Analysis of Microplastics in Water and Biofilm Matrices in Lahor Reservoirs, East Java, Indonesia

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Abstract

One of the aquatic ecosystems that are very susceptible to contamination is the reservoir ecosystem. Reservoirs have different characteristics from other water bodies because they receive continuous water input from the river that flows through them. The river water contains organic and inorganic materials that can cause pollution caused by various kinds of human activities. The pollutant that is currently getting more attention is microplastics. Hence, various ways are developed to monitor the presence of microplastics in environments. The biotic component that may adsorb and accumulate microplastics is microbes that formed biofilm matrices as a predominant habitat. This study analyzes the presence of microplastics in water and biofilm matrices in Lahor Reservoir. The water parameters (pH, dissolved oxygen, temperature, and flow velocity) were also measured. Samplings were carried out at three stations, namely station A (inlet channel), station B (middle), and station C (outlet channel). This study revealed that water quality parameters were still relatively good according to the environment's quality standards. The average value of microplastic abundance in water at station A was 0.0013 particles.mL^{-1}, station B was 0.00083 particles.mL^{-1}, and station C was 0.00072 particles.mL^{-1}. The average abundance of microplastics in the biofilm at station A, station B, and station C was 7.55 particles.g^{-1}, 7.26 particles.g^{-1}, and 4.59 particles.$gram^{-1}$, respectively. This study indicates that the abundance of microplastics in the biofilm in the Lahor Reservoir was thousands of times higher than in the water. According to the results of this study, the biofilm can be used as a biological agent in monitoring the presence of microplastics in aquatic ecosystems such as the Lahor Reservoir, East Java, Indonesia.

Keywords: Aquatic Ecosystem, Biofilm, Microbial Ecology, Microplastics, Water Pollutant.

INTRODUCTION

The increase in population and urban development promote the change in people's consumption patterns. Along with the increasingly limited land for activities, the increase in human activities will put more significant pressure on the environment. Human activities in meeting their daily needs, such as agriculture, industry, and household activities, can produce waste that contributes to the decline in water quality [1].

One of the aquatic ecosystems prone to decreasing water quality is the reservoir ecosystem. A reservoir is an example of artificial freshwater created by damming a river. One of the reservoirs in Indonesia is the Lahor Reservoir. This reservoir is part of the Brantas River area development project, carried out in an integrated manner by the Brantas Project Agency. Lahor Reservoir is fed by three rivers: the Lahor River, the Leso River, and the Dewi River. Reservoirs have different characteristics from other water bodies because they receive continuous water input from the river that flows through them [2]. This river water contains organic and inorganic materials, which have great potential to cause pollution caused by various kinds of human activities [3]. One of the pollutants that are currently getting more attention is microplastics [4].

Microplastics are microscopic plastics found in all of the world's oceans, from beaches and coastlines to subtropical oceans, polar ice caps, and even the deepest parts of the oceans. The size of microplastics is less than 5 mm. However, the lower limit of the particle size included in the microplastic group has not been defined with certainty. The minimum limit often used as a microplastic size is 300 μm [5]. The existence of microplastics can provide various impacts, such as pollution. The impact of microplastic pollution can affect aquatic biota, enter the food chain, and ultimately impact human health. Microplastics can also be carriers of other harmful contaminants, both inorganic and organic [6].

Various attempts have been made to monitor the presence of microplastic contaminants in the ecosystem. One method that can be an alternative is biomonitoring [7]. Biomonitoring is

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a method for monitoring pollutants in an ecosystem using biological agents. The selection of biological agents is one of the primary keys to success in biomonitoring. Biological agents in biomonitoring must be able to accumulate pollutants effectively, easily found in aquatic ecosystems, and easily formed [8,9].

Biomonitoring technology has been developed using biofilms. Biofilms are the dominant form of microbial habitat in aquatic ecosystems [10]. Almost all microbes (> 95%) that live in nature live by forming biofilms [11]. Biofilms are abundant in the aquatic environment and play a variety of essential functions, including the accumulation and purification of pollutants [12,13]. This study used natural biofilm matrices composed of various microbes, such as bacteria, diatom, and algae [14]. The biofilm matrices can be used as biomonitoring agents for several heavy metals such as Pb, Cu, and Cr, Zn in aquatic ecosystems. However, research related to biofilms as biomonitoring microplastics in aquatic ecosystems is still scarce. However, several studies propose that biofilm can accumulate or adsorb microplastics from aquatic ecosystems [15]. Hence, the biofilms showed the potential of biomonitoring agents to monitor microplastics in aquatic ecosystems.

This study aimed to analyze the microplastic content in the water and the biofilm matrix in the Lahor Reservoir. The results of this study were expected as essential knowledge in the use of biofilm as a biomonitoring agent for microplastic pollution in aquatic ecosystems.

MATERIAL AND METHOD

Sampling Area

This research was conducted in the Lahor Reservoir, Sumberpucung Sub-district, Malang Regency, East Java Province, in September 2021. The Lahor Reservoir was built in 1972, has been operating since November 1977, and is part of the Brantas River Basin development project implemented in an integrated manner by the Main Implementing Agency for the Development of the Brantas River Basin.

The Lahor Reservoir is fed by three rivers: the Lahor River, the Leso River, and the Dewi River. Lahor Reservoir has an area of 260 Ha [2]. Water samples in this study were taken from three station points (Fig. 1). Station A is the channel near the inlet (inflow area) of the Lahor Reservoir. Station B is the middle part of the Lahor Reservoir. Station C is the channel near the outlet (outflow area) of the Lahor Reservoir. At each station, the sample was repeated three times. This study used the purposive sampling method to obtain various microplastics.

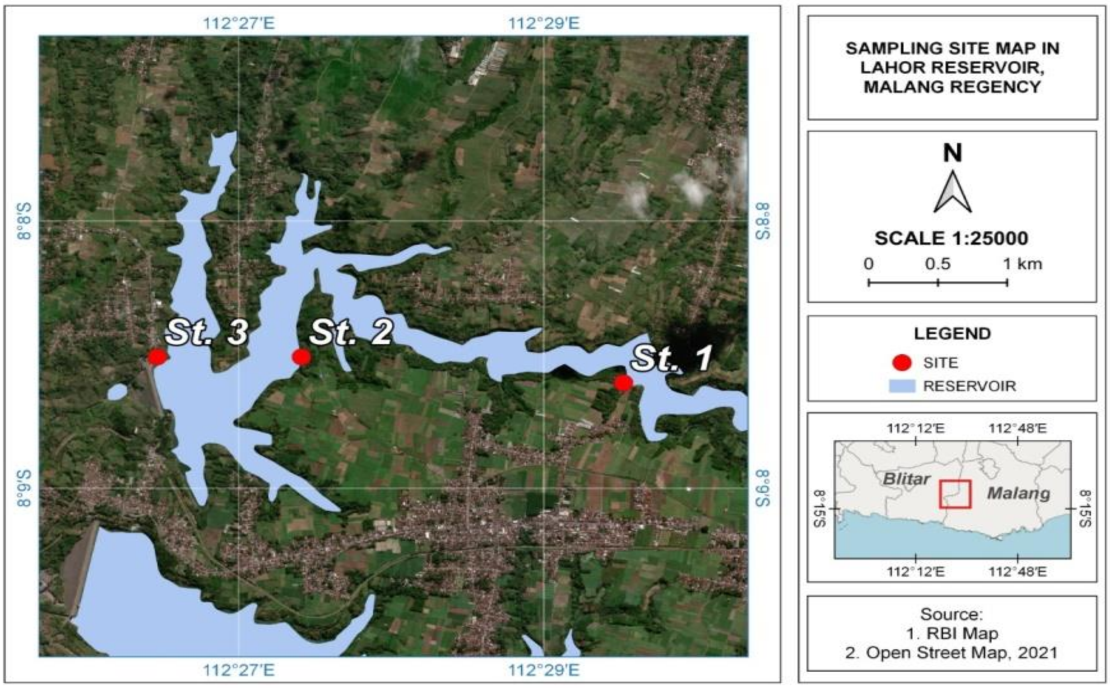

Figure 1. Sampling location

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Sampling Procedure

The samples in this study were water and biofilm taken from the Lahor Reservoir. Water samples were taken by filtering 16 L of reservoir water to 250 mL using a plankton net. The plankton net was rinsed with water after all the water samples were filtered so that no microplastic was left on the plankton net. The biofilm used in this study is a biofilm that naturally grows on rocks in the Lahor Reservoir. The biofilm on the rock surface was taken using the brushing method and suspended in 50 mL of sterilized distilled water [16]. Water and biofilm samples were stored in labeled sample bottles and put in a coolbox (± 4°C) for analysis in the laboratory.

Water Parameter Measurement

The environmental parameters measured in this study were temperature, pH, dissolved oxygen (DO), and water flow velocity. The temperature was measured using a thermometer. pH was measured using a Lutron PH-201 pH meter. Dissolved Oxygen (DO) was measured using a DO meter Lutron DO-5509. Water velocity was measured using JDC Flowatch (FL-03) Flowmeter.

Microplastics Analysis

The identification process of microplastic particles taken from water and biofilm in Lahor Reservoir, Sumberpucung sub-district, Malang Regency, East Java Province, was carried out using a modified method based on Masura et al. [17]. The first stage is a wet filtering process to obtain microplastic samples <5 mm using stacked 5 mm and 0.3 mm stainless steel mesh sieves.

The second stage was carried out by oxidizing water samples with the Wet Peroxide Oxidation (WPO) method or wet peroxide oxidation. It aims to remove organic material contaminants in the sample that interfere with the identification and characterization of microplastics. This step was carried out by adding 20 mL of 0.05 M $Fe_2SO_42H_2O$ solution to separate the microplastic sample from the metal, then 20 mL of H_2O_2 to dissolve the organic matter.

The mixture of solutions and samples was heated to 75°C on a hot plate to boil for ± 30 minutes. In the case of biofilm samples, after the WPO process was carried out, 6 g of NaCl.20 mL^{-1} of the sample was added to increase the density. The separation of organic matter and microplastics in biofilm samples was done using a density separator.

After the process, the third step was to filter the sample by vacuum filtration using Whatman filter paper. After the filtration process, Whatman filter paper was allowed to dry with the help of an oven at a temperature of ± 90°C.

The fourth stage was to identify microplastics filtered on Whatman paper filter media based on size, shape, and color through microscopic observation. Finally, the abundance of microplastics was calculated and shown as identified particles per volume that was calculated based on the below equation:

$$\textit{Microplastics abundance} = \frac{\text{Particle of microplastics observed (particle)}}{\text{Volume of water (mL)}}$$

RESULT AND DISCUSSION

Water Parameters

The environmental parameters measured were temperature, pH, DO, and water flow velocity in this study. These parameters are measured because they can affect the abundance and distribution of microplastics in the aquatic environment. The results of the measurement of these environmental parameters can be seen in Table 1.

The water temperature range of Lahor Reservoir was 28-30°C, pH ranges from 7.6 – 8.5, DO is 7.7 – 8.3 $mg.L^{-1}$, and current velocity is 0.06 – 1 $m.s^{-1}$. The water temperature in the Lahor Reservoir shows a value that is classified as optimum for the growth of organisms, including biofilm-forming microbes in the aquatic environment, which is ± 30°C [3].

The power of hydrogen is one of the environmental parameters that are very influential on the life of organisms in the waters, especially bacteria. A good environment usually has a pH value ranging from 6.5-8, while the ideal pH value in waters is 7-8.5. Concerning microplastics, the pH value affects the number of microbes, especially microbes that form biofilms and degrade microplastics, so that normal pH conditions in waters can support the life of microplastics degrading or adsorbing microbes [18].

The DO value in the Lahor Reservoir shows an optimum value. The ideal DO value for living organisms in the waters is at least 3 to 7 $mg.L^{-1}$. Dissolved Oxygen (DO) concentrations in waters also play an essential role in the life of biofilm-forming organisms and microplastic degrading agents. So, DO concentrations in waters will affect the degradation and adsorption processes of microplastics in waters [19].

Table 1. The results of water parameter measurements

No	Stasiun	Temperature (°C)	pH	DO ($mg.L^{-1}$)	Water Flow ($m.s^{-1}$)
1.	A (Inlet)	28	7.6	7.7	0.06
2.	B (Middle Area)	30	8.5	8.3	0.06
3.	C (Outlet)	28	7.9	8.1	1

Based on the speed of the water flow in the Lahor Reservoir, this reservoir is included in the category of reservoirs with prolonged water flows because the speed is not more than 1 $m.s^{-1}$. The spread of microplastics in waters depends on several factors, one of which is the speed of water currents. The speed of water currents becomes a medium for transporting microplastics in water. The movement of water makes the number of microplastics uncertain because it continues to change. Water currents will carry microplastic particles from one place to another in the waters. Microplastics will accumulate more in aquatic sediments because the water's currents are generally low at the bottom [20]. The slow speed of water flow makes the possibility of microplastic adsorption by biofilms higher.

Type of Microplastics in Lahor Reservoir

The observed water samples and biofilms found four types of microplastics based on their shape, namely fiber, fragment, film, and bead. The types of microplastics found can be seen in Figure 2. Primary and secondary microplastics dominated the microplastics found in the Lahor Reservoir. Primary sources refer to particles produced in small sizes, such as cosmetics and skin scrubbers. The primary source of microplastics found was in the form of beads. Secondary sources are microplastics produced by the breakdown or fragmentation of larger plastics due to exposure to solar ultraviolet radiation, weathering, or gradual weight loss due to physical damage [19]. The secondary sources of microplastics found were films, fragments, and fibers.

Microplastics' origin and route of entry determine the shape of these microplastics [20]. Fiber microplastics can come from the fragmentation of monofilament fishing nets, ropes, and synthetic fabrics. Microplastic pollution from anthropogenic sources such as household waste accounts, the largest microplastic is in the form of fragments [21]. The shape of the film has the physical characteristics of being flexible and thin [22]. Film microplastics are thought to come from pieces of degraded single-use plastic bags. Bead-shaped microplastics are produced from the rest of the raw materials for industrial activities, toiletry materials, soaps, and facial cleansers. Microplastics that float with irregular shapes tend to be attracted to water bodies and retained in water bodies [23]. Hence, microplastic particles in films and fragments tend to be found in this study. The previous study had been reported that the general type of microplastics found in the aquatic ecosystem was fragments, fiber, and film [24].

Abundance of Microplastics in Water

The abundance of microplastics in the water surrounding the biofilm in the Lahor Reservoir was analyzed in this study (Figure 3). The average abundance of microplastics in water at station A was 0.0013 $particles.mL^{-1}$, station B was 0.00083 $particles.mL^{-1}$, and station C was 0.00072 $particles.mL^{-1}$. The value of the abundance of microplastics in the waters measured in this study seems to be strongly influenced by the amount of waste input disposed near water sampling time.

The high and low abundance of microplastics in the waters is influenced mainly by the amount of microplastic waste input into the waters. The results of the measurement of the abundance of microplastics in the water presented in Figure 3 show that the highest abundance of microplastics is at the inlet location of the Lahor Reservoir at 0.0013 $particles.mL^{-1}$ (Station A).

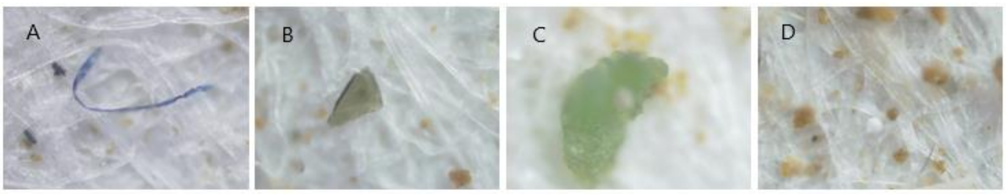

Figure 2. Type of microplastics identified in Lahor Reservoir (A: Fiber, B: Fragment, C: Film, D: Bead)

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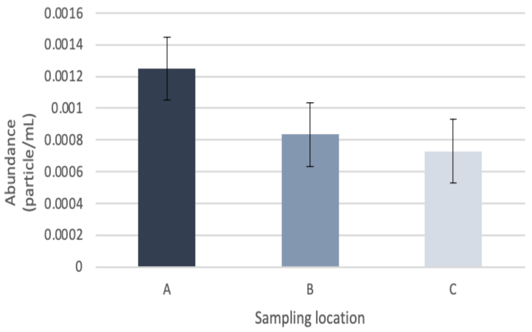

Figure 3. Abundance of microplastics in water of Lahor Reservoir

There was a decrease in the abundance of microplastics in the water along with the increasing distance from the inlet, which is the flow of three rivers, namely the Lahor River, Leso River, and Dewi River. It is suspected that this river flow contains organic and inorganic materials that have great potential to cause pollution caused by various human, domestic activities, anthropogenic activities that produce microplastic pollutants [2].

The second highest abundance of microplastics is in the Lahor Reservoir tourist location, 0.00083 particles.g^{-1} (Station B). There is a tourist activity in that area that can produce microplastic pollutants. The lowest abundance of microplastics was at the outlet channel of the Lahor Reservoir, which was 0.00072 particles.g^{-1} (Station C). The liquid waste from Station A will be carried by water flow from Station B to Station C. Along with this flow of water, microplastics can accumulate into various ecosystem components, including biofilms along the river. This accumulation process can result in a decrease in the abundance of microplastics in water as the distance from the input source of microplastic pollutants increases [25].

Abundance of Microplastics in Biofilm

The abundance of microplastics in the biofilm in the Lahor Reservoir was also analyzed in this study (Figure 4). The average value of the abundance of microplastics in the biofilm at station A, station B, and station C were 7.55 particles.g^{-1}, 7.26 particles.g^{-1}, and 4.59 particles.g^{-1}, respectively. These measurements indicate that the abundance of microplastics in the biofilm is thousands times higher than the abundance of microplastics in the water of the Lahor Reservoir. The high abundance of microplastics in the biofilm can be caused by the accumulation of microplastics from water into the biofilm [14]. The accumulation mechanism of microplastics into biofilms occurred through the interaction of electric charges between biofilms and microplastics [23].

The high abundance of microplastics at stations A and B indicated that the adsorption process of microplastics into the biofilm at this station occurred more than at other stations. This more adsorption could be since the current velocity is slower in this area than in other stations, thus allowing the contact time between microplastics in water and biofilm to be longer. This increased contact time allows more microplastics to accumulate in the biofilm [24].

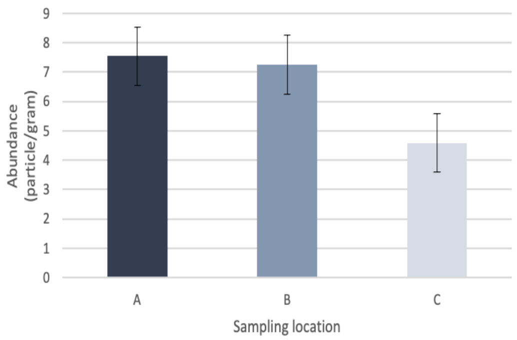

Figure 4. Abundance of microplastics in biofilm matrices formed in Lahor Reservoir

The decrease in the abundance of microplastics at station C maybe because most of the microplastics were tied in the biofilm's interstitial water as the largest part of the biofilm matrix (up to 98%) [26]. Microplastics adsorbed into the biofilm matrix do not completely adhere to the electrically charged sites but can be retained in the inter-polymer space in the biofilm. These results indicate that the microplastics adsorbed in the biofilm are very likely to be released back into the water around the biofilm depending on the abundance of microplastics between the biofilm and water [27]. If the abundance of microplastics between the biofilm and the surrounding water decreases, the microplastics will be desorbed from the biofilm.

Biofilm as Biomonitoring Agent

Based on the comparison of the abundance of microplastics in water and biofilm in the Lahor Reservoir, it is shown that at all stations (assuming 1 mL of water is equivalent to 1 mg) [14], the abundance value of microplastics in biofilms is thousands of times higher than the abundance values of microplastics in water. Therefore, the results of this study indicates that the abundance of microplastics in the biofilm is

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more than in water. Under these conditions, microplastics in the biofilm matrix may indicate that the microplastics have been present in reservoir water. Hence, biofilms can be used as biomonitoring agents for microplastic water pollution.

Measurement of the abundance of microplastics in water cannot fully reflect the conditions of pollution or the input of microplastics into the waters. This condition is mainly because, in aquatic ecosystems, the waste disposed of will immediately flow along with the movement of water. Suppose waste disposal is carried out at a difficult time to detect. In that case, it will be difficult for us to detect the presence of discarded waste. In order to evaluate the microplastic content in water, monitoring should represent the occurrence of waste input into the water even though it occurred long before sampling was carried out. Monitoring like this can be done by utilizing ecosystem components in the waters, both biotic and abiotic components [12]. One of the potential alternatives to be developed is the use of biofilms as biomonitoring agents. Plastic-biofilm interactions potentially influence the physical and chemical properties of the polymer, thereby leading to its degradation [28]. Hence, to mitigate the foreseen nuisance of microplastic contamination, biofilm is being looked upon as a potential candidate for remediation and or monitoring of aquatic environments.

CONCLUSION

This study indicates that the abundance of microplastics in the biofilm matrix in the Lahor Reservoir is thousands of times higher than in the reservoir water. It seems that biofilms can accumulate microplastics from the surrounding water. The abundance of microplastics in the biofilm and reservoir water around the biofilm indicates the activity of dumping waste containing microplastics into rivers that flow to the Lahor Reservoir or directly to the Lahor Reservoir. This study indicates that biofilms have the potential to be used as biological agents in monitoring the presence of microplastics in aquatic ecosystems such as the Lahor Reservoir, East Java. In order to develop biofilms in the management of aquatic ecosystems, it is necessary to conduct further research on the level of ability of microplastic accumulation by biofilms in various aquatic ecosystems to monitor microplastic pollutant waste in waters. Further research is needed to determine the adsorption process of microplastics in biofilms and determine the value of the quality standard for the concentration of microplastics in biofilms as a reference for evaluating the health level of the aquatic ecosystem.

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