Microplastics Abundance and Uptake by *Meretrix lyrata* (Hard Clam) in Mangrove Forest

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Highlights:

- The correlation between microplastics concentration and their uptake by *Meretrix lyrata* is highlighted.
- The uptake of microplastics by *Meretrix lyrata* is highly dependent on the duration of exposure and the microplastics concentration.
- Microplastics particles at smaller sizes tend to be easily uptaken by *Meretrix lyrata*.
- PET is the type of microplastics that is most commonly uptaken by *Meretrix lyrata* as compared to other types of plastics.
- Microplastics particles can migrate to the tissue of *Meretrix lyrata*.

Abstract. The aim of this study was to determine the abundance and distribution of microplastics in a mangrove ecosystem, while investigating its uptake by *Meretrix lyrata.* Microplastics were extracted from 10 L of mangrove sediment using a floatation method. Soft tissues of *M. lyrata* were digested and the microplastics were filtered and observed under a light microscope. Microplastics ranging from 21 μm to 100 μm were the most abundant in the mangrove layers at 936 ± 34 particles/kg (dry sediment) to 1,227 ± 55 particles/kg (dry sediment) (27.19% to 31.16% of the total quantity of recovered microplastics). The most abundant microplastics, with size from 5 μm to 1000 μm, were found in the deepest layer. *M. lyrata* accumulation of microplastics averaged at 0.35 ± 0.08 particles/g tissue and 0.23 ± 0.07 particles/g tissue (wet weight) before and after three-day depuration, respectively. Microplastics with size from 5 to 20 μm were the most commonly uptaken by the clams, averaging at 58% to 72% for pre-depuration and post-depuration, respectively. This study revealed that the most predominant size of microplastics in the sediment was 5 μm to 20 μm, which concurs with the results of the microplastics most commonly accumulated by *M. lyrata*. It is believed that microplastics accumulation in mangrove areas increases over time. On the other hand, the uptake rate of microplastics by *Meretrix lyrata* decreases over time, flushing microplastics from its system more frequently.

Keywords: *bioaccumulation; hard clam; microplastics; mangrove; Meretrix lyrata; uptake.*

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1 Introduction

In the last 60 years, the production of plastics has grown rapidly. According to the statistics presented by the PlasticsEurope Market Research Group [1], global plastics production in 2015 reached up to 322 million tonnes. Per capita plastics consumption in 2015 reached 136 kg in Western Europe and 139 kg in North America, as highlighted in *Plastics Insight* [2].

Approximately 10 to 20 million tonnes of plastics end up in the ocean each year due to improper disposal and inadequate waste management, as reported by Fauziah, *et al*. [3]. Chen [4] predicts that in the next ten years, the amount of plastic waste entering the ocean annually will be more than doubled. Lambert, *et al.* [5] have reported that about 80% of marine plastic debris comes from land, highlighting the importance of proper waste management.

Under the influence of mechanical action, bio-degradation, photo-degradation, photo-oxidation degradation, and other processes, large plastic fragments gradually degrade into smaller pieces which will remain in the ocean for centuries, as stated by Rochman, *et al.* [6]. Plastics that disintegrate into smaller fragments and plastics that are manufactured at micro-size are referred to as microplastics, with particle diameter less than 5 mm, as mentioned by Fauziah, *et al*. [7].

Microplastics make up more than 92% of plastics waste and have been spotted in marine and coastal environments all over the world (Fauziah, *et al.* [3], Claessens, *et al.* [8] and Thompson, *et al.* [9]). Table 1 lists the reported number of microplastics items per kg of sediment worldwide.

Table 1 Abundance of microplastics in marine ecosystems (items/kg) [10].

Country	Location	Concentration (items/kg)
Italy	Sicily	160 ± 31
Spain	Barcelona	148 ± 23
France	Cassis	124 ± 36
Turkey	Dikili	248 ± 47
Denmark	Kalundburg	88 ± 33

Microplastics consist mainly of polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), polyethylene terephthalate (PET), polystyrene (PS), polyamide (PA) and nylon (Fauziah, *et al.* [3]). They come in different forms: line or fiber, fragments, pellets, micro-beads, and foam. The varying sizes and colors of microplastics resemble food residues or plankton. Thus, microplastics are commonly mistaken for food by many marine species.

The presence of microplastics has been reported in lots of organisms at different trophic levels in the marine food web, including various zooplanktonic organisms at the base of the food web as well as invertebrates and vertebrates at higher trophic levels, as explained by Moos, *et al*. [11].

The results of the survey by Kühn, *et al*. [12] showed that the number of sea turtles, marine mammals, and seabirds that are severely affected by plastics (including microplastics) has increased from 86%, 43% and 44% in 1997 to 100%, 66% and 50%, respectively in 2015.

Derraik [13] and Thompson, *et al.* [10] report that ingested microplastics may cause mechanical damage, blocking of the esophagus, or a false sense of satiety, followed by reduction in feeding efficiency, lack of energy and even death, and transmit persistent organic pollutants to the animals' tissue.

Marine microplastics are a global concern because their trans-boundary movement is impossible to regulate. As a result, microplastics can migrate from areas with high anthropogenic activity and improper waste systems to isolated and secluded areas, far removed from human civilization. The review by Fauziah, *et al.* [3] implies that there are no areas that are devoid of microplastics.

Mangrove areas, which have significant ecological and economic impacts on human life, have repeatedly been reported to be polluted with microplastics. Since mangrove areas are the nursery ground for the majority of marine species, it is imperative to understand the level of microplastics pollution and its impact on the fauna.

This study was conducted to determine the abundance and distribution of microplastics in mangrove sediment. In addition, the uptake of microplastics by marine fauna, namely *Meretrix lyrata*, or the hard clam, was also investigated.

2 Methodology

2.1 Study Area

The study area is located on Carey Island (Pulau Carey), Selangor (101º22' E and 2º52' N) on the west coast of Peninsular Malaysia. The area of the mangrove forest reserve is 1,876.85 ha and it has two main types of mangrove sediments (Figure 1) namely, sandy and clayey. For the purpose of this study, the clayey type mangrove area was chosen to collect the sediments for our analysis.

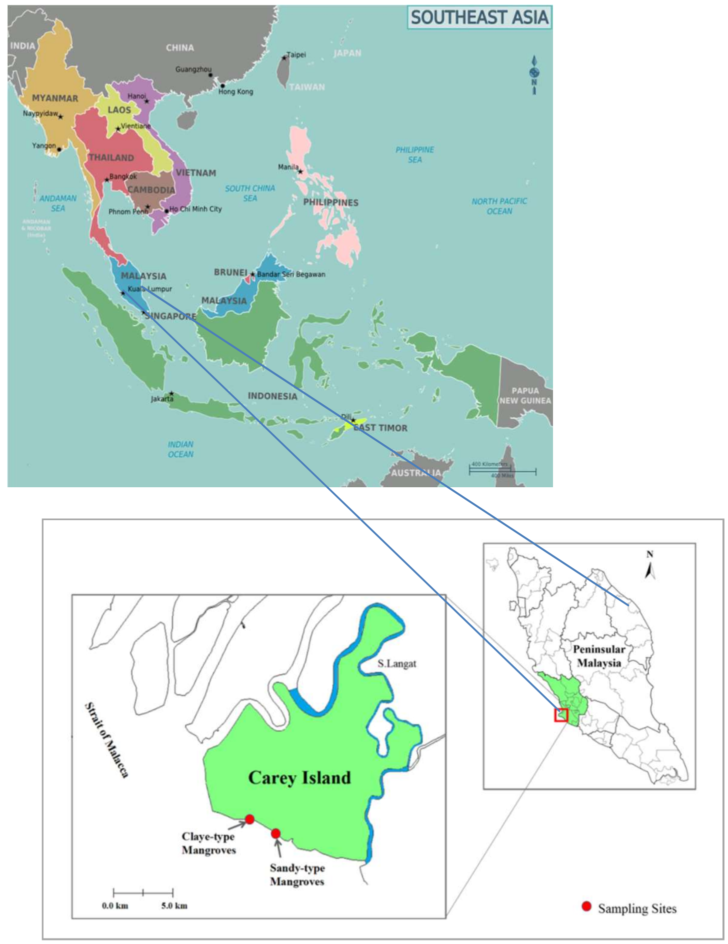

Figure 1 Map of the study area.

2.2 Sampling of Mangrove Sediment

The sediment samples were collected at the low tide line using 10 × 10 × 10 cm^3 sampling drawers. Sediments were taken from nine separate points, located 8 m apart in undisturbed areas (Figure 2) to give a total of nine replicates. The samples from each point were separated into three layers and labeled as top layer (0-3 cm), middle layer (3-6 cm), and deep layer (6-10 cm). In total, 27 samples were collected at each sampling event during each month from February 2017 to January 2018.

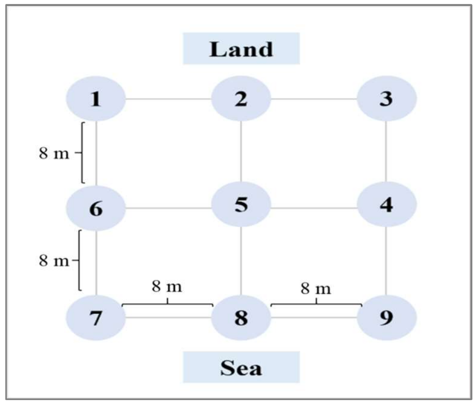

Figure 2 Location distribution of sampling points.

The sediment samples were placed into zip-lock bags. Wet sediment samples were dried in an oven at 60 °C to complete dryness prior to gentle crushing using mortar and pestle. Microplastics were extracted from the sediment sample using the improved flotation method from Claessens, *et al.* [14]. 100 mL of 5 M NaCl (sodium chloride) solution was added to 60 g dry clayey type sediment in a 250 mL conical flask. 2 mL of Tween-80 was added to prevent the aggregation of soil particles during mixing.

The mixture was shaken on a shaker at 200 rpm for 2 minutes and allowed to settle for 6 hours. The supernatant was then sieved using a 1-mm pore size sieve. The particles remaining on the sieve were subjected to further analysis. The supernatant was filtered through a glass fiber filter (Merck AP4004700) using a vacuum filtration unit. Recovered microplastics particles on the filter glass were examined using an optical microscope (Olympus BZ40 at 10× magnification).

Microplastics recovery efficiency was conducted to ensure the accuracy of the results from the extraction method. Microplastics that were identified visually were counted and measured. The microplastics within a size range of 1001 to 5000 μm were classified according to particle type, i.e. line, pellet or film, as categorized by Fauziah, *et al.* [7] and Eerkes-Medrano, *et al.* [15].

2.3 Sampling of *Meretrix lyrata*

For the bioaccumulation of microplastics by *M. lyrata*, 20 adult-size individuals were collected and digested to determine the concentration of microplastics in the organisms.

2.4 Experimental Set-up for Microplastics Uptake by *Meretrix lyrata*

For the purpose of the microplastics uptake by *M. lyrata*, 0.5 g polyethylene powder varying in size from 53 µm to 75 µm (Sigma-Aldrich) were introduced after three days of depuration. *M. lyrata* with 22.51 ± 1.00 mm width, 32.80 ± 1.18 mm height, and 40.42 ± 1.29 mm length were used in the exposure experiments. Four independent exposure experiments were performed in total, one for every exposure time.

The set-up consisted of six aquariums (six replicates) with 600 mL of filtered artificial seawater with 18 individual clams. The water in every aquarium was filtered with a glass fiber filter (Whatman, Grade 1, 55 mm, pore size 11 µm) using a vacuum filtration unit after 3, 6, 9, and 12 hours. The total body weight (g) of the clams was measured with a precision balance (0.01 g precision). The shell length (cm), shell width (cm), and shell height (cm) were determined with a vernier caliper (0.1 mm precision). The amount of microplastics uptake by *Meretrix lyrata* was calculated using the following formula

$$\text{Amount uptaken by } \textit{Meretrix lyrata} = MP_{\text{initial}} - MP_{\text{final}}$$

where:

$MP_{initial}$ = Total amount of microplastics added in the aquarium at the beginning of the exposure time

MP_{final} = Total amount of microplastics remaining in the aquarium at the end of the exposure time

3 Results and Discussion

Based on the results of all sampling events, the abundance of microplastics ranged from 404 ± 23 particles/kg (dry weight) to 532 ± 41particles/kg (dry weight). One-way ANOVA indicated that there was no significant difference ($P > 0.05$) in the microplastics abundance between the different sampling months.

3.1 Microplastics in Sediments

The average number of microplastics with a size range from 21 µm to 100 µm ranged from 936 ± 34 particles/kg (dry sediment) to 1,227 ± 55 particles/kg (dry sediment), which contributed 27.19% to 31.16% of the total quantity of recovered microplastics in the different layers. Figure 3 illustrates the distribution of microplastics according to size at different layers of the mangrove sediment.

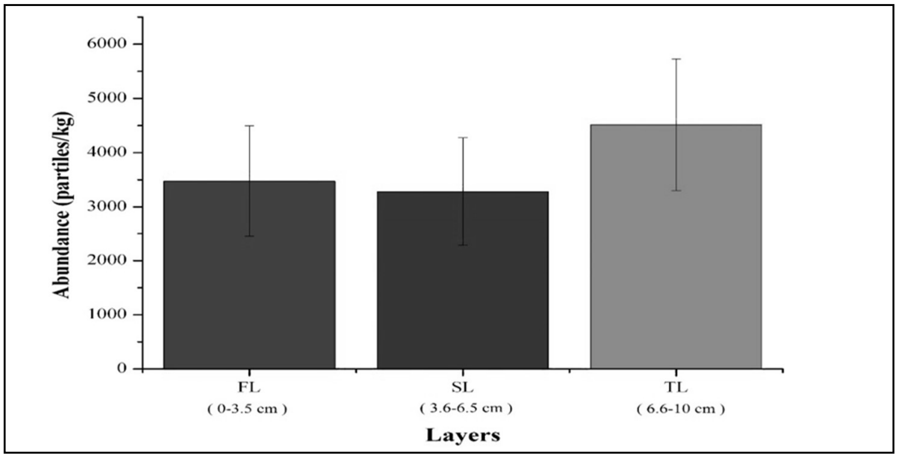

Figure 3 The abundance of microplastics in mangrove sediment at different layers (FL: first layer; SL: second layer; TL: third layer).

The One-way ANOVA indicated that there was a significant difference ($P < 0.05$) between the microplastics abundance and the depth of the sediment layers. The most abundant microplastics were found accumulated in the third layer (TL) with an average abundance of 4,513 ± 81 particles/kg (dry sediment), which accounted for 40.05% of the total recovered microplastics.

Microplastics with size from 5 μm to 1000 μm were found most abundantly in the deepest layer. The cause of this phenomenon may be that plastics buried at 6.5-10 cm depth are less affected by the tide and currents. The longer residence time allows the degradation of the plastics, increasing the number of microplastics particles. Also, some microplastics may penetrate from the surface to deeper soil because of the eroding effect of the environment. Figure 4 and Figure 5 highlight the abundance of microplastics according to size and type, respectively. Finer microplastics particles tended to accumulate more in the deeper layers than in the surface layer, which is probably due to the structure of the sediment, which only allows movement of finer particles within its interstitial space. There is a significant correlation ($P < 0.05$) between size and depth of the sediment.

The most common type of microplastics found in clayey-type mangrove sediments were line microplastics, followed by pellet and film microplastics. The same trend was recorded in all sediment layers namely, FL, SL, and TL. This result is similar to other microplastics studies, conducted in the UK by Thompson, *et al.* [10], in Belgium by Claessens, *et al.* [14] and in Singapore by Mohamed & Obbard [16].

These studies showed that line or fiber microplastics were the most common type of microplastics particles. The line microplastics found on Carey Island may originate from the degradation of some human-made fiber products, such as clothing and bags that have been discarded in the coastal area. These line microplastics may also be introduced to mangrove soils by municipal wastewater containing numerous microfibers and broken fishing nets.

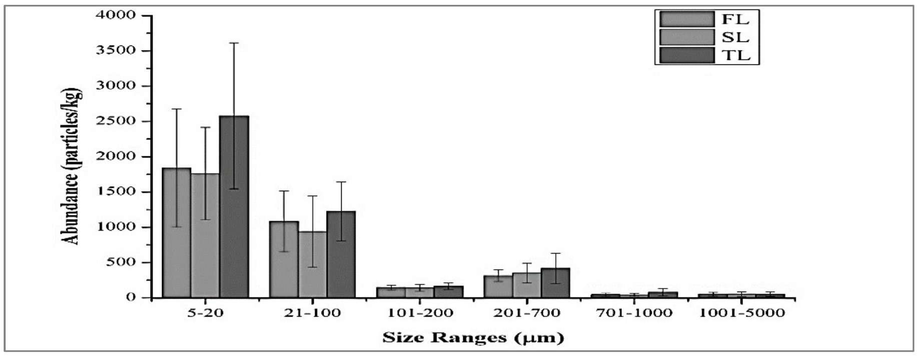

Figure 4 Abundance of microplastics according to size.

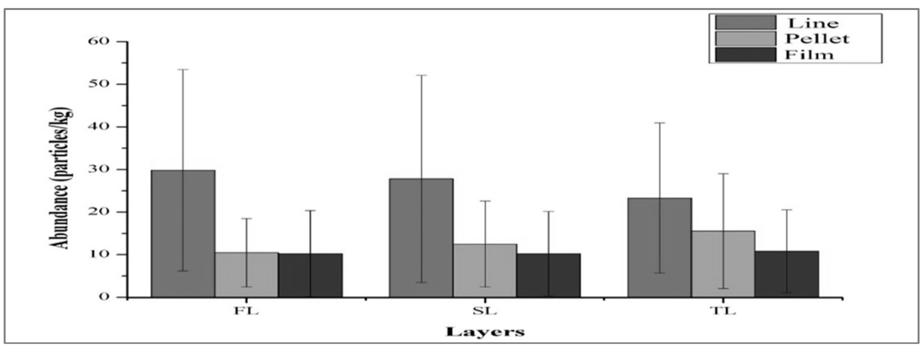

Figure 5 Abundance of microplastics according to type.

3.2 Microplastics in *Meretrix lyrata*

Figure 6 shows the microplastics present in the tissues of *M. lyrata* with and without depuration, while Figure 7 shows the average size range in *M. lyrata* before and after depuration. The average microplastics concentration in *M. lyrata* was 0.35 ± 0.08 particles/g tissue (wet weight) before three-day depuration (Figure 7). After three-day depuration, the average microplastics concentration in *M. lyrata* was 0.23 ± 0.07 particles/g tissue (wet weight). After three days of depuration, the microplastics body burden decreased. This indicates that some microplastics were present in the digestive tract of the hard clams. The results revealed that the most predominant size of microplastics recovered from *M. lyrata* was between 5 µm to 20 µm. This coincided with the microplastics size frequency distribution in their living environment.

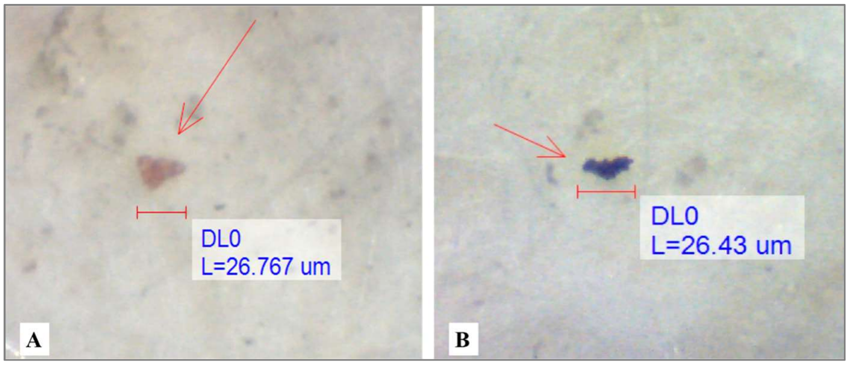

Figure 6 Selected microplastics specimens recovered from *M. lyrata* (A: red particle recovered from *M. lyrata* with depuration; B: blue particle detected in *M. lyrata* without depuration).

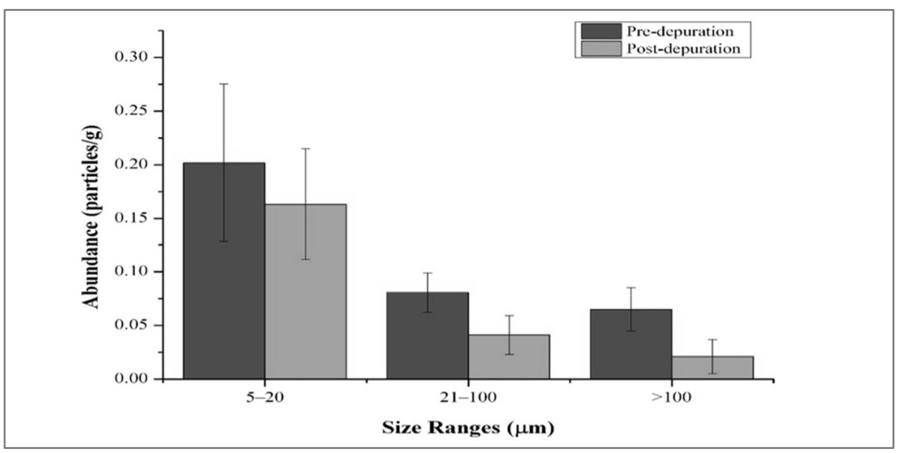

Figure 7 Average microplastics abundance in the tissues of digested *M. lyrata.*

3.3 Microplastics Uptakes by *Meretrix lyrata*

Microplastics were taken up and accumulated in the hard clams after experimental exposure. Figure 8 indicates the microplastics accumulation over exposure time. When exposed for nine hours, the microplastics accumulation was

27.44 ± 1.53 mg/individual. Microplastics accumulated to 28.29 ± 1.05 mg in one individual hard clam after exposure for twelve hours. Figure 9 shows that the uptake rate of microplastics decreased over time.

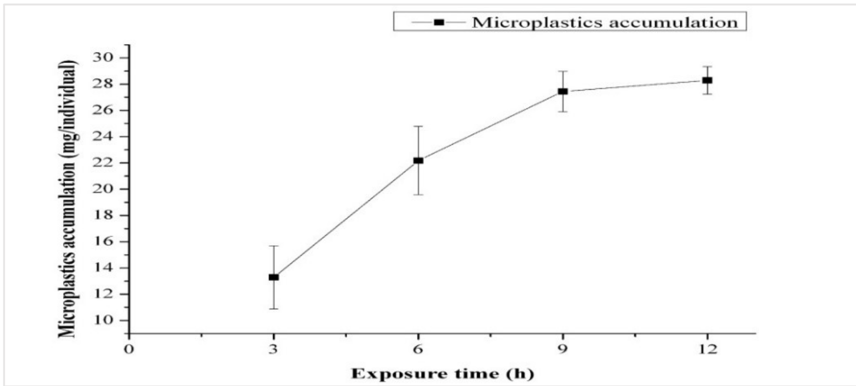

Figure 8 Microplastics accumulation during the experimental exposure.

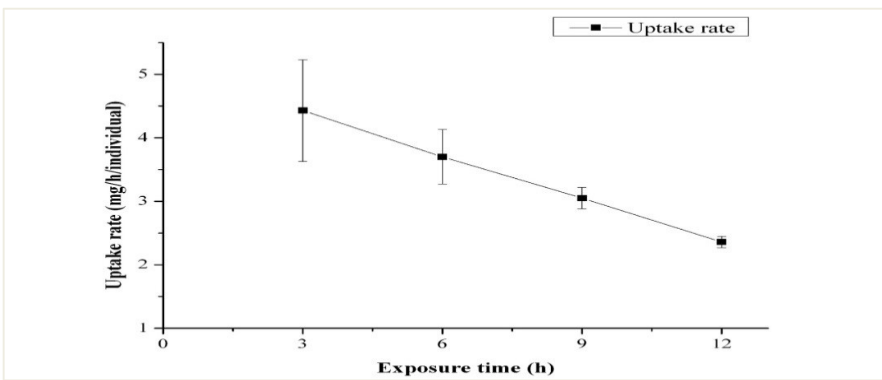

Figure 9 Uptake rate of microplastics during experimental exposure.

The decrease in uptake probably results from the increased number of microplastics that has been accumulated since the beginning of exposure. At the end of the experiment, the experimental individuals did not differ significantly (length = 40.42 ± 1.29 mm; height = 32.80 ± 1.18 mm; width = 22.15 ± 0.99 mm). This experiment showed that microplastics could enter into the clams via the gill surface through ciliary movement, which transfers the particles to the stomach, intestine, and the primary and the secondary digestive tract. Some of this microplastics uptake by the hard clam may adhere to the gills or be retained in the digestive system for hours or days, or migrate to cells. The same finding have also been reported by Browne, *et al.* [17]. This explains the increase of microplastics accumulation in the hard clams over time. On the other hand, microplastics that are retained in the digestive system may contribute to satiety, which slows down the speed of ingestion and reduces the uptake rate over time. The same observation has also been reported by Cauwenberghe & Janssen [18].

4 Conclusion

This study revealed that the most predominant size of microplastics was from 5 µm to 20 µm, which accounted for 52.89% to 57.12% of the total quantity of recovered microplastics from the mangrove sediment. The most common type of microplastics was line microplastics, followed by pellet and film microplastics.

Microplastics were found in *M. lyrata*, which averaged at 0.35 ± 0.08 particles/g tissue (wet weight) before three-day depuration. The largest-size microplastics found in the hard clams ranged from 5 µm to 20 µm, which concurs with the results of the abundance of microplastics in the area.

The microplastics accumulation in a mangrove area increasing continuously will result in higher accumulation of microplastics particles in clam tissue. Microplastics retained in the digestive system of the clam may contribute to satiety, slowing down the speed of ingestion and reducing the uptake rate over time

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