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Do microplastic contaminated seafood consumption pose a potential risk to human health?

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ABSTRACT

Microplastics are present in all parts of the ocean and can have deleterious effects on marine resources. The aim of this work was to map the presence of microplastics in commercial marine species such as bivalves (mussels *Mytilus galloprovincialis* and clams *Scrobicularia plana*), crabs (*Carcinus maenas*) as well as fish (*Mullus surmuletus*) to relate microplastics levels to pollution sources, assess possible impact on marine food chains and on human health. These species were collected from several sites of the Ria Formosa lagoon and along the south coast of Portugal. A quantitative assessment (number, size and color) and typology of microplastics were made in these species. Only one green fragment of polypropylene was detected in the gills of the crabs, while a blue polyethylene fragment was detected in the hepatopancreas of the mullets. Moreover, no microplastics were present in *S. plana* nor in the crabs whole soft tissues. Among mussels, 86% of microplastics were present from all sites and the number, size and color were site specific. Mussels from the west side of the coast (Sites 1–3) had the highest levels of MPs per mussel and per weight compared to the other sites, probably related to the impact of touristic activity, fishing gears, fresh water and sewage effluents along with the hydrodynamics of the area.

1. Introduction

The ocean is the main source of oxygen of our planet and a source of marine life with a vast biodiversity. However, over the last 50 years, the ocean has been threatened by anthropogenic activities. One of these challenges is the impact of marine litter. Plastics account for around 80% of marine litter and constitute a global environmental problem. The manufacture and consumption of plastics expanded since 1940s (Jambeck et al., 2015), due to its low cost, versatility, wide durability, and mechanical resistance that facilitate its application in many activities of modern human life, and it is estimated that 360 million tons of plastics produced in 2018 will reach around 1800 million tons in 2050 (Plastics Europe, 2019). Moreover, it is estimated that 4.6 to 12.7 million tons are introduced annually in the ocean (Jambeck et al., 2015), 80% of which are from land-based sources (Rios et al., 2007; Frias et al., 2014). In addition to visual pollution, marine plastic litter poses a physical and chemical threat to marine life, affecting the entire food web, from small organisms, such as zooplankton to mammals and seabirds (GESAMP, 2016).

Assessment of plastic pollution in the ocean emerged in the beginning of the 70's (European Commission, 2018a, 2018b) a few years after being detected. Once in the ocean, plastics fragments due to several environmental factors such as physical abrasion, exposure to UV radiation (photo-degradation) and hydrodynamics (Barnes et al., 2009) weather into smaller particles, of less than 5 mm in size, known as microplastics (MPs) (NOAAA, 2017) that are a threat to the marine environment (Besseling et al., 2012). MPs can reach the marine environment directly from industrial and wastewater treatment plants effluents and coastal zones due to the proximity of urbanized areas as well as from touristic activities where they tend to accumulate (Cole et al., 2011; Marques et al., 2021). Primary microplastics are originally manufactured as microspheres employed in cosmetic products like exfoliating creams (Fendall and Sewell, 2009), toothpastes (Anderson et al., 2016), as well as microfibers used in the textile industry and pellets in production of other plastics (OSPAR, 2018) while secondary microplastics result from the fragmentation and degradation of macroplastics.

Some plastics contain additives that are toxic such as BPA (Bisphenol A) and phthalates (GESAMP, 2019) whose leaching from plastics can pose additional risks to marine organisms. On the other hand, MPs due to their large surface area in relation to their volume, can adsorb and concentrate various pollutants present in the marine environment including metals (Betts, 2008; Ashton et al., 2010; Cole et al., 2011),

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persistent organic pollutants (POPs) (Ma et al., 2016; O'Donovan et al., 2018, 2020), polychlorinated biphenyls (PCBs) (Rios et al., 2007; Cole et al., 2011), polycyclic aromatic hydrocarbons (PAHs) (Avio et al., 2015b; Pittura et al., 2018), dichlorodiphenyltrichloroethanes (DDTs) (Sharma and Chatterjee, 2017) and PFOs (Islam et al., 2021), that are known to have endocrine, mutagenic, and carcinogenic effects and therefore MPs can act as an important vector for the presence of these toxic substances (Ziccardi et al., 2016; Thompson et al., 2004; Ng and Obbard, 2006; Cole et al., 2011) and the desorption of these contaminants from the plastic can be an additional risk to marine organisms.

MPs are bio-available and end up ingested by marine organisms such as bivalves, crustaceans and fish either of being confused as food or just by ingestion of water contaminated with MPs (Koelmans, 2015; Barboza et al., 2020). Therefore, MPs were detected in various commercial fish species, such as common mackerel (*Trachurus trachurus*), Atlantic hake (*Merluccius merluccius*), seabass (*Dicentrarchus labrax*) and striped red mullets (*Mulus surmuletus*) (Neves et al., 2015; Alomar et al., 2017; Barboza et al., 2020; Pequeno et al., 2021), bivalves such as mussels (*Mytilus* spp.) and oysters (*Crassostrea virginica* and *Ostrea edulis*) (Avio et al., 2015a, 2020; Li et al., 2016; Prata et al., 2020; Ward et al., 2019; Marques et al., 2021) and sea salt (Karami et al., 2017) used for human consumption. In Portugal, MPs have been detected in beaches (Antunes et al., 2018), coastal waters and sediments (Frias et al., 2016; Lechthaler et al., 2020), zooplankton (Frias et al., 2014), mussels (Marques et al., 2021) and clams *S. plana* (Pequeno et al., 2021). However, no study has yet been conducted to identify the presence of microplastics in seafood from the south coast of Portugal, where seafood consumption is the 4th highest in the world. In European countries it is estimated that the possible transfer of MPs to humans will be around 11,000 MPs per year (Van Cauwenberghe and Janssen, 2014), with seafood representing a possible intake of 0.5 g of MPs per week (Dalberg and Bigaud, 2019). Therefore, the aim of this study was to characterize (number, size and color) and quantify the typology of MPs ingestion in high commercial value marine species such as bivalves (clams *Scrobicularia plana* and mussel *Mytilus galloprovincialis*), crabs (*Carcinus maenas*) and striped red mullet (*Mullus surmuletus*) collected in the Ria Formosa lagoon and assess its impact on the food web and human health and identify hotspots of MPs contamination in mussels collected along the South coast of Portugal and relate MPs levels with potential sources of pollution. The hypothesis was that areas with high population densities and anthropogenic activities will be more susceptible to MPs contamination than the ones protected. The selected species besides being ecologically and economically relevant, have also been used as sentinel organisms to assess the quality of the marine environment and of MPs, in particular (Li et al., 2019) since they have a wide geographical distribution, are easy to capture and easily handled in laboratory, have a high resistance towards environmental conditions, and represent an important food source. Mussels are filter feeding organisms relevant to MPs assessment since they can ingest microparticles suspended in the water column and as they are a common prey of crabs were also chosen to evaluate possible MPs transfer along the food web (Li et al., 2016; Van Cauwenberghe and Janssen, 2014; Wang et al., 2020), while clams *S. plana* are deposit-feeders endobenthic bivalves that capture microplastics form water and sediments (Ribeiro et al., 2017; Pequeno et al., 2021). Crabs are omnivore scavengers crustaceans that can take up MPs through the gills as well as from pre-exposed food (i.e., mussels) (Farrell and Nelson, 2013; Watts et al., 2014). and mullets that besides omnivorous are demersal fish that can take up MPs from contaminated sediments (Cheung et al., 2018) and also bioindicators to assess the presence of MPs in the marine environment (Pannetier et al., 2020; Wang et al., 2020). These species have different feeding strategies and can directly ingest MPs through the gills mistaking them for natural food. Once ingested, MPs can induce physical and chemical injuries, accumulate in the epithelial cells of the digestive track and travel from the digestive system to the circulatory system where they induce effects such as inflammation (Van Cauwenberghe et al., 2015), reduce energy intake (Xu et al., 2017) and transfer across trophic levels (Wang et al., 2020). Gastrointestinal tracts and/or gills are tissues where MPs accumulate (Baechler et al., 2020a, 2020b) and different feeding strategies may give a better incite of MPs presence in the marine environment and through trophic transfer.

2. Materials and methods

2.1. Samples collected in the Ria Formosa lagoon

Shellfish species such as clams *S. plana* (3.2 ± 0.2 cm; n = 15), mussels *M. galloprovincialis* (5.5 ± 0.2 cm, n = 30) and crustaceans *C. maenas* (n = 20) were collected, in 2016, from two sites (4 and 6) of the Ria Formosa Lagoon (Table 1 and Fig. 1) while fish (*Mullus surmuletus*) (18.0 ± 0.7 cm; n = 5) were obtained from local fisherman. The Ria Formosa lagoon is a coastal lagoon system in the South of Portugal with 55 km long and 6 km wide (at its widest point), permanently connected to the ocean. It is an important ecological, economic and social system that provides valuable ecosystem services. The catchment area is important for food security and 80–90% of total bivalves produced in Portugal grow and breed in this lagoon but fish catch is also economically important. The Ria Formosa is a privileged place for the spawning and motherhood of many aquatic species, and its productivity is evidenced by the abundance of flora and fauna, together with the production originated in aquaculture. The area surrounding is increasingly urbanized with some intense agriculture activities with green houses and therefore the lagoon is directly influenced by different pressures. Stressors and hazardous substances are introduced through atmospheric deposition, river discharges, agriculture, industrial, urban effluents and emissions from harbors, marinas and boats. In addition, marine litter is transported from land or from fishing and tourism activities. All these anthropogenic activities are degrading the quality of the lagoon which is a concern for its sustainable development (Bebianno et al., 2019). The South coast of Portugal is a highly touristic region. Therefore, population increases 10-fold during the summer as well as the amount of sewage and maritime activities and some of these sites were already known as hotspots for other contaminants (Cravo et al., 2009).

2.2. Samples collected in the South Coast of Portugal

Mussels *M. galloprovincialis* (4.0 ± 0.1 cm shell length, n = 100) were collected in 1018 from several sites along the South Portuguese coast namely: Sagres (site 1), Lagos (site 2), Vilamoura (site 3), Faro (site 4), Olhão (site 5), Tavira (site 6) and Vila Real de Santo António (site 7) as shown in Table 1 and Fig. 1.

2.3. Sample treatment

After sampling, all species were wrapped in aluminum foil to avoid possible contamination, and transported alive to the laboratory where clams, mussels, crabs and fish were cleaned, measured and weighted. The whole soft tissues of clams and mussels were separated from the shells and the sex of mussels determined. From the 20 crabs collected, the whole soft tissues were separated from the shells and half of them were kept for determination of MPs in the whole soft tissues while for the other half (n = 10) the gills and hepatopancreas were dissected to assess MPs in tissue levels. In relation to fish, the hepatopancreas was dissected and analyzed for MPs detection. All samples were wrapped up in aluminum foil and stored at −20 °C until further analysis.

For MPs detection, all samples were analyzed. The tissues were first defrosted and then freeze dried. Clams, mussels, crabs whole soft tissues and fish hepatopancreas were all individually analyzed. For the crab gills and hepatopancreas (n = 10), two pools of five tissues each (two pools of five gills and two of five hepatopancreas) were made for MPs detection. The presence, morphology, type size and color of MPs were

Table 1
Sites geographic coordinates and area and cover percentage of seaport, urban and riparian zones within a 2 km zone buffer.

Sites	Geographic coordinates		Area (km^2)			Cover percentage (%)		
	Latitude	Longitude	Seaport zone	Urban zone	Riparian zone	Seaport zone	Urban zone	Riparian zone
1	37°00′36.2"N	8°55′44.3″W	25.46	202.28	–	5	36	–
2	37°06′23.14"N	8°40′26.46″W	34.73	566.58	–	3	56	–
3	37°04′2821"N	8°07′31.8″W	45.10	387.65	–	7	59	–
4	37°00′30.3"N	7°59′38.5″W	–	79.40	296.67	–	11	43
5	37°01′25.1"N	7°50′15.8″W	28.15	387.64	140.46	2	31	11
6	37°06′57.60"N	7°37′48.16″W	–	138.93	48.16	–	14	5
7	37°12′8.5"N	7°24′57.93″W	33.83	297.02	–	3	24	–

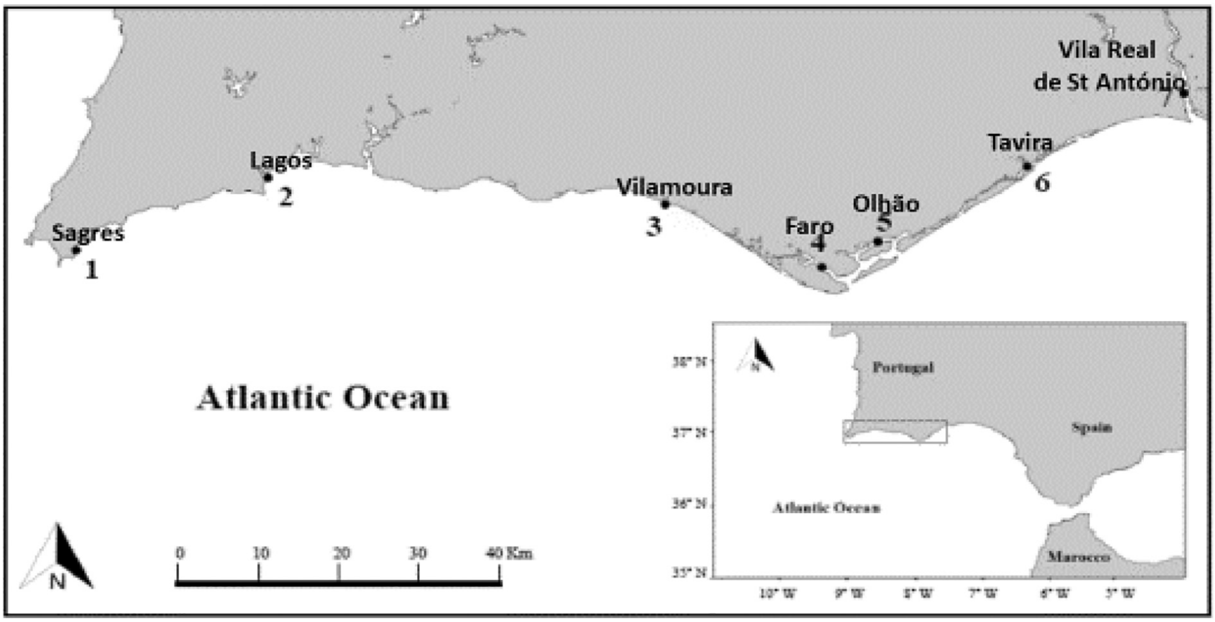

Fig. 1. Sampling sites where organisms were collected in the Ria Formosa Lagoon and in the South Coast of Portugal.

detected according to the method described below.

2.4. Microplastics detection

The detection of MPs in these marine species was made based on a slight modification of the method described by Avio et al. (2015a) (Bour et al., 2018). A solution of 10% KOH (1:5 weight:volume) was added to each sample and placed in an oven at 50 °C for 48 h to digest the tissues. After cooling, 100 ml of a NaCl hypersaline solution (1.2 g/cm^3) was added to each sample to separate MPs present in solution. The mixture was vigorously shaken, introduced in a 50 ml measuring cylinder and allowed to settle for 10 min. The supernatant was collected and filtered through a filter of 5.0 μm porosity. Filters were then observed through a compact stereo microscope (Zeiss Stemi Dv4 model) and MPs quantified and measured by visual assessment, identifying their color and morphology (fragment bead, sphere, pellets, line or flake).

2.5. MPs characterization

Polymer typology of extracted particles was assessed using a μFT-IR microscope (Spotlight i200, Perkin Elmer) coupled to a spectrometer (Spectrum Two, Perkin Elmer). All the measures were made using the μATR mode. Following background scans, 32 scans were performed for each particle, with a resolution of 4 cm^{-1}. Spectrum 10 software was used for the output spectra and the identification of polymers was performed by comparison with libraries of standard spectra both commercial and personally made. Polymers matching with reference spectra for more than 70% were validated, while a deeper interpretation was performed for spectra with a match comprised between 60% and 70% (Avio et al., 2020).

2.6. Quality control/quality assurance

Cotton lab coats were used to reduce the chance of contamination and nitrile gloves were worn at all times from the sampling to the analysis. All the glassware and other tools were rinsed with Milli-Q water three times before use. To avoid contamination, all the solutions were prepared with ultrapure water and filtered through Whatman GF/F Glass Microfiber Filters (pore size: 1.0 μm) before use. The experimental procedure was performed under clean air conditions carefully avoiding the use of any plastic material to prevent contamination. During microplastic extraction, all materials were covered with aluminum foil.

In addition, blanks were prepared with only reagents using the same procedure and run along each batch of samples to assess potential contamination by microfibers or MPs. Textiles microfibers were detected in control samples but not MPs. There was a high contamination of textiles microfibers across all blank replicates (mean 78). NaCl and KOH solutions were also contaminated (39 and 4 microfibers respectively). Because blank correction was high, according to Horton et al. (2021) it was decided not to quantify the fibers in this study.

2.7. Quantification of land cover and land use

Anthropogenic activities are directly reflected in land cover/land use (LC/LU) which is a key factor affecting water quality (Findell et al., 2017). Thus, land cover/land use (LCLU) data for the south coast of

Portugal was assessed using CORINE Land Cover 2018 (CLC) and Riparian Zones 2012 (RZ). CLC and RZ are two mapping programs that provide spatial data of the European environmental LCLU based on the interpretation of satellite imagery freely available online at the platform of the Copernicus Land Monitoring Service (CLMS): https://land.copernicus.eu/.

To quantify LCLU among sites of the South Portuguese coast, different CLC classes were aggregated into three main areas already known to influence water quality: seaport zone (Ng and Song, 2010; Jahan et al., 2019); urban zone (McGrane, 2016; Glinska-Lewczuk et al., 2016; Xu et al., 2021) and riparian zone (Tabacchi et al., 2000; Anbumozhi et al., 2005; Chua et al., 2019). Seaport and riparian zones included only one CLC class "port areas and salt marshes", respectively, while urban zone combined the following CLC classes: continuous and discontinuous urban fabrics, industrial or commercial units, airports and sport and leisure facilities. The analysis of LCLU was limited to a 2 km buffer terrestrial coastal zone within each sampling site. Quantum Geographic Information System (QGIS 3.10, 2021) was used to calculate the area of LCLU polygons within each buffer. The percentage of LCLU coverage of each sampling site was obtained by dividing the respective LCLU areas by total buffer area (≈12.57 km^2) (view Table 1).

2.8. Statistical analysis

To access significant differences of MP concentration per mussel (MP/mussel) and per gram of soft tissues (MPS/g) between sites a General Linear Models was tested followed by the post hoc Dunn's test of pairwise multiple comparisons based on rank sums to test the differences between sampling stations (no multiple-comparison correction was needed). Differences of MP concentration between mussel sex were also checked using the unpaired two-samples Wilcoxon test. Residual normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test) were checked when necessary. To group the sampling sites according to LULC similarity, k-means Cluster analysis was performed. Davies–Bouldin index (DBI) was used to validate the number of clusters (3 clusters) (Liu et al., 2010). To access if there was a relationship between LULC and the levels of MPs both Pearson's and Spearman's correlation tests were performed depending on data distribution. The significance level (α) was specified as 5% ($\alpha = 0.05$) except for the post hoc Dunn's test ($\alpha = 0.025$). All statistical analysis were carried out using the Package RCommander (Fox and Bouchet-Valat, 2018), present in R Software (R Core Team, 2017).

3. Results

3.1. MPs in shellfish in the Ria Formosa Lagoon

No MPs were detected in *S. plana* nor in *C. maenas* whole soft tissues or crab hepatopancreas. However, a blue fragment of polypropylene (PP) of 280 μm in length was detected in one of the gills pooled crab samples from site 4 (Faro). Moreover, a green polyethylene (PE) fragment of 250 μm in length was found in the hepatopancreas of one *Mullus surmuletus*.

In contrast, MPs were present in mussels from both sites. At site 4 (Faro) from all the mussels analyzed only one mussel had one MP and another two which corresponded to a percentage of 17% while in mussels from site 6 (Tavira) only one mussel had two fragments (6%) which corresponded to a range of 0.1–0.2 MP/mussel. The percentage of MPs fragments, lines and pellets detected in mussels are present in Fig. 2A. Fragments were the most common shape representing 60%, while lines and pellets had a similar percentage of occurrence (20%). Color shape and typology of MPs are in Fig. 3. A polyvinyl acetate (PVA) green fragment (290 μm) along with a transparent pellet (390 μm) of PE and a brown line (1148 nm in length) of expanded polystyrene (EPS) were detected in mussels from site 4 (Faro) while a blue fragment (390 μm) of ethylene-vinyl acetate (EVA) and a transparent 490 μm fragment of polystyrene (PS) was detected from site 6 (Tavira) with PE being the dominant type of polymer in the Ria Formosa lagoon (40%).

3.2. MPs in mussels from the South Coast of Portugal

In the Southern Coast of Portugal, MPs were detected in 86% of all mussels collected from all sites (54% in females). Wilcoxon test showed that the number of MPs ingested by females was significantly higher than by males ($p < 0.05$). The number of MPs ranged from 0 to 5 MPs per mussel, but the majority contained only one MPs per mussel (43%) while 23% contained two, 11% contained three, 6% had four and 3% five particles, respectively. Table 2 and Fig. 4A–B show the number of MPs ingested per mussel and per weight of soft tissues and the respective box plots showed that MPs ingested by mussels varied among sites. Mussels from sites 1 (Sagres) to 3 (Vilamoura) had the higher number of MPs ingested while at site 4 (Faro) only 60% of the mussels had MPs ingested with an average number of MPs per mussel ranging from 0.6 (site 4) to 2.6 (site 3) between sites. Regarding the average number of MPs per tissue weight (Table 2 and Fig. 4B), the highest levels were also between sites 1–3 and the lowest at site 4. Sites 1 and 3 had significant higher levels of MPs ($p < 0.05$). Dun's test pairwise comparisons showed significant differences on MPs per mussels weight of soft tissues between sites 1–4 ($df = 2.19$, $p = 0.0141$) and sites 3–4 ($df = 2.38$, $p = 0.0087$). Differences on MPs ingested per mussel were also detected between sites 1–4 ($df = 2.29$, $p = 0.0111$) and 2–4 ($df = 2.14$, $p = 0.0161$) as well as sites 3–4 ($df = 2.58$, $p = 0.0049$). Wilcoxon test showed that the number of MPs ingested by females was significantly higher than by males ($p < 0.05$). These results indicate that there is a higher contamination of MPs in the western part of the South Portuguese Coast.

A large variety of forms, shapes and types of MPs were detected in mussels collected along the South Coast of Portugal are in Fig. 2B. The most abundant forms were flakes (75%), followed by fragments (18%)

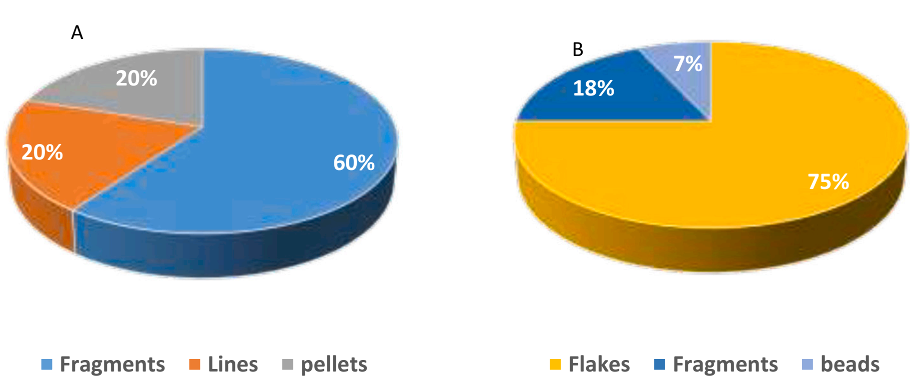

Fig. 2. MPs shapes in (A) Ria Formosa lagoon; (B) South Coast of Portugal.

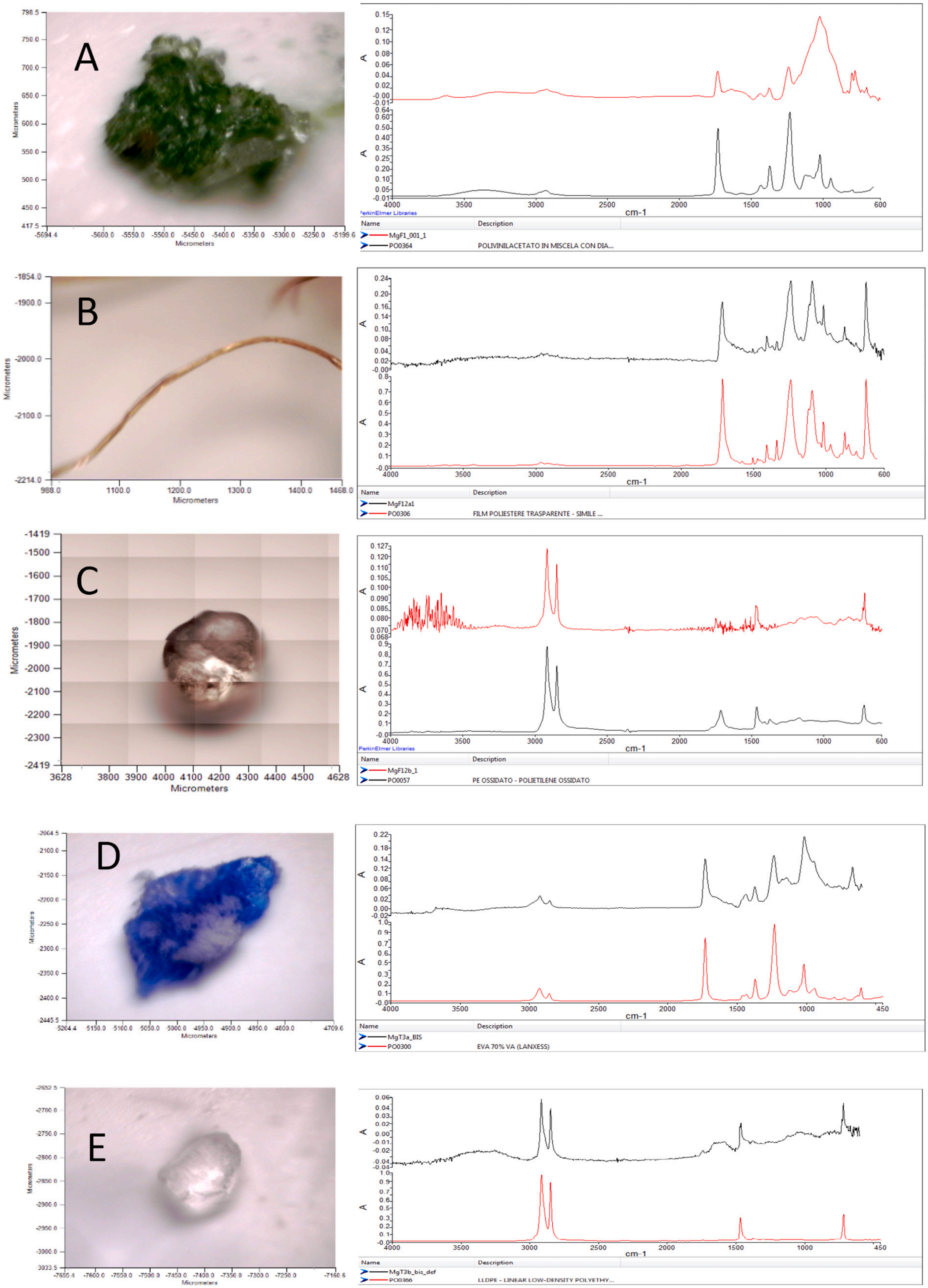

Fig. 3. *M. galloprovincialis*. Shapes, color size and types of MPs by stereomicroscope photographs and FTIR spectrums of MPs: A - PVA, green fragment (250 μm); B - PE, transparent (390 μm), C - PE oxidated transparent (490 μm); D - EVA, blue (490 μm) and E - LDPE (250 μm). A–C Mussels collected from site 4 and D–E from site 6. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Number (mean ± s.d.) of MPs per mussel and per gram of soft tissues.

Site	1	2	3	4	5	6	7
No. MPs/mussel	2.2 ± 1.3	1.8 ± 0.5	2.6 ± 1.8	0.6 ± 0.5	1.4 ± 0.5	1.2 ± 1.1	1.2 ± 1.1
No. MPs/g w.w.	0.89 ± 0.47	1.05 ± 0.48	1.29 ± 0.62	0.45 ± 0.08	0.56 ± 0.25	1.00 ± 0.59	0.44 ± 0.41

and lastly pellets (7%). The size range and color of MPs extracted from the mussels are shown in Fig. 5 and ranged from 3.6 to 540 μm. The most abundant size class was >25 μm, representing 49% while 18% were of the size class between 6 and 10 μm and 22% between 16 and 20 μm. The color of MPs was blue, brown, white, transparent, and red. Blue was the dominant color across all the size ranges and represented 69% of total colors. Brown (16%), and red ranged from 4 to 25 μm while white MPs were >25 μm. Regarding MPs typology PE was the main polymer type (76%) followed by polypropylene (PP) and polyamide (PA).

3.2.1. *Quantification of LCLU*

The clustering of LCLU among sites identified three clusters: cluster I (sites 1, 5–7); cluster II (site 4) and cluster III (sites 2 and 3) (Fig. 6). Cluster I grouped sites characterized by a low coverage of seaports, riparian zones, and moderate urban areas. Cluster II grouped only site 4 representing a zone of relatively low urban activity and large riparian area, while cluster III grouped sites with higher seaport activities and urban areas (Fig. 6). MPs levels in mussels were highly related with the extent of seaports and urban areas ($p < 0.05$), indicating that those areas tend to be more contaminated by MPs. Furthermore, sites with larger riparian zones, such as site 4, were less contaminated (albeit not significant, $p > 0.05$).

4. Discussion

Contamination of the ocean by plastic, and particularly by MPs, is a global environmental problem that impacts marine life and consequently humankind. MPs tend to accumulate near the coast because the major inputs are from land-based sources and when in the ocean they are influenced by physical and chemical processes including tidal movements that favor their accumulation in these areas (Baechler et al., 2020a, 2020b) where they can be ingested by marine organisms and pass through the food web. Simultaneously, coastal areas are an important zone of seafood and aquaculture production and therefore prone for MPs ingestion. Up to date MPs were identified in more than 1400 species (Claro et al., 2019) some of which may pose a risk to human health. So, it is important to understand the risks posed to humans by consumption of commercial seafood contaminated with MPs. It is estimated that in Europe, seafood can be a possible human intake of 0.5 g of MPs per week (Dalberg and Bigaud, 2019), probably higher in Portugal but the effects of MPs in human health are still scarce. Therefore, this study highlights the ingestion of MPs in several important

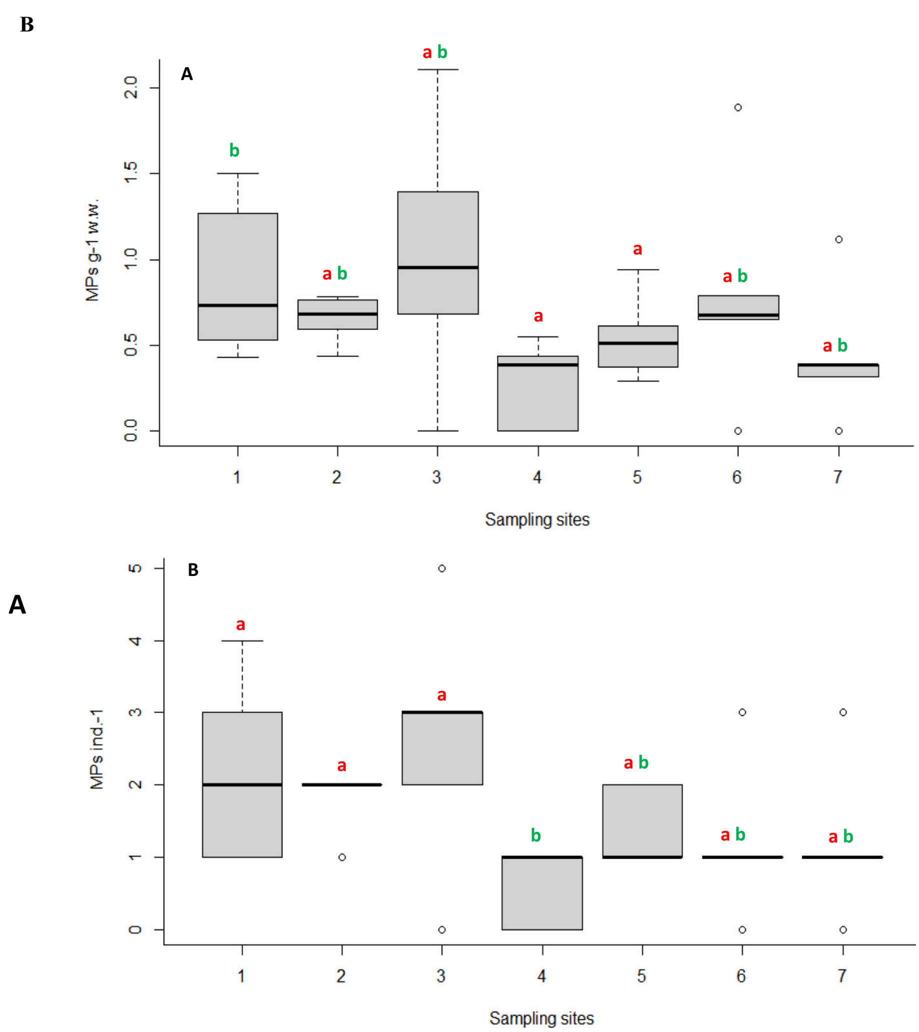

Fig. 4. Boxplot and results of the Dunn's Multiple Comparison test post-hoc test of MPs concentration per A) individual (ind) and B) wet weight of mussels whole soft tissues (g w.w.). The boxes enclose data falling between the 1st and 3rd quartile and the lines in bold represent the median in each location, while the whiskers represent the minimum and maximum value. Data points falling outside these ranges are plotted individually. Different letters indicate statistically significant differences between sites ($p < 0.05$).

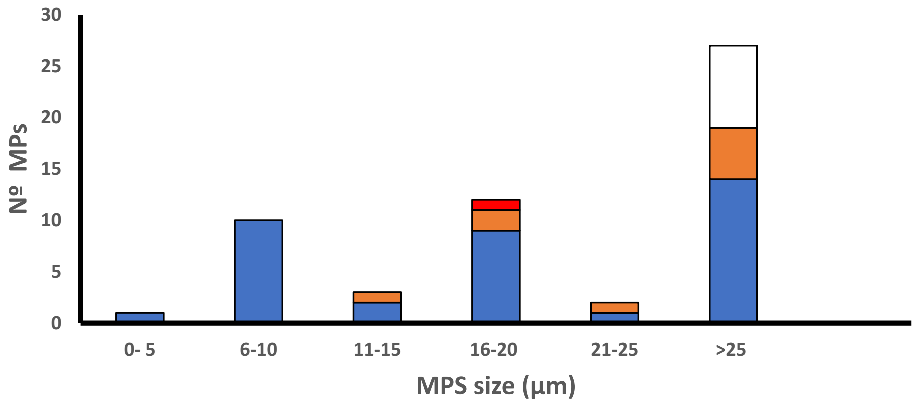

Fig. 5. Color and size classes of MPs detected in *M. galloprovincialis* along the South Coast of Portugal.

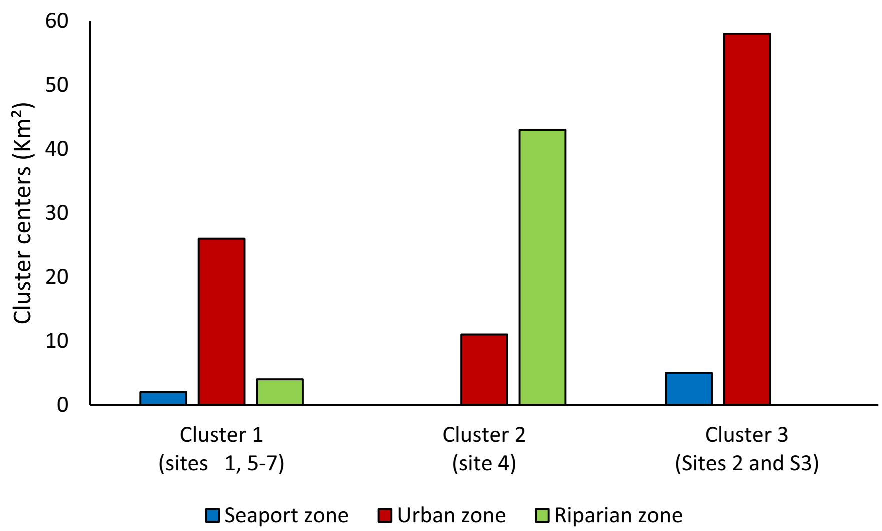

Fig. 6. Cluster centers (means) of seaport, urban and riparian zones. Under each cluster, the correspondent sites are indicated in parentheses.

commercial shellfish and fish collected from the Ria Formosa lagoon and in mussels from the South coast of Portugal and accesses possible risk to human health.

4.1. Presence of MPs in different commercial species in the Ria Formosa Lagoon

Stressors such as MPs in commercial seafood species identified in the lagoon constitute a risk to humans if MPs are internalized in seafood intended for human consumption (Van Cauwenberghe and Janssen, 2014). Molluscs represent a risk because, they are suspension-feeder organisms normally attached to hard substrates that passively ingest MPs via the gills. They are eaten whole and are a possible source of MPs transfer across trophic levels (Baechler et al., 2020a, 2020b). Although lower than in the adjacent coastal area (see Section 4.1), MPs were detected in *M. galloprovincialis* in the Ria Formosa lagoon (Fig. 3) at sites 4 (Faro) and 6 (Tavira). The origin of MPs at Faro (site 4) is linked to the proximity of Faro beach, a zone of relatively low urban activity and large riparian area where nets and other plastic items have been detected. Site 6 (Tavira) is directly affected by the Gilão River estuary (the only important freshwater input to the lagoon) where there is a harbor for small fishing vessels and nurseries for the cultivation of clams and oysters and an effluent of a wastewater treatment plant that serves a population of about 30,000 inhabitants. At this site fishing gear and bivalve farming nets were present. MPs levels in mussels per weight from site 6 were of the similar order of magnitude of those detected from the same area by Marques et al. (2021) (median 0.8 items g^{-1}) where 43% were fibers while <1% were pellets. At site 6 (Tavira), PE was the dominant MPs polymer while Marques et al. (2021) found in mussels collected from the same area (PE, PP, polyester, PA/viscose blend, cotton, viscose).

Mussels are known to be a common prey for crabs, but the levels of MPs detected in crabs (only one MP in the gills) from the same site, indicate that biomagnification do not occur probably due to the capacity of crabs to eject MPs. Wang et al. (2020) compared the ingestion of PS MPs (5 µm) in the crab (*Charybdis japonica*) exposed between water and mussel *Mytilus coruscos* as food borne and no biomagnification was observed probably due to the egestion of MPs. Moreover, Leslie et al. (2017) did not detect MPs in *C. maenas* while Waddell et al. (2020) detected in the blue crab *Callinectes sapidus* stomach 0.87 MP items per crab. This suggests that marine organisms might have a threshold capacity to counteract the effects of MPs above which detrimental effects may occur. Conversely, MPs effects may be organ and concentration dependent which might explain the low level of MPs detected in *C. maenas* gills. However, MPs can be transported to the gills by endocytosis and be detected in the gills surface (von Moos et al., 2012). Watts et al. (2014) detected that when *C. maenas* were exposed to PS microbeads oxygen consumption decreased, thus reducing the energy intake. Therefore, more research is needed to clarify if prey-predation between mussels and crabs depend on the type of MPs because MPs are expecting to increase in the ocean in the years to come.

The fact that no MPs were detected in the clam *S. plana* indicates that the levels of MPs in the water, mud and sand sediments at site 6 (Tavira) were low and not bioavailable for this deposit-feeder species. But when *S. plana* was exposed to PS MPs (20 μm), MPs were accumulated in the gills and digestive gland (Ribeiro et al., 2017; O'Donovan et al., 2018, 2020; Islam et al., 2021) and were not eliminated from any of these two tissues even after a week of depuration (Ribeiro et al., 2017). This indicate that at this site MP levels bioavailable to this species were low.

Crabs and mussels are important diet of the red mullet *Mullus surmuletus* a species of important economic and ecological value that lives in coarse and muddy gravel substrate areas. The levels of MPs detected in mullets were around 20% and of the same order of magnitude of the same species in other parts of Portugal (Neves et al., 2015) and from the Spanish Atlantic Coast and Mediterranean Sea (18.8%) (Bellas and Gil, 2020). The fact that MPs in fish digestive tract have low residence time (Pannetier et al., 2020) along with the feeding strategy might explain the low levels of MPs present in this species and indicate a low risk to humans because the hepatopancreas is not normally eat.

The low levels of MPs detected in the Ria Formosa Lagoon might be related to the fact that this lagoon is a natural park since 1987, and thus management and conservation efforts related to plastic pollution to preserve the quality of this ecosystem seems effective.

4.2. Presence of MPs in the South Coast of Portugal

MPs were present in *M. galloprovincialis* from all sites across the South coast of Portugal (86%) (54% in females) that are type, size, shape, and color dependent. Although there are a few environmental data about sex-related differences in MPs ingestion this topic is still often neglected (Fraser et al., 2016; Kögel et al., 2020). Making a paralysis between the accumulation of MPs and other contaminants the sex difference of MPs ingestion may be due to the variability of biological factors between sexes (sexual maturity, reproduction stages, seasonal growth cycles) (Richir and Gobert, 2014; Blanco-Rayón et al., 2020) so further research is needed to test MPs gender variability in marine organisms. In bivalvia, contaminants can bioaccumulate differently between male and female due to its variability of biological factors (sexual maturity, reproduction stages, seasonal growth cycles) (Richir and Gobert, 2014; Blanco-Rayón et al., 2020). Though our results evidence higher concentrations of MPs in female mussels, further work should be done to test gender induced variability on MPs concentration in aquatic organisms.

The average number of MPs ingested per mussel and per weight (1.6 MPs per species and 0.65 MPs/g w.w.) in the South Coast of Portugal (Table 3) is of the same order of magnitude of those detected in the Tagus estuary and in other areas of the Portuguese coast where MPs levels ranged from 0.54 to 3.0 MPs/g w.w. (Prata et al., 2020; Marques et al., 2021) as well as in other parts of the world (Table 3) in particular in those detected in *Mytilus* spp. off the coast of Norway where the ingestion was 76.6% (Lusher et al., 2017). However, MPs in mussels were higher than those detected in similar species by Vandermeersch et al. (2015) in European Areas (0.12 ± 0.04 MPs/g w.w.) and lower than those reported in Italy, Spain, United Kingdom, South Africa and China (Table 3).

MPs are particularly important in costal zones near urbanized areas because most of MPs are originated from land-based sources, runoff, wastewater, and atmospheric deposition. High human population and pressures due to tourism are also linked to the increase of environmental levels of MPs (Hantoro et al., 2019). Limited site-specific differences in MPs ingestion were detected in mussels. And this to our knowledge is the first report of MPs in mussels from this area. Regarding geographic distribution, mussels from sites 2 (Lagos) and 3 (Vilamoura) had the highest numbers of MPs per gram of soft tissues, with an average of 1.05 and 1.29 pieces of MP/g soft tissue respectively (Table 2). Site 2 (Lagos) has besides tourism, considerable fishing, and recreational activity, with a marina with capacity for 460 small boats. Site 3 (Vilamoura) is in the central region of the South Coast and besides being a highly touristic area where large amounts of plastic waste are generated, there is also a recreational marina considered the largest in Portugal and a port of small fishing boats. The highest number of MPs can also be related with the intense maritime traffic (from the marina and port area) and also from a small river (Ribeira de Quarteira) and from the beach alongside known to have the highest amount of marine litter and it may act as a temporary sink of MPs. At this site fishing gear and macroplastics were present. In the case of sites 2 and 3, these MPs levels might be related to the tourist activity along the coastline producing large amounts of plastic waste that most of the time end up in the sea.

Site 1 (Sagres) (one of the sites where the numbers of MPs per mussel where higher) (Fig. 5) is located on the west end of the coast in an upwelling zone where, a cold filament is recurrently formed off Cape São Vicente, the South West end of the Iberian Peninsula (Monteiro et al.,

Table 3
Levels of MPs (mean ± standard deviation) in mussels from various sites around the world.

Species	Geographical area	Location	No. MP ind.$^{-1}$	No. MPs/g w. w.	Reference
M. galloprovincialis	Portugal	South Coast	0.6–2.2	0.83 ± 0.31	Present study
M. galloprovincialis		Tagus Estuary		0.34 ± 0.33	Vandermeersch et al., 2015
M. galloprovincialis		Tagus estuary and Porto Covo	0.45 ± 0.67	0.18 ± 0.31	Pequeno et al., 2021
M. galloprovincialis		Portuguesa Coast		0.54–3.0	Marques et al., 2021
M. edulis	Germany	North Sea		0.36 ± 0.07	Van Cauwenberghe and Janssen, 2014
M. edulis	French-Belgian-Dutch coastline	French-Belgian-Dutch Coastline		0.2 ± 0.3	Van Cauwenberghe et al., 2015
M. edulis	French Atlantic coast	Area around the Loire Estuary	0.60 ± 0.56	0.23 ± 0.20	Phuong et al., 2018
M. edulis	United Kingdom	Coast of England and Wales	1.1–6.4	0.7–2.9	Li et al., 2018
Mytilus spp.		West coast of Scotland	3.2 ± 0.52	3 ± 0.9	Catarino et al., 2018
Mytilus spp.	Norway	Norwegian Coast (from South Swedish boarder to north Russian border)	1.85	1.84	Lusher et al., 2017
Mytilus spp.		All Norwegian coast	0.97	1.5	Bråte et al., 2018
Mytilus spp.	North Coast of Spain	Ria of Vigo	2.19 ± 1.57	1.59 ± 1.28	Reguera et al., 2019
Mytilus spp.		Cantabrian Sea	2.81 ± 2.80	2.55 ± 2.80	Reguera et al., 2019
M. galloprovincialis	Italy	Central Adriatic Coast	3.0–12.4	4.4–11.4	Renzi et al., 2018
M. galloprovincialis	Turkey	Turkish Coast - Mediterranean Sea	0.6–2.47	0.02–1.12	Gedik and Eryasar, 2020
M. galloprovincialis	South Africa	Cape Town	4,27	2.33	Sparks, 2020
M. edulis	United States of America	Avery point	0.4 ± 0.6	0.6–1.2	Zhao et al., 2018
Mytilus spp.	Canada	Halifax harbor	34–75		Mathalon and Hill, 2014
M. edulis	China	Whole Chinese Coast	1.5–7.6	2.7	Li et al., 2016
M. edulis	China	Whole Chinese Coast	0.77–8.22	1.52–5.36	Qu et al., 2018

2015) although less populated than sites 2–3, there is extensive fishing activity involving long line aquaculture with large amounts of shellfish farming nets, pots and traps, fishing nets, cables, ropes and fishery cooling resources. All this material is made of plastic, and over the years it is weathered and fragmented, giving rise to small plastic particles that become available to aquatic organisms. Site 1 is also highly influenced by maritime traffic because is located in one of most important traffic separation schemes between the Mediterranean Sea and the Atlantic Ocean.

Although site 4 (Faro) located inside the Ria Formosa lagoon had some signs of plastics contamination, such as nylon fishing nets and wires, it was the site were the mussels had the lowest MPs ingestion (0.45 ± 0.08 pieces of MPs/g soft tissue) with levels slightly higher than in the previous survey. Similarly, MPs detected in zooplankton along the Portuguese coast four years before revealed the lowest number of MPs in zooplankton from that site (0.32 ± 0.30 cm^3 m^{-3}, Frias et al., 2014). This may be related to the capacity of retention of suspended sediments and contaminants present in the water column by riparian vegetation such as *Spartina maritima* and *Sarcocornia fruticosa*, present in the Ria Formosa lagoon (Moreira da Silva et al., 2015). Cozzolino et al. (2020), detected that vegetated habitat, within this lagoon, could trap macro and MPs in the sediments at variable degrees (1.3–17.3 macroplastics 100 m^{-2}, and 18.2–35.2 MPs kg^{-1}). The fact that marshland plants of Ria Formosa lagoon function as MPs filters contribute to lower the concentration of these hazard materials is an interesting hypothesis that needs to be confirmed.

Although located in the coastal area with the number of MPs per species similar like site 6 (Tavira), site 7 (Vila Real de Santo António) showed the lowest number per weight (0.44 MPs/g w.w.). This site is located in the Guadiana River that defines the border between Portugal and Spain and it is well established that rivers are sources of MPs to the marine environment (Zhao et al., 2018). Regarding geographical distribution the western part of the south Portuguese coast seems more affected by MPs either due to local-based and shipping sources but also due to specific oceanographic conditions.

In this survey, a wide variety of shapes, colors, size and types in MPs were detected ingested by the *M. galloprovincialis* of the south coast of Portugal. Similarly, MPs of different sizes, shape and colors detected in *Mytilus* spp. was also demonstrated in various parts of the world such as China (Li et al., 2015), Belgium (Van Cauwenberghe et al., 2015), Germany (Van Cauwenberghe and Janssen, 2014) and the United Kingdom (Li et al., 2018) (Table 3). The most abundant forms of MPs were flakes, corresponding to a total of 75%, then fragments (18%) and lastly pellets (7%) (Fig. 2B). On the other hand, it is important to highlight that microfibers although not quantified in the present study, are predominant particles in aquatic organisms (Li et al., 2018), indicating that the number of MPs ingested by these species could be higher if microfibers had been quantified. In the present case, among the several colors of MPs detected, blue was the dominant (67%) (Fig. 4) which was similar off the coast of Norway (39%) (Lusher et al., 2017). However, in the pacific oyster (*Crassostrea gigas*) from the Oregon coast blue represented only 21% of the most common MPs colors (Baechler et al., 2019). Flakes were the most common MPs form and blue was the most observed color. The prevalence of blue as the most common color of MPs is in accordance with other studies (Lusher et al., 2017; Bessa et al., 2018; Reguera et al., 2019; Cozzolino et al., 2020; Pequeno et al., 2021).

In relation to size, mussels seem to be, at the same time, selective and non-selective suspension-feeders (Browne et al., 2008) indicating that smaller particles are retained more easily than larger ones as these are eliminated as pseudofeces (Hantoro et al., 2019) and they might also be selective regarding color, but this needs further confirmation. In the present case, the size of MPs ingested by mussels ranged from 3.6 to 54 μm (Fig. 5), Zhao et al. (2018) detected that MPs ingestion increase asymptotically with the increase in size and von Moos et al. (2012) detected that PE in the range of 0–80 μm induce inflammation in mussels and even at a lower range MPs interfere with the energy uptake and larval development. Moreover, when particle size was higher (>100 μm) there was a decrease in ingestion efficiency which indicates that mussels are selective for particle ingestion (Baechler et al., 2020a, 2020b). In the laboratory, mussels eliminate 63% of MPs in feces and pseudofeces after 6 h (Woods et al., 2018) but were unable to eliminate MPs of size range (49.1 ± 1.3 μm) after 2 h (Rist et al., 2018). In *M. edulis* a reduction from 0.36 ± 0.07 to 0.27 ± 0.07 MPs/g w.w. was also detected in mussels from the North Sea after three days of depuration. Similarly, MPs in *C. gigas* collected from Britanny (France) also decreased (from 0.47 ± 0.16 to 0.31 ± 0.05 MPs/g w.w.) after the same depuration time (Van Cauwenberghe and Janssen, 2014). This capacity of egestion of MPs by mussels needs further research because total removal of MPs from bivalves by depuration either from the field or produced in aquaculture can be an important and cheap solution to reduce the risk to human health.

Quantification and identification of MPs is relevant because it provides not only quantitative data on their abundance and characteristics, but also allows the identification of the possible sources, enabling the implementation of management strategies to minimize the impact of plastic marine litter (Frias et al., 2016). Regarding typology, PE, PP, PA, EVA, PES and PVA were the dominant polymers detected in these marine species indicating the presence of different types of MPs in the south coast of Portugal. Marques et al. (2021) detected similar MP polymers in mussels off the coast of Portugal. PE was the most common polymer (76%) followed by PP and polyamide (PA). These compounds are widely used in textiles and in fishing gears and along with the expanded form of PS used in packaging and all of them have a density lower than seawater becoming thus more bioavailable by suspension-feeders. These types of plastics are among the most common typology of MPs polymers ingested by marine organisms (Lusher et al., 2013). But the toxicity of MPs depends on the microplastic composition, and thus it is important to understand the ecotoxic effects of MPs in these commercial marine species because this species is eaten whole and can be a source of MPs ingestion by humans.

To improve and preserve the health of the ocean, the European Commission adopted strategies to reduce plastics pollution through the implementation of the EU Marine Strategy Framework Directive, to regulate the health of the ocean and encourage the implementation of good environmental status (European Commission, 2017; Gago et al., 2016) and throughout a more circular economy regarding the manufacture and consumption of plastics (European Commission, 2018a, 2018b) with the aim that by 2030 all packaging plastic materials are recyclable, the consumption of disposable plastics reduced and the use of microplastics restricted (EGSRA, 2018) but this approach can only be possible with the involvement of all social actors in management, policies, industry, fishing and maritime activities, educators, scientists and the general public (Frias et al., 2016).

A possible way to improve the quality of Portuguese coastal waters regarding reducing of MPs sources, is to provide effective management of marine ecosystems, establish a monitoring program to support and improve decisions and rise population awareness, beach cleaning actions, reduction of manufacture and consumption of disposable plastics, particularly single used ones. It is also crucial to do a follow up to see if the management goals are being effective (Hill and Wilkinson, 2004; Galgani et al., 2013).

5. Conclusion

A wide array of sizes, shapes, color and types of MPs were detected in commercial shellfish and fish species in the south coast of Portugal. MP levels were generally low in the Ria Formosa lagoon compared to the coast. MPs levels ingested by mussels tend to be related to pollution sources that can cause adverse effects in these commercial species. This data can be considered baseline levels from which trends can be assessed because it is essential to understand the potential risk to human health posed by the consumption of seafood contaminated with MPs.

CRediT authorship contribution statement

Sara Vital – Mussels sampling and tissues digestion; Cátia Cardoso – crabs and fish sampling and tissue digestion; C. Avio – MPS FTIR identification in mussels; L Pittura – MPs polymer identification in crabs and fish; F. Regoli – Interpretation and analysis and project Coordinator; Maria João Bebianno: Ideas; formulation or evolution of overarching research goals, aims and funding acquisition and discussion of data.

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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