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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 detect microplastics in commercial marine species (mussels clams, crabs and fish) to relate microplastics levels to pollution sources and assess impact on food chain and human health. Species were collected from several sites. A quantitative assessment (number, size and color) and typology of microplastics was made. One green fragment of polypropylene was detected in crab gills and a blue in mullet hepatopancreas. No microplastics were present in clams nor in crabs whole soft tissues. 86% of mussels had microplastics and the number, size and color were site specific. Mussels from the west coast (Sites 1 – 3) had the highest levels of MPs per mussel and per weight, probably related to touristic activity, fishing gears, fresh and sewage effluents along with the hydrodynamics of the area.
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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.

***Keywords*:** Marine Pollution, mussels, clams, crabs, fish, microplastics, *Mytilus galloprovincialis*.

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 348 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, 2018) 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) (NOAA, 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). 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), 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) and 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 either of being confused as food or just by ingestion of water contaminated with MPs (Koelmans A.A., 2015). Therefore, MPs were detected in various commercial fish species, such as common mackerel (*Trachurus trachurus*) and Atlantic hake (*Merluccius merluccius*) (Neves et al., 2015), bivalves such as mussels (*Mytilus spp.*) and oysters (*Crassostrea virginica and Ostrea edulis*) (Avio et al., 2015a, 2020; Li et al., 2016; Ward et a., 2019) and sea salt (Karami et al., 2017) used for human consumption. In Portugal, MPs have been detected in coastal sediments (Frias et al.,

2016) and zooplankton (Frias et al., 2014). 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 11000 MPs per year (Van Cauwenberghe and Janssen, 2014), with seafood representing a possible intake of 0.5 g of MPs per week (Dalberg & Bigaud, 2019). Therefore, the aim of this study was to quantify the presence (number, size and color) and typology of MPs ingested in commercial marine species such as bivalves (clams *Scrobicularia plana* and mussel *Mytilus galloprovincialis*), crabs (*Carcinus maenas*) and fish (*Mullus surmuletus*) collected along the South coast of Portugal and relate MPs levels with potential sources of pollution and assess its impact on the food web and human health. These species are ecologically and economically relevant and have been used as sentinel organisms to assess the quality of the marine environment 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, in particular, 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 (Li et al., 2016; Van Cauwenberghe and Janssen, 2014; Wang et al., 2021), while clams are suspension-feeders that capture microplastics form water and sediments (Ribeiro et al., 2017). Crabs and mullets are also used as bioindicators to assess the presence of microplastics in the marine environment (Pannetier et al., 2020; Wang et al., 2021). These species have different feeding strategies and can directly ingest microplastics through the gills mistaking them for natural food. Once ingested, these 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., 2021). Gastrointestinal tracts and/or gills are tissues where microplastics accumulate (Baechler et al., 2020) 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 of the Ria Formosa Lagoon (Figure 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 bivalve produced in Portugal grow and breed in this lagoon but fish are also 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 harbours, 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).

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 - 37°00'36.2"N 8°55'44.3"W), Lagos (site 2 - 37°06'23.14"N 8°40'26.46"W), Vilamoura (site 3 - 37°04'2821"N 8°07'31.8"W), Faro (site 4 -37°00'30.3"N 7°59'38.5"W), Olhão (site 5 - 37°01'25.1"N 7°50'15.8"W), Tavira (site 6 - 37°06'57.60"N 7°37'48.16"W) and Vila Real de Santo António (site 7 - 37°12'8.5"N 7°24'57.93"W) as shown in Figure 1.

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 the sites selected for mussels sampling were already known as hotspots of other contaminants (Cravo et al., 2006).

2.3. Sample treatment

After sampling, all species were wrapped in aluminum foil to avoid any 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 dissectedto 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 ºC 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 analysed. 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 colour of MPs were 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 / cm³) 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 minutes. 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 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 to assess potential contamination by microfibers or MPs. Microfibers were detected in control samples but not MPs and for this reason, it was decided not to quantify the microfibers in this study.

2.7. Statistical analysis

Data were checked for normality and homogeneity using Shapiro-Wilk and Levene´s test, respectively. In order to assess whether there were differences in the number of MPs per individual and per weight among the selected sites, the non-parametric Kruskal-Wallis test was performed, followed by Dunn´s post-hoc test as the multiple comparison procedure to assess if there were significant differences between sites and number of ingested MPs. Wilcoxon test was used to determine statistical differences between the number of ingested MPs and gender. To ascertain the relationship between the number of MPs ingested by the species and biometric parameters (soft tissue weight) a nonparametric test - Spearman's correlation test was used. All statistical analysis were carried out using the Package RCommander (Fox and Bouchet-Valat, 2018), present in R Software (R Core Team, 2017). The significance level was 5% ($\alpha = 0.05$).

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 fragment polyethylene (PE) 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 (n=15) 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 Figure 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 Figure 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 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). 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 1 and Figure 4A-B show the number of MPs per mussels and per weight of soft tissues and the respective box plots. 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 1 and Figure 4B), the highest levels were also between sites 1-3 and the lowest at site 4. However, no significant differences on MPs ingested per mussel nor per weight of soft tissues was detected between sites ($p > 0.05$). Conversely, the number of MPs ingested by females was significantly higher than by males ($p < 0.05$).

A large variety of forms, shapes and types of MPs were detected in mussels collected along the South Coast of Portugal. The forms of the MPs detected in mussels are in Figure 2B. The most abundant forms were flakes (75%), followed by fragments (18%) and lastly pellets (7%). The size range and color of MPs extracted from the mussels are shown in Figure 5 and ranged from 3.6 to 54 µm. The most abundant size class was > 25 µm, representing 49% while 18% were of the size class between 6 - 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 67% of total colors. Brown and red ranged from 4-25 µm while white MPs were > 25 µm. Regarding MPs typology PE was the main polymer type (76%) followed by polypropylene (PP) and polyamide (PA).

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., 2020) 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 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 commercial shellfish and fish collected from the Ria Formosa lagoon and from the South coast of Portugal and 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 species identified the presence of MPs in the lagoon and constitute a risk if MPs are internalized in seafood intended for human consumption (Van Cauwenberghe and Janssen, 2014). Molluscs in particular, represent a risk because, they passively ingest MPs via the gills (Ribeiro et al., 2017), are eaten whole and are a possible source of MPs transfer across trophic levels (Baechler et al., 2020). Although lower than in the adjacent coastal area, MPs were detected in *M. galloprovincialis* in the Ria Formosa lagoon (Figure 3) at sites 4 (Faro) and 6 (Tavira). The origin of MPs at Faro (site 6) is linked to the proximity of Faro beach. Site 6 (Tavira) is directly affected by the Gilão River estuary where there is a harbor for small fishing vessels and also 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.

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 er al. (2021) 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*. This suggest 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. (2016) 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 suspension-feeder *S. plana* indicate that the levels of MPs in the water and sediments at site 6 (Tavira) were not bioavailable for this suspension-feeder species. But when *S. plana* was exposed to PS MPs (20 µm), MPs were accumulated in the gills and digestive gland 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.

Moreover, the levels of MPs detected in mullets were around 20% and of the same order of magnitude in other parts of Portugal (Neves et al., 2015) and of those detected in red mullets from the Spanish Atlantic Coast and Mediterranean Sea (18.8%) (Bellas et al. 2016). The fact that MPs in fish digestive tract have low residence time (Rennetier et al., 2020) along with the feeding strategy might explain the low levels of MPs present in this specie and indicate a low risk to humans due to the fact that the hepatopancreas is not normally eaten.

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 difference of 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 needs to be done to test gender induced variability on MP concentration in aquatic organisms.

On the other hand, the levels of MPs are of the same order of magnitude of those detected in mussels (*Mytilus spp.*) from other parts of the world (Table 2), particular, off the coast of Norway where the ingestion was 76.6% (Lusher, 2017). The average number of MPs detected per mussel and per weight (1.6 MPs per species and 0.65 MPs /g w.w.) in the South Coast of Portugal (Table 2) are similar to those detected in the Tagus estuary and of the same order of magnitude from other parts of the world (Table 2). 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., 1999). 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 located in the central region of the South Coast and besides being a highly touristic 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 from a small river (Ribeira de Quarteira) and from the beach alongside known to have the highest amount of marine litter in the area and it may act as a temporary sink of MPs.

Site 1 (Sagres) (one of the sites where the numbers of MPs per mussel where higher (Figure 4) is located on the west end of the coast. This site is located in an upwelling zone where, a cold filament is recurrently formed off Cape São Vicente, the SW of the Iberian Peninsula (Monteiro et al., 2015) and 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) 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 (Silva, 2015). 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 to 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 (Zao et al., 2017). Regarding geographical distribution the west 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 2). The most abundant forms of MPs were flakes, corresponding to a total of 75%, then fragments (18%) and lastly pellets (7%) (Figure 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%) (Figure 4) which was similar off the coast of Norway (39%) (Lusher, 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).

In relation to size, mussels seem to be, at the same time, selective and non-selective feeders (Brown 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 (Figure 5), Zhao et al. (2017) detected that MPs ingestion increase asymptotically with the increase in size and Von Moos (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., 2020). In the laboratory, mussels eliminate 63% of MPs in feces and pseudofeces after 6 h (Woods et al., 2017) 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. PE was the most common polymer (76%) followed by polypropylene (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 filter feeders. These types of plastics are among the most common typology of MPs polymers ingested in marine organisms (Lusher et al., 2013). But the toxicity of MPs depends on the microplastic composition, and thus important to understand the ecotoxic effects of MPs in these commercial marine species.

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, 2018) 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, 2015).

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. It is also crucial to do a follow up on the work done 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 in these commercial species. Ths 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.

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Table 1. 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

Table 2. Levels of MPs (mean ± standard deviation) in mussels from various sites around the world.

Species	Geographical area	Location	Quantity (MP ind.$^{-1}$)	Nº MPs g^{-1} 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. edulis	Germany	North Sea		0.36±0.07	Van Cauwenberghe and Janssen, 201
M. edulis	French-Belgian-Dutch coastl	French-Belgian-Dutch Coastline		0.2±0.3	Van Cauwenberghe et al., 2015
M. edulis	French Atlantic coast	Area around the Loire Estuar	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 Soutl	1.85	1.84	Lusher, 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, Viñas and Gago, 2019
Mytilus spp		Cantabrian Sea	2.81±2.80	2.55±2.80	Reguera, Viñas and Gago, 2019
M. galloprovincialis	Turkey	Turkish Coast - Mediterranea	0.6-2.47	0.02-1.12	Gedik & Eryasar, 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			0.77–8.22	1.52–5.36	Qu et al., 2018

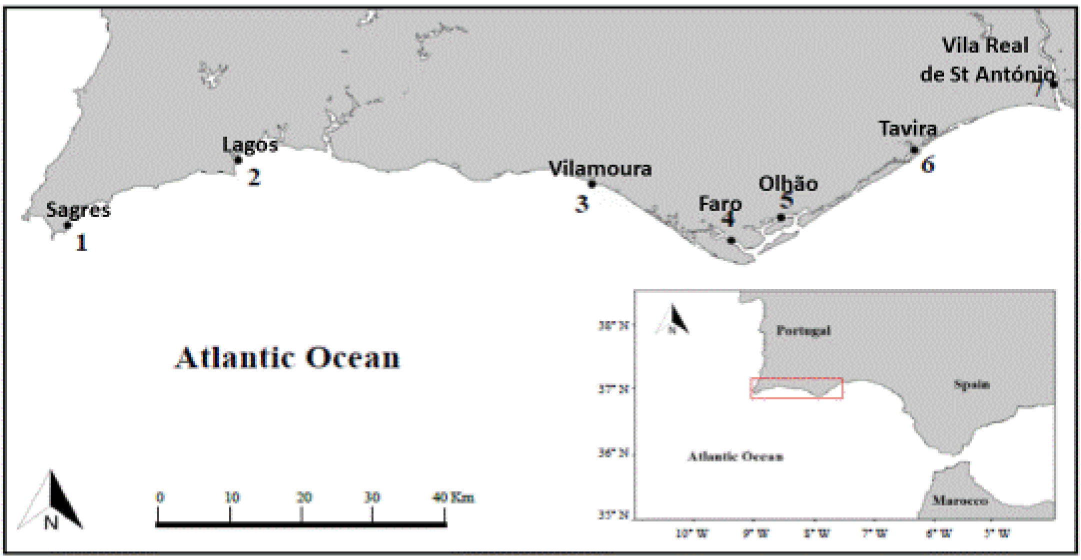

Figure 1. Sampling sites where organisms were collected in the Ria Formosa Lagoon and in the South Coast of Portugal

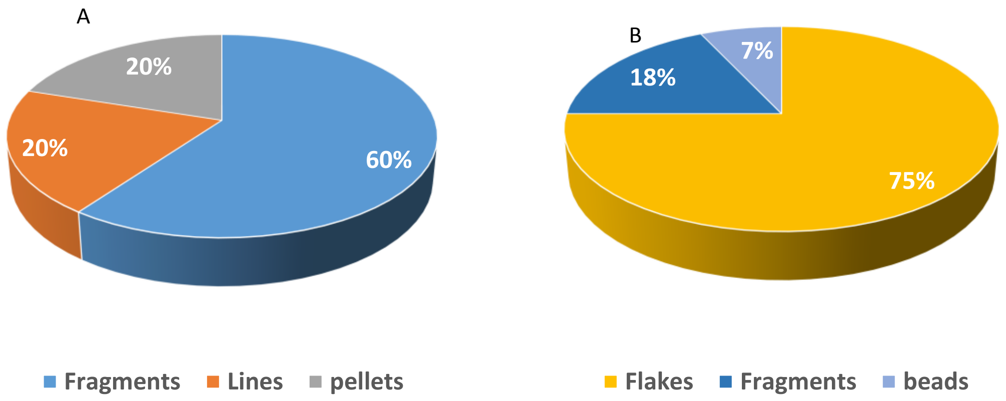

Figure 2 – MPs shapes in (A) Ria Formosa lagoon; (B) South Coast of Portugal

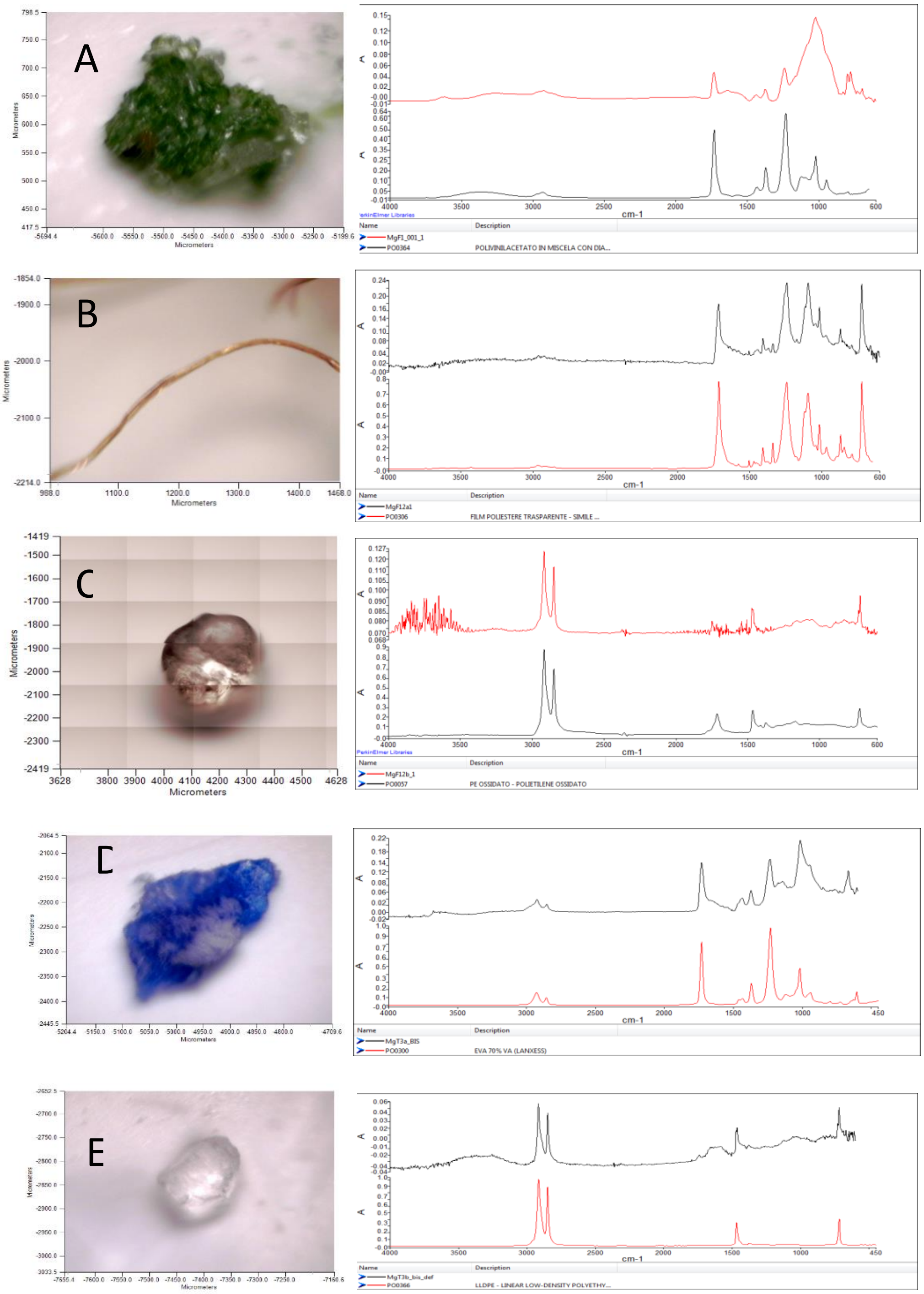

Figure 3 - *M. galloprovincialis*. Shapes, color size and types of MPs by stereomicroscope photographs and FTIR spectrums of MPs: **A-** PVA, green fragment (250 µm); **B -** PE, transparent (390 µm), **C-** PE oxidated transparent (490 µm); **D** - EVA, blue (490 µm) and **E-** LDPE (250 µm). **A-C** Mussels collected from site 4 and **D-E** from site 6

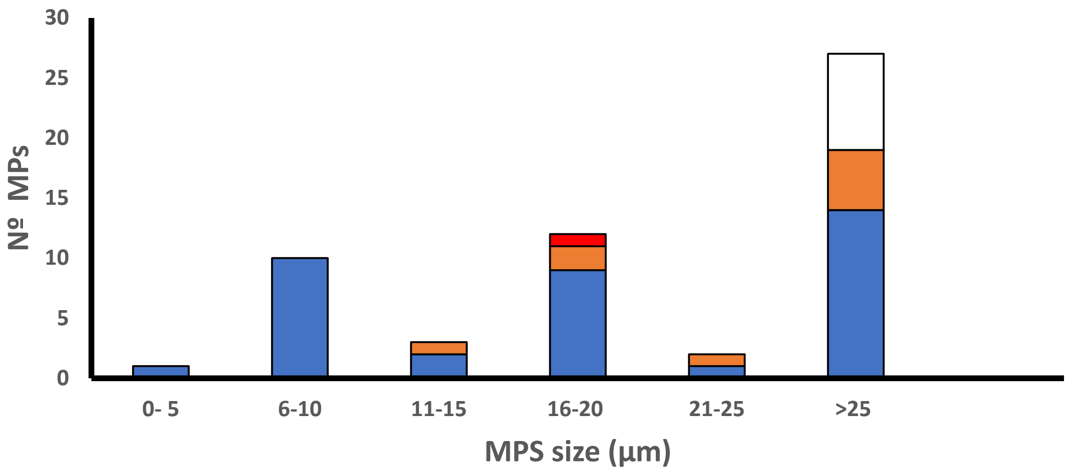

Figure 4. Color and size classes of MPs detected in *M. galloprovincialis* along the South Coast of Portugal

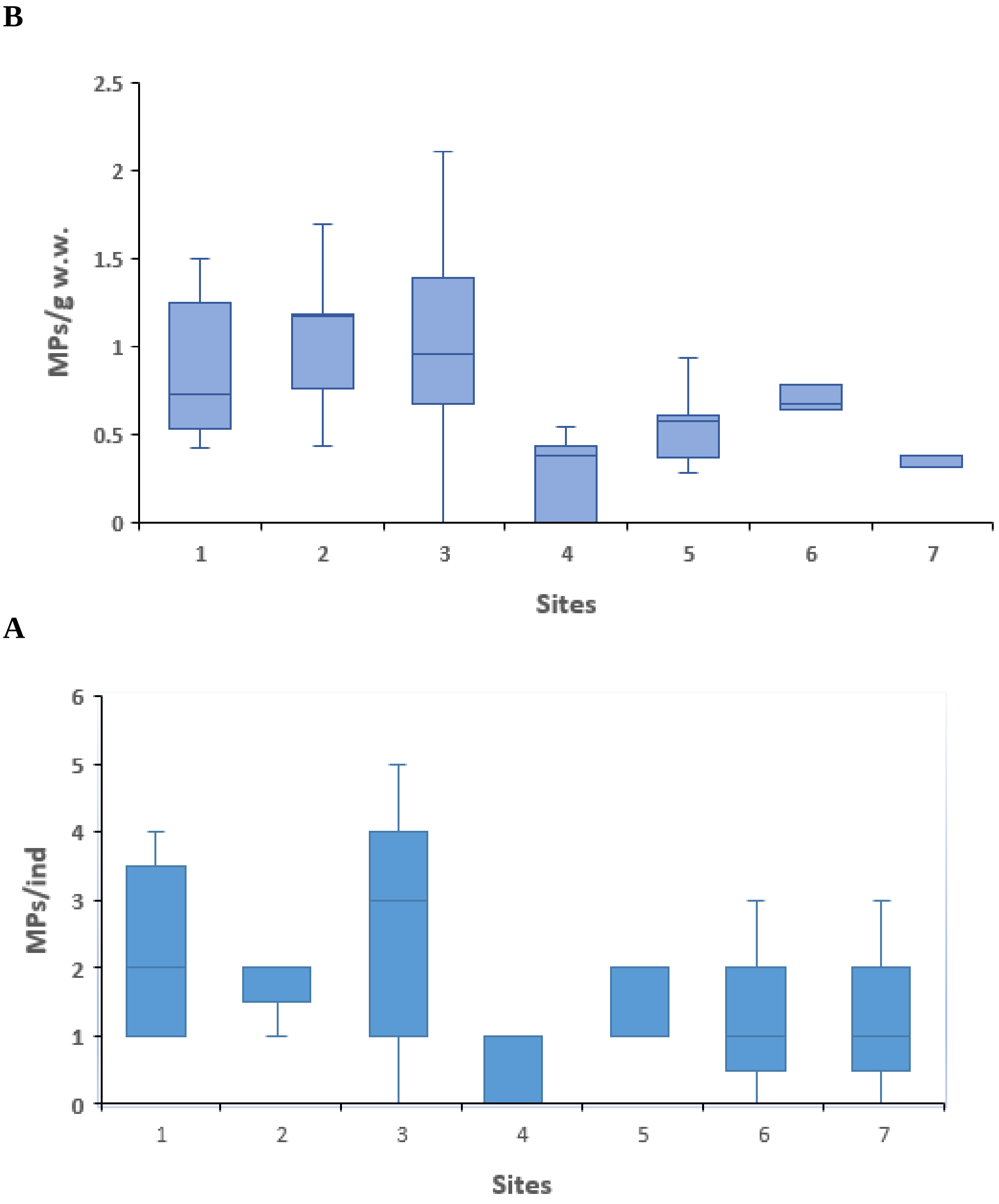

Figure 5 – Box plots of number of MPs (A) per mussel and (B) per wet weight of mussels whole soft tissues

Vila Real de Santo António

Vila Real de Santo António (VRSA) is located at the mouth of the Guadiana River estuary, where there is a port area and a marina. The river has an area of 66,960 km2, being the fourth largest river in the Iberian Peninsula and represents an important system from the socio / geo / ecological point of view, both for Portugal and Spain, not only for the development of various activities such as aquaculture , agriculture and tourism, but also due to the presence of a wide variety of species, some of them endemic, rare and even threatened (Costa et al., 1983; Guerreiro, 1989).

Thus, in the Guadiana River basin area, agriculture, industry, mining, wastewater discharge, landfills are the main point and diffuse sources of contamination / pollution. In the last decades, the increase in the use of water accompanied by the increase in effluent discharges and the greater use of it in agricultural activities associated with long periods of drought have caused a greater impact of point and diffuse sources of anthropic contamination. There was also a change in the natural regime of this river, also conditioned by the damming / storage of water in dams. The entry into service of the Alqueva dam in the 1st quarter of 2002, with a capacity to store »4,150 hm3 of water (the largest artificial lake in Europe) will certainly contribute to the worsening of some environmental problems, particularly in the lower section of the estuary (Brandão and Rodrigues, 2000). The impact of these changes on the biotic compartment of the Guadiana estuary is unknown and it is urgent to establish a reference situation.

In the city of VRSA, there is a small fishing port where 2,246 tonnes of fishery products are unloaded annually (DGPA, 2004) and a small recreational port and some small shipyards for the construction and repair of small boats (BOATS, 1997) . In VRSA the influence of the discharge of wastewater is also felt.

Declaration of Interest Statement

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: