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"DIFENDIAMO IL MARE"
2020 Campaign

"MICROPLASTICS IN THE MARINE ENVIRONMENT ALONG THE TYRRHENIAN COAST – AN UPDATE"

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1. Scope

The Institute for the Study of Anthropic Impact and Sustainability in the Marine Environment (IAS) of the National Research Council (CNR), hereafter CNR-IAS, has been involved, in collaboration with UNIVPM-Università Politecnica delle Marche, in "Difendiamo il mare" sampling campaign. The campaign performed from the 15 to the 28 of July 2020, was promoted by Greenpeace Italia, with the support of different partners (i.e. Fondazione Exodus Onlus). The main goal was to monitor plastic contamination in the Mediterranean Sea, with regards to the Tyrrhenian and Ligurian coasts (Figure 1).

The campaign aimed at:

a. Monitoring the presence of microplastics (MPs) along the Tyrrhenian and Ligurian coasts in three different marine compartments: water column (from surface to deeper layer), seabed and biota;
b. Confirming the presence of MP hotspot areas identified in the previous Greenpeace campaigns performed in 2017 and 2019;
c. Optimizing the sampling methods for MP and potentially nanoplastics (NPs) extraction in water and sediment samples.

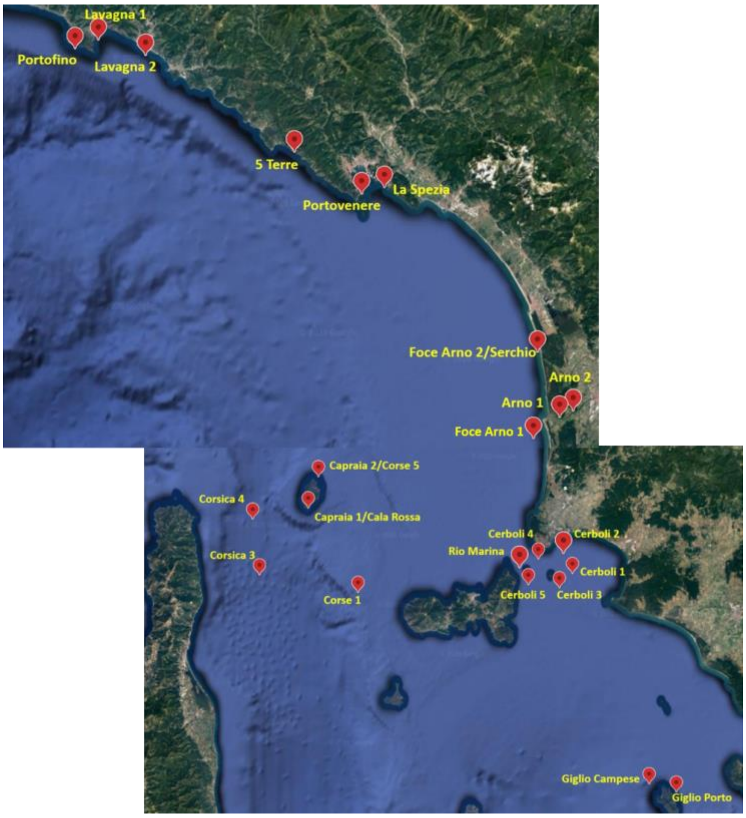

Figure 1. Tour station 2020 of the sampling campaign "Difendiamo il mare"

The present report shows the results obtained from the analysis, performed by CNR-IAS (Genoa, Italy), of sea water and sediment samples.

2. General Overview

Disposal and accumulation of anthropogenic litter has been reported in the marine environment, being one of the fastest-growing threats to the health of the world's oceans (Pham et al., 2014; Bergmann et al., 2015). The Mediterranean Sea is subjected to several anthropogenic pressures (Liubartseva et al., 2018; Macias, 2019), resulting as one of the most affected area in the world (Jambeck et al., 2015; Lebreton et al., 2017; Schmidt et al., 2017; Liubartseva et al., 2018; Chang et al., 2020; Sharma et al., 2021). High amounts of anthropogenic litter are accumulating in the Mediterranean Sea either in the sea surface and in the water column (Cincinelli et al., 2019; Llorca et al., 2020) due to its character of a semi-enclosed basin, with limited outflow of surface waters and a high density populated coastline and site of a series of intensive activities (tourism, fishing, shipping, industrial activities). In this respect, the Mediterranean Sea receives a total annual input of 100,000 tons of plastics, estimated to be 5-10% of the global plastic mass (Cozar et al., 2015). About 50% of such amount are likely to originate from land-based sources, 30% from riverine systems and the last 20% from maritime navigation (Liubartseva et al., 2018). Overall, land-based sources (e.g. sewage treatment plants, urban and agricultural runoff), river discharge, marine activity, and atmospheric dust represent the main sources of marine litter (GESAMP, 2015; Thompson, 2015; Li et al., 2016; Auta et al., 2017). When the plastic reaches the sea, ocean and atmosphere dynamics regulate their behaviour and fate (Atwood et al., 2019). The combination of physical, chemical and biological actions make plastics susceptible to mechanical abrasion (Barnes et al., 2009; Zhang, 2017; Frias and Nash, 2019), promoting their fragmentation into small plastic particles (0.1 µm-5 mm), known as microplastics (5 mm-100 nm, MPs Law and Thompson, 2014) and nanoplastics (<100 nm, NPs) (Rai et al., 2021) that potentially represent the most harmful fraction of plastic waste in the ocean. MPs can occur in the marine environment also by a primary source as manufactured for applications including resin, pellet, microbeads associated with industrial spillage and cosmetic (Cauwenberghe et al., 2015; Coyle et al., 2020). The origin of these emerging pollutants as well as their transport, floating, sinking, ingestion and removal rates influenced by their shape, size and chemical composition, in particular polymer density, promote MP presence and abundance in all compartments of the marine environment, from sea surface to water column, from seabed (including deep sea) to biota (Llorca et al., 2020). Due to their small size, MPs are bioavailable for a variety of marine organisms, such as zooplankton, mussels, fish including seafood species, seabirds, and marine mammals (Botterell et al., 2019; Hantoro et al., 2019; Garrido Gamarro et al., 2020). Marine organisms may confuse MPs with food or indirectly ingest MP through already contaminated prey. MP ingestion may obstruct and compromise the functionality of the digestive system (Gal and Thompson, 2015);

even colour may play a role in the likelihood of ingestion, due to prey item resemblance (Wright et al., 2013). Plastic particles can contain additives and other anthropogenic contaminants, such as organic chemicals that are adsorbed from surrounding seawater. These pollutants include persistent, bio-accumulative, and toxic substances (PBTs), such as polychlorinated biphenyls (PCBs) and dioxins (Alfaro Núñez et al., 2021). Thus, upon ingestion, MPs could lead to toxicological harm, since these contaminants can be released to digestive fluid and can be transferred to other tissues (Rochman et al., 2013; Teuten et al., 2009; Costa et al., 2020; Mistri et al., 2020). Once introduced into the marine food web, MPs could have potential implications and risks not only to the marine life and ecosystems but also to the human health (Carbery et al., 2018).

The recognition of the magnitude of this issue has given rise to several national and international initiatives (i.e. the European Marine Strategy Framework Directive (MSFD 2008/56/EC), the National Oceanographic and Atmospheric Administration (NOAA) Marine Debris Program; Cole et al., 2014) to protect the marine environment against marine litter and MPs. The MSFD included marine litter and its impact on the marine environment and biota as one of the eleven key descriptors of marine environmental status quality. According to the Descriptor 10 (Marine Litter), the assessment of distribution and abundance of MPs in the European waters is mandatory. In this regard, EU member States must monitor MPs and promote research initiatives in order to reduce their environmental levels (Llorca et al., 2020). In this respect a great attention has been given to monitoring MP levels in the Mediterranean Sea surface (i.e. Collignon et al., 2012; De Lucia et al., 2014; Suaria et al., 2016; Baini et al., 2018), while other marine compartments, including water column and sediments, are still poorly investigated.

3. Methods

3.1 Sampling Areas

The campaign “Difendiamo il mare” performed by Greenpeace, CNR-IAS and UNIVPM, took place in the Mediterranean Sea in 2020, between 15 and 28 of July. The sampling campaign was performed in the Tyrrhenian and Ligurian Coasts in the following areas: Giglio Island, Follonica Gulf, Corsica Channel and Capraia Island, Arno river mouth, and Ligurian coast (Figure 2 a-b-c-d-e and Table 1).

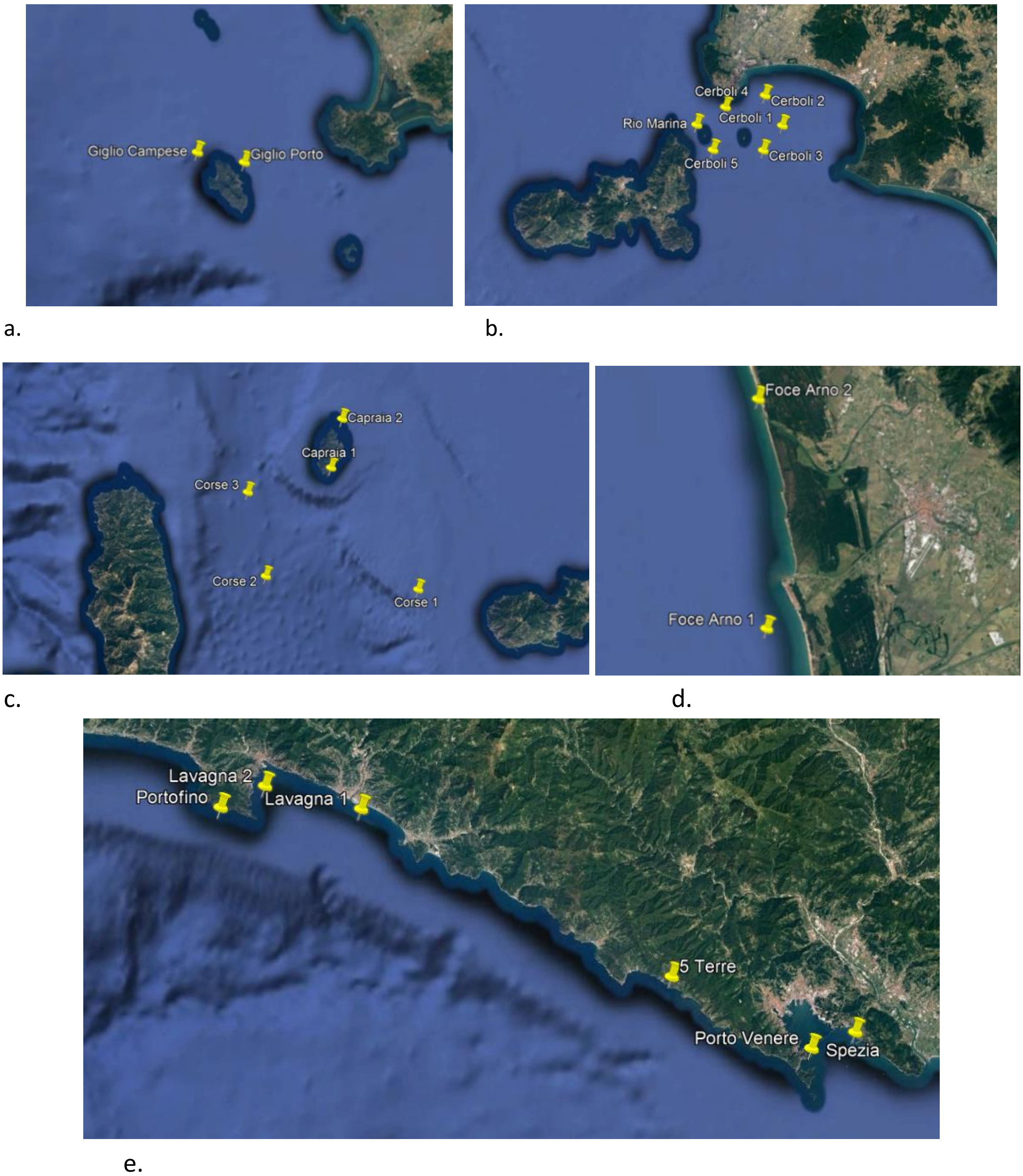

Figure 2. Geographical areas sampled during the 2020 Tour “Difendiamo il mare”. a) Giglio Island, b) Follonica Gulf, c) Corsica Channel and Capraia Island, d) Arno river mouth, e) Ligurian coast.

Table 1 Sampling stations of the "Difendiamo il mare" Tour 2020

Geographical area	Station	Sampling Date	Latitude	Longitude
Giglio Island	Giglio Porto	16/07/2020	42,3711 N	10,9258 E
	Giglio Campese	16/07/2020	42,3902 N	10,8438 E
Follonica Gulf	Cerboli 1	17/07/2020	42,8579 N	10,6147 E
	Cerboli 2	17/07/2020	42,9013 N	10,5878 E
	Cerboli 3	21/07/2020	42,8262 N	10,5748 E
	Cerboli 4	21/07/2020	42,8902 N	10,5114 E
	Cerboli 5	22/07/2020	42,8331 N	10,4809 E
	Rio Marina	18/07/2020	42,8698 N	10,4546 E
Corsica Channel	Corse 1	20/07/2020	42,8157 N	9,9652 E
	Corse 2	20/07/2020	42,8533 N	9,6656 E
	Corse 3	20/07/2020	42,9774 N	9,6434 E
	Capraia 1	19/07/2020	43,003 N	9,8126 E
	Capraia 2	19/07/2020	43,0725 N	9,8429 E
Arno River Mouth	Foce Arno 1	25/07/2020	43,6417 N	10,2484 E
	Foce Arno 2	25/07/2020	43,7879 N	10,2588 E
Ligurian Coast	Spezia	26/07/2020	44,0675 N	9,9009 E
	Porto Venere	26/07/2020	44,0569 N	9,8475 E
	5 Terre	26/07/2020	44,1275 N	9,6893 E
	Lavagna 1	27/07/2020	44,2905 N	9,3375 E
	Lavagna 2	27/07/2020	44,3162 N	9,2256 E
	Portofino	27/07/2020	44,30093 N	9,171 E

3.2 Sampling methods

The sampling strategy was carried out by collecting different types of sea water samples from sea water surface, water column and sediments, as reported in Table 2.

Table 2. Sampling strategy performed during the "Difendiamo il mare" Tour 2020

Geographical area	**Sample**	**Water**			**Sediments**	
		Sea surface	**Water Column**			
		MantaNet 330 µm	**Filtration column 300-100-20 µm**	**Depth of sampling (m)**	**VanVeen grab (1L of sediment)**	**Depth of sampling (m)**
Giglio Island	Giglio Porto	✓	✓	10	✓	14,6
	Giglio Campese	✓	✓	10	✓	13,4
Follonica Gulf	Cerboli 1	✓				
	Cerboli 2	✓				
	Cerboli 3	✓	✓	10		
	Cerboli 4	✓				
	Cerboli 5	✓			✓	9,8
	Rio Marina	✓	✓	10	✓	12,6
Corsica Channel	Corse 1	✓				
	Corse 2	✓	✓	10		
	Corse 3	✓				
	Capraia 1	✓	✓	10	✓	13,1
	Capraia 2	✓				
Arno River Mouth	Foce Arno 1	✓	✓	10	✓	12
	Foce Arno 2	✓			✓	10
Ligurian Coast	Spezia	✓				
	Porto Venere	✓				
	5 Terre	✓	✓	10		
	Lavagna 1	✓				
	Lavagna 2	✓		10		
	Portofino	✓	✓	10		

3.2.1 Water samples

Surface water

Surface water samples were collected using a 300 µm Manta net (Figure 3) (Mouth opening: W0.70xH0.40m) trawled on the water surface at 1-2 Knots for 10/20 minutes. Manta net was towed on the port side of the vessel. The towing point was situated approximately 3 meters away from the hull (Fig.3) to avoid the turbulence induced by the ship. The GPS start and stop positions were recorded in order to define the filtered volume, by calculating the distance covered during the tow. After each sampling event, the whole net was rinsed thoroughly from the outside using a deck hose in order to concentrate all materials to the cod-end, according to Gago et al. (2018). Then, the cod-end sampler was removed and rinsed with sea water on board. All collected samples were carefully transferred into plastic bottles (previously rinsed with filtered seawater) and then appropriately fixed for subsequent laboratory analysis.

Figure 3. Manta net (300 µm)

Water column

Samples from water column were collected by using an Innovative Sequential Filtration System developed by the Danish National Institute of Aquatic Resources - DTU Aqua.

This device (Figure 4) consists in a stainless-steel apparatus with a set of stainless-steel filters of different mesh size mounted in series (300-100-20 µm). It was used to sample water column in 9 stations, as reported in Table 2. The sea water (about 1m^3) was pumped at a depth of 10 meters. After the filtration, the stainless-steel filters were removed from the device and stored into glass Petri dish at room temperature in dark conditions to prevent the photodegradation. Samples were then analyzed in laboratory.

Figure 4. Water column filtration device and different mesh size filters

3.2.2 Sediments

Sediments were sampled by using a Van Veen Grab (5 Kg). Surface sediment samples were collected in 7 stations (Table 2) at depth ranging between 10 and 20 meters.

3.3 Laboratory analysis

3.3.1 Surface water samples

Once in the laboratory, water samples were filtered on 80 µm mesh filter and digested in hydrogen peroxide (15%) in a crystallizer to oxide and digest all biological organic material. The mixture (sample and Hydrogen peroxide) was placed in a temperature-controlled oven at 50 °C until visible organic materials was digested or, alternatively, up to a maximum of 72 hours. During this period the crystallizers were covered with aluminum foils to prevent the environmental contamination. After digestion, samples were filtered through 1 or 0,45 µm Nitrate cellulose filters using a filtration system coupled with vacuum pump. The same procedure was also performed for small size (< 80 µm) samples, not subjected to the digestion process.

3.3.2 Water column samples

The stainless-steel filters (300 and 100µm) were cleaned with deionized water in order to collect all materials. Then, the filters were transferred separately in a glass Petri dish (Ø 150 mm) by adding a solution of Hydrogen peroxide (15%) to digest organic matter and to clean the filters. The Petri dishes containing the filters were placed in a temperature-controlled oven at 50° C until visible organic material was digested or up to a maximum of 48 hours to prevent filters degradation. Then, the filters were cleaned with deionized water and all digested material (without the filter) was collected in the glass crystallizer. After that, the samples collected from 300 and 100 µm stainless-steel filters were filtered through 1 µm Nitrate cellulose filters using a filtration system coupled with vacuum pump.

3.3.3 Sediment samples

All collected samples were defrost at room temperature before the analysis. Sediments underwent density separation using a Sediment-Microplastic Isolation (SMI) developed at CNR-IAS laboratory (Minetti et al., 2021) modified from Coppock et al. (2017). In detail, a transparent 63 mm PVC piping and ball valve, secured to a PVC plate with PVC welding rod for stability have been used to build the SMI. Samples (50 g dry sediment per extraction, for a total of about 100g for sample) were dried at 50 °C in an oven for approximately 72 hours. Dry samples were transferred into the SMI unit with 700 mL of NaI (1,5 g/cm^3) to allow the density separation. After one hour, the SMI valve was closed, the supernatant was collected and transferred onto a 5 µm Nitrate cellulose filters for vacuum pump filtration.

3.3.4 Microplastic analysis

Sorting

The filters obtained from water (surface and column) samples and sediments were analysed for their plastic content under a stereomicroscope (Olympus BX41). Items that were presumed plastics were manually sorted out from the sample, and categorized by colour, shape (fragment, fiber, pellet, film and foam), and size (macroplastics: > 5 mm; and MPs: 5-3 mm; 3-1 mm and < 1 mm), according to Imhof et al. (2012) and Gago et al. (2018) (Figure 6 and Table 3). Then, each particle was transferred onto a microscope slide for the subsequent chemical analysis.

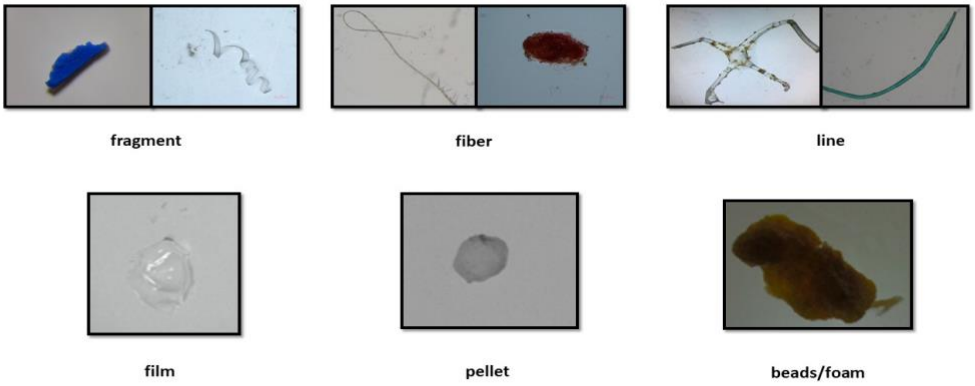

Figure 6. Microplastic type according to shape

Table 3. Size classes

MPs size classification			
Small microplastics	Medium microplastics	Large microplastics	Macroplastics
<1 mm	1-3 mm	3-5 mm	>5 mm

Chemical characterization

Polymer typology (e.g. Table 4) was assessed using a PerkinElmer *Spectrum Two* Fourier Transform Infrared (FT-IR) spectrometer, equipped with Universal ATR (UATR) accessory with a 9-bounce diamond top-plate (Wave number range: 4000 and 450 cm-1 resolutions; 32 scans). After measurement, each spectrum was compared to reference spectra through libraries supplied by Perkin Elmer, with a > 70% similarity threshold. In addition, the fibers isolated from the sediment samples have been analysed to confirm the polymer type by the µ-FTIR (instrument that combines conventional light microscopy and chemical identification by FT-IR spectroscopy).

Table 4. Most frequently identified polymers

Polymer Abbreviation	Name
PE	Polyethylene
PP	Polypropylene
PVC	Polyvinyl chloride
EVA	Ethylene vinyl acetate
PVA	Polyvinyl acetate
PET	Polyethylene terephthalate
PS	Polystyrene
PA	Polyamide
EPDM	Ethylene-Propylene Diene Monomer

3.3.5 Contamination control

To verify airborne contamination during all sampling activities, a jar with deionized water (50 ml) was kept open to the air as control sampling. Several precautionary measures were applied in laboratory to mitigate contamination from external sources (i.e. airborne fibers): laboratory workspace was frequently wiped down; all glassware were washed thoroughly, oven-dried and kept covered (i.e. with a watch glass) when not in use and were heated in a burnout furnace (<600°C) before use (GESAMP, 2019). In addition, cotton laboratory coats and clothing and powder-free examination gloves were worn during the analysis. Consumables were taken directly out from their packaging and all equipment was always rinsed with tap water before and after use. As the laboratory is a busy environment and it is difficult to control contamination from nearby activities (Blair et al., 2019), glass filters (Whatman GF/C), namely procedural blanks, were run in parallel to verify background airborne contamination during laboratorial procedures. Particles detected on filter blanks were analyzed for color, size and chemical composition and compared to particles from environmental samples to avoid false results.

4. Results and Discussion

In the following section, results relative to MP presence in the surface water, water column and sediment samples, collected during the 2020 Tour "Difendiamo il mare", are shown in details. For each environmental matrix, a global MP abundance related to each sampling station is reported. In addition, the MP results are represented according to the global size, shape distribution and chemical composition for each sampling station. Particular concern is given to the fibers, being the most prevalent type of anthropogenic particles found in the ocean (Gago et al 2018; Suaria et al 2020).
In this regard, the results collected from water columns and sediments were reported distinguishing microplastic (mainly, fragments) from microfibers.

4.1 Surface water samples (330 μm Manta net)

Abundance
A total of 3361 items were isolated from surface water samples collected using the 330 μm Manta net. Plastics were found in all sampling stations. The number of plastics found in water samples are expressed as items/m^3 and items/Km^2. Results from the different stations are reported in Table 5, and in the following figures (7-8).

Table 5. Abundance of Microplastics (MPs) reported as items/m^3 and items/Km^2

Geographical area	Sample	Items/m^3	Items/Km^2
Giglio Island	Giglio Porto	0,16	63.354,04
	Giglio Campese	0,09	37.698,41
Follonica Gulf	Cerboli 1	0,22	88.345,86
	Cerboli 2	0,23	91.743,12
	Cerboli 3	0,29	114.285,71
	Cerboli 4	0,19	75.630,25
	Cerboli 5	0,14	56.422,57
	Rio Marina	0,90	361.344,54
Corsica Channel	Corse 1	0,89	266.806,72
	Corse 2	4,13	1.653.061,22
	Corse 3	2,84	1.136.712,75
	Capraia 1	0,91	362.244,90
	Capraia 2	0,74	297.909,41
Arno River Mouth	Foce Arno 1	0,31	125.482,63
	Foce Arno 2	0,63	252.551,02
Ligurian Coast	Spezia	0,20	81.632,65
	Porto Venere	0,20	79.102,72
	5 Terre	0,05	21.739,13
	Lavagna 1	0,16	65.810,59
	Lavagna 2	1,34	401.284,11
	Portofino	0,39	157.349,90

MPs abundance ranged between 4,13 items/m^3 (165.3061,22 items/Km^2) found in Corse 2 Station and 0,05 items/m^3 (21.739,13 items/km^2) in 5 Terre Station, with an average of 0,72 ± 1,00 items/m^3, 275.738,68 ± 398.525,29 items/km^2.

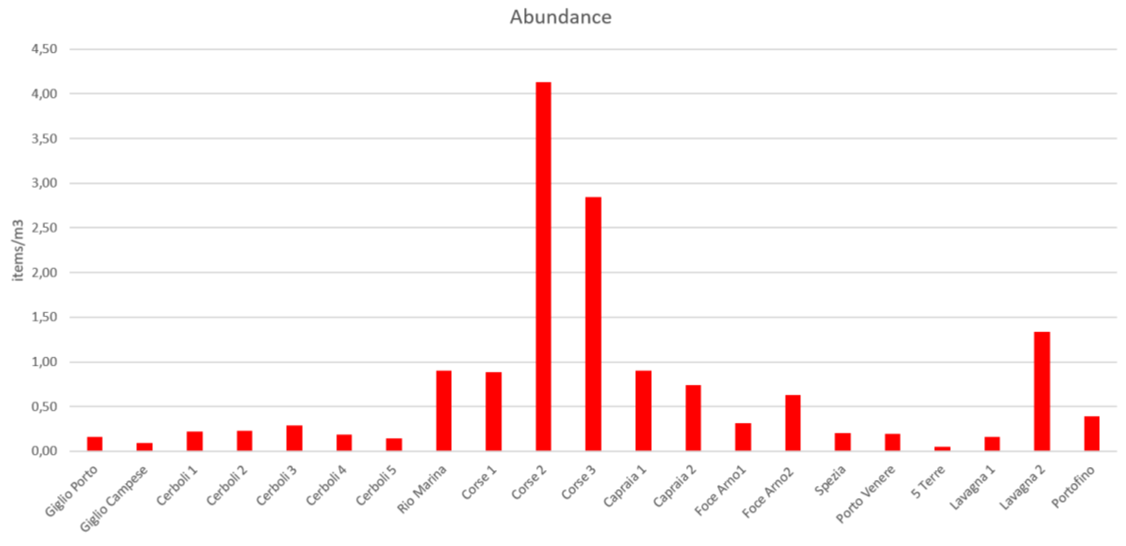

Figure 7. MPs abundance (items/m^3) in surface water samples from different "Difendiamo il mare" Tour sampling Stations

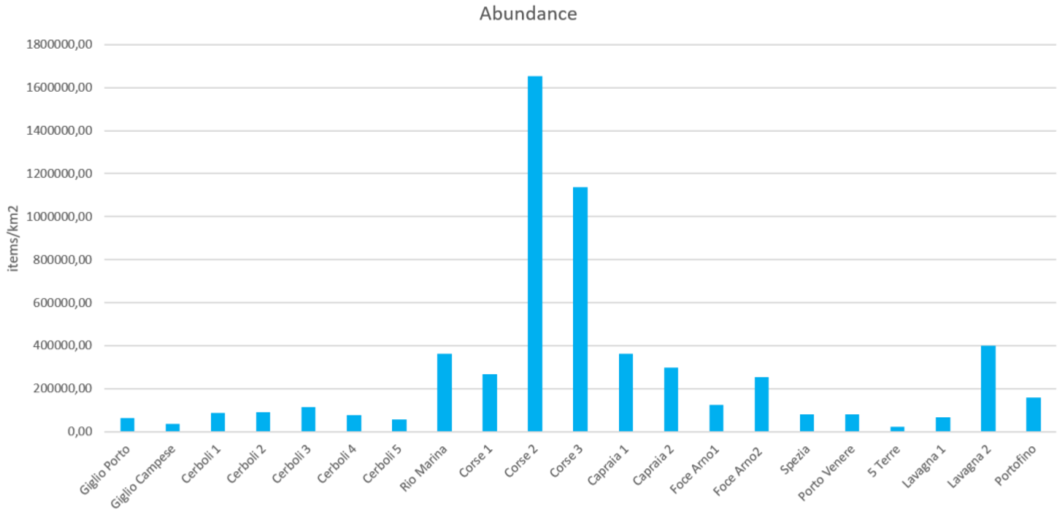

Figure 8 MPs abundance (items/km^2) in surface water samples from different "Difendiamo il mare" Tour sampling Stations

Shape distribution in surface water samples

Considering the results as a whole, on a total of 3361 items found, fragments are represented by 71%, followed by fibers 10% and others shapes (films, lines, foam/beads and pellets) 19% (Figure 9).

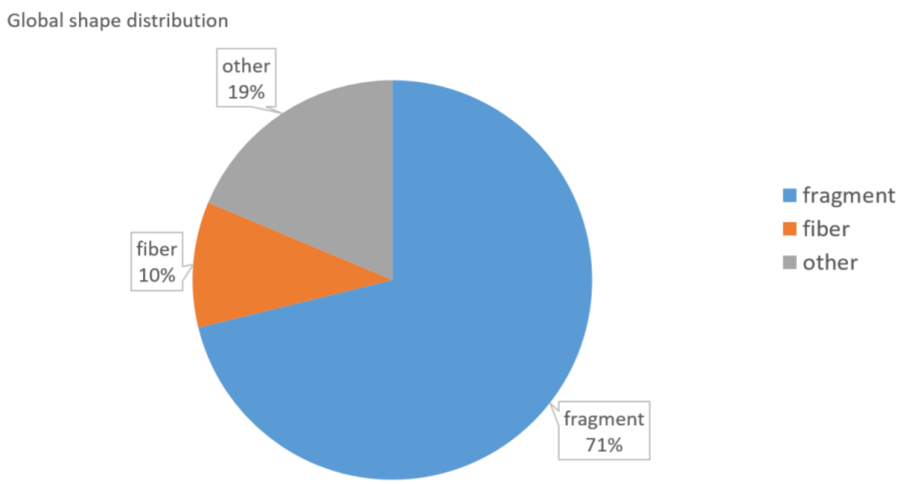

Figure 9 Global Shape distribution in seawater surface samples

In detail (Figure 10), a similar distribution of MPs shape was observed in the collected samples showing fragments as predominant in 16 stations out of 21 representing more than the 50% of analysed items. Fibers account for a significant part of the investigated items in 3 stations (Giglio Campese, Cerboli 4 and 5 Terre). Others shapes (films, lines, foam/beads and pellets) were found from 3 to 41% in all the samples.

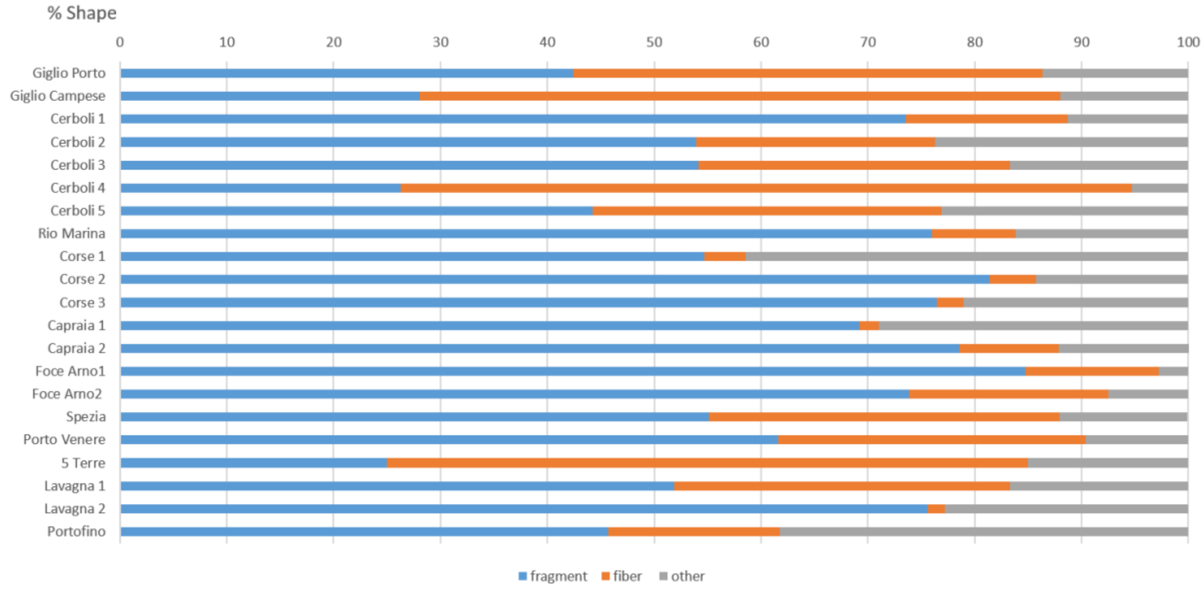

Figure 10. Shape distribution in seawater surface sample collected in each of stations of the tour "Difendiamo il mare"

Size distribution in seawater surface samples

Overall, on a total 3361 items, the most representative size classes were medium MPs (1-3 mm) with a total percentage of 47%, followed by large MPs (3-5 mm) and macroplastics (>5 mm) with 22% and 21% respectively. Small MPs (<1 mm) were the less represented class size 10% (Figure 11).

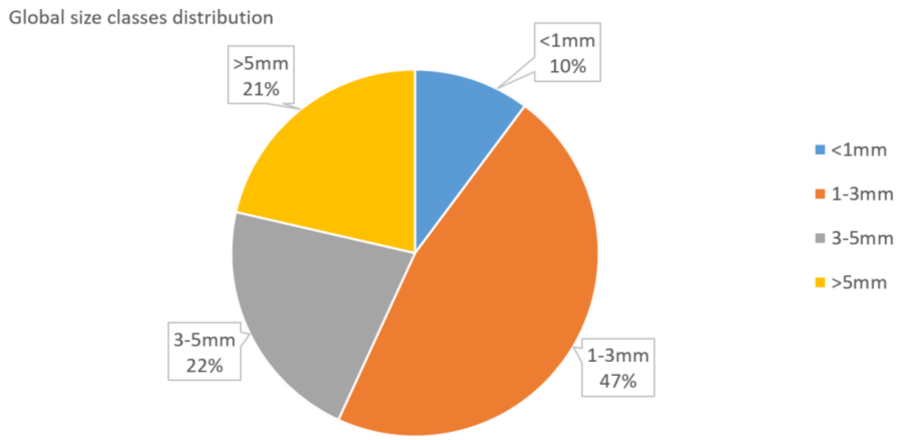

Figure 11. Global size classes distribution in seawater surface samples

As shown in Figure 12, a similar distribution of MPs size classes was observed in all collected samples, where the majority of items fall in the range of 1-3 mm in 16 stations out of 21 representing more than 40%, of analysed items. This size class is most represented with 60% for samples in Giglio Campese and Foce Arno 1 stations. Only in Cerboli 1 30% of the total were smaller than 1 mm, while in Portofino 53% of the total were larger than 5 mm.

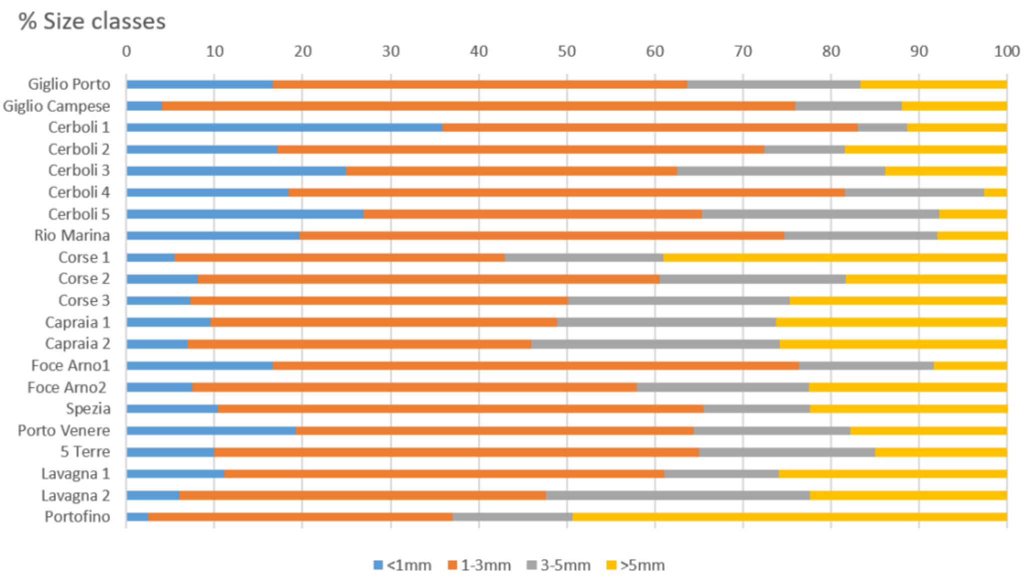

Figure 12. Size classes distribution in seawater surface samples collected in each of stations of the tour "Difendiamo il mare"

Overall, 93% of the total items isolated from the surface water sample (3361), were confirmed to be plastics, 3% was not identified and 4% was detected as not plastic.

The most abundant plastic polymers were polyethylene (PE, 67,5%), followed by polypropylene (PP, 20,0%) and polyethylene terephthalate (PET, 1,8%). Other polymers like ethylene vinyl acetate (EVA), polyamide (PA), ethylene-propylene diene monomer (EPDM) were represented by only 1%, while other polymers (i.e. PVC, PVA, PS, PL, Nylon, Polyisoprene) were less than 1% (Figure 13).

In some case, due to overlapping of interfering signals, items were not identified and were labeled as NI.

Among the items classified as not plastic, the most abundant material resulted to be cellulose with 2,9%, followed by natural materials as wool (0,7%) and cotton (0,1%), cellulosic material as viscose (0,2%), mineral material (0,1%), organic material and latex (0,001%).

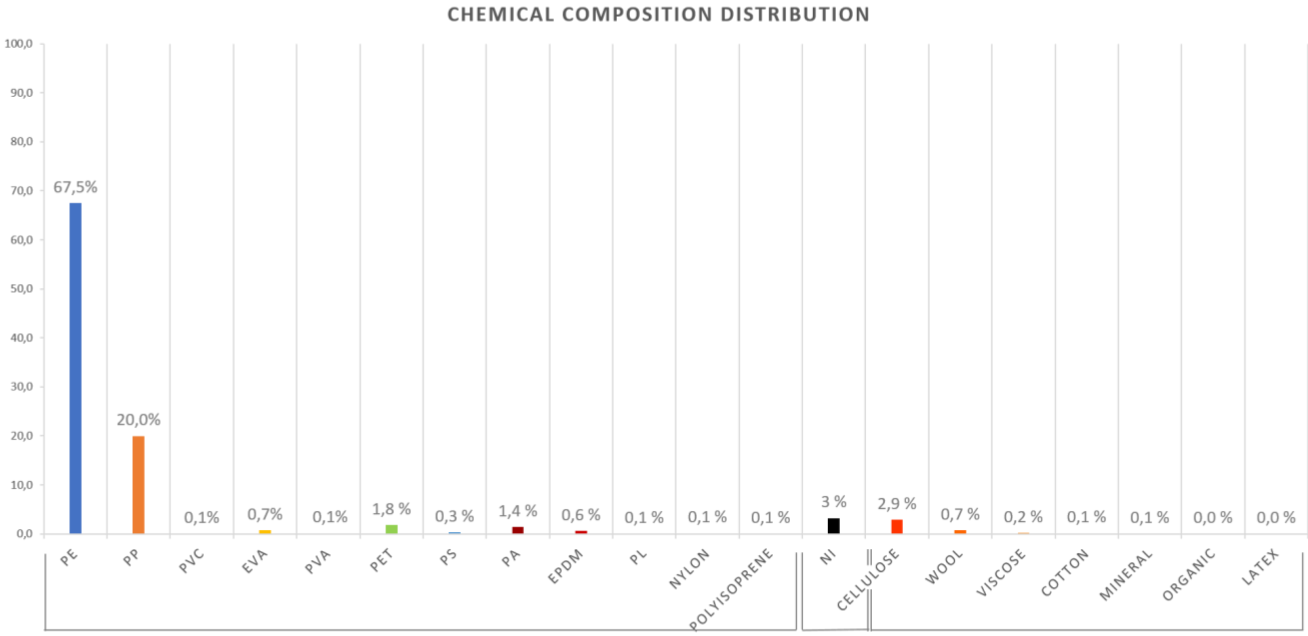

Figure 13. Global chemical composition of MPs in superficial sea water samples

In detail (Figure 14), a similar distribution of polymers was observed in the collected samples showing PE as predominant polymer (blu bars) in 11 stations out of 21 representing more than the 60% of analysed items. The second polymer PP (orange bar) was mainly found as a 30-40% of the total items identified in most stations except Foce Arno (station 1 and 2), Portovenere and 5 Terre where it accounted for less than 10%. An elevated heterogeneity of chemical polymer was assessed in Capraia 2 and Foce Arno 2 where a total 9 different polymer were identified respectively (Capraia 2: PE, PP, EVA, PVA, PET, PS, PA, EPDM, PL; Foce Arno 2: Pe, PP, EVA, PVA, PET, PS, PA ,EPDM, nylon).

The gap, (white area) showed in Figure 14 represent the number of items that have not been identified (namely NI). Most of the not identified items were reported in Giglio Campese with 20% of NI respectively.

Furthermore, as shown in Figure 14 the grey bars represent the items identified as not plastic, where cellulose account for a significant part (20%) in 3 stations (Cerboli 4, 5 Terre and Lavagna).

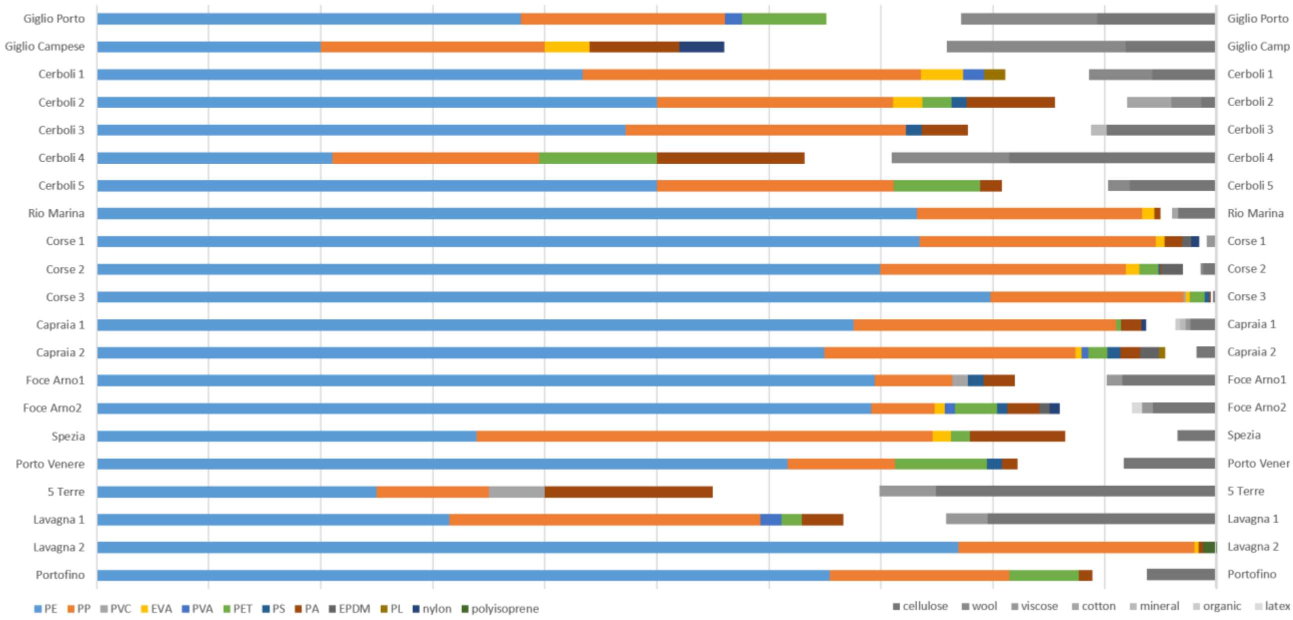

Figure 14. Chemical composition in seawater surface sample collected in each of stations of the tour "Difendiamo il mare". Colours bars (contribution of polymer types), grey bars (not plastic), NI (white area).

Considering results from samples collected using the Manta net, MP abundance mean values found in Mediterranean surface water during the Sea 2020 Tour "Difendiamo il mare" was 0,72 ± 1,00 items/m^3 (275.738,67 ± 398.525,28 items/Km^2). Overall, this data resulted to be in line compared to previous studies in Mediterranean Sea (0,15 items/m^3, de Lucia et al. 2014; 0,31 items/m^3, Fossi et al. 2016; 0,26 items/m3, Baini et al. 2018; 28.376 ± 28.917 items/Km2 Caldwell et al. 2019) and also with the results from the 2017 Tour "Less Plastic More Mediterranean" and 2019 Tour "SOS MAYDAY PLASTICA".

The results obtained in this tour highlight a high plastic accumulation level in the central area of the Thyrrenian Sea (Figure 7), where the majority of MPs was found in Corse channel and Capraia Island with a mean value of 1,90 ± 1,5 items/m^3 (743.347,00 ± 623.109,3 items/km^2). Considering the Corsica channel our results resulted to be higher than those reported last year during the 2019 Tour "MAYDAY SOS Plastic" (0,25 ± 0,12 items/m^3 and 100.797,25 ± 48591,87 items/Km^2) with a concentration of microplastic reaching 4.13 and 2.84 items/m^3 in the Corse 2 and Corse 3 stations, respectively. These findings confirm this area can be considered a hot spot for MP pollution as previously reported by other authors (Collignon et al., 2012; Suaria et al., 2016; Fossi et al., 2017; Kane et al., 2020). The high abundance of MPs may be related to the water circulation in this area; the latter is driven by East Corsica Current divided in the Atlantic Water (AW) in the upper 150 and 200 m and the Intermediate Water (IW), below AW. IW is the saltiest water mass of the whole Mediterranean Sea and originates in the eastern Mediterranean Sea; AW comes from the Atlantic Ocean, crossing the Strait of Gibraltar and flowing into the Mediterranean Sea. Both water masses enter the Tyrrhenian Sea from the South and then follow a cyclonic circulation along the Italian peninsula (Guerra et al., 2019). All these current features promote the site of the Corsica Channel as a possible hotspot of accumulation of floating and deep debris, above all for what concern plastic pollution. The abundance of MPs found in Capraia Island 0,83 items/m^3 is similar to that reported "MAYDAY SOS Plastic" Tour in 2019 (0,80 items/m^3). Therefore, our findings can confirm the presence of a temporary accumulation area of MP around Capraia Island, caused by the presence of the so called "Capraia gyre", a zone characterized by an anticyclonic circulation (Suaria et al., 2016; Fossi et al., 2017; Caldwell et al. 2019).

Regarding the central area of the Thyrrenian Sea, during this tour the sampling activities have been performed in proximity of Giglio Island, due to the great attention that this area has received in the last decade for the Costa Concordia shipwreck. Since 2012, this area is subjected to continuous monitoring studies (Regoli et al., 2014; Avio et al., 2017; Penna et al., 2017). In this respect, the values of MP abundance found in the surface water (average value:0,13 ± 0,05 items/m^3) are in line with those reported in 2017 Tour "Less Plastic More Mediterranean" (0,19 ± 0,1 items/m^3) and in the 2019 Tour "MAYDAY SOS Plastic" (0,12 ± 0,1 items/m^3 items/m^3).

During this tour, surface water samples collected in the Follonica Gulf, around Cerboli Island, were analysed. This area is of great interest since in 2015 the Ivy cargo ship dispersed a load of 56 eco-bales (compressed non-recyclable garbage) thus inducing the Italian Government to declare the Emergency Status last July 2020 allowing the recovery operation by the Civil Protection. The abundance of MPs found in seawater samples show low values if compared with those reported for all other stations of the 2020 Tour (Table 5). The higher value of MP abundance in the Follonica Gulf (Table 5) has been found in Rio Marina (0,90 items/m^3), probably due to the anthropogenic pressure and specifically to the intense marine traffic in the summer time between Elba Island, in the Tuscan Archipelago, and the Italian Peninsula coasts. This area may prevent the entrance of the Tyrrhenian mass of water in the Ligurian Sea (Caldwell et al. 2019), causing plastic accumulation. These results are in accordance with the general findings that indicate areas along shipping routes as characterized by a high MP presence (Caldwell et al., 2011). Thanks to this first assessment of the microplastics level in this area it will be possible to monitor the evolution of microplastic abundance in relation with the presence of the eco-bales in the area.

Among the sampled areas, one of the most MP impacted site results to be the Arno river mouth, with a mean MP abundance of 0,47 ± 0,2 items/m^3. These data are in line with those reported during the previous Greenpeace campaign performed in 2019 in the area closed to the Tevere River (0,65 ± 0,00 items/m^3), one of the most MP polluted Italian river (de Lucia et al. 2018). Thus, this preliminary study represents a starting point for assessing the status of the Mediterranean Sea in this area, considering that Arno river, one of the major Italian rivers, with its densely populated coast and industrialized zone, may represent a significant source of plastic litter pollution in the Tyrrhenian Sea (Guerranti et al., 2020).

The maximum MP value in terms of abundance was not always found in areas in proximity to anthropogenic pressures. For instance, a high MP abundance was found in the Ligurian Sea in proximity of Marine Protected Areas (MPA). Portofino MPA has been defined an "hot spot" of MPs due to the high values of particles per cubic meters reported (Collignon et al., 2012; Fossi et al., 2012). Although Portofino MPA is not subjected to anthropic pressures, the high presence of MPs may be related to the specific configuration of this area. In this regard, the promontory of Portofino may act as an obstacle to the Northern Current, causing a possible storage of floating garbage including MPs. Moreover, other factors (i.e. hydrodinamism) may contribute to MP accumulation in the MPAs: this may explain the differences in MP abundance found in Portofino and 5 Terre MPA (0,39 item/m^3 and 0,05 item/m^3 respectively). However, the majority of MPs items in term of abundance has been found in Lavagna 2, a station located in front of the East Coast of Portofino promontory (Figure 7). In this contest, the MPs accumulation probably is due to the obstacle caused by promontory of Portofino to Northern Current (NC), but further studies are required to confirm this hypothesis.

4.2 Seawater column samples

Abundance

A total of 17,32 fragments and 1.015,66 fibers per m^3 were collected at 10 m depth using the innovative Sequential Filtration System with 300 and 100 µm stainless filters.

Results highlighted a strong unbalance between fragments and fiber, for this reason they will be showed separately representing them as microplastics (fragments) and microfibers.

The proportion of fibers analyzed per sample varied from 9,5 to 95,3 % (mean 43%), but typically, a fixed number of fibers were extracted from each filter, unless the sample lacked sufficient fibers to do so (Table 6).

Table 6. Abundance in term of items/m^3 of all fragments and fibers observed under stereomicroscope (Collected items/m3), sorted and % of analysed in the 300 and 100 µm filter mesh for all stations sampled (seawater column samples).

Sample	Filtered volume	Mesh (µm)	Collected (items/m3)		% Analysed	
			Fragments	Fibers	Fragments	Fibers
Giglio Porto	0,75	300	4,00	113,33	100	49,4
		100	1,33	44,00	100	45,5
Tot			**5,33**	**157,33**		
Giglio Campese	0,75	300	0,00	113,33	/	35,3
		100	0,00	86,67		30,7
Tot			**0,00**	**200,00**		
Cerboli 3	0,75	300	4,00	0,00	100	/
		100	0,00	28,00	/	61,7
Tot			**4,00**	**28,00**		
Rio Marina	1,00	300	3,00	21,00	100	95,2
		100	1,00	60,00	100	10
Tot			**4,00**	**81,00**		
Corse 2	0,75	300	1,33	28,00	100	95,3
		100	0,00	28,00	100	81
Tot			**1,33**	**56,00**		
Capraia 1	0,75	300	0,00	28,00	/	47,6
		100	0,00	20,00	/	33,4
Tot			**0,00**	**48,00**		
Foce Arno	0,75	300	0,00	253,33	/	9,5
		100	0,00	86,67	/	37,2
Tot			**0,00**	**340,00**		
5 Terre	0,75	300	1,33	20,00	100	33,4
		100	0,00	13,33	/	80
Tot			**1,33**	**33,33**		
Portofino	0,75	300	1,33	38,67	100	38
		100	0,00	33,33	/	36
Tot			**1,33**	**72,00**		

In Figure 15, the results of abundance in term of fibers and fragment per m^3 from 300 and 100 µm filters are reported.

Overall an high level of particles, both microplastic (fragments) and microfibers, were found in all the 10 m depth sampling stations; the highest abundance in term of total items per m^3 (Table 6 and Fig. 15), was found in the Arno River mouth (253,33 items/m^3 for 300 µm filter; 86,67 items/m^3 for 100 µm filter) followed by Giglio Campese (113,33 items/m^3 for 300 µm filter; 86,67 items/m^3 for 100 µm filter) and Giglio Porto (117,33 items/m^3 for 300 µm filter; 45,33 items/m^3 for 100 µm filter). Conversely, Cerboli 3 station showed, the lowest abundance in term of items analysed per m^3 of 4 items for 300 µm mesh filter and 28 items/m^3 for 100 µm mesh.

Most of the collected items were microfibers while microplastics (fragments), despite being represented with significant abundance values for what is generally reported in the literature to date, vary from a maximum of 5,33 items/m^3 in Giglio Porto followed by Cerboli 3 and Rio Marina, both with 4 items/m^3.

Microplastics were absent in 10 m depth water from Giglio Campese, Capraia 1 and Arno River mouth.

Conversely, fibers were found in all samples, except in Cerboli 3.

Considering the very high abundance the trend of microfibers abundance was the same reported above for the total items/m^3.

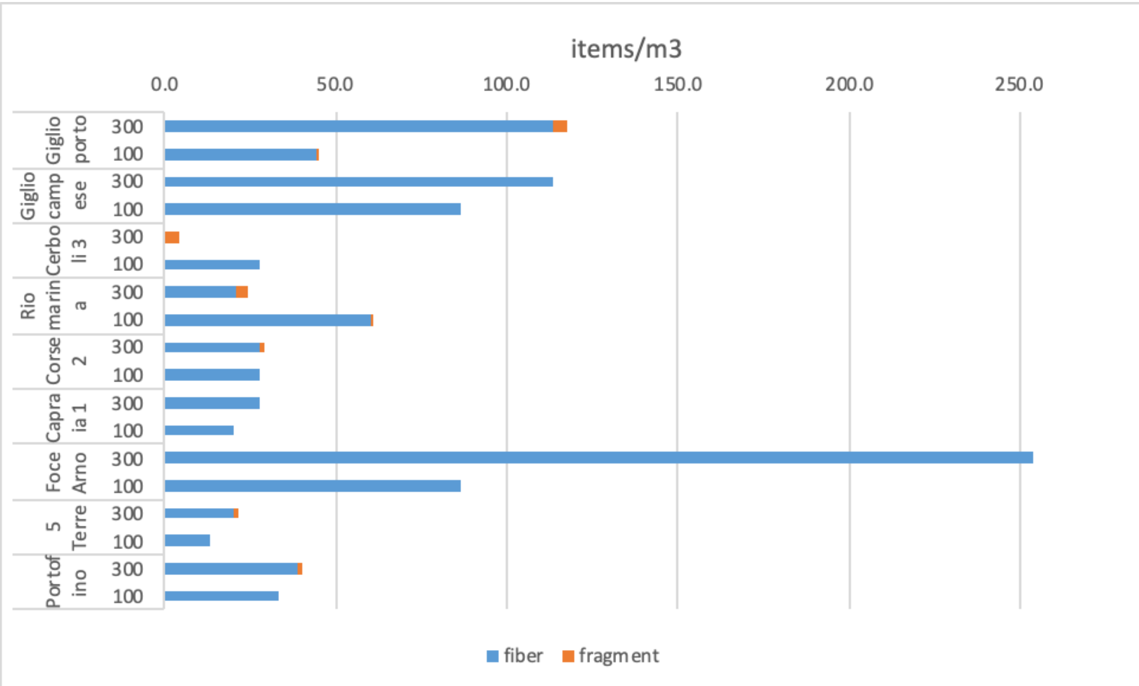

Figure 15 MPs abundance in term of fibers (blu bars) and fragments (orange bars) per m^3 found in water column samples collected from 300 µm mesh filter

Size distribution in sea water column

Considering both microfibers and microplastics the most abundant size class found in the water column samples was represented by that less than 1 mm, followed by the small MPs (1-3 mm) (Figure 16). Medium (3-5 mm) and macroplastic (> 5) were represented by a small fraction of the total items isolated (5%).

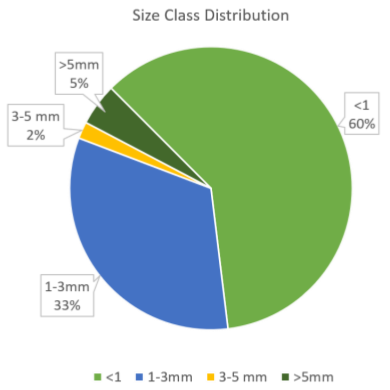

Figure 16 Global size classes distribution in water column samples

Chemical distribution in sea water column

Considering microfibers as whole, 37% was confirmed to be plastic, showing a great variability of polymer types. PE was resulted to be predominant polymer in all stations sampled, followed by PP considering both 300 and 100 µm filters. However, 23% of the total fibers analysed were confirmed to be cellulose, while 40% was not identified (NI) by the FTIR

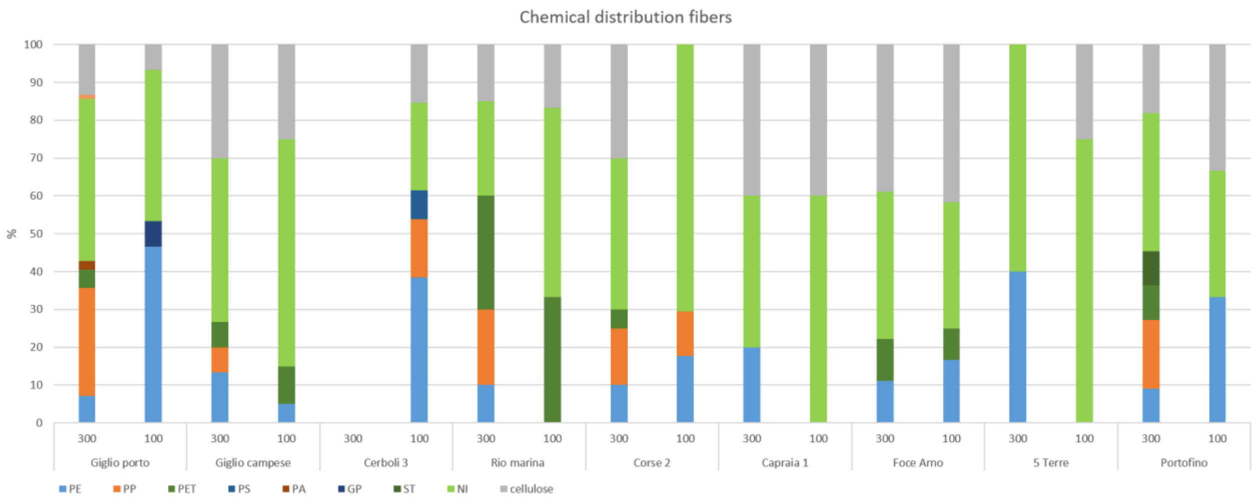

Figure 17 Chemical composition of microfibers found in water column samples collected in each of 10 m depth stations of the tour "Difendiamo il mare" with 300 and 100 µm mesh filter.

Conversely, the total of fragments subject to the analysis were confirmed to be plastic. PE resulted to be the predominant polymer type in all the sampled stations.

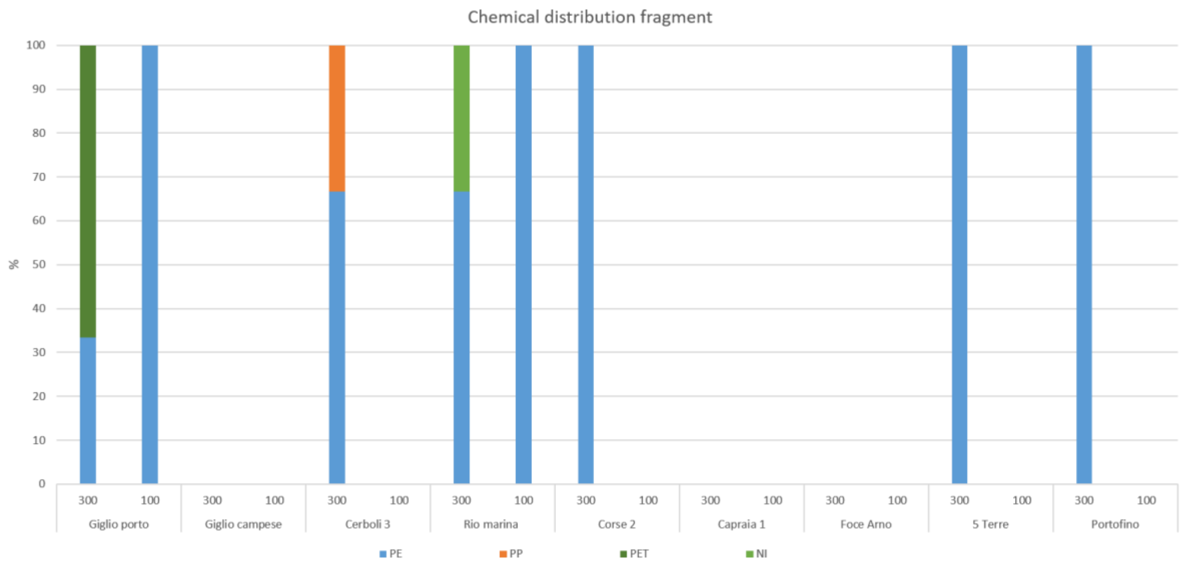

Figure 18 Chemical composition of microplastics (fragments) found in water column samples collected in each of stations of the tour “Difendiamo il mare” with 300 and 100 µm mesh filter.

4.3 Surface water samples *vs.* 10 m depth water column samples

The activity performed in this sampling campaign allow a very new comparison of data from samples collected on the sea surface using the Manta net with those deriving from the water column (-10 m) collected using the innovative sequential filtration system. Being Manta net mesh size 330 µm, the comparison is performed considering only items found in the 300 µm filter of the sequential filtration system (Table 7).

Table 7 Abundance (items/m^3) in term of microplastics (fragments) and fibers found in surface water (Manta net 300 µm) and water column (Multi-mesh filtration device 330 µm filter)

Sample	Manta net 330 µm_surface water (items/m^3)		Multi-mesh filtration device 300 µm _-10 m water column (items/m^3)	
	Microplastcis (fragments)	Microfibers	Microplastcis (fragments)	Microfibers
Giglio Porto	0,08	0,05	4,00	113,33
Giglio Campese	0,03	0,04	0,00	113,33
Cerboli 3	0,17	0,07	4,00	0,00
Rio Marina	0,71	0,04	3,00	21,00
Corse 2	3,41	0,13	1,33	28,00
Capraia 1	0,63	0,01	0,00	28,00
Foce Arno1	0,28	0,02	0,00	253,33
5 Terre	0,02	0,03	1,33	20,00
Portofino	0,17	0,06	1,33	38,67

Considering the water column results, the particles per m^3 resulted to be overall, significantly higher than those found in the surface water samples.

In particular, microplastics trend vary from stations to station. In Giglio Porto, Cerboli 3, Rio Marina, Corse 2, Cinque Terre and Portofino those found in 10 m depth samples resulted to be one order of magnitude higher than those present in the sea surface, conversely, in Giglio Campese, Capraia 1 and at the Arno River mouth they were absent in the water column samples.

If only the microfibers are considered, the difference between what is present on the seasurface and that present at 10 meters depth is even more marked.

In fact, microfibers in the water column are one or two order of magnitude higher than those reported in surface water. This finding is particularly evident in Arno river mouth and Giglio Porto and Giglio Campese with 253,33, 113, 3 113,33 fibers/m^3 in the water column compared with 0,02, 0,05 fibers/m^3 and 0,04 fibers/m^3 in surface water, respectively.

To better explain our findings, we can hypothesize that the vertical mixing may affect the number, mass, and size distribution of buoyant plastics captured by surface nets as reported in previous study (Hidalgo-Ruz et al., 2012; Kooi et al. 2016). Generally, our results do not seem to agree with literature data since Fossi et al. (2012) did not detect microplastic particles in water column samples (items/m^3) collected in the Pelagos Sanctuary and Baini et al. (2018) observed an average concentration of 0.26 items/m^3 in the Tuscany coast (Italy). However, to date very few studies have focused on the quantification of microplastics in the water column in the Mediterranean Sea and at European level a single study on the Baltic Sea shows an average concentration of microlitter of 0.40 items/l, (Bagaev et al., 2018). In addition, microplastic concentrations have been observed to decrease with depth, and depending on sea state, particle characteristics and from the different vertical transport mechanisms (Kooi et al., 2016).

The most abundant size class (47%) found in surface water was represented by particles with a medium size (1-3 mm) while in the water column the majority particles (60%) resulted to be smaller than 1 mm (Figure 19).

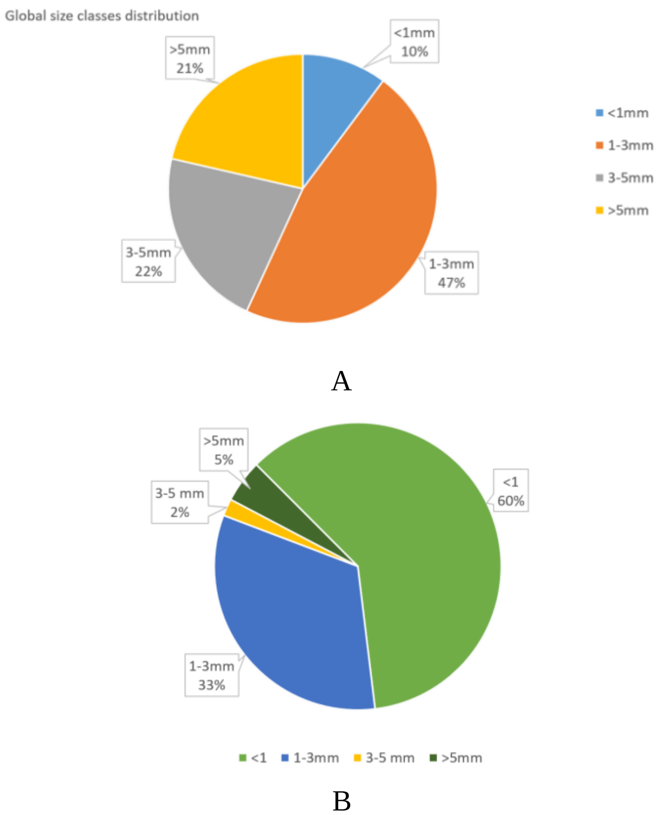

Figure 19: Global size classes distribution in A) surface water samples (Manta net) *Vs.* B) water column sample

In addition, another interesting finding concerning microfibers is that, in agreement with a recent study reported by Suaria et al. 2020, a significant amount of those found in the water column resulted not to be a plastic but dyed cellulose.

4.3 Sediment samples

Table 8 and Figure 20 reports the sampling site depth and the MPs abundance in term of items per Kg of dry sediment collected in 7 stations. Microplastics and microfibers are presented separately also in this case.
The highest value of MPs abundance was observed in Giglio Porto with a total of 53,59 items/kg, while the lowest was reported in Rio Marina with 7,75 items/kg. No plastic items were isolated from sediment samples collected at the Arno River mouth.

Table 8 Average Microplastics (MPs) concentration reported as items/Kg

Geographic area	**Sample**	**Abundance (items/Kg) Total**	**Abundance (items/Kg) Microplastics**	**Abundance (items/Kg) Microfibers**
Giglio Island	Giglio Porto	53,59	10,72	42,87
	Giglio Campese	19,65	0,00	19,65
Follonica Gulf	Cerboli 5	14,02	7,01	7,01
	Rio Marina	7,75	0,00	7,75
Corsica Channel	Capraia 1	23,77	5,94	17,83
Arno River Mouth	Arno 1	0,00	0,00	0,00
	Arno 2	0,00	0,00	0,00

The abundance of microfibers resulted to be significantly higher than microplastics in all the station except in Cerboli 5 where the same number of items for each class have been found. The highest microfibers abundance resulted to be in Giglio Porto with 42,87 items/Kg followed by Giglio Campese and 17,83 items/Kg.

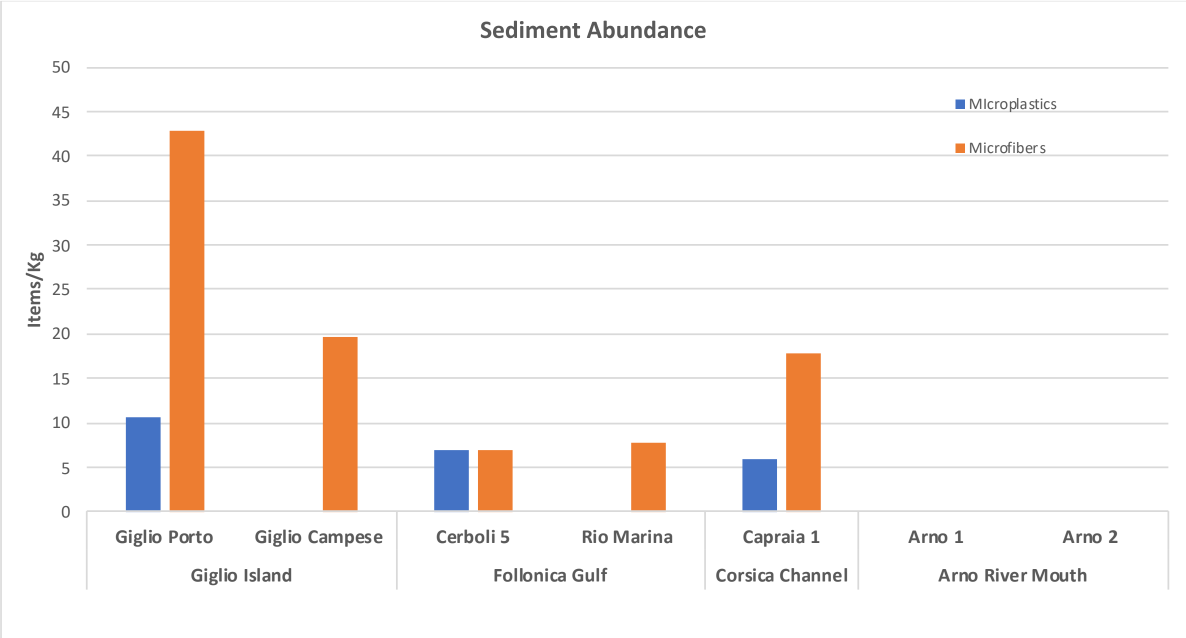

Figure 20 Abundance of microplastic and microfibers found in sediment samples collected during the tour "Difendiamo il mare" (items/Kg)

<u>Shape and size distribution in sediment samples</u>

The ratio between microplastics and microfibers is reported in Figure 21 with a total of 45% and 55% of the different type in the samples as a whole. Most of the items belong to the medium size class (1-3 mm) corresponding to 89%, followed by small MPs (<1 mm), 11% (Figure 21).

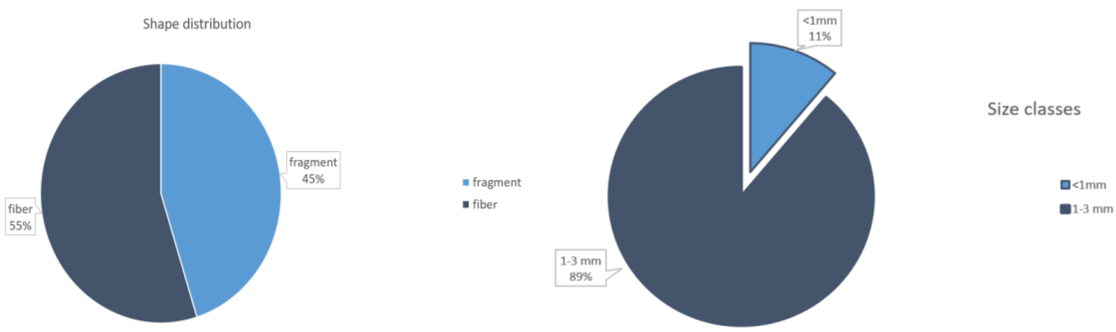

Figure 21 Shape distribution in sediment samples

In Figures 22 and 23 the chemical composition of microplastics (fragments) and microfibers found in the sediment samples are reported separately.

16% of total fibers subject to the analysis was confirmed to be plastic with PVC and PVDF resulting to be the polymers found mainly in all station sampled, expect Cerboli 3 and Rio Marina plastic fibers where not detected. In addition, 39% of the total fibers analysed were confirmed to be cellulose (Figure 22). All the microfibers detected in Cerboli 5 and Rio Marina were made of cellulose and no plastic microfibers where found in these stations. Giglio Campese showed the highest percentage of plastic microfibers (50%).

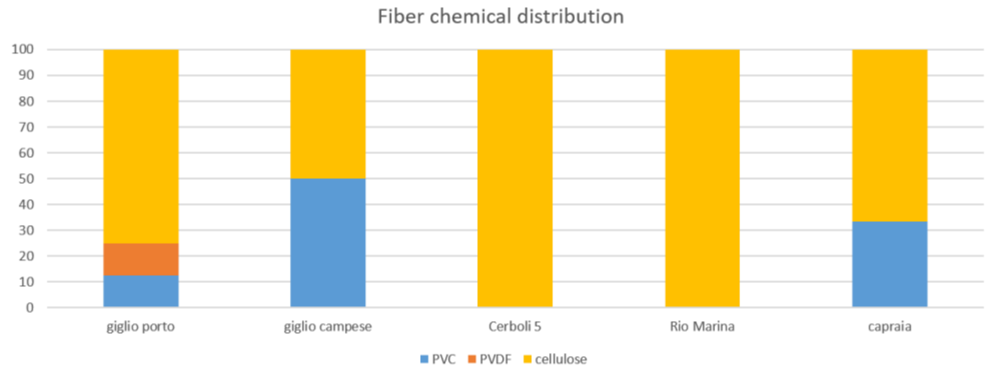

Figure 22. Chemical composition of fibers found in sediments samples collected in each of stations of the tour "Difendiamo il mare".

Considering microplastics (fragments), the polymers type resulted to be station dependent with Cerboli and Giglio Porto characterized by the presence of PVC items and Capraia by Polymeric gum.

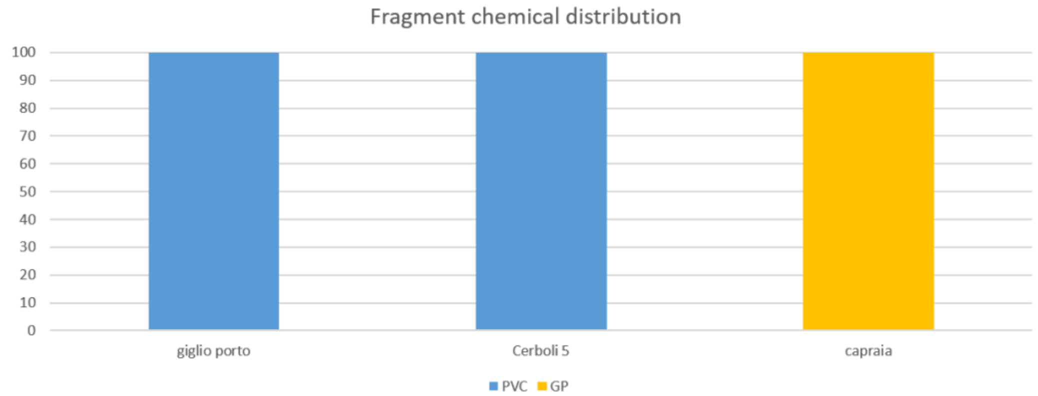

Figure 23. Chemical composition of fragments found in sediments samples collected in each of stations of the tour "Difendiamo il mare" .

The mean values of 17 ± 18,5 items/kg reported for all station sampled during the 2020 Tour "Difendiamo il mare" resulted to be generally lower than those reported for other areas of the Tyrrhenian Sea (e.g 88 items/Kg in Talamone by Cannas et al., 2017 and 45-1069 items/Kg in Ombrone river mouth by Guerranti et al., 2017). Conversely, the concentration of plastic particles in term of items per weight of sediment it resulted to be higher than what reported by Mistri et al. (2020) that found 0,43-4 items/kg in sediment sampled in Piombino Channel. Once in the sea, MPs sink in the water column after a change of density driven by physical, chemical and biological interactions that to date is not fully understood yet, but for these mechanisms the majority of MPs have been found in sediments (Näkki et al., 2019; Phuong et al. 2021).

Logically, plastics with a density that exceeds that of seawater (>1.02 g cm^{-3}) will sink and accumulate in the sediment, while low-density particles tend to float on the sea surface or in the water column. Within this contest, polymer such as PE and PP with low-density (0.90–0.99 g cm-3; 0.85–0.92 g cm-3), have longer residence time at the sea surface, while heavier polymers are prone to rapid sinking. (i.e. PVC: 0.38–1.41 g cm^{-3}) according to our results where PVC has been the polymer more frequently found in the sediment sampled collecting during the 2020 Tour "Difendiamo il mare".

In addition, our findings suggest that a standardized protocol for extraction of MPs from sediment samples is required, since the lacking of conformity among the studies for the MP quantification, regarding the sampling, preparation of samples, extraction, identification and treatment of results makes the results difficult to be compared.

5. Conclusions

Our findings highlighted different pattern of MPs distribution along the water column reporting significantly different concentrations of MPs in surface water, water column with a very high prevalence of microfibers in the latter (Figure 24).

Moreover, our results pointed out that the water column and sediment samples contained more plastic particles than surface water suggesting the need to perform more carefully sampling strategies towards these two marine compartments where the possible interaction between plastic and marine biota is more probable and ecologically relevant.

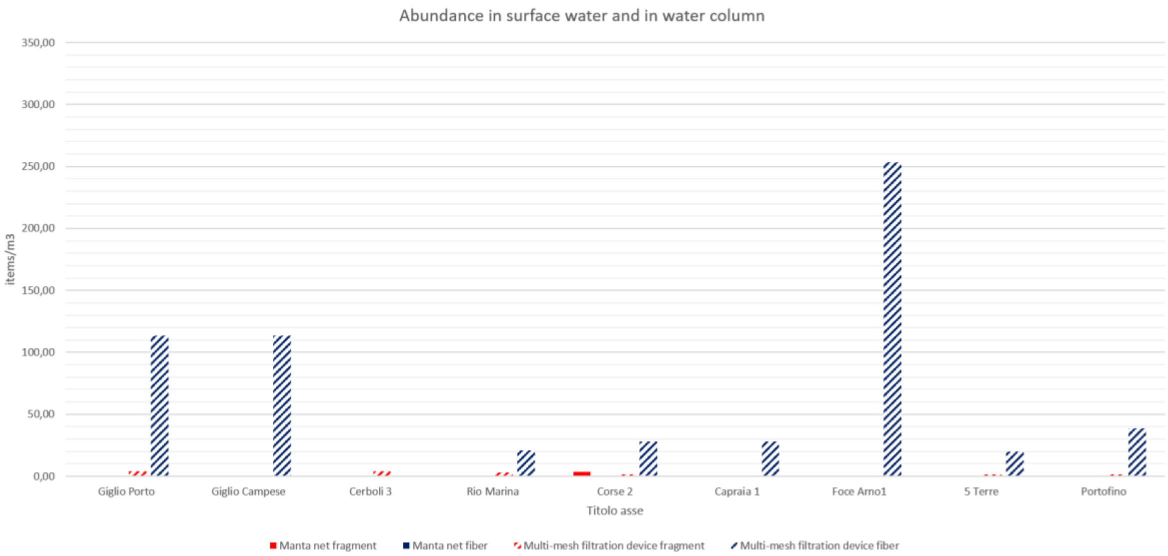

Figure 24. Abundance (items/m^3) in term of fragments and fibers found in surface water (330 µm Manta net) and water column (300 µm filter Sequential Filtration System).

Fibers were the dominant shape in water column and marine sediments, while fragments were mainly dominant in surface water.

However, according to Suaria et al. (2020) a very significant amount of fibers found in the samples were not plastic but dyed cellulose.

Global size distribution of items found in the samples showed that the size range between 3-5 mm (large microplastics) and > 5 mm (macroplastics) were the lowest proportion among the three environmental compartments.

The abundance of microplastics of less than 3 mm in size (small microplastics and microplastics), increases proportionally with sea depth while in sediment a condition similar to sea surface has been found (Figure 25). This dimensional pattern has also been previously observed in studies conducted in the Mediterranean Sea and in other ocean basins (Baini et al. 2018; Caldweell et al 2019).

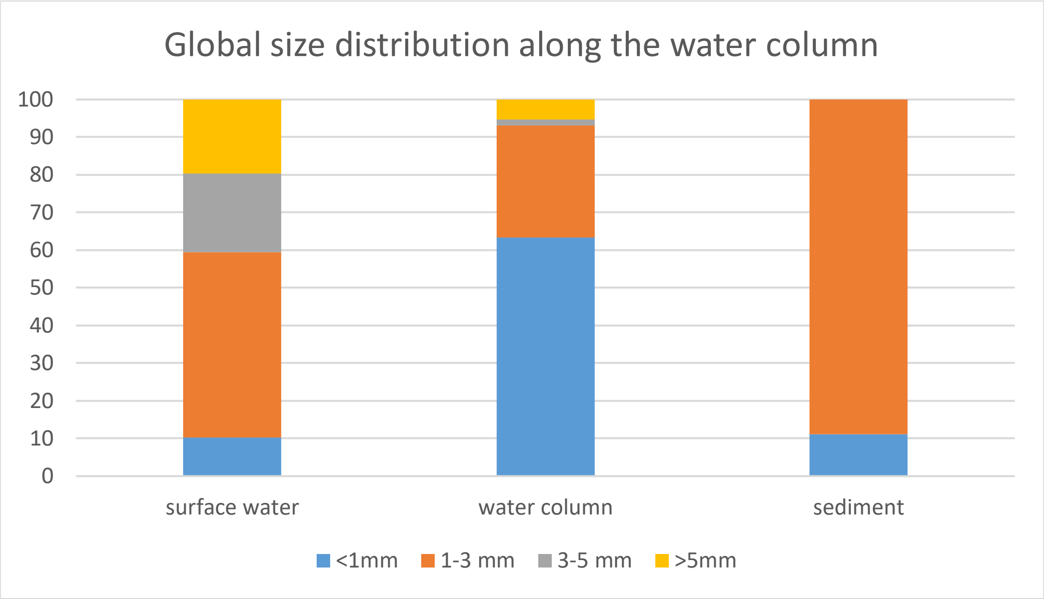

Figure 25. Global size distribution in surface water, water column and sediment samples.

Considering chemical composition, the most industrially produced polymers were the most abundance: namely, PE and PP for surface water and sea water column and PVC for sediments.

These findings confirm the vulnerability of the Mediterranean Sea to plastic pollution due to its semi-closed configuration and the extreme anthropogenic pressure related to high population density, tourism and all marine activities, that make this basin the major hotspot for plastic litter, with 7% of global MPs (Suaria et al., 2016; Baini et al., 2018; Sharma et al., 2021).

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