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Assessing anthropogenic pollution in an oriented nature reserve using *Anemonia viridis* as a bioindicator

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ABSTRACT

In this study, the feasibility of using the Mediterranean snakelocks anemone *Anemonia viridis* to monitor anthropogenic microparticles (APs) was evaluated for the first time at two sites within an Oriented Nature Reserve in the Mediterranean Sea (Italy): Faro Lake and Beach Rock (Capo Peloro). Both surface-adhered and ingested APs were isolated and characterized by size, shape, colour, and polymer type using micro-Raman and FT-IR spectroscopy. Also the risk assessment was performed to evaluate the ecological and health implications. Overall, 191 APs were detected (mean 5.30 items/specimen; 0.70 items/g; frequency of occurrence (FO%) 88.89%. Fibres (90.58%) were the predominant shape. Among identified APs ($n = 88$), cotton (28.41%), polyethylene terephthalate (PET; 14.77%), and cellulose acetate (12.50%) were the most prevalent. *A. viridis* is a sensitive bioindicator, reflecting local pollution profiles and providing a valuable tool for coastal plastic monitoring and management. Furthermore, the detection of APs in *A. viridis*, a species traditionally consumed in certain Mediterranean coastal communities (e.g., fried or in soups), raises potential concerns regarding human dietary exposure to microplastics and associated toxicological risks during cooking processes.

1. Introduction

Plastic pollution, especially microplastics (MPs; 100 nm to 5 mm), has emerged as a major anthropogenic threat to marine ecosystems worldwide due to its persistence, ubiquity, and ecological impacts (Derraik, 2002; Cole et al., 2011; Macleod et al., 2021; Gunasekaran et al., 2026). MPs originate from both primary sources, where plastics are intentionally manufactured at microscopic sizes, and from the secondary fragmentation of larger plastic debris, facilitating to their widespread dispersal (Song et al., 2017). Although coastal and open-ocean waters are heavily impacted, efforts to conserve biodiversity and ecosystem health have led to the creation of Marine Protected Areas (MPAs). However, MPs are readily dispersed over extensive spatial scales through hydrodynamic and atmospheric processes, enabling their infiltration into MPAs and thereby undermining conservation objectives (Luna-Jorquera et al., 2019; Zachello Nunes et al., 2023).

The Mediterranean Sea, a semi-enclosed basin subject to dense coastal populations and intense maritime activity, is recognized as a

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hotspot for MP contamination (Sharma et al., 2021). Extensive pollution has been documented across beaches (Vlachogianni et al., 2020; Bouzekry et al., 2024; Mghili et al., 2024; Branca et al., 2025), seawater (Suaria et al., 2016; Adamopoulou et al., 2021), sediments (Cózar et al., 2015; Kazour et al., 2019; Bouzekry et al., 2024), and marine organisms (Sharma et al., 2021; Soto-Navarro et al., 2021; Bouzekry et al., 2023; Bottari et al., 2024; Fabrizi et al., 2025), raising significant ecological and toxicological concerns. Ingestion of MPs has been linked to adverse effects on reproduction, feeding behaviour, and energy metabolism in marine organisms (Ali et al., 2021). Moreover, MPs can act as vectors for other hazardous contaminants (Ali et al., 2025), underscoring the necessity for robust environmental monitoring (Rossatto et al., 2023; Maaghloud et al., 2025; Bottari et al., 2026). Traditional biomonitoring has primarily focused on fish (Parolini et al., 2023) and filter-feeding invertebrates such as mussels, oysters, and sea cucumbers (Porter et al., 2023). Recently, however, new sessile organisms, including sea anemones, have emerged as promising complementary bioindicators of microplastic pollution due to their wide geographical distribution, ranging from tropical to polar seas and from shallow coastal habitats to the deep sea (Sun et al., 2022; Parolini et al., 2023; Aksun Tümerkan et al., 2024).

As predatory invertebrates, sea anemones feed on a broad spectrum of organisms, including fish, crustaceans, zooplankton, and molluscs, making them ecologically relevant for understanding pelagic–benthic interactions (Menezes and Thakur, 2022; Sun et al., 2022). Their proximity to coastal sources of pollution and their non-selective suspension-feeding behaviour expose sea anemones directly to MPs through both ingestion and external adhesion, with potential physical and chemical impacts.

The Mediterranean snakelocks anemone, *Anemonia viridis* (Forskål, 1775), is not only highly nutritious and beneficial for cholesterol levels (González et al., 2001), but is also considered a culinary across several European regions. In Italy, for instance, it is found in Sardinia, Sicily, Calabria, and Apulia, where it is known as “orziadas”, “beccafico di mare”, and “pupiddi di mare”. Preparation methods reflect local traditions: in Apulia, the anemones are typically floured and fried until crisp, while in Calabria they are also enjoyed in hearty soups. In Spain, particularly Andalusia, they are called “ortiguillas de mar” and feature in a variety of traditional dishes, showcasing a unique and surprising taste of the Mediterranean.

As they are eaten whole, human consumption of *A. viridis* harvested from contaminated areas could represent a health risk owing to both the toxicity of MPs and the pollutants adsorbed onto their surfaces. This highlights the importance of monitoring *A. viridis* as a bioindicator of environmental and food-safety risks. The present study aimed to conduct a pioneering investigation into the feasibility of employing the snakelocks anemone as a bioindicator of anthropogenic microparticle (here in after APs) pollution within a Mediterranean-Oriented Nature Reserve.

To assess the potential environmental risks associated with microplastic contamination in sea anemone samples, three ecotoxicological indices (PLI, H, and PRI) were calculated, together with an analysis of polymer hazard scores, in order to quantify pollution levels and to identify the plastic materials most responsible for the observed impact. By examining both AP ingested and those adhered to external body, this research offers a comprehensive evaluation of AP interactions at the specimen level. This study directly supports the objectives of the EU Marine Strategy Framework Directive (MSFD 2008/56/EC), particularly Descriptor 10 on marine litter, which emphasizes the need for effective monitoring and mitigation of marine litter and pollution in coastal ecosystems.

It also aligns with the United Nations Sustainable Development Goals (SDGs), 14 – Life Below Water, by contributing to the conservation and sustainable use of marine ecosystems through the development of innovative monitoring tools. The novelty of this research lies in its dual-level analysis of plastic contamination and its implementation in a Mediterranean-Oriented Nature Reserve. By assessing the levels of micro- and mesoplastic contamination in *A. viridis*, this study contributes to a deeper understanding of the vulnerability of benthic organisms to plastic contamination, even in conservation zones, and underscore the need to integrate such pollutants into routine monitoring protocols for MPAs.

2. Materials and methods

2.1. Study areas and samplings

This study was conducted in two different areas: Faro Lake, (38°16′07” N, 15°38′24″ E), which is connected to the Ionian Sea through the Faro Channel (425 m long and 8 m wide) and Beach Rock (38°15′29” N, 15°37′06″ E), located in the hamlet of Ganzirri on the Strait of Messina (Fig. 1, Fig. S1 A,B). Faro Lake and Beach Rock, both located within the Capo Peloro Nature Oriented Reserve in the northernmost part of the Peloritani Mountains in Messina, are part of a protected natural area of significant ecological and conservation interest (Bottari et al., 2017; Mancuso et al., 2023; Iaria et al., 2025). The reserve includes a lagoon system with sandy dunes and rich wetlands connected to both the Tyrrhenian and Ionian Seas. The Capo Peloro Oriented Nature Reserve has long been centre for aquaculture activities, including the farming of mussels, oysters and clams. Moreover, the perimeter and the shores of the lake are highly urbanized and anthropized, being bordered by paved ramps, roads, and little structures directly facing the water bodies (Somma et al., 2024). Messina's Beach Rock, located along the northeastern coast of Sicily, is an irregularly shaped intertidal rocky bench extending for nearly two kilometers. It is characterized by tidal ponds of varying shapes and degrees of connection to the sea, which, together with tidal oscillations and marine weather conditions, strongly influence their biotic and abiotic characteristics (Fazio et al., 2013; Cosentino and Giacobbe, 2015). Table S1 provides a description and main characteristics of the two studied sites. In May 2025, a total of 36 specimens of snakelocks anemones were collected by snorkelling at the two study areas (Fig. 1). The collection of specimens within the Capo Peloro Nature Reserve 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). Specifically 17 from Faro Lake (Fig. 1 D,E) and 19 from Beach Rock (Fig. 1 F,G). All specimens were randomly collected to ensure representative sampling of the local populations. To remove the organisms without causing damage, the entire body of each individual was carefully detached from the substrate using either manual methods or a metal spatula, paying particular attention to separate the pedal disc intact. Once collected, each specimen was immediately placed in an individual glass container filled with seawater taken directly from its respective sampling site to minimize stress and maintain environmental conditions as natural as possible. Then, the containers were promptly transferred to a portable field cooler to ensure temperature stability during transport to the laboratory. Upon arrival, the glass containers were drained of seawater and subsequently frozen at −20 °C to ensure the preservation of specimen integrity prior to further processing and analysis.

2.2. APs isolation

All samples were thawed overnight at 4 °C. Before digestion, each individual was weighed and thoroughly rinsed with Milli-Q water. The rinsing water from each anemone was collected in separate glass beaker, which was covered with aluminium foil and kept at room temperature (∼24 °C) to isolate any adhered anthropogenic particles (APs). Adherent APs found on the external surface were isolated by filtering the rinsing Milli-Q water through fiberglass filters (1.6 μm pore size, GF/A Whatman) using a vacuum pump (Rocker). All suspected microparticles were counted, classified based on size, shape, and colour, and subsequently photographed for the analysis.

Each individual was then placed in graduated glass flasks for the

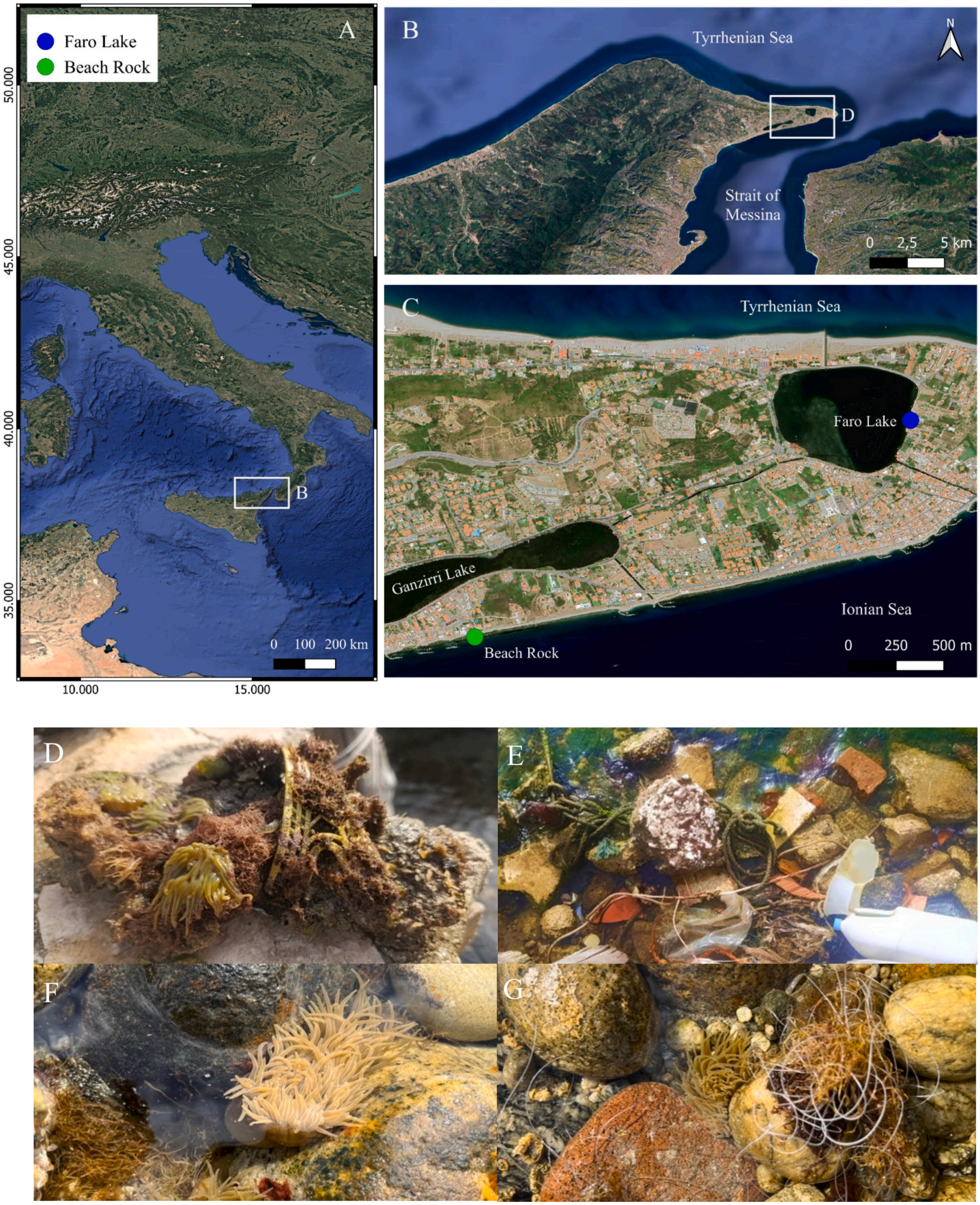

Fig. 1. Geographic location of the study area (A) showing the two sampling sites: Faro Lake (B) and Beach Rock in Capo Peloro Nature Reserve (C). Representative *Anemonia viridis* individuals from Faro Lake (D,E) and Beach Rock (F,G).

digestion process, following Micalizzi et al. (2024) protocol. The extraction of APs ingested by *A. viridis* was performed by digesting the biological material in a 10% potassium hydroxide (KOH) solution at a 1:5 (*w*/*v*) ratio incubated in an oven at 50 °C for 48 h. All soft tissues were completely digested, resulting in a homogeneous, greenish solution. The digestion protocol using 10% KOH (1:5 w/v) was selected based on widely validated methods for biological samples, shown to effectively remove organic matter while preserving polymer integrity; previous studies have demonstrated complete dissolution of fish tissues under similar conditions (Rochman et al., 2015; Kühn et al., 2017; Thiele et al., 2019).

Following digestion, a density separation was performed with a 15% sodium chloride (NaCl) solution at a 1:2 (w/v) ratio. The mixture was left to settle for 3 h to allow phase separation and facilitate the collection of floating microparticles. The resulting supernatant was carefully filtered. The effectiveness of tissue dissolution and the complete release of embedded particles were verified by visual inspection of settled fraction under a stereomicroscope. The isolated APs were analysed by size, shape, and colour, following the same protocol used for the rinsing water samples.

2.3. Quality control

To prevent any airborne contamination during laboratory procedures, several control measures were adopted: i) access to the laboratory was restricted, and only cotton coats worn; ii) work surfaces and tools, composed entirely of glass or stainless steel, were thoroughly cleaned with ethanol and filtered deionized or ultrapure water before use; iii) all containers, including digestion and filtration samples, were covered with aluminium foil; iv) all operations (sample preparation, weighing and filtration) were conducted under a fume hood; v) all solutions (NaCl and KOH) were filtered before use using glass fibre filters (1.6 μm pore size, Millipore) in vacuum system (Rocker). To monitor and detect airborne microparticles and fibres, blank filters moistened with Milli-Q water were exposed near work areas (e.g., under the hood, near the stereomicroscope). Control blanks were filtered every two samples. The APs found on sample filters that matched the shape, size, and colour of those in blank filters were subtracted from the final counts (Micalizzi et al., 2024).

2.4. Polymer characterization by micro-Raman and FTIR-ATR spectroscopy

In this study, 88 APs out of a total of 191 collected items (46.07% of the sample) were subjected to micro-spectroscopic characterization. The subsample was selected randomly. APs were identified using micro-Raman and Fourier Transform Infrared (FT-IR) spectroscopy. For the micro-Raman spectroscopy, the APs were analysed using an HR Evolution micro confocal system (Horiba Scientific) equipped with a 532 nm laser diode, either a 20× or 50× objective lens, an aperture ranging from 50 to 500, and a grating with either 600 or 1800 grooves/mm, depending on the samples and the resulting fluorescence for micro-Raman spectroscopy. Micro-Raman signals within the 200–4000 cm^{-1} range were gathered using a Charge Coupled Device (CCD) detector that was cooled with liquid nitrogen. Depending on the micro-Raman activity of the sample, the integration time ranged from 5 to 10 s and the number of accumulations varied from 2 to 100. With the aim of preventing the degradation of the sample, the laser power was modified to fall within the range of 5 to 30 mW. Individual samples underwent repeated measurements to ensure accuracy, and various sections of the same AP were analysed to remove the effect of local impurities on the micro-Raman spectra. In the first step of spectral data processing, Labspec 6 software was employed to remove fluorescence background, perform baseline subtraction, and filter out noise. Data analysis used the Bio-Rad KnowItAll informatic system, and the KnowItAll Raman Spectral Library or SLoPP/SLoPP-E (Munno et al., 2020) were utilized to determine the polymeric composition of the samples. In addition, the KnowItAll Raman Spectral Library was accessed through the 2024 trial version, which includes the standard polymer-related Raman sub-collections bundled with the trial package (synthetic polymers, monomers, fibres, and common anthropogenic materials) together with the freely accessible SLoPP and SLoPP-E libraries. The Hit Quality Index (HQI) was used to quantify the correlation between the spectra of unknown samples and those of known compounds in reference databases. In line with the methods of Micalizzi et al. (2024), a confidence level of at least 80% was established for identifying positive polymers.

Regarding the FTIR analysis, the chemical composition of the suspected particles was examined using an Agilent Cary 630 spectrometer in combination with specific polymer libraries (ST Japan Libraries for Cary 630/5500, Agilent Polymer Handheld ATR Library, and Agilent Elastomer O-ring and Seal Handheld ATR Library). Prior to each measurement, a background scan was recorded and subsequently subtracted from the sample spectra. The spectral range analysed spanned from 400 to 4000 cm^{-1}, with each spectrum averaged over 64 scans at a resolution of 4 cm^{-1}.

2.5. Data analysis

Snakelocks anemones were grouped in 4 weight classes based on the total weight in gram: class I: 0.1–4.9 g, class II: 5–9.9 g, class III: 10–14.9 g, class IV: > 15) and, for each class, the following abundance indices were calculated:

- 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).
- The mean number of APs was calculated as the ratio of the number of APs (found in internal tissue, externally adhered, and in total) to the total number of individuals (N. APs / N. total individuals).
- The mean number of APs was also calculated as the ratio of the number of APs (found in internal tissue, externally adhered, and in total) to the weight of individuals (N. APs /g).
- To assess differences in AP abundance (items/specimen and items/g) between adherent and ingested forms, a non-parametric Kruskal–Wallis test was conducted. Differences in AP size between adherent and ingested forms were evaluated using the Mann–Whitney *U* test. Additionally, Pearson correlation analysis was performed to investigate the relationship between AP abundance and the total weight of snakelocks anemones. Differences were considered significant at $p < 0.05$. The above statistical analyses were carried out using the PAST software (Hammer et al., 2001).

To compare anthropogenic particles composition associated with *A. viridis* across sites in term of size, shape, colour, and polymer, the data were further analysed using multivariate analysis implemented in the Primer 7 package (Anderson, 2024). The dataset was used to compute a Euclidean distance resemblance matrix. Analysis of Similarities (ANOSIM) was performed to assess the significance of differences in micro-litter composition between sampling sites. Multi-Dimensional Scaling (MDS) ordination was then applied to visualize the degree of similarity.

2.6. Risk assessment: Pollution load index and polymer hazard index

In the two sampling sites, the abundance of plastic was used to estimate the environmental risk. Following Tomlinson et al. (1980), the pollution load index (PLI) for an individual (i) sea anemone was estimated using plastic concentrations as follows:

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

$$PLI_{area} = \sqrt[n]{CF1 \times CF2 \times CF3 \times \ldots \times CF_n} \tag{2}$$

where Ci represents plastic abundance per specimen (n items/specimen), $C_{background}$ the background concentration of plastic in sea anemones, and n refer to the number of samples examined in this work. Since no reference values for this species exist in the study region (Nakano et al., 2024), the lowest plastic abundance measured in this research is considered equivalent to $C_{background}$. The regional MP pollution load index (PLIarea) is calculated as the nth root of the product of all individual MP pollution load indices.

The polymeric risk index (H) and the pollution risk index (PRI) developed by Kabir et al. (2021) were calculated as follows:

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

$$H_{area} = \sqrt[n]{H1 \times H2 \times H3 \times \ldots \times H_n} \quad (4)$$

$$PRI_i = H_i \times PLI_i \quad (5)$$

$$PRI_{area} = \sqrt[n]{PRI1 \times PRI2 \times PRI3 \times \ldots \times PRI_n} \quad (6)$$

where m is the number of identified polymers, j refers to a polymer type, P indicates the amount of each single plastic polymer (j) identified in each specimen (i), and Sj correspond to the hazard score for each polymer based on the classifications reported by Lithner et al. (2011). Hi represents the sum of polymeric risk indices for a sea anemone individual (i). Harea and PRIarea denote, respectively, the MPs polymeric risk and MPs pollution risk for the study area, obtained as the nth root of the product of the total polymeric and pollution risk scores.

3. Results

3.1. APs abundance and polymeric composition

A total of 36 snakelocks anemones were collected from the two sampling sites. The weights, reported in Table 1, ranged from less than 1 g to 33 g. A total of 191 suspected APs were detected in *A. viridis* with an average abundance of 5.30 items per specimen (0.70 items/g) and a FO % of 88.89%.

Most microparticles were between 1 and 4.9 mm (52.13%), followed by smaller than 1 mm (43.62%), and a few APs between 5 and 24.9 mm (4.25%) (Fig. 2A). Fibres were the most common shapes (90.58%), followed by fragments (7.85%), and films (1.57%) (Fig. 2B). Transparent and blue were the predominant colours, accounting for 39.27% and 30.89% respectively, followed by grey (11.52%), light blue (5.76%), red (5.24%), black (4.71%). Brown and violet (1.05%) and white (0.52%) (Fig. 2C). For what concerns the adherent APs, a total of 170, ranging from 1 to 45 items per specimen was found. The abundance was 4.72 items per specimen and 0.6 items per gram with a FO of 86.11% (Table 1). The mean length of the adherent APs was 1.42 mm (range: 0.1–6.2 mm). Most particles measured between 1 mm and 4.9 mm (52.7%), while 43.1.% were smaller than 1 mm. Only 4.20% exceeded 5 mm in length (Fig. 2A). Fibres were the predominant shape category, accounting for 92.94%, followed by fragments (5.88%), and films (1.18%) (Fig. 2B). Transparent (41.76%) was the most common colour, followed by blue were colours (27.06%), grey (12.94%),light blue (5.88% each), red (5.29%), black (4.12%). Other colours, such as brown, violet, and white were also found in smaller proportions (Fig. 2C). Regarding ingested APs, a total of 21 microparticles, ranging from 1 to 45 items per specimen, was found. The abundance was 0.60 items per specimen and 0.10 items per gram, with a FO of 38.89% (Table 1). A significant positive correlation (Pearson: 0.65, $p < 0.01$) was found between the weight of the anemones and the number of ingested APs.

The mean length of ingested APs was 1.63 mm. Most APs measured <1 mm and between 1.0 mm and 4.9 mm (47.62%) followed by APs between 5.0 and 24.9 mm (4.76%) (Fig. 2A). Fibres were the dominant shape (71.43%), followed by fragments (23.81%), and films (4.76%) (Fig. 2B). Blue was the dominant colour (61.90%), followed by transparent (19.95%), black (9.52%), red and light blue each accounted for 4.76% of the particles (Fig. 2C).

A comparison between adherent and ingested APs revealed that the abundance - number of items per specimen and number of items per gram- of adherent APs was significantly higher than that of ingested APs ($H = 6.9$, $p < 0.01$). Analysis by weight class revealed the presence of APs across all weight classes (Table 1). The lengths of APs for each weight class are shown in Fig. 3. Adherent APs were not significantly larger than ingested APs ($U = 1539.5, p < 0.05$). The number of particles per specimen increased from 26 in weight class I to 59 in weight class IV.

The frequency of occurrence (FO%) ranged from 78.57% in class I to 100% in class IV. Notably, weight class II exhibited the highest level of contamination, with an average of 0.93 APs per gram (Fig. 3). Overall, cotton (28.41%) was the most abundant AP, followed by polyethylene terephthalate (PET, 14.77%), cellulose acetate (CA, 12.50%), PES (11.36%), polyethylene vinyl acetate (PEVA, 10.23%), polyethylene (PE, 9.09%), polyurethane (PU) and polyamide (PA) each accounted for 4.55%, and polypropylene (PP) and acrylic each accounted for 2.27% (Fig. 2D, Fig. S2).

3.2. APs per area

In Faro Lake, the number of APs was 2.10 items per specimen (0.6 items per gram), with an FO of 82.35%. (Fig. 4 A, B). Samples collected at Beach Rock showed a higher number of items per specimen (8.20) and per gram (0.70) compared to Faro Lake, and exhibited a FO equal to 94.74% (Fig. 4 A, B). Size, shape, and colour of APs found associated to *A. viridis* in the two sites are shown in Fig. 5 (A, B, C). Chemical identification in each sampling area revealed 10 different material types at the Beach Rock sites, and 9 at Faro Lake (Fig. 5 D). Overall, the two sites exhibited a high degree of similarity in polymer composition, with frequent occurrences of both natural and synthetic materials such as cotton, PET, CA, PES, PEVA, PE, PU, PA, and PP.

Regarding the results of the multivariate analysis, the one-way ANOSIM indicated that there were no statistically significant differences between sites in terms of APs composition (Global $R = 0.095$; $p >$

Table 1
Number of examined Anemonia viridis specimens and total weight range (TW, g) for each weight class. Data include abundance indices and frequency of occurrence (FO%) of anthropogenic particles (APs).

Compartment	Weight Class (g)	Sample Size	Total weight (g)			N. APs	Items/specimen	Items/g	FO%
			Range	Mean	SD				
Adherent APs	TW_0	14	<1–4	3.14	1.03	20	1.43	0.45	78.57
	TW_5	11	5–9	6.91	1.45	65	5.91	0.86	90.91
	TW_10	7	10–14	11.86	2.04	31	4.43	0.37	85.71
	TW_15	4	>15	16.75	1.71	54	13.5	0.81	100
	Total	36	⟨1–⟩15	7.5	4.82	170	4.72	0.63	86.11
Ingested APs	TW_0	14	<1–4	3.14	1.03	6	0.43	0.14	35.71
	TW_5	11	5–9	6.91	1.45	6	0.55	0.08	63.64
	TW_10	7	10–14	11.86	2.04	4	0.57	0.05	28.57
	TW_15	4	>15	16.75	1.71	5	1.25	0.07	50
	Total	36	<1 – >15	7.5	4.82	21	0.58	0.08	44.44
Total APs	TW_0	14	<1–4	3.14	1.03	26	1.86	0.59	78.57
	TW_5	11	5–9	6.91	1.45	71	6.45	0.93	100
	TW_10	7	10–14	11.86	2.04	35	5	0.42	85.71
	TW_15	4	<15	16.75	1.71	59	14.75	0.88	100
	Total	36	<1 – >15	7.5	4.82	191	5.31	25.47	88.89

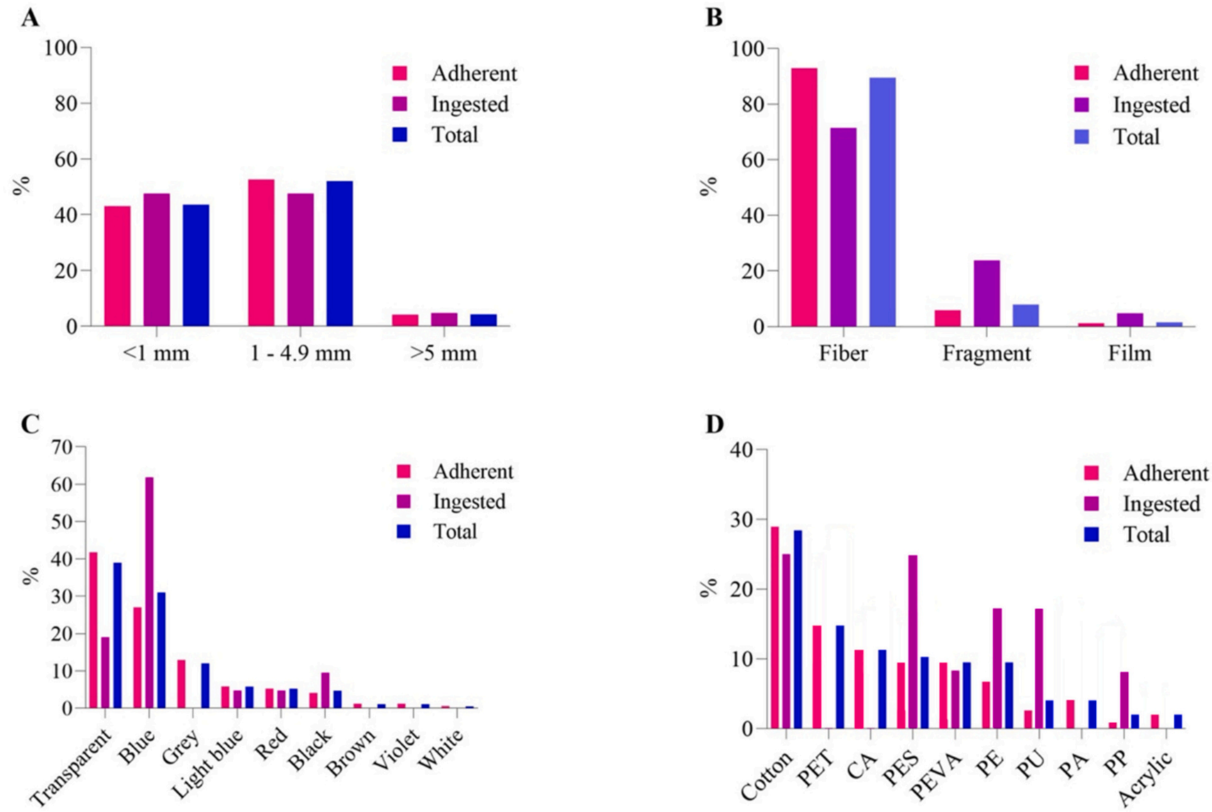

Fig. 2. Size (A), shape (B), colour (C), and chemical composition (D) of adherent, ingested, and total APs found in *Anemonia viridis* specimens.

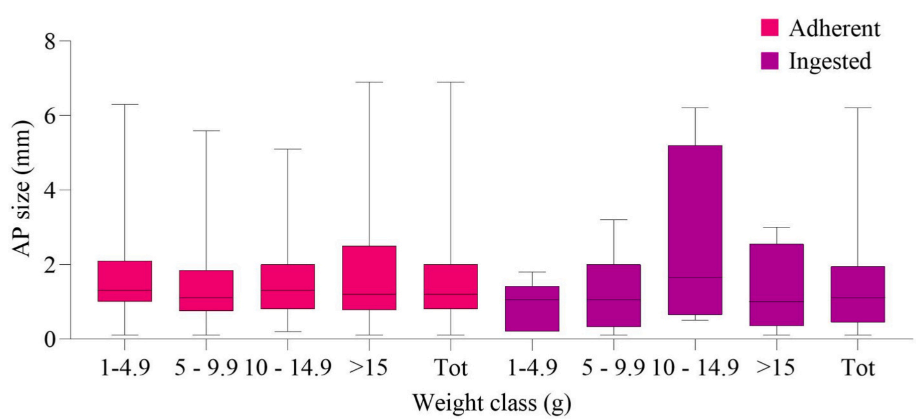

Fig. 3. Box-and-whisker plot showing the size (mm) of anthropogenic particles (APs) isolated from *Anemonia viridis*, grouped by weight class (g). Center lines represent medians, boxes indicate interquartile ranges, and whiskers represent minimum and maximum values.

0.05). The corresponding nMDS plot is shown in Fig. 6.

3.3. Risk assessment

The PLIi values ranged from 0 to 5 in Faro Lake and from 0 to 45 for Beach Rock, with PLIarea 1.91 and 5.07 respectively, indicating that all two area are polluted (Fig. 7 A). H values in Faro Lake and Beach Rock indicated pollution levels ranging from I to IV, with values of 0.5–4614.67 and 0.20–1667.60. The Harea varied from 75.88 and 26.59 respectively in the two sites (Fig. 7 B). Likewise, PRIi values in the two sampling sites showed low to very high pollution levels, with values ranging from 1 to 16,676, with PRI area of 194.60 and 213.26 respectively from Faro Lake and Beach Rock, corresponding to medium pollution level (Fig. 7 C). The most hazardous polymer found was PU (13,844 hazard score), followed by PES (1414), and PA (63) present in varying percentages within the two sites. Specifically, PU showed the highest associated Hi value 3194.77 in Faro Lake, while in Beach Rock was 922. 93, followed by PES with 217.54 and 329.93 respectively in Faro Lake and Beach Rock. PA with 14.54 in Faro Lake and 2.10 in Beach Rock (Table 2).

4. Discussion

4.1. APs abundance

The analysis of APs occurrence in snakelocks anemones provided the first data on plastic contamination in two sites within two Mediterranean Nature Reserve. When comparing our findings with those of

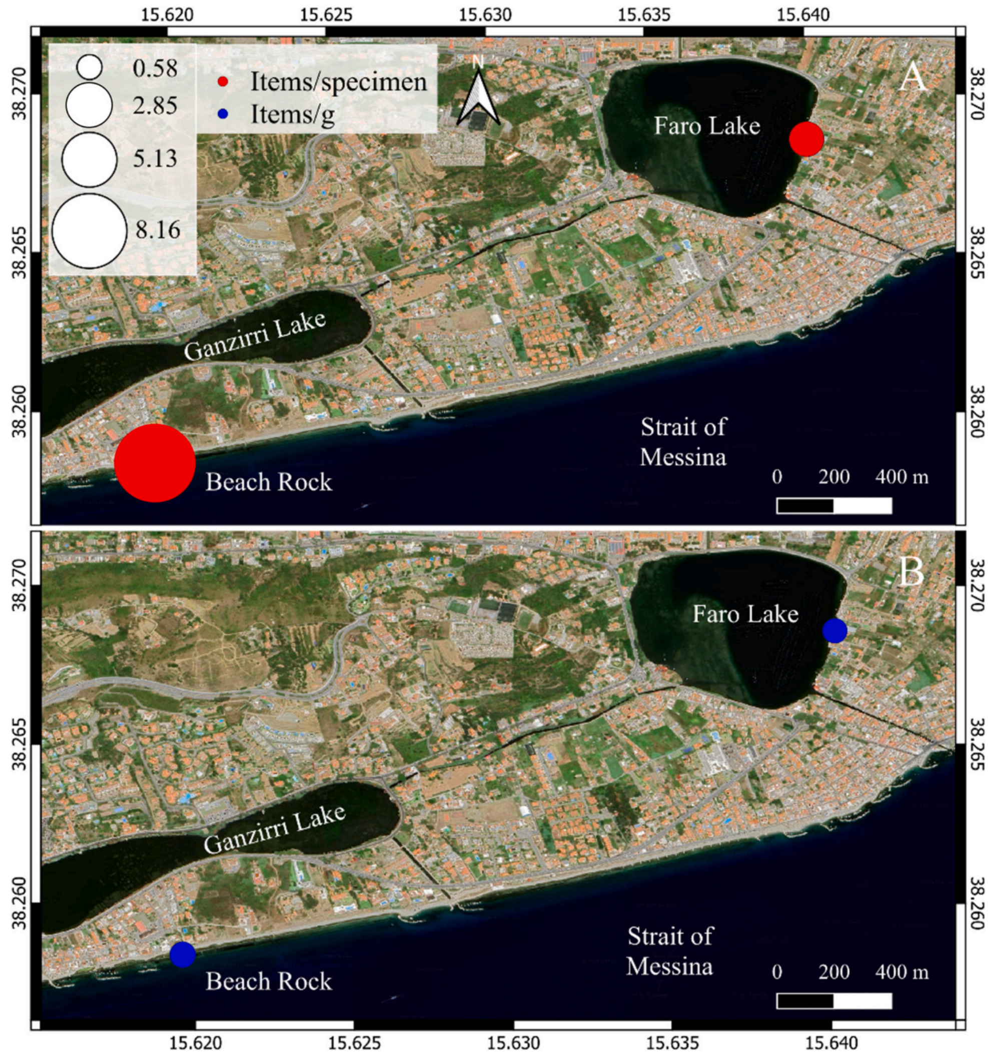

Fig. 4. Variations in APs concentrations at Faro Lake (A; B), and Beach Rock (A; B). Circle size represents values in items/specimen (A) and items/g (B).

Vencato et al. (2024) on *A. viridis* from Sardinia, our values are lower. In contrast, Vencato et al. (2024) reported higher values for both ingested items/specimen (13.3) and items/g (0.79). Regarding the FO of particles ingested by the organisms, Vencato et al. (2024) reported 100%, whereas our results showed lower FOs: Faro Lake 47.06% and Beach Rock 31.58%. Vencato et al. (2024) reported, in *Anemonia equina* specimens from Sardinia, an abundance of 12.35 items per specimen and 0.70 items per gram, with a 100% FO. Janssens and Garcia-Vazquez (2021) found higher abundances in *A. equina* individuals from Spain, ranging from 15.75 to 30.45 items per specimen and from 16.15 to 21.82 items per gram, values higher than those reported by both Vencato et al. (2024) and Aksun Tümerkan et al. (2024), who observed 1.95 items per gram in *A. equina* specimens from Turkey.

Microplastic contamination in *Bunodosoma cangicum* from the Brazilian Amazon coast was documented by Morais et al. (2020, 2025). In the 2020 study, microplastic abundances ranged from 1 to 2 items per specimen, with a FO between 70% and 83.3%. The 2025 study reported similar findings, showing an average abundance of 1.7 items per specimen and an FO of 70%. The abundance of fibres and fragments found in this study suggests that most of the APs isolated in the Capo Peloro Oriented Nature Reserve are of secondary origin, resulting from the breakdown of larger APs from both marine and land-based sources. In our study, transparent and blue APs were predominant. Similarly, Vencato et al. (2024) reported that in *A. viridis* the most abundant colour was transparent, followed by blue. The presence of blue fibres can be associated with nets, ropes, and fishing lines made of synthetic fibres, which degrade over time and release microfibres (Gago et al., 2018), but they may also originate from polyester, nylon, or acrylic clothing, which release fibres during machine washing (Henry et al., 2018).

4.2. Chemical composition and sources

Micro-Raman and FT-IR analyses identified 10 materials, including 1 man-made microparticles (cotton), 8 synthetic polymers (acrylic, PA, PE, PES, PET, PEVA, PP, and PU), and 1 semisynthetic particle (CA). Regarding the APs, cotton was the most abundant anthropogenic AP detected in all sites. Its high occurrence together with co-occurrence acrylic and PES suggests a source originating from textile products (Cesa et al., 2017).

The pronounced occurrence of textile-derived APs points to an additional source of pollution, most plausibly associated with domestic wastewater effluents. Furthermore, microfibers originating from textiles and transported through urban runoff constitute an additional pathway for APs contamination in coastal systems (Almroth et al., 2018; Bouzekry et al., 2023; Fabrizi et al., 2025; Mancuso et al., 2026). Textiles are widely recognized as the predominant environmental source of microfibers, with synthetic fabrics alone contributing an estimated 0.5 million tonnes of microplastics to the marine environment annually (Manshoven et al., 2022). Likewise, cotton, cellulose, and PES fibres have been identified in *A. equina* collected from the Spanish Atlantic

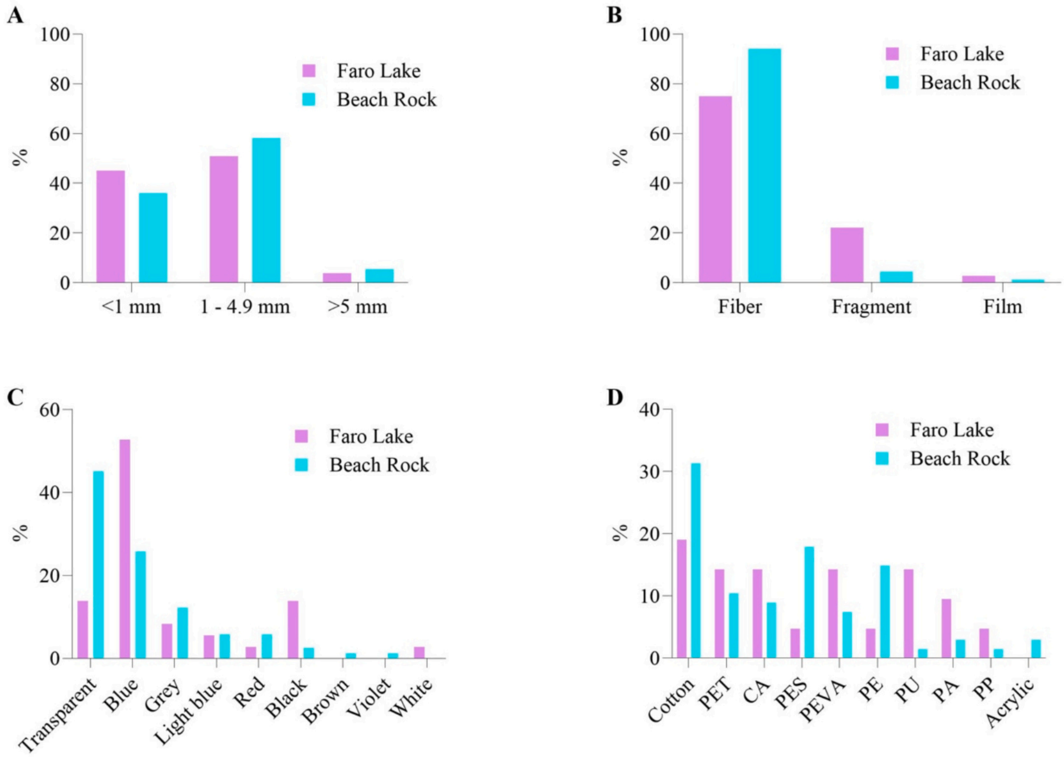

Fig. 5. Size (A), shape (B), colour (C), and chemical composition (D) of APs found associated to *Anemonia viridis* in the different sampling areas.

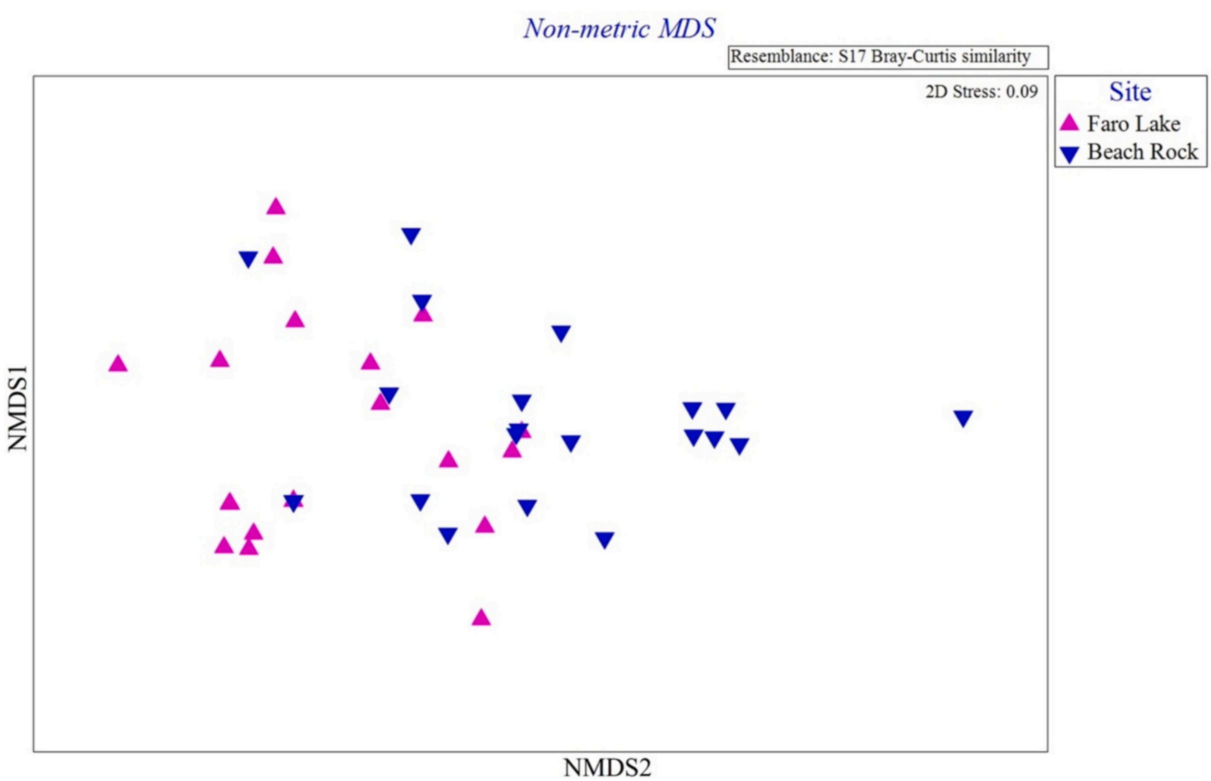

Fig. 6. Non-metric multi- dimensional scaling (nMDS) ordination of the *Anemonia viridis* according to anthropogenic particles composition in investigated sampling sites (violet triangles triangles = Faro Lake, blue triangles = Beach Rock). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

coasts (Janssens and Garcia-Vazquez, 2021; Table S2). PET, PE, PP, and PEVA originate from single-use plastics (SUPs) such as packaging, bags, plastic films, and other disposable products, including domestic, industrial, commercial, and urban activities (Ryan et al., 2021; Kabir et al.,

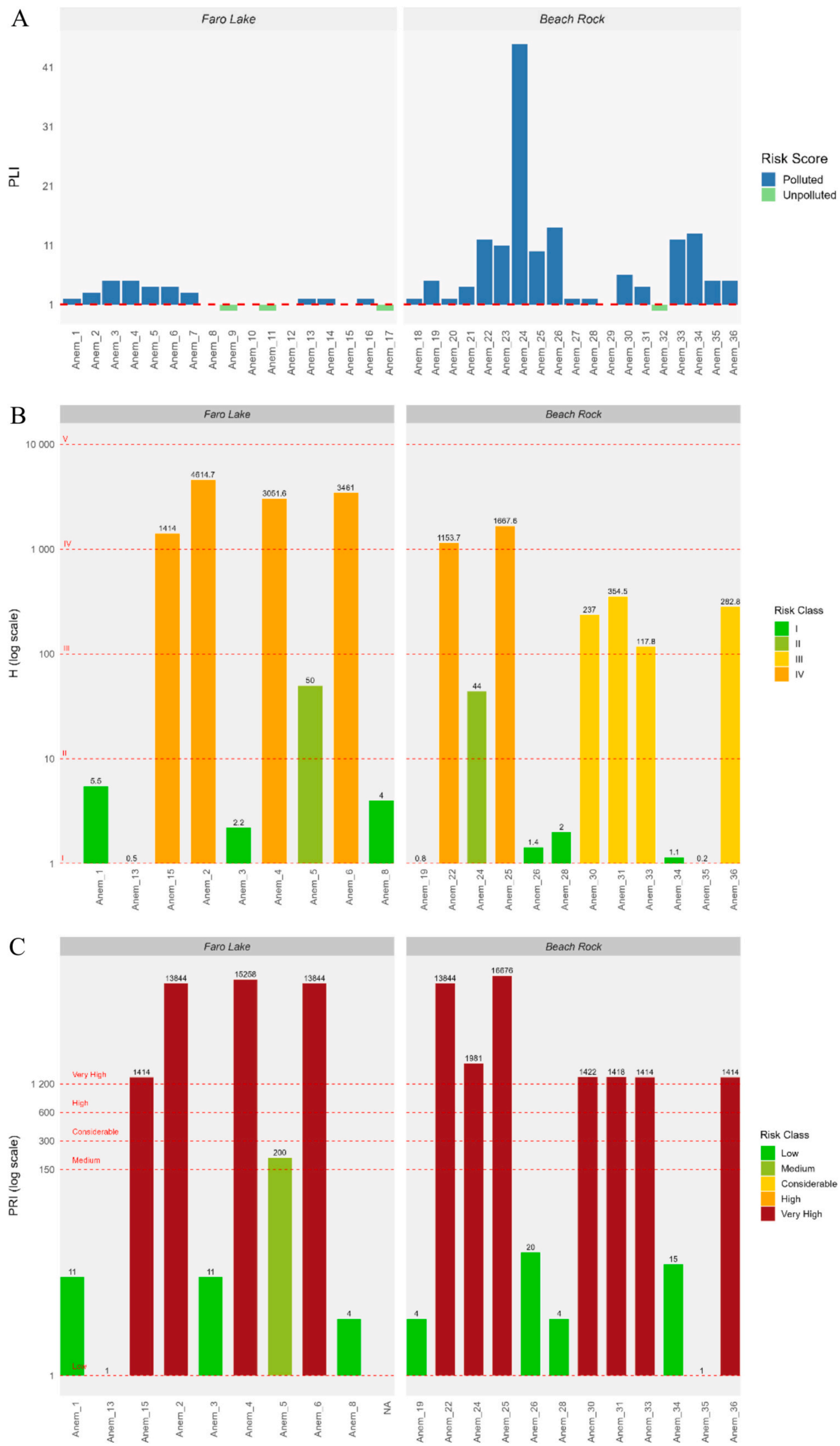

Fig. 7. Estimated values of plastic-related risk for *A. viridis* individuals: (A) Individual Pollution Load Index (PLIi); (B) Polymeric Risk Index (H); (C) Pollution Risk Index (PRI).

Table 2
Hazardous polymers detected in *A. viridis* across sampling sites, with hazard scores, polymer types, total counts (∑Pij), frequency of occurrence (FO%), and associated polymeric risk indices (Hi).

Faro Lake				
Hazard Score	Polymer	∑Pij	FO%	Hi
13,844	PU	3	23.08	3194.77
1414	PES	2	15.38	217.54
63	PA	3	23.08	14.54
11	PE	3	23.08	2.54
4	PET	1	7.69	0.31
1	PP	1	7.69	0.08
Beach Rock				
Hazard Score	Polymer	∑Pij	FO%	Hi
13,844	PU	2	7.14	922.93
1414	PES	7	25	329.93
63	PA	1	3.57	2.1
11	PE	5	17.85	1.83
4	PET	12	42.85	1.6
1	PP	1	3.57	0.03

2021). CA largely originates from cigarette butts, a frequently encountered pollutants in coastal environments (Abbasi et al., 2025). It also comes from textile fibres released during washing or through fabric wear and tear. PA (nylon) is typically associated with fishing nets, technical fabrics, and durable materials used in marine and industrial settings (Mishra et al., 2019). This study's findings are consistent with those reported for *A. viridis* from Sardinian waters, where PE and PP were identified together with CA, cellulose, PES, PET, and PA (Vencato et al., 2024). The occurrence of PU—commonly employed in marine coatings to prevent corrosion and biofouling on vessel hulls (Wang et al., 2022)—is likely associated with boating activities in both the marine site (Beach Rock) and the brackish waters of the Cape Peloro Nature Reserve (Fabrizi et al., 2025). PU has also been reported in *A. viridis* from Sardinian waters (Vencato et al., 2024), in *A. equina* from the Mediterranean Turkish coasts (Aksun Tümerkan et al., 2024), and in *B. cangicum* from the Atlantic Ocean (Morais et al., 2020; Table S2).

The two examined sites, located within two Oriented Nature Reserve, were found to be contaminated. Local hydrographic conditions and anthropogenic pressures are likely to play a key role in regulating the availability and distribution of APs within the study area. Hydrodynamic processes—such as coastal currents, circulation patterns, and winds—combined with human-driven pressures, including maritime traffic, wastewater discharges, and tourism-related activities, can substantially increase the local input of APs. This dynamic increases the availability of floating debris within the water column, promoting both accidental ingestion and surface adhesion to anemone tissues.

Faro Lake is situated within a highly urbanized coastal area and influenced by multiple anthropogenic activities. Bivalve aquaculture facilities may also represent localized sources of plastic inputs, as operational activities and the degradation of farming equipment can contribute to the release of synthetic particles into surrounding waters (Wu et al., 2023). Faro Lake is also characterized by limited water circulation, making it a highly vulnerable site to plastic pollution (Bottari et al., 2024). Beach rock in the study area is directly exposed to intense maritime traffic within the Strait of Messina, one of the busiest navigation corridors in Italy and the Mediterranean Basin, characterized by high densities of ferries and cargo vessels (Marino et al., 2023). The Strait is also influenced by strong currents that can generate convergence zones, favoring the accumulation of floating plastic debris. Previous studies have documented the widespread presence of plastic debris on the seafloor and have identified the submarine canyon of the Strait of Messina as a hotspot (Pierdomenico et al., 2019). Hydrodynamic processes such as upwelling may further enhance both the vertical and horizontal redistribution of microplastics throughout the water column, increasing their environmental availability. In addition, the rocky substrate—characterized by small cavities and depressions—acts as a natural trap for microplastics, which can subsequently be remobilized by wave action and storm-induced turbulence. The interaction between these hydrodynamic dynamics and anthropogenic pressures likely explains the observed spatial variability in debris abundance between the two study sites.

4.3. Risk assessment

To assess preliminary ecological risk in marine environments, the PLI and PRI are frequently applied (Kabir et al., 2021; Tomlinson et al., 1980). In this study, plastic contamination levels in *A. viridis* were assessed to gain insights into the potential risk of plastic in an Oriented Nature Reserve.

Results from PLI indicated that the two studied areas were polluted. Faro Lake showed higher Harea value (75.88) than Beach Rock Harea value (26.59). Faro Lake showed a PRIarea (194.60) lower than Beach Rock that showed a PRIarea (213.26), although both sampling sites resulted in pollution level II (Harea) and medium level of pollution (PRIarea). Thus, no significant difference is shown. However, high hazard score polymers such as PU and PES highly contribute to the pollution of the area despite their low representation, suggesting that even a small amount can be decisive. Moreover, H and PRI values are likely underestimated because several polymers collected in the study are not present in Lithner et al. (2011) classification eventually excluding many potentially hazardous polymers from the computation and affecting the accuracy of the calculation. Nevertheless, the use of these indices is particularly valuable since relying exclusively on polymer abundance can be misleading: a polymer present in low concentration might seems to have little impact, whereas, due to its high hazard score, it can present a greater hazard than a more abundant polymer with a lower hazard score.

4.4. Insights into human health risks associated with the consumption of microplastic contaminated sea anemones

The consumption of sea anemones remains an integral part of the traditional cuisine of Southern Italy and Andalusia (Spain). In both regions, sea anemones are usually eaten whole, most commonly fried, either floured or battered. Alternatively, they may be marinated or cooked into soups. However, the high temperatures reached during frying (approximately 170–190 °C) can cause the polymers present within these organisms to degrade and form hazardous compounds, including dioxins (Smith et al., 2018; Eshun and Pobee, 2022; Li et al., 2022). The formation and release of toxic compounds are strongly influenced by the specific chemical properties of the materials exposed to heat (Li et al., 2022). This underscores the importance of greater awareness regarding the potential health hazards associated with traditional cooking methods involving marine species that may contain plastic debris. Janssens and Garcia-Vasquez in 2021 highlighted that specimens of *A. equina* caught in the Bay of Biscay contained microplastics with compounds potentially harmful to public health and the environment. These species are not completely safe for consumption if harvested from contaminated areas.

5. Conclusion

The snakelocks anemones are sessile, benthic cnidarians that inhabit shallow coastal rocky substrates, often in areas influenced by anthropogenic activity. Their feeding strategy—based on passive prey capture using nematocyst-bearing tentacles and mucus-mediated particle retention—combined with continuous exposure to the water column and resuspension of fine particles, makes them particularly prone to intercepting and retaining suspended particulate matter, including anthropogenic particles.

The characteristics of snakelocks anemones (sessility, direct contact with both the water column and resuspended sediments, and efficient particle-trapping structures) support their potential role as site-specific bioindicators of anthropogenic particle pollution.

Our results underline the vulnerability of benthic organisms like *A. viridis* and the insufficiency of current conservation boundaries in fully mitigating plastic contamination. Ecological risk analysis indicated a medium pollution level within the Capo Peloro Oriented Nature Reserve highlighting the limitations of current conservation boundaries and the necessity for further implementation measures. The ecological traits of *A. viridis*, including its sessile lifestyle and non-selective feeding, enable spatially explicit assessments of localized pollution, offering an integrated view of ambient microparticle abundance and bioavailability (Sun et al., 2022; Aksun Tümerkan et al., 2024). Importantly, *A. viridis* is a culinary species consumed in Mediterranean communities, raising concerns about human dietary exposure to microparticles and related health risks.

Beyond physical accumulation, *A. viridis* also exhibits alterations in its associated microbiota in response to human-induced stressors, indicating microbial dysbiosis as an additional sensitive biomarker. These observations point to the need of improving monitory programs, stricter plastic waste regulations and increased consumer awareness of seafood preparation to minimize human exposure risks from microplastic contamination.

CRediT authorship contribution statement

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

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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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.2026.119818.

Data availability

Data will be made available on request.

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