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"Less Plastic More Mediterranean"
2017 Campaign on board the Rainbow Warrior Greenpeace Ship

"MICROPLASTIC INVESTIGATION IN WATER AND TROPHIC CHAIN ALONG THE ITALIAN COAST 06/24/2017 – 07/15/2017"

Activity Report

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1. Scope

The Campaign started in June 24, 2017 from Genoa Liguria, the first Italian stop of the "Less Plastic, More Mediterranean" tour that Greenpeace carried out on board the Rainbow Warrior ship (Fig. 1) in southern Europe and finished in Ancona in July 9 with a stopover in Naples. "Less Plastic, More Mediterranean"has been a research and awareness tour - organized in Italy with the scientific collaboration of the Institute of Marine Sciences of CNR in Genoa and the Polytechnic University of the Marche - to gather direct data and testimonies on pollution from plastic that afflicts our seas and informs public opinion about this serious problem.

We have been invited to join the Campaign and we performed a comprehensive monitoring of the status of microplastic contamination along the Italian Coasts.

The main goals of the monitoring campaign were to:

A - Establish the extent of microplastic litter in the Mediterranean Sea along the Italian Coast both investigating the surface and vertical distribution to obtain information on the sinked plastics.
B- Assess the microplastics bioaccumulation in the food chain (mesozooplankton, macroinvertebrate and fishes)
C – Investigate polymeric composition of particles present both in water and organisms.
D – Produce data to support the standardization and harmonization of develop the existing protocols to isolate and detect microplastics in water and marine organisms to enable accurate quantification, qualification and comparison between studies

In detail, the aforesaid goals have been pursued as follow:
Goal A
MPs analyses in water:
Surface sampling has been performed using the Manta trawl along different transect located both nearby Marine Protected Areas (MPAs) and urban Centre/river mouths.
In addition, at the transect starting and end points, a vertical sampling has been performed using a 200 µm plankton net.

Goal B
MPs analyses in organisms:
B1- Surface plankton sampling has been performed, in parallel with the one for goal A, using a 200 µm plankton net. At the transect starting and end points a vertical sampling has been performed using a 200 µm plankton net (sampling shared with A). Mesozooplankton samples have been processed in Ismar CNR and UNIVPM laboratories to verify and characterize MPs in the organisms.

B 2 - Depending on the shipping route, different sites (including Genova and Naples ports, Giglio island etc.) have been selected for the marine organisms collection. Vertebrate and invertebrate organisms have been obtained by fishermen or at the local market and dissected on the boat; gastrointestinal tracts of fish or entire tissues of invertebrates have been immediately frozen at

-20°C, until their transfer to the UNIVPM laboratories. Depending on the availability, almost two species have been selected among the following list: *Mullus barbatus*, *Scorpaena* sp., *Sardina pilchardus*, *Merluccius merluccius*, *Solea solea*, *Mytilus galloprovincialis*, *Squilla mantis*, *Ostrea edulis*, *Penaeus kerathurus*, *Nephrops norvegicus.* Further species have been analyzed according to the catch on each location.

Goal C
Particles found in water and organisms have been characterized using FT-IR and μmFT- IR analysis.

Goal D:
MPs presence in seawater have been investigated in both Manta trawl and plankton net samples in order to verify if the sampling method could influence the results.

Ismar activity has been focuses the water and zooplankton samples analysis. Due to the huge amount of samples obtained from the Campaign, this report focuses on the main Goal (A and C) and address the principle need to understand the extent of microplastic litter contamination in the Mediterranean Sea along the Italian Coast and to identify particles polymeric composition.

Fig. 1: Rainbow Warrior ship at Genoa’s harbor

2. General overview

Plastics are synthetic organic polymers and although they have only existed for just over a century, their production have exponentially increased with years, exceeding 300 million tons in 2015 (Plastic Europe, 2015). Every year, around 8 million tons of plastic end up the ocean (Jambeck et al., 2015), accounting for the great majority of marine litter.
Plastic items are present in the environment in a wide variety of sizes, ranging from meters to micrometers. Those smaller 5 mm are called microplastics (MPs).
MPs originate from a variety of sources, according to which they are classified into "primary" or "secondary". Primary MPs are used as specific personal care products (hand cleaners, facial cleaners and toothpaste) or as raw materials used for the fabrication of plastic products, namely plastic resin pellets or flakes and plastic powder or fluff. Secondary microplastics result from the fragmentation of larger plastic materials (Duis and Coors, 2016).
In the last decade, MPs have been reported on a global scale, from poles to the equator. MPs presence has been assessed in ocean waters, bodies of freshwater, marine sediments, as well in biota.
Because of the small dimensions, MPs become available for ingestion by organisms commonly not affected by the larger marine debris. Little is known about the chronic effects of MPs exposure on individuals and populations.
Floating MPs can act as a vector of non-indigenous marine species that can be transported over long distances (**Fig.2**). Lastly, several studies have suggested that MPs are often mixed with additives (flame-retardants, phthalates, colorants). If ingested, the uptake of associated chemicals may cause adverse physiological effects that, up to date are still largely unknown. Moreover, once ingested, MPs may accumulate through the food web, thereby posing a further threat to marine biodiversity.
It is of primary concern to gather as many data as possible about MPs presence in marine environment in order to get a comprehensive knowledge of the issue and to understand the possible impact to human health.

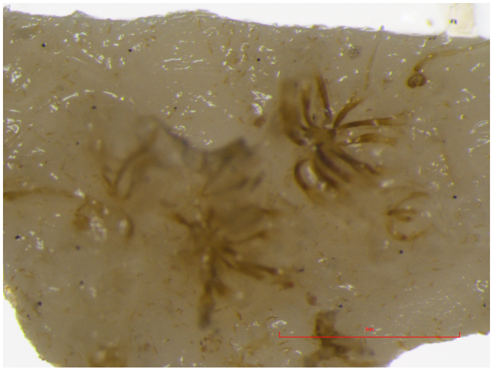

Fig. 2: Algal growth on plastic debris isolated in this study

The Mediterranean Sea is a semi-enclosed basin with coastal zones densely populated and high level of commercial and recreational marine traffic. The limited water exchange with Atlantic Ocean enable the accumulation of floating anthropogenic litter in the area.
These conditions well justify the definition of "plastic soup" (Suaria et al., 2016) that has been recently propose for the Mediterranean Sea, where MPs level are similar to those of the 'Great Pacific Garbage Patch' in the middle of the Pacific Ocean.
The present report presents data about the analysis carried out at the laboratory of ISMAR-CNR (Genoa, Italy) about MPs presence in surface water samples collected with the Manta net along the Italian coast, from June 24, 2017 (Genoa, Liguria) to July 9, 2017 (Ancona, Marche).

3. Methods

3.1. Field activities: study area and samples collection

In detail, water Sampling stations are reported in Figure 3. All the operational details related to each Sampling station are reported in Annex 1.

		Lat	Long
1	AMP 5Terre	44°05.253'N	9°40.005'E
2	Livorno	43°36.4'N	10°14.2'E
3	Arcipelago Toscano	43°22.567'N	9°31.828'E
4	Giglio 1	42°22.7'N	10°56.1'E
5	Giglio 2	42°20.6'N	10°52.3'E
6	Civitavecchia	42°03.322'N	11°47.050'E
7	Tor Paterno	41°35.660'N	12°14.34'E
8	Ventotene	40°50.0'N	13°27.6'E
9	Punta Campanella	40°33.846'N	14°20.463'E
10	Portici	40°48.1'N	14°19.0'E
11	*La Gaiola*	*40°46.4'N*	*14°11.4'E*
12	*Bagnoli*	*40°48.2'N*	*14°08.4'E*
13	Lipari	38°27.528'N	14°58.246'E
14	Messina	38°06.198'N	15°35.825'E
15	SIC *C. caretta*	37°54.3'N	16°04.1'E
16	Capo Rizzuto	38°50.9'N	17°02.3'E
17	Torre Guaceto	40°45.643'N	17°46.492'E
18	Bari	41°09.5'N	16°51.88'E
19	Tremiti	42°10.1'N	15°36.7'E

Fig. 3: Water sampling stations position

3.1.1. Horizontal sampling of microplastics on the sea surface with manta net:

Manta detail

Manta net with 330 µm mesh size have been used. Technical details are reported in figure 4.

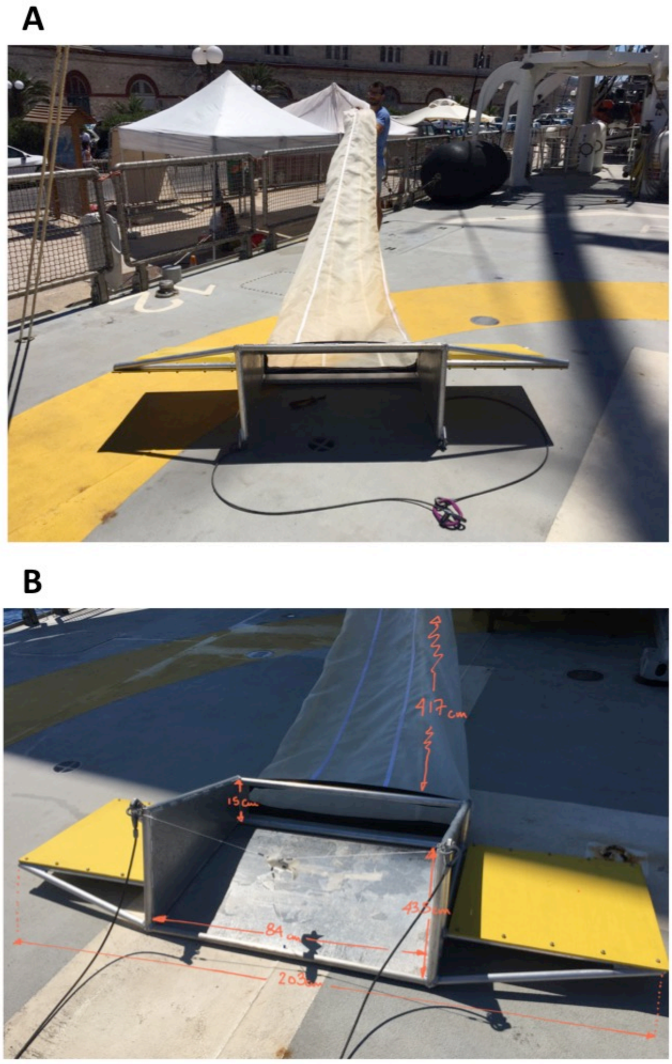

Fig. 4: Manta net with size details. Photo by Alonso Munoz Alcaide

Sampling has been performed as follow:

- Deploy the manta net from the side of the vessel using a spinnaker boom (Fig. 5 A)
- Deploy the manta net out of the wake zone (approx. 3 - 4 m distance from the boat) in order to prevent collecting water affected by turbulence inside the wake zone.
- Start to move in one straight direction with a speed of approx. 1-2 knots for 20 min
- After 20 min stop the boat and lift the manta net out of the water (Fig. 5 B)
- Rinse the manta net accurately using a submersible pump or water from the boat water reservoir (Fig. 5 C).
- Safely remove the cod end and transferred the content into 1l bottle and stored for laboratory analysis (Fig. 5 D)

Fig. 5: Different operative steps of the Manta Net sampling. Photo by CNR-Ismar and Lorenzo Moscia for Greenpeace.

3.2. Laboratorial activities (CNR-ISMAR): MPs investigation on Manta's samples

3.2.1. Plastic identification from surface water samples collected using Manta net

The water samples were analyzed for their plastic content at the CNR-ISMAR laboratory in Genoa (Italy). All samples were visually examined and sorted under a stereomicroscope (Olympus BX41). Putative plastics were photographed (Fig. 6) and categorized according to shape and dimension (Lusher et al., 2015). In order to identify the polymer composition of the items, PerkinElmer Spectrum Two Fourier Transform Infrared Spectroscopy (FT-IR) spectrometer equipped with Universal ATR (UATR) accessory with a 9-bounce diamond top-plate was used.

3.2.2. Contamination control

To mitigate sample contamination in the laboratory, all equipment and consumables were taken directly from packaging. Samples and equipment were covered where possible to minimize periods of exposure; clothing made of cotton were worn during the analysis. Filter blanks were run in parallel to verify contamination during both water sample processing.

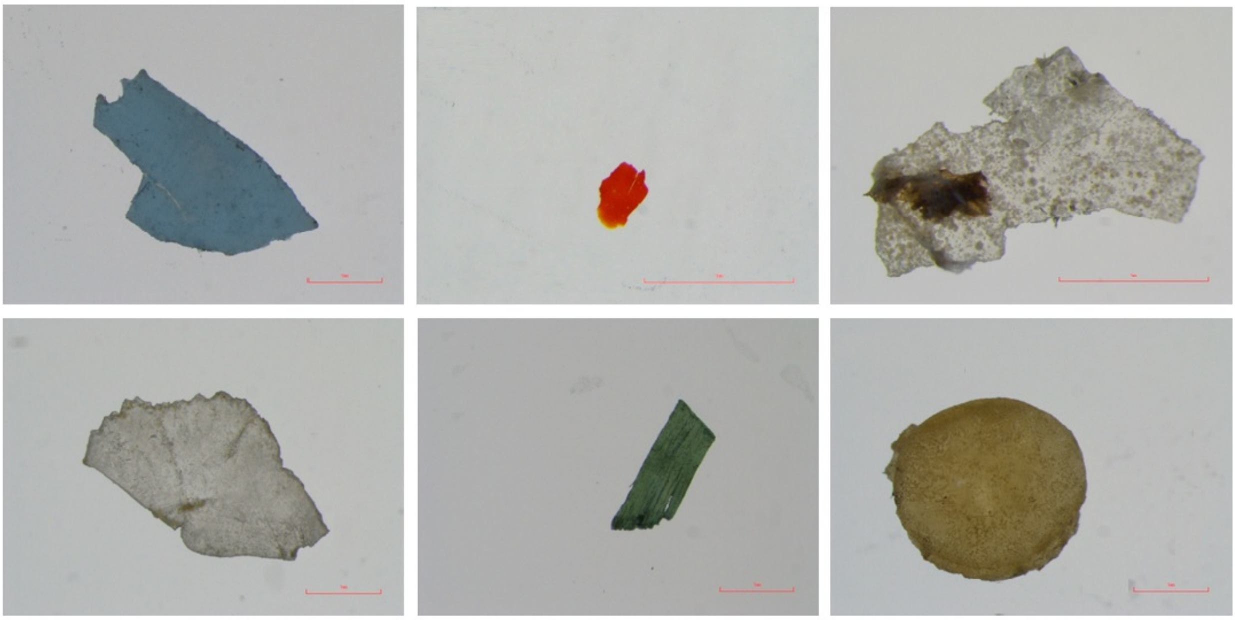

Fig. 6: Selection of MPs of different size, shape and color from water samples analyzed during laboratorial procedures

1. Results and Discussion

Plastics were found at all the stations sampled. The number of plastics found in water samples was expressed as items/m^3 and reported in Table 1. A mean value of 0,52 items/m^3 was found. The maximum value (3,56 items/m^3) was observed in station 10 Portici, while the lowest concentration (0.06 items/m^3) was still found in station 16 (Capo Rizzuto).
Figure 8 reports the results related to polymeric composition, class size and shape of items isolated from each sample expressed as percentages.

Table 1: Microplastics concentration expressed as items/m^3 reported at each sampling statio

Station	item/m^3
1 Cinque terre	0,12
2 Livorno	0,07
3 Arcipelago Toscano	0,18
4 Giglio 1	0,09
5 Giglio 2	0,28
6 Civitavecchia	0,21
7 Tor Paterno	0,68
8 Ventotene	0,09
9 Punta Campanella	0,29
10 Portici	3,56
13 Lipari	0,3
14 Messina	0,12
15 Caretta	0,17
16 Capo Rizzuto	0,06
17 Torre Guaceto	0,14
18 Bari	0,2
19 Tremiti	2.2
Mean	**0,52**

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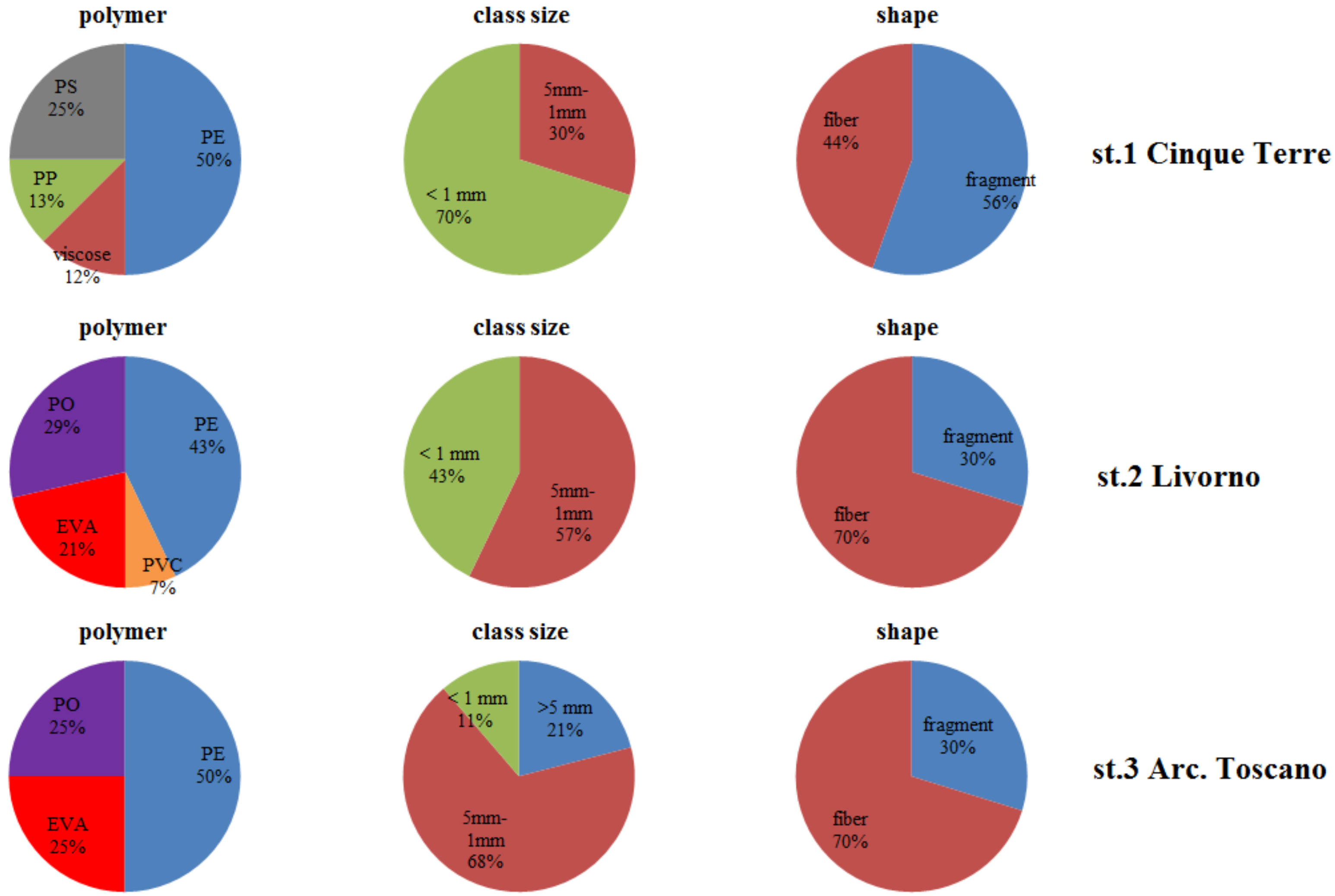

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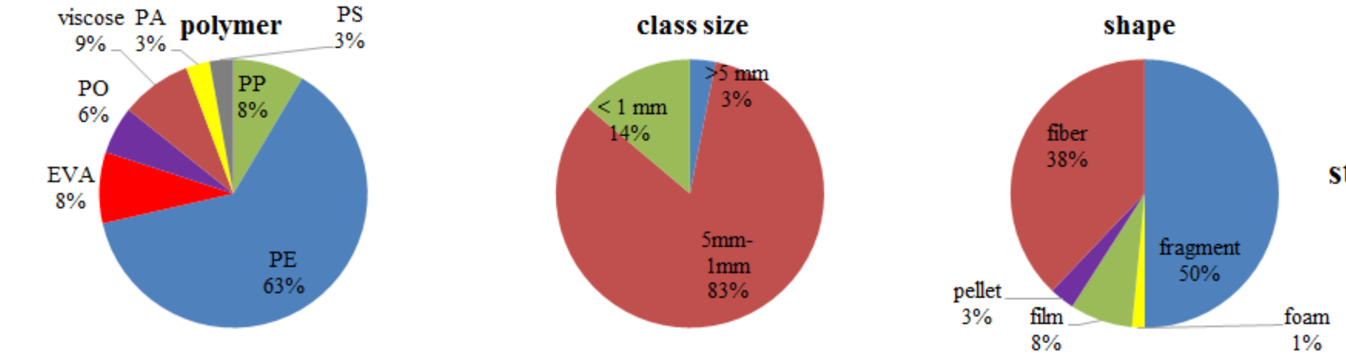
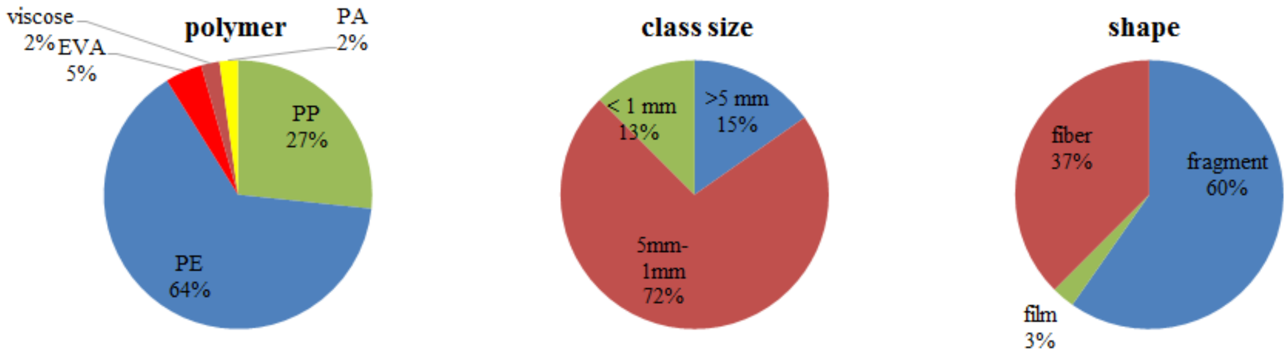
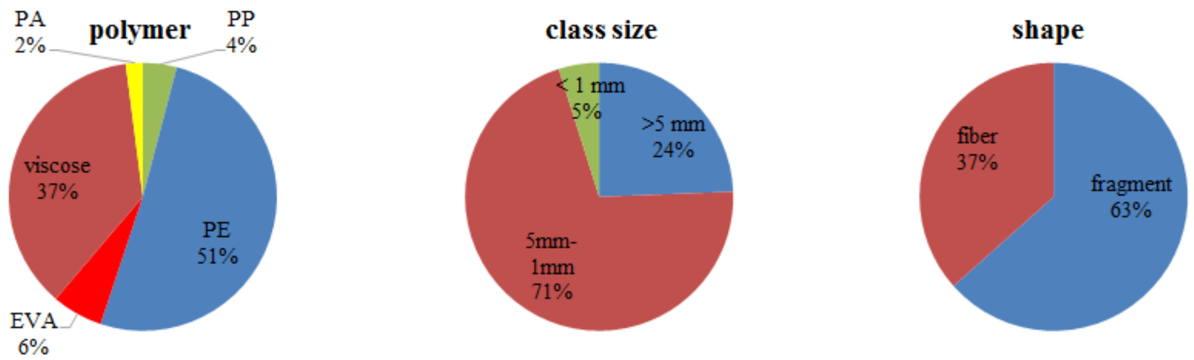


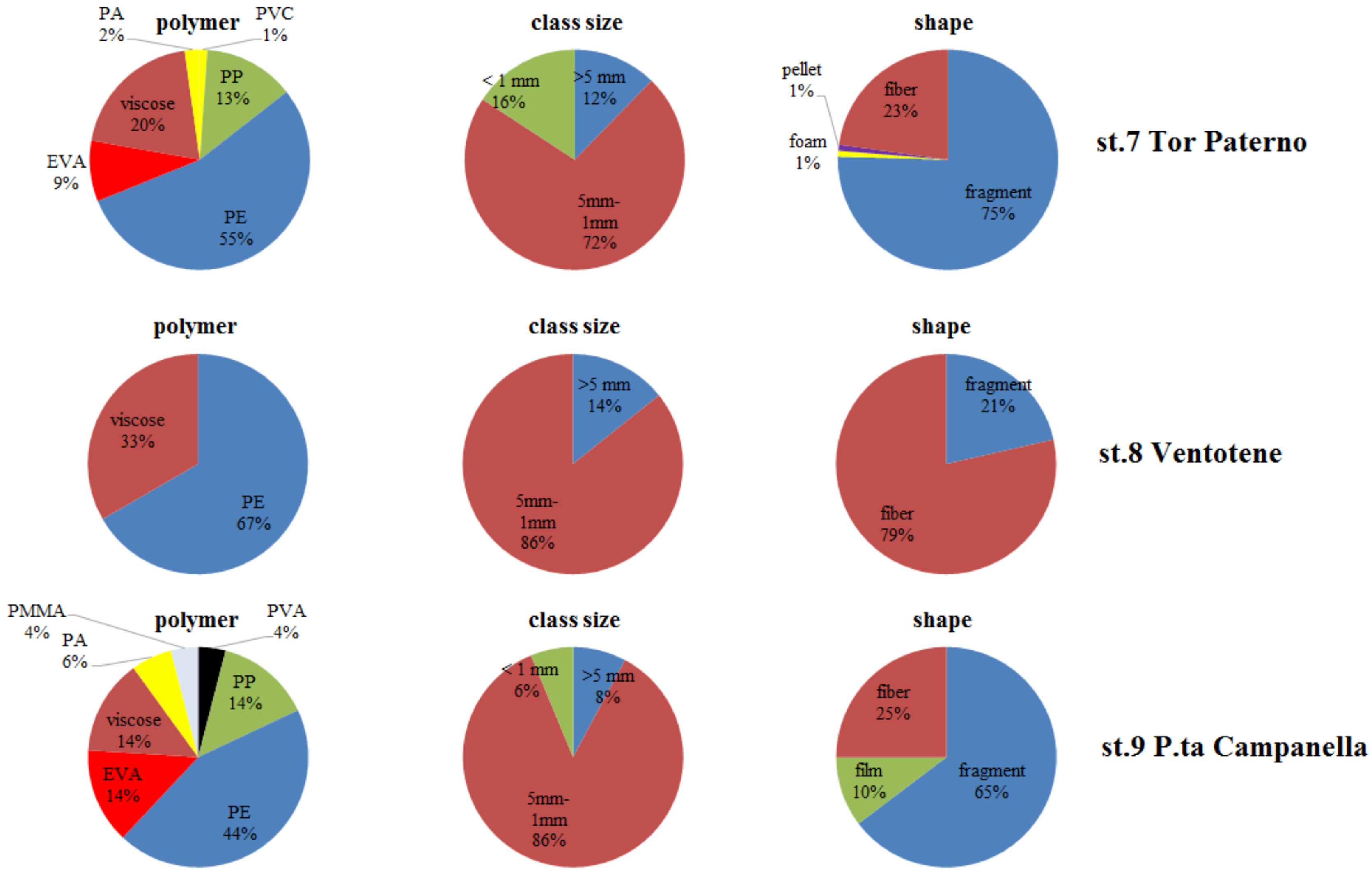
st.4 Giglio 1

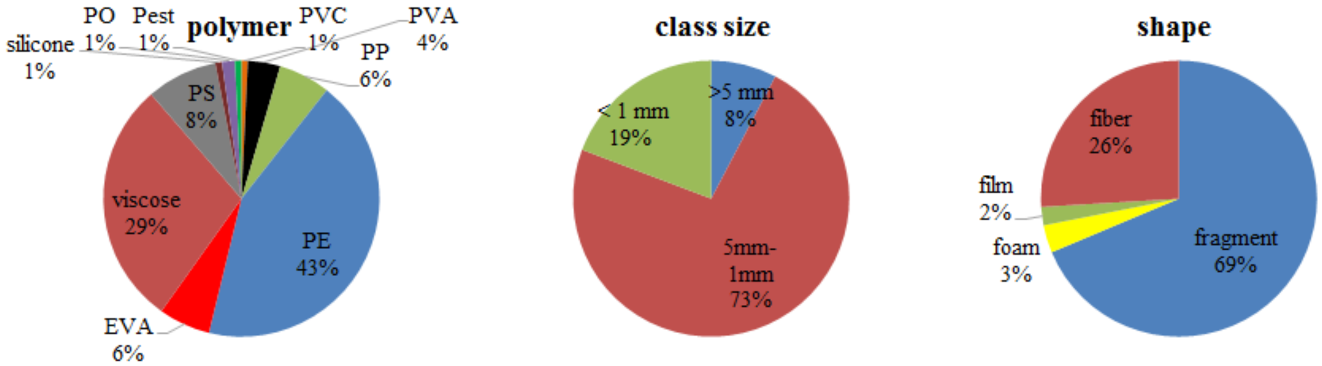


st.5 Giglio 2

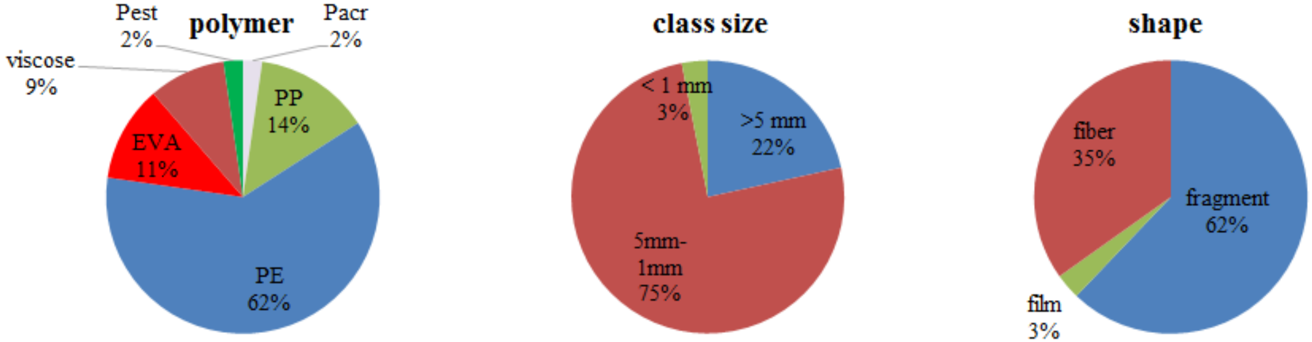


st.6 Civitavecchia

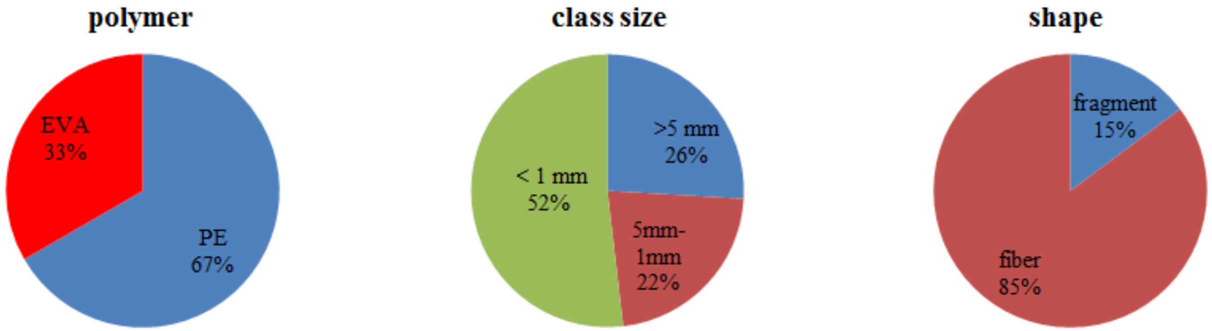

polymer
PA 2%
PVC 1%
PP 13%
viscose 20%
EVA 9%
PE 55%
class size
< 1 mm 16%
>5 mm 12%
5mm-1mm 72%
shape
pellet 1%
foam 1%
fiber 23%
fragment 75%
st.7 Tor Paterno
polymer
viscose 33%
PE 67%
class size
>5 mm 14%
5mm-1mm 86%
shape
fragment 21%
fiber 79%
st.8 Ventotene
polymer
PMMA 4%
PA 6%
PVA 4%
PP 14%
viscose 14%
EVA 14%
PE 44%
class size
< 1 mm 6%
>5 mm 8%
5mm-1mm 86%
shape
fiber 25%
film 10%
fragment 65%
st.9 P.ta Campanella



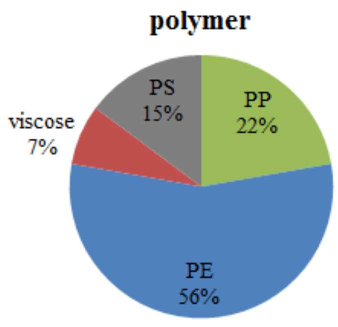
st.10 Portici

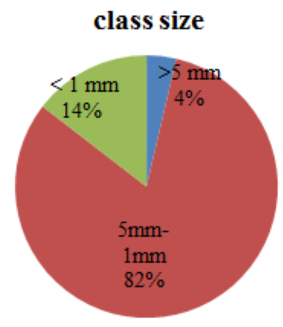


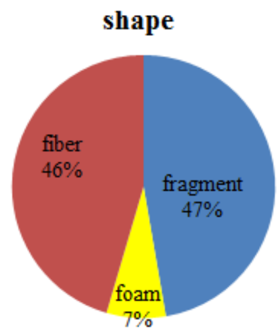
st.13 Lipari



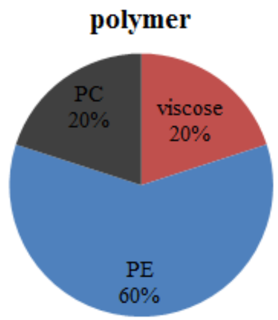
st.14 Messina

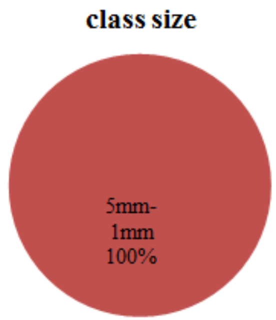


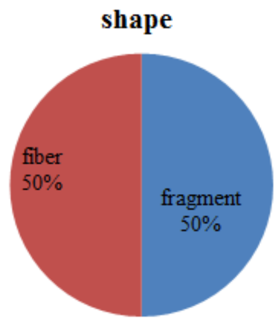




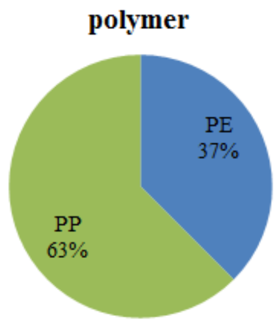
st.15 Caretta

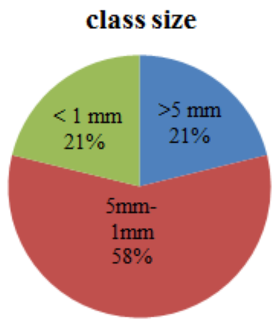


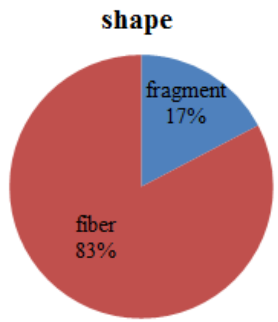




st.16 C.po Rizzuto







st.17 Torre Guaceto

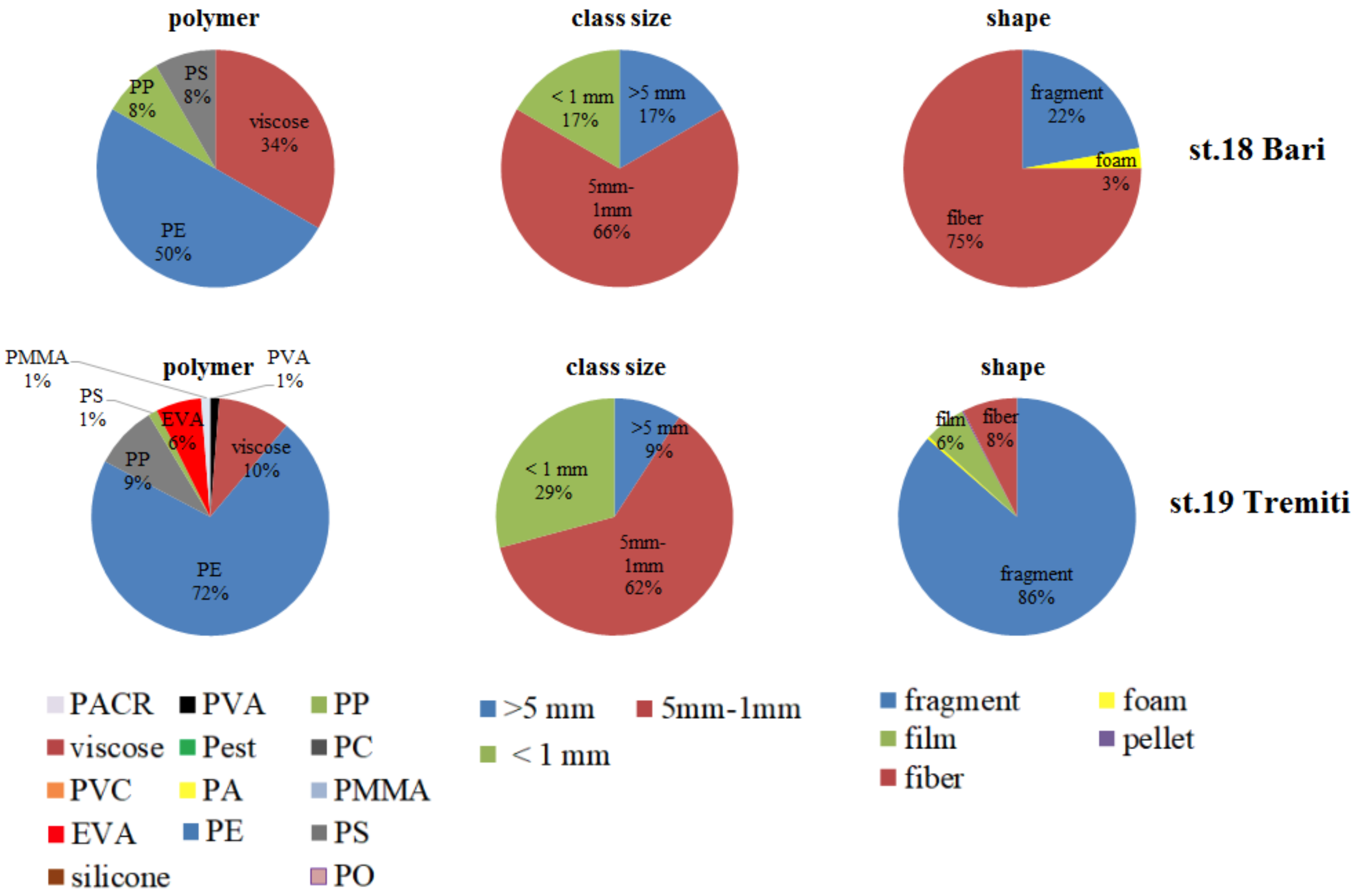


Fig. 8: polymer, class size and shape percentage of MPs collected along the sampled stations. In the legend: PE-Polyethylene; PP-Polypropylene; EVA-ethylene vinyl acetate; PS-Polystyrene; PACR-polyacrylate, PVA-polyvinyl alcohol, PEST-polyester; PC-polycarbonate; PVC-polyvinyl chloride; PA-polyammide, PMMA- poly methyl methacrylate; PO- polyolefin (different from PP and PE).

In station 1 (Cinque Terre, AMP) and station 2 (Livorno), polyethylene (PE) was found to be the most abundant polymer type. Plastic items were mainly smaller than 5 mm, with a high percentage (70%) of items smaller than 1mm (station 1). Only fragment- and fiber-shape were identified. PE was found to be the prevalent polymer in station 3 (Arcipelago Toscano), station 4 (Giglio I) and station 5 (Giglio II). Plastic debris were in the range of 1-5 mm, mainly made of fragment and fibers. In both the Giglio's stations, foams, films and pellets were also isolated. In all these stations (1,2,3,4 and 5), the MPs abundance did not exceed the value of 0.2 items/m^3. A significant number of MPs was isolated in station 7 (AMP Tor Paterno): 0.68 items/m^3. Such value was three times higher than the near station 6 "Civitavecchia" (0,21 items/m^3). MPs were mainly made up of PE-fragment, ranging from 1-5mm. In station 8, 9 and 10, an increasing abundance of MPs was recorded, reaching a maximum value of 3.56 items/m^3 (station 10, Portici). In these stations, the items were mainly represented by 1-5 mm fragment of polyethylene. A great variety of polymer was characterized in the water sample collected from station 10. Several different polymers were also identified in water sample of station 13 (Lipari), where MPs abundance was 0.3 items/m^3. MPs confirmed to be mainly in the form of fragment with a range size of 1-5 mm. The number of MPs identified in station 14-15-16 range between 0,06-0,17 items/m^3. Station 16 (Capo Rizzuto) presented the lowest MPs content found among all the stations sampled. Polyethylene was the most abundant polymer type. In station 14, the 52% of items identified were smaller than 1 mm, whereas items collected from station 15 and 16 ranged between 1 and 5mm. Polypropylene was found to be the prevalent polymer type in station 17 (Torre Guaceto), whereas in station 18 (Bari) polyethylene made up the 50% of items isolated. In both the sample, the items were mainly fibers, ranging from 1 and 5 mm. Plastic items isolated in water samples collected from station 19 (Tremiti) were mainly made up by polyethylene fragment, ranging from 5-1 mm

Microplastics were found in all the stations sampled, with 0,52 items/m^3 as mean value. This value is quite higher but still in the same order of magnitude than those reported by previous studies in Mediterranean regions (0,31 items/m^3, Fossi et al., 2016; 0,17 items/m^3 Panti et al., 2015).
The plastic content found in this study definitely confirms the massive presence of plastic in the Mediterranean Sea that is comparable with MPs level in the North Pacific Gyre by Captain Moore (0,334 particles/m^2, Moore et al., 2001).
The overall class size distribution of plastic items showed a prevalence of small particles. 67% of MPs isolated ranged between 1 and 5 mm, followed by particles even smaller than 1 mm (21%). Items higher than 5 mm account for 12%. This finding confirms MPs (items smaller than 5mm) as the most abundant type of marine debris in sea water (Collignon et al., 2012; Desforges et al., 2014; Lusher et al., 2014; Moore et al., 2001).
14 different polymer typologies were identified. Polyethylene was the most abundant, with an overall frequency of 52% followed by polypropylene and viscose (17%), EVA-ethylene vinyl acetate (6%), polystyrene (3%). Other less frequent polymers (<1%) included: PACR-

polyacrylate, PVA-polyvinyl alcohol, polyester, PC-polycarbonate, PVC-polyvinyl chloride, silicone, PA-polyammide, PMMA- poly methyl methacrylate and PO- polyolefin.
The most common polymer type was polyethylene and polypropylene. The high occurrence of these plastic types in surface waters is not surprising since they have a positively buoyant (their density are lower than seawater) and belong to the largest product group of plastics (Polyolefin), widely used as packaging material and discarded after a short lifetime (PlasticsEurope, 2015).
Fragment (65%) was the most abundant plastic-shape isolated, followed by fiber (31%). In this respect, several researchers raised doubts about considering fiber-items in the final plastic estimate, since they can be confounded with post-sampling contamination in the laboratory. Fibers contamination of sample during analytical procedures is definitely a crucial point that need to be prevented for reliable analysis, adopting specific protection measures. In our study, during laboratorial MPs analysis, we strictly followed a series of measures and methods recommended in the scientific literature that include: wearing clothing made of cotton, avoiding air circulation, cleaning work spaces and tools. Therefore, we can reasonably assume that there has been no air-borne contamination during MPs analysis. During our analysis, the epibiotic community grown on fibers (**Fig. 9A**) and the plastic lines twisted around the plankton (**Fig. 9B**) suggest a long-term stay of items in the sample that cannot be the consequence of a (recent) laboratorial contamination.

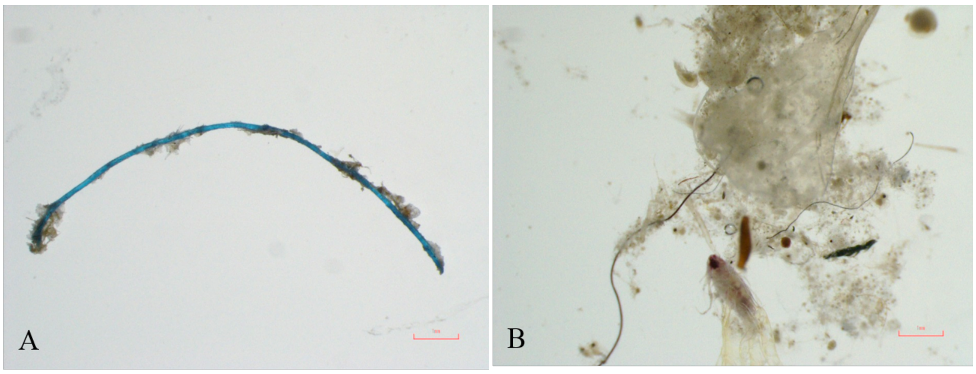


Fig. 9: Example of plastic fiber with epibiotic community (A) and fibers and plankton (B) found out during MPs water samples analysis

In this study, the stations found with the highest level of MPs (upper than average MPs concentration) were: station 10 Portici (3,56 items/m^3); station 19 Tremiti (2,2 items/m^3); and station 7 Tor Paterno (0,68 items/m^3).
The highest MPs concentration was assessed in the water sample collected from Portici. Portici town is located in the Gulf of Naples (8 km southeast of Naples), an area subjected to an intense anthropogenic pressure, from dense urban settlements to industrial activities on the coasts. This might reasonably explain the very high level of plastic pollution in the bay.
Beyond station 10, our survey highlights high content of MPs in the sites of Tremiti (Isole Tremiti-MPA) and Tor-Paterno (Tor Paterno-AMP). Both the sampling stations are located in marine protected areas (MPAs) with strict regulations and limitations on marine and maritime activities. Nevertheless, we found significant level of anthropogenic pollution. In the

impossibility to estimate the sources, we may hypothesize that plastic debris have reached and accumulated in these areas drifted by the current. Indeed, MPAs are often located in proximity of islands and archipelagos. These are isolated systems in the sea subjected to water flow dynamics carrying nutrients, producers that attract consumers (primary and secondary), enriching local biodiversity. On the other hand, water currents also transport anthropogenic particles, including plastics that in turn accumulate in the area. Therefore, MPAs potentially represent area of accumulation for plastic debris. This may suggest the need to plan MPs monitoring programs in these areas, since the key role that MPAs play in marine biodiversity.
Furthermore, the ubiquitous nature of MPs pollution is revealed, from which even a MPA, which is supposed to be unpolluted and pristine, is not exempt.

2. References

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Not only a research but also an awareness tour!

During the "Less Plastic, More Mediterranean" tour, interactive teaching workshops were set up at the ports of Genoa (24-25 June) and Ancona (8-9 July), to raise awareness on the emerging issue of pollution of the microplastics and to contribute to the scientific dissemination of the results of EPHEMARE project, underlining finally the importance of the new collaboration between the Research Institutions involved and the non-governmental organization Greenpeace (Fig 10-13).

Fig. 10: A moment of the Press conference held in Genoa on board the Rainbow Warrior: from the left, Peter Willcox-RW Captain, Serena Maso - Serena Maso, GP Italy, Alessandro Giani, GP Italy, Gabriele Salari GP Italy, Marco Faimali – CNR-Ismar Genoa, Stefania Gorbi – UNIPM and Christophe Brunet – SZN. Photo by CNR-Ismar.

Fig. 11: A moment of the Press conference held in Ancona on board the Rainbow Warrior: from the left, , Marco Faimali – CNR-Ismar Genoa, Sauro Longhi– UNIPM, Giuseppe Onufrio – GP Italy, Francesco Regoli– UNIPM and Peter Willcox-RW Captain. Photo by CNR-Ismar.

Fig. 12: Interactive teaching workshops set up at the Old Ports of Genoa. Photo by Lorenzo Moscia for Greenpeace.

Fig. 13: Interactive teaching workshops set up at the Old Ports of Ancona. Photo by UNIPM.

In addition to the educational workshops on the impact of microplastics, during the "Open-Boat" activities of the Rainbow Warrior in Genoa, CNR-ISMAR organized, in collaboration with the Ziguele cooperative, a particular scientific dissemination activity entitled:

"UnderWaterfront: journey into the submerged life of the port of Genoa"- July 23 - 9:30 pm - Calata Molo Vecchio - Genoa

An extraordinary virtual immersion to discover the amazing biodiversity present between the seabed and the submerged infrastructures of the Port of Genoa was organized.
Thanks to an innovative video and audio signal transmission technology, an underwater diver has been sent live, on the big screen set up in front of the Rainbow Warrior ship moored in the Old Port of Genoa, images and sounds documenting the most characteristic and peculiar biological associations that colonize the seabed and submerged infrastructure of a port. Participants have been also able to interact with the diver, listen to his voice and guide the video footage, thus creating a real "live documentary" of the marine biodiversity of the Port of Genoa (Fig. 14).

Fig. 14: Playbill of the event

Campaign CNR-ISMAR Scientific Manager

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Italy, April 18, 2018