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Anthropogenic particles accumulation in sea cucumbers: insights from a transitional environment

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ABSTRACT

Detritivorous organisms, such as sea cucumbers, are key components of marine benthic ecosystems, contributing to sediment bioturbation and nutrient recycling, and thereby playing a pivotal role in ecosystem functioning. However, due to rising plastic production and inadequate waste management, their sediment-ingesting feeding strategy makes them particularly susceptible to anthropogenic particle (AP) contamination, potentially influencing trophic transfer and benthic-pelagic coupling. This study represents the first investigation on AP occurrence in holothuroids, *Holothuria sanctori* and *H. tubulosa*, inhabiting the transitional coastal waters of the Capo Peloro Natural Reserve (Mediterranean Sea). Raman and FT-IR analyses identified 12 synthetic polymers, three elastomers, one rubber, one petroleum derivative, and one semisynthetic particle. Additionally, cotton and dyed cellulose were also identified. Overall, our findings reveal a broader spectrum and higher level of complexity of APs than previously reported in sea cucumbers from marine environments, with 18 types identified in *H. sanctori* and 17 in *H. tubulosa*. Neoprene and cotton were the most frequently detected APs. The results underscore the suitability of the studied invertebrates as sentinel species for assessing plastic contamination in transitional environments.

1. Introduction

The accumulation of marine plastic litter in aquatic environments represents a major ecological issue in today's world. In the marine environment, 60–80% of marine litter items are plastics (Galgani et al., 2015) affecting marine biodiversity (Kühn and van Franeker, 2020; Gunasekaran et al., 2024; Bottari et al., 2024a), as well as contaminating human food sources (Rochman et al., 2015; Nalbone et al., 2021; Borriello et al., 2023; Bouzekry et al., 2023). Microplastics (MPs), defined as plastic particles smaller than 5 mm, have raised significant environmental concerns. Their minute size, heterogeneous chemical composition, and high persistence facilitate their bioavailability and potential accumulation within marine ecosystems and biota. In this context, the ingestion of plastic litter by marine organisms constitutes a primary route for the uptake and bioaccumulation of plastic particles and their associated contaminants, thereby enabling their transfer and potential biomagnification throughout the trophic web (Koelmans et al., 2016; Hermabessiere et al., 2017). Species' feeding strategies, behavioural traits, and the level of environmental contamination in their habitat influence the uptake of MPs.

Following ingestion, plastics can act as vectors for the release of intrinsic additives, including phthalates and bisphenol A, as well as

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exogenous contaminants adsorbed under environmental conditions, such as persistent organic pollutants and trace metals (Hermabessiere et al., 2017; Barrick et al., 2021). The leaching of these compounds, many of which are recognized for their toxicological effects and endocrine-disrupting potential, poses significant risks to marine organisms through altered physiological and biochemical pathways (Koelmans et al., 2016; de Sá et al., 2018; Chen et al., 2021).

Sea cucumbers (Phylum: Echinodermata; Class: Holothuroidea) are invertebrates of benthic marine ecosystems ecologically important due to their multifaceted roles in maintaining ecosystem function. As deposit-feeders and bioturbators, they contribute to sediment health by mixing and oxygenating sediments and facilitating the recycling of organic compounds (MacTavish et al., 2012), which enhances nutrient regeneration (Purcell et al., 2016) and promotes the growth of seagrass (Watkins et al., 2023). Their feeding activity results in the excretion of inorganic nitrogen and phosphorus, thus supporting primary productivity (Uthicke, 2001; Purcell et al., 2016). Moreover, through their digestive processes and the release of ammonia, sea cucumbers can locally increase seawater alkalinity, potentially mitigating the impacts of ocean acidification (Schneider et al., 2013). They additionally serve an essential function in sustaining marine biodiversity, hosting over 200 symbiotic species across multiple phyla (Eeckhaut et al., 2004).

Sea cucumbers, as deposit- and suspension-feeding organisms, are particularly susceptible to bioaccumulating marine pollutants (Graham and Thompson, 2009). Due to this sensitivity, sea cucumbers are valuable bioindicators of microplastic pollution (Miliou et al., 2016). Recently, several findings have reported the occurrence of plastic items in the gastrointestinal tract, coelomic fluid, respiratory trees, and occasionally pseudo-fecal samples of species such as *H. tubulosa* (Lombardo et al., 2022; Rios-Fuster et al., 2022; Cocci et al., 2025), *Holothuria scabra* (Riani and Cordova, 2022; Fong et al., 2025; Shukhairi et al., 2025), and *Holothuria forskali* (Rios-Fuster et al., 2022; Menéndez et al., 2024). They incorporate microplastics from the environment, including sediments and surrounding water (Riani and Cordova, 2022). MPs are both ingested through feeding (Mohsen et al., 2021) or absorbed during respiration via the cloacal opening (Iwalaye et al., 2020).

MPs may adversely affect ingestion, growth, reproductive performance, and overall viability in marine invertebrates (Murphy and Quinn, 2018; Reichert et al., 2019; Contino et al., 2023). Their presence in the gastrointestinal tract of sea cucumbers has been associated with reduced feeding efficiency and alterations in both histological and behavioural traits (Bhuyan et al., 2024). Moreover, Lombardo et al. (2022) reported increased antioxidant enzyme activity and elevated glutathione levels in *H. tubulosa* exposed to MPs.

This study investigates the abundance and composition of anthropogenic particles (APs) — including plastics, elastomers/rubbers, semi-synthetic materials (e.g., cellulose acetate), and natural fibres of anthropogenic origin (e.g., cotton and dyed cellulose) — in two sea cucumber species, *H. tubulosa* and *H. sanctori*, from the Capo Peloro Natural Reserve. The main goal is to assess and categorize the APs consumed by these species, focusing on identifying potential interspecific differences in ingestion rates and the chemical composition of the particles. Furthermore, this study investigates spatial variations in contamination across the reserve channels, which are essential to maintaining the ecological balance of its lakes by regulating salinity, water levels, and overall ecosystem health.

The uniqueness of the present study also lies in the distinctive features of the Capo Peloro Natural Reserve, a transitional ecosystem characterized by shallow depths, strong temperature fluctuations, pronounced spatial and temporal salinity variations, high productivity, limited water circulation, and low hydrodynamism (Leonardi et al., 2009). These characteristics, together with the human pressures affecting the reserve, make it a potential hotspot for APs accumulation compared to open marine systems, as has already been reported for other transitional environments in the Mediterranean Sea (Simon-Sánchez et al., 2019; Pellegrini et al., 2023).

2. Materials and methods

2.1. Study area and samplings

Capo Peloro Nature Reserve, a protected natural area of significant ecological and conservation interest, is located on the northeastern margin of the Peloritani Mountains in Messina (Sicily, Italy) (Fig. 1). Recognized by the European Union's Natura 2000 network, the area is designated as a Site of Community Importance (SCI) and a Special Protection Area (SPA) for migratory birds. Capo Peloro is recognized as a "Heritage of Ethnic-Anthropological Interest" (decree 1342/88) and it has a long-standing aquaculture tradition. The reserve includes a lagoonal system that opens toward both the Tyrrhenian and Ionian Seas (Geographical Sub-areas 10 and 19). The reserve encompasses two brackish water bodies, known as Ganzirri Lake (GL) and Faro Lake (FL). The area is highly anthropized and is characterized by road traffic and the presence of a mussel farming facility. This research examines two distinct sampling sites, situated within the coastal canals of the reserve: Due Torri Canal (302 m long, 10 m wide) and Faro Canal (425 m long, 8 m wide). The mean depth of the two canals is 85 cm. The bed of the Due Torri Canal is composed mainly of fine sediments (fine sand), while its banks consist of coarse material, typically medium-sized boulders. The bed of the Faro Canal is characterised by muddy/detrital and sandy sediments. These canals link Ganzirri Lake and Faro Lake to the Ionian Sea, playing a crucial role in maintaining the ecosystem functioning of the lakes and influencing salinity, water levels, and the overall health of the aquatic ecosystems (Leonardi et al., 2009).

A total of 57 sea cucumbers were randomly collected using a sling at depths not exceeding 60 cm (Fig. S1). Regarding *H. sanctori*, 19 specimens were sampled in the Due Torri canal and 8 in the Faro canal. For *H. tubulosa*, 9 specimens were sampled in the Due Torri Canal and 21 in the Faro Canal (Table 1). The sea cucumbers were collected in spring, between March and April 2025. The collection of specimens was carried out under authorization issued by the reserve's managing authority, the Metropolitan City of Messina (Prot. No. 0006758/23 of 24/02/2023).

Each individual was anesthetized with clove oil after capture to reduce stress (Lombardo et al., 2022). To prepare the clove-oil solution, clove oil was first diluted at a 1:9 ratio in 100% ethanol (as clove oil is poorly soluble in water). An appropriate volume of this stock solution was then added to seawater to reach a final concentration of 0.125 mL L^{-1} (Lewbart and Mosley, 2012). Each specimen was exposed for 10 min. After collection, the specimens were preserved at −20 °C prior to analysis. Before the necropsy, the samples were thawed overnight at 4 °C. Before dissection, each sample was assessed for the total length (TL, mm) and total weight (TW, g) and thoroughly rinsed with Milli-Q water. Dissections were performed via a longitudinal incision along the dorsal surface in accordance with Peñalver-Soler et al. (2025). After exposing the internal structures, the gastrointestinal tract (GIT), coelomic fluid (CF), and, only for *H. sanctori*, the Cuvierian tubules (CT) were carefully extracted using stainless-steel forceps and scissors.

2.2. APs isolation

AP extraction was performed by digesting the biological material (GIT, CT, and CF) in potassium hydroxide (KOH, 10% sol., 1:5 w/v) and placed at 50 °C for 48 h.

Following digestion, a density separation was carried out using a 15% sodium chloride (NaCl) solution at a 1:2 (w/v) ratio and settled for 3 h to allow phase separation and facilitate the collection of floating microparticles. The resulting supernatant was carefully filtered through glassfiber filters (1.6 μm pore size, Ø: 47 mm; GF/A Whatman) under vacuum (300–660 mbar, Rocker). The effectiveness of tissue digestion and particle release was verified by stereomicroscopic inspection of the settled fraction. Putative APs were counted, categorised by dimension, morphology and colour, and photographed using a Leica EZ4 W stereomicroscope. APs were categorized following the Marine Strategy

Fig. 1. Map of sampling area, showing the sampling stations within the Capo Peloro Nature Reserve (Messina, Sicily, Italy).

Framework Directive (MSFD) protocol (MSFD Technical Subgroup on Marine Litter, 2013).

2.3. *Characterisation of polymers by Raman and FTIR-ATR spectroscopic techniques*

The isolated microparticles were identified using Raman and Fourier Transform Infrared (FT-IR) spectroscopy. The particles examined by Raman analysis, were investigated with an HR Evolution micro-confocal system (Horiba Scientific) equipped with a 532 nm laser diode, and either a 20× or 50× objective lens, together with a grating of 600 or 1800 grooves/mm, depending on the sample and the fluorescence emitted. Raman signals in the 200–4000 cm^{-1} range were acquired using a liquid nitrogen-cooled Charge Coupled Device (CCD) detector. Integration times ranged from 5 to 10 s, with the number of accumulations varying between 2 and 100, depending on the Raman activity of the sample. To avoid sample degradation, the laser power was maintained between 5 and 30 mW. Multiple measurements were performed for each sample to ensure spectral reproducibility, and different regions of the same AP were analysed to minimise the impact of local contaminants on the Raman spectra.

Spectral data were processed using Labspec 6 software to remove fluorescence background, perform baseline correction, and reduce noise. Data analysis was carried out with the Bio-Rad KnowItAll informatics system, while the KnowItAll Raman Spectral Library or SLoPP/SLoPP-E (Munno et al., 2020) were utilised to determine the polymeric composition of the samples. The similarity between the spectra of unknown samples and reference compounds in the databases was assessed using the Hit Quality Index (HQI). A minimum confidence level of 80% was set for positive polymer identification, following methodologies from Micalizzi et al. (2024).

For FTIR analysis, the chemical composition of the suspected items was identified using the Agilent Cary 630 spectrometer equipped with specific polymer libraries (ST Japan Libraries for Cary 630/5500, Agilent Polymer Handheld ATR Library, Agilent Elastomer Oring and Seal Handheld ATR Library). A background scan was recorded prior to measurement and subtracted from the sample spectra. The spectral region analysed ranged from 400 to 4000 cm^{-1}, with each spectrum averaged over 64 scans at a resolution of 4 cm^{-1}. As for the Raman data, a Hit Quality Index (HQI) exceeding 80% was considered indicative of a reliable match between the unknown and the reference spectra.

2.4. *Quality control*

To minimise airborne contamination during laboratory procedures, several precautionary measures were used: i) access to the laboratory was restricted, and only cotton lab coats were worn; ii) work surfaces and tools, made entirely of glass or stainless steel, were meticulously cleaned with ethanol and filtered deionised or ultrapure water prior to use; iii) all containers, including digestion and filtration samples, covered with aluminium foil, and solutions were pre-filtered using glass fibre filters (1.4 μm pore size, Millipore) in vacuum system; iv) all operations (sample preparation, dissection, organ extraction, weighing) were conducted under a fume hood; v) all solutions (NaCl and KOH) were filtered before use. To monitor and detect airborne microparticles and fibres, filters moistened with Milli-Q water were exposed near work areas (e.g., within the hood, beside the stereomicroscope) (Micalizzi et al., 2024). Laboratory air monitoring blanks were positive in four cases, and the corresponding particles were excluded from the counts. Procedural blanks were run every two samples, resulting in a total of 29 blanks. Across all blanks, 4 anthropogenic particles were detected; these were categorized by shape, colour, and size and subtracted from the corresponding sample categories. The average blank rate was therefore 0.14 items per blank filter.

2.5. *Data analysis*

Sea cucumbers were categorised into length classes (LC) according to their total length in mm (LC_90; LC_120; LC_150; LC_180; LC_210), and for each LC, abundance indices were calculated as follows:

- Frequency of occurrence (FO%) was calculated as the proportion of individuals in the total sample from which anthropogenic particles (APs) were isolated (FO% = number of individuals with APs / total number of individuals × 100).
- Mean number of items found in the GIT, CT, and CF, was calculated from the total number of individuals (N. APs/N. total individuals).

A Chi-square test was used to compare the frequency of occurrence of APs between species. As the data did not satisfy the assumptions

Table 1

Total length (TL, mm) and total weight (TW, g) ranges of *Holothuria sanctori*, and *Holothuria tubulosa* average abundance (items/specimen), and frequency of occurrence (FO%). GIT: gastrointestinal tract; CF: coelomic fluid; CT: Cuvierian tubules.

Species	Sampling site	Sample size	Total length			Total weight			N items				Items/specimen				FO%
			Range	Mean	SD	Range	Mean	SD	GIT	CF	CT	Total	GIT	CF	CT	Total	
Holothuria sanctori	Due Torri Canal	19	90–209	150.53	30.37	29–107	72	20.60	96	0	4	100	5.05	0.00	0.21	5.26	78.9
	Faro Canal	8	94–148	113.88	19.93	28–80	52.38	18.55	26	3	5	34	3.25	0.38	0.63	4.25	100.0
	Due Torri and Faro Canals	27	90–209	139.67	32.20	28–107	66.19	21.67	122	3	9	134	4.52	0.11	0.33	4.96	85.2
Holothuria tubulosa	Due Torri Canal	9	110–306	170.00	68.17	78–383	176.00	104.43	26	2	–	28	2.89	0.22	–	3.11	77.8
	Faro Canal	21	90–228	170.38	35.36	17–237	133.19	65.49	60	11	–	71	2.86	0.52	–	3.38	81.0
	Due Torri and Faro Canals	30	90–306	170.27	46.31	17–383	146.03	79.78	86	13	–	99	2.87	0.43	–	3.30	80.0

necessary for a parametric analysis of variance (ANOVA), the non-parametric Kruskal-Wallis test was employed to evaluate whether significant differences existed in abundance (items per specimen) among species, as well as in plastic particle size between species and across sea cucumber length classes.

Kendall's rank correlation was performed to evaluate the correlation between: i) AP abundance and sea cucumber body size; ii) AP abundance and sea cucumber weight; iii) AP size and sea cucumber body size. Differences were considered statistically significant at $p < 0.05$. All statistical computations conducted with PAST 5 software (Hammer et al., 2001).

Multivariate statistical analyses were performed to compare the composition of anthropogenic particles between species in terms of size, shape, colour, and polymer type. Analyses were conducted using the Primer 7 package (Anderson, 2024). A Bray-Curtis resemblance matrix was constructed from the dataset, and a two-way crossed Analysis of Similarities (ANOSIM) was carried out to assess the significance of differences in micro-litter composition between species and sampling sites. Multi-Dimensional Scaling (MDS) ordination was subsequently applied to visualise the degree of similarity among samples.

2.6. Risk assessment

The environmental risk was evaluated based on the abundance of plastic in *H. sanctori* and *H. tubulosa* from the Capo Peloro Natural Reserve. Plastic concentrations, as suggested by Tomlinson et al. (1980), were used to calculate the *pollution load index* (*PLI*) as follows:

$$PLI_i = \frac{C_i}{C_{background}} \tag{1}$$

$$PLI_{area} = \sqrt[n]{CF_1 \times CF_2 \times CF_3 \times \ldots \times CF_n} \tag{2}$$

where PLI_i represents the pollution load index for an individual sea cucumber (i), C_i is the number of plastic items for specimen, $C_{background}$ is the background plastic concentration, and n is the total number of individuals analysed. To ensure comparability with other research and in the absence of a reference value for this species in the study area, Tomlinson et al. (1980) suggest assigning the minimum baseline abundance observed in this study to $C_{background}$ (Nakano et al., 2024).

The use of these methodologies has been formerly tested to calculate the potential hazards derived from the presence of APs and their ecological risks within different environmental compartments, including biota, water and sediments (*PLI*) (Baycan et al., 2026; Pedà et al., 2025; Savuca et al., 2026; Zhou et al., 2024). Thus, polymer composition and their concentration must be considered (*PHI*), and the abundance of APs has to be joined with their toxicity (*PRI*) (Zhou et al., 2024).

The PLI_{area} refers to the area-level microplastic pollution load index, calculated as the nth root of the product of all individual MP pollution load indices.

The polymeric-related hazard of plastic was evaluated using a *polymeric risk index* (*PRI*) proposed by Kabir et al. (2021) and calculated as follows:

$$H_i = \sum_{j}^{m} \left\{ \left(\frac{P_j}{C_i} \right) \times S_j \right\} \tag{3}$$

$$H_{area} = \sqrt[n]{H_1 \times H_2 \times H_3 \times \ldots \times H_n} \tag{4}$$

$$PRI_i = H_i \times PLI_i \tag{5}$$

$$PRI_{area} = \sqrt[n]{PRI_1 \times PRI_2 \times PRI_3 \times \ldots \times PRI_n} \tag{6}$$

where j is a polymer type, m is the total number of polymer types identified, P is the quantity of each individual polymer j found in each specimen, and S_j is the hazard score assigned to each polymer according to the classifications provided by Lithner et al. (2011). H_i represents the sum of plastic polymer risk indices for sea cucumber individual I, while PRI_i denotes the plastic pollution risk index for that individual. The area-level PRI (PRI_{area}) corresponds to the plastic pollution risk for the study area, calculated as the nth root of the product of all individual pollution risk scores.

3. Results

A total of 57 sea cucumbers were collected, including 27 *H. sanctori* and 30 *H. tubulosa*. Length and weight of the collected specimens are reported in Table 1.

3.1. *Holothuria sanctori*

A total of 134 APs (range: 0–24) were found in *H. sanctori*, with a mean of 4.96 items per specimen and a frequency of occurrence (FO%) of 85.2%. The average abundance of APs was greater in the GIT (4.52 items/specimen) than in the CT (0.33 items/specimen) and CF (0.11 items/specimen) (Table 1). No significant correlation was observed between the AP abundance and sea cucumber body size ($p > 0.5$) or weight ($p > 0.5$), nor between AP size and sea cucumber body size ($p > 0.05$), as evaluated using the Kendall's rank correlation test. The length of the APs ranged from 0.1 to 28 mm. Most of them were microparticles smaller than 5 mm (89%), a small number measured between 5 and 25 mm (10.2%), and just 0.8% was longer than 25 mm. Fig. 2 presents the distribution of AP sizes. Fibres and fragments were the predominant shapes, representing 53% and 45% of items respectively, followed by sheets (1%) and spheres (1%) (Fig. 3). Blue (30.4%), white (19.6%) and black (18.6%) were the most frequently observed colours, while red, green, light blue, and other colours were also detected (Fig. 2). Chemical identification of particles isolated from the GIT ($N = 45$) and CT ($N = 4$) identified 18 distinct types of APs. The most abundant AP was neoprene (CR, 24.5%), followed by cotton (12.2%), polypropylene (PP, 12.2%), polyurethane (PU, 10.2%), polyethylene terephthalate (PET, 6.1%), asphalt (4.1%), polyethylene (PE, 4.1%), polyethylene vinyl acetate (PEVA, 4.1%), and polyester (PES, 4.1%). Other APs, each accounting for 2.0%, included acrylic, cellulose acetate (CA), dyed cellulose (CE), polyacrylonitrile (PAN), polystyrene-*co*-divinylbenzene (PS-DVB), resin, rubber, styrene-ethylene-butylene-styrene (SEBS), and styrene-isoprene-styrene (SIS) (Fig. 2).

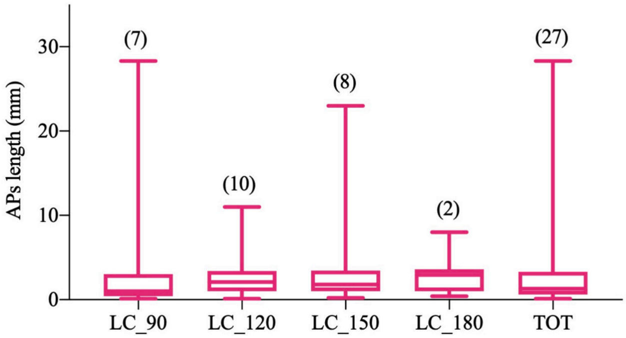

Fig. 3. Box-and-whisker plot showing the size (mm) of anthropogenic particles (APs) isolated from *Holothuria sanctori*, grouped by length class (LC, mm). Center lines represent medians, boxes indicate interquartile ranges, whiskers show minimum and maximum values, and () denotes the number of sea cucumber per length class.

APs were observed in all four LCs of sea cucumbers (Table 2). The average number of particles per specimen increased from 6 in LC180 to 55 in LC120. Fig. 3 illustrates the length distribution of anthropogenic particles (APs) across the different LCs. No statistically significant differences were observed among the sample medians (Kruskal-Wallis test: $H = 6.32, p > 0.05$). Neoprene (47.8%) was the most frequently detected anthropogenic particle in LC90, with PP accounting for 21.7%. PE and PU were the main polymers in LC120 (13.3% each). Cotton and PU were the dominant particles in LC150 (22.2%). PET and PAN were identified as the primary polymers present in LC180, each accounting for 25.0% (Table 2).

3.2. *Holothuria tubulosa*

A total of 99 APs (range: 0–15) were recorded in *H. tubulosa*, with a

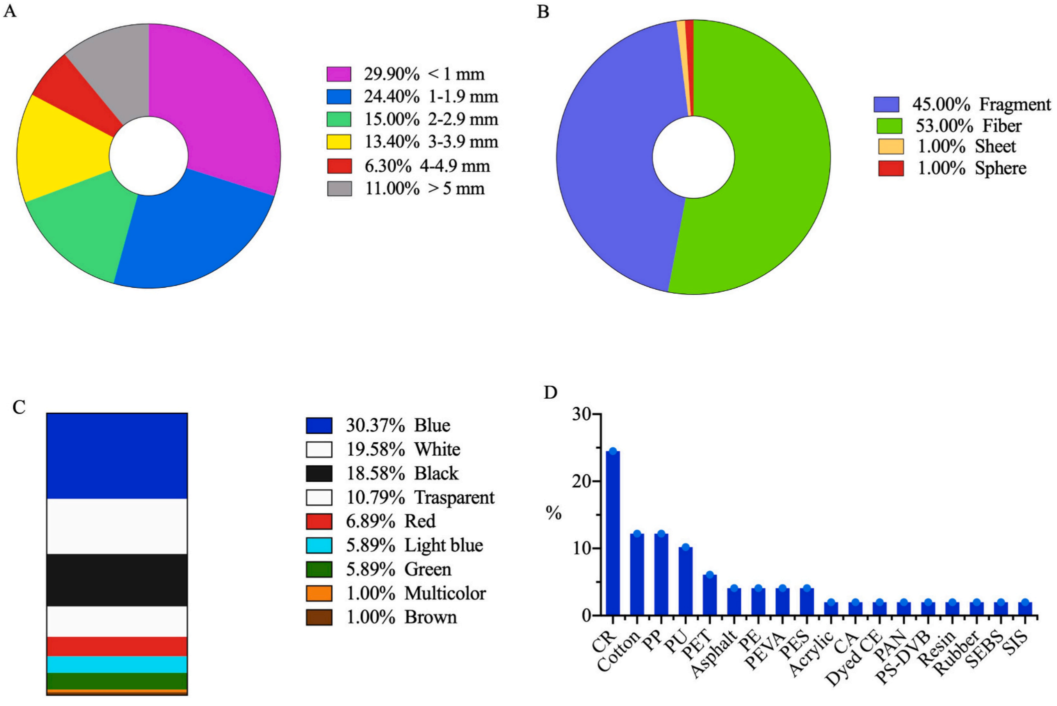

Fig. 2. Size (A), shape (B), colour (C), and chemical composition (D) of APs found in *Holothuria sanctori*.

Table 2
Number of examined sea cucumber specimens and their total length (TL, mm) ranges for each length class (LC, mm). Abundance indices of anthropogenic particles (AP) and frequency of occurrence (FO, %) are also reported.

Species	LC	Sample size	TL (mm)			N. AP	N items/ specimen	FO %	Anthropogenic particle identification
			Range	Mean	SD				
Holothuria sanctori	LC_90	7	90–114	100.57	8.32	47	6.71	100	Neoprene, PP, PU, PES, PEVA, CA, dyed cellulose
	LC_120	10	121–148	133.4	9.73	55	5.5	90	PE, PU, cotton, asphalt, neoprene, SEBS, acrylic, resin, PS-DVB, PET
	LC_150	7	155–178	163.14	8.3	26	3.71	71.4	PU, cotton, PES, rubber, PEVA
	LC_180	3	180–209	197	15.13	6	2	66.7	PP, PEVA, PET, cotton
	Total	27	90–209	139.67	32.2	134	4.96	85.2	
Holothuria tubulosa	LC_90	4	90–114	106.75	11.3	8	2	75	PES, rubber, EPDM
	LC_120	5	130–135	132.2	2.17	12	2.4	80	PP, PET, CA
	LC_150	9	150–178	162.22	10.11	38	4.22	100	Cotton, PE, PES, neoprene, PEVA, dyed cellulose, acrylic, PET, CA
	LC_180	7	182–200	190.57	6.58	32	4.57	71.4	SEBS, PE, PU, PEVA, PES, cotton, dyed cellulose, PA, PVC
	LC_210	5	210–306	245.2	38.01	9	1.8	60	Cotton
	Total	30	90–306	170.27	46.31	99	3.3	80	

mean of 3.30 items per specimen and a FO% equal to 80.0%. The mean abundance of APs was greater in the GIT (2.87 items/specimen) than in CF (0.43 items/specimen).

No significant correlation was observed between the abundance of APs and sea cucumber body size or weight ($p > 0.5$), nor between the size of APs and sea cucumber body size ($p > 0.05$), as determined by the Kendall rank correlation test.

The length of the APs ranged from 0.1 to 9 mm. Most of them were microparticles smaller than 5 mm (87.9%), a small number measured between 5 and 25 mm (12.1%) (Fig. 4). Fibres and fragments were the predominant shape categories, accounting for 57% and 40% respectively, followed by sheets (3%) (Fig. 3). Blue (37.1%), transparent (13.5%), black and light blue (10.1%) were the most common colours observed (Fig. 2). Chemical analysis of the particles ($N = 42$) indicated the presence of a mixture comprising 17 distinct types of APs. The most abundant AP was cotton (19.0%), followed by polyethylene (PE, 14.3%), polyester (PES, 11.9%), polyethylene vinyl acetate (PEVA, 9.5%), and styrene-ethylene-butylene-styrene (SEBS, 7.1%). Cellulose acetate (CA), dyed cellulose, polyethylene terephthalate (PET), and polyurethane (PU) each accounted for 4.8%. Acrylic, ethylene propylene diene monomer (EPDM), neoprene (CR), polyamide (PA), polyetherimide (PEI), polyvinyl chloride (PVC), and rubber each contributed 2.4% (Table S1). Representative images of selected items, along with their corresponding Raman or FT-IR spectra, are presented in Fig. S2. Regarding the comparison between LC groups, APs were found in all five LCs (Table 2). The number of AP per specimen increased from 8 in LC90 to 38 in LC150. Fig. 5 shows the length of APs for each LC. No statistically significant differences were observed among the sample medians (Kruskal-Wallis test: $H = 4.08$, $p > 0.05$). PES, rubber, and EPDM were the main anthropogenic particles found in LC90 (33.3% each). PP, PET, and CA were the main APs in LC120 (33.3% each). In LC150, the most common APs were cotton (26.3%), PE (26.3%), and PES (15.8%). In LC180, SEBS (20.0%), PE, PU, and PEVA (13.3% each) were the most frequent.

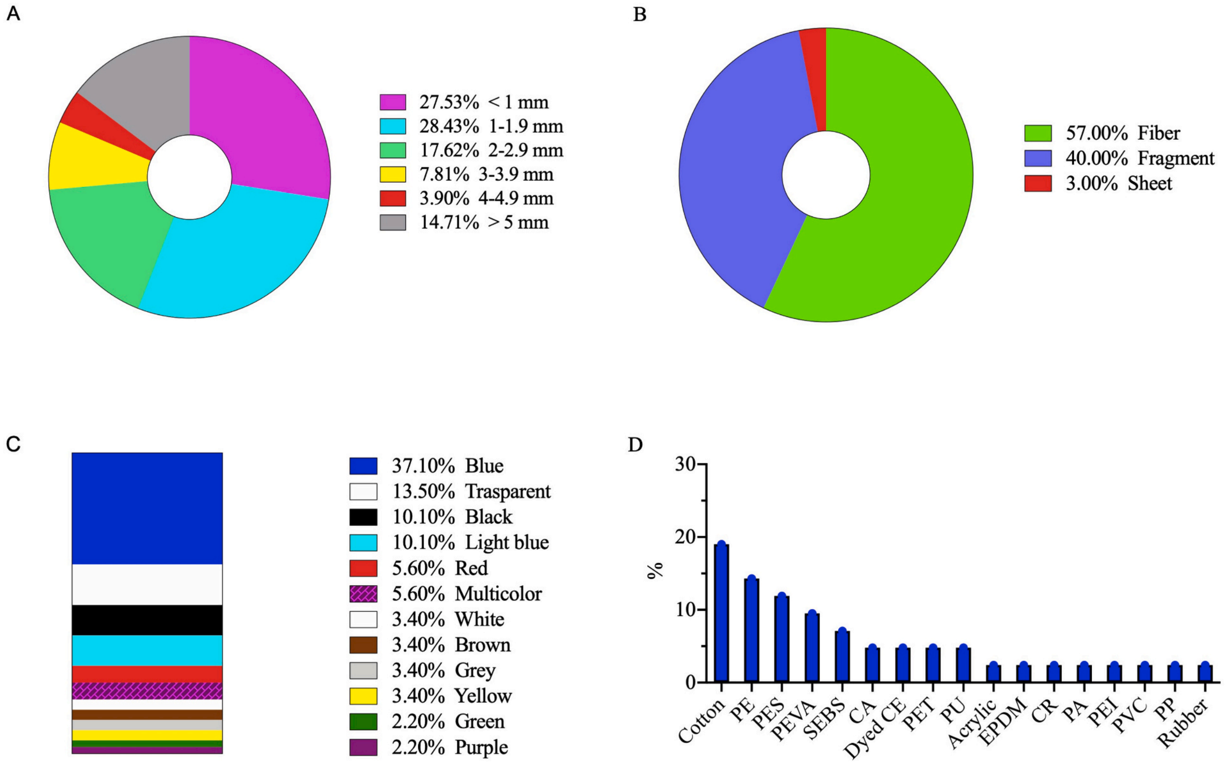

Fig. 4. Size (A), shape (B), colour (C), and chemical composition (D) of APs found in *Holothuria tubulosa*.

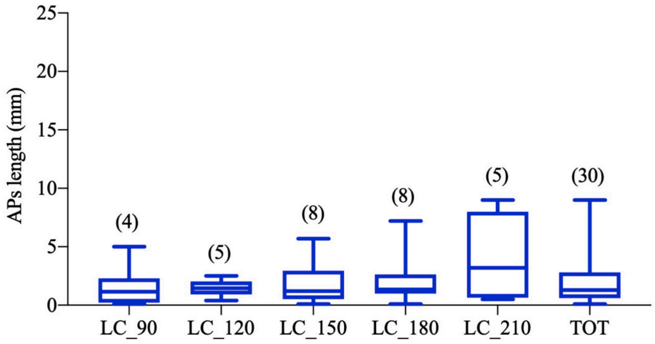

Fig. 5. Box-and-whisker plot showing the size (mm) of anthropogenic particles (APs) isolated from *Holothuria tubulosa*, grouped by length class (LC, mm). Center lines represent medians, boxes indicate interquartile ranges, whiskers show minimum and maximum values, and () denotes the number of specimens per length class.

3.3. Comparison between species and sampling area

The frequency of occurrence did not differ significantly between the two species (Chi-square test: $\chi^2 = 0.5$, $p > 0.05$, df $= 1$). Likewise, the mean abundance (items per specimen) was similar between species ($H = 0.08$, $p > 0.5$). The size of the APs found in *H. sanctori* shows a wider range compared to *H. tubulosa* (Figs. 3, 4); however, the median length of the APs showed no significant differences between the species ($H = 0.01$, $p > 0.05$).

Overall, the two sites exhibited a degree of similarity in polymer composition, with frequent occurrences of both natural and synthetic materials such as neoprene, cotton, PP, PE, PES, and PU. The two-way crossed ANOSIM revealed no significant differences in the composition of anthropogenic particles between the two species and sampling areas (Global $R = 0.04$; $p > 0.05$). The corresponding nMDS plot is shown in Fig. 6.

3.4. Preliminary risk assessment: pollution load index and polymer hazard index

According to Kabir et al. (2021), the resulting indices were graded in pollution levels given in Table S2. PLI_i values ranged from 1 to 24 for *H. sanctori* and from 1 to 15 for *H. tubulosa*, with corresponding PLI_{area} values of 3.3 and 2.4 respectively, both indicating polluted areas (Table S2, Fig. 7A).

H_i for individual *H. sanctori* ranged from 0.20 to 7683.33 (pollution levels I to IV) with a H_{area} value of 407.12 (level III), while for *H. tubulosa*, H_i values ranged from 0.57 to 6882.4 (levels I to IV) with a H_{area} value of 35.96 (level II) (Table S2, Fig. 7B).

The PRI_i for individual *H. sanctori* ranged from 1 to 69,225 and for *H. tubulosa* from 1 to 103,236 —both spanning low to very high pollution levels— while the PRI_{area} values were 2448.33 and 183.33, corresponding to very high and medium pollution levels, respectively (Table S2, Fig. 7C).

4. Discussion

Our analysis shows the presence of AP within the sea cucumbers and supports previous global findings in marine environments (Mohsen et al., 2019; Muhammad Husin et al., 2021; Mazlan et al., 2023; Fong et al., 2025; Peñalver-Soler et al., 2025; Santana et al., 2025; Shukhairi et al., 2025; see Table S3), indicating that this pollutant reaches even small, sensitive transitional zones like Capo Peloro Natural Reserve.

4.1. Abundance levels

At present, the study by Villanova-Solano et al. (2024) in the Eastern Atlantic Ocean (Canary Islands) is the only documented research on microplastic ingestion in *H. sanctori*. The authors documented a frequency of occurrence (FO% = 100%) that exceeded the values observed in the current study (FO% = 85.2% and 80.0%). Additionally, they reported abundance values that were more than twice as high, at 11.8 items per specimen. Conversely, several studies have investigated

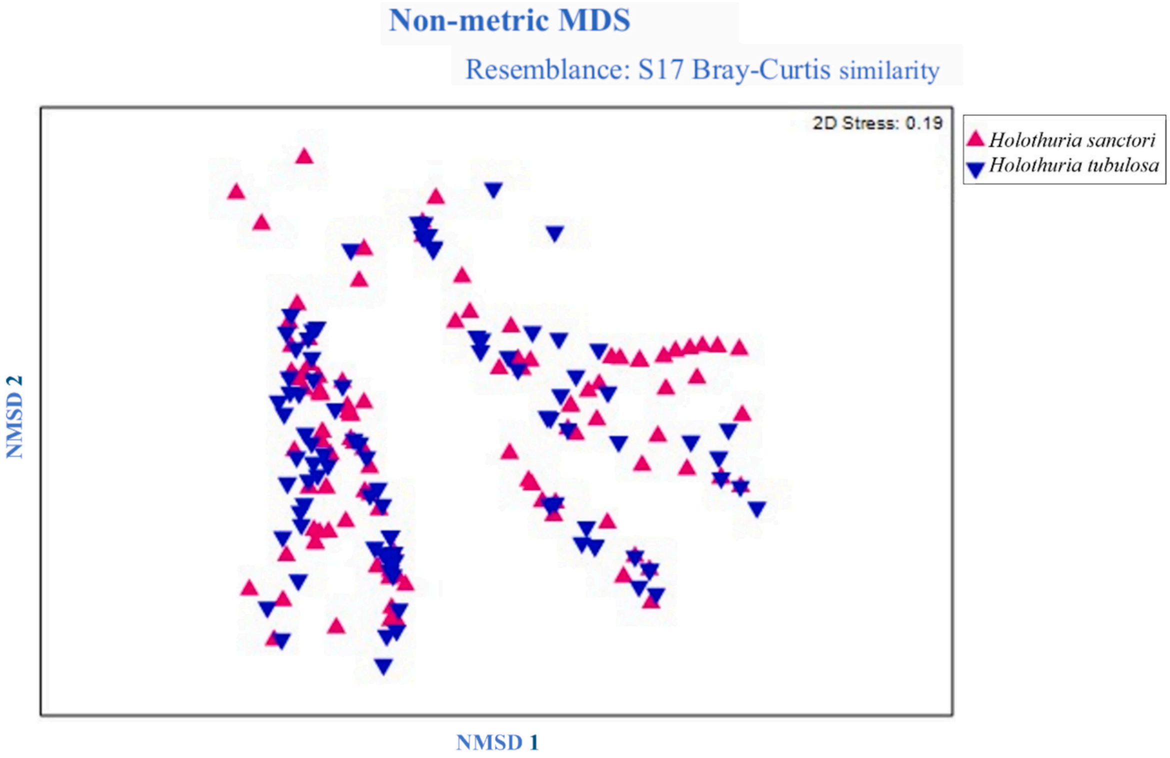

Fig. 6. Non-metric multi- dimensional scaling (nMDS) ordination of the sea cucumbers according to anthropogenic particles composition.

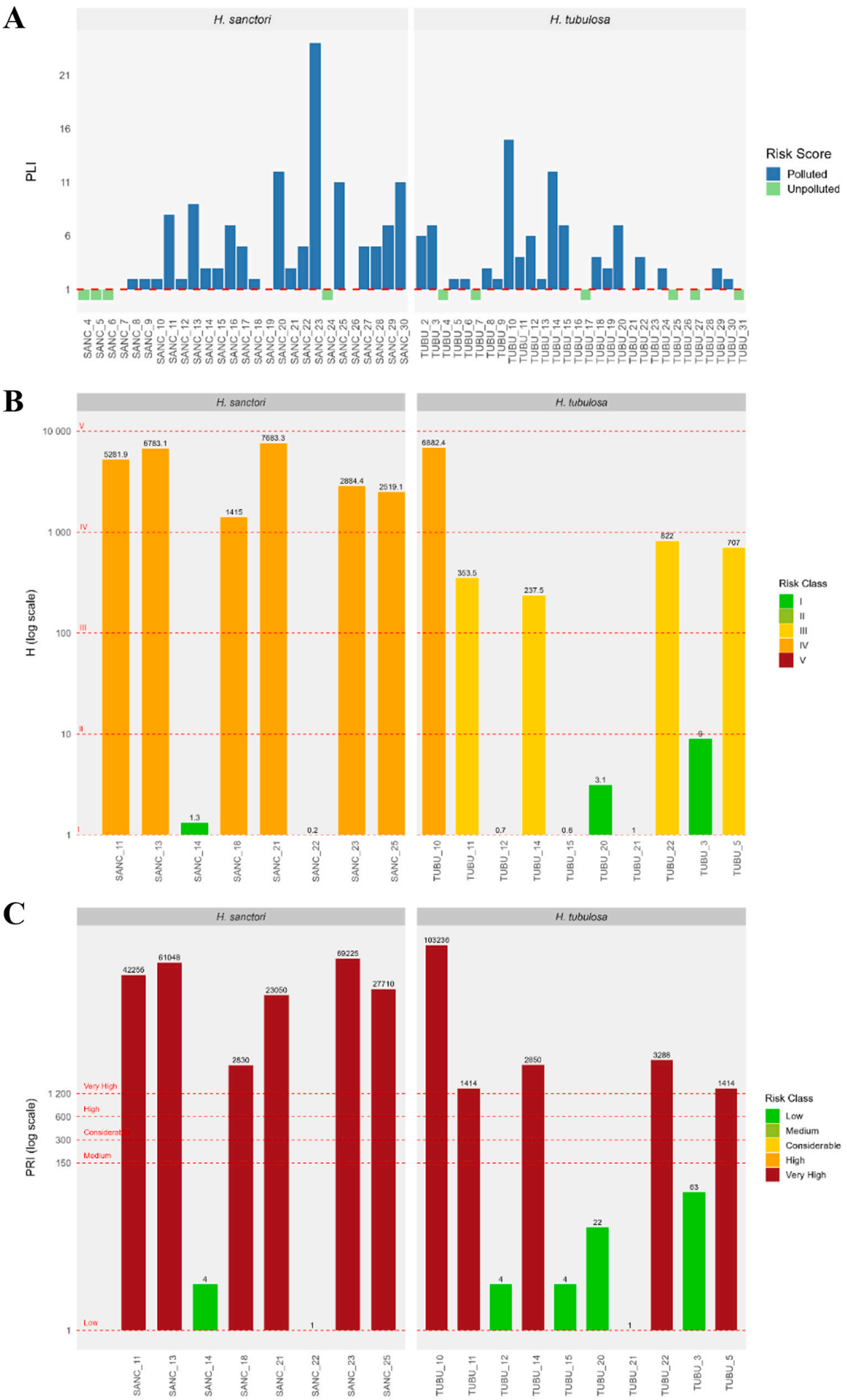

Fig. 7. Estimated value of plastic risk assessment: A) Pollution load index (PLI_i); B) polymeric risk (Hi); cC) pollution risk index (PRI_i) for *Holothuria sanctori* and *H. tubulosa*.

microplastic contamination in *H. tubulosa* across the Mediterranean Sea (Table S3). Renzi et al. (2020) and Renzi and Blaskovic (2020) reported 100% frequency of occurrence (FO%) in the Tyrrhenian and Adriatic Seas, with 1.3–3.8 and 0.74–4.5 particles per individual, respectively. In the Balearic Islands, Lombardo et al. (2022) found an average of 3.5 particles per individual (FO% = 83.3%), while Rios-Fuster et al. (2022) reported significantly higher values, with all individuals contaminated (FO% = 100%) and an average of 12.67 particles per specimen. The differences observed between our results and those reported in other studies may be attributed to several factors. Environmental conditions and local pollution levels can vary significantly among study sites, influencing both the frequency and abundance of microplastics ingested by sea cucumbers. Methodological differences, such as sample size, particle extraction techniques, and size ranges considered, can also contribute to discrepancies among studies. Temporal factors, including seasonal variation in microplastic availability, may play a role.

The prevalence of fibres and fragments identified in this study indicates that most APs present in the canals of the Capo Peloro Natural Reserve are of secondary origin, deriving from the breakdown of larger plastic materials originating from both terrestrial and marine sources. Land-based sources include illegal wastewater discharge-recorded in the area in the recent past-runoff processes and improper waste management in the area. This phenomenon is further exacerbated by inadequate plastic waste handling in the region. Marine-sourced APs may also originate from activities such as mussel farming and fishing, both of which have been established activities in the region. They may also be introduced into the lake from the sea through the hydrodynamic processes of the Strait of Messina (Fabrizi et al., 2025). Colour analysis further supports these source attributions. In our study, blue was the most frequently observed colour. The detection of blue fibres may be indicative of polyester ropes commonly utilised in mussel aquaculture, suggesting potential degradation of farming equipment. Black fibres were most prevalent and are probably associated with tubular mussel farming nets. These results indicate that also aquaculture activities, alongside terrestrial sources, represent a notable contribution to the observed microplastic pollution. The prevalence of transparent and opaque white APs may be attributable to the degradation of single-use plastic (SUP) items, including bags, bottles, or various types of packaging (Bottari et al., 2024b), and through fragmentation of fishing gear, including lines and nets.

4.2. APs composition

Raman and FT-IR analyses identified 12 synthetic polymers (PP, PU, PET, PE, PAN, PEVA, PES, acrylic, PS-DVB, PA, PEI, and PVC), three elastomers (SEBS, SIS, and EPDM), one rubber (neoprene), one petroleum derivative (asphalt discharges), and one semisynthetic particle (CA). Additionally, two man-made microparticles (cotton and dyed cellulose) and were also identified. Overall, our findings reveal a higher diversity and complexity of APs than previously reported in sea cucumbers from marine environments, with 18 types identified in *H. sanctori* and 17 in *H. tubulosa*, 12 of which were shared between the species. Neoprene was the most commonly detected AP in the analysed samples, indicating a significant contribution from sources such as wetsuits, industrial seals, gaskets, or marine equipment. To our knowledge, neoprene has been reported only once in the marine environment, specifically in *Scomber scombrus* from the Atlantic Ocean (Nelms et al., 2018). Based on the available literature, this appears to be the first report of neoprene in transitional waters. This study provides thus the first record of neoprene occurrence in transitional waters, highlighting its potential as an emerging contaminant in these ecosystems. Cotton was the second most abundant AP detected in the samples. The predominance of this polymer, alongside lower quantities of PAN, PES, acrylic, and dyed CE, indicates an additional pollution source, most likely associated with domestic wastewater discharges. This distribution pattern may reflect previous episodes of illegal wastewater releases reported around the lake's perimeter (Fabrizi et al., 2025). Furthermore, textile-derived microfibres transported via urban runoff represent a well-established source of microplastic contamination in coastal and freshwater environments (Gavigan et al., 2020; Fabrizi et al., 2025). PE, PP, and PET are widely used in packaging and SUPs due to their low cost and relatively short functional lifespan. Furthermore, the occurrence of PE and PP in the analysed specimens may reflect the breakdown of discarded fishing gear and waste associated with intensive mussel aquaculture and fishing activities within the reserve's lakes. PA, another synthetic polymer commonly used in fishing lines and nets, likely shares a similar origin. The presence of PU, a polymer commonly employed in marine coatings to prevent corrosion and biofouling (Zhang et al., 2023), is likely associated with vessels operating within these lakes (Fabrizi et al., 2025).

PVC enters the environment primarily through the degradation and improper disposal of construction materials, packaging, medical devices, and consumer goods (toys and footwear and clothing accessories).

In addition to plastic polymers, this study identified synthetic particles classified as elastomers, including SEBS, SIS, and EPDM. These materials are widely used in industrial and consumer applications: SEBS is commonly found in soft-touch coatings, medical devices, and adhesives; SIS is used in packaging materials, sealants, and pressure-sensitive adhesives; while EPDM is prevalent in automotive weather stripping, roofing membranes, and electrical insulation. The presence of these elastomer-based microparticles in marine organisms suggests contamination from both urban sources, highlighting the broad spectrum of anthropogenic materials entering marine environments.

Finally, asphalt, a petroleum-derived material composed primarily of bitumen, is commonly used in road construction. Asphalt particles, likely derived from road surface wear, are introduced into the marine environment via runoff processes (Zou et al., 2024), consistent with the heavy vehicular traffic and urban discharge in the Capo Peloro area (Somma et al., 2024).

In this study, manmade materials (cotton and dyed cellulose) were identified in sea cucumbers. Documenting their presence is important, as they may still pose risks to both environmental and biological health in transitional ecosystems. These materials can be harmful due to the presence of dyes and chemical additives commonly used in textile and manufacturing processes to enhance durability (Bikker et al., 2020; Islam et al., 2025).

4.3. Species and area comparison

Univariate and multivariate analyses revealed no significant differences between the two species in either the abundance of anthropogenic particles or the types of materials found. This indicates that both *H. sanctori* and *H. tubulosa* encounter and ingest similar quantities of these particles.

Likewise, no significant differences were detected within the same species between the two canals of the reserve. This is likely due to the similar environmental conditions, sediment properties, and particle availability in both channels, which probably result in comparable exposure and ingestion patterns.

4.4. Ecological risk

To evaluate preliminary ecological risk in the marine environment, the PLI and the PRI are commonly used (Tomlinson et al., 1980; Kabir et al., 2021). In this study, plastic contamination levels in *H. sanctori* and *H. tubulosa* were assessed, to gain insights into the potential risk of plastic in the canals of Cape Peloro Natural Reserve. PLI indicated that area was polluted. Regarding the results for *H. sanctori*, higher H_{area} and PRI_{area} values were recorded compared to *H. tubulosa*. These values are linked with a greater number of isolated plastic polymers with a high polymeric risk level such as PU and PAN, in *H. sanctori* compared to *H. tubulosa*. However, it should be noted that the H and PHI values are likely underestimated. In fact, several categories of synthetic materials (e.g., rubbers) identified in the samples are not included in the H index calculation, as no corresponding scores are available in the Lithner et al. (2011) classification. As a result, this affects the accuracy of the estimates.

5. Conclusions

This study provides a comprehensive assessment of anthropogenic particle contamination in *H. tubulosa* and *H. sanctori* within the Capo Peloro Natural Reserve, a transitional ecosystem of high ecological value. The high frequency of occurrence of APs and the wide diversity of particle types observed in both species underscore the pervasive nature of plastic contamination, even in such a small and protected brackish water environment. Chemical analyses revealed an unexpectedly high diversity of APs, pointing to a broader and more complex spectrum of anthropogenic materials than previously recognized in marine invertebrates.

The present study represents a first step toward a more comprehensive assessment of plastic pollution within the Natural Reserve. Future studies should extend our analysis to include surrounding water and sediment, as well as consider the seasonal variability of contamination. Given the ecological role of sea cucumbers in sediment bioturbation and nutrient cycling, plastic contamination affecting these organisms may result in broader impacts on the health of benthic ecosystems. These findings should inform future research and policy measures related to plastic pollution in Mediterranean transitional environments. In particular, the results highlight the need for targeted monitoring strategies in transitional reserves and for management actions aimed at identifying and reducing local sources of plastic contamination.

CRediT authorship contribution statement

Monique Mancuso: Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Chiara Anastasia Bruno:** Investigation, Methodology, Writing – review & editing, Formal analysis. **Ilaria Guardamagna:** Investigation, Methodology, Writing – review & editing, Writing – original draft, Formal analysis. **Bilal Mghili:** Writing – review & editing, Formal analysis, Data curation. **Francesca Fabrizi:** Investigation, Writing – original draft, Methodology, Formal analysis. **Valeria Conti Nibali:** Writing – review & editing, Formal analysis. **Kannan Gunasekaran:** Writing – review & editing, Formal analysis. **Caterina Branca:** Writing – review & editing. **Gian Marco Luna:** Writing – review & editing, Formal analysis. **Giovanna D'Angelo:** Writing – review & editing, Supervision. **Teresa Bottari:** Writing – original draft, Project administration, Funding acquisition, Data curation.

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Declaration of competing interest

The authors declare that they have no known financial or personal conflicts of interest that could have influenced the work reported in this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2026.119341.

Data availability

The raw data are available from the corresponding author upon request.

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