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MICROPLASTICS DESORPTION FROM GREEN MUSSELS (*Perna viridis* L., 1758) USING OXIDIZING AGENT $Ca(OH)_2$

DESORPSI MIKROPLASTIK DARI KERANG HIJAU MENGGUNAKAN AGEN OKSIDASI $Ca(OH)_2$

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Abstract

Microplastics (MPs) are currently one of the main pollutants in the aquatic environment and translocation to the human body will cause health problems. This study aims to find the desorption formula for MPs from green mussels (*Perna viridis*) with the oxidizing agent $Ca(OH)_2$ or whiting lime. The results will be compared with the use of HNO_3 which is commonly used for MPs analysis. There were 2 variations of $Ca(OH)_2$, namely 10 and 15 mL, and soaking for 15; 30; and 60 minutes. The abundance and characteristics of MPs (shape, size, and color) were observed in 30 samples with 3 repetitions. The results of this research indicate that all samples have been contaminated with MPs. The highest number of MPs was found in the immersion of $Ca(OH)_2$ for 30 minutes at a concentration of 15 mL of 1% $Ca(OH)_2$. The oxidizing agent $Ca(OH)_2$ was able to remove MPs much higher than HNO_3 with an effectiveness of 72.98–1,120% or 2–12 times, which was supported by statistical results that showed significant differences ($P <0.05$). Thus, the conclusion is that the use of $Ca(OH)_2$ is effective in removing MPs from green mussels with an optimal ratio of 1:3.8 (g/mL).

Keywords*:* Desorption; Green mussel; Microplastics; Oxidizing agent $Ca(OH)_2$

Abstrak

Mikroplastik (MPs) saat ini menjadi salah satu polutan utama di lingkungan perairan dan translokasinya ke dalam tubuh manusia akan menimbulkan masalah kesehatan. Oleh karena itu, penelitian ini dilakukan bertujuan untuk menemukan formula desorpsi MPs dari kerang hijau (Perna viridis) dengan penggunaan oksidator $Ca(OH)_2$ atau kapur sirih. Hasilnya akan dibandingkan dengan penggunaan HNO_3 yang umum digunakan untuk analisis MPs. Terdapat 2 variasi penggunaan $Ca(OH)_2$ yaitu 10 dan 15 mL, serta perendaman selama 15; 30; dan 60 menit. Kelimpahan dan karakteristik MPs (bentuk, ukuran, dan warna) diamati pada 30 sampel dengan 3 kali pengulangan. Hasil penelitian ini menunjukkan bahwa semua sampel telah terkontaminasi MPs. Jumlah MPs tertinggi terdapat pada perendaman $Ca(OH)_2$ selama 30 menit pada konsentrasi 15 mL Ca(OH)2 1%. Oksidator $Ca(OH)_2$ mampu menyisihkan MPs jauh lebih tinggi dibandingkan HNO_3 dengan efektivitas 72.98–1120% atau 2–12 kali, didukung oleh hasil statistik yang menunjukkan perbedaan signifikan ($P <0.05$). Dengan demikian, kesimpulannya adalah penggunaan $Ca(OH)_2$ efektif dalam menyisihkan MP dari kerang hijau dengan rasio optimal 1:3.8 (g/mL).

Kata kunci*: Desorpsi; Kerang hijau; Mikroplastik; Oksidator $Ca(OH)_2$*

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INTRODUCTION

The release of plastic pollutants from various industries and anthropogenic activities into water bodies causes various problems for humans and aquatic ecosystems. Plastic waste that is dumped into the environment, due to physical and chemical factors, such as high UV radiation and mechanical abrasion, will decompose into microplastics (Wright & Kelly, 2017). Microplastics (MPs) include particles measuring 1 µm to <5 µm. Based on previous research results, MPs that enter marine biota can cause slow growth, weaken the immune system, and irritate internal organs (Von Moos et al., 2012; Watts et al., 2015; Welden & Cowie, 2016; Galloway et al., 2017). The presence of MPs can pose a food safety risk if polluted marine biota is consumed by humans. MPs contamination in humans can cause digestive, circulatory, reproductive, respiratory problems, and others (Carbery et al., 2018). Furthermore, the physical hazards of very small MPs allow for the translocation of MPs in marine biota to human organs that ingest MPs (Hollman et al., 2013) through the food chain which is ultimately consumed by humans (Romeo et al., 2015). Thus, microplastics in waters have the potential to pose a major threat to marine biota for consumption. MPs contamination in seafood causes humans to be potentially exposed, causing various health problems (Carbery et al., 2018).

Seafood contaminated with MPs is certainly not safe for consumption. Based on the Republic of Indonesia Law No. 18 of 2012 (2012), food safety is a condition and effort required to prevent food from possible biological, chemical, and other contaminants that can harm, disrupt, and endanger human health and do not conflict with religion, beliefs, and culture of the community so that it is safe for consumption. Therefore, the presence of MPs in seafood is categorized as a contaminant that has the potential to endanger health so that it does not meet food safety standards. Early prevention must be carried out by further studying the MPs that contaminate seafood and the technology for releasing both contaminants in marine biota for consumption. Therefore, this research will conduct a desorption or release test of MPs from the *Mollusca* phylum, namely green mussels which are bottom feeders, using the desorption agent $Ca(OH)_2$. The selection of marine biota from the *Mollusca* phylum because the types of animals from this phylum are widely consumed by humans. In addition, the nature of this animal is to swallow all dissolved materials that enter with food, so, likely, MPs are also carried in with the food that is swallowed. Meanwhile, the use of $Ca(OH)_2$ desorbent is a type of lime that is relatively safe because it is often used in fisheries as a pH neutralizer and binds particles contained in mud that floats in. Therefore, this research is important to find a solution to overcome MPs contamination in marine biota so that it is safe to consume by soaking with whiting lime.

MATERIALS AND METHODS

The research was conducted experimentally and was conducted on a laboratory scale. There were 4 stages of research, namely 1) sample preparation, 2) sample destruction, 3) soaking with $Ca(OH)_2$ desorbent to release MPs from biota, and 4) identification of MPs. The raw materials used were $Ca(OH)_2$ and HNO_3, while the samples used were green mussels. Variations in the use of $Ca(OH)_2$ were distinguished from 2 concentrations, namely 10 and 15 mL of $Ca(OH)_2$ and soaking was carried out with variations of 15; 30; and 60 minutes. The abundance and characteristics of MPs (shape, size, and color) were observed in 30 samples with 3 repetitions. The results were compared with the analysis using HNO_3.

Sample Collection and Preparation

The samples used were green mussels with a weight ranging from 3–7 g and collected from the fish market at Muara Angke, North Jakarta. The samples were first cleaned using distilled water. The length of each individual shell was measured. The shells of marine biota samples were then separated from the inside of the organ using needle tweezers. All parts of the organ were weighed using an analytical balance and their weight was recorded.

Sample Destruction

The destruction method is based on Lusher et al. (2017) and also adopted by Firdausya (2022). The mussel organs were soaked in 70% HNO_3 overnight at room temperature in a fume hood with a ratio of 1:5 of the weight of the body tissue (g) to HNO_3 (mL) in a beaker glass. It is chosen HNO_3

because it has been proven to reduce the density of biological tissue by >98% (Claessens et al., 2013). Furthermore, in the same way, digestion was carried out, but using different variations of 1% $Ca(OH)_2$ (Harsanto & Saputra, 2023), namely 10 and 15 mL. The samples were then soaked in a desorbent solution ($Ca(OH)_2$) with a ratio of 1:5 (g/mL), and soaking time of 15; 30; and 60 minutes.

Separation of Microplastics from Organic Materials

The destructed sample suspension was then added with a saturated NaCl salt solution with a ratio of 1:4 volume of HNO_3 (mL) to NaCl (mL). The purpose of NaCl is to separate MPs particles contained in the mussel body tissue (Claessens et al., 2013; Lusher et al., 2017). The same method is also carried out with sample suspensions that have been soaked with $Ca(OH)_2$ desorbent. The results will be compared between digestion using HNO_3 and those using $Ca(OH)_2$ desorbent.

Microplastics Identification

The diluted sample was then taken using a 1 mL dropper pipette and repeated 3 times. The sample was placed on a glass slide and then observed. MPs were identified based on Hidalgo-Ruz et al. (2012) with several characteristics, namely not having a visible cellular or organic structure, fiber-type MPs must be the same thickness throughout their length, particles must have a clear color, and if the particles are transparent or white, they must be examined at high magnification.

Identification of MPs includes the shape, color, and size of the particles. The shape of MPs is divided into fragments, films, and fibers (Jiang et al., 2018). The color of MPs is divided into transparent, blue, red, black, green, and yellow (Hastuti et al., 2019). The size of MPs is divided into 5, namely <0.1 µm; 0.1–0.5 µm; 0.5–1.0 µm; 1.0–5.0 µm; and >5.0 µm (Digka et al., 2018). Identification of the shape and color of MPs was carried out using a light microscope, while the measurement of MPs was carried out using an Olympus BX51 microscope.

Data Analysis

Microplastics found in the shellfish body were classified based on shape, color, and size which were analyzed descriptively. The abundance of MPs is presented as particles/mL which is calculated using the formula $MPs\ abundance = \frac{number\ of\ MPs}{total\ volume} \times 100$.

Data analysis to see the difference in total MPs in different types of desorbent treatments using the t-test with a significance level of 5%. In addition, the effect of desorbent administration will be analyzed using the t-test method, using Microsoft Excel software and the Software Package used for Statistical Analysis (SPSS) 25.

RESULTS

The results of microplastic desorption in green mussels are shown in Figure 1. From all samples, it shows that the use of $Ca(OH)_2$ as a desorbent of MPs is very effective when compared to HNO_3. This is also supported by statistics showing a significant difference ($P < 0.05$) among the variation of $Ca(OH)_2$ concentration and soaking time. From Figure 1, it is clear that the amount of MPs that can be desorbed or removed from all samples is greater when using $Ca(OH)_2$ compared to HNO_3. This further emphasizes that soaking green mussels with whiting lime can remove MPs and prevent MPs from being eaten by humans.

Data related to MP characteristics, including shape, size, and color are shown in Figures 2–6. The results of observations under the microscope are shown in Figures 3 and 5. The results of observations under the microscope indicate the presence of actual MP in the samples used and this means that the green mussels have been contaminated by MPs. The desorption ratio of MPs with and without $Ca(OH)_2$ based on the body weight of green mussels is shown in Table 1.

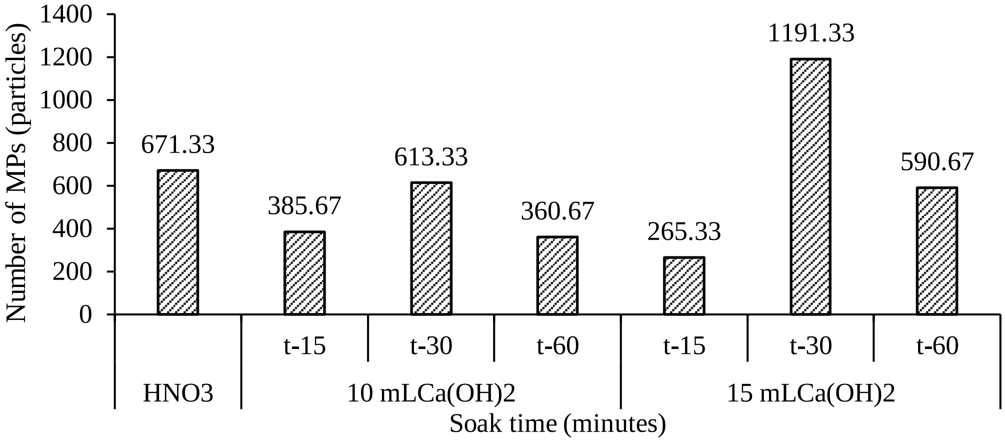

Figure 1. Average amount of MPs in green mussels

Table 1. The ratio of MPs desorption with and without $Ca(OH)_2$

Treatment	Body weight average (g)	Number of MPs (particle/bw)	Ratio (g/particle)
Without $Ca(OH)_2$	4.4	671.33	1:151.43
With $Ca(OH)_2$	4.4	1191.33	1:268.72

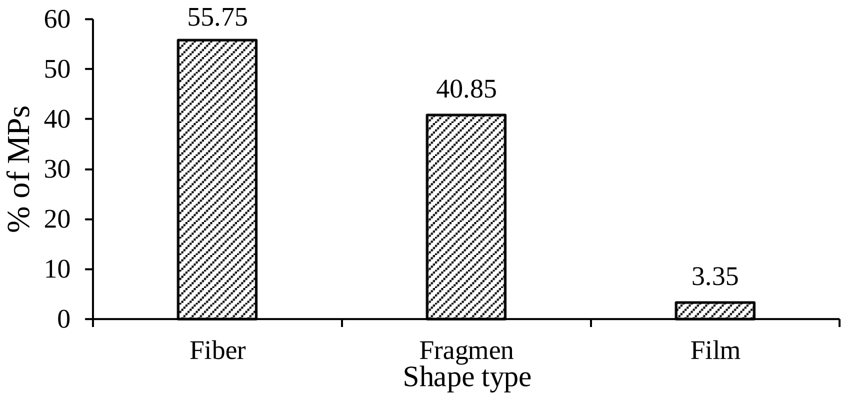

Figure 2. Percentage of MPs based on shape in green mussels

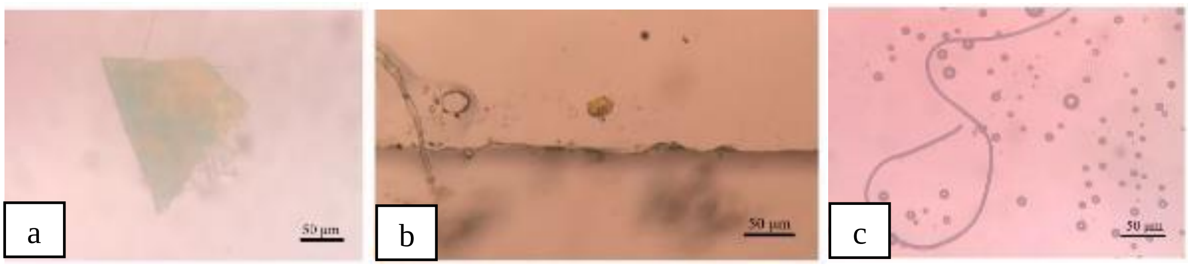

Figure 3. The shape of MPs in green mussels, film (a), fragment (b), and fiber (c)

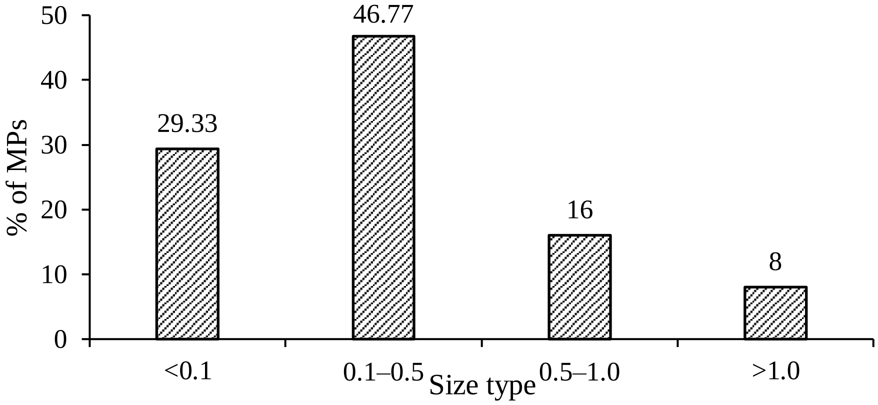

Figure 4. Percentage of MPs based on size in green mussels

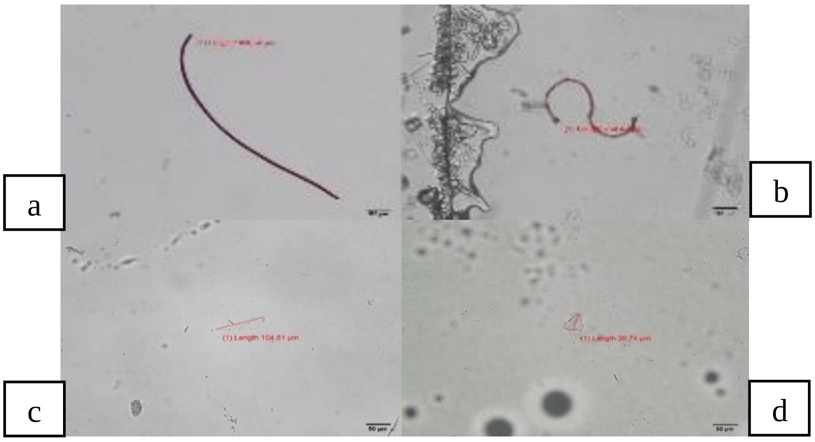

Figure 5. The size of MPs in green mussels, >1 mm (a); 0.5–1 mm (b); 0.1–0.5 mm (c); <0,1 mm (d)

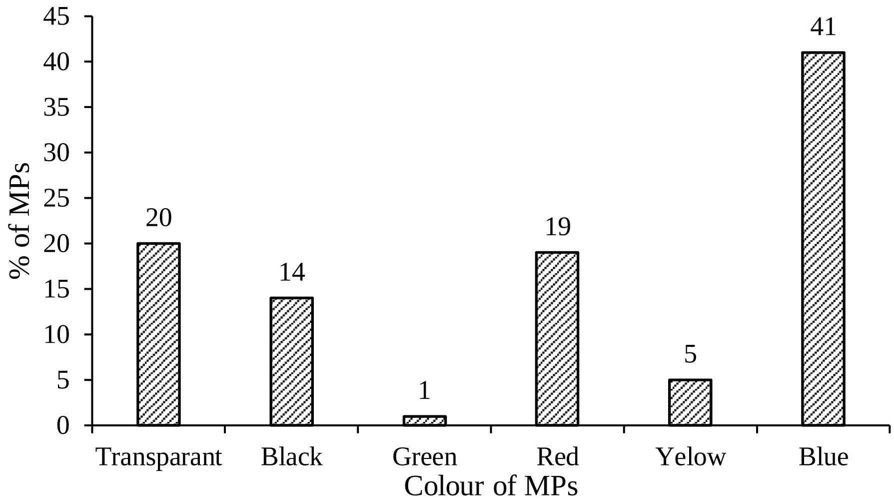

Figure 6. Percentage of MPs based on color in green mussels

DISCUSSION

This study aims to obtain an effective formula for releasing MPs from green mussels using $Ca(OH)_2$ oxidizer. This experiment was compared with the previous research method conducted by Firdausya (2022) which analyzed the MPs content in green mussels using 70% HNO_3. From these results, the optimal single concentration was then used for further testing and compared with the use of $Ca(OH)_2$. The use of $Ca(OH)_2$ is expected to release more MPs from the body of green mussels.

Desorption of MPs

The safety of seafood food which is a current issue can be achieved with one of the innovations produced, namely the use of whiting lime as an adsorbent of MPs from green mussels. Carrying out initial treatment in the form of soaking with whiting lime, it can help translocate more MPs into the human body. Thus, the safety of consuming green mussels is more guaranteed and avoids the accumulation of MPs translocation from mussels into the human body.

For other types of biotas, including fish, shrimp, crabs, and other *Molluscs* phylum, it is assumed that they also contain a lot of MPs. By using whiting lime adsorbent, it is assumed that MPs from other marine biota will also be removed from their bodies more so that they are safer to consume. Further research for other types of biotas is still very much needed so that the safety of seafood can be maintained from MPs contamination.

The results of this study indicated that the highest MPs desorption is soaking for 30 minutes with 15 mL of $Ca(OH)_2$, which reached 1,191.33 particles. The number of MPs successfully released from green mussels was very high when compared to the use of HNO_3 which only released 671.33 MPs particles. The effectiveness of desorption reached 77.43%. This shows that soaking with $Ca(OH)_2$ before consuming green mussels must be done as an early preventive health effort. This can be explained from the results of previous research which shows the dangers of microplastics. Microplastics measuring <1 µm can enter the systemic blood circulation through the transepithelial mechanism and it can cause microplastic accumulation in the spleen, lymphatic duct (nasal-associated lymphoid tissue), and intravascular (Eyles et al., 2001). Other research shows that microplastics with sizes up to 240 nm can also penetrate the placental barrier (Wick et al., 2010). In mice, an experiment involving the administration of 0.1 mg of microplastics in 0.5 mL of drinking water resulted in a decrease in liver mass, reflecting impaired liver metabolic function (Deng et al., 2017). Although no studies have yet found how much toxic impact microplastics have on human health, we must remain cautious.

Based on previous research results, microplastics were found in almost all green mussel samples (Falahudin et al., 2020; Ukhrowi et al., 2021; Nabila & Patria, 2021; Firdausya, 2022; Cordova et al., 2020). This shows that green mussels are generally contaminated by MPs. The characteristics of green mussels as filter feeders, all contaminants in the waters including MPs will be ingested into their bodies (Wang et al., 2014). The number of MPs found in green mussels indicates the level of pollution in the waters (Goh et al., 2021; Li et al., 2018). MPs pollution can be caused by two important factors, namely the living behavior of biota and the level of pollution in the surrounding environment due to anthropogenic activities. Water conditions also affect MPs pollution, one of which is the tidal backflow. The Java Sea with an average current speed of 0.04–0.32 m/s has a major influence as a trap for various plastic waste in the long term (Handyman et al., 2019). Thus, various marine biota in the waters of the Java Sea have a high risk of being contaminated by MPs, which comes from the disposal of community waste from land.

Green mussel is a marine biota that obtains food by filtering seawater that enters its body (filter feeder) and microplastics enter at the same time as the mussel absorbs food (Wang et al., 2014). According to Browne et al. (2015), MP that is swallowed will settle in the digestive tract, some are excreted through feces or undergo translocation to the intestinal epithelial layer to other body tissues. In addition, green mussels are animals that do not move much so the risk of being contaminated by MP is even greater (Yaqin et al., 2014).

Characteristic of Microplastics

There are several potential scenarios for the fate of plastics in the environment. Once plastics enter the environment, they can be transported through various environmental compartments, for example, through wastewater treatment plants (Gündoğdu et al., 2018) and then discharged into rivers and eventually into the sea. Waves can wash some of the plastics to the shore (Crichton et al., 2017) In addition, biotic (enzymatic processes of microorganisms) (Tsiota et al., 2017) and abiotic (photolysis, thermal stress, hydrolysis, and mechanical stress) (Mael et al., 2019) aging processes can cause changes in the chemical and physical properties of plastics transformed into microplastics. Regarding the environmental scenario of plastic waste on the shore, ultraviolet (UV) irradiation results in autocatalytic photooxidation processes, and hydrolysis (Andrady, 2011), which can lead to chemical changes of plastics. In some cases, hydrolysis can lead to the release of low molecular weight water-soluble organic matter. The appearance of cracks on the particle surface followed by surface ablation results in the release of secondary microplastics and nanoplastics fragments.

The aging of microplastics starts from the surface directly exposed to environmental stress (Andrady, 2017). It can then trigger the formation of nanoplastics through two pathways are cracks can penetrate to the deeper part of the particle resulting in the breakage of most of them (Andrady, 2017; Koelmans et al., 2015). On the other hand, fragmentation of the degraded surface layer can release microplastics and nanoplastics fragments. In addition, mechanical stress can also cause the fragmentation of plastics due to increased abrasion in the wave zone (Song et al, 2017; Wright et al., 2021).

The transfer of MPs from the environment to marine biota occurs indirectly, namely by consuming animals at the bottom of the food chain. Due to their small size, microplastics can be ingested by various aquatic animals, either accidentally (entering with seawater) or through active selection (microplastics disguised as prey). Ultimately, MPs will accumulate in the body of the biota, and humans as end consumers will also be exposed to these microplastics secondarily (Smith et al., 2018).

In this study, microplastic fragmentation in green mussels is discussed through the specific characteristics of microplastics, namely size, color, and shape, so that the origin or source of the plastic can be predicted. The characteristics of microplastics found in green mussel samples include film, fragment, and fiber forms (Figures 2 & 3). The most dominant form of microplastics found in green mussel samples was fiber (56.52%). Generally, it comes from boat ropes and fishing nets. According to Ayuningtyas et al. (2019), fragment-type microplastics can come from bottles, plastic bags, and pieces of PVC pipe. Furthermore, Barnes et al. (2009) stated that the fragment type is the result of the breakdown of various everyday plastic products. The high level of fragment microplastics is because this type is easily spread and can survive for centuries in sediment (Derraik, 2002).

According to Kor et al. (2020), fiber microplastics are most widely used in the textile industry made from polypropylene (PP), polyethylene (PE), and fishing gear used by fishermen. Browne et al. (2015) also stated that fiber microplastics can come from laundry waste and capture fisheries. Washing machine wastewater can produce >1,900 fibers per wash. The increase in human population has also resulted in increased contamination of fiber microplastics through household waste that enters the waters.

Meanwhile, the fragment form can come from bottles, plastic bags, and pieces of PVC pipe (Ayuningtyas et al., 2019). Barnes et al. (2009) stated that the fragment type is the result of the breakdown of various everyday plastic products. The high fragment type of microplastics is because this type is easily spread and can survive for centuries in sediment (Derraik, 2002).

Film-type microplastics were the least found in the four sample biota. This is because the size of the film type of microplastics is generally very thin and small so it is easily carried away by the current. Film-type microplastics also have low density so they tend to float on the surface and can easily move from one place to another (Ayuningtyas et al., 2019).

Identification of microplastic sizes found in green mussel samples included sizes <0.1; 0.1–0.5; 0.5–1.0; and 1.0–5.0 µm (Figures 4 & 5). The most dominant microplastic size found in green mussel samples was size 0.1–0.5 µm (46.77%). According to Claessens et al. (2013), the fragmentation of macro-sized plastics into micro-sized ones is caused by ultraviolet radiation, mechanical forces from sea waves, and oxidative materials from plastics. Digka et al. (2018) and Li et al. (2018) also reported that small-sized MPs dominate more in mussels when compared to the environment. One factor that can cause this is that large-sized MPs are easily carried away by tidal currents, reducing the possibility of large-sized MPs settling on the seabed (Tubagus et al., 2020).

The size of MPs is a major factor because it can affect environmental bioavailability (Hastuti et al., 2019). MPs particles can block the digestive tract based on their size. Boerger et al. (2010) stated that ingested MPs that are not excreted can accumulate in the digestive tract, resulting in starvation, malnutrition, and even death. The size of MPs particles can also determine the level of toxicity of MPs (Kor et al., 2020). Organic pollutants can be adsorbed on the surface of MPs due to their hydrophobic nature. The adsorption capacity of organic pollutants on the surface of MPs is reported to increase with increasing surface area of MPs (Aliabad et al., 2019).

Microplastic characteristics are important information that needs to be identified so that the origin of the plastic and the level of danger of the plastic contaminant will be known. Microplastic characteristics consist of identifying the colour, shape, and size of microplastics. Identification of the colour of microplastics found in green mussel samples includes transparent, black, yellow, green, red, and blue (Figure 6). The most dominant microplastic colour found in green mussels was blue (41%), and generally, comes from boat ropes or fishing nets. The color of microplastic particles is caused by the colour of the original plastic that has been physically or oxidatively degraded, exposure to UV and infrared rays, weather, sunlight (photodegradation), or absorption of chemicals. The color of microplastics also indicates the length of residence time on the sea surface and the level of weathering. The yellowish colour indicates a longer stay at sea and is oxidized. Black and dark colours (brown, purple, and green) indicate polystyrene (PS) and polypropylene (PP) types which are suspected of also containing pollutants such as adsorbed polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) (Hidalgo-ruz et al., 2012). Examples of PS polymers are cutlery or food containers and types of PP polymers are rope, nets, and plastic bottle caps.

The color variability of microplastic particles can be associated with the source of microplastics (Sathish et al., 2019). Based on the colour of MPs found in green mussels, they broadly come from shopping bags, boat ropes, fishing nets, and mineral water bottles.

The Ratio of Desorption

So far, microplastic desorption analysis has used the standard developed by Roch and Brinker (2017), using HNO_3. The use of eco-friendly materials such as whiting lime has not been studied before. The use of natural desorbents is very important for marine biota consumed by humans. Thus, this study has a novelty related to the eco-friendly desorbent agent of MPs.

Based on the findings of MPs in green mussels, the ability of $Ca(OH)_2$ or whiting lime as a desorbent of MPs can be determined based on the weight of the biota (Table 1). The ratio of MPs desorption from green mussels using $Ca(OH)_2$ or whiting lime is presented in Table 1 below. For every 1 g of green mussels eaten without soaking, it is assumed that human MPs intake from green mussels consumption is 151.43 MPs particles. However, if someone consumes 1 g of green mussels by pre-treating $Ca(OH)_2$, then it will avoid swallowing 268.72 MPs particles.

Several studies that have been conducted are mostly by soaking green mussels in HNO_3 and obtained as much as 23.11 MPs/g body weight (Fathoniah & Patria, 2021), 14 MPs/g body weight (Irnidayanti et al., 2023), which is relatively less in releasing MPs when compared to soaking using $Ca(OH)_2$ which can release 280.18 MPs/g body weight. This research also proved that the effectiveness of soaking with $Ca(OH)_2$ reached 177.41% compared to HNO_3.

Of course, this is very worrying if initial treatment is not carried out first, even though there are no research results that prove that MP is dangerous to health. However, precautions need to be taken, one of which is pre-treatment to remove MP from seafood. Pre-treatment with $Ca(OH)_2$ adsorbent agent is an important alternative to remove MP from seafood before consumption, especially green mussels.

Thus, this comparison can be used as a guideline for making an initial processing formula before consuming green mussels. Soaking green mussels in 1% whiting lime as much as 15 mL for 30 minutes can release almost 77.45% of MPs from the body of green mussels compared to soaking with nitric acid. In addition, nitric acid is toxic, so it is dangerous for health if it contaminates foods. If you do not soak at all or directly consume green mussels, it is much more dangerous because MPs are also swallowed directly.

CONCLUSION

Based on the data obtained, it can be concluded that the use of desorption agents using $Ca(OH)_2$ or whiting lime is very effective in reducing microplastic content in green mussels. The optimal ratio of 1% $Ca(OH)_2$ desorbent to release MPs in green mussels was 1:268.72 (g/particle), which reached almost 77.45% release of MPs compared to HNO_3. Based on the research results, it is recommended to soak seafood first with 15 mL of 1% $Ca(OH)_2$ with a set formula for 30 minutes. By soaking seafood before consumption, it can prevent microplastic contaminants from entering or being eaten by humans. Whiting lime as a safe, cheap, and easy-to-do desorption agent can be used as an alternative to avoid microplastic contamination in seafood, and food safety from the sea can be addressed appropriately.

Further studies are needed to test other marine biotas that are often consumed by the community. This study is very important to enrich the MPs pollution database in marine waters and other ecosystems. The problem of plastic waste is an important and urgent problem to be addressed seriously and to find alternatives for mitigation and appropriate technology to handle it.

Nevertheless, the most important thing is to reduce the use of plastic and not to throw plastic waste into the environment, especially into the waters. The small efforts we make are environmental conservation efforts for the future. In addition, the security of the marine ecosystem and the biota that live in it will be maintained sustainably.

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