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The concentration of microplastics in epibiont of green mussel (*Perna viridis*) from Maccini Baji waters, Pangkajene Kepulauan

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Abstract. The discharge of microplastics into marine waters harms aquatic ecosystems, especially for filter feeder organisms such as green mussels. The purpose of this study was to analyze the shape, size, and color of microplastics and the concentration of microplastics in the epibiont associated with green mussels (*Perna viridis*) and the effect of the presence of epibionts on the concentration of microplastics in green mussels as a substrate. Sampling was done using the purposive random sampling method. Determination of the number of samples using Lemeshow's formula and obtained 99 green mussels associated with epibiont. The samples were divided into three groups, namely mussel associated with epibiont with microplastic found in epibiont, mussel associated with epibiont with microplastic found in mussel, and mussel without epibiont with microplastics in mussel. The results showed that the number of microplastics contaminated to the mussel group associated with epibiont with microplastic in the epibiont was 46 samples, to the mussel group associated with epibiont with microplastic in mussel was seven samples, and the mussel group without epibiont with microplastic in mussel there were 11 samples. The results also showed that if the microplastic were present in the epibiont, the green mussels associated with the epibionts did not contain microplastics. Conversely, if microplastics contaminated the green mussels, then epibionts were not contaminated by microplastics. The microplastics found ranged from 0.1-4.9 mm in length. The microplastic forms found were fibers and fragments. The microplastic colors found were red, blue, white, and black. The mean values of microplastic concentrations in epibiont, mussel associated with epibiont, and mussel without epibiont were 3.629 items/g, 0.7160 items/g, and 1.032 items/g, respectively.

1. Introduction

Plastics are synthetic polymers that are difficult to decompose naturally, so it takes a long time for plastics to decompose completely. Meanwhile, the increasing use of plastic can have a bad impact on the environment, especially marine waters, because the time the plastic decomposes with the amount of plastic waste is not balanced, resulting in the accumulation of plastic [1]. Thompson (2006) stated that 10% of plastic produced will be dumped into rivers and ended up in the sea [2]. This resulted in 165 million tonnes of plastic per year entering Indonesia's marine waters. Almost all habitats in the ocean, both benthic and pelagic, are found in plastics waste of various sizes. The degradation of plastics in marine waters will produce small particles called microplastics [2].

Microplastics are plastic particles whose diameter or length is less than 5 mm [3]. The large number of microplastics scattered in marine waters will also have an impact on consumption animals originating from marine waters. Its persistent nature means that microplastics that enter the water are not easily lost [4]. Nearly 85% of microplastics are present on sea surface. The particle size of microplastics found in many water areas around the world is <5 mm [5]. According to Sun et al., (2017) microplastics are found in marine biota [6]. Microplastics are also found in bivalves which are filter feeder organisms [7]. Epibionts that live attached to bivalves make them susceptible to contamination by microplastics.

Epibionts are organisms that attach to and grow on the surface of abiotic and biotic substrates in waters [8]. The presence and amount of epibionts usually vary depending on the time of day and water conditions [9] However, the existence of epibiont is often considered as a fouling organism that can damage the place where they attach. Therefore, they are often also called biofouling. One of the epibiont substrates is green mussels (*Perna viridis*) [10]. Research on the microplastic content of green mussels has been carried out in various waters. The results of research conducted in the Java Sea showed that the microplastic content in green mussels with a concentration of 4-20 particles/gram with the type of microplastic found in the form of fibers, fragments [11]. Even in Sulawesi waters, there have been several studies explaining the findings of microplastics in green mussels. One of them is a study that found microplastics in green mussel from Maccini Baji Waters, Labakkang District, Regency Pangkajene Islands, South Sulawesi [12]. Maccini Baji waters are one of the waters that produce green mussels which are usually sold by fishermen for public consumption.

However, there has been no research on microplastics in epibionts associated with green mussels and the effect of the presence of epibionts on green mussels in filtering microplastics. In this regard, it is necessary to research to analyze the concentration, shape, size, and color of the microplastics in the epibionts associated with green mussels and the effect of epibiont on green mussels as a substrate in filtering microplastics to obtain information on the association between epibionts and green mussels in microplastics polluted conditions.

2. Materials and methods

2.1. Sampling

The research was conducted from November 2019 to March 2020. Sampling was carried out in Maccini Baji waters, Labbakang District, Pangkajene Islands Regency, and continued with sample analysis at the Laboratory of Aquatic Animal Physiology and Water Quality Laboratory, Department of Fisheries, Faculty of Marine and Fisheries Sciences, Hasanuddin University, Makassar.

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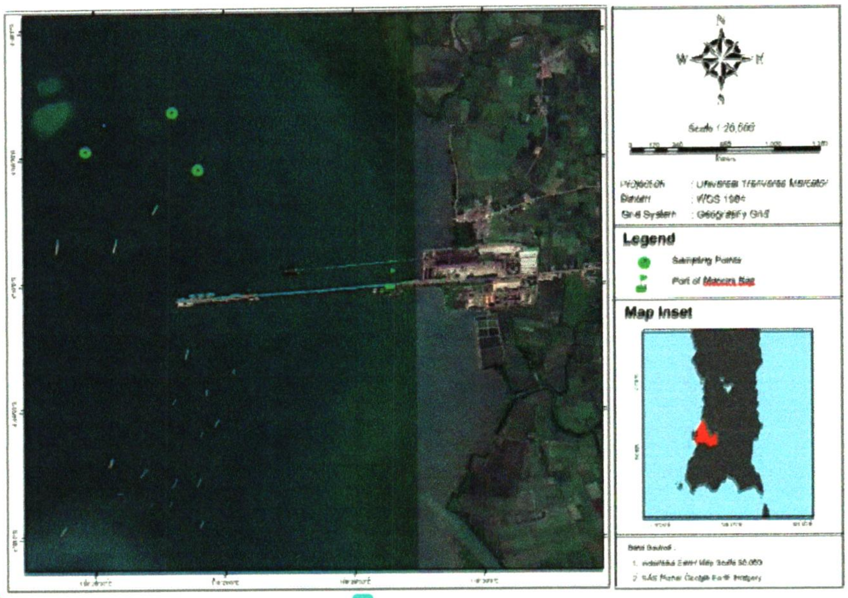

Figure 1. Map of sampling locations for green mussels (*Perna viridis*) in Maccini Baji, Labakkang District, Pangkajene Islands Regency.

The purposive random sampling approach was used in this study. Green mussels were picked up by hand at a seaweed farm area. To find out the number of samples to be taken, the Lemeshow's formula was used whose population was unknown [13], as follows:

$$n = \frac{Z^2 \times P(1-P)}{d^2}$$

n = Number of samples
Z = Z score at 95% confidence = 1.96
P = Maximum estimation = 0,5
d = alpha (0,10) or sampling error= 10%

Based on Lemeshow's formula, it was known that the minimum sample taken was 96 samples, but we took 99 samples of green mussel. The 99 samples were then divided into three groups based on the length of the green mussel shell, which were, the mussel group associated with epibiont with microplastic on epibiont, the mussel group associated with epibiont with microplastic in mussel's tissue, and the group of mussel contained microplastics that was not associated with epibiont.

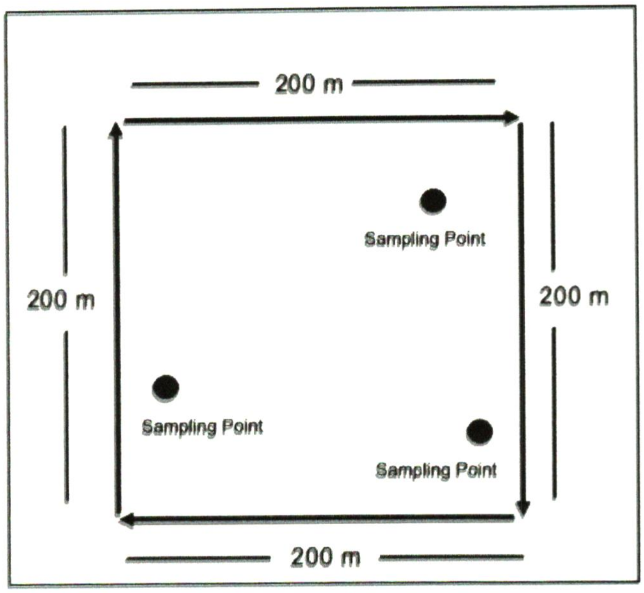

Figure 2. Water sampling illustration.

A sampling of microplastics in the waters was carried out using a neuston net (330 μm) which was towed by a boat with a towing length of 200 m [14]. After that, the water sample in the neuston net bottle was put into the sample bottle and given a station marker. After that, the water sample was stored in a cool box for further analysis in the laboratory.

2.2. Tissue digestion
All equipment used in the digestion process was sterilized with aquabidest. Before the epibiont is separated from the green mussel, identification was performed using the identification book [15]. After the epibiont was separated from the mussel, the weights of epibionts (sponge and macroalga) were measured using a digital scale. The green mussels were measured in length, width, height, and total weight using a caliper and a digital scale. The mussel tissues were separated from the shell using a scalpel and then the tissues were weighted before put into the sample bottle. The shellless epibiont was cut into pieces and put into the sample bottle. Epibiont which has a shell such as barnacles, the tissues were removed from the shell and put into a sample bottle. After that, 10% KOH solution was added to each sample bottle with a volume of three times the volume of samples [16].

2.3. Microplastic identification
After the samples were digested with KOH for three weeks, the sample solutions were filtered using a Whatman cellulose nitrate membrane filter with a pore size of 0.45 μm and a diameter of 45 mm. After that, the identification of microplastics was carried out using the visual observation method under a stereomicroscope. The parameters observed were the number, shape, size, and color of the microplastic particles. The length of the microplastic was determined using the Image J application.

3. Variable analysis

3.1. Frequency of occurrence (FO)

The frequency of occurrence of microplastics in sentinel organisms (epibiont and green mussel) is the percentage of the number of sentinel organisms identified as containing microplastics. The frequency of occurrence is calculated based on the Frequency of occurrence formula [17] as follows:

$$FO : \frac{\text{The number of sentinel organisms containing microplastics}}{\text{The total number of sentinel organisms observed}} \times 100\%$$

3.2. Microplastic concentration

The concentration of microplastics in sentinel organisms is the microplastic content found in sentinel organisms and is expressed in units of item/g. The concentration of microplastics is obtained by dividing the number of microplastic particles in sentinel organisms by the weight of the sentinel organisms [11]:

$$\text{Microplastic concentration (items/g)} = \frac{\text{The number of microplastics in sentinel organisms}}{\text{The weight of the sentinel organism}}$$

3.3. Statistical analysis

Statistical analysis was performed using the one-way ANOVA test to analyze the comparison of microplastic concentrations in sentinel organism groups. To analysis between two shapes of microplastics, the Student T-test was performed. The ANOVA and the student T-test were run using the Graphpad Prism software.

4. Results and discussions

4.1. Microplastics in the epibiont of green mussel (*Perna viridis*)

Epibionts associated with green mussels are sponges, *Clathria (Microciona) atrasanguinea*, macroalgae, *Gracilaria* sp, and barnacles, *Balanus* sp. Bernacles are the predominant organisms found in research as epibionts. On the island of Barangglompo, the bernacles were also found to be the dominant epibionts [18]. These epibionts were contaminated with microplastics. The microplastics found in epibionts associated with a green mussel in Maccini Baji Waters, Labbakang District, Pangkajene Islands Regency were microplastics in the form of fibers and fragments in black, white, blue, and red colors which can be seen in Figure 3. The contamination of the epibionts with microplastics is a result of plastic waste from community activities around Maccini Baji waters which are disposed of by the community. The existence of plastic waste in these waters has been around for a long time so that macro plastic waste was degraded into microplastics. Most of the community work as fishermen and seaweed farmers. The activity of seaweed farmers who use plastic ropes and bottles in the seaweed cultivation process was also one of the contributors to microplastics in the waters. In addition, the existence of a port which, was used as a place for crossing between islands was also very potential as a contributor to microplastic pollution.

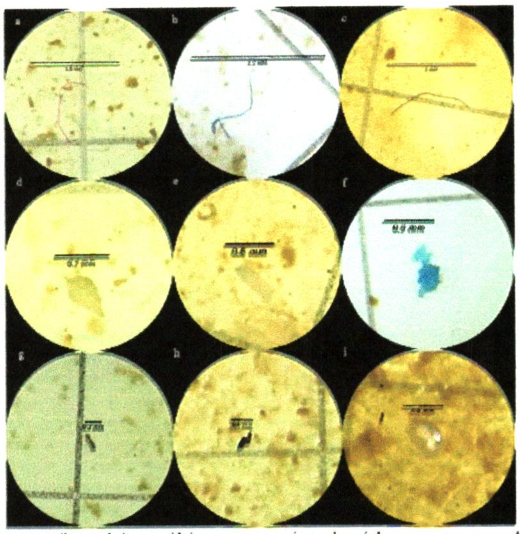

Figure 3. Microplastics we found in epibionts associated with green mussels (*Perna viridis*). (a-c) microplastic in the form of fibers, (d-i) microplastic in the form of fragments.

The length of the microplastics found in the entire study sample in Maccini Baji waters, Labakkang District, Pangkajene Islands Regency was in the range of 0.1 mm - 4.9 mm. This indicates that epibionts can absorb small and large microplastics. Microplastics were found in epibionts due to the feeding behavior of epibionts such as barnacles and sponges by filtering the water so that the microplastics in the waters can enter the body. According to research by Yu and co-workers, microplastics were found in the larvae of barnacles [19]. The discovery of microplastics in epibionts from seaweed species confirmed the research of Li and co-workers who have observed microplastic contamination in the production of seaweed *Pyropia* spp [20].

4.2. Frequency of occurrence (FO)

The frequency of occurrence (FO) of microplastics indicates the number of identified green mussels and epibionts containing microplastic particles. The total numbers of samples of green mussels in this study were 99 samples. Green mussel samples associated with epibionts were 83 samples and 16 samples were not associated with epibionts. Of the 83 samples, it was found that 53 samples contained microplastics with 7 samples whose microplastics were found in the tissue of green mussel and 46 samples whose microplastics were found in epibionts. 16 samples of green mussels that were not associated with epibionts, 11 of which contained microplastics.

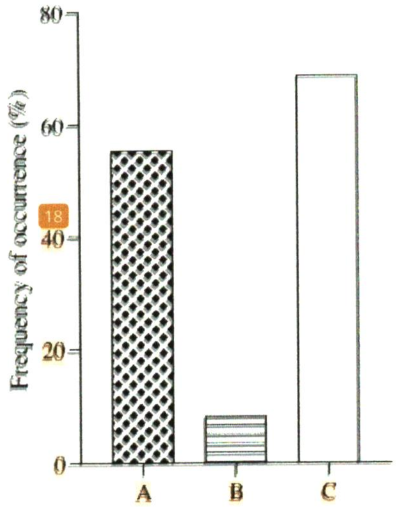

Figure 4. The percentage of frequency of occurrence of microplastics in various groups of green mussel-epibiont association. Green mussels associated with epibionts with microplastics in epibionts (A); Green mussels associated with epibionts with microplastics on mussels tissue (B); Green mussels not associated with epibionts and contain microplastics (C).

The frequency of occurrence of microplastics in the group of green mussels associated with epibionts with microplastics in epibionts (A) was found to be 55.42% contaminated with microplastics. Meanwhile, the group of green mussels associated with epibionts with microplastics on mussels tissue (B) was found to be 8.43% contaminated with microplastics. This indicates that microplastics are filtered more by epibiont than by green mussels if calculated based on the green mussels group associated with epibiont alone. From the data, it can also be seen that the FO percentage of microplastics in green mussel without epibiont (C) was the highest among all groups, 68.75 %. This indicates that epibionts can reduce microplastic contamination in green mussels. In addition, this FO data showed that ecologically the presence of epibionts in green mussel indicates interspecific competition for food between the two entities [21,22].

4.3. Microplastic concentration
Microplastic concentration data on epibionts can be seen in Figure 5. The average value of microplastic concentrations in the three groups; green mussels associated with epibionts with microplastics in epibionts (A), green mussels associated with epibionts with microplastics on mussels tissue (B), green mussels not associated with epibionts and contain microplastics (C) respectively 3,629 items/g, 0, 7160 items/g, 1,032 items/g. The results of statistical tests showed that the concentration of microplastic groups A, B, and C showed significant differences ($p < 0.005$). Group A was different from B and C. However, there was no statistically significant difference between B and C. This showed that epibionts have a major role in reducing microplastics that will enter or expose green mussels.

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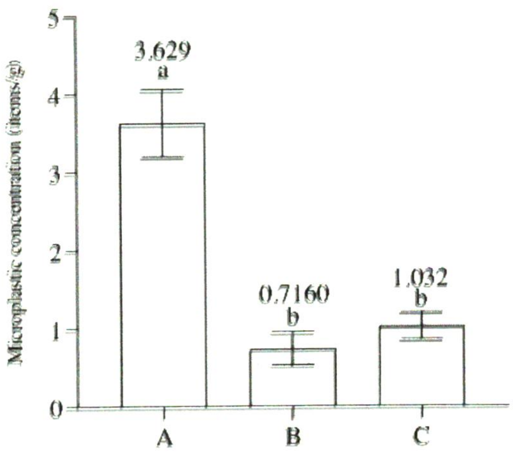

Figure 5. Comparison of microplastic concentrations in various groups of green mussel-epibiont association. Green mussels associated with epibionts with microplastics in epibionts (A); Green mussels associated with epibionts with microplastics on mussels tissue (B); Green mussels not associated with epibionts and contain microplastics (C). Different letters indicate significant differences ($p < 0.05$).

4.3.1. Shape of microplastic.

Based on the observations of all samples, it was found that there were two forms of microplastic particles, namely fiber, and fragments. The average value of the concentration of the microplastic form in the green mussels group associated with epibionts with microplastics in epibionts (A) in the fiber was 1,845 items/g and the fragments were 4,435 items / g, green mussels associated with epibionts with microplastics on mussels tissue (B) the fiber of 0.7833 items/g and fragments of 0.232 items/g, and green mussels not associated with epibionts and contain microplastics (C) in the fiber of 1.2012 items/g and fragments of 0.9772 items/g.

In group the fragment concentration was greater than the fiber concentration statistically ($p < 0.005$). In group B the data could not be analyzed statistically because the data did not meet the requirements in terms of the amount of data. Descriptively it appears that there were more fibers than fragments. This depicted that green mussels filter the fibers more than fragments because the fragments have been filtered by epibiont. This analysis was supported by data on group C, which in group C microplastics were counted on green mussel only. The results showed that there were no significant differences between fiber and fragment concentrations. This means that the presence of epibionts in green mussels affected the type of microplastic that was filtered by green mussels. In addition, if we looked closely at the data for group A, the data also supported the data for group B that showed epibionts reduce green mussel filtration of microplastics in the form of fragments. Therefore, it could be emphasized that the existence of epibiont affected the filtration activity of green mussels on microplastics, which ultimately reduced the concentration of microplastics in green mussels.

The research of Wahdani and co-workers found microplastics in the form of fibers and fragments in the bivalve body, *Venerupis philippinarum* which was found in Maccini Baji Waters [7]. Fiber that has a long and thin form can come from ropes, clothing fibers, fishing gears such as fishing gears, and fishing rods [5]. Fragments are hard particles with irregular shapes [23]. Fragments come from plastic waste, such as in plastic packaging materials, plastic bags, and agricultural tools.

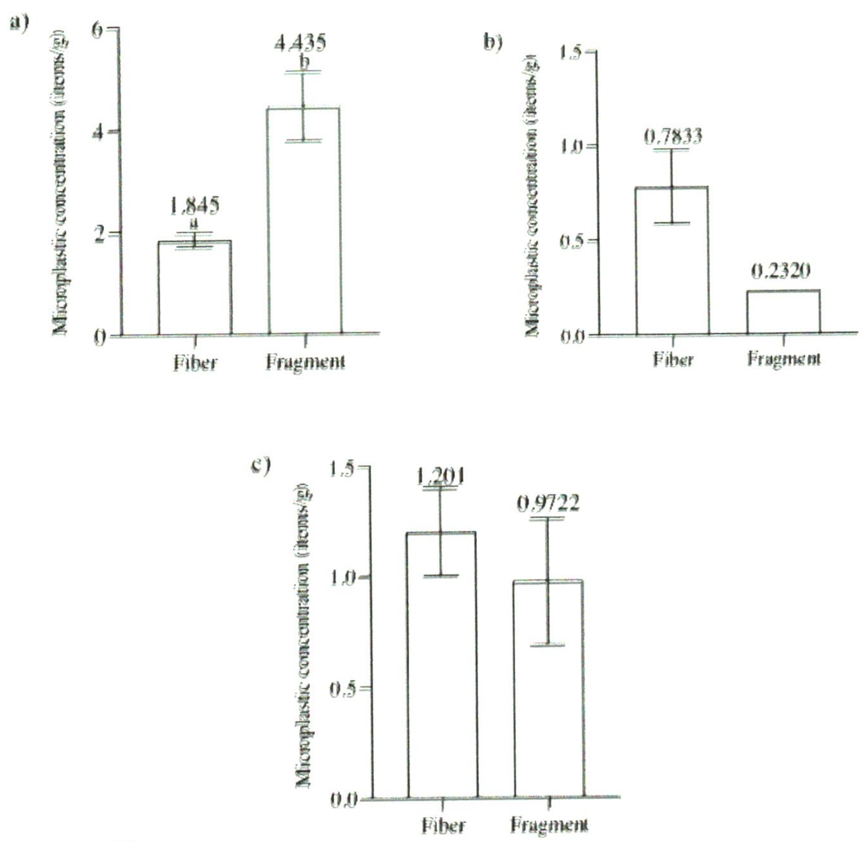

Figure 6. The concentration of microplastics is based on the shape of the microplastic. Green mussels associated with epibionts with microplastics in epibionts (A); Green mussels associated with epibionts with microplastics on mussels tissue (B). Statistical analysis was not performed on data B, because the data were not sufficient for statistical analysis; Green mussels are not associated with epibionts and contain microplastics (C). Different letters indicate significant differences ($p < 0.05$).

4.3.2. Microplastic Color.
The color of microplastic particles found in the green mussels group associated with epibionts with microplastics in epibionts (A) was red, blue, black, and white, with an average value of microplastic concentrations respectively 3.025 items/g, 5.865 items/g, 0.853 items/g, and 7.075 items/g. From the results of statistical analysis, there was only a significant difference between white and black. This was consistent with the research of Jiang and co-workers who found that white microplastic is the color more than black, red, and blue [24].

The average value of the concentration of color-based microplastics in the green mussels associated with epibionts with microplastics on mussels tissue (B) group was 0.256, 0.432, 0.439, and 232 items/g, respectively for red, blue, black, and white. In group B, no statistical analysis was carried out because the data did not meet the requirements for statistical analysis in terms of the amount of data

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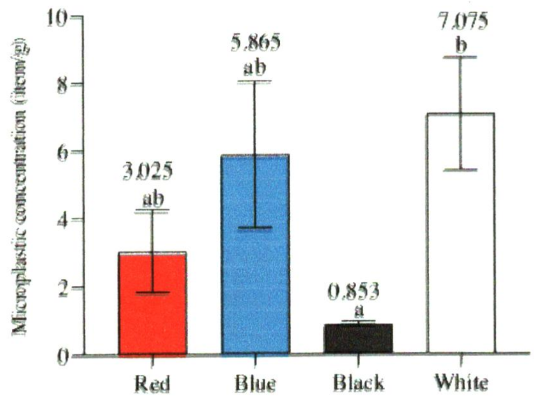
on the white color of microplastics. Then the average values of green mussels are not associated with epibionts and contain microplastics (C), namely 0.761, 1063, 0.811, and 0.729 items/g respectively for red, blue, black, and white. The results of statistical analysis depicted that there were no significant differences between the colors of the microplastics. The absence of a significant difference in the concentration of color-based microplastics was not related to the preference of green mussels and epibionts on the color of the microplastics, because the two types of organisms carried out the filtration activity on whatever was in front of their inhalant.

The color of the microplastic can provide information about the source of plastic waste or the condition of the microplastic [23]. The difference in the color of the microplastics that was found was influenced by the plastic waste around the sampling location. The blue and red colors are artificial colors that are produced from the production of plastic factories [25]. The results of Manalu's research indicate that the microplastic color found in the study could be the original color of the microplastic or the color of the degradation that occurs due to the release of the original color caused by photochemical processes and other processes that occur in the water [26].

a)



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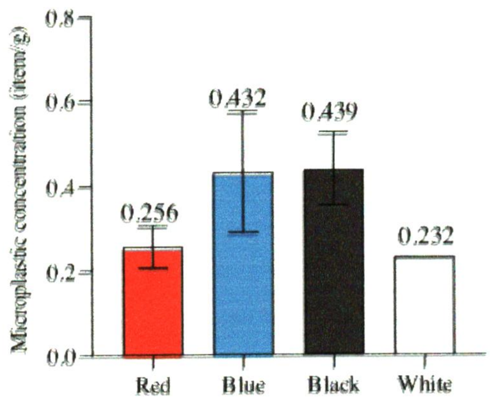

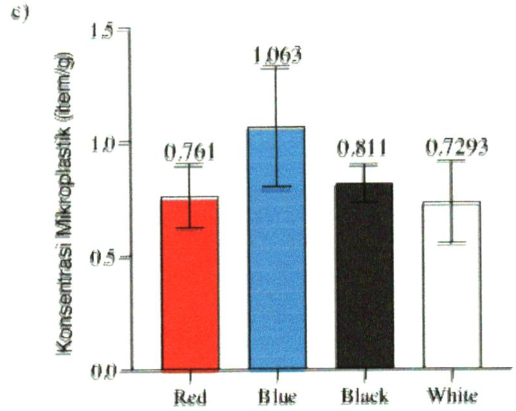

Figure 7. Microplastic concentration based on color. Green mussels associated with epibionts with microplastics in epibionts (A); Green mussels associated with epibionts with microplastics on mussels tissue (B). Data B was not subjected to statistical analysis, because the data did not meet the requirements for statistical analysis; Green mussels are not associated with epibionts and contain microplastics (C). Different letters indicate significant differences ($p < 0.05$).

4.4. Microplastics in water

The results of observing water samples from four different points indicated that the waters of the research location contained microplastics. Figure 8 shows examples of microplastics found in water samples. The forms of microplastic particles found in water samples were fibers and fragments in blue, red, white, and black. The average value of the concentration of microplastics in the water sample is 1.0848 items/m^3. The microplastic particles found in the water samples showed similarities to the microplastic particles found in epibiont and green mussel, both in terms of shape and color.

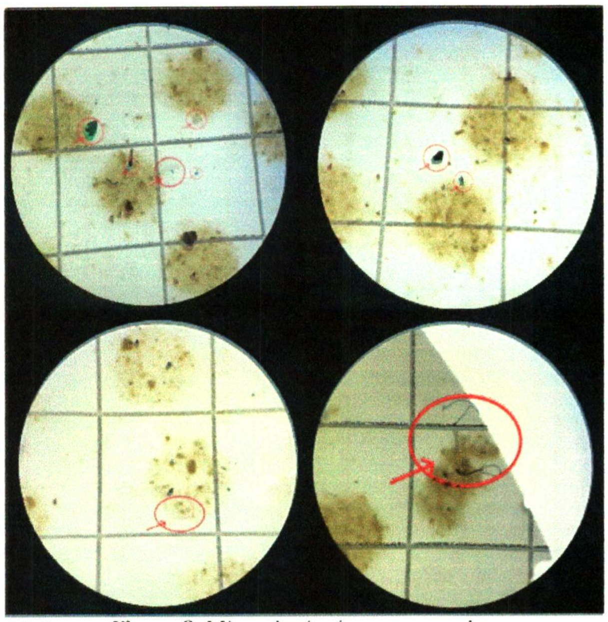

Figure 8. Microplastics in water samples.

5. Conclusion

Based on the research results, it could be concluded that:
(1) Epibionts associated with green mussels in Maccini Baji Waters, Labakkang District, Pangkajene Archipelago Regency were contaminated with microplastics. Microplastic particles found in water, epibionts, and green mussels were fibers and fragments in red, blue, black, and white colors. The microplastics found ranged from 0.1 mm to 4.9 mm in size.
(2) The frequency of occurrence of microplastics in the group of green mussels associated with epibionts with microplastics in epibionts (A); green mussels associated with epibionts with microplastics on mussels tissue (B), green mussels not associated with epibionts and contain microplastics (C) were 55.42, 8.43, and 68.75% respectively.
(3) The presence of epibiont in green mussel shells is ecologically a competitor for green mussel, but ecotoxicologically it can reduce exposure to green mussel microplastics.

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