

The existence of microplastic in Asian green mussels

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Abstract. Due to resistance of polymer as basic properties of plastic, several studies have been conducted to understand the fate of plastic debris in the marine environment. Degradation is the most important process to control distribution of plastic debris along the marine environment until the existence of plastic in the food chain. The physical and chemical changes of plastic because of degradation process will lead to the release of polluted substances which eventually more toxic for the environment. Furthermore, when plastic degraded become a microplastic will lead to easy ingested by biotic such as mussel which commonly consumed by humans. The aim of this research was to investigate the concentration of microplastic adsorbed and ingested by mussels considering characteristic of sea water. About 30 samples have been collected from 3 different locations that is brackish water (31 ppb), high salinity (36 ppb) and low salinity (33 ppb) for measuring a number of microplastic in mussels on three different salinity. The result of microstructure analysis by microscope showed that mussel evaluated from the marine environment contaminated by microplastic with average size of 211.163 μm . In high salinity sea water, microplastic found in mussel was greater than low salinity and brackish water. The SEM/EDX analysis showed the presence of SiO_2 0.14 % (w/w), Na_2O 24.27 % (w/w) and Al_2O_3 0.27 % (w/w) in the microplastic obtained in the mussel indicating the components which are mostly found in the plastic industries. The amount of microplastic in mussel could be used as pollution indicator in the marine environment by plastic waste.

1. Introduction

Since plastics debris has been found as a major component of the mix of debris in the oceans [1] and also contributed to 80% of all anthropogenic debris in the oceans [2], many studies had been conducted to investigate the fate of the plastic debris. One of the most important phenomena occurs in the marine environment is plastic degradation due to chemical, physical and biological processes. The degradation will lead to bond scission of chemical chains and then change its molecule structure [3].



However, when the plastic debris discharged to the marine environment, they are eventually exposed to a combination of chemical and physical components as well as substances produced during degradation process [4]. The plastic degradation is also producing chemicals which is potentially harmful and toxic. The toxicity is not only because the release of organic pollutants from plastic surface and chemicals additives leaching out of the plastic, but it is also due to the release of chemical produced by degradation of the plastic itself such as microplastic.

Law and Thompson [5] categorized microplastic as a plastic materials or fragments in diameter less than 5 mm. Microplastic originated from various sources that directly discharged into the oceans as small particles is defined as primary microplastic i.e. powder and pellets, and microplastic as results of the degradation processes of macroplastic into smaller fragments is categorized as a secondary microplastics [6-7].

Microplastic has been found in almost every marine species due to plastic composition and marine environmental conditions which significantly affecting their distributions [8]. Pollution by microplastic have a negative effect related to entanglement and ingestion of lower trophic level prey [9]. Furthermore, Lambert et al,[4] has found that non-selective and filter-feeding consumers like fish, shell and some sea species could be susceptible to ingest microplastic which lead to the potential passage up the food chain to secondary and tertiary consumers.

Asian green mussels (*Perna viridis*) is one of the very common bivalves belonging to the Mytilidae family that have high economic value [10]. As a highly nutritious food, green mussels is very popular to people in Indonesia and it is cultivation extensively in Tambak Lorok coastal area. The cultivation of green mussels in Tambak lorok is done by installing bamboo rumpon as a place to stick and growth of the green mussels. Green mussels is considered as feeder filter that is able to filter organic particles, vegetable plankton, animal and microorganisms in water. The activity of green mussels is influenced by water temperature, salinity, and concentration of food particles in water. The growth is attached to various substrates in water using a tool of fibers called byssu. The ability of green mussels to accumulate various types of pollutants making green mussels is used as bioindicators for pollution of an environment in the waters of the sea. This research has purposed to investigate the existence of microplastic in mussels that considering characteristic of sea water, to prove the assumption that the product of primary plastic degradation involved in the food chain.

2. Materials and Methods

2.1. Sample collection

Location of sampling is along the Java Sea in Tambak Lorok coastal area. Sample were acquired directly from 3 locations in September 2017. Sampling locations are based on salinity levels of sea water including high salinity (36 ppb) is about 5 km from coastal area, low salinity (33 ppb) is about 0,5 km from coastal area and brackish water (31 ppb). 10 mussels are collected from each location. The level of contamination from seawater also becomes one of the considerations in choosing the sampling location. All mussels acquired are adult mussels with size 4 - 5 cm that cultivated and stick on bamboo *rumpon* at a depth of 2 meters. Fresh mussels are then stored in ice boxes to be transferred to the laboratory and kept in refrigerators for analysis preparation.

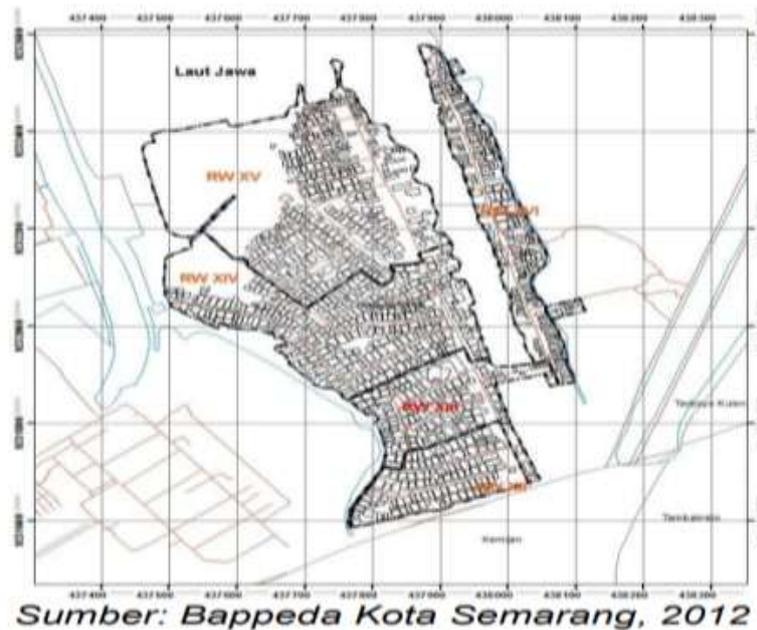


Figure 1. Sampling location in Tambak Lorok Coastal area, Java Sea.

2.2. Preparation sample

The initial step was to ensure that H_2O_2 as a solvent has capability in separating organic compounds from soft tissue [11]. To make sure that H_2O_2 only leach the organic compounds but will not dissolved microplastic, the method was done by dissolving Polyethylene Terephthalate (PET) plastic that has been cut at the size of 1 mm x 0.5 mm into the glass container containing 30% H_2O_2 solution, seawater and distilled water. Furthermore, mussels were carefully removed from their skin and fed into 200 mL of 30% H_2O_2 to digest the organic matter of the soft tissue. The saturated saline solution (1.2 g/mL) was added to dissolve liquid of the soft tissue to separate the microplastic. The solution was then mixed and the bottle was stood for some times. The overlying water was filtered with filter paper and then the filter was placed in the petri-disc for next analysis step.

2.3. Identification and validation of microplastic

Prior to identifying the samples, samples containing microplastic with size of 1 mm x 0.5 mm was used as control in determining microstructural visualization of microplastic through microscope. Filter were visually analyzed using a microscope (Olympus Model MD 50 at magnification 10 x). Microplastic were classified into four morphotypes i.e. fiber, sphere, flake and fragment [11]. After obtaining a filter containing microplastic, the filter was then dried using a freeze dryer at a temperature of 0 - 5°C for 3 days. The dried sample was used for morphology examination using Scanning Electron Microscopy (SEM) as well as confirmation of the element composition of the particles. The result of both SEM and Electron Dispersive X-Ray (EDX) were used to verify that the items were microplastics.

3. Results and Discussion

From the results of visual analysis using microscope (Figure 1), it can be seen that there was a significant variation of both microplastic size and shape based on green mussel location during the sampling. The morphological form of microplastic particles inside mussels in high salinity can be seen in Fig.1. A total of 30 green mussels taken from different locations gave different results that were influenced by the location of the breeding mussels. In mussels that breed in high saline waters (36 ppb) where the location is located about 5 km from the coastline, microplastic particles size of 51.31 to 87.43 μm are found and the number of plastic particles was in average of 5 particles per 0.25 gram of sample weight.

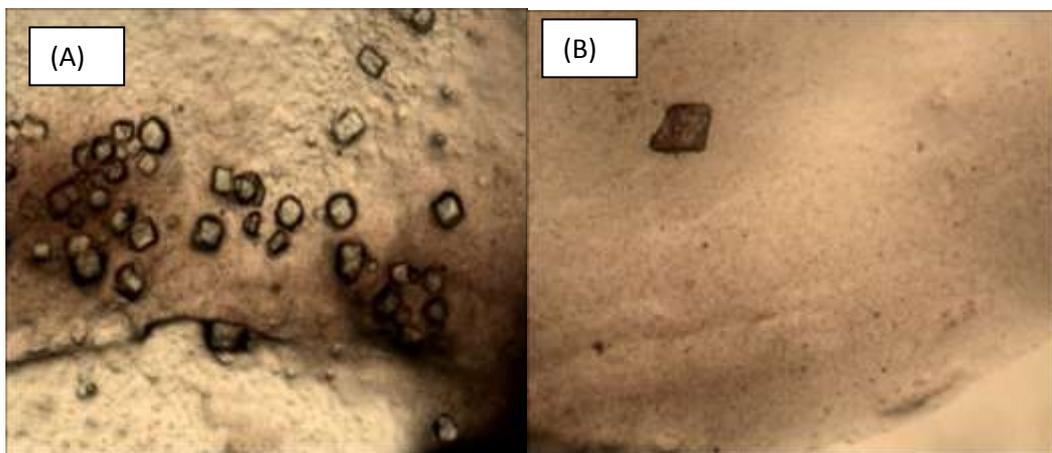


Figure 2. (A). Microplastics detected in green mussels grown in the high salinity sea water, (B). In the low salinity sea waters.(at magnification of 10x)



Figure 3. Microplastics detected in the brackish water green mussels. (at Magnification of 10x)

The microplastic found in mussels living in the sea waters at lower salinity (33 ppb) was fewer than microplastic particles found in high salinity sea water mussel. The microplastic sizes found in the low salinity water was in the range of 45.33-124.74 μm and has concentration of 2 particles per 0.25 gram. On other hand, at brackish environment (31 ppb), the microplastic sizes showed larger than other environments (154.76-232 μm). The average concentration of microplastic in brackish water was 1 particle per 0.25 gr sample. Figure 2 shows the microplastic detected in brackish mussel. Furthermore, in order to support the microscope analysis, the SEM analysis was employed to evaluate microplastic morphology form in mussels. From the SEM/EDX result, the identified tissue showed components of Silica (SiO_2), Alumina (Al_2O_3) and Sodium Oxide (Na_2O) which are mainly used in plastics industry. This also strengthen the previous observation by microscopic analysis.

Table 1. Size range and average concentration of microplastics in green mussels tissue.

Location by degree of salinity	Microplastic size (μm)	Exposure concentration (particles/0.25 g)
High Salinity	51.31 – 87.42	5
Low Salinity	45.33 – 124.74	2
Brackish	154.76 – 232.00	1

In overall, this study (Table 1) shows that location of sampling greatly affected the number of microplastic in the mussels. At longer distance from the costal line (high salinity) there was less sizes of microplastic founded in the mussel, however the concentration showed higher. This is probably due to high salinity water will degrade more plastic than the lower salinity. Moreover, at high salinity water, the accumulation of plastic debris will also larger which consequently increase the number of microplastic particles in the mussels. The chemical, physical and biological degradation processes in higher saline water will greatly contribute to the increasing number of microplastic outcomes from primary plastic degradation [4, 7]. Salinity levels of seawater also play an important role in the accumulated microplastic concentrations in mussels. This is because the ingested microplastic has the potential to be taken by epithelial cells from the intestinal tract and even translate through the intestinal wall to the open mussels system [12]. All the energy used for the digestive system metabolism in mussels is strongly influenced by the salinity of the waters where mussels grow. The salinity of the waters is also one of the physiological factors that affect the utilization of feed and growth of mussels [13] and this becomes the main cause of the amount of accumulated microplastic in higher salinity mussels than lower salinity mussels.

4. Conclusion

This study discovered the presence of microplastic inside the mussels grown in Tambak Lorok coastal area. It also showed that the level of pollution by microplastic in marine waters has exceeded the limit of tolerance of inside living biota. Furthermore, this study also depicts that green mussels become potential medium for microplastic transfer from seawater to the food chain and lead mussel can be considered as one of the bioindicator of pollution level of microplastic in the aquatic environment.

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