



Ubiquitous vertical distribution of microfibers within the upper epipelagic layer of the western Mediterranean Sea

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ABSTRACT

The abundance of microplastics (plastic particles of less than 5 mm) along the sea surface and in seafloor sediments have been extensively documented worldwide; however, little is known in terms of the vertical distribution of microplastics in the water column, especially in the epipelagic zone. Considering the biological importance of this area, the quantification of microplastics available here is essential to identify potential impacts for marine organisms. This study reports the vertical distribution of microplastic abundances throughout the water column in two Marine Strategy Framework Directive (MSFD) demarcations from the western Mediterranean Sea during July 2019. Three concatenated 5-L Niskin bottles were used for sampling at 5, 15 and 25 m from the sea surface in stations with a total depth smaller than 50 m and at 5, 25 and 50 m from the sea surface in stations with a total depth greater than 50 m. This study demonstrates the ubiquitous abundance of microfibers, 96% of the microplastic items identified in the upper epipelagic layer of the western Mediterranean Sea. Microplastics exhibit a heterogeneous vertical and horizontal spatial distribution. Fragments had a very low representation (4% of the items) but showed a similar frequency of occurrence along all sampling depths. In terms of size, 68% of the microplastics were less than 2 mm in length. Microplastics quantified within the study area were mainly composed of low-density polyethylene (LDPE) and polypropylene (PP) (20% each) followed by cellulose acetate (CA) (16%) and polystyrene (PS) (14%). Regarding the spatial distribution of microplastics, higher abundances were found at intermediate distances (5–10 km from the coast) with mean values of 2.41 ± 1.90 items L^{-1} and further away (>20 km) from the coast, with mean values of 2.11 ± 1.80 items L^{-1} . A slight decreasing trend in the abundances of microplastics from the sub-surface to deeper waters was also observed. Stations within MPAs waters showed no significant differences in microplastic abundances when compared to non-MPAs stations. Overall, the results of this study highlight the ubiquitous presence of microplastics, primarily microfibers, along the epipelagic layer of the Spanish Mediterranean continental shelf.

1. Introduction

Microplastics are defined as plastic pieces with a size range between 5 and 0.3 mm, and their ubiquitous presence in the marine environment is a reality. Of these items, microfibers, considered primarily of natural or synthetic fibers (e.g., cotton, nylon, polyester) have a length falling in the size range described above (Brander et al., 2020) and their presence in marine ecosystems is increasingly being reported in studies assessing environmental pollution (Barrows et al., 2018). The abundance of microplastics reported throughout the seas and oceans indicate its heterogeneous horizontal (Cózar et al., 2014) and vertical (Bagaev et al., 2017; Dai et al., 2018) distribution. Its presence in the marine

environment is attributed to continuous releases from land-based sources including cities (Jambeck, 2015), sewage (Kazour et al., 2019) and rivers (Guerranti et al., 2020) and even from the atmosphere (Dris et al., 2016; Gasperi et al., 2018). In addition, the spatial distribution of microplastics in the marine environment is subjected to several factors such as oceanographic processes (currents, waves, etc.) (Wang et al., 2016), physicochemical properties of the water mass (salinity and temperature) (Kowalski et al., 2016), distance to source areas (Jambeck et al., 2015) and exposure to sources and origin of plastics (Soto-Navarro et al., 2020). On the other hand, density (Dai et al., 2018) and shape (Kowalski et al., 2016) of plastic polymers have been highlighted as determining factors defining the behavior of microplastic particle distribution within the water column.

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Polymer abbreviations

Acrylonitrile butadiene styrene ABS
 Butadiene rubber NBR
 Cellulose acetate CA
 High-density polyethylene HDPE
 Low-density polyethylene LDPE
 Polycaprolactone PCL
 Polycarbonate PC
 Polyester Polyester
 Polyethylene PE
 Polyethylene terephthalate PET
 Polyoxymethylene POM
 Polystyrene PS
 Polytetrafluoroethylene PTFE
 Polypropylene PP
 Styrene-acrylonitrile SAN

Numerical simulations estimate that every year up to 9.4 million tons of floating marine plastic debris sink to seafloor areas (Koelmans et al., 2017), indicating that plastic is transported through the water column settling in seafloor sediments. Microplastics within the water column become available to a wide range of species that live in this zone, especially those that perform vertical migrations, which are more exposed to this kind of pollution as they are at risk of encountering these particles along their entire home range. The ingestion of these particles has been widely reported by different fish species with a frequency of occurrence ranging from 0.3 to 77% (Compa et al., 2018; Rios-Fuster et al., 2019) which serves as a biological sink for these items (Kvale et al., 2020). There is evidence that microplastics could act as vectors of different pollutants (Rios-Fuster et al., 2021a) and they may cause physical (Wright et al., 2013), physiological (de Sá et al., 2018) and behavioral (Angiolillo and Fortibuoni, 2020; Rios-Fuster et al., 2021b) consequences for marine organisms. In this sense, knowledge of the vertical distribution of microplastics together with the polymeric characterization of these particles present in the water column will allow us to estimate the exposure of microplastic pollution to which marine organisms are exposed to.

Over the past decade, efforts to document, monitor and mitigate microplastic abundances in the marine environment have increased through the establishment of different governmental requirements such as the Marine Strategy Framework Directive (MSFD/2008/56/EC) (MSFD, 2013). Taking into account the increased reporting of litter abundances in the marine environment, marine debris corresponds specifically to Descriptor 10 of this directive which aims to define and reach a Good Environmental Status (GES) in European seas and oceans through the implementation of 11 descriptors. Descriptor 10 of the MSFD includes a specific indicator to study and quantify marine debris' trends in the water column (indicator 10.1.2). Furthermore, monitoring and identifying microplastics in the water column could improve the ability to predict their sources, fates and distribution (Dai et al., 2018) as well as providing empirical data to improve models focused on the generation of scientific knowledge that helps to understand the dynamics of debris in marine ecosystems (Soto-Navarro et al., 2020).

The western Mediterranean Sea is highly urbanized and populated, with high maritime traffic, touristic and industrial activities, large harbors and rivers that constitute important sources of marine debris (Guerranti et al., 2020). Within this area, some of the most important Marine Protected Areas (MPAs) of the Spanish Mediterranean Sea are found, such as the Delta of the Llobregat Natural Park, the Columbretes Islands and Cabo de Gata. In relation to the MSFD, this area comprises two of the five Spanish demarcations: the Levantine-Balearic (LEBA) and the Estrecho-Alboran (ESAL) demarcations. For this study, we

hypothesized that the abundance of microplastics in the water column is affected by the sampling depth and by the distance to the coast and that the distribution reported provides important data concerning the potential availability of microplastics for marine biota living in the upper epipelagic layer of this area. Consequently, the aims of the study are i) to analyze the latitudinal distribution of microplastics along the continental shelf of the Iberian Peninsula coast (Western Mediterranean Sea), ii) to determine the microplastic vertical distribution along the water column and iii) to study how vertical sampling depth and distance to the coast affect microplastic variability in this area.

2. Material and methods

2.1. Study area and on-board sampling

The study was carried out along the continental Iberian shelf of the Levantine and Alboran Seas in the western Mediterranean Sea (Fig. 1). A total of 129 samples from 43 stations were collected in July 2019 on board of the research vessel (R/V) Miguel Oliver (70 m length) during the scientific oceanographic survey: MEDiterranean International Acoustic Surveys (MEDIAS). These surveys are carried out annually with the aim of assessing the abundance and distribution of sardine (*Sardina pilchardus*) and anchovy (*Engraulis encrasicolus*) through acoustic techniques. The northern part of the study area is influenced by the Northern current (Monserrat et al., 2008) while the southern region is affected by the entrance of the Atlantic water mass from the Strait of Gibraltar (Vargas-Yáez et al., 2002). Both currents can affect the distribution of marine debris along the study area.

Samples were collected at 43 stations in an effort to obtain a spatial representation of the distribution of microplastics throughout the water column throughout the study area and at a latitudinal gradient. Stations were pre-established within transects of the MEDIAS surveys which are distributed 8 nautical miles apart from each other along the continental shelf (e.g. Delta del Ebro) and 4 nautical miles apart of each other in the narrow platform zones (e.g. Alboran Sea). Considering the bathymetric range of the study area, a distinction was made between stations classified as shallow stations, when the bathymetry was above 50 m depth, and as deep stations, when the bathymetry was below 50 m depth. At each station, water samples were collected at three different sampling depths with 5L Niskin bottles. Water samples were collected from the sub-surface (5 m from the surface) at all stations. At the shallow stations, samples were also collected at 15 m (mid waters) and 25 m (deep waters); while at the deep stations, samples were collected at 25 m (mid waters) and at 50 m (deep waters). At each of the stations, oceanographic variables for temperature and salinity were determined by means of a conductivity, temperature and depth (CTD) profile. In addition, wind intensity data was recorded *in situ* and classified according to the Beaufort scale. Furthermore, to assess the distance from the coast to the open sea, the stations were classified taking into account the following distances from the coast: < 5 km; 5–10 km; 10–20 km; and >20 km.

All sampling stations were classified according to whether they were located inside the MPA boundaries and following the MSFD criteria: Levantine-Balearic (LEBA) or Estrecho-Alboran (ESAL) demarcations.

2.2. Sampling and laboratory analysis

Once on board, samples were immediately filtered through glass microfiber filters (1.2 μ m pore size and 47 mm of diameter) using a vacuum pump. The filters were stored in Petri dishes at -20°C on board. Once in the laboratory, each filter was dried at room temperature inside the glass Petri dishes to remove any residual humidity from the samples prior to visual identification using a stereomicroscope (B&Crown model Ultralyt M-5100, 40x magnify). The identified items were measured and categorized according to color and type (fragments, fibers, film, rope and pellets) and length was measured and classified into 6 size classes

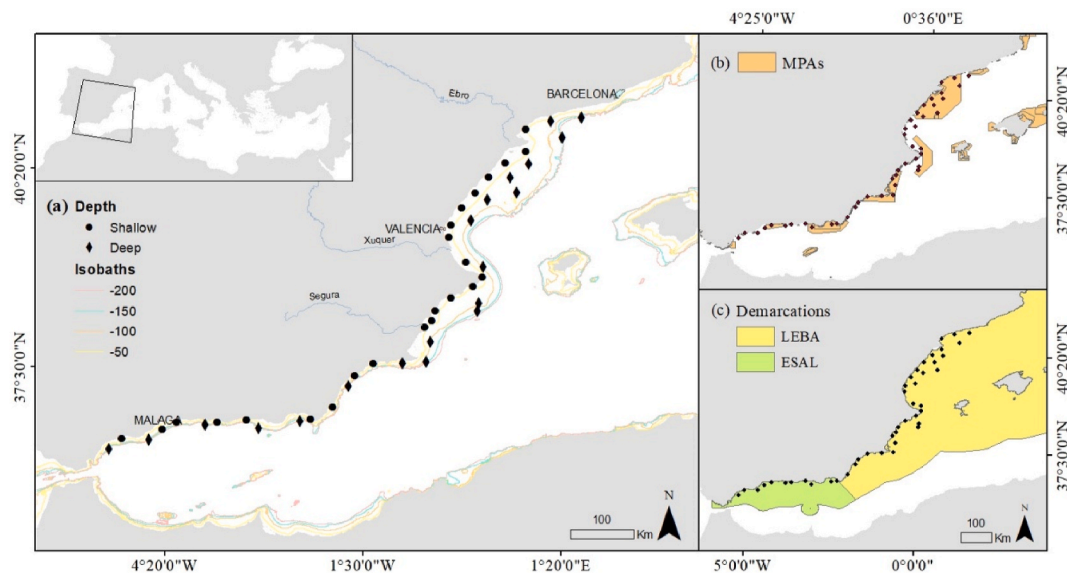


Fig. 1. Study area of the sampling locations in the Mediterranean Sea to assess abundances of microplastics in the water column along the Iberian Peninsula coast. Stations are classified according to a) the total depth regarding two types: < 50 m (circle) and > 50 m (rhombus); b) the allocation inside an MPA; c) the Mediterranean Spanish MSFD demarcations: Levantine-Balearic (LEBA) and Estrecho-Alboran (ESAL).

(<1, 1–2, 2–3, 3–4, 4–5, >5; mm).

Several measures were considered to minimize airborne contamination. On board, before sampling, all equipment was cleaned three times with filtered seawater through a 0.125 μm membrane. Although field blanks of the water sample collection at each depth was not feasible, strict measures were taken to minimize contamination. Standard non-plastic equipment e.g. metal and glass, was used whenever possible. In addition, air currents and exposure time of samples were reduced to a minimum during collection, processing and analyses. A total of 15 items were found in the laboratory blanks, resulting in a mean ($\pm\text{SD}$) 0.12 ± 0.45 items of contamination per sample, indicating that there was little aerial contamination during visual processing. Due to the small quantity of items, we did not make corrections to the item counts.

Furthermore, 30% of the samples from each station, depending whether they were shallow or deep stations, were randomly selected to be analyzed by micro-Fourier-transform infrared spectroscopy (FT-IR). For each sub-set of samples, 10% of the total items identified were further analyzed to determine the polymer type. Glass microfibers filters were directly analyzed using the ATR crystal unit of the Tensor 27 spectrometer (Bruker, Germany) coupled to the FT-IR microscope.

The wavenumber range of 400–4000 cm^{-1} was used for the measurements and 8 scans per item were performed. Each spectrum was compared with spectra from a customized polymer library integrating different databases (Löder et al., 2015, BASEMAN D1_2 FTIR reference database) and an in-house library generated with virgin and weathered reference polymers including various natural and synthetic materials. Only samples with a hit quality index >700 (max. 1000) were accepted as confirmed polymers. Spectra comparison was done with the Opus 6.5 software.

2.3. Statistical analyses

A generalized linear model (GLM) with a Gaussian distribution was performed to identify the main factors that affect the vertical and spatial distribution of the microplastic abundance along the Levantine and Alboran Sea areas from the coast of the Iberian Peninsula coast (western Mediterranean Sea). Physical and geographical variables were introduced into the model: distance from the coast and sampling depth as categorical variables, and wind speed, bathymetry, temperature and salinity as continuous variables. The best-fit model was selected

corresponding to the lowest AIC value (Akaike Information Criterion) by applying the stepAIC function from the MASS package (Brian et al., 2020). Normality was assessed upon inspection of the residuals of the model. Moreover, a Mann-Whitney-Wilcoxon test was performed to compare significant differences in microplastic abundance between stations inside or outside MPAs and between both Spanish Mediterranean demarcations of the MSFD.

Differences in abundances of polymer type between stations and sampling depths were analyzed separately using a Permutational multivariate analysis of variance (PERMANOVA). This analysis was done using the Bray-Curtis-based matrix of the abundances of plastic polymer categories. Prior to this, the abundance matrix was transformed with the square-root transformation. The experimental design incorporated three factors: ‘Total depth’ [fixed with two levels: shallow (<50 m) and deep stations (>50 m)], ‘Sampling depth’ (fixed with six levels: 5, 15 and 25 m for shallow stations and 5, 25 and 50 m for deep stations) and ‘Distance from the coast’ (fixed with four levels: < 5 km, 5–10 km, 10–20 km and >20 km). In addition, to compare the variability in polymer composition amongst depths and coastal distances, a permutation test for homogeneity of multivariate dispersions was performed using the betadisper function of the vegan package in R. Further, a post hoc analysis with a Tukey contrast method (TukeyHSD.betadisper) was performed to determine where the pairwise differences according to intragroup variability were given.

All statistical analyses were performed in R version 1.2.1335.

3. Results

A total of 1199 items of microplastics were found in 129 samples obtained with a 5-L Niskin bottle collected from 43 sampling stations along the Iberian Coast. Only one water sample located at 25 m from a shallow station from the northern Levantine area had no microplastics, indicating that 99.22% of the samples contained microplastics.

3.1. Spatial and vertical distribution of microplastic abundance

An overall abundance of 1.86 ± 1.43 items L^{-1} was found along the studied continental shelf of the Iberian Peninsula and the highest abundance (8.4 items L^{-1}) was observed on the northern Levantine coast at 50 m depth, specifically at an intermediate distance to the coast (5–10

km) (Fig. 2). Most deep stations located at >5 km from the coast showed almost twice as many items as shallow stations, while within the first 5 km of the coast, both shallow and deep stations had similar concentrations (Fig. 2). Regarding the spatial distribution, a decreasing trend in microplastic abundance was observed from north to south (Fig. 3). The shallow stations had an overall average of 1.61 ± 1.06 items L^{-1} , and the sub-surface samples (5m) had the highest abundance with an average of 1.78 ± 1.37 items L^{-1} (Table 1). Similar results were found for deep stations with an overall average of 2.14 ± 1.73 items L^{-1} and the sub-surface layer showing the highest concentrations with 2.42 ± 1.77 items L^{-1} (Table 1).

3.2. Microplastic abundance in MPAs and MSFD demarcations

Results from the Mann-Whitney-Wilcoxon tests showed no significant differences regarding microplastic abundances between stations located inside or outside MPAs (MW, $p > 0.05$). However, differences were detected between the MSFD demarcations (MW, $p < 0.05$) with the highest mean abundance values of microplastics along the Levantine-Balearic (LEBA) demarcation (2.00 ± 1.35 items L^{-1}) compared to the Estrecho-Alboran (ESAL) demarcation (1.46 ± 0.64 items L^{-1}) (Fig. 3).

3.3. Microplastics typology

According to identified microplastic typology, fibers were the most common type with 1154 identified items making up 96.1% of the total microplastics characterized, followed by fragments (43 items; 3.58%) and finally by films and ropes of which only one item of each category was found (0.08% in both cases) and in both cases smaller than 1 mm in size (Table 3; Fig. 4). No pellet items were found in the analyzed water samples. Regarding size, the smallest microplastic particle had a maximum length of 0.1 mm and was found in shallow waters (<50 m; Table 1). On the other hand, the largest microplastic particles were found in deep stations (>50 m) with maximum lengths ranging from 7 to 8.4 mm (Table 1). In terms of sizes classes (<1, 1–2, 2–3, 3–4, 4–5, >5;

mm), items smaller than 2 mm made up 67% of the identified items (818 items) and meso-debris items (larger than 5 mm) made up 3.59% of the identified items (44 items) (Fig. 4; Table 2).

Based on FT-IR analyses, a total of 162 items have been characterized and 17 different polymers have been identified. Low-density polyethylene (LDPE) and polypropylene (PP) were the two main polymers found with a total of 33 (20.4%) and 32 (19.8%) identified items respectively, followed by the semi-synthetic polymer cellulose acetate (CA; 26 items; 16.1%) and polystyrene (PS; 23 items; 14.2%). The rest of the identified polymers were observed in percentages lower than 10% and were represented by polyethylene terephthalate (PET), polycarbonate (PC) or polyoxymethylene (POM) among others. FT-IR analysis revealed a low misidentification rate during visual sorting, since less than 4% of all analyzed particles were reported as non-plastic particles of organic nature and composed of cotton (5 items; 3.1%) and hemp (1 item; 0.6%) (Table 3).

According to identified colors, the most prevalent were blue (441 items; 36.75%) and black (285 items; 23.75%), followed by total or partial transparent items (146 items; 12.17%), pink (140 items; 11.67%) and purple (78 items; 6.50%). The rest of the colors were observed in a lower incidence representing less than 5% of the observed colors.

3.4. Statistical model

In order to explain the spatial distribution of microplastics along the study area, results from the GLM model selection indicated that the best-fit model included the explanatory variables of distance from the coast, sampling depth and bathymetry (GLM, AIC = 458.76). Salinity, temperature and wind speed were not found to be significant contributing factors in the best-fit model. According to sampling depth, shallow stations showed statistically lower microplastic abundances than the deeper stations (GLM, $p < 0.05$; Table 4).

On the other hand, in the FT-IR analysis no differences were detected between stations and sampling depths in polymer type (PERMANOVA, $p > 0.05$) but statistical differences according to the homogeneity of the

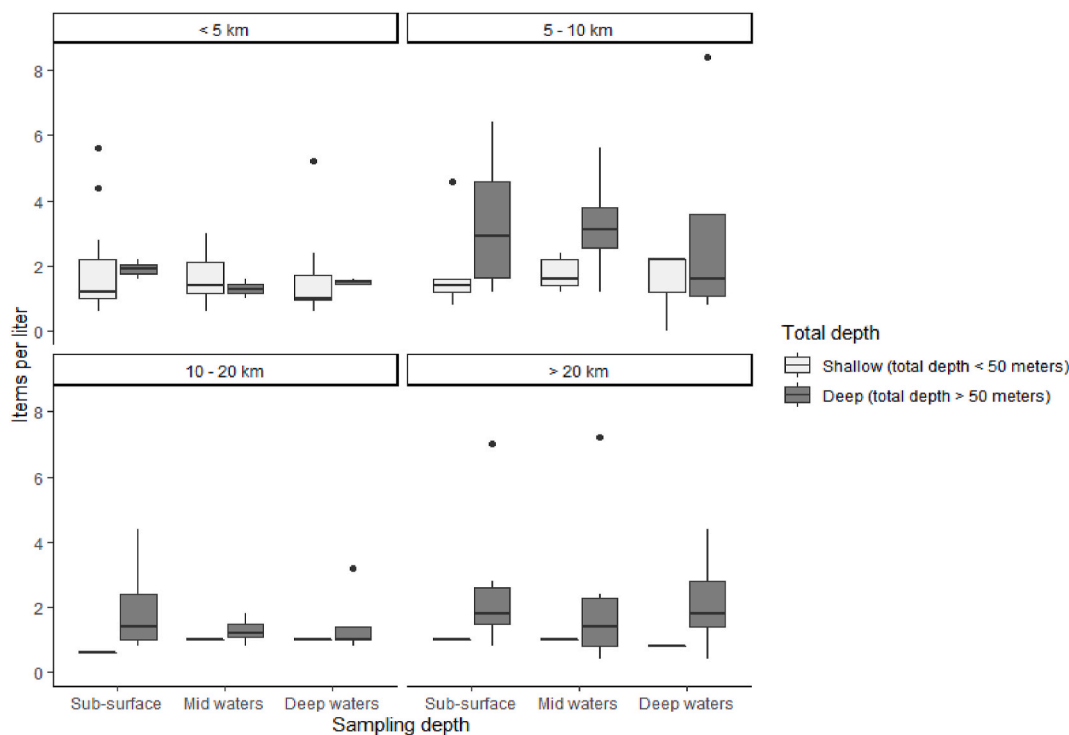


Fig. 2. Boxplot of microplastic items classified according to station (shallow and deep) and by sampling depth (sub-surface, mid and deep waters) and in terms of distance from the coast (<5, 5–10, 10–20 and > 20 km). The central line refers to the median value, the lower and upper lines represent the 25th and 75th percentiles, respectively, and whiskers extend to extreme data points and outliers are plotted individually as circles.

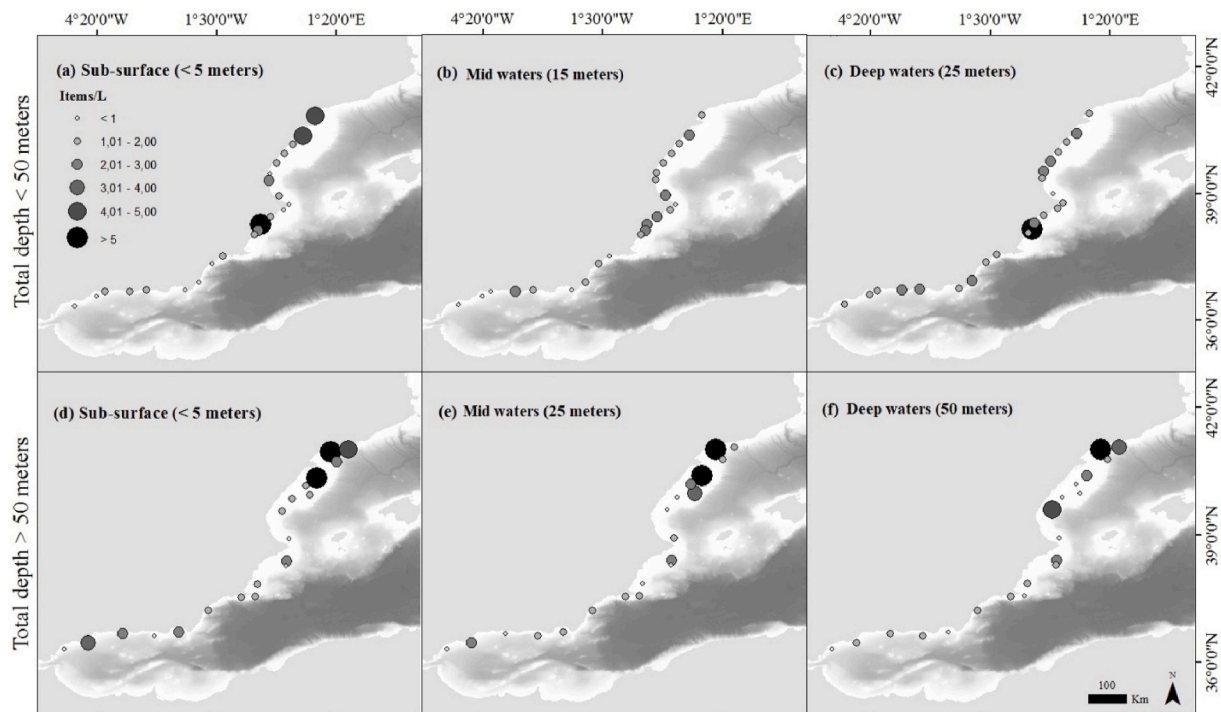


Fig. 3. Microplastics abundances defined as the total number of items per liter of water filtered (items L⁻¹) obtained at different depths according to sampling station (shallow and deep) and sampling depth (5, 15, 25 and 50 m).

Table 1

Microplastic abundances (items L⁻¹) according to station and sampling depth. Mean value (±SD) of temperature (°C), salinity (PSU), total number of microplastic items, mean value of microplastics (items L⁻¹ ± SD) and size range (minimum and maximum; mm).

Station depth	Sampling depth	Total number of samples	Temperature °C	Salinity	Total number of microplastics	Average microplastics	Size range microplastics (mm)
< 50 meters		69	22.31 ± 3.17	37.70 ± 0.70	557	1.61 ± 1.06	0.1 – 5.6
Shallow	Sub-surface (5m)	23	24.84 ± 2.02	37.56 ± 0.62	205	1.78 ± 1.37	0.6 – 5.6
	Mid waters (15m)	23	22.29 ± 2.46	37.67 ± 0.65	183	1.59 ± 0.70	0.6 – 3
	Deep waters (25m)	23	19.79 ± 2.77	37.88 ± 0.80	169	1.47 ± 1.03	0.1 – 5.2
> 50 meters		60	19.62 ± 4.09	37.71 ± 0.63	642	2.14 ± 1.73	0.4 – 8.4
Deep	Sub-surface (5m)	20	24.34 ± 1.87	37.57 ± 0.65	242	2.42 ± 1.77	0.8 – 7
	Mid waters (25m)	20	19.25 ± 2.13	37.61 ± 0.72	199	1.99 ± 1.70	0.4 – 7.2
	Deep waters (50m)	20	15.25 ± 0.36	37.96 ± 0.41	201	2.01 ± 1.79	0.4 – 8.4
Total general		129	21.02 ± 3.86	37.72 ± 0.66	1199	1.86 ± 1.43	0.1 – 8.4

polymer composition were detected in terms of the distance of the stations from the coast (permutation test for homogeneity of multivariate dispersions, $p < 0.05$). In this sense, the posterior pairwise comparisons detected differences between the polymer characteristics in stations located >20 km and stations located at < 5 km and between 10 and 20 km from the coast ($p < 0.05$).

4. Discussion

Microplastics were found in 99% of the stations sampled that covered a large spatial and vertical distribution along the continental Iberian shelf of the Levantine and Alboran Sea in the western

Mediterranean Sea. The highest abundances were observed in sub-surface waters (5 m depth) of deep stations, along the northern Levantine coast and in stations located at 5–10 km from the coast.

4.1. Spatial distribution

4.1.1. Latitudinal distribution

In this study, the majority of microplastics were found along the northern Levantine coast which is characterized by a large number of cities and rivers which potentially can generate important inputs of marine debris to the Mediterranean Sea (Liubartseva et al., 2018). Previous studies have already demonstrated that locations closer to large

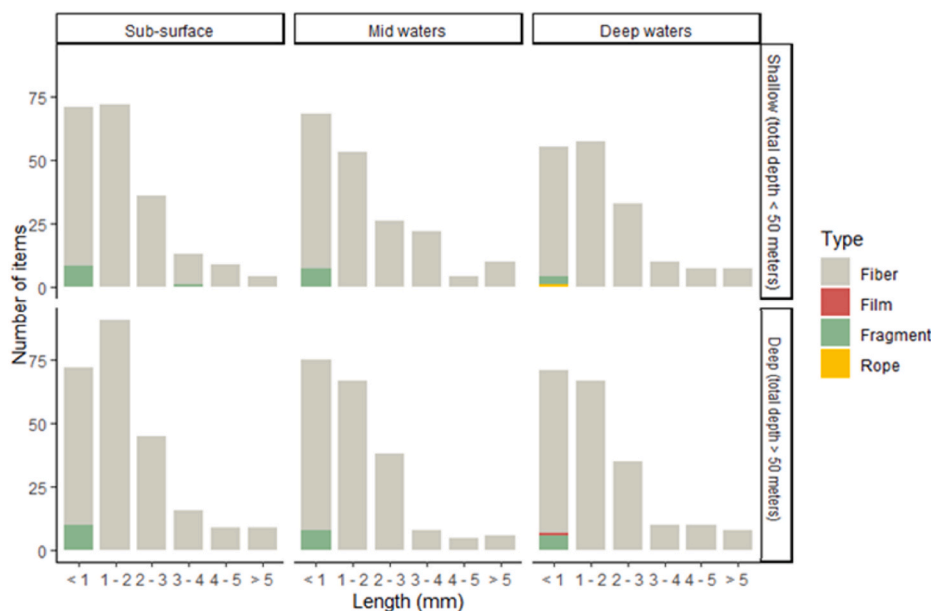


Fig. 4. Barplot for the size-frequency distribution according to type of microplastics and station (shallow and deep) and sampling depth (sub-surface and mid- and deep waters).

Table 2

Summary of the total number of items identified from all samples collected with 5-L Niskin bottles (129) and the percentage of occurrence (%) of items classified by type and by size range in millimeters (mm).

	Total items	Percentage (%)
Type		
Fiber	1153	96.09
Fragment	43	3.58
Film	1	0.08
Rope	1	0.08
Size range (mm)		
<1	411	34.27
1-2	407	33.97
2-3	213	17.56
3-4	79	6.34
4-5	44	4.20
>5	44	3.59

cities are usually highly polluted areas (Jambeck et al., 2015). However, populated cities are not the only direct source of microplastics in coastal areas. Rivers have already been previously considered direct corridors for microplastics from the land to the marine environment (Simon-Sánchez et al., 2019). The Levantine-Balearic (LEBA) demarcation is home to several main rivers of Spain: the Ebro, the Xúquer and the Segura rivers. In this sense, the Ebro river, houses the highest values of microplastics in their surroundings compared to other rivers that flow into the Mediterranean Sea (Guerranti et al., 2020). On the other hand, the Alboran Sea located in the Estrecho-Alboran (ESAL) demarcation is considered one of the most energetic regions of the Mediterranean Sea due to the fact that it is affected by the entrance of Atlantic waters through the Gibraltar Strait, determining this zone as a ‘transit area’ with sub-basin water exchanges (Mansui et al., 2015). In this sense, a recent model that considers population centers, river discharges and maritime traffic has already demonstrated that the Alboran Sea is one of the regions with the lowest concentrations of microplastics from the Mediterranean Sea and acts as a dispersion area (showing concentrations lower than the average) jointly to Ligurian and Tyrrhenian seas (Soto-Navarro et al., 2020). These results are in agreement with *in situ* abundance values from this study and are in consonance with a previous study documenting lower abundances of anthropogenic particles

ingestion in fish species in this area when compared to more northern areas along the Iberian Peninsula coast (Rios-Fuster et al., 2019).

The monitoring of marine debris at Marine Protected Areas (MPAs), considered as reference sites, allows quantifying levels of marine debris in areas with low human influence. Results from this study show that microplastic abundances in MPAs were similar than those reported in the other areas with no protection. In this sense, previous studies revealed high abundances of marine debris on beaches (Giovacchini et al., 2018), sediments (Alomar et al., 2016), sea surface (Fagiano et al., 2022) and water column (Panti et al., 2015) from protected areas. These scientific results provide with further evidence of transferred contamination to MPAs from anthropogenized areas and should encourage member states to develop joint measures at a basin scale which help to reduce marine debris at the source area and consequently mitigate the impacts of these in MPAs. On the other hand, strong maintenance measures for MPAs should be encouraged to avoid the accumulation and subsequent degradation and fragmentation of plastics that accumulate on their surrounding waters.

4.1.2. Vertical distribution

Understanding the real implications of the presence of microplastics in the water column is important as this zone houses a wide range of species that are at risk of ingesting microplastics. In this sense, for zooplankton abundance, a depth-related decrease from epipelagic to mesopelagic waters has been reported (Stefanoudis et al., 2019), highlighting the richness of the epipelagic layer in biota. Furthermore, the Levantine-Balearic demarcation corresponds to an important fishing area for commercial pelagic fish species of special interest, such as *Sardina pilchardus* and *Engraulis encrasicolus* (Brosset et al., 2017). In general terms, our study shows a slight decreasing trend of the abundances of microplastics from sub-surface to deeper waters. Previous studies have already reported higher abundances of microplastics near the sea surface and showed a decrease in the water column (Dai et al., 2018; Song et al., 2018). Moreover, some studies have found a direct correlation of the physical features of the water mass, temperature and salinity, with the density of plastic particles (Dai et al., 2018; Van Sebille et al., 2020). However, from the results of our study, temperature and salinity do not appear to affect the spatial distribution of plastic particles in the waters of the Iberian Peninsula. In general, particles denser than seawater (e.g. most plastic polymers such as PVC) can still be

Table 3
Total number of items according to polymers identified by Fourier-transform infrared spectroscopy (FT-IR) along the different sampling depths. Abbreviations: ABS, acrylonitrile butadiene styrene; HDPE, high-density polyethylene; LDPE, low-density polyethylene; NBR, butadiene rubber; PC, polycarbonate; PCL, polycaprolactone; PE, polyethylene; PET, polyethylene terephthalate; POM, polyoxymethylene; PP, polypropylene; PS, polystyrene; PTFE, polytetrafluoroethylene; SAN, styrene-acrylonitrile.

Station depth	Sampling depth	Polymers																	
		ABS	Cellulose acetate	Cotton	HDPE	Hemp	LDPE	NBR	Paint	PC	PCL	PE	PET	Polyamide	Polyester	POM	PP	PS	PTFE
< 50 m		1	13	3	1	1	15	3	2	1	1	1	1	1	1	21	17	-	4
Shallow	Sub-surface (5m)	-	2	2	1	-	4	1	2	-	-	-	-	2	-	10	2	-	2
	Mid waters (15m)	1	5	-	-	-	6	-	-	1	-	1	-	1	1	6	9	-	1
	Deep waters (25m)	-	6	1	-	1	5	2	-	-	1	-	-	3	-	5	6	-	1
> 50 m		-	13	2	-	-	18	2	-	2	1	1	-	5	1	11	6	2	6
	Sub-surface (5m)	-	4	-	-	-	6	-	-	1	-	-	-	3	-	4	-	-	2
	Mid waters (25m)	-	4	2	-	-	9	1	-	-	-	-	-	1	-	1	4	2	3
Deep	Deep waters (50m)	-	5	-	-	-	3	1	-	-	-	1	-	1	1	6	2	-	1
	Total general	1	26	5	1	1	33	5	2	3	1	1	2	11	2	32	23	2	10

Table 4

Summary of the results of the best-fit generalized linear model considering distance from the coast, sampling depth and total depth as coefficients, with transect of <5 km of distance to the coast and deeper waters in the intercept. GLM, **** $p < 0.001$, *** $p < 0.01$, ** $p < 0.05$, ' $p < 0.1$.

Coefficients:	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	3.15	0.61	5.130	1.14e-06***
5–10 km distance from the coast	0.49	0.34	1.435	0.154
10–20 km distance from the coast	-0.77	0.42	-1.817	0.072.
>20 km distance from the coast	-0.47	0.44	-1.055	0.294
Sub-surface of deeper stations	0.41	0.43	0.947	0.346
Mid waters of deeper stations	-0.02	0.43	-0.046	0.963
Deep waters of shallow stations	-1.53	0.58	-2.630	0.010**
Sub-surface of shallow stations	-1.21	0.58	-2.091	0.039*
Mid waters of shallow stations	-1.41	0.58	-2.421	0.017*
Total depth	-0.01	0.00	-1.976	0.050.

transported through the seas by underlying currents due to the different density (Engler, 2012). On the other hand, particles lighter than seawater (e.g., polyethylene or polypropylene) are expected to move within the uppermost layers of the water column (Reisser et al., 2015). Other authors also suggest that the density stratification of water impacts the vertical distribution of microplastic particles (Song et al., 2018). The physical shape of microplastic particles could also affect their position in the water column due to a different sinking velocity and fibers are expected to achieve lower velocities than fragments (Kooi et al., 2016). However, no spatial distribution was observed according to the shape of microplastics in the study area.

4.1.3. Distance to the coast

Our study evidenced higher microplastic abundances in stations located between 5 and 10 km from the coast and with total depth higher than 50 m (deeper stations). Previous studies have shown that debris abundances nearshore are higher than at offshore (Dai et al., 2018) possibly linked to the influence of coastal urbanizations (Littman et al., 2020). However, it is not clear how anthropogenic factors determine the fate of microplastic transport from land to the ocean (Su et al., 2019) and an indication of this is that higher amounts of seafloor macrodebris have also been reported farther away from the coast rather than near it (García-Rivera et al., 2017). Our data show no clear trend of microfiber distribution in relation to the distance from the coast, as it has also been observed for other parts of the Mediterranean Sea (Suaria et al., 2020). The higher abundances at intermediate distances from the coast (between 5 and 10 km and further than 20 km) highlight the importance of the assessment of other marine human activities linked to oceanographic parameters to better understand the spatial distribution and accumulation rates of debris in relation to the distance from the coast. In addition, the polymer composition evidenced a higher homogeneity in stations located within the first 5 km from the coast and located at a distance between 10 and 20 km from the coast highlighting that the distance to the coast affects the distribution of the different polymers. Further investigation is needed regarding the factors affecting spatial distribution of microplastics.

4.2. Microplastic typology

Our study documented that 68% of the collected items are smaller than 2 mm in length and the lowest reported size is 0.1 mm. This result is similar to the percentage (62%) reported by Barrows et al. (2017) for the small category of plastics particles (100 μm-1.5 mm) along the coast of Maine which were sampled with a grab sampler. Characterization of the types (size, shape, polymer) of accumulated debris in marine ecosystems can provide an indication of the human activities impacting the location where plastic has been quantified (Pham et al., 2014). Microfibers have

been the most prevalent type of microplastics in the study area corresponding to 96% of the identified items. Previous studies in the Mediterranean Sea reported that fibers are the most prevalent type of microplastics on the sea surface (Faure et al., 2015; Suaria et al., 2020), sediments (Sanchez-Vidal et al., 2018), water column (Dai et al., 2018) and also within the stomach contents of fish species (Compa et al., 2018; Rios-Fuster et al., 2019). In this sense, the disposal of municipal wastewater from washing clothes is reported to be a major source of fibers, with approximately 1900 fibers per wash produced by a single garment (Bayo et al., 2016; Browne et al., 2011). These results provide further evidence of the need to implement technologies developed to reduce the release of fibers into the marine environment.

Regarding the polymer characteristics of the plastic particles studied, the present study highlights the predominance of polyethylene (mainly LDPE) and polypropylene (PP) microfibers (22% and 20%, respectively) over the rest of the identified polymers (<16% each). These polymers have been previously found in wastewater treatment facilities indicating a source of laundry and textile washing (Naji et al., 2021). In addition, considering synthetic polymers these were a major source compared to natural fibers, which might be due to the rapid degradation process of natural fibers compared to synthetic fibers (Royer et al., 2021). On the other hand, a predominance of polyethylene terephthalate (PET) microfibers in the water column and in the gastrointestinal tracts of two important pelagic species with percentages ranging from 30% to 71% have also been reported in the study area but not in our study providing further evidence of the variability of plastic polymers in marine ecosystems (Compa et al., 2018; Lefebvre et al., 2019). Another relevant observation is the different percentage of items of semi-synthetic and non-synthetic materials corresponding to cellulose acetate (CA), cotton and hemp items (in total 20% of the items identified in the present study) in comparison with 92% of occurrence reported in other areas of the Mediterranean (Suaria et al., 2020). Other studies reporting synthetic microfibers in sub-surface waters have found a preponderance of polypropylene (PP), polyester (PS), polyamide (nylon), acrylic and polyvinyl alcohol (PVA) (Desorges et al., 2014).

4.3. Sampling methodology

In terms of sampling methodologies, water column sampling has been recommended to be performed with bongo nets or bulk water pump (GESAMP, 2019). However, these tools are at high risk of sample contamination (GESAMP, 2019). In addition, previous studies have detected an underestimation of the smaller particles when samples are performed with different nets (Barrows et al., 2017; Covernton et al., 2019; Lindeque et al., 2020).

There are several advantages of using Niskin bottles for microplastic quantification including its lower risk of airborne and cross contamination in comparison to other sampling procedures. This methodology has the ability of identifying all plastic size ranges and other advantages such as that it is easy to handle and it can sample different depths simultaneously. In this study, we highlight the importance of using Niskin bottles for sampling the water column in addition to other methods such as using stainless steel buckets for sampling the sea surface (Ryan et al., 2020; Suaria et al., 2020). The combination of the two methods could be used together to provide a general vision of the vertical distribution of microplastics as both methodologies have the advantage that all water collected is sampled directly, which is not the case when using a mesh net which may produce an overall underestimation of the smaller size particles, as they are trapped within the net and not quantified.

5. Conclusions

In conclusion, this study gives evidence of the ubiquity presence and distribution of microfibers in the upper epipelagic layer of the Iberian Peninsula coast in the western Mediterranean Sea. A heterogeneous

spatial distribution of the microplastic distribution has been detected without a clear spatial distribution. According to depth, microplastics were equally distributed as no significant differences were observed among sampling depths, suggesting that species living in the water column are exposed to plastic contamination and are at risk of ingesting microplastic throughout the water column. It is important to highlight that lower abundances of microplastics were found in the Alboran Sea which is considered as one of the most energetic regions of the Mediterranean Sea and it is under the influence of the entrance of Atlantic waters. Moreover, MPAs and non-MPAs stations are equally affected by the presence of microplastics, suggesting once again that microplastics are transferred from more anthropogenized areas to protected areas, and indicating the importance of developing mitigation measures for plastic pollution at a global scale. Finally, the predominance of microfibers suggests that textile waste is an important source of marine microplastics highlighting the importance to develop technology which prevents the entrance of microfibers in the marine environment through water treatment effluents.

CRediT authorship contribution statement

BR: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Review & Editing, Visualization, **MC:** Conceptualization, Validation, Formal analysis, Investigation, Writing - Review & Editing, **CA:** Validation, Investigation, Writing - Review & Editing, **VF:** Methodology, **AV:** Validation, Writing - Review & Editing, **MI:** Validation, Resources, Writing - Review & Editing, **SD:** Conceptualization, Validation, Investigation, Resources, Writing - Review & Editing, Supervision.

Declaration of competing interest

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

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