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The Role of Biofilm on Microplastics as A Vector for Heavy Metals in the Waters of Sendang Biru, Malang Regency

Ahmad Nuril Fuad Al Fatih[1*], Andi Kurniawan[2], Maharani Pertiwi Koentjoro[3]

[1]Environmental Resource Management and Development Master Program, Brawijaya University, Malang 65145, Indonesia
[2]Departement of Fisheries and Marine Resources Management, Faculty of Fisheries and Marine Science, Brawijaya University, Malang 65145, Indonesia
[3]Laboratory of Integrated Science and Technology, The Graduate School of Universitas Brawijaya, Malang 65145, Indonesia

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ABSTRACT

Microplastic contamination in water provides an ideal surface for biofilm formation. It facilitates other pollutants, such as heavy metals, to attach to their surface. This interaction leads to biological and environmental problems. We tested this phenomenon by investigating the presence of microplastics in water, biofilms on surfaces, and heavy metal accumulation during September 2024. It further examines the relationship between microplastic and their contamination in fish. Samples were collected from two stations, namely Kondang Buntung Fish Auction Place (TPI) and the Indonesian Navy Post, water from Sendang Biru, using a purposive sampling method. A total of 20 liters of water and 6 Banyar fish (*Rastrelliger kanagurta*) specimens were analyzed. In contrast, biofilm morphology and heavy metal content were analyzed using Scanning Electron Microscopy with energy-dispersive X-ray. The result found that fibre-shaped microplastics, with fragments and filaments, dominated water samples. Microplastics 1-5 mm in size show that their colors included black, blue, red, and transparent. Five biofilm morphologies on the microplastic surfaces were identified: platelet, thin film, solid film, sporous, and diatoms. Heavy metals (Pb, Hg, Cd, Al, Mg, Fe) were detected with weight percentages ranging from 0.473 to 2.533%. Microplastics are found in the digestive tracts of fish, predominantly microplastic as filaments followed by fragments and fibers. We found that biofilm-coated microplastics accumulate heavy metals, posing a risk of pollutant transfer to aquatic organisms.

1. Introduction

Microplastics are not only present as passive particles but also play an active role in influencing the chemical and biological dynamics of the aquatic environment (Hale *et al.* 2022). The presence of biofilm on the surface of microplastics further strengthens the role of microplastics as a vector of pollution because the biofilm structure allows heavy metals to be adsorbed and concentrated on microplastics (Wright *et al.* 2021). This process increases the risk of exposure to heavy metals, both directly in organisms that consume microplastics and indirectly through the food chain.

Biofilms formed on the surface of microplastics play an important role as vectors of heavy metals in the aquatic environment. Biofilms are complex layers consisting of microorganisms, including bacteria, algae, and fungi, embedded in an extracellular matrix (Wright *et al.* 2021). The biofilm structure creates a highly reactive surface that is capable of adsorbing various pollutants, including heavy metals such as mercury (Hg), cadmium (Cd), and lead (Pb) (Li *et al.* 2021). In this context, microplastics not only function as a place for the development of biofilms but also as vectors that enable the widespread distribution of pollutants in aquatic ecosystems.

The main mechanism that makes biofilms effective as heavy metal scavengers is the ability of the extracellular matrix to interact with metal ions through chemical and physical bonds. Biofilm components, such as

* Corresponding Author
E-mail Address: alfatihhh50@gmail.com

polysaccharides, proteins, and lipids, have functional groups that can bind to heavy metals, thereby increasing adsorption efficiency (Flemming & Wuertz 2019). Once heavy metals bind to biofilms attached to microplastics, they can spread passively through water currents, expanding the area of pollution to areas far from the initial pollution source (Xu *et al.* 2023).

In aquatic ecosystems, biofilms on microplastics are also an entry point for heavy metals into the food chain. Small organisms, such as zooplankton and invertebrates, often consume microplastics because their size resembles natural food particles (Zhang *et al.* 2022). When microplastics contaminated with heavy metals enter the body of an organism, heavy metals can accumulate in their body tissues through a biomagnification process, and the concentration of these heavy metals increases at higher trophic levels, including large predatory fish that humans often consume (Li *et al.* 2023). This indicates that the role of biofilms on microplastics is an important link in the transfer of heavy metals from the aquatic environment to biota and even to humans.

Sendang Biru is one of the strategic water areas in Malang Regency, East Java, Indonesia. It has high biodiversity and is an important area for fishing activities. However, the intensity of human activity in this area increases the potential for microplastic contamination, which can have negative impacts on the ecosystem (Rochman *et al.* 2020). Research on the relationship between microplastics, biofilms, and heavy metals in this region is still very limited. Therefore, it is crucial to understand the role of biofilm as vectors in the distribution of heavy metals in the Sendang Biru aquatic ecosystem.

Previous research shows that heavy metals such as Pb, Hg, and Cd can stick to the surface of microplastics, creating new vectors for the spread of contamination (Rochman *et al.* 2013). Biofilms formed on microplastics are known to play an important role in the adsorption process of heavy metals (Wright *et al.* 2018). Based on this, this research offers a new approach by focusing on the relationship between microplastics, biofilm formation, and heavy metal accumulation in Sendang Biru waters. Apart from that, this research also utilized microplastic analysis in Banyar fish (*Rastrelliger kanagurta*) to confirm microplastic contamination in the food chain. The novelty of this research lies in the following: 1. Analysis of biofilm morphology on microplastic surfaces using Scanning Electron Microscopy (SEM), which is rarely carried out in similar studies in Indonesia. 2. Study of the quantity of heavy metals that accumulate in microplastics in coastal waters. 3. Analysis of microplastic integration in fish to identify potential transfer of contaminants through the food chain.

This study aims to analyze the relationship between microplastics, biofilm formation, and heavy metal accumulation in the waters of Sendang Biru, Malang Regency. Specifically, the objectives are to identify the types and morphology of microplastics present in the water, examine the biofilms formed on microplastic surfaces using Scanning Electron Microscopy (SEM), and measure the concentrations of heavy metals such as Pb, Hg, Cd, Al, Mg, and Fe accumulated on microplastic surfaces. Additionally, this research seeks to confirm the presence of microplastics in the digestive tracts of Banyar fish (*Rastrelliger kanagurta*), providing evidence of potential microplastic transfer within the food chain.

2. Materials and Methods

2.1. Location and Sample Collection

Water and fish samples were collected on waters of Sendang Biru, Malang Regency, at two stations with three replications for each location (Figure 1), namely Kondang Buntung Fish Auction Place (TPI) (8º25'38.27"S 112º42'4.18"T) and the Indonesian Navy Post (8º26'30.91"S 112º40'43.93"T).

2.2. Sample of Preparation

Sample collection was carried out using a purposive sampling technique, where samples were selected based on certain criteria that were relevant to the research subject. Therefore, the analysis was in accordance with previously determined parameters (Serra *et al.* 2018). Sample collection was carried out in the dry season in September 2024. Sample identification and analysis were carried out at the Ecology Laboratory of UIN Maulana Malik Ibrahim Malang and the Biosciences Laboratory of Brawijaya University.

(a) Preparation of Microplastic Samples in Water

Preparation of microplastic samples in water uses the method from Lusher *et al.* (2018), which begins with sampling using a tool such as a plankton net 20 µm with a certain mesh size to capture microplastic particles. Furthermore, the water sample was filtered using Wattman paper no 42 mesh to separate solid particles from liquid. In order to separate the microplastics from other particles, a high-density solution such as a NaCl solution with a density of 1.2 g/cm^3 was added in order to obtain microplastics easily for further analysis.

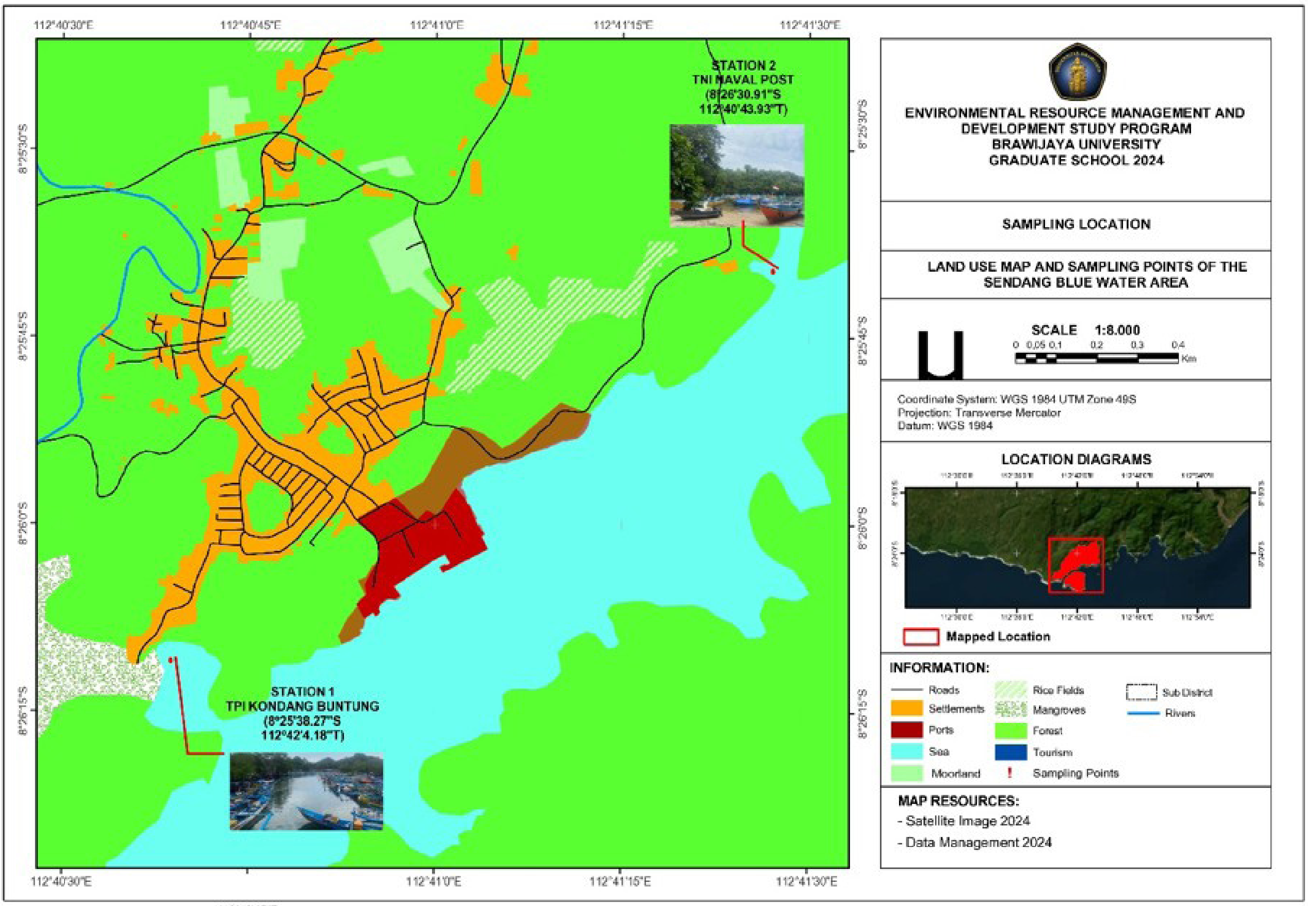

Figure 1. Sampling site location in Sendang Biru Waters

(b) Preparation of Microplastic Samples in Fish Digestive Tract

The fish used in this study were Banyar fish (*Rastrelliger kanagurta*), chosen for their ecological relevance in local marine ecosystems and their potential to accumulate microplastics, making them suitable for this research. A total of six fish were collected to ensure a representative sample size that was adequate for statistical analysis while maintaining sample integrity. The fish were stored under cold conditions to preserve their quality. The digestive tract of each fish was extracted through dissection and processed following the NOAA method developed by Masura *et al.* (2018). Organic tissue was dissolved using a 0.05% H_2O_2 and $FeSO_4$ solution for 48 hours (2 × 24 hours) to ensure complete removal of organic material without compromising the microplastic particles. The samples were then filtered using Whatman Qualitative Filter Paper no. 42 and dried in an oven at 50-60°C to prepare them for further analysis.

(c) Identification and Characterization of Microplastic Samples

Microplastic samples were collected from the water and the digestive tract of the fish, specifically from the stomach and intestines, as these areas are most likely to contain ingested microplastics. All parts of the digestive tract were not tested, as the focus was on the stomach and intestines, where microplastics are typically retained. The samples were observed using a stereo microscope (Olympus SZX16, magnification range: 40× to 100×), which allows for three-dimensional observation of microplastic particles. In this study, we referred to the methods outlined in "Microplastic Analysis: Methods and Protocols" by Richard *et al.* (2018) to identify the shape, size, and color of the microplastics.

2.3. Scanning Electron Microscopy with Energy Dispersive X-ray (SEM-EDX) Analysis

We performed SEM-EDX to observe the microplastic samples and visualize their surface morphology, focusing on surface texture, shape, and any alterations due to biofilm attachment, as well as to assess the presence of heavy metals. The samples were attached to carbon tape, coated with a conductive layer to enhance conductivity, and then placed in the SEM vacuum chamber (Zhang 2020).

3. Results

3.1. Occurrence of Microplastics in Water Samples on Sendang Biru Waters

Based on the results of the analysis carried out on water samples from Sendang Biru waters, Malang Regency, the microplastic exhibited diverse characteristics. As many as three types of microplastics were observed based on their shape, namely fiber, fragment, and filament microplastics (Figure 2).

Based on the analysis conducted, it was found that the water samples collected from the waters of Sendang Biru, Malang Regency, contained microplastics of various sizes, as presented in Figure 3. The microplastic sizes detected were: 1-5 mm (64%), 0.1-1 mm (25%), and <0.1 mm (11%). This data shows that the predominant microplastic size in samples is within the 1-5 mm range.

The characteristics of microplastics based on color, including transparent, black, white, and blue, are presented in Figure 4. It emphasizes that transparent microplastics are most commonly observed.

3.2. Biofilm Morphology Analysis on Microplastic Surfaces

Biofilm morphology on microplastic surfaces collected from the waters of Sendang Biru was performed using Scanning Electron Microscopy (SEM). Through this technique, various biofilm structures were observed, providing insights into the composition and development of biofilms on microplastics. Based on the SEM analysis (Figure 5)

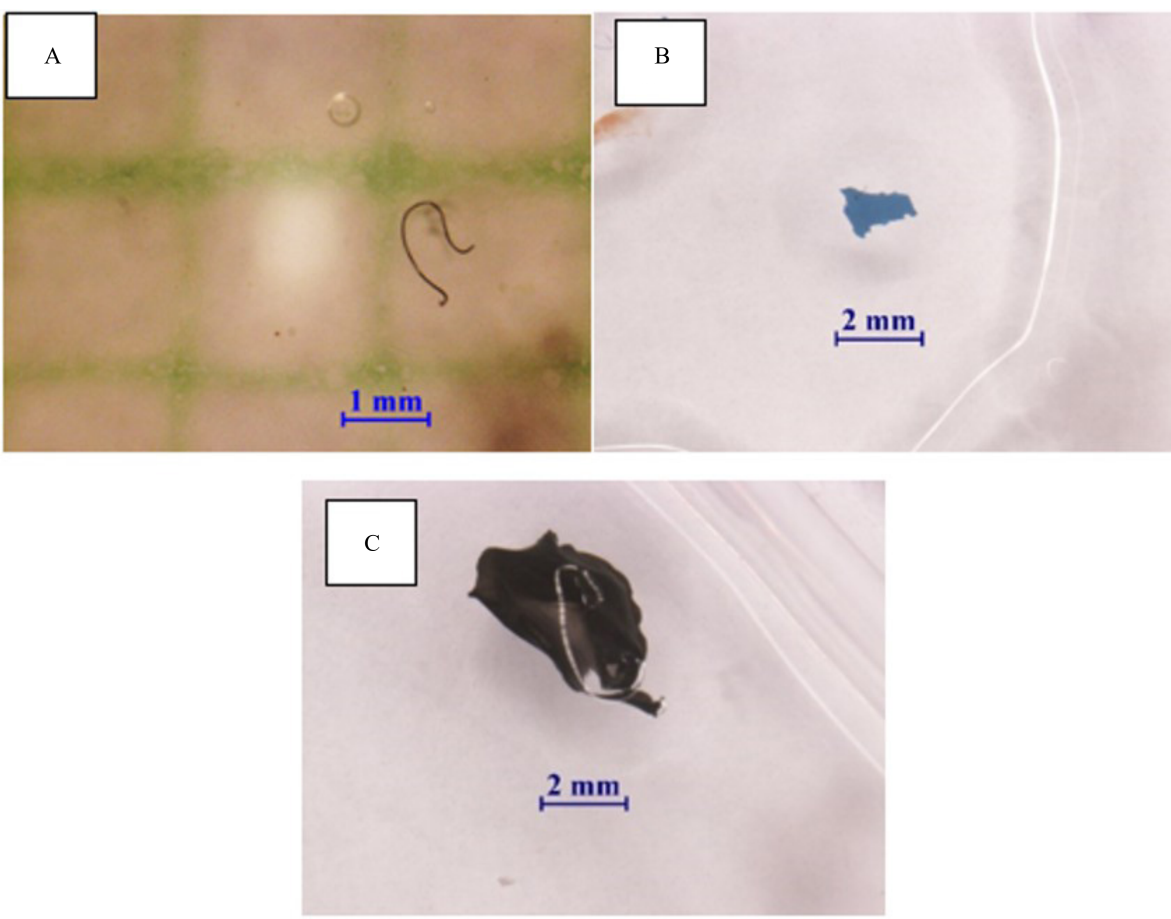

Figure 2. Identification of microplastics in water Samples Based on 100× Magnification. Information; (A) fibre, (B) fragment, (C) filament

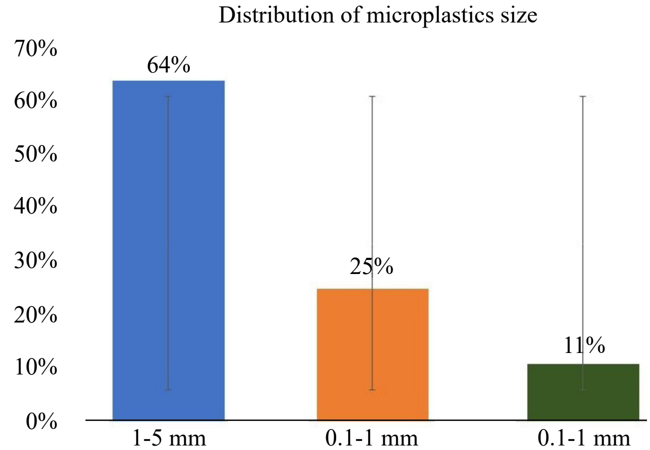

Figure 3. Distribution of microplastics sizes

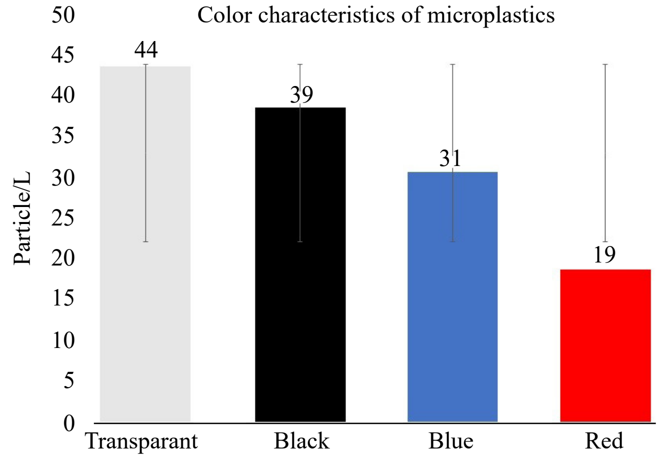

Figure 4. Color characteristics of water sample mcroplastics

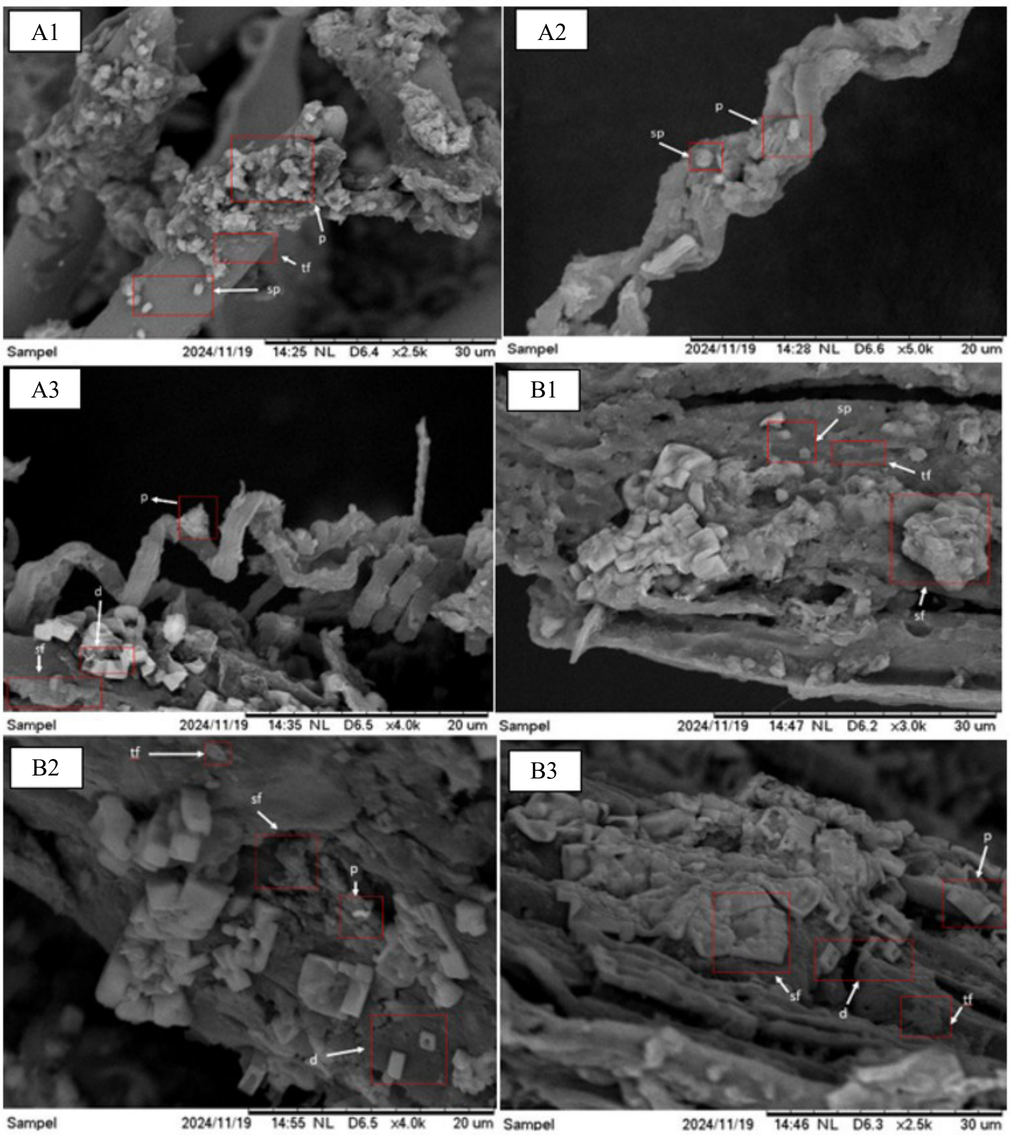

Figure 5. (A & B) SEM test results on microplastic samples magnification × (2.5k 3.0k 5.0k), description: A (TPI Kondang Buntung Station); B (TNI Navy Post Station). 1-3 (Repetition). p (Platelet Structure), tf (Thin Film), sf (Solid Film), sp (Sporus), d (Diatom)

revealed the presence of various biofilm morphologies on the surface of microplastics, including platelet structures, solid films, thin films, diatoms, and spores.

3.3. Analysis of Heavy Metal Content in Biofilm

EDX analysis was conducted on two selected suspected microplastics for heavy metal adsorption on biofilms. Based on the EDX analysis shown in Figure 6, the biofilms found on the surface of microplastics were identified to contain several heavy metal elements, including Pb, Hg, Cd, Al, Mg, and Fe. Furthermore, Table 1 indicates that the highest weight percentage values were predominantly associated with elements found at the TPI Kondang Buntung station.

3.4. Identification of Microplastics in Fish Digestive Tracts

Identification of microplastics was carried out on samples of Banyar Fish collected from the waters of Sendang Biru. Based on the identification results, several forms of microplastics were found, including fibers, fragments, and filaments (Figure 7). These microplastics were detected in the digestive tracts of the fish, indicating plastic contamination in their aquatic habitat. The presence of microplastics in the fish suggests potential risks to marine ecosystems and human health, particularly for those consuming fish from these waters.

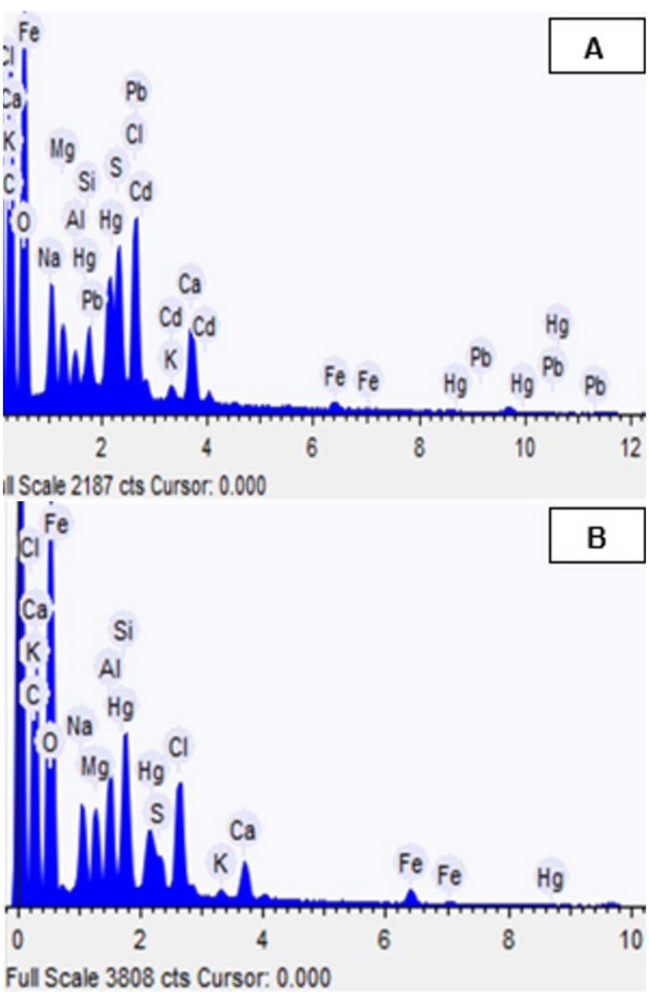

Figure 6. EDX Results of Biofilm Samples on Microplastic Surfaces. Description: (A) (TPI Kondang Buntung Station), (B) (Navy Post Station)

Table 1. Results of identification of heavy metal elements (weight%) using EDX

Station	Types of heavy metal elements (weight %)					
	Pb	Hg	Cd	Al	Mg	Fe
TPI Kondang Buntung	0.473	0.576	0.492	2.533	1.494	1.962
Navy post station	-	0.203	-	0.710	1.955	1.001

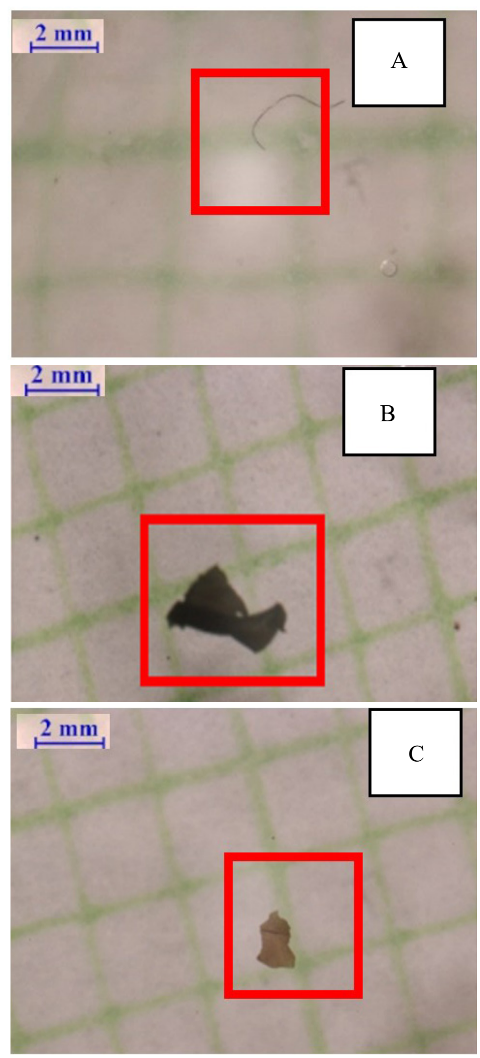

Figure 7. Identification of Microplastics Based on Shape 100 x Magnification. Information; (A) fibre, (B) fragment, (C) filament

Some of the colors of microplastics that have been identified include transparent, black, blue, and red. The number of microplastic colors found at each station is presented in Figure 8.

The results of the study indicate the characteristics of microplastics based on their size distribution as follows: microplastics measuring 1-5 mm accounted for 64%, those measuring 0.1-1 mm accounted for 30%, and those smaller than 0.1 mm accounted for 13%. These results are presented in Figure 9. This data shows that the predominant microplastic size in Banyar fish (*Rastrelliger kanagurta*) samples is within the 1-5 mm range.

4. Discussion

4.1. Microplastic Contamination in Sendang Biru Waters

The size distribution of microplastics in Sendang Biru waters shows a general pattern that is also found in other coastal areas. Microplastics of larger sizes (1-5 mm) tend to be located closer to coastlines, where human activity is more intense. Research by Lusher *et al.* (2017) showed that large microplastics often accumulate in the intertidal zone due to tidal processes and human activities such as coastal tourism and fishing. These large plastic particles may come from consumer products, such as plastic bottles and bags, which undergo physical degradation processes.

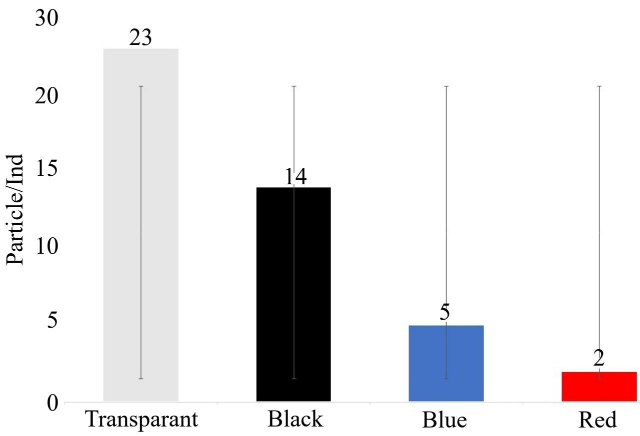

Figure 8. Color characteristics of microplastics in fish digestive tracts

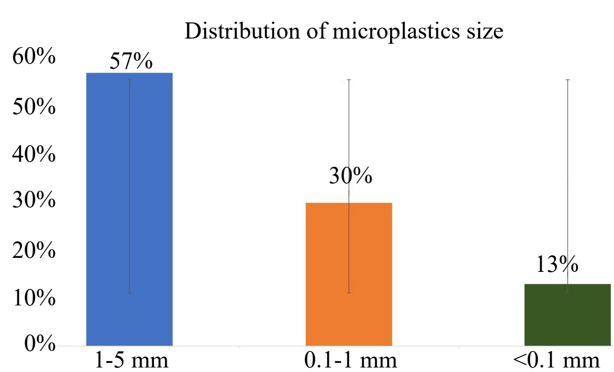

Figure 9. Size of microplastics in fish digestive tracts

The dominant type of microplastic is fiber. Several studies show that microplastic fibers often come from textile fibers, which are widely used in the fashion and household appliances industries. According to a study by Browne *et al.* (2018), microplastic fibers can form through the washing process of synthetic clothing, where fine fibers are released and enter the waste system.

In this study, it revealed that the color of the most commonly found microplastics, namely 44 particles, was transparent. These microplastics often come from more commonly used plastic products, such as food packaging and plastic bottles. This source can be closely related to the consumption habits of local people and the intensive tourism activities in this region. According to a study by Andrady (2017), transparent plastic products, such as packaging, have a high level of use, thereby increasing the potential for pollution when the waste is not managed properly.

4.2. Biofilm Morphological Characterization

The results of SEM analysis on the surface of microplastics visualized five different biofilm morphologies, namely platelet structure, thin film, solid film, sporous, and diatom. Platelet structures form organized layers that stick together like plates, often found in stable environments without much physical or chemical disturbance (Wang *et al.* 2023). Thin films have a thin and flexible morphology, forming in areas with fast water flow, thus allowing microorganisms to adapt to water movement (Li *et al.* 2023). Meanwhile, solid films have a denser and harder structure due to microorganisms, which produce a strong EPS matrix in response to polluted and unstable environments (Nguyen *et al.* 2023). Sporous morphology is formed as a survival strategy for microorganisms amidst adverse conditions and high pollution, where bacteria such as Clostridium difficile form spores to survive (Zhang *et al.* 2023). Finally, diatoms, which have silica-based cell walls, play an important role in biofilm formation and generally grow in good-quality waters (Wang *et al.* 2022).

Biofilm formation on microplastic surfaces begins with the process of adsorption of nutrients in the form of organic compounds such as amino acids, lipids, carbohydrates, and proteins suspended in water through hydrophobic interactions; this attracts microorganisms to colonize (Khan *et al.* 2023). Then, pioneer microorganisms such as bacteria attach to the microplastic substrate using adhesion structures such as flagella and fimbria, which facilitate initial interactions with the microplastic surface (Nguyen *et al.* 2023).

The presence of a variety of biofilm morphologies reflects the adaptation of biofilms to microplastic surfaces and local environmental conditions. On the other hand, previous research also revealed that microplastics with biofilms can be a transport vector for heavy metals in aquatic ecosystems, contributing to the bioaccumulation of metals in the food chain (Chen *et al.* 2023). This indicated that the biofilm on microplastics not only functions as a microbial habitat but also as an important link in the dynamics of heavy metal pollution in the aquatic environment. Microplastics covered by biofilms can increase the adsorption capacity of heavy metals thanks to the ability of biofilms to interact with these elements, allowing microorganisms to bind heavy metals through chemical and biological mechanisms (Zhao *et al.* 2019).

4.3. Heavy Metal Content

Based on the results of the EDX analysis, it is known that several types of heavy metals, including mercury (Hg), lead (Pb), and cadmium (Cd), were found to have high toxicity in waters. According to Zhang *et al.* (2021), heavy metals in the aquatic environment have varying levels of toxicity depending on their chemical properties, biological availability, and interactions with living organisms. The generally accepted order of toxicity is that Hg is more toxic than Cd, followed by Pb. This order is based on the chemical characteristics and biological effects of each heavy metal on ecosystems and the organisms within them (Wang *et al.* 2023). The higher toxicity order of Hg (mercury) compared to Cd (cadmium) and Pb (lead) reflects the extent of ecological and biological impacts these heavy metals have on aquatic ecosystems (Li *et al.* 2021). Mercury has the greatest toxic potential, followed by cadmium and then lead, each of which can disrupt ecosystem balance and the health of aquatic organisms in different ways, such as damaging tissues, interfering with metabolism, or even causing death (Zhang *et al.* 2021).

The process of adsorption or absorption of heavy metals by biofilms on microplastic surfaces begins when microorganisms begin to produce EPS. Heavy metals dissolved in water, such as Pb and Cd, are then adsorbed on the EPS through electrostatic bonding mechanisms and ligand binding to carboxylic and phosphate groups contained in the matrix (Xu *et al.* 2023). This process allows EPS to function as a site for heavy metal attachment, where the negative properties of the groups on EPS attract positively charged heavy metal cations. In addition, the complex EPS structure provides a large surface area for heavy metal binding (Zhang *et al.* 2021).

The ability of biofilms on microplastic surfaces to adsorb heavy metals also has a negative aspect, particularly in the process of removing these pollutants. As biofilms accumulate heavy metals, they can make it more difficult to eliminate these contaminants from the environment, potentially leading to a prolonged presence of harmful substances in aquatic ecosystems. According to Wang *et al.* (2021), it is explained that the potential for microplastics coated in biofilms to accumulate heavy metals carries ecological risks when fish or other aquatic organisms eat these microplastics. This process of removing pollutants can lead to the accumulation of heavy metals in the bodies of higher organisms, which has the potential to disrupt the food chain and overall ecosystem health. This is in line with the findings in this study, whereas six samples of Banyar Fish collected from the waters of Sendang Biru, Malang Regency contained microplastics.

4.4. Microplastic Contamination in Fish Digestive Tract

Our observation clearly revealed that filament-type microplastics are the most dominant in aquatic ecosystems because of their flexibility, ability to degrade into small fragments, and their similarity to plankton (Chen *et al.* 2021). These findings highlight the significant presence of microplastics as a contaminant in aquatic ecosystems, with fish being particularly vulnerable to ingestion. The small size and characteristics of microplastics, such as flexibility and the ability to degrade into even smaller particles, make them easily consumed by fish, either through accidental ingestion during feeding or via filtration (Koelmans *et al.* 2019)

In this condition, microplastics are easily swallowed by fish (Jabeen *et al.* 2017). The most commonly found microplastic colors are transparent and blue because they are often used in commercial plastic products. In contrast, black and red colors usually come from industrial waste, vehicle tires, or textiles (Chen *et al.* 2021). Microplastics measuring 1-5 mm dominate because they can still be ingested by fish through the feeding or filtration process (Andrades *et al.* 2022).

Microplastics measuring <0.1 mm are more potential risk because they can penetrate digestive tissue and accumulate in fish organs (Cole *et al.* 2023). This small size also increases the surface area of the particles, allowing the binding of harmful pollutants (Koelmans *et al.* 2019).

Recent studies have highlighted the pervasive presence of microplastics in various food sources, underscoring the urgent need to address this contamination due to potential human health risks. A study published in Frontiers in Toxicology by researchers from Portland State University's Applied Coastal Ecology found that microplastics and microfibers are prevalent in fish and crustaceans commonly consumed by humans. The study analyzed six species from the Oregon coast or local markets and found anthropogenic particles (APs) in almost all samples, with only the lingcod and herring being exceptions. Pink shrimp showed the highest concentrations of APs, presumably because they live near the surface where a significant amount of floating plastic is found. The researchers stress that microplastics' widespread contamination extends beyond seafood to other consumables like bottled water, beer, and vegetables. Assessing the broader implications of microplastic consumption for human health is crucial, given their potential negative effects on various bodily systems (Barboza *et al.* 2021).

4.5. Potential for Bioaccumulation of Heavy Metals in Fish Through Biofilms on Microplastics

This research indicates that the biofilm formed on the surface of microplastics contains high concentrations of heavy metals, such as Pb, Cd, and Hg, which are adsorbed from the aquatic environment. This could be a potential bioaccumulation of heavy metals in the fish's body because the fish samples studied in this study were confirmed to contain microplastics. In this case, microplastics act as a "base" for microorganisms that interact with the surrounding heavy metals. The adsorption process of heavy metals on microplastic surfaces can occur through physical-chemical interactions such as van der Waals forces and ionic bonds (Kumar 2020). When fish consume microplastics contaminated with biofilm, the heavy metals in the biofilm can enter the fish's body through the digestive tract. This has the potential to increase the concentration of heavy metals in fish body tissue, which in turn can lead to bioaccumulation, namely the build-up of heavy metals in fish body tissue over time (Rochman 2018).

Previous research has demonstrated that microplastics contaminated with heavy metals can be a significant source of exposure for aquatic organisms, including fish. For example, biofilms that develop on microplastics can increase the availability of heavy metals to organisms that consume them because these biofilm layers can act as storage for heavy metals that are easily available for absorption (Wang 2018). In this case, fish that eat biofilm-contaminated microplastics are at risk of accumulating heavy metals in their bodies, which can cause long-term health problems, such as organ damage and metabolic dysfunction (Pérez-Guevara 2020).

Apart from that, the presence of microplastics in the fish's body also has the potential to increase physiological stress because indigestible microplastics can cause inflammation or damage to the fish's digestive system (Anderson 2016). Therefore, the potential for bioaccumulation of heavy metals through biofilms on microplastics is an environmental issue that needs to be watched out for because it can contribute to pollution of the aquatic food chain and impact the health of the ecosystem as a whole.

Future research should focus on exploring the long-term physiological impacts of microplastics and associated biofilm-derived heavy metals on aquatic organisms, particularly fish. Investigations could delve into the molecular and cellular mechanisms underlying inflammation or damage in the digestive system caused by indigestible microplastics. Additionally, studies should assess the cumulative effects of heavy metal bioaccumulation from biofilm-coated microplastics on fish health, reproduction, and survival rates.

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