Microplastic assessment in Seagrass ecosystem at Kodingareng Lompo Island of Makassar City

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Abstract. Microplastic pollution is so widespread and rising a great concern all over the globe. The seagrass ecosystem is commonly known to play significant roles in biodiversity support. The occurrence of microplastics (MPs) in sediments, surface water, fish, and benthos collected from the seagrass ecosystem at Kodingareng Lompo island of Makassar City were observed. From 29 sediment samples collected the range of MPs abundance was 2.96-28.3 item.kg⁻¹ dried sediment. Meanwhile, MPs abundance in surface water was 0.023 item.m⁻³, exceptionally lower compared to the sediment value. Of 4 fish species observed, Siganus canaliculatus was the species with the highest number of ingested MPs. On the other hand, sea urchin Tripneustes gratilla ingested more MPs compared to other benthos species. There was no consistent MPs abundance in sediments found at present studies. Similarly, there were no statistically significant differences seen on MPs abundance within all components examined using non-parametric Kruskal-Wallis analysis. However, the presence of MPs in all components observed has clearly shown a wide dispersion of MPs contamination in the marine food web, as seagrass is a well-known productive ecosystem with high marine biodiversity assemblage in the tropical region.

1. Introduction
Mass production and usage of plastics combined with waste disposal and mismanagement have resulted in the accumulation of immense amounts of plastic litter in marine environments. Ocean plastic pollution has resulted in a substantial accumulation of microplastics (MPs), a predominant fraction that is usually considered as plastic particles with a size less than 5 mm in the marine environment [1,2]. Due to the small size of MPs, organisms such as fish and invertebrates can mistakenly ingest them as food, and through bioaccumulation, MPs ingested by zooplankton can end up in the guts and bodies of larger organisms [3]. Moreover, MPs is well-known to accumulate in sediments and water column of world oceans [4] with estimated maximum concentration up to 100,000 items/m³ [5] and have been detected several years ago in marine organisms [6-8].

The seagrasses [9] and coral reefs [10] of Indonesia hold a greater diversity of marine species compared with any other country in the world. This renders to globally significant biological productivity and biodiversity of seagrass and reefs communities. The consequences of losing these ecosystems can include reduction or loss of food security and ecosystem functionality, and one of the drivers of coral reef and seagrass ecosystem degradation is the input of anthropogenic plastic [11,12]. In addition, the location of Indonesia, in particular, the Indonesian Through-flow (ITF-2) at Makassar Strait with respect to world currents, has been known for transporting enormous water mass from the Pacific Ocean [13]. This means that accumulated plastic debris can readily be carried into Indonesian seas [14], including the Spermonde archipelago. Being a tiny particle will ease for MPs to be carried
by currents and waves that are easily trapped in the ecosystem of the sea, one of which is the seagrass ecosystem [15]. These conditions may perhaps be interconnected to the fact of Indonesia is positioned as the second-highest contributor of plastic debris to the marine environment worldwide [16], and places Indonesia at a high risk of environmental impacts from marine pollution, including marine debris and MPs in particular. The Spermonde Archipelago in the Makassar Strait, Indonesia, comprises many small islands with abundant multispecies-seagrass beds [9, 17,18].

Seagrasses are the only angiosperms able to withstand a saline existence, with almost all genus capable of completing their lifecycle in a submerged marine environment, making possible of a seagrass ecosystem to play significant roles in water filtration, habitat provision, fish nursery grounds and biodiversity support [19]. There are multispecies seagrass beds found in Kodingareng Lompo Island (KL) with a moderate abundance of species such as Halophila ovalis, Cymodocea rotundata, Enhalus acoroides, Thalassia hemprichii, Syringodium isoetifolium and Halodule uninervis [20]. In addition, recently published studies [21, 22] have clearly demonstrated the presence of MPs on seagrass leaves.

2. Materials and methods
2.1. Study area
This study was conducted in KL (5º8’57”S; 119º15’42”E), an island in the Spermonde Archipelago located about 15 km off Makassar City, on the western coast of South Sulawesi, Indonesia (Fig. 1). Makassar is the fourth biggest city in Indonesia with population ca. 1.8 million people and being the main hub for the eastern part of Indonesia.

![Figure 1](image_url)

Figure 1. Sampling location at Kodingareng Lompo Island within seagrass percent.

2.2. Sampling techniques
Sediment, fish, and benthic animal samples were collected in 6-7 December 2017 at Kodingareng Lompo Island of Spermonde Archipelago, within different seagrass percent cover [23] and water depth ranging from 4 to 6 m. Purposive random sampling was conducted on sediment at each seagrass percent cover with total of 27 sediment samples using Wildco hand-corer. Water samples were collected twice during the low and high tides using manta-net (L: 1 m; W: 1 m; H: 30 cm) containing 100 µm mesh size, towed by a small boat that encircling the island at a speed of 4 km.hr⁻¹ with a total
distance of 985 m, respectively. Fish were caught using bottom gillnets assisted by local fishers, and benthos was collected by snorkeling during the low tide.

2.3. Sample processing
Sediments were dried in an oven at 125°C, and 100 g of dried sediments were separated with sieve-net with two gradient mesh sizes of 2 mm and 0.063 mm [24]. MPs specific gravity values were determined with ZnCl$_2$ solution made to 1.4 g.cm$^{-3}$ of solution density [25], followed by shaking on a flat form-shaker at 150 rpm for 10 min. Supernatants were carefully removed with glass pipette from the top of the beaker glass and further subjected to Buchi vacuum pump filtration through nitrocellulose filter (Whatman WCN Type 7141-104), then observed with a stereo microscope Euromex SB1902 with magnification 4.5 x 10. Moreover, a 100 ml seawater from cod-end of the manta-net was poured into a sample bottle, added with 10 ml of 10% KOH and left overnight at room temperature. On the following day, water samples were subjected to vacuum pump filtration and observed for the MPs occurrence with the stereomicroscope.

Fish digestive tracts and the whole body of benthic animals were all subjected to 10% KOH 3 times of samples’ volume [26] and left at 60°C overnight to digest all organic materials. On the following day, samples were poured into respective clean Petri dishes, followed with MPs identification under the stereomicroscope. Fish identification followed Allen [27], while the identification of benthos was performed according to Brueggeman [28] and Leal [29]. The size and morphological descriptors of microplastics were according to GESAMP [30]. All microplastic observed were pictured and recorded for their form and color.

All MPs discovered were mixed with 10x ZnBr then subjected to Fourier transformed infrared spectroscopy (FTIR) Shimadzu Prestice-21 for plastic polymer identification. Pellets were read in room temperature using a wavelength of 340-4500 cm$^{-1}$, resolution 4 cm$^{-1}$ with 300 times of scan numbers for every 5 minutes read per-sample.

2.4. Statistical analysis
Non-parametric Kruskal-Wallis statistical analysis was performed to test any possible differences on MPs abundance in sediments collected from different seagrass percent coverage, and to compare MPs abundance found in fish and benthos samples.

3. Results and discussion
3.1. Sediment and water
Microscope observation on the MPs occurrence in 9 sediment samples from each seagrasses percent coverage resulted in 1 MPs in low, 5 MPs in medium, and 8 MPs in high percent covers. Statistical analysis has revealed no significant differences in MPs abundance between sediments collected from all seagrass percent cover (Fig.2a), and from a total of 295.5 m$^3$ water volume towed at both high and low tides, there were 19 MPs items discovered (11 items from high and 8 items from low tides, respectively) resulted in 0.023 item.m$^{-3}$ of water (Fig.2b). Of the 27 sediment sampling points, the range of average MPs numbers observed was 2.96-28.3 item.kg$^{-1}$ dried sediment. High spatial variability in MPs occurrence on sediments was also recorded by previous works in different parts of the world [31-33], and surface sediments from mangrove area contained higher MPs compared to other areas in eastern water of Java Sea [34]. Recent findings on MPs abundance in surface water of Kodingareng Lompo were lower than those previously estimated value of 0.33 item.m$^{-3}$ for surface water at the Bohai Sea, China [35], and an estimated value of 0.26 item.m$^{-3}$ in the coastal waters of Tuscany, Italy [36]. High disparity in MPs abundance in sediments and surface water is presumably due to relatively low density of certain types of plastic [37], making them drifted at the water surface or for the types of plastic with higher density deposited at the deeper part of the ocean [38-39]. Statistical analysis showed no significant difference in MPs abundance in water and sediments.
3.2. Fish and benthos

From four species of fish with 46 of total individuals, there were 21 items of MPs discovered (Table 1). Rabbitfish (*Siganus canaliculatus*) is the fish species with the highest number of MPs ingested compared to other species. Although the number of fish individuals with MPs is the same with silver biddy fish (*Gerres longirostris*), there were more ingested MPs in the rabbitfish (Fig. 3). The higher number of MPs in the rabbitfish is presumably due to its feeding habits as herbivores regularly feed on the seagrass leaves, which is comparable with previous studies where *S. canaliculatus* was also found as one species with highest ingested MPs from commercial fish collected at Pantai Indah Kapuk Jakarta [40]. A higher number of ingested MPs in *S. canaliculatus* was presumably favored by recent findings [20, 21] that clearly demonstrated the presence of MPs in epiphytes stick to seagrass leaves.

With respect to MPs colors and forms, blue (71.35%) and line (85.71%) were predominantly observed in fish. Ingested MPs in animals could provide a possible new route for POPs to enter the food chain [41] and supposedly capable of evoking a biological response through both physical and chemical mechanisms [42, 43]. Meanwhile, from 42 benthos individuals examined, there were 16 MPs items detected. Black and blue were dominant colors, with the line as the only form found in all observed MPs (Figs. 4-5). There were 7 MPs items observed in four individuals of sea urchin *Tripneustes gratilla*, making them the highest benthos species with the number of MPs ingested (0.5 item.individual⁻¹). In other studies from the shelf of Bering and Chuci seas, the mean abundance of MPs was 0.04-1.67 item.individual⁻¹ had been discovered from benthic animals [43]. The impact of ingested MPs in sea urchin *Lytechinus variegatus* where toxins carried by MPs caused anomalies during embryonic development of the invertebrates has been demonstrated previously [44]. Non-parametric Kruskal-Wallis has revealed no significant difference in MPs abundance both in fish and benthos.

**Figure 2.** Microplastic abundance in sediments (left; n=9) from different seagrass percent coverage and surface water (right; n=2) at Kodingareng Lompo Island.
Table 1. Occurrence and percentage of total contamination of MPs in fish and benthos collected from the seagrass ecosystem at Kodingareng Lompo Island.

| Organism | Species                  | No. of samples | No. of Individual with MPs | No of MPs | % of species MPs contamination |
|----------|--------------------------|----------------|---------------------------|----------|-------------------------------|
| Fish     | *Siganus canaliculatus*  | 10             | 4                         | 8        | 40%                           |
|          | *Gerres longirostris*    | 10             | 4                         | 5        | 40%                           |
|          | *Selaroides leptolepis*  | 14             | 3                         | 6        | 21.43%                        |
|          | *Lethrinus ornatus*      | 12             | 2                         | 2        | 16.67%                        |
| Benthos  | *Tripneustes gratilla*   | 14             | 4                         | 7        | 28.57%                        |
|          | *Cyprea tigris*          | 14             | 4                         | 5        | 28.57%                        |
|          | *Pinctada sp.*           | 14             | 3                         | 4        | 21.43%                        |

Figure 3. Microplastic abundance in fish and benthos samples collected at seagrass ecosystem of Kodingareng Lompo Island (n= 10-14).

3.3. Color, form and types of MPs polymer

Colors of MPs in water were predominated by blue (57.89%) and red (36.84%), while in sediments blue was dominant color (28.57%) followed by red, black and transparent with a respective percentage of 21.43% (Figure 4). With respect to the MPs forms observed, the line was the most predominant form and found in all samples examined (Figure 5). FTIR readings performed on discovered MPs resulted in low-density polyethylene (LDPE) as a dominant polymer with a frequency of 50%, followed with nylon (38%) and polystyrene (PS) with 12% rate of incidence (Figure 6). A high percentage of those polymers is not surprising since the polymers are widely used to manufacture various plastic applications such as plastic bags, plastic bottles, food containers, and fishing ropes. Kodingareng Lompo is one of the Spermonde Island where the waste management is still inadequate, and the inhabitant is mainly fishers. In addition, recent finding on polymer types was in agreement with general types of polymers found in the sea that mainly consisted of polyethylene, polystyrene, polypropylene [45-46].
Figure 4. Proportion of microplastic colors from different samples collected at Kodingareng Lompo Island (Water n= 19; Sediment n=14; Fish n=21; Benthos n=16).

Figure 5. The proportion of microplastic forms from different samples collected at Kodingareng Lompo Island (Water n= 19; Sediment n=14; Fish n=21; Benthos n=16).

Figure 6. Polymers of microplastic from FTIR spectroscopy reads.
4. Conclusions
The presence of microplastic in all examined components of the seagrass ecosystem in these studies have clearly demonstrated the pervasiveness of this newly emerging marine pollutant. It is obvious from detected microplastic occurrence in all samples observed, although with varying levels on the microplastic abundance in each component. However, particularly when the presence of microplastic in seagrass blades by previous studies were also put into consideration, then there is no space left in the seagrass ecosystem without the microplastic presence. The widespread distribution of MPs has simultaneously signaled a strong message on how microplastic will be inescapable by all seagrass food web components and pose a clear threat to the marine living resources security.

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Conflict of Interest
"The authors declare no conflict of interest".

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