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Characteristic of Microplastic on Coral Reef Sediment and Sea Urchin (*Diadema* sp.) in Tidung Island, Jakarta Bay, Indonesia

Sulistiowati[1,5], Neviaty P. Zamani[2,6]*, Dietriech G. Bengen[2], Cheng Ling Lim[3],
Muhammad R. Cordova[4]

[1]Marine Science Postgraduate Student, Faculty of Fisheries and Marine Science, IPB University
[2]Department of Marine Science and Technology, Faculty of Fisheries and Marine Science, IPB University
Jl. Raya Dramaga, Kampus IPB Dramaga, Bogor, West Java, 16680, Indonesia
[3]Centre for International Law, National University of Singapore
Bukit Timah Campus, 469A Bukit Timah Road, 259770, Singapore
[4]Research Center for Oceanography, National Research, and Innovation Agency Republic of Indonesia
BRIN Kawasan Jakarta Ancol, Jalan Pasir Putih I, Ancol Timur, Jakarta, 14430, Indonesia
[5]Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada
Jl. Flora, Bulaksumur, Yogyakarta, 55281, Indonesia
[6]Center for Transdisciplinary and Sustainability Science, IPB University
Kampus IPB Baranangsiang, Bogor, 16144, Indonesia
Email: neviaty@apps.ipb.ac.id

Abstract

Microplastics are recognized as common contaminants of coral ecosystem in Tidung Island, affecting both sediment and sea urchins residing in this environment. Therefore, this study aimed to determine the characteristics of microplastics found in sediment, the mouth, and the digestive system of sea urchins (Diadema sp.), assessing the relationship between coral cover percentage and microplastic type and size, and the transfer of microplastics from sediment to sea urchins. Sampling was conducted twice, namely in October 2021 and October 2022. Microplastics in sediments were extracted using $ZnCl_2$. The destruction process of sea urchins used 30% H_2O_2 and $FeSO_4.7H_2O$, while the microplastics were identified with Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR). The results showed that the microplastics found in the sediments and sea urchins were similar in terms of shape, color, size, and plastic polymers. The forms of microplastics found in this study were fibre, fragment, and foam with fibre predominance, based on the results obtained. The size of microplastics found in sediments and sea urchins was dominated by sizes >1000 µm. Fibres were found in hard coral (HC) and dead coral (DC) conditions while fragments and foams were present in turf algae (TA), sponge (SP), and soft coral (SC) conditions. The microplastics found in sea urchins originate in part from sediments because they have similar characteristics. Sea urchins ingested microplastics from sediment, particularly those grown by algae as a food source. The increase in the number of microplastics found in sediment could potentially result in higher abundance in the biota.

Keywords: *coral reef, microplastic, Tidung island, sediment.*

Introduction

The issue of microplastic pollution has existed since the 1970s (Carpenter *et al*., 1972) and is becoming increasingly prevalent. Microplastics originate from both primary sources deliberately created by industries and secondary sources resulting from the degradation of plastic debris (Cole *et al.,* 2011; Eriksen *et al.,* 2014; Zaki *et al.,* 2020). Microplastic pollution can be found in all ecosystem matrices, including mangroves (Cordova *et al.,* 2021; Duan *et al.,* 2021; Li *et al.,* 2019), seagrasses (Bonanno and Orlando-Bonaca 2020; de los Santos *et al.,* 2021; and Dahl *et al.,* 2021), and coral reefs (Tan*et al.,* 2020; Patti *et al.,* 2020; Jeyasanta *et al.,* 2020). The ingestion of microplastics by marine organisms is caused by several factors, including their size, density, abundance, and color. Smaller ones have a significant potential for ingestion, while a higher density in a given area increases the possibility of ingestion or absorption. Additionally, greater abundance and a wider variety of forms attract organisms. Specific colors have also been found to attract certain groups of organisms (Ugwu *et al.,* 2021).

Microplastics contain additive substances that can serve as carriers of other pollutants, which may have the capability to severely impact the biota that consumes them. The effects of microplastics

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*) Corresponding author

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consumed by biota can include decreased metabolism, consumption levels, energy use, and nutritional status, which can result in a decrease in body weight (Urbina *et al.,* 2023). Berlino *et al.* (2021) explained that microplastics affect the capacity of benthic organisms to assimilate energy. Microplastics ingested in biota can cause physical effects (such as blockage and morphological changes in the digestive tract), chemicals (such as molecular and cellular effects, seismic effects), cancer, and death (Pirsaheb *et al.*, 2020).

In the aquatic environment, microplastics found in coral reefs can enter coral bodies through active incorporation by consumption and passive adhesion through attachment to the surface of the coral body (Corona *et al.,* 2020). Adhesion has an increased likelihood of up to 40 times the possibility of absorbing microplastics compared to active incorporation (Martin *et al.,* 2019). This adhesion affects marine ecosystems, especially coral reef ecosystems. However, only a few studies have been conducted on this topic. Plastic waste encourages the colonization of pathogenic microbes, which can increase the potential for disease by up to 89% when corals come into direct contact with plastic (Bidegain and Paul-Pont, 2018). Dark plastics such as black, grey, or other colors that block and cover coral from solar systems can cause bleaching, necrosis, and inhibit the growth of coral with the potential to cause death within 60 days (Mueller and Schupp, 2020). Moreover, coral reef ecosystems provide essential ecosystem services to humans through fisheries, economic activities, and protection from storms (Eddy *et al.,* 2021). This is especially the case for Indonesia, as it is at the center of the world's coral reefs (coral triangles) with reefs that contain high species richness and endemicity. The coral triangle stretches from the Philippines to the Solomon Islands, with Indonesia's reefs containing the greatest coral reef wealth (Veron, 2009).

Sea urchins are one of the biotas found in coral reef ecosystems. They were abundant on Tidung Island. Sea urchins have a wide range of life niches, not only in coral ecosystems but also in seagrass ecosystems. The morphology of sea urchins is unique; they have an upside-down mouth adjacent to the sediment. Therefore, we chose sea urchins as our research material because sea urchins are not endangered or commercial species because of their gonads. This study aimed to determine the characteristics of microplastics in sediments and the mouth and digestive system of sea urchins (*Diadema* sp.), assessing the relationship between coral cover percentage and microplastic type and size, and the transfer of microplastics from sediment to sea urchins. Jakarta, the capital city of a densely populated country, has coral reefs that are spread across the Seribu Islands. The large population of Jakarta results in high production of plastic waste. We conducted this research on Tidung Island, the Seribu Islands, and Jakarta Bay. The Tidung Island is a marine conservation reserve. However, tourism and residential activities in the region produce plastic waste that impacts upon the area threatens the coral reef ecosystem.

Materials and Methods

Tidung Island, located approximately 50 km from Jakarta Bay, spans 200 m in width and 5 km in length, with two distinct parts namely Tidung Besar and Kecil. Tidung Kecil Island is a marine-protected area, while Besar Island prioritizes tourism and settlement. Approximately 4000 individuals reside on Tidung Besar Island. This study conducted sampling twice, in October 2021 and October 2022. During this period, Indonesia was still experiencing the impacts of Covid-19 with different restrictions in place. In October 2021, large-scale activities, including those of the tourism industry, started to operate again after being banned, and in October 2022, tourism activities were readily carried out with no restrictions. Data obtained from the Transportation Agency of DKI Jakarta showed that approximately 28,842 people visited Tidung Island in 2021, with the number of visitors increasing to 59,658 in 2022. Waste management was carried out by the government sector, including cleaning trash in tourist areas and near settlements every morning. This waste not only comes from resident activities but also from ocean currents and the surrounding area (Hayati *et al.,* 2020). Transboundary wastes from other areas included various toxic materials such as microplastics. The sampling location was selected near the tourism center on Tidung Besar. Therefore, the sampling point (six sites) was in the area between Tidung Besar and Tidung Kecil (Figure 1).

Coral cover percentage data collection and analysis

Sampling at each station used a modification of the Underwater Photo Transect (UPT) method. Each station was repeated three times along a 30 m roller meter parallel to the shoreline. UPT was performed with a 50×50 cm^2 quadratic transect placed on a rolling meter every 1-meter length placed in a zigzag manner. The results of the transect photos were analyzed using Coral Point Count with Excel extension (CPCe) software.

Field sampling

Surface sediment samples taken at a depth of 5 cm using an aluminium scoop were collected from six different locations (Figure 1.) for microplastic assessment. Sea urchins (*Diadema* spp.) were collected from coral reef ecosystems near the sampling sites. A total of 25 sea urchins were obtained by diving at all sampling sites. The samples were then preserved in 96% alcohol.

Sample treatment and microplastic identification

The sea urchin samples were dissected for their mouth and digestive system to be investigated. These samples were subjected to drying at a temperature of 40°C (sediment and sea urchin) (Patterson *et al.,* 2022). To initiate a density separation, 50 g of dry sediment were employed, utilizing a high-density solvent known as $ZnCl_2$ (with a density of 1.5 $g.ml^{-1}$, 200 ml) (Díaz-Jaramillo *et al.,* 2021). To ensure through mixing, the samples were homogenized for 10 min at a speed of 200 rpm. Subsequently, the samples underwent a 24-hour extraction process, and the resulting supernatant particles were filtered using a vacuum pump and passed through sterile paper (specifically, Merck Whatman TM Cellulose Nitrate paper) with a diameter of 47 mm and a pore size of 0.45 µm. For sea urchin samples, density separation occurred when the sample was murky after digestion. This digestion procedure was facilitated using a fenton reagent, a mixture containing H_2O_2 (30%, 20 ml) and $Fe (II) SO_4$ (20 ml) (5 gr dried sea urchin, supernatant of sediment extraction) (Cordova *et al.,* 2022). The samples were subjected to a temperature of 40˚C in a water bath for 24 h to eliminate the organic matter. After this step, the samples were filtered again through a sterile filter paper using a vacuum pump, similar to the previous process. The filtrate obtained from the filtering procedure was placed in a petri dish. Subsequently, the dried particles were examined using a microscope (Olympus CX-31) and camera (Nikon IMX307) that connected to computer with S-EYE software. Particles were identified based on their color, size, and absence of organic or cellular characteristics. For a selected representative sample, 74 particles from sediment and 41 particles from sea urchins were investigated using Fourier Transform Infrared (FTIR) spectrometer (Agilent Cary 630, with attenuated total reflectance diamond). The analysis was conducted using the Microlab FTIR software, with the FTIR settings adjusted to a resolution of 4 cm^{-1}, 32 scans, and a spectral range spanning from 650 to 3000 cm^{-1}, following the methodology outlined by Cordova *et al*. (2022). The sediment and sea urchin results were averaged to provide a summary.

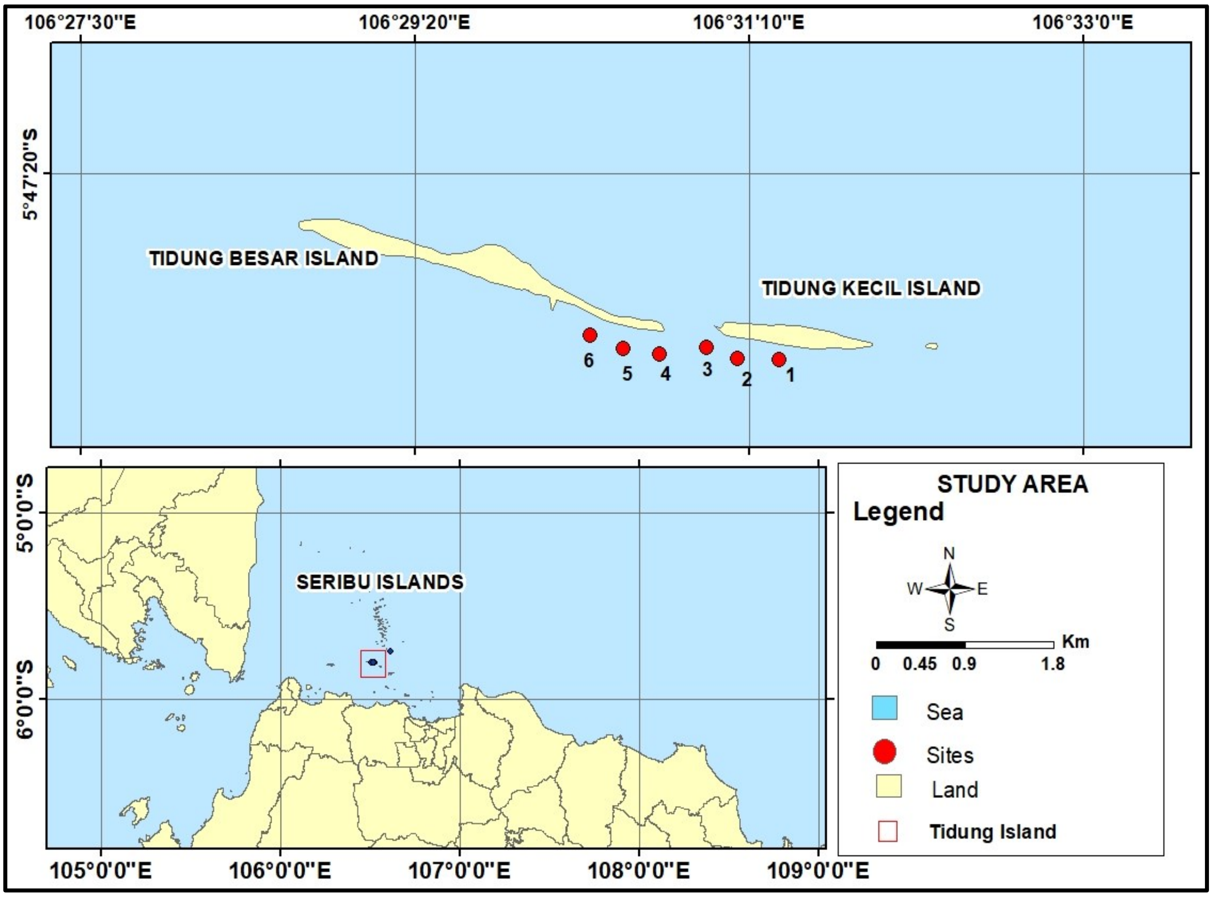

Figure 1. Study Area of Tidung Island

Quality Assurance and Quality Control (QA/QC)

To prevent cross-contamination during sampling, all laboratory equipment was sterilized with sterile water before and after use. A procedural blank was performed simultaneously during field sampling and MP extraction to analyze airborne contamination. Cotton robes and gloves were worn to prevent contamination during experimental procedures. All chemical solutions were filtered using a sterile filter paper (Merck Whatman TM Cellulose Nitrate, sterile, 47 mm in diameter and 0.45 m in pore size) to remove any residual microplastics.

Statistical analysis

Origin 2018 software was used for data interpretation to create the graphs. Visual representation is an interpretation of the shape, color, and size of microplastics. Statistical analysis of Krusskall Walli's and Correspondence Analysis (CA) using PAST4 and ExcelStat 2018 software was conducted to process data on microplastic characteristics and their relationship with coral cover percentage. The Kruskal–Walli's test was conducted to identify significant differences in the shape, color, and size of microplastics in the sediment, mouth, and sea urchin digestive tract.

Results and Disscussion

Abundance of Microplastics

In the first year of the study, the average abundance of microplastics in the sediment was 180 ±90.09 while in the second year, it decreased to 116.67±49.55. However, this decrease was not consistent with the increase in visitors from 2021 to 2022. This was presumably because the study location was in coral reef waters, far from the dense visitors on Tidung Island. Microplastics in sediment are deposited during processing when the density of the polymer is greater than water (Yuan *et al.,* 2023). Biofilm attachment also increases their weight, leading to sinking under calm environmental conditions (Galloway *et al.,* 2017). Currents at the bottom easily distribute microplastics in sediment, influencing their abundance (Yu *et al.,* 2022). Although this study was conducted in the same month, the power of the current to transfer microplastics could be different.

The abundance of microplastics at the mouth in the first year was 0.552±0.42 particles.gr^{-1} while in the second year, none were found (Figure 3). The absence was attributed to the sampling time, conducted in the afternoon when sea urchins were no longer feeding, approaching night (Shulman, 2020). Therefore, the difference in the sampling time affected the results. Ng *et al*. (2022) found microplastics fibres to be dominant in macroalgae talus. The digestive system also experienced a decrease from 0.6±0.16 particles.gr^{-1} to 0.16±0.19 particles.gr^{-1}. This decrease was also due to differences in sampling time, potentially leading to the removal of digestion contents. This study did not consider the age of sea urchins, limiting the hypothesis about the accumulation of microplastics.

Microplastic form

This study found three forms of microplastics namely fibres, fragments, and foams in the sediment and digestive system of samples collected in October 2021. In the first year, sediment sample was dominated by fibres (87 %), while in the second year, fragments dominated (65.71%) (Figure 4.). Fibres were most likely derived from rope and synthetic fabric fragmentation due to increased washing and laundry activities, as well as fragmented microplastics from macro waste (Priyambada *et al.,* 2023). Meanwhile, fragments resulted from the physical breakdown of plastic debris, such as containers, bottles, bags, food packets, wrappings, ropes, and fishing nets (Seeruttun *et al.,* 2023). In 2021, the COVID-19 pandemic led to increased hygiene practices such as washing of clothes, contributing to fibres dominance. Conversely, in 2022, with the reopening of joint activities, such as tourism, offices, and others, increased outdoor activities led to the presence of more fragments. In the first and second sampling, the shapes of microplastics in the sediment and digestive system were similar but differed from those of the mouth. There were significant differences in the shapes of microplastics (Kruskal-test, Wallis's $P< 0.001$; Dunn's Post Hoc, $P< 0.05$).

Fibres dominated microplastics in sea urchins, both in the mouth (84% in 2021) and digestive system (73% in 2021). In 2022, no microplastics were found in the mouth, but 60% were present in the digestive system. This suggested that fibres entered the digestive tract through the ingestion of both macroalgae and sediment.

This study was in line with Sawalman *et al.* (2021) showing consistent forms of microplastics in sediment and sea urchins. Several studies on the contamination of microplastics in sea urchins and the habitats also reported similar results (Fen *et al.,* 2020; Sawalman *et al.,* 2021; Sevillano-González *et al.,* 2022).

Microplastic Color

The color of microplastics in sediment and biota varied significantly, with black dominating in the first year, while red and transparent dominated in the second year. This variation in colors was consistent with the respective origin of microplastics. For instance, predominant fibres source suspected to be from the washing of clothes and ropes corresponded with the discovery of a diverse range of striking colors. In addition, the colors of plastic wrappers, bottles, and packaging varied.

In sea urchins, the mouth and digestive system were dominated by black (31%, 31%) in 2021, while in 2022, the digestive system was dominated by blue (50%) (Figure 5). These colors were reflective of the original plastic source before degradation. The first sampling conducted in October 2021 showed similarities among the colors of microplastics in sediment, the mouth, and digestive system.

All colors of microplastics found in the mouth and digestive tract were also present in those sampled from sediment. These included red, transparent, black, pink, blue, green, and gray. Microplastics sampled from the sediment had more color variations, including gold, yellow, and white, which were not found in the mouth or digestive system. The second sampling conducted in October 2022 also showed similarities in the color of microplastics found in digestive system and sediment, including black, blue, and green. Moreover, the number of colors in sediment samples was greater than in digestive system, as also observed in the first sampling.

All the colors found in the biota were also found in the sediment. Hennicke *et al.* (2021) and Sawalman *et al*. (2021) obtained similar results stating that all colors of microplastics found in biota were also present in the sediment, including red, transparent, black, blue, green, and brown (Hennicke *et al.,* 2021; Sawalman *et al.,* 2021). The color of microplastics in sediment was more varied than in biota. It was assumed that the microplastics found in biota had come partly from sediment, because benthic organisms upon reaching adulthood, have the opportunity to ingest these particles (Ugwu *et al.,* 2021).

Statistical analysis showed a significant difference in the color of microplastics, with various combinations such as red and gold, red and yellow, transparent and gold, transparent and yellow, gold and black, gold and blue, black and pink, black and yellow, black and brown, black and white, pink and blue, blue and yellow, blue and brown, blue and white (Kruskal-Wallis's test $P<0.001$; Dunn's Post Hoc $P<0.05$).

Size of Microplastics

The size of microplastics was categorized into three groups namely <100-500 μm, 500-1100 μm, and >1100 μm based on the general size classification derived from the sampling data, which was less than 2000 μm (Figure 6.). In the first sampling, three size categories of microplastics found in sediments were found in the mouth and digestive tract. The largest percentage of microplastics in the sediments was >1100 μm (43% and 55%, respectively). There are many large sized microplastics in sediments because they have just been degraded by the environment and have just come from the source of origin (human activity). Cordova *et al.* (2020) stated that microplastics with sizes >1000 μm are predominant. The microplastics that accumulate in sediments have the potential to enter benthic biota. Cordova *et al.* (2020) stated that the variation in size of microplastic in sediment was caused by solar radiation and wave action, so it could also happen in this research because our research did in small island that under the influence of sunlight and waves. The mechanisms of microplastic degradation using ultraviolet radiation are regulated by the cutting and breaking of chemical bonds and radical oxidation reactions that cause cracks, wrinkles, and protrusions. Changes in the chemical properties, hydrophobicity, and morphology can also be observed on the surface of microplastics after UV induction pre-treatment (Golmohammadi *et al.,* 2023). The wave action from water breaks the polymer bonds into smaller sizes (Shah *et al*., 2008).

Microplastics in the mouth were dominated by sizes >100-500 μm. This was attributed to the cutting of these particles during the chewing process of sea urchins. The mechanical movement of chewing food facilitated by the sharp teeth leads to the fragmentation of microplastics. On the other hand, samples found in sediment and digestive system were predominantly > 1100 μm in size. There was no significant difference in the size at each station (Kruskal-Wallis test, $P< 0.001$; Dunn's Post Hoc test, $P< 0.05$).

Polymer of Microplastics

There were similarities in microplastic polymers in sediments and sea urchins during the first and second sampling periods. In the first sampling, polybutylene, ethylene, polyethylene, and polypropylene polymers were present, while in the second sampling, only polyethylene polymer was found. This similarity suggested that sea urchins ingested microplastics from the environment, in this case, sediment. The polymers found in sea urchins, but not in the surrounding sediment, are assumed to have originated from water or prey contaminated by microplastics. Polyamide and polyurethane detected in sea urchins were also discovered in coral reef ecosystem (Saliu *et al.,* 2019). Polyethylene and polypropelene polymers found in sediments have been found in several coral reef environments (Tan *et* *al.,* 2020; Lei *et al.,* 2021; Raguso *et al.,* 2022; Saliu *et al.,* 2022, Patterson *et al.,* 2022). Moreover, polyethylene, polyester, polypropylene, polyamide, and polyurethane polymers have also been identified in sea urchins (Feng *et al.,* 2020).

The anthropogenic origin of microplastics was also supported by the results of FTIR analysis, which identified polyethylene, polyester, and polypropylene, similar to Cordova (2020). Ethylene (32.14%) was the dominant polymer in the sediment and sea urchins in the first sampling, while polyethylene (50%) polymer dominated in the second sampling.

Distribution of Microplastics in Sediments Based on Coral Cover

Figure 7 shows the distribution of the shape and size of microplastics in the sediment based on the percentage of coral cover. The results showed that coral cover, shape, and size concentrated along two main axes, namely, F1 and F2 (total variation of 65.29%). The data identified four major groups. The first (F1 positive) showed that stations 5 and 6 (year 2 of study) featured fibres measuring 501-1000 um, 1001-2000 um, and >2000 μm, respectively. These were found in corals that had recently died (DC) and

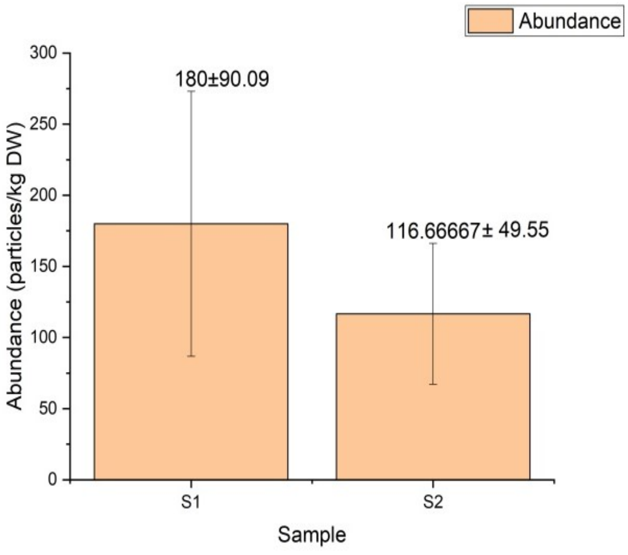

Figure 4. The percentage of microplastic forms

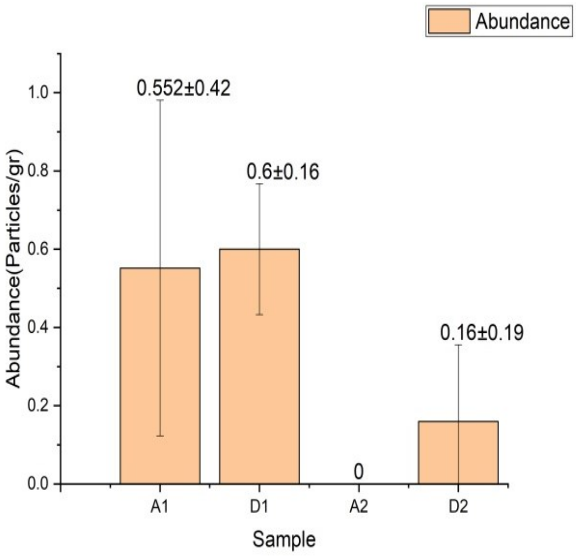

Figure 5. The percentage of microplastic colors.

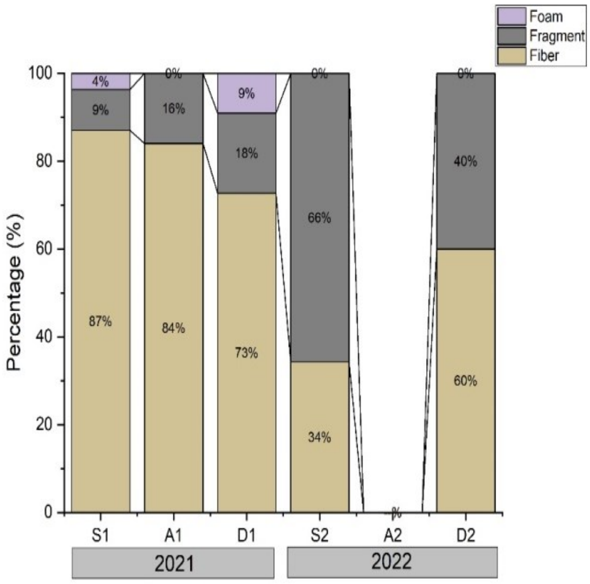

Figure 2. The abundance of microplastic in sediment

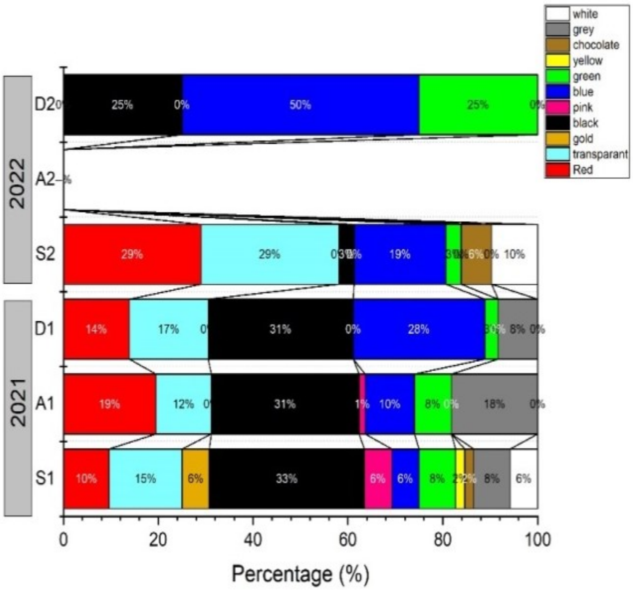

Figure 3. Abundance of microplastic in sea urchin

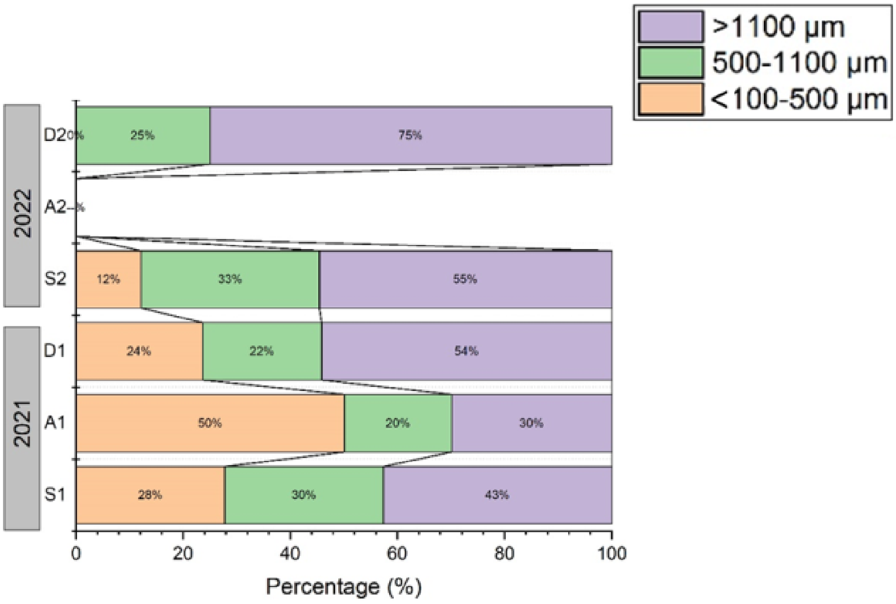

Figure 6. The percentage of microplastic sizes.

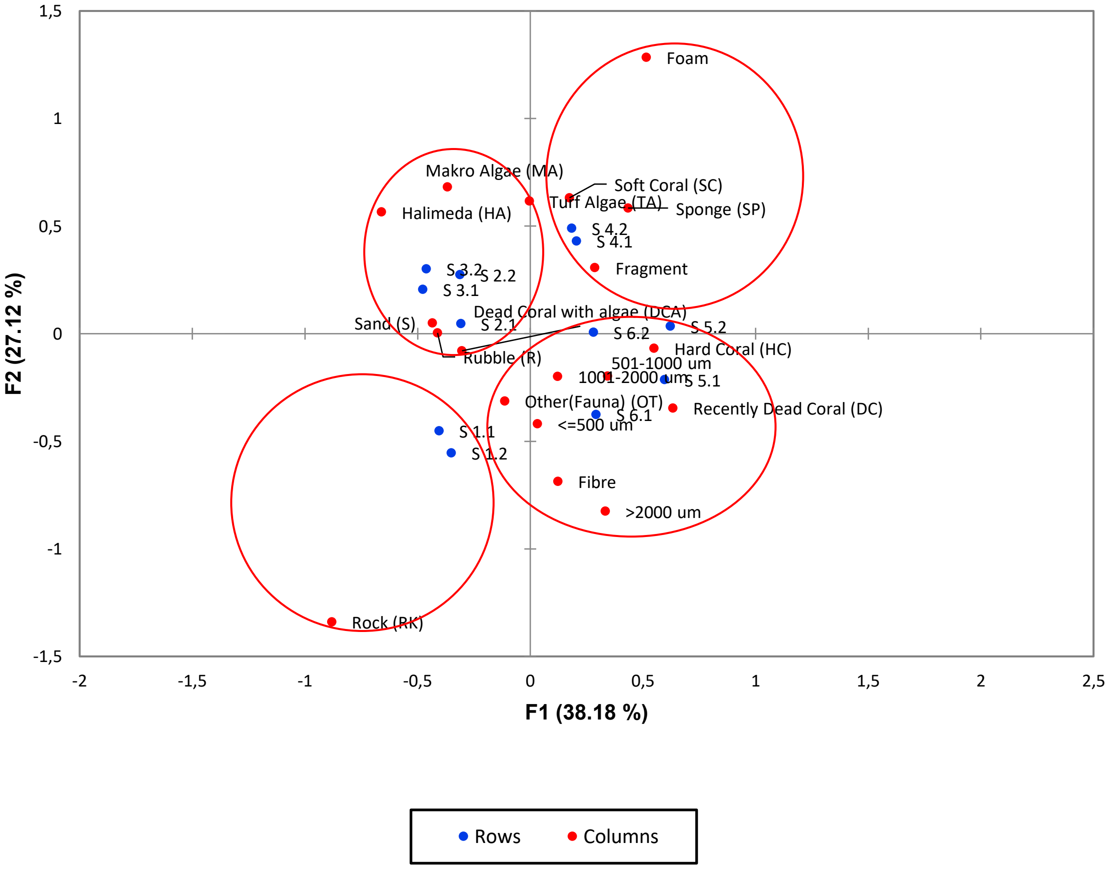

Figure 7. Results of CA analysis based on percentage of coral cover, shape, and size of microplastics

Note: S1= Sediment in first sampling of the research; S2= sediment in the second sampling of the research; D1= digestive system of sea urchin in first sampling of the research; A1= Mouth of sea urchin in first sampling of the research; A2= Mouth of sea urchin in second sampling of the research; D2= digestive system of sea urchin in second sampling of the research

Table 1. Polymer analysis of microplastics in sediment and biota

Polymer Types	S1 (%)	A1 & R1 (%)	S2 (%)	A2 & R2 (%)
Polybutylene	10.50	3.84	7,14	-
Polyethylene	31.60	11.54	50	66,66
Polypropylene	36.85	11.54	35,72	-
Polyester	21,05	-		33,33
Polyvinyl	-	23.08	7,14	-
Polyamide (nylon)	-	11.54		-
Polyurethane	-	3.84		-
Rubber	-	34.62		-

(S1: Sediment in first sampling of the research; S2: sediment in the second sampling of the reasearch; D1: digestive system of sea urchin in first sampling of the research; A1: Mouth of sea urchin in first sampling of the research; A2: Mouth of sea urchin in second sampling of the research D2: digestive system of sea urchin in second sampling of the reasearch;)

hard coral (HC). The second group (F1 negative) explained that station 4 (years 1 and 2 of study) was splattered by microplastics in the form of foams and fragments found in tuft algae (TF), soft coral (SC), and sponge (SP) conditions. The third group (F2 positive) showed that stations 2 (year 2 of study) and 3 (years 1 and 2 of study) were characterized by sandy conditions (sand), rubble (R), macroalgae (MA), Halimeda (HA), and dead coral with algae (DC). The fourth group (negative F2) explained that station 1 (years 1 and 2 of study) faced contamination under rock (RK) conditions.

Transfer of Microplastics from Ecosystem to Sea Urchin

Sea urchins, as benthic biota, primarily forage on grazers that usually eat algae (Livore and Connell, 2012). The unique morphology includes the mouth being at the bottom, in contact with the sediment. Microplastics in sediment are stirred by currents or waves, causing the settlement in the water column and increasing the potential of entering the mouth by ingestion. Ng *et al*. (2022) found that macroalgae were capable of retaining microplastics, due to the morphology (filamentous and non-filamentous). Filamentous algae retain 2.35 times higher amounts of microplastics than non- filamentous algae when considering units per biomass. Sediments collected from vegetated areas contained significantly higher levels of microplastics than those collected from non-vegetated areas (3.39 times higher). Moreover, sediment in proximity to filamentous algae showed a higher abundance than those near non-filamentous algae. The observed microplastic retention in macroalgae suggested that the macroalgal system serves as a focal point for the short-term accumulation of microplastics. The temporal increase was influenced by the seasonal patterns.

Conclusion

In conclusion, microplastics found in sediment and sea urchins were in the form of fibres, fragments, and foams. The colors present were very diverse, but the sediment had a greater variety. In sediment, the size of microplastics spanned all size categories, while in sea urchins, not all categories were found. Fibre-type microplastics with sizes of 500-1000 μm, 1001-2000 μm, and >2000 μm were found in hard coral (HC) and recently dead coral (DC), while fragments and foams-type were present at conditions with turf algae (TA), sponge (SP), and soft coral (SC). The similarities in shape, color, size, and polymers suggested the transfer of microplastics in sediment to sea urchins through ingestion. This transfer occurred due to the adherence to algae which were subsequently ingested by sea urchins. The increase in the number of microplastics found in sediment could potentially result in higher abundance in the biota. However, this study did not conduct sampling for sea urchins at the same hour, suggesting that future biota sampling should be synchronized at the same time frame for consistency in data collection.

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