



# Are research methods shaping our understanding of microplastic pollution? A literature review on the seawater and sediment bodies of the Mediterranean Sea<sup>☆</sup>

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## ABSTRACT

The lack of standardization on the definition and methods in microplastic (MP) research has limited the overall interpretation and intercomparison of published data. This has presented different solutions to assess the presence of these pollutants in the natural environment, bringing the science forward. Microplastics have been reported worldwide across different biological levels and environmental compartments. In the Mediterranean Sea, numerous research efforts have been dedicated to defining the MP pollution levels. The reported MP concentrations are comparable to those found in the convergence zone of ocean gyres, pointing to this basin as one of the world's greatest plastic accumulation areas. However, to what extent are the data produced limited by the methods? Here, we present the results of a systematic review of MP research methods and occurrence targeting the seawater and sediment bodies of the Mediterranean Sea. Based on this dataset, we 1) assess the discrepancies and similarities in the methods, 2) analyze how these differences affect the reported concentrations, and 3) identify the limitations of the data produced for the Mediterranean Sea. Moreover, we reaffirm the pressing need of developing a common reporting terminology, and call for international collaboration between Mediterranean countries, especially with North African countries, to provide a complete picture of the MP pollution status in this basin.

## 1. Introduction

The presence of small plastic particles, microplastics (MPs), in the marine environment has been documented since the 1970s (Buchanan, 1971; Carpenter and Smith, 1972; Morris, 1980; Ryan and Moloney, 1990; Shiber, 1979). However, scientific and public concern have increased over the last 20 years once the accumulation and environmental effects of plastics in the environment were evident (Barnes et al., 2009; Derraik, 2002; Thompson et al., 2004). Since then, growing efforts are being devoted to characterize the presence and risk that these pollutants might pose to the natural environment and human health (Vethaak and Legler, 2021; SAPEA, 2019). Consequently, the MP research field is constantly evolving and different definitions and methodologies are being applied. Microplastics are generally defined as plastic particles smaller than 5 mm (Hidalgo-Ruz et al., 2012; Moore, 2008). This definition, restricted to the size criterion, overlooks one of

the most valuable character of plastic materials: their diversity. Recent proposals to standardize the MP definition consider their physicochemical properties, shape, size, and origin (Frias and Nash, 2018; Hartmann et al., 2019), although authors even differ on the size criterion (1 to <5000  $\mu\text{m}$  - Frias and Nash, 2018; 1 to <1000  $\mu\text{m}$  - Hartmann et al., 2019). Despite the lack of standardization, the MP research community is aware of the pressing need of working towards the harmonization of the data, reporting results using a common terminology (Hartmann et al., 2019; Provencher et al., 2020; Rochman et al., 2019) that would allow the large-scale interpretation and comparison of current knowledge.

In the Mediterranean Sea, numerous research efforts have been dedicated to define the plastic pollution issue (Fig. S1). The reported MP concentration, modeled (Eriksen et al., 2014; Lebreton et al., 2012; Van Sebille et al., 2015) and empirically gathered (Cózar et al., 2015; Suaria et al., 2016), are comparable to those found in the convergence zone of

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the subtropical gyres, indicating that this basin is one of the world's greatest plastic accumulation areas (Cózar et al., 2015; Lebreton et al., 2012). The high levels of MP pollution and its accumulation are likely driven by a combination of high anthropogenic pressure and the hydrodynamic conditions of the Mediterranean Sea (Morris, 1980). The Mediterranean region is inhabited by 480 million people, where one-third is concentrated along the coast, and about one-half is living in the surrounding hydrological basins (European Environment Agency, 2014). The basin supports intensive fishing, shipping, and industrial activities, and it is one of the world's top tourist destinations (UNWTO, 2018). Furthermore, the anti-estuarine circulation of the Mediterranean Sea contributes to this phenomenon by its limited outflow (Rohling et al., 2009). The Strait of Gibraltar is characterized by the inflow of Atlantic surface waters of relatively lower salinity, while the outflow of more saline and denser waters is restricted to deeper depth. Thus, floating plastic debris entering into, or generated within the Mediterranean Sea, will be trapped within the basin with few possibilities of escape (Aliani et al., 2003; Lebreton et al., 2012; Morris, 1980).

The high spatio-temporal variability of the Mediterranean Sea circulation prevents permanent accumulation areas of floating MP debris (Mansui et al., 2015). Most of the plastics are assumed to float at sea because of their positive buoyancy, as their average specific density ( $\rho = 0.9\text{--}1.0 \text{ g cm}^{-3}$ ) is lower than seawater ( $\rho = 1.027 \text{ g cm}^{-3}$ ); except for few denser polymers (i.e., PET,  $\rho = 1.38 \text{ g cm}^{-3}$ ; or PVC,  $\rho = 1.39 \text{ g cm}^{-3}$ ) which are expected to sink once entering into the aquatic environment. Ocean turbulence induces the vertical transport of positively buoyant MPs along the water column (Kooi et al., 2016; Kukulka et al., 2012), yet despite this, the seafloor is considered the long-term sink of MP pollution in the marine environment (Woodall et al., 2014). The export of MPs from the sea surface to the deep sea can be facilitated by the formation of biofouling on the particle's surface, the sequestration of these pollutants into organic and inorganic aggregates, or transportation through the vertical migration of numerous organisms (Van Sebille et al., 2020 and references therein). In a recent study, Kaandorp et al. (2020) estimated the floating plastic budget of the Mediterranean Sea: their results showed that 37–51% of the plastic input to the basin tend to settle on the seafloor and that 49–63% of these inputs end up beaching. Hence, the role of beaches cannot be neglected, as these transitional environments between terrestrial and marine systems act simultaneously as pathways and dynamic storage of MP pollutants.

Previously, authors have provided a general overview of the abundance of MPs in Mediterranean rivers (Guerranti et al., 2020), surface waters (Cincinelli et al., 2019), sediments (Martellini et al., 2018), and their interaction with biota (Llorca et al., 2020). However, none of the studies have discussed in depth the discrepancies in the methodologies and how these might be defining the current understanding of MP pollution in the Mediterranean basin. In this context, our approach for this paper is to: 1) Review and compile the available literature of MP occurrence in the seawater and sediment bodies of the Mediterranean Sea and 2) Describe the methods used for sampling, extracting, and identifying MPs, including the measures to prevent airborne contamination and quality control procedures. The main aims are to summarize how the differences and similarities in the methods influence the observed abundances of MP in the Mediterranean Sea, to outline where the research efforts are focused, and to identify the pressing research needs that will enhance our understanding of the fate of MP within this basin.

## 2. Methods

A systematic literature review was conducted to integrate the MP pollution data and the associated methods in the seawater and the sediment compartments of the Mediterranean Sea. Two scientific databases (Web of Science, [www.webofknowledge.com](http://www.webofknowledge.com); SCOPUS, [www.scopus.com](http://www.scopus.com)) were consulted, integrating logical operators, through the specific string search: (“microplastics” OR “microplastic pollution”

AND “Mediterranean”). The search was limited to English peer-reviewed articles published before January 2021. The first article selection was performed after a screening of both title and abstract. Only the articles investigating the concentration of MPs in sediment and seawater bodies were considered for full-text review. This set of articles was then classified according to the environmental compartment they investigated: sea surface water, seawater column, marine sediments, and/or beach. For the articles in which more than one category was investigated, each compartment was included in the dataset as an independent study. The meeting criteria for inclusion after the full-text revision was that the study provided primary results on the concentration of MPs in at least one of the four targeted compartments of the Mediterranean Sea (Fig. 1).

From each included study, the following information was extracted: the environmental compartment targeted, the description of the methods for MP sampling [e.g., date, instrument and its characteristics, flowmeter, wind correction, number of samples, sample preservation], extraction [e.g., sample's volume, purification treatment, flotation treatment parameters-brine solution, mixing and settling time, repetition], identification [visual, spectroscopy methods, % of particles analyzed, % of particles confirmed as MPs], quality control and contamination protocols, the registered MP occurrence per study [e.g., geographical area, mean abundance, if available, min. and max. abundance] and per sampling station [coordinates and abundance]. When data were missing in the publication, the Online Portal for Marine Litter: LITTERBASE (<https://litterbase.awi.de/>) was used to complete the dataset to acquire the geographical coordinates and abundance per station, or the corresponding authors were contacted for further information.

We used QGIS Desktop 3.12 ‘București’ (QGIS Development Team, 2020) to map the extracted information. Sampling stations were classified as “coastal” if they were located within 12 nm from the coast, and classified as “open-sea” for those exceeding 12 nm from the coast (UNCLOS and Nations, 1982). The nearest distance between sampling stations and the coast was calculated using the NNJOIN plugin (Tveite, 2019). For data visualization, figures were produced in R-3.5.3 (RStudio Team, 2020), using ggplot (Wickham, 2016), gcalluvial (Brunson, 2020) packages, and post-edited in Adobe Illustrator CC 2021 (Adobe Inc, 2021).

## 3. Results

The present literature review draws data from 85 articles obtained from our initial search that identified a total of 641 articles. From this first set of articles, 545 were discarded because they were duplicates or

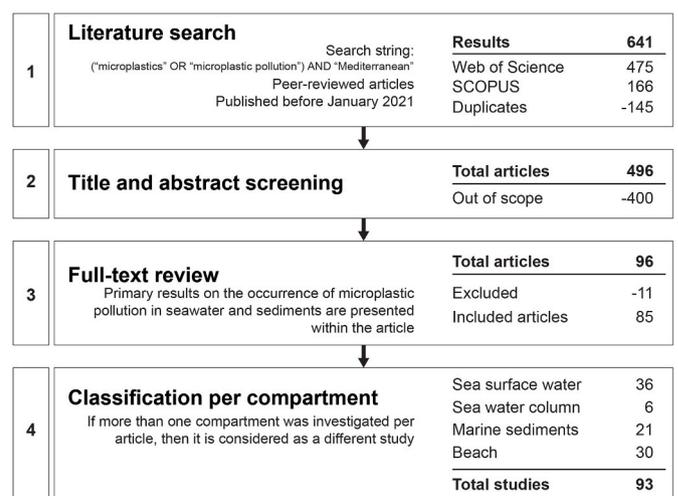


Fig. 1. Flow diagram illustrating the selection process and systematic review of the published literature.

out of the scope of this review. After the full-text review, another 11 articles were discarded as they were methodological or review articles, or did not provide primary results specifically on MPs. Within the suitable articles, eight presented data from more than one compartment, extending our final dataset to 93 independent studies (Table S1), from which 36 investigated the occurrence in sea surface water, 6 within the water column, 21 in marine sediments, and 30 in beaches.

### 3.1. Microplastic data distribution in the Mediterranean Sea

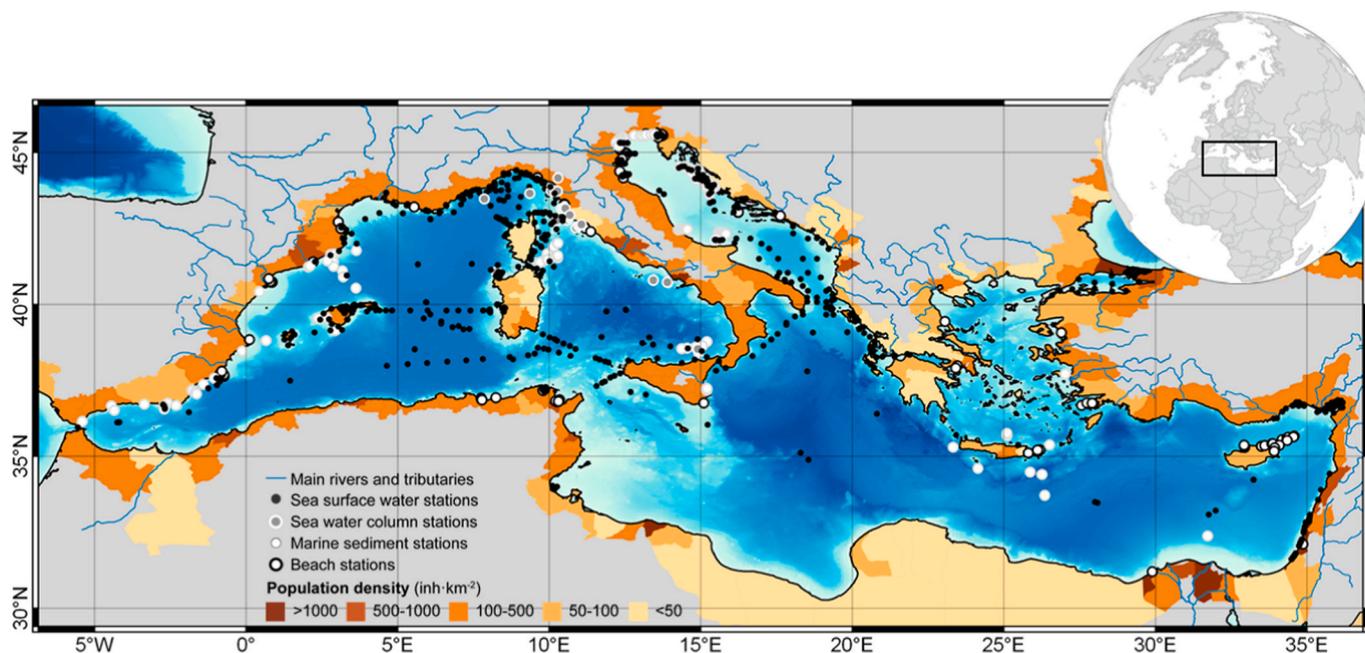
Although MP observations are available for the entire basin (Fig. 2), there is a clear bias towards the Western Mediterranean Sea, with fewer studies (25.8%) investigating MP occurrence in the Eastern Mediterranean Sea. Altogether, and depending on the precision of the sampling description in the reviewed literature, there were 3077 samples in the Mediterranean Sea used to assess the status of MP pollution in its abiotic compartments (Fig. S2). Most of the sampling efforts focused on the presence of these pollutants on the sea surface. Thirty-six studies collected a total of 1200 samples (40.0%). While less attention was devoted to defining the abundance of MPs within the water column, six studies collected a total of 76 samples (2.5%) restricted to the upper 100 m of the epipelagic layer. A similar picture was observed in the sediment compartment: sample scarcity is observed as the sampling sites deepen. Sediments from beaches and the neritic zone have been more extensively studied with 30 and 17 studies, collecting respectively 1302 (42.3%) and 452 (14.7%) samples. In contrast, only four studies reported MP concentration from 47 (1.5%) deep-sea sediment samples.

### 3.2. Seawater

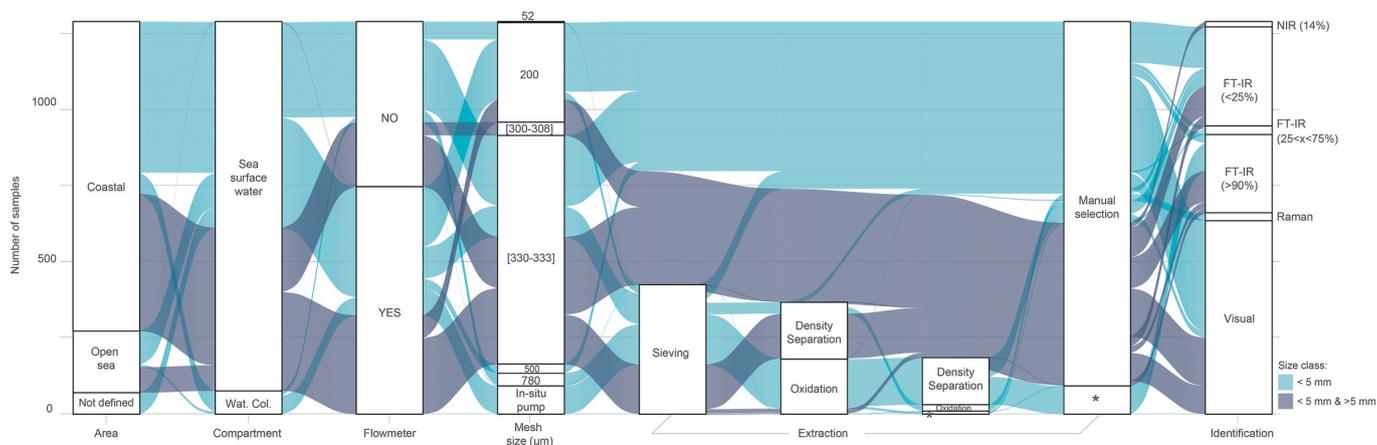
In the seawater compartment (surface water and water column: Fig. 3), most of the studies collected coastal samples (77.4%), and higher efforts have been devoted to reporting the MP abundance in sea surface water (94.0%) rather than their dispersion within the water column

(6.0%). Most of the samples were collected using a net (92.8%) and were volume reduced (i.e., concentrated in the cod-end of the net). Trawling speed depended on the sampling devices, currents, and weather conditions, but generally, it was adjusted between 1 and 4 knots. Reported trawling times ranged from 15 min up to 1 h. Depending on the use of a flowmeter (55.4%) or not (44.6%), the absolute abundances were expressed respectively as MPs/unit of volume or MPs/unit of surface. In the latter, the trawling distance was considered, and multiplied by the width opening of the net, under the assumption of constant seawater flux and ship speed. The authors generally reported concentration of MPs per unit of surface area (71.6%), rather than MPs per unit of volume (32.4%). Additionally, the mass of plastic debris was measured in 43.2% of the samples, and the concentrations are expressed as g MPs/unit of volume or g MPs/unit of surface (Table S2). For water column sampling, vertical or oblique tows were performed to report integrated values of MP abundance. Different mesh sizes were used to collect the seawater samples: 52  $\mu\text{m}$  (0.3%), 200  $\mu\text{m}$  (27.4%), 300  $\mu\text{m}$  (3.6%),  $\approx 333$   $\mu\text{m}$  (335, 333 and 330  $\mu\text{m}$ ; 62.8%), 500  $\mu\text{m}$  (2.5%) and 780  $\mu\text{m}$  (3.5%). Once the net is on-board, it is carefully rinsed from the outside to concentrate the sample into the cod-end. The cod-end content is emptied over a steel sieve with the same or smaller mesh size than the sampling net to avoid the loss of particles. The sample is then resuspended and transferred into the storage container. The authors reported different preservation methods. Frequently, samples were resuspended in pre-filtered seawater or deionized water, and fixed with 4% formalin.

Once in the laboratory, the prevalent protocol for MP extraction consisted of manual separation of the putative MP particles under the stereomicroscope (67.0%). The identification of MPs primarily relied just on visual identification (49.3%). Further identification on the polymer composition was conducted by different spectroscopy methods (Attenuated total reflectance (ATR) - Fourier transform infrared (FTIR; 47.3%), Raman (2.0%), and Near-infrared (NIR; 1.3%)), analyzing a subsample that represented 1.2–100% of the putative MP particles selected under the stereomicroscope (Fig. 3). When mentioned, the



**Fig. 2.** General map of the Mediterranean Sea. The location of sampling stations is shown in black dots for sea surface water stations, grey dots for water column stations, thin white circles for marine sediment stations, and thick black and white circles for beach stations. The population density of coastal areas is represented using an orange gradient. The population density data were obtained from several sources: Eurostat and National Statistical offices using the database City population [<http://www.citypopulation.de>]. NUTS3 regions and Mediterranean rivers were reprinted from Natural Earth free vector and raster map data [[www.naturalearthdata.com](http://www.naturalearthdata.com)]. The background bathymetric map was retrieved from the GEBCO 2019 grid [[https://www.gebco.net/data\\_and\\_products/gridded\\_bathymetry\\_data](https://www.gebco.net/data_and_products/gridded_bathymetry_data)]. Globe earth projection map showing the location of the Mediterranean Sea was created using the World Borders Dataset retrieved from thematic mapping [<http://thematicmapping.org>]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



**Fig. 3.** General overview of the sampling areas and methods for sampling, extraction processing, and characterization of microplastics in the seawater compartment of the Mediterranean Sea. The figure draws data from the 42 studies included in this review. The abbreviation DS refers to density separation, the asterisk (\*) refers to the filtration process, and in the identification column, percentages are indicating the number of particles analyzed within the studies depending on the method used. The different size of plastic debris investigated by the authors is indicated in light blue (<5 mm) and dark blue (<5 mm and >5 mm). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

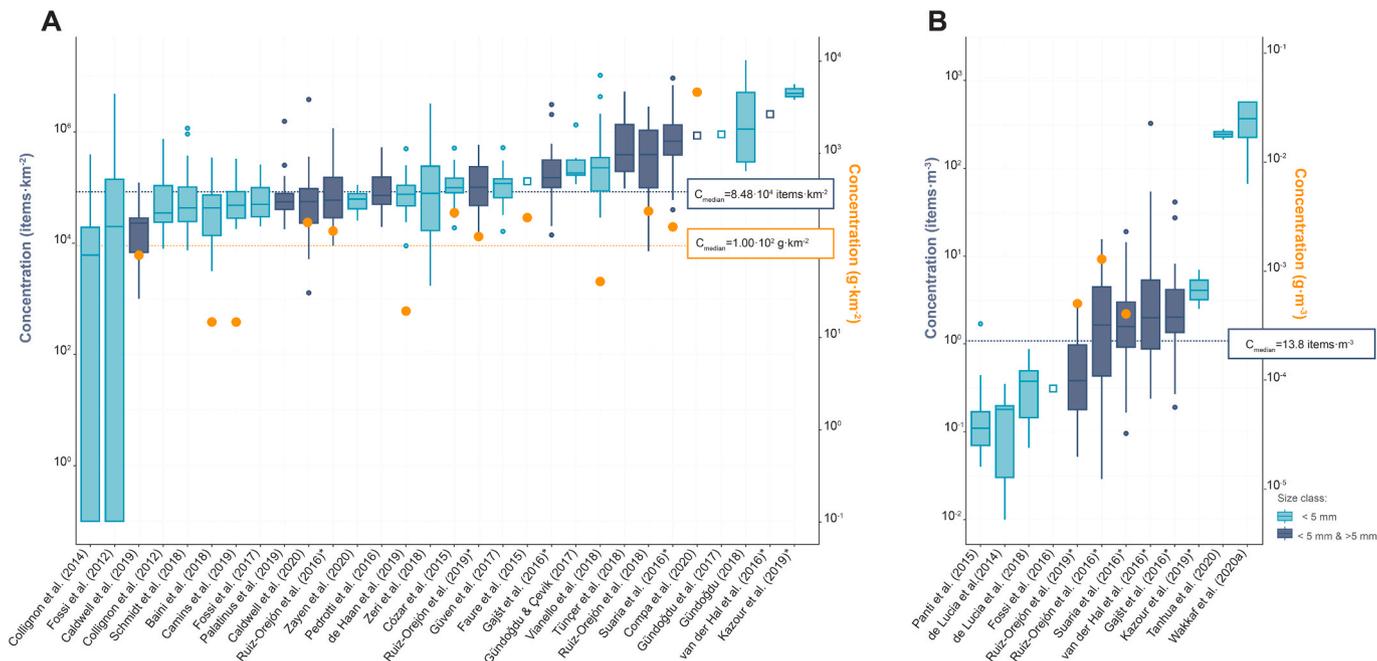
value of putative MP particles confirmed as MP particles after spectroscopy analyses ranged from 58.0% to 95.6%.

Beyond the methodological approaches, high variability was reported for the MP pollution levels in the sea surface waters (Fig. 4), the minimum concentration (200 µm mesh size, median  $6.25 \cdot 10^3$  items  $\text{km}^{-2}$ ;  $Q_1$ - $Q_3$ ,  $0$ - $1.94 \cdot 10^4$  items  $\text{km}^{-2}$ ) was reported in the Bay of Calvi, Corsica, NW Mediterranean Sea (Collignon et al., 2014). In contrast, the highest values were reported in the Levantine Basin, in the coastal waters of Lebanon (52 µm mesh size, median  $2.24 \cdot 10^6$  items  $\text{km}^{-2}$ ;  $Q_1$ - $Q_3$ ,  $1.85 \cdot 10^6$ - $3.20 \cdot 10^6$  items  $\text{km}^{-2}$ ; Kazour et al., 2019), Israel (333 µm mesh size, mean  $1.52 \cdot 10^6$  items  $\text{km}^{-2}$ , van der Hal et al., 2017), and Turkey (333 µm mesh size, median  $1.15 \cdot 10^6$  items  $\text{km}^{-2}$ ;  $Q_1$ - $Q_3$ ,  $2.94 \cdot 10^5$ - $5.12 \cdot 10^6$  items  $\text{km}^{-2}$ ; Gündođdu et al., 2018). In the compiled

dataset (Table S2), we estimated that the MPs median concentration in the surface water of the Mediterranean Sea is  $8.48 \cdot 10^4$  items  $\text{km}^{-2}$  ( $Q_1$ - $Q_3$ ,  $2.89 \cdot 10^4$ - $2.68 \cdot 10^5$  items  $\text{km}^{-2}$ ) and the median weight is  $1.0 \cdot 10^2$  g  $\text{km}^{-2}$  ( $Q_1$ - $Q_3$ ,  $2.79 \cdot 10^1$ - $3.63 \cdot 10^2$  g  $\text{km}^{-2}$ ).

### 3.3. Marine sediments

In the Mediterranean Sea, most of the MP observations were recorded within the coastal areas (94.4%), with the remaining 5.6% in the open sea. For shallow and coastal sediments (<30 m), scientific scuba divers (26.7%) collected surface sediments manually (77.4%) or used hand cores (22.6%). In contrast, when the sampling was conducted from a research vessel (73.3%), Van Veen grab (76.5%), boxcoring (6.8%),



**Fig. 4.** Overview of the concentration of plastic debris reported in the sea surface water studies (n = 36). (A) Studies reporting the concentration in items  $\text{km}^{-2}$  and  $\text{g km}^{-2}$ . (B) Studies reporting concentration in items  $\text{m}^{-3}$  and  $\text{g m}^{-3}$ . The studies marked with an asterisk (\*) were plotted for both panels. The different sizes of plastic debris investigated by the authors are indicated in light blue (<5 mm) and dark blue (<5 mm and >5 mm). The squares represent the average concentration reported within the studies when data per station were not available. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

multicorer (7.4%), or a mixed combination of these different devices (9.3%) were used. From these bulk sediment samples, a large volume of sediment is available, however, researchers collected a subsample of the first top centimeters (1–5 cm) and preserved it by freezing (−20 °C) or cooling (4 °C). It is worth to note that authors generally do not report to homogenize the sample before to further proceed with the MP extraction.

Microplastic extraction is characterized by multi-step procedures including sieving, purification, density separation, and filtration or concentration of the sample for further identification of MP particles (Hidalgo-Ruz et al., 2012). Some of these steps were applied in different combinations or individually by the authors (Fig. 5). Density separation was the prevalent procedure and used 77.4% of the samples. However, different settings for separation by density need to be considered: the type and volume of the brine solution, the mass of the processed sample, the mixing and settling times, and if the extraction is consecutively repeated to enhance the recovery rate. The most common brine solution was saturated sodium chloride (NaCl,  $\rho \approx 1.16\text{--}1.20 \text{ g cm}^{-3}$ ; 88.6%), followed by distilled water ( $\text{H}_2\text{O}$ ,  $\rho \approx 0.99 \text{ g cm}^{-3}$ ; 6.2%), zinc chloride ( $\text{ZnCl}_2$ ,  $\rho \approx 1.6\text{--}1.9 \text{ g cm}^{-3}$ ; 4.7%) and sodium iodide (NaI,  $\rho \approx 1.8 \text{ g cm}^{-3}$ ; 0.5%). The mass of the sample treated varied from 10 g up to 1000 g for seabed sediment. The volume of the brine solution added to the sediment varies from 200 mL to 1000 mL. The mixing and settling time widely varied among the studies, from 30 s to 3 h, and from 2 min to overnight periods, respectively. The extraction was repeated up to three times in 43.8% of cases. In general, the supernatant solution containing the MPs was collected and vacuum-filtered onto a filter (93.8%). In the studies where the 1–5 mm fraction was targeted (22.6%), MP particles were manually selected from the sediment.

In 24.4% of the samples, identification solely relied on visual identification; in 27.3% of the samples, a subsample was selected for spectroscopic analyses, and in 43.3% of the samples, all the putative MP particles were chemically characterized. Only Vianello et al. (2013) performed the identification of MPs via chemical mapping (5.0%) on a subsample of the filter.

The plastic debris concentration in the Mediterranean marine sediments presented high variability (Fig. 6), the median concentration reported is  $3 \cdot 10^2 \text{ items kg}^{-1}$  (Q1-Q3,  $1.49 \cdot 10^2\text{--}7.70 \cdot 10^2 \text{ items kg}^{-1}$ ). The lowest MP concentration (mean  $\pm$  SD;  $1.66 \pm 1.77 \text{ items kg}^{-1}$ ) was reported in the Augusta Harbour, Central Mediterranean Sea (D'Alessandro et al., 2018). The highest concentration (mean  $\pm$  SD;  $2.43 \cdot 10^3 \pm 2 \cdot 10^3 \text{ items kg}^{-1}$ ) was reported in the coastal sediments of Lebanon

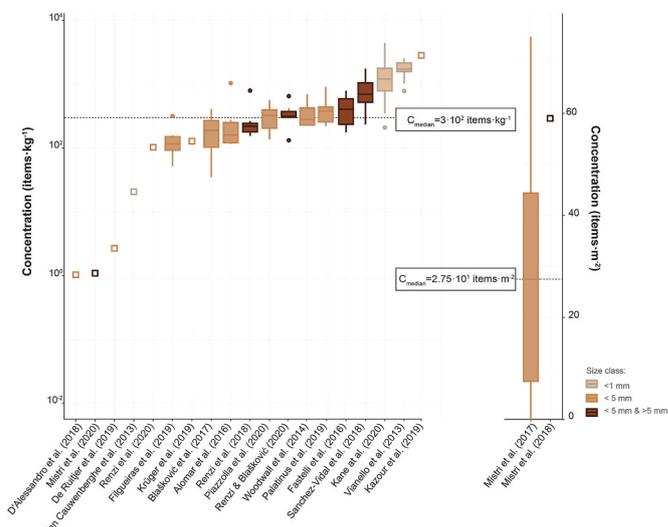


Fig. 6. Concentration of plastic debris reported in the marine sediment studies (n = 21). On the left side of the figure, studies reporting the concentration in items  $\text{kg}^{-1}$ , and on the right side, studies reporting concentration in items  $\text{m}^{-2}$ . The different sizes of plastic debris investigated by the authors are indicated in light brown (S-MPP; < 1 mm), brown (<5 mm), and dark brown (>5 mm and >5 mm). Note that the left y-axis is on a logarithmic scale. The squares represent the average concentration reported within the studies when data per station were not available. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(Kazour et al., 2019). The highest concentrations were found in the studies where the occurrence of S-MPP was investigated (Lagoon of Venice, Italy, median  $1.48 \cdot 10^3 \text{ items kg}^{-1}$ ; Q1-Q3,  $1.35 \cdot 10^3\text{--}1.86 \cdot 10^3 \text{ items kg}^{-1}$  - Vianello et al., 2013; North Tyrrhenian Sea, median  $1.04 \cdot 10^3 \text{ items kg}^{-1}$ ; Q1-Q3,  $6.90 \cdot 10^2\text{--}1.54 \cdot 10^3 \text{ items kg}^{-1}$  - Kane et al., 2020).

### 3.4. Beaches

The relatively easy access required for beach sampling has allowed researchers to widely collect sediment samples in this compartment and characterize the MP abundance along the Mediterranean coastline. However, there is considerable variability in the sampling strategies.

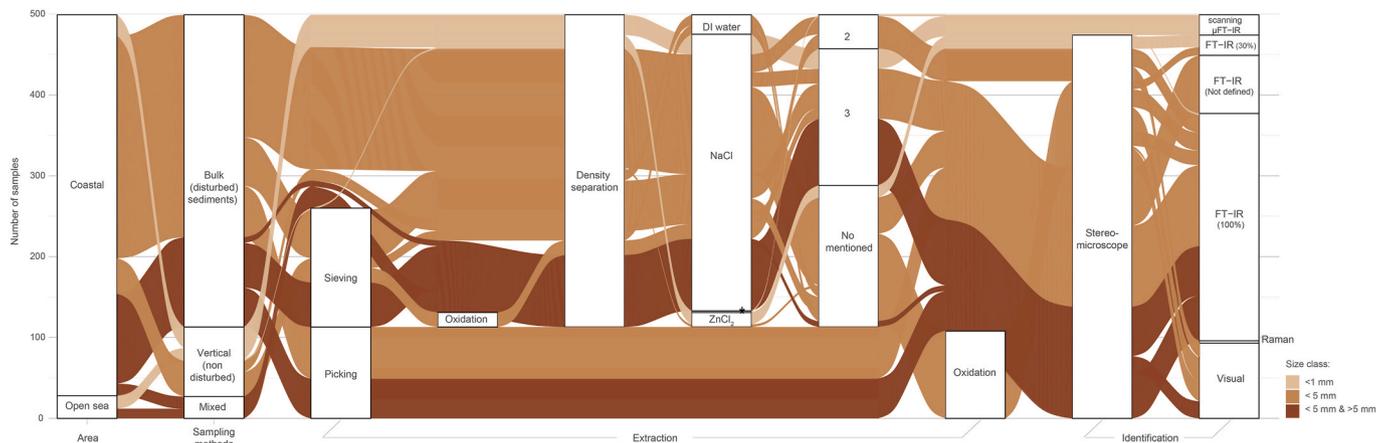


Fig. 5. General overview of the sampling areas and methods for sampling, extraction processing, and characterization of microplastics in the marine sediments compartment of the Mediterranean Sea. The figure draws data from the 21 studies included in this review. From left to right, the sixth column represents the solution used for the flotation treatment, the asterisk (\*) refers to NaI and DI water to de-ionized water, the seventh column represents the consecutive number of extractions performed during the density separation step, and in the tenth column, percentages are indicating the number of particles analyzed within the studies depending on the method used. The different size of plastic debris investigated by the authors is indicated in light brown (S-MPP; < 1 mm), brown (<5 mm), and dark brown (>5 mm). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

The number of beaches sampled ranges from one to 23 in a single study that relied on citizen science (Lots et al., 2017). One study covered the entire extent of the beach, while others targeted accumulation zones or pooled samples from different tidal areas. However, most studies focus on the intertidal zone (37.0%) and the high tide line (29.6%). In the studies where the aim was to report the presence of plastic pellets (10.0%) or large microplastic particles (L-MPP; > 1 mm, 6.7%), selective sampling (3.3%), and volume-reduced sampling (13.3%) were conducted. The rest (83.3%) collected bulk sediment samples for further MP extraction in the laboratory. Samples were collected using a quadrat (56.6%), ranging from 0.04 m<sup>2</sup> to 1 m<sup>2</sup>. From the surface down to a maximum of 15 cm in-depth, the top centimeters were scraped using a metal spoon, spatula, or shovel. In contrast, six studies out of the 30 collected the samples using a corer (16.6%) or boxcorer (3.3%).

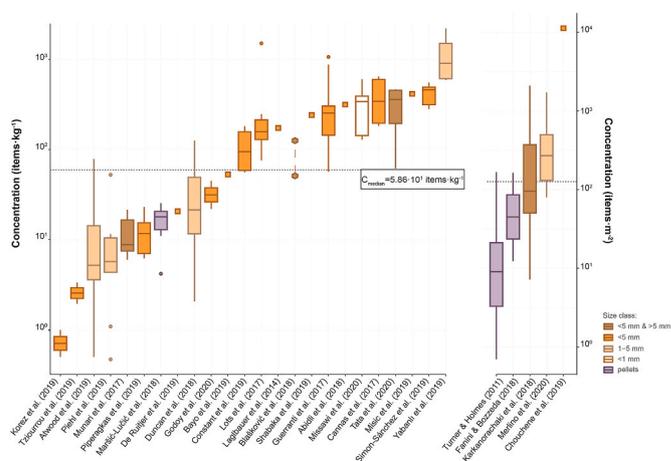
Sieving was used as a pre-treatment of the sample to reduce the total volume, eliminating the particles larger than the MP size fraction (>5 mm) in 15 studies. The majority of the studies (76.6%) used density separation to extract MPs from the sediments. The mass of sediment processed ranged from 50 g to 1000 g. Again, researchers prioritized the use of NaCl (78.3%), followed by ZnCl<sub>2</sub> (13.3%), while two studies (8.7%) combined the use of different brine solutions in the consecutive extractions to assure the recovery of denser polymers (Misić et al., 2019; Piperagkas et al., 2019). Besides those two studies, consecutive extractions, from 2 up to 5, were performed to increase the recovery rate of particles (30.4%). The MPs particles were manually picked from the supernatant in 5 studies (21.7%), whereas in 18 studies (78.3%), the supernatant was concentrated onto a filter for further identification under the stereomicroscope. Additionally, two out of the 30 studies included a digestion step in their protocol, applied at different stages of the MP extraction process. Misić et al. (2019) performed acidic digestion, adding 100 mL of HCl to the supernatant collected at the density separation step. Chouchene et al. (2019) applied alkaline digestion to the sample concentrated onto the filter, which was immersed into 50 mL of a KOH (20%) solution. When no density separation was performed (23.3%), the samples were sieved, and particles were visually selected (20%), or in one study (3.3%), a solvent extraction was performed to determine the content of polymeric and polymer-derived materials by spectroscopic techniques (Pyrolysis-gas chromatographic-mass spectroscopy (Py-GC/MS) - Ceccarini et al., 2018).

The identification of MPs relied on visual characterization in 9 studies (30.0%), differential scanning calorimetry (DSC) in one study (3.3% - Shabaka et al., 2019), and spectroscopy techniques were applied in 19 studies (63.3%). In the latter, 18 studies (60%) used ATR-FTIR on a subsample of particles; when mentioned, it ranges from a few particles to 100%, being confirmed as MPs between 11.3% and 100% of the particles. Lots et al. (2017) analyzed a subsample of particles (221 units) using the Raman spectrometry technique. Here, only 4.5% of the particles were confirmed as MPs, while the rest of the particles did not provide discernible peaks (42%), were categorized as dyes (18%), or did not provide a reliable match with the library used (36%).

In the compiled dataset, the median concentration of MPs along Mediterranean beaches was 58.6 items kg<sup>-1</sup> (Q<sub>1</sub>-Q<sub>3</sub>, 11.6–2.49·10<sup>2</sup> items kg<sup>-1</sup>) (Fig. 7), one order of magnitude lower than the median concentration found in marine sediments (3·10<sup>2</sup> items kg<sup>-1</sup>). The lowest abundance (particle size: <5 mm; median 7.5·10<sup>-1</sup> items kg<sup>-1</sup>; Q<sub>1</sub>-Q<sub>3</sub>, 6.3·10<sup>-1</sup> – 8.8·10<sup>-1</sup> items kg<sup>-1</sup>) was reported in Slovenian beaches (Korež et al., 2019). In this study, the authors reported a low rate of identification success, only 11.6% of the putative MPs were confirmed as MPs. The highest abundance (L-MPP; median 9.75·10<sup>2</sup> items kg<sup>-1</sup>; Q<sub>1</sub>-Q<sub>3</sub>, 6.16·10<sup>2</sup>–1.55·10<sup>3</sup> items kg<sup>-1</sup>) was reported in the Daçca Peninsula, North of Turkey (Yabanlı et al., 2019).

### 3.5. Quality control

To prevent airborne and cross-contamination of samples, especially if S-MPP and fibers are investigated, preventative measures are required,



**Fig. 7.** Concentration of plastic debris reported in the beach studies (n = 29). On the left side of the figure, studies reporting the concentration in items kg<sup>-1</sup>, and on the right side, studies reporting concentration in items m<sup>-2</sup>. The different sizes and types of plastic debris investigated by the authors are indicated in purple for pellets, white (S-MPP; <1 mm), light brown (L-MPP; 1–5 mm), orange (<5 mm), and dark brown (<5 mm and >5 mm). Note that the y-axes are on a logarithmic scale. The squares and hexagons represent, respectively the average concentration and the full range of value (min. and max.) reported within the studies, when data per station were not available. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

including the prioritization of using cotton clothes while sampling, the use of plastic-free and pre-cleaned storage containers, and pre-filtered solutions to resuspend the samples. The use of blanks and procedural blanks is still not standardized in MP studies. Generally, each study attempted to comply with at least some appropriate measures to minimize contamination. For example, researchers wore cotton lab coats at the laboratory, used glass materials or stainless-steel materials, covered the samples to avoid airborne contamination, and all tools and surfaces were rinsed/cleaned (HCl (1 M), ethanol or MilliQ water) before use.

For the studies on the seawater compartment, 38.1% reported the exclusion of fibers from their results due to the high risk of background contamination with this type of particle. To minimize this issue, 23.8% of the studies conducted the analysis below a laminar flow, 26.2% run blank controls (i.e., Petri dish or blank filters are exposed to laboratory conditions), and 7.1% reported to run procedural blank (i.e., MilliQ aliquot is treated as an environmental sample) to assess the cross-contamination while processing the samples.

In the marine sediment studies, 15.8% of studies performed the analysis inside of a specialized clean laboratory designed for microplastic analysis, which minimizes airborne contamination through pre-filtration of the air in the room. Blank controls were run in 42.9% of the studies, and 28.6% of the studies ran procedural blanks along with the environmental samples, to assess the potential contamination from laboratory background. Additionally, despite the use of procedural blanks, one study reported excluding fibers (<500 μm) in order to avoid potential overestimation.

In beach studies, 36.7% did not mention following any specific measure to prevent contamination. These measures were not relevant in 18.5% of studies due to the MP size investigated (L-MPP and pellets). Blank controls and procedural blanks were run in 20.0% and 23.3% of the studies, respectively. Three studies reported running spiked samples (virgin polymers) to assess the recovery rate of their protocols.

## 4. Discussion

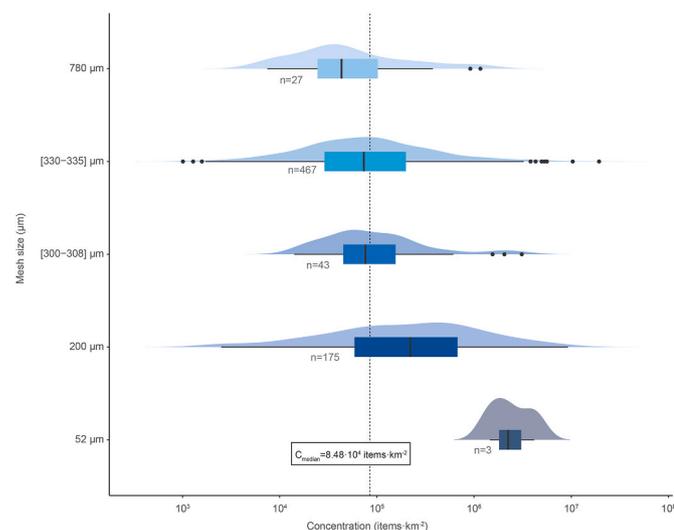
In the Mediterranean Sea, numerous research efforts are focused on reporting MP occurrence in different abiotic compartments. Synthesis

and integration of these data are limited by the lack of standardization of the methods and reporting units. Notwithstanding the researchers' different approaches, similarities are found in the sampling, extraction, and identification protocols. Here, we discuss how these different approaches, rather than solely the prevention for intercomparison among studies, shape our current understanding of the Mediterranean Sea's MP pollution levels. After an extensive literature review, we have identified pressing research needs to be addressed within this basin.

#### 4.1. The effect of sampling methods

##### 4.1.1. Surface waters

In the Mediterranean Sea, most of the sea surface samples were volume-reduced using a net. This approach, preferably using a mesh size of 333  $\mu\text{m}$ , was recommended by the Marine Strategy Framework Directive (MSFD) for the monitoring of MPs (Gago et al., 2016). The main advantage is that large volumes of water are sampled in a relatively short time. However, the mesh size of the net determines the size range and diversity of the particles. In a study of the Seine River (Paris, France) surface waters, Dris et al. (2015) found that MP concentrations measured with a mesh size of 80  $\mu\text{m}$  were 30-fold greater than those collected with a 330  $\mu\text{m}$  manta trawl, as a larger mesh size allows small size-class fibers (100–500  $\mu\text{m}$ ) to pass through it more easily. Kang et al. (2015) reported that floating MP (<2 mm) concentrations were two orders of magnitude higher using a 50  $\mu\text{m}$  hand net when compared to a 330  $\mu\text{m}$  mesh size net in the Southern Sea of Korea. In a comparison experiment on the efficiency of sampling devices along the North Atlantic coastal waters, Lindeque et al. (2020) demonstrated that MP concentrations collected with a 100  $\mu\text{m}$  mesh size net were up to 2.5 and 10-fold greater than with a 333  $\mu\text{m}$  and a 500  $\mu\text{m}$  mesh size nets, respectively. Despite the large-scale-temporal variability of the compiled Mediterranean dataset (715 datapoints), we observed that lower MP abundances in the surface water were reported for larger mesh sizes (Fig. 8). The smallest mesh size (52  $\mu\text{m}$ ) was used by Kazour et al. (2019) to collect samples from the coast of Lebanon ( $n = 3$ ). The predominance of waterborne MP samples collected with  $\approx 333$   $\mu\text{m}$  (68.2%) and 200  $\mu\text{m}$  (27.4%) mesh size nets indicate that smaller MPs (<333  $\mu\text{m}$  and <200  $\mu\text{m}$ ) have been systematically underestimated in the surface waters of the Mediterranean basin. This is particularly relevant when considering that there is a negative relation between MP size and their abundance in the natural environment, with an increase in the number



**Fig. 8.** Violin graph showing plastic particle concentration (items  $\text{km}^{-2}$ ) reported for 715 Mediterranean seawater locations, logarithmically transformed and plotted in relation to the mesh size of the net used to collect the seawater sample.

of particles accompanied by a decrease in size (Isobe et al., 2017; Pabortsava and Lampitt, 2020). Similarly and importantly, the potential environmental risk is negatively correlated with the size of the particles (Covernton et al., 2019; Ma et al., 2019).

Other sampling approaches are required to account for the smaller fraction of floating MPs and potentially nanoplastics (NPs). Barrows et al. (2017) suggested implementing combined sampling, which involves collecting volume-reduced and bulk samples to ensure the characterization of a wide size spectrum of MPs (1–5000  $\mu\text{m}$ ). The pattern observed is that greater MP concentrations were reported in bulk water samples (Barrows et al., 2017; Covernton et al., 2019; Green et al., 2018). Additionally, this approach allows for the assessment of fibers that generally are overlooked or discarded in the net sampling approach (Suaria et al., 2020). However, the volume and number of replicates required to provide a relevant statistical evaluation remain unclear. Ryan et al. (2020) reported that sampling a larger volume of water may increase the reproducibility of the measurement, but at the cost of underestimating the environmental concentration.

In addition to sampling devices, other factors may influence the accuracy of the measurements and possibly explain the differences between studies, such as using a flowmeter or considering the sea state. During sampling, the use of a flowmeter is critical, as it measures the water effectively passing through the net. Suaria et al. (2016) reported that the sampled area was on average two times higher when computed from GPS data compared to the same area when estimated using the flowmeter, making the GPS method a less reliable approach. During sampling, the weather and environmental conditions affect the mixing layer and, hence, MP's vertical distribution along the first meters of the water column (Collignon et al., 2012; Kooi et al., 2016; Kukulka et al., 2012). Manta trawls and neuston nets cover, respectively, the first 15–25 and 50 cm of the water column. Thus, accounting just for the surface tow concentration may underestimate MPs' total load in the surface waters, especially during high wind speed conditions (Kukulka et al., 2012).

##### 4.1.2. Water column

Despite the numerous investigations on seawater, to date, subsurface data are still scarce. Depth integration models (Kooi et al., 2016; Kukulka et al., 2012) and multi-level trawls in the North Atlantic (150  $\mu\text{m}$  mesh size, Reisser et al., 2015; and 330  $\mu\text{m}$  mesh size, Kooi et al., 2016) indicated that MP concentration exponentially decreases within the first meters of the water column, yet reporting smaller sizes in deeper waters (Kooi et al., 2016; Reisser et al., 2015). In the Mediterranean Sea, few studies ( $n = 6$ ), always restricted to the upper 100 m of the photic zone, have investigated the MP occurrence within the water column. de Lucia et al. (2018) found higher MP concentration in coastal surface waters ( $0.32 \pm 0.24$  items  $\text{m}^{-3}$ ) of minor Italian islands compared to the abundance within the first 20 m of the water column ( $0.18 \pm 0.10$  items  $\text{m}^{-3}$ ), although no significant variability was found. Along the Tuscany coastline, Bainsi et al. (2018) reported similar average concentrations between the water column (down to a maximum of 100 m;  $0.16 \pm 0.47$  items  $\text{m}^{-3}$ ) and the floating MPs concentration at the surface waters ( $0.27 \pm 0.33$  items  $\text{m}^{-3}$ ), with the predominance of particles <1 mm. In the Gulf of Lion, Lefebvre et al. (2019) performed vertical tows from the bottom (max. depth of 100 m) to the surface, and solely fibers were found with an average abundance of  $0.23 \pm 0.20$  items  $\text{m}^{-3}$ . In the lagoon of Bizerte, Tunisia, seawater samples were collected using a submersible pump (300  $\mu\text{m}$  mesh size; Wakkaf et al., 2020a, 2020b). The authors reported relevant high concentrations in the sea surface waters ( $453 \pm 335$  items  $\text{m}^{-3}$ ) and benthic waters ( $400 \pm 200$  items  $\text{m}^{-3}$ ). Outside the Mediterranean Sea, when similar sampling approaches were conducted (i.e., in-situ pumps) and deeper layers were investigated, the MP concentrations reported varied between 1–4 orders of magnitude higher (Choy et al., 2019; Pabortsava and Lampitt, 2020). At the Bay of Monterrey (California, USA), Choy et al. (2019) filtered a large volume of seawater (1007–2378  $\text{m}^3$ ) in depths ranging from 5 to 1000 m, and

they found the highest MP (>100–5000  $\mu\text{m}$ ) concentration in the mesopelagic zone (200–600 m; 15 items  $\text{m}^{-3}$ ). In a latitudinal transect in the Atlantic Ocean, Pabortsava and Lampitt (2020) reported much higher concentrations of MPs (PE and PS, 32–651  $\mu\text{m}$ ;  $1114 \pm 542$  items  $\text{m}^{-3}$ ) in the mesopelagic zone. These studies point to the water column as a major reservoir of MPs and highlight the importance of understanding the ecological and physical processes responsible for the export of these pollutants from the surface to deep-sea sediments. Specifically, the presence of smaller particles in the mesopelagic layer (Pabortsava and Lampitt, 2020) suggests the progressive degradation of MPs in the seawater environment, highlighting the existing incongruity of the oceanic plastic budget (Eriksen et al., 2014; Pabortsava and Lampitt, 2020; Thompson et al., 2004; Van Sebille et al., 2015).

#### 4.1.3. Marine sediments

The most common sampling approach was collecting bulk sediment samples, with a significant constraint related to the extraction and purification protocols (See section 4.2). Thus, the use of different sampling devices does not imply such substantial variability as it does for the water compartment. The reviewed studies ( $n = 21$ ) investigated the MPs presence in surface sediments, except for the study from De Ruijter et al. (2019), which investigated the vertical distribution of MPs in intertidal sediments cores. Despite the collection of sediment cores, palaeo-oceanographic approaches investigating historical accumulation patterns in sediment archives (Banccone et al., 2020) are absent from the Mediterranean literature. Future studies would need to address the role of geophysical processes (i.e., sediment and organic accumulation rate) and anthropogenic pressures in the area (i.e., bottom trawling) on the fate of MPs once they reach the seafloor. These aspects need to be taken into account to gain knowledge of MPs' sequestration within marine sediments, their dilution and accumulation patterns, and potential resuspension.

#### 4.1.4. Beaches

The sampling strategy to assess the occurrence of MPs in the Mediterranean beaches encompasses a wide range of methodologies/approaches, depending on the main goal of a given study. For example, reduced volume samples were employed in studies where pellets or specifically L-MPP (>1–5 mm) were targeted (Atwood et al., 2019; Grelaud and Ziveri, 2020; Maršić-Lučić et al., 2018; Merlino et al., 2020; Piehl et al., 2019; Turner and Holmes, 2011). However, the collection of bulk sediment samples was the most common (83.3%). Like in marine sediments, the whole MP size spectrum can be potentially characterized in this sample type, but the limitations are defined by the laboratory procedures (See section 4.2). The main differences in sampling protocols' relied on the number of replicates, volume of sand collected, depth of sampling, beach area sampled, and the ratio of the sampled surface area to the beach's surface. Different protocols have been published for providing guidance on the monitoring of microlitter, targeting MPs particularly. The MSFD technical report recommended monitoring the presence of MPs above the strandline, collecting a minimum of five samples targeting the top 5 cm in a stratified random manner to cover the entire beach or a specific area (MSFD Technical Support group on marine litter, 2013). In an attempt to standardize the sampling protocol, Besley et al. (2017) investigated the sample size, sampling depth, and sampling location required to achieve statistical representativeness. Their proposed protocol developed a formula to calculate the number of samples required per 100 m transect and recommended collecting at least 100 g of sand targeting the top 5 cm. In contrast, the findings of Karkanorachaki et al. (2018) highlight the importance of collecting subsurface samples (down to 10 cm) to truly characterize the concentration of MPs in beaches. Their results showed that the concentration of MPs fragments and pellets was up to one order of magnitude higher in subsurface samples ( $339.8 \pm 104.4$  items  $\text{m}^{-2}$ ) than in surface samples ( $35.3 \pm 11.5$  items  $\text{m}^{-2}$ ). Future studies monitoring MP pollution on beaches need to strategically design their sampling to consider the

morphological characteristics of the beaches. Generally, beaches are dynamic systems with changing conditions due to environmental and anthropogenic causes. Among these factors, for the Mediterranean Sea, studies should consider the low-tide amplitude, hydrodynamic forces, beach morphology, wind exposure, seasonal visitor pressure on the beach, proximity to MP sources, cleaning events, erosion after storm events, and potential maintenance works (i.e., restocking of sand).

#### 4.2. The effect of extraction protocols

In seawater samples taken from the Mediterranean Sea, the extraction of MPs predominantly relies on physical separation processes – sieving, flotation, and manual selection under the stereomicroscope (Fig. 3). Although most of the samples were collected within the continental margins, which are recognized as high productivity areas, pre-treatment for organic matter (i.e., phyto- and zooplankton) oxidation protocols were rarely reported in the reviewed studies. Purification processes (i.e., enzymatic, digestion, oxidation) may reduce the processing time, enhance MPs' recovery, and simplify the subsequent step of identification (Cole et al., 2011; Löder et al., 2017). However, the protocol selection should be cautious, as temperature and different reagents may damage MPs (Enders et al., 2017; Munno et al., 2018). Following the manual MPs selection approach, the extraction efficiency highly depends on the user's experience, the particles' size, microscope's magnification, and the sample's complexity (e.g., rich biota-samples; Löder and Gerdt, 2015). Manual selection of particles is time-consuming and inevitably introduces a bias towards selecting larger and color particles because these are easier to recognize and isolate (Song et al., 2015). The predominance in the use of nets to sample the seawater compartment sets the lower size cut of the collected MPs, generally above 200–300  $\mu\text{m}$ . The manual selection of the particles introduces a wide range of variables that undoubtedly affect the MPs detection limits and compromises the accuracy of the isolation. Such variability should be addressed by systematically implementing quality assurance practices (i.e., spiked samples, interlaboratory comparison exercises between Mediterranean laboratories) to validate the extraction's protocol and define the extraction rate and detection limits.

The MP extraction from marine sediments is challenging. For example, the organic content of the sediments, although less than surface water samples, may retain the MPs, complicating their separation. To address that, samples are subjected to a purification process to facilitate and enhance the recovery of MPs (Hurley et al., 2018; Löder et al., 2017; Masura et al., 2015). In the Mediterranean Sea, most of the samples were collected in the coastal margins. These areas represent sites of significant importance for many biogeochemical processes, including organic carbon burial and remineralization (Muller-Karger et al., 2005). Thus, higher organic content is expected in these types of samples. In the reviewed studies, the predominant extraction protocol consisted in sieving and separation by density (Fig. 5), with few studies ( $n = 2$ ) performing oxidation treatment of the samples (De Ruijter et al., 2019; Krüger et al., 2019).

The wide variety of existing polymers differ on their specific density, which is generally used for the particles' physical separation. Due to the higher density of sediments (quartz,  $\rho = 2.65$  g  $\text{cm}^{-3}$ ) than the plastic materials, Thompson et al. (2004) proposed a flotation approach to extract the MPs from the sample matrix, using NaCl as a brine solution. Researchers used NaCl predominantly for the density separation in the Mediterranean studies because it is the most common brine solution in MP studies (Hidalgo-Ruz et al., 2012), it is environmentally friendly, and it is cost-effective. The limitation of using this brine solution is that plastics materials with a higher density (i.e., PET,  $\rho = 1.38$  g  $\text{cm}^{-3}$ ; or PVC,  $\rho = 1.39$  g  $\text{cm}^{-3}$ ) might be underestimated. Different brine solutions with a higher density, as NaI ( $\rho \approx 1.8$  g  $\text{cm}^{-3}$ ), sodium polytungstate (SPT,  $3\text{Na}_2\text{WO}_4 \cdot 9\text{WO}_3 \cdot \text{H}_2\text{O}$ ,  $\rho \approx 1.4$  g  $\text{cm}^{-3}$ ), zinc bromide ( $\text{ZnBr}_2$ ,  $\rho \approx 1.71$  g  $\text{cm}^{-3}$ ), sodium bromide (NaBr,  $\rho \approx 1.37$ – $1.40$  g  $\text{cm}^{-3}$ ), calcium chloride ( $\text{CaCl}_2$ ,  $\rho \approx 1.3$ – $1.5$  g  $\text{cm}^{-3}$ ) have been proposed

as an alternative to enhance the recovery of denser polymers from sediment samples. While there is no standard recommendation, the selection of the appropriate brine solution should consider the polymers' density targeted in the study, hazard, and economic variables (Frias et al., 2018).

Besides the brine solution, other setting parameters may affect the rate of MP extraction from sediment samples. However, little attention was devoted to investigate these parameters' effect on the extraction efficiency (i.e., the mass of the sample processed, the ratio of the sediment/brine solution, mixing and settling times, and the number of consecutive extractions needed). Besley et al. (2017) addressed this question on beach sediment samples, suggesting that for 50 g dry weight of sediment samples, 200 mL of brine solution should be added to the sample, the mix stirred for 2 min and then left to settle for at least 6 h. This process needs to be repeated three times, reaching a recovery rate of 83.0%. Similarly, Simon-Sánchez et al. (2019) reported a recovery rate of 79.4% after three out of five consecutive extractions from sandy samples using NaCl.

The lack of validation on the extraction protocols prevents a quantitative assessment of the effects of these diverse approaches on the reported MP concentration. In the current status of the research field, it is difficult to recommend the correct extraction protocol, as this should be chosen depending on the study's objective, the environmental matrix investigated, and the economic and time limitations. Under this context, the inter-comparison of results between studies will remain jeopardized by the researchers' numerous research approaches to characterize MP pollution. However, the implementation of good practices and different quality assurance measures, which can be easily fulfilled, can help to provide more accurate comparisons (e.g., providing a clear and concise description of the protocol, acknowledging the size limitation of the methods, analyzing spiked samples to characterize the recovery rate of the protocol, control and procedural blanks while sampling and analyzing the samples, presenting the results separately per type of MP pollutant and size fraction).

#### 4.3. Identification

In the Mediterranean Sea, the identification of the particles relied on visual characterization in 49.3% of seawater samples, 27.3% of marine sediment samples, and 30.0% of beach studies. There are general recommendations to visually identify putative MP particles (Hidalgo-Ruz et al., 2012; MERI, 2012) or fibers (Stanton et al., 2019). This method is time-efficient but highly depends on the user and the microscope's magnification (Löder and Gerdt, 2015). Visual characterization may overlook smaller and transparent particles, significantly impacting the underestimation of the MP concentration (Song et al., 2015). Regarding size, the recommended limit for a correct identification is 1 mm for the naked-eye, 500–100  $\mu\text{m}$  using optical microscopy (highly dependent of the magnification; Primpke et al., 2020 and references therein).

Infrared (IR) and Raman are the most common techniques for the chemical characterization of the particles. These analytical methods are recommended by the MSFD (MSFD Technical Support group on marine litter, 2013) and the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP, 2019). These spectroscopic techniques are typically non-destructive, provide particle number (units) and morphological characteristics of particles larger than 10  $\mu\text{m}$  and 1  $\mu\text{m}$  in size for FTIR and Raman spectroscopy, respectively (Löder and Gerdt, 2015). The analysis is relatively simple, as once the spectrum is retrieved, the polymer type can be determined by comparing it against a reference library. However, the purification and extraction methods are critical to isolate MPs from the environmental matrix (Löder et al., 2017). In the Mediterranean Sea, the general approach was that after visual sorting of the putative plastic particles, a subsample (1.2–100% of the particles) was selected for characterizing its chemical composition. In general, these subsamples were chosen randomly, with scarce reference to the statistical weight of the subsample to validate or

correct the reported concentrations. Similarly, the rate of successful identification was often not reported in the studies, and when this was clearly stated, it ranged from 5% to 100%. The most common technique within the Mediterranean studies was the characterization of particles by ATR-FTIR. In this approach, the particle is pressed against a crystal and subjected to the IR laser beam to record its IR spectrum. This approach requires hand-picking the particles and manually analyzing them, which is time-consuming, and limits the minimum particle size to that can be manually isolated ( $\approx 300 \mu\text{m}$ ; Primpke et al., 2020). As mentioned above, in the seawater compartment of the Mediterranean Sea, the knowledge is predominantly restricted to plastic particles  $>200\text{--}300 \mu\text{m}$  due to the sampling mesh size. In a few studies ( $n = 4$ ), the Raman spectroscopy technique was used to characterize the MP particles. Lots et al. (2017) reported a low success for the identification of particles (4.5%). The authors indicated that spectra had low quality due to fluorescence that may result from biological material on the surface of the particles. In the same study, the authors reported the interference of additives and dyes that masked the spectrum, preventing polymer recognition.

Exclusively, Vianello et al. (2013) applied reflectance  $\mu\text{FTIR}$  based on chemical imaging of the filters. Under this approach, as the analysis is automatized, there is no need for a visual pre-sorting of the particles, minimizing the human bias. However, the analysis is time consuming, and requires a large initial investment for the acquisition of the instrument (Primpke et al., 2020). Additionally, the authors reported difficulties in the analysis caused by the use of Glass Fiber filters to concentrate the sample. To minimize this issue, researchers reported concentrating the samples onto an IR transparent or reflectance surface (i.e., suitable membrane; Löder et al., 2015) or window (Simon et al., 2018) to record the spectrum. In the study of Vianello et al. (2013), the predominant size was in the range of 30–500  $\mu\text{m}$ , and the smallest particle size reported was 15  $\mu\text{m}$ .

Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS) was used to characterize MPs ( $<2 \text{ mm}$ ) in Marina di Vecchiano (Tuscany, Italy) (Ceccarini et al., 2018). To our knowledge, this is the only study in the Mediterranean Sea that applied Py-GC-MS. This technique is a destructive method, which provides accurate data on mass concentration. However, it does not determine the number of particles (units) or their morphological characteristics by itself, which makes the comparison between studies in the region difficult.

#### 4.4. Microplastic pollution in the Mediterranean Sea

While the hydrodynamic conditions and wind-driven processes influence the redistribution of MP debris (Collignon et al., 2012; de Haan et al., 2019; Fossi et al., 2017; Suaria et al., 2016), even far from their sources (Ruiz-Orejón et al., 2018), higher MP presence is related to areas under high anthropogenic pressure and proximity to (micro)plastics land-based sources (Pedrotti et al., 2016). Coastline morphology may also affect the dispersion of these pollutants as the rugosity of the coast may facilitate the MP retention in nearshore areas (Compa et al., 2020), while smooth and large bays may induce the exports of MPs to off-shore waters (Brennan et al., 2018). Most of the sea surface samples from the Mediterranean Sea were collected in coastal areas (78.2%). Few studies specifically investigated the relationship between MP occurrence and the proximity to the coast. In the Balearic Islands of Mallorca (Spain, NW Mediterranean), Compa et al. (2020) found high heterogeneity of the MP levels ( $8.58 \pm 4.08 \cdot 10^6 \text{ items km}^{-2}$ ) within the first km of coast, although MP concentration significantly decreased with increasing distance to the coast ( $<1 \text{ km}$ ). The compiled dataset in Pedrotti et al. (2016) found higher MP concentration ( $1.58 \cdot 10^5 \pm 1.57 \cdot 10^5 \text{ items km}^{-2}$ ) in the first km adjacent to the coast, with a decrease in waters between 1 and 10 km from the coast ( $8.0 \cdot 10^4 \pm 3.80 \cdot 10^4 \text{ items km}^{-2}$ ), and again reaching high values in waters further away from the coast ( $>10 \text{ km}$ ;  $1.76 \cdot 10^5 \pm 2.16 \cdot 10^5 \text{ items km}^{-2}$ ). In the coastal waters of Tuscany, Bainsi et al. (2018) found a correlation between MP abundance, which

significantly increased with distance from land. In the dataset compiled for this literature review, we observed that 61.5% of the sampling stations were located within the first 10 km of the coast (Fig. 9). No relation was observed between plastic debris concentration (items km<sup>-2</sup>) and distance to the coast (km), and this observation is drawn using data from 636 Mediterranean locations (Fig. 9). These findings agree with previous modelling studies (Liubartseva et al., 2018; Mansui et al., 2015) on the effective plastic dilution trend along the Mediterranean Sea's surface waters, facilitated by the basin's high surface dynamics, that prevent the formation of permanent accumulation areas (Mansui et al., 2015).

The geographical distribution of surface water stations (Fig. 2) indicates a gap of knowledge on the MP pollution levels in the southern coast of the basin and in off-shore waters, specifically in the Central Mediterranean and the Levantine basin. Equally important as the spatial distribution of the sampling efforts is the temporal trend. Few studies investigated the seasonal variability of floating plastic debris in the Mediterranean Sea (van der Hal et al., 2017; Bainsi et al., 2018; Compa et al., 2020). Seasonal patterns, at local and regional scales, on the temporary retention of floating plastic debris were predicted by Mansui et al. (2020), who described three larger retention areas located east of the Balearic Islands, in the central Tyrrhenian Sea, and along the Tunisian and Lebanese coast during summer and autumn. Seasonal and interannual variability is critical to understand the physical transport and accumulation patterns of floating MPs. The detection of main circulation features (i.e., permanent and transient cyclonic and anticyclonic gyres) during sampling efforts may also contribute to our understanding of the accumulation and dissipative trends of these pollutants (Brach et al., 2018) in the surface waters of the Mediterranean Sea.

We observed a decrease in the sampling efforts in the marine sediment compartment with depth. Few studies investigated MP occurrence in deep-sea sediment, while most of the efforts focused on the continental margins. In the Mediterranean Sea, continental shelf areas play an essential role, representing up to 20% of the basin's surface (Pinardi et al., 2004). These areas are characterized by highly dynamic biogeochemical processes, the presence of critical endemic ecosystems, and tremendous biodiversity (Coll et al., 2010; Muller-Karger et al., 2005). Thus, it can be expected that plastic pollutants in these areas may pose a higher environmental risk. The nearness to populated areas represents a continuous input of plastic debris to the marine environment. From these inputs, high sinking plastic flux (1 kg km<sup>-2</sup> day) occurs close to the

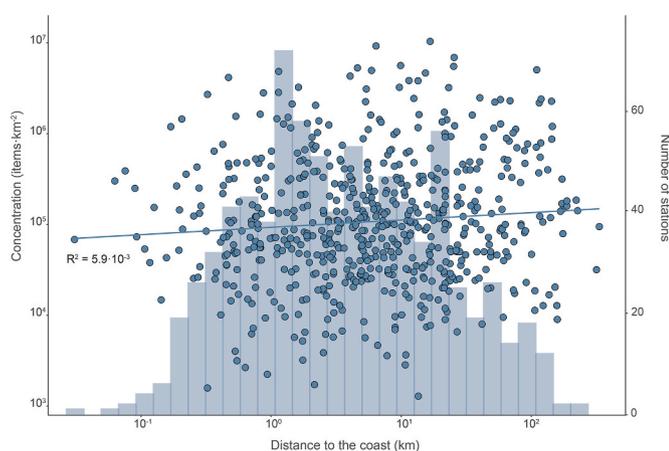
coast (Kaandorp et al., 2020) in the Mediterranean Sea. Biogenic habitats likely sequester those particles in coastal ecosystems (de Smit et al., 2021). For example, seagrass meadows (*Posidonia oceanica*) trap plastic and MP debris and contribute to their removal by aggregating these pollutants within vegetal fibers that are washed up back to the coast (Sanchez-Vidal et al., 2021).

In the Mediterranean Sea, submarine canyons play an essential role in the exchanges between the continental shelf and the deep sea. These geomorphic features of the continental margins are subjected to ephemeral gravity currents, responsible for transporting terrestrial sediments, organic carbon, and recently also MPs to the deep-sea floor (Fernandez-Arcaya et al., 2017; Pohl et al., 2020). High loads of plastic litter were reported in the submarine canyons of the Mediterranean Sea, which are transported down-slope and expected to accumulate at depth (Ramirez-Llodra et al., 2013; Tubau et al., 2015). However, once on the deep-sea floor, bottom currents lead to the erosion, transport, and deposition of sediment (Stow et al., 2019). In the Tyrrhenian Sea, Kane et al. (2020) showed the strong influence of near-bed thermohaline currents on the fate of MPs. Therefore, focusing research efforts to coastal areas may bias our understanding of MPs occurrence in the Mediterranean sediments. Future studies need to understand the MP presence within a bathymetric gradient, considering the Mediterranean physiographic settings, vertical settling fluxes, and deep-sea currents that may lead to the final accumulation of MPs in this environment. In the Mediterranean Sea, the deep sea represents the 80% of the basin, MP impacts to this environment may occur imperceptibly, as the remoteness of the deep sea still limits our knowledge on the biodiversity inhabiting it (Danovaro et al., 2010).

## 5. Perspectives

Detailed assessment of MP pollution is challenging, and while the lack of standardized methods persists in the field, researchers should be responsible for providing standardized results, and to highlight the limitations of their study (Gago et al., 2016; Hartmann et al., 2019; Provencher et al., 2020). Under this context, to advance the knowledge of MP pollution in the abiotic compartments of the Mediterranean Sea and to understand the environmental risk that these pollutants pose to its biodiversity, major research questions/challenges need to be addressed.

- Combining efforts. Here, we have discussed the limitations of characterizing the whole spectrum of MPs while acknowledging the vast amount of work developed in the past years. Ideally, studies should target the whole MP spectrum, however temporal and economic constraints are evident. Thus, MP pollution research in the Mediterranean region will be highly benefited if the data produced – sampling parameters (i.e., coordinates, sea state, wind, depth, etc.), abundances, particle's size, polymer, shape distribution – will be made available in open data repositories.
- Quantifying MP pollution. The numerous research efforts conducted in the Mediterranean Sea provide an excellent opportunity for future monitoring of the temporal trend on the abundance of these pollutants within the basin. Future studies need to consider within their objectives to apply similar approaches for the comparison of data already produced for the Mediterranean Sea. Notwithstanding, implementing good quality practices and quality control protocols will further validate the accuracy of the future knowledge on MP occurrence in the basin.
- Spatial distribution on marine MP data. Future sampling efforts need to consider sea surface features, and seasonal and interannual variability within the Mediterranean general circulation. There is also an urgent need to define the MP pollution levels in the North African coast and the Tyrrhenian, Aegean, and Levantine basins.
- Assessing MP sinking/export in the water column. Very little is still known about the dynamics of MPs from the sea surface to the



**Fig. 9.** Plastic particle concentration (items·km<sup>-2</sup>) reported for 636 Mediterranean Sea surface water data points, logarithmically transformed (left Y-axis, blue dots) and plotted in relation to the distance (km) logarithmically transformed. The barplot indicates the number of sampling stations (#unit, right Y-axis) in relation to the distance to the coast. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

seafloor. The role of the water column is indubitable. Holistic approaches should be considered to gain comprehensive knowledge on the physical and biological processes distributing these pollutants across different environmental compartments. Understanding the MP abundance in the epipelagic and mesopelagic layers provides baseline knowledge to the MP exposure that fish stocks inhabiting these layers of the ocean are facing. This is particularly relevant, as crucial commercial fishing species for human consumption thrive in these layers.

- Assessing the accumulation of MPs. The deep-sea sediments are considered a major reservoir of MP pollution in the marine cycle. In the absence of studies linking geophysical processes with MP occurrence in sediments, the data is restricted to presenting only a snapshot of the potential exposure. However, to further understand the risk of these pollutants buried in the deep ocean, we need to gain knowledge of their patterns (accumulation, dilution, resuspension) and the hazard they might represent to the Mediterranean benthic communities.
- Caring about the macro-sized fraction. The effect of plastic pollutants is detrimental in all its size distribution, from macro to nano. The role of macroplastics as a source of MPs cannot be overlooked, as well as the socio-economic impacts that this size fraction represents.

Our growing knowledge in the last decade shows the ubiquitous presence and high MP pollution levels in the Mediterranean Sea. The floating MP concentrations point to this basin as one of the most plastic polluted regions (Cózar et al., 2015; Suaria et al., 2016), already surpassing levels that pose an environmental risk (Everaert et al., 2020). As long as business continues as usual, without a significant reduction on single-use plastics and relevant investment in minimizing waste mismanagement, we can expect plastic pollution levels to increase (Lebreton and Andrady, 2019). Even if the methods constrain our understanding of the MP pollution issue in the Mediterranean basin, there is an urgent need to dedicate research efforts to produce quality, open, and comparable data on the occurrence of these pollutants, promoting broad basin-scale international collaboration. Only through quality science, well-informed and engaged politics, stakeholders, and society, effective measures, actions and regulations can be implemented to tackle the challenge of plastic pollution.

#### Author statement

LSS, MG and PZ conceived the study; LSS reviewed the literature, collected the data, performed the data analysis, elaborated the figures, and wrote the primary manuscript; MF collected the data from the sea surface water studies in the North-Western Mediterranean; MG contributed to the data analysis; MG and PZ provided supervision, contributed to the writing and editing of the final manuscript.

#### 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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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2021.118275>.

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