Microplastic pollution in Surabaya River Water and Aquatic Biota, Indonesia

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Abstract. Microplastics (MPs/MP) have been considered as an emerging environmental threat worldwide. It occurs due to its persistent, ubiquitous presence, and potential ecotoxicological risks to almost all aquatic environments. The MPs could release chemical additives and adsorb persistent organic pollutants. It could also be ingested mistakenly by aquatic biota due to its similarity with their original prey’s size and colour. This situation could impact water resource quality, aquatic biota biodiversity, even human health. Additionally, rivers have been estimated as the main pathway of plastic transport from the land to the ocean. Surabaya River, is the main lower part of the Brantas, which is one of the top 20 plastic polluted rivers globally. The Surabaya River takes essential role in clean water supply for Surabaya City, aquatic biota habitat, and irrigation. This study aimed to investigate the distribution and characteristics of the MPs in water and aquatic biota in Surabaya River. Fish and bivalve were selected as the representative aquatic biota due to their specific habitat, movement, and feeding behavior. Water and biota samples were collected from five sampling sites in the river. The MP abundance in the water ranged from 9.66 ± 8.87 to 21.16 ± 19.35 particles/m³. The highest MP abundance (21.16 ± 19.35 particles/m³) was found at Joyoboyo. The MP abundance in the fishes of Oreochromis niloticus, Barbonymus gonionotus, and in the bivalves of Elongaria orientalis were 105.25 ± 45.07 – 155.50 ± 61.96; 62.13 ± 20.33 – 155.00 ± 81.71; and 36.00 ± 13.67 – 76.17 ± 29.46 particles/individual, respectively. The MPs in the water were dominated by film shaped, transparent colour particles. Meanwhile, the MPs in the fishes and the bivalves were generally found in transparent and black fiber particles. Only small amounts of film particles were discovered in the biota. Moreover, most of the MPs in the water and the biota were in large sized (1 – 5 mm) particles.

1. Introduction

Plastics are synthetic or semi-synthetic organic polymers that are persistent, durable, lightweight, versatile, resistant to corrosion, and low-cost production [1-3]. These characteristics make plastics become commonly used materials for daily products [4]. Annual global plastic production has reached up to 322 million tonnes in 2016 [4]. It is estimated that the global plastic production will be increased in double by 2025 due to plastic versatility in various daily products [3, 5]. About 75 – 90% of global plastic demand is limited to several types of plastic: high-density polyethylene (HDPE), low-density polyethylene (LDPE), polypropylene (PP), polyethylene terephthalate (PET), polyvinyl chloride (PVC),...
polystyrene (PS), and polyurethane (PU) [1, 6]. Meanwhile, plastic waste could be released and polluted the environment due to improper solid waste management and insufficient service coverage [6 – 11].

Plastics can be fragmented and degraded in the environment due to photodegradation, weathering, mechanical-physical, water current, and biodegradation mechanisms [12 – 13]. One of the emerging environmental concerns of plastic degradation results is microplastics (MPs/MP), plastic particles smaller than 5 mm in size [12 - 15]. The MPs can be originated from primary (household product application and personal care products) or secondary sources (breakdown of larger plastic particles) [15 - 18]. The MPs can be identified based on size, shape, colour, and polymer density [15]. These characteristics could affect its distribution in different environmental compartments and make the particle susceptible to be ingested by the biota [19 – 23].

The MPs have been found in rivers, coastal area, marine, even in aquatic biota in the global [24 – 31]. This condition could give various potential impacts to the environment and the biota [32]. The MPs also has an ability to adsorb environmental pollutants from their surroundings [32 – 33]. Furthermore, biofilm growth on the surface of the MP particles may become a source of microbial pathogens [34]. However, the MPs have been found in commercial edible fishes and mussels, tap and bottled drinking water, table salt, honey, and beer [35 – 37]. This condition could give potential ecotoxicological effects not only to the aquatic biota food web, but also a risk to food security and human health [38 – 40].

Rivers are predicted as the main pathway of plastic waste transport from the land to the marine [41 – 42]. Regrettably, Brantas River, the largest river in East Java Province, Indonesia, was included as one of the top 20 plastic polluted rivers in the global [25, 43]. It has been estimated that about 3.23 – 6.37 x 10^4 tonnes per year of mismanaged plastic waste originating from the Brantas flowing into the marine [25]. However, this river has an essential function as the primary source of clean water supply for 15 densely populated cities in East Java Province [44]. One of the main lower parts of the Brantas, Surabaya River, is also used for clean water supply for Surabaya City, aquatic biota habitat, flood control, and recreation [43, 45]. However, the Surabaya River has suffered from water quality deterioration due to 215 point sources of domestic effluent and ten industrial outlets that discharged the wastewater into the Surabaya River [46 - 48]. Former study investigated 92 – 590 MP particles per kg dry weight sediment contained in the Surabaya river mouth and the Jagir Estuary [49]. This evidence showed that MP had polluted the Surabaya River. Therefore, this study aimed to investigate the distribution of the MPs in the water and biota of the Surabaya River. Fish and bivalve were selected as representatives of edible biota due to their high potential to be contaminated by the MPs [21]. Fish is highly potential to ingest the MPs due to its feeding behavior and active movement in the water [22]. Meanwhile, the bivalve as a sedentary organism could adsorb and accumulate the MPs due to its feeding behavior as a filter feeder [50]. Therefore, this study could contribute for further ecological risk evaluation of MP pollution [26]. More importantly, a better understanding of the MP pollution study in the freshwater, especially in the urban river, is urgently needed for effective prevention of the MP pollution in aquatic environment [51].

2. Materials and Methods

2.1 Study area and sampling sites

The sampling sites were: S1 in Driyorejo, S2 in Bambe, S3 in Karang Pilang, S4 in Joyoboyo, and S5 in Jagir Districts (Figure 1). The water samples were collected during the rainy season from February to March 2019. Meanwhile, the biota samples collection was conducted from the end of September to early October 2019. Both sampling activities were conducted in two replications. The water and biota sampling activities were conducted at different times as this study did not consider time variation, which might influence the MP distribution. The particular reason for this situation was also related to technical challenges (limited sampling accessibility, the availability of supported equipment, and human resources) and hydraulic conditions (river discharge fluctuation). However, both sampling activities were still carried out within the same range of the rainy season in Indonesia.

Water samples collection was done by using a modified Manta trawl with pore size of 333 µm [52, 53]. The dimension of the manta trawl net was 3 m, 61 cm, and 16 cm in length, width, and height,
respectively [53]. A cod-end at the end of the Manta trawl net was placed for collecting the MP particles from the filtered water with an average volume of 55.34 m³. The Manta trawl net was placed in three stratified depths to collect the samples from the surface, middle, and bottom of the river water column. This method was meant for obtaining average MP abundance and avoiding under- or over-estimation of the actual MP abundance [54]. The trawl was towed for approximately 20 minutes in the opposite direction of the river current alongside the boat [55, 56]. Afterward, the cod end, which retained and collected the MP particles, was carefully released from the trawl and preserved in aluminum foil zip lock containing 70% alcohol.

![Figure 1. Map of sampling sites](image)

The fishes of *Oreochromis niloticus* and *Barbonymus gonionotus*, and the bivalves of *E. orientalis* samples were collected by manual random sampling in each site. The *O. niloticus* was found in all sites, while *B. gonionotus* was only found at S1, S4, and S5. Meanwhile, the bivalves were only found at S1, S2, and S4. Only 15 individuals of *O. niloticus*, 6 individuals of *B. gonionotus*, and 16 individuals of *E. orientalis* were selected for their similarities in size. Each sample was preserved in an aluminum zip lock containing 70% alcohol and placed in storage box.

2.2 Sample preparation and extraction

2.2.1 Water samples. The MP extraction from water samples in this study was done based on National Oceanic and Atmospheric Administration (NOAA) method [57]. First, the cod end of the trawl was rinsed by using distilled water. Then, it was wet filtered by two stratified stainless steel sieves with 5.6 mm and 0.3 mm pore sizes. Afterward, the samples were dried at 90°C for 24 h. The next step was wet peroxide oxidation (WPO). The WPO step aimed to remove organic material in the samples. In WPO, the samples were oxidized by adding 20 mL H₂O₂ 30% and 20 mL Fe(II) solutions, then heated until 75°C for 30 minutes. Hereafter, the samples were treated by density separation. About 6 g NaCl per 20 mL solution was added into the samples in this process to increase the solution density to 1.15 g/mL, so that the MP particles could be buoyant and easily separated [57]. After 24 h settling process in density separator, the solution was filtered by 0.3 mm filter. The particles resembling the MPs were manually separated from the filter into a clean petri dish under dissecting microscope (Sunshine SZM45T-B1).

2.2.2 Biota samples. First of all, characteristics of each individual biota sample were measured for obtaining similar size of the samples. Then, MP extraction steps for the biota samples followed by dissection, WPO, density separation, and visual sortation [57 – 59]. For fish samples, the dissection was done by separating the gill and the gastrointestinal tract [58]. Afterward, the wet weight of the gill and the gastrointestinal tract of each individual were measured [58]. Meanwhile, for the bivalve samples,
the whole soft tissue (ST) of each individual bivalve was taken from the shell. Then, the wet weight of whole ST of each individual bivalves was measured and placed in a 500 mL beaker glass [59]. Hereafter, all dissection results were measured in wet weight (ww) [58].

Afterward, all the samples were treated with the same WPO step above using 200 mL H2O2 30% for removing and digesting the organic material. After the WPO step, the biota samples were treated with the same density separation process for 24 h. Then, the solution was filtered by 0.3 mm filter until potential MP particles were obtained. These particles were separated from the filter, identified, and characterized under dissecting microscope.

2.3 Identification and characterization of the MPs

Identification of MP particles can be done based on three fundamental rules: 1) the particles can not be ripped apart by tweezer, 2) the particles have a distinct colour, and 3) the particles are cleared from the bound organic structure [15]. All potential MP particles then were observed and identified visually under a dissecting microscope based on the shape, size, and colour [15, 60]. The MP shapes commonly observed as fragment, film, foam, fiber, and pellet [60]. Based on its size, the MPs can be divided into small (SMP) in sized of 1 µm - 1 mm and a large one (LMP) in sized of 1 – 5 mm. The MP colours can be categorized into blue, black, red, yellow, transparent, and white [15]. Representative MP particles were photographed by dissecting microscope (Olympus SZX16). All particles found in this study could be categorized and considered as the MPs according to the fundamental rules of MP identification [15].

3. Results and Discussion

3.1 MP abundance

3.1.1. MP abundance in the water. Average MP abundance at all sites ranged from 9.66 ± 8.87 to 21.16 ± 19.35 particles/m³ (Figure 2). The highest MP abundance was found in 21.16 ± 19.35 particles/m³ at S4 (Joyoboyo), which located before the Surabaya River branches, namely the Jagir and the Mas Rivers. The average MP abundance at other sites (S1, S2, S3, S5) were obtained as 20.62 ± 12.32; 9.66 ± 8.87; 12.32 ± 7.90; and 16.03 ± 5.29 particles/m³, respectively. This situation showed a fluctuating trend of MP abundance along the river (Figure 2). These results were in accordance to several previous studies, which stated that the MP abundance was spatially and temporally heterogeneous in the urban river [28, 61]. This condition might be affected by several causes, such as population density, domestic and industrial discharges, wastewater effluent, land use, hydrodynamic condition, water velocity, turbulence, drainage system, dams, and stream geomorphology [27, 28, 51, 62, 63]. The population density from the upper to the lower part of the Surabaya River increased up to 150,608 inhabitants/km² [64]. In addition, the water velocity condition might become a crucial factor that contributes to MP distribution in the river [42].
3.1.2 MP abundance in the fishes. All of the fish samples contained MP particles (Table 1). The highest MP abundance of *O. niloticus* was found at S3 (155.50 ± 61.96 particles/individual). Meanwhile, the highest MP abundance of *B. gonionotus* was found at S1 (155.00 ± 81.71 particles/individual). These results showed that the MP abundance in *O. niloticus* was higher than in *B. gonionotus* based on average MP particles/individual (Table 1). This condition might be affected by the different feeding behavior of each fish species [65]. Based on their feeding behavior, *O. niloticus* is categorized as omnivorous with carnivorous tendency [68], while *B. gonionotus* is categorized as herbivorous [66, 67]. This condition could explain the higher MP abundance in *O. niloticus* than in *B. gonionotus*.

### Table 1. MP abundance in the fishes.

| Site |  *O. niloticus* |  *B. gonionotus* |
|------|----------------|------------------|
|      | MP particles/g ww | Average MP particles/individual | MP particles/g ww | Average MP particles/individual |
| S1   | 53.13 ± 53.13 | 8.10 ± 8.10 | 123.25 ± 123.25 | 16.88 ± 16.88 | 10.29 ± 10.29 | 155.00 ± 155.00 |
| S2   | 303.59 ± 293.09 | 265.71 ± 133.34 | 138.83 ± 86.03 | ND | ND | ND |
| S3   | 43.78 ± 43.78 | 53.21 ± 53.21 | 155.50 ± 155.50 | ND | ND | ND |
| S4   | 21.42 ± 21.42 | 34.64 ± 34.64 | 105.25 ± 105.25 | 35.16 ± 35.16 | 7.95 ± 7.95 | 99.00 ± 99.00 |
| S5   | 8.05 ± 8.05 | 15.12 ± 11.01 | 108.50 ± 56.21 | 32.70 ± 9.83 | 17.32 ± 12.59 | 62.13 ± 20.33 |

*ND (no data)*

The MP abundance was generally found higher in the gill than in the gastrointestinal tract of the fishes (Table 1). These results were in accordance to the previous research results [69, 70], in which this condition might be affected by fish interaction and MP pollution levels in their aquatic environments. Furthermore, investigation of the MPs in specific organs of the fishes is essential due to its potential translocation and effects within the body, and further risk of indirect transfer to human [31]. The MPs in the gill could occur as the result of the respiratory process of the fish [69]. Meanwhile, the MPs in the gastrointestinal could origin from ingestion in the food chain, in which large MPs might retain and accumulate in the gastrointestinal [31]. The average MP abundance per individual fish was determined by averaging the amount of the MPs in the gill and the gastrointestinal, so that it can represent the MP abundance of both organs per individual fish.
3.1.3 MP abundance in the bivalves. The MP abundance in *E. orientalis* varied from 20.25 ± 9.60 to 47.28 ± 8.65 particles/g ST, or from 36.00 ± 14.15 to 76.17 ± particles/individual (Table 2). The average MP abundance in *E. orientalis* was 31.33 ± 10.75 particles/g ST or 61.14 ± 21.91 particles/individual. The highest MP abundance of the bivalves was found at S2 (26.47 ± 17.91 particles/g ST or 71.25 ± 59.75 particles/individual). These results showed that MP particles have been found and contaminated the bivalves of *E. orientalis* in the Surabaya River. Furthermore, the bivalves are potential to become bioindicator for the MPs in aquatic environments due to their sedentary movement [59, 71, 72].

**Table 2. MP abundance in the bivalves.**

| Site | MP abundance | MP particles/g ST | MP particles/individual |
|------|--------------|-------------------|------------------------|
| S1   | 7.28 ± 8.65  | 76.17 ± 29.46     |                        |
| S2   | 26.47 ± 17.91| 71.25 ± 59.75     |                        |
| S4   | 20.25 ± 9.60 | 36.00 ± 13.67     |                        |
| Average | 31.33 ± 10.75 | 61.14 ± 21.91 |                        |

*ND (no data)*

3.2 MP characteristics

3.2.1 MP characteristics in the water. The MP shapes found at all sampling sites comprised film (69.17 – 88.50%), fragment (3.63 – 19.57%), fiber (0.67 – 7.20%), foam (0.67 – 8.33%), and pellet (0.20 – 1.00%) (Figure 3a). The film particles were the most dominant found of MP shapes in this study. These results were consistent with research findings in the Qing and the Pearl Rivers, China, which found film as dominant MP particles [74, 75]. The MP shapes also might predict its potential sources. In this study, the film particles morphology has similarly resembled used plastic packaging ruins. Former studies at Singapore’s coastal mangrove and in the Gulf of Mexico, Texas, estimated that the MP particles in film-shaped were originated from the breakdown of used plastic packaging and bags [76 - 78]. This condition might relate to the high demand for plastic packaging [3].

![Figure 3. The MP characteristics in the water: a) shape, b) size, and c) colour.](image-url)
Based on the size, the MP particles were dominantly found as LMP (61.07 – 78.63%). Only a small proportion of SMP was found (15.07 – 38.93%) (Figure 3b). The particle size could affect the vertical distribution of the MPs in the aquatic environment and potentially trigger the particles to be more susceptible ingested by the biota [79 - 81]. The transparent particles were the most abundant MP color in the water (Figure 3c). The MP colour comprised transparent (38.79 – 60.86%), white (7.02 – 27.16%), blue (11.05 – 17.95%), black (5.23 – 10.02%), red (3.75 – 8.18%), and yellow (2.31 – 4.34%) (Figure 3c). Besides, the colour, as plastic additives substances in the MPs could cause potential ecotoxicological risk to the biota as it could leached out to the environment [82].

### 3.2.2 MP characteristics in the fishes
Fibers were the most dominant MP shape found in both fish species. Fibers were found about 73.13 – 100% in the gill and 94.7 – 100% in the gastrointestinal of *O. niloticus* (Figure 4a - b). In *B. gonionotus*, fibers were also found sdominant in the gill (98.17 – 100%) and in the gastrointestinal (73.79 – 99.74%). These results were similar to the finding research from commercial fishes in Hangzhou Bay and Yangtze Ezutary, China, which found fibers as dominant MPs [81]. Other shapes of MP particles were discovered only in a small amount (Figure 4a - b). It might be caused by the fiber size, in which the smaller the particles, the more easily to be uptaken or ingested by the biota [81, 83].

![Figure 4](image)

**Figure 4.** The MP shapes in: a) the gill and b) the gastrointestinal of the fishes.

Based on the size, the LMP was the most commonly found MP particles in the gill and the gastrointestinal of both fish species (Figure 5a - b). The proportion of the LMP was 64.81 – 76.87% in the gill and 59.73 – 82.73% in the gastrointestinal of *O. niloticus*. Meanwhile, the LMP proportion in *B. gonionotus* was found about 57.80 – 70.34% in the gill and 67.02 – 71.47% in the gastrointestinal. Only small amount of the SMP was found in the gill and the gastrointestinal of both fishes (Figure 5a – b). Furthermore, the transparent MPs were the most commonly discovered particles in the gill (29.94 – 59.34%) and the gastrointestinal (27.52 – 58.80%) of *O. niloticus* (Figure 6a - b). However, black MP particles were observed as the most dominant ones in the gill (28.52 – 45.19%) and the gastrointestinal (28.72 – 47.93%) of *B. gonionotus* (Figure 6a - b). Different feeding behavior of the fishes might influence this situation, in which *O. niloticus* is omnivorous, while *B. gonionotus* is herbivorous [65]. Representative MP particles in the fishes were photographed and presented in Figure 7.

![Figure 5](image)

![Figure 6](image)
Figure 5. The MP size in: a) the gill and b) the gastrointestinal of the fishes.

Figure 6. The MP colours in: a) the gill and b) the gastrointestinal of the fishes.

Figure 7. The MP shapes in: a) black fiber, b) transparent film, c) blue film particles (Figure 7b was affected by microscope light)

3.2.3 MP characteristics in the bivalves. The MPs in the bivalves were mostly discovered as black fibers in the sized of LMP (Figure 8a - c). Fiber was found about 98.54 – 99.31% of the MPs in the bivalves at all sites, and only small amount of film particles (0.69 – 1.46%) (Figure 8a). These particles were categorized as the LMP (56.53 – 78.61%) and the SMP (16.04 – 43.37%) (Figure 8b). The MP colour in the bivalves were dominated by black particles (Figure 8c). This condition were similar to the finding results in commercial bivalves from Qingdao, China [84]. The deposition process and settling velocity of the fibers made the particles more easily to be settled than other shape particles in nature [85].

Figure 8. The MP characteristics: a) shape, b) size, c) colour in E. orientalis.
The MP particles were found in the water and the aquatic biota of the Surabaya River (Figure 2, Table 1 - Table 2). These results showed that MP had polluted not only the water, but also contained in the fishes and the benthic bivalves of the Surabaya River. The highest MP abundance in the water was found at S4 (Figure 2). Meanwhile, the highest MP abundance of *O. niloticus* and *B. gonionotus* was found at S1 and S3, respectively (Table 1). Besides, the highest MP abundance in the bivalves was found at S2 (Table 2). These results indicated that the relationship of MP distribution in the water and the biota has remained unclear [73]. This situation could be affected by several environmental conditions, even MP characteristics itself [19 – 21]. The dominant characteristics of MP particles in the water and the biota were dissimilar (Table 3). In the water, the MPs were dominated by transparent film particles. Besides, the MP particles in the gill and the gastrointestinal of *O. niloticus* were transparent fibers. Meanwhile, black fibers were dominant found in *B. gonionotus* and *E. orientalis*. The only similarity of MP characteristics in the water and the biota was particle sized of LMP. These results have confirmed that MP distribution in the environment can be affected by their characteristics itself, which is also supported by other environmental conditions [20, 21].

**Table 3.** Comparison of dominant MP characteristics in the water and the biota

| Sample                  | Dominant MP Shape | Dominant MP Size | Dominant MP Colour |
|-------------------------|-------------------|------------------|--------------------|
| Water                   | film              | LMP              | transparent        |
| *O. niloticus* (gill)   | fiber             | LMP              | transparent        |
| *O. niloticus* (gastrointestinal) | fiber          | LMP              | transparent        |
| *B. gonionotus* (gill)  | fiber             | LMP              | black              |
| *B. gonionotus* (gastrointestinal) | fiber         | LMP              | black              |
| *E. orientalis*         | fiber             | LMP              | black              |

4. Conclusion

Microplastics have polluted the Surabaya River water and the aquatic biota. The MP pollution in the water showed a fluctuating trend along the river. The highest MP abundance in the river water was found at Joyoboyo (S4). Meanwhile, the highest MP content in the fishes was found at Driyorejo (S1) and Karang Pilang (S3). Besides, the highest MP content in the bivalves was found at Bambe (S2). The MP abundance was also found higher in the gill than in the gastrointestinal of the fishes. Based on their characteristics, the MPs were mostly discovered as transparent film particles in the water, while transparent and black fibers were dominant in the biota. The film particles in the biota were only found in small amount. Polymer identification of MP particles is needed for plastic type identification.

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