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Microplastics in Wild Clams Harvested from Coastal Waters of Lamongan, Indonesia

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Highlights:

- A first assessment of microplastics present in different species of wild clams in Indonesia.
- Almost all clams (99.23%) had ingested microplastics.
- The average concentration of microplastics in all clam samples was 3.5 ± 2.8 items/individual and 3.2 ± 2.52 items/g.
- Fibers dominated the microplastics present in the wild clams (80.77%).

Abstract. Contamination by microplastics has been reported in a wide array of marine organisms, including bivalve mollusks. This study aimed to investigate the occurrence and abundance of microplastics in bivalves from coastal waters of Paciran, Lamongan, East Java, Indonesia. The high human population and anthropogenic activities in the area may lead to contamination by microplastics in marine organisms. Microplastics were recovered from the soft tissues of three species of wild clams, *Gafrarium tumidum*, *Anadara antiquata*, and *Venerupis philippinarum*. For each species, the microplastics found were grouped based on type and size. The extraction of microplastics was done using 30% H_2O_2, while the flotation method with NaCl was used to separate the dissolved liquid from the clam's soft tissues. Microplastic ingestion was found in 99.23% of the clam samples with an average concentration of 3.5 ± 2.8 items/individual and 3.2 ± 2.52 items/g. *V. philippinarum* had the highest microplastics concentration (4.9 ± 2.80 items/g and 5.6 ± 3.22 items/individual). Microplastic types from all samples were dominated by fibers (80.77%), where 57% of them were less than 200 µm in size. The ubiquitous contamination of microplastics in clams from the coastal waters of Lamongan, Indonesia is a serious concern for marine food webs and human health.

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1 Introduction

The widespread use of plastic products drives the mass production of plastics [1-3]. Since the 1960s, global plastics production has been growing steadily by approximately 8.7% annually [4]. It was estimated that over eight million metric tons of plastics enter the oceans annually [5], and plastics are now found in all marine environments [6], from coastal areas [3,7] to deep sea sediments and polar seas [8]. Once present in the marine environment, natural forces like wave action and ultraviolet radiation will eventually degrade plastic waste into microplastics (defined as plastic particles less than 5 mm in diameter) [5].

The first paper to describe microplastics was Buchanan [9], which reported synthetic fibers with densities of up to 105/m^3 in water samples from the North Sea. Carpenter & Smith [10] noted a high density of polystyrene pellets in coastal waters off southern New England. In the early 2000s, confirmation that microplastics in the form of fragments and fibers are a ubiquitous form of marine pollution has stimulated public awareness and research interest in microplastics [11].

Microplastics as significant marine pollutants have been found in multiple marine organisms [1,12,13]. The adherence and ingestion of microplastics have been reported in a wide range of planktonic organisms, such as krill, zooplankton, copepods, and other planktonic crustaceans [14-16]. Microplastics have also been identified in fish, turtles, marine mammals, large crustaceans, and echinoderms [17].

Mussels and other bivalves have been reported to contain and ingest multiple types of microplastics in their tissues [12,18-20]. Fibers were the most common types of microplastics in bivalve tissues of commercial bivalves from China and two bivalve species from Oregon, USA [19,21], while fragments were the majority of ingested microplastics in mussels from the Northern Ionian Sea [12]. Microplastics contamination also affected Asian green mussels, *Perna viridis*, cultured in the coastal areas of Central Java, Indonesia [22].

Mussels and clams are among the favorable seafood products in Indonesia. The coastal communities of Lamongan, East Java, for example, harvest, sell, and consume marine bivalves. Ingesting contaminated seafoods could affect human health [2,23]. In the present study, a survey on microplastic pollution in clams from the coastal waters of Lamongan was conducted. The aim was to determine the abundance and type of microplastics present in wild clam tissues from intertidal sediment of the area.

2 Materials and Methods

2.1 Study Site and Samples Preparation

Samples were collected from Lorena Beach (112.349439°, -6.867448°), Paciran in June 2019, at the beginning of the dry season. The area is part of the coastal areas of Lamongan, East Java with approximately more than 150,000 people living in an area of only 120 km^2 [24-25]. According to the Köppen-Geiger climate classification, the study area has a tropical savannah climate with temperature and precipitation averages at 27.4 °C and 1465 mm respectively [26].

In addition to its high population along the coastline, the area is situated between the Sedayu Lawas River and the Ujung Pangkah River. Both rivers are part of the delta of the Bengawan Solo River, the longest river on the Indonesian island of Java (600 km in length). There are also many industrial and port activities around the study area, which may increase the level of heavy metal pollution in its waters, sediment, and cockles [27]. A previous study on microplastics in the area also revealed high levels of microplastics, mainly fibers, in sediment from the Sedayu Lawas River delta and across the coastal areas of Lamongan [7].

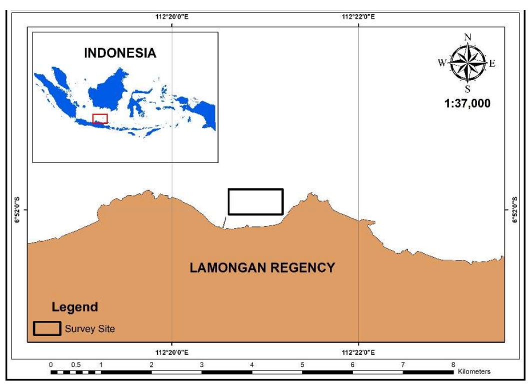

Figure 1 The location of sample collection, Lorena Beach, Lamongan, East Java, Indonesia.

Clams were collected in the intertidal area at low tide, 1 km along the beach and 300 meter perpendicular to the coastline (Figure 1). A total of 80 wild clams were sampled by wading and dredging the substrate at a maximum depth of 20 cm below the surface of the sediment. The samples were separated according to their species and were directly transferred to the laboratory and stored at -18 °C until further analysis. Prior to microplastics extraction, only clams with meat and closed valves were considered for extraction, yielding 15, 8, and 15 clams from the *G. tumidum*, *A. antiquata*, and *V. philippinarum* species, respectively.

2.2 Quality Control of Experiments

All liquids (natrium chloride solution, hydrogen peroxide and tap water) were filtered prior to use with 0.45 µm filter paper to avoid sample contamination. All glass bottles and other equipment used in the experiment were washed three times with filtered tap water. Blank extraction procedures (n = 38 replicates) without clam tissues and seawater were run to evaluate the background contamination. There were 3 microfibers in all blank samples, representing 2.3% of all microplastics extracted from the samples. Therefore, the blank contamination was not subtracted from the results of this study. Furthermore, a probe and hot needle test were performed when plastics were not able to be distinguished from organic pieces.

2.3 Hydrogen Peroxide Treatment of Soft Tissue

The digestion method using hydrogen peroxide (H_2O_2) as oxidizing agent is increasingly implemented. It is considered the most efficient method for the destruction of organic matter for a wide variety of sample matrices [28]. H_2O_2 as destruction agent has been used for the destruction of bivalve tissues [19,29,30], as well commercial fish [31], anchovies [32], and even guano from Arctic seabird colonies [33]. Furthermore, Al-Azzawi, *et al.* [28] compared preparation methods for microplastic analysis and found that H_2O_2 digestion is the most effective in terms of removal of organic matter from microplastic samples. In this study, the extraction method of microplastics from clams was based on Li, *et al.* [29] with minor modification. The shell weight, length, and width of each clam were first recorded. The shells were then opened, and the soft tissue from each individual clam was weighted and placed into a 750-mL glass bottle. In total, there were 15, 8, and 15 bottles for the clam species *G. tumidum*, *A. antiquata*, and *V. philippinarum*, respectively. Approximately, 150 mL of 30% H_2O_2 was gently poured into each bottle to digest the organic matter from each clam tissue. All bottles were covered with foil to prevent airborne plastic contamination and placed in an oscillation incubator at 65 °C at 80 rpm for 12 h. The samples were then settled at room temperature for 24 to 36 h depending on the digestion status of the clam tissue.

2.4 Floatation and Filtration with Natrium Chloride (Nacl) Solution

The floatation method according to Li, *et al.* [29] was used to separate the microplastics from the dissolved liquid from the soft tissues. Approximately 500 mL of filtered NaCl solution was poured into each bottle and mixed thoroughly. The solution was retained overnight and directly filtered over Whatman filter paper (No. 42) using a vacuum system and was then placed into a petri dish with a cover for further microplastics observation.

2.5 Microplastics Observation

The microplastics were observed and identified using an Olympus CX41 microscope. A visual assessment was applied to identify and classify the microplastics particles according to their physical characteristics. The particles were then classified as either fragment, film, fiber, or pellet according to Di & Wang [34].

2.6 Statistical Analysis

Statistical analysis and graph design were performed using GraphPad Prism 9.0.0. The abundance of microplastics per species and the type of microplastics per species were analyzed using two-way ANOVA. Šídák's multiple comparisons test was then used to compare the abundance of microplastics between two species both in terms of items/individual and items/g. Also, the microplastics types per species were compared using the same multiple comparisons test.

3 Results and Discussion

3.1 Bivalve Measurements

The shell length and weight varied from 2.7 to 5.5 cm and 4.9 to 22.73 cm, respectively, where *G. tumidum* had both the shortest shell length and the lowest shell weight (3 ± 0.09 cm and 6.9 ± 0.33 g/individual, respectively). This species also had the lowest soft tissue weight, 0.8 ± 0.04 g/individual (Table 1).

Table 1 Length, width, weight, and soft tissue weight of clams from coastal waters of Lamongan, Indonesia.

Species	Number	Shell length (cm)	Shell width (cm)	Shell weight (g/individual)	Soft tissue weight (g/individual)
Gafrarium tumidum	15	3 ± 0.09	2.4 ± 0.07	6.9 ± 0.33	0.8 ± 0.04
Anadara antiquata	8	4.4 ± 0.44	10	10	3.1 ± 0.58
Venerupis philippinarum	15	3.7 ± 0.05	10	10	1.1 ± 0.05

Mean ± standard error (n = 8-15)

3.1 Abundance of Microplastics in Bivalves

The number of total microplastics in the samples varied from 0 to 10 items/individual and 0 to 8.7 items/g (wet weight), while the total average of microplastics concentration for all species was 3.5 ± 2.8 items/individual and 3.2 ± 2.52 items/g. Šídák's multiple comparisons test of microplastics abundance between two species showed significant differences ($P < 0.001$), where *A. antiquata* had the lowest microplastics concentration (0.5 ± 0.37 items/g and 1.6 ± 1.51 items/individual), while, *V. philippinarum* had the highest microplastics concentration (4.9 ± 2.80 items/g and 5.6 ± 3.22 items/individual) (Figure 2).

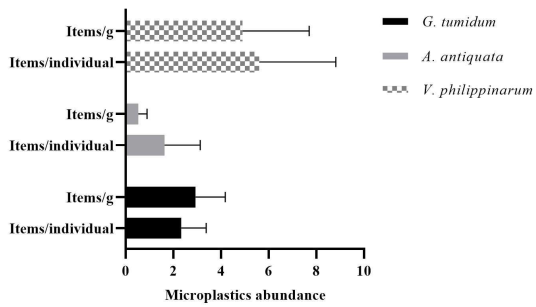

Figure 2 Abundance of microplastics (items/g and items/individual) in clams from the coastal waters of Lamongan, East Java, Indonesia.

3.2 Types of Microplastics in Bivalves

There were 130 microplastics extracted from the clams, where fibers accounted for 80.77% of microplastics contamination. Meanwhile, both films and pellets showed only a small percentage (0.77%) of microplastics ingestion. In *A. antiquata*, 92.31% of microplastics were in the form of fibers and the remaining microplastics were films. The highest share of fragments was in the *G. tumidum* samples (22.86%), while no fragments were found in the *A. antiquata* samples (Figure 3).

There were significant differences in the abundance of fibers and fragments in all clam species ($P < 0.001$). The concentrations of both types of microplastics were highest in *V. philippinarum*, 4.5 ± 2.59 items/individual and 1 ± 1 items/individual respectively, or 3.9 ± 2.39 items/g and 0.8 ± 0.78 items/g respectively. Meanwhile, the lowest abundance of fibers was found in *A. antiquata*, 1.5 ± 1.51 items/individual and 2.2 ± 1.56 items/g. Pellets and films were only found in *G. tumidum* and *A. antiquata* respectively, with concentrations of 0.07 ± 0.25 items/individual and 0.09 ± 0.35 items/g for pellets and of 0.12 ± 0.35 items/individual and 0.37 ± 1.04 items/g for films (Figure 4).

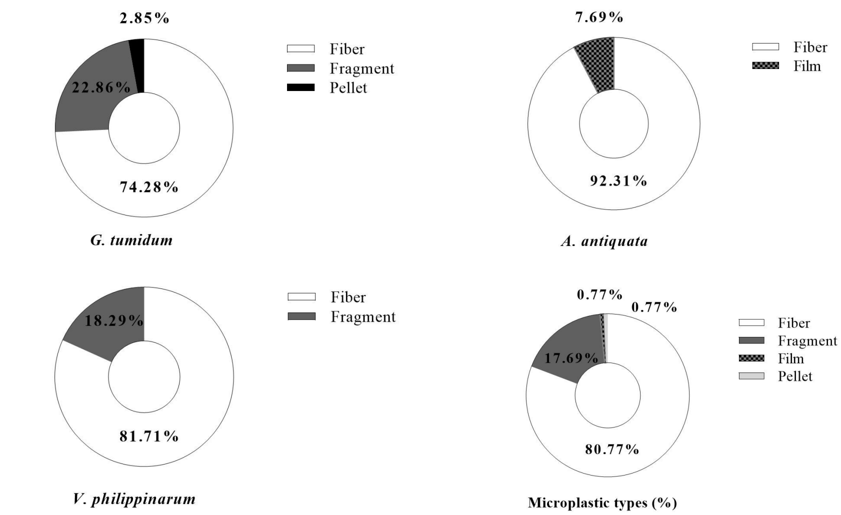

Figure 3 Composition of microplastics types per clam species from the coastal waters of Lamongan, East Java, Indonesia.

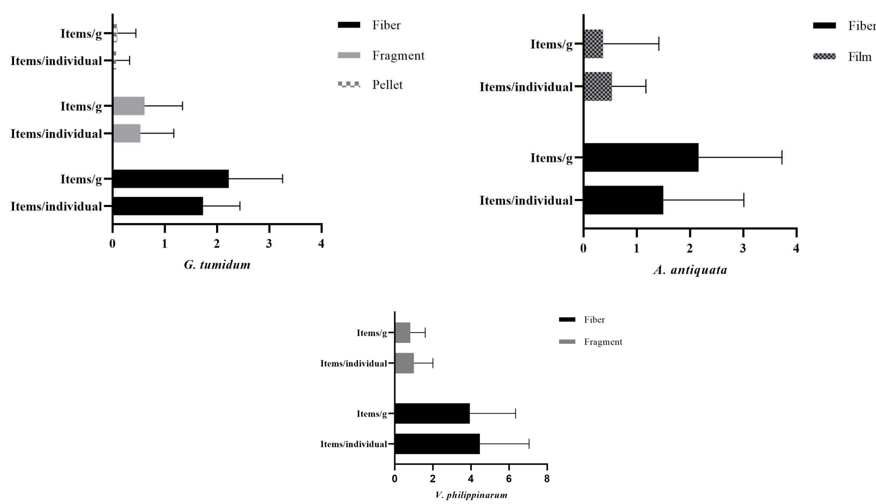

Figure 4 Composition of microplastics types per clam species from the coastal waters of Lamongan, East Java, Indonesia.

3.3 Size of Microplastics in Bivalves

The particle size for all microplastics, either in the form of fragments, pellets or films, was less than 50 µm. Moreover, slightly more than half of the fibers (57%) were in the size range of 3 to 50 µm, although 5.7% of fibers had a particle size greater than 200 µm (Figure 5).

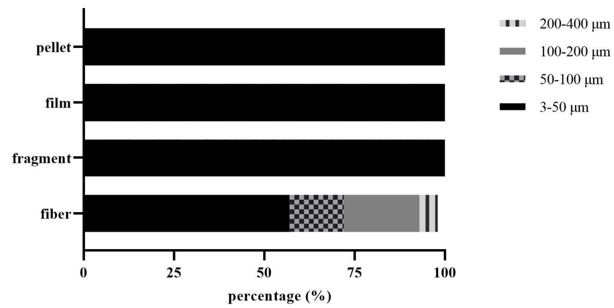

Figure 5 Particle size distribution of microplastics in the clams.

3.4 Discussion

3.4.1 Microplastics Ingestion by Clams

Microplastics ingestion in clams is widespread in the coastal waters of Lamongan. Almost all samples (99.23%) were contaminated with microplastics, ranging from 1 to 10 items in each clam. This indicates that the coastal waters and sediment in the area are highly contaminated with microplastics particles. *G. tumidum*, *A. antiquata*, *V. philippinarum* and most clam species are sediment dwelling bivalves [35,36] and are filter feeders, which means that they feed by straining suspended matter and food particles from the water in their surrounding habitat [24,35]. The sediment of intertidal areas of Lamongan hold microplastics at more than 200 items/kg [7]; therefore, the clams inevitably ingest microplastics via the filter-feeder mechanism as microplastics within sediments are naturally transported to bottom water [37].

Interestingly, despite its highest shell and soft tissue weight, *A. antiquata* ingested far fewer microplastic particles than both *G. tumidum* and *V. philippinarum*, not only by weight but also per individual. As the clams live in the same habitat, a possible explanation for the differences in microplastics ingestion capacity are species-specific traits. Bivalves, including clams, are selective particle feeders and have the specific ability to select among microplastics particles and eject microplastics in the form pseudo-feces and feces [38]. The difference in particle selection depends on the gill architecture and the structure of the cirri [39].

3.4.2 Types of Microplastics in The Clams

The overwhelming majority (>80%) of microplastics identified in the clam samples were in the form of fibers. Fibers are the main type of microplastics, not only in clams but also in their relatives, such as oysters, scallops, and mussels [29,40-43]. Fibers have also been reported as the dominant type of microplastics in other type of marine organisms, such as anchovies [32], zooplankton [44,45], sea cucumbers [46], and sea turtles [47].

As filter feeders, bivalves, including clams, can reflect the variability of microplastics types in water and sediment to an extent. In the sediment of the intertidal area of Lamongan, fibers constituted 87% of microplastics particles [7]; therefore, the microplastics that were extracted from the clam samples were mostly in the form of fibers. In mussels sampled from coastal waters of the United Kingdom, fibers were also the main microplastic type identified in both seawater and mussels, ranging from 50 to 90% [48]. However, no fibers exceeded a size of 400 μm in the clams (Figure 5). Li, *et al.* [49] state that the limit of microfiber internalization in clams is 500 μm.

Furthermore, in addition to the dominance of fibers in the coastal sediment of Lamongan [7], the shape of microplastics may limit the internalization of fragments in clams [50]. Microplastic fragments are derived from the breakdown of larger plastic debris, which is mostly caused by physical, chemical, and photodegradation processes over prolonged time scales [43,51]. Typically, the shape of the fragments is often irregular, cracked, or semi-spherical [51]. The internalization of microplastic in clams is also related to the softness and smoothness of the microplastic materials; smoother fibers are easier to bend and pass through the narrow openings of the clam's gills [49].

3.4.3 Comparison of Microplastic Pollution in Bivalves

Microplastics ingestion in the clams collected from coastal waters of Lamongan was widespread and showed a great variation among individuals and species (Figure 2). Compared to the abundance reported in other studies (Table 2), *G. tumidum* in this study had much lower microplastics content than in Pari Island, Jakarta, Indonesia (13.44 to 17.33 items/individual, 9.1 items/g) [52]. In Jakarta Bay, other bivalve species, *Perna veridis*, were also found to contain a high number of microplastics, 5.39 to 39 items/g [53], while the total average of microplastics concentration from all bivalve species from the Lamongan coast was only 3.2 ± 2.52 items/g. Pari Island is located in Jakarta Bay. The sediment of the bay holds at least 18,405 items/kg of microplastics [54], while the coastal sediment of Lamongan sequestered only 206 items/kg [7].

Although a considerably high number of microplastics, mainly fibers, was observed in *V. philippinarum* from Baynes Sound, British Columbia, Canada (0.07 to 5.47 items/g), *V. philippinarum* in the coastal area of Lamongan, Indonesia had more ingested microplastic particles (Table 2). Clams from other parts of the world also showed lower microplastic abundance; *Donax faba* from the Gulf of Mannar, *Cerastoderma edule* from the Channel Coast, France, and *Siliqua patula* from the Oregon coast, USA held a microplastics concentration of 0.6 to 1.3 items/g, 0.15 to 0.74 items/g, and 0.16 ± 0.02 items/g, respectively [21,55,56].

Table 2 Comparison of microplastics contamination reported by this and other studies.

Species	Location	Type of microplastics	Abundance of microplastics	References
Perna veridis	Jakarta Bay	Fiber (46%), film (40%)	5.39-39 items/g	[53]
Siliqua patula	Oregon coast, USA	Fiber (99%)	0.16 ± 0.02 items/g	[21]
Cerastoderma edule	Channel Coast, France	Fiber (50.2%)	0.15-0.74 items/g	[55]
Donax cuneatus	Gulf of Mannar, India	Fiber (53%), fragment (22%), film (21%)	0.6-1.3 items/g	[55]
Venerupis philippinarum	Baynes Sound, British Columbia	Fiber (90%)	0.07-5.47 items/g	[57]

4 Conclusions

Microplastics ingestion in clams from the coastal waters of Lamongan, Indonesia is widespread, as almost all clam samples were contaminated with microplastics. Therefore, this study provided further evidence that microplastics contamination impacts marine organisms and can potentially be transferred to higher trophic levels through the food chain. Furthermore, as the most ubiquitous microplastics type ingested in the clams, fibers are an underestimated threat to aquatic environments. Policy and regulations need to be implemented to reduce the release of synthetic fibers in the marine environment.

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