Presence of Microplastics in Windowpane Oyster *Placuna placenta* and the waters from the Tambak Lorok Coastal Area in Central Java, Indonesia

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Abstract

Indonesia is the second-largest contributor of plastic debris in the world. The abundance of plastic debris and the bioavailability of its fragmented form known as Microplastics (MPs; < 5 mm of size) can be dangerous for marine environments. This study investigates the presence of MPs in the sea water and marine organisms (Placuna placenta) at Tambak Lorok Waters located at the coast of Semarang city, Central Java, Indonesia. All of the microplastics collected from both samples were identified by their shape, color, size (using Olympus Stereo Microscope), and polymer type (using Fourier Transform Infra-Red Spectroscopy/FTIR). The study found that the Microplastic concentration in the water samples had an average of 12.6 particles.L^{-1}, where the color and size distribution were predominantly blue colored fragments sized less than 50 µm. Examination of the study area showed that microplastics concentration in P. placenta was 1 item and 0.033 items.g^{-1} for each of the organism. Whereas for physical aspects of microplastics found in the oysters showed the blue colored fibers with range size 300-1000 µm were mostly present. Majority of the polymer types of microplastics found in this study were low-density polyethylene, nylon, polystyrene, and polypropylene. Data from this study shows that microplastics were present in the Tambak Lorok coastal waters. However, there was no significant statistical correlation between the concentration of microplastics in the seawater and the P. placenta, the presence of microplastics in the seawater were found to be ingested by the P. placenta.

Keywords: *FT-IR, Microplastics, P. placenta, Tambak Lorok*

Introduction

Marine pollution, namely the presence of Microplastics, causes loss of biodiversity and extinction of marine organisms (Ambariyanto, 2017). In the marine environment, plastic waste pollution is a global problem that is attributed to the marine or land actvities of humans where used plastics are discarded into the marine coastal area (Nerland *et al.,* 2014). Plastics Europe (2015) reported that the world plastic production increased from 1.5 million tonnes to 299 million tonnes from in 1950 to 2013. The report also indicated that there is a significant increase of 4% per year from 2012, in which more than 10 million tons of terrestrial plastic waste are found in the oceans each year (Löhr *et al.,* 2017). Khoironi *et al*. (2020) study indicated that approxiately 2 tons of plastic waste can be found flowing into this coastal area every day.

Indonesia has about 3.22 million tons of unmanaged plastic in which approximately 0.48–1.29 million tons of plastic waste from this country are potentially entering the sea (Jambeck *et al.,* 2015). The large population in Indonesia and the lack of adequate waste management contributes to this large amount of plastic waste (Syakti *et al.,* 2017). Some of this plastic waste is in the form of macro and microplastics (<5 mm), which deposits onto the seabed and polluting the marine environment (Browne, 2015).

There are two types of microplastics based on their source: primary microplastics, which can be attributed to manufactured plastics in microscopic sizes including scrubbers and pellets (Isobe, 2016) and secondary microplastics, which can be derived from the degradation of macroplastic such as fragments, fibers, or films (Lusher *et al.,* 2017). The presence of microplastics its impact on ingestion

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(Cole *et al.* 2013), adherence (Kolandhasamy *et al.,* 2018), and trophic transfer (Farrell and Nelson, 2013) in marine organisms has been of particular concern of late.

Steer *et al.* (2017) said that different life stages of certain species can ingest plastics from their habitat or environment. Many species of shellfish have been widely used in microplastics biomonitoring in marine environments due to several advantages such as broad geographical distribution, easy accessibility, and high tolerance to a considerable range of salinity (Kusnoputranto *et al.,* 2014; Li *et al.*, 2018: Tubagus *et al.,* 2020).

The World Bank Group (2018) reported that the city of Semarang in the island of Central Java, Indonesia, generated 1387.9 tons of municipal solid waste (MSW) daily with a total population of 1,595,267. Among this solid waste amount, 300.5 tons were unmanaged with 35.3% of plastics waste composition found in the non-tidal zone of the city. One plausable explanation for this finding is the existance of rivers from within the city that floats plastic waste out to the estuary and coastal areas. One particular area identified for this study is the Tambak Lorok coastal area, which is currently used as the main hub for selling and supplying marine products from and to the city of Semarang.

This study investigated the abundance of microplastics in the Tambak Lorok coastal area and in a marine organism, windowpane oyster *Placuna placenta,* taken also from the same coastal reagion. The oyster was chosen for this study due to its popularity as a food source for coastal community daily diet.

Materials and Methods

This study was conducted at Tambak Lorok Coastal Area, Semarang City, Central Java, Indonesia, which is known as the largest marine product market in the city (Figure 1.). All samples used in this study (water and *P. placenta*) were collected in October 2019. Microplastics were collected from seawater with plankton nets (Hidalgo-Ruz *et al.,* 2012; Barrows *et al.,* 2017). The net has a 25 cm diameter circular opening, attached with a 85 cm long-net that has a 25 μm mesh opening. The net was attached to the side of the motorboat travelling at a constant speed of 3 knots (1.6m/s) for a duration of 1 min for approximately 100 m. Volume of water that flowed and filtered through the net was approximately ± 5m^3 for each point sampling. The collected water sample from this procedure were kept in bottles and sent to the laboratory for analysis.

Sampling were done at 5 (five) points at Tambak Lorok Coastal area (Figure 1.). Point 1 was located close to the mainland. Point 2 was selected due to its vicinity to the main water canal that flows from the center of the city of Semarang out to as estuary (East Banjir Canal estuary) at Tambak Lorok coast. Point 3 was located 500m from point 1 to determine possible variances in microplastic concentrations from point 1. Point 4 was selected to determine if there were significant differences in the microplactics as this point is closest to both the main electrical power plant (Indonesia Power) for Semarang City and a very large garment factory. Point 5 is selected furthest away from the coast and had the least number of community and commercial activity in Tambak Lorok coastal area. These five collection points were chosen as the to the representative of Tambak Lorok coastal area.

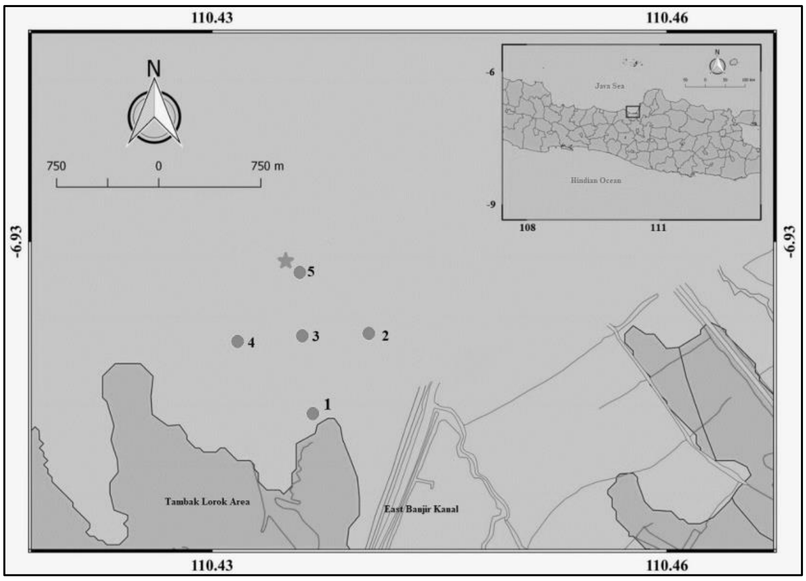

Figure 1. The sampling site in Tambak Lorok Coastal Area, Semarang City, Indonesia.

P. placenta samples were collected using a "*Garok*" net fishing gear operated by local fishermen in the Tambak Lorok Coastal area. *This oyster* can be found at the waterbed of this coastal area which is about 5m deep from the water surface. The samples were put in the cool box, transferred to the laboratory, and kept in -20ºC refrigerator for microplastic analysis. The star shape point in Figure 1 was the location of *P. placenta* sampling.

Before separation and analysis, distilled water, NaCl, and H_2O_2 solution were treated in order to reduce contamination of the microplastics from other sources, and all the instruments were rinsed three times with filtered distilled water (Li *et al.,* 2015). For each microplastic separation, water samples were filtered using sterile Whatman® cellulose nitrate filter papers (diameter 47 mm; pore size 0.45μm) (Cordova and Hernawan 2018). Gast Vacuum Pump was used to accelerate the filtration process. After filtering, Whatman paper was then dried in the oven at 105ºC for 30 mins (Alam *et al.* 2019).

The procedure by Li *et al.* (2016; 2019) was used to separate microplastics from *P. placenta* samples. The shell length/weight of each sample was recorded. The 5g of one individual soft tissue were placed in a 1000 mL glass bottle covered by aluminum foil and counted as one replicate. Six replicates were used for this study. Next, 200 mL of 30% H_2O_2 was used to digest the soft tissues in the bottles. The magnetic stirrer bar was used at 65 °C at 80 rpm for 24 h to accelerate the digestion process. The bottles were then placed at room temperature for 24-48 h (depending on the digestion process) until the solution was clear and no more soft tissues were visible. Density separation by 1.2 g.mL^{-1} NaCl 800 mL was used to separate the microplastics from each bottle that contain the dissolved liquid of the soft tissue overnight. The overlying water was gently discarded and filtered with Whatman® cellulose nitrate filter papers (diameter 47 mm; pore size 0.45μm) using a Gast Vacuum Pump (Model: DOA – P504 – BN, USA) on 20 BAR pressure value. The filter was then placed into clean covered Petri dishes for analysis.

The microscope and FTIR spectroscopy were used to characterize the microplastics that have been separated from the water and *P. placenta* samples (Li *et al.,* 2016; 2017; 2018). The microscope was used to determine the shape, color, and size of the microplastics. Olympus SZX10 Research High-Class Stereomicroscope (Olympus Corporation, Japan) and an Olympus UC30 digital camera were used to observe the filter. ImageJ software was also used for analysis. Polymer type of microplastics was identified using Perkin Elmer Frontier FT-IR with KBr Pellet preparation. Microplastic particles were transformed into powder and homogenized with the KBr (potassium bromide) pellet. The homogenized sample was pressed in a hydraulic instrument for 5 mins. The pressed samples were then ready to be analyzed on the FTIR. The polymer types were determined within the wavelength range of 4000–400 cm^{-1}. Microsoft Excel 2013 was used to process and present data findings.

Results and Discussion

According to Khoironi *et al.* (2020), mismanagement of the plastic waste by the city and its residents directly contributes to high level of microplastics present in the Tambak Lorok coastal area. In this study, microplastics were found in all the water and *P. placenta* samples. The abundance of microplastic in Tambak Lorok Water is shown in Table 1. The abundance of microplastics in *P. placenta* were 6 particles. Although microplastics were found in all sampling points of Tambak Lorok waters and *P. placenta samples*, the data indicates that there are relatively low in numbers. However presence of microplastics in both seawater and biota is very closely related to local community activities, which contribute to the dumping of plastic waste in the sea (Khoironi *et al.,* 2020).

The abundance of microplastics in this study was classified by shape, color, size, and type of polymer that it is made. The number of microplastics found in this study is still relatively low when compared to previous studies related to the abundance of microplastics. This could be due to differences in methods used when sampling microplastics in the water (Besley *et al.,* 2017). Other research studies states that contributions from streams is one of major contirbuting factor of microplastic pollution (Alam *et al.,* 2019; Zheng *et al.,* 2019), and some estuaries have been identified as microplastic hotspots (Wright *et al.*, 2013).

Table 1. Microplastic abundance in the water sample from Tambak Lorok Coastal

Point	The abundance of Microplastics (Particle per 5m^3)
1	17
2	16
3	15
4	9
5	6
Total	63
Average	12.6

Collection point 1 was the highest number of microplastics. See Table 1. This was due to the location of this collection point in which it is nearest to the mainland where there is a high level activity by the residences of the local community. The presence of microplastics is often associated with the amount of population and resident activities (Desforges *et al.,* 2014).

Fragment, fiber, and granule were detected in the seawater sample which is shown in Figure 2. Fragment was the most dominant shape of the microplastics in the water samples. The source of these shapes was most likely from the plastics used daily by the nearby communities and mismanaged plastic waste. This study had similar results with a survey that was conducted by Syakti *et al.* (2018) in Bintan Water, Riau Islands, in which is the most dominant shape of the microplastic found in that area was Fragment. However, the microplastics concentrations of the Tambak Lorok waters found in this study were lower than those of the Bintan waters (Syakti *et al.,* 2018).

The most dominant shape found in *P. placenta* was fiber shaped. See Table 3. Other studies also found that fiber shaped was the most dominant form found in bivalve/mussels (Li *et al.,* 2019). One possible explanation is that the fibers in mussels are due to the result from long-term accumulation in the marine environment as compared to the other shapes (Li *et al.,* 2019). The delay in the egestion of synthetic fibers was also evident since only fibers were detected in mussels after gut clearance period (De Witte *et al.,* 2014). The presence of Fiber forms could be the result from clothes washing of residents directly and indirectly on the coastal waters and the existence of textile industries in the vicinity of the sampling points in this study. The water samples had more of a fragment shape where the *P. placenta* had predominately the fiber shaped microplastic.

Table 2. Total Concentration of Microplastics in water sample based on the shape

Shape	n particle	Total Concentration Average ± SDEV
Fragment	42	8.4 ± 4.67
Fiber	20	4.0 ± 1.41
Granule	1	0.2 ± 0.45

Table 3. Shape of Microplastics Total Number found in *Placuna placenta*

Number of Organisms	Total Weight (g)	Shape Category	Total Number of Particle
6	30	Fiber	5
		Fragment	1

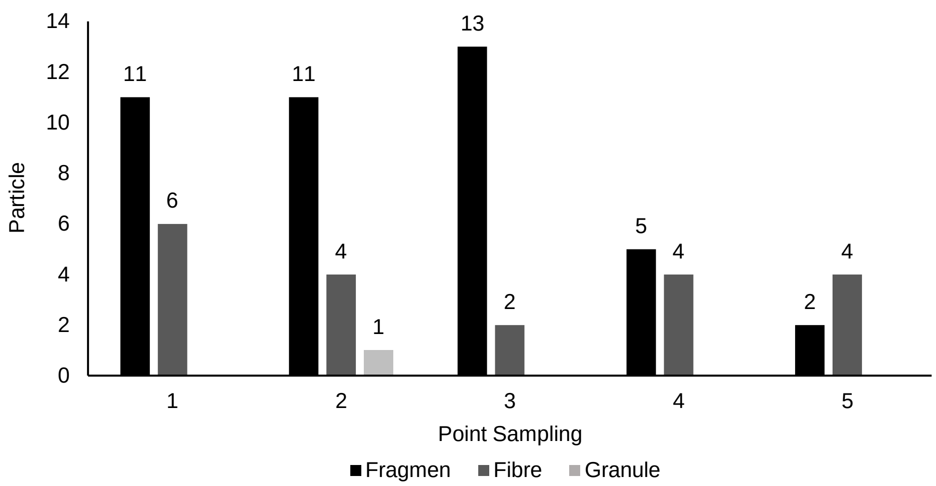

Figure 2. The shape of Microplastics from Waters

Table 4. Size of Microplastics in Tambak Lorok water

Size	n particle	Total Concentration Average ± SDEV
< 50 µm	35	7.0 ± 4.41
50-100 µm	8	1.6 ± 0.89
100-500 µm	2	0.4 ± 0.89
500-1000 µm	5	1.0 ± 0.70
> 1000 µm	13	2.6 ± 1.14

Table 5. Size of Microplastics in *Placuna placenta*

Number of Organisms	Total Weight	Size Category	Total Number of Particle	Particle.Individual^{-1}	Particle.gr^{-1}
6	30	< 300 µm	1	0.167	0.033
		300-1000 µm	4	0.667	0.133
		> 1000 µm	1	0.167	0.033

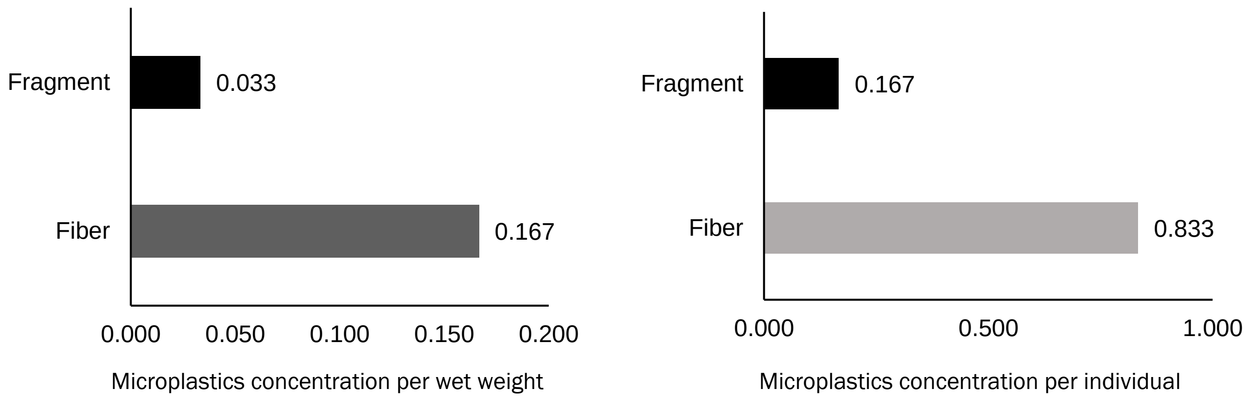

Figure 3. The shape of Microplastics in Windowpane Oyster *Placuna placenta*

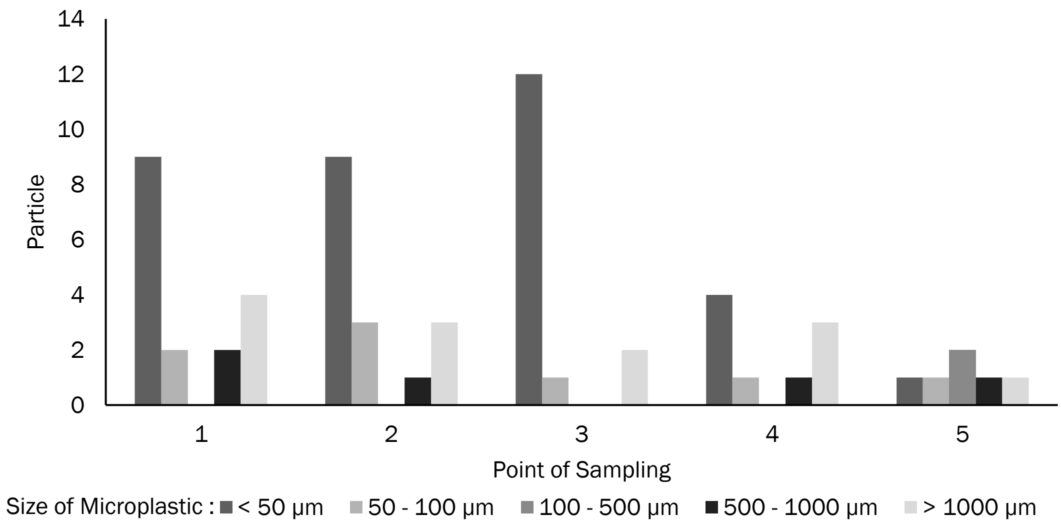

Figure 4. Size abundance of microplastics in Tambak Lorok Waters

Microplastic abundance in both samples was closely related to human activity, and the result from areas with intensive human activities contain significantly higher numbers (Li *et al.,* 2016).

The microplastic size is an important characteristic since it will determine the possibility of being ingested by various marine organisms (Cole *et al.,* 2013). It is also possible that microplastic could be digested in humans through the food chain where the microplastics are ingested by the marine organism. Small size (0.05–0.25 mm) microplastics can be ingested by the mussels (Kolandhasamy *et al.,* 2018). In this study, microplastic size was divided into five classes for the seawater sample which is presented in Table 4, and three classes for *P. placenta*.This study found that class <50 µm of microplastics was mostly found in the seawater sample. This can be due to small microplastics with low densities that will float in water. Particle size showed a significant effect on the modelled fate and retention of microplastic (Besseling *et al.,* 2017).

In this study, microplastics obtained from *P. placenta* sample had a size range of <300 µm, 300-1000 µm, and >1000 µm. See Table 5. Compared to

Table 6. The Color of Microplastic in *Placuna placenta*

Number of Organisms	Total Weight	Color Category	Total Number of Particle	Particle.Individual^{-1}	Particle.gr^{-1}
6	30	Blue	5	0.833	0.167
		Tosca	1	0.167	0.033

Table 7. Polymer Types found in both samples

Sample	Polymer Type	(n)
Tambak Lorok Waters	PS	2
	Nylon	5
	PP	2
P. placenta	Nylon	1
	PP	2

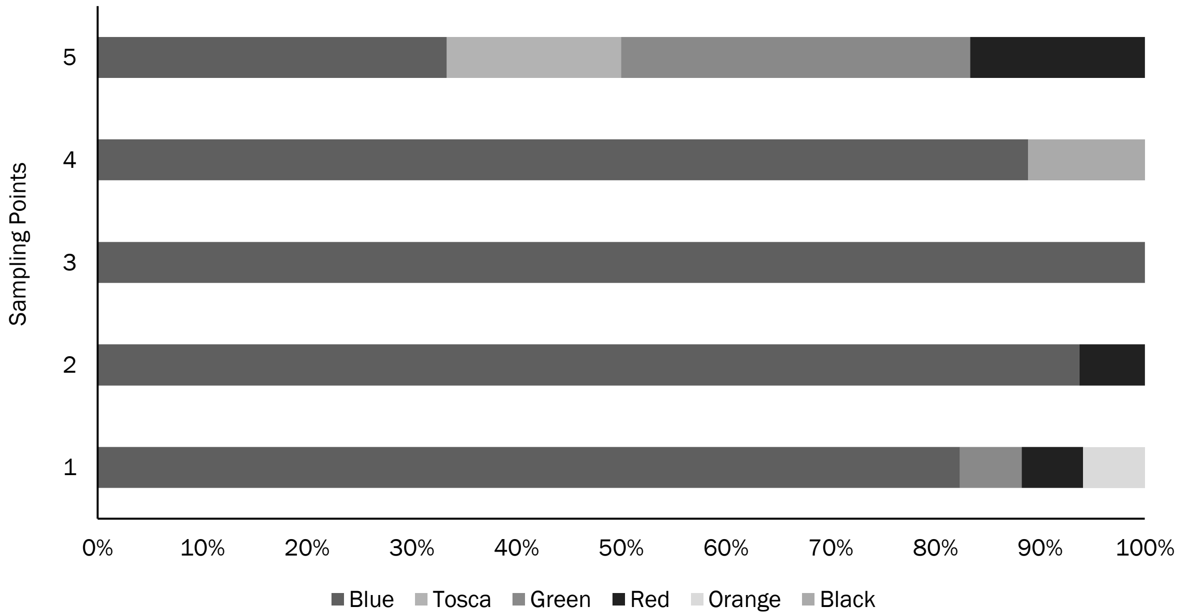

Figure 5. The color distribution of microplastics in Tambak Lorok Water

the study conducted by Kolandhasamy *et al.* (2018), the microplastic size obtained from *Mytilus edulis* is 0.05-0.25 mm (50-250 μm) (>70%). However, these authors also suggested that the bigger size of microplastics can be found in mussels because of its adherence to specific organs of bivalve, rather than ingestion.

The color of microplastics can provide insight as to the level of contamination and its potential to be consumed by aquatic biota (Syakti *et al.,* 2018). Based on the results of this study, Blue microplastic was the most dominant microplastic found in all the 5 sampling points (86%), followed by red and green (5%), black (2%), orange and tosca (1%) (Figure 5.) We assumed that the color of these microplastics was derived from its origin as well as colors that have been degraded. The color of microplastics may have implications for possible ingestion by pelagic organisms such as pelagic fish. Light-colored plastics may appear and account for a majority of microplastics in marine environments (Syakti *et al.* 2018). The color of microplastics found in the *P. Placenta* samples, which is presented in Table 6, were most possibly from the living conditions in their immediate ecosystem.

Among the microplastic found in both samples, selective microplastic samples were further analyzed to determine the type of polymer using FTIR. The results of the polymer spectrum of the samples were compared to the plastic-used polymer spectrum FTIR analysis in Jung *et al.* (2018). FTIR analysis showed three types of polymers (Table 7.), nylon (1389, 1535, 1640, and 2856 cm^{-1}), polystyrene (PS; 2852,1034, 1638, and535 cm^{-1}), polypropylene (PP; 2925, 1454, 1377, 1161 and 841 cm^{-1}). The most common polymer found from both samples were polypropylene (PP) and nylon. Similar results were also obtained by Khoironi *et al.* (2020). Polypropylene (PP) found in this study is suspected to be mainly from food and beverage packaging. PS type was suspected to be derived from fragmentation of food containers and or styrofoam cool-boxes used by fishermen in Tambak Lorok as storage containers for marine catch.

Conclusion

This study concludes that Tambak Lorok Coastal Waters and the oyster *P. placenta* have been contaminated by microplastic debris. Fragments and fibers microplastics were the most common shapes found in both samples. Polypropylene and nylon were most found polymer types in both samples. Even though the numbers of microplastics in this study were low compared to other studies, the presence of plastic waste contributes to the unhealthy contamination of the ocean ecosystem, degrading the marine environment and marine wildlife.

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