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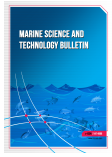

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RESEARCH ARTICLE

Microplastic contamination of *Holothuria (Thymiosycia) arenicola* Semper, 1868, *Holothuria pardalis* Selenka, 1867, sediments and seawater from Karachi Coast, Northern Arabian Sea, Pakistan

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ABSTRACT

Microplastics (MPs) are posing an increasing threat to the marine environment, affecting marine ecosystems and posing potential risks to human health through the food chain. This study aims to investigate the MP contamination of *Holothuria (Thymiosycia) arenicola* Semper, 1868, *Holothuria pardalis* Selenka, 1867, sediments and seawater from Buleji coasts of Karachi, Pakistan. The MP contamination was determined in the different body parts of sea cucumber species and both seawater and sediment samples were contaminated with microplastics. The results show that *H. arenicola* contains more microplastics than *H. pardalis*. In terms of shape and colour, fibres were the most common form of MPs (>99%), with black being the predominant colour. The highest to the lowest amount of MPs was determined as gut (52±26 pieces/individual in *H. arenicola* and 31±14 pieces/individual in *H. paradis)*, coelomic fluid (18±10 pieces/individual in *H. arenicola* and 26±15 pieces/individual in *H. paradis*), respiratory tree (22±11 pieces/individual in *H. arenicola* and 14±9 pieces/individual in *H. paradis* and tentacles (13±8 pieces/individual in *H. arenicola* and 10±5 pieces/individual in *H. paradis)*, respectively. Determination of MP pollution in these sea cucumbers and their surrounding environment is very important in terms of the importance of these organisms in the marine ecosystems. The biological impacts of MPs on sea cucumbers and other marine organisms can eventually affect humans through the food chain. Therefore, the paper advocates for the development of policies to monitor and reduce MP pollution in marine ecosystems.

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Introduction

Anthropogenic pollutants, especially microplastics, which have become a major part of the world's marine litter, are posing an increasing threat to the marine environment. MPs, defined as plastic particles measuring less than 5 mm, originate from various sources, including the breakdown of larger plastic items and the shedding of synthetic fibres (Mathalon & Hill, 2014; Isobe et al., 2017; Concoli et al., 2019; Cincinelli et al., 2019).

Their widespread presence in marine ecosystems not only increases the risk to marine organisms but also raises concerns about their eventual entry into the human food chain. The Arabian Sea, a vital marine ecosystem (Zafar et al., 2018), is no exception, and the coastal waters of Karachi have been reported to harbour significant amounts of plastic pollution, which is likely to have detrimental effects on local marine biota (Ahmed et al., 2023; Tariq et al., 2024).

Among the numerous species affected by MPs, sea cucumbers, especially *Holothuria (Thymiosycia) arenicola* Semper, 1868 and *Holothuria pardalis*, are of significant ecological importance (Bat et al., 2020; Bhuyan et al., 2024). Sea cucumbers play an important role in maintaining benthic health through their feeding activities, which facilitate nutrient cycling, sediment bioturbation, and organic matter recycling (Sezgin et al., 2008; Ahmed & Bat, 2015, 2020; Ahmed et al., 2017, 2023). Additionally, these echinoderms act as biological indicators of coastal ecosystem health due to their sensitivity to environmental changes and pollutants (Ahmed et al., 2015, 2017, 2018, 2019). Their placement at the lower trophic levels of the marine food web also raises implications for larger marine and human consumers, making them critical to studying the effects of MPs in marine environments (Ahmed et al., 2023).

Despite their ecological importance, there is a scarcity of research specifically assessing the accumulation and potential health effects of MPs in sea cucumbers, particularly in the context of the Arabian Sea. Understanding the concentration of MPs in species *H. arenicola* and *H. pardalis* is essential, not only for assessing the overall health of coastal ecosystems but also for evaluating the risks posed to human health through seafood consumption. Given the rising trend of MP pollution globally, coupled with the increasing demand for seafood, there is an urgent need to investigate and characterize the level of contamination within these economically and ecologically significant organisms.

The accumulation of MPs in sea cucumbers, particularly in regions such as the Arabian Sea, poses significant ecological concerns. Sea cucumbers, as deposit feeders, play a crucial role in marine ecosystems by recycling nutrients and enhancing sediment health. However, their feeding habits make them particularly vulnerable to MP ingestion. This study aims to fill a critical gap in the current understanding of MP pollution in marine food webs by analysing samples of *H. arenicola* and *H. pardalis* collected from the intertidal zone of the Buleji coast in Karachi. Through this investigation, we seek to elucidate the extent of MP contamination in these sea cucumbers and discuss the broader implications for marine ecosystems and food safety. The results of this research will contribute to the growing body of literature on MP pollution, inform conservation strategies, and raise awareness about the urgent need for regulatory frameworks to mitigate plastic waste in the Arabian Sea.

Material and Methods

Sampling and Analysing

Sea water, sediment and sea cucumbers (*H.arenicola* and *H. pardalis*) samples were collected from Buleji (Lat: 24°50'20.41" N Long: 66°49'24.15" E), Karachi coast in 2022 (Figure 1). Sea cucumbers were collected on seasonally as North-east monsoon (Nov-Feb), inter-monsoon_1 (Mar-Apr), South-west-monsoon (May-Sept), Inter-monsoon_2 (Oct). Sea water and sediments for also collected for analysis of MP monthly from January to December 2022.

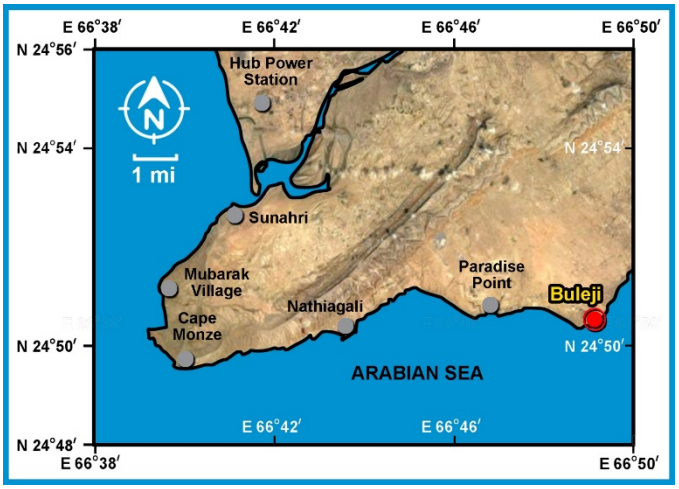

Figure 1. Study area map

The sea cucumbers (total 80 specimens) were randomly collected from intertidal zone The collection took place during low tide to minimize disturbance to the natural habitat and to ensure that the sea cucumbers were accessible for measurement. Length (cm) and weight (g) of sea cucumbers

were measured. The body parts of sea cucumbers were removed separately and microplastic analysis in sea cucumbers was carried out in gut, respiratory tree, tentacles, and coelomic fluid. Different body parts placed in separate beakers and digestion process were applied with 30% H_2O_2. After all organic matter was digested, ZnCl was added to solutions, for the MPs to float on the surface. The particles on the surface of the solution were taken and filtered on filter paper (Whatman glass microfiber GF/C filter discs 1.2 µm pore size), and the filters were dried and stored for future analysis (Ahmed et al., 2021).

Sea water and sediment samples were collected from the Buleji coasts. 1 litre surface water samples were collected in glass bottles, which were submerged in water with floats attached to their exterior to maintain the top at a depth of 5 cm. Sediment samples were collected with a metal spoon from the top 5-10 cm of the sediment during low tide. Sediment samples were dried for three days at 65°C to reach a stable weight. A density separation process was then applied to the sediment samples. In a glass beaker, 200 g of dried sediment was mixed with 500 mL of saturated NaCl solution (density 1.2 g/cm^3) and manually agitated for 2 minutes before being allowed to settle for 2 hours and floating solids were taken clean beakers. Digestion processes were applied to seawater and sediment samples. The digestion process was conducted using 20 mL of 30% H_2O_2 at 65°C for 24 hours for remove organic materials in the solutions. The final solutions were then filtered (Whatman glass microfiber GF/C filter discs 1.2 µm pore size), and the filters were dried and stored for future analysis (Ahmed et al., 2021).

The filter papers were investigated under a stereo microscope to determine the presence of MPs as well as group them according to MP groups based on shape and colour (Idris et al., 2022).

Quality Control and Quality assurance

Precautions were taken to detect and prevent the potential risk of contamination. To avoid contamination, cotton lab coats and gloves were worn during each stage of research from sampling to analysing. All tools and surfaces were washed with distilled water. Additionally, during every step, aluminium foil was used to cover the filters, beakers and solutions in order to minimize airborne contamination. Contamination control filters were used for detect airborne contamination.

Data Analysis

The number (pieces/individual for sea cucumbers, pieces/kg for sediment, pieces/L for seawater), shape (fibre, fragment, film, and bead), and colour of MPs were recorded after each filter was examined under a stereo microscope (50X magnification).

All obtained data were processed and maintained using MS Excel (Microsoft 365) and presented as mean and standard deviation (SD). The Kruskal-Wallis test was used to evaluate MP amounts among the groups (seasons in different body parts) and Mann Whitney U test was used to evaluate MP amounts between the species because the data did not have a normal distribution. The relationship between MP amounts in sediment and seawater samples and additionally sediment/seawater and different organs of sea cucumbers were investigated with Pearson correlation. The p-value was accepted as 0.05.

Results

The mean length and weight of the sea cucumber species were 23±5.30 cm and 190.18±36.41 g in *H. arenicola* and 11.30±1.87 cm and 37.58±11.14 g in *H. paradis*. The MP amounts in different parts of the *H. arenicola* and *H. paradis* and also statistical differences were shown in Figure 2 ($p<0.05$).

MP abundance in the gut of sea cucumber species was 52±26 pieces/individual in *H. arenicola* and 31±14 pieces/individual in *H. paradis*. The MP abundance of gut in *H. arenicola* was higher than in *H. paradis* and this was statistically significant ($p<0.05$). MP abundance in the tentacles of sea cucumber species was 13±8pieces/individual in *H. arenicola* and 10±5 pieces/individual in *H. paradis*. The respiratory tree of sea cucumber species contained 22±11 pieces of MP in *H. arenicola* and 14±9 pieces of MP in *H. paradis*. The MP abundance of respiratory tree in *H. arenicola* was higher than in *H. paradis* and this was statistically significant ($p<0.05$). Finally, MP abundance in the coelomic fluid of sea cucumber species were 18±10 pieces/individual in *H. arenicola* and 26±15 pieces/individual in *H. paradis*. The MP abundance of coelomic fluid in *H. paradis* was higher than in *H. arenicola* and this was statistically significant ($p<0.05$).

MPs were found in all seawater and sediment samples obtained from Buleji (Figure 3). MP abundance in sediment samples was a mean of 293±111 pieces/kg in sediment and 78±28 pieces/L in seawater. MP concentration was found as a maximum in August in both seawater and sediment samples. The lowest MP abundance was found in November in seawater and February in sediment samples. There was no relationship between microplastic abundance in seawater and in sediment samples ($p>0.05$).

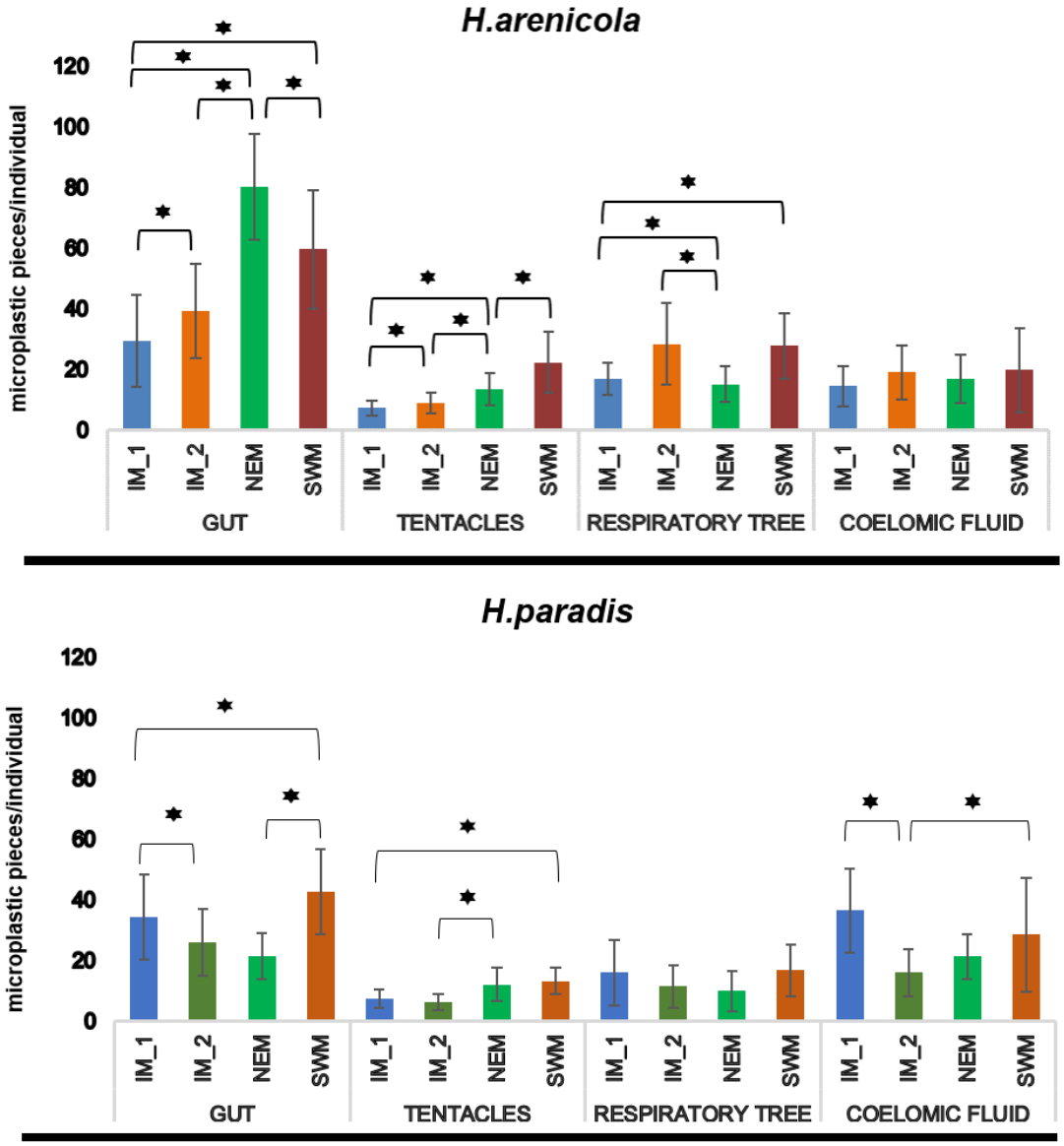

Figure 2. The MP concentrations in the different body parts of *H. arenicola* and *H. paradis*. Asterix upper the vertical bars in each graph indicate the values are significantly different ($p<0.05$) between seasons

There was a correlation between the amount of MP in the gut (R: 0.78), respiratory tree (R: 0.73), coelomic fluid (R: 0.65), and the number of MPs in the sediment ($p<0.05$) and there was no correlation between the amount of MP in the seawater and the different organs of the sea cucumber species.

Four different shapes of MPs were found in samples (fibre, fragment, film and bead). Fibre was found as dominant MP shape in all samples. Fibre ratio was found over 99% in sea cucumber species and seawater samples and over 97% in sediment samples (Figure 4). Bead was found in only seawater.

Black was the dominant colour of MP detected in sea cucumber species followed by red, blue, and green (Figure 5).

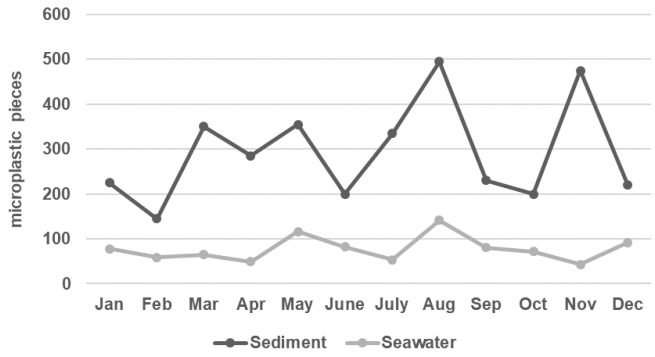

Figure 3. MP abundance in seawater (pieces/L) and sediment (pieces/kg) samples in Buleji coasts

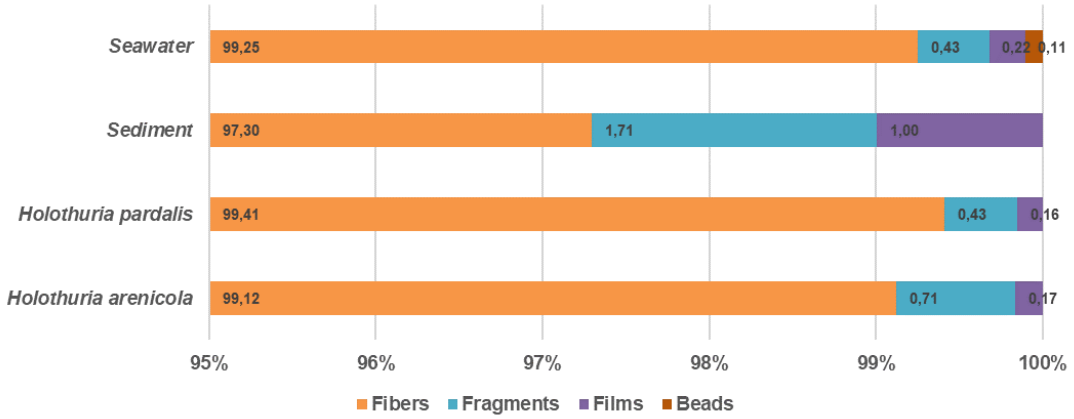

Figure 4. The shapes of MP in seawater, sediment and sea cucumber samples

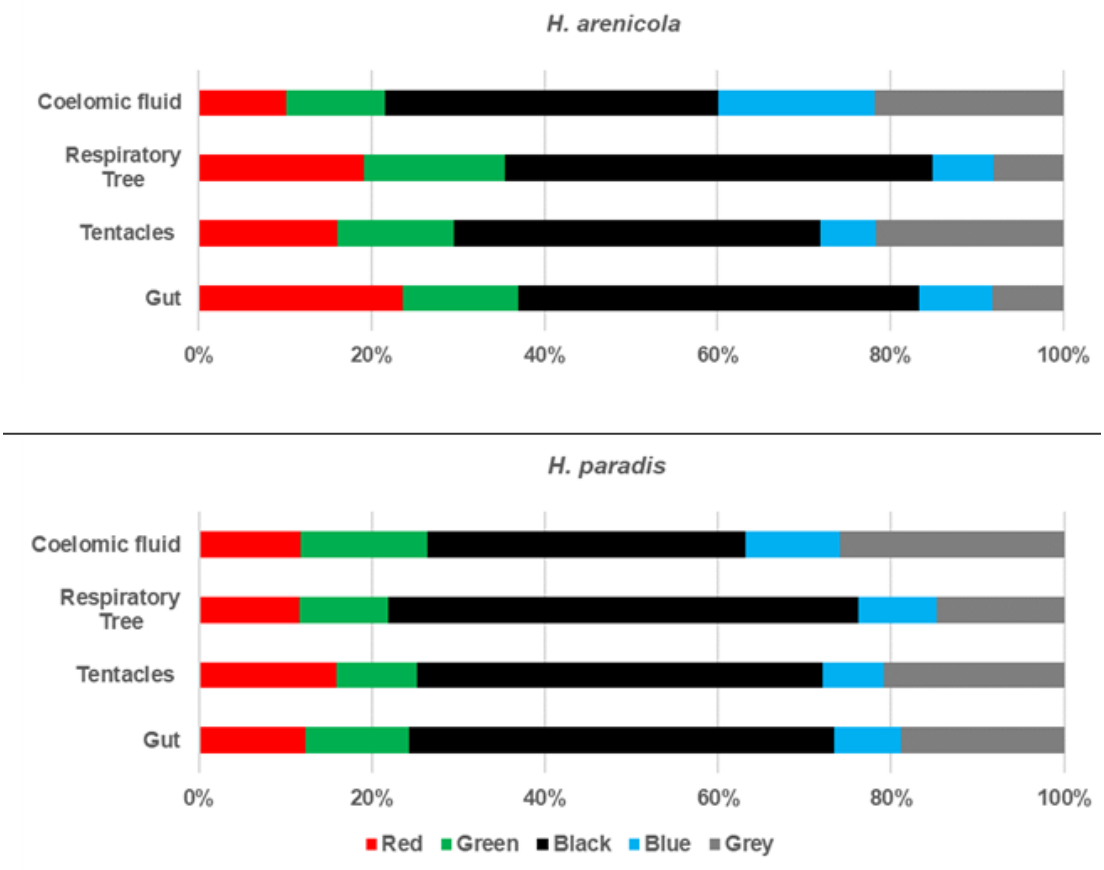

Figure 5. The colours of MPs in sea cucumber species

Discussion

Despite the growing body of literature on marine litter and MP pollution globally (Aydın et al., 2023), there remains a significant knowledge gap regarding its impact on marine life within the Arabian Sea, particularly along the coasts of Karachi (Ahmed et al., 2023; Tariq et al., 2024). Currently, there are very few studies focused on the accumulation of MPs in local marine organisms, especially keystone species like the sea cucumbers. This lack of targeted research emphasizes not only the novelty of our results but also the urgency to understand how MPs influence the health of marine ecosystems in Karachi coasts.

The results show that *H. arenicola* contains more microplastics than *H. pardalis*. This situation could be related to the size of the organisms. Generally, larger individuals can accumulate more microplastic particles because their bodies are bigger, allowing them to ingest more food, and in this process, environmental pollutants, including microplastics, have a greater accumulation potential (Welden et al., 2018). Additionally, larger individuals typically spend more time and

cover larger areas, which could result in more exposure to microplastics (Porter et al., 2023). Furthermore, larger sea cucumber species may forage over wider areas for food, which increases the likelihood of ingesting microplastic particles. However, to fully understand this, more data and research are needed. Besides size, factors such as age, differences between species can also influence microplastic accumulation.

MP accumulation in sea cucumbers, particularly in the Arabian Sea, is an emerging area of concern due to the ecological implications of MPs on marine life. Sea cucumbers, as deposit feeders, are particularly susceptible to MP ingestion, which can occur during their feeding processes as they sift through sediments. Studies have shown that MPs can be found in the digestive tracts of various sea cucumber species, indicating a significant level of exposure and potential bioaccumulation (Mohsen et al., 2019; Idris et al., 2022; Ahmed et al., 2023; Sulardiono et al., 2023). The presence of MPs in *H. arenicola* and *H. pardalis* raises immediate concerns regarding the bioavailability of MPs within the marine food web and their potential transfer up the trophic levels, impacting larger marine fauna and human consumers alike. The results obtained from our study reveal the extent of MP contamination in these vital sea cucumber species, thereby serving as a critical indicator of the broader health of the marine environment in which they reside. Such results underscore the interconnectedness of marine ecosystems, where MPs in sea cucumbers may cascade through food webs, ultimately affecting biodiversity, and human health.

Research conducted in different marine environments has documented the presence and types of MPs ingested by sea cucumbers. For instance, a study on *H. atra* in Indonesia with fibres being the dominant type (Sulardiono et al., 2023). Similarly, another study reported a high abundance of MPs in sea cucumbers from the seagrass ecosystem of Bintan Island, Indonesia, again with fibres being the dominant type (Idris et al., 2022) and also similarly fibre was dominant and all sea cucumber individuals contaminated with MPs in *H. leucospilota* in the Karachi coasts of Pakistan. These results showed the similarity with our results.

In the context of the Arabian Sea and Karachi, the accumulation of MPs can be attributed to various anthropogenic activities, including coastal development and tourism, which contribute to marine pollution (Ahmed et al., 2023). The ingestion of MPs by sea cucumbers can lead to adverse health effects, including potential toxicity and disruption of physiological processes. For example, MP fibres can breach the tissues of the respiratory tree during respiration, leading to their presence in the coelomic fluid, which may affect the overall health of the organism (Mohsen et al., 2020, 2023). Furthermore, the ingestion of MPs has been linked to the transfer of harmful additives and pollutants, posing additional risks to marine ecosystems (Sharma et al., 2021).

The implications of MP accumulation in sea cucumbers extend beyond individual health, as these organisms play a crucial role in marine ecosystems. They contribute to nutrient cycling and sediment turnover, and their consumption of MPs raises concerns about the potential transfer of these contaminants through the food web (Courtene-Jones et al., 2017; Mohsen et al., 2019). As such, monitoring MP levels in sea cucumbers can serve as an effective method for assessing the health of marine environments and the extent of plastic pollution (Courtene-Jones et al., 2017; Mohsen et al., 2019).

The sources of MPs in the Arabian Sea and Karachi are multifaceted, including urban runoff, sewage discharge, and the degradation of fishing gear (Ahmed et al., 2023; Jawad Al-Shaikh Ali et al., 2023). In this study, synthetic fibres are a major contributor to MP pollution, in previous studies conducted in the region, it was generally assumed that they were released into the marine environment through wastewater discharge (Ahmed et al., 2023). Additionally, physical processes such as UV degradation can fragment larger plastics into MPs. This is particularly concerning given the high levels of MP contamination reported in sediments along the Arabian coast, which directly correlates with the levels found in local marine fauna, including sea cucumbers (Idris et al., 2022; Ahmed et al., 2023).

Research has demonstrated that MPs can accumulate in the digestive systems of sea cucumbers, with studies revealing a positive correlation between MP concentrations in sediments and those found in the organisms themselves (Ahmed et al., 2023). This suggests that sea cucumbers could serve as bioindicators for monitoring MP pollution in marine sediments. The ingestion of MPs can lead to various physiological impacts, including potential toxicity and disruption of normal feeding, digestive processes and growth (Mohsen et al., 2020, 2023). Furthermore, the presence of MPs in sea cucumbers raises concerns about the transfer of these pollutants through the food web, ultimately affecting higher trophic levels, including humans who consume seafood (De-la-Torre et al., 2019).

The presence of MPs in various anatomical parts of sea cucumbers, including the gut, respiratory tree, tentacles, and coelomic fluid, has become a critical area of research due to its

implications for marine ecosystems and food safety. The highest to the lowest amount of MPs was determined as gut (52±26 pieces/individual in *H. arenicola* and 31±14 pieces/individual in *H. paradis)*, coelomic fluid (18±10 pieces/individual in *H. arenicola* and 26±15 pieces/individual in *H. paradis*), respiratory tree (22±11 pieces/individual in *H. arenicola* and 14±9 pieces/individual in *H. paradis* and tentacles (13±8 pieces/individual in *H. arenicola* and 10±5 pieces/individual in *H. paradis)*, respectively in this study. Results showed statistical differences.

In the gastrointestinal tract, studies have shown that sea cucumbers can accumulate different types of MPs, including fibres, fragments, and pellets (Mohsen et al., 2019; Idris et al., 2022; Ahmed et al., 2023; Sulardiono et al., 2023). For instance, research on MP abundance in the gut content of sea cucumber showed that MP content ranged from 0-30 pieces per individual in *Apostichopus japonicus* in China (Mohsen et al., 2019), 72.3 pieces per individual in *Stichopus horrens* in Malaysia (Husin et al., 2021), 15.3 to 40.45 pieces per individual in *H. leucospilota* in Pakistan (Ahmed et al., 2023). The present study is similar to the studies mentioned above and MP abundance in the gut were found 52±26 pieces/individual in *H.arenicola* and 31±14 pieces/individual in *H.paradis*.

The respiratory tree of sea cucumbers also serves as a site for MP accumulation. MPs can enter the respiratory system during the process of respiration, where water is drawn in through the anus and filtered through the respiratory tree (Mohsen et al., 2023). Studies have reported MP concentrations in the respiratory trees of sea cucumbers, with findings indicating that these organisms can ingest between 8.9 to 9.55 MP pieces per individual in Karachi (Ahmed et al., 2023), MP abundance in the tentacles of sea cucumber species was 13±8pieces/individual in *H.arenicola* and 10±5 pieces/individual and consequently, similar results were obtained in nearby regions. The inhalation of MPs can lead to potential physiological impacts, including respiratory distress and impaired gas exchange, which are critical for the survival of these organisms (Mohsen et al., 2020, 2023).

Moreover, MPs have been detected in the tentacles of sea cucumbers, which are essential for feeding and sensory perception. The tentacles can inadvertently capture MPs while the sea cucumber feeds, further contributing to the overall burden of MPs within the organism (Ahmed et al., 2023). Studies have reported MP concentrations in the respiratory trees of sea cucumbers (*H. leucospilota*) were 8.9 to 9.55 MP pieces per individual in Karachi (Ahmed et al., 2023), MP abundance in the tentacles of sea cucumber species was 13±8pieces/individual in *H.arenicola* and 10±5 pieces/individual in *H.paradis*, similar results were obtained in nearby regions. The presence of MPs in the tentacles may also affect the organism's ability to detect food and interact with its environment, potentially leading to reduced feeding efficiency and altered behaviour (Sulardiono et al., 2023; Ahmed et al., 2023).

The coelomic fluid, which serves as a medium for gas exchange and nutrient transport in sea cucumbers, has also been found to contain MPs. Research indicates that MPs can transfer from the digestive tract into the coelomic fluid, with studies reporting among the 0-19 MP particles per individual in this fluid (Mohsen et al., 2019, 2020). MP abundance in the coelomic fluid of sea cucumber species were 18±10 pieces/individual in *H.arenicola* and 26±15 pieces/individual in *H.paradis* in this study. This transfer raises concerns about the systemic effects of MPs on the health of sea cucumbers, as they may interfere with physiological processes and lead to toxicological effects (Mohsen et al., 2019, 2023).

The composition of microplastics were evaluated in the current study. The size and shape of MPs ingested can vary significantly, with fibres often being the most prevalent due to their widespread presence in marine environments (Mohsen et al., 2023; Ahmed et al., 2023; Sulardiono et al., 2023). The feeding behaviour of sea cucumbers, which involves the selective ingestion of sediment particles, contributes to the accumulation of MPs in their digestive systems (Widianingsih et al., 2023). Black was the dominant colour of MP detected in sea cucumber species. Microplastics primarily arise from the degradation of plastics and specifically, tire wear particles, which are predominantly black, contribute significantly to aquatic and terrestrial microplastic pollution (Han et al., 2024). The runoff from the road can be a source of microplastics as the sampling location is on the roadside. Additionally, wastewater effluents containing black microplastics from textile industries further augment their environmental concentrations. Wastewater infrastructure is inadequate in Pakistan's major cities, especially in metropolitan areas such as Lahore, Karachi, and Islamabad. So, these plastic particles may be present in the research areas from wastewater effluents.

There are seasonal fluctuations in the microplastic abundance of sea cucumbers, and they found statistically significant. However, no clear seasonal increase or decrease in each body part was observed. Changes in environmental conditions during different seasons can impact the sources and transport mechanisms of microplastics (Anastasiou, 2020). For

instance, increased rainfall during certain seasons may lead to higher runoff from urban and agricultural areas, consequently enhancing the availability of microplastics in adjacent marine environments. Despite the observed seasonal fluctuations in the surrounding environment, studies have indicated that there is no clear, uniform pattern regarding microplastic abundance in various body parts of sea cucumbers.

A positive relationship was found between in the different organs and the amount of MPs in the sediment in this study. Similar results were found by Ahmed et al. (2023) and Mohsen et al. (2019). Determination of MP pollution in these sea cucumbers and their surrounding environment is very important in terms of the importance of these organisms in the marine ecosystems. This research can be used as an indicator for the sea cucumber might be used as a bioindicator of MP pollution in the sediment (Mohsen et al., 2019: Ahmed et al., 2023). Sea cucumbers are one of the indicator biotas that play an important role in ecology, contribute to sediment turnover and nutrient cycling, and help fertilize the substratum by stirring the bottom (Husin et al., 2021; Idris et al., 2022). The implications of MP accumulation extend beyond individual species; they threaten the overall health of marine ecosystems.

Conclusion

In conclusion, the accumulation of MPs in seawater, sediment, and sea cucumbers in the Arabian Sea is a significant environmental issue that warrants further investigation. Understanding the extent of MP ingestion and its ecological consequences is essential for developing effective management strategies to mitigate marine pollution and protect marine biodiversity.

MPs in the different parts of sea cucumbers highlight the pervasive nature of plastic pollution in marine ecosystems. The implications of such accumulation are profound, affecting not only the health of sea cucumbers but also the broader marine food web and human health through seafood consumption. Continued research is essential to understand the full extent of MP impacts on marine organisms and to develop strategies for mitigating plastic pollution in marine environments.

Our research highlights the pressing need for continued monitoring and investigation of MP pollution along the Karachi coast and beyond. Future studies should aim to include a broader range of marine organisms, evaluating not only their MP loads but also the biological effects on their physiology, behaviour, and reproductive success. Longitudinal studies could provide critical insights into seasonal and spatial variations in MP accumulation, enhancing our understanding of source and sink dynamics within this marine environment. Additionally, findings of this study can inform policymaking and conservation efforts aimed at mitigating plastic pollution. By raising awareness of the ramifications of MP contamination on marine ecosystems and food safety, stakeholders—including policymakers, conservationists, and the fishing community—can be encouraged to implement strategies aimed at reducing plastic waste and enhancing ecosystem resilience. While our study lays a foundational understanding of MP presence in local sea cucumber *H. arenicola* and *H. pardalis* populations, it also serves as a clarion call for increased scientific inquiry into the implications of MPs on marine biodiversity and ecosystem health. By fostering a greater understanding of these issues, we hope to contribute to the development of effective strategies to protect our marine environments for future generations.

Compliance With Ethical Standards

Authors' Contributions

QA: Conceptualization, Investigation, Writing – review & editing;

AÖ: Writing – original draft, Formal Analysis, Data visualization;

QMA: Conceptualization, Writing – review & editing;

LB: Writing – review & editing, Supervision

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Funding

Not applicable.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

AI Disclosure

The authors confirm that no generative AI was used in writing this manuscript or creating images, tables, or graphics.

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