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DETECTION OF MICROPLASTICS IN BLOOD CLAM (*Tegillarca granosa*) AND GREEN MUSSEL (*Perna viridis*) FROM BERINGHARJO MARKET, YOGYAKARTA CITY

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ARTICLE INFO

ABSTRACT

Article history		Plastic is a major ocean pollutant due to its persistent
Submission	2023-12-31	nature. When plastics break down into pieces smaller
Revision	2024-03-05	than 5 mm, they are classified as microplastics, which
Accepted	2024-04-20	can enter the bodies of aquatic biota such as shellfish.
Keywords:		The objectives of this research were to analyze the
Characteristics of		abundance and identify the characteristics of
microplastics		microplastics in blood clam and green mussel from
Fragments		Pasar Beringharjo, Yogyakarta City. The methodology
Polyamide		involved sampling shellfish from Pasar Beringharjo,
Shellfish		removing the meat and soaking the samples in 10%
		KOH for 3 days. The resulting mixture was filtered, and
		the microplastics were observed under a light
		microscope to determine their abundance and
		characteristics. The polymers comprising the
		microplastics were analyzed using FTIR. The difference
		in abundance and characteristics of microplastics
		between the two shellfish species were assessed using
		the Mann Whitney test. The result revealed that the
		abundance of microplastics in blood clam and green
		mussel did not differ significantly $(p < 0.05)$. The
		dominant form of microplastics in both types of shellfish
		was fragmenting. The most common colors of
		microplastics found in both types of shellfish were black
		and brown. The size range of microplastics in both
		shellfish species was predominantly 0-100 μ m. FTIR
		analysis identified the microplastics as nylon polymer
		(polyamide). The results concluded that both blood
		(porjannae). The results concluded that both bloba

clam and green mussel from Pasar Beringharjo, Yogyakarta City, contained microplastics with similar abundance and characteristics.

INTRODUCTION

Plastic is an indispensable product in everyday life due to its practical, versatile, and cheap affordability (Hale et al., 2020). However, plastic waste can degrade into particles ranging from 0.1 to 5,000 μ m in size, known as microplastics (EFSA, 2016). Despite these particles being small, their persistent nature results in pollution of both land and water (Lusher et al., 2017; Salsabila & Ulfah, 2017). Microplastic pollution in marine waters is projected to double by 2030 (Hale et al., 2020). Microplastics in water can enter the bodies of aquatic biota directly through ingestion of seawater or indirectly when they consume prey containing microplastics (Lusher et al., 2017; Vendel et al., 2017; Yona et al., 2020). These particles can cause various effects in aquatic biota, such as digestive tract blockage leading to false satiety (Gall & Thompson, 2015).

Physiological problems may also arise from plastic additives, which are carcinogenic and disrupt hormonal functions (Wright et al., 2013). Accumulated microplastics in the human body have been associated with chromosomal changes, trigger cancer, infertility, and obesity (Sharma & Chatterjee, 2017), as well as the transfer of pathogens (Barboza et al., 2018) and chronic inflammatory lesions (Prata et al., 2020). This underscores the role of microplastics as emerging contaminants in food safety. Therefore, it is important to monitor the presence of microplastics in aquatic organisms consumed by humans by considering the potential risk they pose to human health. Microplastics in aquatic biota have been extensively studied across various species such as fish, crustaceans, molluscs, and echinoderms (Danopoulos et al., 2020).

Blood clams, along with green mussels, are highly valued marine commodities widely consumed by humans. However, as filter feeders, shellfish are particularly susceptible to contamination by microplastics present in their surrounding waters. This contamination poses a significant food safety concern for human health (Hermabessiere et al., 2019). A recent study has identified microplastics in aquatic biota, including economically important species like blood clams (Tegillarca granosa) from the North

Coast of Java (Widianarko & Hantoro, 2018). Typically, microplastics are studied from the digestive tract of organisms (Lusher et al., 2017).

However, shellfish are consumed whole. Beringharjo Market in Yogyakarta City, within the Special Region of Yogyakarta Province, was a major hub for various fishery commodities. There has been no previous research on microplastics in shellfish in this region. Therefore, it is essential to investigate the presence of microplastics in blood clams and green mussels sourced from Beringharjo Market, Yogyakarta City. Detecting microplastics in shellfish is crucial to minimizing the risk of microplastics entering the bodies of shellfish. Additionally, this research is important for plastic waste management.

MATERIALS AND METHODS

Research Design

Materials and Methods This exploratory research involved sampling blood clams (Figure 1.a) and green mussel (Figure 1.b) samples (10 individuals) using the quota sampling technique. from Beringharjo Market, Yogyakarta City. Data collection included observations on the abundance, shape, color, size, and type of microplastic polymer found in the samples

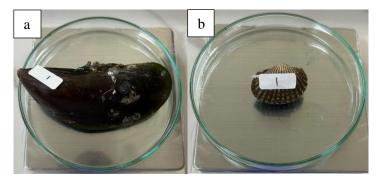


Figure 1. Samples of this research: a. Blood Clamp (*Tegillarca granosa*), b. Green Mussel (*Perna viridis*) from Beringharjo Market, Yogyakarta City

The purchased samples were placed in a box with ice cubes and transported to the Ecology and Systematics Laboratory, FAST UAD Biology Study Program. Upon arrival, the sample was measured for length using a digital caliper and weighed using a digital scale. The extraction procedure followed modifications from previous research (Suwartiningsih et al., 2020). To prevent contamination, all tools were sterilized with distilled water and 70% alcohol prior to the procedure. Each tool was then individually

wrapped in aluminum foil and dried in an oven at 50 °C for 12 hours. During extraction, shells were opened and all the internal organs were removed. The samples were placed in flacon bottles and submerged in a KOH 10% solution to a volume approximately three times that of the sample. The samples were then incubated at room temperature for three days to degrade biogenic materials. The sample was filtered using filter paper to obtain the pellets, which were collected at the top of the filter paper and transferred to a petri dish. The pellets from the petri dish were moved onto a glass object, dripped with enough distilled water, and covered with a cover glass. The samples were observed under a light microscope at a maximum magnification of 1000x, and the detected microplastics were documented using a microscope camera. Microplastic measurements were conducted using the ImageRaster application. The type of polymer in the microplastics was analyzed using Fourier transform infrared (FT-IR) (Ibrahim et al., 2017).

Data Analysis

The data on microplastic abundance were analyzed using a mean difference test (Ttest) to compare the abundance of microplastics between the different types of shellfish. The relationship between length, weight, and abundance of microplastics was assessed using a correlation test.

RESULTS AND DISCUSSION

Abundance of Microplastics

The result revealed that the abundance of microplastics in blood clam (839.25 \pm 144.24 microplastics/individual) and green mussel (703.75 \pm 159.21 microplastics/individual) (Table 1) did not differ significantly (p<0.05). Although blood clams are smaller in size compared to green mussels, they were found to contain more microplastics. The smaller size of shellfish typically results in a higher filtration rate compared to larger ones (Tantanasarit et al., 2013). The higher the filtration rate, the greater the likelihood of microplastics entering their systems.

Sample	Amount of Sample	Length (mm)	Width (mm)	Height (mm)	Weight (g)	Weight of Meat (g)	Range of Microplastics	Average of Microplastics
Blood clam	5	29,68	23,19	22,18	8,90	2,964	654-988	839,25±144,24ª
		±0,94	±0,71	±1,00	$\pm 0,65$	±2,71		
Green	5	88,15	35,84	25,44	33,07	5,938	528-913	703,75±159,20ª
mussel		±2,66	±1,10	±0,87	±3,99	±1,81		

 Table 1. The Average Abundance of Microplastics in Blood Clam (*Tegillarca granosa*) and Green Mussel (*Perna viridis*) from Beringharjo Market, Yogyakarta City

Note: superscript with the same letter in the same column indicate there is no significant difference (p>0.05).

The results of the correlation test indicated that the abundance of microplastics was not correlated with the length, width, height, and weight of the shellfish. This finding aligns with a previous study by Kawung et al. (2022), which similarly reported no correlation between microplastics content and bivalve body morphometry. A similar study also reported that microplastics abundance does not correlate with shell length (Rahim et al., 2022). Additionally, studies on shellfish from the Atlantic coast of France (Phuong et al., 2018) and green mussels from Thailand (Chinfak et al., 2021) have shown the absence of a correlation between microplastics and shellfish body morphometry.

Shape of Microplastics

Figure 2. The Morphology Forms of Microplastics found in Blood Clamp (*Tegillarca granosa*) and Green Mussel (*Perna viridis*) from Beringharjo Market, Yogyakarta City: a. fiber, b. film, c. fragment, d. pellets (blue circles)

The research results revealed four forms of microplastics present in shellfish organs: fragments (Figure 2.a.), fibers (Figure 2.b.), films (Figure 2.c.), and pellets (Figure 2.d.). Fragments were the predominant form found in both the blood clam (412.5 \pm 128.79 microplastics/individual) and green mussel (320.5 \pm 139.19

microplastics/individual). The least frequently found form was pellets, with blood clams containing 3.25 ± 2.63 microplastics per individual and green mussels 0.5 ± 1 microplastics per individual (Figure 3). The distribution of microplastics did not show a significant difference between the two types of shellfish (p > 0.05).

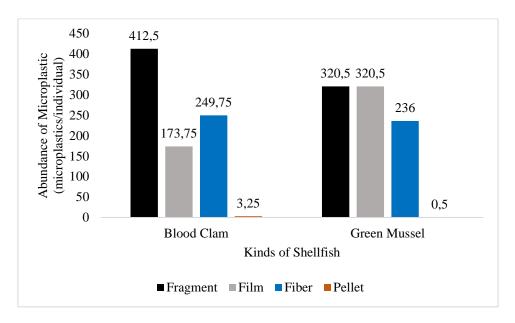


Figure 3. The Forms of Microplastics found in Blood Clamp (*Tegillarca granosa*) and Green Mussel (*Perna viridis*) from Beringharjo Market, Yogyakarta City

The dominance of fragment form (71.98%) was also observed in Mediterranean mussels (Mytilus galloprovinciali) on the Apulia Coast, Italy (Dalvand & Hamidian, 2023). Similarly, green mussels from the Thai market were reported to be predominantly composed of fragments (75.4%) (Imasha & Babel, 2021). Fragments originate from plastic waste generated by local community activities such as pipes, buckets, or bottles (Mauludy et al., 2019). These fragments are large plastic flakes with high density (Wright et al., 2013). Secondary microplastics result from the fragmentation of larger plastics due to mechanical factors and oxidation in the environment (Dalvand & Hamidian, 2023). Due to their high density, fragments settle to the bottom of the water and are filtered by shellfish during their feeding activities (Wahdani et al., 2020).

In contrast, pellets were found to be the least abundant form because they are primary microplastics, produced in microsize, and are rarely found in the environment (Hiwari et al., 2019). Pellets often wash up on shorelines and are less likely to settle on the bodies of shellfish (Zhang et al., 2018).

Colors of Microplastics

This research identified five colors of microplastics in both blood calm and green mussels: black, transparent, blue, red, and yellow (Figure 4). However, there was no significant difference in color distribution between the two types of shellfish (p > 0.05). in both clam Black microplastics dominated blood (491.75 ± 130.59 microplastics/individual) and green mussel (410.5 ± 147.884 microplastics/individual) (Figure 4), primarily due to their prevalence in fragment shapes (Hastuti et al., 2019). This finding aligns with previous research that reported black microplastics as dominant (50%) in blood clams from the South Coast of Thailand (Goh et al., 2021). The black color often indicates the microplastics' ability to absorb pollutants (Hiwari et al., 2019).

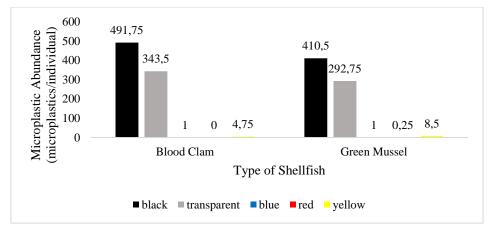


Figure 4. The Color of Microplastics found on Blood Clam (*Tegillarca granosa*) and Green Mussel (*Perna viridis*) from Beringharjo Market, Yogyakarta City.

After black, transparent is the second-most dominant color after black. observed in microplastics from Blood Clam (Tegillarca granosa) and Green Mussel (Perna viridis) at Beringharjo Market, Yogyakarta City. Transparent colors suggest the prevalence of polypropylene (PP) polymers in the water (Pedrotti et al., 2014). Additionally, transparent microplastics often exhibit color fading due to UV exposure, indicating prolonged exposure to the environment (Hiwari et al., 2019). Blue and red microplastics typically indicate the presence of polyethylene (PE) polymers, maintaining their color density (GESAMP, 2016). Yellow microplastics are attributed to an increase in the carbonyl index (Stolte et al., 2015).

Size of Microplastics

The study results indicated that particle sizes from 0 to 100 μ m were predominant in both blood clamp (33%), and green mussel (31%). In contrast, the smallest particle size

category, 901–1000 μ m, was present at only 3% in both blood clamp and green mussel (Figure 5).

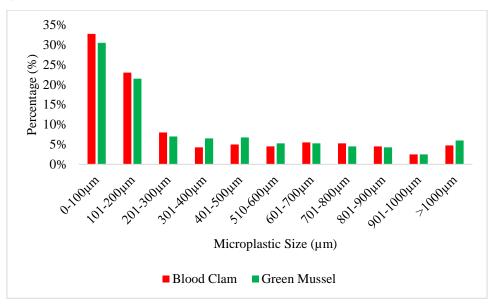


Figure 5. Size of Microplastics in Blood Clam (*Tegillarca granosa*) and Green Mussel (*Perna viridis*) from Beringharjo Market, Yogyakarta City.

This finding aligns with research on green mussels from the Java Sea, where particle sizes of $51.31-87.42 \mu m$ were predominant (Khoironi et al., 2018). Similarly, a study on yellow clams (Meretrix casta) from India reported a dominance of particle sizes in the range of 1-100 μm (51.36%) (Naidu et al., 2022). In contrast, research on blue mussels (Mytilus edulis) from the Atlantic Coast, France, found a predominance of particles sized 20–100 μm (83%) (Phuong et al., 2018). The result of this study shows a dominant particle size range of 0-100 μm in both types of shellfish. This pattern is consistent with the filter-feeding behavior of shellfish (Hermabessiere et al., 2019) and the size of their food, which ranges from 7–11 μm , facilitating the entry of microplastics sized 0-100 μm (Strohmeier et al., 2012).

Types of Microplastics

FTIR analysis of a blood clamp from Beringharjo Market, Yogyakarta City, identified the presence of N-H groups (3307.97 cm1), C-H2 groups (2108.68 cm1), C-O groups (1634.32 cm1), and N-H bending groups (1387.86 cm1) (Figure 6.a.). For green mussel, FTIR results revealed the N-H group (3324.88 cm1), C-H2 group (2126.97 cm1), C=O group. (1633.97 cm1), and N-H bending group (1361.80 cm1) (Figure 6.b.). These results indicate that the microplastics present are likely nylon (polyamide) polymers. This

is consistent with findings from a study of green mussels from Thai beaches, which also identified the presence of nylon at 40% (Chinfak et al., 2021). Given that nylon has a density greater than seawater (Tang et al., 2021), it tends to sink, making it susceptible to being filtered by shellfish (Phaksopa et al., 2023).

Consuming shellfish sold at Bringharjo Market in Yogyakarta City is still permissible, as no safety limits for microplastic content in food, including shellfish, have been established. However, given the high levels of microplastics found in this study, consumption should be significantly limited, and diversifying protein sources is advisable. Moreover, urgent action is needed to address plastic waste.

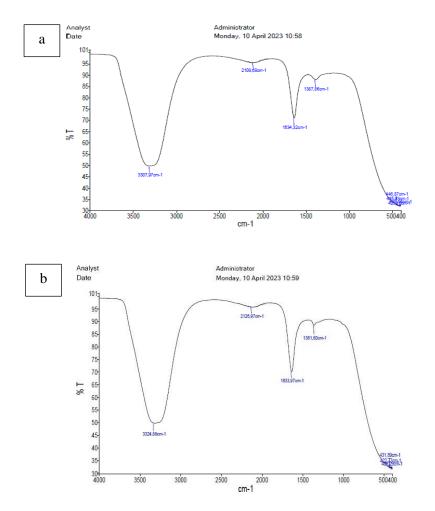


Figure 6. FTIR analysis of microplastics: a. Blood Clam (*Tegillarca granosa*), b. Green Mussel (*Perna viridis*)

CONCLUSION

Based on the research results, it can be concluded that there are no significant differences in the abundance, shape, color, size, and type of microplastic polymers between blood clams and green mussels from Beringharjo Market, Yogyakarta City. The abundance of microplastics in blood clams (839.25 \pm 144.24 microplastics/individual) was not significantly different (p > 0.05) from green mussels (703.75 \pm 159.21 microplastics/individual). In both types of shellfish, microplastics were predominantly in the form of fragments, with black being the most common color. The most abundant microplastic size range in both shellfish was 0-100 µm. The polymer identified in both shellfish was nylon (polyamide).

CONCLUSION

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