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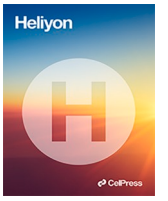

Research article

# Microplastics determination and quantification in two benthic filter feeders *Sabella spallanzanii,* Polychaeta and *Paraleucilla magna*, Porifera

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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

Plastic pollution is a worldwide problem especially in the marine environment. Plastic items once fragmented into microplastics (MPs), can be captured by different marine species. Benthic filter feeders like sponges and polychaetas, due to their trophic strategy, are highly exposed to MPs pollution. Herein a simple but effective method to digest the fan worm *Sabella spallanzanii* and the calcareous sponge *Paraleucilla magna* is presented: a solution with KOH and $H_2O_2$ was able to remove quantitatively (more than 98 %) the organic matter in 3 h while an acid treatment dissolved most of spicules and chaetes in less than 30 min. MPs were easily identified both microscopically and spectroscopically on filters. Quantification in animals collected from the same environment showed that, on average, sponges accumulate fewer MPs than polychaetes (66 ± 31 and 117 ± 46 particles/g dry weight, respectively). The plastic recovery of the method was validated using three different approaches (spiking of standard PS microspheres, of common-use plastic objects, and of microplastics already weathered in marine environment). This procedure can make it easier and cost-effective to process biota in monitoring studies, providing information about bioindicator/bioremediation species.

## 1. Introduction

From the early 20th century to the present day, plastic has been a very useful material, with its market explosion in the 60s and exponential growth to date. Plastic is an advantageous material perfect for making light, cheap, and durable products, that meet consumer demands. However, these same characteristics transform those objects into a dangerous threat to ecosystems and wildlife due to their durability over time in the environment [1]. Even if plastic contamination of the marine environment is a worldwide problem, at the Mediterranean level the situation is very alarming because this biodiversity hot spot is now considered one of the most

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polluted areas [2] with great concern for marine fauna. In fact, due to their size and shape, microplastics (MPs) can be easily captured by a wide range of marine organisms from zooplankton to marine mammals [3–7]. Ingestion of MPs can result in direct and indirect effects on organisms, such as physical damage [8] or potentially transferring chemical pollutants hosted on their surface along the trophic chains [9]. Marine animal forests (MAF) benthic communities, composed mainly of sessile filter feeders (such as sponges, polychaetes, ascidians, and corals) that build three-dimensional structures increasing the complexity and functionality of ecosystems [10] are also affected by this problem [5,11,12]. Benthic suspension feeders accumulate seston, thereby integrating pollutants from a variety of sources [10]. Their feeding strategy makes them sentinels of changes in the water column over time [13]. Increasing knowledge of the presence of MPs in different organisms will contribute to a better understanding of the distribution and movement of plastics in the marine environment, and to estimates of the true extent of MPs pollution [14]. Polychaetes and sponges are active suspension feeders able to remove organic and inorganic particles from the enriched seawater [15,16] or organic and microbial waste from the water column [17,15,18,19]. These animals have, in fact, been suggested as potential bioindicators of micropollutants in biota due to their widespread distribution, high tolerance to pollutants, sessile behavior, and high abundance [17,20,21,22,23–26]. The use of higher eukaryotes as bioremediators and/or bioindicators remains an under-investigated alternative [22,27–29]. Various methods to digest these animals while preserving plastic integrity have been proposed, with the most commonly used being acidic, alkaline, enzymatic, and oxidative [7,30–36]. In some papers [21,22,37,38] the same methodology is used to digest tissues of organisms of different phyla, but not always the digestion efficiencies are reported: there is still no common procedure, leaving the way open for different approaches to be tested. In this work a simple and cost-effective procedure is presented: it allows both the quantitative digestion of the polychaete *Sabella spallanzanii* and the calcareous sponge *Paraleucilla magna* and the quantitative recovery of microplastics. The different accumulation abilities of these two animals in the same environment have also been studied, improving knowledge of their interaction with MPs. The data presented in this paper will contribute to the definition of a common method that can be used for routine analysis in environmental monitoring.

## 2. Materials and methods

### 2.1. Sample collection and preparation

Animals were sampled in November 2020 at the Mariculture Facility Mar Grande in the Mar Grande of Taranto (Ionian Sea) in the Apulian region, Italy (40°25′60.0″ N 17°14′18.2″ E), from the Integrated Multi Trophic Aquaculture system that use natural coconut rope collectors as a substrate to facilitate the growth of organisms such as those used in this study.

Eight specimens of similar size per species were collected by divers from the coconut fibre rope collectors near the mariculture cages at a maximum depth of 10 m, without gloves to avoid possible contamination. On the boat, the animals were individually stored in Al foils, then in laboratory were frozen and kept at – 20 C°. Before analysis, animals were defrosted and measured: *Sabella spallanzanii* was extracted from the tube gently pressing along its shape until they fell on aluminum foil, the animals were positioned as straight as possible, and the length measured. *Paraleucilla magna* surface was rinsed with 0.1 μm pre-filtered MilliQ water to remove any trace of detritus and any adherent particles, then the animal was placed on an Al foil and measured. The average length for polychaetes was 18.6 ± 1.53 cm, whereas for sponges was 6.3 ± 1.53 cm. The animals were treated individually. Each sample was dried in an oven at 50 °C for 48 h, and the dried tissues, friable and easy to pulverize, were gently crushed and weighed. The average dry weight (DW) was 0.35 ± 0.16 g for polychaetes and 0.90 ± 0.31 g for sponges. The samples were then individually placed into each glass bottle for digestion.

### 2.2. Chemicals and materials

Potassium hydroxide (KOH) pellets, 30 % hydrogen peroxide ($H_2O_2$) solution, Formic acid ($CH_2O_2$) (96 %), and Sodium citrate were purchased from Sigma-Aldrich (Milano, Italy). All the working solutions were prepared with ultrapure water prefiltered on 0.1 μm pore size PES filters. PES filters (Steriltech, 47 mm 0.1 μm pore size) and Glass fibre filters (Whatman 47 mm, 0.7 μm pore size) were supplied by Sigma-Aldrich. Red and Blue spherical polystyrene microparticles (ø = 6 and 10 μm respectively) were purchased from Histoline (Milano, Italy).

### 2.3. Digestion procedure

The multi-reagent digestion solution, consisting of 5 % KOH and 10 % $H_2O_2$ aqueous solution, was freshly prepared every day. 20 mL of this alkaline solution were added to the dried animal in a glass bottle. Then the bottle was capped and kept in a bath at 75 °C for 3 h. After cooling at room temperature, the solution was centrifugated for 5 min at 3000 rpm, and the supernatant was filtered on a 0.7 μm glass fiber filter. Then 5 mL of 25 % formic acid and 10 % sodium citrate aqueous solution was added to the residue and allowed to react at room temperature to dissolve spicules (in *P. magna*) and chaetes (in *S. spallanzanii*). After 30 min, spicules and chaetes were no longer visible, and the solution was filtered on the same filter. Each container/tool used to perform the procedure was rinsed three times with 0.1 μm pre-filtered MilliQ water, and the rinsing water was then filtered to recover all the particles. When the filtration was completed, the filter was gently placed in a Petri dish and left in a dryer until constant weight.

As the MPs weight is negligible respect to the tissue weight, the digestion efficiency (%DE) was calculated for each sample following the following formula [39],

$$\%DE = \left(1 - \frac{(W_{fa} - W_{fb})}{W_i}\right) \times 100$$

where: $W_i$ = sample DW; $W_{fb}$ = filter DW before digestate filtration; $W_{fa}$ = filter DW after digestate filtration.

### 2.4. Microscopic identification of MPs

To evaluate MPs presence in the digested organisms, the filters were analysed using a Nikon Eclipse 80i microscope equipped with a Nikon camera. Images were taken in a dark field at 20x magnification with ACT-2U software. MPs were classified according to their chemical composition, shape, color, and size. Size measures were performed using ImageJ software [40].

### 2.5. Micro Raman spectroscopy

Raman analyses were performed using Renishaw InVia spectrometer equipped with a Leica microscope with a 5x/20x/50x magnification lens and a 785 nm diode laser. Spectra were collected in 500–1800 $cm^{-1}$ range using different laser power: 1 % laser power (about 1 mW on the sample) for dark (blue, black, brown) particles that could be more prone to be damaged by laser irradiation, up to 10 % laser power (10 mW on the sample) on clear ones. Exposure time and the number of accumulations were 20 s and 4, respectively. Raman spectra were acquired using WiRe software and spectra were compared with SloPP and SloPP-E libraries [41], with commercial Renishaw polymer database [42,43], and literature [44,45]. Moreover, some objects and materials (e.g. gloves, coatings, textiles …) were collected from the sampling site (the IMTA facility at Mar Grande of Taranto) and analysed by Raman spectroscopy.

### 2.6. Quality control

In the laboratory and during all the procedures, the samples were kept in closed glass containers or covered with Al foil, and the analysis were performed in glass bottles under a laminar flow cabinet to minimize airborne contamination. All laboratory staff wore cotton lab coats and nitrile gloves. Work surfaces and tools were cleaned using paper wet with pre-filtered 0.1 μm MilliQ water. All the solutions were freshly prepared using prefiltered 0.1 μm Milli-Q water. All these precautions were effective: method blanks showed the presence of 5 ± 1 transparent fibres, soi an average of 5 transparent particles were subtracted from each filter.

### 2.7. Method validation

Method validation was carried out following three approaches and using plastic with different polymer compositions, shapes, and sizes:

1) MPs items recovery: Polystyrene (PS) red beads (6 μm) standard solution was diluted to have a spiking solution with a final concentration of ca. $2.3 \times 10^4$ particle/mL. Before digestion 100 μL of PS beads solution was added to samples (3 polychaetes and 3 sponges). The recovery %R was calculated using the following equation:

$$\%R = 100 \times \frac{\text{beads counted on filter after alkaline and acidic digestions}}{\text{beads spiked}}$$

2) Chemical, color, and shape stability: common use objects like clothes pieces (viscosa and polyester), bottles (PET), bottle cap (PE), mussel nets (PP) and plastic wires (PA) were selected to perform this test. Each item was manually cut in the μm range. Different shapes were evaluated: fibres from textiles (polyesther and viscosa), fragments from bottle cap (PE), mussel net (PP)), and plastic cable (PA), and film from water bottles (PET). As to spherical shape, 10 μm beads (PS) from a standard suspension were used. Five pieces of each polymer and about 200 PS beads were placed in glass vial and treated following the same procedure used for the animals. Chemical stability was assessed by acquiring Raman spectra of each polymer tested before and after treatment, while colour and shape stability was assessed by comparing photographs of each plastic item taken before and after treatment.
3) Mass recovery of MPs: several plastic items collected on a beach in southern Italy, were ground to a powder and sieved at 250 μm; 100 mg of this powder were weighed and treated following the procedure already described. The mass recovery was assessed by weighing the dried filter before and after filtration. The recovery was measured as:

$$\textit{mass recovery}\% = 100 \times \frac{\mathrm{WFb} - \mathrm{WFa}}{\mathrm{Wi}}$$

$WF_b$ = weight of filter before procedure; $WF_a$ = weight of filter after procedure; $W_i$ = plastic items weight.

## 3. Results

### 3.1. Method description and validation

Polychaetes and sponges required a modified extraction protocol compared to the protocols used for bivalves because the presence of spiculae and chaetes. These could be removed by an acid step at room temperature for 30 min with a 25 % formic acid and 10 % sodium citrate solution, however the digestion of *S. spallanzanii* and *P. magna* demonstrated more difficult than that of bivalves: the optimized conditions for digesting mussels (2.5 % KOH, 5 % $H_2O_2$, and 2.7 % methanol at 60 °C for 3 h), in fact, brought to a suspension that could not be filtered on the 0.7 μm glass fiber filters. On considering the adverse effect of methanol on plastic recovery with hydrogen peroxide and KOH increase, the digestion was performed with 5 % KOH and 10 % $H_2O_2$ at 60 °C for 3 h without methanol. The resulting suspension could be filtered but the digestion efficiency was as low as 70 %. An increase of the digestion temperature up to 75 °C was effective: high yields were reached for both organisms, as digestion efficiencies (%DE) were on average 98.0 ± 0.8 % and 98.8 ± 0.5 % for *S. spallanzanii* and *P. magna,* respectively.

The validation of the whole procedure (digestion and acid attack) was carried out as follows:

1) **MPs items recovery**: samples (3 sponges and 3 polychaetes) in glass bottles were spiked with about 2000 red PS microparticles. After alkaline and acid steps, the solutions were filtered and the red PS beads were collected on filters and counted: the average recovery obtained for P. Magna and S. Spallanzani in these experiments was 96.8 % ± 0.5 %. The applied protocol permitted the elimination of almost all the organic matter without damaging spiked particles which can be easily found and counted on filters (Fig. S1).

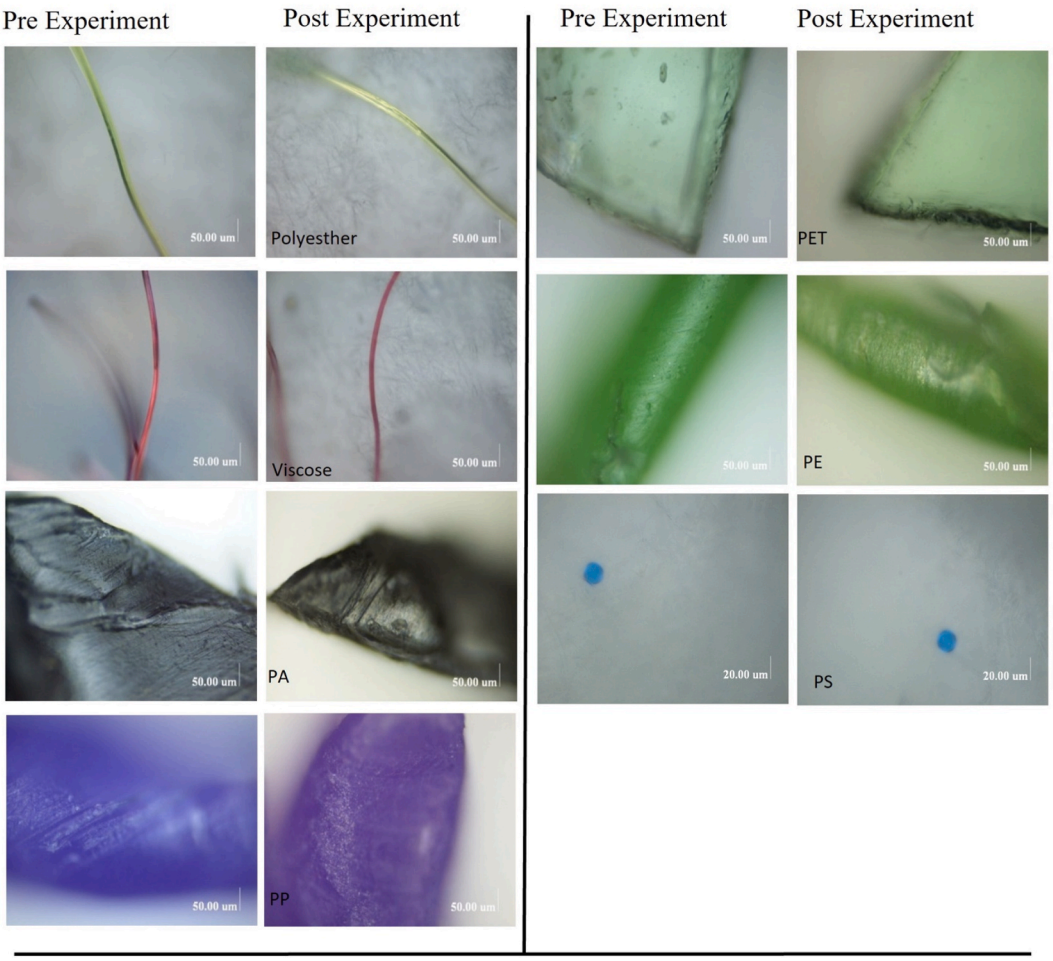


**Fig. 1.** The microplastics used to validate the method were photographed before and after the alkaline and acidic digestion experiments. The fragments (PA; PP; PE), the film (PET) and the fibres (polyester and viscose) were photographed at 20x magnification whereas the spheres (PS) at 50X magnification.

2) **Shape, color, and chemical stability:** Photographs of fragments, fibres, spheres and films taken before and after the whole procedure were compared and no significant changes to the color or appearance of the surfaces were found (Fig. 1). The Raman spectra of the standard polymers collected before and after the treatment were also compared and all the spectra are almost superimposable (Supplementary materials Fig. 2S). These experiments demonstrated that, following the alkaline and acidic digestion steps, there are no modifications that can interfere with microscopic or chemical identification of MPs.
3) **Mass recovery**: The microplastics used in this test was constituted by macro and mesoplastics collected on a beach and powdered. The recovery was 95.4 % ± 1.1 % (average of 3 experiments). As a result, these experiments permitted to assess the effectiveness of the method on plastic already weathered in marine environment.

### 3.2. Analysis of microplastics in S. spallanzanii and P. magna from Mar Grande (Taranto, Italy)

#### 3.2.1. Microscopic identification and morphological classification

The entire filter was analysed, and the particles were photographed, counted, and classified according to shape, colour, and size. Fig. 2 shows the number of particles (p) per gram of dry weight (DW) tissue and the distinction between shape and colour. The average number of MPs is 66 ± 31 p/g DW in sponges and 117 ± 46 p/g DW in polychaetes which corresponds to 52 ± 15 p/individuals and 38 ± 20 p/individuals, respectively.

In the entire study, fibers were more abundant than fragments: the total proportion of fibers was 88 %. Almost all the particles (92 %) in the tissues of *S. spallanzanii* were fibers, the majority of them were blue followed by transparent ones, while in *P. magna* tissues, 85 % of particles were fibers, most of them were transparent followed by the blue ones. Fragments were present in all the samples except for S3 and P1, representing 15 % of particles in sponges and 8 % in polychaetes, and most of these items were light blue in colour. Microplastics were classified according to their size (Fig. 3). On average, the most frequent size range in *P. magna* is 500–1000 μm (Fig. 3a), while both 150–300 μm and the 500–1000 μm fractions are frequent (Fig. 3b) in *S. spallanzanii*.

#### 3.2.2. Spectroscopic (chemical) characterization

All the particles on the entire filter were analysed by Raman spectroscopy. The MPs were spectroscopically characterized, and Nylon, Polyethylene (PE), Polypropylene (PP), Alkyd resins (AR), Polyethylene terephthalate (PET), Polyurethane (PUR) were the identified polymers. Cellulose fibers were also identified in both species, but since the animals were collected from natural cellulosic ropes, these fibers were excluded from the final counting. As a result, Nylon was 16 and 34 %, PE both 6 %, PP 2 and 3 %, Alkyd resins 30 and 14 %, PET 8 and 16 %, PUR 38 and 27 %, for sponges and polychaetes, respectively. The unidentified spectra were 34 % in sponges and 27 % in polychaetes. The plastic type distributions of these polymers in *P. magna* e *S. spallanzanii* are presented in Fig. 4A and B, respectively.

Due to the similarity in colour, the Raman spectrum of the IMTA fishermen's boat varnish, collected at the sampling site (Fig. 5a), was compared with the spectrum of the light blue fragments found in the animals' tissues (Fig. 5b), and both were chemically identified as phthalocyanine blue pigmented alkyd resin: in fact, almost superimposable spectra have been obtained (Fig. 6).

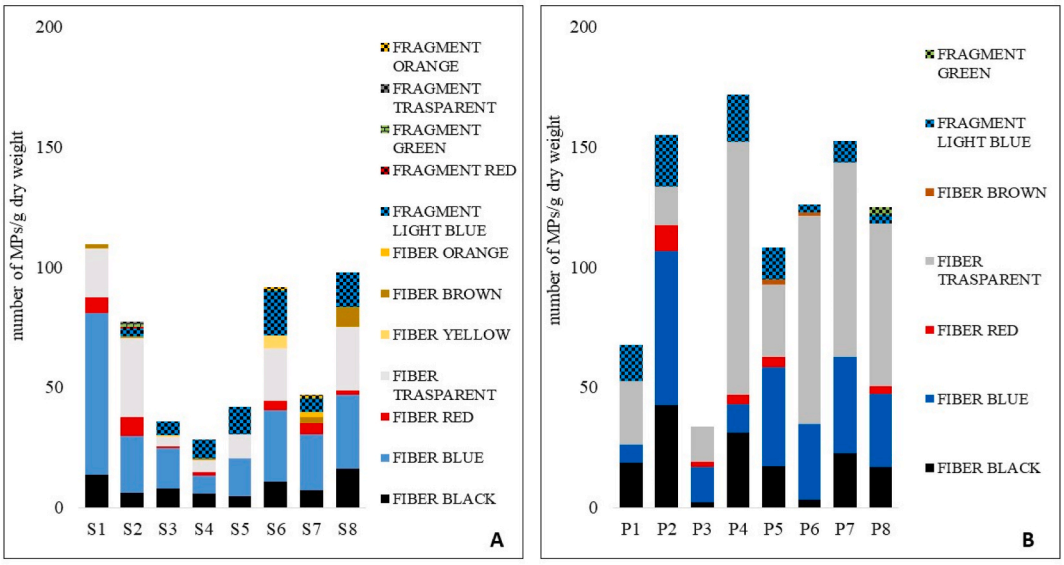


**Fig. 2.** Number of microplastics/g dry tissue according to their shape and color. Panel A (left) shows results relevant to each *P. magna* sponge (S), while panel B(right) shows those relevant to *S. spallanzanii* polychaetes (P). Full colors correspond to fibers while squared patterns indicate fragments. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

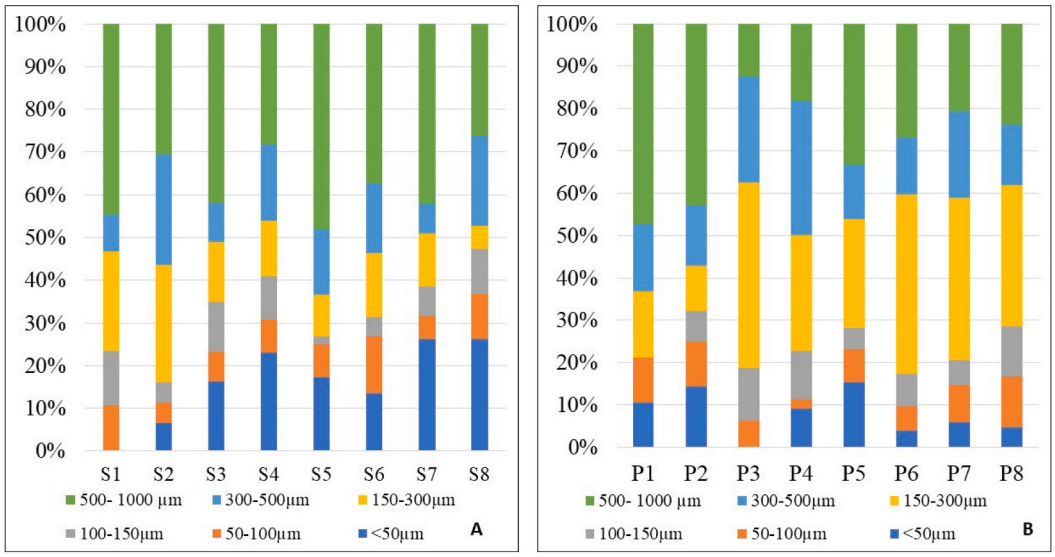


**Fig. 3.** Percentage of microplastics counted in all samples for each size fraction: sponges (a) and polychaetes (b).

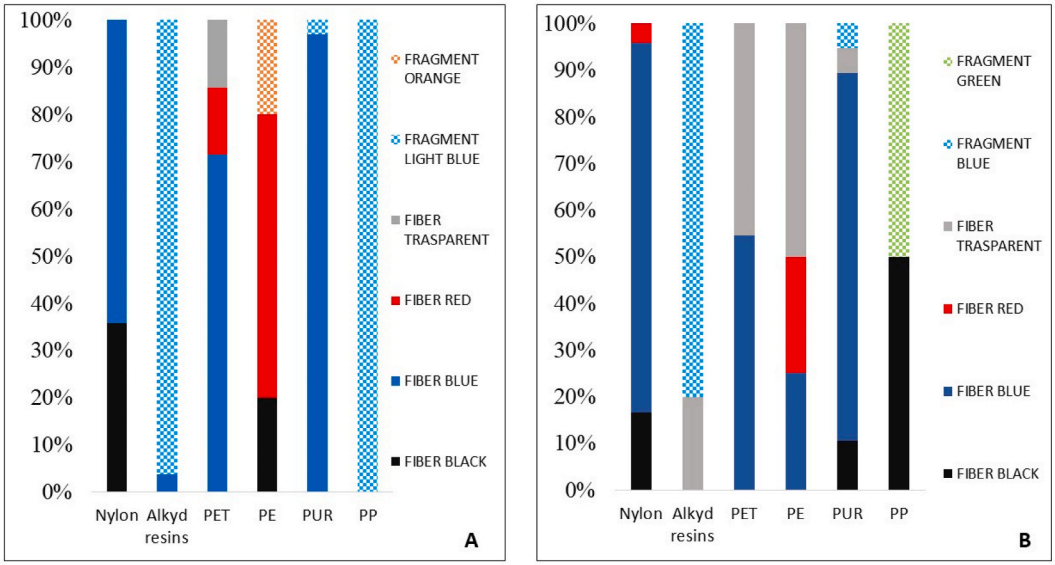


**Fig. 4.** Plastic type distribution of fragments and fibres identified in *P. magna* (A) and *S. spallanzanii* (B). Full colour corresponds to fibers while fragments are indicated with squared pattern. Polymer identified are Nylon, Alkyd resins; PET (Polyethylene terephthalate); PE (Polyethylene); PUR (Polyurethane); PP (Polypropylene). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

## 4. Discussion

*P. magna* and *S. spallanzanii* are two organisms widely distributed in the Mediterranean Sea [46]. The former is a calcareous sponge [47] belonging to class Calcarea subclass Calcaronea and is characterized by the presence of calcareous free spicules, blended, or inserted in a surrounding calcareous matrix [48]. *S. spallanzanii* is one of the largest Sabellids in the Mediterranean Sea and, as polychaete, is rich in proteins and lipids [49]. Both organisms are common in mesotrophic-eutrophic environments [47], and can tolerate the presence of pollutants [46]. These two animals are part of the fouling community growing on the natural rope collectors in the IMTA of Mar Grande of Taranto and have been already tested and proposed as MPs bioremediators in a manipulative feeding experiment [22].

In the last years, several different procedures to digest biota tissues have been proposed without reaching a standardization [50]. Moreover, the interest in studying not only commercially relevant marine species, but animals with different feeding strategies or

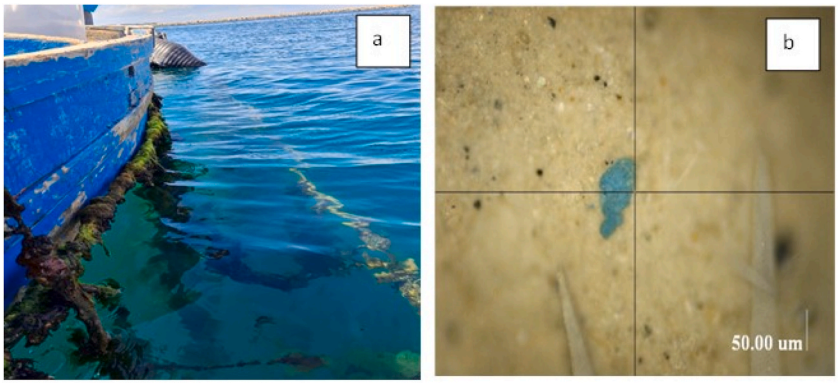


**Fig. 5.** Picture of a fishing boat used in the Mar Grande Mariculture, whose paint is visibly deteriorated (a), and a fragment identified in the animals with a very similar colour (b) and spectroscopically identified as alkyd resin. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

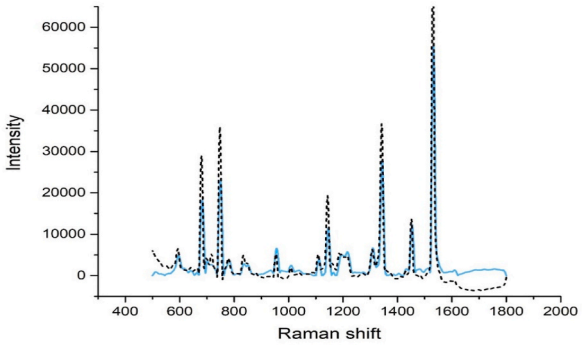


**Fig. 6.** Raman spectra of the blue fragments on the filters (solid line) and fisherman boat varnish (dashed line) relevant to a phtalocyanine blue pigmented alkyd resin. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

biological features has increased over time, expanding the knowledge about MPs contamination in the marine environment [11,21,51]. A literature search combining the keywords "microplastics" and "sponges" or "microplastics" and "polychaetes" in the Scopus database returned approx. 60 papers per each search, among them just the ones focused specifically on the MPs extraction from sponges and polychaeta collected from the field have been summarized in Supplementary materials, Table S1. The reported protocols are quite different: some studies visually inspected sponge tissue or performed histological analyses [5,20,52], others carried out chemical digestion using different reagents (acids or alkaline solutions); some authors adopted a short and easy procedure [53] while other followed multiple steps to extract MPs [54]; the chemical procedures lasted from 2 h [53] to two or even three days [11]. Polychaetes' tissues instead, were always chemically digested, with long procedures lasting up to the complete tissue digestion [55] and in general not less than 12 h (Protocol 3) [21], in most of the cases using KOH (10 %) but also using $HNO_3$ [56,57].

Some authors have summarized the different chemical digestion approaches (acid; alkaline; oxidizing) focusing on the plastic recovery after treatment, in order to assess the riskiest or safest procedures [58–61]. In many cases acids (in particular $HNO_3$) have been considered dangerous to use in MPs extraction procedure: they can damage plastics causing melting and discoloration or alter their FTIR and Raman spectra causing problems during spectroscopy identification [60–62]. It has been recently reported that reducing concentration of $HNO_3$ improves the plastic stability even for prolongated exposure time (18 h) at high temperature 80 °C [58]. On the other hand, recently, has been assessed the effect of different not diluted reagents (HCl, KOH, NaOH, $H_2O_2$, $H_2SO_4$) and digestion conditions (1 or 7 days at 25 °C or 70 °C) on MPs [59]. From these experiments HDPE, LDPE, PP and PET MPs resulted stable from morphological and spectroscopical point of view in presence of HCl, $H_2O_2$, KOH, and NaOH at all reaction conditions, while PS showed some abrasion when exposed to bases at 70 °C for 24 h or 7 days [59]. In another study PS showed swelling if exposed to 10 % of KOH at 90 °C for 48 h [61]. In the procedure presented here, time, temperature and reagent concentration were lower than in these studies and our results are in agreement with the literature in terms of plastic recovery and spectroscopic identification. This method has some limitations, such as the possible formation of foam due to the presence of $H_2O_2$, which can cause particle loss. To avoid this problem, large glass bottles have been used: this allows the foam produced to expand in the bottle without leaking out. In addition, *S. spallanzanii* females undergo vitellogenesis in summer [63], this can cause the formation of a lipid orange layer made of oocytes on the filters that can slow down the filtration process or obstacle the visual inspection of filters: a few mL of methanol (20 %) [64] can be added to overcome this issue.

Sponges and polychaetes require the removal of spicules and chaetae, respectively, to recover MPs after digestion of organic tissue. Two different approaches have been proposed: the density separation using hypersaline solution [38] and the acid digestion [12,21]. The former could be advantageous if the organic digestion efficiency is not high, but the latter approach is definitely faster: in the procedure here proposed this step takes up to 30 min (for larger animals) and is performed at room temperature, thus below the temperature threshold (60 °C) that some authors [41,65] have found to affect polymer integrity. Interestingly, the quantitative alkaline-oxidative digestion of organic tissues and the acidic removal of inorganic carbonate material also allows the filtration of the whole of the alkaline and acidic solutions giving a further advantage: no discrimination based on polymer density, as the entire sample is filtered and MPs are recovered on the filter.

MPs found in the animals were measured in size. In both species, the MPs bigger than 150 μm were the most abundant. Despite sponges have inhalant pores (ostia) of tenths of μm, fibers up to 1000 μm (Supplementary Materials Fig. S3), were found in *P. magna* as in other studies [20,53]. There are some possible explanations to this phenomenon, endocytose can be the process responsible for the presence of big particles (up to 2 mm) in sponges tissues [53,66]; these particles could be blocked in sponges canals after filtration [20] or stuck on the animal surface [52], which in the case of *P. magna* is particularly rough and irregular. Regarding *S. spallanzanii*, instead, these results are not surprising as this tube worm can catch particles in a wide size range from 1 μm to 1 mm and beyond [10]. Some authors suggested that smaller particles were trapped into the crown, those having intermediate size are stored in the ventral part of the animal, and the bigger ones are excreted [25]. Overall, these results are consistent with those of other authors [38] who identified MPs from 10 μm up to 5 mm and found that fibers were more abundant than fragments in different filter feeders, *M. galloprovincialis*, *O. edulis*, *S. spallanzanii*, and *Actinia* sp., from different areas of the Adriatic Sea.

From a quantitative point of view, filter feeders are considered to be the most polluted by MPs compared to other taxa with different feeding strategies [67,68]. In the present work the number of MPs counted in sponges is comparable to those reported in other studies [20,53], or even higher [69]. At the best of our knowledge there is only a study on Mediterranean sponges, which were much more contaminated [57]. Polychaetes also showed an amount of particles higher than usually reported [21,37,38,26]. These discrepancies may be due to study site, as the contamination of the surrounding environment plays a key role in the particle uptake [11,57]. The animals of this work, in fact, were sampled on the natural rope collectors in the IMTA facility in the Mar Grande of Taranto and it is well known that mariculture activities contribute to MPs pollution [70]. Moreover, if we compare the absolute amounts of MPs in these animals taken from the same natural environment, it is evident that *S. Spallanzanii* has a greater capability to capture these particles, and this is a key result supporting bioremediation by polychaetes.

The spectroscopic characterization of MPs showed that the most abundant polymers present in the organisms' tissues were PUR and Nylon. These materials are widely employed in mariculture sector, to build ropes, to fill inner boat layer, and in working clothes [7]. Numerous publications underlined how the biota contamination mirrors their habitat contamination [11,21,38]. Our data seems to corroborate this statement: a correlation between the particles present in the animal tissues (light blue fragments) and some materials (boat varnish) from the sampling site was found confirming the connection between the anthropic pollutant's sources and the presence of those pollutants in organisms living in the same area.

## 5. Conclusions

Several protocols have been published in response to the need to reduce time, cost, and complexity of MPs determination in invertebrates. Aiming at developing a method with the potential to be employed for routine analysis of different marine animal tissues, we used a multi-reagent digestion solution, consisting of 5 % KOH, and 10 % $H_2O_2$, which quantitatively (>98 %) digested the organic matter of sponges and polychaetes in 3 h at 75 °C. Subsequent acid treatment of the residue with 25 % formic acid and 10 % sodium citrate solution at room temperature dissolved the carbonate spicules and chaetes in less than 30 min, facilitating both filtration and identification of the MPs on the filter by microscopy. Both steps did not alter the chemical stability of common polymers, so the spectroscopic characterisation of the microplastics was unaffected. This work also extends knowledge of the interaction between MPs and marine organisms and contributes to the need for information on less studied animals such as sponges and filter-feeding fan worms in the context of research into the occurrence and distribution of MPs in the marine environment.

## Data availability statement

Data is included in the article and in the supplementary material.

## Funding statement

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## CRediT authorship contribution statement

**Giuseppe E. De Benedetto:** Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization. **Silvia Fraissinet:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Nicoletta Tardio:** Methodology, Investigation. **Sergio Rossi:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Cosimino Malitesta:**

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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

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