), Japan (MOEJ, 2021). For references, the first class in the Indonesian standard (the AA grade in the Japanese standard), which means a drinking water supply (a first-class water supply and the conservation of the natural environment is required), should be shown >6 (7.5) mg/L DO, <2 (1.0) mg/L BOD, <10 mg/L COD, <1000 (50) MPN/100 mL total coliform (Indonesia Government, 2021; MOEJ, 2021). This poor water quality

implied that the inadequate wastewater treatment systems are the primary cause resulting in heavy plastic wastes in the Citarum River Basin (Honingh et al., 2020; Izza

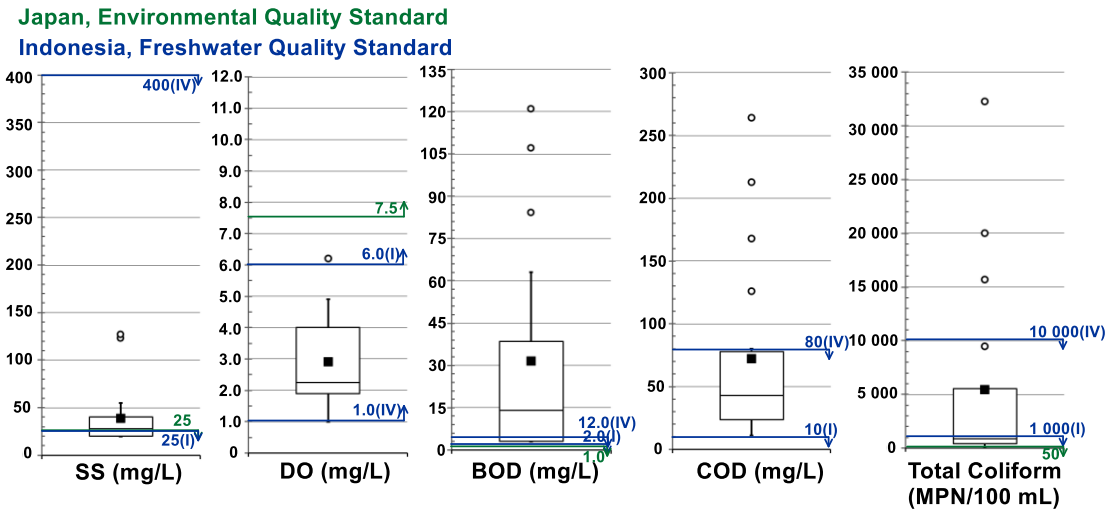

Figure 29. The figure assessed water quality parameters on 18 sampling stations following the mainstream of Citarum River in 2018 (DLH, Bandung District, Indonesia, 2018).

Indah Afkarina et al., 2020; Kataoka et al., 2019).

Additionally, this plastic richness in the freshwater was also found in the developing countries (Table 4), such as Saigon River Vietnam and a semiarid region of Mexico, which were stated insufficient wastewater treatment systems in the local area (Lahens et al., 2018; Piñon-Colin et al., 2020). The effectiveness of removing MPs from wastewater treatment systems is reported to be 64-99% globally (Edo et al., 2020; H. J. Park et al., 2020; Sugiura et al., 2021). Although WWTPs are likely to release tiny MPs that could not be removed in the wastewater treatment systems, it is an essential source of microplastic reduction (Edo et al., 2020; Kataoka et al., 2019; H. J. Park et al., 2020; Sugiura et al., 2021). The MPs abundances in developed countries such as Korea (H. J. Park et al., 2020) and Japan (Sugiura et al., 2021), where there are well-known high wastewater treatment levels (H. J. Park et al., 2020; Sugiura et al., 2021), were remarkably fewer than those in developing countries. It is necessary to establish the management plan for MPs in this river, including the effectiveness of WWTPs in Bandung city (Honingh et al., 2020; Izza Indah Afkarina et al., 2020).

Compared with the other studies in Indonesia (Table 4), MPs pollutions in the Citarum River were over two orders of magnitude higher than the other media such as the rivers (Alam et al., 2019; Lodo Pe et al., 2020; Sembiring et al., 2020), seawaters (Falahudin et al., 2019; Syakti et al., 2018) except for aquaculture ponds (Priscilla & Patria, 2019). Except for Alam et al. (2019) and Sembiring et al. (2020) mentioned above, Lodo Pe et al. (2020) reported MPs in the freshwater, Cimandiri watershed toward Palabuhan Ratu bay, West Java. This previous study area was located near the Sucabumi city, which had about 300,000 populations. However, there is no intensive

industrial activity and not a vast urban district like Bandung City, which has about 2.4 million inhabitants. In the seawater (Syakti et al., 2017, 2018), the low MPs abundance might be caused by the studied areas with the characteristics of open sea areas objected to the semi-closed bay, which has a non-exchange of seawater and a long residence time of MPs.

On the other hand, MPs numbers in the water can be easily affected by various factors, not only environmental factors but also the sampling methods, pre-treatment, and analytical technics. Firstly, as mentioned above, the minimum detected size due to mesh size might result in decreased MPs numbers (Hidalgo-Ruz et al., 2012). Many other Indonesian studies employed the Volume-reduced method (Priscilla & Patria, 2019; Syakti et al., 2017, 2018), and this might show the significant numerical differences between this study and other studies. Secondly, the results should be carefully discussed in this study because filtered water volume was only 20 mL for whole samples in 2018 and the tributary (St.3-a) sample in 2020. Although the estimation that raw data were multiplied fifty times to calculate for liter unit had no mathematical problems, it remains to inquire whether the estimated particles exist in a liter of real water. Finally, several researchers in Indonesia have observed MPs visually using a microscope. Microscopic counting has the advantage that samples with relatively many particles can be quickly identified (Li et al., 2018). It provides an overall picture of MPs numbers in a short time and at a low cost (Li et al., 2018).

However, in the actual marine environment, overestimated fragments and underestimated fibers were reported through a comparison between Micro FT-IR and the stereomicroscope (Song et al., 2015). Significantly, the ultrafast mapping method of the FT-IR microscope does not need to select the plastic candidates using a microscope, unlike the other identification methods. It rapidly and accurately covers most particles on wide inspected areas without pre-treatments for measuring polymer (T. J. Park et al., 2020).

This viewpoint also could applicate a case on the 355 μm mesh because the MPs collected by the large mesh are challenging to inspect the polymer identification using the Micro FT-IR due to large particle diameter. The essential step of picking plastic candidates by MPs in the previous FT-IR ATR and Raman spectrometer, may make the microplastic abundance overlook significantly (Song et al. 2015).

Compared with other studies, numerical MPs abundance on the Bulk water sampling method revealed the same order of magnitude as other rivers in which the inadequate wastewater treatment system was pointed out (Lahens et al., 2018; Piñon-Colin et al., 2020) or the influent of WWTPs (H. J. Park et al., 2020; Sugiura et al., 2021). It indicated that the bulk water sampling method was valid in the MPs abundance perspective.

Table 4. The comparison of microplastics abundance between this study and other studies

Location	Size* (µm)	Sampling Method**	Min-Max (particles/L)	Mean±SD (particles/L)	reference
2018 Citarum River, Indonesia	100	Bulk	0 - 350	210±130	this study
2018 Citarum River, Indonesia	100	Bulk	0 - 550	140±275	this study
Nakdong River, Upstream – downstream, Korea	20	Bulk	-	0.293±0.083 - 4.760±5.242	Eo et al., 2019
Jul. 2017, Pearl River, China	20	Bulk	0.379 - 7.924	2.724	Lin et al., 2018
Dec. 2017, Pearl River, Urban section, China	50	Bulk	8.725 - 3.250	19.869	Yan et al., 2019
Twenty-nine rivers, Japan	335	Volume	0 - 0.012	0.001±0.002	Kataoka et al., 2019
Wuhan Lake, China	50	Bulk	-	1.660±0.639 - 8.925±1.591	W. Wang et al., 2017
Yangtze Estuary, China	500	Volume	0.500 - 10.200	4.137±2.461	Zhao et al., 2014
50 WWTPs, Korea, Influent	45	Bulk	10 - 470	-	H. J. Park et al., 2020
50 WWTPs, Korea, Effluent	100	Volume	0.004 - 0.51	-	
STP, Japan, Influent	10	Bulk	420	-	Sugiura et al., 2021
STP, Japan, Effluent	10	Bulk	8.7	-	
Saigon River, Fiber, Vietnam	50	Bulk	172 - 519	-	Lahens et al., 2018
Tijuana, Semiarid region, Mexico	25	Bulk	12 - 2054	88±42 - 289±584	Piñon-Colin et al., 2020
Ciwalengke River (Upstream of Citarum River)	50	Bulk	-	5.85±3.28	Alam et al., 2019
Maura Gembong area (Downstream of Citarum River)	125	Bulk	$0.038x10^{-3}$-$0.086x10^{-3}$	-	Sembiring et al., 2020
Cimandiri Watershed area	20	Bulk	0.685 - 7.444	-	Lodo Pe et al., 2020
Muara Kamal Agriculture Pond	300	Volume	-	103.8±20.7	Priscilla & Patria, 2019
Marunda Agriculture Pond	300	Volume	-	90.7±17.4	
Small Islands of Bintan Seawater	100	Volume	$0.13x10^{-3}$ - $0.94x10^{-3}$	$0.45x10^{-3}$±$0.25x10^{-3}$	Syakti et al., 2018
Cilacap Coast Seawater	2500	Volume	$0.27x10^{-3}$ - $0.54x10^{-3}$	-	Syakti et al., 2017

The '-' means that there was no information. *A minimum detection size of target microplastics in each study. ** Bulk (Bulk water Sampling method), Volume (Volume-reduced sampling method).

5.2.3 Perspective 3: Microplastic Features

The characteristics of PP and PE polymers were stated above Chapters. Briefly, there are very susceptible to photo-oxidation resulting in physical and chemical changes, are easy to break down as nano plastics (Rodrigues et al., 2018). There is a viewpoint that microplastic polymer degradation is correlated with their tensile strengths (Kameda et al., 2021).

These PP and PE polymers have respective 0.91 and 0.94 g/cm^3 densities, which are lower than freshwater (Prata et al., 2019), resulting in high portions of PP and PE polymers in the surface water (Eo et al., 2019; Lin et al., 2018). However, the relatively shallow depth in the freshwater is likely to make the numerical microplastic abundance be equally dispersive in vertical (Dris et al., 2018). This results in that the light-density polymer types exist in the water column and provide the opportunities that those polymers are bioaccumulated via accidental ingestion.

The nano plastics have high ingestion potential enhancing bioaccumulation (Andrady, 2011; GESAMP, 2019; Rodrigues et al., 2018). It indicates that PP and PE polymers, which are prone to breakdown (Kameda et al., 2021; Rodrigues et al., 2018), are crucial polymer types implying the potential indicators of MPs bioaccumulation.

The 100 μm mesh and the Bulk water sampling method revealed these PP and PE distributions in the surface freshwater (Table 5). The distributions were also shown in Japan, Indonesia, and other studies globally. However, 355 μm mesh captured polymer compositions different from 100 μm mesh in the Japanese river. Thus, sampling microplastic with 100 μm mesh and the Bulk water sampling method were more valid than another mesh.

Contrary to this, 335 μm mesh in the 29 Japanese rivers collected PP and PE as major polymer types, consistent with the 100 μm mesh result in Chapter 3 (Kataoka et al., 2019). This implied that the distribution in which PP and PE polymers were not collected by 355 μm mesh in Chapter 3 might be caused by the pristine T River characteristics.

Additionally, microplastic shape and polymer types are also primary factors to trace the MPs origins (Conkle et al., 2018; Isobe, 2016; J. H. Kang et al., 2015; Katsumi et al., 2021). However, Lindeque et al. (2020) revealed similar fragment and fiber compositions in seawater in 100, 333, and 500 μm meshes (Figure 30).

Previous two results (Kataoka et al., 2019; Lindeque et al. 2020) indicated that the improper fragment and film distributions by 355 μm mesh in shape and polymer type perspectives in this present study are likely due to the specific T River characteristics in which the few large fragment and film numbers existed in the pristine water.

Table 5. The comparison of microplastics abundance between this study and other country studies

Location	Size* (μm)	Sampling method**	Main types (%) ***	reference
2020 T River, Japan	100	Volume	PE (40), PP (39), PS (10)	this study
2020 T River, Japan	355	Volume	PET (71), polyester (28)	this study
2018 Citarum River, Indonesia	100	Bulk	PP (52), PE (16), PA (12)	this study
2020 Citarum River, Indonesia	100	Bulk	PP (82), PE (18)	this study
Nakdong River, Upstream – downstream, Korea	20	Bulk	PP (42), polyester (23)	Eo et al., 2019
Jul. 2017, Pearl River, China	20	Bulk	PP (36), PE (29), PET (29)	Lin et al., 2018
Dec. 2017, Pearl River, Urban section, China	50	Bulk	PA (26), cellophane (23), PP (13), PE (10)	Yan et al., 2019
Twenty-nine rivers, Japan	335	Volume	PP + PE (80)	Kataoka et al., 2019
Wuhan Lake, China	50	Bulk	PET (18), PP (13), PE (6), PA (5), PS (2)****	W. Wang et al., 2017
50 WWTPs, Korea, Influent	45	Bulk	PE (40), PP (26), PET (21)	H. J. Park et al., 2020
50 WWTPs, Korea, Effluent	100	Volume	PE (14), PP (63), PET (13)	
Saigon River, Fiber, Vietnam	50	Bulk	PET (70)	Lahens et al., 2018
Maura Gembong area (Downstream of Citarum River)	125	Bulk	PE (70), PP (30)	Sembiring et al., 2020
Small Islands of Bintan Seawater	100	Volume	PP (54), PE (17), LDPE (18), PS (10)	Syakti et al., 2018
Cilacap Coast Seawater	2500	Volume	PP (68), LDPE (11)	Syakti et al., 2017

* A minimum detection size of target microplastics in each study. ** Bulk (Bulk water Sampling method), Volume (Volume-reduced sampling method). ***Only the polymers accounted for over 10% of total compositions were exhibited. **** The study utilized the microscope and analyzed the Raman spectrometer for only twenty-one of plastic candidates. The numbers in parentheses mean the number of polymers, not proportion.

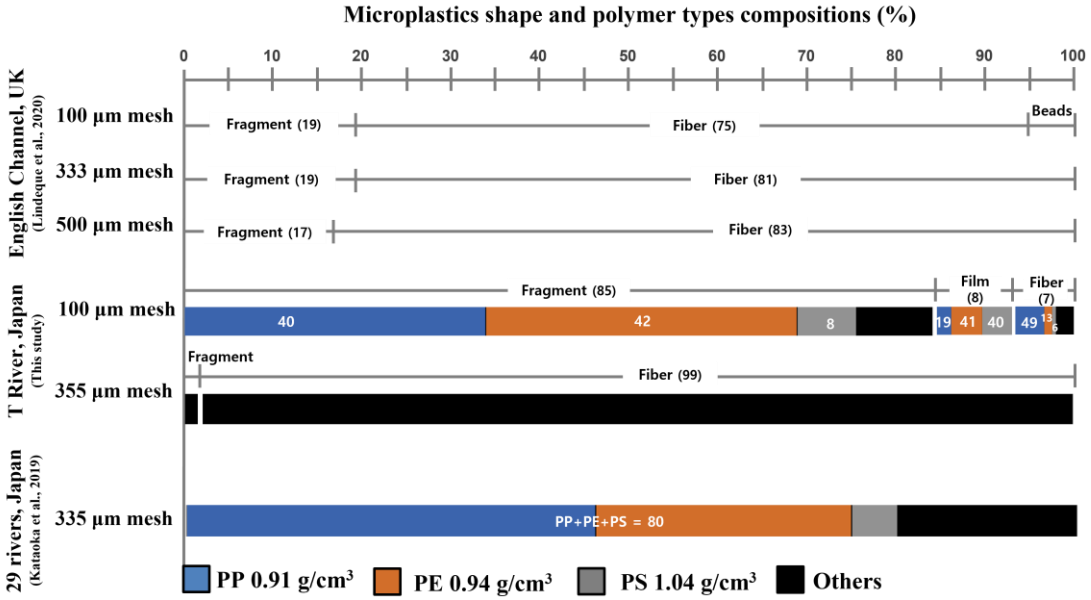

Figure 30. Compared validities of sampling mesh in the MPs features perspective with Kataoka et al. (2019) and Lindeque et al. (2020).

5.2.4 Perspective 4: River characteristics

In Chapter 4, the extremely highest MPs abundance (11 particles/20 mL) was revealed in the tributary (St.3-a), Citrarum River, Indonesia 2020 (Figure 23). Figure 31 exhibits the investigation pictures at the Cisirung (St.3) and tributary (St.3-a) on 23rd January 2020.

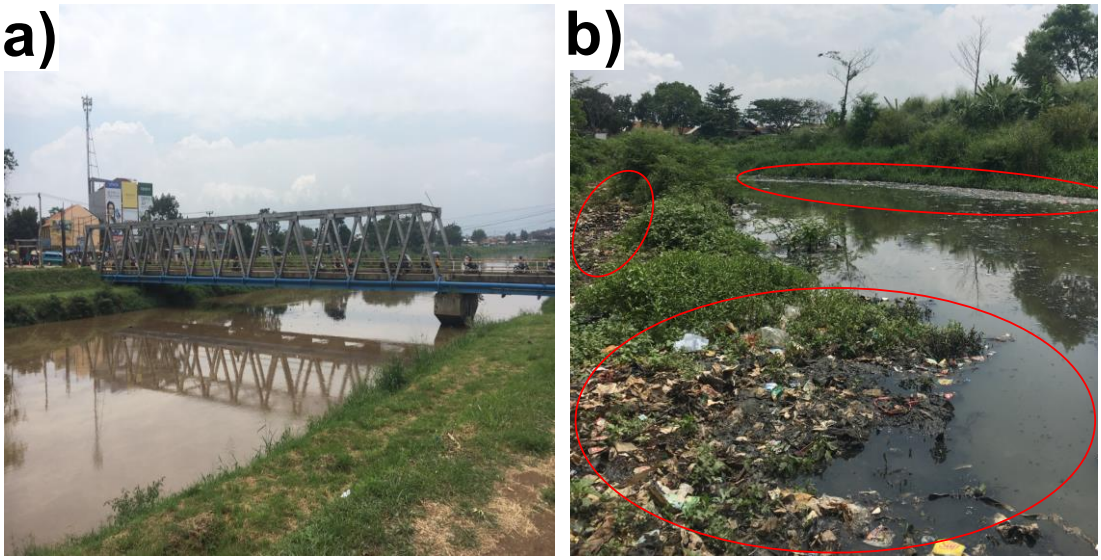

Figure 31. The sampling pictures in this study a) Cisirung (St.3), b) Tributary (St.3-a) in Citarum River, West Java, Indonesia, on 23rd January 2020. Red circles indicate the river plastic debris.

During sampling in Cisirung (St.3), Nanjung (St.4), Jatiluhur (St.5), the water level was relatively high, and there was a lot of suspended matter (Figure 31a). Contrary to this, in the tributary (St.3-a), no suspended matter like sand or mud was observed, and the water flows was also stationary (Figure 31b). These sampling pictures indicated that the additional flow to this tributary (St.3-a) was not generated, though the precipitation occurred in the investigation date (Figure 23), and the water condition was not exchanged with the mainstream.

A visually distinct substantial debris in the picture (Figure 31b) also implied that unknown sources of plastic had been presented continuously in this tributary (St.3-a), and the accumulated plastics might result in remarkable MPs abundance in Chapter 4. Notably, the numerous fragment shapes (83%) of MPs in this tributary were likely derived from this debris (Figure 23b). It is urgent to identify specific pollutant sources, the features of water flow, and the management

These two factors in 2020, *i.e.*, stagnant water flow and numerous microplastic abundance, made the Bulk water sampling method reveal the significant MPs distribution in the tributary (St.3-a) (Figure 23). The MPs abundance was acceptable

based on comparing the numerical abundance and features with other studies (Tables 4, 5).

Therefore, the Bulk water sampling method was regarded as a powerful tool to present MPs distribution in stagnant water flow and heavily polluted freshwater environments. However, the Bulk water sampling method captured no MPs in other sampling stations, it also indicated that the method was susceptible in fluid water. This can be probably linked to the weather condition perspective.

5.2.5 Perspective 5: Weather Conditions

Many researchers reported the increasing numerical MPs abundance depended on the rainfall in the river (Eo et al., 2019; Piñon-Colin et al., 2020), the estuary (Cheung, Fok, et al., 2018), and the seawater (Kataoka et al., 2013) excepted for the bay that is clearly separated from inland pollutant sources (Tsang et al., 2017).

In the river, the main factors of the increase were caused by following,

- ✓ The MPs become wash-off during rain events from the adjacent city of the river (Piñon-Colin et al., 2020).
- ✓ The huge amounts of river water discharge and the fast water velocity re-float the previously deposited MPs on the river bottom (Hurley et al., 2018).
- ✓ The atmospheric MPs are fallen by the precipitation (Dris et al., 2015).

Contrary to this, cases in which MPs concentrations were decreased after rainy events were also reported (Lin et al., 2018; Moore et al., 2011; Yan et al., 2019). For example, the MPs were collected in a station with three consecutive samplings during 21 mm of rain, after the 46 mm of rainfall occurred within two days in Lam Tsuen River, Hongkong (Cheung, Hung, et al., 2018). The result revealed approximately ten-fold lower numerical MPs abundance in the last sampling than the first one (Cheung, Hung, et al., 2018). In this tendency, the main reasons can be assumed by following,

- ✓ The increased MPs already flow into the sea (Cheung, Hung, et al., 2018; Moore et al., 2011).
- ✓ The massive water discharge dilutes the numerical microplastic abundance.
- ✓ The MPs are attached to suspended matter floating in the surface water. Therefore, the fate of the combined particles is tremendously complicated by the fast water velocity and is not transported to the surface water.

On the other hand, we established the Bulk water sampling method in Chapter 4. However, despite filtering water volume was increased from 20 mL (2018) to 1000 mL (2020), the MPs were not collected in 2020. The sampling in 2020 was implemented with precipitation (Figure 24). It indicated that the water volume should be more grabbed or that the Bulk water sampling method is unsuitable in the intermediate or post-rain events. Therefore, the Volume-reduced sampling method is recommended for the sampling with the rain events.

In this regard, we selected randomly and measured the sizes of three hundred relatively large suspended matters in which the sampling mesh may be clogged in the 2020 Nanjung (St.4) sample (Figure 32). The range of the lengths was 12-90 (mean

32±12, median 32) µm. Using the sampling mesh >100 µm may be proper because the maximum size was 90 µm.

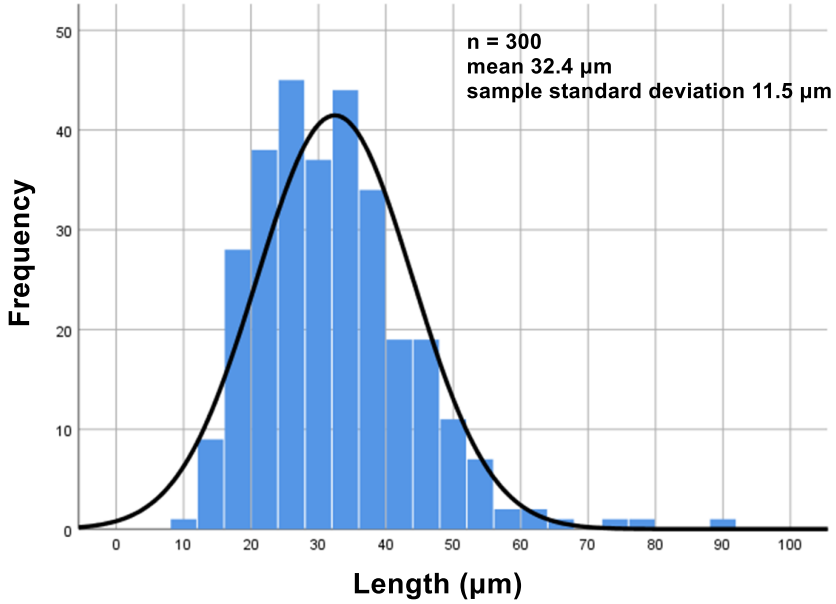

Figure 32. The histogram for the sizes of suspended maters in surface water in Nanjung (St.4) 2020, in Citarum River, West Java, Indonesia.

However, Michida et al. (2019) recommended the 350 µm mesh for the collection of microplastics in the surface seawater because floating fish eggs interrupted the collection by 100 µm mesh. In this regard, several studies employed 335 µm mesh in the surface freshwater; the interrupting factor of 100 µm mesh was suspended matter in those studies (Kudou et al., 2019; Kataoka et al., 2019; Nihei et al., 2020). Thus, the proper mesh size >100 µm may be recommended in Citarum River, West Java, Indonesia. It indicated that 355 µm mesh size is more valid in clogging the mesh aspects (rainfall events and numerous suspended matters) than 100 and the Bulk water sampling method.

5.3 Summary

Chapter 5 determined comprehensive validities on 100, 355 μm meshes and the Bulk water sampling in five perspectives: MPs sources, abundances, features, river characteristics, and weather conditions (Table 6).

The 100 μm mesh in the pristine Japanese freshwater is certainly recommended in the abundances, sources, features perspectives. However, the mesh was susceptible to clogging of the mesh. Additionally, the pristine freshwater in the T River made the different mass abundance ignore between 100 and 355 μm meshes. Although 355 μm mesh was unsuitable in microplastic features, other studies showed no difference between 100 and 355 μm meshes. In particular, the 355 μm mesh had the advantage to utilize in specific conditions, *i.e.*, rainfall events and numerous suspended matters. On the other hand, the Volume-reduced sampling method denoted the possibilities of underestimating MPs distributions, and the Bulk water sampling method validly recommended in the slow/stagnant water and heavily polluted freshwater environments in abundance, source, and feature perspectives.

It indicates that the desirable mesh selection in a research plan establishment step should be considered depending on the research purposes, river characteristics, and weather conditions.

Table 6. Summary of Chapter 5

Perspectives	100μm mesh	355μm mesh	Bulk water sampling
MPs Sources	Suitable	Unsuitable	Suitable
MPs Abundances	Suitable	Moderate*	Suitable
MPs Features	Suitable	Moderate**	Suitable
River Characteristics	Pristine river	Numerous suspended matter (Terrestrial plant debris /Mud/ Silt)	Slow or stagnant water flow, Heavy MPs pollution
Weather Conditions	Susceptible	Rain events	Susceptible
Comprehensive Disadvantages	Possibility underestimating 67% of MPs	Possibility underestimating 97% of MPs	Numerous water volumes for filtering

**Although the mass MPs abundance between the 100 and 355 μm meshes was statistically different, the pristine characteristics in T River made the difference ignore. **The mesh was not suitable in the present study, but other studies showed validity in the corresponding perspective.

CHAPTER 6

Conclusions

6 Conclusion

The present study determined the validities of the 100, 355 μm meshes and the Bulk water sampling method in five perspectives: MPs sources, abundances, features, river characteristics, and weather conditions.

Apart from the numerical abundance that is naturally increased, mass abundance in 100 μm mesh was six times higher than 355 μm mesh. Additionally, 355 μm mesh was challenging to trace MPs origins and estimate bioaccumulation potentialities in the MPs features perspective. The microplastic numerical abundances in 100 μm mesh showed a gradually increased distribution toward the estuary direction in which the urban area is located, whereas 355 μm showed a different distribution due to accidental irregulars in analytical steps. Therefore, the 100 μm mesh in the pristine Japanese freshwater is certainly recommended in the abundances, sources, features perspectives. However, the mesh was susceptible to clogging of the mesh.

Although sampling with 355 μm mesh in the freshwater environment was insufficient in the MPs abundances aspect, the pristine freshwater in the T River, Japan, made the different mass abundance neglect. Moreover, 355 μm mesh was unsuitable in MPs features (shape and polymer types), but previous studies descript similar shape and polymer type compositions between 100 and large mesh sizes >300 μm. In this regard, the 355 μm mesh may be recommended in rainfall events and numerous suspended matters characteristics.

However, the cumulative probabilities for the minimum Feret diameter of particles were divided into three parts, indicating 96% and 67% of potential underestimation in the 355 and 100 μm meshes, respectively. Underestimating tiny MPs is indispensable in the volume-reduced method based on mesh sizes.

The Bulk water sampling method validly revealed numerical microplastic abundance derived from inadequate wastewater treatment systems in Citarum River, West Java, Indonesia. Additionally, in 2018, polymer composition in Wangisagara was distinct from other upstream stations, and it indicated different MPs sources with other stations. The high MPs pollutions were distributed in the Cisirung and Nanjung near the Bandung City with intensive human populations and in the Koyod, which had intensive industrial activities related to textiles, in 2018. The primary water source (Jatiluhur) of the capital city had no MPs in both years. Moreover, the bioaccumulation via fish was concerned due to the frequently detected PP, PE in the 100-1000 μm group, indicating the general microplastic polymer distribution. Thus, the Bulk water sampling method was validly recommended in the slow/stagnant water or heavily polluted freshwater environments in abundance, source, and feature perspectives.

In conclusion, the 100 μm mesh and the Bulk water sampling method revealed more specific MPs distributions than 355 μm in the present study. However, the three methods denoted the respective advantages and disadvantages. Therefore, proper sampling method in a research plan establishment step should be considered depending on the research purposes, river characteristics, weather conditions.

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