Note

Chemical composition of microplastic in sediments and protected detritivores from different marine habitats (Salina Island)

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A R T I C L E I N F O

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A B S T R A C T

This study estimates chemical composition of microplastic in sediments and benthic detritivores (sea cucumbers) collected from different marine rocky bottom habitat types (bank, landslide, cliff) of Salina Island (Aeolian Archipelago, Italy). Also, species richness and bottom coverings by benthic species were recorded at each sampling station. Correlations among chemical composition of microplastic in sediments and in detritivores were explored linking recorded variability to the factor "habitat type". Results evidence that the habitat types considered in this study are characterized by wide species richness and by high percentages of bottom coverage by protected species by international conventions. In spite of the high ecological value of habitats considered in this study, microplastics were recorded both in sediments (PVC, PET, PE, PS, PA, PP) and in stomach contents of sea cucumbers (PET, PA) collected in all sampling sites, confirming the exposure of benthic species to microplastic pollution.

Plastics are the principal component of marine litter and microparticles originated from their degradation could represent a significant risk for marine ecosystem conservation. For this reason, Horizon 2020 identifies marine litter among the principal objectives of Marine Strategy Framework Directive (2008/56/EC). Cetaceans (Tonay et al., 2007), large pelagic fish species (i.e. *Xiphias gladius*, Thunnus sp.; Romeo et al., 2015), and small pelagic planktivorous fishes (i.e. sardines; Avio et al., 2015; sardine and anchovies; Renzi et al., 2019) ingest microplastics (hereinafter abbreviated as MPs) that are able to penetrate the marine trophic web (Ivar do Sul and Costa, 2014; Setala et al., 2014). It is reported by the literature that pelagic species prefers particles while benthic ones prefers fibres (Neves et al., 2015). Recent studies showed as benthic species are more impacted by MPs stored in sediments as Pellini et al. (2018) reported in benthic flatfish *Solea solea* MPs percentages of presence in stomach contents at 95%.

This research represents an advance of recent studies performed in the same marine protected area (Fastelli et al., 2016; Renzi et al., 2018a). Previous studies reported that over than 85% of the recorded marine litter was represented by green and black MPs fibres (20–28%). The lower MPs levels were found in sediments from Stromboli and Salina Islands while Lipari showed the highest level among studied Island from the Aeolian Archipelago (Fastelli et al., 2016). Salina Island in particular, is characterized by different marine habitat types on rocky bottoms (cliff, landslide, bank) of particular scientific interest as documented by previous researches (Renzi et al., 2018a). In these habitat types, the holothurians are representative of the marine benthic species with food habits of deposit-feeding or suspension-feeding (Purcell et al., 2016). Furthermore, under in vitro conditions, this species is reported to actively ingest plastics (Graham and Thompson, 2009). This species is considered a key species in linking sediment pollution to the marine food web. In fact, it undergoes predation by stars, crustaceans, gastropods, and fishes (Francour, 1997; Dance et al., 2003), and ingested microplastic could be transfer towards the upper trophic web by such ecological interaction with other marine species. Further studies could be useful to better understand if the series of ingestions and digestions of MPs by animals connected via a trophic web produces an increase or a decrease of the microplastic toxicity on biota. A recent study showed a relation between both the quantity and the morphological aspects of particles recorded in sediments and in stomach contents of the sea cucumbers *Holothuria tubulosa* (Renzi et al., 2018a). In landslides habitat type, a preference towards plastics within 100–2000 µm of size and a selective intake of fragments were, also, evidenced.

Starting from a solid basis represented by previous researches

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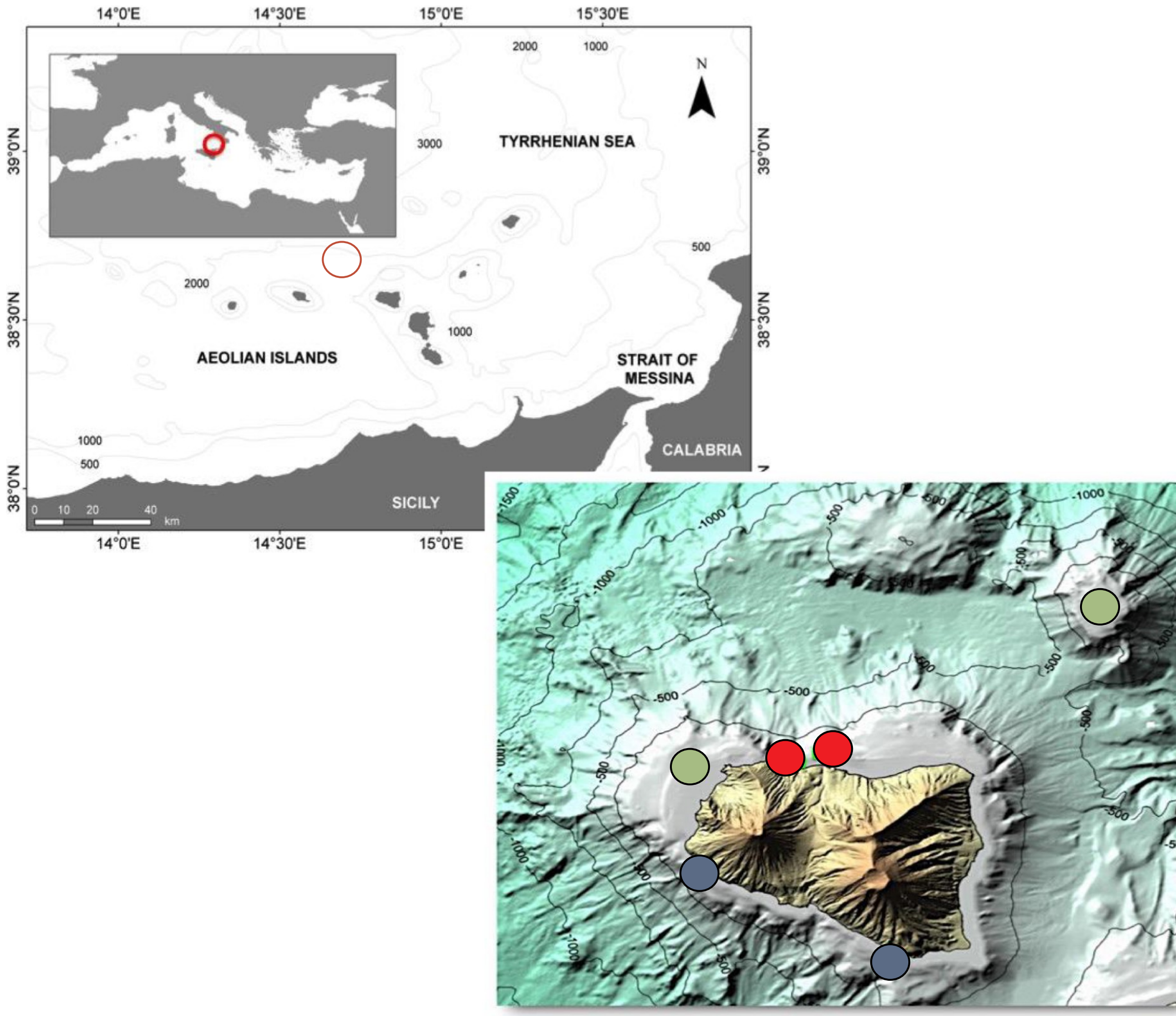

Fig. 1. Study area and sampling sites in Salina Island. Sampling sites are reported with coloured dots to highlight different position of considered habitat types. Red dots = Cliffs; Blue dots = Landslides; Green dots = banks. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

performed on Salina Island, this study aims to improve general knowledge on cliff, landslide, and banks habitats and on dynamics involving microplastic transfer towards trophic web in these ecosystems. This study determines the chemical composition of microplastic items collected in sediments and benthic species coming from three rocky bottom habitat types to evaluate significant differences among them on a statistical basis (Bosman et al., 2013). Also, sampled habitat types were characterized by a taxonomical point of view to evaluate species richness and percentages of bottom coverage by benthic species associated to studied rocky-bottom habitats. Furthermore, the presence of marine species subjected to some levels of protection was recorded to highlight the ecological relevance of the selected study area.

Three different rocky bottom habitats (bank, landslide, cliff) were sampled in September 2017 (Fig. 1) by scuba divers marine biologists according to a nested model that included three levels (habitat types = three level, fixed; site replicates = two levels, fixed; sampling replicates, three levels, random; n = 18). Samples were collected following the same sampling protocols and at the same water depth (bathymetrical range of −30 ± 1 m) of literature (Renzi et al., 2018a) to allow comparisons among our results to previous studies performed. Five animals (*H. tubulosa*) were collected per each sampling replicates and store as a single analytical pool to increase total amount of microparticles recorded (n = 5 per each sampling replicates). Taxonomical identification of collected sea cucumbers was performed following literature (Tortonese, 1996). Animals were collected by scuba divers accurately and gently cleaned on site to remove superficial sediments and external particles that could be adhere at the outer surface of the animals (i.e. sediment particles, shells, cuticula). Cleaned animals were placed in a glass bottle directly on the marine bottom. Also, sediments were collected and directly put in a glass bottle. Collected samples were stored and transported to the laboratory at +4 °C. In all tested habitat types, sands represent over than 75% d.w. while silt and clay represent lower than 1% of the total weight (Renzi et al., 2018a). Furthermore, habitat types were characterized to determine the percentage of bottom coverage and a complete taxonomic list using a square sampling unit of 50 × 50 cm of length. In this case, the same logic model was applied but random sampling replicates were ten (n = 60). Collected sediments were extracted using 200 mL of saturated NaCl pre-filtered solution (Fastelli et al., 2016), while, species tissues were extracted using 20 mL of H_2O_2 (30% per gram of tissue) incubating samples at 50 °C till the complete digestion (adapted by Nuelle et al., 2014 as reported in Renzi et al., 2018a). In both cases, extracts were filtered on a paper fiber filter disk (0.45 μm pore size) by a filtering apparatus. All activities were performed under fume hood equipped with HEPA filters to reduce cross-contaminations of samples by airborne pollution. Experimental blanks were performed per each matrix to evaluate false-positive occurrence due to indoor plastic pollution of laboratory.

Filters were dried overnight at 40 °C in oven using glass Petri dishes. After that, microparticles items on filter discs were identified by stereomicroscopy (Nikon SMZ-800 N) highlighting possible particles of interest using Bengala pink. Targeted particles were collected by micro tweezers for the successive analysis by microscopy associated to Fourier Transform Infrared Spectroscopy (μFT-IR). Nicolet iN10 MX (μFT-IR, Thermo Fischer Scientific) was equipped with liquid nitrogen cooled MCT-A operating within the spectral range 7800–650 cm^{-1} and with OMNIC Picta™ (Thermo Scientific, Waltham, MA, USA) users' interface. The "tip and shoot" operating mode was applied to improve focusing resolution. Particles with a thickness > 35 μm, were analysed by ATR (Attenuated Total Reflection) detector using a germanium crystal (spectral range 3000–1300 cm^{-1}). Particles smaller than 35 μm were analysed by transmission mode. On each field several points per particle were selected, and each collected spectrum were processed and identified using the specific reference libraries for normal and aged microplastics available in the OMNIC™ Picta™ software expanded with the in-house collected spectral library. The threshold for IR spectra back-recognition on similar spectral fingerprints was fixed over 65% of match. Limit of detection was 10 μm of particle size. Results on chemical analyses performed are reported as percentages of each plastic type in respect to the total amount recovered. Multivariate analysis was performed by Primer v6.0 (Primer-E Ltd., Plymouth Marine Laboratory, UK) routines to evaluate difference of chemical composition of microplastic in sediments and animals according to the factor of variability "habitat type". Raw data on mean values (items/kg for sediments and items/animal for sea cucumbers) per each chemical type of microparticles recorded were pre-treated by square root and successively normalized before running the multivariate analysis. Principal Component Analysis (PCA) was performed on loadings values to evaluate similarities according chemical composition of detected particles. ANOSIM tests (one-way) were performed on the Euclidean matrix of distance calculated on treated data, in order to highlight significant effects on the variability according to the factor "habitat type".

The Italian Law defines Salina island as a marine protected area of future designation (L. 979/82). Salina Island is characterized by lower microplastics levels in sediments compared to values recorded in other Aeolian Archipelago Islands (Fastelli et al., 2016). Rocky bottom habitat types considered in this study are characterized by the presence of a large variety of benthic species, also, protected ones. A taxonomic list of protected and non-protected species recorded per each habitat type is reported respectively in Table 1 and 2; data are expressed as mean percentages of coverage of bottom (+standard deviation, SD). By an ecological point of view, studied ecosystems resulted typical of marine environments of medium-high depth and are characterized by the presence of different ecological niches due to abiotic factors (i.e. light availability, geomorphology, etc.). Photophilic macroalgal species such as *Acetabularia acetabulum*, *Padina pavonica*, *Halopteris scoparia*, and *Caulerpa prolifera* were recorded in all sampled habitat types. Nevertheless, the presence of sciaphilic species such as *Flabellia petiolata* and *Palmophyllum crassum*, confirmed the occurrence of a notable microhabitat variability associated to a wide biodiversity. All tested rocky bottom habitats recorded the presence of coralligenous communities supported by the presence of *Leptopsammia pruvoti*, *Eunicella cavolini*, *Centrostephanus longispinus*, and *Ophidiaster ophidianus* (True, 1970; Ballesteros, 2006).

Protected species on the basis of a general overview are listed in IUCN, CITES and Berna Convention, and EU Habitat Directive (European protection level); while ASPIM represents a Mediterranean overview of the level of protection. It is to notice that, in Salina Island, a significant number of protected species listed in IUCN, CITES, EU Habitats Directive, Bern Convection, ASPIMIM are reported in all sampled habitat types (cliff, landslide, bank). Vulnerable species according to IUCN classification are, also, recorded in this study. Biocoenosis composition is different concerning the habitat types analysed in this study. Cliffs evidencing the presence of the largest number of protected species compared to other habitat types even if, considering the coverage of bottom, banks result covered at the highest percentage. Landslides account for the highest number of photophilic species and, consequently, for the lower number of coralligenous assemblages that are light limited (Ballesteros, 2006). Cliffs and banks show the largest number of coralligenous species such as *Peyssionnella sp.* (Balata et al., 2005), and *Meosphyllum sp.* (Ballesteros, 2006). Banks are largely populated by cnidaria (*Corynactis viridis*), echinoderms (*Antedon mediterranea*) and porifera (*Spirastrella cunctatrix*, *Haliclona sp.*).

Trends of microplastic litter recorded in both sediments and holothurians are consistent with previous researches evidencing a dominance of fibres. Nevertheless, this study records lower abundances compared to literature (Fastelli et al., 2016; Renzi et al., 2018a). This study evidences a mean number of total MPs in sediments of 49.0 ± 1.4 items/kg (landslides), 153.5 ± 41.7 items/kg (cliff), and 106.0 ± 104.7 items/kg (banks). Holothurian species evidence a mean stomach content of MPs of 1.3 ± 0.9 items/animal (landslides), 3.8 ± 0.7 items/animal (cliff), and 2.47 ± 0.3 items/animal (banks). Considering that the total amount of sediment present inside the holothurian species is included within 10–20 g, the total amount of microplastic items recorded per animal is consistent with levels measured in sediments. Lower levels recorded in sediments are probably related to the fact that μFT-IR was used to confirm the whole amount of recovered microplastics in this matrix. Extraction performed by NaCl induces larger matrix interference by the natural component of the organic matter present in this matrix that could produce overestimations reported by the literature. The largest difference recorded among items targeted as MPs by stereomicroscopy and those confirmed by μFT-IR resulted due to fibres. Almost the 85% of fibres < 500 μm targeted by stereomicroscopy resulted to be, after the chemical analyses, cellulose or cellulose acetate. These results evidence as chemical analysis should not be avoided for particles identification lower than 500 μm as also reported by the literature (Eriksen et al., 2013; Wirnkor et al., 2019).

Table 1
Protected species and bottom coverage in habitat types. Data on bottom coverages are reported as percentage of bottom coverage by the species compared to the total surface of bottom sampled. Notes: Levels of protection associated to the listed species: 1 = IUCN; 2 = CITES; 3 = EU Habitats Directive; 4 = Bern Convection; 5 = ASPIM Control. SD = standard deviation; sampled surface = square units 50 × 50 cm; random replicates per sampling sites (n = 100).

		Level of protection	Landslides		Cliff		Banks	
Protected species	Phylum		Mean	SD	Mean	SD	Mean	SD
Balanophyllia europaea	Cnidaria	1 (LC), 2	0.00	0.00	0.15	0.41	0.00	0.00
Cladopsammia rolandi	Cnidaria	2	0.00	0.00	4.50	11.21	8.00	10.83
Eunicella cavolini	Cnidaria	1 (NT)	2.77	7.90	0.25	1.00	0.00	0.00
Leptopsammia pruvoti	Cnidaria	1 (LC), 2	0.00	0.00	0.75	3.00	2.40	7.59
Palinurus elephas	Arthropoda	1 (VU)	0.00	0.00	0.38	1.50	0.00	0.00
Centrostephanus longispinus	Echinodermata	3,4,5	0.00	0.00	0.38	1.50	0.40	1.26
Ophidiaster ophidianus	Echinodermata	4,5	0.31	1.11	0.50	1.15	0.00	0.00

Table 2
Non-protected species and bottom coverage in habitat types. Data on bottom coverages are reported as percentage of bottom coverage by the species compared to the total sampled surface. SD = standard deviation; sampled surface = square units 50 × 50 cm; random replicates per sampling sites (n = 100).

		Landslides		Cliff		Banks	
Specie	Phylum	Mean	SD	Mean	SD	Mean	SD
Acetabularia acetabulum	Chlorophyta	0.08	0.28	0.15	0.41	0.20	0.63
Caulerpa prolifera	Chlorophyta	0.00	0.00	0.63	2.03	2.40	5.06
Codium bursa	Chlorophyta	4.15	5.57	0.00	0.00	0.00	0.00
Flabellia petiolata	Chlorophyta	2.92	4.59	9.75	8.29	0.60	1.35
Halimeda tuna	Chlorophyta	0.92	1.55	1.20	2.24	0.32	1.01
Palmophyllum crassum	Chlorophyta	0.00	0.00	1.50	6.00	2.00	3.40
Cystoseira sp.	Ochrophyta	0.13	0.50	0.00	0.00	0.00	0.00
Halopteris scoparia	Ochrophyta	5.85	16.86	0.00	0.00	0.00	0.00
Padina pavonica	Ochrophyta	8.92	15.22	0.25	1.00	1.20	2.70
Sporochnus pedunculatus	Ochrophyta	2.15	5.26	0.00	0.00	4.00	12.65
Zonaria tournefortii	Ochrophyta	0.92	2.40	0.25	1.00	0.00	0.00
Mesophyllum sp.	Rhodophyta	0.62	1.50	3.88	5.68	4.40	13.91
Peyssonnelia sp.	Rhodophyta	1.38	2.75	1.25	2.82	2.40	5.06
Seirospora sp.	Rhodophyta	2.15	6.66	0.00	0.00	7.60	13.53
Dysidea sp.	Porifera	0.00	0.00	0.13	0.50	0.00	0.00
Haliclona sp.	Porifera	2.00	5.03	0.75	3.00	6.00	13.76
Ircinia sp.	Porifera	1.85	6.66	0.00	0.00	0.00	0.00
Phorbas tenacior	Porifera	0.00	0.00	0.75	3.00	0.00	0.00
Spirastrella cunctatrix	Porifera	0.31	1.11	1.13	2.53	8.00	25.30
Aiptasia mutabilis	Cnidaria	0.25	0.89	0.00	0.00	0.40	1.26
Alicia mirabilis	Cnidaria	0.31	1.11	0.00	0.00	0.00	0.00
Corynactis viridis	Cnidaria	0.00	0.00	0.00	0.00	9.60	30.36
Umbraculum umbraculum	Mollusca	0.00	0.00	0.13	0.50	0.00	0.00
Bonellia viridis	Anellida	0.00	0.00	0.25	1.00	0.00	0.00
Anseropoda placenta	Echinodermata	0.00	0.00	0.25	1.00	0.00	0.00
Antedon mediterranea	Echinodermata	0.00	0.00	0.00	0.00	1.00	2.54
Hacelia attenuata	Echinodermata	0.31	1.11	0.00	0.00	0.00	0.00

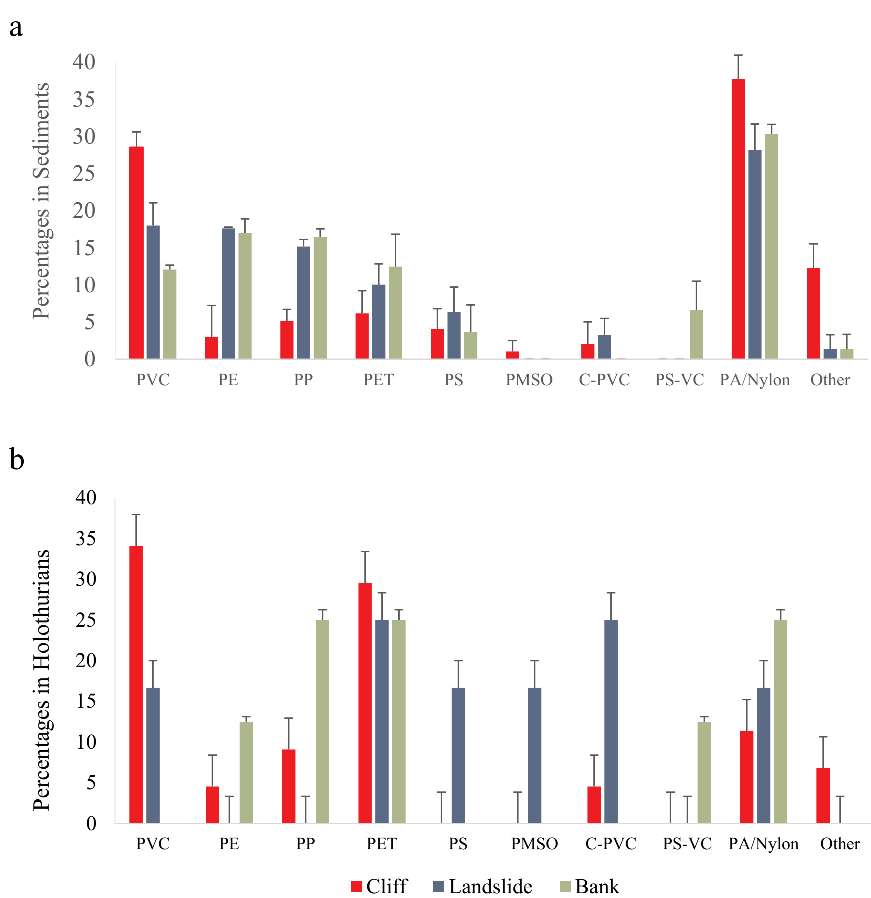

Fig. 2. Chemical compositions percentage of microplastic recorded in sediments and holothurians. Data are expressed as percentage of recorded chemical type over the total amount recorded. Mean results (+standard deviation) are grouped according to the factor of variability "Habitat type" considered.

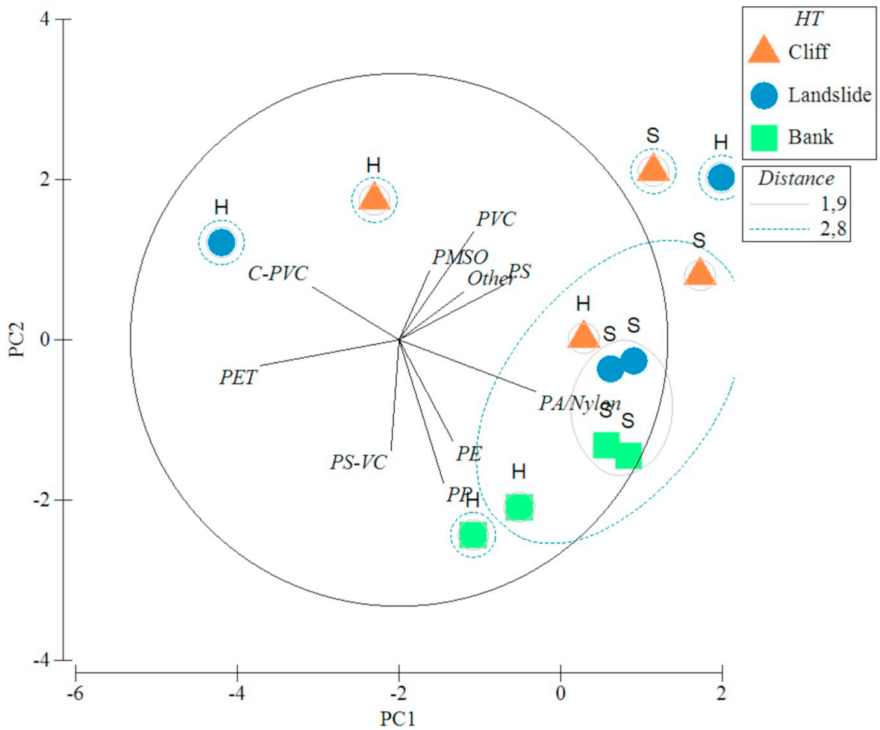

Fig. 3. Principal component analysis (PCA) performed on the chemical composition of microplastics. PCA is performed on pre-treated (square root) and normalized mean data. Results are targeted according to the matrix (H = holothurian; S = sediment) and habitat type (cliff, landslide, bank). Cluster analyses (Simprof test) significant results are superimposed to the two-dimension PCA projection and distances are highlighted.

In Fig. 2a, and b, the chemical compositions respectively of MPs recorded in sediments and in holothurians are reported. Principal component analysis (PCA) performed on pre-treated and normalized data is reported in Fig. 3. The first three axes accounted for the 73.4% of the total variability. The first axis was positively related to PA/Nylon (0.509), and negatively related to PET (−0.517). On the contrary, the second axis was positively related to PVC (0.404) and negatively related to PP (−0.537) and PS-VC (−0.415). Chemical analyses performed on MPs recovered from sediments and sea cucumbers from different habitat types evidencing a different MPs fingerprint among banks and other habitat types. The ANOSIM (one-way) test performed on the factor of variability "habitat types" evidenced significant differences (Global R = 0.181; significance level of sample statistic P = 2.7%). The pairwise test performed to evaluate differences between couples of habitats evidenced significant differences between cliff and banks (Global R = 0.406; P = 2.9%) and between landslide and banks (Global R = 0.198; P = 2.9%); on the contrary cliffs and landslides resulted not significant (Global R = −0.031; P = 57.1%).

Our study evidences that chemical composition of plastic in sediments is different according to the habitat types. Exception made for PA/Nylon that resulted to be the principal fraction of the MPs amount recorded; cliffs resulted largely represented by PVC. On the contrary, landslides and banks are represented by almost all the other recorded materials. In sediments the largest part of recorded items are PA and nylon fibres in almost all tested habitat types confirming data recorded by the literature showing that the largest part of MPs in sediments are fibres (Renzi et al., 2018a, 2018b). Microfibres recorded in marine environments are, in large part, originated by household washing of synthetic fabrics (Corami et al., 2019). This research shows a different chemical composition of MPs recorded in sediments from banks compared to cliffs and landslides and this is probably due to the higher distance of banks from the coastline of Salina Island compared to other habitat types that are located closer to the Island.

As regards as chemical composition of MPs recorded in holothurians, animals from cliffs are largely represented by PVC and PET. Animals from landslides evidenced percentages within 15–25% for PET, PVC, C-PVC, PS, PMSO, and PA/Nylon. Animals from banks are largely represented by PP, PE, PET, PA/Nylon. By a comparison between the chemical composition of MPs recorded in sediments and those recorded in holothurians, resulted a higher percentage of PET in animals' stomach contents than in the surrounding sediments. This fact could be related to the feeding behaviour of this species. The occurrence of a selective predation behaviour of some colours and shape of microplastic (i.e. black and blue filaments and fragments) was suggested by literature for sardines and anchovies (Renzi et al., 2019). Graham and Thompson (2009) reported that holothurians preferably predate plastic fragments selecting them by other plastic shapes by tentacles. Results reported in this study evidenced a significant effect on MPs chemical composition in both sediments and holothurians. These results could be due both to different ecological characteristic of the habitat types (i.e. currents, distance from coastline, etc.) and to different attitudes of holothurians to selectively intake some MPs types than others that could produce recorded differences. Furthermore, also the occurrence of fragmentation during digestion of ingested particles could represent a possible cause of recorded differences among abundances. These aspects need specific and opportunely focused studied are object to further researches to be better explained.

Due to the key role of holothurians in marine ecosystems these animals are now protected species by the Italian Law n. 156 (27/02/2018, Ministry of Agriculture and Forestry) till 31/12/2019 to avoid human overexploitation. Results from our study could be of particular interest as they analysed microplastic exposure of a marine protected taxa of particular concern for benthic trophic web in highly relevant ecosystems by an ecological point of view such rocky bottom habitats analysed.

CRediT authorship contribution statement

Monia Renzi: Conceptualization, Project administration, Supervision, Writing - original draft, Writing - review & editing, Funding acquisition. **Andrea Blašković:** Resources. **Andrea Broccoli:** Data curation, Formal analysis. **Giulia Bernardi:** Investigation, Conceptualization. **Eleonora Grazioli:** Data curation, Formal analysis. **Giovanni Russo:** Conceptualization, Supervision.

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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