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Anthropogenic fibers in the Mediterranean sea: Methods and monitoring of an overlooked category of microparticles in the water column

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ABSTRACT

Anthropogenic particles (APs) are widespread in the marine environment, but knowledge gaps remain regarding anthropogenic fibers. This study aimed to evaluate the presence of APs, including natural and synthetic fibers, in the water column. A literature review on fibers in Mediterranean seawater revealed that current sampling methods are underdeveloped. Two sampling methods were compared to determine the best approach for collecting fibers: a new *in-situ* pump (20 μm mesh filter) and a WP2 plankton net (200 μm). The *in-situ* pump was the most effective method and was applied in three areas of the Western Mediterranean Sea (Gulf of Asinara, Capraia Island, Capo Carbonara). The predominant APs, characterized by μFTIR, were cellulose and polyester fibers, reflecting the global textile fiber production. The Asinara area was the most impacted area (average of 393.7 items/m^3). This study highlights the ubiquitous presence of fibers in the water column and underscores the need for further investigation of potential impacts on marine biota.

1. Introduction

Anthropogenic fibers are emerging pollutants widely distributed in the air, soil, and water. Within the marine environment, they can be detected in the water column, on the sea floor, and throughout marine ecosystems (UNEP, 2021). It is estimated that approximately 5 million tonnes of fibers enter the oceans each year (Carr, 2017; Mishra et al., 2019). The primary sources of anthropogenic fibers in the marine environment include household laundry, textile, and tire industries, the fragmentation of large plastic items, illegal dumping, landfills and fishing gear (Mishra et al., 2019; Samal et al., 2024).

Recent studies have highlighted anthropogenic fibers as an underestimated threat to the marine environment, as they are often not considered in the data analysis of many marine litter studies (Athey and Erdle, 2022; Rebelein et al., 2021). Due to limitations in on-field sampling, isolation, from biological samples and chemical characterization, coupled with potential overestimation linked to airborne contamination during both on-site and laboratory activities, fibers have frequently been omitted from analytical data and/or insufficiently characterized (Avio et al., 2015; Concato et al., 2023; Waldschläger et al., 2020). Despite that, when detected fibers have been identified as the most prevalent category of anthropogenic particles (APs) both in different environmental compartments and organisms (Carlotti et al., 2023; Compa et al., 2018; Fagiano et al., 2023; Gago et al., 2016; Giani et al., 2019; Mathalon and Hill, 2014; Santonicola et al., 2023; Scotti et al., 2023; Suaria et al., 2020). Studies have shown that fiber pollution is widespread globally (Compa et al., 2018; Suaria et al., 2020; UNEP, 2021), with the Mediterranean Sea identified as a particularly affected area due to its semi-enclosed configuration.

Techniques for detecting anthropogenic fibers and other microparticles in the marine environment often involve sampling with nets, such as the Manta trawl and WP2 plankton net, which enable the collection of particles from both the sea surface and the water column. Studies have shown that using nets with smaller mesh sizes results in higher quantities of marine litter being collected, due to the net's increased capacity to retain smaller particles, particularly fibers (Covernton et al., 2019; Dris et al., 2015; Lindeque et al., 2020; Simon-Sánchez et al., 2022). For this reason, Lindeque and colleagues (2020) suggest that traditional

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sampling methods, which typically have mesh sizes of 200 and 300 μm, may underestimate the levels of marine litter, especially fibers, present in seas and oceans. Covernton et al. (2019) recommend that sampling techniques designed to detect microlitter in seawater should include filtration at sizes smaller than the width of many fibers, typically between 10 and 20 μm (Suaria et al., 2020). This approach is crucial, considering that the environmental risk posed by marine litter increases as particle size decreases; smaller particles are more likely to come into contact with a greater number of organisms (Fossi et al., 2018). New sampling methodologies have been developed to include particles smaller than 200 μm while limiting the samples' exposure to air and handling before laboratory analysis. However, a unique, standardized protocol facilitating comparable data has yet to be defined. Harrold et al. (2022) recently proposed a new method for sampling anthropogenic particles, including fibers, through *in-situ* pumped water filtration. Dong et al. (2023) highlighted this technique, comparing various sampling strategies, and identified it as one of the most effective for collecting marine litter from the water column. This method employs a peristaltic pump linked to a sealed filtration system with in-line filters of different mesh sizes, reducing air exposure and sample handling; this sampling approach has not yet been applied to detect anthropogenic particles, including fibers, in Mediterranean waters.

Moreover, the lack of chemical characterization of fibers may lead to inaccurate identification of pollution sources, often mistakenly attributed solely to plastic materials (Athey and Erdle, 2022; Rebelein et al., 2021). Some researchers have begun differentiating synthetic fibers from natural ones (Avio et al., 2020; Capillo et al., 2020; Compa et al., 2018; Savoca et al., 2019). Notably, Suaria et al. (2020) report that most fibers analyzed in the Mediterranean Sea are of natural origin, such as cellulose.

Marine organisms are exposed to anthropogenic fibers, but the understanding of the effects associated with the biological risk of fibers on organisms in natural populations is very limited (Kwak et al., 2022). Some studies have linked higher ingestion of anthropogenic particles to reduced individual growth (Compa et al., 2018; Hipfner et al., 2018), increased oxidative stress (Alomar et al., 2017), and a negative correlation between trophic level and AP ingestion in fish species (Giani et al., 2023). Additionally, APs may act as carriers for plastic additives within organisms (Sambolino et al., 2023; Syberg et al., 2015). The limited laboratory studies that have compared the toxicological effects of fibers have reported similar impacts on organisms exposed to natural, semi-synthetic, and synthetic fibers (Athey et al., 2022). For instance, Kim and colleagues (2021) observed a reduction in growth in *Daphnia magna* specimens exposed to both synthetic and natural fibers. The significant knowledge gap regarding the threats posed by anthropogenic fibers to marine organisms highlights the urgent need for further research, including the effects of natural fibers, which remain largely understudied (Concato et al., 2023).

This study aimed to assess the presence of anthropogenic fibers, both natural and synthetic polymers, as well as microparticles, in the water column across various regions of the Western Mediterranean Sea. This involved employing an innovative methodology designed for the accurate detection of fiber presence. Specifically, the following subobjectives were pursued: (i) to conduct a literature review on the presence of fibers in the Mediterranean Sea water to evaluate the methods used and identify knowledge gaps; (ii) to compare two methodologies for sampling anthropogenic fibers from the water column (the WP2 plankton net and a new method employing an *in-situ* pump with a series of filters) to assess which one is most suitable for sampling anthropogenic fibers, including microparticles, in the water column; (iii) to apply and validate the most efficient methodology for detecting and characterizing anthropogenic fibers in three distinct study areas with different protection levels in the Western Mediterranean Sea (Capraia Island, Gulf of Asinara, and Capo Carbonara), with the additional goal of identifying the primary sources of pollution in these areas.

2. Material and methods

Nomenclature

Given the different types of classification that can be made for particles isolated from organisms and the environment, in describing the results obtained, particles have been referred to as 'anthropogenic particles' when they include both plastic (synthetic origin) and non-plastic (semi-synthetic and natural origin) particles of all shapes and sizes. The nomenclature used is described in Fig. 1.

2.1. Bibliographic search

Systematic literature searches were conducted from January to September 2024, using general search engines such as Google Scholar and Scopus, to collect all studies conducted in the Mediterranean Sea that detected fibers in seawater compartments. Specifically, the following keywords were used for the search: fibers (or synonyms such as fibers, microfibers, anthropogenic microfibers, microplastic fibers, textile fibers, lines, filaments, and threads) in the Mediterranean Sea. In total, 900 studies were examined, and all articles on research conducted in the Mediterranean Sea (starting from 2012, when the first study was found) that identified the presence of fibers in seawater were selected to gather information on the sampling and analysis methods used, as well as the data obtained.

2.2. Study area and sampling strategy

Water column sampling was conducted in three distinct areas of the Western Mediterranean Sea (Fig. 2): The Island of Capraia in the Tuscan Archipelago National Park, and the Marine Protected Areas (MPAs) of Asinara Island and Capo Carbonara. These study areas were chosen based on their levels of protection and exposure to various potential anthropogenic impacts, including ports, urban centers, and naval traffic. Additionally, the area surrounding the Island of Capraia has been identified by several studies as a possible marine litter hot spot due to a combination of various factors, such as hydrodynamics and the circulation of currents (Fossi et al., 2017; Galli et al., 2023; Schroeder et al., 2011; Suaria et al., 2016). Sampling activities in each study area were carried out at sites located both inside and outside the protection zone boundaries.

Sampling around the Island of Capraia was conducted along its west coast at five different points: four within the area with the highest level of protection and one near the island's port, outside the protected zone. A novel methodology involving *in-situ* pump water filtration was used for water column sampling. Additionally, at four of the five points, water column sampling was also conducted using a WP2 zooplankton net. Data collected from these two different methodologies were compared to identify the most effective technique for sampling anthropogenic particles, including fibers, in the water column. In the Gulf of Asinara (Sardinia), sampling was carried out using the most effective methodology at three points: two within the MPA of Asinara Island and one near the heavily impacted area of Porto Torres. Near the Capo Carbonara MPA (Sardinia), sampling was also conducted using the most effective methodology, with two points within the MPA and two outside. The data collected from all three areas were then compared.

2.3. In-situ pump sampling

A new technique for sampling anthropogenic particles from the water column was applied in the three study areas. This method involves the use of a peristaltic pump to propel water through a sequence of in-line stainless-steel mesh filters (Fig. 1 in the Supplementary Material). The water is sampled using a 12-V peristaltic pump (Osculati, Self-Priming bilge pump) and passes through a sequential filtration apparatus milled in aluminum. This apparatus was equipped with three

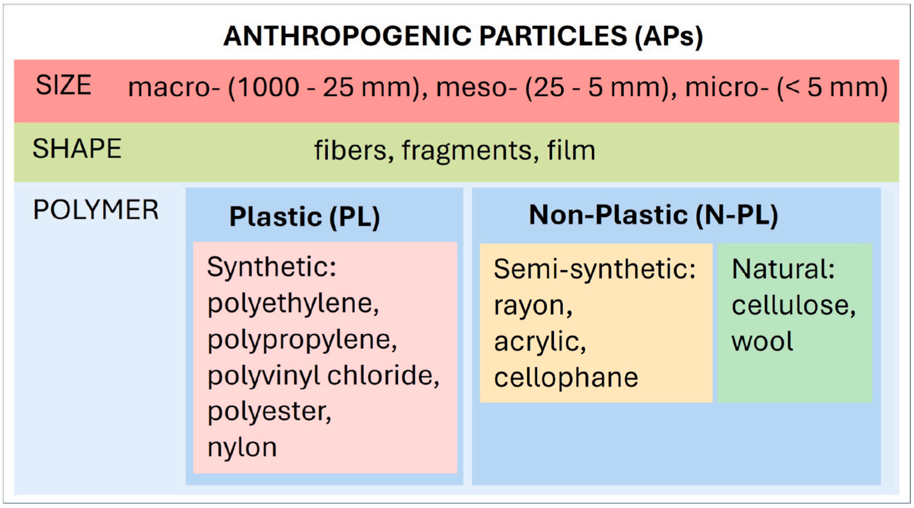

Fig. 1. Nomenclature used to classify anthropogenic particles based on size, shape, or polymer. Each category is described with few examples according to the main results obtained in the present study.

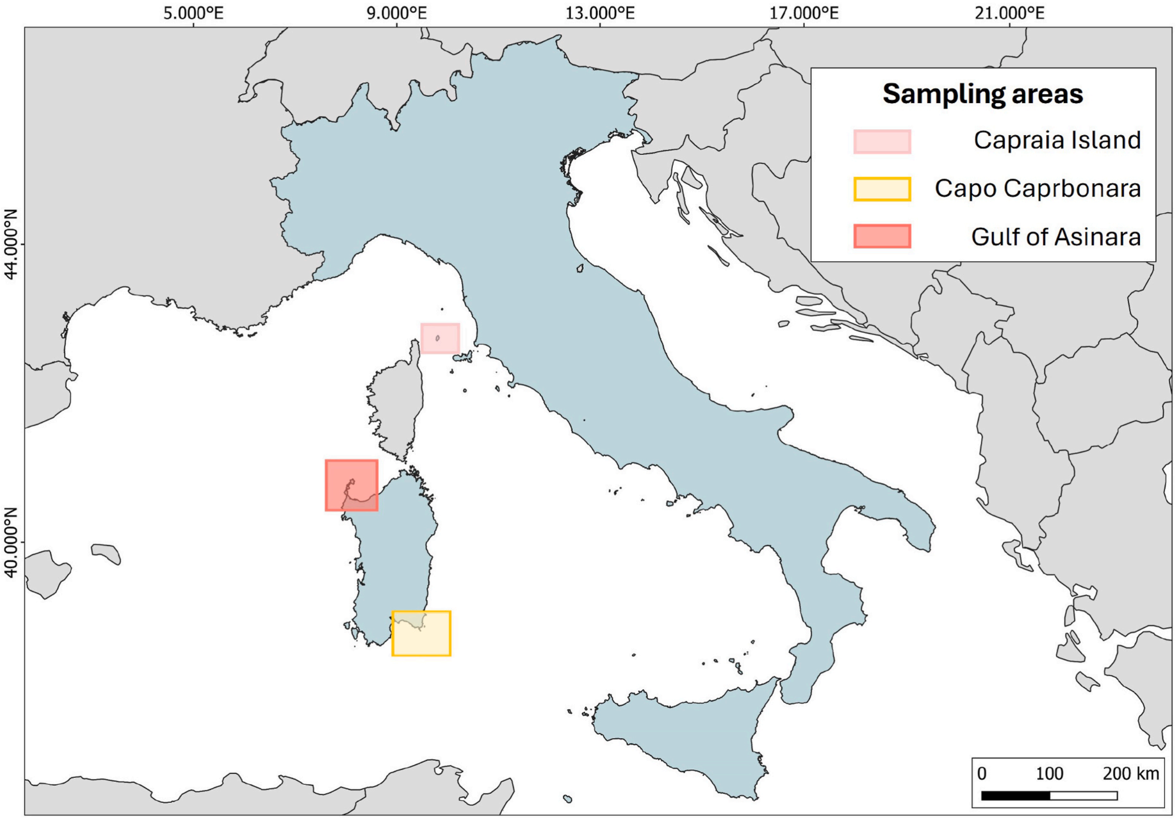

Fig. 2. Sampling areas selected for the investigation of APs, including fiber, presence in the water column. The map highlights the three study locations: Capraia Island (pink), Capo Carbonara (yellow), and the Gulf of Asinara (red). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

stainless steel filters with different mesh sizes (100 μm, 50 μm, and 20 μm; filter diameter 142 mm) provided by DTU Aqua National Institute of Aquatic Resources. A flowmeter was used for the quantification of the sampled water volume. The water was sampled at a depth of approximately 10 m, and at least 300 L of seawater were filtered at each sampling point. At the end of the sampling, the filtration apparatus was opened, and the filters were thoroughly rinsed with 20 μm-filtered water into glass jars previously rinsed with the same water. Subsequently, the jars were hermetically sealed and stored at 4 °C, until laboratory analysis.

2.4. *WP2 sampling*

During the survey campaign near Capraia Island, a sampling methodology using the WP2 plankton net was also employed to sample anthropogenic particles from the water column. A standard WP2 plankton net (mesh size: 200 μm, mouth diameter: 57 cm) was used to perform four vertical hauls, from 10 m to the sea surface, at the same locations as the sampling points of the *in-situ* pump, except for the southern point. To quantify the water filtered the net was equipped with a flowmeter. After the samplings, the nets were thoroughly rinsed with pre-filtered water from the outside to the inside to ensure all particles were washed toward the end of the net and to prevent any contamination. The sample, once collected at the bottom of the net, in the cod end bucket, was then gathered into glass jars, and stored at 4 °C until the laboratory analysis.

2.5. *Sample filtration and characterization of anthropogenic particles*

To isolate the APs, the samples contained in the jars were filtered through a nylon net with a mesh size of 20 μm using a vacuum pump. Subsequently, the sample container was washed with a solution of microfiltered water (0.22 μm) and ethanol (1:1) to accurately collect all the particles. The samples were then observed under a stereomicroscope (ZEISS SteREO Discovery.V8), equipped with a Cold Light Source (Zeiss CL 1500 HAL), and with the help of tweezers, all particles considered potentially anthropogenic were isolated. According to Cadiou et al. (2020), the isolated particles were categorized into dimensional classes (0.02–0.05 mm; 0.05–0.1 mm; 0.1–0.2 mm; 0.2–0.5 mm; 0.5–1 mm; 1–2.5 mm; 2.5–5 mm; 5–25 mm; >25 mm), with slight modifications to the classes, as well as into primary shapes (fibers, fragments, films, spheres, expanded materials) and main colors (black, blue, green, red, white, yellow, transparent, and other colors). Subsequently, all the isolated particles were processed for polymer composition analysis.

2.6. *Polymer composition analysis*

The polymer composition analysis was performed on all particles isolated using a micro-Fourier-transform infrared (μFTIR) Nicolet iN10 MX Infrared Imaging Microscope (Thermo Scientific). Reflection and Transmission modes were used for characterizing the polymer of the particles isolated including fibers. The particles were placed on specific supports (silicone filter, MakroPor PZM5-500 with a porosity of 5 μm). In the presence of larger particles, the ATR mode was used, which allows for determining the polymer of thicker particles. Each spectrum was generated through 16 scans of the particle, lasting 3 s each. A spectral range from 4000 to 650 cm^{-1} was measured, and the spectral resolution was set at 8 cm^{-1}. The obtained spectra were compared with a data library using OMNIC Picta Software (Thermo Fisher), and the polymer was identified only when the spectra of the isolated particle showed a similarity, with a Hit Quality Index (HQI) of more than 70 %, to the library spectra.

2.7. *Contamination control (QA/QC)*

To prevent airborne contamination during sampling activities, all materials used, including the WP2 net, filters, and other instruments employed during the *in-situ* pump survey, were thoroughly rinsed with filtered water at 0.22 μm and several procedural blanks were always performed by rinsing cleaned filters and the WP2 net and collecting the resulting washing water in glass jars for subsequent laboratory analysis for QA/QC contamination control.

To prevent airborne contamination, the laboratory analyses were conducted in a draft-free room. Throughout all stages, the samples were kept covered with aluminum foil in glassware.

To prevent contamination during all laboratory procedures, glassware and other instruments were meticulously cleaned using a washing solution composed of filtered water at 0.22 μm and ethanol (in a 1:1 ratio). The use of this type of solution is expected to promote the detachment of all particles, including fibers, from the surfaces of the objects used, thereby preventing contamination. The washing solution was also used to prepare other procedural blanks to monitor laboratory contamination during the analysis of WP2 samples.

Procedural blanks (from the *in-situ* pump and WP2) then underwent the same laboratory procedures as the samples. The particles isolated from the blanks were subtracted from the samples based on the number of isolated items, as well as their shape, color, and polymer type. Furthermore, during all procedural activities, detailed records were kept of the types of clothing worn by the operators to account for potential contamination sources. This information was carefully considered during the blank subtraction phase to ensure accurate correction for any fibers or particles introduced from operator clothing.

2.8. *Data elaboration and statistical analysis*

The results of the literature review were graphically represented using a Sankey diagram (SankeyMATIC, 2022, open-source software available at https://sankeymatic.com). In this type of flowchart, the width of the arrows is proportional to the flow rate.

To compare the data on water column samples obtained using the two sampling methodologies, the number of APs isolated was normalized to m^3 and L of water filtered. When comparing the two methodologies, only particles larger than 200 μm (the lower limit of the WP2 net mesh size) were considered to highlight differences in the abundance of particles isolated between the WP2 net and the *in-situ* pump technique. All statistical analyses for the field data were performed using RStudio (version 2023.3.1.0) (RStudio Team, 2020), with a significance level of 0.05. The Shapiro-Wilk test was employed to assess the normality of the obtained data. The variance in the number of particles isolated per cubic meter was compared between the two sampling techniques using a two-tailed F-test; subsequently, the mean abundance of isolated items was compared using Welch's *t*-test. The Wilcoxon-Mann-Whitney test was used to compare the number of particles divided by size class or shape type, isolated from Capraia Island. One-way ANOVA tests were performed to assess significant differences in particle abundance and compositions in the three study areas.

3. Results and discussion

3.1. *Studies identifying the presence of fibers in the mediterranean sea*

To compare the data obtained in the present work with the data available in the literature and to evaluate the methodologies used to determine the presence of fibers, bibliographic research was conducted, as reported in Table 1 in Supplementary Material and Fig. 2.

In most samplings conducted in the Mediterranean Sea, the Manta net, and WP2 plankton net were the methods applied for sampling seawater. In both cases, the nets typically used have a mesh size of 330–300-200 μm, which does not allow the isolation of smaller particles.

Most of the studies identified have focused on the determination of anthropogenic particles, microplastics, and fibers on the sea surface, often starting from the assumption that the majority of these particles are plastic and therefore float (Fig. 2). In the Mediterranean Sea, only 3 studies focused on sampling marine litter, including fibers, throughout the water column (Lefebvre et al., 2019; Rios-Fuster et al., 2022; Sayed et al., 2021), and another 3 studies identified fibers in both at the surface and in the water column (Carlotti et al., 2023; de Lucia et al., 2018; Güven et al., 2017). Also in the review by Simon-Sánchez et al. (2022), the sea surface was identified as the water compartment most frequently studied concerning marine litter, accounting for 70 % of the analyzed studies. It was estimated that sampling surface water resulted in underestimations of marine litter abundance, including fibers, ranging between 3.4 % and 97 % (Rios-Fuster et al., 2022). Understanding the real implications of the presence of anthropogenic fibers in the water column is important because this zone hosts a wide range of species that could have dangerous interactions with marine litter (Rios-Fuster et al., 2022).

Research has shown that one of the main challenges in determining the presence of anthropogenic particles, in particular fibers, in the marine environment is controlling contamination, especially during the

Table 1
Technical characteristics of the two sampling methods.

Sampling method	Average volume of water sampled (L)	Average sampling time (min)	Depth (m)	Contamination control onboard (yes/no)	Minimum mesh size (mm)	Mean particle abundance (items/L)	Mean particle abundance (items/ m^3)
WP2	2899	3	from 10 m to surface	yes	0.2	0.01 ± 0.01	15 ± 9
in-situ pump	404	47	10 m	yes	0.02	0.15 ± 0.06	153 ± 66

sampling phases using the net sampling approach. During this process, airborne fibers can enter the sample uncontrollably, and there are also difficulties in creating field blanks. For this reason, many studies conducted in the Mediterranean Sea that collect marine litter in seawater have opted to exclude fibers from data processing (Alomar et al., 2020; Baini et al., 2018; Cincinelli et al., 2019; de Haan et al., 2019; Galli et al., 2023; Ruiz-Orejón et al., 2018; Schmidt et al., 2017; Suaria et al., 2016). Only 6 studies detecting fibers in the Mediterranean Sea reported taking precautions for contamination control during the sampling phases (Caldwell et al., 2019; Carlotti et al., 2023; Kazour et al., 2019; Lefebvre et al., 2019; Rios-Fuster et al., 2022; Suaria et al., 2020). Guidelines for creating blanks that adequately monitor contamination during the sampling phases have not yet been established; this represents a methodological issue that needs to be addressed in future research.

Additionally, considering that the average width of fibers is between 15 and 20 μm (Suaria et al., 2020; UNEP, 2021) and that the literature review indicated only four studies have used methodologies capable of isolating particles smaller than 200–300 μm (Expósito et al., 2021; Rios-Fuster et al., 2022; Sayed et al., 2021; Suaria et al., 2020), the presence of fibers in the Mediterranean Sea may be underestimated. The studies that isolated particles smaller than 200–300 μm used employed different methodologies, including stainless-steel buckets, neuston nets, and glass containers for sampling surface water, as well as Niskin bottles for sampling the water column. Moreover, polymer composition analysis was not performed in 18 % of the studies, and anthropogenic fibers of natural origin remain understudied (Fig. 3), despite Suaria and colleagues (2020) finding that natural fibers accounted for approximately 92 % of the isolated fibers in samples collected from the Mediterranean Sea. The study and monitoring of this type of particles should be further investigated, as the effects on marine organisms are not yet known.

3.2. Comparison of two sampling methodologies

Sampling around Capraia Island was conducted using the *in-situ*

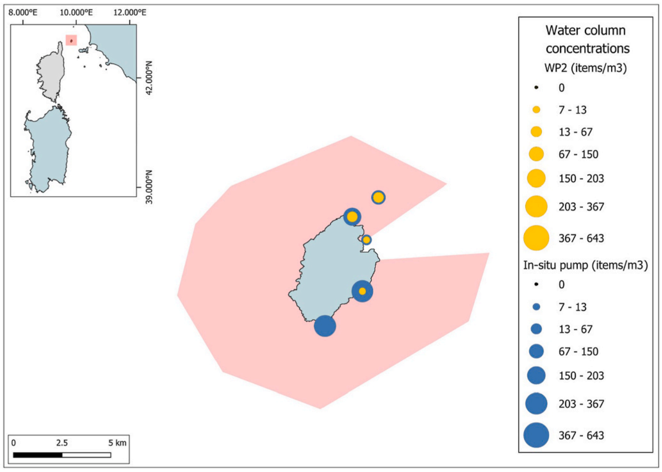

Fig. 4. Concentrations of APs in the water column around Capraia Island were detected using two different methodologies. Sampling points are represented by circles of varying sizes corresponding to the number of items per square meter isolated with the in-situ pump (blue circles) and the WP2 net (yellow circles); the area with the highest level of protection is indicated in pink. More details on the number of items/m^3 at each sampling point are provided in Table 2S of the Supplementary Material. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

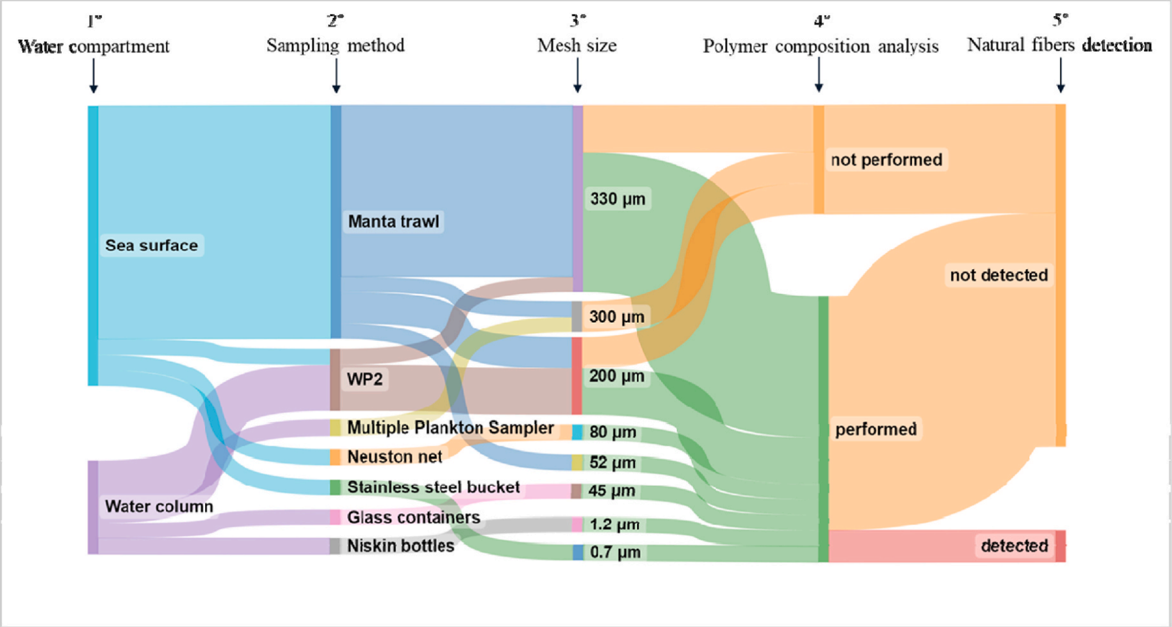

Fig. 3. Sankey diagram showing the results of the literature review on studies that sampled marine litter in the marine environment and included fibers in their data analysis. The main environmental compartments and sampling methods applied are reported.

pumped water filtration technique and the use of the WP2 plankton net (Fig. 4), to determine the most suitable method for detecting anthropogenic particles in the water column, including both natural and synthetic fibers. The technical characteristics of the two sampling methods are listed in Table 1. A comparison was made for the average number, shape, and dimensions of isolated particles, as literature research revealed a potential underestimation for certain categories of shapes and size classes when using the WP2 equipped with a 200-mesh net.

A total of 184 particles were isolated using the *in-situ* pump, resulting in an average of 153 ± 62 items/m^3 or 0.15 ± 0.06 items/L. From samplings conducted using a WP2 plankton net, it was possible to isolate a total of 162 particles: with an average AP concentration of 14.9 ± 8.84 items/m^3, equivalent to 0.01 ± 0.01 items/L. Thanks to the use of smaller mesh filters in the *in-situ* pump methodology, a greater number of particles (10 times higher) longer than 200 μm were isolated (the limit of the WP2), as shown in Fig. 5a (Welch's *t*-test, p-value = 0.03). As expected, the difference in the mean number of isolated particles aligns with findings from various studies, confirming that smaller mesh sizes used during sampling result in higher quantities of anthropogenic particles being collected (Covernton et al., 2019; Dris et al., 2015; Lindeque et al., 2020; Simon-Sánchez et al., 2022). This can be attributed to the fact that most of the isolated particles were fibers (90 %) with an average diameter of less than 200 μm (WP2 mesh size), approximately 20 μm, as previously mentioned. Consequently, a significantly greater number of these particles can be isolated using the *in-situ* pump (Fig. 5b). The comparison of isolated APs shapes (considering only particles longer than 200 μm) between the two methodologies revealed statistically significant differences (Welch's *t*-test, p-value = 0.02) in the average number of fibers per cubic meter sampled with the *in-situ* pump compared to those collected with the WP2 plankton net (Fig. 5b). However, no statistically significant differences were detected in the average number of fragments or films per cubic meter between the two sampling methods. These results support the assertion made by Carlotti et al. (2023) that larger mesh sizes and towed nets are not suitable for fiber sampling.

Regarding the size class of particles sampled using both methodologies, the majority were smaller than 2.5 mm (Fig. 6a), considering all isolated particles. However, differences can be observed in the number of particles per cubic meter when divided into size classes (Fig. 6a).

The WP2 net tends to underestimate the abundance of anthropogenic particles (AP) in the water column, particularly for smaller particles, which aligns with the findings suggested by Lindeque and colleagues (2020). Statistically significant differences in the number of particles per cubic meter isolated by the two methodologies were observed across various size classes using the Wilcoxon-Mann-Whitney test. These differences specifically concerned particles smaller than 2.5 mm, with significant variations found in the following size ranges: 0.05–0.1 mm (p-value = 0.026), 0.1–0.2 mm (p-value = 0.029), 0.2–0.5 mm (p-value = 0.029), 0.5–1 mm (p-value = 0.006), and 1–2.5 mm (p-value = 0.028). In contrast, no differences were observed between the two methodologies for particles larger than 2.5 mm, suggesting they are comparable for this size range.

Finally, no significant differences were detected concerning the color of the isolated particles; in both cases, over 80 % of the APs were either blue or black. The same applies to the origin and composition of the polymers (Fig. 6b); this analysis revealed that approximately 69 % of the isolated particles were of natural origin, specifically cellulose. Particles of synthetic origin constituted around 20 % of the total, with polyester, PVC, and nylon being the most frequent polymers identified. About 9 % of the particles were made of semi-synthetic polymers, predominantly rayon.

Based on observations made during the samplings, the primary advantages and disadvantages of the two tested methodologies have been identified and are presented in Table 2. Considering this information alongside the obtained results, the *in-situ* pump technique emerges as the most suitable method for sampling APs in the water column, particularly fibers, despite its longer sampling duration. It should be noted that the size class of a particle is assigned based on its larger dimension (length), without considering its width. When there is a significant difference between the length and width/diameter of the particles (as in the case of fibers), they could be collected also by a tool which has a smaller mesh size rather than the larger dimension of them. The observations made during sampling are consistent with those reported by Harrold et al. (2022), who first proposed and evaluated this sampling methodology. They demonstrated through laboratory tests that this method minimizes contamination by reducing sample exposure to air and achieves a particle recovery rate of over 70 %. In the present study, the analysis of the procedural blanks allowed to corroborate the differences in the levels of contaminations between the two methods: the mean numbers of particles in the blanks of the *in-situ* pump technique was less than 20 % of the mean numbers of particles isolated in the samples, on the contrary, in the blanks of the WP2 net it was, instead, more than 20 %. Additionally, the *in-situ* pump technique minimizes sample handling and accommodates various filter types based on mesh size. Furthermore, Dong and colleagues (2023) compared different methodologies for sampling anthropogenic particles from various environmental compartments and identified this method as one of the most effective for sampling anthropogenic particles from the water column.

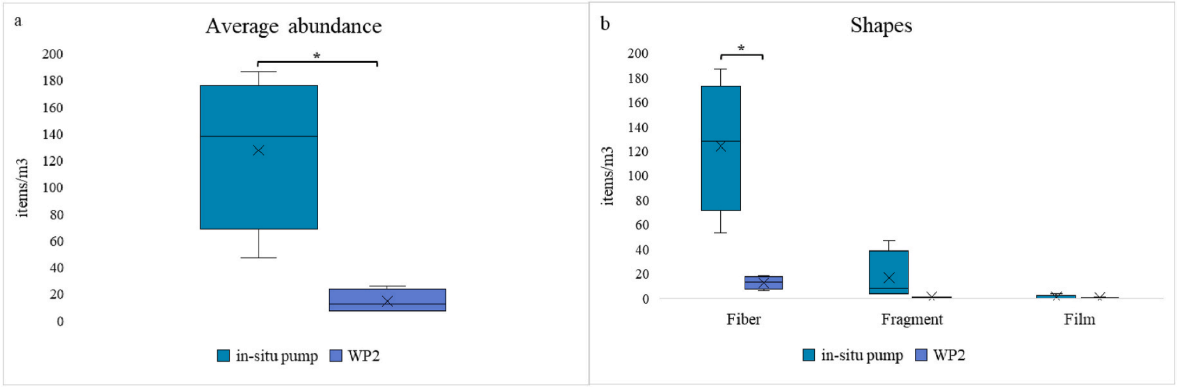

Fig. 5. Comparison of results obtained using the in-situ pump methodology and the WP2 plankton net, expressed in items/m^3. In Fig. 5a, a box plot illustrates the mean abundance of anthropogenic particles, while Fig. 5b shows a box plot depicting the mean number of items/m^3 based on the different shapes found (considering only particles longer than 200 μm). Asterisks highlight cases where the Wilcoxon-Mann-Whitney or Welch's test indicated a significant difference between the two methodologies, with a p-value <0.05.

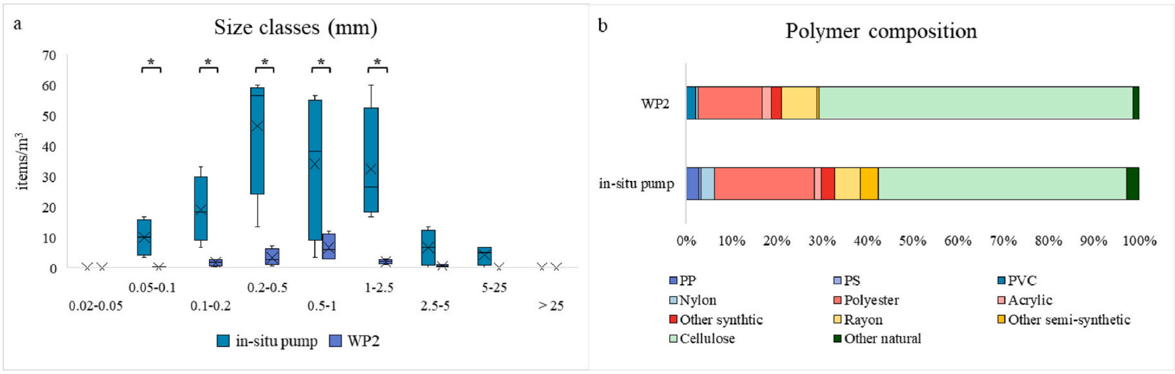

Fig. 6. The average number of particles for each size class is reported in Fig. 6a, and in Fig. 6b, the polymer compositions of the isolated items are shown. Asterisks highlight cases where the Wilcoxon-Mann-Whitney indicated a significant difference between the two methodologies, with a p-value <0.05.

Table 2
Advantages and disadvantages of the two methodologies used to sample anthropogenic particles, particularly fibers in the water column near the island of Capraia.

Sampling method	Advantages	Disadvantages
in-situ pump	reduces sample handling reduces the risk of contamination supports a variety of filter types possibility to create procedural blanks during sampling possibility of sampling at different depths	longer sampling times need for electric current
WP2 net	shorter sampling times a larger volume of sampled water	most commonly used net has a lower limit set at 200 µm unable to sample at specific water depths unable to differentiate vertical variation sample exposed to air during sampling

3.3. Anthropogenic particles in different areas of the Western Mediterranean Sea

3.3.1. Quantification and characterization of anthropogenic particles

The results obtained by applying the new sampling technique in three areas of the Western Mediterranean Sea were examined and compared to evaluate the amount and composition of anthropogenic particles, including fibers, in the water column (Fig. 7). Examples of isolated particles are shown in Fig. 2 of the Supplementary Material. Using the *in-situ* pumped water filtration technique, a total of 294 anthropogenic particles were isolated and characterized from samplings conducted near Capraia Island. The average number of particles was 196 ± 109.6 items/m^3, corresponding to 0.2 ± 0.1 items/L. From the Gulf of Asinara samples, a total of 537 anthropogenic particles were isolated, with an average number of 393.7 ± 95.4 items/m^3, corresponding to 0.4 ± 0.1 items/L. In the proximity of the marine protected area of Capo Carbonara, 245 particles were isolated, and the average number of items per cubic meter was 142.4 ± 25.1, corresponding to 0.14 ± 0.03 items/L. No differences in the number of particles per cubic meter were observed among sampling sites monitored inside and outside the marine protected area boundaries (Fig. 7).

The average abundance of anthropogenic particles isolated in the three study areas is illustrated in the box plot in Fig. 8a. Statistically significant differences were observed in the mean number of particles per cubic meter isolated from the Gulf of Asinara compared to the other two sampling areas. Samples from the Gulf of Asinara appear to be the most contaminated by anthropogenic particles, whereas those from Capo Carbonara are the least contaminated. One-Way ANOVA test confirmed a statistically significant difference from the comparison between these two areas (p-value = 0.01). As for the samples from Capraia Island, the test demonstrated a significant disparity from those collected near Asinara (p-value = 0.03) (Fig. 8a). Nevertheless, there was no statistical difference found when comparing samples from Capraia to those collected in proximity to Capo Carbonara.

The obtained data indicate that the employed methodology has successfully allowed the isolation of anthropogenic particles from all the analyzed water column samples. This sampling and analysis methodology differs from the approaches commonly used to isolate APs, including fibers, in the Mediterranean Sea (Table 1 of Supplementary Material). A comparison with the literature is challenging because few studies utilize methodologies that include particles smaller than 200 or 300 µm. Only one study, conducted on the surface water of the Mediterranean Sea, by Suaria and colleagues (2020), did not employ the net approach for sampling surface water, but instead utilized stainless steel buckets, enabling the collection of smaller particles (down to approximately 20 µm). The data reported in the publication relate to the surface water of the Mediterranean Sea, encompassing the same areas as this study, with an average of isolated particles per liter equal to 4.6, including both synthetic and natural fibers; this is higher than the 0.13 ± 0.13 items/L found in this study. Regarding water column sampling methods, the net sampling approach is the most commonly used; only one study sampled the water column using a methodology allowing the isolation of particles smaller than 200 or 300 µm, in the Mediterranean Sea. Rios-Fuster and colleagues (2022) utilized 5-L Niskin bottles to sample the water column at different depths (5, 15, and 25 or 50 m) along the Iberian Peninsula; the samples were then filtered through filters with a mesh size of 1.2 µm. In this case, as well, the average concentration of particles per liter, corresponding to 1.86 items/L, was lower compared to that found in this study.

The area around the Island of Capraia is considered a potential accumulation zone for APs due to specific oceanographic conditions that result in the formation of a transient gyre of currents in this area, known as the "Gyre of Capraia" (Fossi et al., 2017; Guerrini et al., 2019; Schroeder et al., 2011); despite this none of the studies available in the literature reported in Table 1 of Supplementary Material, have determined the presence of APs, including fibers, in the water column. Among the studies that have also determined the presence of fibers, Caldwell and colleagues (2019), sampling the surface water of the eastern sector of Capraia island between May and June 2018, recorded a density of 0.4 items/m^3, values lower than those determined in this study. Also, from samplings conducted in the spring of 2019 (Caldwell et al., 2020), they determined approximately 23.84 particles per m^3, including both meso- and microplastics; this data is lower than that obtained in the present study using the *in-situ* pump methodology.

The number of isolated particles from the Gulf of Asinara emerged as

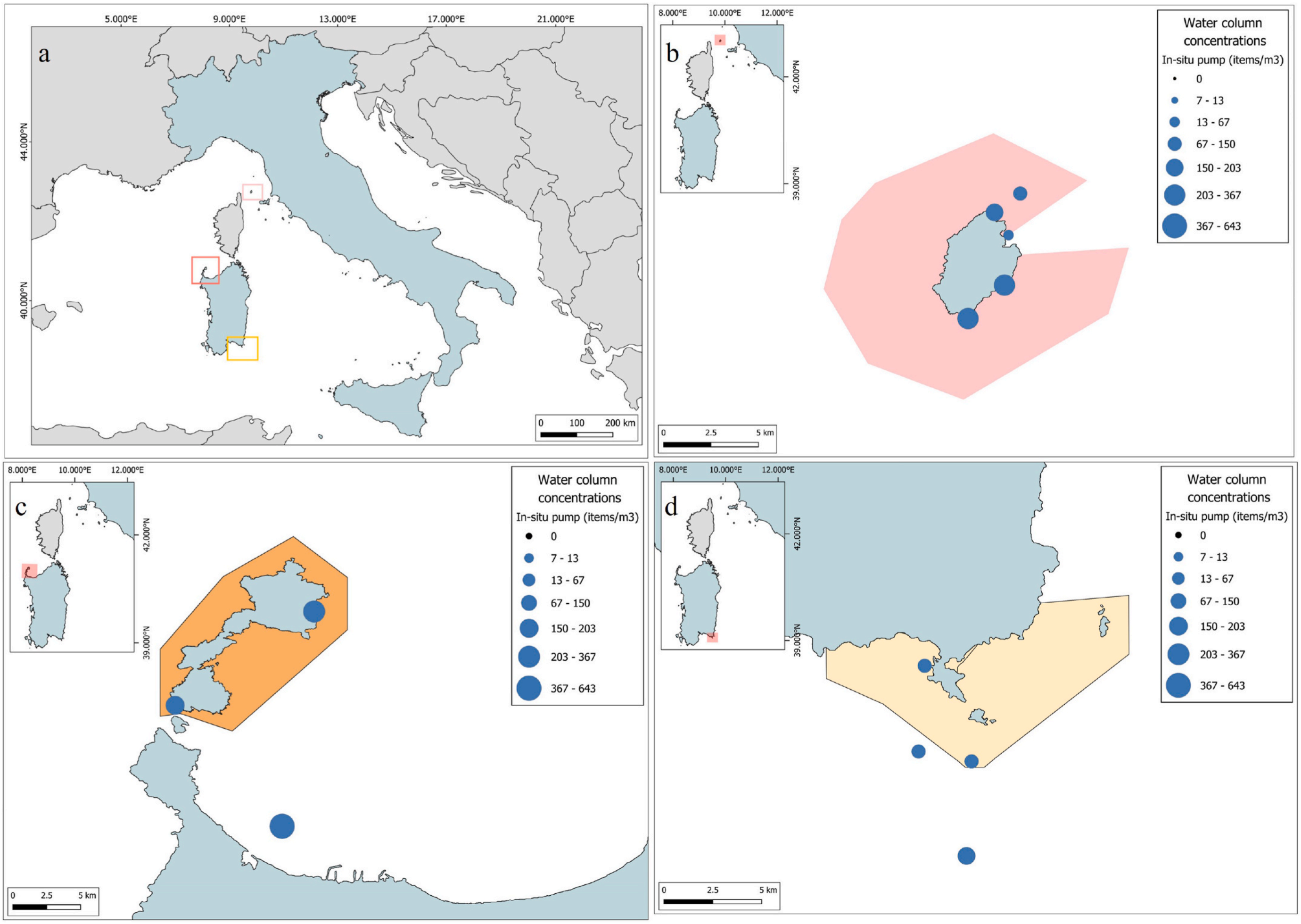

Fig. 7. Concentrations of anthropogenic particles in the water column across the three study areas. The three areas of study have been indicated (a): in pink, the Island of Capraia (b); in orange, the Island of Asinara (c); in yellow, Capo Carbonara (d). For each sampling area, sampling points are represented by circles of varying sizes corresponding to the number of items per square meter isolated with the in-situ pump (blue circles). The marine protected areas have been indicated (pink for Capraia, orange for Asinara, yellow for Capo Carbonara). More details on the number items/m^3 at each sampling point are provided in Table 2S of the Supplementary Material. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the highest among the surveyed areas. Previous research by de Lucia and collaborators (2018) had conducted sampling in this area of Sardinian using both a Manta trawl and a WP2, covering the surface and the initial 20 m of the water column. Their findings reported an average of 0.12 ± 0.04 items/m^3, which is lower compared to the values discovered in the present study. Panti and colleagues, in monitoring campaigns performed in 2012 and 2013, also investigated this area and found higher values, averaging 0.17 ± 0.32 items/m^3, though still less than those identified in the current research (Panti et al., 2015).

Among the studies conducted in the Mediterranean Sea, only one reported sampling data in an area near Capo Carbonara. Suaria et al. (2016) monitored the presence of APs on the sea surface southeast of Sardinia, and fibers were not considered. The reported data align with the findings of the current work, indicating a lower concentration of APs southeast of Sardinia compared to the concentrations found in Capraia during the same monitoring campaign. Furthermore, these data are consistent with models of AP distribution in the Mediterranean Sea, estimating a lower concentration of APs in this area compared to the areas around the Island of Asinara (northwest Sardinia) and the Island of Capraia, where a concentration of APs three times higher is estimated (Fossi et al., 2017).

The isolated APs were classified based on size class, shape, and color (Fig. 8b, c, d). Approximately 90 % of the isolated particles were fibers, which is consistent with the average found in samplings conducted by Suaria and colleagues in the same areas (Suaria et al., 2020). Lower values were reported by Caldwell et al. (2020, 2019) and de Lucia (de Lucia et al., 2018), both applied the net approach for sampling. Regarding the data characterization of APs based on size class, the majority of isolated particles in the water column in this study were smaller than 2.5 mm (Fig. 8b). This data aligns with results from samplings done on the sea surface in this area of the Pelagos Sanctuary by Caldwell et al. (2020) and other studies that, however, did not consider the presence of fibers (Baini et al., 2018; Fossi et al., 2017; Galli et al., 2023; Panti et al., 2015). In particular, particles in the 0.2–0.5 mm size category were the most frequently observed in samples from Capraia (33 % of the total) and Asinara (40 % of the total) (Fig. 8b). However, Capo Carbonara samples did not contain particles smaller than 200 μm, and the most abundant size class, comprising 33 % of particles, had lengths ranging from 1 to 2.5 mm (Fig. 8b). As previously mentioned, the One-Way ANOVA test revealed statistical differences between the Asinara and Capo Carbonara samples, specifically for particles ranging from 0.1 to 0.2 mm (p-value = 0.02), 0.2–0.5 mm (p-value = 0.006), and 0.5–1 mm (p-value = 0.02); and between the Asinara and Capraia samples, specifically for particles ranging from 0.2 to 0.5 mm (p-value = 0.001) and 0.5–1 mm (p-value = 0.03).

3.3.2. Polymer origin and composition of anthropogenic particles

In all study areas, the majority of particles (approximately 60 %)

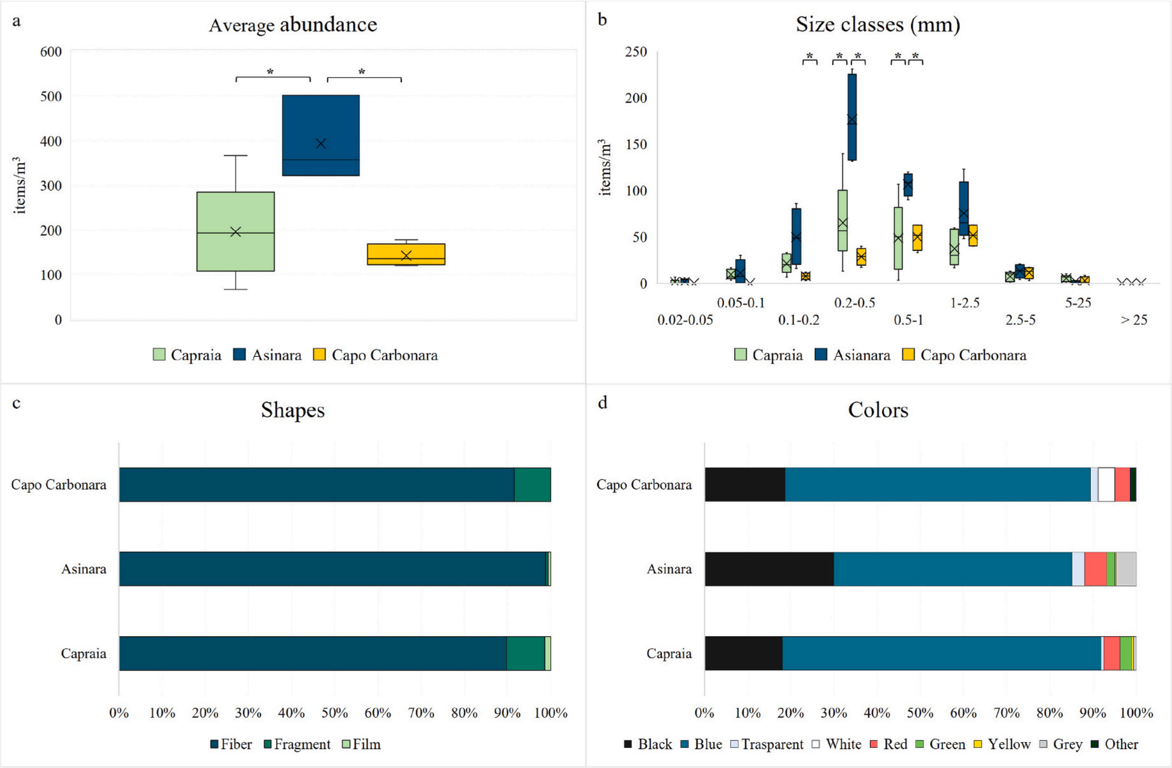

Fig. 8. Comparison of results obtained using the in-situ pump methodology in the three sampling areas: the average isolated items per square meter (a), and the characterization of particles according to size class (b), shape (c), and color (d); "Other" colors included transparent and bi-colored items. The asterisk highlights cases where the One-Way ANOVA test indicated a significant difference in the average number of particles isolated between the studied areas, with a p-value <0.05. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

were of natural origin, predominantly composed of cellulose fibers (Fig. 9), as previously reported in other studies, which have shown that cellulose fibers account for more than 60–80 % of all fibers in the sea floor, sea surface, marine organisms, wastewater, and airborne fiber pollution (Avio et al., 2020; Dris et al., 2017, 2018; Sanchez-Vidal et al., 2018; Suaria et al., 2020). Isolated synthetic plastic particles accounted for 26 %, with polyester being the predominant polymer. This contrasts with findings from other studies conducted on the sea surface of the Mediterranean Sea, where higher percentages of polyethylene (PE) or polypropylene (PP) were observed, while polyester was generally present in lower proportions (Baini et al., 2018; Caldwell et al., 2019, 2020; Fossi et al., 2017; Suaria et al., 2016).

This finding could be explained since the polyester found in this study was in the form of fibers, a particle typology not considered in the studies cited above. Furthermore, PE and PP polymers have a lower density than water and therefore tend to accumulate more on the sea surface; with density ranging from 0.89 to 0.98 g/cm^3 for PE, and from 0.83 to 0.92 g/cm^3 for PP (Kooi and Koelmans, 2019). On the other hand, polyester, having a higher density (from 0.96 to 1.45 g/cm^3), could accumulate below the marine surface (Kershaw, 2015; Kooi and Koelmans, 2019) as well as cellulose (density ranging from 1.54 to 1.63 g/cm ^{3}) (Suaria et al., 2020). Other polymers with a higher density than water have also been identified, such as PVC, polyvinyl acetate (PVA), and nylon (or polyamide), and only 1.37 % of the particles isolated were PP and 0.07 % PE. Semi-synthetic particles, including rayon and cellophane, accounted for 7 % of the total APs isolated. Overall, the abundance of the types of polymers isolated from the samples of this study differs from those reported in other studies for two primary reasons: the

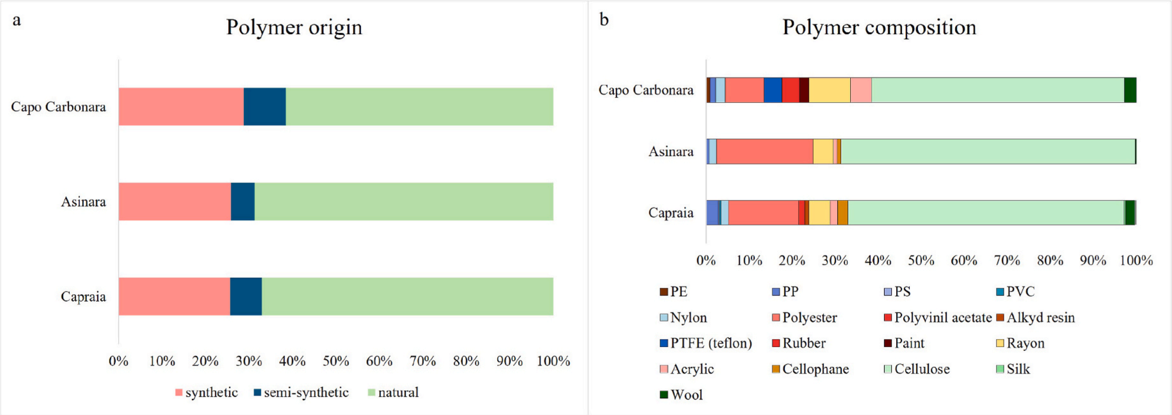

Fig. 9. Polymer origin (a) and composition (b) of anthropogenic particles isolated from *the water column.*

first is that we sampled an environmental compartment that has been relatively unexplored, and the second is that fibers, both of natural and synthetic origin, were considered. If we consider the current fiber production, the abundance of cellulose constituted 30 % of fibers in 2021 and represented the majority of production until the year 2010. However, the production of polyester fibers has been steadily rising; since 2010, it has become the most widely produced type of fiber (Textile Exchange, 2022), accounting for 6.2 % of globally produced plastic polymers in 2022 (Plastics Europe, 2023). Polyester and cellulose are the first and second most widely produced fiber types globally, and they represent the majority of isolated fibers in this study (19 % and 66 %, respectively), followed by rayon (viscose) at 5 % and nylon (polyamide) at 1.8 %. Nevertheless, in this study, cellulose fibers have been sampled in a greater quantity compared to polyester fibers. This can be explained by considering that cellulose was the most produced fiber type historically until 2010, the year when polyester fibers became the most produced. Additionally, it has been demonstrated that cotton textiles, composed of cellulose, release more fibers than polyester during laundering (Cesa et al., 2020; Sillanpää and Sainio, 2017; Zambrano et al., 2019). Currently, the implications of the presence of these particles in the environment are not known, and future studies will need to clarify this issue.

4. Conclusions

The *in-situ* pump technique proved to be the most suitable methodology for representative sampling of anthropogenic particles, particularly fibers, present in the water column. Future applications of this methodology to understand the fate of fibers should verify the presence of APs at other depths beneath the sea surface, examining the vertical distribution of APs in the water column.

From the comparison with the obtained data and literature, it emerged that the most prevalent APs in the Mediterranean Sea have dimensions smaller than 2.5 mm. This suggests that sampling efforts should focus on smaller particles, especially fibers, which are still underexplored, particularly in the water column where they seem to accumulate more than other APs, considering that this environmental compartment is a fundamental habitat for the marine ecosystem.

The Gulf of Asinara seems to be the most affected by the issue of APs amongst the three analyzed, which is consistent with the higher anthropogenic impact in the area due to the presence of a larger urban center and commercial port with a significant influx of tourists, compared to the other areas considered. The analysis of the polymer composition of particles revealed that textile fibers are the primary type of anthropogenic particles affecting the water column. Precautionary measures to reduce the release of fibers and limit their presence in the sea have not yet been officially adopted, some solutions have been proposed, as reported in a recent publication (Rathinamoorthy and Balasaraswathi, 2024). These measures primarily focus on reducing domestic fiber release during washing and include the use of bags or filters in washing machines. Additionally, specific treatments and additives for textile products that are currently being tested aim to decrease fiber release into the environment during both the use and washing of textile materials.

Overall, the results obtained indicate the widespread presence of these particles even in ecologically significant areas, distant from pollution sources but potentially exposed to this type of contamination due to factors such as sea currents. Future studies aimed at determining the presence of APs in the marine environment should always consider fibers, including those of natural origin, to elucidate their distribution dynamics. Additionally, the impact of fibers on marine organisms should be investigated, given that the small size of these particles makes them easily assimilable by a wide range of organisms, and they could act as vectors for contaminants adsorbed onto these particles.

CRediT authorship contribution statement

Margherita Concato: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Cristina Panti:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Matteo Baini:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **Matteo Galli:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Michela Angiolillo:** Writing – review & editing, Project administration, Funding acquisition. **Maria Cristina Fossi:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization, Methodology.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2025.107138.

Data availability

Data will be made available on request.

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