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Research Article

Analysis of Microplastic in *Holothuria (Mertensiothuria) leucospilota* (Echinodermata-Holothuroidea) and Sediments from Karachi coast, (Northern Arabian Sea)

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Abstract

Microplastics are found in all aquatic environments, and they can have several negative impacts on marine life. In this study, the distribution of microplastics in the sediment and in the organs (gut, respiratory tree, and tentacles) of sea cucumber [*Holothuria leucospilota* (Brandt, 1835)] collected from Mubarak Village and Cape Monze on the Karachi coast of Pakistan were investigated. The microplastic amount in different organs of *H. leucospilota* was found as 15.3-40.45 pieces/individual in the gut, 15.45-23.9 pieces/individual in the respiratory tree, 8.9-9.55 pieces/individual in tentacles. The microplastic amount of sediment samples was 57.40-129.35 pieces/kg. Fibers were the dominant microplastic type, up to 99% of all samples. The results of the present study show that the region is contaminated with microplastics both in biota and in sediments.

Keywords: Microplastic, Pollution, Pakistan, Karachi, Fiber

Introduction

Microplastics are small pieces of plastic that are less than 5 mm in size and these particles are found in the oceans and other aquatic environments, where they can have several negative impacts on marine life(Gregory, 2009; Mathalon and Hill, 2014; Isobe et al., 2017; Cincinelli et al., 2019; Concoli et al., 2019). One of the main ways that microplastics are harmful to marine life is by being ingested. Many marine species, including fish, birds, and mammals, can mistake microplastics for food and ingest them (Neves et al., 2015; Nelms et al., 2017; Carlin et al., 2020). This can lead to a range of health problems, including digestive issues, reduced growth, and reproduction, etc. and even death (Gregory, 2009; Wright et al., 2013). In addition, microplastics can absorb chemicals from the environment, such as pollutants and pesticides, and can be transferred to the aquatic environment and aquatic life (Brennecke et al., 2016; Jaaffar et al., 2021).

In addition to the harmful effects of microplastics on marine life, there are several other negative impacts of these tiny particles on the marine environment. Microplastics in the marine environment has impact on the food chain (Okeke et al., 2022). Microplastics can be interacted by a wide range of marine species, including phytoplankton and zooplankton (Desforges et al., 2015; Long et al., 2015). They are found in the marine food chain, and the ingestion of microplastics can have a cascading effect on the entire ecosystem. For example, it can affect the feeding rate and fecundity of these species, which can in turn impact the health and abundance of other species that rely on them for food (Yu et al., 2020). Plastics can also harm the marine environment by acting as a vector for the spread of invasive species (Miralles et al., 2018; Ibabe et al., 2020; Garcia-Gomez et al., 2021). These particles can act as rafts, carrying invasive species from one location to another. This can introduce new species into an ecosystem, which can have negative impacts on the native species and the overall health of the ecosystem. Plastics are harmful to marine life by entangling or smothering them, many marine species can become entangled in plastics, which can restrict their movement and lead to injury or death (Stelfox et al., 2016; Carvalho-Souza et al., 2018; Consoli et al., 2019). In addition, microplastics can clog the gills of fish and other aquatic animals, making it difficult for them to respiratory (Barboza et al., 2020). Overall, the presence of microplastics in the oceans is a major concern for the health of the marine environment. These tiny particles can have a range of negative impacts on the health and well-being of many marine species (Consoli et al., 2019; Yu et al., 2020).

Microplastics can also contribute to the pollution of the seas. As these particles break down, they can release chemicals and other pollutants into the water, which can have negative impacts on the health of marine life and the overall quality of the marine environment (Gewert, 2018; Gewert et al., 2021). The distribution of microplastics in the oceans is complex and dynamic, and it is influenced by a variety of factors, including ocean currents, winds, and the actions of marine life (Kim et al., 2015). Microplastics can be blown onto beaches and shorelines by the wind, and they can also be transported by currents (Shim and Thompson, 2015). This can result in the accumulation of microplastics in coastal areas. Holothurians, also known as sea cucumbers, are particulate-feeding invertebrates (Roberts et al., 2000). They feed on dead plants, algae, and other organic debris. They live in shallow habitats and are found mostly on outer and inner reef flats, back reefs, and shallow coastal. To feed, sea cucumbers use their tentacles to collect food particles from the surrounding environment, which they then transfer them their pharynx (Roberts et al., 2000). Once the food is in their mouth, sea cucumbers use their digestive system to break it down and extract the nutrients. Some species of sea cucumbers are also able to filter feed, meaning that they can collect small particles of food suspended in the water column.

In this study, the distribution of microplastics in the organs (gut, respiratory tree, and tentacles) of *H. leucospilota* and sediment samples collected from Mubarak Village and Cape Monze on the Karachi coast of the Arabian Sea was investigated.

Materials and Methods

Microplastic surveys were conducted from January to December 2021 (except June and July) along Mubarak Village (MV) and Cape Monze (CM) on the Karachi coast of Pakistan (Figure 1). Pakistan's coast has nearly 1000 km of coastline to the Arabian Sea, from the Indian border to the Iranian border. In all of Pakistan's coast, microplastic pollution was reported in previous investigations in Pakistan (Irfan et al., 2020a,b; Ahmed et al., 2022; Yousuf et al., 2022).

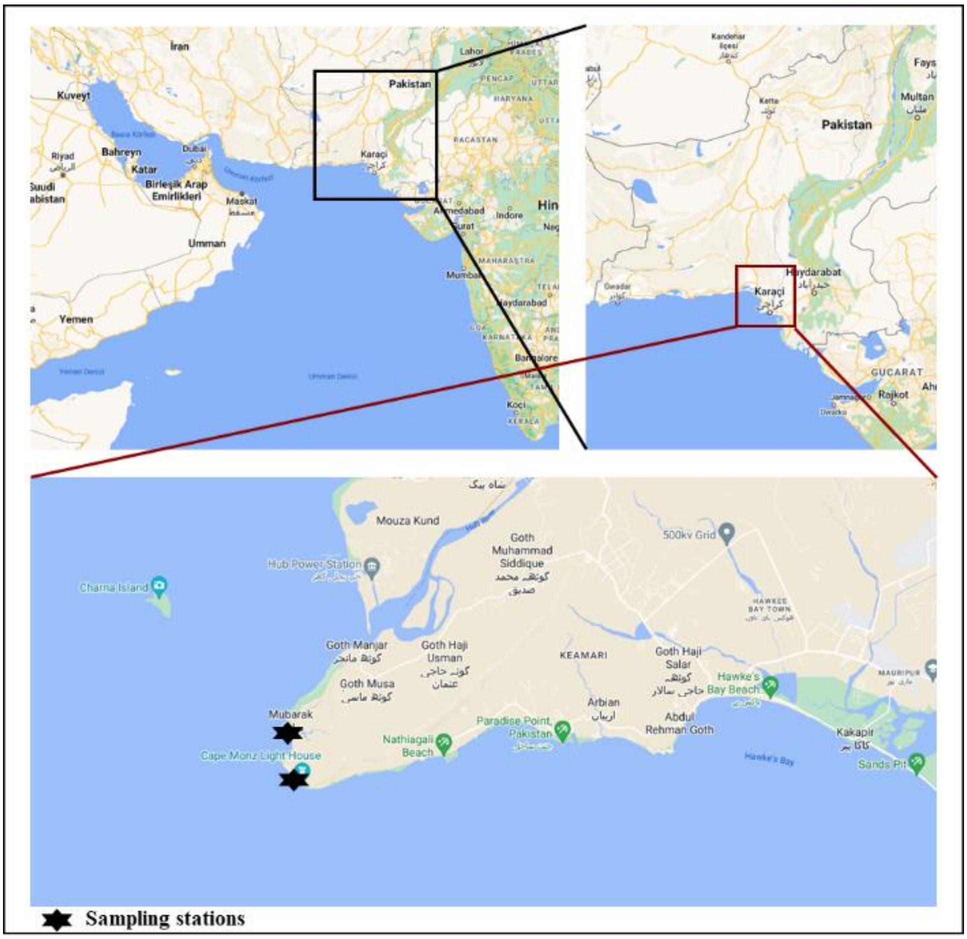

Fig. 1. Survey area

H. leucospilota Brandt, 1835 has one of the widest distributions of all holothurians (Figure 2), and it can be found in most tropical localities in the western central Pacific, Asia, Indian Ocean (Purcell et al., 2012). This species lives in shallow habitats and is found mostly on outer and inner reef flats, back reefs, and shallow coastal lagoons. The body wall of *H. leucospilota* is consumed by Asians and the entire animal or its gut and/or gonads may be consumed as a delicacy or as protein in traditional diets or during difficult times (Purcell et al., 2012).

A total of 40 specimens of *H. leucospilota* and 40 sediment samples were collected from MV and CM from January to December 2021.*H. leucospilota* samples were dissected, and the gut, respiratory tree, and tentacles were removed. The sea cucumber's gut, respiratory tree, and tentacles were oxidated by a 69% nitric acid solution for 24 hours and samples were filtered on filter paper.

Fig. 2. *Holothuria (Mertensiothuria) leucospilota* (Brandt, 1835)

Sediment samples were taken at a depth of 0-10 cm from the sediment surface. 1 kg of sediment was collected with the help of a shovel for each station. All samples were wrapped in aluminum foil and immediately frozen for storage at −20°C until further analysis. A modified method from Thompson et al. (2004) was used to extract microplastics from sediments. After homogenizing sediment samples, subsamples were taken for analysis (25 g). A saturated NaCl solution was added to the sediment and mixed. This allowed the sample material to suspend and enabled density separation of the sediment and microplastics. Post-stirring, the suspension was left for 1-h and floating samples were then filtered using filter paper.

All, equipment and working surfaces were cleaned with distilled water, and gloves and cotton laboratory coats were worn to prevent contamination. Also, the filters and solutions were covered with aluminum foils during all procedures to reduce airborne contamination.

Each filter was observed using a Stereo Microscope, and the amount (pieces/individual and pieces/kg sediment), type (fiber, fragment, and film), and color of microplastics were reported.

T-test was conducted to compare the MP abundance in the sediment, gut, respiratory tree, and tentacle between the sites. Additionally, the Pearson correlation was used to investigate the relationships between the amount of microplastic in the different organs of sea cucumbers and the amount of microplastics in the sediment. All tests were performed in IBM SPSS and the p-value was accepted as 0.05.

Results

The mean length and weight of *H. leucospilota* individuals were 31.85±3.82cm and 336.20±33.55 g in MV and 31.15±3.18 cm and 325.25±41.56 g in CM.
Microplastics were found in all *H. leucospilota* samples (Figure 3). Microplastic amounts in the gut of *H. leucospilota* samples were mean 40.45±16.95 pieces/ind. in MV and 15.30±7.65 pieces/ind. in CM. The microplastic concentration of gut in MV was higher than in CM and this was statistically significant ($p<0.05$). The microplastic concentration of guts was found as a minimum in December and maximum in October in MV (19-57 pieces/ind.), in CM, was a minimum in September and maximum in December (7-26 pieces/ind.).

The amount of microplastic in the respiratory tree of *H. leucospilota* was mean 23.90±10.93 pieces/ind. in MV and 15.45±5.46 pieces/ind. in CM. The microplastic concentration of the respiratory tree in MV was higher than in CM and this was statistically significant ($p<0.05$). The amount of microplastic in the respiratory tree was found as a minimum in October and maximum in August in MV (13-33 pieces/ind.), in CM, was minimum in October and maximum in December (8-20 pieces/ind.).

The amount of microplastic was mean 8.90±4.93 pieces/ind. in MV and 9.55±5.21pieces/ind. in CM in the tentacles of *H. leucospilota*. The microplastic concentration of tentacles in CM and MV was similar. The amount of microplastic in the tentacles was found as a minimum in March and maximum in February in MV (4-13 pieces/ind.), in CM, was minimum in August and maximum in March (4-15 pieces/ind.).

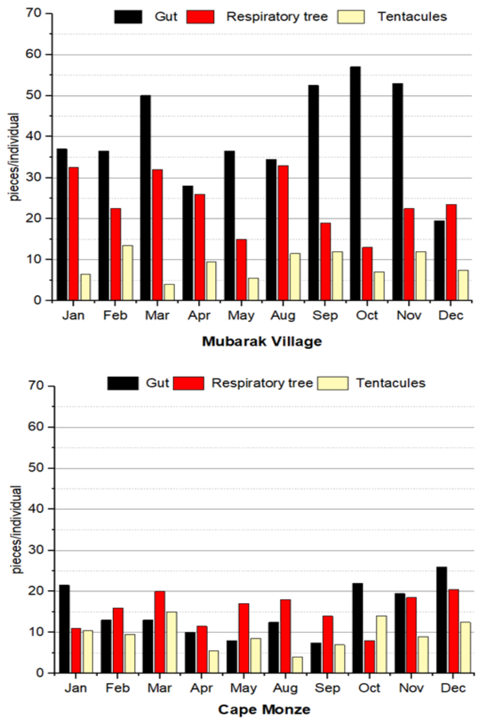

Fig. 3. The amount of microplastic in different organs of *H. leucospilota* (pieces/ind)

Microplastics were found in all sediment samples (Figure 4). Microplastic concentration was found as a minimum in December and maximum in March in MV (81-145 pieces/kg sediment). Microplastic concentration was found as a minimum in August and maximum in December in CM (27-79 pieces/kg sediment). Microplastic abundance in sediment samples was a mean of 129.35±44.17 pieces/kg in MV and 57.40±35.84

pieces/kg in CM. The amount of microplastic in MV sediment samples was higher than in CM sediment samples and this was statistically significant ($p<0.05$).

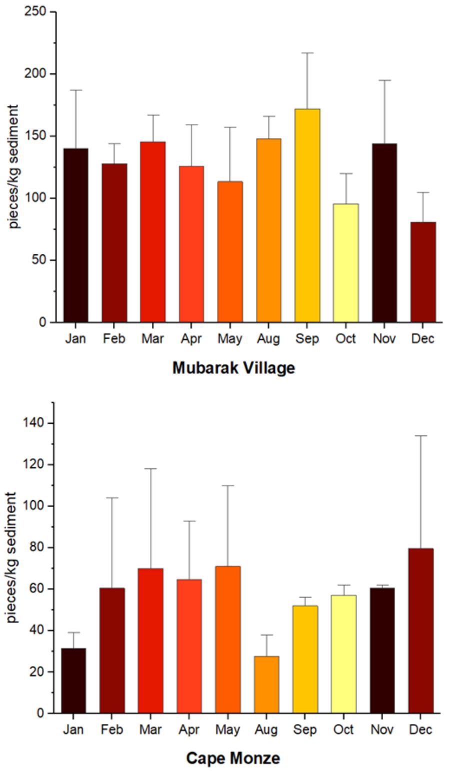

Fig. 4. The amount of microplastic in sediment samples

There was a correlation between the amount of microplastic in the tentacles and the amount of microplastics in the sediment (R: 0.60) and between the amount of microplastic in the tentacles and the amount of microplastics in the gut (R:0.61) in CM ($p<0.05$). However, there was no relationship between the amount of microplastic in the different organs of sea cucumbers and the amount of microplastics in the sediment in MV. Three different types of microplastics were found in the sediment samples. Fibers were dominant (99.14%) and a low percentage of fragments (0.64%) and films (0.21%) were also found (Figure 5). Fibers were found in samples over 99% of both MV and CM. Thus, the percentage distribution graphs are not given separately.

The types of microplastic in different organs of *H. leucospilota* were like sediment and three different types of microplastics were found. Fibers were found in over 99% of all examined organs, also, this situation was similar both in MV and in CM. So, the percentage distribution graphs are not given separately. The fiber was dominant (99.34%) and a low percentage of fragments (0.57%) and films (0.09%) were also found in *H. leucospilota*.

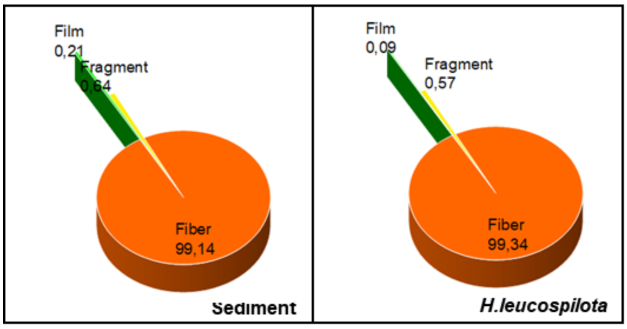

Fig. 5. The types of microplastic in sediment and *H. leucospilota*

The five different colors of microplastic were detected in sea cucumbers and red was the dominant color in all organs (Figure 6). The dominant microplastic color of sediment samples was black (49.58%), and followed by red, blue, and green.

Discussion and Conclusion

The present study is the first investigation on the abundance of microplastic in sea cucumber and sediments along the coast of Pakistan. Microplastic amounts [pieces/individual (ind.) in sea cucumber and pieces/kg in sediment samples] and types (fibers, fragments, and films) were investigated in Mubarak Village and Cape Monze in Karachi, Pakistan. Investigations on sea cucumber and sediments around the world was shown in Table 1.

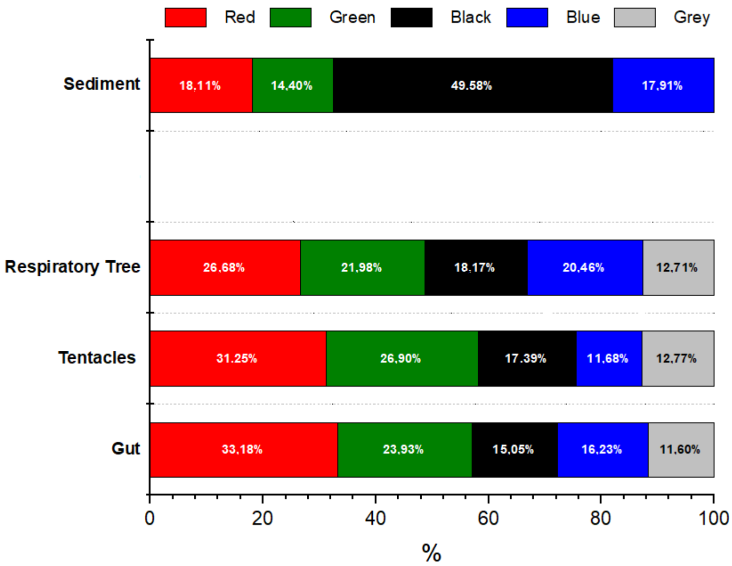

Fig. 6. Color distribution of microplastics in different organs and sediment

Table 1. Investigations on sea cucumber and sediments

Region	Research area	Abundance of microplastics	Types of microplastics	Color	References
Placencia Lagoon, Belize, Carribean	Sea cucumber (*Holothuria floridana*)	8.4±4.5 pieces/ind (digestive tract)	Fragments:93% Fibers:7%	-	Coc et al., 2021
	Sediment	0.15 pieces/g			
Pulau Pangkor, Perak, Malaysia	Sea cucumber (*Stichopus horrens*)	72.3 pieces/ind (digestive tract) (total 1446 in 20 specimens)	Fibers:90.87% Fragments:8.23% Film:0.9%	Black/Grey: 59.13%, Blue:27.04%, Red:9.9%, Green:0.96%, Transparent/ White:0.9%, Pink/Purple:0.83%, Brown:0.76%, Yellow/orange:0.48%	Husin et al., 2021
Bintan Island, Riau Archipelago	Sea cucumber (*Holothuria atra, Holothuria scabra, Sticopus variegatus*)	32-52 pieces/ind (digestive tract)	Fibers>fragments>films>foams	White:30%, Black:23%, Red:16%, Blue:13%, Brown:11%, Green:7%	Idris et al., 2022
Bohai Sea and Yellow Sea, China	Sea cucumber (*Apostichopus japonicus*)	0-30 pieces/ind (intestine) 0-19 pieces/ind (coelomic fluid)	Fibers: 91% Fragment: 8%	Blue:59%, Transparent:24% Black: 6%, Red:5% Purple: 4%, Brown:1% Green:1%	Mohsen et al., 2019
	Sediment	20-1040 pieces/kg dry sediment	Fibers: 97% Fragments and films: 3%	Blue: 48%, Transparent:27% Black: 14%, Red: 5% Purple: 5%, Brown: 1%	
Mubarak Village and Cape Monze, Karachi, Pakistan	Sea cucumber (*Holothuria leucospilota*)	Gut: 15.3-40.45 pieces/ind Respiratory tree: 15.45-23.9 pieces/ind Tentacle: 8.9-9.55 pieces/ind	Fiber: 99.34% Fragments: 0.57% Films:0.09%	Gut: Red: 35.64%, Green: 20.79%, Black: 7.92%, Blue: 17.16%, Grey: 18.48% Respiratory tree: Red: 23.62%, Green: 19.74%, Black: 18.45%, Blue: 21.36%, Grey: 16.83% Tentacle: Red: 28.95%, Green: 24.74%, Black: 17.37%, Blue: 16.32%, Grey: 12.63%	This study
	Sediment	57.40-129.35 pieces/kg	Fiber: 99.14% Fragments: 0.64% Films:0.21%	Red: 18.11%, Black: 49.58%, Blue: 17.91%, Green: 14.40%	

There is limited investigation on microplastic pollution in the Pakistan coast (Balasubramaniam and Phillott, 2016; Irfan et al., 2020a,b; Ahmed et al., 2022; Yousuf et al., 2022). In sediment investigations in Pakistan, Hawkesbay Beach was researched, and the results of this study showed that there were 12 microplastic fibers in 25 g sand (480 fibers/kg). The abundance and distribution of microplastics in freshwater systems were researched, and the results showed that sediments were also contaminated with microplastics (Irfan et al. 2020a,b).

The research conducted by Ahmed et al. (2022) showed that the microplastic amount of sediment samples in the Karachi coast of Pakistan was mean 987.40±617.06 particle/kg. In the same research, Mubarak Village and Cape Monze was sampled in 2019 and the result of the research microplastic amount in these two-sampling point was 645 particle/kg in Cape Monze and 1170 particle/kg in Mubarak Village. In this study, the microplastic amount of the sediment samples was 57.40±35.84 pieces/kg in Cape Monze and 129.35±44.17 pieces/kg in Mubarak Village and these results were lower than the previous study. In both investigations, Mubarak Village had a higher microplastic amount than Cape Monze. Mubarak Village is highly polluted than Cape Monze due to fishing activity and visitors. However, Cape Monze is not active or not a disturbance of fishing activity as well.

In the present study, the microplastic amount in different organs of *H. leucospilota* was found as 15.3-40.45

pieces/ind. in the gut, 15.45-23.9 pieces/ind. in the respiratory tree, 8.9-9.55 pieces/ind. in tentacles.

The microplastic amount of *Holothuria floridana* digestive tract was 8.4±4.5 pieces/ind. in Placencia Lagoon, Belize, Caribbean (Coc et al., 2021). In total 1446 microplastic in 20 specimens' digestive tract of *Stichopus horrens* were reported in Pulau Pangkor, Perak, Malaysia (Husin et al., 2021). Digestive tract of three different sea cucumber species (*Holothuria atra, Holothuria scabra, Sticopus variegatus*) was investigated by Idris et al. (2022) and microplastic amounts of these species were found between 32-52 pieces/ind. in Bintan Island, Riau Archipelago. The microplastic amount in the intestine of *Apostichopus japonicus* was reported as 0-30 pieces/ind. and 0-19 pieces/ind. in the coelomic fluid in the Bohai Sea and Yellow Sea, China. Microplastics have been found in edible tissue (Mohsen et al., 2022).

There was a positive relationship between in the tentacles and the amount of microplastics in the sediment in Cape Monze. Similar results were found by Mohsen et al. (2019) in the farmed sea cucumber's (*Apostichopus japonicus*) digestive tract and sediment samples in China. The high correlation between the amount of microplastic in the sediment and the amount of microplastic in the sea cucumbers showed that the sea cucumber might be used as a bioindicator of microplastic pollution in the sediment (Mohsen et al., 2019).

Fibers were the dominant microplastic type, up to 99% of all samples in this study. Similar results were reported by various researchers in different sea cucumber species and also different sediment samples (Husin et al., 2021; Idris et al., 2022; Mohsen et al., 2019). Fragment was the dominant only in the *Holothoria floridana* in Placencia Lagoon, Belize, Caribbean (Coc et al., 2021). The microplastic concentration of the surrounding environment affected the plastic uptake and investigations on the microplastic contamination of Pakistan coast (including Karachi) and different Scombridae species (in Karachi), the microplastics were mostly fibers (>%99) (Ahmed et al., 2022; Yousuf et al., 2022).The similar result was reported in Hawkesbay Beach in Pakistan (Balasubramaniam and Phillott 2016). So, the fiber density found both in sediment and in different organs of sea cucumber samples in the region and the results of this study supported by the investigation which was conducted in the region (Ahmed et al., 2022; Yousuf et al., 2022). Anthropogenic activities in land are the main source of microplastics in the marine environment and one of these main sources is sewage systems and fibers is discharged form washing of synthetic clothes (Napper and Thompson, 2016; Boucher and Friot, 2017). This high fiber density in this study may be due to the condition of the region because, Karachi has major commercial sectors of Pakistan and the region's intensive agricultural, household, and urbanization activity, the harbor may receive enormous amounts of untreated agricultural and domestic sewage (Ahmed et al., 2015).

Similar colors were observed in different organs of sea cucumber in this study. The dominant microplastic colors were red and green in the gut, red and blue in the respiratory tree, and red and green in the tentacle. Red was the dominant microplastic color in all examined organs. The microplastic colors of the sediment samples were red, black, blue, and green and these colors were similar to the dominant colors in the examined organs. Similarly, the colors of microplastics in sea cucumber (*Apostichopus japonicus*) digestive tract and in sediment samples were reported as parallel by Mohsen et al. (2019).

Iwalaye et al. (2020) investigated the routes by which *H. cinerascens* could take up microplastics from the environment via laboratory study. They reported that *H. cinerascens* has two ways (through respiration and eating) to access and absorb microplastics from the environment, increasing their chances of accumulating more microplastics and potentially harmful compounds absorbed from the environment. This puts sea cucumber (*H. cinerascens*) consumers and predators at risk of ingesting microplastics (Iwalaye et al., 2020). Microplastics are present in all types of environments around the world and consumption has been studied a variety of aquatic organisms (Lusher et al., 2015; Karlsson et al., 2017; Daniel et al., 2020). Ingestion of plastic can cause various adverse effects, plastics can adsorb and concentrate potentially dangerous toxic compounds from the aquatic environment and can be treated with a variety of chemicals during production (Gregory, 2009; Teuten et al., 2009; Wright et al., 2013; Napper and Thompson 2016; Bai et al., 2021; Wang et al., 2021). Because of this, people are getting more worried about organisms eating microplastics. Even though only the sea cucumber's body wall is eaten by people, there needs to be more research done on the potential health risks associated with sea cucumber consumption because the contaminants may move from microplastics to sea cucumbers.

There were large quantities of microplastics all samples and fibers were the dominant microplastic type like many other regions all over the world. Investigations in Pakistan's coasts showed that the region was contaminated by microplastics both marine environment and biota. Limited investigations have been conducted in the region and the problem of microplastic pollution has not yet been adequately recognized in Pakistan, so more extensive and further research are needed.

Plastic waste can harm marine life, degrade water quality, and create unsightly beach conditions that discourage visitors. In response, action is needed to address plastic pollution at both the local and global levels. Locally, improved waste management practices, such as recycling and proper disposal, can reduce the amount of plastic waste that enters the sea.

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