Science of the Total Environment 902 (2023) 166043

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Marine sponges as bioindicators of pollution by synthetic microfibers in Antarctica

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HIGHLIGHTS

- Microplastics are present in Antarctic waters and biota.
- First quantitative data on contamination of Antarctic sponges by microplastics
- Aramide fibers or micro-bioplastics found by mass-based depolymerization protocol
- Local sources could be main contributors to microplastics pollution in Antarctica.
- Systematic monitoring of microplastics in Antarctic waters is urgently needed.

GRAPHICAL ABSTRACT

ARTICLE INFO

Editor: Damià Barceló

Keywords:
Microplastics
Wastewater
Porifera
Biofiltrators
Human impact
Ocean

ABSTRACT

Different marine sponge species from Tethys Bay, Antarctica, were analyzed for contamination by polyester and polyamide microplastics (MPs). The PISA (Polymer Identification and Specific Analysis) procedure was adopted as it provides, through depolymerization and HPLC analysis, highly sensitive mass-based quantitative data. The study focused on three analytes resulting from the hydrolytic depolymerization of polyesters and polyamides: terephthalic acid (TPA), 6-aminohexanoic acid (AHA), and 1–6-hexanediamine (HMDA). TPA is a comonomer found in the polyesters poly(ethylene terephthalate) (PET) and poly(butylene adipate co terephthalate) (PBAT), and in polyamides such as poly(1,4-*p*-phenylene terephthalamide) (Kevlar™ and Twaron™ fibers) and poly (hexamethylene terephthalamide) (nylon 6 T). AHA is the monomer of nylon 6. HMDA is a comonomer of the aliphatic nylon 6,6 (HMDA-*co*-adipic acid) and of semi-aromatic polyamides such as, again, nylon 6 T (HMDA-*co*-TPA). Except for the biodegradable PBAT, these polymers exhibit high to extreme mechanical, thermal and chemical resistance. Indeed, they are used as technofibers in protective clothing able to withstand extreme conditions as those typical of Antarctica. Of the two amine monomers, only HMDA was found above the limit of

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https://doi.org/10.1016/j.scitotenv.2023.166043
Received 16 June 2023; Received in revised form 2 August 2023; Accepted 2 August 2023
Available online 5 August 2023

quantification, and only in specimens of *Haliclona* (*Rhizoniera*) *scotti*, at a concentration equivalent to 27 μg/kg of nylon 6,6 in the fresh sponge. Comparatively higher concentrations, corresponding to 2.5–4.1 mg/kg of either PBAT or PPTA, were calculated from the concentration of TPA detected in all sponge species. Unexpectedly, TPA did not originate from PET (the most common textile fiber) as it was detected in the acid hydrolysate, whereas the PISA procedure results in effective PET depolymerization only under alkaline conditions. The obtained results showed that sponges, by capturing and concentrating MPs from large volumes of filtered marine waters, may be considered as effective indicators of the level and type of pollution by MPs and provide early warnings of increasing levels of pollution even in remote areas.

1. Introduction

Concerns about the pollution by macro- and microplastics (MPs) in Antarctica have resulted in increasing attention of the scientific world over the last years, since the first reports on the presence of these pollutants in surface marine waters (Barnes et al., 2010), and sediments (Thompson et al., 2009), and later even in freshwaters (Citterich et al., 2023). Contamination by plastic debris in subsurface Antarctic waters (sampled at a 5 m depth) was confirmed more recently by a study of Cincinelli et al. (2017) concerning the coastal and open sea areas of the Ross Sea and Tethys Bay. The collected polymer microparticles were identified by means of micro FT-IR. The most abundant polymer types were polyethylene (PE), polypropylene (PP), polyesters (presumably microfibers of polyethylene terephthalate, PET), polytetrafluoroethylene (PTFE), poly(methyl methacrylate) (PMMA), and polyamides (PAs). It was also found that the highest concentration of fibers was present in subsurface water samples taken in the Ross Sea near the wastewater effluent of the Italian research base Mario Zucchelli, facing the Terra Nova Bay. The presence of micro- and macroplastics, in the form of fibers, films and fragments with size between 0.3 and 22.0 mm, was also detected at decreasing concentration in seabed sediments sampled at increasing distance from the same research base (Munari et al., 2017). Various polymer types, including PE, PP, nylon 6,6 (a polyamide), poly(styrene-butadiene-styrene) (SBS and/or SBR), polyvinyl chloride (PVC), polystyrene (PS), thermoplastic polyurethane (TPU), polyvinyl alcohol (PVA), and ethylene-propylene rubber (EPR) were identified by ATR FT-IR spectroscopy. Among them, SBS (typically as tire wear particles) was the most abundant at the sampling site closest to the base, while nylon and TPU (possibly as textile fibers) were the most abundant at the farthest sampling sites. It was hypothesized that the origin of the pollution was the gray water of the purification plant of the American base. Given the importance of the Antarctic continent for the preservation of natural environments not altered by human activities, the actual origin of plastic debris pollution has been highlighted as a critical issue to be addressed (Obbard, 2018; Rota et al., 2022). Data available in Antarctica are still so limited that the Scientific Committee on Antarctic Research has recently created an action group to coordinate more systematic investigations and plan appropriate measures against MPs pollution (https://www.scar.org/science/plastic/about/).

Sampling surface seawater alone, e.g., with neuston nets and similar devices, can lead to large underestimates of MPs abundance, since those in the accumulation zones, i.e. seabed sediment and water column, are not accounted for (Gallo et al., 2018). Indeed, it has been postulated that only one twentieth of the total number of MPs polluting the oceans are to be found floating at or close to the surface, with about the same fraction distributed in the water column, and the remaining deposited in seafloor and beaches (Andrady, 2011; Pabortsava and Lampitt, 2020). To be positively buoyant plastics must have a specific gravity lower than that of seawater (≈1.025 g/mL). Denser varieties of plastics such as polyesters and PAs tend to sink in the water column and down to the sediment, unless transported to coastal deposits. Wright et al. (2013) noted that benthic suspension and deposit feeders may be exposed to high-density MPs and other lower density but biofouled ones that sink to the benthos. Microalgae and biofilm attached to MPs are then assumed to make such particles more easily captured by filter feeders than free MPs (von Moos et al., 2012; Farrell and Nelson, 2013; Goldstein and Goodwin, 2013).

The nearly ubiquitous presence of MPs in the marine environment and their ingestion by filtering and detritivorous marine organisms are determining the diffusion of micro and nano-plastics along the trophic chain, from the zooplankton (Absher et al., 2019) up to the tissues of marine vertebrates (Bessa et al., 2019), to humans. Such contamination poses an emergent threat to the stability of the ecosystems, as it is not limited to the direct effect of the particulate but it may also act as a source of molecular pollutants. Indeed, MPs are potential adsorbers and concentrators of toxic pollutants from contaminated environments (Fu et al., 2021; Hartmann et al., 2017) as well as direct sources of toxic or otherwise biodisruptive low molecular weight degradation products (Biale et al., 2022; Khan and Jia, 2023).

The environmental contamination level by MPs could be efficiently studied using bioindicators, and among them by filtering and detritivorous ones that can act as concentrators of contaminants from sediments and water (Belfroid et al., 1995). Much of the existing research on MPs ingestion has revolved around aquatic vertebrates, with fish being the most studied group of organisms (de Sá et al., 2018), although an increasing number of investigations focuses on MPs ingestion by marine invertebrates (Sussarellu et al., 2015; Hankins et al., 2018; Rotjan et al., 2019).

Contamination by plastic debris in Antarctica has been ascertained in benthic organisms (Sfriso et al., 2020) and evaluated as potential food availability for pelagic amphipods (crustacea) (Jones-Williams et al., 2020). In the first case, the study included 12 different species characterized by different filtration feeding rates, sampled along with the embedding sediment with a van Veen grab in the Ross Sea. According to the micro-FTIR analyses, 83 % of the samples showed some level of contamination by MPs (from 10 to 3290 particles/g, ranging in size from 33 to 1000 μm, with a maximum of between 50 and 100 μm). Polyamides, both aromatic (typical of technical fibers and filaments) and aliphatic, accounted for 86 % of the polymer particles, the remaining ones consisting of PE (5 %) and other polymers identified as polyoxymethylene, phenolic resin, PP, and PS (overall around 3 %). In the second study, aimed at assessing the amount of micro- and meso-plastics as a potential food contaminant for pelagic amphipods, an average concentration of 0.019 particles/m^3 of water was estimated at Antarctic Peninsula sampling sites compared to an average value three times lower at sampling points in the open ocean. Comparable contamination values had previously been observed on the surface waters of the Southern Ocean (Isobe et al., 2017; Lacerda et al., 2019; Suaria et al., 2020). While low-density MPs (PP and PE) are likely of remote origin, and thus characterized by prolonged exposure to environmental degradation conditions, high-density MPs (e.g., acrylics, epoxy, polyurethane, polycarbonate, etc.) are most probably from local sources (Jones-Williams et al., 2020), including naval and fishing activities. Transport of MPs towards Antarctica may occur by means of oceanic currents from the northernmost regions (Waller et al., 2017), as it is the case for other types of pollutants.

Poriferans, commonly known as sponges, phylum Porifera, are very effective filtering organisms. They colonize all types of aquatic environments: from polar areas to tropical ones, from mesophotic infralittoral environments to bright coral reefs, generally preferring shallow

waters of the continental shelf, but colonizing even at depths around 7000 m (Hooper and van Soest, 2002; van Soest et al., 2012; de Sá et al., 2018). Sponges often exhibit high water pumping rates (0.005–0.6 $L{\cdot}s^{-1}{\cdot}L^{-1}$ of sponge tissue) through their aquiferous systems for feeding, breathing, and eliminating waste (Pawlik et al., 2018). Because of their widespread distribution, ability to retain small particles (typically smaller than 70 μm as the sponge ostia rarely exceed 60 μm preventing intake of larger particles) (Ribes et al., 1999) as potential food source (Kowalke, 2000), and their high volume seawater filtering capability, they have been central in the biomonitoring "Sponge Watch Program", which aims at evaluating the effects of various types of pollutants on the sponges selected as bioindicators (Belfroid et al., 1995).

Few studies have examined the ingestion of MPs by marine sponges, either to assess the effects of this class of pollutants on the health of porifera (Baird, 2016) or to evaluate the sponges as bioindicators of environmental pollution (Karlsson et al., 2017; Celis-Hernández et al., 2021; Girard et al., 2021; Saliu et al., 2022). In a laboratory study Baird (2016) did not find any detectable impact on sponge respiration or food particle retention in the temperate sponges *Tethya bergquistae* and *Crella incrustans* exposed to 1 and 6 μm plastic beads at two different concentrations (200,000 and 400,000 beads mL^{-1}). Later on Girard et al. (2021) found up to 612 foreign particles g^{-1} of dry sponge tissue in samples from North Sulawesi, Indonesia; the particles, including PS MPs, were incorporated in both skeletons and other internal tissues. Indeed, Modica et al. (2020) found microfibers embedded on the surfaces of preserved museum sponge specimens, representing 31 families, collected off the northern coast of Spain >20 years ago. Saliu et al. (2022) adopted a multi-step protocol to isolate by flotation and sequential microfiltration the MPs from Maldivian sponges. The MPs with size >25 μm were analyzed by number and type using the micro-FTIR technique, while those in the size range 0.2–25 μm were recovered from the microfilters through pressurized solvent extraction and analyzed by Pyrolysis-Gaschromatograpy-Mass Spectrometry. The latter technique provides information on the total mass of MPs, mainly (but not limited to) those based on fully carbon main chain polymers such as PVC, PS, PE and PP.

In this study, we have analyzed the contamination of sponges collected in Antarctica in two subsequent expeditions and three different sampling sites in Tethys Bay (Terra Nova Bay, Ross Sea). Given the extremely low concentration, if any, of MPs expected in such remote sites, the study was focused on the highly accurate and sensitive total mass quantification of condensation polymers according to the Polymer Identification and Specific Analysis (PISA) protocol (Castelvetro et al., 2021a). The section of the PISA procedure aiming at condensation polymers entails their hydrolytic depolymerization, followed by purification and HPLC analysis of characteristic monomers such as terephthalic acid (TPA, as in the polyester PET, but also in aromatic PAs containing terephthalic acid as a comonomer) and the polyamide monomers 6-aminohexanoic acid (AHA, from nylon 6) and 1,6-diaminohexane or hexamethylenediamine (HMDA, as in nylon 6,6). The polyester PET and the PAs nylon 6 and nylon 6,6 are the most common polymers for textile and technical fibers, while extreme performance technical fibers are typically based on semi-aromatic or fully aromatic PAs. To the best of our knowledge, this is the first work to report the presence of microplastics in sponges inhabiting Antarctica, a continent considered, until a few years ago, pristine.

2. Materials and methods

2.1. Sponge specimens

The quantitative determination of polymers characteristic of synthetic microfibers, such as PET (an aromatic-aliphatic polyesters) and PAs in general, was carried out on samples of Antarctic Porifera belonging to different species. The samples were collected in two different sites (inner, INN; outer, OUT) in Thetys Bay during the 33rd (2017–2018, in red in Fig. 1) and 34th (2018–2019, in green in Fig. 1) Italian expeditions to Antarctica. The details on sampling sites and time of sampling, physical and chemical parameters of seawater during sampling, and identification of the sampled porifera, are reported in Table 1.

The collection of animal specimens was carried out by gently removing the porifera from their substrate, taking care not to harm the animal's body. The collected individuals were carefully washed with distilled water to remove detritus from the surface, wrapped in aluminum foil previously decontaminated by washing with hexane and acetone, and then stored at −25 °C prior to their analyses. A fragment of each sponge was preserved in 70 % ethanol for the subsequent taxonomic identification and deposited in the National Antarctic Museum (MNA) Section of Genoa (Italy), assigning a museum code (Table S1). Analyses were performed in Italy at the Department of Chemistry and Industrial Chemistry of the University of Pisa for the determination of MPs types and content.

The sponge samples were freeze dried and gently homogenized in a ceramic mortar. For the analyses, 1–5 g of dried powder from each sample was placed in a sonication bath and extracted with 30 mL n-hexane/acetone 1:1 mixed solvent for 40 min at 45 °C. The residue was recovered by centrifugation and the supernatant set aside, then the residue was further extracted twice as in the first step, but using 30 mL of pure n-hexane in each step. The supernatant fractions were filtered at 0.22 μm pore size PVDF filter and the collected particulate was added to the residue, to exclude any loss of particles above 0.22 μm which was therefore the lower detection threshold in the adopted procedure. This extraction procedure was carried out to ensure the removal of most of the low molecular weight organic pollutants, including the nearly ubiquitary phthalate esters that might act as interferents in the subsequent analyses.

Single species sponge samples have been reconstituted by combining freshly collected individual specimens (also from different sampling sites and time in the case of *D. antarctica*) from the 33rd and 34th Italian expeditions in Antarctica. In Table S1 of Supplementary Material are reported the details of the reconstituted sponge species.

2.2. Instrumentation

HPLC analyses were performed with an Agilent 1260 Infinity Binary LC instrument equipped with diode array (DAD VL+ 1260/G1315C) plus fluorescence (FLD 1260/G1321B) double detector. ATR FT-IR spectra were recorded with a Perkin Elmer (PerkinElmer Italia Spa, Milano, Italy) Spectrum GX spectrometer equipped with a MIRacle TM ATR accessory (angle of incidence 45°) and a germanium crystal. The spectra were recorded over the wavenumber range of 600–4000 cm^{-1} using a spectral resolution of 4 cm^{-1}. Automatic baseline and smooth corrections were applied after 128 scans. All spectra were processed using Spectrum 5.0 software (PerkinElmer).

2.3. Microscopic inspection of freeze-dried sponge specimen and general procedural precautions

The freeze-dried sponge samples were inspected with a Konus Cristal Pro 5424 stereomicroscope to exclude the presence of any plastic fragments larger than 500 μm. All laboratory operations were carried out with particular care in order to avoid external contamination: operators wore exclusively cotton lab coats; exposure of open surfaces (solids and solutions) to the lab environment was limited to a minimum; glassware and other lab equipment was previously rinsed with water and acetone to remove any contaminating fibers or particulate.

2.4. Chemicals

Methanol (99.9 %, Sigma-Aldrich, Milan, Italy), and n-hexane (99.9 % Sigma-Aldrich, Milan, Italy), were used for the preliminary extraction

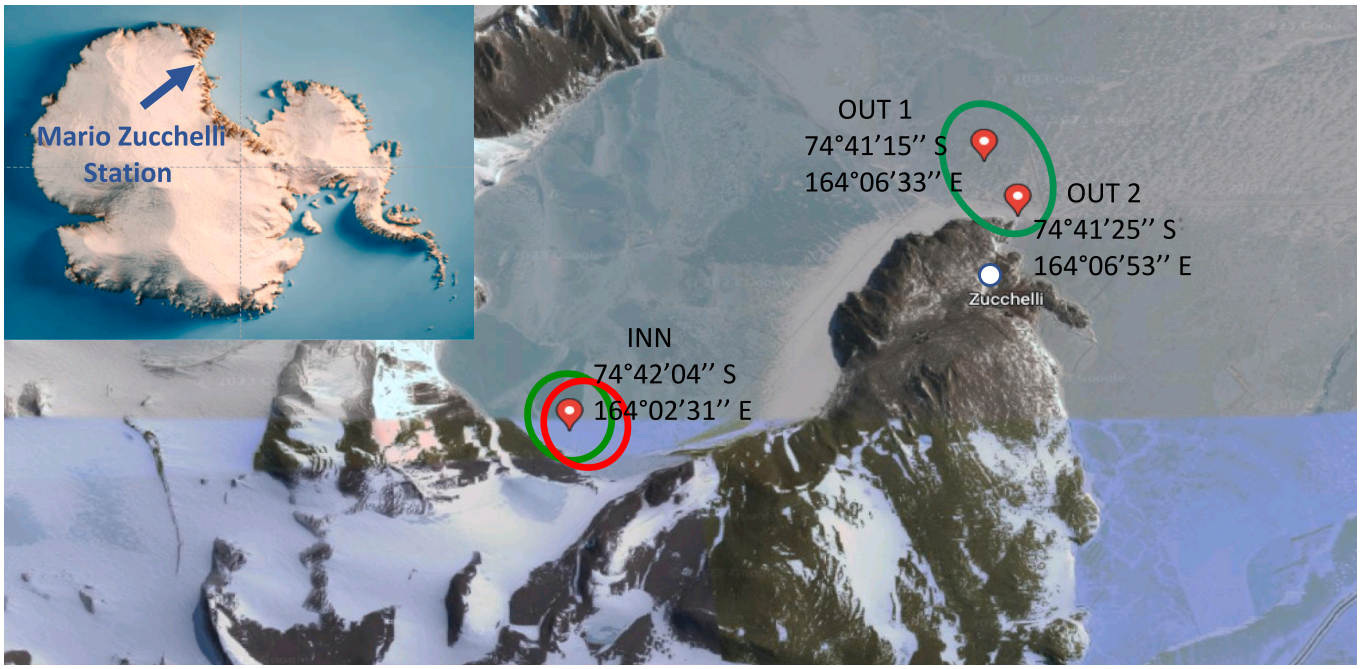

Fig. 1. Geographic map of sampling sites. Red and green circles indicate the 2018 and the 2019 campaign, respectively.

Table 1
Collected Porifera species, sampling sites, and seawater chemical-physical parameters at sampling time.

Site/ expedition	Coordinates	Sampling depth (m)	Sponge taxonomy	Sample ID	MNA code	pH	Conductivity (mS/cm)	T (°C)	O_2 (ppm)
INN (33rd)	74°42,067′ S 164°02,518′ E	25	*Dendrilla antarctica* Topsent, 1905	A1	MNA 11764	–	–	–	–
			Dendrilla antarctica Topsent, 1905	A2	MNA 11765				
			Dendrilla antarctica Topsent, 1905	A3	MNA 11766				
OUT1 (34th)	74°41,252′ S 164°06,548′ E	29	*Dendrilla antarctica* Topsent, 1905	1Sp3a	MNA 11748	6.85	43.8	1.6	10.45
			Haliclona (*Rhizoniera*) *scotti* (Kirkpatrick, 1907)	1Sp1a	MNA 11744				
			Haliclona (*Rhizoniera*) *scotti* (Kirkpatrick, 1907)	1Sp1b	MNA 11745				
			Haliclona (*Rhizoniera*) *scotti* (Kirkpatrick, 1907)	1Sp1c	MNA 11746				
OUT2 (34th)	74°41,416′ S 164°06,880′ E	29	*Dendrilla antarctica* Topsent, 1905	1Sp3c	MNA 11758	7.03	54.3	−1.6	11.61
			Dendrilla antarctica Topsent, 1905	1Sp3b	MNA 11757				
			Microxina sarai Calcinai & Pansini, 2000	1Sp2c	MNA 11756				
			Microxina sarai Calcinai & Pansini, 2000	1Sp2b	MNA 11755				
INN (34th)		25	*Dendrilla antarctica* Topsent, 1905	2Sp3a	MNA 11749	6.9	47.4	−1.6	15.50
			Dendrilla antarctica Topsent, 1905	2Sp3b	MNA 11750				
			Dendrilla antarctica Topsent, 1905	2Sp3c	MNA 11754				
			Mycale (*Oxymycale*) *acerata* Kirkpatrick, 1907	2Sp4a	MNA 11751				
			Mycale (*Oxymycale*) *acerata* Kirkpatrick, 1907	2Sp4b	MNA 11752				
			Mycale (*Oxymycale*) *acerata* Kirkpatrick, 1907	2Sp4c	MNA 11753				

of sponge tissue. HPLC-MS grade water (99.9 %) used to prepare the 6 N hydrochloric acid (37 %) and 1.9 N sodium hydroxide (98.0 %) solutions for hydrolytic depolymerization of PAs and polyesters, respectively, and the phase transfer catalyst hexadecyl-tributyl-phosphonium bromide (TBHDPB 97 %) were all purchased from Sigma-Aldrich. Hydrogen peroxide (30 % *v*/v, Pancreac, Nova Chimica, Cinisello Balsamo, Italy) and 2 N sulfuric acid (95–98 %, Sigma-Aldrich, Milan, Italy) were used in the pre-treatment of alkaline hydrolysates to remove biogenic

material. The fluorophore 5-dimethylaminonaphthalene-1-sulfonyl-chloride (dansyl chloride, DNSCl, 96 %, Alfa Aesar, Kandel, Germany), acetone (99 %, Sigma-Aldrich, Milan, Italy), potassium carbonate (K_2CO_3 98 %, Carlo Erba Reagents, Milan, Italy), and n-butylamine (99 % Fluka, Milan, Italy) were used for the derivatization of amino compounds.

A commercial PET sample produced by cryogenic micronization (average size 509 μm, a kind gift from Poliplast, Casnigo, BG, Italy) and the two powdery PAs poly(hexamethylene adipate) (nylon 6,6) and poly (6-aminohexanoic acid) (nylon 6), both from Sigma-Aldrich, were used as reference polymers. Terephthalic acid (TPA, 98 %, Sigma-Aldrich, Milan, Italy), 1,6-diamino-hexane (HMDA, 99 %, Fluka, Milan, Italy), and 6-aminocaproic acid (AHA, 99 %, Sigma-Aldrich, Milan, Italy) were used as monomeric reference compounds. Methanol (99.9 %) and acetonitrile (99.9 %) HPLC grade solvents (Sigma-Aldrich, Milan, Italy), triethylamine (99.9 % Fluka, Milan, Italy), and acetic acid were used for the mobile phases in HPLC analysis.

Bond Elut C18 cartridges loaded with 500 mg stationary phase (Agilent Technologies, Santa Clara, CA, USA) were used for the purification and pre-concentration of TPA in the alkaline hydrolysates.

2.5. Acid hydrolysis and polyamides quantification procedure

A weighed amount (1.5–4.0 g) of each sponge sample previously extracted with acetone and hexane is transferred into a 100 mL round-bottomed flask equipped with a reflux condenser and a magnetic stirring bar. After the addition of a known volume (10–50 mL) of aqueous 6 N HCl, the mixture is heated to the reflux temperature of about 105 °C for 24 h under stirring at 500 rpm. At the end of hydrolysis, the mixture is vacuum filtered on a 0.22 μm, 47 mm diameter PVDF filter membrane (Durapore®, Merck, Darmstadt, Germany) to separate the solid residue from the acid solution. The solid residue on the filter membrane is thoroughly rinsed with HPLC/MS grade water and set aside in a closed Petri dish for subsequent treatments. After weighing the acid solution, 5.0 mL of it is taken up, weighed and then neutralized at pH 6.5–7.5 with 6 N NaOH using a burette; the obtained solution is centrifuged (3000 *g* for 5 min) to remove any precipitate and brought to 10 mL with deionized water in a volumetric flask. To enable a highly sensitive quantification, the aminated species present in the solution, including the monomers of nylon 6 (AHA) and nylon 6,6 (HMDA), are then derivatized with the fluorescent amino-tag DNSCl. For this purpose, 500 μL of the neutralized solution is transferred in a 4.0 mL glass vial and added sequentially with 1.0 mL of 18.5 mM DNSCl solution in acetone and 1.0 mL of aqueous 116 mM K_2CO_3 solution. After 30 min of stirring at room temperature in the dark, a slight excess (5.0 μL, 51 μmol) of n-butylamine is added to quantitatively convert the unreacted DNSCl. Finally, the solution is transferred in a 10 mL volumetric flask and brought to the volume with a water/acetone 1:1 mixture for the HPLC analysis.

HPLC analysis for quantification of AHA and HMDA from the PAs was performed using a Phenomenex-Aqua (Phenomenex, Castel Maggiore, Italy) reversed-phase column (length 25 cm, internal diameter 4.6 mm, particle size 5 μm). The injected 20 μL of sample solution were eluted at a flow rate of 1.0 mL/min in gradient mode, by combining an aqueous solution of 2.5 vol% acetic acid and 0.83 vol% triethylamine (phase A) with acetonitrile (phase B). The elution program adopted was as follows: 0 → 20 min isocratic with 60 % phase A and 40 % phase B; 20 → 30 min gradient to reach a composition of 30 % phase A and 70 % phase B; 30 → 40 min isocratic with 30 % phase A and 70 % phase B; 40 → 50 min gradient to reach 60 % phase A and 40 % phase B, followed by further 10 min isocratic for column equilibration. For the analysis of dansylated derivatives, the HPLC instrument was equipped with a fluorimetric (FLD 1260/G1321B, set at excitation/emission wavelength of 335/522 nm) detector.

2.6. Alkaline hydrolysis and PET quantification procedure

For the alkaline hydrolytic depolymerization of PET in the analyzed sponge specimens, the dried solid residue (0.5–2.7 g) from each acid hydrolysis was transferred into a 100 mL round-bottomed flask equipped with a reflux condenser and magnetic stirring bar. After the addition of a given volume (15–50 mL) of 2 N NaOH and 0.3 mL of 75.0 mM hexadecyl-tributyl-phosphonium-bromide (TBHDPB, a phase transfer catalyst) aqueous solution, the mixture was stirred 6 h at 85 °C and 500 rpm. After cooling at room temperature, the suspension was vacuum filtered through a 0.22 μm, 47 mm diameter PVDF filter membrane, and the solid residue carefully rinsed twice with small amounts (2–3 mL) of the same NaOH solution, then the filtered liquid fractions were joined in a volumetric flask (50 or 100 mL, depending on the initial amount of alkaline solution) that was brought to the volume with the same NaOH solution. To remove any excess of residual biogenic material as a potential interferent with the subsequent HPLC analysis, 500 μL of the alkaline hydrolysate was transferred into a 10 mL glass vial followed by addition of 1.0 mL of 30 % H_2O_2 to achieve complete discoloration and/or the end of bubble formation, and then of 1.0 mL of 2 M H_2SO_4. The resulting acid solution was eluted through a reversed-phase SPE cartridge (Bond Elut C18 loaded with 500 mg stationary phase) that had been previously activated by sequential elution of methanol (2.0 mL) and HPLC grade water (2.0 mL). Desorption was then carried out with 0.8 mL of MeOH. Finally, 0.5 mL of the methanolic eluate was taken with a micropipette into a glass vial and 0.75 mL of 1 wt% acetic acid in HPLC grade water was added to obtain a final 40:60 vol% methanol/water mixture.

The HPLC determination of TPA was carried out by injection of 20 μL methanol/water sample solution into a Jones Genesis® AQ reversed-phase column (120 Å, 15 cm length, internal diameter 4.6 mm, particle size 4 μm) and isocratic elution at 1.0 mL/min with a 30:70 vol% mixture of methanol and aqueous phosphoric acid (0.1 wt% in HPLC grade water). The chromatographic trace was recorded with the diode array UV detector (DAD VL+ 1260/G1315C, set at a wavelength of 242 nm).

The above procedure, from the treatment with H_2O_2/H_2SO_4 on, was also carried out on a fraction of the filtered solutions from the acid hydrolysis, which was then analyzed for the possible presence of TPA as an aromatic comonomer of PAs such as those used for high performance technical fibers.

2.7. Quality assurance/quality control

All hydrolytic depolymerization experiments and the subsequent processing treatments were carried out in triplicate (acid hydrolysis) or duplicate when the amount of available sample was deemed too low (alkaline hydrolysis for TPA). HPLC analyses were always run in triplicate. Blank samples were also run through the hydrolysis, processing and HPLC analysis steps in the same way as for the sponge samples and did not result in any detectable peak associated with analytes of interest.

For the HPLC analysis of the amines the response of the DAD and fluorescence detectors was calibrated using five standard solutions of dansylated AHA (concentration range 17.5–140 ng/mL) and HMDA (concentration range 13.25–132.5 ng/mL). The following linear regression equations were obtained: AHA (peak area) = 0.03717 + $0.01241 \cdot C_{AHA}$, $r^2 = 0.99174$; HMDA (peak area) = −0.08077 + $0.01622 \cdot C_{HMDA}$, $r^2 = 0.99466$. The LOD (Limit of Detection) and LOQ (Limit of Quantification) values for the analyte concentration determined by HPLC were calculated as the ratio σ_A/s_{linear} multiplied by 3 for LOD, and multiplied by 10 for LOQ, where σ_A is the standard deviation of the fluorescence values recorded from the triplicate analysis of the most diluted solution used for instrumental calibration, and s_{linear} is the slope of the linear fit. By adopting the same conditions of preparation (hydrolysis conditions and chromatographic set up) as those used for the actual samples, the following values were obtained: $LOD_{AHA} = 5.5 \pm$

0.7 ng/mL, LOQ_{AHA} = 18.2 ± 1.6 ng/mL, LOD_{HMDA} = 2.1 ± 0.3 ng/mL, and LOQ_{HMDA} = 7.0 ± 0.6 ng/mL (concentrations relative to the neutralized solution, before the subsequent dilutions required to carry out the dansylation reaction).

For the HPLC analysis of TPA, a 7-point calibration of the diode array UV detector (DAD VL+ 1260/G1315C, set at a wavelength of 242 nm) was performed by analyzing standard TPA solutions in the concentration range from 0.21 to 1.68 μg/mL plus a blank. The following linear regression equation was obtained: TPA (peak area) = −3.03 + 39.46·C_{TPA}, r^2 = 0.9958. The LOD and LOQ values, calculated as specified for the analysis of AHA and HMDA, and by adopting the same conditions of preparation as those adopted for the actual samples, the following values were obtained: LOD_{TPA} = 0.12 ± 0.05 μg/mL, LOQ_{TPA} = 0.41 ± 0.09 μg/mL.

As a quality control (QC) check, and to assess the effectiveness of the PISA procedure for the quantitative analysis of the contamination of the sponges by the above synthetic polymers, fractions (1.7–4.5 g) of the dried sample of *M. sarai* were spiked with known quantities of nylon 6 (1.0–4.5 mg), nylon 6,6 (2.0–3.5 mg) and PET (1.0–2.0 mg) (the spiking weight range refers to the test performed in triplicate, along with blanks on unspiked samples) micropowders. The recovery rates were quantified by HPLC analysis of the three monomers AHA, HMDA, and TPA, obtained by sequential acid and alkaline hydrolysis followed by purification and, for the amine monomers, by tagging (dansylation), as described in the experimental part. Average recovery rates of 95, 124, and 97 % were calculated for AHA, HMDA and TPA, respectively (Table 2).

To assess the effectiveness of the adopted acid hydrolysis conditions in causing the complete depolymerization of the two TPA copolymers PBAT and PPTA, as it will be discussed in Section 3.2, recovery tests were performed in triplicate on two commercial samples. The tested PBAT consisted of BASF Ecoflex™ pellets kindly provided by the producer, while Kevlar 49 fibers were obtained by disassembling a certified protective sleeve purchased at a local shop of protective working equipment, followed by FTIR analysis to confirm that only the PPAT fibers had been collected. The polymeric materials were treated separately by refluxing 24 h in aqueous 6 N HCl, and then processed and analyzed as in the case of TPA from the alkaline hydrolysate in the procedure for PET quantification. The PBAT pellets were readily solubilized, in spite of the disadvantageously low specific surface area and the absence of a phase transfer catalyst (used instead in the case of TPA from PET); the final recovery of TPA was 92 % ±0.2 % based on an estimated 1:1 mol ratio of the TPA and adipic acid comonomers. The Kevlar fibers were much more refractory, as highlighted by the persistence of yellowish microfibers (as the original ones) in the form of small fragments (typically <0.5 mm in length) and recovery of about 2 % of theoretical TPA after 16 h. However, the progress of the hydrolytic degradation was confirmed by the exhaustive recovery, at the end of the 24 h of acid treatment, of a white crystalline precipitate; the solid was filtered on a 0.22 μm PVDF filter, solubilized in aqueous NaOH, sampled and diluted to avoid supersaturation under acidic conditions (reported TPA solubility of about 15 mg/L in acid aqueous solution) required for the TPA determination by HPLC. The successful depolymerization was qualitatively indicated by the yellowing of the hydrolysate in a few hours, due to the oxidation of the phenylenediamine comonomer. A 94 % ±0.4 % TPA recovery was obtained by HPLC analysis.

Table 2
Monomer recovery as determined by HPLC analysis of sponge samples spiked with the corresponding polymer.

Run	Calculated[a] (μg/mL)			Detected[b] (μg/mL)		
	AHA	HMDA	TPA	AHA	HMDA	TPA
1	0.077	0.078	1.639	0.073	0.100	1.61
2	0.058	0.073	1.681	0.056	0.096	1.70
3	0.092	0.063	1.254	0.087	0.071	1.16
Average recovery (%)				95.3	124.0	97.3

[a] The calculated concentration refers to the hydrolyzate solutions obtained after dilution within the concentration range in which calibration was performed.

[b] The actual content may be higher than the calculated one as it includes the possible contribution by MPs contaminating the sponge samples.

2.8. Data analysis

The concentrations of contaminating polymers in each sample was calculated from the concentrations of the corresponding AHA, HMDA and TPA monomers, as determined by HPLC, according to Eqs. (1)–(3):

$$Nylon\ 6 = C_{AHA}\frac{MW_{PA6}}{MW_{AHA}} \quad (1)$$

$$Nylon\ 6,6 = C_{HMDA}\frac{MW_{PA6,6}}{MW_{HMDA}} \quad (2)$$

$$PET = C_{TPA}\frac{MW_{PET}}{MW_{TPA}} \quad (3)$$

where MW_{PA6} = 113.16 g/mol, $MW_{PA6,6}$ = 226.32 g/mol, and MW_{PET} = 192.2 g/mol are the molecular weights of the repeating units in the corresponding polymers (Fig. 2), and MW_{AHA} = 131.17 g/mol, MW_{HMDA} = 116.21 g/mol, and MW_{TPA} = 166.13 g/mol those of the monomers detected, if any. The above equations are based on the assumption that the original polymers do not include different comonomers. Biogenic amines or diacid (as shown in Fig. 2b–f), corresponding to the monomeric species obtained upon depolymerization, are not expected to contribute to the analytical results as such low molecular weight species would be removed by the washing and solvent extractions preliminary to the hydrolytic depolymerizations.

The concentration of TPA copolymers that undergo complete depolymerization under the acid hydrolysis procedure adopted for the aliphatic PAs, was calculated by assuming that the TPA determined by HPLC analysis of the acid hydrolyzate comes completely from only one of the following copolymers: PA6T, PPTA, and PBAT.

The concentrations of the copolymers are given by Eqs. (4)–(6):

$$\text{polyamide 6T} = C_{TPA}\frac{MW_{PA6T}}{MW_{TPA}} \quad (4)$$

$$\text{PPTA} = C_{TPA}\frac{MW_{PPTA}}{MW_{TPA}} \quad (5)$$

$$\text{PBAT} = C_{TPA}\frac{MW_{PBAT}}{MW_{TPA}} \quad (6)$$

where MW_{PA6T} = 246.32 g/mol, MW_{PPTA} = 238.25 g/mol and MW_{PBAT} = 210.24 g/mol are the molecular weights of the repeating units in the respective copolymers.

3. Results and discussion

3.1. Microscopic inspection of freeze-dried sponge specimen

The samples of freeze-dried sponges were observed under a microscope to evaluate their morphology and the presence of any plastic fragments larger than 500 μm. In the case of *M. sarai* (Fig. 3a), particles not belonging to the organism, including what looked like a textile fiber and a fragment with the shape of a scale of amaranth color (Fig. 3b), were detected and analyzed by ATR FTIR spectroscopy. In particular, the FTIR spectrum of the amaranth fragment shows a combination of absorptions typical of aromatic (3020 cm^{-1}), ester (shoulder at 1730 cm^{-1}) and amide/urea (1627 and 1534 cm^{-1}) groups, as in polyurethane-urea typically used as binders in paints (Fig. 4). The broad absorption at 1000–1100 cm^{-1} suggests the presence of inorganic fillers

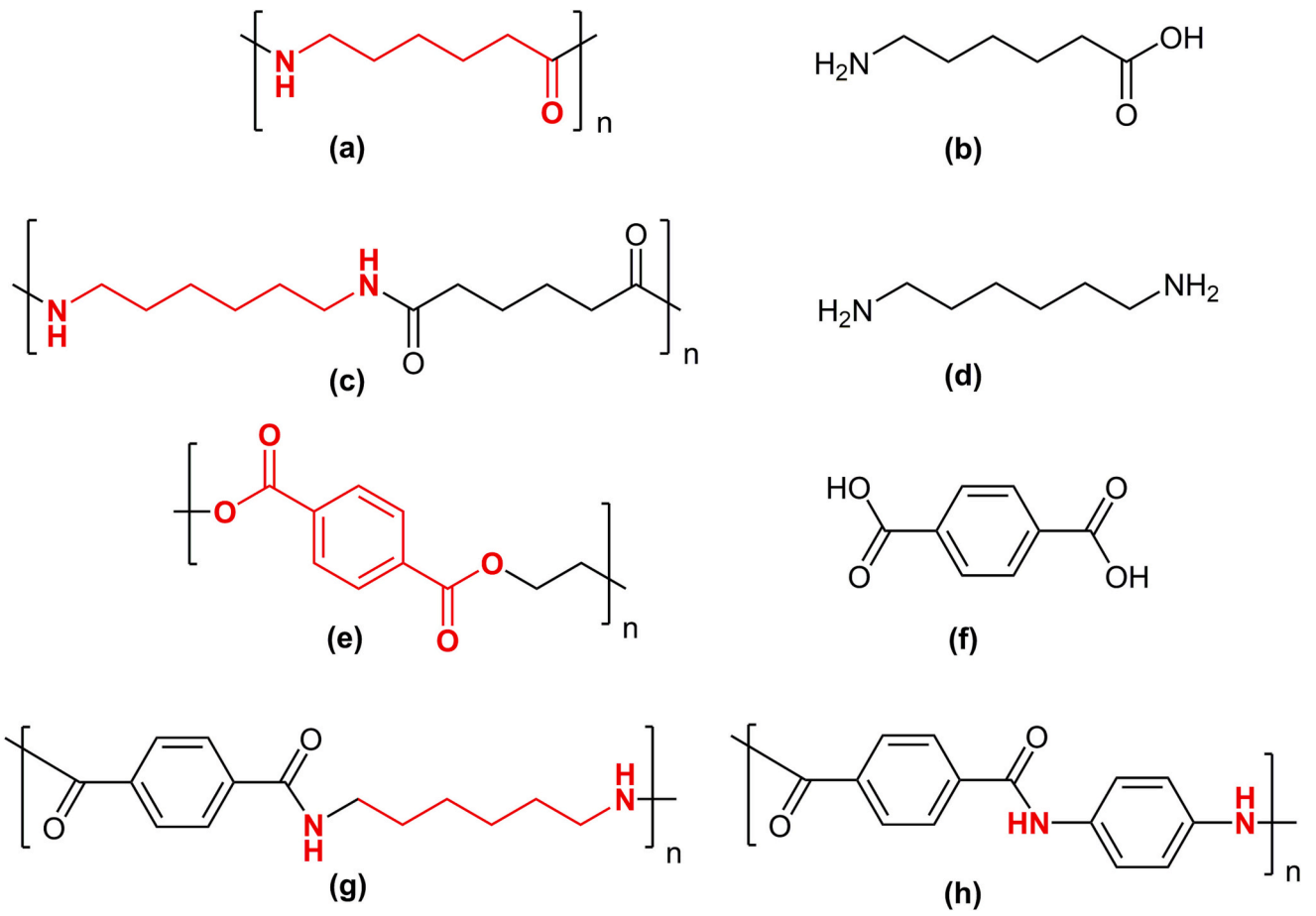

Fig. 2. (a) Nylon 6 (polycaprolactame), the homopolymer of 6-aminohexanoic acid (b); (c) nylon 6,6, a copolymer of 1,6-hexanediamine (d) and adipic acid (not shown); (e) PET (polyethylene terephthalate), copolymer of terephthalic acid (f) and 1,2-ethanediol (not shown); (g) polyamide 6 T, a copolymer of HMDA with TPA; (h) poly(*p*-phenylene terephthalamide) (PPTA), a copolymer of TPA with *p*-phenylenediamine.

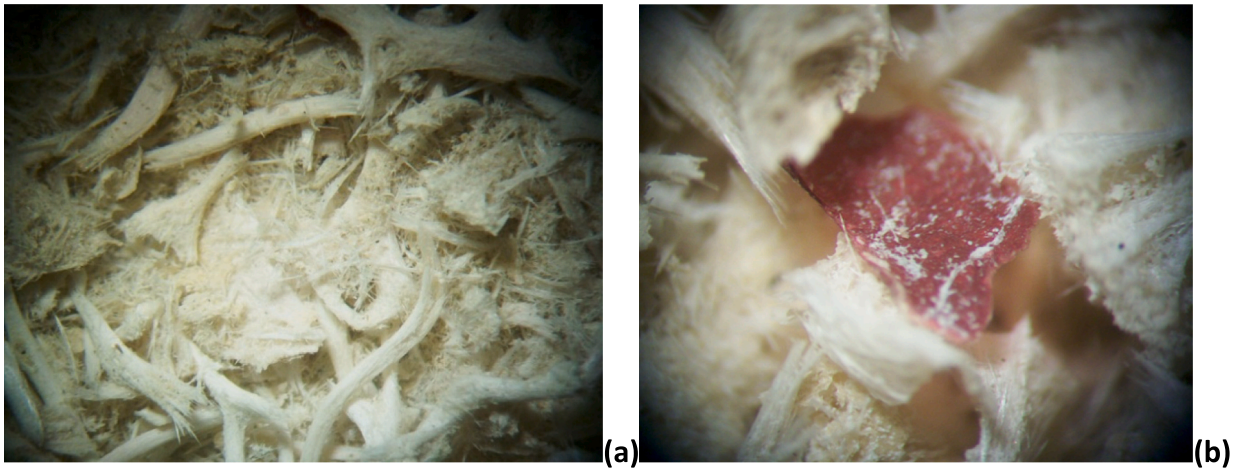

Fig. 3. Freeze dried *M. sarai*: a) magnification 14×, b) magnification 28× showing a fragment of amaranth color.

(e.g., silica). Most likely the fragment originates from local anthropic activity, and in particular from the scaling of protective paints of ship hulls, cargo containers or other equipment used by the personnel in the nearby Mario Zucchelli research station and in other Antarctic research stations (Sfriso et al., 2020).

3.2. Quantification of MPs in the sponge specimens by depolymerization and monomer analysis

The possible presence and extent of contamination of the sampled porifera by microplastic particles was evaluated by means of the PISA protocol (Castelvetro et al., 2021a). The latter allows to calculate the total mass of the individual polymers even in complex environmental matrices with biogenic and inorganic material. Among them, the two

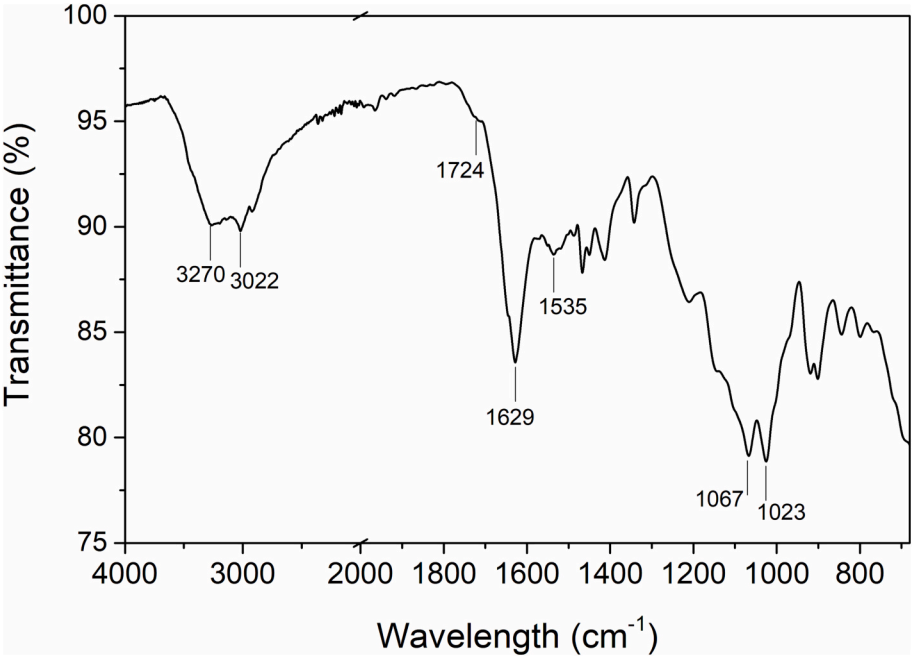

Fig. 4. FT-IR ATR spectrum of the fragment of amaranth color found in *M. sarai*.

aliphatic PAs nylon 6 and nylon 6,6 are quantified from the concentration in their acid hydrolysates of the respective amino-monomers AHA and HMDA (Castelvetro et al., 2021b); PET is quantified from the concentration in the alkaline hydrolysate of its monomer terephthalic acid (TPA) (Castelvetro et al., 2020).

The concentrations of AHA and HMDA from the acid hydrolysate and of TPA from the alkaline hydrolysate indicate a nearly quantitative calculated recovery for the samples spiked with the corresponding nylon 6, nylon 6,6 and PET micropowders. This agrees with previous studies in which TPA could be quantified even in samples with a high content of biogenic material (Castelvetro et al., 2021b; Castelvetro et al., 2021c). On the other hand, the conditions for the quantitative determination of the two aminated monomers of synthetic PAs were more critical, due to the possible interference from natural aminoacids (protein hydrolysates) and the need to combine the chromatographic conditions ensuring *d*AHA peak resolution while avoiding excessively long analytical runs for the elution of the diamine *d*HDMA. The HPLC trace of the dansylated acid hydrolysate reported in Fig. 5 clearly shows the presence of

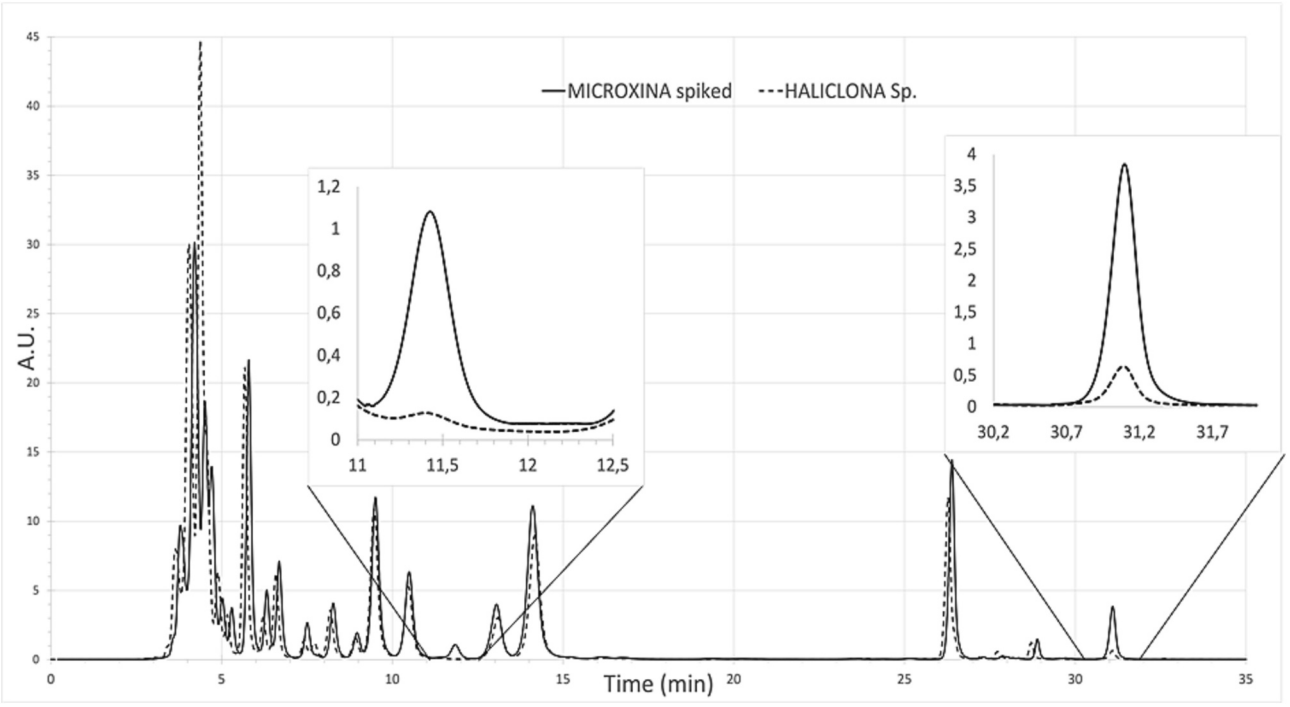

Fig. 5. HPLC traces of the hydrolysates obtained upon acid catalyzed depolymerization of a representative spiked sponge sample (*M. sarai*, solid line) and a representative unspiked sponge sample (*H. scotti*). The inserts show the peaks corresponding to the tagged (dansylated) monoamine *d*AHA at $t = 11.4$ min and the tagged diamine *d*HMDA at $t = 31$ min.

dansylated natural aminoacids, along with well resolved peaks of the tagged *d*AHA and *d*HDMA, with intensity (and thus concentration of the analyte) comparable with those of the aminoacids.

In the case of HMDA, the recovery well above 100 % suggests the presence of endogenous contamination by nylon 6,6 MPs or other HMDA sources (different polymers, as discussed later) in the spiked sponge sample. Indeed, the chromatograms of both spiked and not spiked samples shown in Fig. 5 present a *d*HMDA peak, with a significant signal increase in the case of the spiked sample.

The amounts of polymeric materials, determinated according to the results of the HPLC analyses on the hydrolysates obtained from the sponge samples, pre-treated by freeze-drying and solvent extraction as described in Section 2, are reported in Table 3. In all four sponge samples the concentrations of AHA from the acid hydrolysate and of TPA from the alkaline hydrolysate were lower than the respective LOD values. On the other hand, a definite chromatographic peak for *d*HMDA was found for *M. sarai* (Fig. 6), corresponding to concentrations slightly lower than LOQ and higher than LOD, where, as in case of *H. scotti*, concentrations higher than LOQ were found.

The detected amount of HMDA above LOQ for *H. scotti* would result in a nominal nylon 6,6 concentration of 27 μg/kg sponge. Indeed, the presence of not negligible amounts of HMDA could be anticipated from the nominal recovery >100 % for the spiked *M. sarai* (Table 2). On the other hand, the possible origin from semi-aromatic PAs such as e.g., polyamide 6 T (PA6T, a copolymer of HMDA with TPA) instead of, or along with nylon 6,6 could not be excluded. Indeed, such semi-aromatic PAs are typically used as high-performance materials for protective textile clothing and for various technical items in electrical and mechanical applications requiring extreme mechanical strength along with chemical, thermal, electrical and moisture resistance. HMDA/TPA copolymers undergo acid catalyzed depolymerization like nylon 6 and nylon 6,6, therefore the possible presence of TPA from PAs would not be detected in the alkaline hydrolysate obtained from the residue of the acid hydrolysis step.

To clarify this question, the presence of TPA was investigated also in the solutions obtained from acid hydrolysis. For such purpose, the neutralized acid hydrolysates that had been set aside after withdrawing the small amounts required for AHA and HMDA analyses were submitted to the same purification and analysis steps adopted after alkaline hydrolysis. Thus, the solutions were treated with H_2O_2/H_2SO_4 to oxidize any excess biogenic material, eluted through a SPE C18 cartridge, then the adsorbate was desorbed and analyzed by HPLC for the determination of TPA (see Section 2.5).

Indeed, unexpectedly high concentrations of TPA were detected in all four sponge samples, as reported in Table 3. Such high concentrations of TPA, orders of magnitude higher than that of HMDA in *H. scotti*, could be ascribed neither to the originally postulated PA6T, nor to the nearly ubiquitous phthalic esters as these well-known low molecular weight contaminants would have been removed by the preliminary extraction with hexane/acetone of the freeze-dried sponge samples. On the other hand, TPA could result from acid-sensitive polymers that would undergo depolymerization under the harsh acid hydrolysis conditions of the procedure (24 h in refluxing 6 N HCl); among them, the semi-aromatic polyester PBAT and the fully aromatic PPTA. PBAT is a synthetic polyester, biodegradable under composting conditions but not readily degradable in water (Wang et al., 2021); it is produced in about 100 ktonne/year (Skoczinski et al., 2023) and mainly used in agriculture, in single use packaging items, as paper coating, along with other uses. PPTA, a non-biodegradable copolymer of TPA and *p*-phenylenediamine commercially known as Kevlar™ or Twaron™, is mainly used for super-high performance fibers. Both PBAT and PPTA are completely depolymerized to the respective comonomers under the acid hydrolysis conditions effective for the depolymerization of aliphatic PAs used in the PISA procedure (see Section 2.7 for the recovery tests) (Morgan and Butler, 1992). The maximum (not simultaneous) concentrations of PPTA and PBAT are reported in Table 3. In the case of PBAT the calculation refers to a hypothetical 50:50 mol ratio of adipate and terephthalate diacid *co*-units in the polyester, close to the 53:47 mol ratio for the commercial BASF Ecoflex™ sample. The maximum concentration of PA6T, if present, cannot be based on the detected TPA but must be based on the much lower, limiting concentration of its comonomer HMDA; Therefore, the small amount of HMDA detected in *H. scotti* should be considered here as originating from either nylon 6,6 or PA6T, or both.

4. Conclusions

Antarctic sponge samples were analyzed for their mass content of functional polymers such as PET and PAs as microplastic contaminants. These polymers are typically denser than seawater and are thus likely to travel short distances from the point of entry into the ocean before sinking where benthic filtrators process sediments and water. The analytical procedure employed relies on the hydrolytic depolymerization to convert the polymers in their respective monomers, followed by quantification by HPLC. The alternative, and most common, detection procedures, based on density separation followed by micro-spectroscopic identification and counting of individual particles, can be inaccurate because of: i) loss of particles trapped in the spicules of sponges; ii) missed detection of particles below 10–20 μm in size; iii) partial loss of polyesters and PAs if the MPs are submitted to strong alkaline pre-treatment (typically with 10 % aqueous KOH at 60 °C) aimed at removing biological material but leading to hydrolytic degradation of most condensation polymers.

Out of the total MPs content of up to 5 mg/kg of sponge, neither PET nor aliphatic PAs (nylons) could be detected, with the possible exception of 27 μg/kg of nylon 6,6 in *H. scotti*. On the other hand, through an extension of the PISA procedure it was possible to detect high concentration of TPA in the acid hydrolysate, most likely associated with aramide PPTA fibers and/or not biodegraded semiaromatic polyester PBAT MPs.

Indeed, MPs generically assigned to aromatic polyamides by FT-IR

Table 3
Quantitative determination of AHA, HMDA, and TPA from the acid and alkaline hydrolysates of the sponge samples, as determined by HPLC analysis.

Porifera Specimen	weight (g)		AHA ng	HMDA ng	nylon 6,6 μg/kg[d]	TPA[c] μg	PBAT / PPTA mg/kg[e]
	dry[a]	wet[b]					
Microxina sarai	3.431	21.445	<LOD	<LOD[f]	<LOD	59.6 ± 1.5	3.5 / 4.0
Haliclona (Rhizoniera) scotti	3.472	20.423	<LOD	281 ± 32	27	54.9 ± 1.1	3.4 / 3.9
Mycale (Oxymycale) acerata	3.432	17.874	<LOD	<LOD	<LOD	50.5 ± 1.3	3.6 / 4.1
Dendrilla antarctica	3.529	23.837	<LOD	<LOD	<LOD	46.6 ± 0.9	2.5 / 2.8

[a] Freeze dried sponge sample.
[b] Fresh sponge sample.
[c] From the acid hydrolysate (TPA < LOD for all samples in the alkaline hydrolysate).
[d] Based on fresh sponge; amount of nylon 6,6 calculated by assuming that the measured HMDA found originates uniquely from this polyamide.
[e] Based on fresh sponge; amount of either PBAT or PPTA, calculated by assuming that the measured TPA originates uniquely from either one copolymer.
[f] Detectable HPLC peak from HMDA, although with integral slightly below the calculated LOD.

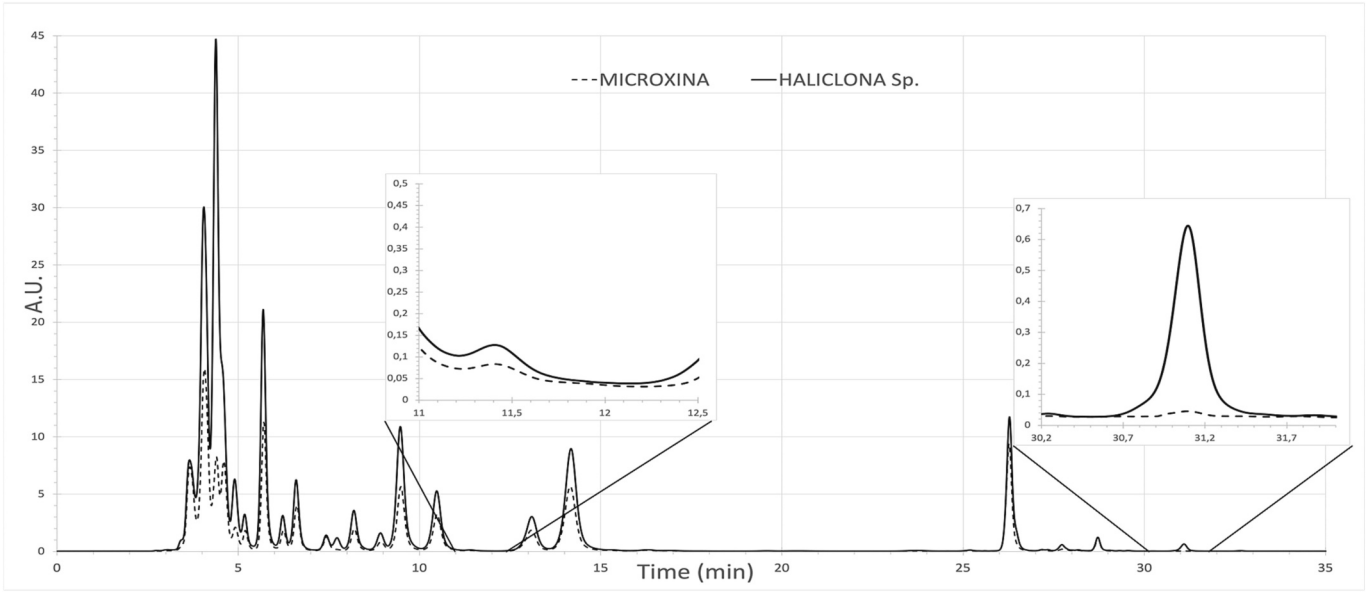

Fig. 6. HPLC trace of the hydrolysates obtained upon acid catalyzed depolymerization of the samples of freeze-dried *M. sarai* (dashed line) and *H. scotti* (solid line). The inserts show the peaks of *d*AHA at $t = 11.4$ min and of *d*HMDA at $t = 31$ min.

identification through spectral database matching had been previously found in Antarctica. In our case, by assuming that 5 mg/kg of TPA exclusively originates from PPTA fibers (e.g., from Kevlar™ or Twaron™ fabrics or ropes) typically 12 μm in diameter and 5 mm in length, a simple calculation results in about 6 microfibers (each one weighing about 0.82 μg) in 1 g of wet sponges, or 6000 microfibers kg^{-1}.

The obtained results showed that sponges may be considered as effective indicators, other than of organic pollutants and heavy metals, of the level and type of pollution by MPs even in remote areas. In particular, the high-density polyamide/polyester MPs found in the sampled marine sponges are likely associated with release of fibers from equipment used in the anthropic activities in Antarctica. Given the fragility of the Antarctic ecosystems, a systematic monitoring of micro- and nanoplastic pollution to be accomplished through the mass-based PISA polymer quantification, extended to hydrocarbon polymers as well as to the whole of environmental matrices (sediment, water, and aquatic organisms) could represent an important tool for a comprehensive understanding of the environmental effect of anthropic activities.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.166043.

CRediT authorship contribution statement

AC: conceptualization, data curation, investigation, methodology, validation, visualization, writing (original draft), supervision; GP: formal analysis, data curation; ALG: resource, funding acquisition; MP: sampling; MA: sampling; CR: sampling; VC: conceptualization, methodology, validation, writing—review and editing; VV: conceptualization, visualization, writing (original draft); SG: financing, supervision; data curation, visualization, writing (original draft), writing—review and editing.

All authors have read and agreed to the published version of the manuscript.

Funding sources

This research was funded by MUR, Programma Nazionale di Ricerche in Antartide (project PNRA 2016_00020): "Poriferi antartici: Hotspots di diversità Procariotica e Potenzialità biotecnologiche - P3".

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Stefania Giannarelli reports financial support was provided by Government of Italy Ministry of Education University and Research.

Data availability

Data will be made available on request.

Acknowledgment

The authors wish to thanks Marco Bertolino and Gabriele Costa (University of Genoa, Italy) for the taxonomic identification of sponge specimens.

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