

Baseline Assessment of Microplastic Ingestion in the Sea Cucumber *Holothuria scabra*, Jaeger 1833 from Pujada Bay, Philippines

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ABSTRACT

Microplastics are major pollutants in marine ecosystems and pose threats to benthic organisms such as sea cucumbers. This study investigated the occurrence, morphotypes, colors, polymer types, and spatial variation of microplastics ingested by *Holothuria scabra* in selected sites of Pujada Bay. A total of 90 individuals were examined, with 35 (38.88%) containing microplastics. Thirty-six microplastic particles were recovered, averaging 0.4 particles per individual. Fibers were the dominant morphotype (75%), while blue particles were the most common color (61.11%). FTIR analysis identified synthetic rubber as the most abundant polymer type. The findings provide baseline information on microplastic contamination in *H. scabra* and highlight its potential as a bioindicator of sediment-associated microplastic pollution in Pujada Bay.

INTRODUCTION

Microplastic (MP) pollution is a global environmental threat, affecting marine and terrestrial ecosystems and posing risks to human health (Lee *et al.*, 2022; Yu & Singh, 2023). MPs are widespread in surface waters and sediments worldwide, with some areas showing alarmingly high concentrations (Kibria, Nugegogda, and Haroon, 2022). Microplastics (MP) are synthetic solid particles or polymeric matrices, either with uniform or erratic shapes, measuring between 1 μm and 5 mm, originating from primary or secondary production, and are resistant to dissolution in water (Pieper *et al.*, 2020). These are fragments from bigger plastics that are disintegrated over time due to use, maintenance, and disposal (Auta, Emenike, and Fauziah, 2017; Fiore *et al.*, 2022). Some of the most usual types of microplastics are polypropylene, polyvinyl chloride, polystyrene, polyethylene, and polyethylene terephthalate (PET) (Yano *et al.*, 2020; Yano *et al.*, 2021). MPs enter marine food webs through various pathways, with ingestion being the primary route for benthic organisms (Pinheiro, do Sul, and Costa, 2020). These pollutants pose significant ecological and toxicological risks to aquatic and terrestrial environments (Bhattacharya & Khare, 2022; Li *et al.*, 2023).

The Philippines is a significant contributor to global plastic pollution, ranking third worldwide and generating 2.7 - 5.5 million metric tons annually (Schachter & Karasik, 2022). Without intervention, mismanaged plastic waste could reach 9 million metric tons by 2040 (Schachter & Karasik, 2022). The implementation of Republic Act 9003 (Ecological Solid Waste Management Act) in the Philippines faces various challenges and successes. While some Local Government Units (LGUs) fully comply with creating Solid Waste Management Boards and submitting management plans (Dalugdog, 2021), implementation effectiveness varies.

Sea cucumbers, the essential contributors to the health of benthic ecosystems via bioturbation and nutrient cycling, (Purcell *et al.*, 2016) are among the numerous marine species affected by microplastic contamination (Markic & Nicol, 2014). *Holothuria scabra* (Jaeger, 1833) is a commercially important sea cucumber species (Junus, Kwong, and Khoo, 2018). They are gonochoric invertebrates belonging to the phylum Echinoderms under the class Holothuroidea (Schoppe, 2000). *H. scabra* is known for its importance due to its wide distribution and abundance in intertidal zones of Indo-Pacific and as a high commercially valued species in Asian markets for being the only species to be mass produced in hatcheries and mainly sold as a dried product (beche-de-mer) (Hamel *et al.*, 2022). *H. scabra* is commonly known as sandfish internationally, and locally known as “kagisan” or “bat kagisan”. These bottom-feeding organisms enhance mineralization and nutrient cycling in coastal sediments, increasing the effluence of organic nitrogen and fueling algal productivity (MacTavish *et al.*, 2012). They redistribute surface sediments, reduce organic load, and excrete inorganic nutrients, contributing to ecosystem productivity and biodiversity (Purcell *et al.*, 2016). Due to their feeding behavior as deposit-feeding detritivore, these marine invertebrates are particularly vulnerable to microplastic ingestion through their feeding tentacles and respiratory trees (Iwalaye, Moodley, and Robertson-Andersson 2020; Markic & Nicol, 2014). Consequently, these MPs can

penetrate the main edible parts of the sea cucumbers (Mohsen *et al.*, 2022) which can pose safety issues for human consumption.

Pujada Bay, located in City of Mati, Davao Oriental, is an ecologically and economically significant coastal area in southeastern Mindanao, covering approximately 21,200 hectares and designated as a Protected Landscape and Seascape and officially declared part of the NIPAS (National Integrated Protected Area System) under Presidential Proclamation No. 431 in 1994 (Pliego, 2015). It harbors rich marine biodiversity, including over 50 genera of corals, 9 species of seagrass, and 16 species of mangroves, which support a variety of marine life such as sea cucumbers, mollusks, crustaceans, and commercially important fish species (DENR-BMB, 2004). Despite its ecological richness, the bay is threatened by overfishing, coastal development, sedimentation, and marine pollution, including microplastics.

Studies on microplastics among marine habitats and organisms across all trophic levels are already present (do Sul & Costa, 2014; Parolini *et al.*, 2023). However, research specifically focused on sea cucumbers in Pujada Bay remains limited, particularly concerning economically important species such as *Holothuria scabra*. Most MP studies focus on pelagic species and water-only exposures, creating significant knowledge gap regarding sediment-associated MPs and their effects on benthic invertebrates (Sandgaard *et al.*, 2023).

Establishing baselines on microplastic contamination in marine organisms is essential for assessing ecological risks, identifying potential pollution sources, and informing mitigation strategies (Ballent *et al.*, 2016; Khaleel *et al.*, 2023). Therefore, this study aims to determine the occurrence of microplastics in *Holothuria scabra* collected from Pujada Bay, Philippines. The findings provide baseline data on microplastic contamination in this commercially important species and contribute to a better understanding of pollution exposure in benthic marine organisms. The results may also have implications for food safety and support evidence-based policies for marine pollution management in the area.

THEORETICAL REVIEW

Deposit-Feeding Exposure Theory

This study is anchored on the concept of *Deposit-Feeding Exposure Theory*, which explains that benthic-deposit feeding organisms are highly susceptible to contaminant ingestions because they continuously consume sediments while extracting organic matter. Since microplastics accumulate in marine sediments, deposit feeders such as *Holothuria scabra* inevitably ingest these particles during feeding activities.

According to Bellasi *et al.*, 2020 and Egbeocha *et al.*, 2018, ingestion is one of the primary pathways by which microplastics enter benthic organisms. Similarly, according to Mancuso *et al.*, 2026, sea cucumbers are particularly vulnerable to microplastic uptake due to their feeding mechanism and close association with sediments. Because *H. scabra* ingests large quantities of sediment as part of its natural feeding behavior, the species serves as an effective indicator of sediment-associated microplastic contamination.

This theory supports the assumption that the occurrence and characteristics of microplastics recovered from *H. scabra* reflect the level and nature of microplastic pollution present in the benthic environment of Pujada Bay.

H1: Microplastics are present in the gastrointestinal tract of *Holothuria scabra* collected from Pujada Bay.

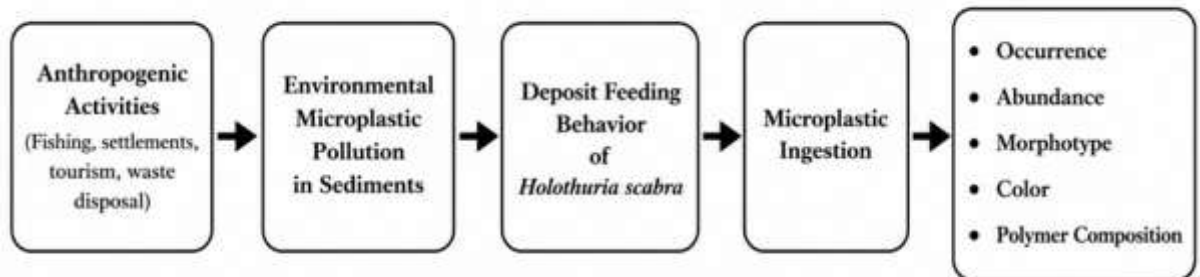


Figure 1. Conceptual Framework

METHODOLOGY

Study Sites

The study was conducted in three coastal barangays within the Pujada Bay in the City of Mati, Davao Oriental, Philippines, namely: Lawigan, Badas, and Dahican (Figure 1). The study site in Barangay Lawigan is located at about 6°48' North, 126° 20' East. The coast is located at the mouth of the bay and is composed of a long strandline of white sand with small patches of mangroves. Study site in Barangay Dahican is located at 6.9182° North, 126.2592° East, which is a small cove bay, surrounded by mangrove forests and close to shrimp ponds, features various substrates including sandy, sandy-muddy, sandy coral line, and muddy areas. In contrast, Tagawisan, is situated at 6.9356° North, 126.1826° East and characterized by a muddy substrate, located perpendicular to a national highway, adjacent to residential areas, near a busy fish port, and within the more populated section of Pujada Bay.

Sample Collection and Processing

Thirty-three adult-sized individuals of *H. scabra* are collected using opportunistic sampling in each site. Thirty samples are used for analysis while the other three are used for spiking. The sample collection was done at different time points until completed and are taken through hand collection with the aid of the local gleaners and the presence of the researcher. Each sample was washed with pre-filtered water and placed in an airtight glass container individually to avoid contamination and placed in an ice box until lab processing. For 10% KOH solution preparation, 100g of lab grade KOH pellets is dissolved in 1 liter filtered distilled water.

The samples are dissected individually. The isolated guts of the samples are transferred to a washed glass jars and digested using the 10% KOH solution. The added solution is three times more than the tissue volume for digestion of tissues (Karami et al., 2017) and is set aside for 7 to 14 days or until all the tissues are fully digested. After thorough digestion, the solution was filtered through a

47mm microfiber filter paper through vacuum filtration. The filter paper is then stored in a clean petri dish or sterile aluminum cups and covered in aluminum foil for storage until quantification. One air contamination control and blank sample are placed during dissection and another one during filtration.

Isolated microplastics from sea cucumbers were examined for visual observation using a stereo microscope with 10x to 40x magnification and photographed using a mobile phone camera. The confirmed MPs were sorted based on their morphological category, color, and plastic polymer. Stereomicroscopy is utilized for initial MP identification and morphological classification (Ricardo et al., 2024). The microplastics collected were characterized into visual categories according to their shape and color. Shape classification is based on the FAO technical report (Lusher, Hollman, and Mendoza-Hill, 2017) as fragments, beads, films, fibers, or foams. Color classification is based in the ISCC-NBS (Inter-Society Color Council and National Bureau of Standards) system. The FTIR (Fourier Transform Infrared Spectroscopy – Agilent Cary 630 FTIR spectrometer) was used to analyze the presumed microplastic particles and to identify their chemical compositions. Comparison between the generated spectra from FTIR spectroscopy and the established spectra of different polymer standards is conducted to determine the type of polymer of each sample. MP samples are determined as Polyethylene (PE), Polypropylene (PP), Polystyrene (PS), Polyethylene terephthalate (PET), Polyvinyl chloride (PVC), nylon, or Polyurethane, among others (Gola et al., 2021). The Raman spectra is utilized to identify the polymer matrix of detected particles (Dong et al., 2020). A Motic microscope was used to observe the microplastic particles, and representative particles were selected for measurement. The size of these representative microplastics was determined using Motic Images Plus version 3.0.

Strict measures and procedures are essential in preventing sample contamination in microplastic research (Belontz & Corcoran, 2021). For quality assurance, this study adapted quality control measures from the study of Osorio, Tanchuling, and Diola (2021) to guarantee finding reliability and validity. To alleviate the possibility of cross-contamination from external MPs, the use of plastic containers in sample storage is strictly avoided. All samples are stored in sterile non-plastic containers, carefully labeled, safely stored in a box, and transported to the laboratory for processing.

All materials, equipment, samples, and laboratory surfaces used were properly cleaned and decontaminated using pre-filtered water. Sample handling and processing are conducted with utmost care. Air contamination/quality controls and blank samples are placed around all sample processing (sample collection, dissection, weighing, solution preparation, filtration, microscopy, and FTIR analysis) to monitor airborne contamination and prevent false-positives. Samples were always covered with aluminum foil before, during, and after processing. Laboratory gowns (100% cotton), safety masks, and nitrile gloves are worn all throughout the experiment and analysis to ensure the reduction of potential sample contamination.

Extracted MP samples in glass petri dishes and aluminum cups were covered at all times to limit the period of exposure to airborne contamination.

Glassware and other apparatus used in the process were rinsed repeatedly with distilled water. This was done to minimize sample loss due to adhesion of MPs on the walls of the filter apparatus. All washing solutions from this rinsing procedure are also subjected to filtration to guarantee the retention of all possible microplastics. These stringent methods will establish a strong foundation for dependable and replicable research results.

RESULTS

A total of 90 individuals of *Holothuria scabra* (30 per site) were examined for microplastic ingestions. Among these, 35 individuals contained microplastics, representing 38.88% of the sampled population. A total of 36 microplastic particles were recovered from all specimens, yielding an average of 0.4 particles per individual across the entire sample.

$$\begin{aligned} \text{Average particles per individual} &= \frac{\text{Total microplastic particles recovered}}{\text{Total number of individuals examined}} \\ &= \frac{36}{90} = 0.4 \end{aligned}$$

Microplastics Morphology

The analysis of the gastrointestinal tract of *H. scabra* collected from the three sites of Pujada Bay revealed the presence of microplastics. Figure 2 shows the morphological characterization of identified microplastics, which highlights the prevalence of fibers and fragments across the three sites. Fibers are long thread-like structures, while fragments are irregularly shaped particles with rough random edges. Data indicates that Fibers are long thread-like structures, while fragments are irregularly shaped particles with rough random edges. Data indicates that fibers are the most dominating particle (27 total items), accounting for 75% of all the microplastics recovered, compared to fragments (9 total items) across three sites.

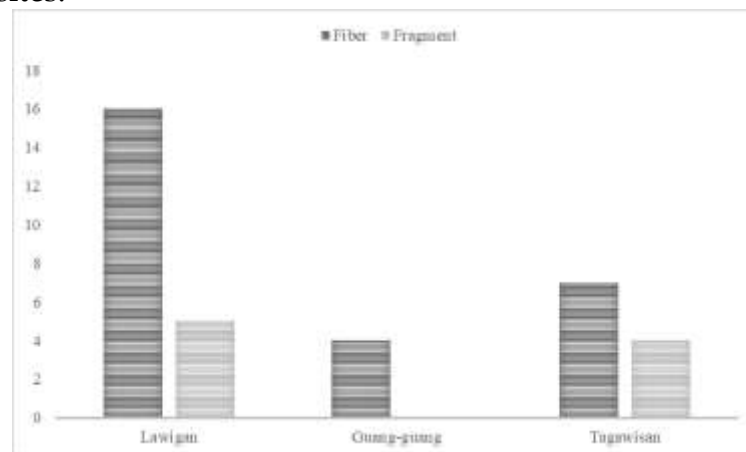


Figure 1. Distribution of microplastic morphotypes (fibers and fragments) recovered from *Holothuria scabra* across sampling sites in Pujada Bay, illustrating variation in morphotype abundance.

having a total of 16 particles recovered, corresponding to an average of 0.53 particle per individual species. Tagawisan has the second highest number of

fiber totaling to 7 particles, equivalent to an average of 0.23 particle per individual species. Lastly, Guang-guang shows the lowest abundance, with 4 fiber particles, resulting to an average of 0.13 particle per individual species.

Fragments, on the other hand, has considerably lower abundance with only 9 particles recovered. The distribution varied among sites, with Lawigan having the highest occurrence, followed by Tagawisan, but no fragments observed in Guang-guang. Overall, fragments accounted for 25% of the total microplastics recovered.

Microplastics Color Distribution

The thirty-six (36) microplastics recovered from the gastrointestinal tracts of *H. scabra* exhibited a variety of colors. The colors identified include blue, black, pink, light blue, purple, green, red, and yellow. Figure 4 shows the color distribution of microplastics across three sites.

Among the identified colors, blue was the most dominant, accounting for 22 total particles or 61.11%, followed by black (8.33%). Pink, light blue, purple, green, and red each accounted for 5.56%, while yellow represented the lowest proportion of 2.78%. The distribution of colors varied slightly across the three sampling sites, although the blue particles remained the most prevalent in all locations.

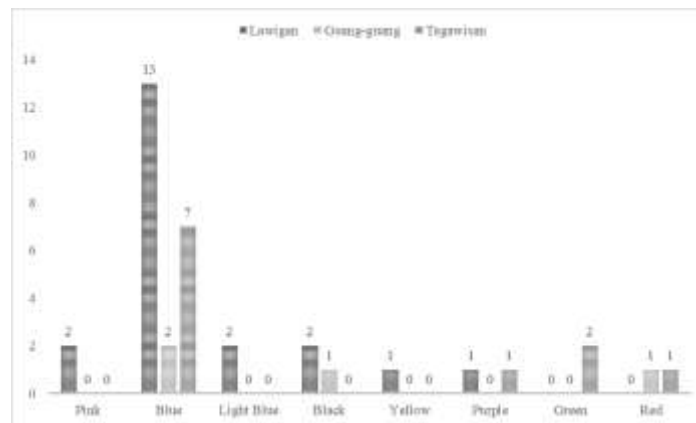


Figure 2. Color distribution of microplastics recovered from *Holothuria scabra* in Pujada Bay, highlighting the relative abundance of different color categories.

Polymer composition of microplastics (FTIR analysis)

All recovered microplastic particles are subjected to FTIR analysis to determine their polymer types. The analysis identified several polymer types including synthetic rubber, synthetic fiber, copolymer, polypropylene (PP), polyethylene (PE), and nylon. Figure 5 presents the distribution and prevalence of different polymers across three sites. Among the analyzed particles, synthetic rubber is the most prevalent, accounting for the highest proportion of all samples, representing 66.67% followed by nylon (11.11%). Other polymer types were detected in equivalent proportions, accounting to 5.56%. Representative FTIR spectra of the identified polymers exhibited characteristic absorption peaks

consistent with their respective reference spectra. These spectral analyses confirmed the polymer identity of the MP particles analyzed.

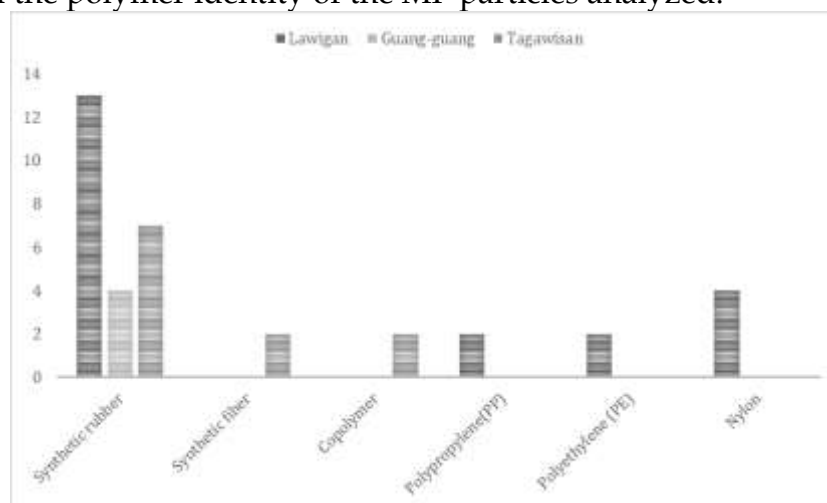


Figure 3. Relative abundance of polymer types identified by FTIR in microplastics from *Holothuria scabra* across sampling sites in Pujada Bay, indicating variation in chemical composition.

Although the distribution of polymer types varied among the sampling sites, synthetic rubber being the most abundant, was also detected consistently in all locations, indicating its prevalence in the study area.

DISCUSSION

This study confirms the presence of microplastics in the gastrointestinal tracts of *Holothuria scabra* collected from Pujada Bay, indicating the incidence of microplastic contamination within the benthic environments. The ingestion of microplastics by the species is probably influenced by its deposit-feeding behavior (Riani & Cordova, 2022), wherein sediment is constantly ingested for the extraction of organic matter (Mathieu-Resuge *et al.*, 2020).

The detection of microplastics in *H. scabra* indicates that plastic pollution did not end in waters but extended to the benthic organisms and their primary habitats (Shukhairi *et al.*, 2025). This finding is coherent with related studies reporting microplastic ingestions in benthic invertebrates, which is considered highly susceptible to sediment-associated contaminants. Highlighting the potential roles of deposit-feeder macroinvertebrates as bioindicators of environmental pollution such as microplastics in coastal environments (Ben-Haddad *et al.*, 2022; Costa *et al.*, 2023).

Microplastic morphology

The prevalence of fiber microplastics in the gastrointestinal tracts of *H. scabra* may indicate that fibrous materials represent a major component of microplastic pollution in the study area. Microplastic fibers are the most common morphotype reportedly dominating the environments (Acharya *et al.*, 2021). These particles are also highly associated with synthetic textiles (Weiss & De Falco, 2022) and fishing ropes (Song *et al.*, 2025). These particles enter the coastal ecosystems through water

discharges, laundering (Browne *et al.*, 2011), and fishing activities (Das & Das, 2024).

The greater abundance of fibers compared to fragments may be attributed to their elongated shape and overall physical characteristics, which affect their settling velocity (Nguyen *et al.*, 2022). As a result, fibers tend to remain suspended in the water column for longer periods, allowing them to be widely transported before eventually accumulating in sediments where they become available for ingestion by benthic organisms such as *Holothuria scabra*. Its distribution is highly influenced by hydrologic dynamics (Wang *et al.*, 2022). The relatively lower abundance of fragments in this study indicates that secondary microplastic formation through the degradation of larger plastics may be less dominant than fiber.

The occurrence of both fibers and fragments in *H. scabra* further signifies that multiple sources of microplastic pollution exist within Pujada Bay. As a deposit-feeder, this species consumes sediments in large quantities along with detrital materials (Indriana & Kunzmann, 2018), leading to the ingestions of various microplastic particles present in the habitat. Similarly, other findings on other benthic organisms noted that fibers also dominate over other morphotype (Pagter *et al.*, 2021), reinforcing the extensive nature of fiber pollution in coastal ecosystems.

Color distribution

The high abundance of blue-colored microplastics in the gastrointestinal tracts of *H. scabra* may be an implication that blue-colored plastics may represent a major source of microplastic contamination in Pujada Bay. Fishing materials such as ropes and fishing lines, like nylon, are the most common items that are highly associated with blue-colored microplastics (Ferreira *et al.*, 2018). The occurrence of other colors such as black, pink, light blue, purple, green, red, and yellow indicates high variation of plastic pollution within the study area, indicating multiple possible anthropogenic sources including household waste (Khan & Bose, 2024), packaging materials (Kadac-Czapska *et al.*, 2023), and other degraded plastics.

Polymer composition

The polymer types of microplastics recovered from the gastrointestinal tracts of *H. scabra* in Pujada Bay suggests potential sources of the pollutants both from marine and land-based activities that contribute to microplastic pollution. The results indicate a varying type of polymer is present across the sampling sites, with Lawigan showing the highest abundance of identified polymers, followed by Tagawisan and Guang-guang.

Among the identified particles, rubber is the most abundant, especially in Lawigan. These are polymers typically like tires and is considered one of the most prevalent microplastics in the ecosystem (Klun, Rozman, and Kalčíková, 2023). Typical polymer types, such as polypropylene and polyethylene were also detected across the three sampling sites. These are common plastics widely used in packaging, containers, and fishing-related applications and are among the commonly reported polymers in marine ecosystems. Due to their durability and

resistance to degradation, these polymers can be broken down to microplastics and be introduced into the food chain (Gallo *et al.*, 2018).

The detection of nylon also supports the effects of fishing activities in the sites as a significant source of microplastics (Timmers *et al.*, 2005) in Pujada Bay. Nylon is the main materials of fishing nets, ropes, and fishing lines, which are prone to degradation due to mechanical stress and environmental exposure. The occurrence of nylon in the gastrointestinal tracts of *H. scabra* implies that fragmented fishing gears contribute to the microplastics deposited in the benthic environments.

The difference of polymer compositions across the three sampling sites may be influenced by environmental factors like hydrodynamic conditions, sediment compositions, and proximity to human activities. The higher diversity and abundance of polymers observed in Lawigan suggest that this site may be subject to greater anthropogenic pressure and microplastic accumulation compared to the other locations.

Generally, the detection of a wide range of polymer types, both common plastics and synthetic rubbers, highlight the complicated nature of microplastic pollution in Pujada Bay. As a deposit-feeder, *H. scabra* is vulnerable to sediment-associated pollutants from different sources, making it an essential indicator of environmental microplastics, highlighting the need to consider both plastics and rubber-based materials in monitoring and management focusing on the mitigation of microplastic pollution in coastal environments.

The detection of microplastics in the gastrointestinal tracts of *H. scabra* from Pujada Bay emphasizes the potential ecological risk linked with plastic contamination in benthic environments. As a deposit-feeding organism, *H. scabra* plays an essential role in bioturbation and nutrient recycling (Purcell *et al.*, 2016). Microplastics may actually harm benthic organisms like sea cucumbers since they may carry toxic substances, such as heavy metals and some organic pollutants, which can adsorb to plastic surfaces (Kinigopoulou *et al.*, 2022). This raises concerns regarding the long-term exposure of benthic organisms to microplastics pollutants on the marine environment.

While sea cucumbers are not considered a major component of a higher trophic, the presence of microplastics in benthic organisms still raises expansive ecological concern. The displacement of microplastics across different trophic levels, from predation to environmental process, may have implications towards ecosystems health and biodiversity. This paper highlights the need for continued and much improved monitoring and management of macroplastic and microplastic pollution in coastal ecosystems such as Pujada Bay.

CONCLUSIONS AND RECOMMENDATIONS

The study confirmed the presence of microplastics in the gastrointestinal tracts of *Holothuria scabra* from Pujada Bay, with 38.88% of examine individuals containing microplastics, at an average of 0.4 particles per individual. Fiber is the most dominant morphotype, while fiber on the other hand exhibited lower abundance but showed a relatively larger particle sizes compared to fiber. The prevalence of blue-colored particles and the detection of synthetic rubber as the

most dominant polymer emphasizes the impact of anthropogenic activities, especially those related to coastal usage and material inputs, on microplastic contamination in the study site.

Spatial difference was evident among the three sampling sites, with Lawigan having the highest microplastic abundance, signifying localized environmental and anthropogenic influences. The occurrence of different microplastic particles in a deposit-feeding organisms underscores the role of sediment as sinks for plastic pollution and supports the utilization of *H. scabra* as a bioindicator of benthic contamination.

These findings emphasize the need for continued monitoring and improved management strategies and implementations to reduce the introduction of plastics into marine environments, particularly in protected areas such as Pujada Bay, where microplastic contamination is already evident.

FURTHER STUDY

The current study is only focused on the investigation of microplastic ingestions in sea cucumber, particularly *Holothuria scabra*. There is still a limited study focus on the market-relevant marine organisms such as gastropods and echinoderms (sea urchins). Further investigations may be conducted in these organisms in order to establish a more panoramic view on the extent of microplastic contamination in marine organisms.

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