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Abstract

*Marine ecosystems face growing threats from microplastic pollution, necessitating detailed investigations of its ecological impact. This study examined microplastics (MPs) in sediments and sea cucumbers (*Holothuria atra*) from coastal areas in Puerto Princesa City, Palawan, comparing a Marine Protected Area (MPA) and a non-marine protected area (NMPA). MPs were detected in all analyzed compartments: sediments, gut, and body wall of sea cucumbers. Sediments had the highest MP concentration, with 14 particles in the MPA and 9 in the NMPA. The gut contained 5 MPs in the MPA and 12 in the NMPA, while the body wall had none in the MPA and 2 in the NMPA. Most MPs were fibers (76.19%), primarily blue (76%), likely from synthetic textiles and fishing gear. Statistical analysis revealed significant differences in sea cucumber body weight between MPAs and NMPAs, though MP abundance did not correlate with body weight. These findings highlight the widespread presence of MPs and their potential ecological impact. Conservation efforts and waste management strategies are crucial to mitigating contamination. Future studies should employ advanced techniques like Fourier-transform infrared (FTIR) spectroscopy for polymer identification and investigate the long-term physiological effects of MPs on marine organisms.*

Key Words: Environmental impact, Marine pollution, contamination, bioindicator

Introduction

In recent decades, plastics have triggered a significant transformation in our daily lives. On a global scale, people utilize more than 260 million tonnes of plastic annually, constituting approximately 8% of the world's oil production [1]. The pervasive issue of plastic pollution stems from human activities affecting coastal and marine ecosystems worldwide. The continuous and unprecedented introduction of plastic contaminants into aquatic ecosystems by human sources directly and/or indirectly disrupts ecosystem structure, functions, and consequently, services and values. The primary avenues through which these contaminants, originating from both land-based and sea-based sources, enter the ocean have been identified.

Plastics are in high demand due to their lightweight, resilient, corrosion-resistant, transparent, and easy-to-produce properties. However, their high demand and improper disposal have resulted in severe environmental pollution and management issues

[2]. Plastic production is projected to triple by 2050 and account for a fifth of global oil consumption [3, 4]. Polypropylene, polyethylene, polyvinyl chloride, polystyrene, polymethyl methacrylate, and other thermoplastic polymers are the most commonly used. Plastics often break down into smaller and smaller particles, also known as microplastics, which can be ingested by both animals and people.

When natural mineralization processes slow down and the chances of successful engineered remediation solutions are low, the accumulation of plastic pollution in a specific environmental zone is termed as "poorly reversible." In these cases, any negative effects resulting from plastic pollution become almost irreversible. Potential outcomes of poorly reversible plastic pollution encompass changes in carbon and nutrient cycles, shifts in habitats within soils, sediments, and aquatic ecosystems, simultaneous biological impacts on endangered or keystone species, ecotoxicity, and associated societal repercussions [5].

Microplastics (MPs), defined as plastic particles smaller than 5 mm, are now recognized as widespread pollutants in marine environments. These particles originate from the breakdown of larger plastics and from direct sources such as tourism, municipal discharge, and industrial effluents [6]. Once introduced into the marine environment, MPs exhibit a heterogeneous distribution, with sediments acting as long-term sinks. Coastal waters and semi-enclosed seas, especially those with high biological productivity, often record the highest concentrations [7]. Sediments play a crucial role in the fate and transport of microplastics, as these particles settle and accumulate on the seafloor, particularly in low-energy environments. As such, studying sediments provides a valuable indication of long-term plastic pollution and potential exposure risks to benthic organisms [8]. Additionally, marine organisms influence microplastic movement by redistributing or burying them through their natural behaviors, further integrating these particles into the benthic ecosystem [9].

Sea cucumbers are benthic invertebrates commonly found in seagrass beds, reef ecosystems, and muddy or sandy marine substrates. They serve important ecological functions such as bioturbation and nutrient cycling, and they are also valuable economically due to their high demand in fisheries [10]. As deposit feeders, sea cucumbers ingest large volumes of sediment, making them particularly susceptible to microplastic ingestion [11]. Because they are non-selective feeders, MPs present in sediments are taken in alongside organic material and detritus, with potential physiological effects such as gut obstruction, reduced nutrient uptake, and exposure to adsorbed pollutants. Their interaction with MPs also has implications for trophic transfer within marine food webs. Moreover, their role as ecological indicators can provide insight into the level of contamination in their habitats [12, 13]. Investigating the presence of MPs in sea cucumbers can therefore contribute to a broader understanding of microplastic pollution in marine sediments and its biological impacts.

Holothuria spp. comprise a diverse group of echinoderms characterized by their soft bodies and thorn-covered skin. They possess an elongated body shape and are commonly found inhabiting shallow tropical waters, particularly around coral reefs, seagrass beds, and dark ocean bottoms [14]. Benthic holothurians, exhibiting detritivore trophic habits and of commercial interest, have been the subject of previous studies regarding their interaction with microplastics in sediment [15, 16]. These studies confirm the presence of plastic in the gut content of analyzed animals, establishing a positive correlation between gut plastic content and sediment plastic concentration, thus indicating their utility as environmental indicators. Due to their low mobility and trophic behavior, detritivore holothurian species can serve as effective sentinels for microplastic pollution in specific areas [17].

Holothurians, as nonselective feeders, intake large quantities of sediment, potentially absorbing plastic leachates and contaminants, posing risks for transfer to higher trophic levels [18, 19]. The translocation of ingested plastic can cause significant harm to holothurians, impacting reproductive success and population abundance in marine ecosystems [20]. However, studies on the

physiological and biochemical effects of microplastics on holothurians remain limited, often conducted in controlled laboratory conditions, which may not accurately represent environmental scenarios [21, 22].

The marine holothurians are spiny skinned invertebrates, which form important commercial group among the echinoderms. In India, about 200 species of holothurians are known of which 75 species are from shallow waters within 20 m depths [23]. *Holothuria atra*, a widely distributed and abundant species of sea cucumber, plays a significant role as an omnivore, consuming detritus, uneaten food, and algae within marine substrates [24]. *Holothuria atra* (Echinodermata: Holothuroidea) is a type of invertebrate animal that lives on the bottom of the rocky coast and has the potential as a nutritious food raw material for humans and pharmaceutical raw materials [25].

Puerto Princesa City, located in Palawan, Philippines, is celebrated for its pristine beauty, boasting clean urban areas nestled amidst stunning beaches and lush hills. However, microplastic pollution is emerging as a serious threat to its coastal waters. A study found microplastics in beach sand across Puerto Princesa, with higher concentrations on the east coast [26]. This pollution could affect key attractions like the Puerto Princesa Subterranean River National Park, Honda Bay, and Ulugan Bay, as well as surrounding forests. As Puerto Princesa City remains a favored destination for nature lovers, it's crucial to address and minimize the effects of microplastics to uphold the city's ecological balance and maintain its reputation as a paradise [27].

This research was conducted to provide crucial data and information on the presence of MPs in the gut of sea cucumbers, which are important organisms in the seagrass ecosystem. This data will serve as a reference for future research and decision-making related to the management of conservation areas. Thus, this study aimed to investigate the amount, type, and color characteristics of microplastics (MPs) in the gut, body walls of sea cucumbers, and sediments in the Central Park Station Puerto Princesa Subterranean River National Park and Barangay Tagburos, Puerto Princesa City. The study also aimed to compare the abundance of microplastics in sea cucumbers and sediments in marine protected areas (MPA) to those in NMPA.

Materials And Methods

Sampling Site

The study was carried out in two distinct areas, each offering unique ecological characteristics and human interactions. The first focal point of research was the Marine Protected Area (MPA), specifically the Central Park Station of Puerto Princesa Subterranean River National Park. This MPA protected a diverse range of marine life and ecosystems. It was designated as a protected area, ensuring that delicate ecosystems were preserved. In contrast, the second area under scrutiny lay outside the protective confines of marine conservation. Located in Hondoy Bay, Barangay Tagburos, this non-marine protected area served primarily as a site for gleaning activities. Here, people interacted extensively with the coastal environment through traditional harvesting practices for their livelihoods (Figure 1).

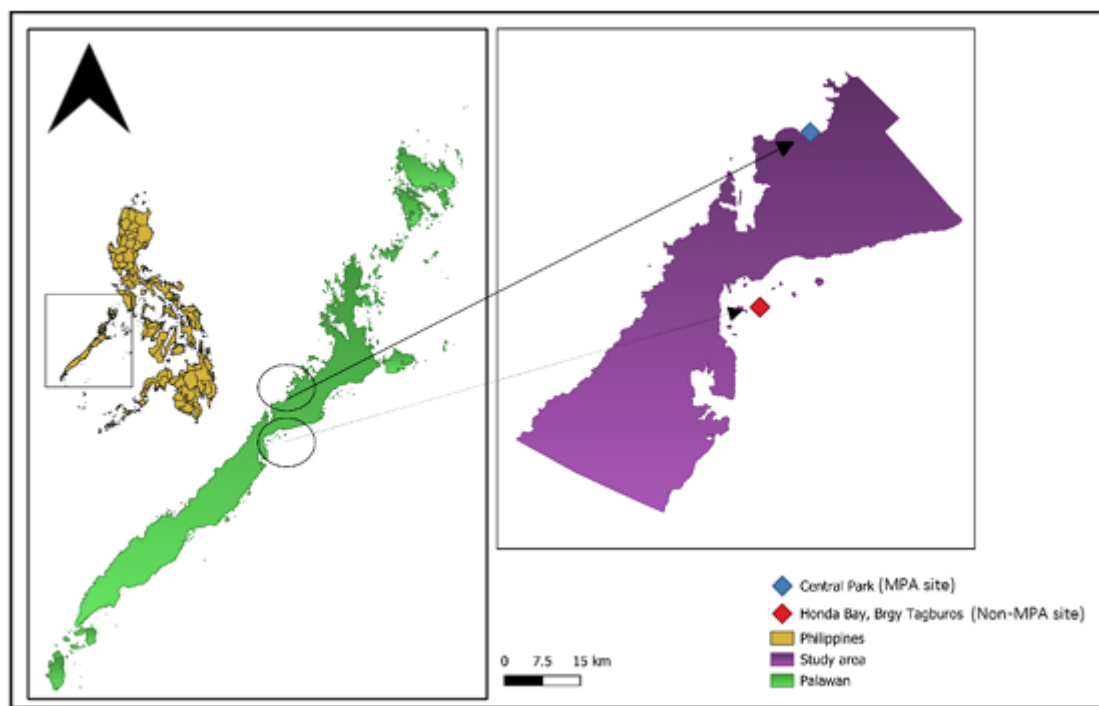


Figure 1: Map of Puerto Princesa, Palawan Showing The Sampling Sites.

Sample Collection

Sea cucumber samples were collected from two research areas randomly, and the location of the collection was marked using GPS coordinates. The survey area for the collection was 100 x 50 m. At each sampling location, a total of 10 sea cucumber specimens (*Holothuria atra*) were carefully collected (Plate 1). Once found, the sea cucumbers were securely placed within a glass jar [28]. Additionally, 3 replicates of sediment samples were taken to a depth of 10 cm from the sediment surface where sea cucumbers were collected. Sediments were collected, amounting to 50-100 g per sea cucumber collection. Sediment samples were taken manually and stored in glass jars [29]. Subsequently, the glass jars containing samples were promptly transferred to a cooler box filled with ice, ensuring optimal preservation until further analysis in the laboratory [30, 31].

Sample Preparation

Before commencing the laboratory work, the researcher utilized filtered distilled water for all stages of preparation and cleaning in the microplastics extraction process. This included washing, rinsing, and cleaning all laboratory apparatus to ensure the elimination of potential microplastic contaminants. The same filtered water was also employed to rinse the samples, maintaining consistency and minimizing the risk of introducing external microplastic particles during the analysis (Plate 2).

Dissection of the gut and body wall of Sea Cucumber

Before dissection, sea cucumber samples were weighed, and their guts were carefully extracted using surgical instruments, following the methodology outlined by Umasangaji [32]. The weights of the extracted guts were recorded [33]. Subsequently,

the body wall of the sea cucumber was scraped and washed to ensure that no gut residue remained, then diced into smaller sections, with portions weighed (Plate 3). These sections were then transferred into clean, dry glass beakers to be used as samples for microplastic extraction.

Microplastics Extraction in Sea Cucumber

After the removal of the gut and cutting of the body wall of sea cucumbers, five samples of gut and ten samples of the body wall were homogenized and placed in a beaker covered by aluminum foil, then dried in an oven at 60°C until it completely dry. The remaining homogenized five samples of sea cucumber gut were processed for microplastic extraction while still fresh. All fresh and dried intestines (gut) and body walls from sampled sea cucumbers were placed in a glass beaker. To facilitate tissue digestion, 10% KOH was applied, followed by incubation at 60°C (Plate 5). This process effectively broke down biological molecules without compromising the integrity of the plastic polymer. To facilitate tissue digestion, 10% KOH was applied, followed by incubation at 60°C. This process effectively broke down biological molecules without compromising the integrity of the plastic polymer. After 5 days of digestion at 60°C, the samples underwent the floatation method by adding 300 mL of analytical grade NaCl saturated solution (358.9 g/L) and homogenized by mixing for 2 min (Plate 6). The digestion solution was carefully extracted and filtered using Whatman filter paper (Plate 7). To ensure result accuracy, three identical blanks were introduced, employing a floatation test [34, 35]. This process was conducted with three replicates for robustness. A positive control with known microplastic particles was included to check extraction efficiency, while a negative control using only reagents ensured

no contamination from laboratory procedures [36, 37].

Microplastics Extraction in Sediments

A 150 g composite sediment sample was placed in a beaker and dried in an oven at 55°C until completely dry. Once dried, the sample was sifted through a 5 mm mesh sieve to exclude particles larger than 5 mm. The portion of the sample that passes through the sieve (less than 5 mm) was used for microplastic (MP) extraction. To remove organic materials, 150 ml of 10% KOH solution was added to the sample and heated in the incubator at 60°C for 40 hours (Plate 5). After oven-drying, the KOH solution was drained, and the samples were rinsed with distilled water and dried in the oven at 60°C until its completely dry. The dried samples then underwent the floatation method by adding 300 mL of analytical grade NaCl saturated solution and mixing for 2 minutes (Plate 6). The mixtures settled for approximately 2 hours to allow the microplastics to float. After settling, the mixture was filtered twice using Whatman filter paper and a filtration system to ensure complete extraction of microplastics (Plate 7). The filter paper was washed with filtered distilled water, placed in a clean Petri dish, and dried in the oven at 40°C for 24 hours [38, 39].

Identification of Microplastics

After drying, the filter paper was observed under a stereo microscope (magnification 40) (Plate 8), and the suspected MPs were subjected to a hot needle test for confirmation (Plate 8). The observed MP particles was photographed, identified, and recorded based on their shape (fibers, fragments, or spheres) and color, following the protocol established [40, 41]. To confirm the pres-

ence of microplastics, the methodology involves a hot needle test on samples suspected of containing microplastics.

Data Analysis

The statistical analysis was conducted using SPSS to assess differences in microplastic abundance and body weight of sea cucumbers between Marine Protected Areas (MPAs) and NMPA sites. A Student's t-test was performed to compare the means of these variables between the two locations, with a significance level set at $p < 0.05$. Additionally, a one-way ANOVA was used to analyze the abundance of microplastics in the digestive tract, body walls, and sediments in MPAs and NMPA. Results were presented using tables and graphs for clarity.

Results

Microplastics in Sea Cucumbers

Microplastics were detected in both the body wall and gut of sea cucumbers (Figure 2). Statistical analysis showed no significant difference in microplastic (MP) count ($p = 0.736$, $t = -0.344$) between body wall and gut of sea cucumber (Appendix 1). Microplastic contamination was higher in NMPAs, with MPs found in both the body wall (0.67 ± 1.16 particles) and gut (4.00 ± 1.00 particles), while MPA had MPs only in the gut (2.00 ± 1.00 particles) (Appendix 3). The t-test confirmed significant differences between NMPA and MPA ($p = 0.015$), reinforcing higher MPs in NMPA. Figure 3 shows various microplastic types identified in the samples, including black fibers (gut samples), blue fragments (gut samples), white fibers (body wall), and silver fibers (gut samples) (Appendix 5).

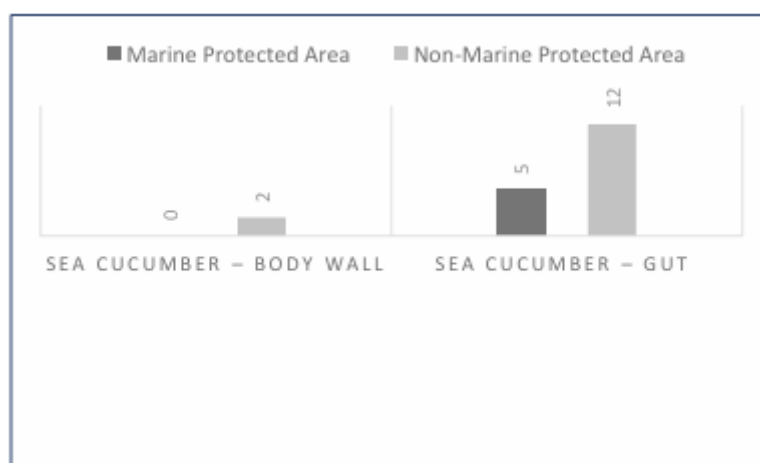


Figure 2. Presence of Microplastics in Sea Cucumbers Collected from Marine Protected Areas (MPAs) and Non-Marine Protected Areas (NMPAs).

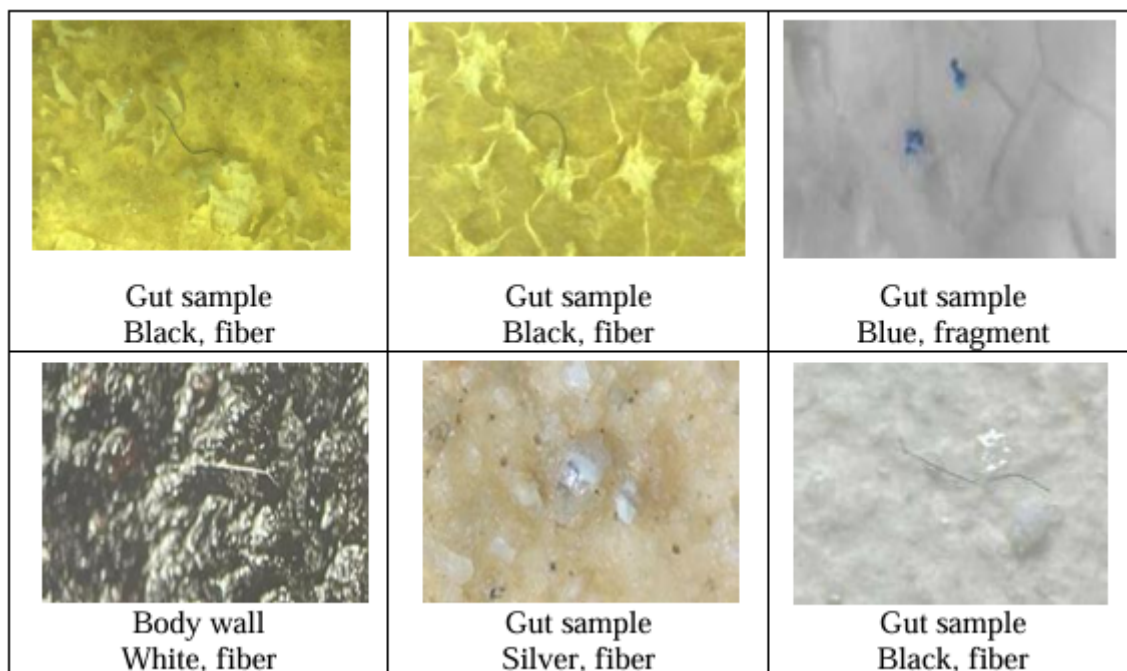


Figure 3: Microplastics Detected in the Body Wall And Gut Of Sea Cucumbers Collected from Central Park Station, Puerto Princesa Subterranean River National Park, and Honda Bay, Barangay Tagburos, Puerto Princesa City.

The analysis of microplastics (MPs) extracted from sea cucumbers showed two distinct shapes: fibers and fragments (Figure 4). Fibers were the dominant form, comprising 84% of the total MPs, which were present in all samples from marine protect-

ed areas (MPAs) (100%) and in a slightly lower proportion in non-marine protected areas (NMPAs) (79%). Fragments accounted for 16% of the total MPs and were detected exclusively in NMPAs (21%).

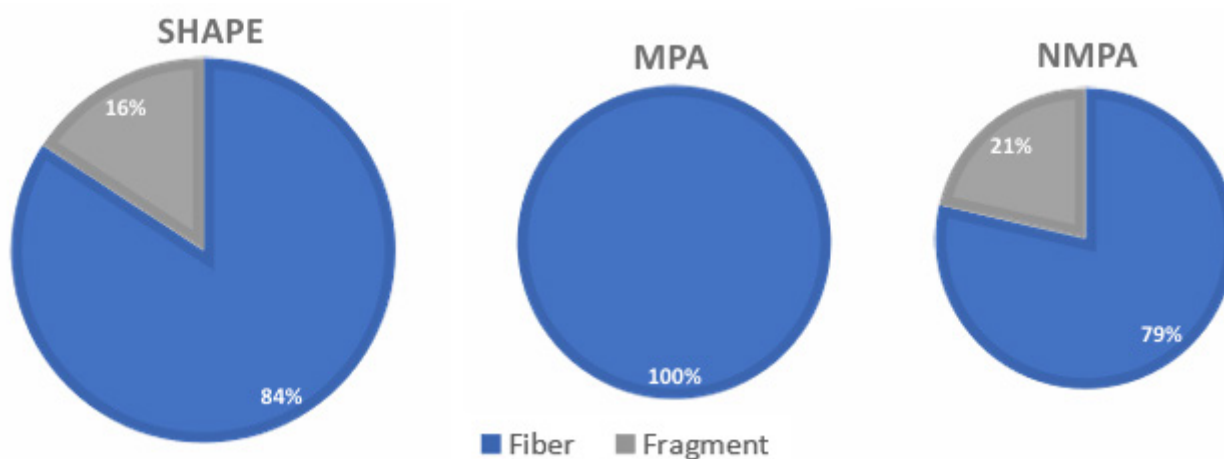


Figure 4. Characterization of microplastics (MPs) in sea cucumbers based on shape across marine protected areas (MPAs) and non-marine protected areas (NMPAs).

For color distribution, blue MPs were the most prevalent, representing 63% of the total MPs, with similar proportions in MPAs (60%) and NMPAs (64%). Black MPs were found exclusively

in NMPAs (29%). Silver MPs (11%) were present only in MPAs (20%). White MPs (5%) were detected solely in MPAs (Figure 5).

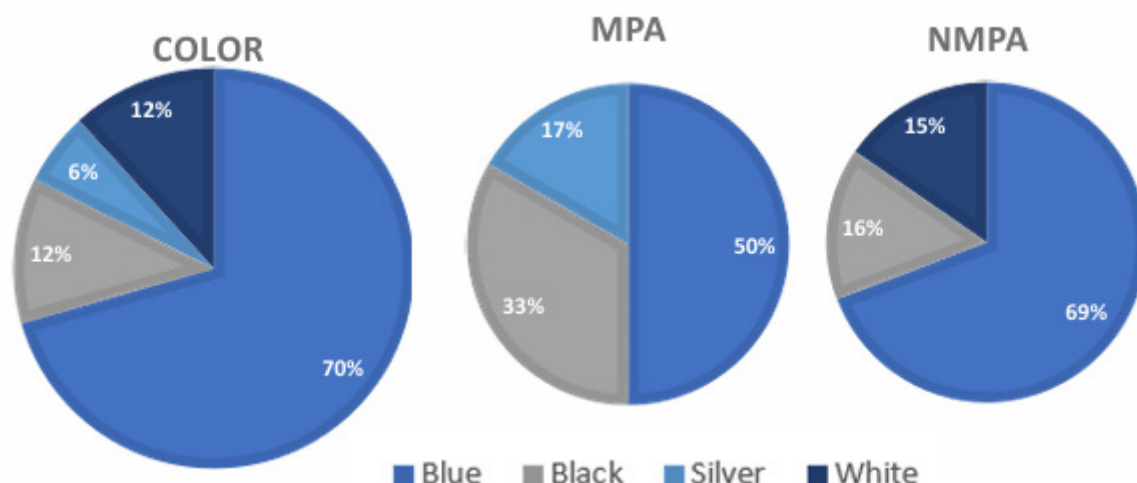


Figure 5: The Figure Presents The Characterization Of Microplastics (Mps) In Sea Cucumbers Based on Color Across Marine Protected Areas (Mpas) And Non-Marine Protected Areas (NMPAs).

Microplastics in Sediments

Figure 6 shows comparison of microplastic abundance between MPA and NMPA sites. The MPA recorded a total of 14 microplastic particles, whereas the NMPA exhibited a lower count of nine MPs. MP concentrations in sediments confirmed their role

as a major MP reservoir. Despite protection, MPAs still accumulated MPs, likely due to ocean currents or external pollution. These results highlight the need for stricter plastic waste regulations and improved pollution management strategies to address sediment contamination.

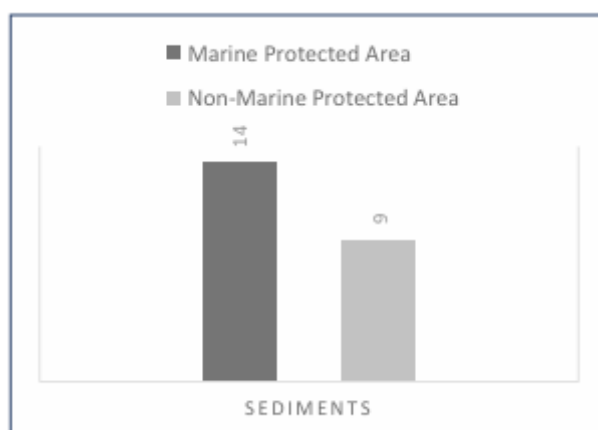


Figure 6: Comparison Of Microplastic Abundance in Sediments From Marine Protected Areas (Mpas) And Non-Marine Protected Areas (NMPAs).

The analysis of microplastics (MPs) extracted from sediment samples identified two distinct shapes, the fibers and fragments (Figure 7 and 8). Fibers were the dominant form, comprising 57% of the total MPs. These were present in a higher proportion

in MPAs (64%) than in NMPAs (44%). Fragments accounted for 43% of the total MPs, with a greater presence in NMPAs (56%) than in MPAs (36%).

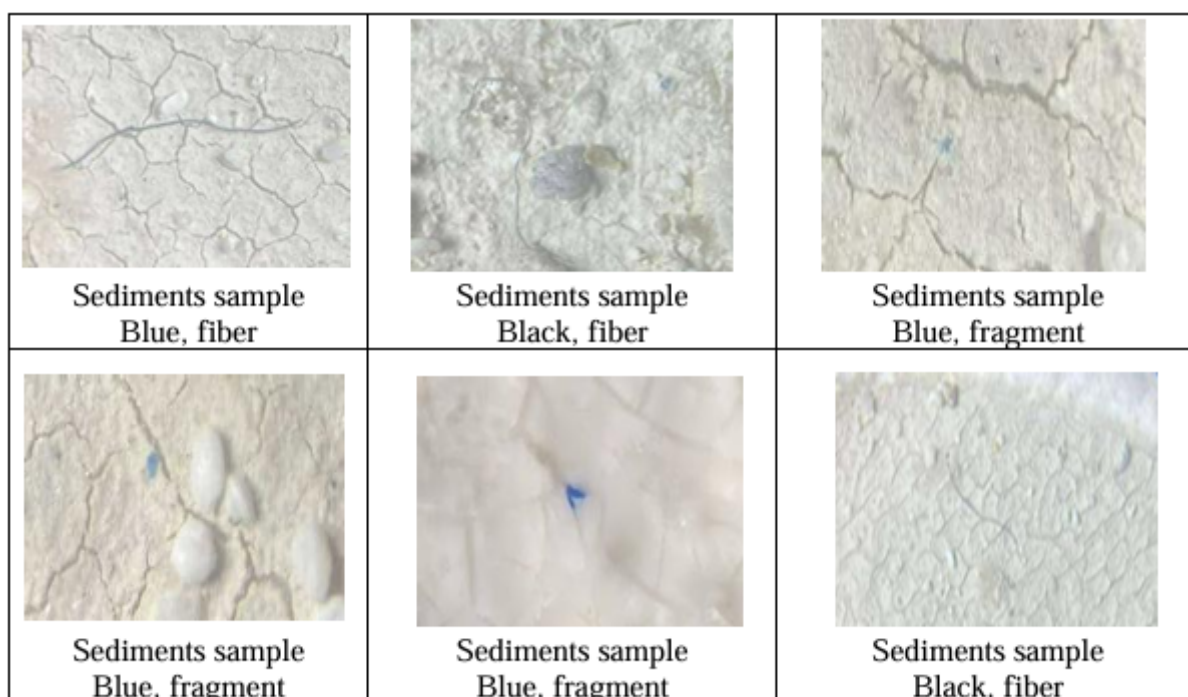


Figure 7: Microplastics Detected in Sediment Samples Collected From Central Park Station, Puerto Princesa Subterranean River National Park, And Honda Bay, Barangay Tagburos, Puerto Princesa City.

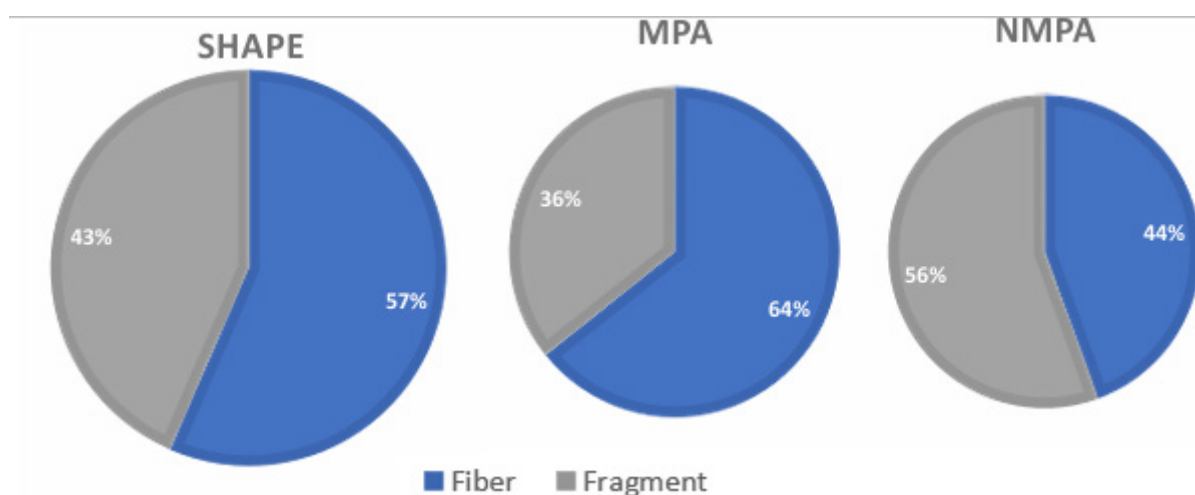


Figure 8: Characterization Of Microplastics (Mps) In Sediment Samples Based On Shape Across Marine Protected Areas (Mpas) And Non-Marine Protected Areas (NMPAs).

In terms of color distribution, blue MPs were the most prevalent in sediment, representing 86% of the total MPs, with similar proportions in MPAs (86%) and NMPAs (87%). Black MPs consti-

tuted 14% of the total MPs, found in MPAs (14%) and NMPAs (13%), with no detection of silver or white MPs in either category (Figure 9).

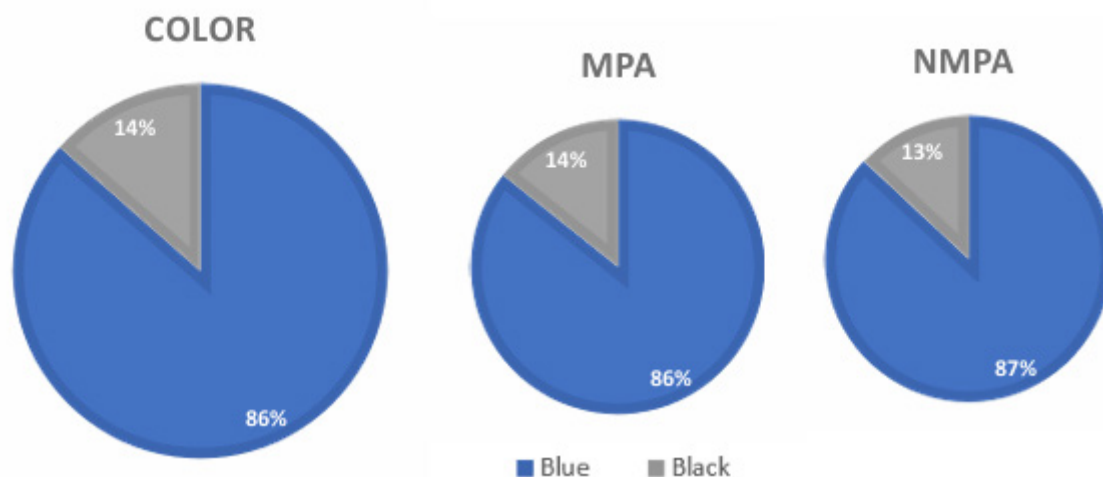


Figure 9: Microplastics (Mps) In Sediment Samples Based on Color (B) Across Marine Protected Areas (Mpas) And Non-Marine Protected Areas (NMPAs).

Comparison of Microplastics in Sediments and Sea Cucumber in MPA and NMPA

The univariate analysis of variance indicated a statistically significant difference in microplastic abundance between sample types ($F(1,14) = 6.572$, $p = 0.023$), with sediments ($M = 3.83$, $SD = 1.72$) containing higher levels of microplastics compared to sea cucumbers ($M = 1.67$, $SD = 1.78$). However, there was no significant difference in microplastic abundance between the

two study areas, MPA and NMPA ($F(1,14) = 0.039$, $p = 0.847$). The interaction between sample type and study area was not statistically significant ($F(1,14) = 3.150$, $p = 0.098$). Levene's test confirmed that the assumption of homogeneity of variance was met ($p > 0.05$). These results are illustrated in Figure 10, which presents the mean microplastic counts across sample types and locations.

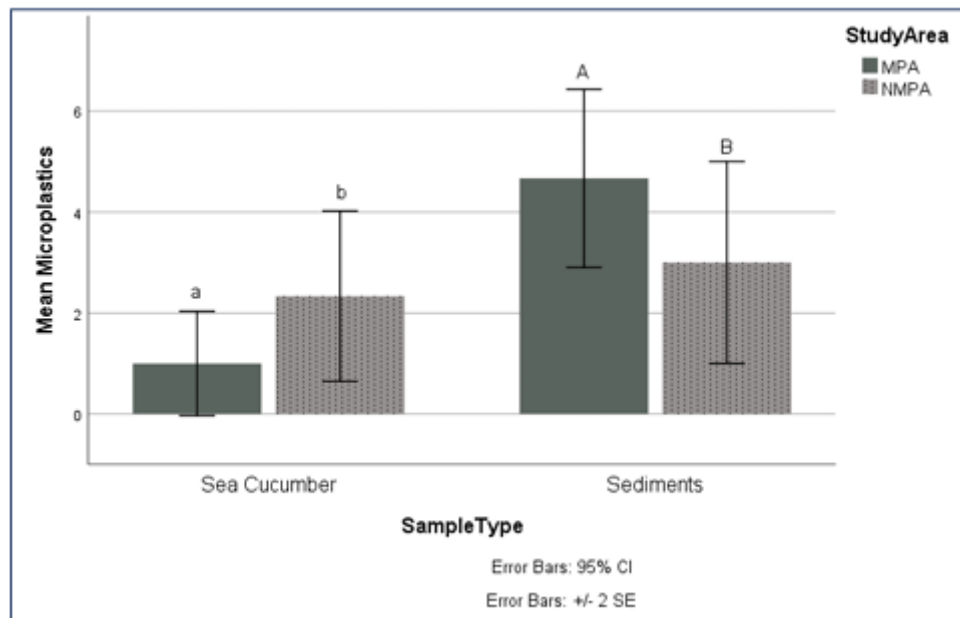


Figure 10: Mean Microplastic Abundance ($\pm$ sd) in Sea Cucumber And Sediment Samples Across Mpa and Nmpa Sites. Letters Signify A Significant Difference.

Discussion

Microplastic Contamination

The findings of this study highlight the pervasive presence of microplastics (MPs) in sea cucumbers and sediments collected from both a Marine Protected Area (MPA) and a Non-Marine

Protected Area (NMPA) in Puerto Princesa City. The results demonstrate that microplastics were detected in both the body wall and gut of sea cucumbers, as well as in sediment samples, with notable variations between the two study sites. While the total number of microplastic particles was higher in NMPA sea

cucumbers than in MPA sea cucumbers, the sediments from the MPA exhibited a greater microplastic concentration compared to the NMPA. These results suggest that microplastic accumulation in sea cucumbers is not solely dependent on protection status but may be influenced by other environmental and anthropogenic factors. Furthermore, previous studies indicated that microplastic pollution is a widespread issue, regardless of conservation status, where factors such as human activities, water currents, and the feeding habits of sea cucumbers likely contribute to the distribution of microplastics in marine environments (Li et al., 2018; Lusher et al., 2017; Cole et al., 2013).

Microplastic Contamination in Sea Cucumbers

Microplastic contamination was observed in both the body wall and gut of sea cucumbers, with the gut exhibiting higher levels of MPs than the body wall in both MPA and NMPA samples. Notably, no microplastics were detected in the body walls of MPA sea cucumbers, while NMPA samples exhibited a low level of contamination. The gut samples, however, showed a significant accumulation of MPs, particularly in NMPA sea cucumbers, compared to MPA sea cucumbers. This suggests that ingestion is the primary route of microplastic exposure in these organisms, likely through sediment ingestion or filter-feeding mechanisms. These results align with findings by Graham and Thompson (2009), who demonstrated that deposit-feeding organisms, including sea cucumbers, accumulate microplastics primarily through ingestion of contaminated sediments.

Sea cucumbers from the Non-Marine Protected Area (NMPA) contained a higher number of MPs than those from the Marine Protected Area (MPA). This suggests that microplastic contamination is more severe in areas with higher human activity and fewer environmental regulations [42, 43]. The presence of MPs in the gut of sea cucumbers confirms ingestion as the primary route of exposure, while MPs detected in the body wall indicate potential external adhesion from waterborne microplastics [44]. The higher MP abundance in NMPA sea cucumbers is consistent with findings who reported that sea cucumbers from more polluted areas contained greater MP concentrations. This trend highlights the influence of local environmental conditions and anthropogenic factors on MP bioavailability in marine organisms [44].

Microplastic Characteristics and Distribution

The analysis of microplastic shape revealed that fibers were the dominant type of MPs. This trend was consistent across both MPAs and NMPAs, indicating a common source of contamination. Fragment-shaped MPs were exclusively detected in NMPA samples, which may suggest differences in pollution sources or degradation processes. In terms of color distribution, blue MPs were the most prevalent, followed by black, silver, and white. Notably, black MPs were exclusively detected in NMPA samples, while silver and white MPs were only found in MPAs. These findings are comparable to results who reported a predominance of fibrous microplastics in marine samples, suggesting that synthetic fibers from textiles and fishing gear are a major source of marine plastic pollution [45].

The predominance of fibers further supports the idea that microplastic contamination in marine environments primarily originates from human activities such as wastewater discharge, fishing, and improper plastic disposal. The dominance of fibers aligns with the findings who reported that synthetic fibers from laundry wastewater and fishing nets are among the primary contributors to marine MP pollution [46]. Fragmented microplastics were detected primarily in NMPA samples, suggesting that plastic degradation is more advanced in these areas due to prolonged exposure to environmental factors such as UV radiation and wave action [47, 48]. These findings emphasize the role of physical and chemical degradation in shaping MP distribution patterns in marine environments.

Microplastic Contamination in Sediments

Sediment samples exhibited the highest microplastic concentrations among all sample types, emphasizing the role of sediments as a major reservoir for microplastics. Interestingly, the MPA sediments had a higher mean concentration than NMPA sediments. This finding aligns with studies which confirm that microplastics accumulate in sediments over time due to their density and hydrodynamic properties. The higher abundance of MPs in MPA sediments compared to NMPA sediments was unexpected, suggesting that factors such as tidal currents, sedimentation rates, and localized human activity may contribute to MP deposition in protected areas [49].

The presence of MPs in sediments is of significant concern because these particles can be reintroduced into the water column through bioturbation or resuspension, increasing their bioavailability to marine organisms. Furthermore, sediments act as long-term pollution reservoirs, slowly releasing MPs into the surrounding environment and posing a prolonged risk to marine ecosystems.

Ecological Implications

Although MPAs enhance biodiversity, they do not prevent MP contamination from external sources such as industrial runoff. MP accumulation in sediments can alter microbial communities and disrupt nutrient cycles, potentially affecting food availability and contributing to weight differences in sea cucumbers. Higher MP concentrations in sediments and sea cucumber guts reinforce that ingestion is a key pathway for MP exposure, posing risks to benthic organisms and marine food webs. These findings highlight the need for stricter waste management, plastic regulations, and public awareness efforts to mitigate MP pollution. Future research should assess the long-term effects of MP ingestion and contamination trends in MPAs and NMPAs.

Comparison of Microplastics in Sediments and Sea Cucumbers in MPA and NMPA

The significant higher microplastic abundance in sediments suggests that sediments may act as primary sinks for microplastic pollutants in marine ecosystems. This supports earlier studies indicating that sediments often accumulate higher concentrations of microplastics due to their ability to trap and retain particles settling from the water column.

In contrast, biota such as sea cucumbers showed lower microplastic counts, possibly due to biological variability in feeding behavior, habitat exposure, and gut retention time. The absence of a significant difference between Marine Protected Areas (MPA) and Non-Marine Protected Areas (NMPA) implies that the presence of protection status alone may not be enough to mitigate microplastic contamination, which can be transported over long distances through ocean currents and atmospheric deposition. While the interaction between sample type and study area was not statistically significant, the observed trend suggests possible site-specific influences that may warrant further investigation, such as proximity to anthropogenic sources or differences in sediment composition. These findings underscore the importance of sediment monitoring as a reliable indicator of microplastic pollution in coastal ecosystems.

Comparison with Previous Studies

The results of this study are consistent with previous research indicating widespread microplastic contamination in marine environments. For example, [1] found that microplastic ingestion was prevalent among benthic organisms, with the gut serving as the primary accumulation site. Similarly, a study by [2] reported higher microplastic concentrations in marine sediments than in biota, supporting the finding that sediments act as a significant reservoir for microplastic pollution. However, our study contrasts with findings who observed a greater prevalence of microplastics in pelagic organisms than in benthic species, suggesting that exposure routes and feeding behaviors play a crucial role in microplastic accumulation.

Several studies have demonstrated that sea cucumbers, as deposit feeders, are particularly susceptible to MP ingestion due to their feeding behavior. The presence of MPs in both the body wall and gut of sea cucumbers is consistent with findings who highlighted that sea cucumbers can serve as bioindicators of microplastic pollution. Additionally, previous research has established that MPs accumulate in marine sediments over time, serving as long-term reservoirs of pollution found that MPs in sediments can be resuspended into the water column, making them available for ingestion by benthic organisms. These findings support the results of this study, which revealed significant MP contamination in both sediments and sea cucumber tissues.

Conclusions

This study confirms the presence of microplastics (MPs) in sediments and sea cucumbers (*Holothuria atra*) from both Marine Protected Areas (MPAs) and Non-Marine Protected Areas (NMPAs) in Puerto Princesa City, Palawan. Microplastics were detected in both gut and body wall tissues of sea cucumbers, with significantly higher concentrations in the gut, indicating ingestion as the primary pathway of exposure. Sediment samples exhibited the highest MP abundance among all sample types, reinforcing their role as major reservoirs for microplastic pollution in marine ecosystems [50-55].

Characterization of MPs revealed that fibers were the predominant shape, followed by fragments, which were found exclusive-

ly in NMPA samples, with blue being the most prevalent color. This pattern suggests potential sources such as fishing gear, synthetic textiles, and wastewater discharge. The study also found that while MPAs provided better ecological conditions, as evidenced by the greater body weight of sea cucumbers collected from protected areas, they did not significantly reduce MP contamination compared to NMPAs.

A comparative analysis of MP abundance between MPAs and NMPAs showed no statistically significant difference, indicating that protection status alone does not mitigate microplastic pollution. The presence of MPs in both areas suggests that external factors, such as ocean currents, atmospheric deposition, and land-based pollution sources, contribute to contamination across marine environments regardless of conservation designation. Statistical analysis further confirmed significant differences in MP concentrations across sample types, with sediments and sea cucumber guts showing the highest contamination levels, supporting the conclusion that ingestion is a key exposure pathway for benthic organisms.

These findings highlight the widespread nature of microplastic pollution and its potential ecological implications for sediment-dwelling organisms and marine ecosystems more broadly. The results provide valuable baseline data for future monitoring studies and conservation management efforts in coastal environments. To effectively address microplastic contamination, an integrated approach beyond spatial protection measures is needed, including improved waste management practices, stricter plastic regulations, enhanced public awareness campaigns, and further research on the long-term physiological effects of microplastic exposure on marine organisms.

Recommendations

To mitigate microplastic contamination and protect marine biodiversity, a comprehensive approach is necessary. First, strengthening regulations on plastic waste disposal and enforcing stricter waste management policies are crucial steps toward reducing pollution at its source. Implementing regular monitoring programs for microplastic pollution in both Marine Protected Areas (MPAs) and Non-Marine Protected Areas (NMPAs) will provide valuable data to track contamination trends and evaluate the effectiveness of conservation measures.

Public awareness campaigns and educational initiatives can foster sustainable practices among communities, industries, and tourists, particularly in coastal areas. Further research employing advanced analytical techniques such as Fourier Transform Infrared Spectroscopy (FTIR) would enable more precise identification of polymer compositions and help trace pollution sources more effectively.

Additionally, promoting the development and adoption of biodegradable materials and sustainable alternatives through policy incentives and market-based mechanisms can significantly reduce long-term microplastic accumulation in marine environments. A coordinated effort involving government agencies, re-

search institutions, industries, and local communities is essential to address this pervasive environmental challenge and preserve the health of marine ecosystems.

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Plates



Plate 1: Sample Collection: (a) Sea Cucumbers from a non-MPA site, (b) Sediment Samples, (c) Sea Cucumbers from an MPA site, and (d) Collection of Sediment Samples.



Plate 2: Sample Preparation: (a) Filtration of Distilled Water, (b) Chemical Weighing, (c) Sample Preparation, and (d) Sediment Drying For Analysis

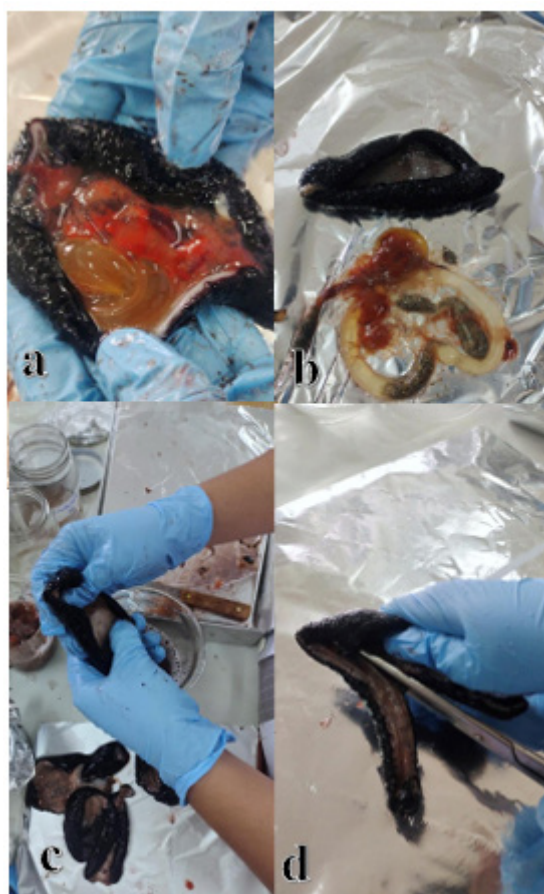


Plate 3: Sea Cucumber Dissection: Cutting the Specimen (a), Separating the Gut from the Body Wall (b). Removing and Scraping of Gut Residues (c), the Body wall is Cut Into Cubes (d).

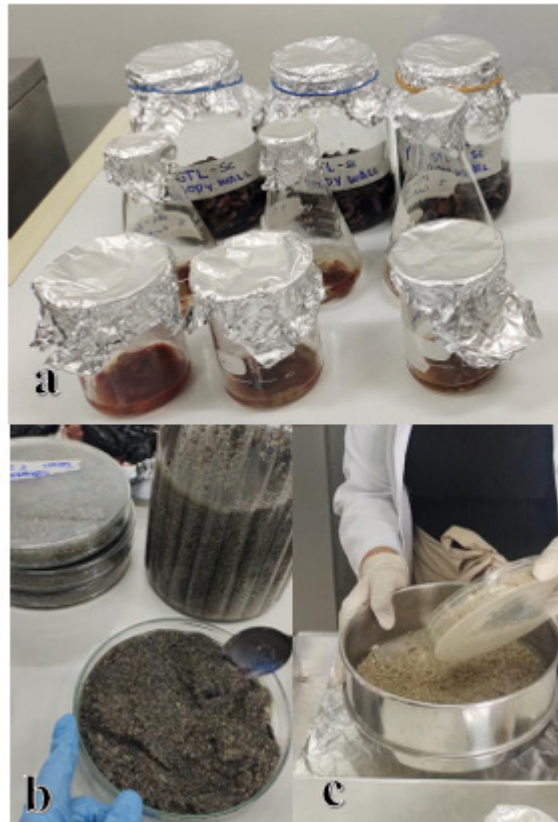


Plate 4: Body wall and Gut Samples in A Glass Jar (for drying) (a). The Sediments in Petri Dishes (b). Sieving of Sediments (c).

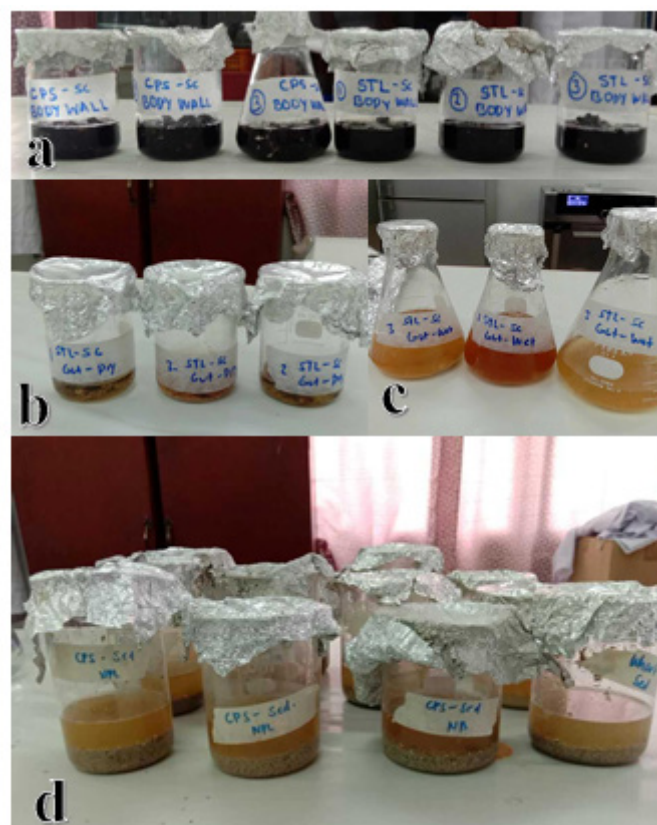


Plate 5: Samples with KOH for Digestion: Body wall (a), gut dry (b), wet gut (c), and Sediments (d).



Plate 6: Samples with NaCl and Homogenizing by Mixing for 2 minutes: Sea Cucumber gut (a), Body wall (b), and Sediments (c)

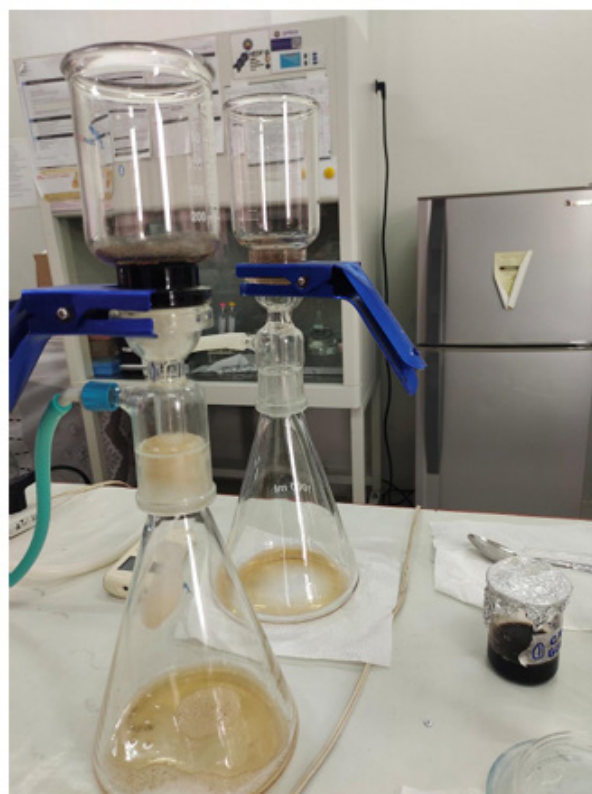


Plate 7: Filtration using Whatman Filter Paper.

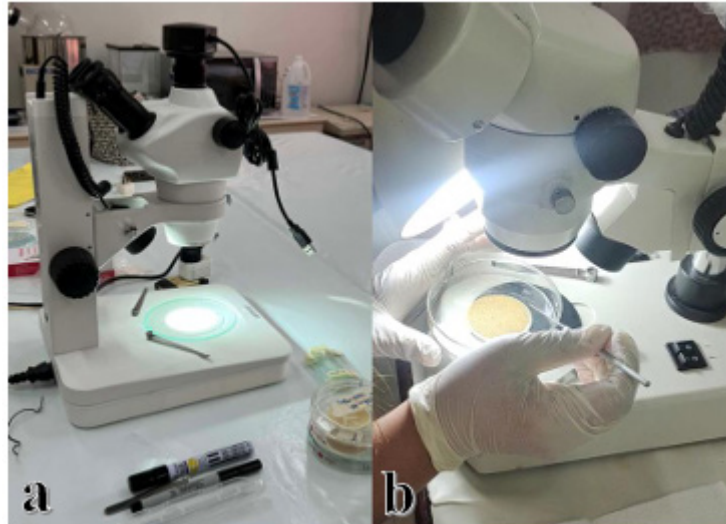


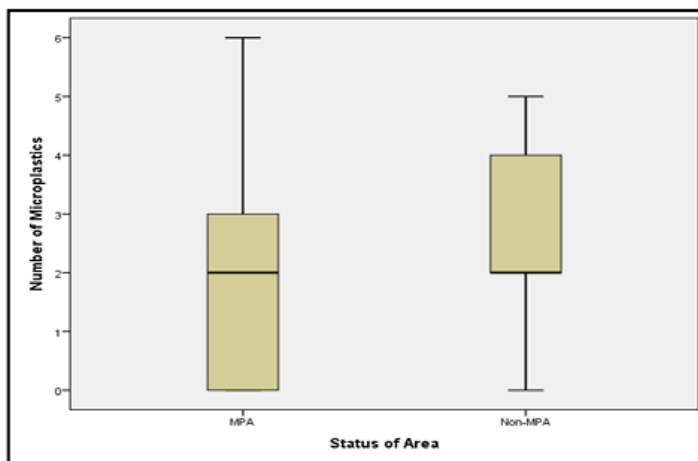
Plate 8: (a) Observing of Filter Paper Under A Stereo Microscope. (b) Hot Needle Testing.

Appendices

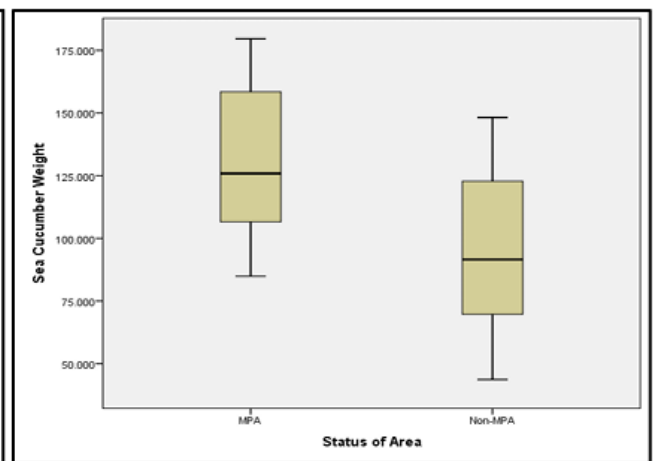
Appendix 1: Differences in the amount of microplastics and body weight of sea cucumbers between MPA and NMPA.

Variable	Test	t	p	Interpretation
Microplastics	Student's t-test	-0.344	0.736	Not significant
Body Weight	Student's t-test	2.632	0.017	Significant

A



B



Appendix 2: Comparison of microplastic abundance and sea cucumber weight between Marine Protected Areas (MPAs) and Non-Marine Protected Areas (NMPAs). (A) presents the microplastic concentrations, while (B) illustrates the weight variations of sea cucumbers collected from both areas.

Appendix 3: Descriptive Statistics Table Illustrate The Clear Comparison Of Microplastic Abundance Across Different Groups.

Sample Type	N	Mean	Std. Deviation	Min	Max
MPA Sea Cucumber Body Wall	3	0	0	0	0
MPA Sea Cucumber Gut	3	2	1	1	3
MPA Sediments	3	4.67	1.528	3	6

NMPA Sea Cucumber Body Wall	3	0.67	1.155	0	2
NMPA Sea Cucumber Gut	3	4	1	3	5
NMPA Sediments	3	3	1.732	2	5

Appendix 4: ANOVA Test Results Which Present The Statistical Significance of Differences in Microplastic Abundance Among the Sample Types.

Source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	50.944	5	10.189	7.054	0.003
Within Groups	17.333	12	1.444		
Total	68.278	17			

Appendix 5: Turkey Hsd Test, Table Identifies Which Groups Differ Significantly In Microplastic Abundance.

Comparison (I -J)	Mean Difference	Std. Error	Sig. (p-value)	95% CI (Lower)	95% CI (Upper)
MPA Sediments –MPA Body Wall	4.667	0.981	0.005	1.37	7.96
NMPA Gut – MPABody Wall	4	0.981	0.015	0.7	7.3
NMPA Body Wall– MPA Sediments	-4	0.981	0.015	-7.3	-0.7
NMPA Gut –NMPA Body Wall	3.333	0.981	0.047	0.04	6.63

Appendix 6: Homogeneity of Variances (Levene's Test) Test Confirms Whether The Variance Across Sample Groups Is Statistically Different.

Test	Levene Statistic	df1	df2	Sig. (p-value)
Based on Mean	2.345	5	12	0.105
Based on Median	0.400	5	12	0.840

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