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Evaluation of Macro Litter and Microplastics in the Ave Estuary

Daniela Sofia do Carmo Padilha

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Freshwater 3cology, 3cotoxicology and 3nvironmental Monitoring Laboratory

MAELSTROM
Smart technology for MArinE Litter SusTainable RemOval and Management

Evaluation of Macro Litter and Microplastics in the Ave Estuary

Daniela Sofia do Carmo Padilha

Mater thesis presented to Sciences Faculty of
Oporto University in
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Evaluation of Macro Litter and Microplastics in the Ave Estuary

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Todas as correções determinadas pelo júri, e só essas foram efetuadas.

O Presidente do Júri,

Porto, ___/________/________

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Those who dream by day are cognizant of many things
which escape those who dream only by night.
- Edgar Allan Poe

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Resumo

Nas últimas décadas, a poluição antropogénica tem-se tornado uma ameaça para os ecossistemas e para a saúde humana, sendo um problema global e amplamente estudado. O lixo marinho é composto por uma grande variedade de materiais provenientes de diferentes fontes, contudo, o plástico, é de longe, o material mais registado. Este é maioritariamente descartado para os ecossistemas aquáticos e transportado para diversas áreas pelas correntes, inicialmente através dos rios, que transportam o lixo gerado nas zonas terrestres até aos estuários, e finalmente até aos oceanos. Deste modo, os estuários podem atuar como uma zona tampão, e evitar que o lixo chegue ao mar. Assim, dados adquiridos nestes ecossistemas, contribuirão para uma melhor compreensão sobre a forma como o lixo proveniente de fontes terrestres entra nos oceanos. Particularmente, o plástico presente no lixo marinho está exposto a stressores físicos, químicos e biológicos, resultando em fragmentos de menores dimensões conhecidos como microplásticos (MPs). Esta realidade resulta em impactos severos nos ecossistemas globais, na saúde humana e na vida marinha. É por isso urgente promover a consciencialização, adoção de boas práticas, comportamentos e escolhas responsáveis, essenciais para uma sociedade e futuro sustentáveis. Este estudo tem como objetivos (i) avaliar e caracterizar os microplásticos e o lixo aquático flutuante (FML) no estuário do rio Ave, e (ii) identificar as principais fontes desses detritos e analisar os principais padrões hidrodinâmicos, e o tempo de residência das massas de água no interior do estuário para compreender como se transporta o lixo no estuário do Ave. Paralelamente, foram organizadas atividades de sensibilização sobre o tema através do envolvimento direto dos cidadãos. A avaliação do lixo aquático flutuante foi realizada através de monitorizações visuais, enquanto as partículas de microplásticos na coluna de água foram amostradas através de arrastos horizontais, utilizando uma rede de plâncton. Ambas as tipologias de monitorização e as amostras recolhidas foram processadas seguindo diretrizes internacionais. Os microplásticos foram identificados, medidos e categorizados por tipo (fragmento, pellet, fibra, film, espuma e tinta) e cor. No geral, os resultados obtidos indicaram que o rio Ave apresenta uma contaminação considerável por lixo aquático (ex. esferovite, sacos, garrafas de plástico), e uma grande diversidade de microplásticos (12325 no total e uma média da concentração de 63.1 ± 4.7 MPs/m^3), com uma prevalência significativa de fragmentos (53%), seguido de fibras (29%). A cor predominante foi o azul (34%), e a maioria dos microplásticos observados mediam entre 0.5 mm e 1 mm (42%). Após a analise dos resultados, o lixo público, o turismo e as atividades piscatórias foram consideradas como possíveis fontes do lixo aquático flutuante e dos microplásticos encontrados no estuário do Ave. Este estudo representa uma contribuição para a necessidade urgente de monitorização temporal e espacial da poluição por plásticos em estuários e outros ecossistemas costeiros. Este conhecimento científico é essencial para uma gestão adequada do lixo e dos ecossistemas marinhos e costeiros.

Palavras-chave: Contaminantes Emergentes, Lixo Marinho, Ecossistemas Costeiros, Consciencialização Ambiental, Hidrodinâmica Estuarina.

Abstract

In recent decades, anthropogenic pollution has become a threat to ecosystems and human health, being a widely studied and global key-topic. Marine debris are composed of a wide variety of materials from various sources, however, plastics are, by far, the most abundant material. These are mostly discharged into aquatic ecosystems and transported by currents to diverse areas, first through the rivers, which channel the waste generated in terrestrial sources, into the estuaries, and finally into the oceans. Thus, the estuaries can act as buffer zones to prevent garbage from reaching the sea. The acquired data on those ecosystems, will contribute to a better understanding on the entry of litter into the ocean from terrestrial sources. Particularly, plastic marine debris are exposed to physical, chemical, and biological stressors, resulting in smaller fragments, known as microplastics (MPs). This reality results in severe impacts on global ecosystems, human health, and marine life. It is, therefore, urgent to promote awareness, adoption of good practices, behaviours, and responsible choices, which are essential for a sustainable society and future. This study aims to (i) evaluate and characterize microplastics and floating macro litter (FML) in the estuary of the Ave river, and to (ii) identify the main sources of such debris and analyse the main hydrodynamic patterns, and the residence time of the water bodies inside the estuary to understand how the litter transport occurs on the Ave estuary. At the same time, awareness-raising activities were organized on the subject through the direct involvement of citizens. The evaluation of floating macro litter was performed through visual monitoring, while microplastic particles in the water column were sampled by a horizontal trawl using a planktonic net. Both monitoring typologies and the samples collected were processed following international guidelines. Microplastics were identified, measured, and sorted by shape (fragment, fibre, film, pellet, foam and paint) and colour. Overall results indicated that the Ave River estuary has considerable contamination by floating macro litter (e.g. styrofoam, bags, plastic bottles) and a great diversity of microplastics (12325 in total, with an annual average density of 63.1 ± 4.7 MPs/m^3), with a significant prevalence of fragments (53%), followed by fibres (29%). The most predominant colour was blue (34%), and most microplastics observed measured between 0.5 mm and 1 mm (42%). After analysing the results, public litter, tourism and fishing activities are considered possible sources for the floating macro litter and microplastics found in the Ave estuary. This study represents a contribution to the urgent need of temporal and spatial monitoring on plastic pollution in estuaries and other coastal ecosystems. This scientific knowledge is essential for adequate management of litter and of marine and coastal ecosystems.

Keywords: Emerging Contaminants, Marine Litter, Coastal Ecosystems, Environmental Awareness, Estuarine Hydrodynamics.

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List of Abbreviations

ADCP – Acoustic Doppler Current Profiler

APA – Agência Portuguesa do Ambiente

CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental

DIVA – Data Interpolating Variational Analysis

ECs – Emerging Contaminants

EPRO – European Association of Plastics Recycling and Recovery Organisations

EU – European Union

FML – Floating Macro Litter

GESAMP – Group of Experts on the Scientific Aspects of Marine Environment Protection

ICBAS – Instituto de Ciências Biomédicas Abel Salazar

ICNF – Instituto para a Conservação da Natureza e das Florestas

IPMA – Instituto Português do Mar e da Atmosfera

KOH – Potassium Hydroxide

MAELSTROM – MArinE Litter SusTainable RemOval and Management project

ML – Marine Litter

MP – Microplastic

MSFD – Marine Strategy Framework Directive

OSPAR – Oslo and Paris Convention for the Protection of the Marine Environment of the North-East Atlantic

PCO – Principal Coordinates Analysis

SEM – Standard Error of the Mean

SNIAmb – Sistema Nacional de Informação de Ambiente

SNIRH – Sistema Nacional de Informação de Recursos Hídricos

TSG-ML – Technical Subgroup on Marine Litter

UNEP – United Nations Environment Programme

WWTPs – Wastewater Treatment Plants

1. Introduction

1.1 General Framework

Pollution can be introduced into the environment naturally (e.g. volcanic ash), or by direct and indirect human activities (e.g. garbage, factory runoff) (Boudreau et al., 2022). There are several types of pollution, the main ones being air pollution, water pollution and soil pollution, but there is also noise pollution and light pollution. In recent decades, anthropogenic pollution has become a threat to ecosystems and human health, being a widely studied and global key-topic (Rhind, 2009). This is because it can spread to remote places far from their sources, carried by air and water currents, causing negative effects on the balance of the environment (Boudreau et al., 2022).

Over the past two decades, the presence of Emerging Contaminants (ECs) in aqueous environments has become a major worldwide concern, as some of these contaminants are endocrine disruptors and others are proven carcinogens and mutagens (Datta et al., 2018; Morin-Crini et al., 2022). These micropollutants can be natural or synthetic substances resulting from anthropogenic activities, which accumulate in aquatic compartments as a result of continuous and uncontrolled release. Additionally, they are persistent, which means that they have resistance to degradation, staying in the environment for longer periods of time, and, subsequently producing stronger effects on the fauna and flora (Radović et al., 2015; Sousa et al., 2019).

ECs include pharmaceuticals (e.g. antibiotics, anti-inflammatory, hormones, psychiatric drugs, illicit drugs, veterinary medicine); personal care products and cosmetics (e.g. bisphenols, parabens, triclosan, UV filters, disinfectants, biocides); industrial substances (e.g. surfactants, alkylphenols, nanoparticles, perfluorinated compounds); pesticides (e.g. fungicides, herbicides, insecticides, bactericides, rodenticides, nematicides); biological agents (e.g. pathogenic bacteria, multi-drug resistant microbes, antifungal resistance, antibiotic resistance genes); unintentional persistent organic pollutants (e.g. dioxins, brominated dioxins, halogenated polycyclic aromatic hydrocarbon, polychlorinated naphthalenes, environmentally persistent free radicals); and other substances (e.g. rare earth elements, selenium, microplastics (MPs), radionuclides, food packaging and containers, corrosion inhibitors) (Morin-Crini et al., 2022).

Due to their widespread use in daily applications, these micropollutants are ubiquitous not only in water and sediments, but also in soils, atmosphere, food products and living organisms, and they were even found in areas where there is no anthropogenic activity (Datta et al., 2018; Routti et al., 2019). Furthermore, conventional urban wastewater treatment plants (WWTPs) are not designed to eliminate this type of pollutants, and end up discharging their effluents contaminated with ECs into aquatic environments, such as rivers (Morin-Crini et al., 2022; Sousa et al., 2019).

Portugal is the top third country in the world with more studies in the field of surface water, monitoring the occurrence of water contaminants included in the European Union guidelines (priority substances of European Directive 39/2013/EU and contaminants of emerging concern of Watch Lists of European Decisions 495/2015/EU and 840/2018/EU) (EU, 2013, 2015, 2018), as well as other specific pollutant, not considered in those European Union documents, and with reported high concentrations and frequencies (Morin-Crini et al., 2022; Sousa et al., 2018, 2019). Even so, the impact of aquatic litter, in particular MPs, in river waters has received little attention in comparison to marine system (Morin-Crini et al., 2022).

Of all the waste produced by humans, plastics constitutes an alarming 54% (Hoellein et al., 2014). Currently, most of the post-consumer plastic waste ends up in recycling or energy recovery processes (75.1%), however, in many European countries, landfill is still the first option (24.9%) (Plastics - the Facts, 2019). Thus, the input of solid waste from terrestrial sources into the oceans has become a major environmental threat, as such, marine litter (ML) and plastic pollution as become a priority issue in the international agenda (GESAMP, 2016; González-Fernández et al., 2021; UNEP, 2016).

If no effective measures are taken, it is expected that, growth in the production and use of plastics will become unsustainable, increasing the amount of waste accumulated in both terrestrial and aquatic ecosystems around the world (Geyer et al., 2017; Lau et al., 2020; Lebreton & Andrady, 2019), potentially causing impacts on biodiversity and human health (González-Fernández et al., 2021; Huerta Lwanga et al., 2017; Rillig & Lehmann, 2020). An example of how quickly these impacts can arise, is the fact that at the time of writing this dissertation, for the first time there were found plastic particles in human blood and placenta (Leslie et al., 2022; Ragusa et al., 2021).

1.2 Marine Litter

1.2.1 Description

According to the Marine Strategy Framework Directive (MSFD), ML is "any persistent solid material discarded, manufactured or processed, disposed of, abandoned or lost in the marine and coastal environment” (Agência Portuguesa do Ambiente, 2021; UNEP, 2021). It consists on items that, after being produced or used by people, are deliberately or accidentally discarded in the sea, rivers or beaches, or brought indirectly to the sea by rivers, sewage, storm water or wind (Galgani et al., 2010).

Marine debris are present in all oceans and higher abundances are frequently found in the main shipping routes and coastal waters near major urban regions, it can be found floating at the surface, in the water column, stranded on coastlines, and/or deposited on the seafloor (Galgani et al., 2015; Sá et al., 2016). Plastic is the predominant material among ML worldwide, in some cases representing up to 95% of the waste that accumulates on shorelines, the sea surface and the seafloor (Galgani et al., 2015; Neves et al., 2015; Sá et al., 2016). At this moment, pollution by ML is recognized as a worldwide problem and a major threat to marine ecosystems (Galgani et al., 2010; Sá et al., 2016). Such is the concern for this type of pollution that the United Nations urges to prevent and reduce ML by 2025 (Target 14.1) as part of the Decade of Ocean Science for Sustainable Development (2021–2030) (Guerrero-Meseguer et al., 2020).

1.2.2 Sources

Marine debris can have land-based sources (which comprises about 80%), or ocean-based sources (20%) (Neves et al., 2015; UNEP, 2005). Land-based sources include mainly recreational use of the coast, general public litter, industry, harbours and unprotected landfills located close to the coast, but also sewage overflows, introduction by accidental loss and extreme events (Galgani et al., 2015; Mordecai et al., 2011). ML can also be transported by streams, rivers, sewers and other industrial discharges and run-offs into the ocean (Galgani et al., 2015). Ocean-based sources of ML include commercial shipping, ferries, commercial and recreational fishing vessels, military and research fleets, pleasure boats, offshore installations such as platforms and aquaculture sites, and legal and illegal dumping at sea (Galgani et al., 2015; Mordecai et al., 2011). Other sources are the result of accidental dumping or disposal of material from ships, offshore platforms and the wear and tear of the implanted equipment (20%) (Galgani et al., 2015; Neves et al., 2015).

The distribution and accumulation of ML are influenced by oceanic and estuarine current patterns, tides, waves, freshwater sources, meteorological conditions, as precipitation and winds, the proximity to areas with anthropogenic activities (e.g. urban, industrial and recreational areas, shipping lanes and fishing grounds) (Galgani et al., 2015; Guerrero-Meseguer et al., 2020), and the chemical characteristics of the materials themselves (Gago et al., 2018a). And, since physical and environmental conditions, as well as anthropogenic activities such as fishing and tourism, often change seasonally, the accumulation of ML can also vary over time (Guerrero-Meseguer et al., 2020).

1.2.3 Impacts

ML impacts the environment in several negative ways, also affecting the economy and human health (Guerrero-Meseguer et al., 2020). Some of the main impacts that ML has on marine fauna are related to entanglement and ingestion, mainly of several types of plastic (e.g. synthetic fibres, plastic sheets) or discarded fishing gear (e.g. rope, nets, lines and trawls) (Sá et al., 2016). Consequences of entanglement, range from restriction of movements, to amputation and death. Ingestion of ML by marine fauna can cause physical blockage in the digestive system and reduced absorption of nutrients, ultimately leading to starvation or digestive injuries and death (Gall & Thompson, 2015; Guerrero-Meseguer et al., 2020; Kühn et al., 2015; Sá et al., 2016). ML can also be entangled in flora, resulting in reduced light levels needed for growth and added weight that can crush the vegetation (Guerrero-Meseguer et al., 2020; Kühn et al., 2015).

ML is transported by ocean currents for substantial periods of time, reaching places far from its source, increasing the potential for invasions of alien species (Guerrero-Meseguer et al., 2020; Kühn et al., 2015; Molnar et al., 2008), and for transporting organic and inorganic contaminants (Gall & Thompson, 2015; Rochman et al., 2013). Marine debris can be dangerous to shipping, and, at the same time they are aesthetically detrimental, generating negative socio-economic consequences (Gall & Thompson, 2015; Mouat et al., 2010).

A large proportion of the marine debris that reach the ocean will accumulate in the oceanic gyres (Fig.1). An ocean gyre is a large system of circular ocean currents formed by global wind patterns and by forces created by the Earth's rotation (Filho et al., 2021). These gyres are a reflection of the main wind patterns. Additionally, some of these surface currents are part of the thermohaline circulation, also known as the ocean conveyor belt, which makes the oceanic water circulate around the entire planet. Ocean gyres circle large areas of stationary, calm waters. Due to this condition, marine debris drifts into these areas, and can accumulate there for years. These regions are known as garbage patches (Filho et al., 2021; National Geographic, 2022).

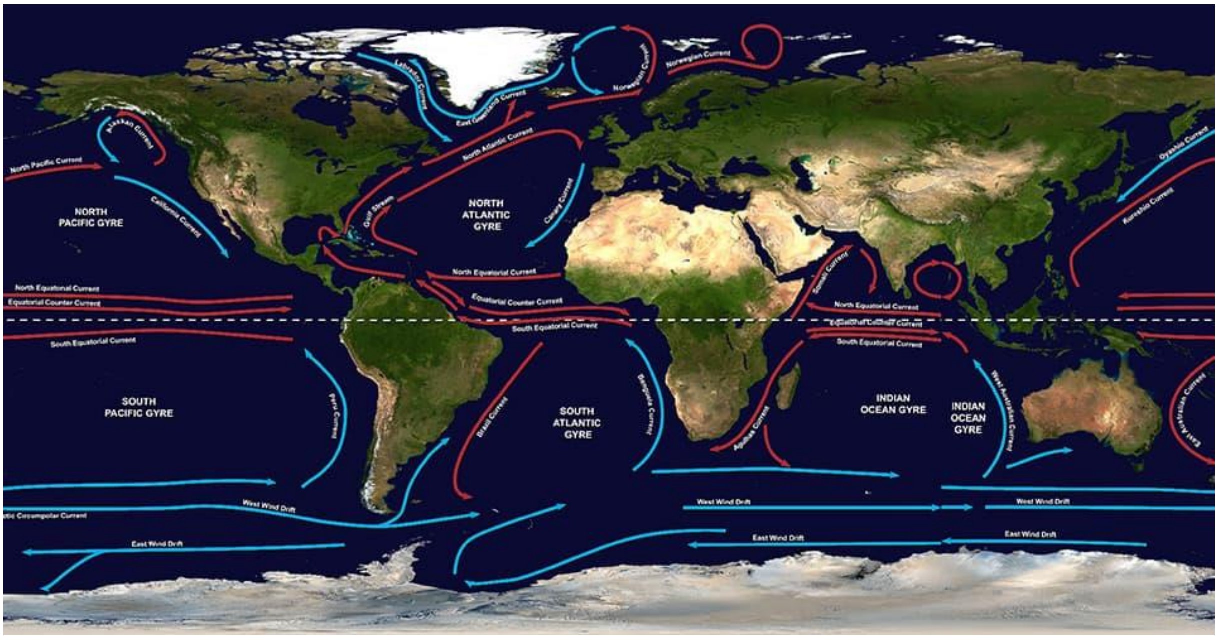


Fig. 1 Main oceanic gyres (Source: National Geographic, 2022).

The world's largest garbage patch is the Great Pacific garbage patch, discovered in 1997 by oceanographer Charles Moore (Rochman et al., 2016). It has an estimated area of 1.6 million km², and approximately 80,000 tons of debris, which is equivalent to 50 mg/m² (Filho et al., 2021; Iberdrola, 2022). The other known garbage patches are the ones found at the North Atlantic (discovered in 2009), the Indian Ocean (discovered in 2010), the South Pacific (discovered in 2011), and the South Atlantic (discovered in 2017) (Iberdrola, 2022). The patches are composed of a wide variety of materials, including wood, glass, metal, rubber, plastic and paper from various sources. However, plastics are by far the most abundant material (Arthur et al., 2008; Cole et al., 2011).

Guerrero-Meseguer et al., (2020) stated that there is currently more garbage than algae on the sandy beaches of northern Portugal, evidencing the need to take urgent action against this contamination.

1.3 Microplastics

1.3.1 Description

Plastic is a cheap, lightweight, robust, durable and, corrosion resistant material with high thermal and electrical insulation properties. In addition, the diversity of polymers and the versatility of their properties are used to make a wide range of products, which enable medical and technological advances, energy savings and other numerous social benefits (Andrady & Neal, 2009). Thus, plastics have contributed to the improvement of human life quality. However, their incorrect use and disposal have led to the accumulation of plastics in the environment, with potential negative effects for ecosystems, organisms, and human health (Thompson et al., 2009). When the plastic reaches the ocean, it suffers a physical and chemically deterioration, creating small fragments due to the conditions to which it is exposed, such as ultraviolet radiation (photodegradation), sand and rocks (abrasion), and interactions with marine organisms (biodegradation) (GESAMP, 2016). Thus, the fragmentation of larger plastics results in smaller fragments, known as MPs (Gago et al., 2018b).

These fragments can be classified regarding their dimension, origin, shape, colour and chemical properties. Plastics with sizes greater than 50 cm are defined as megaplastics, particles between 50 cm and 25 mm are defined as macroplastics, particles between 25 mm and 5 mm as mesoplastics, particles between 5 mm and 1 μm as MPs and particles smaller than 1 μm are considered nanoplastics (Gago et al., 2018b). MPs can be divided into two categories: primary, the ones which are manufactured for a purpose (e.g. abrasives in cosmetics such as facial and hand cleansers, toothpastes, and shower gels; resin pellets; and abrasives from the air-blasting industry), or secondary, which derive from the fragmentation of larger floating plastic items (Browne, 2015).

According to the shape of the fragments, MPs can be classified by shape as fibres, fragments, films, pellets, foams, and paint (Fig. 2). MPs can be coloured (e.g. black, blue, red, green, white, etc.), transparent or multi-coloured (when a fragment has several colours) (Gago et al., 2018b).

The chemical composition of these fragments is also relevant because, depending on the density of the plastic particles compared to the density of the oceanic, estuarine and riverine water masses, they can be settled and become a part of the sediments (in case of denser particles), be in suspension in the water column, or floating on the surface (in case of lighter particles) (Khalid et al., 2021).

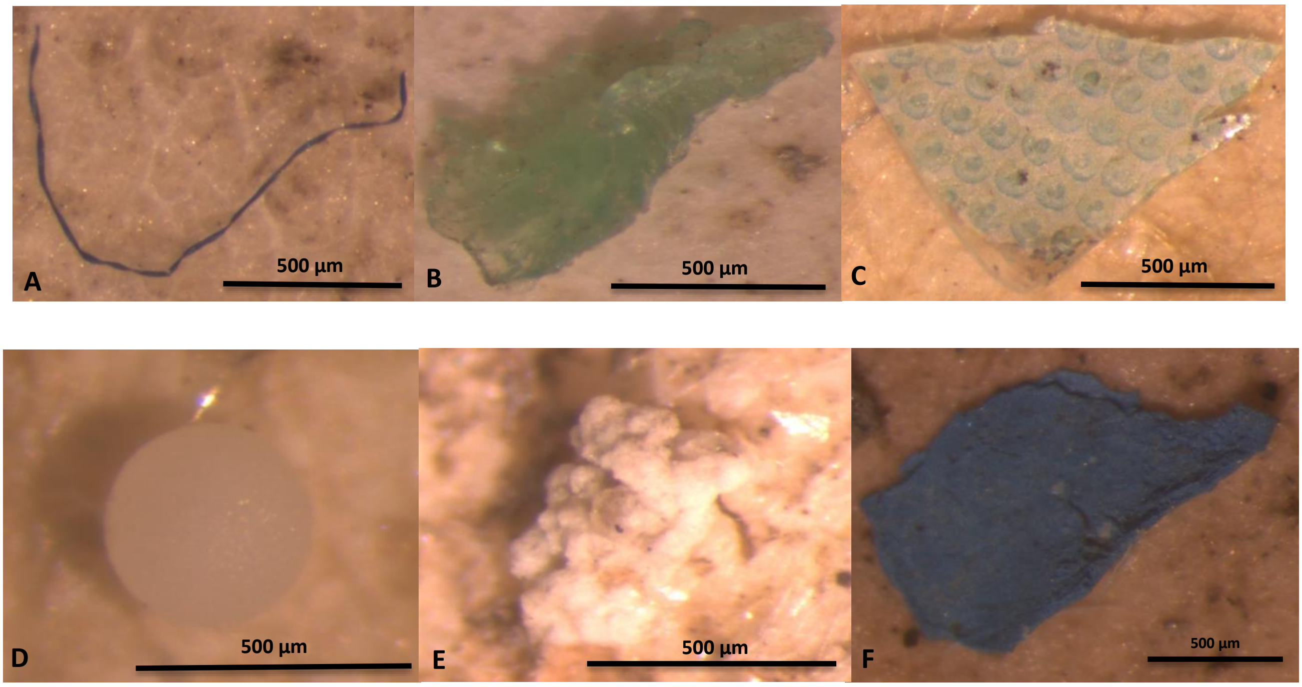


Fig. 2 Different shapes of MPs (A – Fibre; B – Fragment; C – Film; D – Pellet; E – Foam; F – Paint) (Source: the author).

1.3.2 Sources

MPs have various distinct sources, which can be organized in terrestrial sources (e.g. water flow, effluents from WWTPs) or maritime sources (e.g. fishing or transport activities) (Prata et al., 2020). The principal maritime sectors that can be considered MP sources, are fisheries, aquaculture, shipping, offshore industry, and ship-based tourism.

The main terrestrial sectors that generate MPs are:

- Cosmetics and personal care products: which can include small plastic spheres as abrasives and fillers in facial scrubs and shower gels (Andrady, 2011; Cole et al., 2011). These plastic spheres will inevitably be released to wastewater systems, as most WWTPs can´t retain them in the treatment processes (Fendall & Sewell, 2009; Rodrigues et al., 2020a);
- Textiles and clothing: releasing fibres during mechanical washing;
- Terrestrial transportation: emitting plastic particle dust caused by tire wear, which ends up washed away by the rain, into the sewer, surface waters and ultimately the sea;
- Plastic producers and fabricators: which produce and transport plastic resin pellets, and as such, accidental losses occasionally occur during transport or at manufacturing facilities, releasing the pellets into the environment;
- Ship maintenance and ship dismantling: cleaning the ship hulls, which involves air blasting the ships with plastic particles to remove biological growth (biofouling) and old paint. This gives the potential for two types of MPs to enter the environment, the plastic abrasive and the flakes of paint which often contain a polymer base (Andrady, 2011; UNEP, 2016).

Also, proximity to highly urbanized areas or intense anthropogenic activities is generally associated with environments that have a higher level of MP pollution, so it is expected that different environments with different levels of anthropogenic pressure, will have different concentrations of MPs, with different characteristics, coming from different sources (Xue et al., 2020).

1.3.3 Impacts

Due to their high strength, durability and slow degradation, MPs are persistent, being able to travel great distances and reach places very far from their origin, through ocean currents and winds. Therefore, MPs have been found in all types of aquatic environments, even in the most remote areas (Castillo et al., 2016; Rodrigues et al., 2020a), such as isolated oceanic islands (Ivar do Sul et al., 2009), polar regions (Bergmann et al., 2019; Peeken et al., 2018), and also in deep sea sediments (Cauwenberghe et al., 2013).

The major concern caused by MPs is their ability to impact the environment and wildlife in various ways. MPs have sizes similar to the natural food of several organisms that feed on plankton. Therefore, there is a high risk of ingestion of these particles by filtering organisms, and thus entering the food chain (Castillo et al., 2016; Rodrigues et al., 2020a). Since filter organisms are at the base of food chains, the intake of MPs by these organisms may have more severe effects along the trophic chain (Khalid et al., 2021). When ingested by marine fauna, MPs can cause an inflammatory response, physical lesions in the body, changes in eating behaviour, give a false feeling of satiety, or transfer of toxic chemicals (Fu et al., 2020).

Since humans are at the top of the food chain, we can also be exposed to MPs through the consumption of different marine species, such as fish, crustaceans and invertebrates (Khalid et al., 2021). Therefore, MP pollution can also become a risk to human health, since the ingestion of contaminated organisms can expose us to plastics and chemicals with unknown consequences (Prata et al., 2020). These polymers may contain chemical additives, including some known endocrine disruptors, which can be harmful even at extremely low concentrations. However, is worthy to note that, there is not enough information about the impacts that these chemicals can have on human health (Gallo et al., 2018). According to Prata et al., (2020), based on mollusc consumption, each Portuguese citizen can consume up to 1440 MPs per year, and therefore more scientific research is urgent on the potential impacts that the direct or indirect intake of MPs may have on the endocrine system and all human health (Gallo et al., 2018).

In addition to adsorbing various organic and inorganic compounds, due to their large distribution in the oceans, MPs also provide substrates for the colonization of diverse microbial communities (Dussud et al., 2018), allowing exotic species to travel great distances. Therefore, MPs can play an important role in dispersing various marine organisms to new habitats (Andrady, 2011). These MP biofilms are a new ecological niche, known as plastisphere (Khalid et al., 2021). The mechanism by which MPs carry organisms, and how it differs from natural materials, is still unknown and unexplored. The ecological impacts of the transport of species in MPs cannot be established until further research on the subject is developed (Khalid et al., 2021).

1.4 Integration of the study in the MAELSTROM Project

Rivers act as pipelines to the ocean, channelling the waste that is dumped along their curse. Thus, due to the potential negative effects of the ML and MPs, and the ability to reach ecosystems far from their origin, it is increasingly important to carry out continuous monitoring of these pollutants, particularly in the limit of the riverine regions: the estuaries. This will contribute to a better understanding on the entry of litter into the ocean from terrestrial sources. Monitoring the levels and characteristics of this type of pollution can help to better understand its sources and impacts and serve as a basis for the design of effective management plans to reduce and prevent ML and MPs (Rodrigues et al., 2020c).

There are several factors that can influence the accumulation of debris in rivers and estuaries, and their arrival in the ocean, such as the proximity to residue sources, the hydrodynamic conditions, and also social and economic factors. In this way, it is extremely important to carry out integrative and multidisciplinary research projects (Guerrero-Meseguer et al., 2020).

This dissertation is integrated into the MAELSTROM (MarinE Litter SusTainable RemOval and Management) project, a multidisciplinary European project that aims to provide diverse answers and solutions to the complex issue of sustainable removal and treatment of ML. The main goal of MAELSTROM is to integrate sustainable and innovative technologies for the removal of ML in different European coastal ecosystems, before it reaches the sea, combined with a developed circular economy and society-oriented solutions. While at the same time, involving scientists, stakeholders, and citizens, in order to raise awareness to the problem.

To achieve this, MAELSTROM stands in four main pillars, Identify, Remove, Recycle and Marketize. The first step is to identify the litter accumulation hotspots through numerical modelling simulations and experimental campaigns. Then, the litter will be removed from the seabed and water column using one of the MAELSTROM technologies: the bubble barrier or the seabed cleaning platform. Afterwards, the litter collected will be recycled and put back into the industrial chain, becoming a new resource.

Before installing any technology that could help in the ML removal, it is of utmost importance to characterize the area in terms of ML and MP content and hydrodynamic conditions, In this sense, the work here presented is devoted in understanding the necessity of installing these kinds of technologies into a Portuguese estuary. Also, as this will be the first time MAELSTROM will be installing one of their technologies in an estuary, understanding the hydrodynamic conditions of the estuary is critical to adapt the technology to the site. Furthermore, this is the first study to monitor ML and MP in the Ave river, distinguishing itself from similar studies, and contributing to the knowledge in this matter, by having distributed sampling points in the estuary, near the coast and in the upstream region of the estuary.

1.5 Study Site

The final section of a river is called an estuary, which extends from the mouth of the river to the limit of the brackish waters. Estuaries are low-energy coastal areas which are less subjected to the maritime currents, however, still very susceptible to tidal currents. Furthermore, they are in permanent contact with marine water, making these estuaries a very complex ecosystems, both ecologically and morphologically speaking (ICNF, 2008).

Estuaries are one of the most productive ecosystems in the world (Likens, 2010). These nearshore coastal areas are often referred to as important fish larvae and juvenile nurseries (Baptista et al., 2019), as the success of early life stages of marine fish depends on the ability of larvae to reach coastal nursery grounds, which provide shelter and food availability for their growth and survival (Bailey et al., 2008). Estuaries also support several human activities (e.g. marine transportation, harbours, fishing, and tourism), explaining the need for their protection, which has been recognized worldwide (Rodrigues et al., 2020b).

Estuaries are important sediment accumulation zones and, as such, they contribute to a better understanding on the entry of garbage into the ocean from terrestrial sources (González-Fernández et al., 2021). Despite the relevance that transition systems, such as estuaries, have in the study of MP pollution and their consequences to the wildlife, until now in Portugal, only the Douro (Porto) and Antuã (Aveiro) estuaries were sampled (Prata et al., 2020).

The study area of this dissertation was the estuary of the Ave River. The Ave hydrographic basin occupies 1400 km², crossing 19 municipalities (Fig. 3) (APA, 2022). The river has an extension of about 94 km from its source, in Serra da Cabreira, until the mouth in Vila do Conde (Amporto, 2009). It exchanges waters with a large set of smaller rivers and streams, being its largest tributaries the Este River (right margin), which drains 247 km², and the Vizela River (left margin), which drains 340 km² (APA, 2014; *Plano de Ação - Despoluição do rio Ave*, 2015).

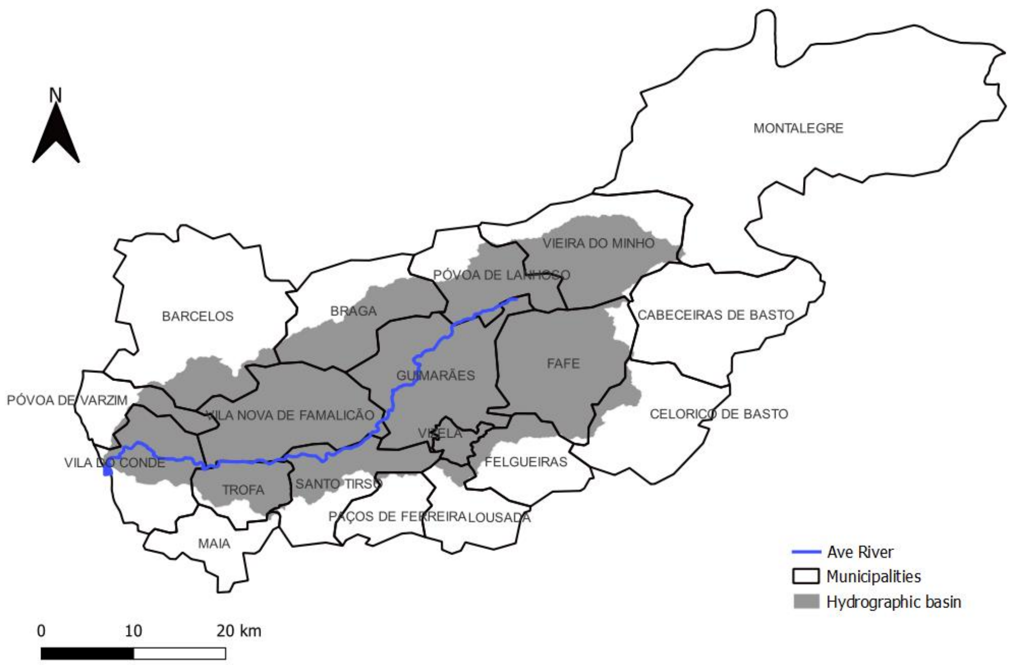


Fig. 3 Hydrographic basin of Ave River (Source: SNIAmb).

The Ave river estuary is small in size, compared with other Portuguese estuaries as the Douro or the Tejo, with an average depth of 3.6 m (Fig. 4), 1.5 km long and an area of 1.2 km² (*Plano de Ação - Despoluição do rio Ave*, 2015). It's classified as an Atlantic estuary (1130pt2) by ICNF, (2008). The river flow of the Ave is small in comparison with other Portuguese rivers, with an average value of 30 m³/s per year, minimum values next to zero, and maximum values around 60 m³/s. Minimum values are observed during, summer, between June and October, when the estuary has a weaker flow. On contrary, maximum river flow values are present during winter months, from November to March when the flow is stronger. The maximum values registered in the Ave river were 450 m³/s, 390 m³/s and 360 m³/s, which were recorded in April of 1979, March of 1990 and January 1982 respectively, coincident with winter months. (Fig. 5).

The study site features a weir located 1.5 km upstream, which limits the estuary extension, and two breakwaters designed along the coast to protect the entrance from the oceanic waves and storms (APA, 2014). An important ornithological natural reserve (Reserva Ornitológica de Mindelo), is located near the river mouth (Ribeiro et al., 2016) (Fig. 6). It's a highly urbanized estuary, with an elevated density of river activities, like fishing, canoeing, rowing, stand up paddle and boat traffic. Along the river, several potential sources of debris were identified, such as 4 port facilities, 5 wastewater treatment plants above the 150.000 population mark, and several marinas, artificial structures, tributaries, intensive agricultural areas, livestock farms, and industries, mainly in the textile sector, but also metal plating, leather tanning, rubber and plastic, cutlery, and metalworking manufactures (APA, 2014; Rocha et al., 2013).

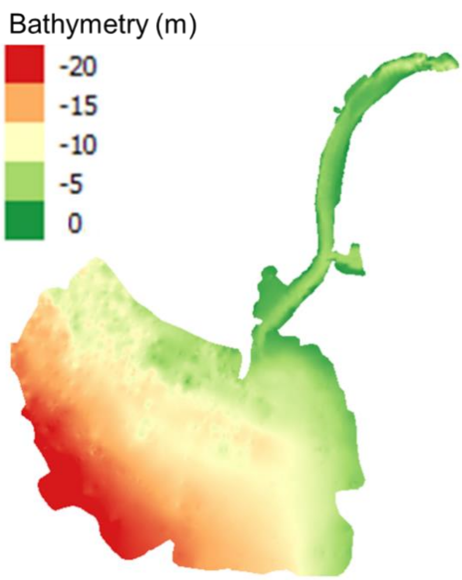
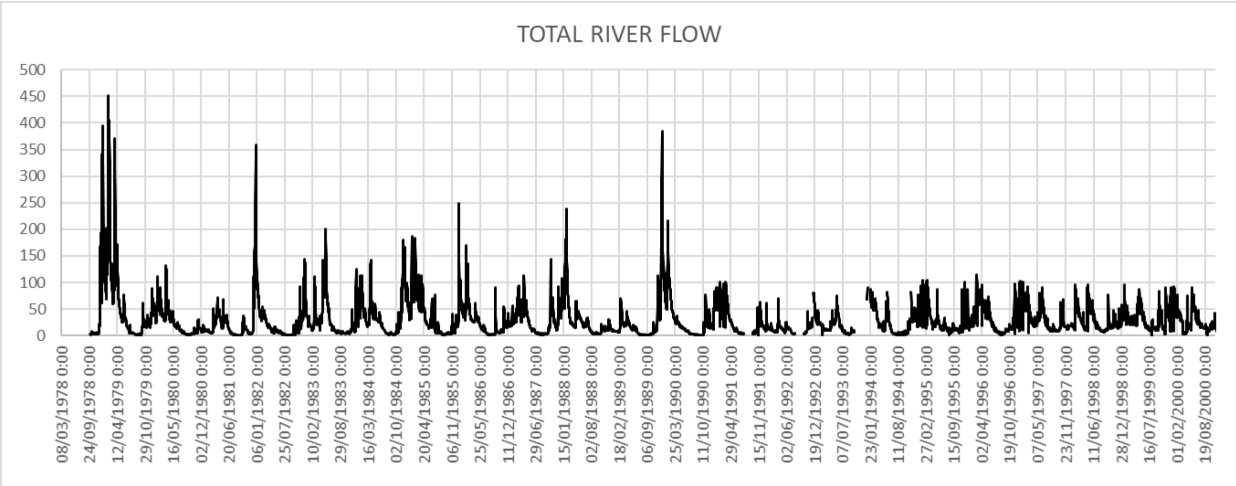
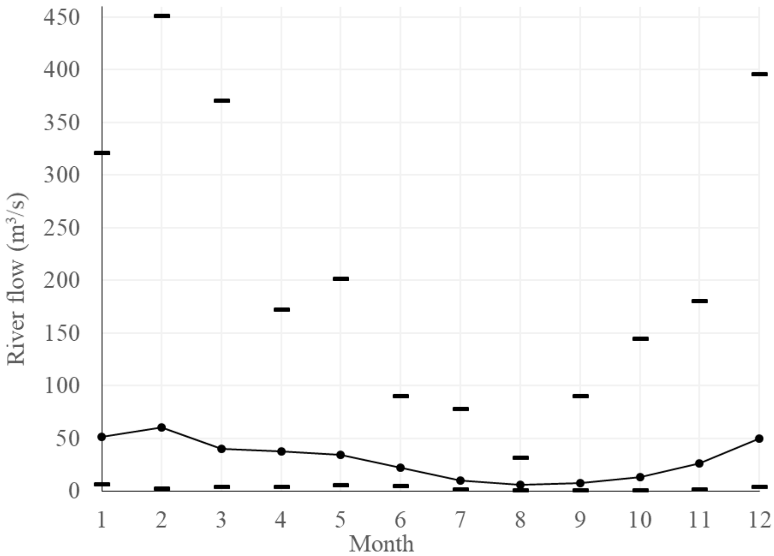


Fig. 4 Bathymetry of the Ave estuary measured in September 2021 under the scope of the MAELSTROM project.



a)



b)

Fig. 5 a) Ave river flow in the estuarine area between 1978 and 2000. b) Monthly average of the Ave river flow (dots) and maximum and minimum values measured for each month (Data Source: SNIRH).

Fig. 6 Estuary of Ave River. 1- Weir; 2- Recreational marina; 3- Fishing port; 4- Shipyard; 5- Ornithological natural reserve: 6- Breakwaters (Source: Google Earth).

Due to multiple untreated or with inadequate treatment domestic and industrial discharges, the Ave was considered one of the most polluted rivers of Portugal, causing the deterioration of the water quality, and resulting in inappropriate water for several uses, such as water supply for domestic and industrial uses, recreational use, fishery, and irrigation (Ribeiro et al., 2016).

Because of this, for the last years, the Ave hydrographic basin has presented serious physical-chemical and biological disturbances. Previous studies have shown that the parameters that presented the worst results were biochemical oxygen demand, dissolved oxygen and the bacteriological parameters, resulting in the degradation of the riparian curtains and poor water quality, which in turn has evident effects on the aquatic communities (Amporto, 2009; APA, 2014).

1.5.1 Site Hydrodynamics

Knowledge of estuary hydrodynamics is critical to understand problems such as water quality, residence time, dispersion rate of pollutants, ecosystem characteristics, sediment transport and erosion processes in coastal and estuarine areas (Dias et al., 2013). The main physical driving forces in an estuarine region are the river flow, the waves, and the tides. The river flow can produce different water discharges, and as such, vastly different water elevation levels, changing the transport patterns depending on the river flow, as well as the physical, chemical, morphological and biological properties of the region. The oceanic and meteorological components of the tides also affect the water elevation of the estuarine areas and the properties of the estuary. If the estuary presents a mouth that is wide enough to let the waves enter the region, the oceanic waves can induce more mixing and oxygenation in the estuarine waters, enhancing the entrance of oceanic water masses. However, it must be taken into account that the estuaries are normally protected by natural structures (e.g. sand splits), or by man-made structures (e.g. breakwaters). These structures protect the estuarine regions from storms coming from the sea, not allowing the big waves to pass through the mouth. An additional parameter that can also condition the estuarine circulation is the wind (Iglesias, n.d.; Iglesias, Avilez-Valente, et al., 2019; Iglesias et al., 2020; Iglesias, Venancio, et al., 2019). The wind acting directly on the estuary only has a substantial effect if the estuary presents dimensions big enough to allow formation of a wind-promoted circulation. However, if the estuary has low river flows, the effect of the wind at oceanic and coastal locations can enhance the entrance of oceanic water and change the circulation patterns and the stratification conditions in the coastal regions, and so in the estuarine mouth, (Mohammedi, 2022). The influence of each one of these factors can vary in different spatial and temporal scales. Spatial variations can be produced at different points of the same estuary or from one estuary to another. Temporal variations can be annual, considering the differences between the winter and summer months, or interannual, with differences between years (Dolgopolova & Isupova, 2010).

The distribution of the oceanic and riverine water masses in estuaries will depend on the freshwater flow, the volume of seawater entering the estuary with the tide, the waves, the precipitation and the effect of the wind. The thermohaline (temperature and salinity) structure of the estuary and the mixing between the water masses will be strongly dependent on these highly variable meteorological and oceanic parameters. The salinity of the water masses will indicate their provenience and the amount of mixing that is produced. The temperature can also provide useful qualitative information on the hydrodynamical processes of a system and its characteristics, presenting highly variable values depending on the season, with the riverine waters being colder during the winter and hotter during the summer. Vertical profiles of salinity and temperature can provide information about the vertical distribution of the water masses, helping find the halocline and the thermocline. These layers define the vertical position of the highest salinity (halocline) and temperature (thermocline) gradient. In the absence of external forces, like wind or waves that could mix the water masses, these layers limit the vertical movements of the water, keeping their properties (Domínguez-Villar et al., 2018).

Additionally, pH values in estuaries are an important indicator of the solubility and availability of nutrients, as well as chemical pollutants (Sousa et al., 2019). The determined values considered as optimum for plankton growth and nutrient availability are between 5 and 8 (Ribeiro et al., 2016). The proximity to textile industries, which produce alkaline effluents, could be related to slightly alkaline pH levels in rivers and estuaries (Sousa et al., 2019).

Estuaries can be classified according to their vertical stratification of salinity as, highly stratified, partially stratified, or well-mixed (Dyer & Taylor, 1973). Highly stratified estuaries (also known as salt-wedge estuaries), usually develop at coastal river mouths, when the flow of the river is higher than the tidal flow, suppressing the tidal mixing. The less dense freshwater extends on top of the heavier saltwater, creating a wedge. This will preserve a strong stratification along the estuary (Dolgopolova & Isupova, 2010; Krvavica et al., 2021). Partially stratified estuaries are generally shallow and, although the mixture of salt and freshwater occurs at all depths, the bottom layers have higher salinity levels than the upper layers at all points in the estuary. Furthermore, salinity is at its highest levels at the mouth of the estuary, decreasing in the upstream direction (McLusky & Elliott, 2006). Finally, well-mixed estuaries develop when the flow of the river is low and the tidal flow is high. In this case, the water salinity is vertically homogenous, being higher downstream and lower upstream (McLusky & Elliott, 2006).

Until this date, and to the best of the author's knowledge, the Ave estuary presents a lack of data regarding its hydrodynamic patterns. To solve that, and under the scope of the MAELSTROM project, CIIMAR performed two short-term campaigns that measured several hydrodynamic parameters of the Ave estuary. These campaigns have a duration of 13 hours each, encompassing an entire spring tide cycle. The equipment selected for these campaigns was an Acoustic Doppler Current Profiler (ADCP), and a multiparametric probe to measure vertical profiles of velocity, salinity, temperature and pH along a transect located in the estuarine region following the next scheme:

- Go: CMIA → SICNAVE: ADCP continuously measuring with a boat constant velocity of 2 knots.
- Return SICNAVE → CMIA: Two multiparametric probe profiles at 1/3 and 2/3 of the transect length and always at the same locations.

The location of the transect and a diagram of the campaigns are provided in Fig. 7. The short-term campaigns were performed in October 2021 and January 2022, and their results were carefully represented and analysed.

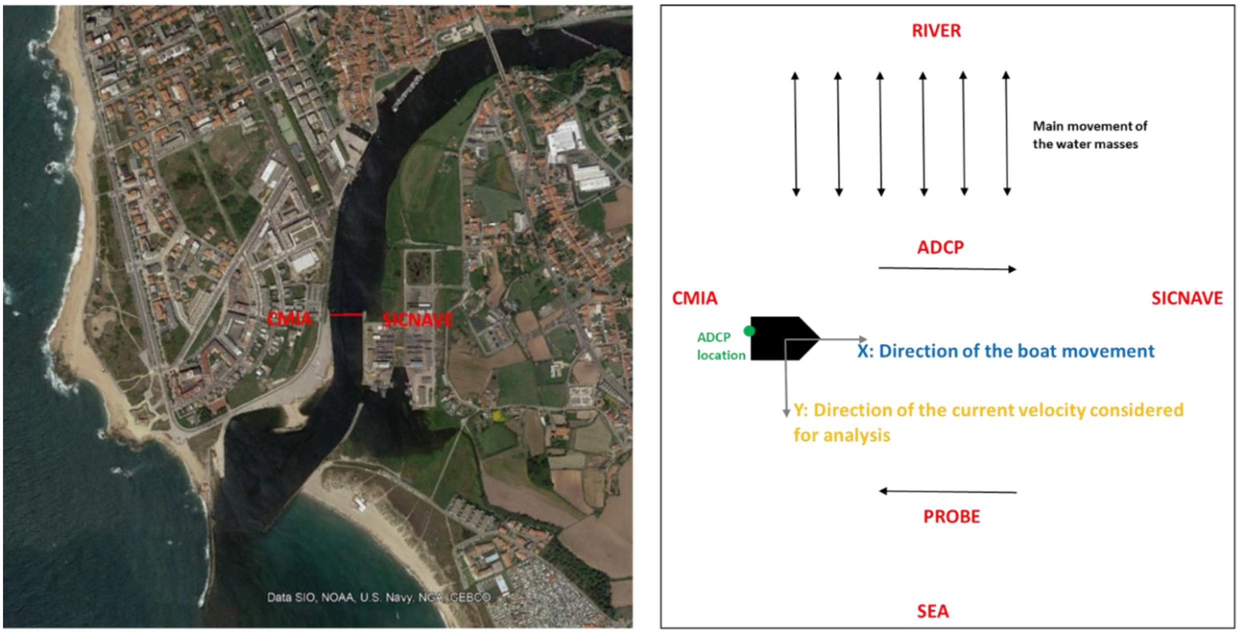


Fig. 7 Transect selected for the short-term campaigns and scheme of the performed campaigns

Through the interpretation of the graphics, it's possible to observe that, for the October campaign, variable velocity currents can be found along the transect, ranging from very low values to spots that can reach the 0.8 m/s (Fig. 8 to 11). The higher velocities (0.8 m/s) were found during high tide conditions (Fig. 11). Note that the sign of the current is an indicative of the direction. Positive (red) values mean movements of the current in the upstream direction (from the sea to the head of the estuary). Negative (blue) values represent downstream movements (from the head of the estuary to the sea). For the October campaign, the velocities range from - 0.4 m/s to 0.2 m/s in the low tide (Fig. 8), where the main direction of the current is downstream, and between -0.8 m/s and 0.6 m/s in the high tide (Fig. 11), where the main direction of the current is upstream. In the low tide, the ADCP reached only 2.5 m deep and during high tide the velocity measurements reached a depth of 5.5 m (Fig. 8 to 11). Despite these main observed directions, there are some dipoles of currents of opposite sign in the same transects (see, for example, Fig. 11). This can represent some recirculation currents that could take place in this estuary, being indicative of some turbulence. It's important to note that, due to the configuration of the ADCP and the protocol of the sampling campaign, the velocity is only measured below 1.5 m depth, losing all the surface circulation.

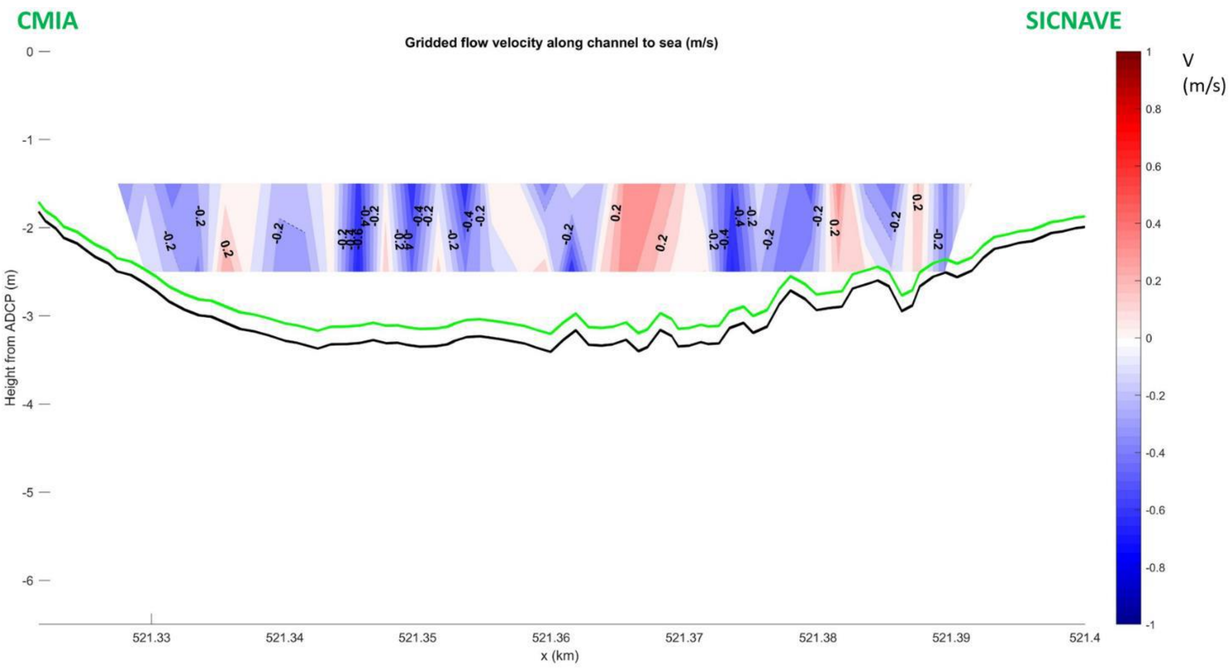


Fig. 8 ADCP data acquired in the Ave estuary, in October 2021 campaign, near the low tide, in North – South direction (transect 2). Red – Positive velocities; Blue – Negative velocities; Black Line – Bathymetry; Green Line – 94% of the depth.

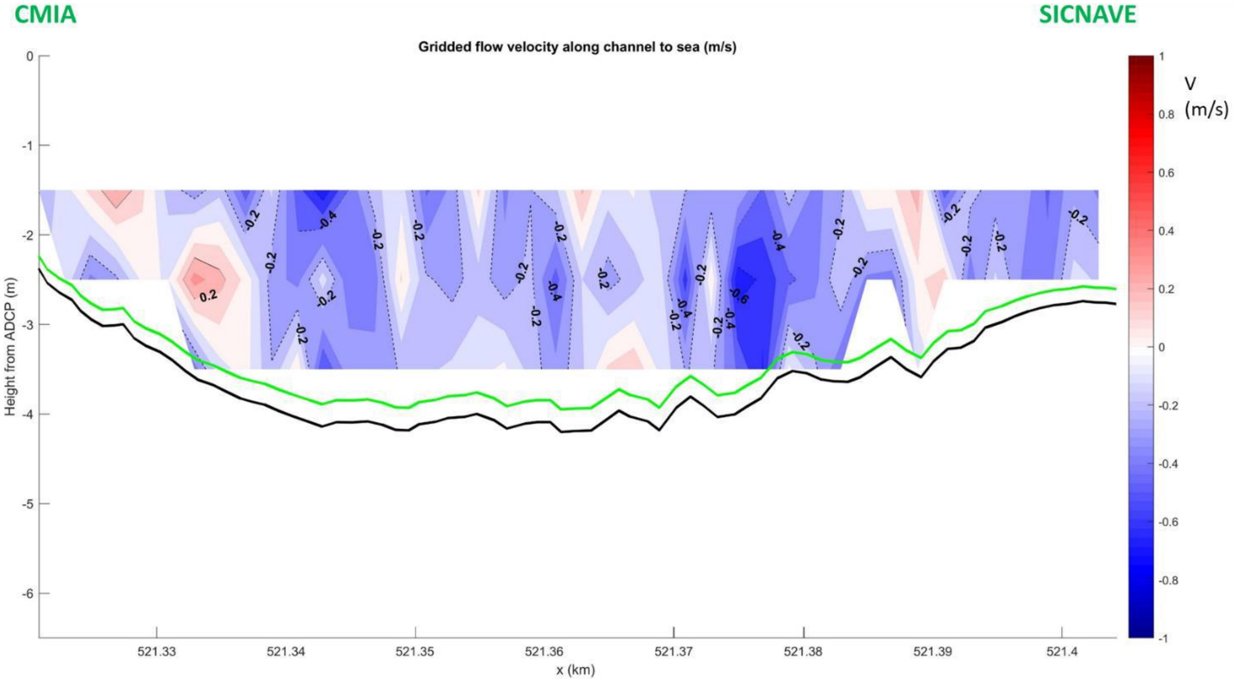


Fig. 9 ADCP data acquired in the Ave estuary, in October 2021 campaign, in the middle of the low tide, in North – South direction (transect 12). Red – Positive velocities; Blue – Negative velocities; Black Line – Bathymetry; Green Line – 94% of the depth.

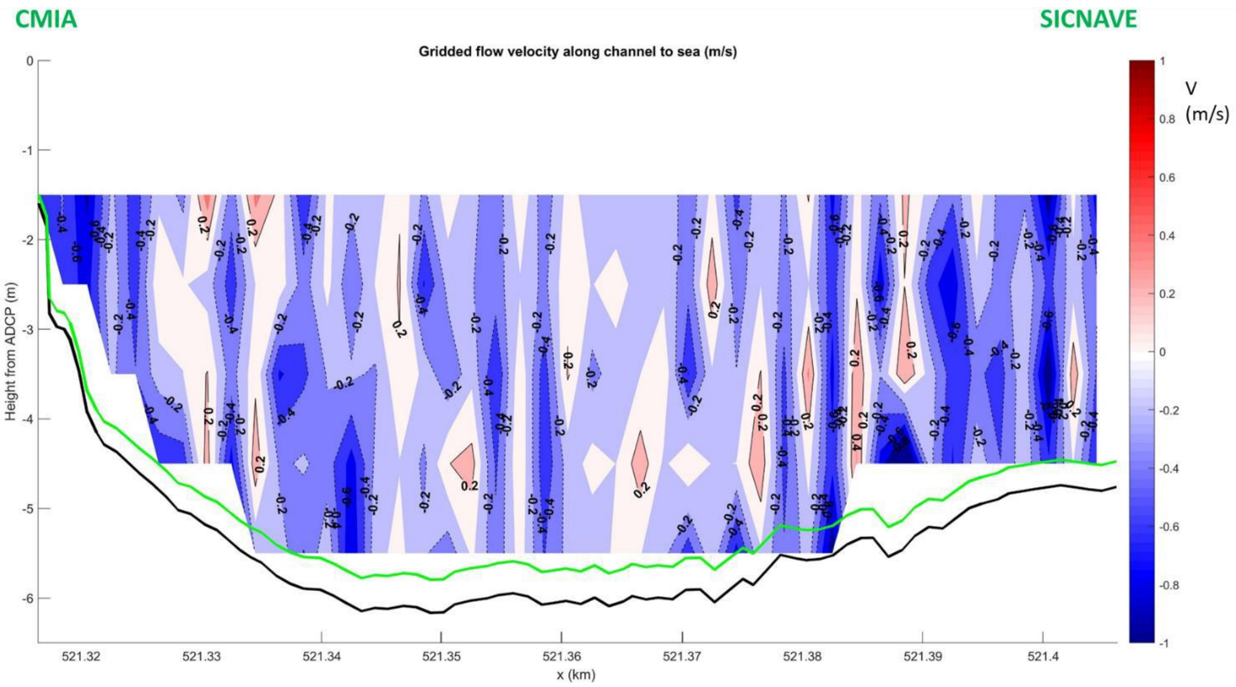


Fig. 10 ADCP data acquired in the Ave estuary, in October 2021 campaign, near the high tide, in North – South direction (transect 7). Red – Positive velocities; Blue – Negative velocities; Black Line – Bathymetry; Green Line – 94% of the depth.

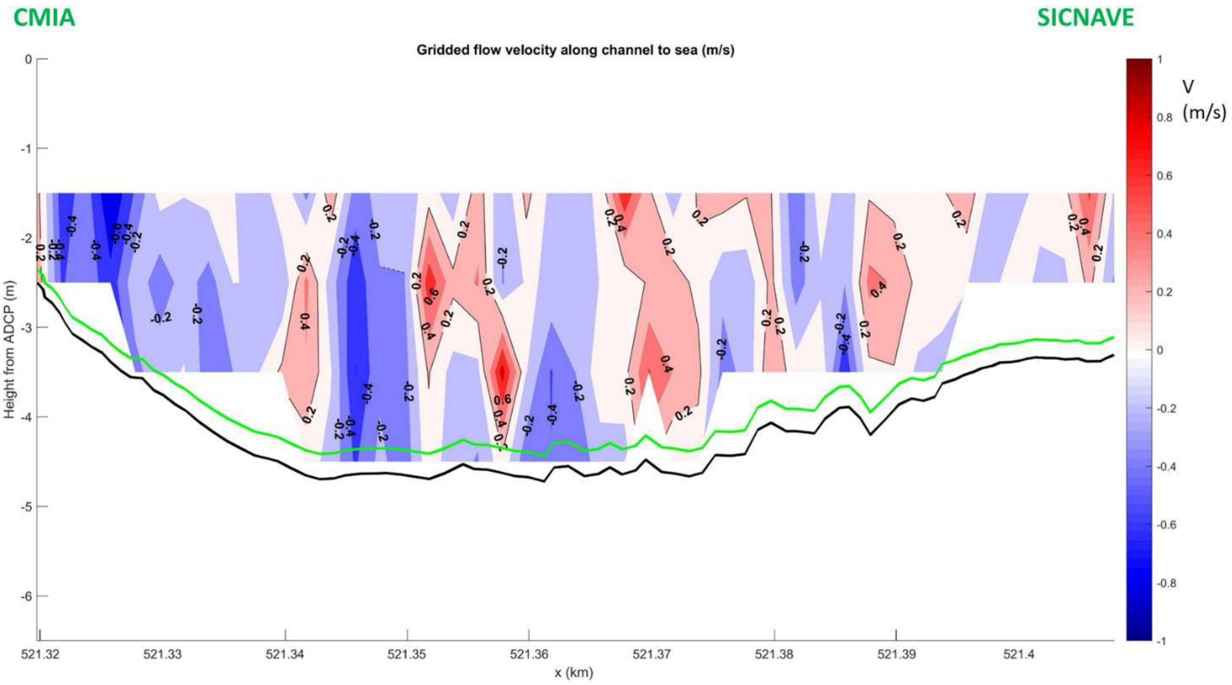


Fig. 11 ADCP data acquired in the Ave estuary, in October 2021 campaign, in the middle of the high tide, in North – South direction (transect 5). Red – Positive velocities; Blue – Negative velocities; Black Line – Bathymetry; Green Line – 94% of the depth.

In the January campaign, the velocities range from -0.2 m/s to 0.4 m/s in the low tide (Fig. 12), where the main direction of the current is upstream, and between -0.4 m/s and 0.8 m/s in the high tide (Fig. 14), where the main direction of the current is downstream. In the low tide, the ADCP measurements reached around 3 m deep and during high tide they reached a depth of 4.5 m (Fig. 12 to 15). The measured velocities seem to present more generalized positive values than the ones represented in the October campaign. This is probably related with wind conditions at coastal regions. Favourable wind directions can pile the oceanic waters next to the coast, enhancing the entrance of oceanic water masses into the estuaries with low rivers flow (Mohammedi, 2022). However, this is a preliminary result, and this behaviour should be carefully analysed to understand the effect of the coastal wind into the hydrodynamics of the Ave estuary, which is out of the scope of this thesis.

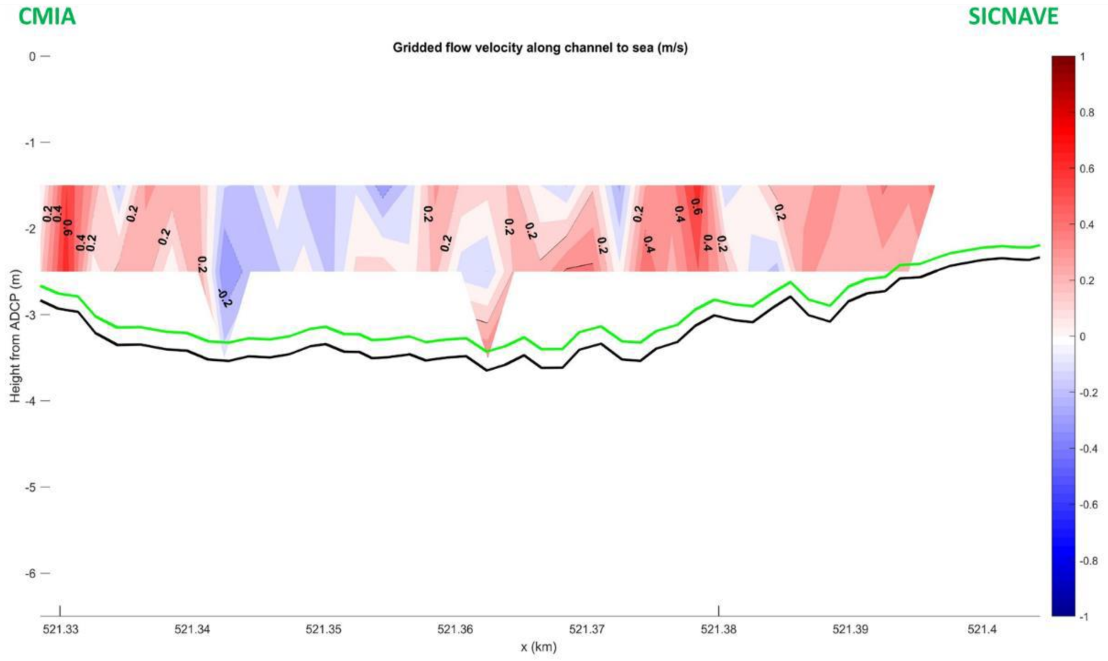


Fig. 12 ADCP data acquired in the Ave estuary, in January 2022 campaign, near the low tide, in North – South direction (transect 12). Red – Positive velocities; Blue – Negative velocities; Black Line – Bathymetry; Green Line – 94% of the depth.

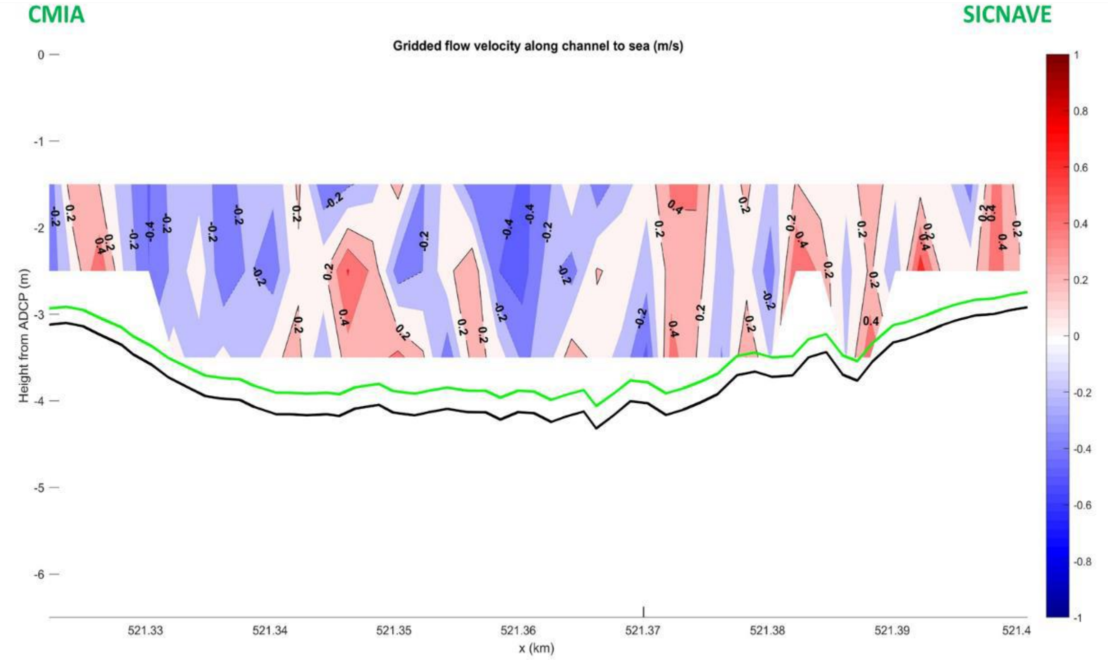


Fig. 13 ADCP data acquired in the Ave estuary, in January 2022 campaign, in the middle of the low tide, in North – South direction (transect 10). Red – Positive velocities; Blue – Negative velocities; Black Line – Bathymetry; Green Line – 94% of the depth.

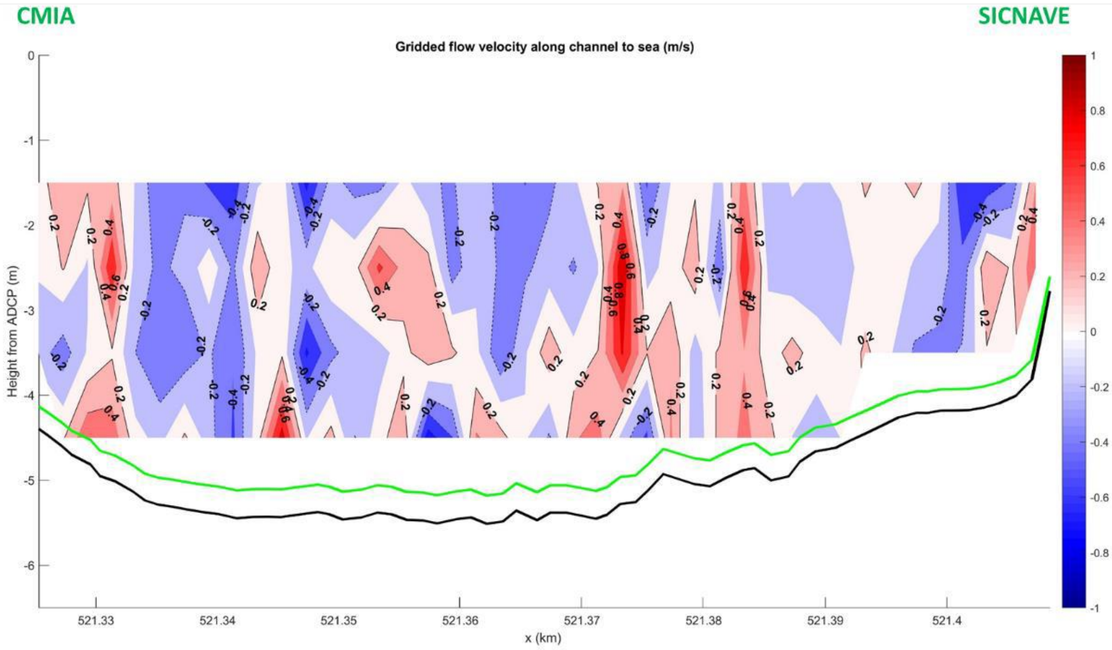


Fig. 14 ADCP data acquired in the Ave estuary, in January 2022 campaign, near the high tide, in North – South direction (transect 6). Red – Positive velocities; Blue – Negative velocities; Black Line – Bathymetry; Green Line – 94% of the depth.

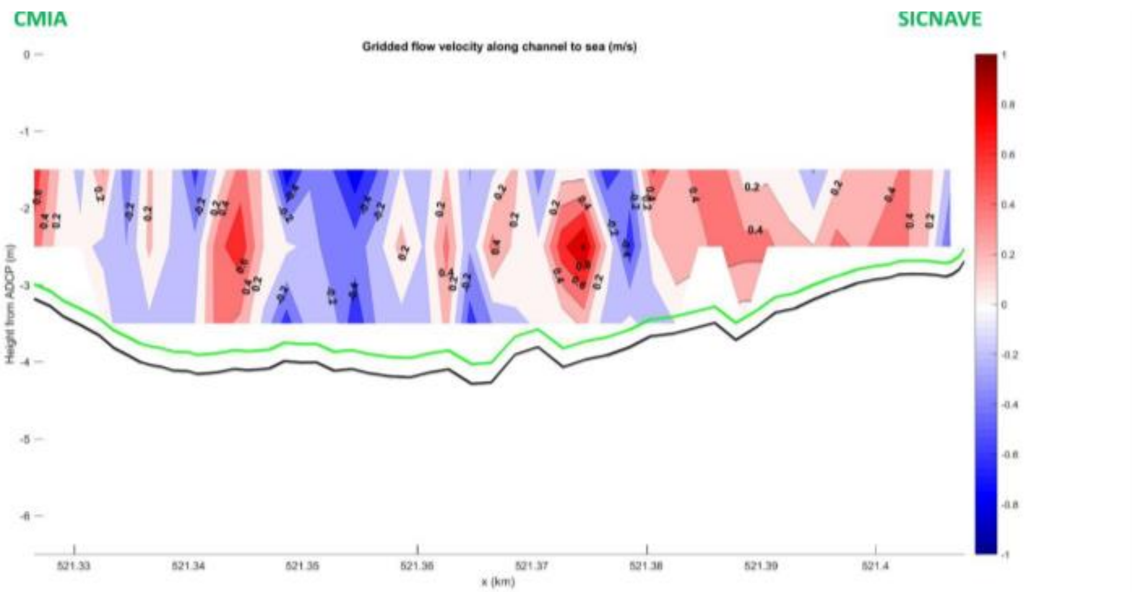


Fig. 15 ADCP data acquired in the Ave estuary, in January 2022 campaign, in the middle of the high tide, in North – South direction (transect 4). Red – Positive velocities; Blue – Negative velocities; Black Line – Bathymetry; Green Line – 94% of the depth.

When the multiparametric probe profiles are analysed, it's possible to observe a clear stratification of the Ave estuary (Fig. 16 to 23). The salinity plots showed a freshwater zone close to the surface, which is deeper during low tide conditions (around 1 m) and shallower during high tide (around 0.5 m). During the January 2022 campaign, the freshwater zone seems to be deeper than during the October 2021 campaign. It is also possible to observe a thick brackish zone where the two water masses interact. In this region was found the halocline, where the maximum gradient of salinity was found. Finally, the deepest layer is associated with oceanic water. The depth of this layer varies with the tide, being higher during high tide conditions and lower during low tide conditions (Fig. 16a to 23a). The three different layers identified in the salinity profiles are coincident with the behaviour observed in the temperature profiles. The halocline observed in the salinity profiles is now replaced by the thermocline, separating the surface freshwater masses from the oceanic deeper water. Comparing both campaigns, we can extract that in October 2021 the river temperature was around 19 ºC and the oceanic temperature was 16 ºC. Meanwhile in January 2022, the river temperature was around 11 ºC and the oceanic temperature was 13 ºC.

Lastly, the pH graphs show that during the October 2021 campaign the riverine pH was around 7 and the oceanic pH was 6. These values didn't suffer great alteration in the January 2022 campaign, where the riverine pH was also around 7 and the oceanic pH was 6. It is important to notice that the multiparametric probe profiles were represented using the Ocean Data View software (*Ocean Data View*, 2022), which allows to interpolate the results between the two measured profiles. The interpolation technique selected was the DIVA gridding, developed at the University of Liege (Beckers et al., 2014).

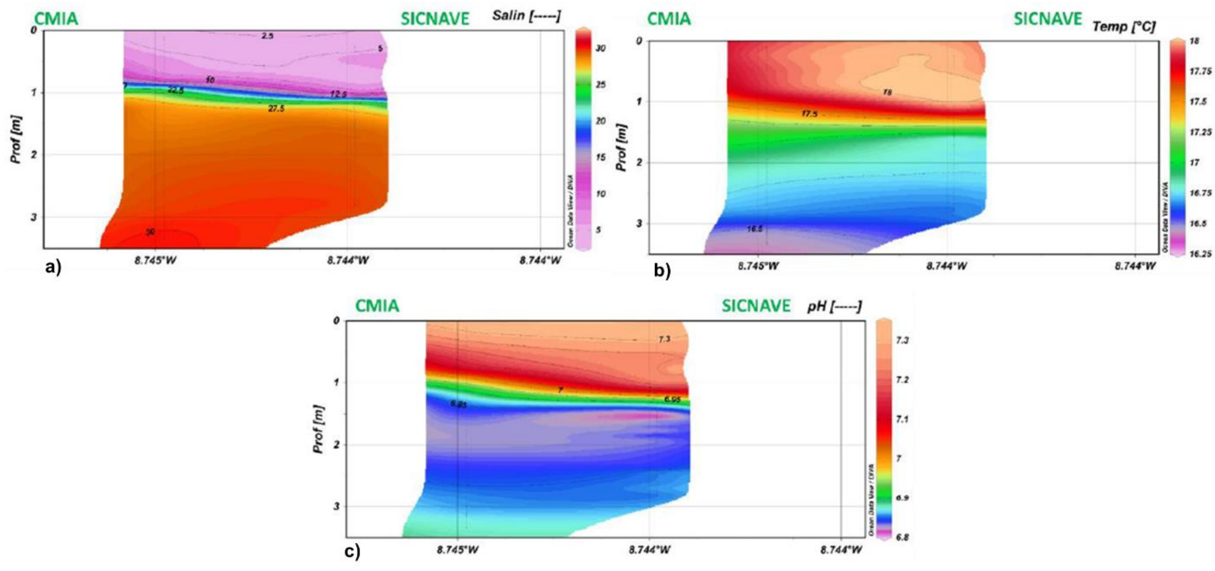


Fig. 16 Multiparametric probe profiles acquired in the Ave estuary, in October 2021 campaign, near the low tide, (transect 2). a). Salinity profile; b). Temperature profile; c). pH profile.

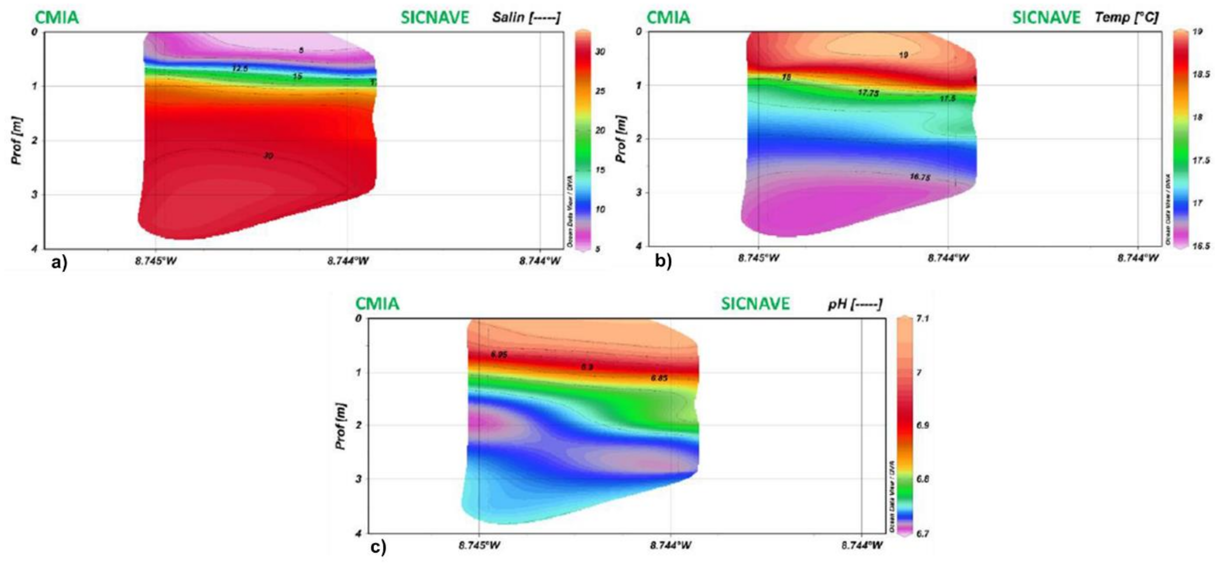


Fig. 17 Multiparametric probe profiles acquired in the Ave estuary, in October 2021 campaign, in the middle of the low tide, (transect 12). a). Salinity profile; b). Temperature profile; c). pH profile.

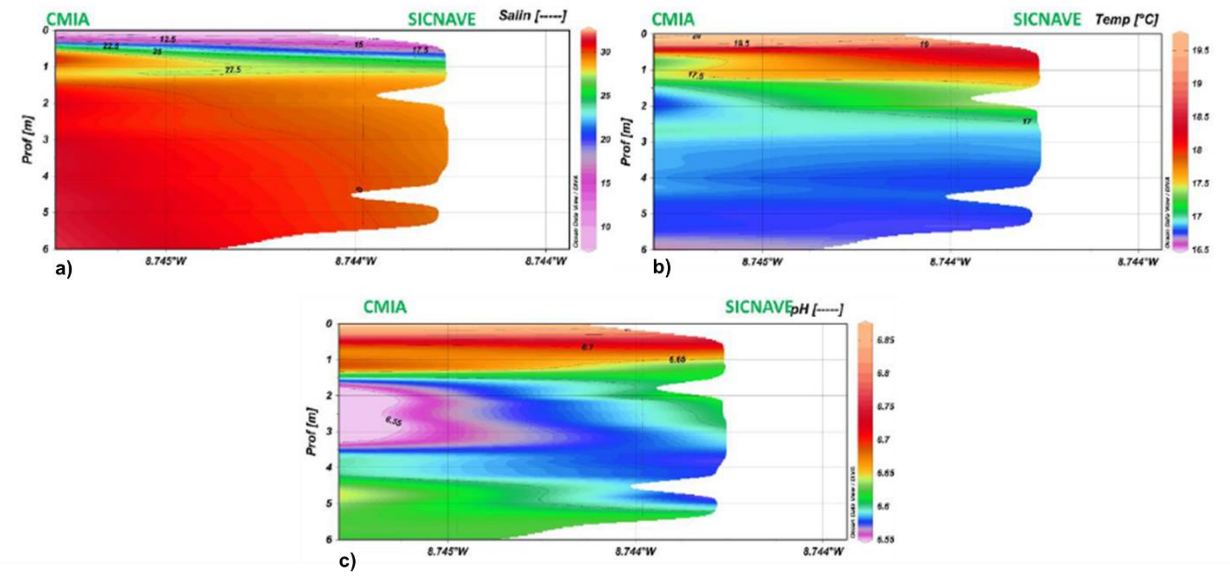


Fig. 18 Multiparametric probe profiles acquired in the Ave estuary, in October 2021 campaign, near the high tide, (transect 7). a). Salinity profile; b). Temperature profile; c). pH profile.

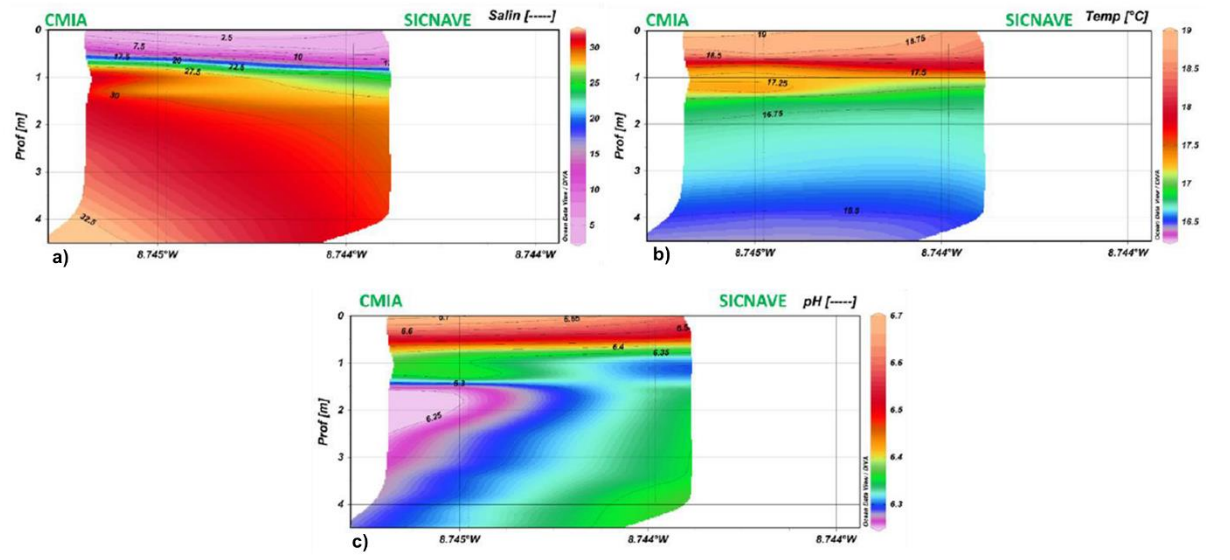


Fig. 19 Multiparametric probe profiles acquired in the Ave estuary, in October 2021 campaign, in the middle of the high tide, (transect 5). a). Salinity profile; b). Temperature profile; c). pH profile.

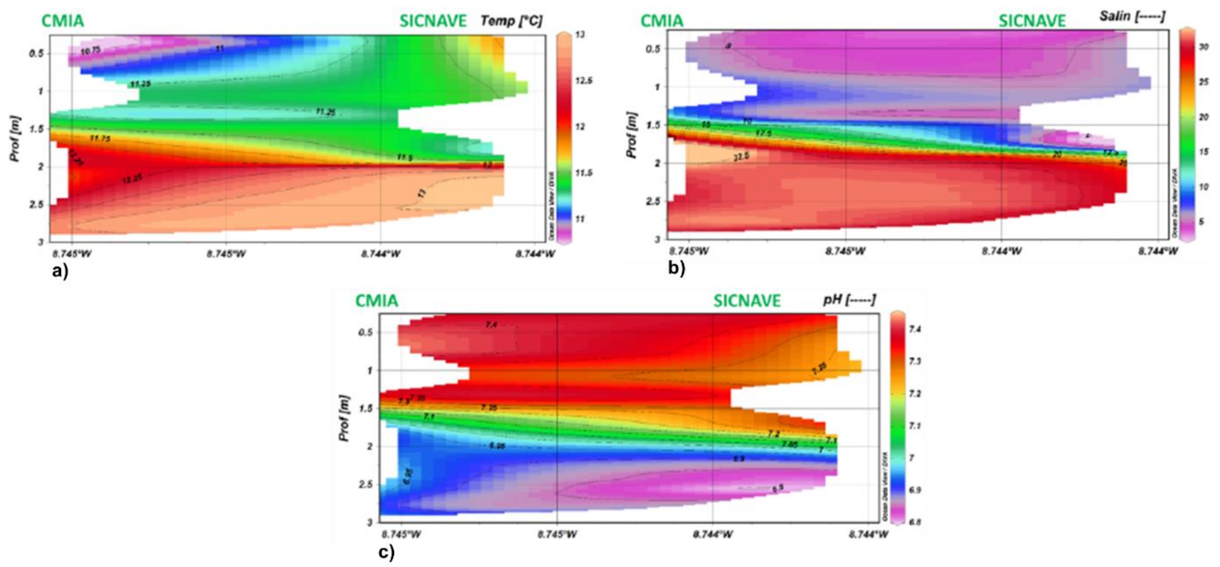


Fig. 20 Multiparametric probe profiles acquired in the Ave estuary, in January 2022 campaign, near the low tide, (transect 12). a). Temperature profile; b). Salinity profile; c). pH profile.

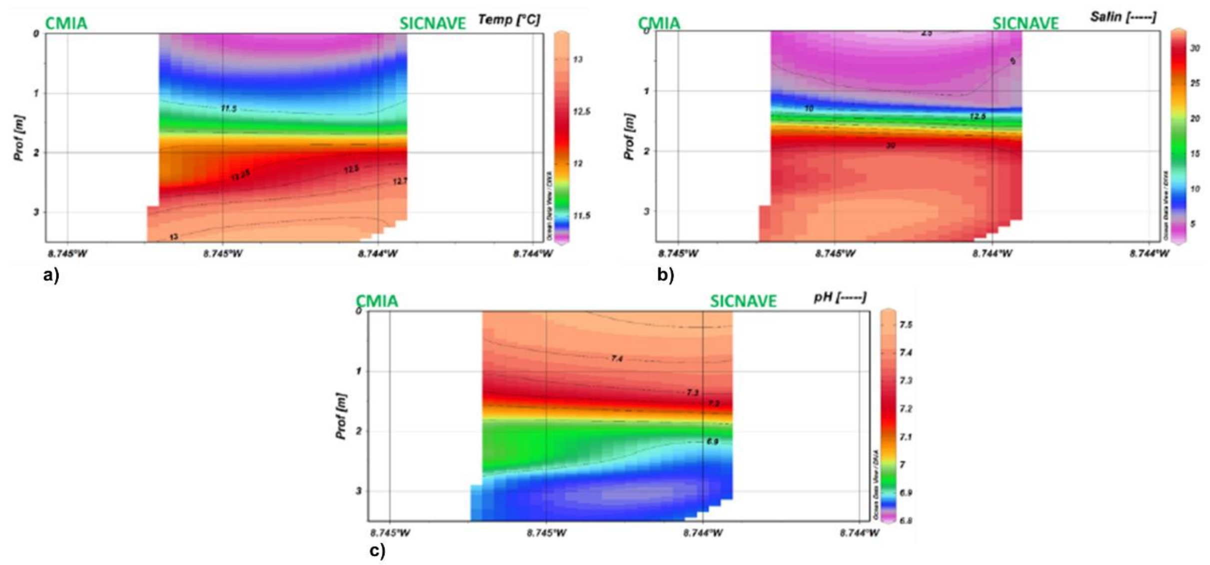


Fig. 21 Multiparametric probe profiles acquired in the Ave estuary, in January 2022 campaign, in the middle of the low tide, (transect 10). a). Temperature profile; b). Salinity profile; c). pH profile.

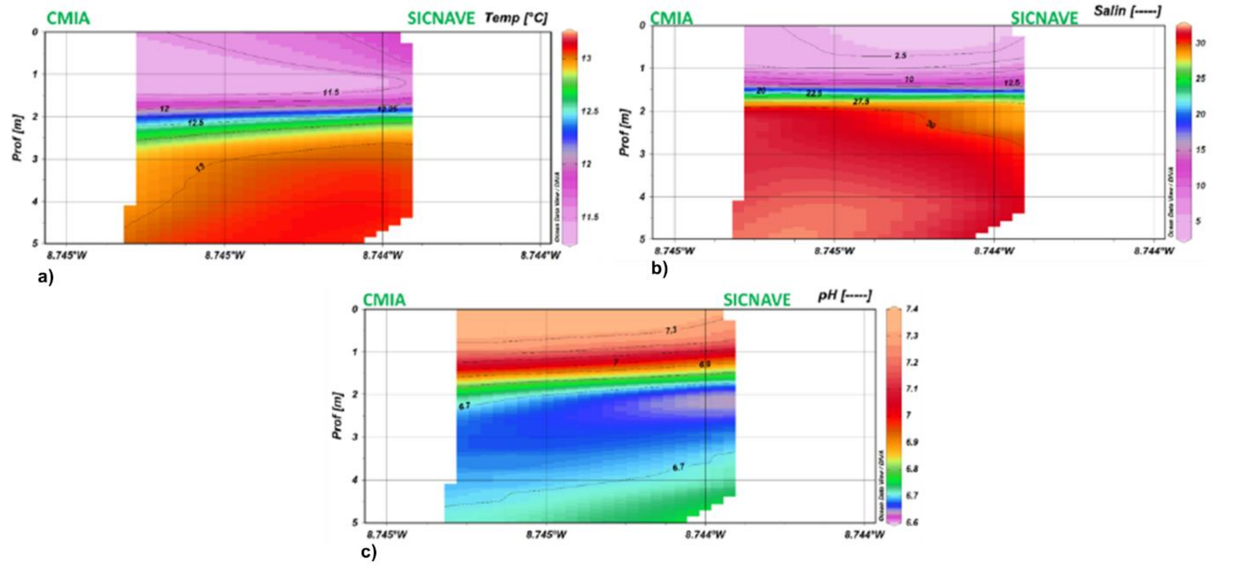


Fig. 22 Multiparametric probe profiles acquired in the Ave estuary, in January 2022 campaign, near the high tide, (transect 6). a). Temperature profile; b). Salinity profile; c). pH profile.

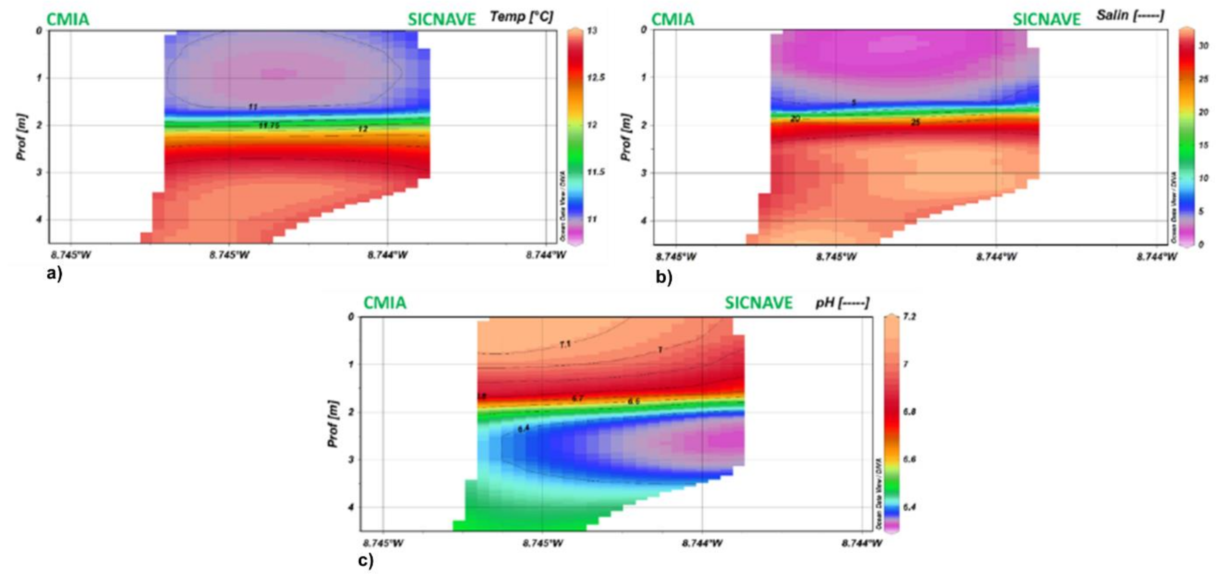


Fig. 23 Multiparametric probe profiles acquired in the Ave estuary, in January 2022 campaign, in the middle of the high tide, (transect 4). a). Temperature profile; b). Salinity profile; c). pH profile.

1.6 Science and Society

On the 18th of September of 2021, the author participated in the Castelo do Queijo Beach CleanUp for World CleanUp Day, organized by CIIMAR and the MAELSTROM project, being part of the organization as one of the monitors of the event.

On the 6th of May of 2022 the author did a poster communication for the Young Investigation of the University of Porto (IJUP), using the preliminary results of the present thesis (see attachment 1).

On the 3rd of June of 2022 the author co-organized a multidisciplinary CleanUp event in Vila do Conde. This event involved several partners, including multisectoral stakeholders, among them several members of the MAELSTROM consortium, including CIIMAR, The Trash Traveller, and ICBAS (see attachment 2). The author was responsible for introducing the MAELSTROM project, highlighting to the participants the main project pillars and goals. It was also an opportunity to test a mobile application, the MAELSTROM App, developed in the project with the objective to, follow the management cycle of ML, including beach cleaning initiatives, through the identification, collection and tracking of litter and their recycling at the end. The App allows the user to record when and where the clean-up was done, how much kilograms of litter were collected, or, if preferred, the exact number and type of items that were collected. In just 30 minutes the participants at the event managed to collect 74.9 kg of litter, and 90 plastic bottles, in one Ave estuarine beach, no longer than 100 m. This was an important way to involve the citizens in the project and to raise awareness for the problematic and the urgency to act on it (see attachment 3).

1.7 Research Aims

This study contributes to several goals of the Agenda 2030 for Sustainable Development, the main ones being, Goal 4: “ensure that all students acquire the knowledge and skills necessary to promote sustainable development, including, but not limited to, education for sustainable development and sustainable lifestyles”; Goal 6: “improve water quality, reducing pollution, eliminating dumping and minimizing the release of hazardous chemicals and materials, halving the proportion of untreated wastewater, and substantially increasing recycling and reuse globally” and Goal 14: “prevent and significantly reduce marine pollution of all kinds, especially from land-based activities, including marine debris and nutrient pollution”. But also Goal 12: “substantially reduce waste generation through prevention, reduction, recycling and reuse”; and Goal 13: “improve education, increase awareness and human and institutional capacity on mitigation, adaptation, impact reduction and early warning measures regarding climate change.”

Thus, the main objectives of this study are, to (i) evaluate and characterize microplastics and macro litter in the estuary of the Ave river, and to (ii) identify the main sources of such debris and analyse the main hydrodynamic patterns, and the residence time of the water bodies inside the estuary to understand how the litter transport occurs on the Ave estuary.

2. Methods and materials

2.1 Sample Collection

The evaluation of floating macro litter (FML) in the estuary was performed through visual monitoring, in which the diversity and relative abundance in the area were evaluated through the respective concentration rates. During the campaigns, any objects sighted in the estuary and on the banks were observed, identified, and recorded, using binoculars (Olympus 8-16x40 Zoom DPS I) (Fig. 25 to 27). For this same monitoring, a ML classification table was used, based on the OSPAR Guideline (OSPAR Commission, 2010).

MPs particles in the water column were evaluated following standardized protocols (Gago et al., 2018b). MPs were surveyed under low tide conditions, through subsurface planktonic trawls, using a manta net with 200 µm mesh size, at a constant velocity of 3 nodes, with an average duration of 7 min each (Fig. 28 and 29). To quantify the filtered water volume, a flowmeter (Hydro-Bios 438115) was attached to the manta net. The planktonic trawls were performed along five different transects, with a total of 20 samples collected (Fig. 24). The sampling was performed seasonally (2021-2022), with dedicated campaigns in summer and autumn of 2021, and winter and spring of 2022.

Fig 24 Location of selected sampling transects (T1 – T5) for microplastic collection, downstream in the Ave estuary (Source: Google Earth).

Fig. 25 Visual monitoring of floating macro litter performed by the author, in the Ave estuary (Source: Mafalda Correia).

Fig. 26 Macro litter found in the Ave estuary (Source: Isabel Iglesias).

Fig. 27 Example of a FML item observed in the Ave estuary (Source: Luis Vieira).

Fig. 28 Planktonic trawl sample, containing MPs, collected in the Ave estuary (Source: Luis Vieira).

Fig. 29 Planktonic trawl using a manta-net, in the Ave estuary (Source: the author).

2.2 Sample Treatment

The samples were transported immediately to the laboratory and initially frozen, to be further treated and analysed, and were later placed in the refrigerator to defrost. The samples were then processed following the procedure described by Gago et al., (2018b).

Since the samples had a high concentration of organic matter, it was necessary to digest them. For this it was prepared a solution of Potassium Hydroxide (KOH 10%), which was added to each sample in a ratio of 1:3 (sample:solution), the samples were then stored in glass vials, covered with aluminium foil and placed in the oven at 40 ºC (Memmert, model 100-800) for a period of 48 hours (Fig. 30).

After this period, the samples were filtered, using glass microfibre filters (47 mm) in a glass filtration system kit (Pall Corporation), which were placed in closed and identified glass petri dishes, and dried in the oven at 40 ºC. To avoid contamination, the protocol was performed inside the fume hood, and cotton lab coats were worn during the entire process. Also, blank samples were placed in open petri dishes, near the filtration system, and subjected to the same drying process to assess any contamination. After being completely dried, the petri dishes were sealed with parafilm for further observation of the filters using the magnifying glass, equipped with a high-definition camera (Leica MZ7.5) (Fig. 31).

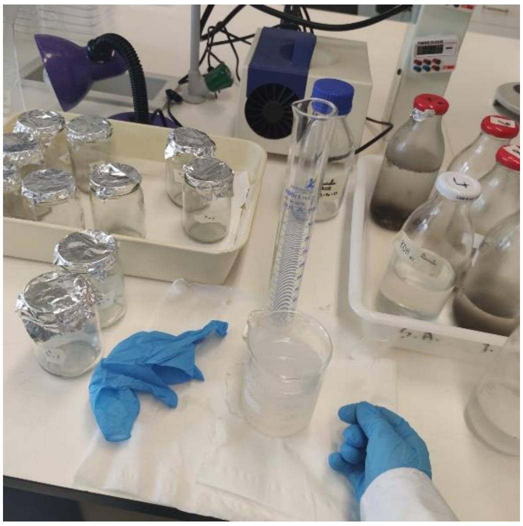


Fig. 30 Organic matter digestion process (Source: the author).

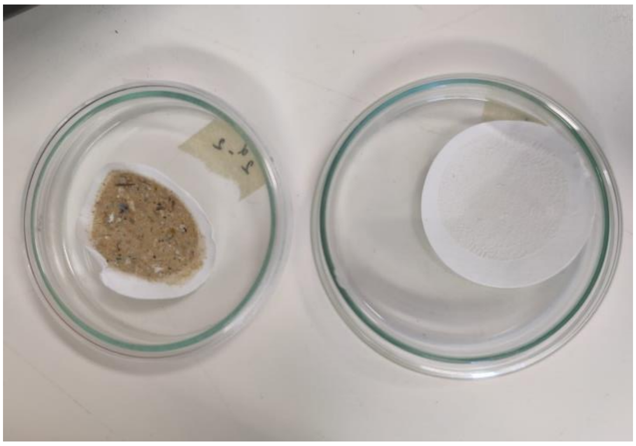


Fig. 31 Filtration filters sealed in the petri dishes (Source: the author).

2.3 Data Processing

FML was identified and characterized according to the OSPAR code, (2010) and the Technical Subgroup on Marine Litter (TSG-ML) General Code (Galgani et al., 2018). FML concentrations were then calculated as the number of items per hour (see attachment 4).

All the MPs collected were visually inspected and identified according to their shape, colour, and size, using the Leica Application Suite (LAS) software. Blank samples were also inspected for MPs. The MPs were classified as fibre, fragment, film, pellet, foam and paint (Table 1). Two different measuring methods were used, one for fibres (Formula 1) and another one for the remaining shapes of MPs (Karami et al., 2017) (Formula 2) (Fig. 32) (Frias et al., 2018; Karami et al., 2017). Six length categories were then defined: < 0.1 mm, 0.1-0.5 mm, 0.5-1 mm, 1-2.5 mm, 2.5-5 mm, and > 5 mm. MP concentrations were calculated as the number of particles per m³ of water collected (Formula 3).

Table 1. Microplastic shape definition (Adapted from: Rodrigues et al., 2020a).

Shape	***Definition***
Fibre	Thin line, equally thick throughout its entire length
Fragment	Hard and irregular particle
Film	Thin and malleable, flimsy particle
Pellet	Spherical particle
Foam	Lightweight, sponge-like particle, usually opaque
Paint	Flake-like particle, similar to film but opaque

$$line\ a + line\ b + line\ c + line\ d + \cdots$$

Formula 1. Used to measure fibres.

$$\frac{lenght + width}{2}$$

Formula 2. Used to measure fragments, films, pellets, foams, and paint.

$$MPs/(net\ opening\ area\ (m^2) \times number\ of\ revolutions \times flowmeter\ calibration\ factor)$$

Formula 3. Used to measure the number of MPs per m³ of water.

2.4 Data Analysis

All collected data was evaluated for distribution and normal homogeneity of variances using the Kolmogorov-Smirnov and Levene tests, respectively. For data that did not follow the normality and/or homogeneity of variances, logarithmic (log (x+1)) and square root transformations were applied, using Microsoft Excel software.

Differences between the total MPs identified, sampled seasons and transects were evaluated using a unifactorial analysis of variance (one-way ANOVA), followed by a Tukey test. To assess the differences between the MPs classification (shape, colour and size), sampled seasons and selected transects, a multifactorial analysis of variance (general linear model, with two factors and interactions) was performed (Zar, 1996). For these analyses, the IBM SPSS Statistics software (Version. 27) was used and the significance level selected was 0.05 ($p < 0.05$). Principal Coordinates Analysis (PCO) was used to visualize the spatial variation of MPs shape, colour and size, with overlapping vectors (Pearson correlations), indicating correlations between these variables and the selected transects and seasons (Anderson, 2001). The PCO analysis was processed using PRIMER software (Version. 6).

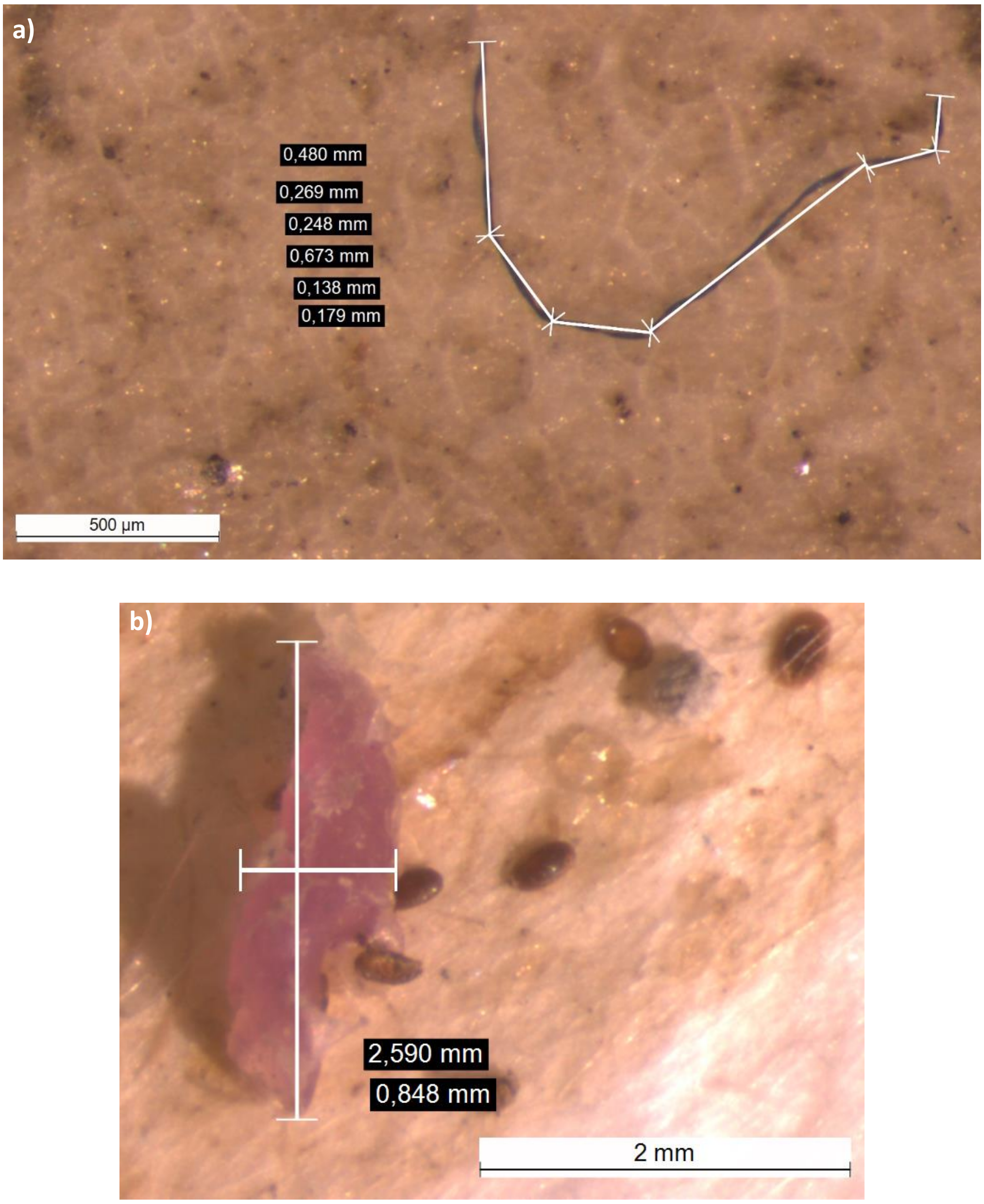


Fig. 32 a) Example of the measurement method of fibres. b) Example of the measurement method of other particles (Source: the author).

3. Results

3.1 Floating Macro Litter

During all the campaigns performed, in total, 166 Floating Macro Litter (FML) items were observed. The campaign where most FML were identified was the one that took place during winter (97 items) (Fig. 33). The overall estimated FML flux was 7.4 items/h. Considering the estimated FML flux per season, 3.8 items/h were estimated in summer and in autumn, 13.9 items/h in winter and 5.6 items/h in spring (Fig. 34).

Table 2. represents the characterization of the observed FML in all seasons, based on both TSG-ML general and OSPAR codes (Galgani et al., 2018). The most 10 representative items (top 10) were styrofoam pieces (41 items), plastic bottles (22 items), small plastic bags (14 items), cigarette butts and filters (13 items), plastic/polystyrene pieces (13 items), plastic caps/lids (10 items), face masks (9 items), string/cord (7 items), other paper items (6 items) and crisps packets/sweets wrappers (5 items) (Tabel 2). Figure 35 represents the items percentage, by category, observed in each season. The most recorded items by season were plastic bottles, cigarette butts and filters and styrofoam pieces during summer (15.79% each), plastic bottles and styrofoam pieces during autumn (15.79% each), styrofoam pieces during winter (36.08%), and small plastic bags during spring (25.81%) (Fig. 35).

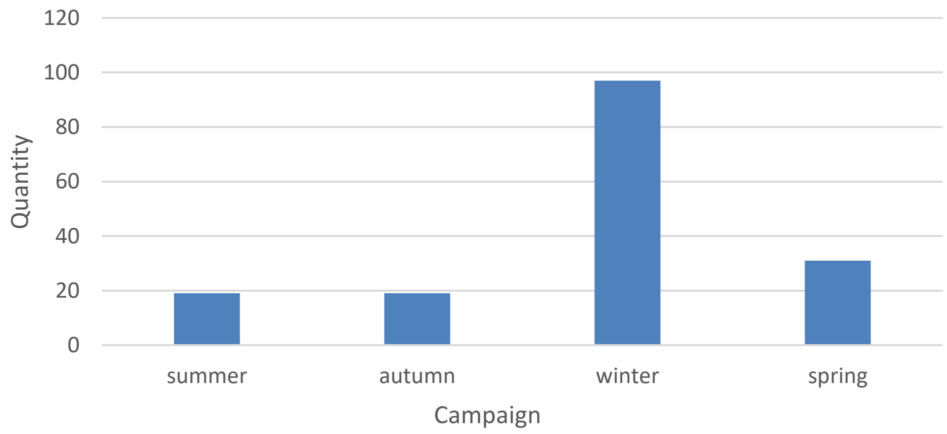


Fig. 33 Total number of items observed in each campaign.

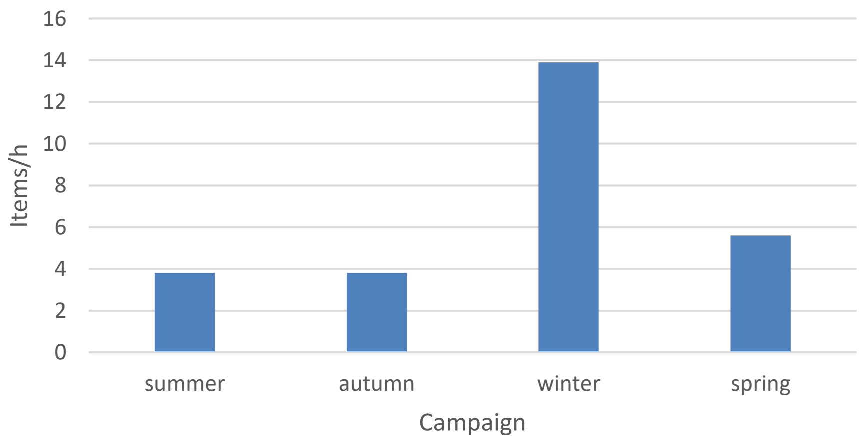


Fig. 34 Total observed FML (items/h) in each season.

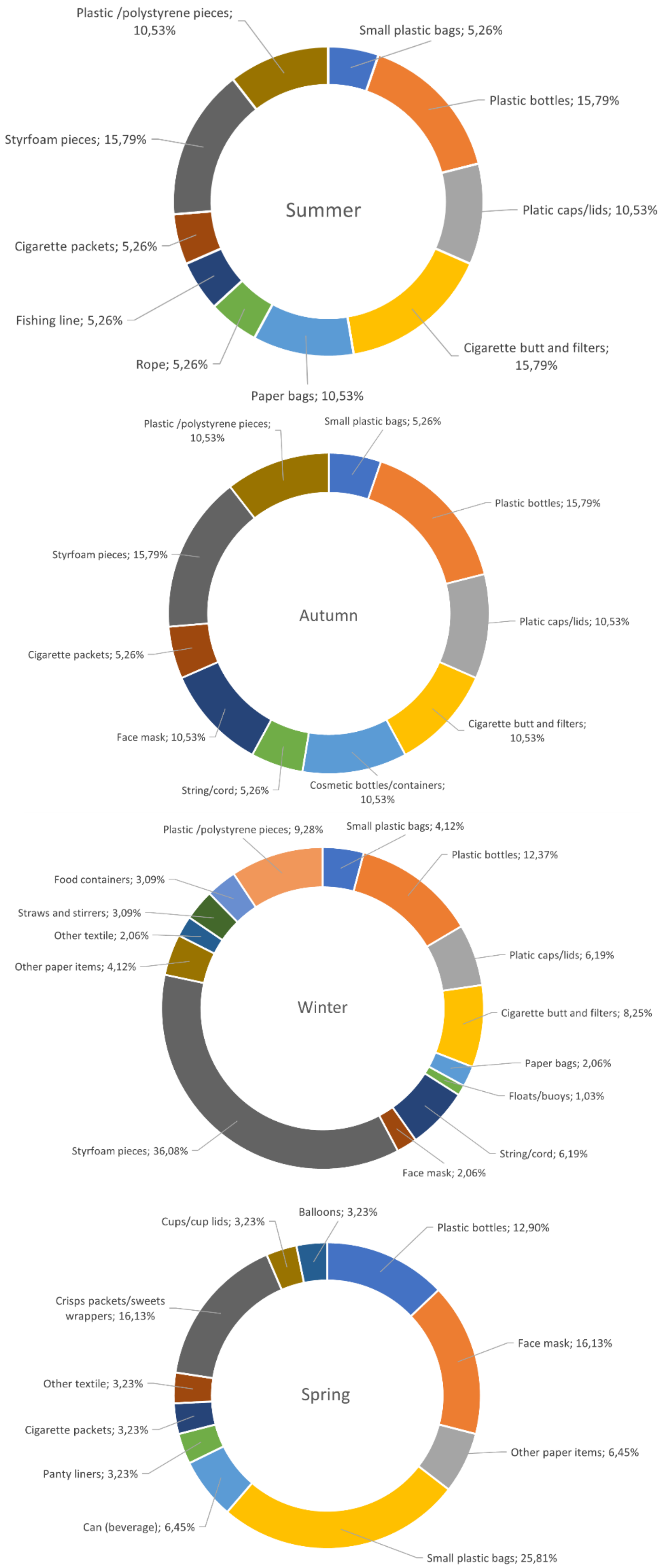


Fig. 35 Percentage of items by category observed in each season.

Table 2. Characterization of the observed FML based on TSG-ML general code and OSPAR code (Galgani et al., 2018).
*The items marked with this symbol don't have a code attributed in the guidelines.

General name	Quantity	Materials	TSG-ML general code	OSPAR code
Styrofoam pieces	41	Artificial polymer materials	G117	*
Plastic bottles	22	Artificial polymer materials	G6	4
Small plastic bags	14	Artificial polymer materials	G4	3
Cigarette butts and filters	13	Artificial polymer materials	G27	64
Plastic/polystyrene pieces	13	Artificial polymer materials	G76	46
Plastic caps/lids	10	Artificial polymer materials	G21	15
Face mask	9	Artificial polymer materials	*	*
String/cord (diameter less than 1cm)	7	Artificial polymer materials	G50	32
Other paper items	6	Paper/Cardboard	G158	67
Crisps packets/sweets wrappers	5	Artificial polymer materials	G30	19
Paper bags	4	Paper/Cardboard	G147	60
Food containers	3	Artificial polymer materials	G10	6
Other textile	3	Cloth/Textile	G137	54
Straws and stirrers	3	Artificial polymer materials	G35	22
Can (beverage)	2	Metal	G175	78
Cigarette packets	2	Paper/Cardboard	G152	63
Cosmetic bottles/containers	2	Artificial polymer materials	G12	7
Balloons	1	Rubber	G125	49
Cups/cup lids	1	Artificial polymer materials	G33	21
Fishing line	1	Artificial polymer materials	G59	35
Floats/buoys	1	Artificial polymer materials	G62	37
Panty liners	1	Artificial polymer materials	G96	99
Rope (diameter more than 1cm)	1	Artificial polymer materials	G49	31

3.2 Microplastics

From the 20 samples collected in the four campaigns, a total of 12325 MP particles were identified (Table 3) with an annual average density of 63.1 ± 4.7 MPs/m³ (mean±SEM). Some MP contamination was observed in the blank samples, but none of it coincided with the MPs found in the other samples, validating the obtained results. No significant differences were observed between the total collected MPs during summer and winter (Tukey test; $p>0.05$) (Fig. 36). The concentrations were found to be significantly lower during autumn and significantly higher during spring (Tukey test; $p<0.05$) (Fig. 36). MPs were found in all selected transects, during all the campaigns (Fig. 37). Comparing the total collected MPs in each transect, higher number of particles were obtained in T4 (30%) during summer, T5 (38%) during autumn, T5 (33%) during winter, and T3 (31%) during spring. Spring campaign represented the season where higher quantities of MPs were collected (56%), followed by winter (19%) (Fig. 37). Comparing the same sampled transects, it was possible to observe that, in the majority of them, MPs concentration (MPs/m³) did vary significantly over the seasons (Tukey test; $p < 0.05$) (Fig. 37), with the exception of T1, T2 and T3 in summer and winter, and T5 in summer and autumn ($p>0.05$) (Fig. 37).

All six shapes of MPs, commonly described in literature, were found, with the most common shape being fragments, representing 53% of the total, followed by fibres (29%) (Fig.38). Fragments were the predominant shape of MPs observed in all seasons. However, in autumn, fibres were the most common typology. Paint was the less abundant MP (0.01%), and it was only observed in the autumn samples for the T5 transect. Foam was only observed in the winter and in the spring samples, pellets were absent from the autumn samples (Table 3 and Fig. 39). The concentrations (particles/m³) of all six identified MP shapes showed to be significantly different between seasons and transects ($p<0.05$; Table 6).

Particles in eleven different colours were found, namely: blue, transparent, red, white, black, green, pink, orange, yellow, purple, and multicolour. The predominant colour was blue (34%), followed by transparent (24%) (Fig.38). In fact, blue was the predominant colour in all seasons and transects, except for spring, where transparent was the most representative colour (Table 4 and Fig. 40). Considering the MPs concentrations per colour, the obtained data showed significant differences between seasons and sampled transects ($p<0.05$; Table 6).

The most representative MPs class size was between 0.5 mm and 1 mm (42%), and the size category with less MPs was between 0 mm and 0.1 mm (Fig.38). The predominance of the 0.5 mm to 1 mm category was observed in all seasons, except summer, where the predominant size of MPs was between 0.1 mm and 0.5 mm (50%). MPs measuring between 0 mm and 0.1 mm were not observed during winter samples (Table 5 and Fig. 41). All differences between size classes were found to be statistically different between seasons ($p<0.05$; Table 6). Only the first 3 size classes were found to be significantly different between transects ($p<0.05$; Table 6).

The interaction analysis between seasons and transects, revealed significant differences in the majority of colours, shapes and sizes ($p<0.05$; Table 6), with the exception of paint shape, orange colour and of smallest and highest size-classes ($p>0.05$; Table 6).

The PCO representation explains 87.7% of total variability by season and transect (Fig. 42). In both representations, the axis 1 is vertical and axis 2 horizontal. The first PCO showed clearly a spring campaign separated from all the other seasons, with a high relation with all MP identified categories. Fibres were found to be associated to winter conditions, with higher concentrations during this period. Considering the second PCO, some interesting associations were observed: the colours blue and purple and were closely related with transect 5, while foam shows a strong relation whit the colour white and transect 3 (Fig. 42).

Table 3. Total number of MPs by shape in each transect (n = 20; 1 to 5 transects; 4 campaigns).

		Fibres	Fragments	Pellets	Films	Paint	Foam	Total	% (by season)
		Number of MPs							
Summer	T1	199	237	0	34	0	0	470	21.57
	T2	107	92	12	12	0	0	223	10.23
	T3	188	135	6	47	0	0	376	17.26
	T4	224	336	2	99	0	0	661	30.34
	T5	168	216	2	63	0	0	449	20.61
Autumn	T1	60	44	0	6	0	0	110	11.78
	T2	47	43	0	5	0	0	95	10.17
	T3	104	51	0	8	0	0	163	17.45
	T4	150	47	0	16	0	0	213	22.81
	T5	255	85	0	12	1	0	353	37.79
Winter	T1	189	209	0	21	0	1	420	18.25
	T2	39	214	2	47	0	5	307	13.34
	T3	179	140	1	14	0	0	334	14.51
	T4	221	228	2	32	0	0	483	20.98
	T5	390	334	4	30	0	0	758	32.93
Spring	T1	150	661	18	144	0	41	1014	14.67
	T2	82	477	6	87	0	90	742	10.74
	T3	230	1241	15	173	0	499	2158	31.23
	T4	259	1086	17	125	0	151	1638	23.70
	T5	299	702	18	104	0	235	1358	19.65
	Total (number of MPs)	3540	6578	105	1079	1	1022	12325	
	%	28.72	53.37	0.85	8.75	0.01	8.29		

Table 4. Total number of MPs by colour in each transect (n = 20; 1 to 5 transects; 4 campaigns).

		Blue	Transparent	Red	White	Black	Green	Pink	Orange	Yellow	Purple	Multicolour
		Number of MPs										
Summer	T1	231	83	58	17	40	22	5	7	3	0	4
	T2	116	46	8	11	13	11	7	1	3	2	5
	T3	218	76	32	7	15	12	3	5	3	0	5
	T4	308	127	57	22	39	44	12	21	10	13	8
	T5	241	74	40	12	32	25	6	13	2	0	4
Autumn	T1	54	27	4	14	7	2	0	2	0	0	0
	T2	45	23	4	15	4	2	0	1	1	0	0
	T3	80	42	6	16	14	4	0	1	0	0	0
	T4	112	43	18	15	21	2	0	1	0	0	1
	T5	210	60	27	16	26	7	0	1	4	1	1
Winter	T1	187	89	40	19	43	25	4	0	10	2	1
	T2	71	107	17	50	26	12	4	2	6	6	6
	T3	154	43	36	12	44	14	3	7	6	2	13
	T4	235	115	35	22	39	17	2	5	5	2	6
	T5	361	151	61	42	87	27	1	4	13	6	5
Spring	T1	240	333	77	159	76	66	12	7	18	1	25
	T2	166	206	49	163	65	44	1	7	16	3	22
	T3	366	559	164	654	122	167	14	15	68	4	25
	T4	420	470	145	259	128	117	11	22	40	1	25
	T5	338	323	92	323	101	94	16	5	26	6	34
	Total (Number of MPs)	4153	2997	970	1848	942	714	101	127	234	49	190
	%	33.70	24.32	7.87	14.99	7.64	5.79	0.82	1.03	1.90	0.40	1.54

Table 5. Total number of MPs by size category in each transect (n = 20; 1 to 5 transects; 4 campaigns).

		Categories					
		]0 - 0.100mm]	]0.100 - 0.500mm]	]0.500 - 1mm]	]1 – 2.5mm]	]2.5 – 5mm]	>5mm
		Number of MPs					
Summer	T1	5	226	126	83	29	2
	T2	0	82	51	61	24	4
	T3	6	153	86	91	37	3
	T4	9	386	147	83	31	6
	T5	2	250	106	71	16	4
Autumn	T1	0	11	47	36	13	3
	T2	0	11	46	29	7	2
	T3	1	39	49	55	14	5
	T4	1	38	75	66	26	7
	T5	0	97	101	116	32	5
Winter	T1	0	96	222	96	5	1
	T2	0	49	139	105	12	2
	T3	0	101	152	71	9	1
	T4	0	146	220	98	16	2
	T5	0	208	376	151	20	3
Spring	T1	0	184	457	334	34	4
	T2	0	140	368	202	30	3
	T3	0	467	1052	549	81	9
	T4	0	335	825	422	50	5
	T5	1	378	537	356	62	10
	Total (Number of MPs)	25	3397	5182	3075	548	81
	%	0.20	27.56	42.04	24.95	4.45	0.66

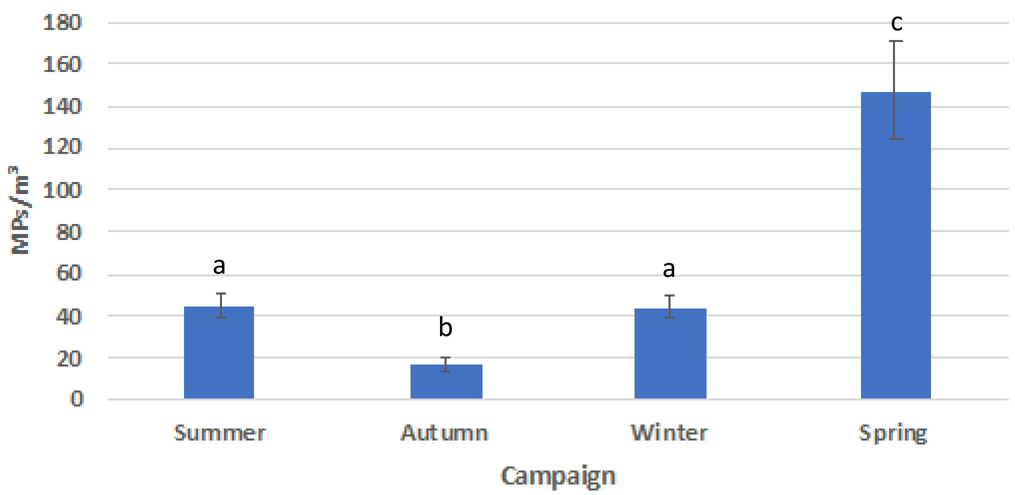


Fig. 36 Average MPs concentration (particles/m^3) by campaign. Different letters represent significant differences (Tukey test; p<0.05) between seasons.

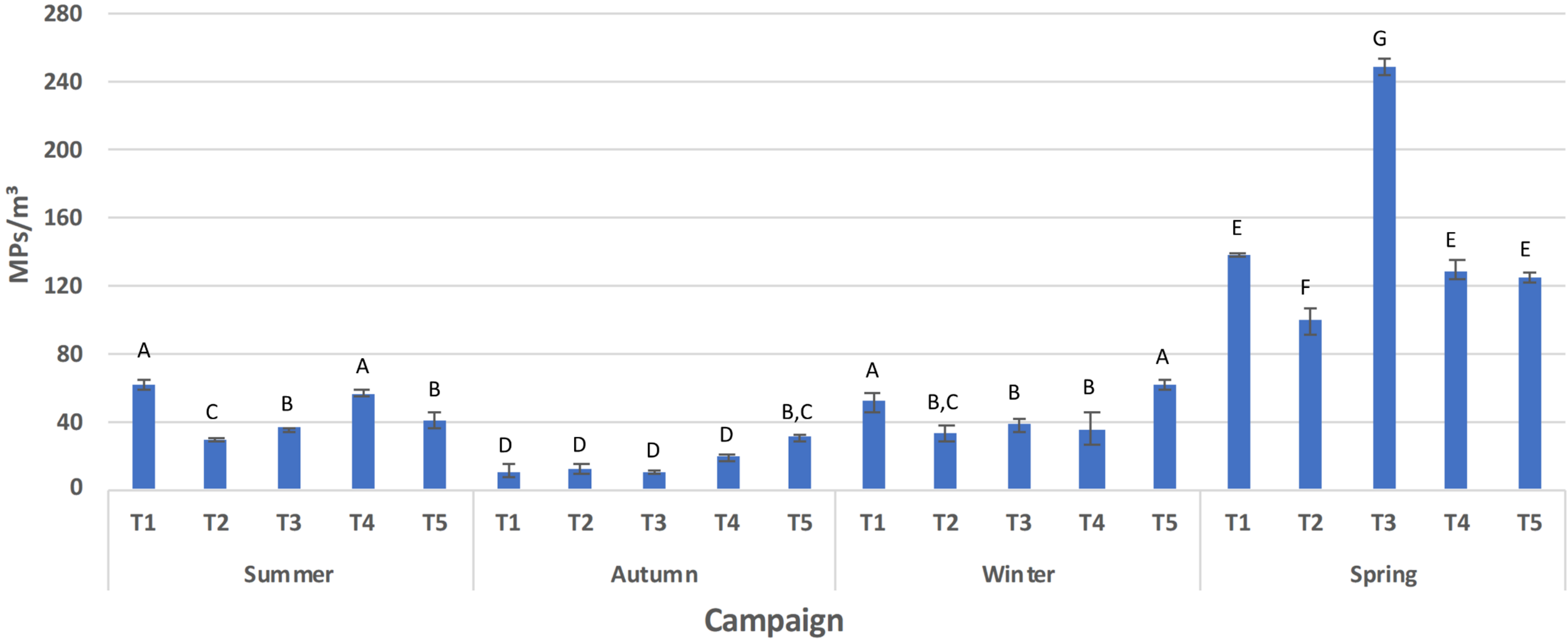


Fig. 37 Average MPs concentration (particles/m³) in each selected transect from each season. Different letters represent significant differences (Tukey test; $p<0.05$) between transects.

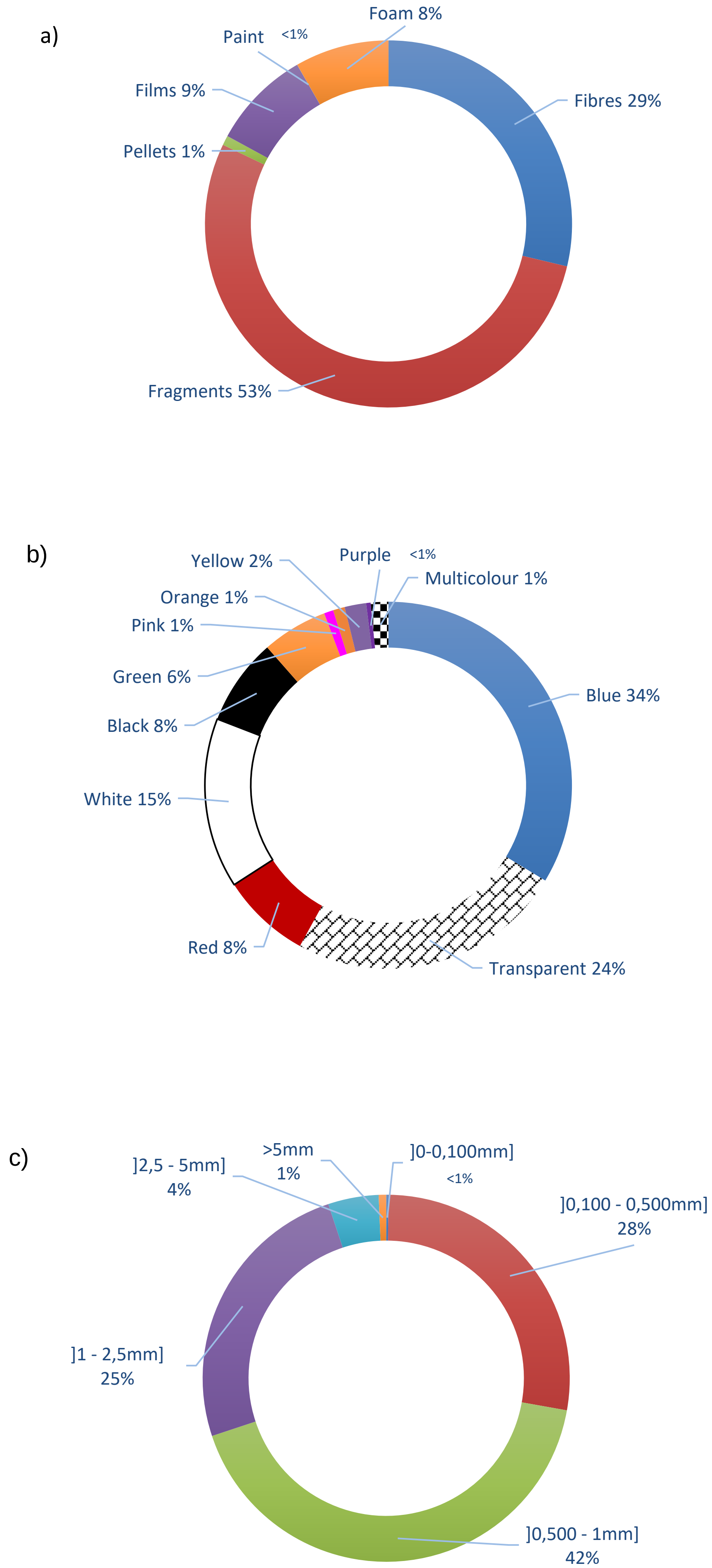


Fig. 38 MPs annual concentration (%) by a) shape; b) colour and c) size.

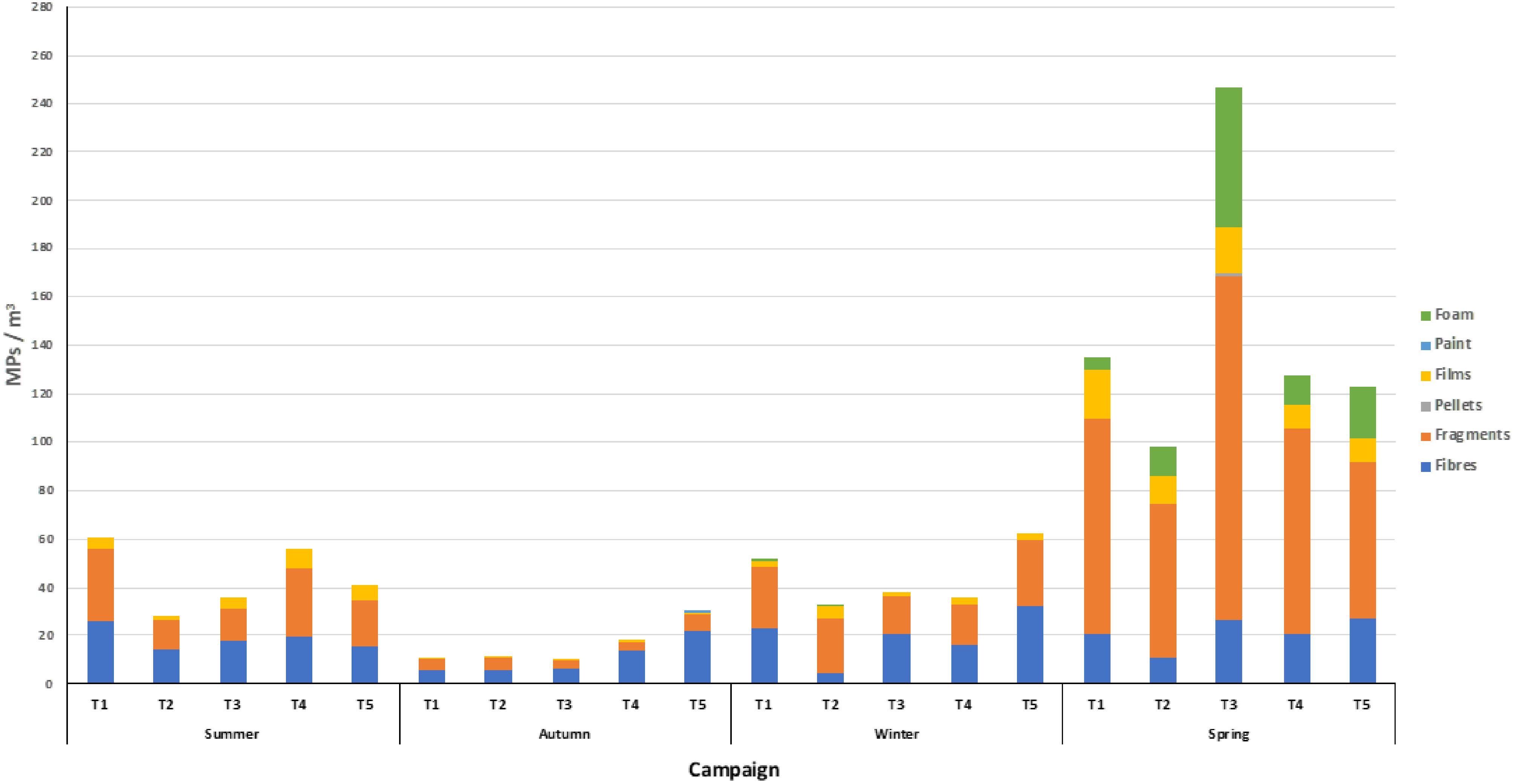


Fig. 39 Total number of MPs by shape in each transect, and respective standard errors (n = 20; 1 to 5 transects; 4 campaigns).

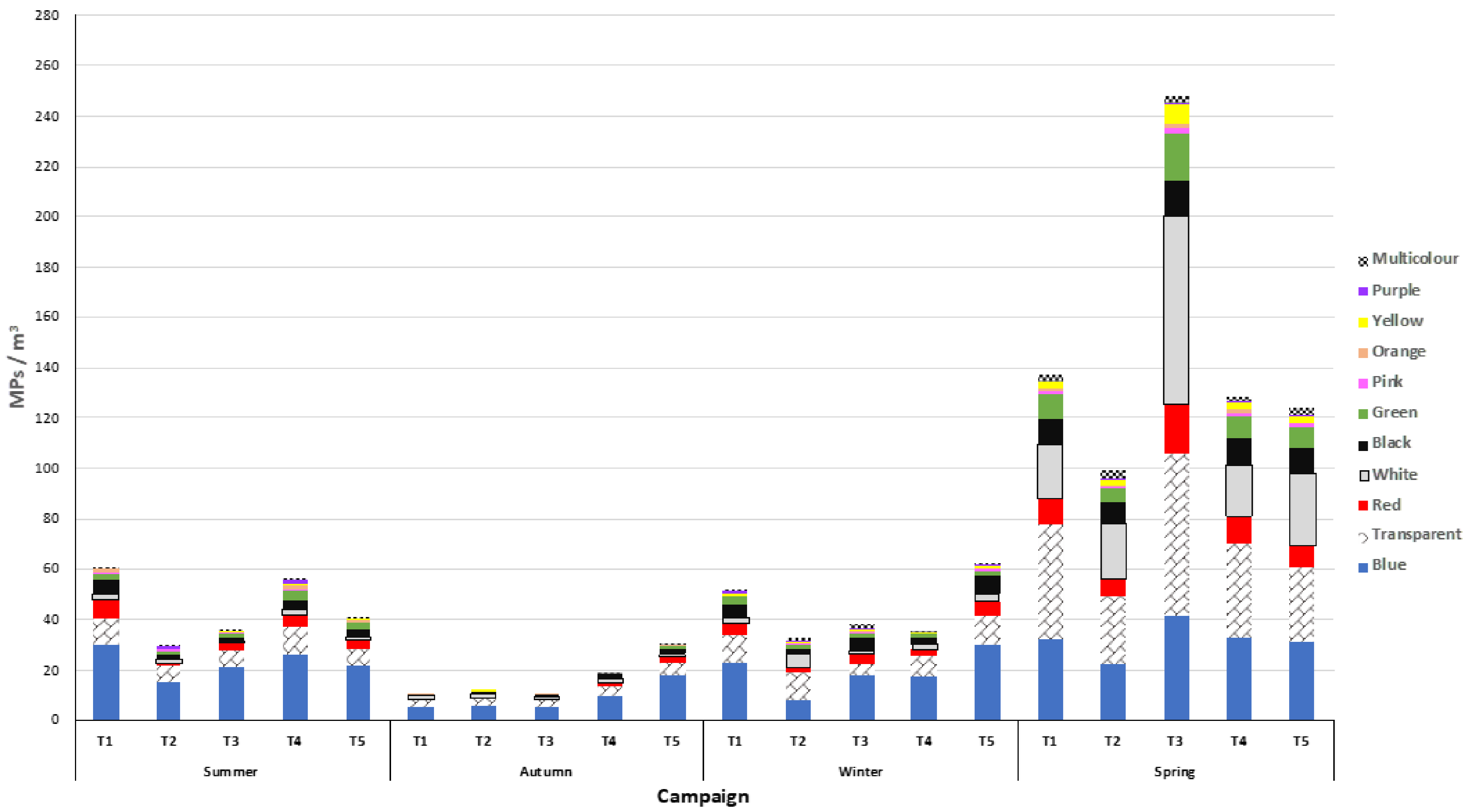


Fig. 40 Total number of MPs by colour in each transect, and respective standard errors (n = 20; 1 to 5 transects; 4 campaigns).

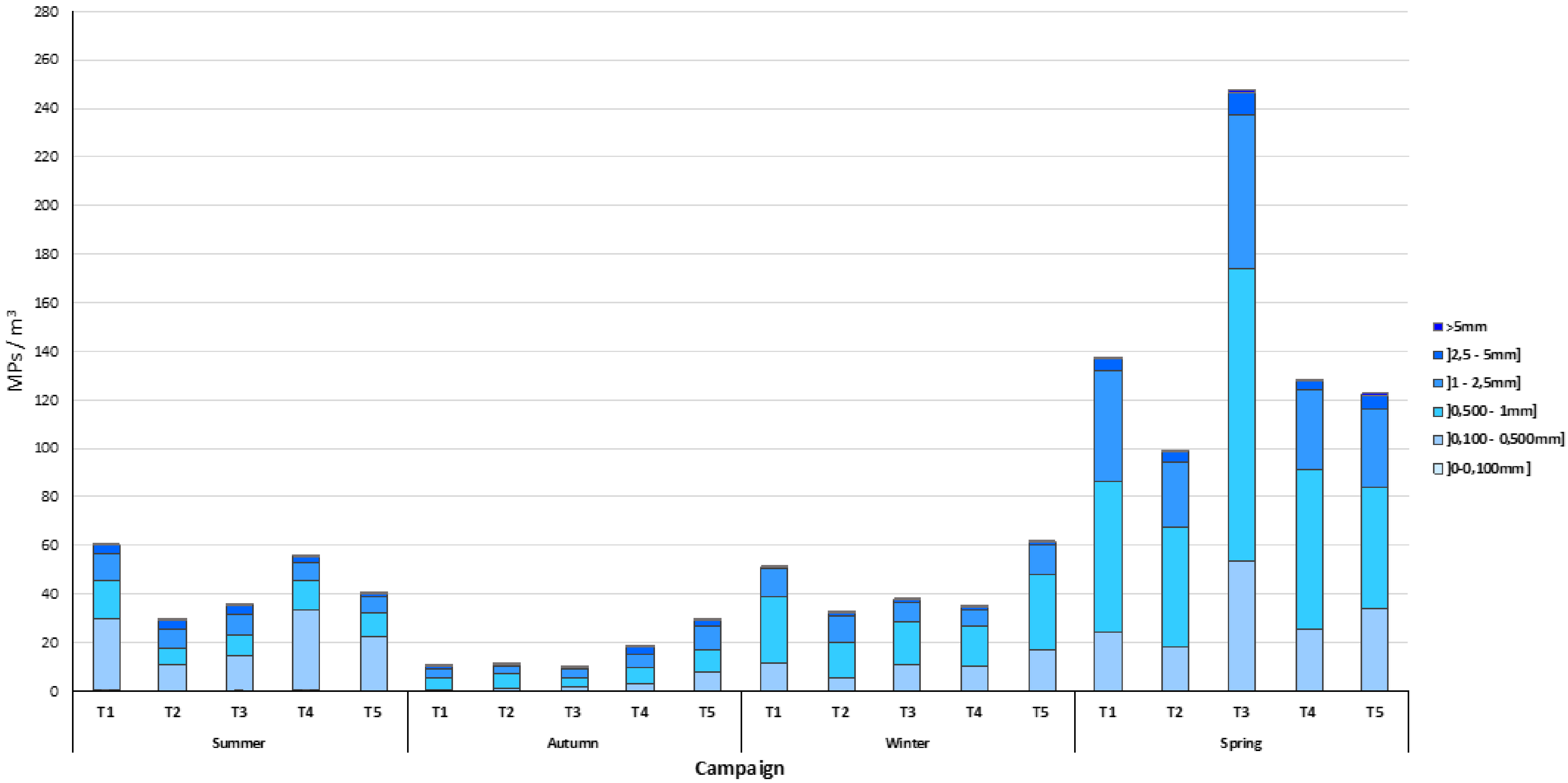


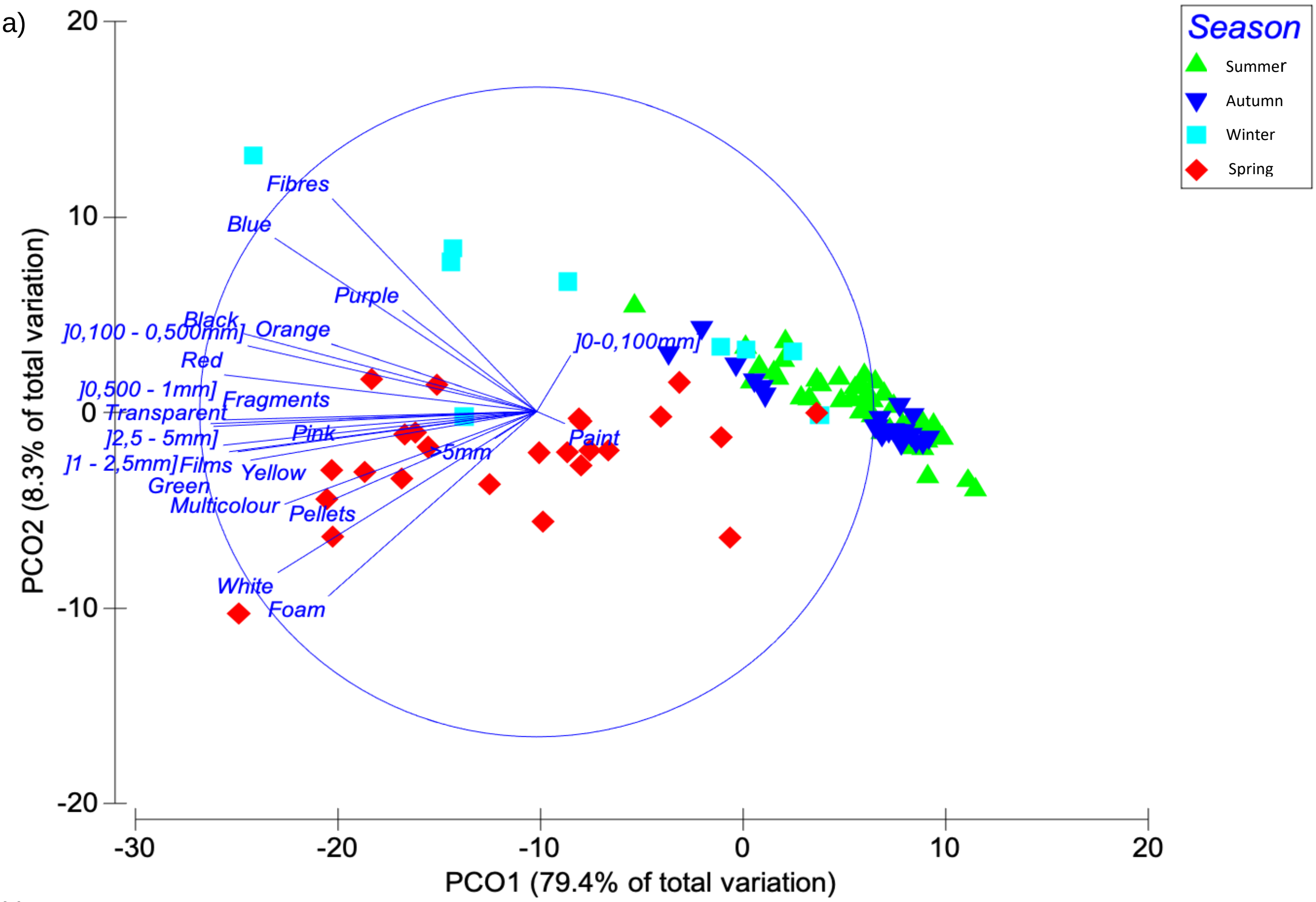
Fig. 41 Total number of MPs by size category in each transect, and respective standard errors (n = 20; 1 to 5 transects; 4 campaigns).

Table 6. Multifactorial analysis of variance results comparing seasons, transects, and seasons and transects.

Factor	Variable	Sum of Squares	df	Sig.
Season	Fibres	5152.4	3	0.020
	Fragments	21912.1	3	0.001
	Pellets	4316.0	3	0.021
	Films	7034.9	3	0.009
	Paint	93432.6	3	0.004
	Foam	28655.8	3	0.001
	Blue	61942.1	3	0.001
	Transparent	84214.1	3	<0.001
	Red	56127.5	3	<0.001
	White	61926.3	3	<0.001
	Black	72048.7	3	<0.001
	Green	24558.1	3	0.002
	Pink	35773.7	3	0.002
	Orange	66346.3	3	0.004
	Yellow	72532.7	3	<0.001
	Purple	64443.2	3	<0.001
	Multicolour	27744.0	3	0.002
	]0-0.100mm]	6950.7	3	0.009
	]0.100 – 0.500mm]	53834.6	3	<0.001
	]0.500 - 1mm]	76530.3	3	<0.001
	]1 – 2.5mm]	72270.6	3	<0.001
	]2.5 - 5mm]	4388.4	3	0.022
	> 5mm	50432.3	3	0.001
Transect	Fibres	21661.0	4	0.001
	Fragments	48723.4	4	0.032
	Pellets	52942.7	4	0.025
	Films	21995.5	4	0.043
	Paint	17553.3	4	0.009
	Foam	14375.0	4	0.013
	Blue	15241.1	4	0.010
	Transparent	1585.5	4	0.031
	Red	3384.6	4	0.012
	White	4762.8	4	0.020
	Black	1370.0	4	0.031
	Green	2184.7	4	0.022
	Pink	943.2	4	0.041
	Orange	5443.1	4	0.008
	Yellow	1044.4	4	0.040
	Purple	1234.0	4	0.042
	Multicolour	1632.4	4	0.045
	]0-0.100mm]	1336.0	4	0.047
	]0.100 – 0.500mm]	8585.0	4	0.008
	]0.500 - 1mm]	1647.1	4	0.035
	]1 – 2.5mm]	1633.0	4	0.422
	]2.5 - 5mm]	1185.6	4	0.123
	> 5mm	62.7	4	0.275

Season * Transect	Fibres	27490.2	12	0.011
	Fragments	84398.1	12	<0.001
	Pellets	1125.6	12	0.030
	Films	2552.8	12	0.001
	Paint	3.2	12	0.888
	Foam	18489.7	12	0.003
	Blue	15163.6	12	0.031
	Transparent	17821.7	12	<0.001
	Red	1555.6	12	0.007
	White	25664.3	12	0.001
	Black	1165.4	12	0.031
	Green	1402.9	12	0.000
	Pink	322.6	12	0.015
	Orange	47.6	12	0.074
	Yellow	318.7	12	0.002
	Purple	39.5	12	<0.001
	Multicolour	953.8	12	0.045
	]0-0.100mm]	4.1	12	0.731
	]0.100 – 0.500mm]	14077.9	12	0.031
	]0.500 - 1mm]	65421.1	12	<0.001
	]1 – 2.5mm]	13516.5	12	0.001
	]2.5 - 5mm]	307.9	12	0.017
	> 5mm	7.3	12	0.300

a)



b)

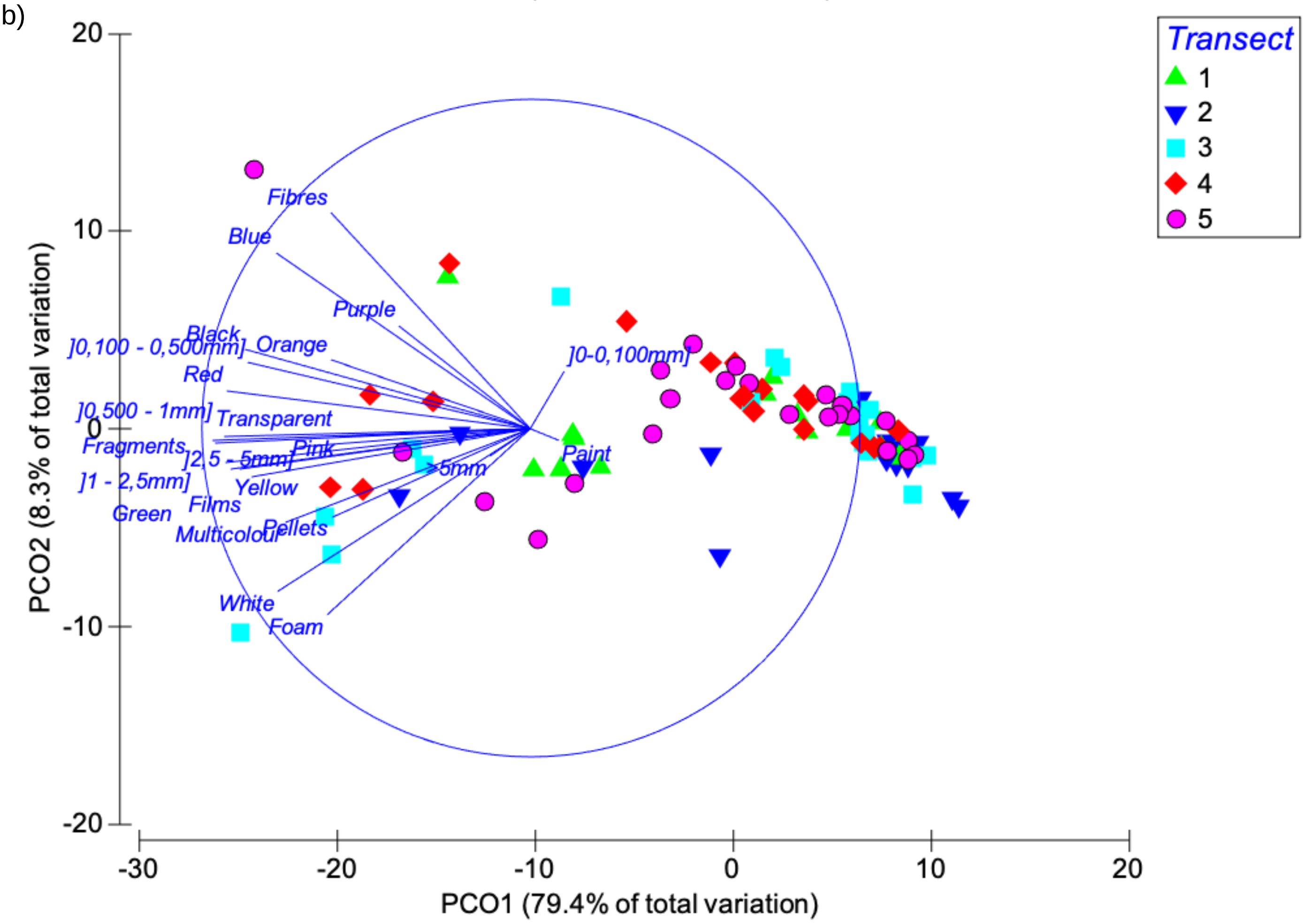


Fig. 42. Principal Coordinates Analysis (PCO), showing similarities between MPs shape, colour and size and relation with a) seasons and b) selected transects. The percentage contribution of both axis is also indicated. First axis is horizontal (PCO 1) and the second vertical (PCO 2); both axis account to a total of 87.7% of total variability.

4. Discussion

FML and MP contamination of aquatic ecosystems has become a major global concern. As a consequence, the number of studies focusing on this type of pollution has been increasing (Rodrigues et al., 2019). However, it is important to focalize the analysis in rivers and estuaries because they are the main conductors of FML and MPs to the oceans.

The present study is the first that aims to characterize and quantify ML and to evaluate and characterize MPs in the estuary of the Ave river. At the same time, this study also intends to identify the main sources of ML that occur in the estuary and to analyse the main hydrodynamic patterns and the typology of the water bodies inside the estuary to understand how the litter transport takes place on this estuarine region. To achieve this, the number and type of FML and MPs observed in each campaign/transect were analysed taking into account the main hydrodynamic patters defined in this estuary. Additionally, FML was classified by typology and material, and MPs were classified by shape, colour, and size category. A comparison was made between transects and between campaigns (seasonality) to look for possible patterns that can help to avoid the aquatic environment contamination by FML and MPs.

4.1 Floating Macro Litter

This study showed that the annual FML flux value in the Ave estuary was approximately 7.4±1.09 items/h (mean ± SEM), which is in accordance with the results of González-Fernández et al. (2021), the only research performed on FML in the Ave river basin so far. This study demonstrated that the Ave river has a FML flux value of 9.97 items/h, in agreement with the observations performed during the execution of this thesis and presented in this document. Furthermore, according to González-Fernández et al., (2021), the Ave river basin has an annual FML loading of approximately 87306 items.

The most common FML material found in the Ave estuary was artificial polymer (plastic), also determined as the top riverine FML material in Europe (82%), by González-Fernández et al. (2021). The most recorded item was styrofoam pieces, followed by plastic bottles, which are part of the top five plastic items found in rivers across Europe by González-Fernández et al. (2021), with 6% and 10% respectively. These results may indicate that possible sources of the FML found in the Ave estuary are public litter and tourism, which are more pronounced in the river mouth due to the several urban activities led out in the northern margin of the Ave estuary, also explaining the abundance of cigarette butts and filters. Another potentially contributing source for the FML observed are fishing activities, which use several items made of polystyrene, such as buoys and cooler boxes for baits and catches, as well as ropes, string/cord and fishing lines. It’s important to mention that face masks are a relatively new item, which wasn’t visible as litter in previous years, having been increasing due to the COVID-19 pandemic. When the campaigns for this study were performed, masks were still mandatory in Portugal, which explains why they were observed as FML. This item doesn’t have a code attributed to them yet (Table 2) , but that might happen soon due to their exponential increase as ML in the last few years.

The results showed that the FML flux value was higher in the winter. This seems to be in agreement with the hydrodynamic analysis and the normal river flow. As winter and spring are the rainiest seasons in the Porto district, they also present the strongest river flows (Fig. 5b and 43), transporting higher amounts of ML.

However, it must be considered that winter 2021/2022 was extremely dry, as it can be seen in Fig. 44 for the month of January. In the Porto district it was accumulated 50 mm when the average amount of precipitation for January is next to 150 mm (Fig. 43). June and October 2021 (Fig. 44) are in the line of the averaged values (Fig. 43), meanwhile April 2022 presented values lower than usual. Lower precipitation can be associated with lower river flows and lower ML transport to the estuary. Despite these differences in the rainfall patterns, the higher FML flux was obtained in winter and the second higher in spring. A more detailed analysis using daily accumulates of precipitation near Ave estuary should be performed to justify the patterns obtained in the FML flux. Unfortunately, this data is not available for the campaign periods and this detailed analysis could not be performed.

During winter, it was observed a higher abundance of styrofoam, probably due to the stronger and more frequent winds that occur during this season, which can drag litter from terrestrial sources into the river and the estuary. The abundance of styrofoam can potentially be explained by fisheries activities, which despite decreasing during winter, are frequently responsible for leaving a great number of styrofoam items near the estuary margin, exposed to adverse weather conditions till the next fishing season (Rodrigues et al., 2020a).

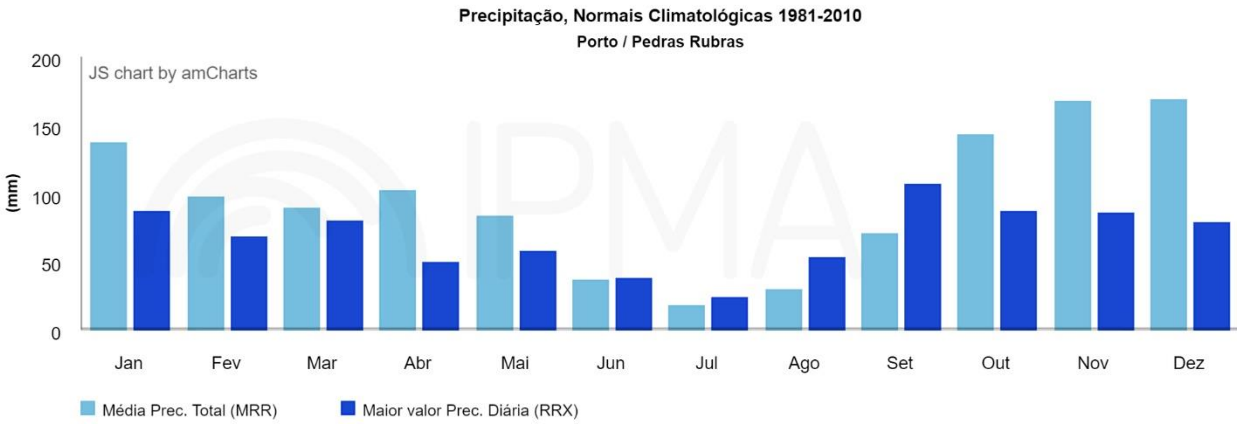


Fig. 43. Average amount of total precipitation (light blue) and highest value of daily precipitation (dark blue) for the period 1981-2010 for the Porto meteorological station (Source: IPMA).

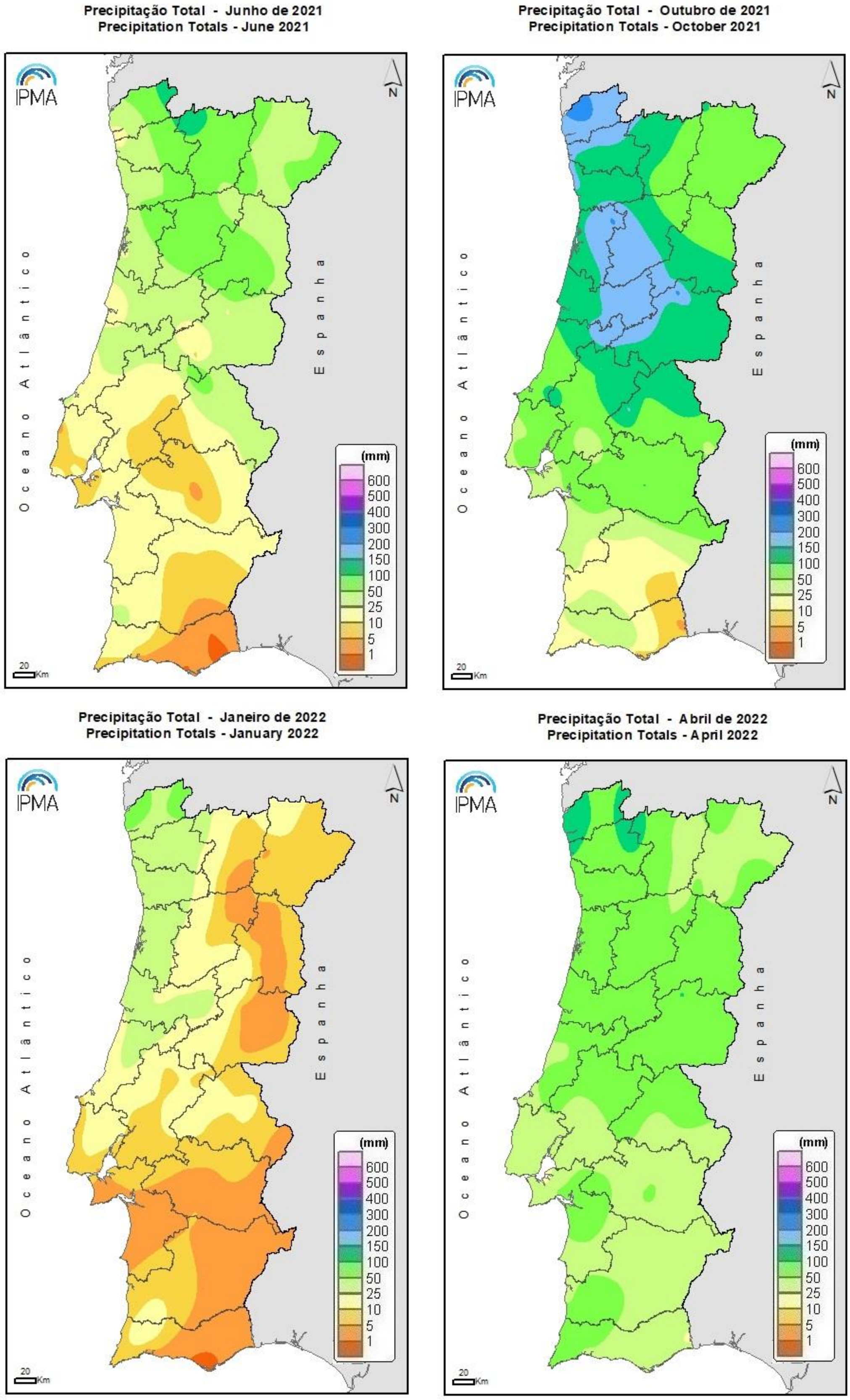


Fig. 44. Total precipitation in June 2021, October 2021, January 2022 and April 2022, the months where the ML and MPs campaigns were performed (Source: IPMA).

4.2 Microplastics

This study showed that MPs were present in all samples collected, with the manta net, with an annual average density of 63.1 ± 4.7 MPs/m³ (mean ± SEM). These values were much higher than those reported by Rodrigues et al., (2019) for the Douro estuary (0.17 ± 0.16 MPs/m³), by Rodrigues et al., (2020a) (0.45 ± 0.52 MPs/m³) in the Sado estuary, and by other studies carried out in Portugal like in Aveiro (0.002 ± 0.001 MPs/m³), Lisboa (0.033 ± 0.021 MPs/m³), Costa Vicentina (0.036 ± 0.027 MPs/m³), Algarve (0.014 ± 0.012 MPs/m³) (Frias et al., 2014), and Mondego estuary (1.53 ± 1.04 MPs/m³) (Bessa et al., 2018). They were, however, closer to the levels of contamination reported in different areas, such as the Seine estuary (37.7 MPs/m³) in Europe (Cocca et al., 2020), and several estuaries in China, like Minjiang (1245.8 ± 531.5 MPs/m³), Jiaojiang (955.6 ± 848.7 MPs/m³), Oujiang (680.0 ± 284.6 MPs/m³) and Yangtze estuary (4137.3 ± 2461.5 MPs/m³) (Zhao et al., 2014, 2015). Comparisons on the average concentration of MPs in different estuaries should, however, be made with caution due to the different sampling and quantification methods, different local environmental conditions and distinct levels of anthropogenic pressure (Lima et al., 2014; Rodrigues et al., 2019).

Along the estuary, MPs showed spatial patterns between transects, which means that the transects were well selected for this study, although there aren't differences globally speaking, which can be explained by the small size of the Ave estuary. MPs are more abundant in the transects closer to the estuary mouth (T3, T4 and T5), which is an area with several river activities, a fishing port and a shipyard. The proximity to urban centres has been suggested by several studies as one of the most important contributors to MPs pollution (Xue et al., 2020). Hydrodynamics can also explain this distribution. Transect 1 is located near the upstream limit of the estuary. Upstream of the region where this transect was performed there are some rocks and the weir that limits the estuary. The presence of the rocks can act by mixing the freshwater masses with the oceanic water from the bottom layers, developing the stratification downstream this transect. Transect 2 is mostly developed in the inner bend of the estuary where the bathymetry is shallower and the velocities are lower. The outer bends of the rivers and estuaries are the regions where the higher currents and stronger erosive processes are found. Thus, it's expected that most part of the MPs are conducted by the surface currents and found in the outer bends regions. On the other hand, transects 3, 4 and 5 present different hydrodynamic configurations that, together with the distribution of the navigational activities at the margin, can explain the augment of the MPs caught on those transects. Transect 3 goes from one margin to another of the estuary in a rectilinear region where the surface currents could be more uniform, and so the MPs distribution. Transect 4 and Transect 5 are at the estuarine mouth. In that region the mouth opens and the currents diminish its intensity. Among that, it's the last part of the estuary, where all the MPs from the entire estuary are accumulated. And, due to the presence of the breakwaters, this zone it's still protected from oceanic waves, still preventing the mixing of the freshwater masses with oceanic waters, keeping the MPs still at the surface. Unfortunately, the hydrodynamic in situ campaign dataset presented in this study is not enough to confirm these hypotheses.

The manta net is the most commonly used and recommended method for MPs sampling in freshwater, ocean and coastal ecosystems, allowing large volumes of water to be sampled relatively quickly under relatively calm water conditions (Pasquier et al., 2022; Gago et al., 2018b; European Commission, 2010). It's important to remember that, the manta-net used in this study only sampled the surface water of the estuary (50 to 70 cm deep), and, as seen before, the Ave estuary is highly stratified, which means that only the riverine water was sampled and not the oceanic water. As such, it's possible to hypothesize that the MPs found in this study were caught in the freshwater layer. This means that their origin could be from upstream regions of the estuary or from the urban conditions around the region under study. It could be expected that the lower/oceanic layer of the Ave estuary has a total different concentration of MPs.

The analysed samples showed a predominance of plastic fragments and fibres, in concordance with other studies (Rodrigues et al., 2020a; Rodrigues et al., 2019), what may suggest that they were mostly secondary MPs. This means, that most of the MPs found in the Ave estuary are probably the result of the degradation of larger plastic debris. Fragments can derive from non-definable sources, public litter and sewage-related debris, and fibres can originate from fishing activities, deposition of airborne and textile industry (Cole et al., 2011). Previous studies (Rodrigues et al., 2018), have reported fibres as the most common MPs shape, however many of those studies didn't apply any procedure for organic matter digestion (e.g. KOH). Therefore, a possible explanation for those results may be that those studies included both the plastic fibres but also the cotton fibres derived from clothes. This shows the importance of establishing appropriated laboratory procedures prior to the observation of MPs. Another possible explanation may be the efficiency of treatment processes of WWTW on the retention of fibres from textile laundry (Rodrigues et al., 2020a).

MPs also showed a very strong seasonal pattern, which can be direct/indirectly related to the hydrodynamics of the Ave river and the potential MPs sources. Similar to what was observed for the FML, the seasons when most MPs were collected were spring (April) and winter (January), coinciding with the months when the river flow values are expected to be higher, due to the precipitation regime. These results were similar to those obtained by Rodrigues et al., (2019) in the Douro estuary, and by Rodrigues et al., (2020a) in the Sado estuary. However, and as already mentioned in the previous section, due to the anomalies in the precipitation regimes observed in 2021 and 2022, the correlation between rainfall, river flow and MPs concentration should be carefully analysed. As demonstrated in section 1.5.1. the Ave is a highly stratified estuary where the less dense freshwater extends on top of the heavier saltwater. Considering the followed protocol for MP collection, it is expected that the collected MPs were in the freshwater layer. Further studies are needed to characterize the MPs distribution in a higher range of depths of the Ave estuary. Paint was the less abundant MP, and it was only observed in the autumn samples, for the T5 transect. One hypothesis may be the fact that the cleaning of the ship hulls to remove biological growth and allow re-painting is done in the low season, and as the shipyard is close to the mouth of the estuary, the currents and flow of the estuary may lead the paint particles to concentrate at the transect 5 location (UNEP, 2016). More studies are needed to understand the distribution and seasonal patterns of these particles in the Ave estuary. Foam showed a distinctive pattern in their distribution, being predominantly collected during the spring campaign. The higher concentrations of foam (expanded polystyrene) in the estuary during spring could be related to fisheries activities, consisting of secondary MPs from the breakdown of buoys and cooler boxes for bait and catches.

The predominant colour found for the MPs was blue, in agreement with other studies (Rodrigues et al., 2018).

The most percentage of MPs particles collected had sizes between 0.5 mm and 1 mm. The lack of MPs of the smallest size class (0 – 0.1 mm) could be related with the net mesh size. Secondly, it could be related with the resident time spent in the estuary, which could enhance biofouling levels and consequently cause smaller particles to sink (Kaiser et al., 2017) or to be ingested, as biofilms are suggested to increase MP palatability (Vroom et al., 2017). Several studies have emphasized that studies monitoring MP concentrations at the surface waters may underestimate them, due to the lower size limit of the range considered (net mesh used, 200 µm in this study). As there is a tendency for plastics to suffer fragmentation over time, studies are missing the size class which is possibly the most abundant. This shows the importance that future studies should have in including a sampling method capable of collecting smaller MP in an efficient way to clarify the abundance patterns found at the Ave estuary.

5. Conclusions and Future Directions

This study represents the first approach on ML and MPs research in the Ave estuary, as well as it's hydrodynamics, contributing to understand the amount of ML and MPs that reach the ocean. Following international protocols and the most direct and objective methodology, this study demonstrated that both ML and MPs identified in the Ave estuary emphasise major concerns on potential impacts on both ecosystem and human health. The main results obtained showed that the Ave River estuary has considerable contamination by FML, with an overall estimated FML flux of 7.4 items/h, where the most common item observed was styrofoam, and being winter the season where most FML were identified (97 items, 13.9 items/h).

A great diversity of MPs was also observed (12325 in total, with an annual average density of 63.1 ± 4.7 MPs/m^3), with a significant prevalence of fragments (53%), followed by fibres (29%). The most predominant colour was blue (34%), and most MPs observed measured between 0.5 mm and 1 mm (42%). MPs were found in significantly lower concentrations during autumn and significantly higher during spring. At the same time, a preliminary work was done about the hydrodynamics of the area, where one interesting result stands out, the fact that the Ave estuary is highly stratified, with possible influence on MPs concentrations and distribution. After analysing the results, public litter, tourism and fishing activities were considered possible sources for the FML and MPs found in the Ave estuary. If no effective measures are taken, it is expected that, unsustainable growth and practices will increase the impacts on the ecosystems and human health. Therefore, the importance of involving citizens in the project, and raise awareness for this problematic and the urgency to act on it becomes even greater.

The results from the present work also highlight the urgent need for more temporal and spatial monitoring of plastic pollution in estuaries and other coastal ecosystems, as well as a research based solution to act on the sources of plastic pollution. This study acts as a contribution to identify and propose new solutions to tackle aquatic litter, by providing essential data for research, international databases and, also to support the installation of an innovative technology to remove the ML in the Ave river estuary (a bubble barrier), in the scope of MAELSTROM.

Some future directions can be considered, based on this thesis, including the need for a long term study in the Ave estuary, to clearly unravel the impact of rainfall and hydrodynamic patterns on MPs dynamics. Furthermore, there is a considerable lack of solutions on MPs prevention and removal, which is why awareness-raising activities through the direct involvement of citizens, which enable capacity-building, are so important. As such, this scientific knowledge is essential for adequate public engagement and political commitment, working together on more effective management of litter and marine and coastal ecosystems.

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7. Attachments

Evaluation of Macro Litter and Microplastics in the Ave Estuary

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Introduction

- Anthropogenic pollution has become a threat to ecosystems and human health.
- Marine debris are composed of a wide variety of materials from various sources; however, plastics are by far the most abundant material recorded [1].
- Rivers act as pipelines to the ocean, channelling the waste that is dumped into the estuaries. Thus, acquired data on those ecosystems contribute to a better understanding on the entry of litter into the ocean from terrestrial sources.
- Plastic marine debris are exposed to physical, chemical and biological stressors, resulting in smaller fragments, known as microplastics [2].

Aims

- To characterize and quantify **macro litter** in the Ave estuary
- To evaluate and characterize **microplastics** in the water column, as well as identifying the main sources of such debris.

Methodology

- The evaluation of macro litter was performed through visual monitoring, with 60min per observation [3].
- Microplastics in the water column were sampled by a horizontal trawl using a 200 µm planktonic net.
- The samples collected were processed following international guidelines [3,4].
- Microplastics were identified, measured and sorted by type (fragment, pellet, fiber, film) and colour [4].

Results

Macro Litter

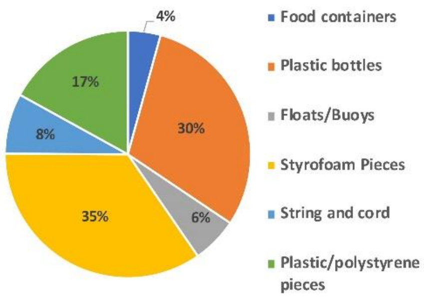

Figure 1. Percentage of the top six macro litter items identified in the Ave estuary

Microplastics

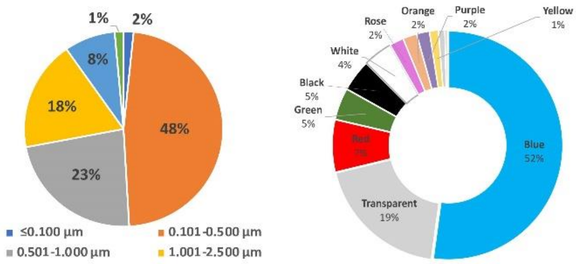

Figure 2. Percentages of microplastics collected per size class and colour

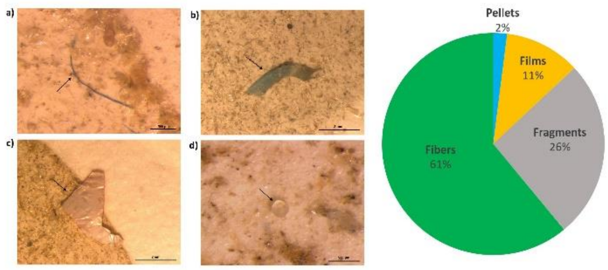

Figure 3. Some examples and percentages of microplastics recovered: a) – Fiber; b) – Fragment; c) – Film; d) – Pellet.

Discussion and Conclusions

- Overall, the results indicated that the Ave estuary has a considerable contamination by macro litter (e.g. styrofoam, bags, plastic bottles), and a great diversity of microplastics > 0.100 µm, with a significant prevalence of fibers, was also recorded.
- This study addresses the urgent need for temporal and spatial monitoring on plastic pollution in estuaries and other coastal ecosystems. This scientific knowledge is essential for adequate management of litter and of marine and coastal ecosystems.

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Attachment 1. Poster presented at IJUP 2022.

Attachment 2. Flyer for the Beach Cleanup in Vila do Conde.

Attachment 3. Location of the Beach Cleanup at Vila do Conde.

TSG- ML General code	OSPAR-Code	UNEP-Code	General Name	Level1 – Materials	Core	Beach	Sea-floor	Float-ing	Biota	Mi-cro
G1	1	PL05	4/6-pack yokes, six-pack rings	Artificial polymer material	x	x				
G2		PL07	Bags	Artificial polymer material	x		x	x		
G3	2	PL07	Shopping Bags incl. pieces	Artificial polymer material		x				
G4	3	PL07	Small plastic bags, e.g. freezer bags in pieces	Artificial polymer material		x				
G5	112		Plastic bag collective role; what remain from rip-off plastic bags	Artificial polymer material		x				
G6	4	PL02	Bottles	Artificial polymer material	x		x	x		
G7	4	PL02	Drink bottles <=0.5l	Artificial polymer material		x				
G8	4	PL02	Drink bottles >0.5l	Artificial polymer material		x				
G9	5	PL02	Cleaner bottles & containers	Artificial polymer material	x	x				
G10	6	PL06	Food containers incl. fast food containers	Artificial polymer material	x	x	x			
G11	7	PL02	Beach use related cosmetic bottles and containers, e.g. Sunblocks	Artificial polymer material		x				
G12	7	PL02	Other cosmetics bottles & containers	Artificial polymer material	x	x				
G13	12	PL02	Other bottles & containers (drums)	Artificial polymer material	x	x				
G14	8		Engine oil bottles & containers <50 cm	Artificial polymer material		x				
G15	9	PL03	Engine oil bottles & containers >50 cm	Artificial polymer material		x				
G16	10	PL03	Jerry cans (square plastic containers with handle)	Artificial polymer material		x				
G17	11		Injection gun containers	Artificial polymer material		x				
G18	13	PL13	Crates and containers / baskets	Artificial polymer material		x	x	x		
G19	14		Car parts	Artificial polymer material		x				
G20		PL01	Plastic caps and lids	Artificial polymer material			x			
G21	15	PL01	Plastic caps/lids drinks	Artificial polymer material		x				
G22	15	PL01	Plastic caps/lids chemicals, detergents (non-food)	Artificial polymer material	x	x				
G23	15	PL01	Plastic caps/lids unidentified	Artificial polymer material		x				
G24	15	PL01	Plastic rings from bottle caps/lids	Artificial polymer material		x				

TSG- ML General code	OSPAR-Code	UNEP-Code	General Name	Level1 – Materials	Core	Beach	Sea-floor	Float-ing	Biota	Mi-cro
G25			*Tobacco pouches / plastic cigarette box packaging*	*Artificial polymer material*		*x*				
G26	*16*	*PL10*	*Cigarette lighters*	*Artificial polymer material*	*x*	*x*				
G27	*64*	*PL11*	*Cigarette butts and filters*	*Artificial polymer material*		*x*	*x*			
G28	*17*		*Pens and pen lids*	*Artificial polymer material*		*x*				
G29	*18*		*Combs/hair brushes/sunglasses*	*Artificial polymer material*		*x*				
G30	*19*		*Crisps packets/sweets wrappers*	*Artificial polymer material*		*x*				
G31	*19*		*Lolly sticks*	*Artificial polymer materials*		*x*				
G32	*20*	*PL08*	*Toys and party poppers*	*Artificial polymer materials*	*x*	*x*				
G33	*21*	*PL06*	*Cups and cup lids*	*Artificial polymer materials*	*x*	*x*				
G34	*22*	*PL04*	*Cutlery and trays*	*Artificial polymer materials*		*x*				
G35	*22*	*PL04*	*Straws and stirrers*	*Artificial polymer materials*		*x*				
G36	*23*		*Fertiliser/animal feed bags*	*Artificial polymer materials*		*x*				
G37	*24*	*PL15*	*Mesh vegetable bags*	*Artificial polymer materials*		*x*				
G38			*Cover / packaging*	*Artificial polymer materials*				*x*		
G39		*PL09*	*Gloves*	*Artificial polymer materials*			*x*	*x*		
G40	*25*	*PL09*	*Gloves (washing up)*	*Artificial polymer materials*	*x*	*x*				
G41	*113*	*3*	*Gloves (industrial/professional rubber gloves)*	*Artificial polymer materials*	*x*	*x*				
G42	*26*	*PL17*	*Crab/lobster pots and tops*	*Artificial polymer materials*		*x*				
G43	*114*		*Tags (fishing and industry)*	*Artificial polymer materials*		*x*				
G44	*27*	*PL17*	*Octopus pots*	*Artificial polymer materials*		*x*				
G45	*28*	*PL15*	*Mussels nets, Oyster nets*	*Artificial polymer materials*		*x*				
G46	*29*		*Oyster trays (round from oyster cultures)*	*Artificial polymer materials*		*x*				
G47	*30*		*Plastic sheeting from mussel culture (Tahi-tians)*	*Artificial polymer materials*		*x*				
G48			*Synthetic rope*	*Artificial polymer materials*			*x*	*x*		
G49	*31*	*PL19*	*Rope (diameter more than 1cm)*	*Artificial polymer materials*	*x*	*x*				
G50	*32*	*PL19*	*String and cord (diameter less than 1cm)*	*Artificial polymer materials*	*x*	*x*				
G51		*PL20*	*Fishing net*	*Artificial polymer materials*			*x*	*x*		

TSG- ML General code	OSPAR-Code	UNEP-Code	General Name	Level1 – Materials	Core	Beach	Sea-floor	Float-ing	Biota	Mi-cro
G52		*PL20*	*Nets and pieces of net*	*Artificial polymer materials*	*x*	*x*				
G53	*115*	*20*	*Nets and pieces of net < 50 cm*	*Artificial polymer materials*		*x*				
G54	*116*	*20*	*Nets and pieces of net > 50 cm*	*Artificial polymer materials*		*x*				
G55		*PL18*	*Fishing line (entangled)*	*Artificial polymer materials*			*x*			
G56	*33*	*PL20*	*Tangled nets/cord*	*Artificial polymer materials*		*x*				
G57	*34*	*PL17*	*Fish boxes - plastic*	*Artificial polymer materials*		*x*		*x*		
G58	*34*	*PL17*	*Fish boxes - expanded polystyrene*	*Artificial polymer materials*		*x*		*x*		
G59	*35*	*PL18*	*Fishing line/monofilament (angling)*	*Artificial polymer materials*	*x*	*x*	*x*			
G60	*36*	*PL17*	*Light sticks (tubes with fluid) incl. pack-aging*	*Artificial polymer materials*		*x*				
G61			*Other fishing related*	*Artificial polymer materials*			*x*			
G62	*37*	*PL14*	*Floats for fishing nets*	*Artificial polymer materials*	*x*	*x*				
G63	*37*	*PL14*	*Buoys*	*Artificial polymer materials*		*x*		*x*		
G64			*Fenders*	*Artificial polymer materials*		*x*				
G65	*38*	*PL03*	*Buckets*	*Artificial polymer materials*		*x*				
G66	*39*	*PL21*	*Strapping bands*	*Artificial polymer materials*	*x*	*x*	*x*			
G67	*40*	*PL16*	*Sheets, industrial packaging, plastic sheet-ing*	*Artificial polymer materials*		*x*	*x*	*x*		
G68	*41*	*PL22*	*Fibre glass/fragments*	*Artificial polymer materials*		*x*				
G69	*42*		*Hard hats/Helmets*	*Artificial polymer materials*		*x*				
G70	*43*		*Shotgun cartridges*	*Artificial polymer materials*		*x*				
G71	*44*	*CL01*	*Shoes/sandals*	*Artificial polymer materials*		*x*				
G72			*Traffic cones*	*Artificial polymer materials*		*x*				
G73	*45*	*FP01*	*Foam sponge*	*Artificial polymer materials*		*x*				
G74			*Foam packaging/insulation/polyurethane*	*Artificial polymer materials*				*x*		
G75	*117*		*Plastic/polystyrene pieces 0 - 2.5 cm*	*Artificial polymer materials*		*x*				
G76	*46*		*Plastic/polystyrene pieces 2.5 cm > < 50cm*	*Artificial polymer materials*		*x*				
G77	*47*		*Plastic/polystyrene pieces > 50 cm*	*Artificial polymer materials*		*x*				
G78			*Plastic pieces 0 - 2.5 cm*	*Artificial polymer materials*		*x*				

TSG- ML General code	OSPAR-Code	UNEP-Code	General Name	Level1 – Materials	Core	Beach	Sea-floor	Float-ing	Biota	Mi-cro
G79			Plastic pieces 2.5 cm > < 50cm	Artificial polymer materials		x		x		
G80			Plastic pieces > 50 cm	Artificial polymer materials		x		x		
G81			Polystyrene pieces 0 - 2.5 cm	Artificial polymer materials		x				
G82			Polystyrene pieces 2.5 cm > < 50cm	Artificial polymer materials		x		x		
G83			Polystyrene pieces > 50 cm	Artificial polymer materials		x		x		
G84			CD, CD-box	Artificial polymer materials		x				
G85			Salt packaging	Artificial polymer materials		x				
G86			Fin trees (from fins for scuba diving)	Artificial polymer materials		x				
G87			Masking tape	Artificial polymer materials		x				
G88			Telephone (incl. parts)	Artificial polymer materials		x				
G89			Plastic construction waste	Artificial polymer materials		x				
G90			Plastic flower pots	Artificial polymer materials		x				
G91			Biomass holder from sewage treatment plants	Artificial polymer materials		x				
G92			Bait containers/packaging	Artificial polymer materials		x				
G93			Cable ties	Artificial polymer materials		x	x			
G94			Table cloth	Artificial polymer materials				x		
G95	98	OT02	Cotton bud sticks	Artificial polymer materials	x	x	x			
G96	99	OT02	Sanitary towels/panty liners/backing strips	Artificial polymer materials		x	x			
G97	101	OT02	Toilet fresheners	Artificial polymer materials		x				
G98		OT02	Diapers/nappies	Artificial polymer materials		x	x			
G99	104	PL12	Syringes/needles	Artificial polymer materials		x	x			
G100	103		Medical/Pharmaceuticals containers/ tubes	Artificial polymer materials		x				
G101	121		Dog faeces bag	Artificial polymer materials	x	x				
G102		RB02	Flip-flops	Artificial polymer materials		x				
G103			Plastic fragments rounded <5mm	Artificial polymer materials						x
G104			Plastic fragments subrounded <5mm	Artificial polymer materials						x
G105			Plastic fragments subangular <5mm	Artificial polymer materials						x

TSG- ML General code	OSPAR-Code	UNEP-Code	General Name	Level1 – Materials	Core	Beach	Sea-floor	Float-ing	Biota	Mi-cro
G106			Plastic fragments angular <5mm	Artificial polymer materials						x
G107			cylindrical pellets <5mm	Artificial polymer materials						x
G108			disks pellets <5mm	Artificial polymer materials						x
G109			flat pellets <5mm	Artificial polymer materials						x
G110			ovoid pellets <5mm	Artificial polymer materials						x
G111			spheruloids pellets <5mm	Artificial polymer materials						x
G112		PL23	Industrial pellets	Artificial polymer materials	x				x	
G113			Filament <5mm	Artificial polymer materials						x
G114			Films <5mm	Artificial polymer materials						x
G115			Foamed plastic <5mm	Artificial polymer materials						x
G116			Granules <5mm	Artificial polymer materials						x
G117			Styrofoam <5mm	Artificial polymer materials						x
G118			Small industrial spheres (<5mm)	Artificial polymer materials					x	
G119			Sheet like user plastic (>1mm)	Artificial polymer materials					x	
G120			Threadlike user plastic (>1mm)	Artificial polymer materials					x	
G121			Foamed user plastic (>1mm)	Artificial polymer materials					x	
G122			Plastic fragments (>1mm)	Artificial polymer materials					x	
G123			Polyurethane granules <5mm	Artificial polymer materials				x		
G124	48	PL24	Other plastic/polystyrene items (identifi-able)	Artificial polymer materials		x	x	x		
G125	49	RB01	Balloons and balloon sticks	Rubber	x	x	x	x		
G126		RB01	Balls	Rubber		x		x		
G127	50		Rubber boots	Rubber		x	x	x		
G128	52	RB04	Tyres and belts	Rubber	x	x	x	x		
G129		RB05	Inner-tubes and rubber sheet	Rubber		x				
G130			Wheels	Rubber	x	x				
G131		RB06	Rubber bands (small, for	Rubber		x				
G132			Bobbins (fishing)	Rubber		x	x			
G133	97	RB07	Condoms (incl. packaging)	Rubber		x	x			
G134	53	RB08	Other rubber pieces	Rubber		x	x	x		
G135		CL01	Clothing (clothes, shoes)	Cloth/textile				x		
G136		CL01	Shoes	Cloth/textile			x			
G137	54	CL01	Clothing / rags (clothing, hats, towel	Cloth/textile	x	x	x			

TSG- ML General code	OSPAR-Code	UNEP-Code	General Name	Level1 – Materials	Core	Beach	Sea-floor	Float-ing	Biota	Mi-cro
G138	57	CL01	Shoes and sandals (e.g. Leather, cloth	Cloth/textile		x				
G139		CL02	Backpacks & bags	Cloth/textile		x				
G140	56	CL03	Sacking (hessian)	Cloth/textile		x				
G141	55	CL05	Carpet & Furnishing	Cloth/textile		x	x	x		
G142		CL04	Rope, string and nets	Cloth/textile		x	x	x		
G143		CL03	Sails, canvas	Cloth/textile		x		x		
G144	100	OT02	Tampons and tampon applicators	Cloth/textile	x	x				
G145	59	CL06	Other textiles (incl. rags)	Cloth/textile		x	x	x		
G146			Paper/Cardboard	Paper/Cardboard			x			
G147	60		Paper bags	Paper/Cardboard		x				
G148	61	PC02	Cardboard (boxes & fragments)	Paper/Cardboard	x	x	x	x		
G149		PC03	Paper packaging	Paper/Cardboard				x		
G150	118	PC03	Cartons/Tetrapack Milk	Paper/Cardboard	x	x				
G151	62	PC03	Cartons/Tetrapack (others)	Paper/Cardboard	x	x				
G152	63	PC03	Cigarette packets	Paper/Cardboard		x				
G153	65	PC03	Cups, food trays, food wrappers, drink con-tainers	Paper/Cardboard	x	x				
G154	66	PC01	Newspapers & magazines	Paper/Cardboard		x		x		
G155		PC04	Tubes for fireworks	Paper/Cardboard		x				
G156			Paper fragments	Paper/Cardboard		x				
G157			Paper	Paper/Cardboard					x	
G158	67	PC05	Other paper items	Paper/Cardboard		x	x	x		
G159	68	WD01	Corks	Processed/worked wood		x				
G160	69	WD04	Pallets	Processed/worked wood	x	x	x	x		
G161	69	WD04	Processed timber	Processed/worked wood		x				
G162	70	WD04	Crates	Processed/worked wood	x	x		x		
G163	71	WD02	Crab/lobster pots	Processed/worked wood		x				
G164	119		Fish boxes	Processed/worked wood	x	x				
G165	72	WD03	Ice-cream sticks, chip forks, chopstic tooth-picks	Processed/worked wood	x	x				
G166	73		Paint brushes	Processed/worked wood		x				
G167		WD05	Matches & fireworks	Processed/worked wood		x				
G168			Wood boards	Processed/worked wood				x		
G169			Beams / Dunnage	Processed/worked wood				x		
G170			Wood (processed)	Processed/worked wood			x			
G171	74	WD06	Other wood < 50 cm	Processed/worked wood		x				

TSG- ML General code	OSPAR-Code	UNEP-Code	General Name	Level1 – Materials	Core	Beach	Sea-floor	Float-ing	Biota	Mi-cro
G172	75	WD06	Other wood > 50 cm	Processed/worked wood		x				
G173		WD06	Other (specify)	Processed/worked wood	x		x	x		
G174	76		Aerosol/Spray cans industry	Metal	x	x				
G175	78	ME03	Cans (beverage)	Metal	x	x	x	x		
G176	82	ME04	Cans (food)	Metal	x	x	x			
G177	81	ME06	Foil wrappers, aluminium foil	Metal		x				
G178	77	ME02	Bottle caps, lids & pull tabs	Metal	x	x				
G179	120		Disposable BBQ's	Metal		x				
G180	79	ME10	Appliances (refrigerators, washers, etc.	Metal		x	x			
G181		ME01	Tableware (plates, cups & cutlery)	Metal		x				
G182	80	ME07	Fishing related (weights, sinkers, lurhooks)	Metal		x	x	x		
G183		ME07	Fish hook remains	Metal					x	
G184	87	ME07	Lobster/crab pots	Metal	x	x				
G185			Middle size containers	Metal			x			
G186	83	ME10	Industrial scrap	Metal		x				
G187	84	ME05	Drums, e.g. oil	Metal		x	x			
G188		ME04	Other cans (< 4 L)	Metal		x				
G189		ME05	Gas bottles, drums & buckets (> 4 L)	Metal		x				
G190	86	ME05	Paint tins	Metal		x				
G191	88	ME09	Wire, wire mesh, barbed wire	Metal		x		x		
G192		ME05	Barrels	Metal				x		
G193			Car parts / batteries	Metal		x	x			
G194			Cables	Metal		x	x			
G195		OT04	Household Batteries	Metal		x				
G196			Large metallic objects	Metal			x			
G197			Other (metal)	Metal			x	x		
G198	89	ME10	Other metal pieces < 50 cm	Metal		x				
G199	90	ME10	Other metal pieces > 50 cm	Metal		x				
G200	91	GC02	Bottles incl. pieces	Glass/ceramics	x	x	x			
G201		GC02	Jars incl. pieces	Glass/ceramics		x	x			
G202	92	GC04	Light bulbs	Glass/ceramics	x	x				
G203		GC03	Tableware (plates & cups)	Glass/ceramics		x				
G204	94	GC01	Construction material (brick, cement, pipes)	Glass/ceramics		x				
G205	92	GC05	Fluorescent light tubes	Glass/ceramics	x	x				
G206		GC06	Glass buoys	Glass/ceramics		x				
G207	95		Octopus pots	Glass/ceramics		x				
G208		GC07	Glass or ceramic fragments >2.5cm	Glass/ceramics		x	x			
G209			Large glass objects (specify)	Glass/ceramics			x			
G210	96	GC08	Other glass items	Glass/ceramics	x	x	x			
G211	10	OT05	Other medical items (swabs, bandaging, adhesive plaster etc.)	unidentified		x				
G212			Slack / Coal						x	

TSG- ML General code	**OSPAR-Code**	**UNEP-Code**	**General Name**	**Level1 – Materials**	**Core**	**Beach**	**Sea-floor**	**Float-ing**	**Biota**	**Mi-cro**
G213	*181 ,109, 110*	*OT01*	*Paraffin/Wax*	*Chemicals*		*x*			*x*	
G214			*Oil/Tar*	*Chemicals*					*x*	
G215			*Food waste (galley waste)*	*Food waste*					*x*	
G216			*various rubbish (worked wood, metal parts)*	*undefined*					*x*	
G217			*Other (glass, metal, tar) <5mm*	*unidentified*						*x*

Attachment 4. TSG-ML General Code and OSPAR-code (Galgani et al., 2018).