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Microplastics Detected in Sediments and Rocks Substrate of Marine Areas with Ghost Nets

Dyana Vitale [1,*], Andrea Spinelli [2] and Yolanda Picó [1]

[1] Food and Environmental Safety Research Group (SAMA-UV), Desertification Research Centre—CIDE (CSIC-UV-GV), University of Valencia, Road CV-315 Km 10.7, Moncada, 46113 Valencia, Spain
[2] Research Department, Fundación Oceanogràfic de la Comunitat Valenciana, Oceanogràfic, Ciudad de las Artes y las Ciencias, 46013 Valencia, Spain
* Correspondence: dyana.vitale@uv.es

Abstract: According to UNEP and FAO reports, the fishing nets abandoned in the seas around the world represent approximately 10% of the plastic waste present in the oceans. These nets, also called ghost nets, can be degraded over time, releasing microplastics and contaminating the environment. Studying the presence and amount of microplastics in an area impacted by ghost nets in the Gulf of Cefalù, northern coast of Sicily, Italy can help to understand the level of contamination in these zones. The planned study methodology has been carried out by sampling sediment and rock substrate, on a quantitative basis, by scuba diving at seven stations selected as the most representative for the presence or absence of fishing nets in the study area. Two different extraction methods for sediment and rock samples were taken from the literature and modified for the present study. Microplastics determination was carried out according to, first, a visual identification and, second, a polymer type identification by ATR-FTIR spectroscopy, demonstrating the presence of polyamide, nylon, or polyethylene in the impacted areas, probably derived from the degradation of fishing nets. The present study reports the first record of microplastics determination in rock substrate samples recollected by scuba diving activities.

Keywords: microplastics; plastic; contamination; sediment; rock substrate; Mediterranean Sea; ghost nets; fishing nets; ATR-FTIR; polymer

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1. Introduction

According to the Plastics Europe Market Research Group (PEMRG) and Conversio Market & Strategy GmbH in 2020 [1], the plastics production in the world reached almost 370 million tons in 2019 and, in Europe, almost 58 million tons. Plastic has found its way to deep seas around the world, including in the Mediterranean Sea [2], suffocating entire ecosystems. Only about 20% of plastics are recycled, while the remaining 80% eventually accumulate in the soil, rivers, and ocean environment [3].

According to UNEP and FAO reports, the fishing nets abandoned in the seas around the world weigh more or less 640 thousand tons, representing approximately 10% of the plastic waste present in the oceans [4]. In the past, natural fibers, such as cotton, were used to fabricate the nets, a material which guaranteed their disintegration in a short period of time and sank to the bottom, preventing further ghost fishing [5]. Currently, synthetic fibers have greatly increased the life of fishing gear, as well as the size and complexity of nets. Modern fishing nets are made from synthetic fibers, such as polyamide, nylon, or polyethylene in different forms, such as braided or twisted [6]. Of greatest concern are the current ghost nets accidentally or deliberately dispersed into the sea that add to marine debris.

Over time, these networks degrade under physical, chemical, or biological action into microplastics (MPs) (<5 mm in size) and further into nano size, with an extremely low rate of degradation that takes several hundred years [5,7], negatively affecting marine fauna, from megafauna (e.g., turtles, dolphins, whales) to fish and benthic communities [8,9].

The MPs are classified according to their origin, where primary MPs are originally manufactured in sizes lower than 5 mm, while the secondary MPs are derived from the larger plastic fragments or primary MPs [10]. The MPs can also accumulate, migrate, and spread in the environment due to their hydrophobic attacks, small particle sizes, and their stable chemical properties with other environmental contaminants (such as antibiotics and heavy metals).

The problem of ghost nets and the microplastics released from them is of concern. However, unlike what happens with plastic pollution in general, there are hardly any studies. The study of MPs is important to evaluate the damage they can cause to the ecosystem, especially the health of marine organisms that are ingesting these pollutants accidentally, which can also affect human health. As a result of this, the fish stock assessment models can also be affected [11,12]. There are several reports that show the presence of MPs in shellfish, in sea salt [13,14], in the gastrointestinal tract of marine animals [15,16], in human intestines [17], and in human blood [18]. The study conducted with researchers from Marche Polytechnic University, Ancona, Italy and CNR-IAS of Genoa showed that 35% of the fish and invertebrates collected in the central Tyrrhenian Sea, during the "MAY DAY SOS Plastica" tour that we conducted in spring 2019, had ingested textile fibers and microplastics [19].

In the present study, the determination of MP in two types of samples taken during the project "Missione Euridice"—a study conducted to evaluate the impact of fishing nets on the marine ecosystem in a specific area of the Gulf of Cefalù, Italy, thanks to the crowdfunding practice—was reported [20]. This study area was chosen to be considered as a fishing ground for local fishermen, taking into account that the Mediterranean Sea is known for the presence of ghost nets [21,22], and Italy, in particular, is one of the countries with the largest number of ghost fishing recorded in the Mediterranean. According to a recent study, Italy was first among the 12 countries analyzed [22].

The objectives of the present study were to: evaluate the presence of MPs in sediment and rock substrate samples collected from underwater activities by visual observation with a stereomicroscope and ATR-FTIR for polymer identifications; evaluate the environmental impact of MPs caused in the area under consideration, in the presence and absence of fishing nets; raise awareness and study some aspects of MPs pollution associated with the presence of ghost nets, which is increasingly frequent.

2. Materials and Method

Sediment (sand) and surface rock substrate samples were taken in the specific area of the Gulf of Cefalù, northern coast of Sicily, Tyrrhenian Sea, Italy, during the summer of 2021, for two weeks, where the ghost nets were located to assess the possible release and accumulation of microplastics. The study area was selected for the specific presence of some underwater rocks extending vertically from a 28 m deep sandy bottom up to 19 m from the surface sea, on the Secca dei Campanari (38.031065° N, 14.098783° E, Figure 1) where the ghost nets were very abundant and extended for many kilometers.

Seven of these rocks were selected as representative sampling stations. The type of ghost net in question is a pattern called trammel nets. These nets consist of three layers of gillnets that are dragged to the bottom with weights and placed in the water with floating plugs. Its peculiarity is that the different layers have meshes of different sizes. If the fish make it past the first layer, they are caught in the narrowest net in the middle. According to data reported by the local coastguard, the abandoned fishing nets considered in the present study had been present on the bottom for more than 10 years. Therefore, a possible degradation with time of these microplastic networks was taken into consideration as a relevant factor for the research.

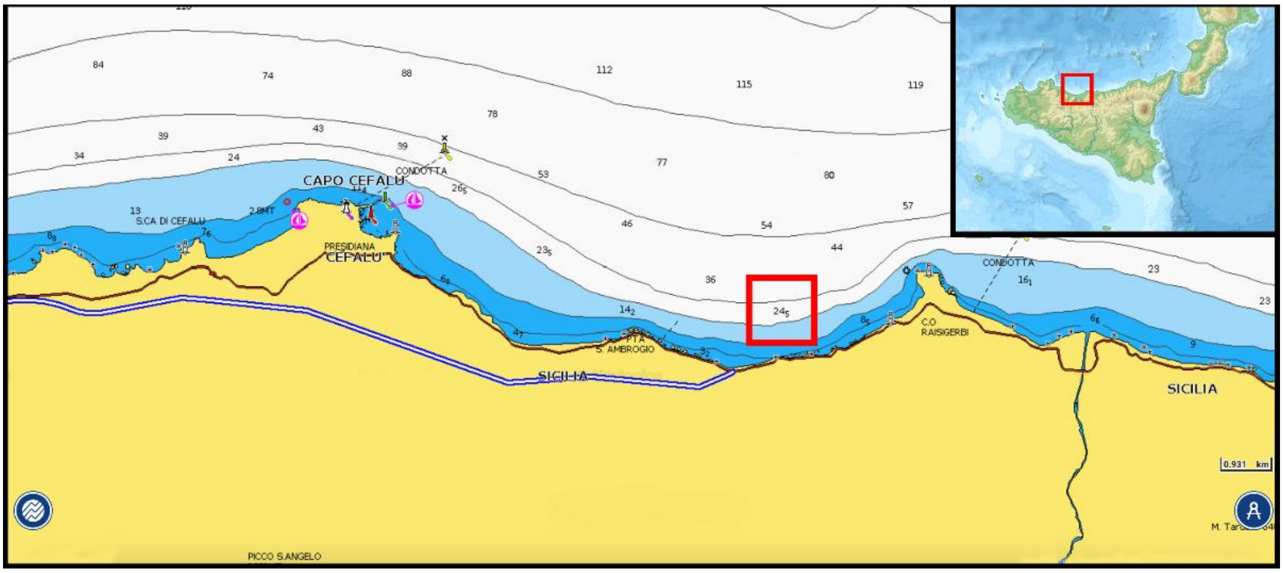

Figure 1. Area of study (empty square) in presence of the ghost nets, in Gulf of Cefalù, northern coast of Sicily, Tyrrhenian Sea, Italy. The geographical coordinates (38.031065° N, 14.098783° E) were reported.

2.1. Sampling

Sediment and rock substrate were collected by scuba diving at different depths in June 2021, at two different times (1st week and 2nd week), chosen according to the weather and sea conditions. The methodology plan for sampling was carried out before the recovery of the fishing nets, on a quantitative basis, of seven more representative stations. Three rocks (numbers 1, 5 and 7) were selected as control stations without fishing nets to compare the non-impacted zones with those affected by the presence of fishing nets. Two sediment samples and two rocky substrates were collected in each of the control station. Other four rocks (numbers 2, 3, 4 and 6) were established within the area impacted by a gosht net. One sediment and one rocky substrate were collected in each one of these stations. Table 1 shows the number of samples taken, the sampling station, the geographical coordinates of the sampling point, and the depth.

Table 1. Data reported for sampling station.

Sampling Station (Rock Number)	Samples Type	Geographical Coordinates	Depth
1 (control)	2 Sediment/ 2 Rock substrate	38.031065°N, 14.098783°E	19 m
2	4 Sediment/ 4 Rock substrate	38.031065°N, 14.098783°E	23 m
3	4 Sediment/ 4 Rock substrate	38.031065°N, 14.098783°E	24 m
4	4 Sediment/ 4 Rock substrate	38.031065°N, 14.098783°E	24 m
5 (control)	2 Sediment/ 2 Rock substrate	38.031065°N, 14.098783°E	26 m
6	4 Sediment/ 4 Rock substrate	38.031065°N, 14.098783°E	28 m
7 (control)	2 Sediment/ 2 Rock substrate	38.031065°N, 14.098783°E	28 m

During the underwater activities, for each type of sample (sediment and rock substrate), one sample (800 g^{-1} kg of wet weight, WW) in each control station and two samples (in total 1–2 kg WW) in each impacted station were taken every week. The same quantities are also reported in [23]. Some studies report the methodology for sampling rock pools and coral fragments [24,25], but not for underwater rocks substrate, specifically. There are no data on the analysis of MPs in rocky substrates sampled underwater to compare methods with the one selected in the present study. Based on the little information found about the sediment, in general, and on earth rocks [26], rock substrates sampling involved the use of a hammer and chisel. Then the substrates were stored in bags (previously labeled) suitable for substrate sampling and, subsequently, were left to dry at room temperature for further analyses.

Sand sediment sampling involved the use of a stainless-steel shovel to take samples, which were then weighed and stored in bags at 4 °C until analysis, as reported in other study [23]. All samples were analyzed and processed in the food and environmental safety laboratory (SAMA-UV) of the CIDE Desertification Research Center, University of Valencia, Spain.

Once at the laboratory, the samples were differentially treated, depending on their characteristics. Specially rock substrate needs to be fragmented to liberate the microplastics that can be occluded in the cavities, while sediments need to be dried in order to obtain a constant weight needed to express the results.

2.2. Rock Substrate Fragmentation and Sieving

Samples were first dried at room temperature (38–40 °C), and only 500 g dry water (DW) of each sample (for week and for station) was taken for further fragmentation and analysis. Fragmentation of the samples was carried out following the other method proposed [27], slightly modified to obtain a finer dust. Briefly, first the rocks were roughly fragmented with a hammer, then the fragments were extended in a tray to obtain finer fragments using a homemade cylindrical metal roller of 6 cm diameter, which breaks the rough fragments into smaller fragments and, subsequently, the resulting fragments were passed through a 2 mm sieve. These sieved particles were further fragmented using the Fritsch Pulverisette 5 instrument planetary mill with a ball of 20 mm diameter dm^3 (FRITSCH, Made, Germany).

2.3. Sand Sediment Lyophilization and Sieving

All of sand sediments samples stored in the laboratory at −20 °C were lyophilysed (−65 °C, with a vacuum between 1 and 4 mTorr) for 72 h with Sentry 2.0 lyophilizer from VirTis SP Scientific manufacturer (Gardiner, NY, USA) to eliminate water to preserve them as recommended [28], and subsequently passed through a 2 mm sieve.

2.4. Extraction

Two different extraction methods have been carried out, according to the different characteristics of the sample, to obtain clean extracts without interferents for MPs. However, both methods used a solution of the same density to separate MPs. Then, the same type of MPs is extracted by both methods. For sand sediment extraction, the Tsang et al., 2017 [29] method, and for rock substrate samples, the Vianello et al., 2013 [26] method was selected and slightly adapted (Figure 2).

In particular, for the extraction of MPs of both types of samples, 400 mL saturated sodium chloride solution was added. For sand sediment samples, two aliquots of 100 g (DW), and for rock substrate, one aliquot of 250 g (DW) were used according to literature [26,29]. Samples were shaken for 1.5 min in a glass flask with concentrated NaCl saline solution (CS, 120 g/L), to reach a final volume of 1 L, and subsequently allowed to settle for 1 h. To reduce interference with the plastic identification, biogenic matter present in the samples was oxidized by adding 30% hydrogen peroxide. After shaking, the whole suspension was allowed to settle for 1 day. The resulting supernatant, with floating particles extracted from the samples, was filtered through a 500 µm steel wire sieve. This procedure was repeated three times, consecutively. The three extracted fractions were finally pooled and resuspended in Milli-Q water, filtered through 0.6 µm (nominal porosity) glass fiber filter paper (ADVANTEC, Made, Japan) to retain the microplastics, and stored in glass Petri dishes.

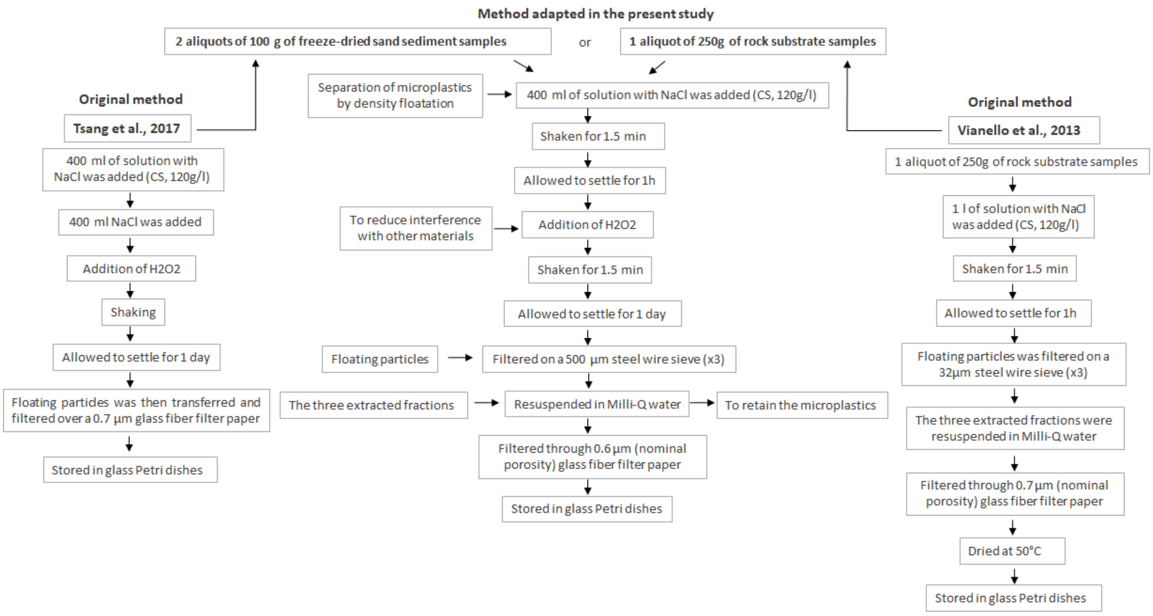

Figure 2. Comparison of methods (from literature Tsang et al., 2017 [29] and Vianello et al., 2013 [26] and the method adapted in the present study).

2.5. Identification

MPs were retained in the filters of 0.6 µm porosity in a glass Petri dish to be identified in size, shape and color by visual observation with a stereomicroscope (Model EZ4; Leica AG, Wetzlar, Germany) and with a digital camera system (Canon, G15) (Figure 3). Furthermore, the chemical composition of microplastics was determined through an Attenuated Total Reflectance Fourier Transform Infrared Spectroscope (ATR-FTIR) for polymer identifications (Nicolet iN10 MX FTIR Microscope; Thermo Fisher Scientific, Madison, WI, USA).

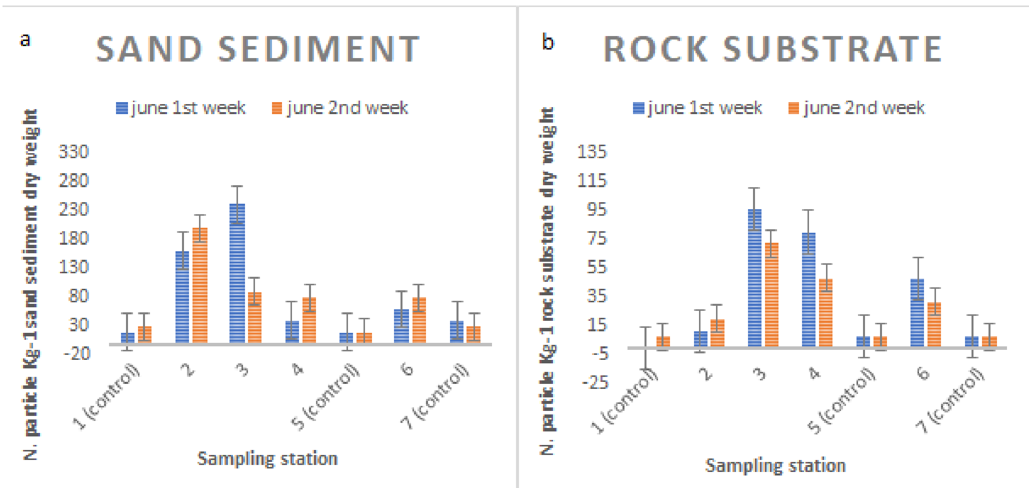

Figure 3. (**a**) sand sediment and (**b**) rock substrate.

3. Results and Discussion

3.1. Abundance, Shape and Size of Microplastics

MPs number depends on the sampling station and the sampling week (Figure 3). The highest abundance of microplastics in sand sediment sample was recorded for sampling station 3, with a concentration of 240 ± 68,604 particles kg^{-1} dry weight sediment. While for rock substrate sample, the highest concentration of MPs was also in sampling station 3, with 84 ± 30,425 particles kg^{-1} dry weight.

A visual observation is one of the most used techniques for the identification and quantification of plastic particles [30]. A careful visual sorting of the residues is required, separating the plastic from other materials, such as organic debris (shell fragments, animal parts, dried seaweed, etc.) remaining despite the extraction steps. A first identification phase was carried out by direct examination of the sample with a stereoscope (Figure 4). According to different studies [31,32], MPs have been identified and classified as fragments, such as hard plastic, as line and line, such as fishing nets and fishing line, and as fiber and fiber, such as clothing or textiles. The results of our observation are reported in Figure 4. The most representative type of MPs in both of the samples and in all of the sampling stations has first been fiber and fiber, followed by lines, according to the studies [33,34].

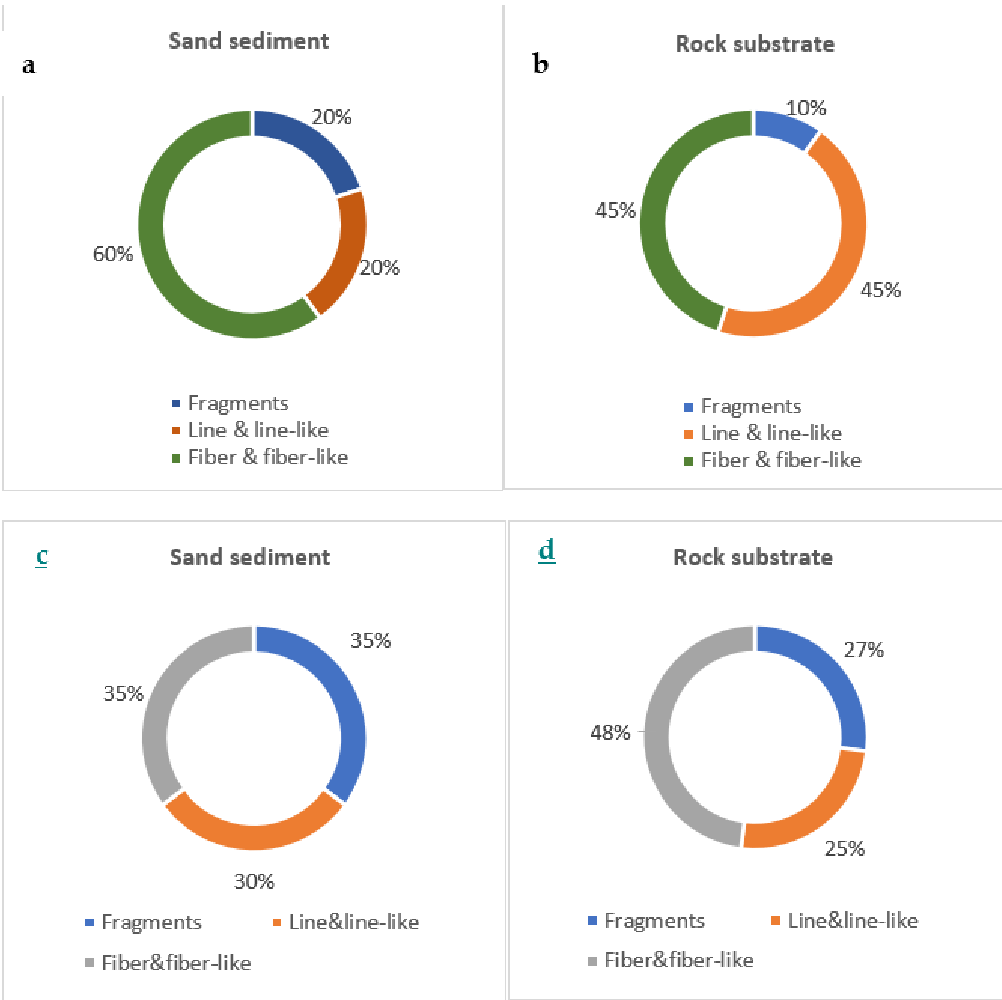

Figure 4. Percentage of MPs observed in sediment samples and in rock substrates in impacted stations (**a**,**b**) and in the control stations (**c**,**d**).

As reported in the Figure 4, MP proportions determined in the impacted stations were 10% fragments, 45% line and 45% fiber for rock substrates samples, and 60% fibers, 20% line and 20% fragments for sandy sediments. In the control stations, MPs proportions were 48% fibers, 27% fragments and 25% line for rock substrate, and 35% fragments, 35% fiber and 30% lines for sand sediment samples. It is evident that in the impacted stations, the minor MPs characterization was fragments for both samples. While, for the control stations (n.1, 5 and 7), the minor MPs concentration was the line and line type for both samples (Figure 4). As reported in the previous literature, fragments and fiber has been the dominant forms in sediment samples according to Zhang et al., 2022 [35], ranging from 85–90%. Another study reports the MP proportion of 44.8% and 44.9% of fragments and fibers, respectively [36].

In the present study, the most frequent MPs sizes range from 500 µm to 2 mm, according to [29]. No relevant size differences were observed for both samples. Other studies report the similar sizes of collected MPs in sediment samples ranging from 0.5 to 2 mm [37]. Furthermore, other studies affirmed the sizes of microplastic fibers ranging from 500 to 1000 µm [35], or from 0.3 to 0.7 mm [23]. For identification, characteristics such as shape, color, appearance and texture were taken into consideration, based on the classification by Razeghi et al., (2021) [32]. Photoshop CC 2019 software was used for size determinations because no micro-FTIR or Raman spectroscopy was accessible on site. The colors of the MPs varied widely, from transparent and white to eye-catching colors, such as orange, red, pink, green and black (Figure 5).

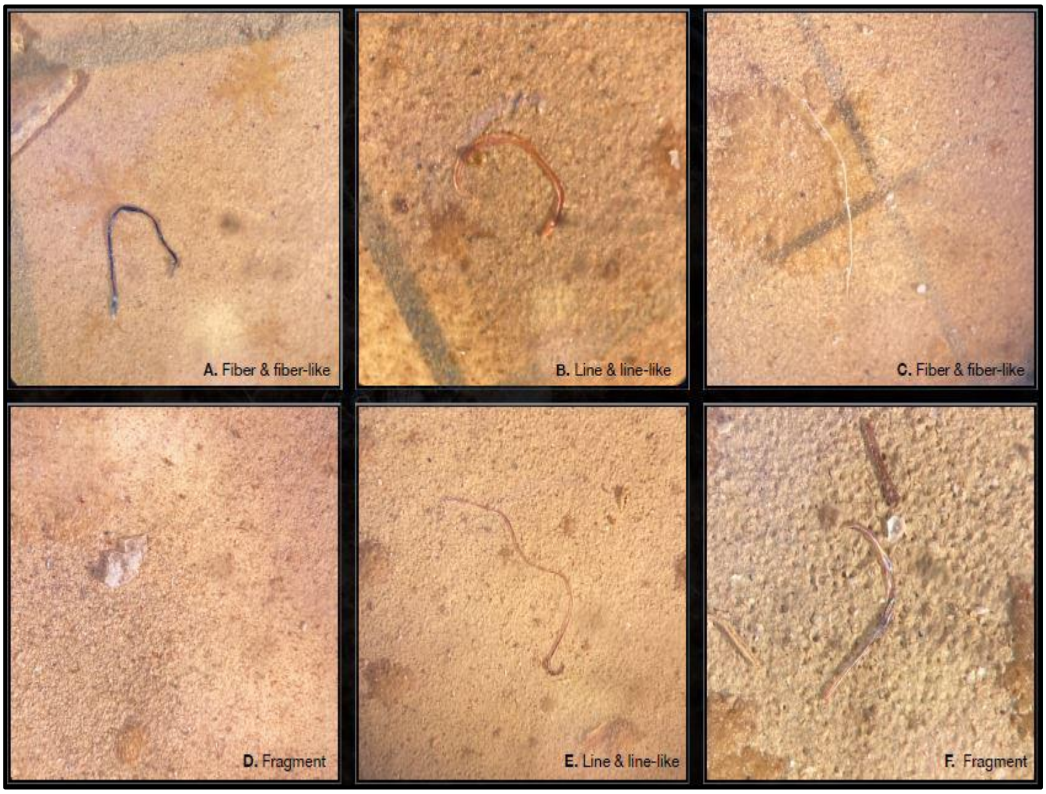

Figure 5. Most common MPs determined by visual observation using stereomicroscopy. Samples of sandy sediments (**A**,**D**,**E**) corresponding to the number 3, 4 and 6, respectively (impacted stations). Samples of rock substrates (**B**,**C**,**F**) corresponding to number 2, 3 and 4, respectively (impacted stations). (**F**) also observed a line type.

As observed in Figure 5, the probable presence of lines can be derived from fishing nets and fishing lines. Plastics that extend over large areas can also be derived from the degradation of packaging bags, films and other products that are deposited in the sediment, according to the existing literature [23,35,38], or, as this study demonstrated, in the rock substrate. Furthermore, in our case study, the area under examination appears to be one of the most fished areas in the Gulf of Cefalù. Therefore, our results could be consistent with the presence of fibers derived from fishing nets, which, over 10 years, have possibly degraded.

3.2. Polymer Types of Microplastics

An attenuated Total Reflectance Fourier Transform Infrared Spectroscope (ATR-FTIR) for polymer types of identification was used for helping us, from the commercial libraries available. The polymer types determined in all station points were Polyvinylidene fluoride (PVDF), Ethylene-Propylene Diene (EPDM), Polyethylene/Ethyl Acrylate copolymer (EAA), Polystyrene atactic, Polyester tere- and iso-phthalate, Polystyrene vinylidene chloride, Polystyrene atactic, Nylon/EVA Tie layer, Nylon (polyamide), Nylon 6 [Poly(caprolactam)] (Figure 6).

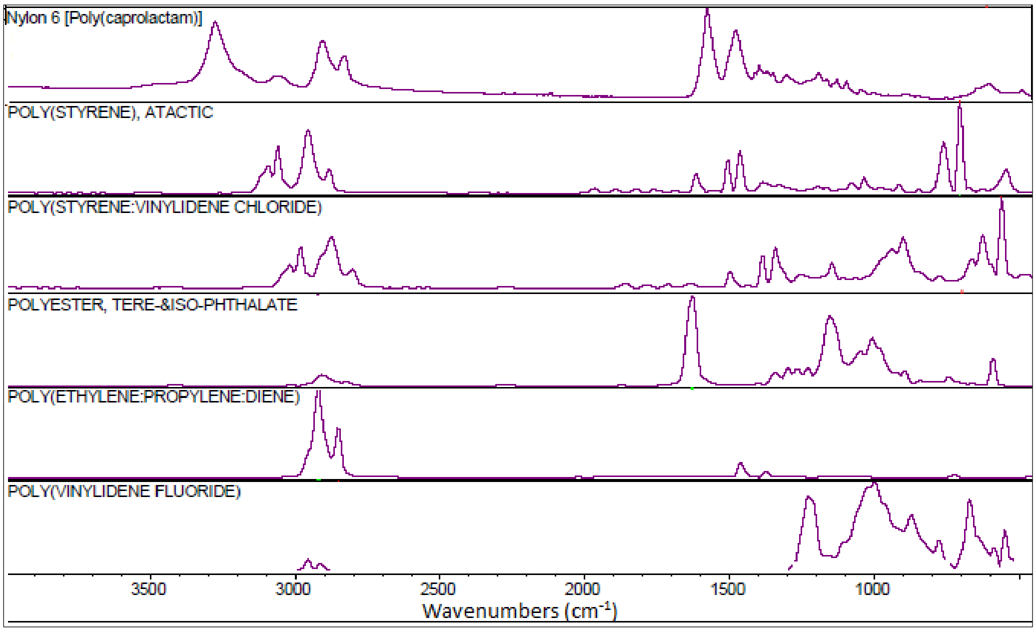

Figure 6. Most common microplastic polymers determined with ATR-FTIR in the present study.

The predominant polymer types obtained in the sand sediments of impacted stations were Polystyrene atactic and Nylon 6 [Poly(caprolactam)]. For the rock substrates samples in the impacted stations, the predominant polymer types were Polystyrene vinylidene chloride and Polystyrene atactic, with the frequent presence of Nylon (polyamide) type. In general, the percentage range reported in the impacted stations including both types of samples were from 44.23% to 80.17%. The highest abundance of polymers was observed in the station sampling n.3, for both samples, as reported previously by the visual observation, and also in the station n.4, but only for the rock substrate samples.

Compared to the control stations, the predominant polymer types determined in sand sediment were Ethylene-Propylene Diene and Polyester tere- and iso-phthalate, and in the rock substrate samples, they were Polyethylene/Ethyl Acrylate copolymer (EAA) and Polyvinylidene fluoride (PVDF). The highest abundance of microplastics was reported in station n.3 for both samples and, in general, the percentage range reported was from 42.66% to 46.23%. Nylon (polyamide) was determined in the control stations with a low percentage of 16.52%. Based on these results, low percentages with ATR-FTIR determinations were obtained at control stations and therefore cannot be taken into consideration. In addition, high contamination from lines made from fishing nets or fishing lines was not evident. It can be deduced that the presence of MPs in the control stations, also confirmed by visual observation, albeit minimal, may be due to their proximity to the impacted stations.

The polymer types determined are commonly observed in other studies as being more representative in the oceans [23,39,40]. For example, the polystyrene, atactic, is used in plastic products, include protective packaging, containers, lids, bottles, trays, tumblers, and disposable cutlery. In general, polyethylene and polyester tere- and iso-phthalate are also widely used. Ethylene-propylene-diene (EPDM) is one of the most important polyolefin materials widely commercialized and used in various industries, in recent year [41], to manufacture high-quality construction and flat roof membranes. Additionally, polystyrene vinylidene chloride is a familiar polymer. Polyester can also come from synthetic fibers, such as those used in the manufacture of clothing or fabrics, which enter the aquatic environment. Especially, nylon was also identified, which—together with the identification of other synthetic fibers and lines such as polyamide, nylon or polyethylene—suggests that it could be related to the presence of fishing nets. It is reported that the modern fishing nets are made with the same synthetic materials [6] determined in this study.

4. Conclusions

MPs were detected in both sample types, sediment and rock substrate, in the study area, represented by three control stations without the presence of fishing nets and four stations impacted by fishing nets. Lower percentages of MPs were determined in the control stations than the impact zones. Probably, these results can be interpreted as an index of a greater accumulation of MPs where the fishing nets were present. The presence of polymer types of MPs and the visual observation demonstrates that our study area selected is contaminated. The average observations in the control stations were $n = 5$ and in impact stations were $n = 24$ for sand sediments, and $n = 3$ (control) and $n = 25$ (impact) for rock substrates. The MP sizes range from 500 μm to 2 mm. MP proportions determined in the impact stations were 10% of fragments, 45% line and 45% fiber for rock substrates samples, and they were 60% of fiber, 20% of line and 20% of fragments for sediments. In the control stations, the percentage of each type was 48% of fiber, 27% of fragments and 25% of line for rock substrate, and it was 35% of fragments, 35% of fiber and 30% of lines for sand sediment samples.

With regards to polymer determinations, polyamide, nylon, or polyethylene were detected to be present at much greater percentages in impacted areas, probably related to the presence of fishing nets. These results demonstrated that the impacted stations where there are abandoned fishing nets are more affected and contaminated by MPs. However, studies conducted on MP derived contamination in the presence of abandoned fishing nets are currently a poorly explored topic. Furthermore, there is no previous literature on the determination of MP on rock substrate sampled from underwater activities, therefore the present study represents a first record. In the future, further studies should focus on the level of MP contamination and the ecotoxicological risks related to the increasingly frequent presence of fishing nets to better understand the environmental impact caused over time, even after the fishing nets have been removed. Often, marine organisms remain trapped between the nets. Furthermore, it would be interesting to investigate aspects of population biomass, comparing before and after the possible removal of the nets (data soon to be published). Any contamination from microplastics could pose a risk to biota health and consequently to human health. Therefore, it is important to focus attention to and carry out studies in sea areas in the presence of fishing nets or highly fished, and try to extract them from the sea with the aim of expanding knowledge and trying to reduce the environmental pollution caused by microplastics.

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