Beatriz Isabel Martins Rodrigues

The use of microplastics to reconstruct dated sedimentary archives:

The showcase of Mondego estuary (Portugal)

UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnologia

2023

Beatriz Isabel Martins Rodrigues
The Use of Microplastics to Reconstruct Dated Sedimentary Archives: The Showcase of Mondego Estuary (Portugal)
2023

Beatriz Isabel Martins Rodrigues

The use of microplastics to reconstruct dated sedimentary archives: The showcase of Mondego estuary (Portugal)

Mestrado em Biologia Marinha

Supervisors:

Dra. Ana Filipa da Silva Bessa

Co-supervisor

Dra. Margarida Ribau Teixeira

UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnologia

2023

The use of microplastics to reconstruct dated sedimentary archives: The showcase of Mondego estuary (Portugal).

Declaração de Autoria de Trabalho

Declaro ser autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam na listagem de referências incluídas.

Declaration of authorship of work

Declaration of authorship of work I declare to be the author of this work, which is original and unpublished. Authors and works consulted are duly cited in the text and are included in the list of references.

Copyright

Abstract

Microplastics (plastics < 5 mm), have become a defining feature of the Anthropocene epoch, representing humanity's profound impact on the planet. Envisioned as "techno-fossils" they provide detailed records of deposition history with dating resolutions from years to decades. This study used the Mondego estuary as a case study to assess whether microplastics can be used as a marker of the Anthropocene. Sediment corers were used to collect 50cm replicates longitudinally. In total, 885 particles were extracted using a saturated NaCl (1.2. g.cm^{-3}) solution and characterised according to type, colour and shape. The polymer of 14 particles were ascertained using FTIR analysis. The sediments retrieved, dated approximately from 1947 to 2019, presented potential microplastics in all layers. The abundance of these particles fluctuated over the years, without a clear pattern, even though, the highest concentration of potential microplastics corresponded to the sediment layer (37) of 1973, where the first Polyethylene terephthalate beverage bottles were introduced and produced in mass. This study also supported the trend of high abundances of fibres, with a total value of 93% of total samples, which is often the most predominant type of microplastics found in aquatic environments and can be linked to point sources in transitional ecosystems and estuaries, such as wastewater treatment plants (WWTPs). This is a preliminary study evaluating the way microplastics tend to accumulate in sediments and how this data can be integrated. During this present work, it was clear that the main amount of potential microplastics present is fibres (93% of the total sample), followed by fragments (6%), and then by films (1%). In these particles, the most predominant colour is blue, with a total of 397 potential microplastics (45%) in a sample of 885 potential microplastics.

Keywords: Microplastics, Anthropocene, “Tecno-fossils”, Sediments, Mondego estuary, Markers.

Resumo

Neste trabalho, o principal objetivo foi investigar o potencial uso de microplásticos como marcadores para o Antropoceno nas camadas de sedimentos do estuário do Rio Mondego. O Antropoceno é uma época geológica recentemente reconhecida, que assinala um período em que as atividades humanas exerceram uma influência profunda nos ecossistemas. Esta influência, impulsionada por fatores como industrialização, agricultura e urbanização, deixam uma marca duradoura nos ecossistemas do planeta. Atualmente, existe uma proposta que considera 1950 como o ponto de partida do Antropoceno, que se baseia na análise da profundidade das diversas camadas anuais, as quais apontam para a marcação do limite proposto referente a 1950. Os plásticos, especialmente os microplásticos, surgiram como marcadores proeminentes desta época, refletindo o amplo impacto humano no ambiente. Os plásticos, por serem uma adição relativamente recente à linha do tempo geológico da Terra passaram a definir a era do Antropoceno. Isto envolve a criação de registos detalhados da história de deposição dos sedimentos, usando microplásticos como "tecno-fósseis", possibilitando a datação relativa, que pode ser desde anos a décadas. Entre os plásticos, destacam-se os microplásticos, classificados como plásticos com menos de 5 mm de tamanho, que ganharam destaque, sendo agora considerados como os principais potenciais representantes para datar sequências estratigráficas. Os microplásticos, frequentemente negligenciados no contexto mais amplo da poluição por plásticos, podem oferecer uma perspetiva única sobre o impacto ambiental das atividades humanas. Neste estudo, os microplásticos foram meticulosamente separados, mais precisamente, foram recolhidas cores de sedimentos, em réplicas de 50 cm longitudinalmente, do interior do estuário onde 885 partículas foram recolhidas, na amostra, usando uma solução saturada de NaCl (1,2 g.cm3) e foram caracterizadas de acordo com o tipo, cor e forma. Foram definidos polímeros de 14 partículas, através de uma análise em FTIR. Este núcleo de sedimentos, que é abrangente (desde 1947 a 2019), oferece um registo temporal único em que cada camada de sedimento corresponde a um período específico, servindo como indicadores de potenciais eventos e desenvolvimentos históricos. Os resultados mostram potenciais microplásticos em toda as camadas do núcleo de sedimentos, em que os mesmo se encontram amplamente distribuídos (100%), com uma média de 18,44 potenciais microplásticos por camada de

sedimento. Com as fibras a corresponder a 93% da amostra, seguido dos fragmentos (6%) e os filmes (1%) Esta ubiquidade destaca o impacto profundo e duradouro da poluição por microplásticos em ambientes aquáticos. No entanto, é essencial reconhecer que a abundância destes potenciais microplásticos apresenta flutuações ao longo dos anos, apresentando um quadro complexo e desprovido de um padrão claro. Uma revelação intrigante, relaciona-se com a maior concentração de potenciais microplásticos (56 potenciais microplásticos), na camada de sedimento 37, que tem como idade correspondente o ano de 1973., que corresponde mais precisamente à introdução e consequente produção em massa, de garrafas de bebidas em tereftalato de polietileno (PET). Esta descoberta levanta questões importantes como, a cor predominante, encontrada nos potenciais microplásticos nas camadas sedimentares em estudo, é o azul, correspondendo a 45% do total da amostra. Este resultado é semelhante a estudos anteriores. É, no entanto, necessário ter em consideração, que este tipo de cor em microplásticos, representa um problema derivada à sua semelhança a vários tipos de plâncton, que é a fonte primária de alimento para peixes de superfície, uma vez que são, na sua maioria, predadores visuais, no entanto ainda não existe muita informação sobre como as cores afetam os ambientes.

Um passo crucial neste estudo, foi a caracterização e distribuição dos tipos de microplásticos dentro do estuário do Mondego. Aquando da análise das amostras, demonstrou-se que a maioria dos potenciais microplásticos recolhidos se encontravam na forma de fibras, constituindo 93% da amostra total. Esta observação vai de encontro a estudos realizados anteriormente, em que fibras são o tipo predominante de microplásticos que se podem encontrar em ambientes aquáticos. A presença destas fibras no estuário do Mondego reflete, ainda, um padrão observado em ecossistemas de água doce e transição. É imperativo investigar as potenciais fontes destes microplásticos, tendo em conta que um dos maiores contribuidores são as estações de tratamento de águas residuais (ETARs). A proximidade de ETARs a ecossistemas de água doce, torna os ecossistemas mais suscetíveis à contaminação com este tipo de partículas, sem esquecer, as fibras que se libertam na lavagem de roupa, vão dar ao esgoto e que facilmente acabam nos ambientes aquáticos. A expansão urbana e industrial ao longo das margens do rio Mondego agravam a poluição sentida no estuário desse mesmo rio. Estas áreas, que incluem a Figueira da Foz e Coimbra, são responsáveis pelo escoamento de águas urbanas e industriais, caracterizadas pela quantidade de microplásticos

que apresentam. Adicionalmente, 32% da área da bacia do rio Mondego é composta por terrenos agrícolas, também contribuem (e.g., revestimento plástico de sementes) para a libertação de microplásticos para as águas do estuário. Apesar de neste estudo se ter obtido informação significativa sobre a presença e distribuição dos microplásticos nas camadas sedimentares do estuário do Rio Mondego, também se suscitam questões críticas e direções que podem ser a vir consideradas em investigações futuras. Um destes pontos, que exige uma exploração aprofundada, é a investigação de diferentes morfologias dos microplásticos, bem como, dos seus processos de sedimentação, que poderá tornar mais fácil a interpretação do comportamento dos microplásticos em ambientes aquáticos. Igualmente importante, é a compreensão dos padrões e fatores ambientais que influenciam a acumulação de microplásticos no sedimento, de forma a desenvolverem-se medidas mais eficazes que possam reduzir ou até mitigar o impacto da poluição plástica nos ecossistemas. Todos estes fatores suprarreferidos influenciam o potencial dos microplásticos como marcadores do Antropoceno, simbolizando o impacto profundo das atividades humanas nos ecossistemas.

Para se obter respostas mais concretas sobre este tema, sugere-se uma investigação mais profunda e detalhada, que deverá contemplar as fontes, o comportamento em ambientes sedimentares, o transporte, a resiliência e a entrada no ambiente dos microplásticos. Algo critico já mencionado em estudos anteriores referia inconsistências relacionadas com a preservação dos microplásticos nos registos sedimentares, e, como tal, é necessário em futuras pesquisas mais rigor e métodos mais avançados.

Palavras-chave: Microplásticos, Antropoceno, “Tecno-fósseis”, Sedimentos, Estuário do Mondego, Marcadores.

Acknowledgements

I would like to thank my supervisor, Filipa Bessa, for her amazing support and motivation. For always giving me hope, believing in me, for her time, patience and calm to teach and help me. And thanks to MAREFOZ, for their time and space. To all the people there who helped me and supported me on this journey. To my co – supervisor, Dra. Margarida Ribau Teixeira, thank you for the help and time.

A big thanks to Yasmina, for teaching me with joy and care, for all the knowledge, motivation and patience. Thank you to Gloria and Cristina. To all my friends in the Azores, I learned a lot, I laughed a lot, it was a great time.

Thank you to my family, to Xana, Zira, Rosa, Paulo e Carolina, for being there. To Cookie, Silvestre and Nikka.

A big thank you to my friend Catarina, the one to blame, the one who challenged me and made me apply. To Joana, who always believes in me, cheers me and makes sure that nothing goes unnoticed. To all my friends, a big thank you.

To Aria, Selina, Tam, Ana, Vincent and Sam, thank you for making life so easy in the hardest times, for being home wherever and whenever. It was so worth it.

To JP, the one that made all the impossible things possible, the kindest soul and my best person, without you this would not have been possible. Lucky me. There is not enough thank you.

To Dandy.

Table of Contents

List of Abbreviations, Acronyms and Symbols

ATR - Attenuated total reflectance

ETARs - Estações de tratamento de águas residuais

FTIR - Fourier-transform infrared spectroscopy

HPGe - High Purity Germanium

MPs – Microplastics

PCBs - Polychlorinated biphenyls

PCDD/ Fs - Polychlorinated dibenzo-p-dioxins and furans

PET - Polyethylene terephthalate

POPs - Persistent organic pollutants

TIC - Total inorganic carbon

TOC - Total organic carbon

WWTPs - Wastewater treatment plants

General Introduction

1.1. Plastics and the Anthropocene

Plastics have appeared as an abrupt occurrence in geological scale time, because in the course of a short time span (Martin et al., 2021), hundreds of millions of metric tons (Mt) of plastics and, consequently, plastic wastes were generated (Geyer et al., 2017). This created the possibility of using microplastics (particles with < 5mm) as a potential proxy material to date stratigraphic sequences and as markers of the Anthropocene (Martin et al., 2021; Ivar and Labrenz, 2021). Specifically, it involves creating highly detailed records of deposition history using microplastics as "techno-fossils" as recently pointed out by some authors (Chen et al., 2022; Martin et al., 2021). This is achieved by attributing specific sediment strata to the different periods of the history of plastic production and use, based on the presence or absence of those types of microplastics, resulting in a relative dating resolution on the scale of years to decades (Ivar and Labrenz, 2021; Martin et al., 2021).

The Anthropocene is a newly recognized geological epoch characterised by the dominant influence of human activities related to several anthropogenic activities, such as agriculture, industrialization, and urbanisation (Crutzen, 2016; Crutzen and Stoermer, 2000). Although still a subject of ongoing debate, the year 1900 is approximately suggested as the commencement of the Anthropocene (Chen et al., 2022). This notable starting point is characterised by the widespread emergence of fly ash resulting from the combustion of coal (Chen et al., 2022; Lewis and Maslin, 2015; Snowball et al., 2014). However, the most recent proposition is to define the Anthropocene as the epoch, that started in 1950, taking into consideration the depth of the different yearly layers of sediments, which indicates that the year 1950 is the starting limit (McCarthy et al., 2023).

Nonetheless, it is important to consider that the possibility of using microplastics as "techno-fossils" (Chen et al., 2022; Martin et al., 2021**)** relies on the assumption that different microplastics show similar transport behaviours between environments and within the sediment column, considering that their decomposition rates do not affect, significantly, the mobility of aged plastics (Bancone et al., 2020; Martin et al., 2021).

1.2. Plastics into Microplastics

Plastics were primarily synthesised in 1907 (Zhou et al., 2022) and industrialised in the 1930s (Chen et al., 2022). Their annual production has grown intensively from 1.5 million tons in the 1950s (Chen et al., 2022), to 365.5 million metric tons in 2018 (Plastics Europe, 2018), and to 390.7 million metric tons in 2021 (Plastics Europe, 2022). Characterised as synthetic organic polymers (Drraik, 2002), these materials are also strong, lightweight, durable and cheap, making them versatile, suitable and appealing for the manufacture of a very wide range of products (Drraik, 2002). However, these very characteristics are part of the reason why plastic is a pollutant, with loss and inadequate disposal from land-based sources being the biggest cause of global plastic pollution (Martin et al., 2021; Zhou et al., 2022).

Plastic debris can be classified according to colour, type, origin, polymer type or even original use but mainly by size (Thompson & Napper, 2019). Microplastics (MPs) are defined as plastic fragments whose size is < 5 mm (Arthur et al., 2009) with a lower limit of 1 µm. However, the boundary can be imposed by constraints such as the mesh or sieve sizes available for sampling at the site (Thompson & Napper, 2019).

Depending on their source, microplastics can be qualified as primary or secondary. If the microplastic was intentionally manufactured in its present size, it is considered to be primary, whereas if its size is the result of fragmentation of larger plastics it is classified as secondary (Cole et al., 2011; Thompson & Napper, 2019).

Primary microplastics are industrially manufactured and produced for direct use, such as microbeads with different sizes in cosmetics, skin care, most commonly exfoliants, and other personal care products ((Thompson & Napper, 2019; Andrady, 2017). Also, as air-blasting media or as building blocks for the production of larger plastic products, such as plastic pallets and plastic powders (Thompson & Napper, 2019). In previous studies (Napper et al., 2015), it has been estimated that from only 9 personal care products, 94 500 microbeads can be released in a single use (Napper et al., 2015). Due to their size and characteristics these particles can go into the environment through different sources, e.g., the household wastewater system (Andrady, 2017; Bajt, 2021).

Secondary microplastics are the most abundant type of microplastics in the ocean, due to their source of degradation and fragmentation of larger macroplastic debris (e.g., plastic packaging, fishing gear and fishnets) (Thompson & Napper, 2019; Andrady, 2017; Zhou et al., 2022), as a consequence of different mechanisms, such as photodegradation by UV-light, weathering, thermal degradation by visible light, biodegradation, thermal oxidation (infrared radiation) and mechanical processes such as abrasion, turbulence and wave action (Thompson & Napper, 2019; Amaral-Zettler et al., 2020).

Secondary microplastics include a variety of different particles (e.g., textiles fibres that detach from synthetic fibres, tires) originated from the abrasion caused by the usage of large plastic items on land, entering the marine environment directly as microplastic particles (Friot & Boucher, 2017; Thompson & Napper, 2019). Secondary microplastic particles are more abundant than primary particles in aquatic systems (Hale et al., 2020). Both primary and secondary microplastics have been found to persist in the natural aquatic ecosystems (Bajt, 2021). Microplastics present a surface area large enough to, while floating, adsorb toxic compounds such as heavy metals or other contaminants (Chen et al., 2022) which in turn become bioavailable to organisms through ingestion. Thus, the ingestion of these materials can be toxic, causing changes in feeding and reproductive behaviour, which can increase organisms' mortality (Bajt, 2021; Cole et al., 2011).

1.3. Marine Plastic Pollution

Marine debris emerges as a consequence of careless disposal of waste materials, which can be conveyed to seas and oceans through both direct and indirect means (Lozano and Mouat, 2009; Ryan et al., 2009). Plastic waste originating from land-based sources accounts for approximately 80% of the plastics detected in marine litter (Cole et al., 2011; Andrady, 2011). In its majority the plastics enter through river systems, whether through direct disposal, wastewater effluent, or leachates from refuse sites, ultimately finding their way into the sea (Cole et al., 2011). Several studies have demonstrated that the strong, one-way flow of freshwater systems propels the movement of plastic debris into the oceans (Browne et al., 2010; Moore et al., 2002). This flow can be further intensified by extreme weather events like flash floods or hurricanes (Chen et al., 2022). Before arriving in the sea, microplastics can make their way to sediments in terrestrial environments, originating from on-site plastic disposal and fragmentation, emissions from off-site sources, or even through airborne pathways (Huang et al., 2020; Cole et al., 2011), such as mulching film in agriculture that is discarded and deposited locally, entering the sediment (Hou et al., 2021). Additionally, microplastics can be transported before their final deposition as demonstrated by previous studies (Nizzetto et al., 2016) that substantial quantities of microplastics have been collected from sewage sludge and wastewater during treatment processes and when used in agriculture (Nizzetto et al., 2016; Cole et al., 2011).

The primary vehicle for the movement of land-based plastics to freshwater and marine environments is the flow of rivers (Mai et al., 2020; Wang et al., 2019). Microplastics originating from activities in rivers, coastal regions, and the open sea, as well as airborne microplastics, can gradually settle through the water column and accumulate in aquatic sediment (Chen et al., 2022; Zhang et al., 2019). The settling rates can vary from a few millimetres per second to hundreds of millimetres per second (Khatmullina and Isachenko, 2017; Kowalski et al., 2016). Consequently, the time it takes for microplastics to settle in offshore seabed generally falls within an annual timeframe (Cole et al., 2011; Chen et al, 2022).

Considering all the types of pollution and transport, sediments are regarded as a reservoir for microplastics (Hidalgo-Ruz et al., 2012; Van Cauwenberghe et al., 2015) and the abundance, type and colour of the microplastics found in the sedimentary records change over time, mirroring the variations in plastic production, types and colours (Chen et al., 2022). Nonetheless, it is important to understand that microplastics display unique transport characteristics when compared to sediments. Namely, they do remain suspended in water for longer durations than the sediments that could contain them (Hale et al., 2020). Despite their small size, microplastics represent a potential hazard as a man-made pollutant that is challenging to mitigate, particularly in intricate environments like sediments (Padervand et al., 2020).

Since sedimentary systems are commonly recognized as the primary storage location for misplaced microplastics (Chen et al., 2022), it becomes paramount to gain a comprehensive understanding of their rates of sequestration, environmental degradation, and potential for re-entry into the environment (Martin et al., 2021). Such insights are essential for devising strategies to combat plastic pollution challenges (Rochman and Hoellein, 2020). Consequently, the level of confidence in the current knowledge regarding microplastics in natural sediment archives remains uncertain (Martin et al., 2021; Chen et al., 2022).

1.4 Mondego River Estuary

Estuaries are considered one of the most valuable aquatic ecosystems (Li et al., 2020), offering a diverse range of benefits and services, including the provision of sustenance, coastal protection, and the creation of habitats for a rich array of species, including seabirds, fish, and mammals (Teixeira, 2016). Notably, estuaries play a crucial role as essential nursery grounds for fish (Bessa et al., 2018).

The Mondego estuary, situated in the central part of Portugal, Europe, is a small, warm temperate, polyhaline intertidal system (Teixeira, 2016). This estuarine system comprises two distinct arms: the northern arm, characterised by depths ranging from 4 to 8 meters during high tide, and the southern arm, where depths range from 2 to 4 meters (Lillebø et al. 2007; Teixeira, 2016). Water flows into the estuary from multiple sources, including the Mondego River, which enters through the Remolha water body, as well as tributaries that merge with the estuary's north and south banks (Teixeira, 2016). Over the years, the Mondego estuary has faced environmental challenges due to eutrophication processes, as documented in various studies (Teixeira, 2016; Baeta et al. 2011). In the 1990s, macroalgae known as Ulva spp. opportunistically replaced the seagrass *Zostera noltii* in the southern arm, prompting concerns about eutrophication in this area (Teixeira, 2016; Lillebø et al. 2005). Ulva remains present in the estuary throughout the year, with its growth season typically commencing in late winter and reaching peak biomass in spring, occasionally experiencing a smaller biomass peak in autumn (Teixeira, 2016).

To address these environmental challenges, several mitigation measures were introduced in 1998. These measures included the expansion of the upstream connection between the two estuary arms to enhance the hydraulic regime and reduce water residence time (Teixeira, 2016). Additionally, efforts were made to decrease freshwater input from the Pranto River by restricting the openings of its sluice and diverting Pranto freshwater to the northern arm using another upstream sluice (Cruzeiro et al., 2016; Teixeira, 2016). Before these mitigation measures were implemented, the circulation of water in the southern arm was primarily influenced by tides and freshwater discharges from the Pranto River (Teixeira, 2016). This small tributary's flow was controlled by a sluice, and it served as a significant source of both organic and inorganic matter to the estuary, largely due to the use of fertilizers in the agricultural land upstream (Teixeira, 2016). Prior investigations into the eutrophication of the Mondego estuary subsequent to the interventions in 1998 have indicated that the nutrient balance and condition of this coastal system are influenced by both biogeochemical mineralization processes (Otero et al. 2013; Teixeira, 2016) and external sources, which may be localized within the southern arm or stem from the northern arm of the Mondego River (Lillebø et al. 2005). Therefore, a comprehensive evaluation of the new eutrophic conditions following the interventions should account for the contributions of riverine nutrient loads (Teixeira, 2016). However, to our knowledge, comprehensive data collection at the mouth of all tributaries of the Mondego estuary has not been conducted thus far. River input data has relied on national databases, but the intermittent nature of the service and the limited number of monitoring stations with pertinent data often hinder the utilization of such environmental information.

Despite the interventions implemented in the Mondego estuary, the system remains impacted by the release of nutrients from agricultural practices, urban drainage, and domestic and industrial wastewater (Cruzeiro et al., 2016). The Mondego estuary receives runoff from agricultural areas covering 15,000 hectares of upstream cropland, predominantly rice and corn fields (Santos & Freitas, 2012). Agriculture represents a primary source of diffuse pollution in the Mondego, and it also contributes to point source pollution through the operation of two sluices, which are regulated based on the water requirements of farmers (Teixeira, 2016; Cruzeiro et al., 2016). Urban and industrial areas account for 3% of the Mondego River basin, with the municipalities of Coimbra and Figueira da Foz being among the most densely populated (Cruzeiro et al., 2016). These areas have expanded along the riverbanks and play a significant role in the dynamics of the Mondego River. They contribute to urban and industrial runoff, reduce soil permeability, lead to increased runoff, and promote diffuse pollution into surface waters. Moreover, they serve as point sources for domestic and industrial wastewater (Teixeira, 2016).

1.5. Objectives of the study

The main objective of this study is to explore the use of microplastics as potential markers in the sedimentary archives, aiming to reconstruct the history of plastic pollution in estuarine conditions and assessing their usage as Anthropogenic markers. The Mondego estuary was used as a case study. Therefore, the objectives of this thesis are:

1. Assessing the presence of microplastics in sediments and characterising them (type, colour, size and polymer) in different depth conditions;

2. Determining how microplastics can vary along time (using sediment layers as dated systems);

3. Assessing if there is a correlation between the levels of particles and the historical environmental changes and human activities described within the Mondego River estuary in the last years.

References of the General Introduction

Amaral-Zettler, L. A., Zettler, E. R., & Mincer, T. J. (2020). Ecology of the plastisphere. *Nature Reviews Microbiology*, *18*(3), 139-151.

Andrady, A. L. (2011). Microplastics in the marine environment. *Marine pollution bulletin*, *62*(8), 1596-1605.

Andrady, A. L. (2017). The plastic in microplastics: A review. *Marine pollution bulletin*, *119*(1), 12-22.

Arthur, C., Baker, J. E., & Bamford, H. A. (2009). Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris, September 9-11, 2008, University of Washington Tacoma, Tacoma, WA, USA.

Baeta, A., Niquil, N., Marques, J. C., & Patrício, J. (2011). Modelling the effects of eutrophication, mitigation measures and an extreme flood event on estuarine benthic food webs. *Ecological Modelling*, *222*(6), 1209-1221.

Bajt, O. (2021). From plastics to microplastics and organisms. *FEBS Open bio*, *11*(4), 954-966.

Bancone, C. E., Turner, S. D., Ivar do Sul, J. A., & Rose, N. L. (2020). The paleoecology of microplastic contamination. *Frontiers in Environmental Science*, *8*, 574008.

Bessa, F., Barría, P., Neto, J. M., Frias, J. P., Otero, V., Sobral, P., & Marques, J. C. (2018). Occurrence of microplastics in commercial fish from a natural estuarine environment. *Marine pollution bulletin*, *128*, 575-584.

Boucher, J., & Friot, D. (2017). *Primary microplastics in the oceans: a global evaluation of sources* (Vol. 10). Gland, Switzerland: Iucn.

Browne, M. A., Galloway, T. S., & Thompson, R. C. (2010). Spatial patterns of plastic debris along estuarine shorelines. *Environmental science & technology*, *44*(9), 3404-3409.

Chen, H., Zou, X., Ding, Y., Wang, Y., Fu, G., & Yuan, F. (2022). Are microplastics the ‘technofossils’ of the Anthropocene?. *Anthropocene Coasts*, *5*(1), 8.

Cole, M., Lindeque, P., Halsband, C., & Galloway, T. S. (2011). Microplastics as contaminants in the marine environment: a review. *Marine pollution bulletin*, *62*(12), 2588-2597.

Crutzen, P. J. (2006). The “anthropocene”. In *Earth system science in the anthropocene* (pp. 13-18). Berlin, Heidelberg: Springer Berlin Heidelberg

Crutzen, P. J. (2016). Geology of mankind. *Paul J. Crutzen: A pioneer on atmospheric chemistry and climate change in the Anthropocene*, 211-215.

Cruzeiro, C., Rocha, E., Pardal, M. Â., & Rocha, M. J. (2016). Environmental assessment of pesticides in the Mondego River Estuary (Portugal). *Marine Pollution Bulletin*, *103*(1-2), 240-246.

Derraik, J. G. (2002). The pollution of the marine environment by plastic debris: a review. *Marine pollution bulletin*, *44*(9), 842-852.

Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. *Science advances*, *3*(7), e1700782.

Hale, R. C., Seeley, M. E., La Guardia, M. J., Mai, L., & Zeng, E. Y. (2020). A global perspective on microplastics. *Journal of Geophysical Research: Oceans*, *125*(1), e2018JC014719.

Hidalgo-Ruz, V., Gutow, L., Thompson, R. C., & Thiel, M. (2012). Microplastics in the marine environment: a review of the methods used for identification and quantification. *Environmental science & technology*, *46*(6), 3060-3075.

Hou, L., Kumar, D., Yoo, C. G., Gitsov, I., & Majumder, E. L. W. (2021). Conversion and removal strategies for microplastics in wastewater treatment plants and landfills. *Chemical Engineering Journal*, *406*, 126715.

Huang, Y., Liu, Q., Jia, W., Yan, C., & Wang, J. (2020). Agricultural plastic mulching as a source of microplastics in the terrestrial environment. *Environmental Pollution*, *260*, 114096.

Lewis, S. L., & Maslin, M. A. (2015). Defining the anthropocene. *Nature*, *519*(7542), 171-180.

Li, J., Huang, W., Xu, Y., Jin, A., Zhang, D., & Zhang, C. (2020). Microplastics in sediment cores as indicators of temporal trends in microplastic pollution in Andong salt marsh, Hangzhou Bay, China. *Regional Studies in Marine Science*, *35*, 101149.

Lillebø, A. I., Neto, J. M., Martins, I., Verdelhos, T., Leston, S., Cardoso, P. G., ... & Pardal, M. A. (2005). Management of a shallow temperate estuary to control eutrophication: the effect of hydrodynamics on the system's nutrient loading. *Estuarine, coastal and shelf science*, *65*(4), 697-707.

Lopez Lozano, R., & Mouat, J. (2009). Marine litter in the Northeast Atlantic Region: assessment and priorities for response.

Ivar do Sul, J. A., & Labrenz, M. (2020). Microplastics into the anthropocene: rise and fall of the human footprint. *Handbook of Microplastics in the Environment*, 1-16.

Khatmullina, L., & Isachenko, I. (2017). Settling velocity of microplastic particles of regular shapes. *Marine pollution bulletin*, *114*(2), 871-880.

Kowalski, N., Reichardt, A. M., & Waniek, J. J. (2016). Sinking rates of microplastics and potential implications of their alteration by physical, biological, and chemical factors. *Marine pollution bulletin*, *109*(1), 310-319.

Mai, L., He, H., Bao, L. J., Liu, L. Y., & Zeng, E. Y. (2020). Plastics are an insignificant carrier of riverine organic pollutants to the coastal oceans. *Environmental Science & Technology*, *54*(24), 15852-15860.

Martin, J., Lusher, A. L., & Nixon, F. C. (2022). A review of the use of microplastics in reconstructing dated sedimentary archives. *Science of the Total Environment*, *806*, 150818.

McCarthy, F. M., Patterson, R. T., Head, M. J., Riddick, N. L., Cumming, B. F., Hamilton, P. B., ... & McAndrews, J. H. (2023). The varved succession of Crawford Lake, Milton, Ontario, Canada as a candidate Global boundary Stratotype Section and Point for the Anthropocene series. *The Anthropocene Review*, *10*(1), 146-176.

Moore, C. J., Moore, S. L., Weisberg, S. B., Lattin, G. L., & Zellers, A. F. (2002). A comparison of neustonic plastic and zooplankton abundance in southern California's coastal waters. *Marine pollution bulletin*, *44*(10), 1035-1038.

Napper, I. E., Bakir, A., Rowland, S. J., & Thompson, R. C. (2015). Characterisation, quantity and sorptive properties of microplastics extracted from cosmetics. *Marine pollution bulletin*, *99*(1-2), 178-185.

Nizzetto, L., Futter, M., & Langaas, S. (2016). Are agricultural soils dumps for microplastics of urban origin?.

Otero, M., Coelho, J. P., Rodrigues, E. T., Pardal, M. A., Santos, E. B. H., Esteves, V. I., & Lillebø, A. I. (2013). Kinetics of the PO4-P adsorption onto soils and sediments from the Mondego estuary (Portugal). *Marine pollution bulletin*, *77*(1-2), 361-366.

Padervand, M., Lichtfouse, E., Robert, D., & Wang, C. (2020). Removal of microplastics from the environment. A review. *Environmental Chemistry Letters*, *18*, 807-828.

PlasticsEurope. (2018). Plastics – The Facts 2018: An Analysis of European Plastics Production, Demand and Waste Data.

PlasticsEurope. (2022). Plastics – The Facts 2022: An Analysis of European Plastics Production, Demand and Waste Data.

Rochman, C. M., & Hoellein, T. (2020). The global odyssey of plastic pollution. *Science*, *368*(6496), 1184-1185.

Ryan, P. G., Moore, C. J., Van Franeker, J. A., & Moloney, C. L. (2009). Monitoring the abundance of plastic debris in the marine environment. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1526), 1999-2012.

dos SANTOS, J. F., & FREITAS, V. (2012). Aproveitamento hidroagrícola do Baixo Mondego actualidade e desafios futuros. In *IV Congresso nacional de rega e drenagem. Direcção-Geral de Agricultura e Desenvolvimento Rural (DGADR)* (p. 14).

Snowball, I., Hounslow, M. W., & Nilsson, A. (2014). Geomagnetic and mineral magnetic characterization of the Anthropocene. *Geological Society, London, Special Publications*, *395*(1), 119-141.

Teixeira, Z. F. G. (2016). *Resilience to land use induced impacts* (Doctoral dissertation).

Thompson, R. C., & Napper, I. E. (2019). Microplastics in the Environment. In *Plastics and the Environment* (pp. 60-81). The Royal Society of Chemistry.

Van Cauwenberghe, L., Devriese, L., Galgani, F., Robbens, J., & Janssen, C. R. (2015). Microplastics in sediments: a review of techniques, occurrence and effects. *Marine environmental research*, *111*, 5-17.

Yin, Z. (2023). The pollution of microplastics in sediments: The ecological risk assessment and pollution source analysis. *Science of The Total Environment*, *859*, 160323.

Wang, T., Li, B., Zou, X., Wang, Y., Li, Y., Xu, Y., ... & Yu, W. (2019). Emission of primary microplastics in mainland China: invisible but not negligible. *Water research*, *162*, 214-224.

Willis, K. A., Eriksen, R., Wilcox, C., & Hardesty, B. D. (2017). Microplastic distribution at different sediment depths in an urban estuary. *Frontiers in Marine Science*, *4*, 419.

Xiaodong, Z., Zhifei, L., Yanwei, Z., & Yulong, Z. (2019). Research progress on source-to-sink transport processes of marine microplastics. *Advances in Earth Science*, *34*(9), 936.

Zhou, C., Bi, R., Su, C., Liu, W., & Wang, T. (2022). The emerging issue of microplastics in marine environment: A bibliometric analysis from 2004 to 2020. *Marine Pollution Bulletin*, *179*, 113712.

THE USE OF MICROPLASTICS TO RECONSTRUCT DATED SEDIMENTARY ARCHIVES:

THE SHOWCASE OF MONDEGO ESTUARY (PORTUGAL)

Beatriz Rodrigues[1*], Filipa Bessa[2],

[1]Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139, Faro, Portugal.

[2] University of Coimbra, MARE – Marine and Environmental Sciences Centre/ARNET – Aquatic Research Network, Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

*Correspondence: beatrizimrod@gmail.com

Keywords: Microplastics, Anthropocene, "Tecno-fossils", Sediments, Mondego estuary, Markers.

Abstract

Microplastics (plastics < 5 mm), have become a defining feature of the Anthropocene epoch, representing humanity's profound impact on the planet. Envisioned as "techno-fossils" they provide detailed records of deposition history with dating resolutions from years to decades. This study used the Mondego estuary as a case study to test whether microplastics can be used as a marker of the Anthropocene. Sediment corers were used to collect 50cm replicates longitudinally. In total, 885 particles were extracted using a saturated NaCl (1.2. $g.cm^{-3}$) solution and characterised according to type, colour and shape. The polymer of 14 particles were ascertained using FTIR analysis. The sediments retrieved, dated approximately from 1947 to 2019, presented potential microplastics in all layers. The abundance of these particles fluctuated over the years, without a clear pattern, even though, the highest concentration of potential microplastics corresponded to the sediment layer (37) of 1973, where the first Polyethylene terephthalate beverage bottles were introduced and produced in mass. This study also supported the trend of high abundances of fibres, with a total value of 93% of total samples, which is often the most predominant type of microplastics found in aquatic environments and can be linked to point sources in transitional ecosystems and estuaries, such as WWTPs. This is a preliminary study testing the way microplastics tend to accumulate in sediments and how this data can be integrated. During this present work, it is clear that the main amount of potential microplastics present is fibres (93% of the total sample), followed by fragments (6%), and then by the film (1%). In these particles, the most predominant colour is blue, with a total of 397 potential microplastics (45%) in a sample of 885 potential microplastics.

1. Introduction

Plastics, a relatively recent addition to the geological timeline, have emerged as a defining feature of the Anthropocene epoch. Over a short period, hundreds of millions of metric tons of plastics and plastic waste have been generated, making them a unique marker of human influence on the planet (Martin et al., 2021; Geyer et al., 2017). Microplastics are now being considered as potential proxies for dating stratigraphic sequences and as markers of the Anthropocene (Martin et al., 2021; Ivar and Labrenz, 2021). This involves creating detailed records of deposition history using microplastics as "techno-fossils," allowing for relative dating resolutions on the scale of years to decades (Ivar and Labrenz, 2021).

The Anthropocene, a recently recognized geological epoch, signifies the era in which human activities related to agriculture, industrialization, and urbanization have had a dominant impact on the Earth's systems (Crutzen, 2016). While the exact start date of the Anthropocene is still debated, it is often suggested to have commenced around 1900, coinciding with the widespread use of coal and the emergence of fly ash (Chen et al., 2022). Plastics, particularly microplastics, have the potential to serve as key markers for this epoch, assuming that they exhibit consistent transport behaviours and do not significantly degrade over time (Bancone et al., 2020; Martin et al., 2021).

Plastics were first synthesized in 1907 and industrialized in the 1930s, leading to exponential growth in production from 1.5 million tons in the 1950s to 390.7 million metric tons in 2021 (Plastics Europe, 2022; Zhou et al., 2022). These synthetic organic polymers are known for their strength, lightness, durability, and affordability, making them versatile for various applications (Drraik, 2002). However, these same characteristics contribute to plastic pollution, primarily from land-based sources (Martin et al., 2021; Zhou et al., 2022). Microplastics are plastic fragments measuring less than 5 mm (Arthur et al., 2009) and are categorized as primary or secondary based on their source. Primary microplastics are intentionally manufactured for specific uses, such as microbeads in personal care products or as building materials (Thompson & Napper, 2019). Secondary microplastics result from the fragmentation of larger plastics, often due to various environmental processes (e.g., UV-light exposure, weathering and mechanical abrasion (Thompson & Napper, 2019; Amaral-Zettler et al., 2020).

Both primary and secondary microplastics persist in aquatic ecosystems and can adsorb toxic compounds, posing risks to organisms through ingestion (Chen et al., 2022). These tiny particles are prevalent in marine environments and are associated with changes in feeding and reproductive behaviour in aquatic life (Bajt, 2021; Cole et al., 2011). Marine debris, including plastics, is a consequence of careless waste disposal and can enter oceans through various pathways (Lozano and Mouat, 2009). Approximately 80% of marine litter comes from land-based sources, primarily transported through rivers (Cole et al., 2011; Andrady, 2011). The flow of freshwater systems, particularly rivers, plays a crucial role in transporting plastics into the oceans, exacerbated by extreme weather events like floods or hurricanes (Chen et al., 2022). Additionally, microplastics can reach sediments in terrestrial environments through local disposal, fragmentation, airborne transport, and even sewage sludge (Huang et al., 2020; Cole et al., 2011). Sediments act as reservoirs for microplastics, and their abundance, types, and colours change over time, reflecting variations in plastic production (Chen et al., 2022). Microplastics have unique transport characteristics compared to sediments; they can remain suspended in water for longer durations (Hale et al., 2020). Understanding the sequestration rates, degradation processes, and potential re-entry of microplastics into the environment is crucial for addressing plastic pollution challenges (Martin et al., 2021; Rochman and Hoellein, 2020).

Estuaries are ecologically valuable systems, providing numerous benefits, including habitat creation, coastal protection, and serving as nursery grounds for various species (Li et al., 2020; Bessa et al., 2018). The Mondego estuary in Portugal is a warm temperate, polyhaline intertidal system with two distinct arms fed by the Mondego River and tributaries (Teixeira, 2016). Over the years, it has faced environmental challenges, including eutrophication due to factors such as the proliferation of Ulva spp. algae (Teixeira, 2016). Mitigation measures were implemented in the 1990s to address these challenges, including changes to hydraulic regimes and freshwater input (Cruzeiro et al., 2016). However, the estuary continues to be impacted by nutrient runoff from agriculture, urban areas, and industrial sources (Cruzeiro et al., 2016). Agriculture, particularly rice and corn fields, contributes significantly to diffuse and point source pollution in the Mondego estuary (Santos & Freitas, 2012). Urban and industrial expansion along the riverbanks further compounds the pollution issues thereby affecting water quality (Teixeira, 2016).

The primary objective of this study is to utilize microplastics as markers in sedimentary archives to reconstruct the history of plastic pollution in the Mondego River estuary and assess their potential as markers for the Anthropocene. Other objectives include, Assessing the presence and distribution of microplastics in the estuarine sediment; Conducting a temporal analysis of microplastics within the sediments to understand how their abundance and types have changed over time; Investigating correlations between microplastic presence, environmental changes, human activities, and pollution impacts within the Mondego River estuary.

2. Materials and Methods

2.1. Study area

The Mondego Estuary (Figure 1.1) is characterised as a warm-temperated, polyhaline and intertidal system being around 7 km long and having an approximate area of 1072 ha. It consists of two arms: the northern arm with depths around 4 to 8 metres at high tide, and the southern arm with depths around 2 to 4 metres. These two arms are separated by Murraceira island, being an alluvium formed island. The estuary is water fed by the Mondego River (Teixeira, 2016).

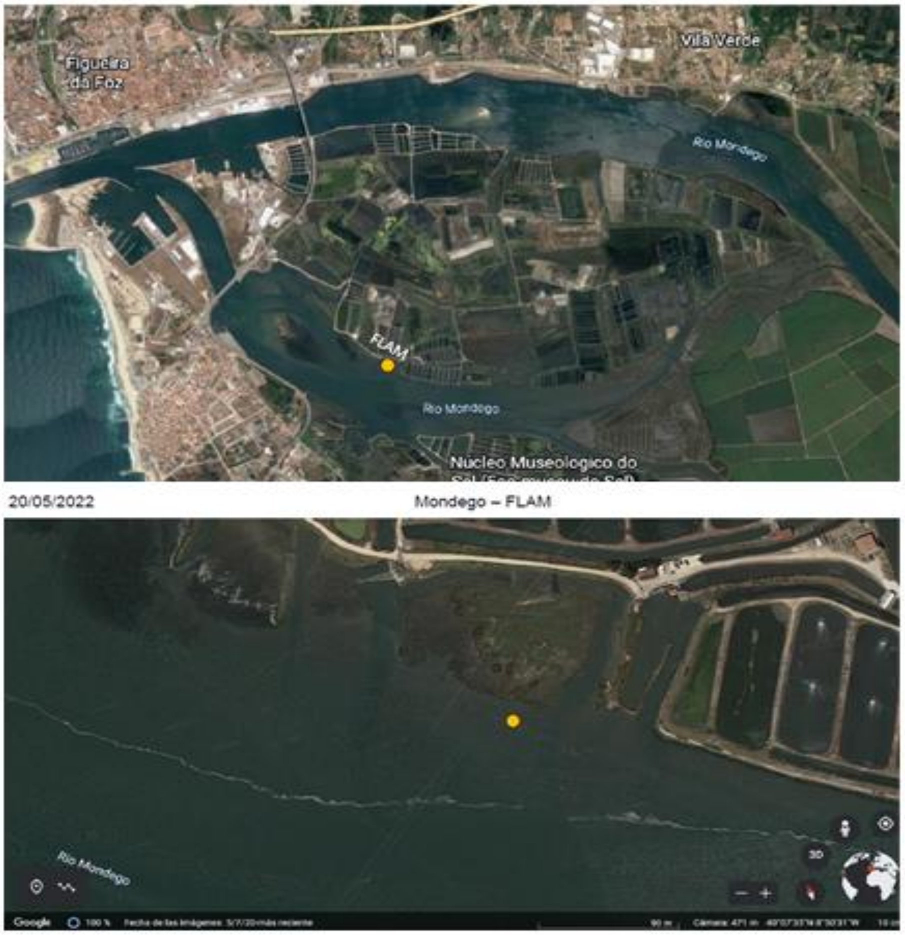

Figure 1.1- Mondego River Estuary, with the sampling site from where the sediment core was collected, in yellow.

The Mondego estuary has a long history of anthropogenic activities and faced environmental stress caused by the eutrophication process that has ecological effects on the system (Baeta et al. 2011, Dolbeth et al. 2003, Sousa et al. 2008). For example, it is documented that in the 1990s, in the upstream area of the estuary, the communication between the two arms was interrupted causing the south arm to be mainly dependent on tides, where, consequently, eutrophication with macroalgal blooms became a long term problem (Dolbeth et al. 2007). In 2006, an intervention occurred to reconnect the two arms, allowing an efficient flow of nutrients, reducing the water residence time and preventing pollution related problems in the estuary (Teixeira, 2016). For over 25 years, the Mondego estuary has been widely studied regarding sediment dynamics, biological communities and more (Veríssimo et al., 2012).

The Mondego River passes through agriculture fields and industrial areas that affect the concentration of the pesticides present in the estuary, represented by studies that show that the pesticide values present in the estuary are above the limits in the European Directives 98/83/EC and 2013/39/EU (Cruzeiro et al., 2016; Nunes et al., 2011). These high concentration components are then more prone to contaminate the ground and surface (Cruzeiro et al., 2016). Even with these results, the studies are only in part of the estuary not allowing extrapolation of the results to the general environment (Nunes et al., 2011; Cruzeiro et al., 2016). The pollution caused by persistent organic pollutants (POPs) such as, polychlorinated dibenzo-p-dioxins and furans (PCDD/ Fs) and polychlorinated biphenyls (PCBs) have been recognized in the estuary (Mandal, 2005). Nevertheless, there is insufficient information regarding plastic pollution and its potential accumulation in the systems, only a study of fish from the estuary is available (Bessa et al., 2018), revealing moderate levels of microplastics in resident species (Nunes et al., 2011).

2.2. Sample collection

In order to analyse the presence of potential microplastics in sediments from the Mondego estuary, an intertidal core was drilled in May 2022 from the inner estuary. A 50 cm long core with an 11.5 cm diameter was extracted from the inner estuary (Figure 1). The core was divided into 1 cm interval replicates (layers), which were stored and frozen for subsequent laboratory analyses. In parallel, another core was collected for geochemical studies (trace metals, TOC and TIC contents and carbon stable isotopes) and a third corer for radiometric and magnetism analyses respectively). These data are not the main focus of this work and are not included here (Gardoki et al., 2023).

However, several natural (Pb and Ra) and artificial (Cs) radiotracers data were used here to indicate the recent sedimentation processes. Moreover, sediment layer dating was performed by analysing the vertical distribution of these radionuclide activities. 210Pb, 226Ra, and 137Cs were determined by gamma spectrometry in samples from cores using an HPGe detector at the University of Cantabria, Spain. These data are presented in the results section.

2.3. Microplastic extraction and characterisation

The procedures for microplastic analyses used were mainly adapted on previous studies and existing protocols (Reeves et al. 2016, Bessa et al. 2018; Gardoki et al., 2023).

The sediment core was divided into segments of 1 cm each. The samples (48 sediment samples) were dried in the oven at 50ºC for 48 h. This temperature range was ideal to remove moisture content without melting the plastic material in the sediments. The samples were then weighed and sieved, with a different mesh size (100 and 500 µm respectively), to arrive at a suitable size range for the identification of microplastics. A chemical digestion was employed for each sediment layer using a Hydrogen Peroxide solution at 10%, being stirred for 5 min and left to settle for 48 h at room temperature. Digestion occurs when the solution is poured into each beaker and left to act for 48 hours. After sieving, density separation was done using a saturated NaCl (1,2. g.cm^{-3}) solution to extract potential microplastics from the sediments. The floating phase was extracted and filtered using a filtration system with the pump through glass microfibre filters with 47mm of diameter. The resulting filters were sealed in Petri dishes, avoiding contamination for further analyses under the stereomicroscope.

2.4. Contamination precautions

To evaluate airborne contamination, control filters known as blanks were employed. These were exposed to the air both during the sampling phase (contained within open glass jars on the boat deck, with one blank for each sampling campaign) and throughout the laboratory work (kept in Petri dishes, one per replicate). Any fibres detected in the samples that matched those in the respective blanks were excluded from the final results. To further minimize potential sources of contamination, glass, stainless-steel, and wooden materials were used during both fieldwork and laboratory procedures.

Inside the laboratory, a controlled and enclosed environment was maintained. All instruments used were made of glass and were meticulously cleaned beforehand. Throughout the procedures, the team wore cotton lab coats and nitrile gloves to prevent any external contamination. Before utilizing working surfaces, they were thoroughly rinsed with distilled water and ethanol. Additionally, it was ensured that all prepared solutions and rinsing liquids were properly filtered before application. In summary, stringent measures were in place to maintain a contamination-free environment. As an additional precaution, control filters were placed on the laboratory bench during all procedures. After the process, a thorough examination of these filters was conducted. Any fibres resembling those found in the control filters were identified and subsequently discarded from consideration in the analysis.

2.5. Observation and Identification of Microplastics

The observation and identification of the microplastics procedure occurred after the digestion of all segments of sediments, examining the filters, placed in the petri dishes.

All glass filters were examined under a stereomicroscope LEICA M80 (Leica Microsystems GmbH, Wetzlar, Germany) at the MAREFOZ laboratory. All the particles classified as potential microplastics were photographed using the image analysis system IC80 HD Camera with Leica Application Suite (LAS) software. Particles were classified and categorised by colour, type/shape and size. Type classification was based on their shape, fibres being elongated, films being thin and translucent and fragments being irregular and angular. Categorization, according to their size classes (≤0.5 mm, 0.5-1 mm, 1-2 mm, 2-3 mm, and > 3 mm), was made using the software *ImageJ* (Bessa et al. 2018).

2.6. Spectral analyses

A subset of 9 particles (1%) of the total particles, were randomly selected for polymer identification. These particles were analysed by Fourier Transform Infrared Spectroscopy in attenuated total reflectance (FTIR ATR), using a Perkin Elmer® Spectrum Two spectrometer. The analysis was performed on the sample surface, sometimes at more than one point, when results were dubious. A background scan was performed before any analysis series. Polymer identification relied on a match of over 80% (Pequeno et al., 2021) between the sample and a referenced database (Primpke et al., 2018). The assignments were confirmed with the analysis of the polymer’s characteristic bands (Brandon et al., 2019).

2.7. Statistical analysis

The analysis comprises counting all the microplastic particles in each segment of sediment to estimate their abundances in each sediment layer (total of 48 layers). The abundances were calculated according to the dry weight and area m², per segment.

A relation of the number of microplastics per dry weight, area and segments is represented through abundance and a positive correlation. Shape, colour frequency and size range are also represented.

3. Results

3.1. Sediment layers and the presence of potential microplastics

A total of 48 layers of depth sediments were collected from the Mondego estuary during the spring of 2022 (Table 3.1). Potential microplastics (e.g., particles extracted that are suspected of being of synthetic origin) were found in all sediment layers (100%), with a total of 885 potential microplastics present in the entire corer. According to the 48 segments an average of 18.438 potential microplastics per sediment was calculated, with a standard deviation of ±10.126. Also, with an average size of 93.662 mm (SD±404.432).

Table 3.1 – Percentage and Average of microplastics per sediment segments collected at the Mondego estuary.

% segments with potential microplastics	*100%*
average number of potential microplastics per segment	*18.438 (SD±10.126)*

When analysing the data of potential microplastics in the layers of the sediment core, relevant differences in the number of potential microplastics, in each segment, were found. In sediment layer 37, a distinctively big amount of potential microplastics were counted, with 56 potential microplastics, representing 6.33% of the total potential microplastics in the sample, with a value of 846.305 potential microplastics per kilogram of dried sediment (MP.DW sediment) and 2.240 potential microplastics per area m^2. This was followed by a total of 46 potential microplastics in sediment layer 10, with values of 515.926 potential microplastics per dry weight and 1.840 potential microplastics per m^2 Contrasting with these values, the sediment layers 29 and 32, represent the smallest amount of potential microplastics per layer, having 4 potential microplastics, representing each, 0.45% of the total potential microplastics sample.

Potential microplastics per area (m^2) (Figure 3.1) values are almost uniform compared with the values of potential microplastics per dry weight (1 Kg) (Figure 3.2), whereas in area there are significant changes through layers, with sediment layers 37 and 10 presenting a bigger amount of potential microplastics compared to other layers. This confirms the significant difference shown previously.

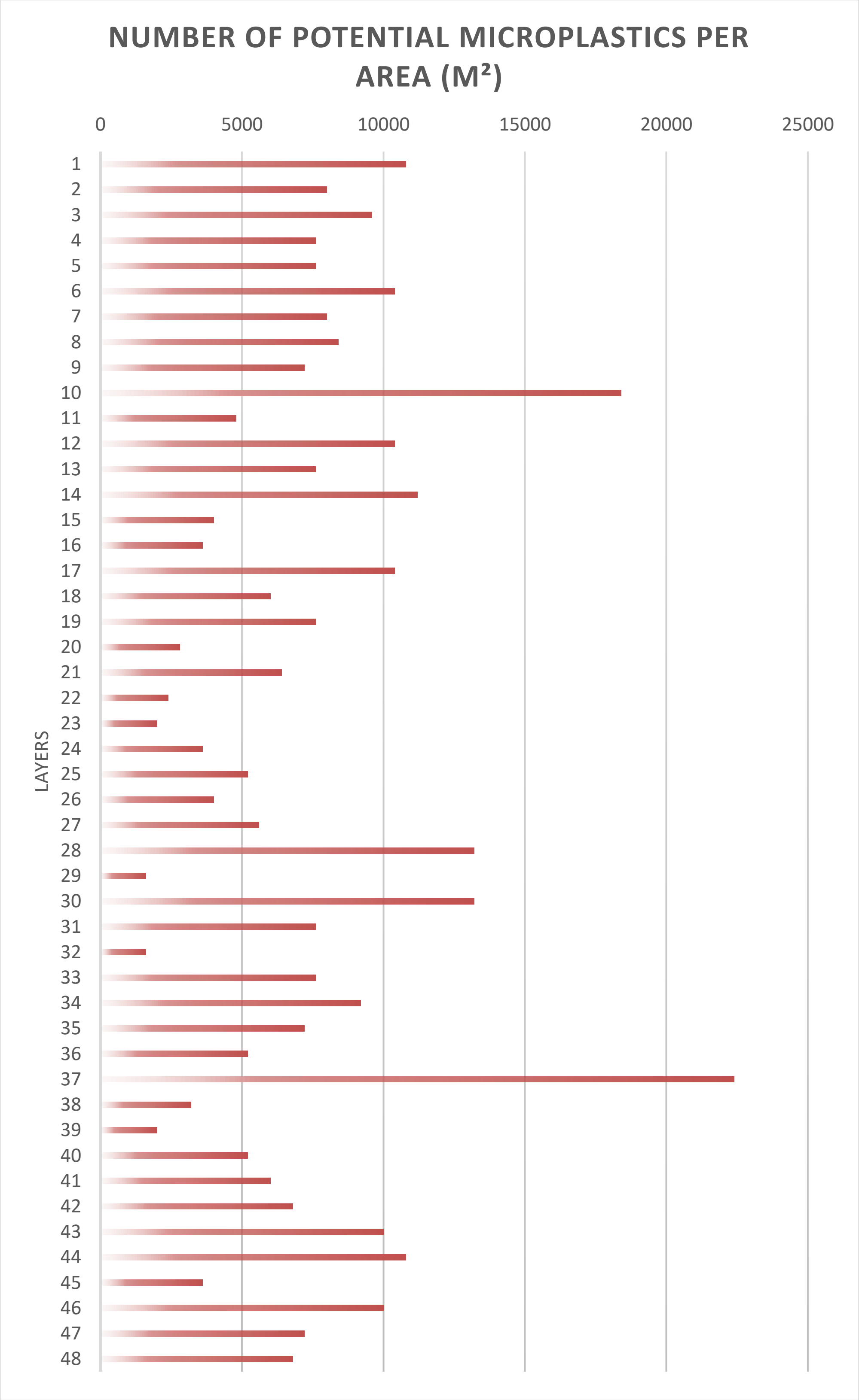

Figure 3.1 - Number of potential microplastics per area (m^2)

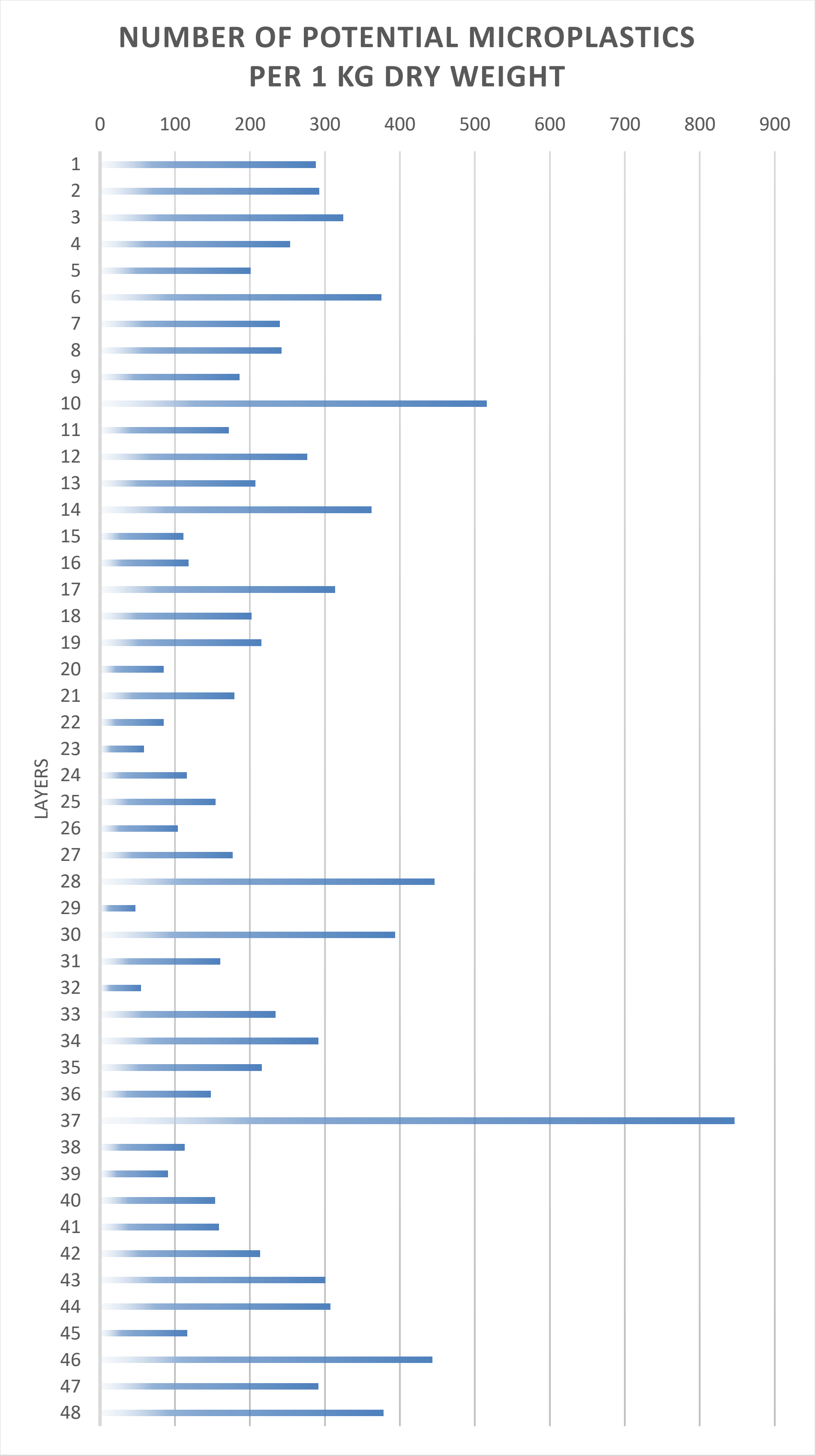

Figure 3.2- Number of potential microplastics per dry weight (1 Kg)

When performing a potential correlation between potential microplastics per dry weight and per area, it was possible to understand that there exists a positive correlation, with a value of 0.898 (Figure 3.3).

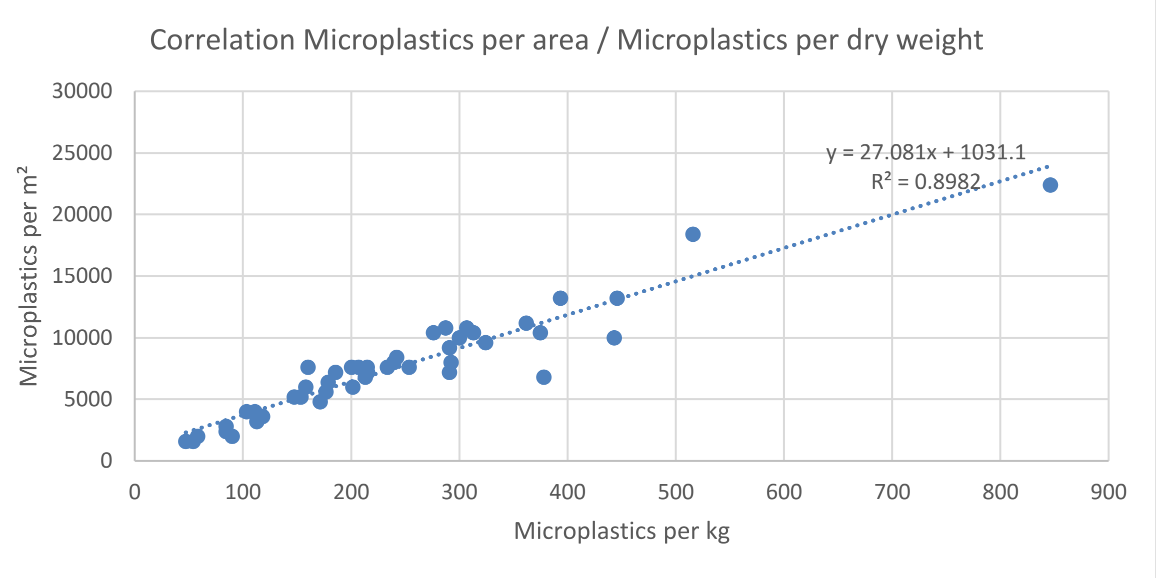

Figure 3.3- Graphic representation of the correlation between potential microplastics per area (m^2) and potential microplastics per dry weight (kg).

In this correlation, it is also considered the significance of the correlation. In this case, the significance of the correlation is based on the *p-value*, with a corresponding value of 0.05. In this instance, the significance of the correlation is positive, being algo significant < to 0.5.

3.2. Sediment layer dating

After analysis of the vertical distribution, it was determined the corresponding age, in years (Table 3.2), of each sediment layer in the core, represented in depth. This revealed that the core used in this study is at its oldest age the year 1947 and the most recent depth layer from the year 2019.

Table 3.2 – Representation of the depth of each sediment layer, of the core, corresponding to their age (in years)

Depth Layer (cm)	**Age (years)**
1	2019
2	2017
3	2015
4	2013
5	2011
6	2009
7	2007
8	2006
9	2004
10	2002
11	2001
12	1999
13	1998
14	1997
15	1996
16	1995
17	1994
18	1993
19	1992
20	1991
21	1991
22	1990
23	1990
24	1989

Depth Layer (cm)	**Age (years)**
25	1989
26	1989
27	1988
28	1987
29	1986
30	1985
31	1984
32	1982
33	1980
34	1980
35	1977
36	1975
37	1973
38	1970
39	1968
40	1966
41	1964
42	1962
43	1959
44	1957
45	1955
46	1951
47	1950
48	1947

3.3. Microplastics abundance and characterisation

A total of 885 potential microplastics recovered from the segments were characterised according to their colour, shape and size (Figure 3.4).

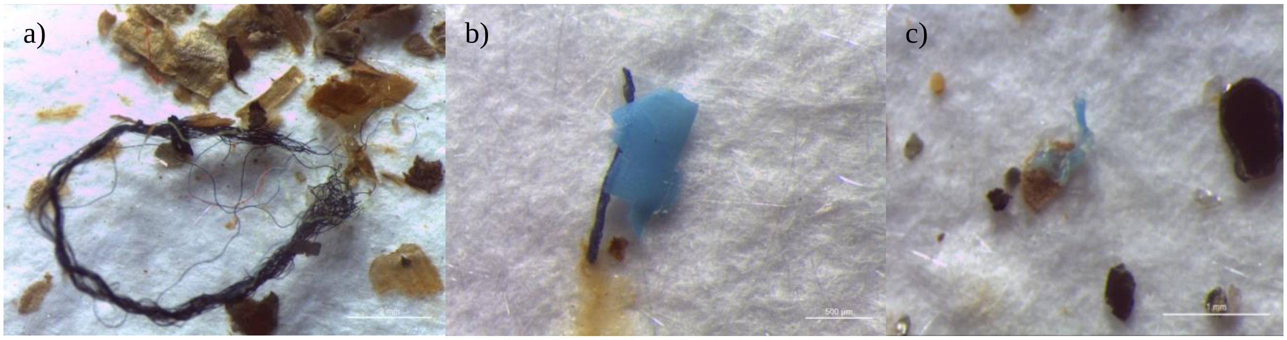

Figure 3.4 - Examples of different types of microplastics found in the sediment samples, from the most abundant to least: a): Fibres, b): Fragment and c): Film.

The particles were categorised as fibres (93%), fragments (6%) and films (1%) (Figure 3.5).

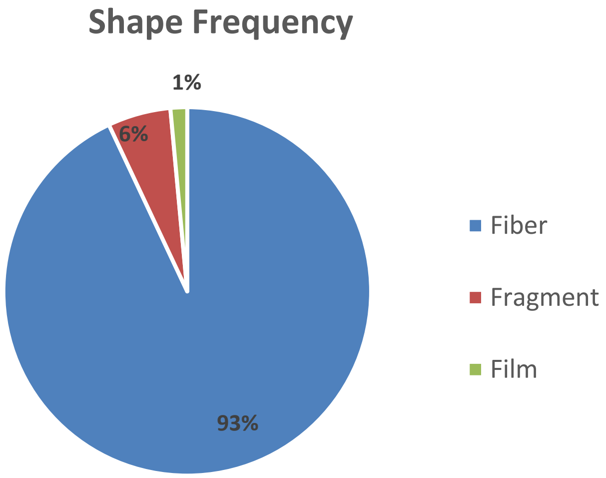

Figure 3.5 – Representation of the Shape Frequency (%) in the potential microplastics found in the sediment samples.

The colour distribution (Figure 3.6) of potential microplastics is very diverse, being blue particles (45%), followed by translucent (27%), red (9%) and black (7%), while other colours such as brown (5%), green (5%), yellow (1%), white (1%), orange and pink were less frequent.

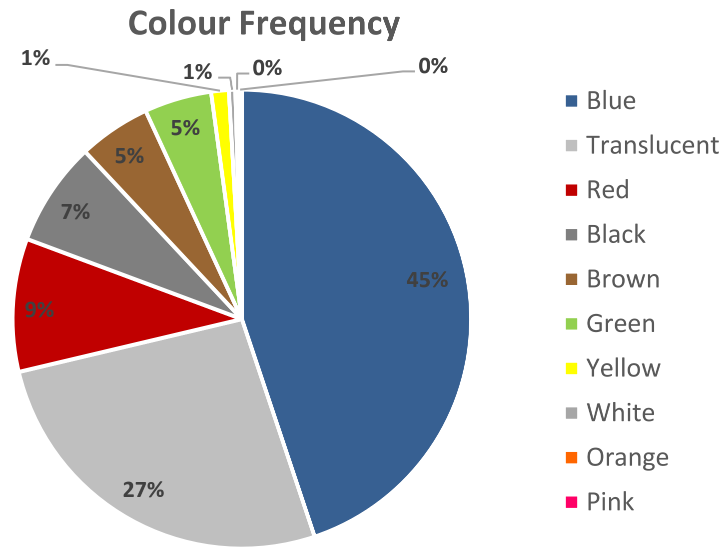

Figure 3.6– Representation of colour frequency (%) in the microplastic found in the sediment samples.

3.4. Analyses of potential microplastics present in the sediment layers.

A subsample of 14 particles (1.54% of the total sample) was analysed using FTIR, to confirm their synthetic origin (i.e., microplastics) and to determine their chemical composition (i.e., polymer type). The particles were randomly selected from all layers of sediments. Five different polymer types were identified in the samples, being polyethylene (64.3%), polypropylene (14.3%), polyester and polyester fibre (PET) (7.1%, each), and a natural linen fibre (7.1%). Examples of microplastics and natural fibre obtained are presented in Figure 3.7.

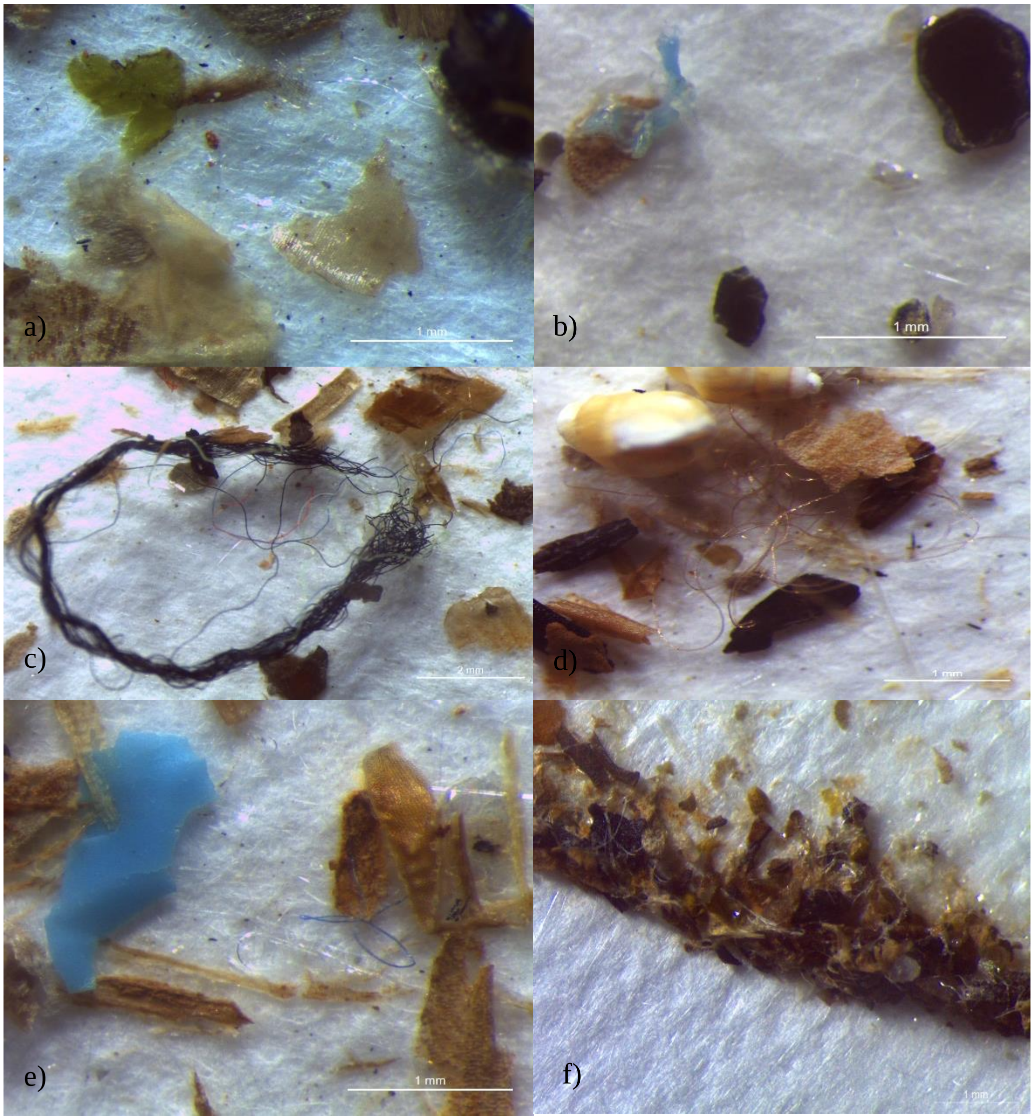

Figure 3.7 - Example of microplastics and natural fibre, , found in the sediment samples: a) and b) polyethylene, c) polyester, d) polyester fibre (PET), e) polypropylene and f) natural linen fibre.

In order, to confirm the synthetic or natural origin of the sampled microplastics, the spectrums obtained through FTIR need to have a match of above 80% between the sample and a referenced database. Examples of these spectrums were obtained in transmittance and are presented in Figure 3.8.

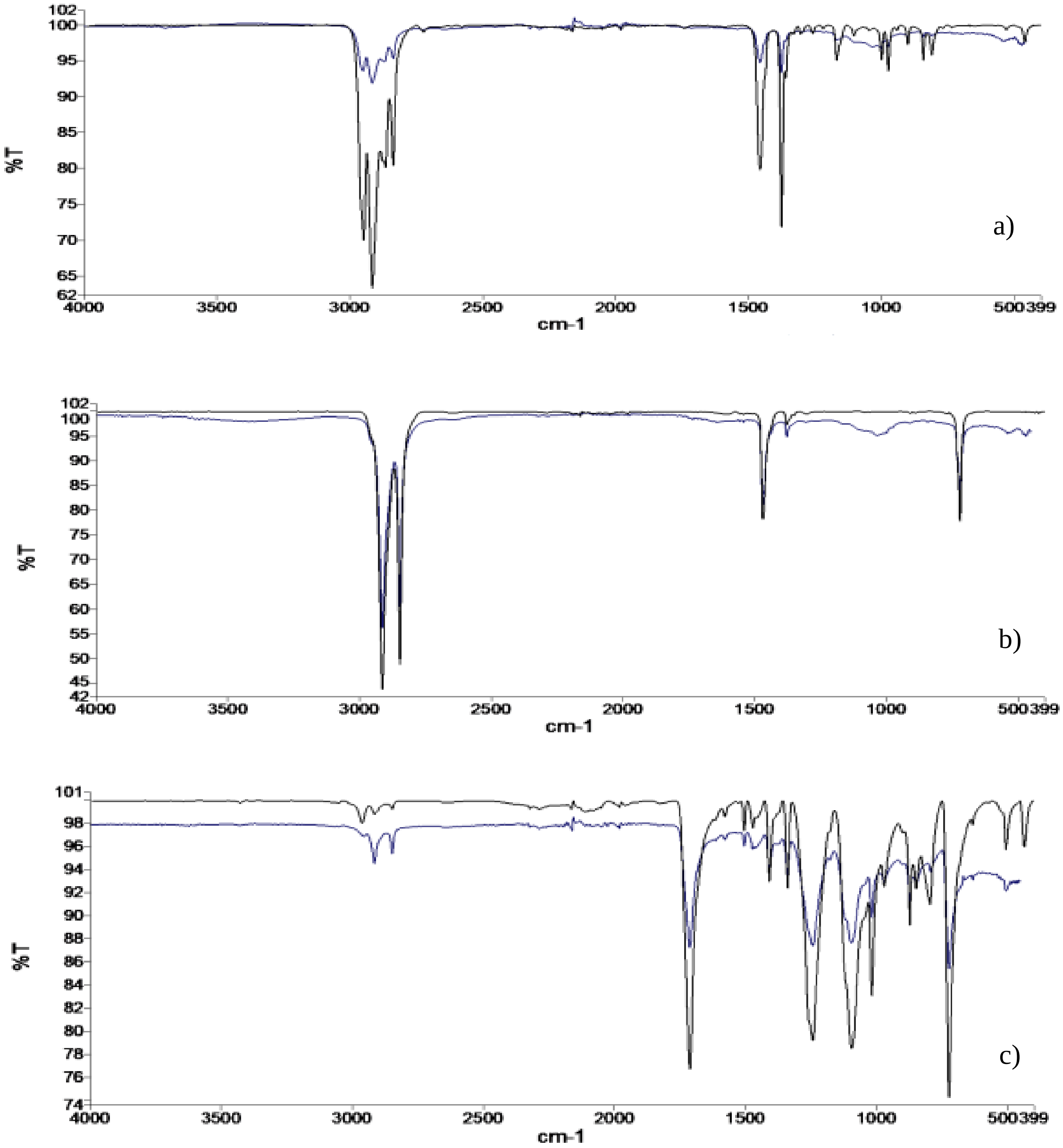

Figure 3.8 – Examples of spectrums, in transmittance, obtained from the particles in the study. With a) representing a spectrum for polypropylene, with a match of 96%; b) representing a spectrum of polyethylene, with a match of 99.6% and c) representing a spectrum of polyester fibre (PET), with a match of 97%.

4. Discussion

4. Main Findings

The main focus of this thesis was to assess if and how microplastics can be used as markers of the Anthropocene in the estuary of the Mondego River, considering sinking and presence in the different layers of the sediment core. According to the results obtained, these samples correspond to a core that ranges its age from 1945 to 2019. The results show that the pollution caused by potential microplastics is very widespread, with the presence of potential microplastics in all layers of the sediment core (100%). Additionally, the results show that the amount of potential microplastics oscillates over the years, with no clear pattern in the amount of microplastics pollution over the years. From the subsample of 14 particles (1.54% of the total) extracted and collected during the study, 92.9% of these particles were identified as synthetic particles, i.e., microplastics, more specifically polyethylene (64.3%) polypropylene (14.3%), polyester and polyester fibre (PET) (7.1%, each), and then, a natural linen fibre (7.1%).

4.1 Amount and characterisation of potential microplastics

In order to understand how microplastics affect the sedimentary layers and how they can be used as markers of the Anthropocene, it is important to account their significance to be considered. In this present study, it is clear that the main amount of potential microplastics present are fibres (93% of total sample), followed by fragments (6%) and then by film (1%). In these particles, the most predominant colour is blue, with a total of 397 potential microplastics (45%) in a sample of 885 potential microplastics. These results are similar to the ones obtained by Napper et al. (2015), that presented a significant high amount of blue microplastics, when extracted from facial products. One of the major problems represented by this type of colour in microplastics is their similarity to many types of plankton. These colours closely resemble the hues of various plankton species, which serve as a primary food source for surface-feeding fish, which are primarily visual predators (Wright et al., 2013).

This work shows that the pollution by microplastics is present in the sediment core of the estuary of the Mondego River. Despite the lack of studies addressing the presence and characterisation of microplastics in the sediments of the Mondego River estuary and other estuaries in Portugal, one of the few studies related to this topic (Bessa et al., 2018), reported the occurrence of microplastics in commercial fish from the Mondego estuary, in which the particles were also mainly in the form of fibres (96%). Comparing this study results with the study mentioned previously, it is observed that the percentage of fibres obtained in this study (93%) is similar to the percentage obtained in (Bessa et al., 2018). In addition, when compared with the results obtained by Napper et al. (2015) the values in this work show that the values obtained are the same, with the majority of microplastics being fibres (93%). According to Rodrigues et al. (2022) this distribution can be related to the high surface area to volume ratio, in contrast with the fragments and also, their slow sinking process which allows them, to travel further away from their potential source.

4.2. Microplastics as markers of the Anthropocene

Another objective of this work is to assess the possibility of the use of microplastics as Anthropocene markers. In this case, the sediment core sampled from the estuary from the Mondego River, corresponds to a core with a temporal time of years from 1947 to 2019 (Table 3.2), with each sediment layer representing an interval of time, the oldest sediment layer (48) corresponding to the year 1947 and, the most recent sediment layer (1) corresponding to the year 2019.

In order to understand how microplastics can act as potential Anthropogenic markers, it is important to understand that the role of microplastics, in this type of research, is highly dependent on their persistence and mobility through the sediments (Chen et al., 2022). This persistence correlates with their physical and chemical properties. The degradation of microplastics is one of the processes that influences the possibility of using microplastics as markers. This happens because it is necessary to consider the oxidation, ultraviolet radiation, weathering and other mechanic processes such as abrasion, turbulence and even wave action (Chen et al., 2022; Thompson & Napper, 2019; Amaral-Zettler et al., 2020). However, in

Chen et al. (2022) it is presented that the physical and chemical effects do not have as much effect when the microplastics are already inside the sediment layers. So, as in Gregory and Andrady (2003) the microplastics that are embedded within the sediment layers are, generally, regarded as well preserved in geological strata and are improbable to deteriorate or break down into smaller particles over time. Furthermore, microplastics have the capacity to endure for extended durations when exposed to anaerobic and anoxic conditions (Chen et al., 2022). However, there is a debate on the mobility of the microplastics, due to their appearance in sediment layers that correspond to years earlier when compared to the creation of those microplastics, reducing the possibility of using some particles as markers (Chen et al., 2022). This is still an open debate and further studies are needed to corroborate these findings.

In this study, the highest amount of potential microplastics in a sediment layer corresponds to layer 37, which corresponds to the year 1973. According to the British Plastics Federation (2014) at the beginning of the 1970s the First Yellow HDPE pressure pipes for gas were introduced and followed by that, in the year 1973, the Polyethylene terephthalate beverage bottles were introduced and then adopted for all types of usage, creating a higher demand of production. Continuously, in the years between 2002 and 2004, the demand for PET was 10% of the total demand for plastic products (Chen et al., 2022), which also corresponds to the second highest value of potential microplastics found in the sediment core analysed in this study, where in sediment layer 10 the presence of potential microplastics has a value of 46 potential microplastics. However, there is no understandable correlation with the lower values presented in the sediment core layers (29 and 32), in this study.

In Chen et al. (2022), one of the considerations in having microplastics as markers of the Anthropocene microplastics being called as potential “tecno-fossils”, meaning that due to the robust preservation of microplastics within the sedimentary layers and their mobility, once within the sediment being limited, these proprieties offer a viable mean to establish the stratigraphic age of the core layers. This approach may present as more convenient than alternative dating methods and eases comparisons of stratigraphy across diverse sedimentary environments, including lakes and oceans. However, Martin et al. (2022) hypotheses that even though there has been an increase in microplastics research area, only a few explain the presence of microplastics in the layered sediment, arguing inconsistent reporting hinders the ability to directly compare findings across studies. Furthermore, the sedimentary sequences exclusively represent the conditions prevailing at the time of coring, and the preservation of microplastic inputs into the environment may be incomplete in any given deposition layer. Challenges related to sediment movement and environmental disruptions are common when reconstructing depositional histories.

4.3. Microplastic in the Mondego

When assessing the number of potential microplastics, this study shows that the majority of potential microplastics found were fibres with a value of 823 (93%) out of the total value of 885 potential microplastics found in this core. The current study's findings of a substantial concentration of fibres in the waters and sediments of the Mondego estuary align with previous research indicating that fibres represent the predominant type of microplastics detected in aquatic settings, as noted in studies by Browne et al. (2011) and Gago et al. (2018). This trend holds true, not only on a global scale but also within estuarine ecosystems, as evidenced by studies conducted by Zhao et al. (2019), Lahens et al. (2018), Miller et al. (2017), and Gallagher et al. (2016). However, in a study conducted by Rodrigues et al. (2020) in the Sado estuary and the Arrábida coastal area (in Setúbal, Southern Portugal) it was found that the sampling station within the Sado estuary exhibited a representativity of approximately 3% for fibres and in comparison, the most abundant type of microplastics present were fragments (70%).

It is important to consider the hypothesis indicated in previous studies (Jabeen et al., 2017 and Bessa et al., 2018), that freshwater and transitional ecosystems may exhibit a higher susceptibility to fibre contamination compared to marine environments. This heightened vulnerability stems from their proximity to potential point sources of human-made fibres, such as wastewater treatment plants (WWTPs) (Teixeira, 2016). Also, Willis et al. (2017) suggest that the majority of microplastic fibres in marine habitats may have sewage as a source, originating from clothes washing.

However, when studying fibres, it is essential to acknowledge that animal and cellulosic fibres may be relatively underrepresented in the current body of environmental pollution research (Suaria et al., 2020). Even though cellulosic fibres should not be classified as synthetic, man-made cellulosic fibres can contain a range of chemicals, including synthetic dyes, additives, and flame retardants (Andrady, 2017). These chemical components have the potential to pose environmental risks, support distinct bacterial communities, and exhibit a slower rate of degradation when compared to natural-based particles (Bessa et al., 2018). While the primary origins and destinations of fibres in aquatic ecosystems are not fully elucidated, it is a common perception that WWTPs effluents often serve as substantial point sources of fibre emissions into aquatic environments. This holds true within the Mondego estuarine area, where two WWTPs are in operation. These facilities offer only secondary water treatment and lack the capacity to manage industrial wastewater, (Teixeira, 2016). This limitation could potentially make them significant contributors to the input of fibres into the Mondego riverine and estuarine waters. Furthermore, these fibres are most likely to have as a source the breakdown of lost and abandoned fishing equipment and recreational sailing gear (Bessa et al., 2018). This additional source underscores the intricate and multifaceted nature of fibre contamination within aquatic ecosystems. The presence of microplastics in the Mondego estuary is not only attributed to the mentioned sources but also to human activities within the Mondego River basin. This river basin encompasses urban and industrial zones, with two of its most densely populated cities, Coimbra and Figueira da Foz, expanding along the riverbanks (Teixeira, 2016). These urban and industrial areas may have a significant role in microplastic contamination since they reduce soil permeability, potentially leading to the runoff of urban and industrial waters containing microplastics into the river (Cruzeiro et al., 2016). Additionally, agricultural land covers approximately 32% of the river basin area (Teixeira, 2016), which could also contribute to the release of microplastics into the waters that ultimately flow into the riverine and estuarine environments (Teixeira, 2016; Bessa et al., 2018).

4.4 Considerations and future studies

The study presented sheds light on various aspects of microplastics and their behaviour in sedimentary environments, with an emphasis on the susceptibility of microplastic, mainly fibres and those in smaller size fractions to remobilization or diffusion downcore (Martin et al., 2022). While it appears that microplastics are accumulating in sediment globally over time, Yin (2023), Lee et al. (2023), and Martin et al. (2022) call for increased methodological rigor and further investigation before confidently establishing historical microplastic loading inventories, highlighting the diversity of microplastics, their sources, and modes of transport, which can question their effectiveness as markers. Therefore, it is important to emphasize the need for further research into the sedimentation processes of different microplastic morphologies (Yin, 2023; Martin et al., 2022) and stress the importance of understanding patterns and environmental factors influencing microplastic accumulation in sediments to develop effective measures for preventing plastic inputs into marine ecosystems (Rodriguez et al. 2022).

Considering the global dispersion and accumulation of microplastics, particularly in populated coastal and urban areas, with potentially negative consequences for marine and aquatic ecosystems, Chen et al. (2022) compare different types of microplastics to “technofossils”, suggesting that they could serve as markers of specific periods in sedimentary records due to their short migration time, good preservation, and limited mobility, giving depth to the argument of this thesis on using microplastics as markers of the Anthropocene. Even though it is important to consider that the fate of microplastics depends on their density (Andrady, 2017) it is important to emphasize the goal of reducing the quantity of larger litter items entering the environment (Thompson and Napper, 2019).

5. Conclusions

This research highlights the pervasive presence of microplastics, predominantly fibres, in the Mondego River estuary's sedimentary layers. One potential ecological implication which arises is the issue of blue being the most prevalent colour, due to its resemblance to plankton, a primary food source for certain fish. Notably, the sediment data aligns with significant events in the plastic industry, alluding to its effectiveness as Anthropocene markers.

However, debate surrounds the mobility of microplastics within sediments, challenging their chronological reliability. Continued research is imperative to refine our understanding of microplastics behaviour, distribution, and historical significance.

6. References of the Manuscript

Amaral-Zettler, L. A., Zettler, E. R., & Mincer, T. J. (2020). Ecology of the plastisphere. *Nature Reviews Microbiology, 18*(3), 139-151.

Andrady, A. L. (2011). Microplastics in the marine environment. *Marine pollution bulletin*, *62*(8), 1596-1605.

Arthur, C., Baker, J. E., & Bamford, H. A. (2009). Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris, September 9-11, 2008, University of Washington Tacoma, Tacoma, WA, USA.

Baeta, A., Niquil, N., Marques, J. C., & Patrício, J. (2011). Modelling the effects of eutrophication, mitigation measures and an extreme flood event on estuarine benthic food webs. *Ecological Modelling*, *222*(6), 1209-1221.

Bajt, O. (2021). From plastics to microplastics and organisms. *FEBS Open bio*, *11*(4), 954-966.

Bancone, C. E., Turner, S. D., Ivar do Sul, J. A., & Rose, N. L. (2020). The paleoecology of microplastic contamination. *Frontiers in Environmental Science*, *8*, 574008.

Bessa, F., Barría, P., Neto, J. M., Frias, J. P., Otero, V., Sobral, P., & Marques, J. C. (2018). Occurrence of microplastics in commercial fish from a natural estuarine environment. *Marine pollution bulletin*, *128*, 575-584.

Brandon, J. A., Jones, W., & Ohman, M. D. (2019). Multidecadal increase in plastic particles in coastal ocean sediments. *Science advances*, *5*(9), eaax0587.

Chen, H., Zou, X., Ding, Y., Wang, Y., Fu, G., & Yuan, F. (2022). Are microplastics the 'technofossils' of the Anthropocene?. *Anthropocene Coasts*, *5*(1), 8.

Cole, M., Lindeque, P., Halsband, C., & Galloway, T. S. (2011). Microplastics as contaminants in the marine environment: a review. *Marine pollution bulletin*, *62*(12), 2588-2597.

Crutzen, P. J. (2016). Geology of mankind. *Paul J. Crutzen: A pioneer on atmospheric chemistry and climate change in the Anthropocene*, 211-215.

Cruzeiro, C., Rocha, E., Pardal, M. Â., & Rocha, M. J. (2016). Environmental assessment of pesticides in the Mondego River Estuary (Portugal). *Marine Pollution Bulletin*, *103*(1-2), 240-246.

Dolbeth, M., Pardal, M. A., Lillebø, A. I., Azeiteiro, U., & Marques, J. C. (2003). Short-and long-term effects of eutrophication on the secondary production of an intertidal macrobenthic community. *Marine Biology*, *143*, 1229-1238.

Dolbeth, M., Cardoso, P. G., Ferreira, S. M., Verdelhos, T., Raffaelli, D., & Pardal, M. A. (2007). Anthropogenic and natural disturbance effects on a macrobenthic estuarine community over a 10-year period. *Marine Pollution Bulletin*, *54*(5), 576-585.

Drraik, J. G. (2002). The pollution of the marine environment by plastic debris: a review. *Marine pollution bulletin*, *44*(9), 842-852.

Gardoki, J., Cearreta, A., Irabien, M. J., Gómez-Arozamena, J., Villasante-Marcos, V., García-Artola, A., ... & Bessa, F. (2023). Modern conditions and recent environmental evolution of the industrialized inner Ría of Ferrol (Galicia, NW Spain). *Continental Shelf Research*, *267*, 105098.

Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. *Science advances*, *3*(7), e1700782.

Hale, R. C., Seeley, M. E., La Guardia, M. J., Mai, L., & Zeng, E. Y. (2020). A global perspective on microplastics. *Journal of Geophysical Research: Oceans*, *125*(1), e2018JC014719.

Huang, Y., Liu, Q., Jia, W., Yan, C., & Wang, J. (2020). Agricultural plastic mulching as a source of microplastics in the terrestrial environment. *Environmental Pollution*, *260*, 114096.

Li, J., Huang, W., Xu, Y., Jin, A., Zhang, D., & Zhang, C. (2020). Microplastics in sediment cores as indicators of temporal trends in microplastic pollution in Andong salt marsh, Hangzhou Bay, China. *Regional Studies in Marine Science*, *35*, 101149.

Lopez Lozano, R., & Mouat, J. (2009). Marine litter in the Northeast Atlantic Region: assessment and priorities for response.

Ivar do Sul, J. A., & Labrenz, M. (2020). Microplastics into the anthropocene: rise and fall of the human footprint. *Handbook of Microplastics in the Environment*, 1-16.

Mandal, P. K. (2005). Dioxin: a review of its environmental effects and its aryl hydrocarbon receptor biology. *Journal of Comparative Physiology B*, *175*, 221-230.

Martin, J., Lusher, A. L., & Nixon, F. C. (2022). A review of the use of microplastics in reconstructing dated sedimentary archives. *Science of the Total Environment*, *806*, 150818.

Nunes, M., Marchand, P., Vernisseau, A., Le Bizec, B., Ramos, F., & Pardal, M. A. (2011). PCDD/Fs and dioxin-like PCBs in sediment and biota from the Mondego estuary (Portugal). *Chemosphere*, *83*(10), 1345-1352.

Pequeno, J., Antunes, J., Dhimmer, V., Bessa, F., & Sobral, P. (2021). Microplastics in marine and estuarine species from the coast of Portugal. *Frontiers in Environmental Science*, 18.

PlasticsEurope. (2022). Plastics – The Facts 2022: An Analysis of European Plastics Production, Demand and Waste Data.

Primpke, S., Wirth, M., Lorenz, C., & Gerdts, G. (2018). Reference database design for the automated analysis of microplastic samples based on Fourier transform infrared (FTIR) spectroscopy. *Analytical and bioanalytical chemistry*, *410*, 5131-5141.

Reeves, S., Davey, A., and Ling, S. (2016). A Method for Extraction and Enumeration of Microplastics from Marine Sediments: Draft Report. Institute for Marine and Antarctic Studies; University of Tasmania

Rochman, C. M., & Hoellein, T. (2020). The global odyssey of plastic pollution. *Science*, *368*(6496), 1184-1185.

dos SANTOS, J. F., & FREITAS, V. (2012). Aproveitamento hidroagrícola do Baixo Mondego actualidade e desafios futuros. In *IV Congresso nacional de rega e drenagem. Direcção-Geral de Agricultura e Desenvolvimento Rural (DGADR)* (p. 14).

Sousa, A. I., Lillebø, A. I., Caçador, I., & Pardal, M. A. (2008). Contribution of Spartina maritima to the reduction of eutrophication in estuarine systems. *Environmental Pollution*, *156*(3), 628-635.

Teixeira, Z. F. G. (2016). *Resilience to land use induced impacts* (Doctoral dissertation).

Thompson, R. C., & Napper, I. E. (2019). Microplastics in the Environment. In *Plastics and the Environment* (pp. 60-81). The Royal Society of Chemistry.

Veríssimo, H., Bremner, J., Garcia, C., Patrício, J., van der Linden, P., & Marques, J. C. (2012). Assessment of the subtidal macrobenthic community functioning of a temperate estuary following environmental restoration. *Ecological Indicators*, *23*, 312-322.

Willis, K. A., Eriksen, R., Wilcox, C., & Hardesty, B. D. (2017). Microplastic distribution at different sediment depths in an urban estuary. *Frontiers in Marine Science*, *4*, 419.

Zhou, C., Bi, R., Su, C., Liu, W., & Wang, T. (2022). The emerging issue of microplastics in marine environment: A bibliometric analysis from 2004 to 2020. *Marine Pollution Bulletin*, *179*, 113712.

6. Annexes

Figure 6.1- Measurements and separation of the collected core before sampling

Table 6.1 – General matrix of results, from the sampled core. In pink, the sediment layers with the highest amount of potential microplastics and, in yellow the sediment layers with the lowest amount of potential microplastics

Layer	Nº plásticos	Massa seca (kg)	Plásticos p/ massa	Plásticos p/ área (1cm2)	Plásticos p/ área (1m^2)
1	27	0,09394	287,4175	1,080	10800,0
2	20	0,0684	292,3977	0,800	8000,0
3	24	0,07401	324,2805	0,960	9600,0
4	19	0,07493	253,5700	0,760	7600,0
5	19	0,09476	200,5065	0,760	7600,0
6	26	0,06937	374,8018	1,040	10400,0
7	20	0,08348	239,5783	0,800	8000,0
8	21	0,08675	242,0749	0,840	8400,0
9	18	0,09697	185,6244	0,720	7200,0
10	46	0,08916	515,9264	1,840	18400,0
11	12	0,06995	171,5511	0,480	4800,0
12	26	0,09417	276,0964	1,040	10400,0
13	19	0,0918	206,9717	0,760	7600,0
14	28	0,07737	361,8974	1,120	11200,0
15	10	0,09005	111,0494	0,400	4000,0
16	9	0,07621	118,0947	0,360	3600,0
17	26	0,08305	313,0644	1,040	10400,0
18	15	0,07437	201,6942	0,600	6000,0
19	19	0,08841	214,9078	0,760	7600,0
20	7	0,08289	84,4493	0,280	2800,0
21	16	0,08953	178,7110	0,640	6400,0
22	6	0,07106	84,4357	0,240	2400,0
23	5	0,08582	58,2615	0,200	2000,0
24	9	0,07781	115,6664	0,360	3600,0
25	13	0,08455	153,7552	0,520	5200,0
26	10	0,09672	103,3912	0,400	4000,0
27	14	0,07922	176,7230	0,560	5600,0
28	33	0,07403	445,7652	1,320	13200,0
29	4	0,08478	47,1809	0,160	1600,0
30	33	0,08385	393,5599	1,320	13200,0
31	19	0,11854	160,2834	0,760	7600,0
32	4	0,0739	54,1272	0,160	1600,0
33	19	0,0813	233,7023	0,760	7600,0
34	23	0,07909	290,8079	0,920	9200,0
35	18	0,08358	215,3625	0,720	7200,0

36	13	0,08808	147,5931	0,520	5200,0
37	56	0,06617	846,3050	2,240	22400,0
38	8	0,07088	112,8668	0,320	3200,0
39	5	0,0554	90,2527	0,200	2000,0
40	13	0,08496	153,0132	0,520	5200,0
41	15	0,09483	158,1778	0,600	6000,0
42	17	0,07976	213,1394	0,680	6800,0
43	25	0,08327	300,2282	1,000	10000,0
44	27	0,08794	307,0275	1,080	10800,0
45	9	0,07755	116,0542	0,360	3600,0
46	25	0,05639	443,3410	1,000	10000,0
47	18	0,06188	290,8856	0,720	7200,0
48	17	0,04497	378,0298	0,680	6800,0
Total	885	3,8759			
Média	18,4375	0,0807	234,263	0,738	
Desvio padrão	10,1261	0,0123	141,756	0,4050	

Table 6.2 – Number of particles by colour

Row Labels	**Count of COLOUR**
Blue	397
Translucent	234
Red	83
Black	65
Brown	45
Green	42
Yellow	11
White	4
Orange	2
Pink	2
Grand Total	**885**

Table 6.3 – Number of particles by shape

Row Labels	**Count of SHAPE**
Fibre	823
Fragment	49
Film	13
Grand Total	**885**

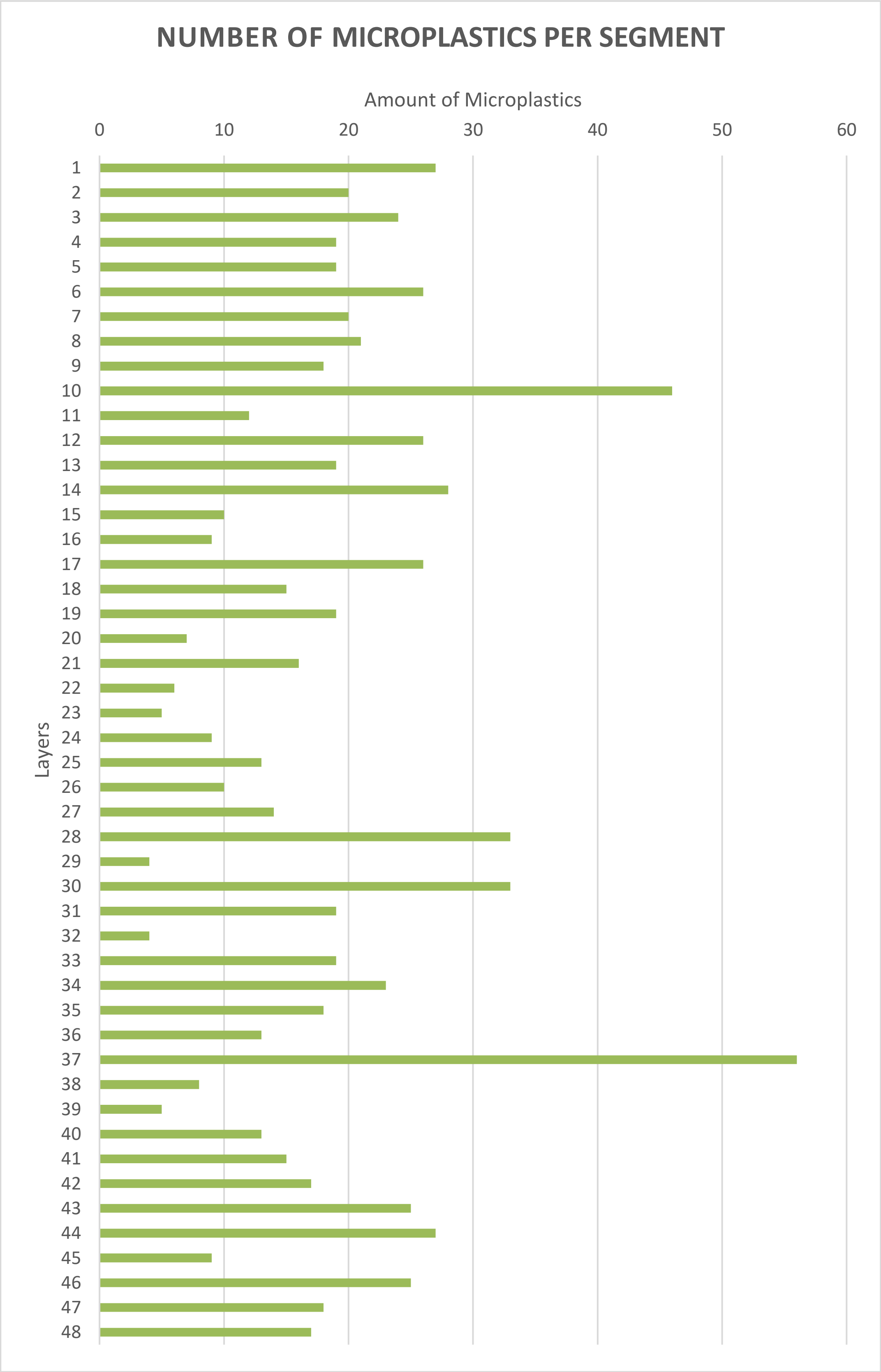

Figure 6.2 – Amount of potential microplastics per sediment layer