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Abundance and characterization of microplastics in the coastal waters of Tuscany (Italy): The application of the MSFD monitoring protocol in the Mediterranean Sea

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ABSTRACT

Monitoring efforts are required to understand the sources, distribution and abundance of microplastic pollution. To verify the abundance of microplastics along the Tuscan coastal waters (Italy), water-column and surface samples were collected in two seasons across four transects at different distances to the coast (0.5, 5, 10 and 20 km), within the implementation of the European Marine Strategy Framework Directive. The results show an average concentration of 0.26 items/m^3 in the water-column samples and 41.1 g/km^2 and 69,161.3 items/km^2 of floating microplastics, with an increase with the distance to the coast The seasonality and the sampling area do not affect the abundance of microplastics. The most abundant size class is 1–2.5 mm as fragments and sheets suggesting that fragmentation of larger polyethylene and polypropylene items could be the main source of microplastics. These data represent the application of a harmonized protocol to make the data on microplastics comparable and reliable.

1. Introduction

Anthropogenic litter on the ocean surface, beaches and seafloor has become an urgent issue in recent decades worldwide, including the Mediterranean Sea that has been described as one of the most affected area in the world (Cózar et al., 2015; van Sebille et al., 2015). The Mediterranean Sea houses around 10% of the global coastal population along its shores (about 100 million people within the 10-km coastal strip) (CIESIN, 2012). To date, a lot of studies carried out in this basin have focused on beach litter (Karapanagioti et al., 2011; Munari et al., 2017; Portman and Brennan, 2017; Prevenios et al., 2018; Turner and Holmes, 2011), on the accumulation of marine debris on the coastal sediments (Alomar et al., 2016; Blašković et al., 2017; Cannas et al., 2017; Fastelli et al., 2016; Guerranti et al., 2017) and on the sea floor (Cannas et al., 2017; Galgani et al., 2000; García-Rivera et al., 2017; Guerranti et al., 2017; Koutsodendris et al., 2008; Mifsud et al., 2013; Ramirez-Llodra et al., 2013). Regarding the abundance of floating macro and mega debris in the Mediterranean waters, studies (Aliani and Molcard, 2003; Carlson et al., 2017; Di-Méglio and Campana, 2017; Faure et al., 2015; Fossi et al., 2017; Gajšt et al., 2016; Suaria and Aliani, 2014), showed densities between 0 and 194.6 items/km^2, with a maximum registered in the Adriatic Sea and Algerian Basin (Suaria and Aliani, 2014). Levels of microplastics have been detected in surface waters using surface net tows (Collignon et al., 2014, 2012; Cózar et al., 2015; Faure et al., 2015; Fossi et al., 2017, 2012; Gündoğdu and Çevik, 2017; Panti et al., 2015; Pedrotti et al., 2016; Suaria et al., 2016; van der Hal et al., 2017) even in areas with low human impact, confirming that floating debris is widespread in the Mediterranean Sea with concentration higher or in the same order of magnitude of those found in oceanic accumulation zones (Cózar et al., 2014; Suaria et al., 2016; van der Hal et al., 2017; van Sebille et al., 2015).

Very few specific studies have focused on the quantification of microplastics in the water column. At European level a single study on the Baltic Sea shows an average concentration of microliter of 0.40 items/l, where the highest concentrations are found in the near-bottom samples from the coastal zone and from near-surface waters (Bagaev et al., 2018) Any study has been published on the Mediterranean sea, apart from few data presented in Fossi et al. (2012).

The density and characteristics of the floating plastics and microplastics sampled in the Mediterranean sea are comparable to other

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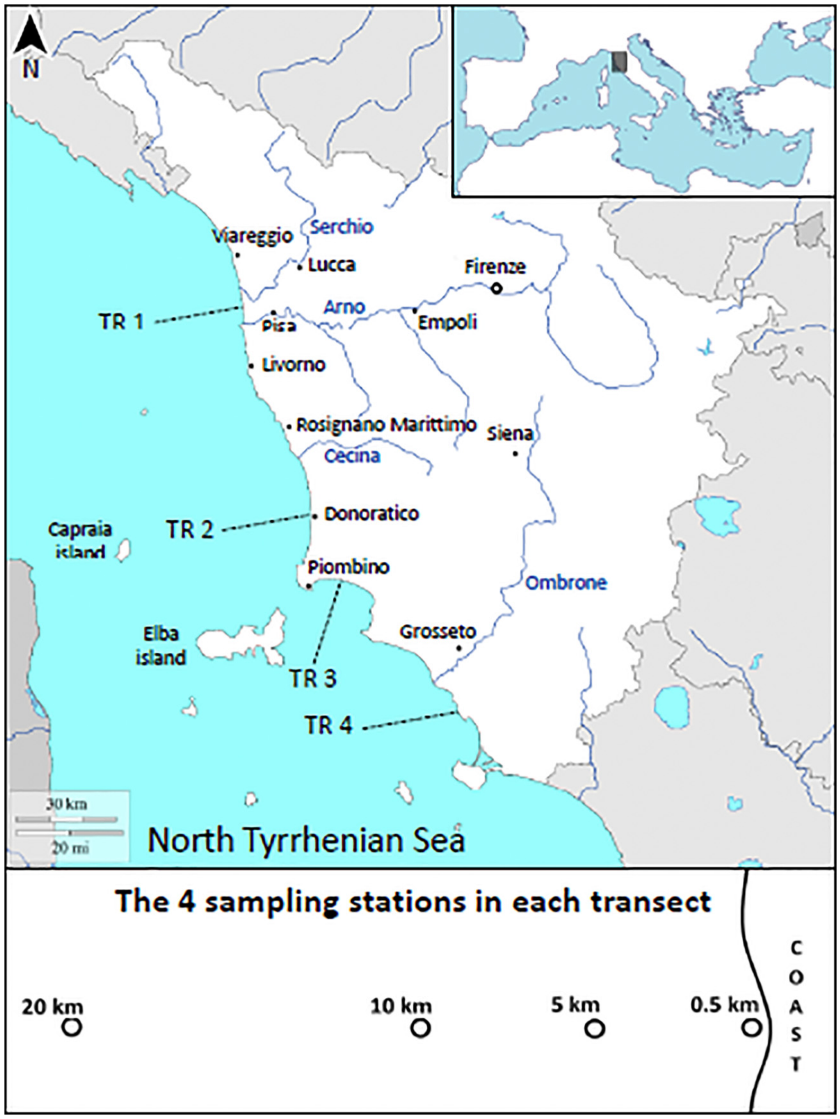


Fig. 1. Map of the study area along the Tuscany coast, in blue the rivers and in black the towns. Sampling plan with four different transects, each one with four stations located at increasing distance from the coast (0.5 km, 5 km, 10 km, 20 km). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

basins and oceans, but the issue about the total mass based on the size of the particles, based both on field and model observation, is still debated (Cózar et al., 2015; van Sebille et al., 2015).

Large plastic items and microplastics distribution vary across the Mediterranean Sea, depending on surface circulation and transient and seasonal formation of fronts, eddies and accumulation areas (Zambianchi et al., 2017). The surface flow temporal variability might play a role in driving marine litter accumulation pattern, as well as the costal kinematics and features influence the distribution of marine litter in the 10–50 km near-shore waters (Liubartseva et al., 2018; Ourmieres et al., 2018). A general tendency of floating litter to accumulate in the southern portion of the basin, and, in particular, a long-term accumulation in the southern and south-eastern Levantine basin, has been observed (Zambianchi et al., 2017).

Permanent structures which retain floating items have not been yet identified since the circulation variability in the basin alters their stable spatial distribution, but these oceanographic features led to consider the entire basin as a great accumulation region of microplastics with concentrations comparable to those found in of the great Subtropical Gyres (Cózar et al., 2015).

Other factors influencing the accumulation of plastic and microplastics items can be related to the distance from input sources (e.g. rivers, waste water treatment plants, urban sewage) as well as the distance to the coast (Pedrotti et al., 2016; Ryan et al., 2016).

The area investigated is characterized by a variety of anthropogenic pressures. On the northern area, there are two main possible pollution sources. The Leghorn commercial, touristic and maritime port is one of the largest Italian ports with 30 million tonnes of cargo and 600,000 twenty-foot equivalent unit and 2 million of people and the mouth of the Arno river, one of the largest Italian river, which during its 240 km of length cross several cities, agricultural sites and industrial area (Cincinelli et al., 2001; Cortecci et al., 2002). Some minor inputs can derive for the Piombino harbour and minor rivers (e.g. Ombrone) and city facing the Tyrrhenian sea (Guerranti et al., 2017; Guidi et al., 2018). Along all the Tuscany costs, maritime traffic for the Tuscan Archipelago islands and the presence of several touristic location makes the area highly anthropized and prone to the plastic and microplastic accumulation.

As underlined by the Barcelona Convention within the Regional Plan for Marine Litter (Barcelona Convention, 2013) "Marine pollution knows no border, pollution in one country affects all other 21 countries"; hence a regional approach is strictly needed. This awareness has resulted in a number of formal and informal initiatives at global, regional (e.g. OSPAR, UNEP Regional Seas Programme), European (e.g. MSFD), national and local levels. The Marine Strategy Framework Directive 2008/56/EC is a European Union directive committing EU Member States to take action aimed at achieving the "Good Environmental status" (GES) of the EU's marine environment by 2020, according to 11 key descriptors of environmental status. Among these, the descriptor 10 focuses the attention on the emerging problem of marine litter and its effects on the marine environment and biota (MSFD Technical Subgroup on Marine Litter et al., 2013). To this end the present study aims to provide background information on the occurrence of floating microplastics in Italian coastal waters, as part of the implementation data of the Marine Strategy Framework Directive requested by descriptor of 10.1.3 "Trends in the amount, distribution and, where possible, composition of micro-particles (in particular microplastics)". The relative abundance, the spatial distribution of microplastic particles along the Tuscany coast (Italy) and their polymeric characterization have been evaluated applying a standardized monitoring protocol to assess variations over space, time and vertical distribution.

2. Methods

The Tuscany Region, in collaboration with ARPAT (Agenzia Regionale per la Protezione Ambientale della Toscana) and the University of Siena, has developed a sampling plan, carried out between November 2013 and May 2014, identifying 4 transects, 100 km far from each other along the Tuscan coast (North Western Mediterranean Sea), to respond to the requirements indicated by the ministerial protocol within the MSFD monitoring program (MSFD Technical Subgroup on Marine Litter et al., 2013). Superficial and water column samplings were realized on board the oceanographic vessel "Poseidon" in two different sampling seasons, winter (November–December 2013) and spring (April–May 2014). The winter campaign was conducted only in two transects (TR 1 and TR 3) due to adverse weather and sea conditions. Each transect has been divided into four stations located at increasing distance from the coast (0.5 km, 5 km, 10 km, 20 km) and sampled one time per season (Fig. 1). A total of 24 surface tows and 24 vertical tows were carried out for quantitative and qualitative analysis of microplastics.

2.1. Water surface and water column sampling

Surface samples were collected using a manta trawl (330 μm mesh size, 25 × 50 cm mouth opening) towed on the water surface at 2–3 knots for 20 min, kept at a distance of about 70 m from the boat to avoid the turbulence induced by the wake of the ship. Vertical hauls with a WP2 standard ring net (200 μm mesh size, 57 cm mouth diameter) were performed along the entire water column depending on the bathymetry up to a maximum of 100 m when this depth was exceeded. At the end of samplings, the nets were rinsed thoroughly from the outside to ensure that both plankton and debris were washed into the end of the net and to prevent any contamination by rinsing water. To quantify the water filtered the nets were equipped with a flowmeter.

2.2. Prevention of external contamination

To prevent contamination throughout the analysis process, all the materials used for sample collection, including the nets, were accurately cleaned and rinsed before any tow. During the laboratory procedures glassware was used and particular care was taken to prevent airborne contamination by performing sample analysis in a clean air flow cabinet and using two glass petri dishes placed at each side of the stereomicroscope as blank control.

Despite the adoption of contamination control procedures, fibres and paint were not considered due to the risk of external contamination during sampling.

2.3. Microplastics count and characterization

For plastic particle analysis, samples were observed under a stereomicroscope Stereo Zoom NBS (mod. NBS-STMDLX-T) with a LED light and measured with micrometer ocular lens. Plastic particles were characterized and classified by colour (white, black, red, blue, transparent, green, other colour), size (≤0.5, 0.5–1.0, 1–2.5, 2.5–5.0 mm) and shape (spherical, filament, fragment, sheet) according to the MSFD guidelines. The items where dried at room temperature and weighted in OHAUS Explorer analytical balance (device error +/− 0.05 g).

The WP2 data were normalized to the total water volume filtered (V), calculated from the following formula and expressed as items/m^3:

$$V = N \times A \times C$$

N is the number of propeller revolutions measured by the flowmeter;
A is the mouth area of the net;
C is a constant value, typical of each flowmeter, and supplied by the manufacturer.

The manta trawl data were normalized to the total water surface filtered (S), calculated from the following formula, and expressed as items/km^2 and g/km^2:

$$S = N \times D \times C$$

D is the horizontal opening of the net.

All data obtained were corrected according to weather and sea conditions considering the possible "wind stress" effect as described by Kukulka et al. (2012). The wind speed was retrieved from an anemometer placed on the vessel at 10 m above the water surface and the wave eight by visual observation. The average correction factor over all collected samples was equal to 1.07 and the max value was 1.5. This could be due to the very soft wind conditions which are measured in the Mediterranean Sea during the sampling period and because the sampling was performed only in calm sea and good weather to avoid any misinterpretation of the data. The polymer identification was performed on a sub-sample of the total particles (20%) isolated in water surface samples. The particles were selected among all the microplastics isolated taking into account the different percentage of abundance of each category (size, colour and shape). The polymer fingerprint was detected by means of the Fourier Transform Infrared (FT-IR) spectroscopy technique using an Agilent Cary 630 FTIR spectrophotometer (Agilent Technologies) and the Agilent MicroLab FTIR software was used for the output spectra elaboration adopting the method proposed by Fossi et al. (2017).

2.4. Statistical analysis

Data were analysed by multivariate analysis techniques. In particular principal component analysis (Johnson and Wichern, 2015) and the correspondence analysis (Benzécri, 1973) were applied to the whole water surface microplastic dataset which includes: abundance, size, shape, distance to the coast and season. The significance of the analysis was tested using the Montecarlo test with 999 permutations. One-way ANOVA test was applied to test the existence of a distance to the coast effect. All statistical analyses were performed using the R software version 3.1.2 (R. Core. Team, 2013) and Ade4 package (Dray and Dufour, 2007).

3. Results and discussion

3.1. Bibliographic work of abundance of microplastic in the Mediterranean Sea

A comparison of the existing data on the abundance of floating

Table 1
Bibliographic data on microplastic abundance in Mediterranean surface waters.

Mediterranean sub-region	N° samples	Net mesh	Sampling nets	Year of sampling	Abundance	Abundance units	References
Western Mediterranean Sea	24	330 µm	Manta trawl	2013–2014	69,161 ± 83,244 0.26 ± 0.33	Items/km^2 ± SD Items/m^3 ± SD	Present study
Western Mediterranean Sea	21	330 µm	High speed manta trawl	2014	82,000 ± 79,000	Items/km^2 ± SD	(Fossi et al., 2017)
Western Mediterranean Sea	6	780 µm	Manta trawl	2014	112,000	Items/km^2	(Schmidt et al., 2018)
Aegean-Levantine Sea	17	330 µm	Manta trawl	2015	140,418 ± 120,671	Items/km^2 ± SD	(Güven et al., 2017)
Aegean-Levantine Sea	108	333 µm	Manta trawl	2013–2015	7.68 ± 2.38	Items/m^3	(van der Hal et al., 2017)
Western Mediterranean Sea	33	200 µm	Neuston net	2013	125,930 ± 132,485	Items/km^2 ± SD	(Pedrotti et al., 2016)
Western Mediterranean Sea and Adriatic Sea	74	200 µm	Neuston net	2013	1.00 ± 1.84	Items/m^3	(Suaria et al., 2016)
Whole Mediterranean	71	333 µm	Manta trawl	2011	62,000	Items/km^2	(Ruiz-Orejón et al., 2016)
Adriatic Sea	17	300 µm	Neuston net	2014	472,000 ± 201,000	Items/km^2 ± SD	(Gajšt et al., 2016)
Western Mediterranean Sea	70	200 µm	WP2	2012	0.31 ± 1.17	Items/m^3 ± SD	(Fossi et al., 2016)
Aegean-Levantine Sea	7	333 µm	Manta trawl	2016	37,600	Items/m^2 ± SD	(Gündoğdu and Çevik, 2017)
Western Mediterranean Sea	27	200 µm	WP2	2012–2013	0.17 ± 0.32	Items/m^3 ± SD	(Panti et al., 2015)
Whole Mediterranean	39	200 µm	Neuston net	2013	243,853	Items/km^2	(Cózar et al., 2015)
Western Mediterranean Sea	41	330 µm	Manta trawl	2012	129,682	Items/km^2	(Faure et al., 2015)
Western Mediterranean Sea	38	200 µm	WP2	2011–2012	115,000	Items/km^2	(Collignon et al., 2014)
Western Mediterranean Sea	30	500 µm	Manta trawl	2013	0.15 ± 0.11	Items/m^3	(de Lucia et al., 2014)
Western Mediterranean Sea	23	200 µm	WP2	2011	0.62 ± 2.00	Items/m^3 ± SD	Fossi et al. (2012)
Western Mediterranean Sea	40	333 µm	Manta trawl	2010	116,000	Items/km^2	(Collignon et al., 2012)

microplastics in the Mediterranean Sea has been carried out to compare the data obtained in the present study (Table 1). The results obtained in this work are in line with the heterogeneity of data present in literature, not only considering the same Mediterranean sub-area, but also at basin scale (Table 1).

Although, floating microplastic comparisons among different studies must not be performed without taking into account the different bias and variables that can affect the abundance and characteristics of the particles sampled such as the type and the mesh size of nets, the protocol used and the number of the samples considered. Bearing this in mind, one of the main aim of the present work is to apply, implement and, possibly, standardize the protocols available at European level to obtain robust data within the monitoring programme of the European MSFD. This approach, which is also one of the main aim of the MSFD, could allow to more easily compare data gained from all countries for all the European body of waters.

Beyond the methodological approach, also oceanographic ocean circulation, the weather condition and different sources of litter from in-land and coastal activities can affect the homogeneity of the data on the abundance of microplastics obtained in a certain area.

In the Mediterranean waters, the formation of transitory open sea aggregation zones identified also by circulation models edited by Fossi et al. (2017) and Mansui et al. (2015), can be caused by the periodic strong wind stress associated with the complex local circulation patterns that interest this area. These temporary zones where floating marine debris have a tendency to aggregate could influence the collected data resulting in different values of plastic particles abundance.

3.2. Microplastics in water column samples

Turbulence in the upper-sea layer can vertically mix buoyant plastic particles, affecting the standardized surface net sampling. In order to evaluate the seasonal occurrence of microplastic along the water column in winter and spring, a total of 24 samples have been collected. Plastic debris was present in 37% out of total water column samples, for a total of 21 items isolated (Table 2). A different abundance was observed between the seasons, only one microplastic was found in the water column samples collected during the winter season contrary to what described in the Baltic Sea by Bagaev and coworkers (2018). The numerical plastic concentration have been demonstrated to decrease exponentially within the first 5 m below the air-seawater interface (Reisser et al., 2015). Fragments were the most abundant type (62%) followed by filaments (29%); the highest abundance appeared in size range < 1 mm (52%), confirming the distribution found by Reisser et al. (2015), which states that the smaller plastics are more susceptible to vertical transport. Microplastic concentrations have been observed to decrease with depth, and depending on sea state and particle characteristics (Kooi et al., 2016). Also, different vertical transport mechanisms should be considered to explain any sinking, due to ingestion-egestion (Cózar et al., 2014), marine snow (Long et al., 2015) and/or biofouling (Fazey and Ryan, 2016) or mixing mechanisms (Kukulka et al., 2012; van Sebille et al., 2015).

A total characterization by shape, dimension and colour are presented in the Supplementary material (Fig. SM1).

3.3. Microplastics in water surface samples

A total of 1586 microplastics was isolated from all 24 superficial samples, with a minimum of 2 and a maximum of 403 (maximum abundance: 347039.8 items/km^2) total particles per sample and an average concentration of 41.1 ± 68.6 g/km^2 (average ± S.D.) and 69,161.3 ± 83,243.9 items/km^2 (Table 3), corresponding to 0.16 ± 0.26 mg/m^3 and 0.27 ± 0.33 items/m^3 (for the normalization of all the values to m^3, see Table SM1).

A Principal Component Analysis (PCA) has been performed as an exploratory data analysis to investigate whether sampling factors (season, distance to the coast and sampling area) could influence the abundance and characteristic of microplastics (shape, size, number of items/km^2 and weight in g/km^2). The analysis shows that sampling area (transect) and season seem to not affect microplastic abundance; however an increasing trend of microplastic concentration from coast to distant waters was pointed out (see Supplementary Material, Fig. SM3). The one-way ANOVA test confirmed that the distance to the coast statistically significant influence the abundance of microplastics in terms of items/km^2 ($F(3,20) = 2.96$ $p = 0.05$).

In a previous study, surface samples showed high concentrations of plastic debris in the 1-km water strip adjacent to the coast, low concentrations in waters between 1 and 10 km from the coast, and again high values towards distant waters 10–100 km (Pedrotti et al., 2016). In the present study, the highest values were found at 20 km (average ± S.D.; 128,034.2 ± 119,597.6 items/km^2) and 10 km (97,456.2 ± 79,935.4 items/km^2) and decrease in the stations closer to the coast (Fig. 2).

This trend may be related to the hydrodynamic of the area where

Table 2
Microplastic number and abundance (items/m^3) in water column samples collected during two seasons (winter and spring) in the four stations for each transect; mean values ± s.d per each transect, season and total.

Winter campaign							
Transect	Dist. coast	Longitude	Latitude	Bathymetry (m)	m^3 filtered	No. items	Items/m^3
TR 1	20 km	43°44.321	10°03.795	44.3	11.1	0	0
	10 km	43°44.058	10°10.102	15.1	3.8	1	0.26
	5 km	43°43.896	10°14.274	8.6	2.2	0	0
	0.5 km	43°43.898	10°16.200	2.3	0.6	0	0
Mean value							**0.07 ± 0.13**
TR 3	20 km	42°46.808	10°34.957	80	20	0	0
	10 km	42°51.698	10°38.421	36	9	0	0
	5 km	42°54.274	10°39.668	26	6.5	0	0
	0.5 km	42°56.621	10°40.846	2	0.5	0	0
Mean value							**0**
Winter mean value							**0.03 ± 0.09**
Spring campaign							
Transect	Dist. Coast	Longitude	Latitude	Bathymetry (m)	m^3 filtered	No. items	Items/m^3
TR 1	20 km	43°44.086	10°09.978	50	12.5	0	0
	10 km	43°44.058	10°10.102	16.8	4.2	2	0.48
	5 km	43°43.896	10°14.274	11	2.8	0	0
	0.5 km	43°43.898	10°16.200	3.5	0.9	0	0
Mean value							**0.11 ± 0.24**
TR 2	20 km	44°09.951	10°17.342	110	27.5	2	0.07
	10 km	43°09.680	10°24.565	71	17.8	1	0.06
	5 km	43°09.767	10°28.242	35	8.8	0	0
	0.5 km	43°09.807	10°31.813	2	0.5	0	0
Mean value							**0.03 ± 0.03**
TR 3	20 km	42°46.995	10°34.819	77.8	19.5	5	0.26
	10 km	42°51.748	10°38.365	35.2	8.8	1	0.11
	5 km	42°54.329	10°39.557	23.6	5.9	0	0
	0.5 km	42°56.633	10°40.833	2	0.5	0	0
Mean value							**0.09 ± 0.12**
TR 4	20 km	42°31.038	10°53.073	120	30.0	1	0.03
	10 km	42°34.659	10°58.436	86	21.5	0	0
	5 km	42°36.282	11°01.137	55.2	13.8	3	0.22
	0.5 km	42°38.049	11°03.861	3.5	0.9	2	2.29
Mean value							**0.63 ± 1.11**
Spring mean value							**0.22 ± 0.57**
Total mean value							**0.16 ± 0.47**

surface convergence, boundary currents and local near-shore circulation features (Vetrano et al., 2010) could influence the accumulation of floating plastics as previously observed in the Pelagos Sanctuary (Fossi et al., 2017) and in the French Riviera (Ourmieres et al., 2018).

Analysis of the size distribution of microplastics in surface samples showed that the most abundant size class is 1–2.5 mm both in the whole dataset (n=742, 47%) and at different distances to the coast (Fig. 3). This finding suggests a potentially higher hazard for zooplanktivorous species, from mesopelagic fish to top predators, that may accidentally ingest microplastics mistaken for natural prey since this is the same size of most zooplankton organisms (Besseling et al., 2015; Collard et al., 2015; Fossi et al., 2017, 2012; Lusher et al., 2015; Rochman et al., 2015).

The other size classes, 0.5–1 mm and 2.5–5 mm, accounted for a comparable number of particles (n = 375 and n = 412, respectively). The items with sizes below 0.5 mm represented the minor portion of the total microplastics (n = 57) (Fig. 3). This dimensional pattern has also been previously observed in studies conducted in the Mediterranean Sea and in other ocean basins.

Plastic fragments (n = 1284, 81%) and sheets (n = 204, 13%) were found to be the majority of plastic items isolated (Fig. 3), suggesting that the possible fragmentation of large plastic manufactured objects could be the main source of microplastics, as also hypothesized by Isobe et al. (2014).

Polyethylene (PE) was the most abundant (> 66%), followed by polypropylene (PP) (28%), polystyrene (PS) (5%), Ethylene-vinyl acetate (EVA) and Styrene butadiene (SBR) (1%) (Fig. SM2). Being PE and PP positively buoyant polymers with low specific weight (0.89–0.95 g/cm^3 and 0.85–0.92 g/cm^3, respectively), and the most abundant in the marine environment worldwide, it was expected that these polymers consistently account for the majority of the plastic particles floating not only in Mediterranean sea but in surface waters worldwide (Eriksen et al., 2014; Hidalgo-Ruz et al., 2012; Reisser et al., 2015; Suaria et al., 2016). Comparing the dimension of the different polymer detected, microplastic dimensions increases with the PE percentage and decrease with the PP percentage, suggesting higher rate of fragmentation of PP compared to PE. Fragments and sheets are equally distributed between PP and PE, filaments are mainly composed by PP whereas the spherical items show the highest percentage of polystyrene (PS) (Fig. 4).

3.3.1. Seasonal variation

During the winter campaign, sampling activities were carried out only in TR1 and TR3. A total of 610 microplastic was sorted by 8 water surface samples. The results show a similar trend for both transect analysed, with the majority of plastic items found in the off-shore sampling sites (> 10 km). Fragments and sheets are the most common shapes (94% of the total) while the size classes between 1 and 2.5 mm and 2.5–5 mm are the most abundant. The 20 km away stations are the ones with the largest number of microplastics collected with a decreasing gradient with the proximity to the coast, with values respectively ranging from 64,096 to 3158 items/km^2 for TR 3 and from 347,040 to 10,645 items/km^2 for TR 1 (Table 3).

The spring campaign was carried out in all selected transects. A total of 976 plastic particles was isolated from the 16 surface samples. The result showed the same characterization for the plastic particles

Table 3
Microplastic number, abundance (items/km^2) and weight (g/km^2) in water surface samples collected during two seasons (winter and spring) in the four stations for each transect; mean values ± s.d. per each transect, season and total.

Winter campaign						
Transect	Dist. coast	Longitude	Latitude	No. items	Items/km^2	Weight (g/km^2)
TR 1	20 km	43°44.321	10°03.795	403	347,039.8	262.4
	10 km	43°44.058	10°10.102	109	102,837.2	43.9
	5 km	43°43.896	10°14.274	12	10,645.4	8.8
	0.5 km	43°43.898	10°16.200	17	20,936.0	14.0
Mean value				**135 ± 184**	**120,364.6 ± 156,645.3**	**82.3 ± 121.1**
TR 3	20 km	42°46.808	10°34.957	51	64,095.5	90.3
	10 km	42°51.698	10°38.421	12	18,490.1	3.8
	5 km	42°54.274	10°39.668	5	8490.8	1.4
	0.5 km	42°56.621	10°40.846	2	3157.7	0.9
Mean value				**18 ± 23**	**23,558.5 ± 27,761.9**	**24.1 ± 44.1**
Winter mean value				**76 ± 137**	**71,961.6 ± 116,292.9**	**53.2 ± 89.9**
Spring campaign						
Transect	Dist. Coast	Longitude	Latitude	No. items	Items/km^2	Weight (g/km^2)
TR 1	20 km	43°44.086	10°09.978	194	33,726.0	26.1
	10 km	43°44.058	10°10.102	188	43,501.9	14.9
	5 km	43°43.896	10°14.274	18	13,928.6	21.9
	0.5 km	43°43.898	10°16.200	8	59,730.3	9.4
Mean value				**39 ± 20**	**37,721.7 ± 19,147.4**	**18.1 ± 7.4**
TR 2	20 km	44°09.951	10°17.342	34	186,673.1	27.9
	10 km	43°09.680	10°24.565	44	228,820.6	240.6
	5 km	43°09.767	10°28.242	14	25,000.0	60.7
	0.5 km	43°09.807	10°31.813	62	13,430.5	1.5
Mean value				**102 ± 103**	**113,481.0 ± 110,301.5**	**82.7 ± 108.1**
TR 3	20 km	42°46.995	10°34.819	67	70,055.8	44.4
	10 km	42°51.748	10°38.365	40	43,061.6	11.9
	5 km	42°54.329	10°39.557	44	51,490.6	10.8
	0.5 km	42°56.633	10°40.833	4	4019.6	1.1
Mean value				**38 ± 26**	**42,156.9 ± 27,813.3**	**17.0 ± 18.9**
TR 4	20 km	42°31.038	10°53.073	46	66,615.0	10.9
	10 km	42°34.659	10°58.436	146	148,025.6	55.2
	5 km	42°36.282	11°01.137	63	85,996.3	20.5
	0.5 km	42°38.049	11°03.861	7	10,104.4	3.7
Mean value				**65 ± 58**	**77,685.3 ± 56,881.6**	**22.6 ± 22.8**
Spring mean value				**61 ± 61**	**67,761.2 ± 65,632.0**	**35.1 ± 57.7**
Total mean value				**66 ± 90**	**69,161.3 ± 83,243.9**	**41.1 ± 68.6**

analysed in each transect. The most abundant size class is the one comprised between 1.01 and 2.5 mm and the predominant shape is the fragment. The high presence of plastic items (76%) was found in the off shore waters between 10 and 20 km from the coast for all the transect, excepted for TR 1 where the major plastic concentration, 59,730 items/km^2, was founded in the water strip adjacent to coast (0.5 km)

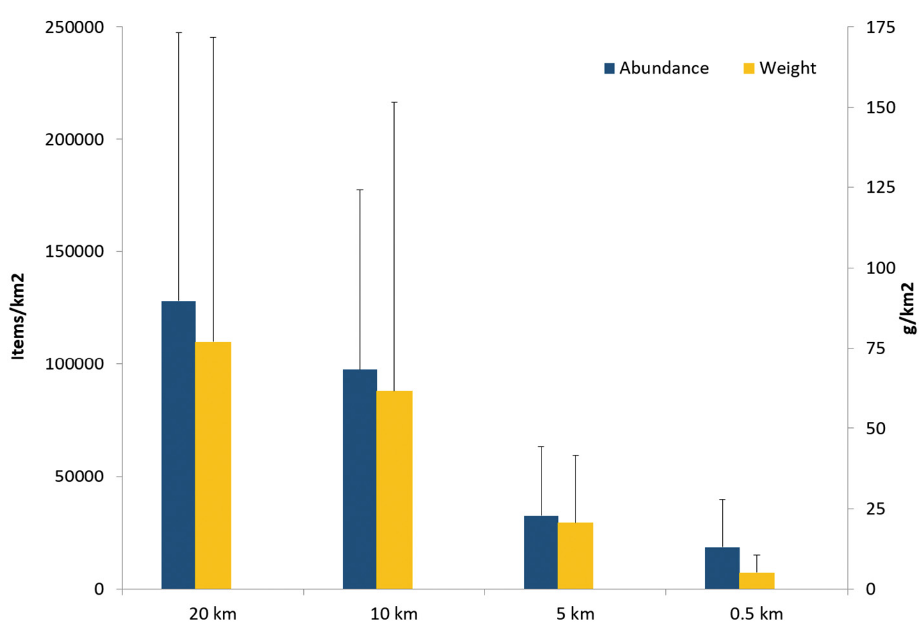


Fig. 2. Average abundance ± S.D. (items/km^2) and weight (g/km^2) of microplastic isolated in water surface samples (n=24) in the four sampling stations (20 km, 10 km, 5 km, 0.5 km).

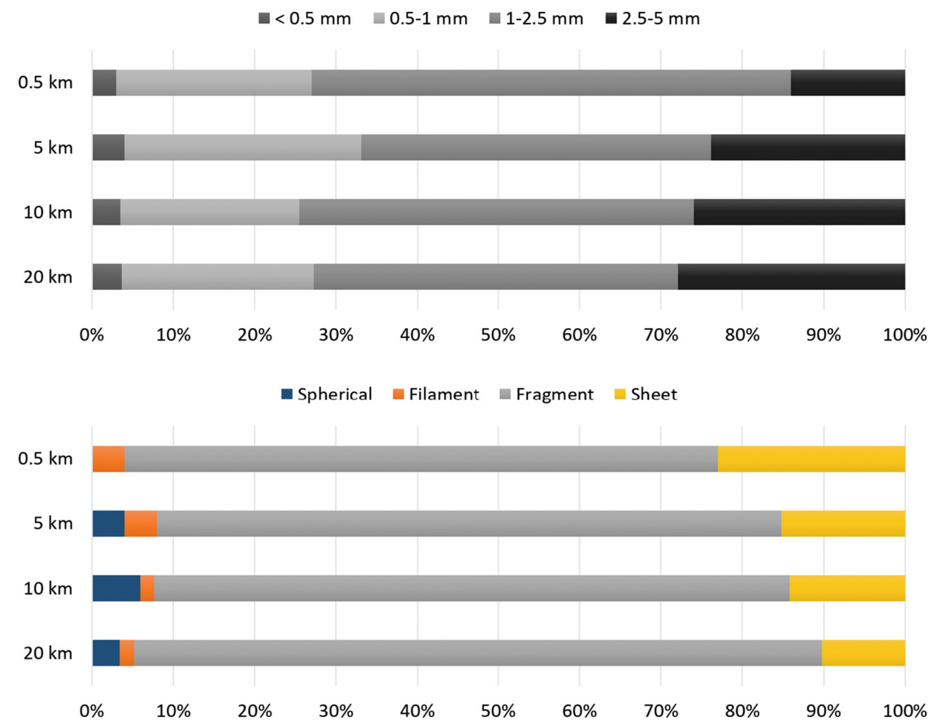


Fig. 3. Average abundance of microplastic isolated in water surface samples (n = 24) in the four sampling stations (20 km, 10 km, 5 km, 0.5 km) depending on the classes size (upper); plastic items shape categorization depending on the distance to the coast (lower).

suggesting a stronger terrestrial inputs of marine debris. The highest plastic concentration (228,821 items/km^2) has been found in TR 2, an area located near the Capraia gyre, described by Fossi et al. (2017) as a potential site where floating debris may accumulate temporarily and confirmed also by Suaria et al. (2016).

Only for the transects TR 1 and TR 3 have been possible to outline a possible seasonal variation in microplastic abundance. In the two transects opposite scenarios have been observed: while for TR 1 the highest values of microplastics collected was registered in winter for TR 3 the highest abundance of microplastics in all four sampling stations was registered in spring (Fig. 5). Seasonal variations in plastic abundance have already been investigated in Mediterranean Sea by Collignon et al. (2014), where very low concentrations were showed during the two seasons here considered. Overall, the spatial heterogeneity found in this study could be probably due to multiple factors influencing the plastic particles distribution in Mediterranean Sea. The presence of anticyclonic gyres, isolated eddies and the combined effect induced by wind and water currents may create unstable plastic retention areas affecting the sampling activities. In addition, the high distribution of coastal populations and land-based pollution scattered sources (e.g. Arno River, harbours, industrial sites, etc) could also contribute to the variability observed in the two sampling campaigns.

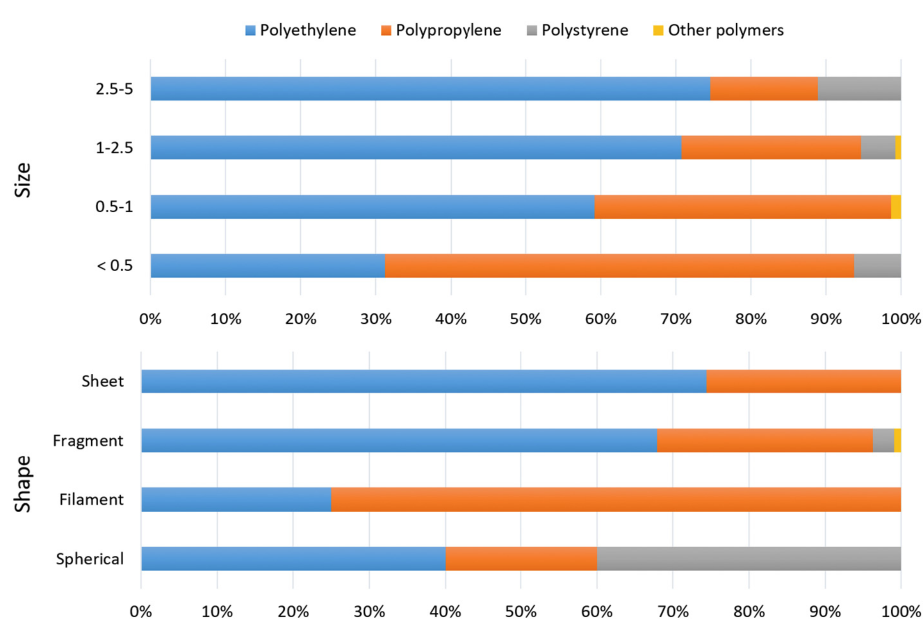
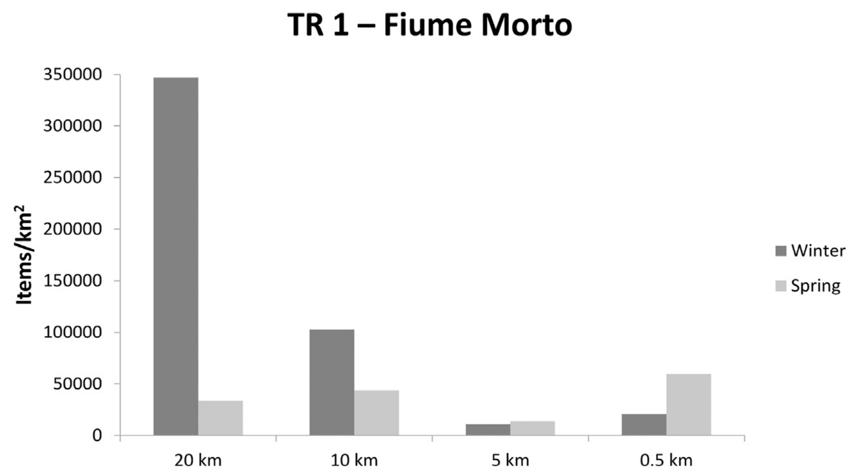


Fig. 4. Plastic polymers % of microplastic isolated in water surface samples in relation to the size (upper) and the shape (lower) of microplastic items analysed. Others polymers included EVA and SBR copolymers.



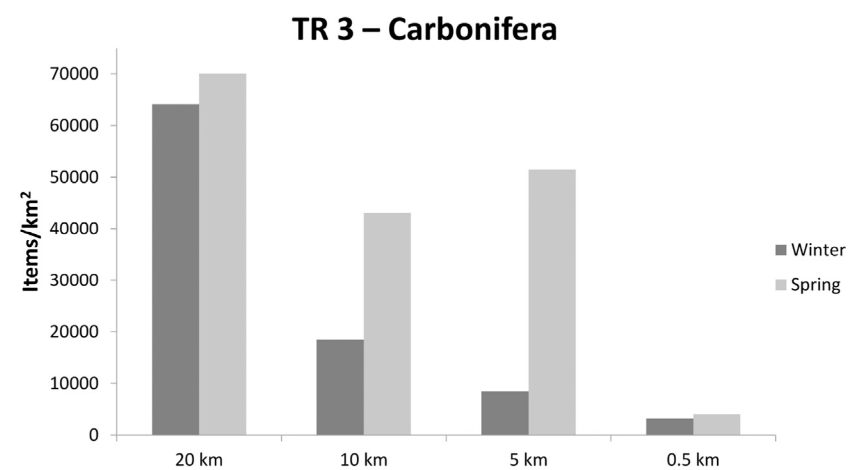


Fig. 5. Comparison of seasonal microplastic abundance isolated in surface water samples collected (items/km^2) in TR 1 (upper) and TR 3 (lower) transects in the four sampling stations.

Focusing the attention on in the TR1, it is important to notice the microplastic abundance trend observed during the spring with the stations at 0.5 km showing the highest value.

In this case, the riverine inputs may represent a significant source of plastic litter pollution for this sampling site, being located near the mouth of Fiume Morto stream and the estuary of Arno River, the longest river in Tuscany which passes through two densely populated cities, Florence and Pisa. A model edited by Lebreton et al. (2017) estimating the riverine plastic mass input to oceans and its seasonality, indicated how this could be one of the main inputs of marine debris presence in the Mediterranean Sea.

4. Conclusion

This paper reports the results of the monitoring program for the implementation of the MSFD in the Mediterranean Sea. These campaigns aimed at establish baseline levels for the future definition of the "Good Environmental Status" by 2020 for the Descriptor 10.1.3. These data represent an attempt to apply a harmonized protocol across Mediterranean European countries, in order to make the data on the quantification of floating microplastics comparable and reliable. Being the Mediterranean Sea an important basin within the European waters and due to its morphological and oceanographic features as a closed basin surrounded by densely populated countries generating important quantities of waste, the evaluation of the presence of floating plastic are mandatory to establish solid data and consequently apply mitigation measures. This study proposes detailed data concerning the present state of microplastic along the Tuscany coast, recognized by plastic dispersion model how a possible transitory retention area, due to the presence of mesoscale anticyclonic eddy structure. The adoption of a six-months monitoring plan, based on the identification of four fixed transect along the Tuscany coast has allowed to evaluate the occurrence of microplastics (including the characterization of plastic polymers) and its variations relating to the distance from land and to the seasonality, for a more comprehensive assessment of this threat. The results showed a higher abundance of items in the waters located at 10–20 km distance to the coast and a heterogeneous distribution between the two seasonal campaigns. At the regional scale, the terrestrial pollution and the riverine inputs seem to influence the presence of floating debris, also related to the scattered distribution of marine litter sources along the coast. In conclusion, in order to respond to MSFD requirements, this work underlines the importance to use a standardized sampling plan to better understand the plastic distribution and transport and identifying the possible microplastics sources to apply mitigation measures to achieve the "Good Environmental Status" of the Mediterranean Sea.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2018.06.016.

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