



## Microplastic accumulation in aquatic biomonitoring agents from Mud Lusi Island

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**Abstract.** Microplastic (MP) in the aquatic environment contaminates the water column and is sedimentated and potentially transferred to the fish through food webs. The aquatic biomonitoring organisms are threatened by MPs, which are undegradable polymers and accumulate in the sediment, interfering with the organisms living on the bottom. Lusi Island has a high potential for accumulating plastic wastes of anthropogenic origin from the main river of Java Island and the Lapindo mud flow. This research aims to investigate the MP accumulation in the benthic organisms living in the waters of Lusi Island. The biotic samples were destroyed and diluted using 30% KOH. The water samples were tested using FeSO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub>, while the sediment samples were saturated in NaCl. The degraded samples were filtered in the Whatman paper for the MP concentration. A visual identification of MPs was carried out using a microscope with a magnification of 10x10. MPs level in the aquatic environment was relatively low, namely about 528 particles L<sup>-1</sup> and 253 particles g<sup>-1</sup> in water and sediment, respectively, while in the living organism, most MPs were found in the mudskipper fish, namely around 1592.44 particles g<sup>-1</sup>, followed by the river catfish, red crab, and horn snail, at significantly lower levels, namely: 476.79 particles g<sup>-1</sup>, 284.26 particles g<sup>-1</sup>, and 173.33 particles g<sup>-1</sup>, respectively. The high concentration of MPs may be due to the feed habits of the mudskipper; as a sediment-dwelling fish, it consumes more sediments than other organisms. High MP levels in the organism indicated that plastics, originating mainly from the sewage system, have already transferred and accumulated into the aquatic food webs. Thus, reducing plastic usage would help solve the MP contamination threat.

**Key Words:** benthic organism, microplastic contamination, invertebrates, vertebrates

**Introduction.** Lusi Island is an artificial island formed by mud sedimentation following the Lapindo mud disaster and serves as a mud disposal facility (Miller & Mazzini 2018). The unique process of the island's formation determined its inclusion on the list of small protected islands in Indonesia. Thus, people tried to promote it as a tourism destination, specifically for silver-fishery activities, due to the opportunities offered by the existing mangrove forests and aquaculture activities. The island is located near the estuary of the Brantas River, one of the major rivers in Java Island. Like other rivers in rural areas, the increasing plastic contamination caused by human activities became a severe issue (He et al 2019; Idris et al 2023). Although natural degradation already processes plastics, plastic contamination is still ubiquitous in the environment and even transferred to living organisms (Gomiero & Strafella 2019; Jambeck et al 2015; Reddy et al 2014). The natural degradation of plastics by physical, chemical, and biological processes takes time and only reduces it to micro-molecules, aka microplastics (MP) (Andrady 2017; Luo et al 2018). Moreover, these MPs are abundant in the sea and difficult to remove because they are constructed from tenacious substances (Sarkar et al 2023). The globally increasing abundance of plastics in the water column is due to the industrial and domestic agents' dumping chemicals into the environment and the long half-life of these chemicals (Dai et al 2018; Kye et al 2023).

The large quantities of MPs in the environment endangered the ecosystem. Several studies reported on their uptake from the water by biomonitoring organisms, determining the food webs (Botterell et al 2019; Cole et al 2013; Setälä et al 2018) and drinking water (Steer et al 2017; World Health Organization 2006) contamination. Among the aquatic biomonitoring organisms, the endemic benthos were the most threatened by the abundance of microplastic in the environment (Reiss et al 2014). The MP substances might accumulate in their bodies, particularly in the digestive and respiratory organs (Setälä et al 2018). Benthos have an essential role as bioindicators of water and environment quality due to their ability to adapt to polluted habitats (Wimbaningrum et al 2016). Benthic vertebrates and invertebrates might indicate the level of environmental quality due to their sensitivity to the MP content in sediment and water column (Jualaong et al 2021; Pervez & Wang 2022).

Although MP contamination in fish and other aquatic organisms is a global issue, there are few reports on the contamination of benthic organisms via mud disposal in an island's waters. The MPs were analyzed in both benthic vertebrate and invertebrate species, such as the mudskipper (*Boleophthalmus* sp.), river catfish (*Mystus* sp.), red crab (*Perisesarma* sp.), and horn snail (*Telescopium* sp.).

## Material and Method

**Sampling.** Sampling was conducted on January 2023 by investigating three sites to collect samples of benthic vertebrates, namely mudskipper fish and river catfish, and invertebrates, namely group red crab and horn snail. Water and sediment samples were also collected from Lusi Island to observe the MP in the environment (Figure 1).

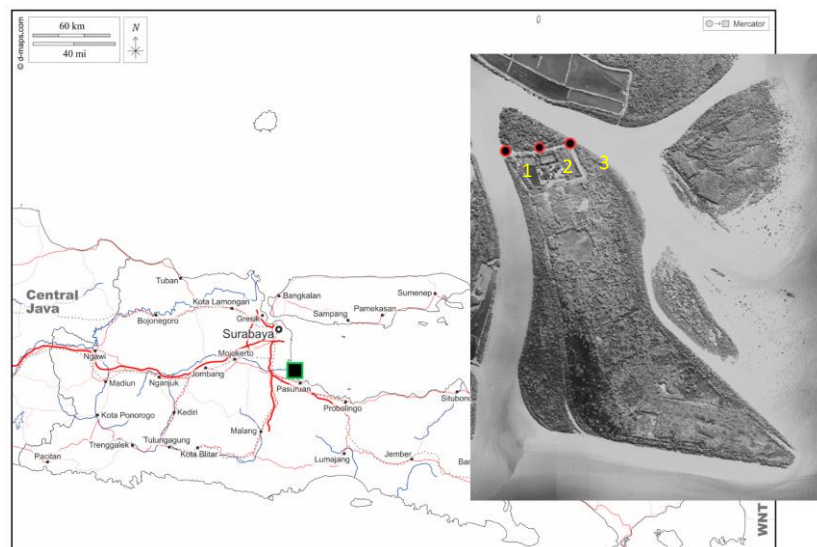


Figure 1. Lusi Island is a small Island in the Sidoarjo, East Java. The sampling sites (red dots) were chosen in the north estuary of Lusi Island.

**Analysis method.** Samples were collected using a purposive sampling method. Benthic vertebrates and invertebrates were gathered using fishing rods and nets; water was collected using plankton nets, and sediment was collected using sediment traps. Samples were stored in a cool box and transported to the marine pollution laboratory. Furthermore, the total length of the sample was measured in the laboratory, and each biota's digestive organs and gills were removed and weighed. The samples were then placed in an Erlenmeyer flask, filled with a 30% KOH solution, and baked at 40°C for 24 hours (Sierra et al 2020). A saturated NaCl solution separated the organ solution from the MPs. The material was filtered using Whatman paper and identified using an electron microscope at a magnification 10x10. Water samples were filtered using sieves with mesh sizes ranging from 60 mm to 250 mm. FeSO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub> were then added to the supernatant. The mixture was then heated for 30 min on a hotplate at 70°C, filtered

using Whatman paper, and identified using an electron microscope with the same magnification. The sediment samples were dried in an oven set to 105°C. The dried sediments were mashed and sieved using a shaker. 50 g of fine sediments were added to 550 mL of saturated NaCl. After stirring for 2 min, samples were filtered and identified using an electron microscope with the same magnification. The calculation of MP using the abundance measurement formula (Despasari et al 2023):

$$K = \frac{n}{v}$$

Where:

K - abundance;

n - the number of microplastics;

v - number of samples.

**Statistical analysis.** The Pearson correlation test used SPSS v.16.0 (IBM, NY, USA) to examine the correlation factors between the total microplastics in the benthic organism and the environment. Each data was presented by mean  $\pm$ SD, and the significant value was  $<0.05$ .

## Results and Discussion

**Identification of the microplastic particles.** The samples contained four MPs: film, fiber/filament, fragment, and granule (Figure 2). MPs of the film type are fragile, low-density plastics with rough structures (Kanyathare et al 2020). The fiber/filament type was formed due to the decomposition of the fabric or nets (made of synthetic materials) (Choi et al 2021). The fragment type is the most abundant fraction, resulting from the extensive physical breakdown (Boettcher et al 2023). The granule type, resembling a droplet or a pellet, is found primarily on the shoreline (Coley & Olivero-Verbel 2015), indicating that the water environment is already contaminated by plastics from the sewage, even if the island is not yet exposed to temporary activities.

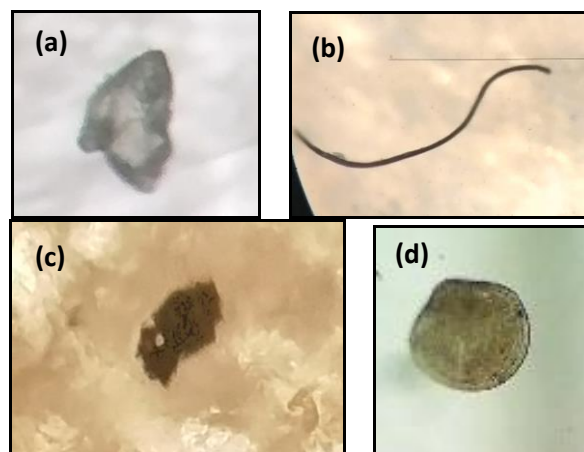


Figure 2. Four MP forms were found in both types of benthic organisms: (a) film, (b) fiber, (c) fragment, and (d) granule.

Interestingly, there were three significant colors of MPs: green (66.67%), brown (16.67%), and black-gray (11.1%), while the other colors (5.56%) were rare, indicating that these plastics originated from the degradation of multiple polymers origin: polypropylene, polyethylene, polystyrene, polyethylene terephthalate, and nylon (Moore 2008; Shim et al 2018).

**Water and sediment.** The abundance of MPs in the water column and sediment is an indicator used to monitor the condition of the aquatic ecosystem (Jualaong et al 2021).

Interestingly, the offshore was less contaminated than the nearshore areas (Shim et al 2018). Around 528 particles L<sup>-1</sup> of MPs were found in the water: 56% of the fiber type, 25% of the film type, 18% of the fragment type, and 1% of the granule type. Even more debris was found in the sediment, with around 253 particles g<sup>-1</sup>: 50% of the fragment type, 41% of the fiber type, and 9% of the granule and film types (Table 1).

MPs sink and settle down under the influence of the physical water properties, which eventually lead to being bound and stacked at the bottom of the water column (Jualaong et al 2021). Higher MP contamination in sediment showed that accumulation of the contaminant has been occurring for decades. It was reported that highly populated areas in Asia faced the issue of the MP threat to the ecosystems due to intensive plastic usage (Shim et al 2018; Vadera 2021).

Table 1

MP content in water and sediment

Microplastic type	Water (particles L <sup>-1</sup> )	Sediment (particles g <sup>-1</sup> )
Film	132±1.1	10±1.97
Fragment	96±1.41	125±9.06
Fiber	294±1.41	104±3.01
Granule	6±1.26	14±163
Total	528±5.18	253±15.67

**MPs content in the benthic invertebrates.** Collected invertebrate specimens were from two benthic species, the red crab (*Perisesarma* spp.) and the horn snail (*Telescopium* spp.), and about 284.26 and 173.33 MP particles g<sup>-1</sup> of body weight, respectively, were found in their organisms. These plastics were observed on their outer body (carapace/shell) and internal organs (Table 2).

Table 2

Characteristic of benthic invertebrates

Sampling site	Red crab		Horn snail	
	Carapace (cm)	Organ weight (g)	Shell (cm)	Organ weight (g)
1	5.5±0.1	4.2±0.81	7±0.2	1.1±0.56
2	5±0.22	4±0.75	6.5±1.4	0.8±1.36
3	4.8±0.81	3.5±0.77	6.5±0.7	0.8±0.48

The types of microplastics found in the samples are films, fragments, fiber, and granules (Table 3). Film-type microplastics were the most common contaminant found in both organisms (Figure 3).

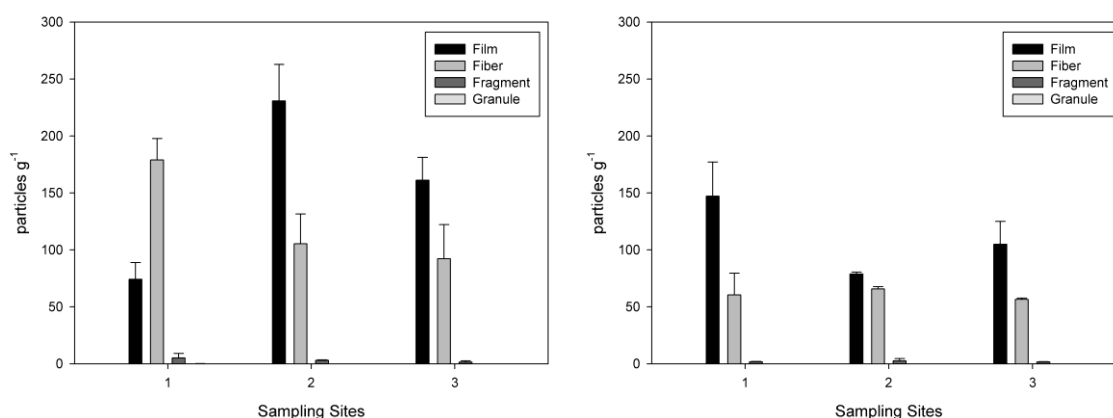


Figure 3. MP content in benthic invertebrates: (a) red crab and (b) horn snails.

The red crab was more exposed to films (59%) and fragments (40%) but less to fibers (only 1%/), and only a single granule was found. There are several intake methods for MPs, like feeding and osmoregulation processes (Watts et al 2016; Wu et al 2023; Zhang et al 2021). Meanwhile, the horn snail was also more exposed to films (64%) and fragments (35%) but less to fibers (1%), with no granules found in their bodies. In mangrove areas, the snail is commonly used as a bioindicator, accumulating MPs (Putri & Patria 2021; Supriatna et al 2023). The benthic invertebrates investigated were detritus-eating organisms in mangrove forests (Zaman & Jahan 2015). MPs have been identified in the plankton's digestion process (Botterell et al 2019; Steer et al 2017; Yu et al 2012) and were also transferred into the higher level consumers, such as gastropods, bivalves, and crustaceans (Hu et al 2017). Higher contaminants were discovered in red crabs than in horn snails due to their size and feed habits, resulting in more significant MPs. The detected MPs of the film type were low primary-density polymers, generally resulting from waste degradation (Kye et al 2023).

Table 3

MP abundance in invertebrate organisms

<i>Microplastic type</i>	<i>Red crab (particles g<sup>-1</sup>)</i>	<i>Horn snail (particles g<sup>-1</sup>)</i>
Film	155.41±62.47	110.32±33.23
Fragment	125.58±46.88	60.99±7.71
Fiber	3.24±1.60	2.02±0.69
Granule	0.03±0.06	0±0
Total	284.26±111.0	173.33±41.63

**MPs on benthic vertebrates.** The light density of polymer plastics causes them to sway in the water column and sink until reaching the seafloor. According to de la Fuente et al (2021), MP particles had 6.2–509.23 m d<sup>-1</sup> of sinking velocities, meaning they would reach the seafloor by 3.1–255 days, based on simulation in the Mediterranean Sea. Thus, MP was repeatedly found in marine ecosystems, suggesting that large populations of aquatic species could be impacted by MP toxicity, from plankton to larvae and mature stages of complex organisms (Cole et al 2013; Galafassi et al 2021; Md Amin et al 2020; Steer et al 2017).

Table 4

Characteristic of benthic vertebrates

<i>Sampling site</i>	<i>Mudskipper</i>		<i>River catfish</i>	
	<i>Total length (cm)</i>	<i>Organ weight (g)</i>	<i>Total length (cm)</i>	<i>Organ weight (g)</i>
1	8±1.21	0.7±0.37	15±2.26	3.4±1.17
2	13±2.18	1.3±1.06	15±2.34	3.5±1.23
3	10±1.66	1±0.12	17±1.58	4.2±0.88

The aquatic vertebrate also indicated a high contamination by MPs. The mudskipper fish (*Boleophthalmus* spp.) and river catfish (*Mystus* spp.) were chosen as representatives for the mud-dwelling fish populating the mangrove ecosystem. The mudskipper is relatively more minor than the river catfish, with a length of about 10.3 cm and a digestive organ of 1 g versus 15.6 cm length and 3.7 g digestive organ weight, respectively (Table 4). All types of microplastics were found in the mudskipper fish: 46% films, 52% fragments, 2% fibers, and a single particle of MP granule (Figure 4, Table 5) Previous reports on MP contamination in mudskipper fish found lower MP contaminant quantities (Kumkar et al 2021; Maghsodian et al 2021; Su et al 2019), possibly due to the continuous impact of the mud flow of Lapindo and the anthropogenic non-degradable wastes on the island. The river catfish was less contaminated with MPs, showing the following breakdown by type: 59% films, 40% fragments, 1% fibers, and no granule type

were found (Figure 3). Most of the freshwater fish, like the catfish, were reported to be susceptible to uptake of small amounts of MPs (Arafat et al 2023), but allegedly with more impact than in open water marine fishes, as shown by increasing HIF (hypoxia-inducible factor) and TNF (tumor necrosis factor) activities (Li et al 2021), but also to transfer MPs to its eggs (Pradit et al 2023).

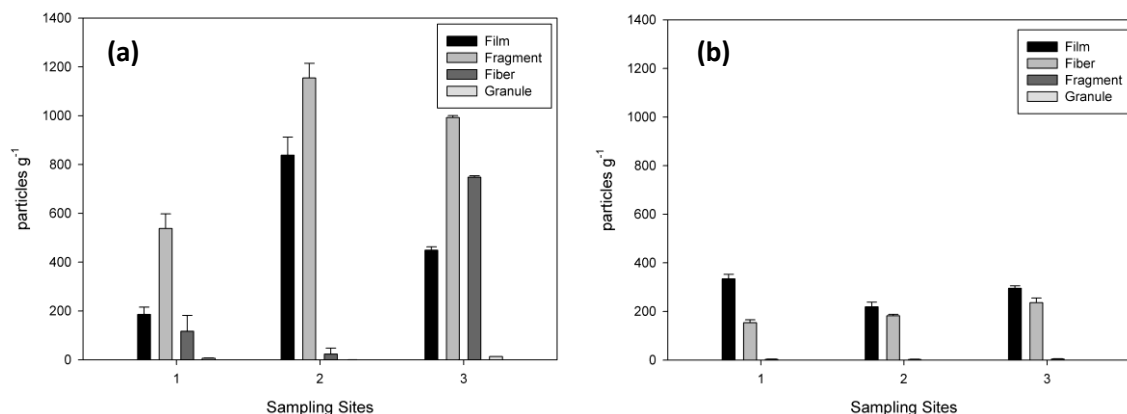


Figure 4. MPs content in benthic vertebrates: (a) mudskipper and (b) river catfish.

Table 5

Microplastic abundance in benthic vertebrates

Microplastic type	Mudskipper fish (particles g <sup>-1</sup> )	River catfish (particles g <sup>-1</sup> )
Film	697.55±89.12	282.93±44.96
Fragment	840.13±102.44	190.72±33.02
Fiber	53.59±9.2	3.13±1.05
Granule	1.18±1.02	0±0
Total	1592.44±192.78	476.79±79.03

High MP abundance in the benthic organisms may have occurred due to the contamination of food webs by the ecosystem's primary producer, especially in the high sedimentation areas. Trapping plastics caused a degradation process in isolated areas, leaving microplastic particles instead. Meanwhile, the plastic sewage has become intense, and the accumulation rate is higher than the natural half-life of plastics. The degradation process produces more micro nano-plastics (Lamichhane et al 2023).

**Conclusions.** A high abundance of MPs was discovered in the estuary environment. They could be identified in the water column, sediment, and benthic organisms commonly used for biomonitoring. The granule was the rarest MP-type found in all bioindicators. The benthic organisms accumulated MPs in a significantly higher proportion than their environment, suggesting that the bioaccumulation has occurred over a long time. Massive contamination was discovered in the digestive organs of then-studied biomonitoring agents, suggesting the MPs transfer across the food webs.

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**Conflict of interest.** The authors declare no conflict of interest.

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