Microplastic abundance in sea urchins (*Diadema setosum*) from seagrass beds of Barranglompo Island, Makassar, Indonesia

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**Abstract.** High levels of anthropogenic activities on Barranglompo Island in Makassar City, South Sulawesi Province, Indonesia increase the amount of plastic waste in the surrounding waters, especially in seagrass beds. Plastic waste becomes fragmented into small particles called microplastics (MPs). Sea urchins (Echinoidea), as organisms associated with seagrass beds, can accumulate microplastics through their feeding habits. This study aimed to determine the abundance and characteristics of microplastics accumulated in sea urchins (*Diadema setosum*), seagrass leaves, and sediment. Samples (urchins, seagrass leaves, and sediment) were collected from a multi-species (*Enhalus acoroides* and *Thalassia hemprichii*) seagrass bed where *D. setosum* was present. The internal organs of the sea urchins were extracted using a KOH 10% solution to degrade organic materials. The seagrass leaves were rinsed using distilled water and stirred using a shaker rotator. MPs in sediment were separated using ZnBr₂. MPs in all samples were identified visually under a stereomicroscope. The results show that all (100%) sea urchins samples (*n=10*), seagrass leaves (*n=24*), and sediment samples (*n=8*) were contaminated by MPs. The average MP abundance was 23.70±2.99 items/individual in *D. setosum*; 0.10±0.02 items/cm² and 0.24±0.05 items/cm² respectively on *E. acoroides* and *T. hemprichii* leaves; and 195±66.98 items/kg of dry weight in the sediment. The MPs found shared similar characteristics such as colour (predominantly blue) and shape (dominated by line).

1. Introduction
Plastic pollution has received a lot of attention over the last few years due to its threat to the marine environment. Plastic debris is ubiquitous in all marine compartments including coastline, surface water, and seafloor [1]. There have been around 4.8 – 12.7 million metric tons (MMT) of plastic debris discharged into the oceans [2]. In Indonesia, the total plastic debris released into the ocean reaches 0.48 – 1.29 MMT/year. The occurrence of marine plastic debris in the ocean is mainly a result of mismanaged and uncontrolled plastic disposal systems [3].

Barranglompo Island, one of the islands in the Spermonde Archipelago, is located to the North West of Makassar City. This area has relatively high ecological and economic potential but also has a high population and anthropogenic activities that continue to increase every year [4,5]. Due to high levels of anthropogenic activities and inadequate waste management, plastic debris scattered across...
several parts of the island ends up in the shallow water seagrass ecosystem. Once plastic debris enters into seagrass beds, it can disrupt the productivity of the ecosystem and will fragment into small particles called microplastic [2,6].

Microplastics (MPs) have a particle size of 1 µm - 5 mm [2]. MPs can either be manufactured deliberately as small plastic pellets (primary microplastic) or formed due to current and wave factors in the ocean when large marine plastic debris is broken down into smaller pieces (secondary microplastic) [7]. Due to their high bioavailability and small particle size, microplastics can easily be ingested by marine organisms [8]. Several studies have reported the presence of microplastics in marine organisms such as mussels [9], fish [10], shrimps [11], lobsters [12], and sea cucumbers [13].

The occurrence and potential exposure to microplastics can pose a major threat to the seagrass beds ecosystem, especially to the marine organisms within this habitat [14]. One type of marine organism associated with seagrass beds is the various species of sea urchin (Echinoidea) [15]. The species of sea urchin most consumed by the people of Barranglompo Island is Diadema setosum. It is known as a marine organism with high economic value because the gonad has a high nutrient content for human consumption [16]. This species exhibits generalist feeding habits as an omnivorous grazer and detritus feeder with a varied diet [17]. The generalist feeding habits of D. setosum may enhance the accumulation of microplastics from the marine environment, especially MPs that are accidentally ingested when D. setosum feeds on seagrass leaves and sediment.

Therefore, this study aimed to determine the abundance and characteristics of accumulated microplastics in sea urchins (Diadema setosum), seagrass leaves, and sediments at Barranglompo Island, Makassar, Indonesia.

2. Materials and Methods

2.1. Sampling Area

The sampling location of Diadema setosum (DS) was determined by their natural environment surrounding Barranglompo Island. The area was divided into four distinct sampling locations, based on the occurrence of multispecies seagrass beds including both Enhalus acoroides and Thalassia hemprichii. The map of the research location in Barranglompo Island can be seen in Figure 1 and the coordinate points of the sampling location can be seen in Table 1.

![Figure 1. Map of the research location in Barranglompo Island.](image-url)
Table 1. The coordinate points of the sampling location.

| Coordinate points | Location points |
|-------------------|-----------------|
| X                 | Y               |
| 119.328905        | -5.051893       | DS I          |
| 119.326474        | -5.047830       | DS II         |
| 119.327707        | -5.045690       | DS III        |
| 119.327918        | -5.044200       | DS IV         |

2.2. Sample Collection

2.2.1. Sea urchin samples. Individuals of *D. setosum* (*n*=10) were collected across all four locations. Morphometric measurements consisted of the diameter and bodyweight of *D. setosum*. The spines of sea urchins were removed using scissors and the test was split using a knife so that the internal organs could be removed.

2.2.2. Seagrass leaves samples. Seagrass samples (*n*=24) consisted of 3 leaves for each species, *E. acoroides* and *T. hemprichii* from every location. Samples from *E. acoroides* were taken from the base of the leaf, above the sediment surface, up to the central part of the leaf, whereas *T. hemprichii* samples were taken from all parts of the leaf. All samples were cut using scissors.

2.2.3. Sediment samples. Sediment sampling was carried out using a hand corer in each sampling area of *D. setosum* to ±5 cm depth beneath the sediment surface (*n*=8).

2.3. Extraction and Separation Processes

2.3.1. Sea urchin samples. The internal organs of *D. setosum* were put into sampling bottles containing KOH 10% solution with a ratio of 1:3. This followed the extraction method described by Karami et al. (2017), where one part of the sample material being dissolved in three parts of KOH 10% was shown to be the most effective method for dissolving biological material of organisms without reacting with plastic polymers [18]. Once placed in solution, the samples were soaked for 2 weeks.

2.3.2. Seagrass leaf samples. The preparation of seagrass leaf samples was conducted by rinsing the entire surface of the seagrass leaves with double distilled water, to remove any attached microplastics ( MPs) from the leaves. The volume of rinse water was adjusted according to the size of the leaf. Distilled water was then added into the remaining seagrass leaf samples and stirred using a shaker rotator (H-SR-200) at 120 rpm for 2 hours.

2.3.3. Sediment samples. The preparation of sediment samples was carried out by drying the sediment samples in an oven at ±65°C for 24 hours. If the samples were not dry then this process was repeated. Up to ± 100 grams of sediment was weighed by using an analytical scale, then put it into an Erlenmeyer flask. Separation density was then conducted using a ZnBr2 solution, to separate any remaining MPs. A ZnBr2 solution was added into the sediment sample following the ratio of 1:3 (one part of sample material being dissolved in three parts of ZnBr2 solution) and stirred using the shaker rotator at 300 rpm for 3 minutes (modification method) [19,20]. After that, MPs of the samples were left floating on the surface of the sample solution. The samples were filtered through Whatman 47 mm filter papers (pore size 0.45 µm) on a vacuum pump (Rocker 410) and roughly ⅙ of the sample solution was then drawn from the surface of the sample using a pipette to complete the separation.
2.4. Data Analysis
All data obtained were expressed as mean±standard deviation (SDs). The length or diameter of the MPs was visualized using ImageJ software (version 1.4551). The difference in average microplastics abundance in D. setosum, seagrass, and sediment for each location was analyzed by One-Way ANOVA. If the results were not homogeneous, they were analyzed by the Kruskal-Wallis test. All statistical analyses were run using SPSS version 22. Microplastics in all samples were identified using a stereomicroscope (Stereo Blue) and characterized by size, color, and shape. The number of MPs present on the seagrass was calculated by dividing the total number of MPs identified by the area of seagrass leaves (multiplied by the two sides of the leaf).

3. Results and Discussions

3.1. The Abundance of Microplastics in Diadema setosum
A total of ten individuals of D. setosum were collected from DS I (3 individuals), DS II (3 individuals), DS III (2 individuals), and DS IV (2 individuals). The abundance of microplastics (MPs) found in D. setosum from each location is depicted in Figure 2.

![Figure 2. Microplastic abundance in D. setosum at each sampling location.](image)

There was 100% contamination of MPs in all samples collected (N=10). The average abundance of MPs in D. setosum was 23.70±2.99 MPs/individual. Based on a one-way ANOVA test, there was no significant difference among MP abundance in D. setosum from each location (F_value=0.336 and P=0.800). Although there was no statistically significant difference, microplastics abundance at DS I and DS III were seen to be slightly higher than the other locations. This was assumed to be because of the fact that these locations were closer to the mainland of Barranglompo island. This may have contributed to the higher MPs found in sea urchins at these locations. Also, the high MPs detection rate (100%) in D. setosum indicates that the sea waters of Barranglompo Island are contaminated by microplastics.

A recent study by Feng et al. (2020) detected the presence of microplastics in four types of sea urchins (S. intermedius, T. reevesii, T. hardwickii, and H. pulcherrimus) collected from Northern Chinese waters, with a detection rate of 89.52% and the abundance of microplastics reaching 2.20±1.50 to 10.04±8.46 MPs/individual, compared to the 100% abundance of microplastics in the present study, ranging from 19.00±11.31 to 27.00±12.73 MPs/individual [21]. The presence of microplastics in seagrass leaves can act as a pathway for the transfer of microplastics to marine organisms, especially grazers on seagrass [22,23]. In this study, the feeding techniques of sea urchins
(D. setosum) as both grazers in seagrass (Enhalus acoroides and Thalassia hemprichii) and deposit feeders in sediments, facilitated the ingestion of microplastics. The pathway for microplastics to be ingested by sea urchins can occur either via food ingestion or through the sea urchin’s water vascular system [24].

The fragmentation process of plastic debris into smaller particles does not only occur through physical processes but also biologically. This has been proven experimentally by Porter et al. (2019), by placing a large piece of plastic in an aquarium containing the sea urchin species Paracentrotus lividus [25]. The results showed that the grazing activity of these sea urchins was able to fragment one piece of macroplastic into 91.7 ± 33.8 small plastic pieces with a size of 118 – 15.797 μm over 10 days.

3.2. The Abundance of Microplastics in Seagrass

A total of three seagrass leaves were collected from each location for each species E. acoroides and T. hemprichii, respectively (N=24). The abundance of microplastics (MPs) found from each location is depicted in Figure 3.

![Figure 3](image-url)

**Figure 3.** Microplastic abundance on seagrass at each sampling location (different letters of the alphabet show the difference on α=0.05).

There was 100% contamination of MPs in all samples collected (N=24). The average abundance of MPs on seagrass leaves was 0.10±0.02 MPs/cm² of E. acoroides and 0.24±0.05 MPs/cm² of T. hemprichii. Based on a one-way ANOVA test, there was no significant difference among MPs abundance of E. acoroides from each location (F_{value}=0.271 and P=0.844) whereas for T. hemprichii MPs abundance from each location did exhibit a significant difference (F_{value}=12.218 and P=0.002). Based on a Post Hoc test, the difference for T. hemprichii was at DS I and DS II-DS III.

The high abundance of microplastics in T. hemprichii compared to E. acoroides was assumed to be due to two pathway mechanisms, namely the attachment of epiphytes to seagrass leaves and the different leaf characteristics of the two types of seagrass in reducing current and wave action. First, the presence of epiphytes on the surface of seagrass increases the rate of both the capture of microplastics suspended in the water column and the attachment of biofilms [14,23]. Second, the differences in the width and height of the canopy of seagrass leaves from each species affect their ability to reduce current and wave energy [26, 27]. Seagrass leaves of E. acoroides are wider and taller than T. hemprichii, so it is strongly assumed that microplastics attached to the leaf surface of E. acoroides will escape to the water column. If the density of microplastics is higher than seawater, microplastic will
sink into the substrate [28]. This facilitates the attachment of microplastic to the shorter and smaller-sized surface leaves of *T. hemprichii*.

A previous study by Datu et al. (2019) showed that the number of microplastics found in *Cymodocea rotundata* on Barrangcaddi Island was 23 items from 15 seagrass leaves [14]. Besides, another study by Goss et al. (2018) on *Thalassia testudinum* in Turneffe Atoll found a total of 71 MP items from 15 seagrass leaves. This represents a lower amount of microplastics than in the present study, which found 88 MP items from 12 seagrass leaves of *E. acoroides* and 70 MP items from 12 seagrass leaves of *T. Hemprichii* [23]. However, this difference in the number of microplastics in the previous study can also be attributed to differences in the leaf size of the different seagrass species being studied.

### 3.3. The Abundance of Microplastics in Sediment

A total of 2 sediment samples were collected from each location (N=8). The abundance of microplastics (MPs) found from each location is depicted in Figure 4.

![Figure 4. Microplastic abundance of sediment at each sampling location.](image)

There was 100% contamination of MPs in all samples collected (N=8). The average of MPs abundance in collected sediment was 195±66.98 MPs/kg DW. Based on a *Kruskal Wallis* test, there was no significant difference among MPs abundance within sediment from each location (*F* value=5.608 and *P*=0.132).

Although there was no statistically significant difference, microplastic abundance at DS I was higher than the other locations. This is assumed to be because of the proximity of this location to the mainland of Barranglombo island. This may also have contributed to the higher MPs found in sea urchins at this location.

A previous study by Falahudin et al. (2020) showed that the number of microplastics found in sediments from Banten Bay, in Northwest Java, Indonesia, was 101 to 431 MPs/kg DW with an average MPs abundance of 267±98 MPs/kg DW for 25 sediment samples [29]. This is a greater amount compared to the present study, where the number of microplastics found was 75 to 490 MPs/kg DW with an average MPs abundance of 195±66.98 MPs/kg DW. The average MPs abundance of this study was, however, higher than MPs abundance reported in the Gulf of Thailand [30].

Although the number of microplastics found in *D. setosum* Figure 2 was lower than sediment, the presence of microplastics in sediment was certainly one of the microplastic routes to transfer into benthic organisms, especially to deposit and detritus feeders [13,21]. Based on the *Pearson correlation* test, significant values (*P*>0.05) were obtained for this hypothesis. Microplastic abundance in *D.*
setosum (R=0.600) has a strong correlation with the number of MPs in sediment when compared to the number of microplastics found in E. acoroides and T. hemprichii. Therefore, it is assumed that the main source of microplastics occurring in D. setosum was from the sediment.

3.4. Microplastic Characteristics

The colour characteristic of microplastics found in all samples was predominantly blue (E.acoroides > D. setosum > T. hemprichii > sediment) with the percentage of blue being in sequence 76.1% > 73.8% > 71.4% > 50.0% (Figure 5a). Meanwhile, the microplastic shape found in all samples was dominated by line (E. acoroides > T. hemprichii > D. setosum > sediment) with the percentage of line being in sequence 97.73% > 97.14% > 79.32% > 69.23 (Figure 5b).

![Figure 5](image)

**Figure 5.** (a) Microplastic colors; (b) shapes composition.

Line has been found as the most common shape of microplastic in various compartments of the marine environment including seagrass [14], sediment [21], seawater [31,32], and marine organisms [21,33]. Likewise, blue is the predominant color found in several studies [9,14,21,31]. Microplastics with small particle sizes have a high bioavailability for ingestion by marine organisms, subsequently accumulating in their body tissues [21]. The negative effects caused by microplastics certainly have an impact on food safety. A study by Nobre et al. (2015) showed that the presence of virgin microplastic pellets has a toxic effect and could increase the gonadal development of embryonic *Lytechinus variegatus* abnormally [34]. Although sea urchins are known as organisms that have high protein content [16], the presence of microplastics in sea urchins raises concerns about the potential risks associated with human consumption.

![Figure 6](image)

**Figure 6.** Microplastics on D. setosum, seagrass, and sediment.
4. Conclusion
There was 100% microplastic contamination in all collected samples of *D. setosum*, seagrass leaves, and sediments. Microplastics found in *D. setosum*, seagrass (*E. acoroides* and *T. hemprichii*), and sediments have similar characteristics in terms of color (predominantly blue) and shape (dominated by line). The occurrence of microplastics in benthic animals, seagrass, and sediment is a threat to food webs in the marine environment. Further study on the presence of microplastics in the water column and also other marine organisms associated with seagrass beds is required to complete the various compartments in the food webs and to produce comprehensive results.

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