Plastic Litter in the Adriatic Basin

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1. Introduction

The biggest landfill of our planet is the ocean. The sea accumulates various types of wastes, their ensemble takes the name of *marine litter* which is defined as "any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment" (UNEP, 2009). Indeed, marine litter was introduced by the Marine Strategy Framework Directive (MSFD - Directive 2008/56/EC) as one of the 11 descriptors to define marine ecosystems' environmental status and to target the Good Environmental Status in 2020 (Galgani *et al*. 2013).

Far from being an aesthetic problem, this massive invasion of marine debris constitutes an environmental and economic threat which, together with other global key issues such as overfishing, global warming and ocean acidification, seriously jeopardize the biodiversity of marine ecosystems and the goods and services they provide (CBD, 2012; Suaria & Aliani, 2014; Sutherland *et al*., 2010).

Types and quantities of marine litter vary considerably across marine regions due to different hydrodynamics, geomorphologic and anthropic factors, being the enclosed seas, such as the Mediterranean Sea, the most affected areas in the world (UNEP/MAP, 2015).

Solid waste enters the seas mostly from land-based sources, and about the 80% of plastic waste is from terrestrial sources and originates from accidental or intentional dumping and poor waste management systems in coastal regions (Andrady, 2011; Bonanno and Orlando-Bonaca, 2018).

Nevertheless, marine-based sources play an important role too through unintentional or illegal unloading of waste from ships, including fishing boats, and accidental losses of fishing gear (Bonanno and Orlando Bonaca, 2018; Ryan, 2015;).

Despite the great variety of waste materials (plastics, metals, glass/ceramics, wood, rubber, textiles, paper, etc.) found in the marine environment, plastics and other artificial polymers are predominant worldwide (Derraik, 2002) and they represent the most common types of marine litter (Fossi *et al*., 2020). The proportion of plastic consistently varies between 60% and 80% of the total marine litter, overcoming 90% in some regions (Derraik, 2002).

Plastic litter comprises such diverse items with different shapes and sizes (ranging from meters to nanometers) which can vary according to their origin and use: fishing gear, bottles, bags, food packaging, taps, straws, industrial pellets. Once dispersed in the marine environment, plastic items can float on the surface, drift in the water column, sink on the seafloor or settle on the beaches, depending on their size and their chemical-physical characteristics. Thus, interacting with different ecological niches and different species, both pelagic and benthic, and posing potential risks to marine biodiversity.

Macro litter sampling are carried out using different methodologies based on the characteristics of the sampling area. On the coastlines, macro litter is usually collected by hand (de Francesco *et al*., 2018; Munari *et al*., 2016; Vlachogianni *et al*., 2017), while at seabed macro litter is usually collected by scubadiving (Macic *et al*., 2017; Vlachogianni *et al*., 2017) or using bottom trawls (Fortibuoni *et al*., 2019; Pasquini *et al*., 2016; Strafella *et al*., 2015; Strafella *et al*., 2019) (Figures 1-2).

Generally, seabed debris is much less investigated in respect to the sea surface and shores due to sampling difficulties and costs (Strafella *et al*.,2019). However, detecting marine benthic litter is fundamental for developing policies aimed at achieving the Good Environmental Status in European Seas by 2020, as requested by the Marine Strategy Framework Directive.

For what concerns the macro litter floating in seawater, some studies report visual census as main sampling methodology (Arcangeli *et al*., 2018; Suaria and Aliani, 2014; Vlachogianni *et al*., 2017).

Instead of visual survey, also retention booms as well as bottom trawling are used to capture floating marine litter (Schneider *et al*., 2018). In addition, in the last years, numerous innovative technologies have been developed in order to effectively recover macro litter from the surface of the sea. For example, the so called "Pelikan" is an ecological vessel designed by Garbage Group (Ancona, Italy) for the collection from the surface of the sea of floating and semi-submerged marine litter (https://garbagegroup.it/).

Macro litter can also mechanically interact with different marine organisms and potentially impact their health status, for example through entanglement or ingestion of plastic debris (Biagi *et al*., 2021; Di Renzo *et al*., 2021; Fossi *et al*., 2020). Some studies also investigated the role of floating marine litter as a raft for drifting voyages for some crustaceans (Tutman *et al*., 2017). In fact, drifting debris has been found also to act as a dispersal vector for invasive marine species (Barnes, 2002; Barnes and Fraser, 2003; Barnes and Milner, 2005; Gregory, 2009), bloom-forming algae (Masó *et al*., 2003), bacterial pathogens (Carson *et al*., 2013; Harrison *et al*., 2011; Zettler *et al*., 2013).

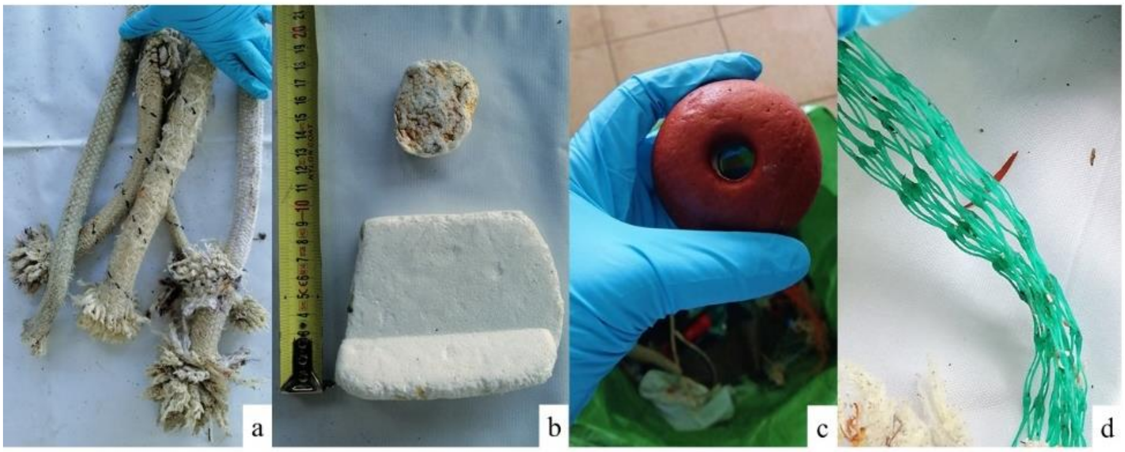

Fig. 1 *Wastes related to marine-based sources, in particular fishing activities, collected during a beach cleaning along the Conero Riviera (AN); a) fishing lines; b) polystyrene; c) fishing float; d) mussel net.*

Fig. 2 *Manual collection of marine litter. Beach cleaning carried out in November 2020 along the Conero Riviera coastline (43°35'11''N 13°33'53''E). a) Polystyrene and "Rapido" trawls; b) mussel net, pacifier, lighter and orange synthetic fabric; c) polystyrene and footwear; d) collected marine litter.*

These persistent plastics, which have an estimated lifetime for degradation of hundreds of years, floating in the sea can be dispersed over long distances even in areas far away from the pollution sources (Bonanno and Orlando-Bonaca, 2018) and for the effect of winds, currents, high temperature and intense sunlight can break up into smaller pieces. When the plastic fragments are smaller than 5 mm, these fragmentation debris are called microplastics (MPs).

In recent years, the growing presence of MPs in marine environments has provoked increasing concern, particularly considering their prevalence in water, sediment and biota (Schmid *et al*., 2021). In fact, the small size of MPs facilitates their uptake by marine biota throughout the food chain, from planktonic communities to large mammals (Avio *et al*. 2015; Collard *et al*. 2017; Dehaut *et al*. 2016; Fossi *et al*. 2016), accumulating through the food web.

Moreover, MPs can release in the environment some plastic additives (flame retardants, phthalates, colorants) including some known endocrine disruptors that may be harmful at extremely low concentrations (Gallo *et al*., 2018) and may cause adverse physiological effects to marine biota.

MPs can also act as a carrier of other environmental pollutants, such as persistent organic pollutants (POPs) which are present in seawater and can efficiently adsorb to plastic items' surface (Engler, 2012; Endo *et al*., 2005; Teuten *et al*., 2009). These pollutants can even have a potential toxic outcome becoming bioavailable or even bioaccumulating in organisms after the ingestion of MPs (Andrady *et al*., 2021). Lastly, MPs can act as a vector of non-indigenous marine species, that can be transported over long distances.

The occurrence of MPs in the marine environment has been investigated both in biota and environmental samples through different sampling methods. The environmental sampling can be conducted in different matrix such as: beach (manual sampling, using sieve), seawater, using Manta trawl (Figure 3-4) (Atwood *et al*., 2019; de Lucia *et al*., 2018; Palatinus *et al*., 2019; Ruiz-Orejón *et al*. 2016; Vianello *et al*., 2018; Zeri *et al*., 2018), Neuston net (Suaria *et al*., 2016), or Niskin Bottle and seabed using for example a box-corer (Vianello *et al*., 2013) or a Van Veen grab (Mistri *et al*., 2017). In Figure 5 are showned different types of meso- and microplastics which are usually collected during beach cleaning and represent some of the most frequently found items, such as: fragments deriving from bigger plastic items (mussel nets, cotton bud sticks, other plastics), pellets, little pieces of expanded polystyrene, pieces of fishing lines and plastic sheets, collected during a beach cleaning in Falconara (AN) in April 2021.

Assessment of plastic ingestion in marine species has become a research priority in the context of the Marine Strategy Framework Directive, MSFD, 2008/56/EC, Descriptor 10, Indicator 10.1.3 (Bellas *et al*., 2016). Ingestion of microplastics has been demonstrated in various marine organisms with different feeding strategies; this phenomenon may negatively influence both the feeding activity and nutritional value of a plankton-based diet, particularly in those species which cannot discriminate the food source (Browne *et al*., 2008; Moore *et al*., 2001).

The UNEP/MAP recognized the importance of defining appropriate bioindicator organisms for measuring the prevalence and effects of (micro)-plastic ingestion (UNEP/MAP, 2019). A panel of seven general criteria has been proposed to select species for assessing the impact of marine litter on Mediterranean biodiversity (Fossi *et al*., 2018). Such criteria are based on background information on the organisms, habitat, trophic level and feeding behaviour, spatial distribution, commercial importance and conservation status, documented ingestion of marine litter (Fossi *et al*., 2018).

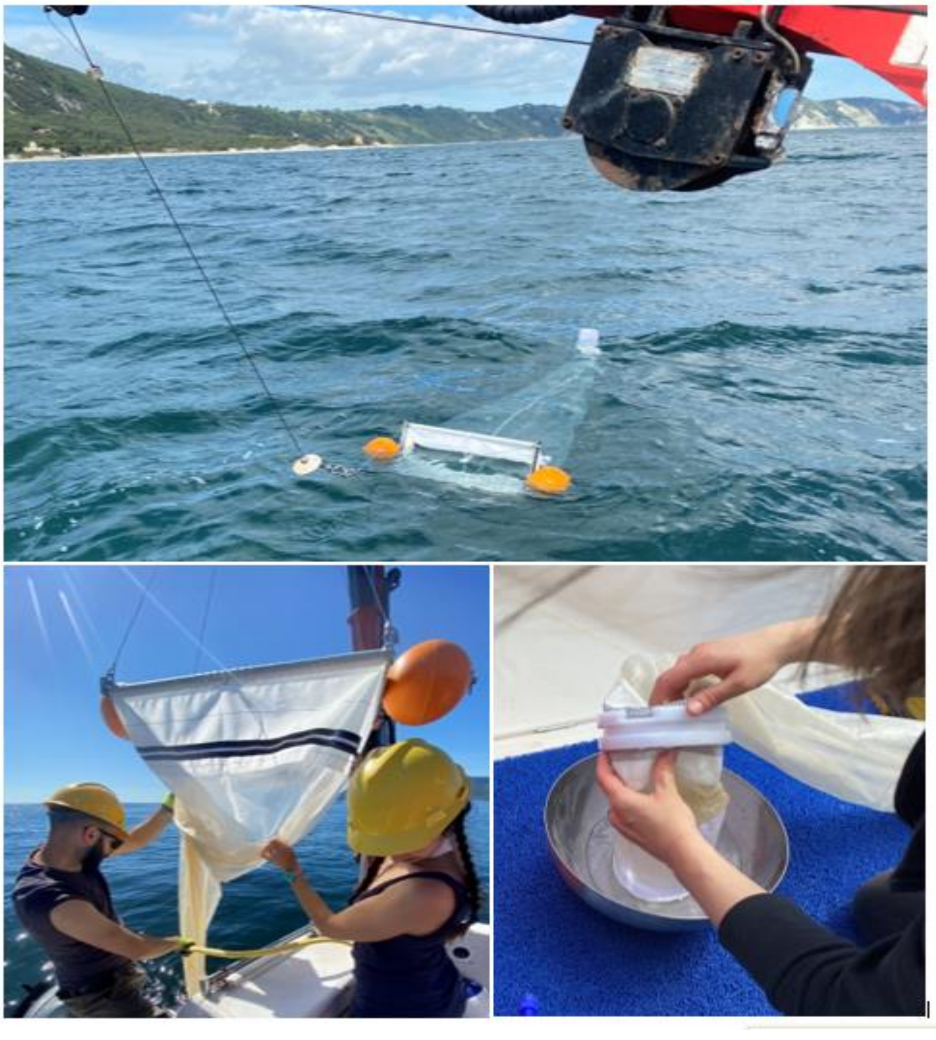

Fig. 3 *Monitoring the occurrence of microplastics in the first 15-25 cm of surface water layer using a Manta trawl. Sampling performed in May 2020, operating transects in several stations located along the Conero Riviera (Ancona coast, Marche, Italy).*

Fig. ***4*** *Cotton bud sticks and mussel nets collected by hand during beach cleanings along the Conero Riviera (Ancona coast, Marche, Italy).*

Fig. 5 *Different types of meso- and microplastics (e. g. fragments, pellets) collected during a beach cleaning in Falconara (AN) in April 2021.*

2. *Plastic litter in Mediterranean Basin*

Mediterranean Sea, whose basin is surrounded by countries with massively waste-generating human activities, represents not only a worldwide biodiversity hotspot, but also one of the areas most impacted by marine litter (Fossi *et al*., 2017; Suaria *et al*., 2016; UNEP/MAP, 2015; van Sebille *et al*., 2015). The marine litter problem in the Mediterranean is exacerbated by the basin's limited water exchanges with other oceans, a highly developed coastal tourism, densely populated coasts, busy offshore waters (with 30% of the world's maritime traffic), waste disposal sites often located close to the coast, high temperatures accelerating litter degradation into difficult-to-collect secondary products, and litter inputs from large rivers.

Marine litter and in particular floating plastics have been found in the Mediterranean Sea in comparable quantities to those found in the five oceanic garbage patches (Fossi *et al*., 2019). In this respect, studies based on global models have proposed the Mediterranean Sea as the sixth greatest accumulation zone for marine litter worldwide (Baini *et al*., 2018; Fossi *et al*., 2017; Panti *et al*., 2015; Suaria *et al*., 2016; van Sebille *et al*., 2015). The abundance of microplastics in marine habitats increases and 115,000–1,050,000 particles/km^2 are estimated to float in the Mediterranean Sea (Fossi *et al*., 2012; Suaria *et al*., 2016; UNEP/MAP, 2015; Zeri *et al*., 2018).

The ingestion of plastics by marine species is one the most documented impacts in the Mediterranean (Fossi *et al*., 2018); in the last 5 years, more than 40 papers on the incidence of marine litter ingestion in marine organisms in this basin have been published (Fossi *et al*., 2019).

The MSFD divides the European marine waters into four Marine Regions (Article 4): the Baltic Sea, the Black Sea, the Mediterranean Sea and the North-east Atlantic Ocean. In the Mediterranean, four subregions have been identified, and Italian seas are included in three of these: The Adriatic Sea (MAD); the Western Mediterranean Sea (MWE); The Ionian Sea and the Central Mediterranean Sea (MIC) (Fortibuoni *et al*., 2021).

Most of the studies conducted to date, have been focusing on beach litter (Ariza *et al*., 2008; Gabrielides *et al*., 1991; Golik and Gertner, 1992; Kordella *et al*., 2013; Martinez-Ribes *et al*., 2007; Shiber, 1982; Shiber, 1987; Shiber and Barrales Rienda, 1991; UNEP/MAP/MEDPOL, 2009), and on the accumulation of marine debris on the sea floor (Galgani *et al*., 1995, 1996, 2000; Güven *et al*., 2013; Mifsud *et al*., 2013; Sánchez *et al*., 2013). Floating debris has received less attention. Four studies dealt with the abundance of neustonic microplastics (Collignon *et al*., 2012; Fossi *et al*., 2012; Hagmann *et al*., 2013; Kornilios *et al*., 1998), and only a few studies have been published on the abundance of floating macro and mega debris in Mediterranean waters (Aliani *et al*., 2003; Morris, 1980; Topcu *et al*., 2010; UNEP/MAP/MEDPOL, 2009).

Moreover, most of the research has been carried out in the Western Mediterranean Sea, whereas the Ionian Sea and the Central Mediterranean Sea, the Adriatic Sea, and the Aegean Levantine Sea are less investigated (Fossi *et al*., 2019).

According to a study conducted by Fortibuoni *et al*., (2021), which investigates the occurrence of marine litter along Italian coasts (Adriatic Sea, Western Mediterranean Sea, Ionian Sea and Central Mediterranean Sea), subregional differences emerged both in terms of litter quantities and composition. In particular, the Adriatic Sea was the most polluted subregion (590 items/100 m), followed by the Western Mediterranean Sea (491 items/100 m) and the Ionian Sea and Central Mediterranean Sea subregion (274 items/100 m). The most common macro-category on Italian beaches was plastic and polystyrene (74%). Specifically, the numbers of cotton bud sticks were extremely high in some beaches of the Western Mediterranean Sea; while the aquaculture-related litter (mainly mussel nets) turned out to be the items more frequently observed during the samplings performed along the Adriatic coastline. In particular, Figure 4 shows some cotton bud sticks and mussel nets, collected during a beach cleaning on the Conero Riviera (AN) coastline in 2021. These specific plastic items represent two of the most abundant typologies of plastic litter that can be easily found on the west coasts' beaches of the Adriatic. Also according to the investigations of Suaria *et al*. (2014), based on visual surveys of marine floating debris in the Mediterranean Sea, the maximum densities of anthropogenic floating debris (>52 items/km^2) were found in the Adriatic Sea, while the lowest densities (<6.3 items /km^2) were found in the Thyrrenian and in the Sicilian Sea. Figure 6 clearly represents a map of the central-western Mediterranean Sea and shows the study area selected by Suaria *et al*. (2014) which is divided in different sectors named by letters from "A" to "N". In particular, the Figure shows the distribution of both anthropogenic marine debris (AMD) represented by black bars and natural marine debris (NMD, e.g. wood: mainly logs, trunks, branches and canes; algae or others: egagropili of *Posidonia oceanica* L. Delile, cuttlebones, bird feathers, sponges or pumice) represented by white bars in all the 167 transects' locations. The marine debris' densities are expressed as number of items/km2 in all surveyed transects. From this Figure is possible to deduce how the two Mediterranean sectors "A" and "B" which correspond to the Adriatic Sea, together with sector "M" (Algerian basin) reveal the maximum densities of AMD.

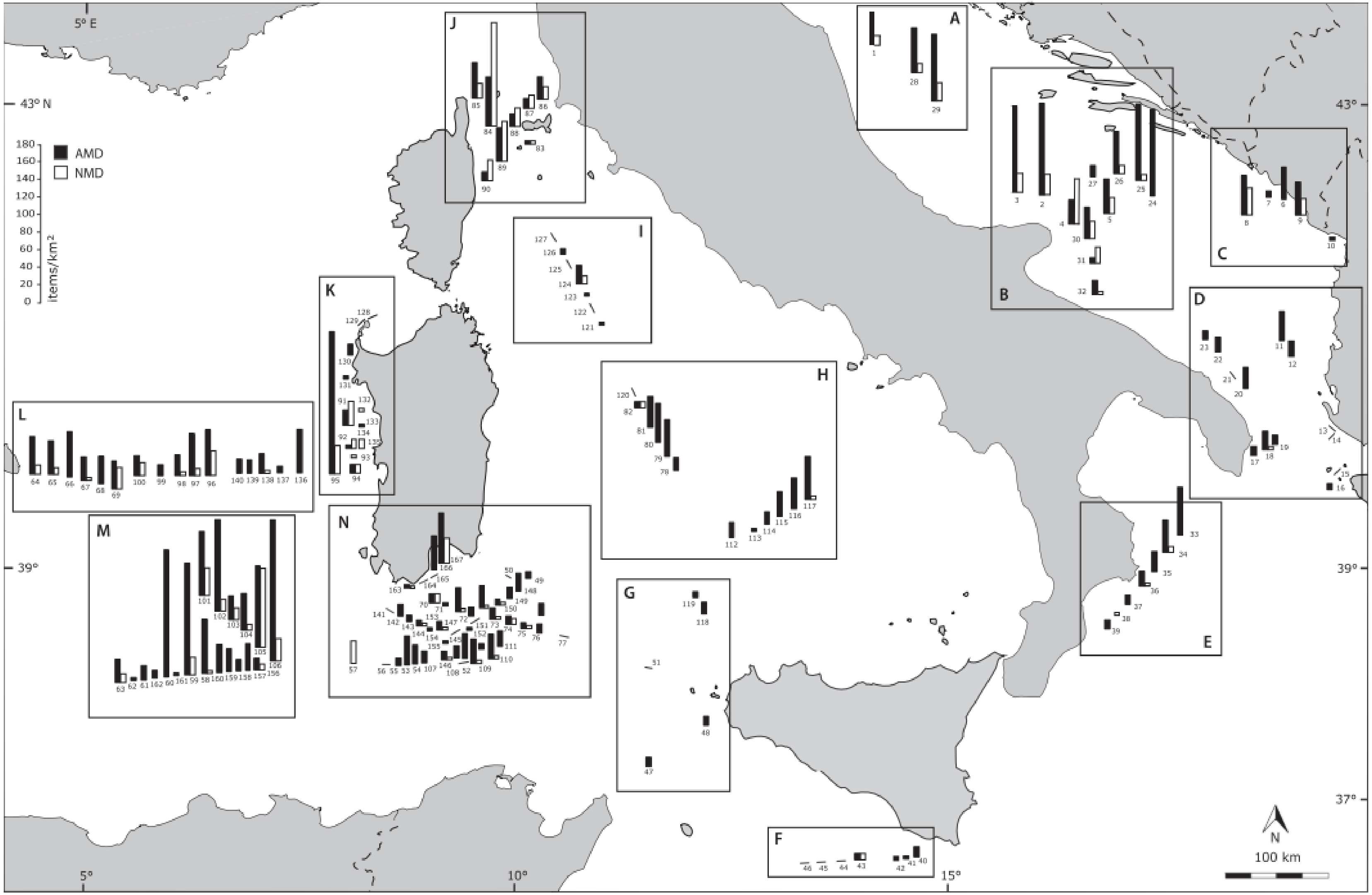

Fig. 6 *Map of the central-western Mediterranean Sea showing the study area, the location of all transects and sectors and the distribution of anthropogenic marine debris (AMD, black bars) and natural marine debris (NMD, white bars) densities (expressed as number of items/km2)in all the visual surveyed transects (Suaria et al., 2014).*

Figure 7 shows in detail the mean densities of AMD and NMD (expressed as numbers of items per km2 ± s.e.) in all surveyed sectors of the study area. The relative proportions of all AMD and NMD type categories are also shown in the pie charts.
As it is possible to see from this Figure, all the sectors of the Adratic basin show not only high level of AMD but also considerably high densities of NMD, that could be due to multiple interacting factors such as: the total river discharge (Po River in the northern basin, Albanian rivers in the southern basin) (UNEP/MAP, 2012), the high density of population of the shores (more than 3.5 million people) with fisheries and tourism acting as significant sources of income all along the Adriatic coast and the Maritime traffic. In all the other sectors mean AMD densities ranged from 10.9 to 30.7 items/km2.

For what concerns the composition of the sighted marine litter, plastic was the most abundant type of debris in all sampled locations, accounting for 82% of all man-made objects and confirming in this way the overwhelming presence of plastic debris on the surface of the Mediterranean Sea (Suaria *et al*., 2014). On the whole, 95.6% of all man-made objects (74.7% of all sighted objects) were petrochemicals derivatives (i.e. plastic and expanded polystyrene). The highest abundances of expanded polystyrene (mean 10.2 items/km2) were observed in the Adriatic Sea (sectors A, B, C and D), with the highest values (peaking to a maximum of 34.75 items/km2) reported from sector B (Southwestern Adriatic). This could be due to the intense fishing and aquaculture activities which characterize this area, which are an important source of expanded polystyrene in the marine environment (Abu-Hilal and Al-Najjar, 2004; Cho, 2005; Fujieda and Sasaki, 2005; Hinojosa and Thiel, 2009). Even if the Adratic basin seems to be, considering the already conducted investigations, a preferential area for marine litter accumulation, (due to the land inputs, impacting this basin such as: rivers runoff, industrialized area along the coasts, intense touristic activities during the summer and aquaculture, fishing, and port activities and also due to its geomorphological characteristics), there is a gap in the current scientific literature regarding the presence of marine litter in this part of the Mediterranean Sea.
Most of the existing studies concern the north-western part of the Adriatic Sea and, in particular, they investigate those areas which are close to the Po delta or to industrialized areas. In this sense, the southwestern area of the Adriatic Sea corresponds to the area where less scientific studies have been conducted.

Consulting the " Online Portal for Marine Litter" (https://litterbase.awi.de/litter), which summarize results from 2,812 scientific studies about marine litter in understandable global maps and Figures, it is possible to have a snapshot of the current investigations about the occurrence of marine litter and microplastics and their interactions with biota, in all over the world. One of the two global maps available on this website shows the distribution of litter and microplastics and their quantities, which were taken from 1,308 publications. Marine litter quantity is expressed using the commonly used units: items / km^2; items / km; items / m^3. For what concerns the Italian Region (Figure 8), it is possible to deduce from the map that: a) plastic is the major type of litter that occurs; b) most of the research are conducted in the Ligurian Sea, in the Northern Tyrrhenian Sea and in the in the North-Western Adriatic; c) the South-western part of the Adriatic Sea is less investigated.

The second global map available on *Litterbase* currently comprises 1,772 scientific publications on interactions of organisms with litter. Interaction refers to encounters between wildlife and litter items. Ingestion was the most frequently observed interaction, followed by entanglement, which affects motility, often with fatal consequences. In the map, four different typologies of interactions are summarized: *colonisation* (purple), *entanglement* (orange), *ingestion* (green) and *other* (yellow). Zooming in on the Mediterranean Sea (Figure 9), it is possible to see, even in this case, how the most investigated areas correspond to the Ligurian Sea, the Tyrrhenian Sea and the the North-Western Adriatic. While very few studies, investigating the interaction between marine litter and biota, were conducted in the central-southern Adriatic.

In fact, few are the studies aimed to investigate the occurrence of marine litter in the Adriatic basin in seabed, in seawater or along the coastline and the presence of microplastics in both environmental samples (Arcangeli *et al*., 2018; Blašković *et al*., 2017; Carlson *et al*., 2017; de Francesco *et al*., 2019; de Lucia *et al*., 2018; Fortibuoni *et al*., 2019; Korez *et al*., 2019; Mistri *et al*., 2017; Munari *et al*., 2016, 2017; Palatinus *et al*., 2019; Pasquini *et al*., 2016) and biological samples (Anastasopoulou *et al*., 2018; Avio *et al*., 2020; Biagi *et al*., 2021; Di Renzo *et al*., 2021; Giani *et al*., 2019; Gomiero *et al*., 2019; Martinelli, *et al*., 2021; Pellini *et al*., 2018; Piarulli *et al*., 2019).

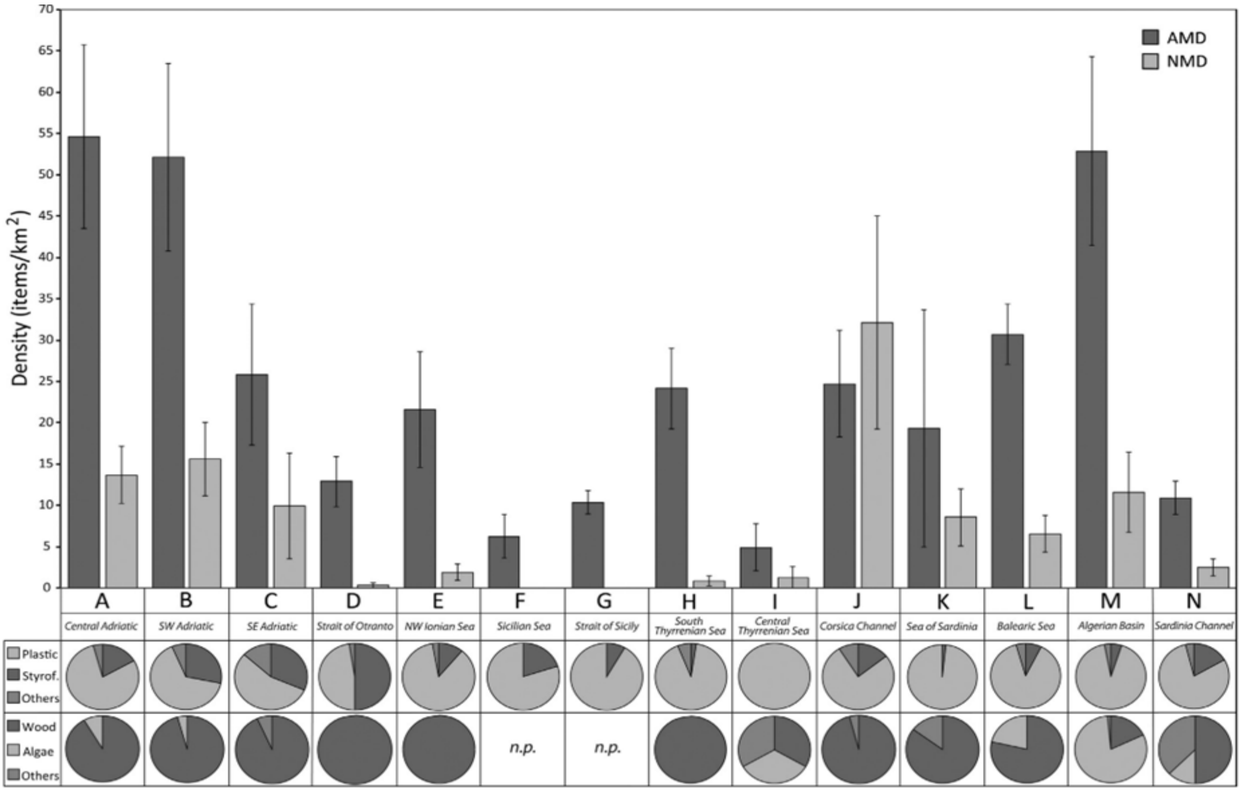

Fig. 7 *Mean densities of anthropogenic marine debris (AMD, dark grey bars) and natural marine debris (NMD, light grey bars) (expressed as numbers of items per km2 ± s.e.) in all surveyed sectors of the study area. The relative proportions of all AMD and NMD type categories are also shown in the pie chart (Suaria et al., 2014).*

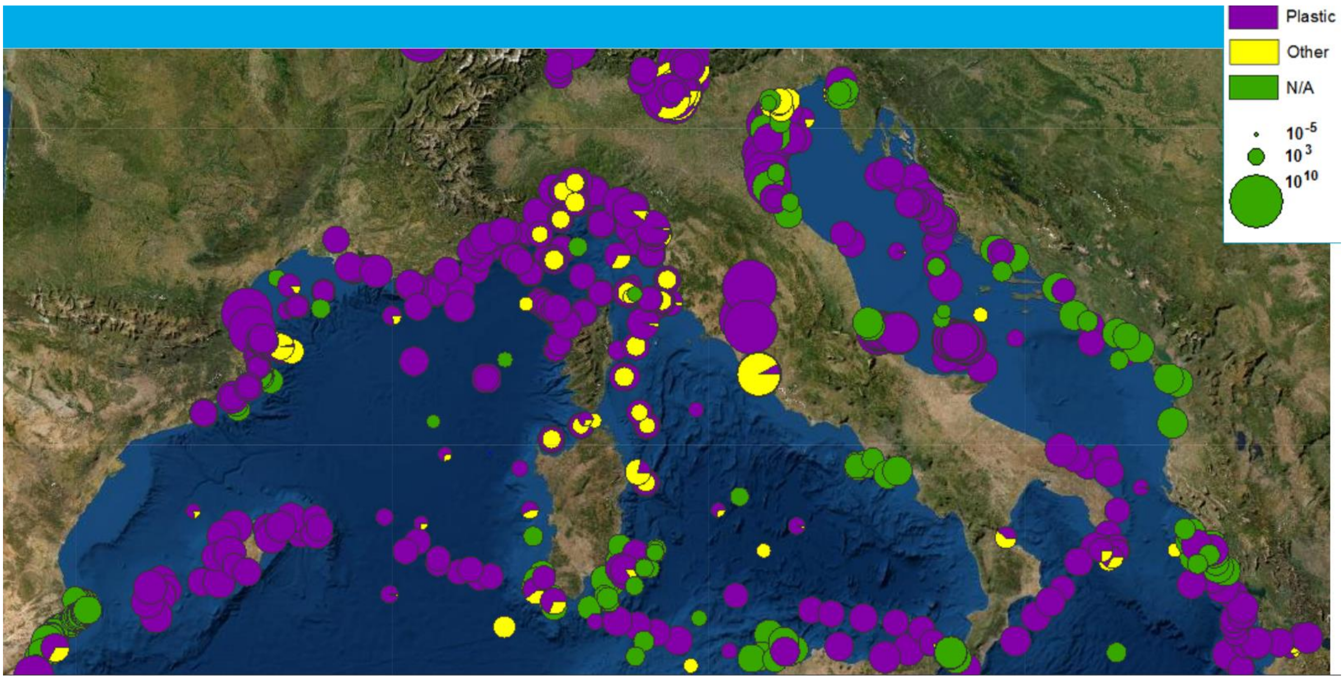

Fig. 8 *Map showing scientific publications on the amount, distribution, density (litter unit: items / km²; items / km; items / m³) and composition of litter in the Mediterranean Sea (https://litterbase.awi.de/litter). Purple circles represent plastic litter, yellow circles other type of litter and green circles not identified litter.*

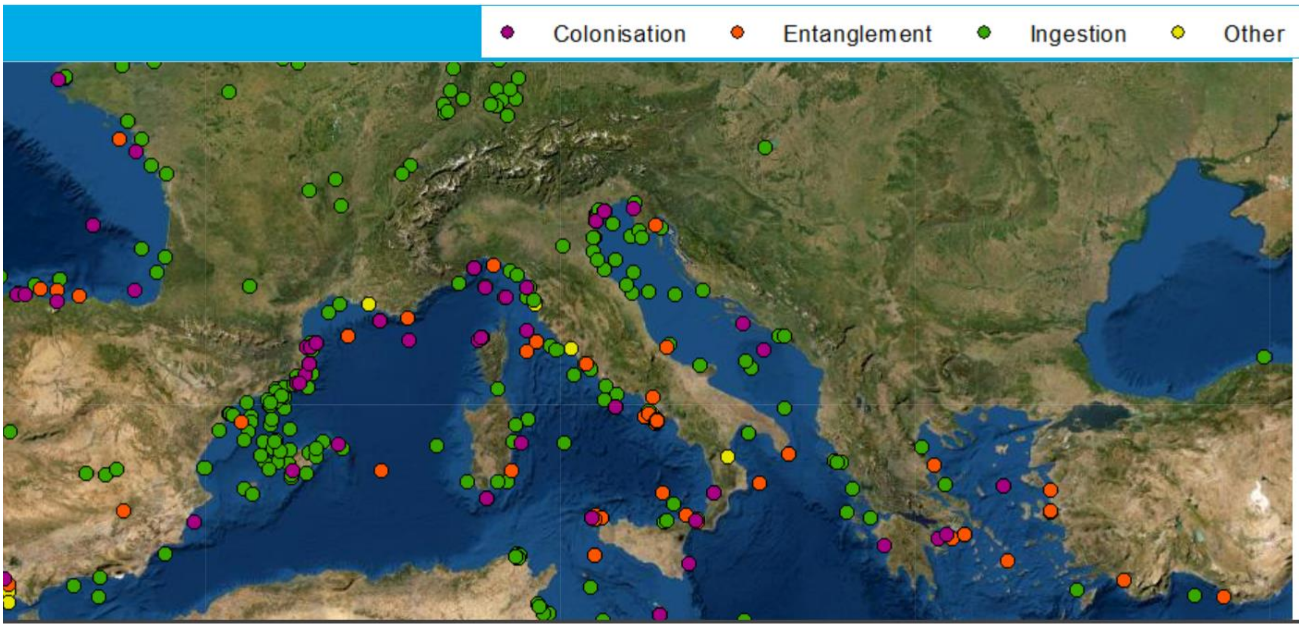

Fig. 9 *Scientific publications on interactions of organisms with litter in the Mediterranean Sea (https://litterbase.awi.de/litter). Interaction refers to encounters between wildlife and litter items. Ingestion was the most frequently observed interaction, followed by entanglement, which affects motility, often with fatal consequences. In addition, many species can settle on floating litter*

3. Focus on plastic litter in Adriatic Basin

The Adriatic Sea, which consists in a narrow elongated and semi-enclosed sub-basin, whose only water exchange with the Mediterranean Sea takes place through the Otranto Channel (Artegiani *et al*., 1997) is predicted as a preferential area of plastics accumulation within the Mediterranean Sea (Avio *et al*., 2020). This could be due to the high land to sea ratio of this basin (Ludwig *et al*., 2009), the eastern coast is generally high and rocky, whereas the western coast is low and mostly sandy (Gomiero *et al*., 2019) which is affected by the input of several rivers along the Italian coast (the Po river being the most relevant). The northern area is very shallow, gently sloping, with an average depth of about 35 m, while the central part is on average 140 m deep, with the two Pomo Depressions reaching 260 m (Strafella *et al*., 2019). The area is subject to a heavy anthropic pressure due to intense coastal urbanization. In fact, several anthropogenic activities coexist such as tourism, heavy marine traffic from commercial and tourist vessels, offshore activities, ferry boats and trawl-fishing. Moreover, a continuous intensification of mussel farms along the Italian coast and fish farms along the Croatian coast is underway (Coll *et al*., 2007; Fabi *et al*., 2009; Gomiero *et al*.,2019; Ponti *et al*., 2007; Pranovi *et al*., 2016; Punzo *et al*., 2017).

The West Adriatic Current (WAC), flowing toward SE along the western coast, and the East Adriatic Current (EAC), flowing NE along the eastern coast together with wind stress and the river discharges, are the main drivers affecting the Adriatic circulation (Strafella *et al*., 2019).

In fact, a large number of rivers discharge into the basin, with significant influence on the circulation, in particular the Adriatic Sea collects a third of the freshwater flowing into the Mediterranean, mainly via the Po River (Gajst *et al*., 2016) in the northern basin, while particularly relevant is the influence of the Albanian rivers in the southern basin (Artegiani *et al*., 1997). These rivers collect wastewater and rainwater from one of most heavily industrialized area of Europe thus contributing toward the anthropogenic pressure through large loadings of organic and inorganic chemicals, nutrients, and garbage including plastic litter (Gomiero *et al*., 2019). The Adriatic sea’s current circulation could have a determinant role in the dispersion and/or accumulation of marine litter in this basin. Visual surveys of marine debris give us crucial information about the density and spatial distribution of floating anthropogenic litter in a basin, but such observations provide just a “snapshot” of local conditions at a given time and cannot be used to deduce the provenance of the litter or to predict its fate (Carlson *et al*., 2017). Few are the studies that use mathematical models in order to evaluate the possible action of marine currents in the dispersion or creation of area of marine litter’s accumulation, such as the study of Carlson *et al*., (2017). In fact, this study combines litter observations (Visual ship transect surveys) with a regional ocean model (Lagrangian particle tracking model) to identify sources and sinks of floating debris in the Adriatic Sea. In particular, the results of the study indicate that anthropogenic macro debris originates largely from coastal sources near population centres. Then, the litter is advected by the cyclonic surface circulation until it strands on the southwest (Italian) coast, exits the Adriatic, or recirculates in the southern gyre. According to Liubartseva *et al.*, (2016) the majority of floating plastic debris (FPD) is supposed to originate from 68 biggest Adriatic rivers, 45 cities with a population of more than 20000, and 76 most congested shipping lanes. These floating debris inputs are well shown in Figure 10, where shipping lanes, rivers, and cities are highlighted with different symbols and colors. The present work combines mathematical model with the Adriatic Forecasting System (AFS) ocean currents simulations and surface wind analyses (ECMWF) to simulate the plastic concentrations at the sea surface and fluxes onto the coastline that originated from terrestrial and maritime inputs. The analysis of statistics obtained, by the simulations of multiple FPD released during the Adriatic Sea, revealed that the Adriatic Sea is a highly dissipative system. The main FPD sink is the coastline and the mean FPD half-life time is about 43.7 days. However, further consideration of sinking plastic debris is necessary to calculate its distribution on the seabed. Distinctive coastal “hot spots” are found: on the Po Delta coastline that receives a plastic flux of 70.1 kg (km day)-1; Venice: 35.8 kg (km day)-1; Chioggia: 32.6 kg (km day)-1; the Reno Mouth: 25.3 kg(km day)-1; and Pula, situated at the southern tip of the Istria Peninsula: 24.9 kg(km day)-1.

As demonstrated by Schmid *et al*., (2021), most of the scientific investigations conducted, in order to assess the occurrence of marine litter in the Adriatic Sea, are focused on the northern Adriatic Sea (FAO-GSA 17) (Fig. 10). In particular, such areas which are closest to the deltas of the major rivers, (such as the River Po) (Atwood *et al*., 2019; Munari *et al*., 2016, 2017; Piehl *et al*., 2019; Vianello *et al*., 2018) and to the most industrialized cities, are the most studied and probably the most impacted by activities related to fishing, aquaculture and tourism. But farther investigations are needed, especially in the southern part of the basin, in order to fill the current lack of information regarding the presence and the nature of marine litter.

The main part of investigations has been carried out in Italy (as shown by Figure 11), followed by Croatia and Slovenia, others in Montenegro and Albania and few in Greece and Bosnia Herzegovina. The graph in Figure 12 (Schimd *et al*., 2021) reveals that most of the campaigns were carried out between 2014 and 2015.

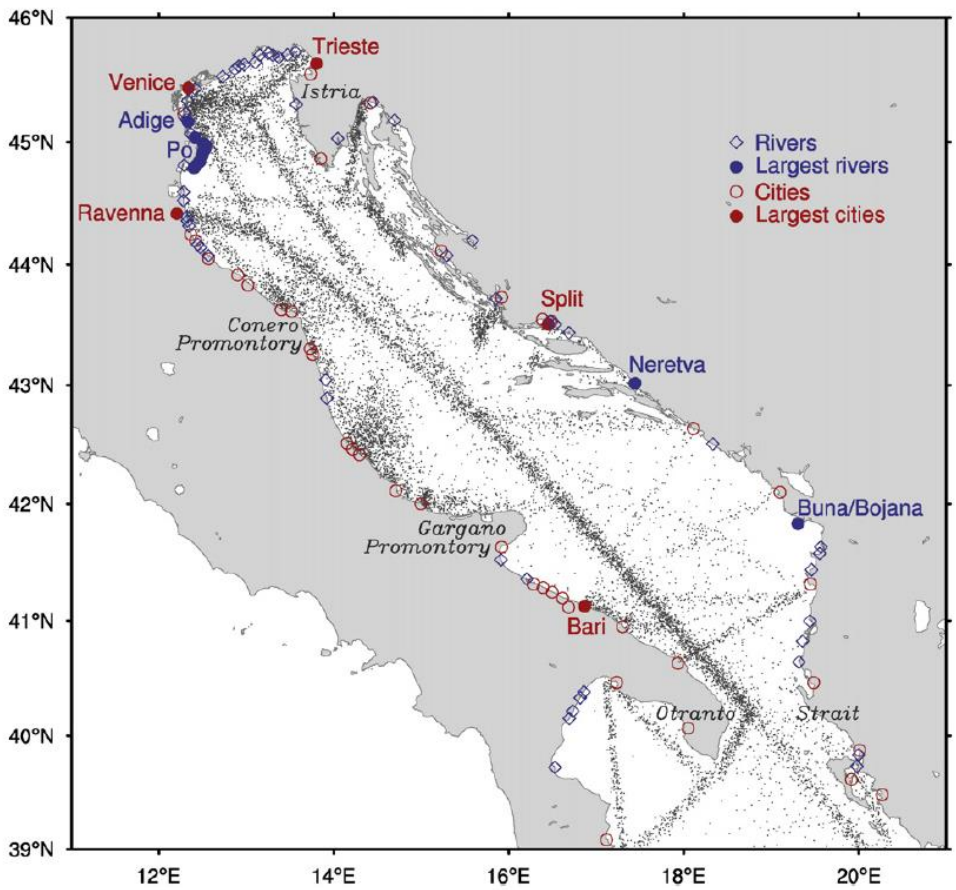

Fig. 10 *Spatial distribution of the floating debris inputs into the Adriatic Basin: shipping lanes (gray scatter plot); rivers (open blue diamonds), the largest rivers (closed blue circles); cities (open red circles); and the largest cities (closed red circles) (Liubartseva et al., 2016)*

Fig. 11 *Geographical distribution of the research concerning beach monitoring campaigns for the presence of marine litter In the Adriatic Sea. The two areas that received more attention are highlighted with rectangles, in particular the blue arrow shows the area of the Po Delta while the black one shows the coasts of Slovenia. (Schmid et al., 2021)*

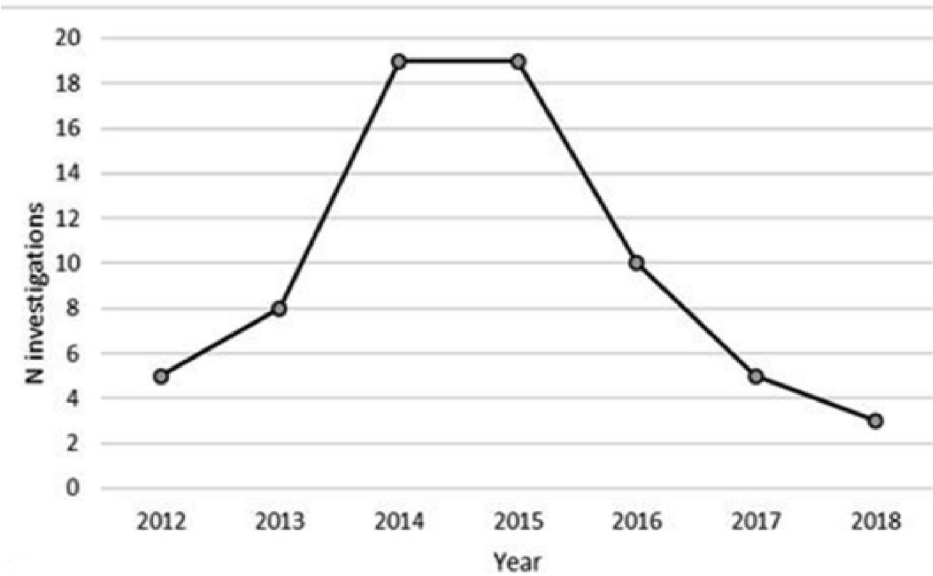

Fig. 12 *Number of investigations published for each year, regarding the occurrence of marine litter in the Adratic Basin (Schmid et al., 2021)*

3.1 Macroplastic in Adriatic Basin

Considering macro litter, samplings in the Adriatic Sea are carried out in different compartment of marine environment, such as: coastline, seawater and seabed.

In particular, for what concerns macro litter on beaches, its collection is usually done by hand (Table 2) following a standardized methodology according to the MSFD protocol (European Parliament and Council, 2008), i.e., detailing the number of objects collected per 100 m. The distinction of marine litter collected on the beach is made by dividing the different typologies of wastes in categories (Galgani *et al*., 2013).

For most of the works, the concentration of marine litter on the beach is expressed as items / m2, and only for some of them the weight of the litter is also indicated (Laglbauer *et al*., 2014; Vlachogianni *et al*., 2017) (Table 2). As reported by the literature in Table 2, the mean of objects per square meter collected on the beach is between 0.12 - 3.5 items / m2.

Plastic represents a percentage always greater than 50%, in terms of number of items, of the total marine litter (Table 2). As reported by Munari *et al*., (2016), in a study conducted near the delta of the river Po, the greater majority (81.1%) of the total marine litter collected is made of plastic; paper and cardboard are the second most abundant group at beaches (7%), followed by glass and ceramics (3.9%), foamed plastic (3.3%), rubber (1.4%) and wood (1.2%). According to Munari *et al*. among the 35 litter categories found, cigarette butts account for the highest percentage (22.9%), followed by unrecognizable plastic pieces (13.5%), bottle caps (9.2%), mesh bags (7.2%) and plastic bottles and cutlery (6.5% and 6.4%, respectively). On the basis of this analysis, it is possible to deduce majority of marine litter came from land-based sources: shoreline and recreational activities, smoke-related activities and dumping, while sea-based sources contributed for less. The abundance and distribution of litter seemed to be particularly influenced by beach users, reflecting inadequate disposal practices (Munari *et al*., 2016).

According to De Francesco *et al*., (2019), most of litter elements collected during the samplings in a coastal area of Molise and Abruzzo regions, were plastic items (85%) and the majority of the debris derived from food packaging, fishing and recreational activities.

Vlachogianni *et al*., (2018) investigated the presence of marine litter in 31 different sites in Adriatic both eastern and western coasts, a total of 180 surveys were performed and 70,581 marine litter items were classified, recorded and removed. Items varied widely in abundance and types. The total abundance of litter items in Croatia (Zaglav, Vis) was found to be extremely high in comparison to the abundance of litter items recorded in the rest of the sites, with the average number of items being 11 items/m2 (1055 items/100 m). The second highest abundance of litter items was recorded in Greece (Ipsos) with the average number of items being 0.91 items/m2 (455 items/100 m), followed by Slovenia (0.83 items/m2 - 828 items/100 m), and Italy (Foce Bevano, 0.55 items/m2 (549 items/100 m). In accordance with the beach litter density results, the beach with highest CCI were Zaglav (CCI=211, ‘Very dirty’). The majority of litter items were made out of artificial/anthropogenic polymer materials. In almost all countries of the Adriatic-Ionian region plastic items were in the range of 74–92% of total items recorded (Figure 13), followed by glass/ceramics (3.2%), items made of metal (1.5%), paper (1.4%) and cloth/textile (1.1%). Of the total litter items collected, 33.4% originated from shoreline sources, including poor waste management practices, tourism and recreational activities.

For macro litter floating in the seawater, there has been no real sampling per se, merely visual survays (Table 2), in particular the majority of the research has relied on ship-board observation. Wastes were differentiated by anthropological and natural origin; it is not surprising that the majority was made of plastic. For most of the works, the reported concentrations of litter are usually expressed as items / km2 (Table 2), with a range moving from 4.7 ± 0.5 items / km2 (Arcangeli *et al*., 2018) to 332 ± 749 items / km2 (Vlachogianni *et al*., 2017).

Arcangeli *et al*. (2018) collected data regarding the occurrence of floating macro-litter monitoring five fixed transects connecting Italy to France, Spain, Greece, and Tunisia: Livorno-Bastia; Civitavecchia-Barcelona; Cagliari-Trapani; Ancona-Patras; Palermo-Tunis-Civitavecchia. According to these investigations, an average density of anthropological litter of 4.7 ± 0.5 items/km2 was reported. In the Adriatic Sea, the highest values of litter density were recorded in all seasons compared to the other areas, as shown in Figures 14 and 15. Furthermore, seasonal variations were observed, with the maximum density being during the winter (Figure 15). According to Suaria and Aliani, (2014) concentrations of marine floating litter are higher then those reported by Arcangeli *et al*., 2018, varying from 22 items/km2 (in the area facing Montenegro) to 52 items/km2 (in the waters between Ancona and Zadar). The difference in this case was probably due to the different speeds of the vessels used for the survey (slower in the case of Suaria and Aliani (2018)) and the different size of items: >2cm in the case study of Suaria and Aliani (2014); > 20 cm in the study of Arcangeli *et al*. (2018).

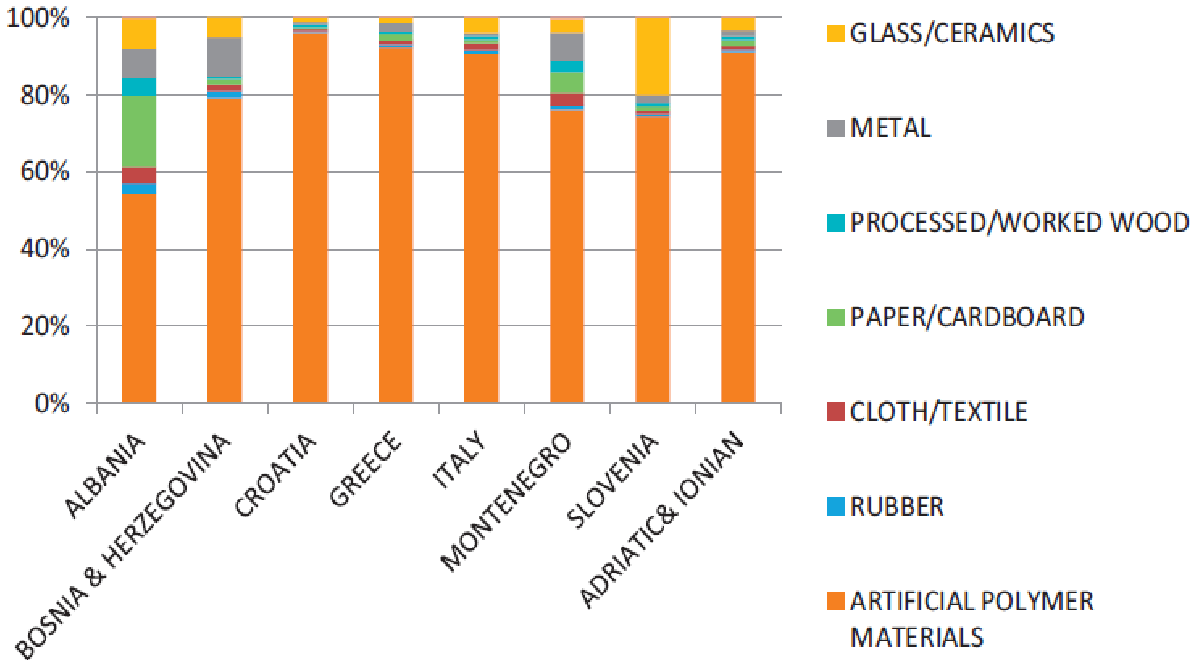

Fig. 13 *Percentage (%) of total litter items per category type (artificial/anthropogenic polymer material; rubber; cloth/textile; paper/cardboard; processed/worked wood; metal, glass/ceramics) collected by hand during coastline sampling in the seven countries of the Adriatic-Ionian macroregion, from October 2014 to April 2016, (Vlachogianni et al., 2018)*

	Total survey length km	Total surveyed area km^2	Average transect length km	Litter items N	Density of litter N/ km^2	Natural items N	Density of natural debris N/ km^2
Adriatic Sea (AS)	6733	661	570	2679	4.7 ± 0.5	225	0.35 ± 0.06
Ionian Sea (IS)	4565	400	170	686	1.9 ± 0.2	205	0.48 ± 0.13
Ligurian Sea (LS)	3724	346	357	701	1.8 ± 0.2	150	0.60 ± 0.1
Sardinian-Balearic basin (SB)	5098	473	498	1162	2.5 ± 0.3	153	0.33 ± 0.06
Bonifacio Strait (Bon)	2303	216	219	441	2.4 ± 0.4	74	0.55 ± 0.17
Central Tyrrhenian Sea (CTS)	2488	233	760	428	2.1 ± 0.4	83	0.46 ± 0.14
Sicilian-Sardinian Ch. (SSCC)	4500	396	150	735	2.8 ± 0.5	24	0.12 ± 0.06

Fig. 14 *Results from a 3-year survey's program (from October 2013 to September 2016) performed by dedicated and trained observers in standard effort conditions. Survey effort data: transect lengths and area surveyed within each area of the study, total number, and density values of anthropogenic and natural items (Arcangeli et al. 2018).*

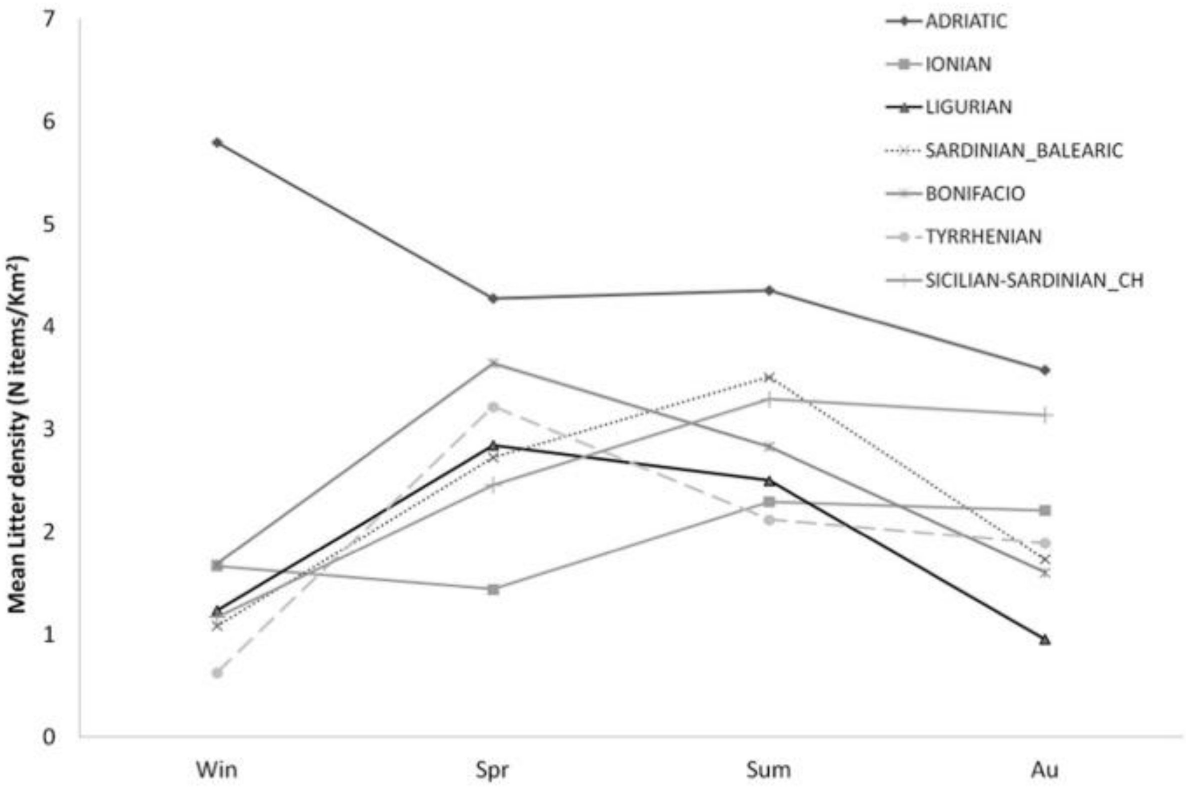

Fig. 15 *Seasonal trends in litter density: number of items observed on the surveyed area (expressed as number of items/ km2) recorded in the seven areas of the study (Arcangeli et al. 2018).*

Carlson *et al*. (2017) compared the data reported by Suaria and Aliani (2014) with that collected in a subsequent campaign in 2015. They also found that the global average density of floating waste varies by the period, specifically 32 ± 31 items/km2 in May 2013, 115 ± 173 items/km2 in March 2015 and 75 ± 74 items/km2 in November 2015. Demonstrating that, even for marine floating debris, as well as for marine litter in the coastline, the highest concentrations of litter are reported during the winter.
For what concerns seabed litter, it can either be collected with bottom trawls or observed when scuba and/or snorkelling (Table 2). What emerges from the analyzed works is that the data gathered through visual inspection during scuba diving or snorkeling show very high concentrations: 27,800 items/km2 and are not comparable to those found during sampling with trawl nets: 510 ± 517 items/km2 (Vlachogianni *et al*., 2017). This could be due to the fact that marine litter tends to concentrate in that area of the seabed that is closest to the coast, while going offshore the concentration of wastes decreases.
For example, the data collected by Strafella *et al*. (2015), concerning the presence of marine litter on seabed, were also divided by sampling depth, as shown by Figure 16, taking into consideration only plastic and rubber. Figure 16 shows a significant decrease in the quantity of marine litter in seabed (expressed in kg/km2) as the depth increased. This may lead to think that the main cause of the occurrence of marine litter in seabed could be the anthropogenic activities from the ground and the river inputs. In fact, the highest density of litter is found at the mouths of rivers, in particular the Po (Strafella *et al*., 2015; Pasquini *et al*., 2016), and in the more urbanized areas, such as the Boka Kotorska (Macic *et al*., 2017).
The fact that the seabed is less dirty offshore is probably due to a less intensive anthropic influence far from the coast (Schimd *et al*., 2021). But further investigations are needed in order to assess the impact of fishing and shipping activities, which can strongly influence the presence of marine litter in seabed offshore, for example with the accidental loss of fishing nets (Figure 17). In fact, according to a more recent investigations, undertaken by Strafella *et al*. (2019), the highest concentration of total litter on seabed was found in stations close to the coast within 30 m depth. The percentage composition of the marine litter recorded showed how the most abundant category is Plastic. In particular, lost fishing nets and mussel culture debris accounted for 50% of the overall plastic litter (32% and 18% respectively), as shown in Figure 18. Moreover, the sub-category “other plastic” (50% of the total plastic) comprised a wide range of objects such as garbage bags, shopping bags, cups, bottles, food packaging, dishes, other kitchen stuffs and industrial packaging.

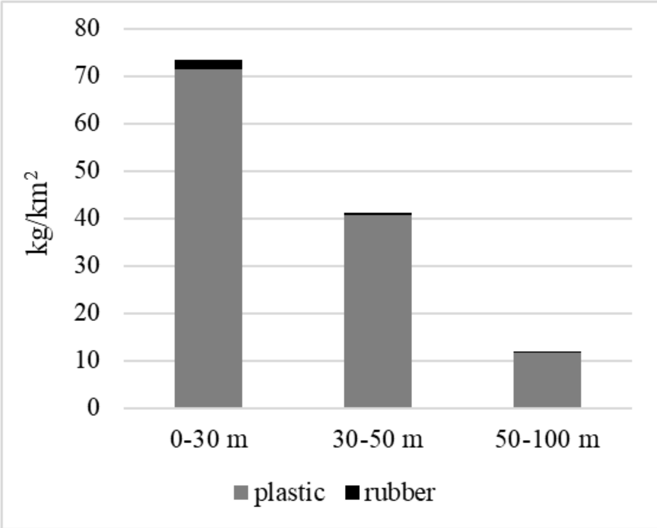

Fig. 16 *Plastic and rubber concentration on seabed vs. sampling depth. According. Significant decrease in the quantity of marine litter in seabed (expressed in kg/km2) as the depth increased (Strafella et al. 2019)*

Fig.**17** *Different types of fishing nets, polyethylene nets, nylon nets and "rapidi" parts of trawling nets. Collected in the seabed of the Conero Riviera (An).*

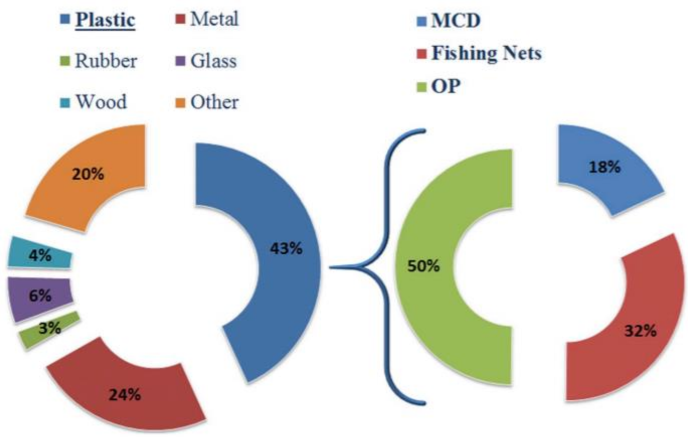

Fig. **18** *Percentage composition of the marine litter recorded on the seabed during the six survey years. The three sub-categories of plastic litter are reported in detail. Marine litter was collected using modified beam trawls called "rapido" in the northern and central Adriatic Sea (GSA 17) (Strafella et al.,2019)*

3.2 Microplastic in Adriatic Basin

Nowadays, the occurrence of microplastics in the Adriatic basin is still poorly investigated. The literature reported in Table 3 analyses the presence of microplastics in different environmental compartments, such as: the coastline, the seawater column and the seawater surface, as well as the seabed. Table 4 shows some studies that analyse the presence of microplastics in biological samples. For what concerns microplastics on the coastline, only six publications are reported in Table 3.

As can be seen, the research has concentrated on three distinct areas: beaches in Slovenia (Korez *et al*., 2019; Laglbauer *et al*., 2014), beaches in Croatia (Maršić-Lučić *et al*., 2018) and beaches around the Po Delta in Italy (Atwood *et al*., 2019; Munari *et al*., 2017; Piehl *et al*., 2019). In almost all the studies, identification was performed via FTIR. Exceptions are the first study of the beaches of Slovenia (Laglbauer *et al*., 2014), in which only the observation by optical microscopy (MO) was made, and that by Maršić-Lučić *et al*., (2018), in which no identification of the polymer was made.

Among the Italian coasts overlooking the Adriatic Sea, only the area of the Po delta has been investigated for the occurrence of Microplastics. Further investigations are needed in order to evaluate, which are the most impacted areas and the possible main inputs related to the presence of microplastics on the coastlines. For what concerns the River Po Delta area, the works of Munari *et al*. (2017) conducted in May 2015 as well as Atwood *et al*. (2019) and Piehl *et al*. (2019) in June 2016 report different average densities, but always in the order of tens of items per kg d.w.

In any case, the most common polymers found were PE, PP and PS (Schmid *et al*., 2021).

While highest concentrations of microplastics are reported in publication of Laglbauer *et al*., (2014), where the microplastics collected are in the order of hundreds of items per kg d.w. in Slovenian beaches.

Table 3 shows 10 publications that investigate the presence of microplastics in water. In all the works, the seawaters' surface layer sampled through the use of manta net and/or epineuston net, while in the publication of Zeri *et al*., (2018), microplastics with size > 2.5 cm are only visually identified from small boats.

There are therefore no works in the literature that investigate the presence of microplastics in the water column (below the first 20 cm of the seawater surface) through the use of specific pumps. Again, further studies are needed in order to evaluate the presence of microplastics in the pelagic domain. Furthermore, a variety of diverse units of measurement were used to calculate the occurrence of microplastics, specifically items/m2, items/m3, items/km2, and merely items. Only in two cases was data in g/km2 also reported (Suaria *et al*., 2016; Zeri *et al*., 2018). For this reason, the comparison between the data of the various publications is not so simple and direct. Nevertheless, the most abundant polymers in all the studies were PE (from 26% to 88%) and PP (from 5% to 30%) (Schmid *et al*., 2021).

Regarding the presence of microplastics in seabed, a total of 5 works is reported in Table 4. The sediment is sampled in different ways through box-corer (Vianello *et al*., 2013), Van Ven grab (Mistri *et al*., 2017; Renzi *et al*., 2018a) and Glass jars by divers (Renzi & Blašković, 2020). Three of these works concern the Italian territory: in particular Venice, Pescara and Pianosa and the Gulf of Triest, while 2 investigate the presence of microplastics in the Islands or Silba and Grebena in Croatia.
According to the data reported, the microplastics concentrations, often expressed in items/kg d.w., are highly variable, depending strongly on the geographical area (Schmid *et al*., 2021). The highest concentrations: 672-2174 items / kg d.w. are reported in the Venice lagoon in the work of Vianello *et al*., (2013). In the stretch of sea between Pescara and Pianosa (Mistri *et al*., 2017) microplastics density ranged from 2.5 to 88 items/m2 (between 2 and 1700 mg/m2. While Renzi *et al*. (2019), in October 2017, found an average concentration of 307 ± 108 items/kg d.w. (mP size >5 mm). Figure 19 shows microplastics with different shapes (Films; fragment and fibres), recovered by Renzi *et al*. (2019) in marine seabed samples. In the same area investigated by Renzi and Blašković (2020), once again in October 2017, found an average MPs concentration of between 113 and 378 items/kg d.w. in the sediment. In this case too, a variety of units of measurement were used, although the majority of the authors do report the density in items/ kg d.w. Surprisingly, no other investigations have since been con- ducted in this area.Where identified, the majority of plastics were found to be PE and PP, even though significant percentages of PA and PVC were found in some cases (Schmid *et al*., 2021)

The presence of microplastics in biological samples is still poorly investigated. Table 4 contains 7 publications that report data on the presence of microplastics in different organisms, such as: fishes (Anastasopoulou *et al*., 2018; Avio *et al*., 2020; Giani *et al*., 2019), Loggerhead turtles (Biagi *et al*., 2021; Di Renzo *et al*., 2021), lobsters (Martinelli *et al*., 2021) and invertebrates (Avio *et al*., 2020; Gomiero *et al*., 2019). The presence of microplastics in the gastrointestinal tract of fishes, reptiles, crustaceans and in the soft tissue of molluscs is respectively due to phenomena of ingestion or filtration of water or phenomena of bioaccumulation within the marine food web.
As Table 4 shows, several approaches to microplastics and biota have been taken, making data not so easy to compare. Different concentrations of MPs on biota can be related not only to geographical areas, but also to the species of organisms, their habitat, their feeding habits (benthic or pelagic organisms) and their biometric values. In many cases, fibres were predominant, but were often not synthetic, including also natural and semi-synthetic ones (Avio *et al*., 2020).

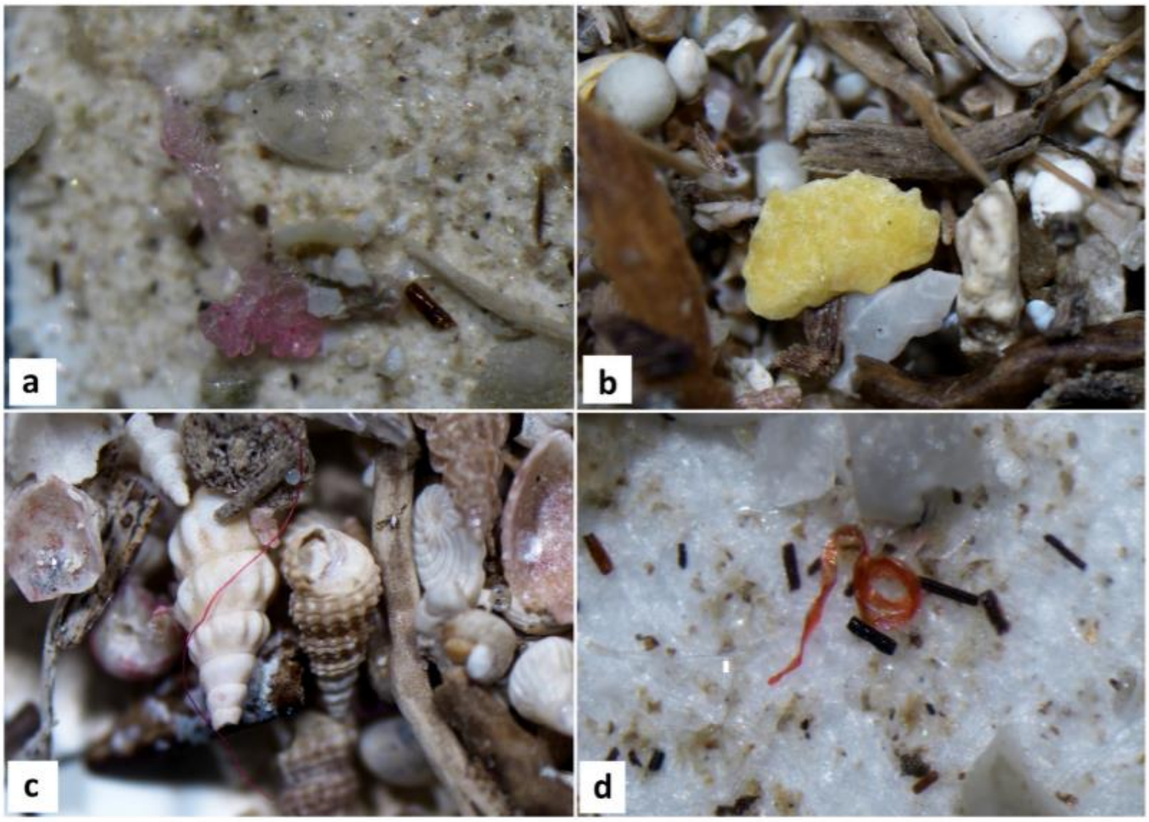

Fig. **19** *Marine litter recovered in sediments form the study area. Pictures reported represent some of the recovered microplastics shapes in analyzed samples (40×) Films (a); fragment (b); fibres (c,d). (Renzi et al.,2019)*

Tab.1 *Recent literature data for marine litter in Mediterranean Sea*

Reference	***Compartment***	***Type of marine litter***	***Location***	***Method***	***Period***	***Results***	***Plastic % numeric***
Arcangeli *et al.*, 2018	Sea surface	floating macro litter	Western Mediterranean Sea, the Adriatic Sea, the Ionian and Central Mediterranean Sea	Visual surveys (binoculars, GPS, range finder, digital camera, and recording data sheet)	October 2013 to September 2016)	2–5 items/km2	> 80%
Fortibuoni *et al.*, 2021	Coastline	Beach litter	Adriatic Sea, Western Mediterranean Sea, Ionian Sea and Central Mediterranean Sea coastlines	visual census	2015-2018	477 items/100 m	74%
Garofalo *et al.*, 2020	Sea floor	macro-litter found on the seafloor	Strait of Sicily between 10 and 800 m depth	bottom trawl surveys	from 2015 to 2019	The five-years average density of seafloor litter was 79.6 items/km2 and ranged between 46.8 in 2019 and 118.1 items/km2 in 2015	58%
Ruiz-Orejón *et al.*, 2016	Sea Surface	Seventy-one neustonic samples	First study from the Balearic Islands to the Adriatic Sea The second study from the Balearic Islands to the Ionian Sea	Manta trawl (a mesh size of 333 mm)	2011 and 2013	average weight concentration 579.3 g dw km^2 (maximum value of 9298.2 g dw km^2) average particle concentration 147,500 items km^2	average value, 579.35 ± 155.92 s. e. g dw km^2; median value, 140.99 g dw km^2
Suaria *et al.*, 2014	Sea surface	floating macro litter	central-western Mediterranean Sea	Visual Survays (Shipboard sighting surveys)	May, June and October 2013	24.9 items/km2 Maximum densities (>52 items/km2) were found in the Adriatic Sea	95.6%

Table 2: Literature data for macro litter on beaches, seawater and seabed.

Reference	*Compartment*	*Location*	*Method*	*Period*	*Results*	*Plastic % numeric*
Laglbauer *et al*., 2014	*Coastline*	Slovenia	By hand	July 2012	0.81-3.5 items/m^2 2.84-19.12 g/m^2	64%
Munari *et al*., 2016	*Coastline*	Po delta	By hand	May-June 2015	0.12-0.57 items/m^2	81% (86%)
de Francesco *et al*., 2018	*Coastline*	Abruzzo and Molise Regions, Italy	By hand	spring 2014-2015	Total 5330 items	57% (88%)
Vlachogianni *et al*., 2017	*Coastline*	Macro region Adriatic-Ionian	By hand	2014-2015	0.67 items/m^2 (0.23-2.9) 65 kg/km^2 (3-339)	91%
Vlachogianni *et al*., 2018	*Coastline*	Macro region Adriatic-Ionian	By hand	October 2014-April 2016	0.37-2.9 items/m^2	74-92%
Vlachogianni, 2019	*Coastline*	Marine Protected Areas: IT Miramare	By hand	December 2017-January 2018	0.86 items/m^2	69%
de Francesco *et al*., 2019	*Coastline*	Abruzzo and Molise Regions, Italy	By hand	April-May 2018	2.2 items/m^2 (max 20)	85%
Suaria and Aliani, 2014	*Seawater*	Central Adriatic sea	Visual survey	May-October 2013	55 items/km^2	96%
		SW Adriatic			52 items/km^2	94%
		SE Adriatic			26 items/km^2	98%
Palatinus *et al*., 2019	*Seawater*	islands of Kvarner-Velebit and Zadar-Šibenik Archipelago	Visual survey and manta net (308 µm mesh)	April 2015	175± 181 items/km^2 (0-689 items/km^2)	Macro litter
Vlachogianni *et al*., 2017	*Seawater*	Macro region Adriatic-Ionian	Visual observation	2014-2015	332 ± 749 items/km^2	92%
Arcangeli *et al*., 2018	*Seawater*	Adriatic sea	Naked eye	October 2013 to September 2016	4.7 ±0.5 items/km^2	90%

Ronchi *et al*., 2019	***Seawater***	15 ports in five countries (Italy, Slovenia, Croatia, Montenegro and Greece)		September 2014 – August 2016	Total marine litter collected 122 t	-
Carlson *et al*., 2017	***Seawater***	Adriatic	Visual survey	March 2015	115±173 items/km^2	-
				Nov.-Dec. 2015	75±74 items/km^2	-
Strafella *et al*., 2015	***Seabed***	North and Central Adriatic sea	“rapido” trawl nets	Fall 2011 and 2012	185 ± 26 kg/km^2	34%
Macic *et al*., 2017	***Seabed***	Montenegro coasts	Scuba diving	2012-2014	2.5 items/1000m^2	54%
Pasquini *et al*., 2016	***Seabed***	North and Central Adriatic	bottom trawl nets	Fall 2014	913 ± 80 item/km^2	80%
Melli *et al*., 2017	***Seabed***	North Adriatic, “Tegnùe of Chioggia”	ROV imaging	Summers 2014 and 2015	3.3 ± 1.8 items/100m^2	
Fortibuoni *et al*., 2019	***Seabed***	Bosnia & Herzegovina, Croatia, Greece, Italy, Montenegro and Slovenia coasts	Bottom trawl // underwater visual surveys	August 2014 to November 2015	Total 2658 items; 10-2145 items/km^2	-
Vlachogianni *et al*., 2017	***Seabed***	Macro region Adriatic-Ionian	Bottom trawl	2014-2015	510 ± 517 items/km^2	91%
			scuba		27800 items/km^2	37%
Palatinus *et al*., 2019	***Seabed***	islands of Kvarner-Velebit and Zadar-Šibenik Archipelago	Van Veen grab	April 2015	36 ±17 particles/100 g d.w.	
Strafella *et al*., 2019	***Seabed***	North and Central Adriatic sea	“rapido” trawl nets	2011-2016	103 ± 42 kg/km^2	43%

Tab.3 *Literature data for microplastics (MPs) in Adriatic Sea*

Reference	***Location***	***Compartment***	***Period***	***Method***	***Type (M / m)***	***Identification***	***Results***
Laglbauer *et al*., 2014	Slovenia beaches	Coastline	July 2012	Shoreline Spatula (first 5 cm); infralittoral 500ml corers and filtered at 250μm	mPs	optical microscopy	133 items/kg shoreline; 156 items/kg infralittoral;
Munari *et al*., 2017	5 beaches around Po delta	Coastline	May 2015	Scraping the first 5cm of sand from 50x50cm2 area	mPs	ATR-FTIR on 20 items for each shape	7.4 items/kg d.w. (calculated)
Atwood *et al*., 2019	9 beaches around Po delta	Coastline	June 2016	25x25cm stainless steel quadrat to a depth of 5cm	mPs	ATR-FTIR / Focal Plane Array -FTIR	0-78 items/kg d.w.
Piehl *et al*., 2019	9 beaches around Po delta	Coastline	June 2016	Metal spatula – as Atwood19	mPs (1-5mm)	ATR-FTIR	2.9±4.9 – 23±45 items/kg d.w.
Maršić-Lučić *et al*., 2018	2 Croatian beaches on Vis island	Coastline	Spring and autumn 2016	by a stainless steel spoon from a surface area of 30×15 cm2, to a depth of approximately 5–7 cm	mPs (2-7mm)	No polymer analysis	24 ± 9 pellets/dm^3 min-max: 6-36 items/dm^3
Korez *et al*., 2019	9 Slovenian beaches	Coastline	March 2017 August 2017	Metal cylinder	mPs	ATR-FTIR of particles > 100μm	0.5±0.5 items/kg d.w. 1±0.8 items/kg d.w.
Ruiz-Orejón *et al*. 2016	Mediterranean sea – data for Adriatic	Seawater	May-July 2011	Manta trawl – 333 μm mesh	M (25-1000mm), meso (5-25 mm), mP (<5 mm)	optical microscopy	Global on Adriatic, average: 0.155±0.27 items/m^2 , (725 ±1149 g/km^2)
Suaria *et al*., 2016	Adriatic Sea between Puglia and Montenegro	Seawater	May 2013	neuston net-200 μm mesh	meso > 0.7 mm	FTIR (100%)	0.15 ±0.18 items/m^2 (299 ±471 g/km^2)
					mPs < 0.7 mm		0.59±0.68 items/m^2
Gajšt *et al*., 2016	Slovenian water	Seawater	December 2012 – August 2014	epineuston net 300 μm mesh	mPs	Near InfraRed spectroscopy on 14%	406000 items/km^2 (5.4 items/m^3)
Vianello *et al*., 2018	in front of: Venice lagoon	Seawater	March and April 2014	manta trawl – 330 μm mesh		μATR-FTIR 100% if n=100; 50% if 100<n<500;	2.7 items/m^2 (5.5±9.5 g/km^2) 0.15 items/m^2 (0.04±0.04 g/km^2)

	in front of the Po delta					10% if n=500	1.7 items/m^2 (0,39±0.33 g/km^2) 0.23 items/m^2 (0.06±0.04 g/km^2)
Bonifazi *et al.*, 2017	Cesenatico, Porto Garibaldi, Lido di Volano	seawater	October 2014	Not reported	mPs	HIS-FTIR, only fragment	643 items
Kovač Viršek *et al.*, 2017	Slovenian water	Seawater	August 2014 May 2015	manta net – 308 µm mesh	mPs	ATR-FTIR on 4.6% in 2014 and 1.5% in 2015	259310 ±57096 items/km^2 1304811 ±609426 items/km^2
Zeri *et al.*, 2018	Adriatic sea	Seawater	2014-2015	Visual by small boats	MPs	optical microscopy + ATR-FTIR (7%)	251± 601 items/km^2
				manta net – 330 µm mesh			315009± 568578 items/km^2 (217±575 g/km^2)
de Lucia *et al.*, 2018	Po mouth, Tremiti	Seawater	2015	manta trawl 333 µm mesh	mPs	µFTIR, Single Reflection-Attenuate Total Reflectance, optical microscopy, GC	0.3 ± 0.04 items/m^3
Palatinus *et al.*, 2019	islands of Kvarner-Velebit and Zadar-Šibenik Archipelago	Seawater	April 2015	manta net 308 mm mesh	mPs	optical microscopy + FTIR on about 15% of total	micro: 127135±294847 items/km^2
Atwood *et al.*, 2019	Po Delta plume	seawater	June 2016	manta trawl 300 µm mesh	mPs	ATR-FTIR / Focal Plane Array -FTIR	1-84 items/m^3
Mistri *et al.*, 2017	between Pescara and Pianosa	*Seabed*	November 2015	Van Veen grab	mPs	FTIR	28 ± 23 items/m^2
Renzi *et al.*, 2018a	between Caorle and the Gulf of Trieste	*Seabed*	March 2016	Van Veen grab	mPs	optical microscopy	137 – 703 items/kg d.w.
Renzi *et al.*, 2019	islands of Silba and Grebena, Croatia	*Seabed*	October 2017	according to the JRC13 guide (217)	mPs ≤5mm	optical microscopy, FTIR	Silba: 180-520 items/kg d.w. Grebena: 267-360 items/kg d.w.
Renzi and Blašković, 2020	islands of Silba and Telašćica, Croatia	*Seabed*	October 2017	Glass jars by divers	mPs	optical microscopy, FTIR	113-378 items/kg d.w.

Vianello *et al*., 2013	Venice lagoon	*seabed*	Not reported	box-corer	mPs	mFTIR + optical microscopy + Scanning Electron Microscopy	672-2175 items/kg d.w.

Tab. 4: Literature data for microplastics (MPs) in biological samples

Reference	***Location***	***Organism***	***Period***	***Method***	***Type (M / m)***	***Identification***	***Results***
Avio *et al*., 2020	Northern, Central and Southern Adriatic	n several fish and invertebrate species	summer 2016	Samples were dried, pottered, separated through a NaCl hypersaline solution (density 1.2 g cm3), filtered, partially digested with hydrogen peroxide (15%),	microplastic particles and textile microfibers	sorting and chemical characterization by mFT-IR	frequency of ingestion, ranging from 13 to 35%
Anastasopoulou *et al*., 2018	Adriatic and NE Ionian Sea macro-region (Mediterranean)	Different fish species	2014 -2015.	1:20 (w/v) 30% H2O2 / heat / dried samples dissolved with Milli-Q, stirred, and filtered stereomicroscope for micro-litter items.	mPs	stereomicroscope for micro-litter items.	“macrolitter” in the gut of fish < 3% in both the NE Ionian and N Adriatic 26% in the S Adriatic Sea. Micro-litter occurrence was 40 for the NE Ionian and increased to 87% in the N Adriatic (Slovenian Sea).
Biagi *et al*., 2021	Northwestern Adriatic Sea	Loggerhead Turtle (Caretta caretta)	2017-2019	fecal samples were collected, and stored at −80◦C until further analysis. Plastic particle extraction procedure reported by Valente *et al*. (2019). 0.2 g fecal material was digested (10% KOH) and incubated overnight at 40◦C under continuous stirring. Samples were pre-filtered (1 mm sieves), and filtered through glass microfiber filter (1.2 µm pore size). Each filter was left to dry and inspected using a digital microscope.	mPs	digital microscope. 4, 10, and 20X magnification with a digital camera Pictures employed for particle counting and their classification according to shape and color. ImageJ image analysis software.	All individuals except one showed particle items in the feces, for an average value of 6 ± 6.09 particles/sample. The maximum number of particles found in a single individual is 34. In all size class categories, data show the prevalence of filaments, followed by unclassified shapes. As to colors, the most represented classes are transparent/white and red particles.
Di Renzo *et al*., 2021	Central Adriatic Coast	Loggerhead Turtle (Caretta caretta)	2019	The intestinal contents were collected and the microplastics until 0.45 µm were extracted.	Mps	ATR-FTIR and Ramanmicrospectroscopy, RMS	Microparticles 0.45 µm - 1 mm were found in all turtles, for a total of 623. Plastic litter > 1mm were found only in 4 specimens. 19 polymers and 10 pigments, including polyester (100% of animals), HDPE (50%) and polypropylene (50%) were identified. BPA, PTA and

							PET were detected in fat and liver tissues of all animals, while PC was found only in 50%.
Giani *et al*., 2019	three different geographical sub-areas of the Mediterranean Sea.	Mullus barbatus and Merluccius merluccius	2019	the GI tract was rinsed, opened and visually inspected with a stereomicroscope to determine the state (full/empty) and only full GIs were analyzed. Digestion method follows the protocol of Rochman and co-workers published in 2015 (10% of KOH solution 1/3 v.v. incubated at 60° overnight). The method was modified by adding a sonication step and by increasing the ratio sample/KOH to 1/20 v.v. The samples were filtered on glass fiber filters (1.6 µm mesh) and filters were visually observed under a stereomicroscope (Mod. NBS-STMDLX-T), allowing the identification of plastic particles > 100 µm according to Lenz *et al*., 2015.	MPs	Ingested microplastics were characterized using a stereo-microscope: observed, photographed, measured and categorized according to size class, shape and colour.	Plastic fragments (ranging from 0.10 to 6.6 mm) were detected in 23.3% of the total investigated fish; a total of 65 plastic particles (66% constituted by fibers) were recorded. The percentage of plastic ingestion shows high variability between the two species and among the different sampling area. The
Gomiero *et al*., 2019	OSR, marine area offshore Rimini; OSP, marine area offshore Pesaro; CP, Conero natural park coastal area; CS, Cesenatico coastal site	native mussels	2013	Each pool (10 individuals) was weighed, transferred in a Pyrex bottle with of a 5% w/v Tween 20 solution. sample was stirred and dried. Then a protease solution was added and the sample was incubated for 48 h at 50 °C. Sample was digested (20%KOH solution), separated through a NaI solution, filtered, partially digested with hydrogen peroxide (15%), dried and analyzed.	MPs	stereomicroscope coupled with a digital camera and characterized by a µ-FTIR system	Coastal organisms: 1.06–1.33 fragments g−1 (wet weight) and 0.62–0.63 fibers g−1 (wet weight) Offshore organisms: 0.65–0.66 fragments g−1 (wet weight) and 0.24–0.35 fibers g−1 (wet weight). Prevalence of smaller particles (20 µm to 40 µm range). Most recurring polymer type in analyzed organisms was PE followed by PP, PET, and equal amounts of PS, PLY, and PVC.
Martinelli *et al*., 2021	GSA 17: the off Ancona fishing ground (partially delimited by the 70 m bathymetric) and the Pomo Pits area (western, central and eastern Pits delimited by the 200 m bathymetric)	Nephrops norvegicus	Spring 2019	Samples of Gut- hepatopancreas-tail were pre-burned, stirred, protease solution was added and incubated. The sample was filtered, and digested (30% H2O2 solution)	mFTIR imaging	Mps	Average of about 17 MPs/individual. Fragments were predominant over fibers with a ratio of about 3:1. The majority of MPs were in the dimensional range 50e100 ?m. The predominant polymers were polyester, polyamide 6, polyvinyl chloride and polyethylene, which together constitute about 61% of all the MPs found.

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