

Microplastics in *H. tubulosa*. A case study to support future assessment of the cost of microplastic pollution

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**CITATION:**

PAUNA, V. H., ANSEMI, S., PICCARDO, M., FRANZESE, P. P., RUSSO, G. F., & RENZI, M. (2025). MICROPLASTICS IN H. TUBULOSA. A CASE STUDY TO SUPPORT FUTURE ASSESSMENT OF THE COST OF MICROPLASTIC POLLUTION. JECO, 3(1), 11-37. [HTTPS://DOI.ORG/10.82010/JECO.BSRC.2025.10](https://doi.org/10.82010/JECO.BSRC.2025.10)

ACADEMIC EDITOR: PAOLO PASTORINO

RECEIVED: 18 SEPTEMBER 2025

REVISED: 13 NOVEMBER 2025

ACCEPTED: 21 NOVEMBER 2025

PUBLISHED: 23 DECEMBER 2025

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Abstract: Large quantities of environmental data are required to carry out a comprehensive Ecosystem Economic Valuation (EEV) in Marine Protected Areas (MPAs). EEV is used to quantify social and economic costs and benefits that can be associated with nature and ecosystem services. This quantification requires translating ecological changes into welfare impacts. Microplastic (MP) levels are not yet considered in the EEV context due to a substantial lack of consistent and comparable data on levels in sediment and within trophic webs. These data are relevant for translating ecological changes into economic terms. This study reports the levels and chemical composition of microplastics in sediment and benthos species (*H. tubulosa*) collected from the Punta Campanella MPA in Italy following harmonised collection, sampling, extraction, and analysis methods. The reported data is compatible for use in a future EEV in this MPA. Results obtained provided a more thorough understanding of microplastic pollution in the Punta Campanella MPA ecosystem, which could be useful in filling knowledge gaps related to data availability in EEV studies of other similar MPA ecosystems. Results showed that samples contained low levels of MPs, mostly PET and nylon fragments or fibres (in sediment). *H. tubulosa* samples showed lower levels than sediment samples.

Keywords: Sea cucumbers; *H. tubulosa*; sediment; Marine Protected Areas (MPA); microplastics; fibres; Ecosystem Economic Valuation (EEV).

1. Introduction

Many studies have been performed in recent years to define microplastics (MPs) in marine ecosystems by measuring quantities found in sediment and bottom species. Despite the large quantity of published literature, harmonised methods of extraction and analyses remain poorly acknowledged and referred to, often resulting in an overestimation of microplastic pollution in marine ecosystems and trophic webs. The lack of robust methods in most of the available literature, today, has also been reported by several experts, including Bakir et al. (2024), Koelmans et al. (2019) and Wootton et al. (2024). Principal methodological problems tend to be related to collection, laboratory air-borne pollution of samples, and chemical identification of plastic items among natural substances. Some published papers report identifications performed with visual identification using stereomicroscopy and/or chemical identifications performed on only a percentage of the identified MP items, which is considered problematic (Enders et al., 2020). As microplastic research continues to evolve in terms of sampling, extraction, and analysis of MP particles, the use of existing recommended guidelines and best practices is essential to ensure that resulting data can be compared across studies (Weis, 2019). There were not yet harmonised methods such as the “Guidance on the Monitoring of Marine Litter in European Seas” for MP quantification in complex matrices (MSFD Technical Group on Marine Litter 2023) at the time of the present work’s sampling and analysis. Nonetheless, guidelines such as the GESAMP 2019 Guidelines for the Monitoring and Assessment of Plastic Litter in the Ocean and the Harmonised Protocol for Monitoring Microplastics in Biota by JPI-Oceans BASEMAN Project had been published and provided effective strategies which could encourage reproducibility in MP sampling and extraction. These suggestions have been reflected in the updated guidance document by the JRC (i.e. MSFD Technical Group on Marine Litter 2023). Despite advancements in this area, there remains a lack of methodological harmonisation in the study of MPs (Wootton et al. 2024), the resulting non-comparative data could be largely responsible in hindering, for example, ecological risk assessment of this pollutant (Weis, 2019). MP research has not yet comprehensively addressed the current lack of knowledge in quantifying attributes critical to assessing the ecological risks of MPs (Everaert et al., 2018; Li et al. 2025). Although this is likely due to the complexity of the topic, i.e. the many environmental functions that must be considered to accurately demonstrate how MPs interact within an ecosystem, it would benefit the scientific community to implement strategies that have already been established in future studies. It is clear from an environmental modelling point of view that the way in which data is “organized, encoded, and described” allows for or inhibits one’s ability to conduct a meaningful scientific analysis (Horsburgh, 2014). Therefore, to reasonably conduct a

risk assessment of MPs on ecosystems, relevant information should be compiled in a structured way (Waldschläger et al., 2020). In hopes of tackling the lack of organized MP data from a bottom-up approach, this study utilized the most recent guidelines regarding MP analysis, at the time of sampling, (i.e. GESAMP, 2019; Bessa et al., 2019) in marine sediment and biota to create an effective and reproducible method for the extraction of MPs from *Holothuria tubulosa* (*H. tubulosa*) and respective sediment samples.

The purpose of this study is to determine MP levels in both sediment and *H. tubulosa* from the Punta Campanella (Italy), marine protected area (MPA) using an optimised, harmonised, and tested method. The study aims to produce reliable and harmonised data to contribute to the development in assessing the impact of MPs in terms of loss of ecosystem service value (i.e. assessed through Ecosystem Economic Valuation (EEV)) in MPAs. The approach proposed here is not limited to laboratory analysis methods, rather it also considers methods that can allow for more effective data acquisition, organisation, and reporting.

2. Materials and Methods

2.1 Method optimisation

In the development of the methodology presented in this work, all aspects of the study were planned with respect to existing research needs, best practices, and guidelines. These aspects include sampling protocol, sample preparation, extraction, recovery, visual sorting, and chemical analyses of MPs. To start, the Punta Campanella MPA (PCMPA) was chosen as a study site for this investigation because, in theory, the area should contain relatively low levels of MPs due to restrictions on human activity. A lower quantity of in situ MPs can be helpful for protocol validation because it reduces the possibility of interference when accounting for spike particles to establish percent recovery and limit of quantification (LOQ). Furthermore, sediment and biota sampled in MPAs can be of value for ongoing monitoring (European Commission: Joint Research Centre, 2023). Next, *H. tubulosa* was chosen as the study species of interest because benthic invertebrates are inherently effective ecological indicators for the assessment of ecosystem health, particularly in the context of MP exposure (Warnau et al., 2006; Bessa et al., 2019; Cocci et al., 2025). *H. tubulosa* also contributes to sediment health, nutrient cycling, and the transfer of organic matter from detritus to higher trophic levels (Purcell et al., 2016). It is important to note that today the collection and commercial exploitation of *H. tubulosa* are currently prohibited in Italy, meaning appropriate authorisation must be obtained before sampling and using sea cucumbers and bioindicator species for MP monitoring (Pasquini et al., 2022), as well as possible species misidentification, as described by Sertic Kovacevic et al. (2022), however, at the time of sampling for this study (October 2019) this prohibition was not yet in place. However, we did acknowledge the trend in overexploitation of *H. tubulosa*, therefore, we sampled as few of the animal as possible to allow us to test the proposed methods. Future studies which aim to provide data of statistical relevance will likely be required to sample more individuals of the species of interest. Studies show that the concentration of MPs found in aquatic sediment can be orders of magnitude larger than that found in the water column, indicating that benthic species are at a considerably high risk of interacting with these particles (Haegerbaeumer et al., 2019). Despite this, *H. tubulosa* has not yet been heavily studied in MP research, which could be due to prohibitions.

The sample preparation process and extraction methods for the sediment and benthic fauna were developed to ensure that loss of MPs and/or contamination was avoided as much as possible (Bour et al., 2018). Therefore, different analytical methodologies, two for sediment and two for biota, were tested in a trial format at the start of this study to

determine the best practice for the extraction of MPs from the *H. tubulosa* samples and their respective sediment samples based on percent recovery and LOQ results. The trial methodologies focused on the percent recovery in the matrices that were spiked with microplastic particles, the existing environmental pollution and cross-over contamination using negative controls (blanks). Furthermore, the efficiency of the digestion step has previously been applied to complex matrices with successful outcomes.

2.1.1 Sample Preparation

2.1.1.a - Sediment

Each sediment sample had been collected in glass jars with tight closing metal lids directly underneath the location in which the *H. tubulosa* was collected. The sediment was frozen in the same way as the biota. To analyse the sediment, the samples were removed from the -40 °C freezer where they had been stored and were then thawed for approximately 48 hours. The sediment and residual sea water were transferred to a 1.5 L glass jar using a metal spatula. The transfer of the samples was conducted underneath a hood and all equipment used was rinsed with microplastic free distilled water to reduce the possibility of contamination. The jars in which the sediment was collected were opened inside of the transfer jars to catch any sea water that could potentially spill out due to the pressure change. Next, the sample jar was quickly inverted directly above and halfway inside of the transfer jar to pour out any seawater, and the sediment was scooped out using a metal spatula. The original sediment jars were rinsed several times with marine primary filtrate (MPF) sea water until there were no particles visible under the lid or inside the sample jar.

2.1.1.b - Biota

Biota samples were weighed and measured prior to being humanely killed using decapitation, then immediately dissected to avoid any stress which may result in the elimination of contents present in the digestive tract. In detail, the digestive tract and respiratory trees of each *H. tubulosa* had been extracted and weighed at the Punta Campanella sampling site and finally transferred to individual 250 mL glass jars with tight closing lids before being frozen at -40 °C (approximately 1.5 hours post dissection time). At the time of the analysis, each sample was thawed at room temperature for approximately 48 hours, then two samples were prepared for enzymatic digestion with Creon (a pancreatic enzyme) (Trial Method Biota 1: TM1B-S10; TM1B-S5) while the other two samples were digested with Potassium Hydroxide (KOH) (Trial Method Biota 2: TM2B-S10; TM2B-S5).

2.1.2 Spiking of samples

Spike particles were used in this study to quantify the loss of MPs and/or contamination during the application of the protocol. Spike particles were made of low-density polyethylene (LDPE) fragments. They were of varying shapes and sizes and in three colours, i.e. white, pink, and blue. Prior to adding the spike MPs to the sediment and sea cucumber samples, they were measured and photographed using a Nikon SMZ-800 N stereomicroscopic and Nikon ACT-1 imaging analysis software. It was noted which

particles were added to which samples to ensure no double counting or counting of contaminant particles that could have entered the samples during the transfer of the spikes occurred.

2.1.2.a - Sediment

Approximately 50 mL of MPF seawater was used to help transfer spike particles to the four sediment samples. Two subsets of spiked samples were prepared: those spiked with 10 irregular pink, white, and blue MP fragments (S10=2) and those spiked with 5 irregular pink, white, and blue MP fragments (S5=2). Each subset was additionally divided into two different trial methods to evaluate the influence of a drying step on the final results. The residual water from the sampling, transfer and spiking processes of one S10 and one S5-sample was removed via filtration through paper filters of 6 µm threshold (pf-6 µm). These filters were saved to be visually analysed under a microscope after being dried in an oven at 40 °C until the weight reached a constant value. This method was called Trial Method 2 (TM2-S10; TM2-S5). The method that did not include the drying step was called Trial Method 1 (TM1-S10; TM1-S5).

2.1.2.b - Biota

Spike MPs were added to biota samples after the tissue digestion procedure was completed following the same protocol as that described in 2.1.2.a for sediment.

2.1.3 - Microplastic extraction

2.1.3.a - Sediment

The wet samples (TM1-S10 and TM1-S5) were pre-filtered through pf-6 µm. Thus, the MP spike particles were extracted from both wet and dry (TM2-S10; TM2-S5) sediment samples using density separation in 200 mL of saturated NaCl solution. All four samples containing the saturated NaCl solution were agitated at 110 rpm for 20 mins then left to rest for at least 5 minutes. After resting, the supernatant was filtered through pf-6 µm, and the filter was changed and collected whenever it clogged, and no more fluid could pass through. The procedure was carried out three more times for each sample allowing for four NaCl “washes”. The dried samples experienced another step during the filtration process in which the material collected on each filter was rinsed with approximately 20 mL of MPF distilled water after all liquid had gone through the filter, but prior to removing the filter from the vacuum pump system.

2.1.3.b - Biota

2.1.3.b.1 - Enzymatic digestion

This method follows the protocol used in von Friesen et al. (2019). Creon 10000 was added to two of the *H. tubulosa* samples at a weight calculated using Equation 1:

$$\text{g wet weight (w.w.) sample} * 0.2 = \text{weight (in grams) Creon 10000}$$

Next, the sample was added until the pH reached a value of 8. The spike protocol described in section 2.1.2.b was then carried out. Next, the samples were agitated at 110 rpm for two hours before being placed in a 37.5 °C incubator for no less than 14 hours. After the 14 + hour period, the samples were removed from the incubator, agitated again at 110 rpm for 2 hours and placed back in the 37.5 °C incubator until the filtration step (through pf-6 µm). The filter was washed twice with 100 mL of saturated NaCl solution and the supernatant which occurred between washes was filtered again.

2.1.3.b.2 - KOH digestion

The protocol presented by Enders et al. (2020) was followed for the KOH digestion approach used on the remaining two *H. tubulosa* samples. Briefly, a saturated KOH stock solution was prepared by dissolving 560 g KOH salt tablets in 0.5 L MPF distilled water. Next, 150 mL of this stock solution was added to 150 mL of NaClO (14% active chlorine) and 700 mL of MPF distilled water in a tightly closing glass bottle. The quantity of solution added was calculated using Equation 2:

$$\text{g w.w. sample} * 5 \text{ mL} = \text{volume (in mL) KOH digestion solution}$$

The solution was used to help in transferring the spike particles to each sample (15 of each colour in sample TM2B-S15 and 10 of each colour in TM2B-S10), i.e. by rinsing the glass petri dishes in which the spike particles had been kept. Next, the jars containing the samples and the digestion solution were shaken gently and left to incubate at 23 °C for 5 hours. The digested *H. tubulosa* material was filtered through pf-6 µm. Finally, the filters were washed twice with 100 mL of saturated NaCl solution and the supernatant between washes was filtered again.

2.1.4 - Visual identification of spike particles

For both sediment and *H. tubulosa*, the extracted samples were recovered on 6 µm paper filter disks with the help of a filtration glass set, vacuum pump, manifold, and funnel. The funnel and glass filter were rinsed thoroughly with MPF filter water before filtering a new sample. The wastewater from the rinse was collected for each sample and filtered at the end of the process to ensure that no particles were lost during the rinsing phase. All filters collected from the 8 samples were dyed with enough 0.025 concentration Rosa Bengala to cover the entire filter. The filters were kept in covered glass petri dishes inside a 40 °C incubator to allow for more rapid drying of the Rosa Bengala dye before stereomicroscopic analysis.

2.2. Application to experimental sample

In this study, sediment (n=4) and biota (n=4) samples were retrieved by research divers from “Parthenope” University of Napoli. The *H. tubulosa* were identified and obtained by hand, then sediment (2 cm depth) within a surface area of approximately 150 cm² from directly below each animal was collected in 250 mL wide mouth glass jars with aluminium lids. Information regarding the sampling was recorded with the help of “Subtidal Sampling Datasheet” templates (provided in the Harmonized Protocol for Monitoring Microplastics in Biota by JPI-Oceans BASEMAN Project, see Frias et al., 2018 pg. 17). Collected samples were transferred to the laboratory for extraction,

purification and chemical analysis of microparticles recovered using micro-FTIR technology. Efficiency of recovery was evaluated by spiking samples with microplastic particles.

Methodological aspects - By following the guidelines and harmonised protocol proposed by GESAMP and JBI-Oceans, respectively, along with the researchers' simplified methods of obtaining and transferring sediment, the sampling and sample preparation methods used in this study appeared to aid in avoiding the potential loss of MP particles. Both methods are simple and require only one instance in which the sample must be transferred from one vessel to another before the filtration process. The results of the TM1 and TM2 for the extraction of MP particles from sediment favour TM2 as a method to be used in future studies. However, the drying of the sediment required approximately 48 hours, and was seemingly an irrelevant step since the sediment was rehydrated with the saturated NaCl washes. Therefore, it is recommended to simply filter the sediment sample after it is transferred to the larger glass jar for the washes, then add 200 mL saturated NaCl. Rinsing the filters with approximately 20 mL of MPF distilled water appeared to greatly improve visibility. This step is highly recommended for any sample extracted via density separation. *H. tubulosa*, has been shown to eviscerate internal organs due to neurological response to stimuli and soon after death (Byrne, 1986). Given this characteristic, it is important to avoid stressing the specimen at any point during the sampling process to avoid losing the contents of the digestive tract. The sampling protocol used in this study appeared to avoid the encouragement of evisceration given that no material appeared to be expelled until the organs were removed via dissection on site.

Percent recovery values obtained following the digestion and extraction procedures, TM1B and TM2B, indicate that enzymatic digestion results in a higher recovery than KOH digestion and is, therefore, recommended to be used in future studies. The enzymatic digestion not only resulted in more effective removal of the organic material than the KOH but also did not appear to affect the visibility of the white and pink spike particles. Finally, enzymatic digestion has been shown to be gentle on MP particles, whereas KOH can impact polymers as shown in the spectral analysis of this study and described by others (von Friesen et al., 2019).

3. Results

3.1. Method standardisation

Each filter that resulted from the extraction procedures described above was individually analysed using a Nikon SMZ-800 N stereomicroscopic. When a spike particle was found, the Nikon ACT-1 imaging analysis software was used to measure the particle and match it to the measurements made prior to the digestion and extractions. Some images of spike particles before and after each method can be found in Figure 1.

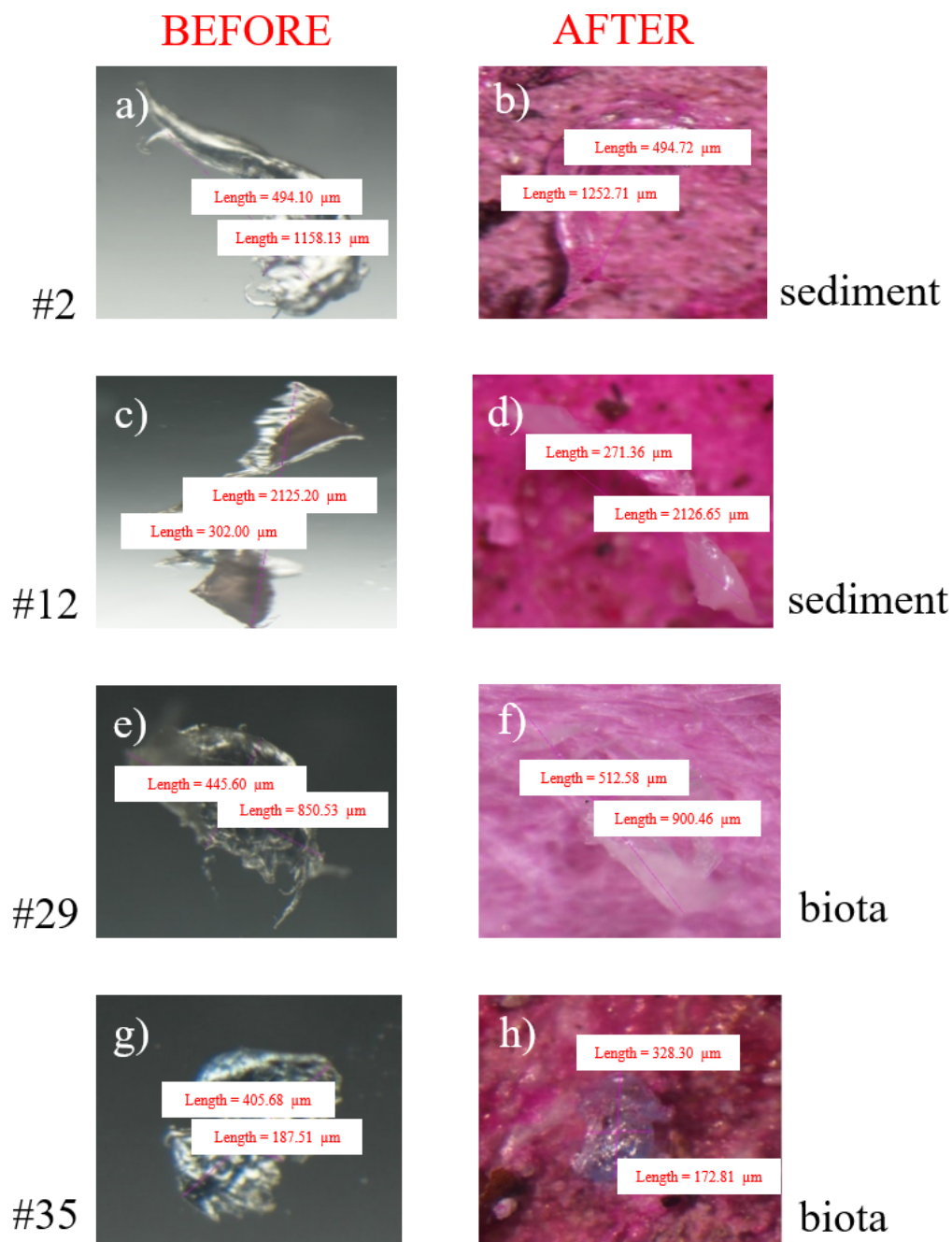


Figure 1. MP spike particles before and after digestion and extraction: a) white particle #2 before being added to TM1-S5/S10; b) white particle #2 from TM1-S5/S10 filter (filtered after first NaCl wash); c) pink particle #12 before being added to TM2-S5/S10; d) pink particle #12 from TM2-S5/S10 filter (filtered before drying) e) white particle #29 before being added to TM2B-S15/S10; f) white particle #29 from TM2B-S15/S10 filter (filtered after KOH digestion); g) blue particle #35 before being added to TM1B-S15/S10; h) blue particle #35 from TM1B-S15/S10 filter (filtered after first NaCl wash)

Regarding sediment samples, TM1 resulted in fibres consisting of low visibility that could be the result of salt crystals forming on the particles from the saturated NaCl solution. The recovery of all particles from all samples is demonstrated in Figure 2.

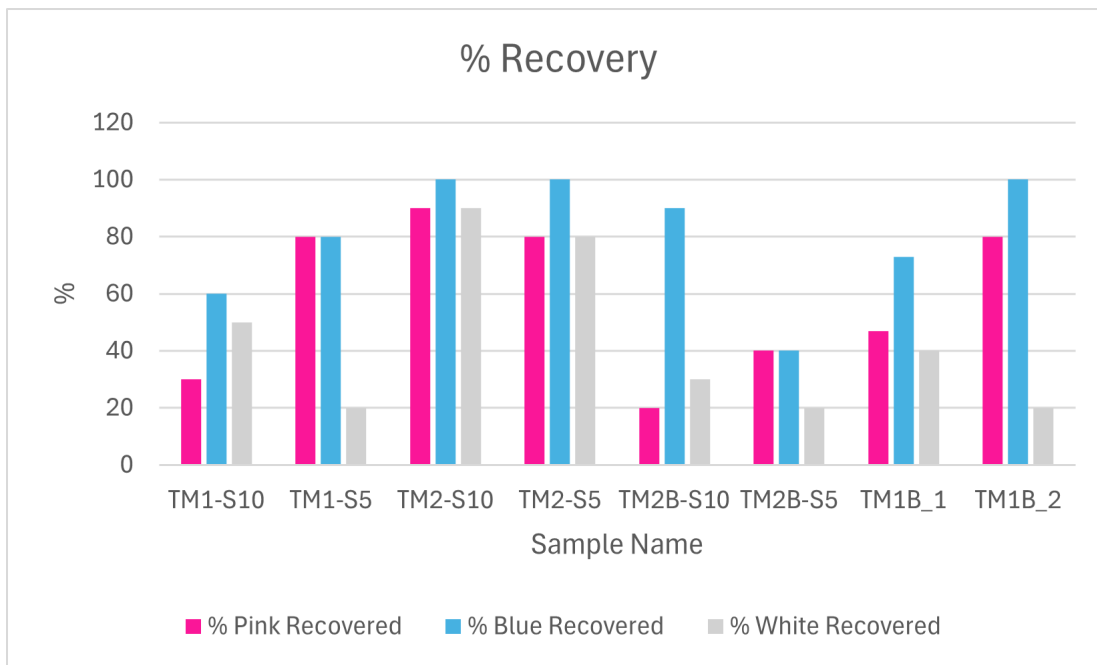


Figure 2. Percent recovery of all samples. Pink bars, blue bars and light grey bars represent the pink particles, blue particles, and white particles, respectively. Exact values can be found in Tables 1-8 along with their respective limit of quantification (LOQ) values

Tables 1 and 2 provide the recovery values of particles in TM1-S10 and TM1-S5, respectively, highlighting the very low recovery rate, particularly for the white and pink particles.

Table 1. Recovery rate of each colour from the MP spikes in sample TM1-S10 (i.e. 10 pink, 10 blue, and 10 white) and respective LOQ values.

	Pink	Blue	White
%	30%	60%	50%
Total Pink Particles Found of 10	3	6	5
LOQ	0.007	0.014	0.012
per kg	6.956	13.911	11.593
Wt. Sample (g w.w.) 431.3			

Table 2. Recovery rate of each color from the MP spikes in TM1-S5 (i.e. 5 pink, 5 blue, and 5 white) and respective LOQ values.

	Pink	Blue	White
%	80%	80%	20%
Total Pink Particles Found of 5	4	4	1
LOQ	0.011	0.011	0.003
per kg	10.724	10.724	2.681
Wt. Sample (g w.w.) 373			

Due to the low recovery rate of TM1, TM2 involved rinsing the filters with approximately 20 mL of distilled MPF water before they were removed from the vacuum pump system. This added step greatly improved the recovery as shown in Tables 3 and 4.

Table 3. Recovery rate of each colour from the MP spikes in TM2-S10 (i.e. 10 pink, 10 blue, and 10 white) and respective LOQ values.

	Pink	Blue	White
%	90%	100%	90%
Total Pink Particles Found of 10	9	10	9
LOQ	0.018	0.019	0.017
per kg	17.442	19.380	17.442
Wt. Sample (g w.w.) 516			

Table 4. Recovery rate of each colour from the MP spikes in TM2-S5 (i.e. 5 pink, 5 blue, and 5 white) and respective LOQ values.

	Pink	Blue	White
%	80%	100%	80%
Total Pink Particles Found of 5	4	5	4
LOQ	0.007	0.009	0.007
per kg	7.472	9.341	7.472
Wt. Sample (g w.w.) 535.3			

Regarding biota samples, the KOH digestion protocol appeared to have decreased the visibility of the spike particles as is shown via the recovery rates listed in Tables 5 and 6.

Table 5. Recovery rate of each colour from the MP spikes in TM2B-S10 (i.e. 10 pink, 10 blue, and 10 white) and respective LOQ values.

	Pink	Blue	White
%	20%	90%	30%
Total Pink Particles Found of 10	2	9	3
LOQ	0.042	0.188	0.063
per kg	41.667	187.5	62.5
Wt. Sample (g w.w.) 48			

Table 6. Recovery rate of each colour from the MP spikes in TM2B-S5 (i.e. 5 pink, 5 blue, and 5 white) and respective LOQ values.

	Pink	Blue	White
%	40%	40%	20%
Total Pink Particles Found of 10	2	2	1
LOQ	0.077	0.0769	0.038
per kg	76.923	76.923	38.462
Wt. Sample (g w.w.) 26			

Furthermore, it is worth noting that the filters often contained a gelatinous layer, covering the particulate matter. Unlike the KOH digestion, the enzymatic digestion resulted in a higher recovery rate also for the white and pink spike particles as shown in Tables 7 and 8.

Table 7. Recovery rate of each colour from the microplastic spikes in TM1B_1 (i.e. 15 pink, 15 blue, and 15 white) and respective LOQ values.

	Pink	Blue	White
%	47%	73%	40%
Total Pink Particles Found of 15	7	11	6
LOQ	0.14	0.22	0.12
per kg	140	220	120
Wt. Sample (g w.w.) 50			

Table 8. Recovery rate of each colour from the microplastic spikes in TM1B_2 (i.e. 5 pink, 5 blue, and 5 white) and respective LOQ values.

	Pink	Blue	White
%	80%	100%	20%
Total Pink Particles Found of 5	4	5	1
LOQ	0.133	0.1667	0.0333
per kg	133.334	166.667	33.333
Wt. Sample (g w.w.) 30			

Sample TM1B_1 was spiked with 15 particles of each colour to allow for a more thorough understanding of the resulting LOQ values.

3.2. Levels in sediment

All tested sediment samples were observed to contain microplastic items (reaching 100% of the total sediment tested), levels recorded were about 0.05 item per gram of sediment extracted. Collected sediment samples showed sizes ranging from 31-17.021 μm with a dominance of black and blue colours and PE (50%), PVC (33%), nylon (15%) plastic types (Figure 3).

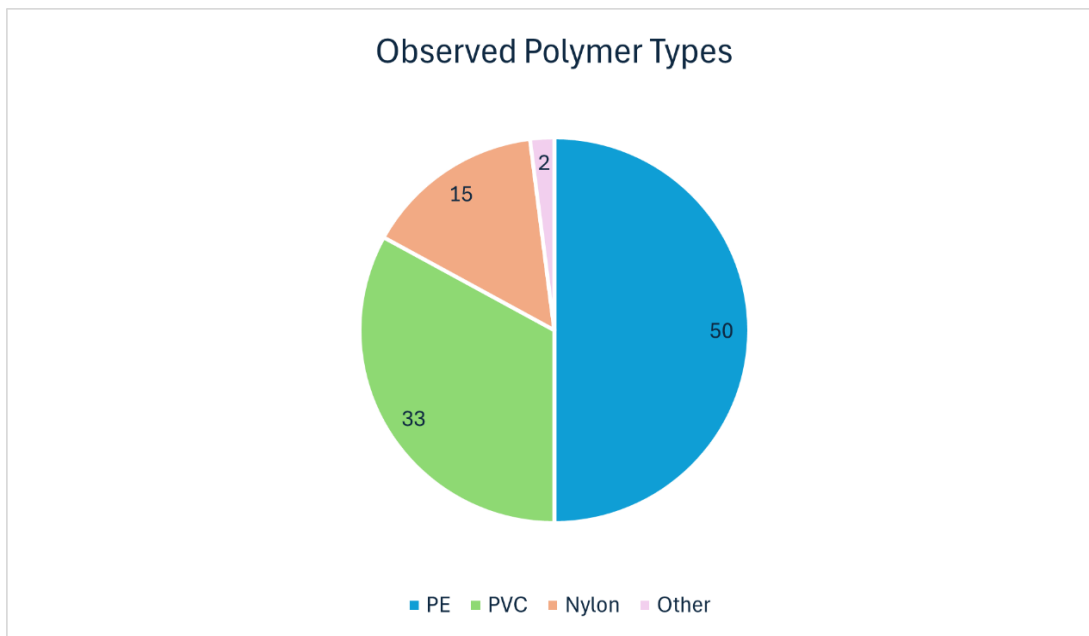


Figure 3. Representation of observed MP polymer types. PE, PVC, Nylon and other shown in blue, green, orange, and pink, respectively

In the sediment samples, about 66% of the recovered microplastic shapes were recorded as fragments while 33% were recorded as fibres. Other shapes were not recorded.

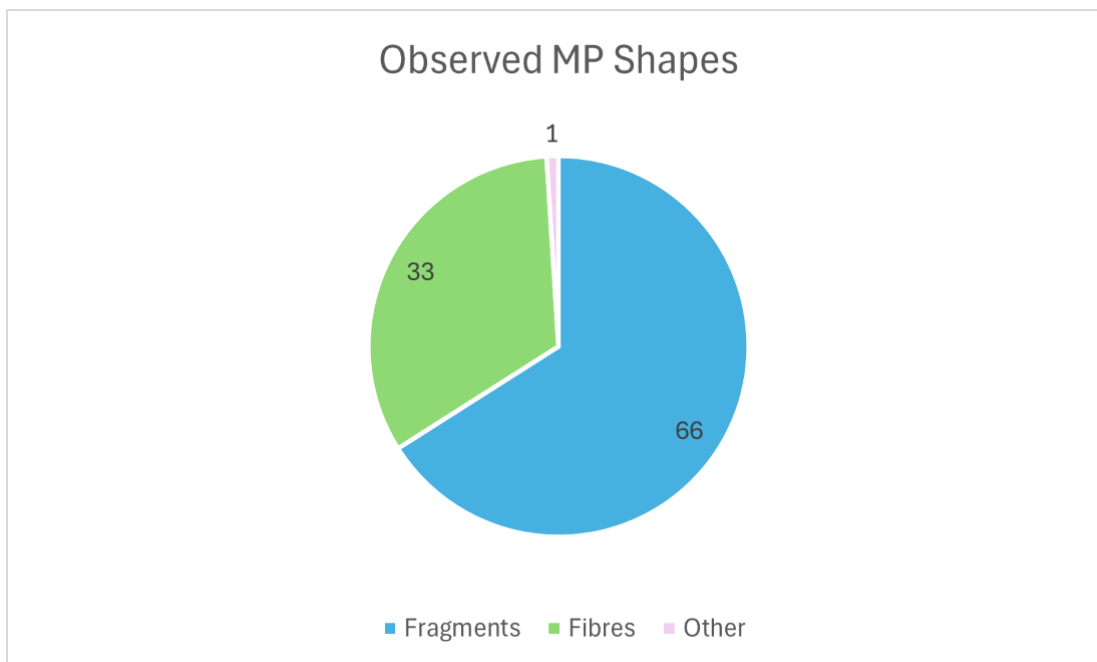


Figure 4. Representation of observed MP shapes. Fragments, fibres, and other shown in blue, green, and pink, respectively

Furthermore, one third of the total amount of recovered fibres were found to be of natural origin (cellulose). It was interesting to observe that the presence of bioclast in sediment seems to actively captures microplastic fibres acting as a sort of filter. Note that this possible scenario was observed on the slides after the complete microplastic extraction protocol and has not been proven with a more thorough investigation (Figure 5).

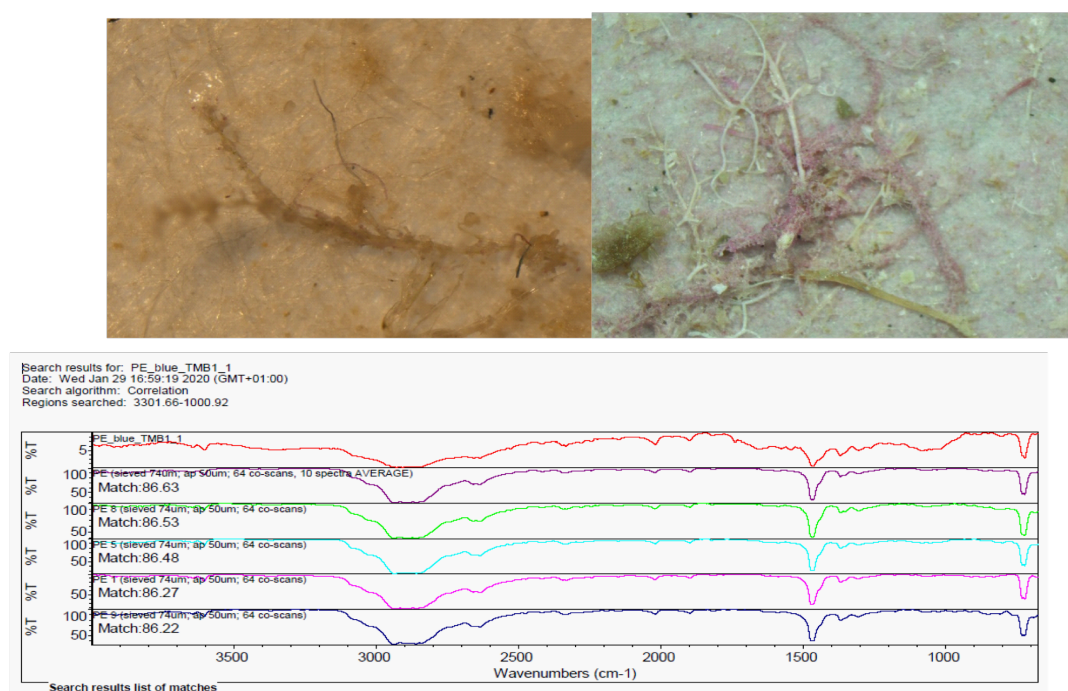


Figure 5. Collecting sediment trapping microfibrils (40X); FT-IR spectra reported as an example

3.3. Levels in sea cucumbers

In the sampled *H. tubulosa*, levels of microplastics were significantly lower than those recorded in the sediment samples. This occurrence could be due to the observed potential trapping activity exerted by bioclast (coralliferous species) as reported in Figure 5. Levels in *H. tubulosa* were on average, 0.5 items/animal. Only PET fibres were recorded in the *H. tubulosa* (100% of types and shapes) showing dimensions lower than 100 μm . In some cases (15% of the total) cellulose/viscose black filament fibres were recorded.

We believe that the results obtained in this study represent reliable data that can contribute to a larger dataset used to determine MPA loss of value caused by MP pollution in both sediment and benthonic species. The tested method of extraction and determination showed high recovery levels of spiking microplastics as shown in Tables 1-8. The efficacy of spiking to control recovery, the efficiency of the extraction, and the fact that MP microchemical analyses are essential in excluding false positive results are helpful analytical tools to generate data needed to more accurately evaluate levels of MPs in the context of EEV. Assessment methods relevant for decision-making, such as EEV, require comprehensive datasets using consistent methodological approaches (Nyadjro et

al. 2023). Proper meta-analysis can only be carried out when there is minimal variability in methodology and reporting of results (Beaumont et al. 2019). Thus, we expect that by following recommended protocols for MP sampling and extraction, our data can be included in future meta-analyses. Furthermore, we demonstrate the feasibility of carrying out sampling, extraction and identification using recommended approaches.

4. Discussion

Coastal areas tend to consist of high biological productivity given their shallow, protected waters and fresh nutritional inputs from rivers (Setälä et al., 2018). In addition, coastal areas are suggested to be at high risk of MP pollution because of their proximity to sources of anthropogenic pollution (Setälä et al., 2018). Furthermore, PCMPA should be relatively clear of MPs contamination given that it is a protected area, therefore, common local sources are expected to exist relatively far from the sampling site. The expected low levels of contamination make the PCMPA optimal when attempting to determine the best practices in sample preparation and analysis because it allows for the researchers to test the applied method without over-saturation of pollution.

MP experts continue to communicate that while there have been several methods used when acquiring biota, sea water, and sediment to analyze MPs, there remains a lack of harmonisation for these methodologies (Barike et al. 2024; Stock et al., 2019; Wootton et al., 2024). However, there do exist guidelines to follow for sampling and analysis (GESAMP, 2019; MSFD Technical Group on Marine Litter, 2023). A combination of referring to published guidelines and personal expert knowledge of the researchers and samplers regarding the PCMPA and *H. tubulosa* was used to carry out this study. In addition, each *H. tubulosa* was sampled along with a respective sediment sample to allow for an investigation of the biota's habitat. We believe this approach is useful in the investigation of the fate of MPs within the marine food web as it ensures a more comprehensive dataset that can be linked to both the species in question and the habitat in question. The method for obtaining samples was based on the GESAMP Guidelines for the Monitoring and Assessment of Plastic Litter in the Ocean which describes that the quantity of litter present in an individual biota will represent a combination of the exposure (i.e. quantity of litter in the surrounding environment) and the residence time in the digestive tract or tissues. According to literature, the biota selected for monitoring should be suitable to act as a bio-indicator of plastic contamination (GESAMP, 2019; Stock et al., 2019). For global monitoring schemes, *H. tubulosa* is an acceptable target specific organism, if it is not considered a protected species at the sampling location, given that it plays a key role in the transfer of organic matter from detritus to higher trophic

levels (Purcell et al., 2016) and is an effective ecological indicator for ecosystem health (Cocci et al., 2025; Warnau et al., 2006).

This study aimed to collect important data on the marine benthic invertebrate, *H. tubulosa*, to obtain more comprehensive data that we expect will be useful in quantifying EEV in the PCMPA and to define intake levels from the bottom to the upper levels of the trophic web. Unlike laboratory MP experiments in which the environment of the study animals can be controlled, in situ environments cannot be controlled. This indicates that valuable information could be missing if components of the sampled animals' habitat are not analysed along with the biota. Furthermore, the sediment obtained can be used to better understand the habitat of the species of interest, *H. tubulosa*.

The approach proposed in this study for the analysis of MPs in *H. tubulosa* aims to highlight the importance of comprehensive research from start to finish. Each aspect of the study was planned from a top-down perspective, to pinpoint where in the bottom-up scientific process there might be room for improvement. Perhaps using this approach in the development of future MP studies could be one way to address complex knowledge gaps that continue to inhibit the scientific community's ability to demonstrate the potential ecological impact of MPs on marine ecosystems.

Although not all researchers who investigate the presence of MPs in the marine environment are experts in extraction methodologies, a harmonised approach could allow scientists from other disciplines to contribute their findings as they conduct their individual research. The goal of the work described here is to demonstrate that reasonable quantification of MPs can be achieved with simple methodologies that do not require specialised personnel. The scientific community needs more high-quality data to accurately predict the impacts of MPs (Waldschläger et al., 2020), particularly with respect to socio-economic assessments, however, the availability of data to this extent could require years if limited to MP specialists. Given that there have already been reasonable guidelines and harmonised protocols proposed by experts in the field of MP research, the use of these could catalyse the development of research approaches that provide experts in environmental modelling with adequate data for risk assessment, thus increasing the presence of data relevant for socio-economic assessment and other ecosystem service-based assessments such as EEV.

5.

Conclusions

In conclusion, as microplastic research continues to move forward in terms of improved methodologies for sampling, extraction and analysis, there remains a lack of understanding the pollutant's impact at the larger scale. While it has been established that MPs are “everywhere” (Barceló and Picó, 2019; Liu et al., 2019), the scientific community continues to wonder what this means for the big picture in terms of risks (Waldschläger et al., 2020). For this reason, this study aims to address the possibility that lack of necessary information for the risk assessment of MPs on marine ecosystems could be partially due to inconsistent approaches used in the sampling, extraction and analysis of in situ samples as well as the format in which findings are reported.

ACKNOWLEDGMENTS

AUTHORS ARE GRATEFUL TO BSRC ADMINISTRATIVE AND TECHNICAL SUPPORT.

AUTHOR CONTRIBUTIONS: VALENTINA H. PAUNA & PIER PAOLO FRANZESE: CONCEPTUALISATION. VALENTINA H. PAUNA & SERENA ANSELM: METHODOLOGY. VALENTINA H. PAUNA: SOFTWARE. SERENA ANSELM & MANUELA PICCARDO: VALIDATION. SERENA ANSELM & MONIA RENZI: FORMAL ANALYSIS. VALENTINA H. PAUNA & SERENA ANSELM: INVESTIGATION. MONIA RENZI & PIER PAOLO FRANZESE: RESOURCES. VALENTINA H. PAUNA: DATA CURATION. VALENTINA H. PAUNA, SERENA ANSELM & MANUELA PICCARDO: WRITING – ORIGINAL DRAFT PREPARATION. MONIA RENZI: WRITING - REVIEW AND EDITING. MONIA RENZI: VIZUALIZATION. PIER PAOLO FRANZESE, GIOVANNI F. RUSSO: SUPERVISION. PIER PAOLO FRANZESE, GIOVANNI F. RUSSO: PROJECT ADMINISTRATION. GIOVANNI F. RUSSO, PIER PAOLO FRANZESE & MONIA RENZI: FUNDING AQUISITION. ALL AUTHORS HAVE REVIEWED AND ACCEPTED THE PUBLISHED VERSION OF THE MANUSCRIPT IN ITS ENTIRETY.

FUNDING: THIS RESEARCH WAS FUNDED BY BIOSCIENCE RESEARCH CENTER, GRANT NUMBER RG_30_2025. SAMPLINGS WERE PERFORMED WITHIN THE RESEARCH AGREEMENT BETWEEN CONISMA AND THE MPA OF PUNTA CAMPANELLA “PROGETTO DI CONTABILITÀ AMBIENTALE NELLE AMP ITALIANE”, PROT NO. 1281/2019 OF 03/10/2019.

DATA AVAILABILITY STATEMENT: DATA IS INCLUDED WITHIN THE MANUSCRIPT AND OTHER SUPPORTING DATA COULD BE ACQUIRED THROUGH THE CORRESPONDING AUTHOR.

AI USAGE STATEMENT: AI HAS NOT BEEN USED FOR THIS WORK.

CONFLICTS OF INTEREST: THE AUTHORS DECLARE NO CONFLICTS OF INTEREST.

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