Microplastic Pollution in the Inlet and Outlet Networks of Rawa Jombor Reservoir: Accumulation in Aquatic Fauna, Interactions with Heavy Metals, and Health Risk Assessment

Rita Rahmayanti, Basith Kuncoro Adji, and Andhika Puspito Nugroho*

Faculty of Biology, Universitas Gadjah Mada, Special Region of Yogyakarta 55281, Indonesia

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*** Corresponding author:**
E-mail: andhika_pn@ugm.ac.id

ABSTRACT

Streams are regarded as a pathway for spreading microplastics from land to various aquatic systems. The contamination of streams connected to the Rawa Jombor Reservoir may increase microplastic concentrations in the reservoir. The water coming out of the reservoir carries microplastics that spread out into the stream networks around the reservoir. Heavy metals have a high affinity for microplastics, increasing metal burdens on the surface of microplastics. The transfer of microplastics along the food chain leads to the possibility of increased adverse effects on organisms, mainly top predators. This research evaluated the accumulation and characterization of microplastics in water, sediment, and aquatic fauna (zooplankton, benthos, and fish); interactions with heavy metals (Pb, Cu, Cd, and Zn); and health risk assessment. Microplastics were collected from six sampling locations. The density, type of polymers, and color of microplastics were analyzed, as well as heavy metal concentrations on the surface of microplastics and a health risk assessment. The results showed microplastic contamination at a moderate level. The accumulation of microplastics in aquatic fauna showed the same pattern as microplastics in the environment. Microplastic concentrations in aquatic fauna showed an increase through trophic transfer and indications of biomagnification. Heavy metals were adsorbed on the surface of microplastics in high concentrations. Based on the health risk assessment, microplastic contamination of fish at the inlet and outlet of the Rawa Jombor Reservoir is still safe, but further monitoring is needed because of the possible long-term health hazards that may arise.

1. INTRODUCTION

Global plastic production continues to increase and become a global threat with the accumulation of plastic waste in marine and terrestrial ecosystems (Cox et al., 2019; Park et al., 2019). Most land-based plastic disposal from urban areas, industry, and agriculture are received directly by rivers and become a vital pathway runoff to marine ecosystems (Eriksen et al., 2013; Park et al., 2019). The increase in the accumulation of plastic waste is exacerbated by poor management of plastic waste disposal (Yuan et al., 2019).

Plastic waste that enters the aquatic ecosystem will undergo mechanical processes (erosion and abrasion), chemical processes (photo-oxidation and hydrolysis), and biological processes (degradation by microorganisms) breaking down into small pieces (micro and nano), which are then transported and spread over long distances following the water flow (Fan et al., 2019; Julienne et al., 2019). Physical, chemical, and biological degradation and fragmentation processes can cause the plastic to be fragmented into plastic pieces smaller than 5 mm, such as microbeads (Godoy et al., 2019; Naqash et al., 2020).

Microplastics (MPs) less than 5 mm in size can migrate and accumulate in water and sediment, and they have the potential to be ingested by aquatic fauna (Amin et al., 2020; Barboza et al., 2020). Aquatic organisms can ingest MPs due to the small particle size

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and confusion with food, which can possibly be harmful to organisms at higher trophic levels, leading to biomagnification (Akhbarizadeh et al., 2019; Barboza et al., 2019). The surface of MPs can adsorb additives and other chemicals such as heavy metals (Baalkhuyur et al., 2018; Barboza et al., 2019; Barboza et al., 2020). Association of MPs and toxic compounds may be harmful to aquatic organisms, causing respiratory disorders, nervous disorders, and damage to lipid oxidation in fish (Barboza et al., 2020; Godoy et al., 2019; Naqash et al., 2020), while in humans, it can cause anemia, hypertension, nervous system disorders, brain damage, oxidative stress, and cell damage (Campanale et al., 2020), thus requiring a quantitative assessment of the human health risks.

Rawa Jombor Reservoir (RJR) plays a vital role in the life of the local communities as it is used for irrigation and tourist destinations (Alina et al., 2015). Several studies related to pollution in RJR waters indicated that the reservoir belongs to the light to moderately polluted category (Alina et al., 2015; Atmawati, 2012; Rina et al., 2020). One study related to heavy metals in the reservoir showed cadmium accumulation in the fish *Tilapia* and sediment that exceeded the applicable regulatory quality standards (Kusumaningtyas, 2015). Meanwhile, the aquatic network system around the Rawa Jombor Reservoir is vulnerable to MP pollution due to plastic waste disposal activities around the waters, floating restaurants, and tourist activity which causes an increase in the accumulation of plastic waste that enters through the inlet through Kali Ujung (KU) and exits through the outlet of Kali Sosrodiningrat (KS). Heavy metals (HMs) can be carried on the surface of microplastics through the adsorption ability of microplastic; this condition has the potential to endanger the RJR ecosystem. In addition, it is feared that there will be long-term effects on public health due to fish consumption from the RJR waters, thus requiring a health risk assessment.

This study evaluated MP contamination in the RJR inlet and outlet water network. It was new and complemented existing research. The abundance and characteristics of MPs were identified in surface water, sediments, and aquatic fauna (zooplankton, benthos, and fish) from the inlet and outlet water networks of the RJR. The relationship between MPs accumulation in aquatic fauna and the environment at each sampling location was analyzed using Principal Component Analysis (PCA). The interaction of MPs with US EPA priority HMs (Pb, Cu, Cd, and Zn) was also studied to determine the human health risk assessment based on Estimated Daily Intake (EDI), Target Hazard Quotients (THQ), Total Target Hazard Quotients (TTHQ), and Target Cancer Risk (TR). The results of this study can also provide essential information for policymakers in environmental management and water resources conservation.

2. METHODOLOGY

2.1 Study area

This study was conducted at the RJR inlet and outlet water networks, Bayat, Klaten, Central Java, Indonesia. The RJR is surrounded by limestone hills and obtains its water source from rainwater and partly from the Ujung River (Kali Ujung), located to the west of RJR (Arumsari, 2019). Sampling was carried out in May 2021. Six sampling stations with three replications in the inlet and outlet water networks were selected based on the assumption of the level of plastic pollution visually and fishing activities around the waters of RJR (Figure 1). Sampling in the river flow that enters the reservoir (inlet) will determine the concentration of MPs in the flow and its possible contribution to MP pollution in the RJR reservoir. In addition, sampling at the outlet station is to determine whether there is microplastic contamination in the reservoir area which can lead to the spread of microplastics outside the reservoir. The selection of sampling points was based on the condition of the level of plastic pollution visually in the RJR inlet and outlet locations.

Station inlet 1 (I1) was the main inlet that Kali Ujung passes and was near the fishing line (7°45’15.9”S 110°37’10.3”E). Station inlet 2 (I2) was an inlet where fishing activities were carried out, and near a floating shop, this inlet received water from underground and was the most polluted location from initial observations (7°44’52.8”S 110°37’32.2”E). Station inlet 1 Kali Ujung (I1 KU) was a river flow, which was a water source for reservoirs located in densely populated areas (7°45’06.4”S 110°36’43.5”E). Station outlet 1 (O1) was a basin outlet with water sourced from RJR and Kali Ujung; the water condition in O1 was relatively clean compared to other stations (7°45’46.1”S 110°37’28.5”E). Station outlet (O2) was an outlet that comes from reservoir water, and this outlet location was rarely a fishing location (7°45’34.4”S 110°38’08.9”E). Station outlet 1 Kali Sosrodiningrat (O1 KS) was an underground outlet

sourced from outlet 1 and was located in a densely populated area (7°46'34.7"S 110°37'18.7"E).

2.2 Sample collection

Water samples from six sampling stations in the inlet and outlet network were collected at a depth of 0-20 cm from the water surface. Samples were collected with three replications on the right, middle, and left sides of the water body under the same conditions through the sampling method described by McNeish et al. (2018). Water was sampled into a 2 L bottle from each stations. Then, the bottle was immediately closed to avoid MP contamination from the air.

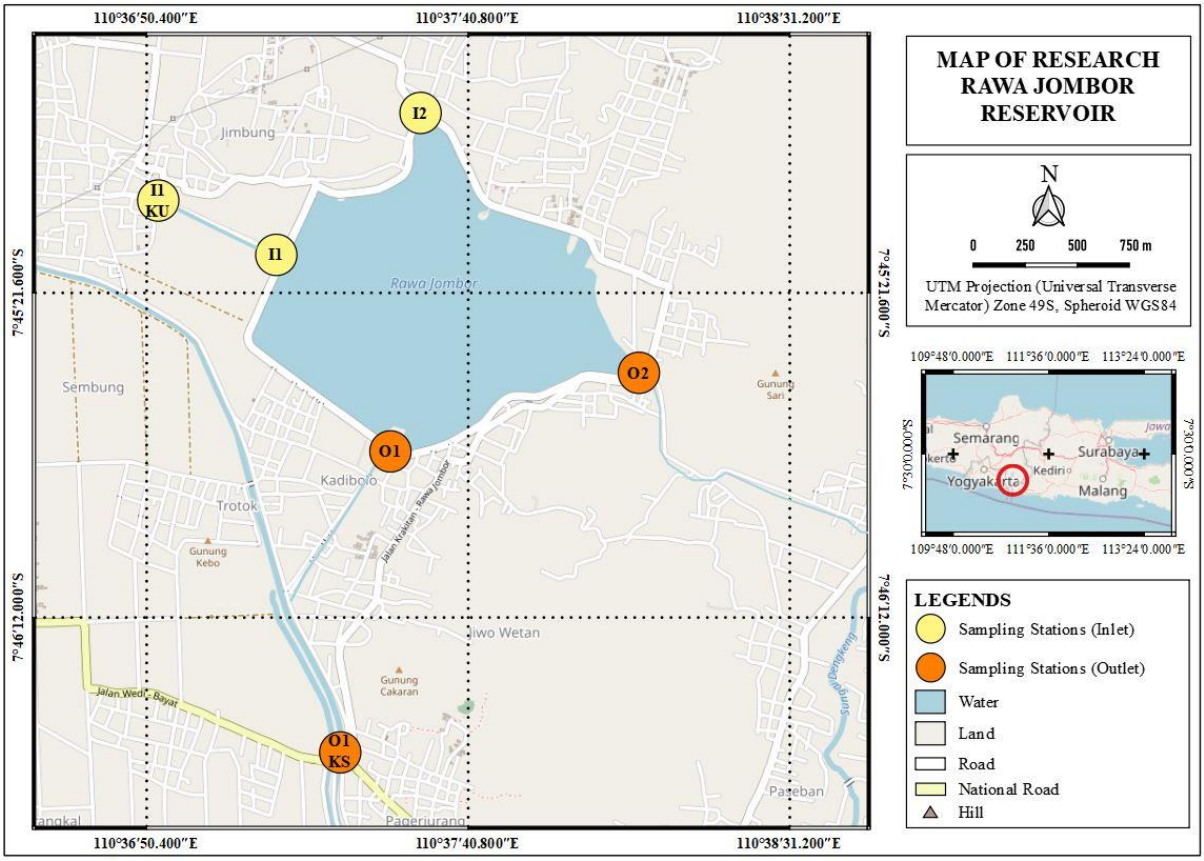

Figure 1. Location of sampling stations in the inlets and outlets of RJR

Sediment samples were taken from the surface water to a depth of 45 cm sediment layer using a 1 L Ekman dredge. In each site, three replications were randomly sampled using the grab sampling method described by Barrows et al. (2016). The filtered substrate was then dried, and density separation was carried out using NaCl to obtain MPs based on the MP particles flotation method (Wang et al., 2017).

Zooplankton samples were collected using a Wisconsin plankton net (200 µm) at a 0-20 cm water surface depth. Water containing zooplankton with a volume of 50 L was taken using a water sampler (10 L) and filtered using a plankton net for three replications at each station. The filter results were preserved with 1 mL of 4% formalin (Cole et al., 2013) and stored in a 10 mL vial.

Benthos samples were selected from the sediment sample collection at each sampling station. The benthos samples with a medium size (>5 mm) were then stored in an icebox in a labeled Ziploc container.

Fish were collected from fishermen around the stations. Several groups of fish commonly found by fishermen with a length of size include C. *batrachus* (21-31 cm), *O. marmorata* (17-26 cm), and *O. niloticus* (11-22 cm). Twenty-four fish were collected from all stations. The fish samples obtained were stored in an icebox. In the laboratory, all collected samples were stored in a freezer at -20ºC.

2.3 MPs extraction

MPs from the collected samples were extracted using the procedure according to the study of McNeish et al. (2018), Wang et al. (2017), and Hidalgo-Ruz et al. (2012), while for zooplankton samples we referred to the study of Amin et al. (2020).

The water sample was filtered using 0.45 µm filter paper (WhatmanTM, UK) to obtain MPs measuring less than 5 mm, referring to the recommendations of Sun et al. (2020). Filter papers containing MPs were stored in Petri dishes and labeled for further identification and characterization of MPs. The concentration of MPs in water samples was expressed in units of particles/L.

The sediment sample was dissolved in 2 L of concentrated NaCl (Merck, Germany), filtered through a 5 mm metal sieve, and left for 1 h to separate MPs based on density. The processed sample containing floating MPs was filtered using 0.45 µm filter paper. The papers were stored in Petri dishes for further identification of MPs. The concentrations of MPs were expressed in units of particles/kg wet weight (ww).

Zooplankton samples were identified using a light microscope to determine functional groups. In this study, copepods were found, then transferred to cavity blocks containing ten individuals each. Approximately 17-20 µL of 65% nitric acid (HNO_3) (Merck, Germany) was dripped into the cavity block (Desforges et al., 2015). The cavity block was then closed and heated at 80ºC for 30 min (Amin et al., 2020). The results of MP digestion were then counted and characterized by light microscopy (40-100X magnification). The concentrations of MPs in zooplankton were expressed in units of particles/10 individuals.

Benthos samples were measured in length and weight. In this study, *Pila ampullacea* was found at each sampling station. The contents of the benthos shell were cleaned with tweezers and transferred to a Beaker glass. We added 10% KOH (Merck, Germany) until the sample was submerged. The samples were then oven-dried at 60ºC for 24 h to dissolve the organic compounds and filtered with 0.45 µm filter paper. The filter papers were stored in a labeled petri dish for further identification. The concentrations of MPs in benthos were expressed in units of particles/individuals.

Fish were dissected and separated into the gills, GIT, and muscles. Each organ was weighed with a semi-analytical balance. For the extraction, each organ was then put into a Beaker glass and 10% KOH was added until submerged. The fish samples were then dried in an oven at 60ºC for 24 h to dissolve the organic compounds. The filtration result was filtered with 0.45 µm filter paper. The filter paper was then stored in a labelled petri dish for further identification. The concentrations of MPs in fish samples were expressed in units of particles/individuals.

The control filter paper was also analyzed to observe microplastic contamination in the laboratory, according to the study of McNeish et al. (2018). Filter paper control was carried out by filtering distilled water with 0.45 µm filter paper. In addition, to anticipate contamination in the digestion and cleaning process, a 10% KOH concentration was carried out with 0.45 µm filter paper. The control filter paper was then dried using an oven at 60ºC for 24 h. Contamination in control samples was used to compare abundances and characteristics found in laboratory samples.

2.4 MPs identification and characterization

MPs were identified using a stereomicroscope (Nikon SMZ745, Japan) and Optilab (Advance V2, Miconos, Indonesia), then characterized based on size, shape, color, and polymer type in each sample. The visual classification of MPs referred to McNeish et al. (2018) and Yuan et al. (2019). The size of MPs was classified into small (<1.5 mm), medium (1.5-3.3 mm), and large (>3.3 mm). MPs were classified into fragments, fibers, films, foams, and pellets based on the shape. Based on the color, MPs were classified into colored, white, black, and transparent.

The polymer type was identified using Fourier-transform infrared spectroscopy (FT-IR) (Nicolet Avatar 360 IR, Thermo Scientific, USA) with the reflectance mode range set at 4,000-400 cm^{-1} with a sensitivity of 50 at a collection time of 16 seconds. FT-IR test samples were taken based on the color and shape characterization of the MPs. The results of the FT-IR spectra were compared with the database in Jung et al. (2018) to determine the type of MPs polymers. Especially for zooplankton samples, the FT-IR test could not be carried out since the sample size was too small. The type of polymer was determined based on estimation by interpreting the physical characteristics of the MPs.

MPs surfaces were identified using Scanning Electron Microscope Energy-Dispersive X-ray

Spectroscopy (SEM/EDX) (Hitachi SU 3500, Japan) on randomly selected water, substrate, and fish samples. For fish samples, as a confirmatory material for heavy metals on the surface of MPs in the muscle, they were imaged using EDX at 10 keV with an SE sensor.

2.5 Heavy metal analysis

Heavy metals (HMs) Pb, Cu, Cd, and Zn were analyzed in the water, sediment, fish muscle, and on the surface of MPs found in water, sediment, and fish muscle.

The water sample in a 1 L HDPE bottle was filtered to separate out debris. The concentrations of HMs in water were determined using Atomic Absorption Spectroscopy (AAS) (Agilent 200 240FS AA Series, USA) and expressed in units of mg/L (Asare et al., 2018).

Dried sediment samples were crushed using a pestle and mortar, then sieved using a multilevel sieve to obtain fine particles (Asare et al., 2018). Sediment samples of as much as 250 g were tested for heavy metal content. The concentrations of HMs in the sediment were determined using AAS and expressed in units of µg/g.

Preparation for detecting HMs in fish muscle referred to the study of Asare et al. (2018). A 0.2 g dry weight of fish muscle was ground with a pestle and mortar and put into a 50 mL Erlenmeyer (Pyrex-Japan). Then, a total of 5 mL of concentrated H_2SO_4 (Mallinckrodt, USA) and 10 mL of concentrated HNO_3 were added to the Erlenmeyer. The sample was then heated at 130ºC for 20 min on a hotplate in a fume hood. After being cooled at room temperature, the sample was filtered with 0.45 µm filter paper to a 50 mL volumetric flask, then distilled water was added until it reached the limit. The concentrations of HMs in fish muscles were determined using AAS and expressed in units of µg/g.

The MPs from the characterization were weighed using an analytical balance and rinsed with distilled water to remove impurities. The samples were transferred into a Beaker glass. The procedure of HMs testing on the surface of MPs referred to the study of Munier and Bendell (2018). A total of 10 mL 10% HNO_3 was added and left for 2 h at 30ºC for metal decay on the surface of the MPs. The sample was then filtered with 0.45 µm filter paper in a 25 mL volumetric flask and added with distilled water until the mark. The samples were then tested for HMs (Pb, Cu, Cd, and Zn) concentration using AAS with the respective HMs limits of 0.5, 0.1, 0.1, and 0.1 mg/L. The concentrations of HMs on the MPs surface were expressed in units of µg/g.

2.6 Health risk assessment

A human health risk assessment was carried out based on an estimated consumption of fish contaminated with MPs and HM pollution. The health risk assessment of EDI referred to the study of Barboza et al. (2020) and Cox et al. (2019). The EDI of MPs was calculated using the equation (1):

$$\text{EDI of MPs} = \text{MP particles}\left(\frac{\text{particles}}{\text{g}}\right) \times \text{consumption rate (g/d/individual)} \quad (1)$$

The EDI of HMs was calculated following the study by Salam et al. (2020) using the equation (2):

$$\text{EDI of HM} = \frac{\text{HM concentration (µg/g) x consumption rate (g/d)}}{\text{body weight (kg)}} \quad (2)$$

The body weight value for Indonesian adults was assumed to be about 61.4 kg (NCD-RisC, 2020). The consumption rate of Indonesian adults was assumed to be about 130 g/d/individual (Firmansyah et al., 2019). RfD was used to evaluate the results of metal EDI calculations in fish muscle (Salam et al., 2020). RfD was the reference dose of HM referring to DeForest et al. (2007) (4.0 µg/kg/d for Pb; 40 µg/kg/d for Cu; 0.5 µg/kg/d for Cd; and 300 µg/kg/d for Zn).

The non-cancer risk assessment was calculated based on the THQ, the ratio between the estimated contaminant and reference doses (US EPA, 2000). THQ was calculated using the equation (3):

$$\text{THQ} = \frac{\text{EF x ED x FIR x C}}{\text{RfD x WAB x TA}} \times 10^{-3} \quad (3)$$

The EF value was obtained from the frequency of exposure (156 d/year) based on an estimate of eating fish about three times a week. ED was the duration of exposure (70 years), equivalent to the mean lifetime. FIR was the rate of food absorption. Based on Firmansyah et al. (2019), the FIR of Indonesian adults was 130 g/d/person. C showed the metal concentration in fish muscle (µg/g wet weight). WAB represented the average body weight of Indonesians, about 61.4 kg (NCD-RisC, 2020), and TA was the average exposure time for non-carcinogens (365 d/year×ED). As a consideration, if the THQ value was greater than 1, it was considered

harmful to the health of the person who consumes the fish (Khan et al., 2008).

TTHQ was calculated using the formula from Li et al. (2013) to determine if more than one HM can cause consumers to have several non-carcinogenic effects. TTHQ was calculated using equation (4):

$$TTHQ = THQ_{Pb} + THQ_{Cu} + THQ_{Cd} + THQ_{Zn} \quad (4)$$

TR, referring to the study of Liu et al. (2013) was calculated to assess the likelihood of cancer being caused by a particular carcinogen during the exposure period. TR was calculated using the equation (5):

$$TR = \frac{EF \times ED \times FIR \times C \times CSF}{WAB \times TA} \times 10^{-3} \quad (5)$$

The CSF value was an oral carcinogenic slope factor. The CSF value for Pb was 8. 8.5×10^{-3} $(\mu g/kg/d)^{-1}$ and for Cd was 6.3 $(\mu g/kg/d)^{-1}$ (US EPA, 2015). The cumulative TR value was also calculated based on the total individual trace metal risk using the equation (6):

$$\sum TR = TR_{Pb} + TR_{Cu} + TR_{Cd} + TR_{Zn} \quad (6)$$

2.7 Statistical analysis

Statistical analysis was conducted using one-way ANOVA to compare the significance of MPs concentrations in water, sediment, and aquatic fauna samples between stations. Tukey-HSD was performed for post-hoc analysis to determine the significance of each station. Principal Component Analysis (PCA) was performed to evaluate the relationship between MPs concentrations, sampling stations, and aquatic fauna.

3. RESULTS AND DISCUSSION

3.1 MPs in water and sediment

MP particles have contaminated water and sediment at all sampling stations, dominated by small (<1.5 mm) and medium (1.5-3.3 mm) sizes (Figure 2(a-b)). MP concentrations in surface water and sediment were significantly different at each station ($p<0.05$). Similar results have been reported in several studies, with MPs found predominantly less than 3 mm in size on the Brisbane River in Australia (He et al., 2020) and along the Han River in South Korea (Park et al., 2019). This finding proved that there is a process of plastic fragmentation and degradation into secondary MP particles. Plastic is decomposed into small particles due to the degradation process and chemical weathering by H_2O and CO_2 (Andrady, 2017).

The MPs observed in water were highest at station I2, about 20 particles/L (Figure 2(a)). The station was the most polluted site, a fishing spot near the floating restaurants and tourism. Fishing activities around the station may increase the concentrations of plastic waste from fishing tools (Stolte et al., 2015) and exacerbate poor plastic waste management (Yuan et al., 2019). The result was similar to the MPs found in the River Rhine, which generally detected 1-10 MP particles/L, but in the Rhine-Rhur metropolitan area, which is the most polluted area, the number of MPs exceeded 25 particles/L (Mani et al., 2015).

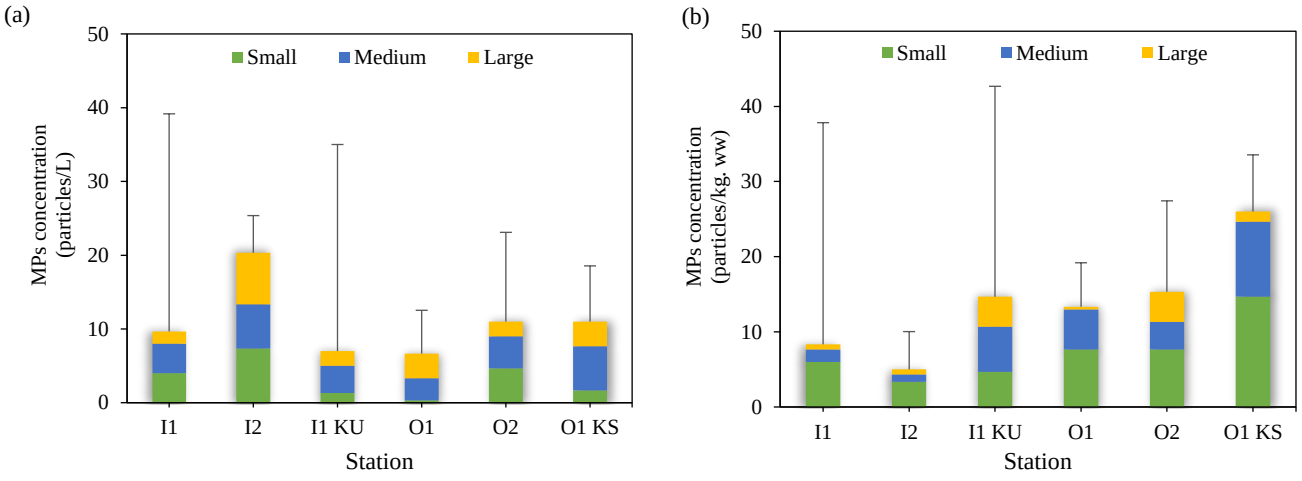

Figure 2. MP concentrations in water (a) and sediment (b) at each station. Different letters indicate significant differenced between sites at $p<0.05$.

In comparing MPs in water at the inlet and outlet area of RJR, this study showed the concentration at the inlet being lower (2.4 particles/L) than that of the outlet (4.5 particles/L), indicating microplastic pollution in the RJR. The result was also consistent with Ziajahromi et al. (2020), who reported 0.9 and 4.0 particles/L at the inlet and outlet of stormwater floating wetlands.

The concentration of MPs in water showed differentiated patterns and variables among the stations. Many factors in the water networks, such as currents, water turbulence, season, and weather, caused variations of MP concentrations in the streamflow (Bellasi et al., 2020; Mani et al., 2015). The influence of these factors is not visible, since the interactions of MPs and hydrological dynamics are very complex.

The MPs in sediment were inversely proportional to the water, lowest at station I2 (Figure 2(b)). The sedimentary structure probably affected the concentration of MPs. Gravels dominated the sediment of station I2, so that the possibility of MPs being trapped is lower than O1 KS dominated by clay and silt. He et al. (2020) reported that the concentration of MPs correlated positively with the content of clay particles. The concentration of MPs in sediments can be affected by current velocity, making it difficult to sink into the sediments (Jiang et al., 2018). Several factors, such as the retention process of MPs in sediments and sedimentation rates for certain particles, also affect the concentration (Mani et al., 2015).

The MPs in the water and sediment indicated that stream ecosystems require great attention to MPs pollution due to the possibility of aquatic fauna ingesting MPs directly from their habitat (water or sediment) or indirectly through trophic transfer.

Identification of polymer types by FT-IR showed that polyethylene (PE) and polypropylene (PP) were common polymer types of MPs (Figure 3(a)). The proportion of polymer PP and PE in water was about 68% and 32%, in sediment 32% and 53%, respectively. The MP pollution of inlet and outlet networks Rawa Jombor Reservoir may result from local input of plastic materials, such as fishery materials and household waste as secondary MPs. PP and PE are the most common polymers in plastic products, such as single-use packaging (Bordós et al., 2019). It is known that the two polymers have a lower density than water, so they are more commonly found on the surface of the water (Amelia et al., 2021; Andrady, 2011). When PP and PE interact with additives and attach to biofilms from the environment, it can increase their density (Pinheiro et al., 2020). Therefore, PP and PE can sink and be found in the sediment; benthic organisms can ingest them (Chubarenko et al., 2016).

In water, the most dominant MP shapes were film (33%) and fiber (28%). In sediment, the film was the most common MP (49%). All of them were dominantly colored MPs (Figure 3(b)-(c)). Similar results were reported by Fan et al. (2019) that the types of film, fiber, and fragment were the most dominant in Pearl River, China. It shows that plastics are degraded and fragmented into smaller sizes with different shapes based on the sources. Furthermore, management and mitigation of MP pollution are essential to reduce MP contamination to aquatic fauna.

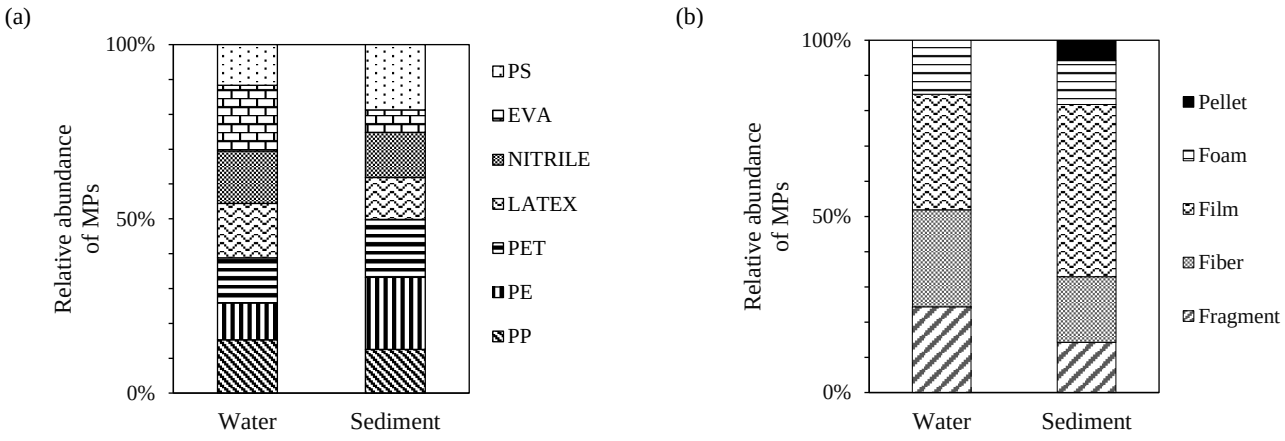

Figure 3. Relative abundance of polymer type (a), shape (b), and color (c) of MPs in water and sediment of Rawa Jombor Reservoir

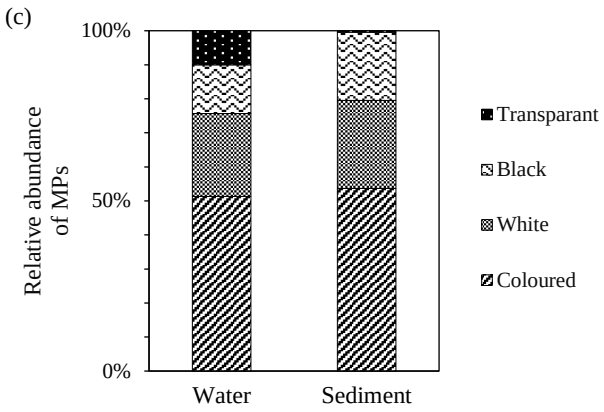

Figure 3. Relative abundance of polymer type (a), shape (b), and color (c) of MPs in water and sediment of Rawa Jombor Reservoir (cont.)

3.2 MPs in aquatic fauna

This study highlights the potential of MPs to be ingested by the food chain. Small MPs dominated the concentration of MPs in copepods (<1.5 mm), since the observed zooplankton sizes ranged from 0.5 to 2.0 mm. The ingestion of MPs is mainly due to the particle size of the MPs and the trophic position of the zooplankton in the water column (Desforges et al., 2015). MP concentration in zooplankton was highest at station I2 (Figure 4). These findings indicate that MPs in copepods tend to be associated with the concentration of MPs in water, the highest MPs in water being at station I2 (Figure 2(a)). In this study, MP concentration in copepods was not significantly different (NS) at each stations ($p<0.05$).

Several studies have reported that copepods are highly susceptible to MP exposure (Costa et al., 2020; Sun et al., 2018). However, studies of zooplankton in lotic waters are very limited. The concentration of MPs ingested by zooplankton was variable and inconsistent. It may be influenced by the region's abundance of MPs and zooplankton (Amin et al., 2020).

Similar to zooplankton, MPs were also found in benthic organisms, except in the O1 KS station. The most common benthic organism found was P. ampullacea. The MP concentrations in the benthos were more variable in size, with the medium MPs (1.5-3.3 mm) predominantly found (Figure 5). Benthic organisms compared to fish are more susceptible to ingesting MPs and have wide distribution, while current velocity can affect the ability of MPs to settle in sediment. Thus, it has been proposed to be used as a potential bioindicator of MP pollution (Li et al., 2020; Wang et al., 2019). The average MP concentration in benthos between stations was not significantly different (NS) at each station ($p<0.05$).

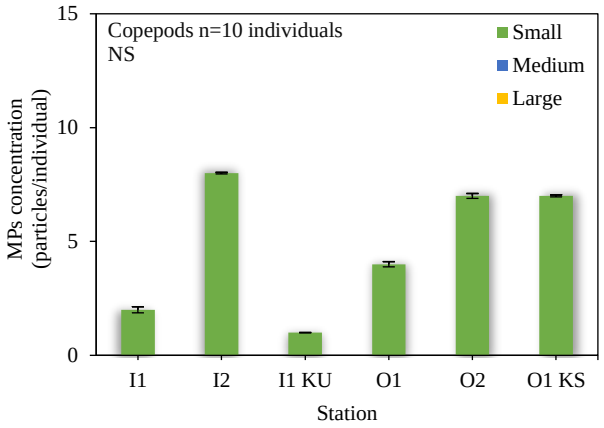

Figure 4. MP concentrations in Copepods at each station

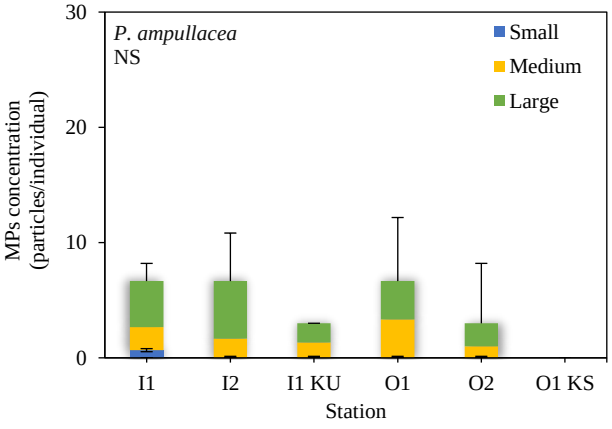

Figure 5. MP concentrations in *P. ampullacea* at each station

Zooplankton and benthos have an essential role in the food chain, affecting the accumulation of MPs at higher trophic levels. Zooplankton and benthos are susceptible to predation, so they can be the main route

for transferring MPs from one trophic to another higher, such as fish.

MPs were found in the gills, GIT, and muscle of fish at each station, small MPs (<1.5 mm) being the most commonly found (Figure 6(a)-(c)). This finding was in line with the study of Park et al. (2019), who found MPs with a size of <0.6 mm were dominant in the gills of *C. batrachus* and other fish. In this present study, MPs were found mainly in the gills. It may be related to direct interaction between the gills and the surrounding environment, MPs being easily trapped in the gills. As a comparison, in the research of Park et al. (2019), who reported MPs in catfish and other species from the Han River, South Korea, MPs in the gills ranged from 1 to 16 particles/fish, and in GIT ranging from 4 to 48 particles/fish, while in muscle no MPs were found. Inversely, this study observed that MPs tended to be lower in GIT. In addition, the average of MP concentration in fish between stations was not significantly different (NS) at each station ($p<0.05$).

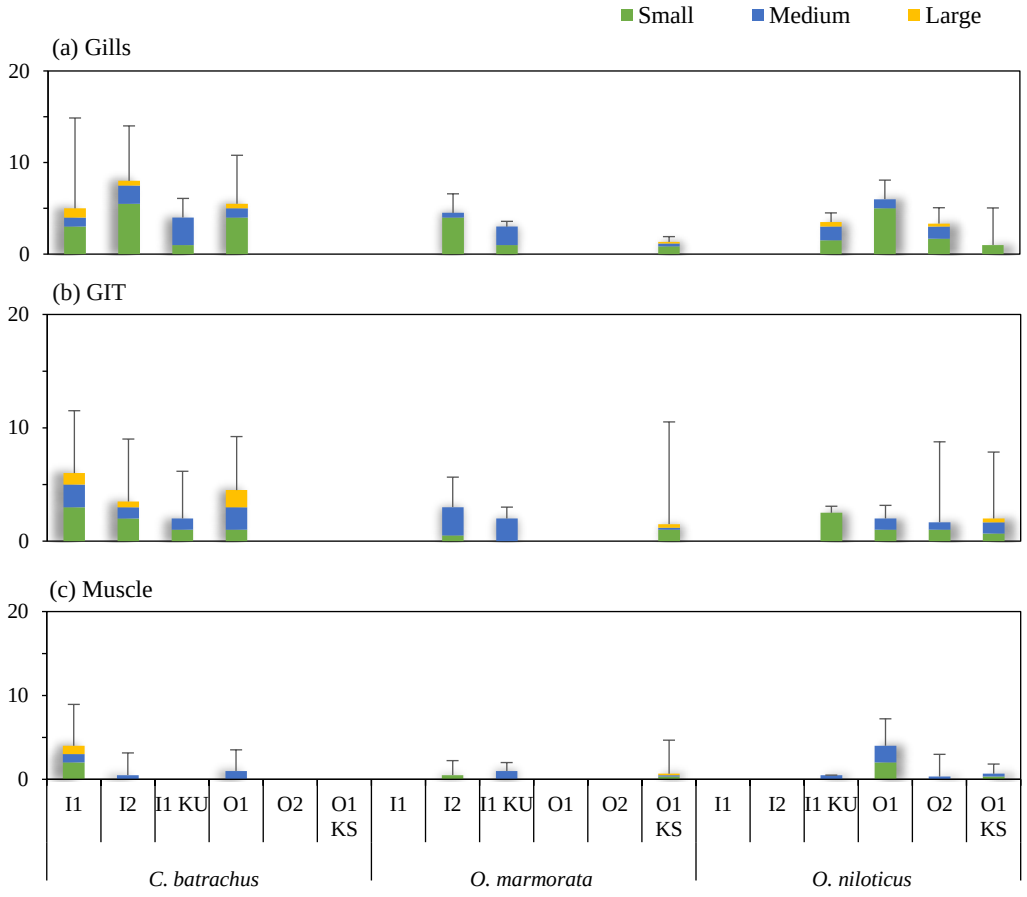

Figure 6. MP concentrations in fish gills (a), GIT (b), and muscle (c)

MPs in the gills resulted from retention during water filtration. It is also influenced by the size of the MP, morphology, and efficiency of the filtering apparatus (Barboza et al., 2020; Collard et al., 2017). MPs may have been ingested by fish directly from the water stream passively (e.g., gills) and actively (i.e., ingested by confusion with prey). The ingestion causes the accumulation of MP in fish organs (Barboza et al., 2020; Lusher et al., 2013). It is also possible that the fish ingested the plastic from the fishing tools used to catch the fish (Lusher et al., 2013). The buildup of MPs in the gills can cause fish to experience hypoxia and decreased respiratory efficiency, causing fish death in severe conditions (Barboza et al., 2020; Jabeen et al., 2018). The difference in the concentration of MPs was also influenced by several factors, such as several ecological characteristics (time spent in MP contaminated areas), physiological differences (e.g., water filtration rate and elimination process) in fish (Barboza et al., 2020), and eating habits (Baalkhuyur et al., 2018).

Some of the risks of plastic consumption depend on a variety of factors, including particle size, abundance, and plastic deposition in the environment

(i.e., similarity to prey), as well as the mode of feeding and the anatomy of the consumer's feeding/digestive organs (Desforges et al., 2015; Kaposi et al., 2014).

Polymer PP and PE, which are floating plastic polymers, showed the highest abundance in zooplankton (Figure 7(a)). This is confirmed by the study of Frias et al. (2014), which also found PE and PP as major polymers used in the last decade. In contrast to zooplankton, the types in benthic organisms were nitrile and EVA. Hoellein et al. (2017) and Hurley et al. (2021) reported that nitrile and EVA polymer types were commonly abundant in benthic organisms. This study also revealed that the dominant polymer type of MPs in fish was nitrile and EVA (Figure 7(a)). This is confirmed by the study of Ferreira et al. (2020), EVA is one of the most common polymers in fish organs.

The majority of MPs ingested by zooplankton was fibers and fragments (Figure 7(b)). This result was consistent with other studies that showed fibers and fragments are the common MP types in zooplankton (Amin et al., 2020; Payton et al., 2020). In benthic organisms, a higher amount of MP was found in the film form, followed by fibers and fragments. As expected, the film was the common type of MP in sediment. Benthic organisms generally ingest MPs, especially fibers, fragments, and some pellets (Li et al., 2016; Lusher et al., 2013; Wang et al., 2019). In fish organs, the colored fiber was also the common type of MP (Figure 7(b)-(c)). In addition, color plays an important role since the colored MPs are more likely to be ingested by fish due to confusion with prey and the color of the MP (Barboza et al., 2020).

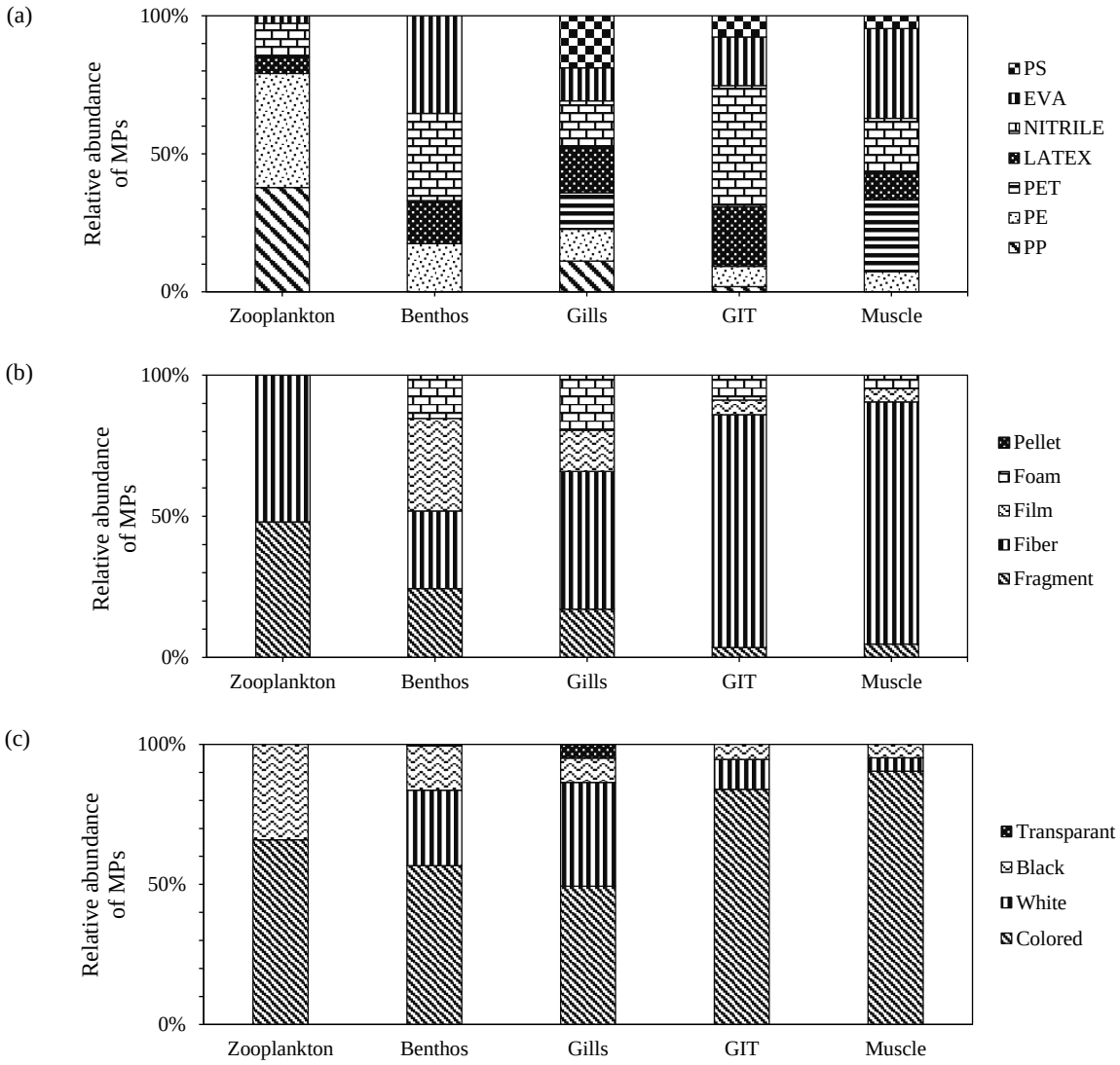

Figure 7. Relative abundance of polymer type (a), shape (b), and color (c) of MP in aquatic fauna of Rawa Jombor Reservoir

MPs found in aquatic fauna are similar to MPs found in water and sediments, with MPs being predominantly small and medium in size. This indicates that the smaller the MPs in the environment, the more likely they are to be ingested by aquatic fauna due to incorrect food preferences or inadvertently ingested by water currents (Barboza et al., 2020; Yuan et al., 2019). In addition, the accumulation of MPs can increase from one trophic to higher trophic levels as a potential for biomagnification and may affect human health risks due to additives adsorbed by MPs (Akhbarizadeh et al., 2019; Kumar et al., 2020).

SEM results showed that the surface of MPs tended to be rough, cracked, and had signs of deformation (Figure 8(a)-(c)). A rough surface can increase the surface area of the MPs and the possibility of metal residing on the plastic surface (Godoy et al., 2019; Wang et al., 2019). The degradation process from physical abrasion and hydraulic friction allows plastic particles to become smaller; this can also increase the potential for ingestion by aquatic fauna (Barboza et al., 2020). Thus, weathered MPs have toxicological effects on ingested aquatic organisms. These patterns of MP weathering were also found in the study conducted in the Pearl River, China (Yan et al., 2019) and in the Atoyac River basin, Mexico (Shruti et al., 2019).

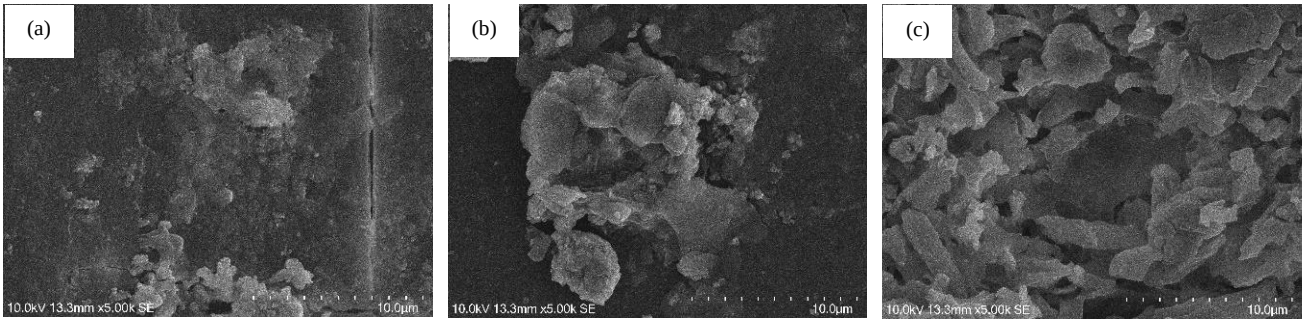

Figure 8. Mechanical weathering of surface MP in water (a), sediment (b), and fish muscle (c) from selected MPs

3.3 PCA Analysis

The analysis of PCA was conducted to identify correlations between the variables that contributed to explain the MPs concentration at each station. PCA results show that of the 13 active variables, there is a spatial distribution at the sampling stations in the four quadrants of the coordinate plane (Figure 9). The PCA biplot revealed an apparent clustering of MPs concentrations with PC1 and PC2, explaining >71.84% variation of the total variance. Station I2, with the condition of the water column being contaminated with MPs had a significant effect on the accumulation of MPs in zooplankton, benthos, and *C. batrachus* (gills). The highest MP contaminated sediment was found at the O1 KS station, and *O. niloticus* (GIT and muscle) accumulated many MPs. The MP concentration variable did not correlate with several stations such as the O1 and O2 station, which were quite clean from MP pollution.

3.4 MP interaction with heavy metal

HMs Pb, Cu, Cd, and Zn, were detected in water, sediment, fish muscle, and the surface of MPs of all samples. The concentration of heavy metals in the water and sediment among the stations varied (Table 1). This study explained that some heavy metals in water and sediment exceeded the permissible limits referring to the US EPA (2002) and WHO (2011).

The HMs were detected in fish muscle, with varying values (Table 2). According to the US EPA (2002) and WHO (2011), some exceeded the allowed limits. Accumulation of heavy metals in fish can vary due to fish size and mass, sex, and ability to bind heavy metals (Ozmen et al., 2008; Salam et al., 2020). Zn metal accumulation was detected higher than other metals. Zn metal was left in fish muscle since it binds to metallothionein proteins, which is an important component of fish muscle cells (Salam et al., 2020). In contrast, Cu was detected at very low levels. However, the long-term possibility is hazardous, such as Wilson disease and other disturbances of copper homeostasis (WHO, 2011).

Human activities are a significant contributor to heavy metal concentrations in the environment, such as agricultural and industrial residues carried into

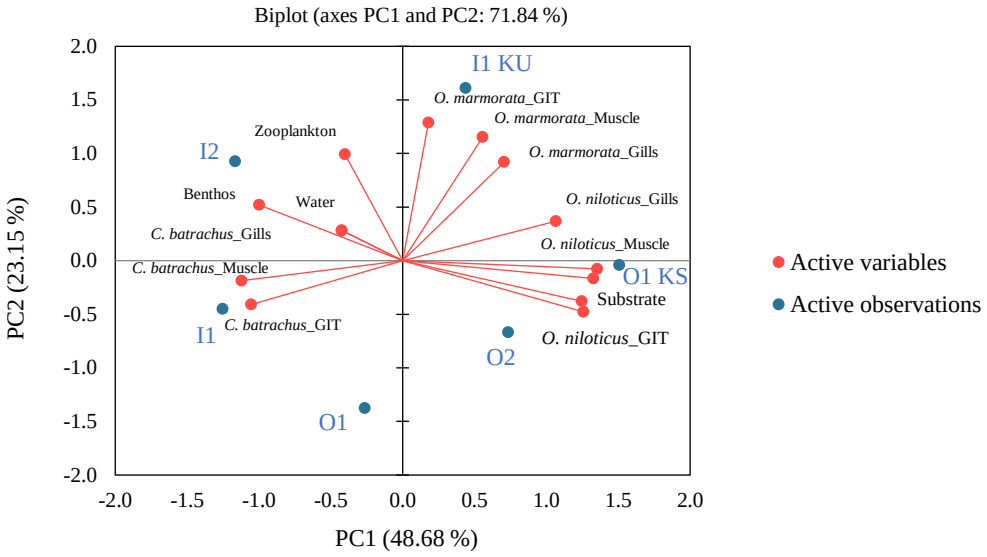

Figure 9. PCA biplot of MPs concentration

water bodies (Asare et al., 2018). Several findings reveal that persistent accumulation of heavy metals in the environment can harm aquatic fauna and possibly interact with MPs (Asare et al., 2018; Naqash et al., 2020). MPs can adsorb heavy metals in the environment. It is known that MPs have a high affinity for heavy metals Pb, Cu, Cd, and Zn, which can transfer to human tissues (Cox et al., 2019; Godoy et al., 2019). Brennecke et al. (2016) showed that MP polystyrene beads and PVC fragments could adsorb heavy metals Cu and Zn.

Additive translocation in MPs can occur along with food webs (Cole et al., 2016). In addition, exposure to MPs is harmful to aquatic organisms and humans in the long term and can cause chronic toxic effects when associated with toxic compounds (Cox et al. 2019; Godoy et al. 2019; Naqash et al. 2020). Unfortunately, the effects of MP consumption on human health are not known for sure, but these findings suggest that when MPs are in the GIT, they can release monomers and can absorb additives and toxins causing physiological damage (Cox et al., 2019; Naqash et al., 2020).

Table 1. Heavy metal concentration in water and sediment at each station

Heavy metal at each station	Heavy metal concentration			
	Water (µg/mL)	MP in Water (µg/g)×10^3	Sediment (µg/g)	MP in sediment (µg/g)×10^3
		Pb		
I1	0.14-0.14*	0.99-24.90	3.25-3.25	1.75-3.62
I2	0.18-0.18*	0.40-0.58	3.25-3.25	0.98-11.02
I1 KU	0.26-0.26*	0.70-14.50	3.25-3.25	0.35-0.64
O1	0.21-0.21*	0.86-6.59	53.38-53.38*	0.46-1.91
O2	0.28-0.28*	1.32-4.60	18.09-18.09	0.14-0.53
O1 KS	0.30-0.30*	0.76-17.20	3.25-3.25	0.18-0.44
		Cu		
I1	0.02-0.02	0.00-0.13	22.64-22.64	0.00-0.24
I2	0.05-0.05	0.00-0.67	26.87-26.87	0.00-0.51
I1 KU	0.05-0.05	0.00-1.13	5.31-5.31	0.00-0.03
O1	0.06-0.06	0.00-0.02	17.80-17.80	0.00-0.17
O2	0.06-0.06	0.00-0.02	13.57-13.57	0.00-0.09
O1 KS	0.06-0.06	0.00-0.14	5.47-5.47	0.00-0.05

*Indicating that the value exceeded the permissible limit by the WHO (2011) and US EPA (2002)

Table 1. Heavy metal concentration in water and sediment at each station (cont.)

Heavy metal at each station	Heavy metal concentration			
	Water (µg/mL)	MP in Water (µg/g)×10^3	Sediment (µg/g)	MP in sediment (µg/g)×10^3
		Cd		
I1	0.03-0.03*	0.00-0.54	0.85-0.85*	0.00-0.78
I2	0.09-0.09*	0.06-0.11	0.85-0.85*	0.00-0.00
I1 KU	0.09-0.09*	0.00-0.10	0.85-0.85*	0.00-0.08
O1	0.10-0.10*	0.00-0.19	0.85-0.85*	0.00-0.05
O2	0.09-0.09*	0.00-0.17	0.85-0.85*	0.00-0.01
O1 KS	0.10-0.10*	0.00-6.35	0.85-0.85*	0.02-0.16
		Zn		
I1	0.00-0.00	0.00-0.42	74.59-74.59	0.00-0.20
I2	0.03-0.03*	1.37-0.15	75.86-75.86	0.33-3.77
I1 KU	0.05-0.05*	0.18-1.28	28.02-28.02	0.07-0.32
O1	0.03-0.03*	0.17-0.58	24.72-24.72	0.21-1.10
O2	0.10-0.10*	0.08-1.22	51.77-51.77	0.12-0.90
O1 KS	0.04-0.04*	0.14-8.20	22.89-22.89	0.00-0.91

*Indicating that the value exceeded the permissible limit by the WHO (2011) and US EPA (2002)

Table 2. Heavy metal concentration in fish muscle and MPs ingested by fish

Heavy metal at each station	Species	Heavy metal concentration							
		Pb		Cu		Cd		Zn	
		Muscle (µg/g)	MP in Muscle (µg/g)×10^3	Muscle (µg/g)	MP in Muscle (µg/g)×10^3	Muscle (µg/g)	MP in Muscle (µg/g)×10^3	Muscle (µg/g)	MP in Muscle (µg/g)×10^3
I1	*C. batrachus*	-	11.95-11.95	0.43-0.43	1.66-1.66	0.02-0.02*	0.00-1.36	0.50-0.50*	0.86-0.86
	O. marmorata								
	O. niloticus								
I2	*C. batrachus*	0.06-0.24*	0-5.80	0-0.06	0-1.83	0-0.30*	0-1.03	0.30-0.37*	0-0.86
	O. marmorata	0-0.26*	0-138.25	-	-	0-1.03*	0-22.25	0.40-0.87*	-
	O. niloticus								
I1 KU	*C. batrachus*	-	-	-	-	-	-	0.24-0.24*	-
	O. marmorata	0.07-0.07*	145-145	-	23.75-23.75	-	23.5-23.5	0.36-0.36*	13.25-13.25
	O. niloticus	0.03-0.06*	0-130	0-0.007	0-23.5	0-0.002	0-28.75	0.26-0.35*	0-20.50
O1	*C. batrachus*	0.07-0.08*	0-56.6	-	0-15.25	0.01-0.13*	-	0.33-0.49*	0-0.70
	O. marmorata								
	O. niloticus	0.05-0.05*	30.38-30.38	-	-	0.14-0.14*	3.69-3.69	0.39-0.39*	0.062
O2	*C. batrachus*								
	O. marmorata								
	O. niloticus	0.05-0.24*	0-118.25	0.03-0.15	0-19	0.22-0.40*	-	0.28-0.77*	0-61.75
O1 KS	*C. batrachus*								
	O. marmorata	0.06-0.25*	0-118.75	0-0.35	-	0-0.35*	0-11.25	0.16-0.43*	0-14.75
	O. niloticus	0.04-0.09*	0-122.25	0-26.25	0-26.25	-	0-3.75	0.33-0.46*	0-166.25

*Indicating that the value exceeded the permissible limit of the WHO (2011) and US EPA (2002).

The SEM/EDX analysis confirmed the presence of heavy metals on the MP surface in fish muscle (Figure 10). These findings validated several studies that stated heavy metals can be adsorbed on the surface of MPs, in which the ability of MPs to increase the concentration of toxic compounds can reach 106 times higher through the adsorption process (Naqash et al., 2020; Wang et al., 2017; Turner and Holmes, 2015).

3.5 Health risk assessment

The health risk assessment was calculated based on the estimated daily intake (EDI) of accumulated

heavy metals and MPs in each fish species in the inlet and outlet networks of the Rawa Jombor Reservoir (Table 3). The calculation of the value of MP accumulation in *O. niloticus* showed a higher EDI value than other species. This value was comparable with a previous study which showed that from 300 g of fish consumed, adults generally would consume an average of 2.29 MP particles/day (Barboza et al., 2020). This study also showed that regular consumption in the studied location would not result in health risks. The EDI value of heavy metal levels in each was more than the RfD value. Long-term accumulation of MPs in humans may cause a health hazard, but evidence is still limited.

All species of fish in this study were commercial fish commonly found and consumed by the residents. Therefore, the average trace concentration in fish was used for the calculation of THQ for residents. THQ and TTHQ values of heavy metals Pb, Cu, Cd, and Zn were lower than 1 for each fish species (Table 4). These results indicated that the health risks associated with metal exposure were not significant, and the types of fish at the inlet and outlet of the Rawa Jombor Reservoir were in the safe category for consumption.

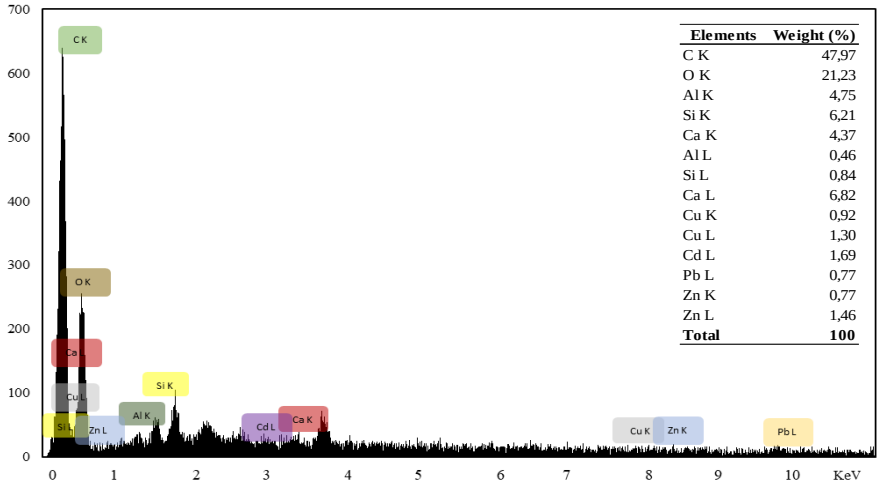

Figure 10. EDX spectra with the surface elemental composition of MPs

Table 3. Estimated daily intake of MPs and heavy metals due to consumption of fish from Rawa Jombor Reservoir

Species	EDI MPs (particles/day)	EDI HM in muscle (µg·kg/day)			
		Pb	Cu	Cd	Zn
C. batrachus	0.95	0.16	0.21	0.16	0.79
O. marmorata	0.79	0.24	0.21	0.14	0.83
O. niloticus	2.14	0.17	0.05	0.24	0.86

Table 4. Target hazard quotient, total target hazard quotient, and target cancer risk of heavy metals due to consumption of fish from Rawa Jombor Reservoir

Species	THQ ($\times 10^{-3}$)				TTHQ ($\times 10^{-3}$)	TCR (L/kg)	∑TR (L/kg)
	Pb	Cu	Cd	Zn		Cd	
C. bartrachus	0.00	0.18	0.02	0.00	0.20	0.0004	0.0004
O. marmorata	0.00	0.18	0.02	0.00	0.20	0.0004	0.0004
O. niloticus	0.00	0.05	0.03	0.00	0.07	0.0007	0.0007

4. CONCLUSION

MP contamination was found to be at moderate levels in the inlet and outlet networks of the Rawa Jombor Reservoir. Variations in polymer, shape, and color of MPs indicated that water is an important source of secondary MPs that can enter and be distributed to reservoirs with PP and PE as the commonly found polymer types. The most polluted

station, Inlet 2, showed high MP accumulation in the aquatic fauna. This finding indicates that the concentration of MPs in the environment is in line with the accumulation of MPs in aquatic fauna. This finding also indicates MPs in aquatic fauna increase through trophic transfer, indicating biomagnification. Furthermore, heavy metals were adsorbed on the surface of microplastics in high concentrations. The adsorption ability of MPs of heavy metals can endanger the health of the ecosystem. It can be dangerous if the accumulation of MPs occurs in trophic transfer and biomagnification. The condition of fish MP contamination in the inlet and outlet of the Rawa Jombor Reservoir was still safe for consumption, but long-term consumption may cause cancer with Cd as the main contributor. However, management and mitigation of MP pollution are important to reduce MP contamination.

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