Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(2): 1559 – 1575 (2025)
www.ejabf.journals.ekb.eg

Contamination of Microplastics in the Gastropod *Sulcospira* sp. from Upstream of the Brantas River in Indonesia

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ARTICLE INFO

Article History:
Received: July 9, 2024
Accepted: Feb. 29, 2025
Online: April 4, 2025

Keywords:
Microplastics,
Gastropod,
Fiber,
Water pollution,
Freshwater

ABSTRACT

Microplastics in river is potentially toxic and carcinogenic to aquatic life. Microplastics consumed by aquatic organisms have a negative impact on survival and endanger the aquatic food chain. The research objective was to determine the type and abundance of microplastics in the waters and gastropod *Sulcospira* sp. in the upper reaches of the Brantas River. The data were collected by sampling at five distinct sites based on land usage. The NOAA standard technique was utilized to detect and determine the quantity of microplastics in gastropods and water samples. The abundance of microplastics in water and sulcospira was determined using two-way ANOVA analysis. Microplastic abundance in water samples is 5600 particles/m^3 and 6.38 particles/individual in sulcospira gastropod samples. The predominant microplastic in the water and sulcospira samples is fiber type. Multivariate testing for various types of microplastics discovered in different samples and collecting locations yielded a $P<0.05$. The abundance of microplastics in water samples and sulcospira samples varied by station.

INTRODUCTION

Human dependence on goods made from plastic in daily activities continues to grow without realizing it because plastic offers several advantages. Plastic, as a commercial material, is more resistant and durable than other materials and is advantageous to a variety of parties, but its influence is viewed on the environment **(Barnes *et al*., 2009)**. Degraded large plastic wastes yield tiny plastics known as microplastics **(Plastics Europe, 2018)**. Microplastics are small plastic particles <5mm in diameter, making it easy for organisms to ingest and accumulate in their bodies **(Wagner *et al*., 2014)**. Microplastics present in freshwater are also a concern for research because microplastics can absorb chemicals and have toxicological effects related to the chemicals they release **(Wright *et al*., 2013; Su *et al*., 2019)**. As demonstrated by toxicological studies on the habitats of fish, benthic invertebrates, and zooplankton, microplastics pose environmental problems associated with their presence in the aquatic environment **(Szymańska & Obolewski, 2020)**. Microplastics not only affect the aquatic environment, but they also make their way into the bodies of fish and other aquatic animals **(Buwono *et al*., 2021a)**.

Gastropods, in general, cannot move swiftly and are easily affected by chemical pollutants **(Fauziah *et al*., 2012)**. Several studies have indicated that invertebrate organisms in aquatic environments consume a large amount of microplastics. Invertebrates in polluted coastal or intertidal zone ecosystems are more likely to ingest micro-sized particles due to their extremely small size **(Thushari *et al*., 2017)**. Bivalve mollusks are reported to have sucked up a lot of microplastics in their natural environment. More than just ingesting microplastics, some bivalves can accumulate microplastics for a long time with the detrimental risk of death **(Egbeocha *et al*., 2018)**. Microplastics were also found in mangrove snail *Littoraria scabra*, mud crab *Metopograpsus quadridentata* in Pramuka Pulau, Jakarta Bay, and gastropod *Nerita articulata* in Batu Karas, Pangandaran, Jakarta **(Patria *et al*., 2020; Azhari *et al*., 2022)**. Microplastic contamination of major rivers in Africa and Europe has been studied, and it has been discovered that gastropods are potential microplastic consumers in freshwater habitats **(Akindele *et al*., 2019)**.

The upstream part of the Brantas River starts from the Batu area in the north to the Malang area **(Lukitasari & Hendrajaya, 2016)**. The Brantas River upstream area is known to have witnessed significant growth beginning in 2000, which was characterized by an increase in population, changes in land use patterns, and the establishment of various sorts of industries **(Yetti *et al*., 2011)**. Waste buildup is thought to increase pollution in the upper sections of the Brantas River in East Java **(Widianto, 2020)**. The Brantas River is currently vulnerable to microplastic pollution.

Data on the presence of microplastics in the waters of the Upper Brantas River in East Java are still few, even though the degree of plastic pollution is considerable. In addition, there is still a lack of research on the analysis of the type and amount of microplastics found in water and gastropods in the upper reaches of the Brantas River, East Java. The study aimed to determine the abundance and type of microplastics in water and *Sulcospira* sp. sampled upstream of the Brantas River. A test was performed on the water from the higher sections of the Brantas River, as well as the creatures that reside in those waters to determine the degree of microplastic contamination in the river. The aquatic animal used as a bioindicator is the gastropod as *Sulcospira* sp. Gastropods are often used as bioindicators because they are suitable for evaluating water quality, namely their sedentary nature in an environment, relatively low levels of mobility, and easy identification **(Afwanudin *et al*., 2019)**.

MATERIALS AND METHODS

Sample collection and identification

This research was conducted using survey method upstream of Brantas River in East Java from June to July 2023. Based on land usage, sampling locations were identified at five stations (Fig. 1). Site 1 is relatively natural, located at the upper reaches of the Brantas River, surrounded by forests and agriculture. Site 2 is dominantly surrounded by residential areas. Site 3 is close to the cooking oil industry and densely populated residential areas. Site 4 is in densely populated areas. Site 5 is located near markets and residential areas. Each station collected ten gastropods *Sulcospira* sp. at random. The frequency of sampling is four repetitions per month.

The NOAA standard method was used to identify and calculate the amount of microplastics in gastropods and water samples. The abundance value of the organism sample is in particles/individuals whereas the abundance value of the water sample is in particles/m^3 in the NOAA Technical Memorandum NOS-OR&R-48 standard procedure **(Masura *et al*., 2015)**. Gastropod sampling was carried out by hand picking and kick net techniques **(Maramis *et al*., 2011; Mustofa *et al*., 2023)**. Water was sampled using a plankton net with a mesh size of 25µm, followed by wet sieving with stainless steel filters with stacked mesh sizes of 0.3 and 5 millimeters **(Buwono *et al*., 2021b)**. Gastropod and water samples were then treated with 2ml of 30% H_2O_2 and incubated in an 80°C water bath for 24h until the samples were clear **(Buwono *et al*., 2021a)**. The material was then filtered using filter paper Whatman No. 42 with the assistance of a vacuum pump. Microplastic examination with a Nikon Olympus CH-2 microscope at a magnification of 40x. Microplastics were classified according to their kind, which includes fiber, film, fragments, and pellets **(Hidalgo-ruz *et al*., 2012; Di & Wang, 2018)**. Quality control of microplastic contaminants was carried out using cotton lab coats and avoiding the use of plastic instruments. Distilled water was used in instrument sterilization procedures, and drying with materials made from synthetic fibers. Covering gastropod samples with aluminum foil reduces contact with air. Temperature (C), dissolved oxygen (mg L^{-1}), pH, and stream velocity (ms^{-1}), and total suspended solid (TSS) were measured as river water quality parameters. National Indonesia Standards (SNI) measurement procedure was used to water quality parameters testing.

Data analysis

Differences in the abundance of *Sulcospira* sp. and water samples were analyzed using two-way ANOVA. For the first phase of ANOVA, normality and homogeneity tests were performed on the data. Post-Hoc analysis using the Tukey test was used as a follow-up test to see which variables have significant differences.

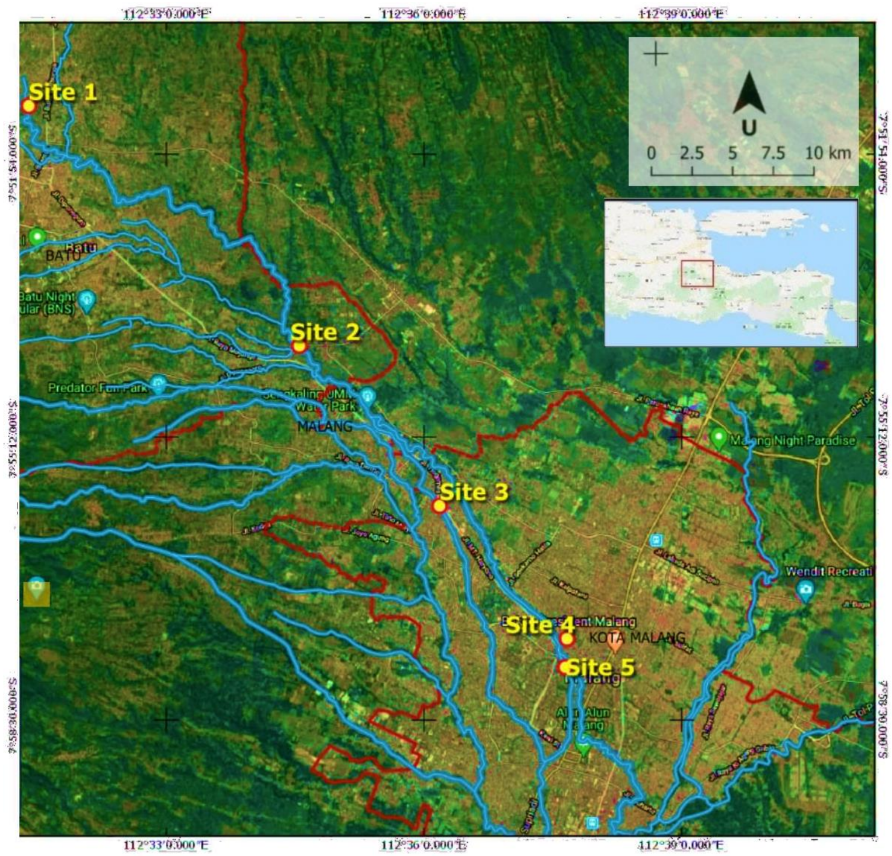


Fig. 1. Sampling areas of gastropod and water samples in upstream of Brantas River

RESULTS

In this study, *Sulcospira* sp. were found at all sites (Fig. 2). *Sulcospira* were mostly found at Site 2 up to 25 ind/m^2, with the least abundance at Site 5 up to 4 ind/m^2. *Sulcospira* sp. belongs to the kingdom Animalia, the phylum Mollusca, the class Gastropoda, the order Caenogastropoda, the family Pachycilidae, and the genus *Sulcospira* **(Molluscabase, 2023)**. The shell is thick, smooth, and opaque with a long conical shape. The outer layer is brown to blackish brown in hue.

The type of microplastics has been identified in water and *Sulcospira* sp. samples from THE upstream of the Brantas River. Based on Fig. (3), the same three types of microplastic were detected in the water samples and sulcospira, namely films, fibers, and fragments.

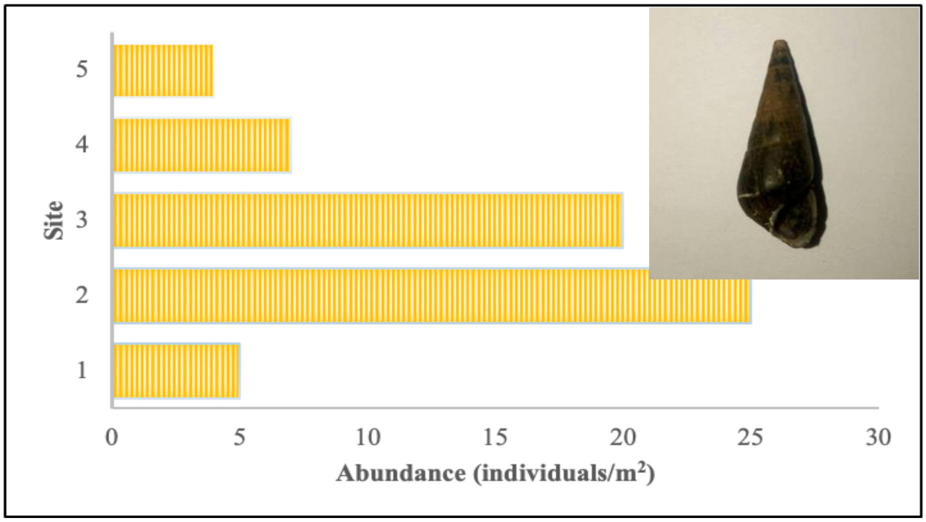
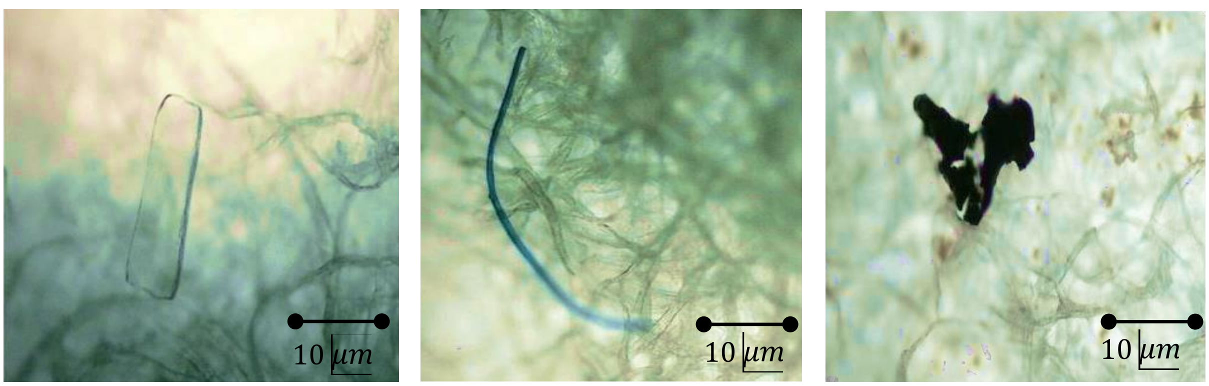


Fig. 2. The abundance of *Sulcospira* sp. at all sites in upstream of Brantas River



A **B** **C**

Fig. 3. Microplastics type in water and *Sulcospira* sp. in upstream Brantas River: **A.** Film; **B**. Fiber; **C.** Fragment

Table (1) shows the proportion of microplastic types in water samples, whereas Table (2) shows the percentage of microplastic types in *Sulcospira* sp. The level of microplastics in water samples varies from film (23.1%) to fiber (38.6%) to fragment (38.3%). In sulcospira, microplastics are distributed as follows: film (27.4%), fiber (40%), and fragment (32.6%).

Table 1. The percentage type of microplastic in water samples

Type	**Particles**	**Percentage (%)**
Film	97	23.1
Fiber	162	38.6
Fragment	161	38.3
Total	420	100

Fiber was the type of microplastic detected the most in the water and sulcospira samples, compared to the other categories. Whereas, film is a type of microplastic with the lowest percentage in both sample. Fragments are in second place after fiber in water and gastropod samples. The average abundance of microplastic in water samples is 5600 particles/m^3 and 6.38 particles/individual in sulcospira gastropod samples.

Table 2. The percentage type of microplastic in *Sulcospira* sp.

Type	Particles	Percentage (%)
Film	175	27.4
Fiber	255	40
Fragment	208	32.6
Total	638	100

The results of microplastic abundance in water samples obtained from the upstream of the Brantas River were subjected to normality and homogeneity tests. The normality test of Shapiro-Wilk with a *P*-value of 0.157 > 0.05 and Kolmogorov-Sminov with a *P*-value of 0.125 > 0.05 showed normal distribution. The homogeneity tests show that the sig 0.917 > 0.05 with decision homogeneous. It indicates that the abundance of microplastics in water samples may be investigated further using the post-hoc test.

Table 3. Post-hoc analysis on water samples at each station

Site		Mean difference	Sig.
1	2	-6.5*	0.02
	3	-7.2*	0.01
	4	-12.5*	0.00
	5	-14.5*	0.00
2	1	6.5*	0.02
	3	-0.7	0.81
	4	-6.0*	0.04
	5	-8.0*	0.01
3	1	7.2*	0.02
	2	0.7	0.81
	4	-5.3	0.07
	5	-7.3	0.01
4	1	12.5*	0.00
	2	6.0*	0.04
	3	5.3	0.07
	5	-2	0.48
5	1	14.5*	0.00
	2	8.0*	0.01
	3	7.3*	0.01
	4	2	0.48

Significance level < 0.05.

Table (3) presents the post-hoc test outcomes for each location by comparing the abundance of microplastics in water samples. According to Table (3), the concentration of microplastics at Site 1 differs from that of Sites 2, 3, 4, and 5. At Site 2, microplastic abundance has a different value from Site 1, Site 4 and Site 5 but has similarities to Site

3. The amount of microplastics detected at Site 3 differs from that reported at Sites 1 and 5, although it is the same at Sites 2 and 4. Furthermore, at Site 4, the value of microplastic abundance is different from Site 1 and Site 2 and has similarities with Site 3 and Site 5. At Site 5, the microplastic abundance values are different from those at Site 1, Site 2 and Site 3 and have similarities with station 4. These results indicate that site 1 has different microplastic abundances from other sites (2, 3, 4, and 5) due to the lowest abundance value compared to other sites.

Table (4) compares the abundance of microplastics based on their types (film, fiber, and fragment) in water samples using the post-hoc test. The quantity of fragments differs from the type of film according to a significance value of 0.05, although it is similar to fiber due to a significance value of 0.926. The abundance of film types differs from the abundance of fragments and fibers. While, fiber abundance is different from film but has similarities with fragment abundance. The results show that the abundance of microplastics fragment and fiber is similar but different from the abundance of film types. This means that the least film types were found in the water samples.

Table 4. Post-hoc analysis on water samples for each type microplastic

Type of microplastic		Mean difference	Sig.
Film	Fragment	-13.1*	0.000
	Fiber	-12.9*	0.000
Fiber	Fragment	-0.2	0.926
	Film	12.9*	0.000
Fragment	Film	13.1*	0.000
	Fiber	0.2	0.926

Significance level < 0.05

The abundance of microplastics in *Sulcospira* sp. samples collected from the upstream of the Brantas River was tested for normality and homogeneity. The normality tests of Shapiro-Wilk (*P*-value of 0.059 > 0.05) and Kolmogorov-Sminov (*P*-value of 0.063 > 0.05) revealed that the distribution was normal. The homogeneity tests show that the sig 0.917 > 0.05 with decision homogeneous. It suggests that the abundance of microplastics in sulcospira samples might be tested further using the post-hoc test.

Table 5. Post-hoc analysis on *Sulcospira sp*. samples at each station

Site		Mean difference	Sig.
1	2	-7.0*	0.00
	3	-5.3*	0.00
	4	-12.2*	0.00
	5	-16.8*	0.00
2	1	7.0*	0.00
	3	1.7	0.52
	4	-5.2*	0.00
	5	-9.8*	0.00
3	1	5.3*	0.00
	2	-1.7	0.52
	4	-6.8*	0.00
	5	-11.5*	0.00
4	1	12.5*	0.00
	2	5.2*	0.00
	3	6.8*	0.00
	5	-4.7*	0.00
5	1	16.8*	0.00
	2	9.8*	0.00
	3	11.5*	0.00
	4	4.7*	0.00

Significance level < 0.05

Table (5) compares the abundance of microplastics in sulcospira samples to show the post-hoc test results for each location. Table (5) shows that the abundance of microplastics in sulcospira at Site 1 differs from that at Sites 2, 3, 4, and 5. Microplastics abundance in sulcospira at Site 2 also has different values from Site 1, Site 4 and Site 5 but have similarities with Site 3. Site 3 has a different microplastic abundance value than Sites 1, 4, and 5, but has similarities with Site 2. At Site 4 the abundance of microplastics shows differences with Site 1, Site 2, Site 3 and Site 5. Furthermore, the microplastic abundance levels in sulcospira at Site 5 differ from those at Sites 1, 2, 3, and 4. The abundance of microplastics in sulcospira at Site 2 is similar to that at Site 3 and vice versa. Overall, the microplastic abundance data at each site vary.

Table 6. Post-hoc analysis on *Sulcospira* sp. samples for each type microplastic

Type of microplastic		Mean difference	Sig.
Film	Fragment	-3.3*	0.001
	Fiber	-8.0*	0.000
Fiber	Fragment	4.7*	0.000
	Film	8.0*	0.000
Fragment	Film	3.3*	0.001
	Fiber	-4.7*	0.000

Significance level < 0.05.

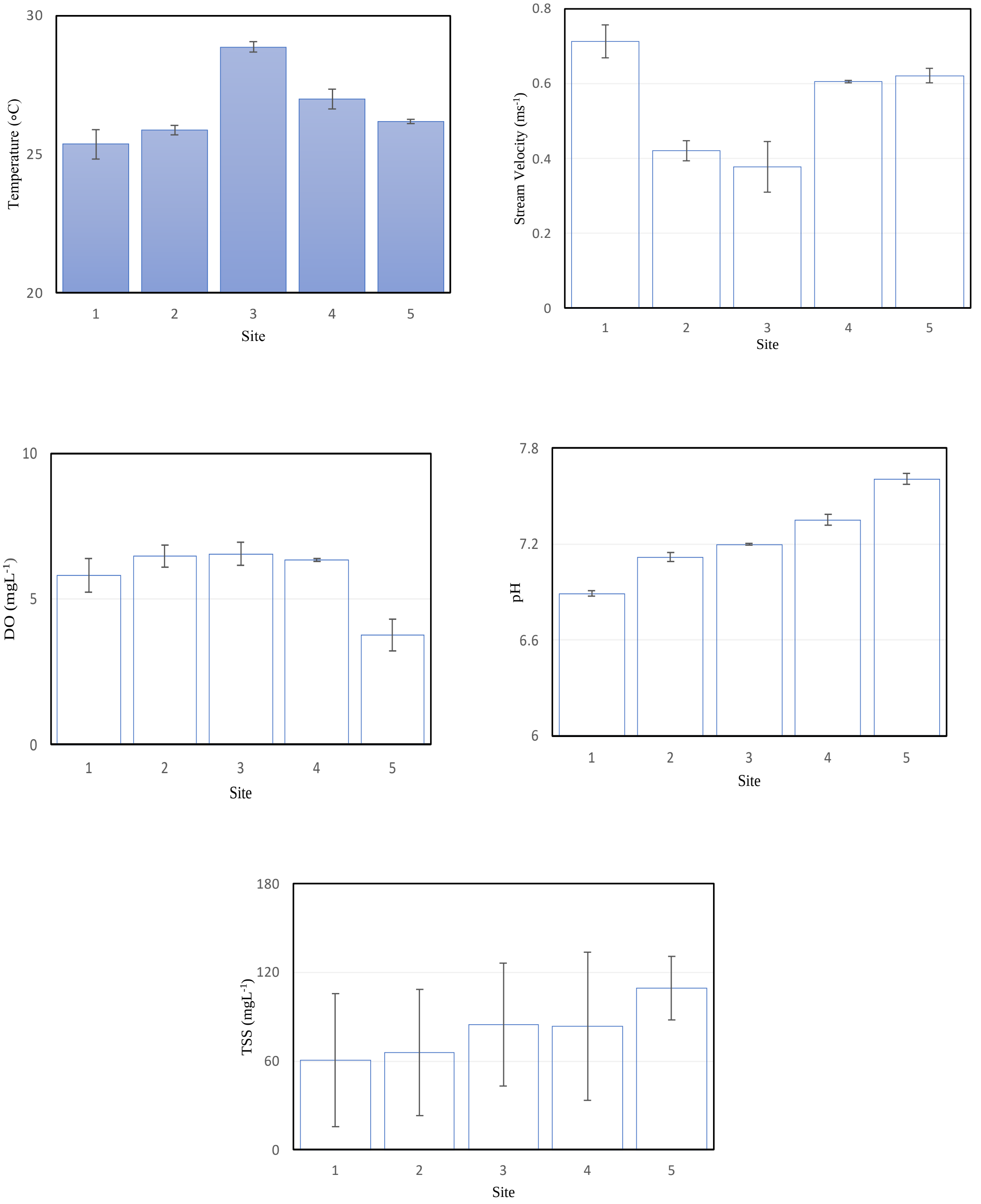


Fig. 4. Result of water quality in upstream of Brantas River

Table (6) compares the abundance of microplastics based on their types (film, fiber, fragment) in sulcospira samples using the post-hoc test. The abundance of fragments found in *Sulcospira* sp. was different from film and fiber because it has a significance value of <0.05. The abundance of films also differs from the abundance of fragments and fibers. Fiber abundance differs from fragment and film abundance.

According to these outcomes, the abundance of types (film, fiber, and fragment) in sulcospira samples varies.

The result of evaluating the parameters of water quality for two months covering the upstream of Brantas River is shown in Fig. (4). The bar chart shows that the water temperature ranges from 25.38±0.53 – 28.88±0.18ºC and stream velocity ranges from 0.38±0.07– 0.71±0.04 ms^{-1}. Dissolved oxygen (DO) was recorded to be in the range of 3.76±0.53– 6.54±0.40 mgL^{-1}, and pH ranges from 6.9±0.02 – 7.6 ±0.03. The last parameter, total suspended solid (TSS) ranges from 60.75±44.90 to 109.44±2.30 mgL^{-1}

DISCUSSION

Sulcospira sp. is a gastropod that has an oval-shaped shell and twists at the ends and has segments in the shell **(Safa'ah *et al*., 2018)**. A flat body is used for walking or moving and includes mucus to aid in walking **(Zulfa, 2022)**. The color of this organism's shell varies widely; some are blackish brown, while others are solid black **(Marwoto & Isnaningsih, 2012)**. When handled, the shell has a silky touch and a pair of tentacles serving as sensors **(Tyas *et al*., 2015)**. Site 2 in this study had the highest abundance of *Sulcospira* sp. among the other locations. Site 2 is a watershed and is close to irrigation canals. *Sulcospira testudinaria* is very common in rivers and creeks also in rice field irrigation canals **(Marwoto & Isnaningsih, 2012)**. *Sulcospira* sp. is a species of gastropod found in waters with slightly sandy substrates, such as mud, or layers of organic matter, such as leaf litter **(Safa'ah *et al*., 2018)**. However, sulcospira abundance was lower in other places. It might be due to a combination of unsuitable habitat types and a scarcity of various food sources. Environmental factors such as pH, water temperature, and air temperature all influence gastropod abundance **(Rudianto *et al*., 2014)**.

Types of microplastic films, fibers and fragments have been identified in water and sulcospira samples. When compared to other forms of microplastic, fiber is the most abundant. Another research found that the most common type of microplastic in snail *Littoraria scabra* was fiber (66.89%), followed by film (32.45%), and fragment (0.66) **(Patria *et al*., 2020)**. Fiber is a long-shaped microplastic made from plastic measuring between 0.1 – 5 millimeters **(Altreuter, 2017)**. Fiber also comes from pieces of nets made from plastic, cloth and rope **(Azhari *et al*., 2022)**. The source is the degradation of synthetic materials caused by household activities such as washing clothes, laundry, and textile industrial waste. Fiber is created by fabric waste that is dispersed in the environment and degrades as a result of natural processes **(Ecoton, 2023)**. Microplastics enter the environment via liquid waste from washing result due to improper processing; the trash enters rivers and ends up/accumulating in the bodies of creatures **(Kiran *et al*., 2022)**.

The analytical results for water samples show that Site 1 has a different abundance of microplastics than the other four locations. This is due to Site 1 having the lowest microplastic abundance value compared to Sites 2, 3, 4, and 5. Site 1 is an area that is far from human settlements and is still relatively natural compared to other sites. Several factors account for the abundance of microplastics in freshwater environments. Some of them are the comparison between the height of the human population and the number of available water sources, the location of urban centers, the residence time of water, water sources, types of waste treatment, and the number of sewers **(Victoria, 2016)**. Microplastic abundance in river waterways is affected by industrial waste disposal, home garbage disposal, and high population activities that generate plastic waste such as drinking bottles, baby diapers, and other single-use plastic goods **(Buwono *et al*., 2021b)**.

There is a considerable quantity of microplastic fiber types in the upper reach of the Brantas River, followed by fragments. According to a study, as many as 15 rivers in East Java contain microplastics, one of which is the Brantas River, which has been dominated by an abundance of microplastic fiber and fragments originating from baby diapers and single-use plastic goods **(Alicia, 2018)**. Based on the wastewater microplastics study, fiber-type microplastics are followed by fragments and films **(Kye *et al*., 2023)**. Because fiber-type microplastics are smaller in size than fragment-type microplastics, they are more abundant in sludge **(Edo *et al*., 2020)**. Most low-density plastics produce fibers or films, whereas high-density plastics produce microplastic fragments and flakes **(Kye *et al*., 2023)**. Microplastic fragments are the product of massive (macro) trash fragmentation induced by ultraviolet (UV) radiation, currents, and chemical reactions from the plastic itself **(Andrady, 2011)**. Plastic shards, buckets, mineral water bottles, plastic food packaging, and other big plastic-based tools are among the debris suspected of entering the waterways from human activities near the sample area in this study. Microplastics in the form of fragments originate from plastic fragments that are thick, stiff, and irregularly shaped **(Kovač Viršek *et al*., 2016)**.

The analytical result for sulcospira samples show that there is a disparity in abundance between each site (1, 2, 3, 4, and 5). This difference indicates that each gastropod at each site has a different abundance of microplastics in its body. Microplastic fibers were the most abundant in the gastropod sulcospira compared to fragments or films. This is consistent with previous research that shows that the majority of the identified fiber predominates. **(Doyle *et al*., 2020; Patria *et al*., 2020; Zaki *et al*., 2021)**. Fibers were the most common microplastics in freshwater gastropods, commonly known as snails (*Filopaludina sumatrensis speciosa* and *Pomacea canaliculata*), in a river flowing into a shallow coastal lagoon in Thailand **(Jitkaew *et al*., 2023)**.

Snails eat a wide range of food sources, including leaf macrophytes, filamentous algae, mangrove tree tissue, microalgae, bacteria, and zooplankton **(Alfaro, 2008)**. This demonstrates that microplastics reach the snail's body via the food it consumes. Snails, for example, feed microplastic-contaminated zooplankton and microalgae **(Patria *et al*., 2020)**. *Sulcospira* is a freshwater snail categorized as a suspension feeder, possessing gills with long filaments that capture and suspend plankton transported by water currents **(Lailiyah *et al*., 2021)**. Food is transported to the mouth by movements of the radula in snails. When the radula is extended, it makes contact with the substrate, and algae particles are scraped off when retractors draw the radula back into the mouth. The radula can also pulverize food particles by grinding them on the roof of the mouth **(Kesler *et al*., 2011)**. A lengthy esophagus connects to the stomach, which is positioned within the visceral bulk. Particles become stuck in mucus and are transported to the stomach via the esophagus. The stomach empties into an intestine that winds through the digestive gland and gonads to a rectum **(Alfaro, 2008)**. Microplastics can readily enter the snail's body due to their tiny size, making it difficult to identify food particles **(Browne *et al*., 2011; Patria *et al*., 2020)**.

The results reveal that the water quality of the upstream Brantas River is generally good, as the values recorded meet the quality criteria established by Government Regulation No.82 of the Republic of Indonesia in 2001. Gastropods can grow and develop appropriately in water temperatures ranging from 20 to 30°C **(Hamidah, 2000; Erlinda *et al*., 2015)**. Snails can withstand dissolved oxygen (DO) levels ranging from 6.2 to 10mg/ L **(Sahin & Albayrak, 2017)**. While, the optimal pH range for gastropods is 6.1-7.2 **(Erlinda *et al*., 2015)**. The distribution of gastropods, which choose the sorts of creatures that live, can be affected by current velocity, such that only the connected species survive against the current **(Hoffman *et al*., 2006)**. Slow current speeds in a body of water also lead it to be dominated by muddy substrates rich in organic materials **(Hartini *et al*., 2012)**. TSS of 25mg/ L has no impact, TSS of 25-80mg/ L has minimal effect, TSS of 81-400mg/ L is not good, and TSS of >400mg/ L is not ideal for gastropod survival **(Lestari, 2009)**. The TSS value also determines gastropod diversity, distribution, and abundance **(Ladias *et al*., 2020)**.

CONCLUSION

According to this study, *Sulcosphira* sp. is a living gastropod found in freshwater habitats that may be employed as a microplastic biomonitoring agent. Microplastics have been found in the waters of the higher portions of the Brantas River and the gastropod *Sulcospira* sp. The distribution of microplastics in water and sulcospira varies depending on land use in each location. The quantity of type (films, fibers, and fragments) in the sulcospira samples varies with the significant kind of fiber. More study is needed to assess the implications and dangers of microplastics in the aquatic environment and creatures regarding their effects on health and toxicity. Microplastic abundance in water bodies and *Sulcospira* sp. is related to water quality parameters in the Brantas River.

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