University of Minho
School of Sciences

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Master thesis in Geosciences: External Dynamics and Global Changes

Work made under supervision of:
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Thank you.

"*A maioria pensa com a sensibilidade, eu sinto com o pensamento. Para o homem vulgar, sentir é viver e pensar é saber viver. Para mim, pensar é viver e sentir não é mais que o alimento de pensar.*"

– Bernardo Soares (Fernando Pessoa)

Statement of Integrity

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration.

I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

Título: Estudo da influência da dinâmica fluvial na distribuição e transporte de microplásticos.

Resumo: A dependência global da água, juntamente com o crescimento exponencial da produção de plástico nas últimas décadas, deu origem a uma preocupação ambiental premente - a poluição da água por microplásticos. A pandemia de COVID-19 e as consequências das crises financeiras agravaram ainda mais esta questão, com o aumento do consumo de plástico a conduzir a uma maior poluição dos ecossistemas aquáticos. Embora os processos de fragmentação dos plásticos gerem microplásticos, partículas de polímero com menos de 5 mm, a extensão total dos seus efeitos em diversos organismos, nomeadamente o ser humano, continua a ser objeto de investigação. Esta dissertação procura compreender a relação entre a dinâmica fluvial, o transporte de microplásticos e a sua distribuição em ambientes aquáticos. Os rios, considerados como os principais agentes responsáveis pelo transporte de partículas para os oceanos e zonas costeiras, desempenham um papel fundamental neste fenómeno. O comportamento dos microplásticos nos sistemas fluviais é uma interação complexa de processos físicos, químicos e biológicos, que exige uma análise meticulosa. Fundamentalmente, esta investigação tem como objetivo dar um passo no sentido de promover uma compreensão mais abrangente e clara desta questão e das suas consequências. O estudo foi efetuado no rio Cávado, desde a Ponte do Porto até à foz, abrangendo diversos ambientes e condições ambientais. Foram selecionados 17 locais de amostragem estrategicamente marcados e foi amostrado o sedimento em cada um deles. As amostras foram submetidas a um processo meticuloso, incluindo a secagem numa estufa e a separação da densidade utilizando uma solução de $CaCl_2$. A amostra sobrenadante resultante foi então recolhida e submetida a uma inspeção visual ao microscópio. Esta análise rigorosa levou à identificação visual de um total de 571 microplásticos. Os locais MP012B, MP009, MP005 e MP003 apresentaram as contagens mais elevadas e o estudo revelou um aumento distinto da prevalência de microplásticos de montante para jusante. Estatisticamente, os valores exibiram normalidade e indicaram diferenças estatisticamente significativas entre os grupos. Como resultado, a hipótese de que a dinâmica fluvial influencia o comportamento dos microplásticos foi conclusivamente corroborada. O estudo também explorou potenciais correlações entre os fatores de pressão fluvial e a abundância de microplásticos em vários locais de amostragem, considerando a presença de urbanizações, ETARs, terrenos agrícolas, áreas industriais e outras infraestruturas. No entanto, é essencial reconhecer potenciais fontes de contaminação cruzada, tais como partículas transportadas pelo ar ou fibras de vestuário, bem como a ausência de digestão da matéria orgânica e subsequente filtragem ou peneiração, o que pode ter induzido erros humanos na identificação das partículas. Por conseguinte, recomenda-se uma reavaliação e re-execução dos procedimentos laboratoriais.

Palavras-chave: Microplásticos, Granulometria, Bacia Hidrográfica do Rio Cávado, Amostragem, Técnica de Extração, Efeitos, Transporte, Distribuição e Fatores de Pressão.

Title: Study of the influence of fluvial dynamics on the distribution and transport of microplastics.

Abstract: The global reliance on water, coupled with the exponential growth of plastic production over the past decades, has given rise to a pressing environmental concern — microplastics water pollution. The COVID-19 pandemic and the aftermath of financial crises have further exacerbated this issue, with increased plastic consumption leading to the heightened pollution of aquatic ecosystems. While the fragmentation processes of plastic waste generate microplastics, polymer particles smaller than 5mm, the full extent of their effects on diverse organisms remains the subject of extensive research. Remarkably, the effects of microplastics on human health remain inadequately understood, underscoring the urgency of further investigation. This thesis embarks on a comprehensive exploration of the intricate relationship between fluvial dynamics, microplastic transportation, and their distribution in aquatic environments. Rivers, regarded as the primary agents responsible for the transportation of microplastic particles to oceans and coastal areas, play a pivotal role in this phenomenon. The behaviour of microplastics within river systems is a complex interplay of physical, chemical, and biological processes, requiring a meticulous examination. Understanding the specifics of how rivers impact the transport and dispersion of these particles is crucial for developing effective strategies to mitigate their adverse environmental and ecological effects. Fundamentally this investigation aims to be a step towards fostering a more comprehensive understanding of the broader issue of plastic pollution and its far-reaching consequences. The study was conducted in the Cávado River, spanning from the Porto Bridge to the river mouth, encompassing a diverse range of environmental conditions. A total of 17 strategically marked sampling sites were selected and the riverbed sediment of each was sampled. The samples underwent a meticulous process, including drying in a laboratory oven and density separation using a $CaCl_2$ solution. The resulting supernatant sample was then collected and subjected to visual analysis under a microscope. This rigorous analysis led to the visual identification of a total of 571 microplastic particles. Notably, microplastics at sites MP012B, MP009, MP005, and MP003 displayed the highest counts, and the study revealed a distinct increase in microplastic prevalence from upstream to downstream locations. Statistically, the values exhibited normality and indicated statistically significant differences among groups. As a result, the hypothesis that fluvial dynamics influence microplastic behaviour was conclusively substantiated. The study also explored potential correlations between fluvial pressure factors and microplastic abundance at various sampling sites, considering the presence of urbanizations, wastewater treatment plants, agricultural land, industrial areas, and other infrastructures. However, it is essential to acknowledge potential sources of cross-contamination, such as airborne particles or clothing fibers, as well as the absence of organic matter digestion and subsequent filtering or sieving, which could lead to human error in particle identification. Therefore, a careful and thorough reassessment of the laboratory procedures is recommended.

Keywords: Microplastics, Particle size, Cávado River watershed, Sampling, Extraction technique, Effects, Transportation, Distribution, and Pressure factors.

Contents

Acronyms and Abbreviations

CO_x – Carbon Compounds

UNEP – United Nations Environmental Program

GI – Gastrointestinal

MP - Microplastics

NP - Nanoplastics

POPs – Persistent Organic Pollutants

MDA - Malon-di-aldehyde

BPA - bisphenol A

BBP - butyl benzyl phthalate

DEHP - di-2-ethylhexyl phthalate

PM particles (PM2.5 and PM10) – Particulate Matter 2.5μm and 10μm

NOAA - National Oceanic and Atmospheric Administration

MSFD - Technical - Marine Strategy Framework Directive

ICM - Intermunicipal Community of Cávado

Plastic types – **Table 3** in Attachments

WWTP – Wastewater Treatment Plant

WTP – Water Treatment Plant

1. The Wonders of Microplastics

From the dawn of human history, our relentless pursuit has been to evolve and acquire knowledge, symbolized by the metaphorical "light" representing progress and inspiration. Medicine, Physics, Chemistry, Philosophy, stand as pillars of knowledge, propelling science, and humanity to remarkable achievements, such as groundbreaking technologies. Yet, we ask ourselves, could we have done it better? We could, but that is not the question we should be asking. With these great technologic achievements emerged even more significant challenges that remained overlooked for a long time, reflecting heavily on the environment and well-being of multiple organisms. (Aristotle, 1908)

As early as 1600 BCE, humans already sought and relied on polymers. Meso-Americans ingeniously transformed natural rubber into figurines and bands Hosler *et al.* (1999) since then we have hungerly chased the perfect material, increasingly depending on natural polymers, such as horn, waxes, natural rubbers, and resins. However, it was not until the 19th century that we witnessed the world future shifting (Andrady & Neal, 2009).

Leo Baekeland, a physicist from the 19 and 20th centuries, who brought us one of the greatest achievements of modern society – plastic! Derived from the Greek "*plastikós"*, meaning "for moulding" or "capable of being moulded" evolved into the contemporary definition as a "synthetic product made from oil derivatives". Dating 1909, Baekeland, motivated by the scarcity of naturally occurring shellac, decided to publish his breakthrough in developing a substance both rigid and malleable. The "*Bakelite*" (**Figure 1**), a thermosetting phenol formaldehyde (pre-)polymer or resin, that undergoes a curing process induced by heat or radiation, typically under high pressure or through the addition of a catalyst, by its own words "*I began to think that the formaldehyde evaporates before it can act and that the proper way would be to impregnate with the viscous liquid which is obtained by boiling* CH_2O+C_6H_5OH *together without a catalytic agent*." (OED, 2023; AMS, 1993).

Baekeland's research continued to evolve, with additional insights found in his notes. It was not until February 8th that he publicly announced his groundbreaking discovery. He noted that previous reactions had yielded slow processes and brittle products. However, he stated, "... by the use of small amounts of bases, I have succeeded in preparing a solid initial condensation product, the properties of which simplify enormously all moulding operations." With this announcement, Baekeland indelibly altered the course of history (AMS, 1993).

Although this change brought a lot of advancements and innovation, it also brought significant challenges, with waste taking one of the spotlights. Waste, defined as (raw) materials without economical value, as shown to be terribly managed (Cashman, 2020). Pollution affects every type of environment, but it is worth

pointing that it was only around the 70's that we started to focus on the ocean's pollution. Concern and interest in the study of ocean pollution gradually evolved, albeit slowly (Carpenter *et al.*, 1972; Colton *et al.*, 1974), Over the years, we have made substantial strides in understanding the critical role of the ocean, upon which we depend on for, fresh water, renewable energy, health, cultural significance, trade, and transport. It is a source of innumerable benefits and ecosystem services (Zhang *et al.*, 2021).

Plastics have become an essential part of our lives, serving a multitude of purposes, whether in scientific and engineering fields or to satisfy our daily domestic needs. For well over a century, plastics have been a subject of concern, not just due to their widespread use, non-degradable nature, and persistence, but also because of their chelation capacity, as elaborated upon in this document (Lam *et al.*, 2018). As mentioned, human reliance on plastics is profound and rather than investing vast resources in costly materials, we have chosen the cheap oil-based option, widely employed in numerous daily activities and across various sectors. This includes packaging, as we illustrated in **Figure 2**, is responsible for 44% of the plastic production in 2021. The other main uses include building and construction, clothing, health care and leisure industries, transportation, electronics, automotive manufacturing, agriculture, and the food industry (Plastics Europe, 2023; Okoffo2021; Sudesh & Iwata, 2008; Andrady & Neal, 2009). However, is it all benefits? Given how well stablished and impactful they are in our lives and since there is little to no studies surrounding fluvial environments, this work aims to further understand the plastic pollution in them, by particularly targeting microplastics, and unravel how they behave.

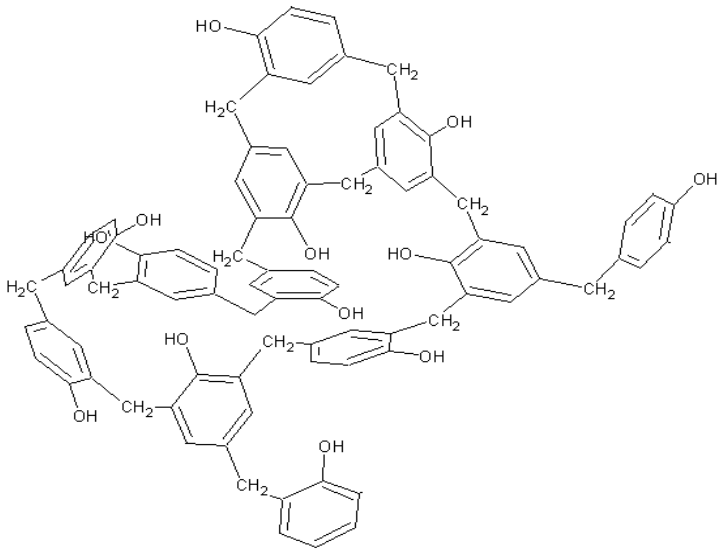

Figure 1 - 3-D Structure of Bakelite, result of the phenol-formaldehyde reaction. Retrieve from "JohnSRoberts99" Wikipedia contributor.

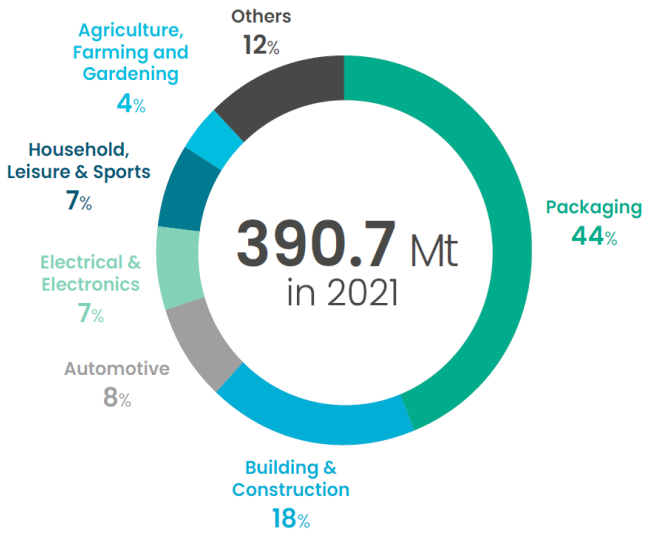

Figure 2 - Distribution of the global plastics use by application for the year 2021 – Retrieved from Plastics Europe, 2022.

2. The problematic (Barriers, Impacts, Numbers and Effects?)

It is an undeniable fact that plastics have revolutionized our way of life. Whether we are willing to acknowledge it or not, our dependency on them is undeniable. In the present scenario, it is a rare occurrence to encounter anything devoid of plastics in some form or another. They have inexhaustible applications and benefits, they are versatile, resistant, persistent, require less energy to produce than alternative materials, and they seamlessly integrate into a wide range of industries. Furthermore, their lightweight nature reduces transportation and construction costs, thereby diminishing carbon dioxide emissions, making it the "XXI true bargain deal" (Worm *et al.*, 2017; Andrady, 2015; Schmaltz *et al.*, 2020).

However, it conceals a substantial underlying issue — elevated levels of COx emissions owing to its fossil fuel origins. Consequently, its sustainability is non-existent, making it an increasingly prominent concern in today's world (Andrady & Neal, 2009; Sudesh & Iwata, 2008).

It is also intriguing that, while the most pressing complications are environmental, plastics and their mismanagement are inflicting significant economic losses on multiple nations (Williams & Buitrago, 2022). Single-use plastics stand out, as they often enjoy a one-way ticket to environmental disposal. Major global economies, including India, China, and the USA, are significant contributors to hindering our progress towards sustainability. Many of these barriers arise from the lack of suitable, sustainable material alternatives, the absence of biodegradable single-use plastics manufacturing facilities (despite their higher value compared to synthetic plastic counterparts), insufficient government regulation and initiatives, taxation policies, plastic usage restrictions, local government and school awareness campaigns, financial support

and incentives, as well as concerns among business owners about the potential loss of customers and profits. Moreover, there is an increasing dependence on single-use plastics, like straws, food packaging, films, and others, by individuals with disabilities or those with motor-restricting diseases/conditions such as Parkinson's (Vimal *et al.*, 2020).

It is vital to acknowledge that environmentalism, while well-intentioned, can have negative impacts and risks, affecting marginalized and vulnerable communities. Therefore, it is crucial to adopt an environmental justice perspective to ensure that measures, policies, and laws do not adversely affect society beyond their economic implications (Jenks & Obringer, 2020).

As mentioned earlier, the use and production of plastics have witnessed astronomical growth. Since 1950, a staggering 7800 million tonnes (Mt) of plastic resin and fibers have been manufactured, with half of this production occurring between 2004 and 2017 (Geyer *et al.*, 2017). In 1950, the annual plastic production stood at approximately 2Mt, and by 2015, it had already surged to 380 Mt (**Figure 3**). In the years leading up to 2019, coinciding with the onset of the pandemic, there was a slight dip in global plastic production, with output reaching 365.5Mt (Plastics Europe, 2023; OWD, 2019). However, after the pandemic, as depicted in **Figure 4**, we witnessed a return to the upward trend, peaking in 2021, at 390.7Mt (Plastics Europe, 2023).

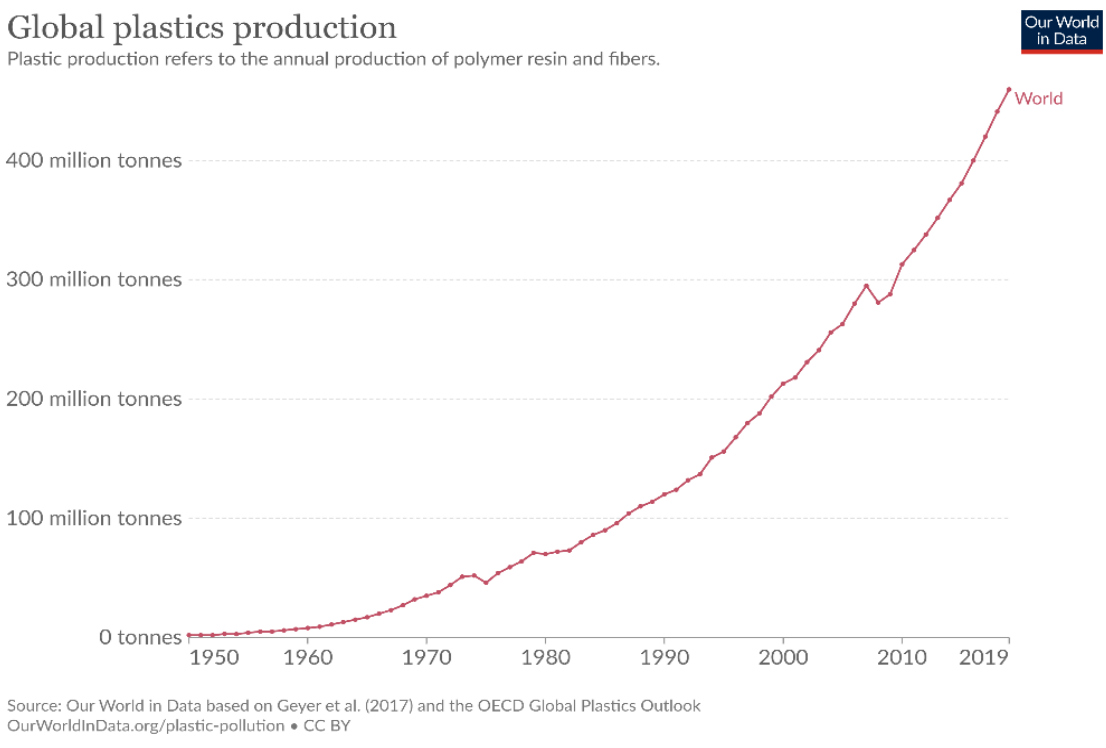

Figure 3 - Global primary plastics production (in million metric tons) according to industrial use sector from 1950 to 2015. – Data obtained from Geyer *et al.,* 2017 and OECD, 2019. Graph retrieved from OWD, 2023.

Although these numbers are the most cited by authors, we see discrepancies between the different statistic sources, with a variation of the number of total global plastics produced between 350 - 450Mt, for the year of 2018 (**Figures 3, 4 and 5**). This could be justified by a multitude of factors, including differences in data sources, the considerations of statistical teams, means and methods of analysis, and the willingness of governments, industries, and enterprises to disclose accurate values. However, there is a common denominator — the overall upward trend. By 2050, we should anticipate plastic production reaching approximately 1100-1400Mt, as illustrated in **Figures 3**, **4**, and **5**. Europe also followed this growth trend, however, in the last 2 - 3 years it has made gradual reductions in the production and consumption of fossil-based plastics, and has been investing more in recycled and bio-based plastics (**Figure 4**), nevertheless we are still far from reaching the desired sustainability.

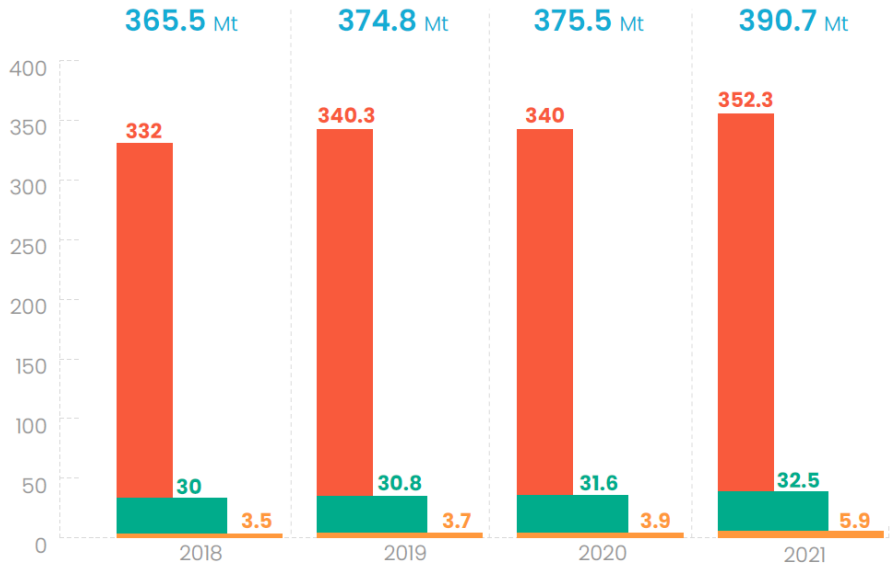

Figure 4 - Global Plastic Production from 2018 to 2021. Red Bar - Fossil-based plastics; Green bar - post-consumer recycled plastics; Orange bar - Bio-based plastics. Retrieved from Plastics Europe, 2022.

One could argue that the plastic problem relies on many distinct factors, its existence for example, but truthfully the problem lies in its End of Life (EOL) solution, which until now as proven to be inefficient (Williams & Buitrago, 2022).

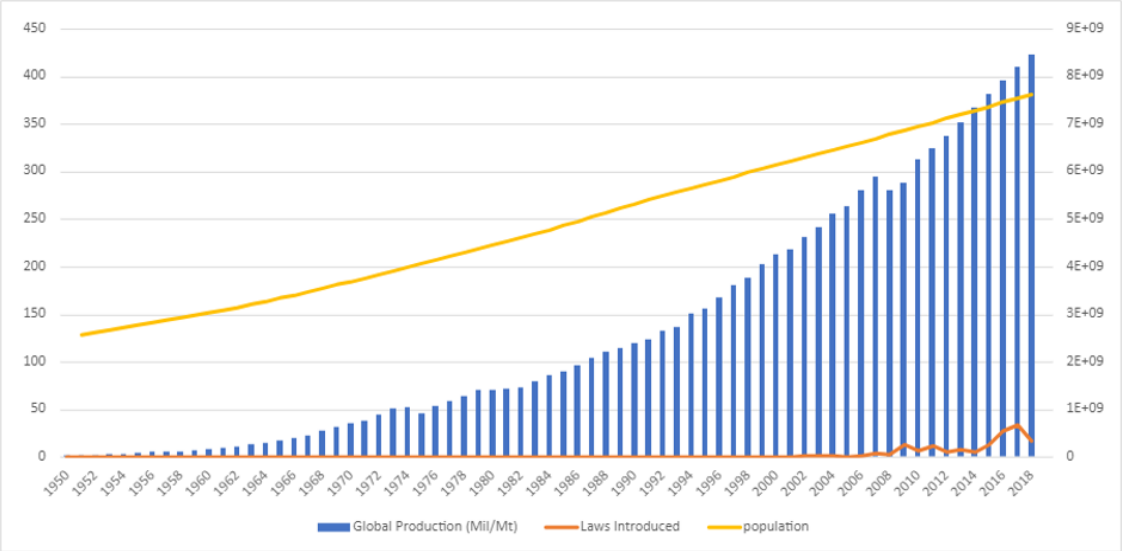

Figure 5 - Global Plastic Production, since 1950, in corelation with population growth and law introduction. Retrieved from Hazlegreaves, 2021.

But how can we target such problem? Well, Ammendolia *et al.* (2021) suggested that we should start by defining a clear line between the terms we use, specifically “litter” and “pollution”. According to their proposal, 'litter' should be linked to individual actions, such as improper garbage disposal. On the other hand, 'pollution' should be associated with companies engaged in the extraction, production, and disposal of plastics. Furthermore, when such pollution poses risks to humans or animals, it should be named “hazardous pollution.”

The authors also emphasized the notion of “culpability.” They highlight a prevalent Western narrative that predominantly addresses the end stages of the plastic life cycle, particularly consumption and disposal. However, this perspective overlooks the various key agents in the process, commencing with the extraction

of crude materials and extending through transportation, refining, production, and distribution, as depicted in **Figure 6**.

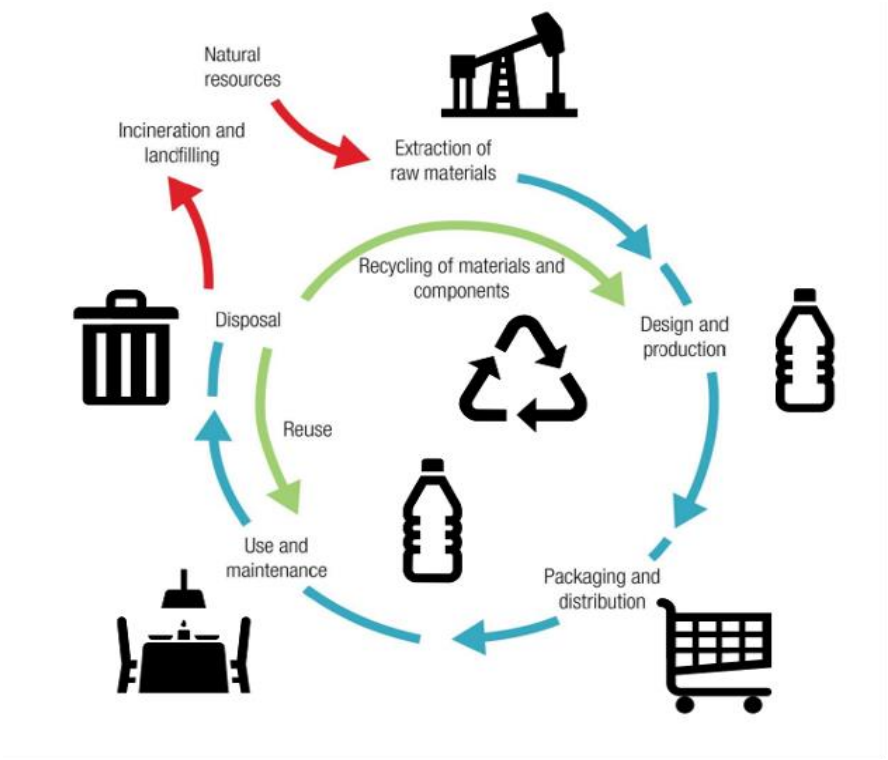

Figure 6 - Plastic Life Cycle. Obtained from Life Cycle Initiative by UN environment programme in October 2023. Retrieve from Life Cycle Initiative, 2023.

This individual focused narrative does not address our current predicament. This approach often results in measures and policies adopted by governments that primarily target the end consumer, overlooking the critical production process.

One commonly employed strategy is the imposition of additional charges on plastic items. For instance, in Portugal, consumers are charged an extra €0.10 for each plastic bag they use, which once again impacts only the end consumer. Another example is the fines for littering, specifically related to cigarettes and improper disposal of plastic waste. While not particularly stringent, these fines can still carry significant financial penalties or societal consequences for individuals.

Nonetheless, it is not to be misinterpreted, as nobody is trying to exonerate the individual responsibility but rather point that focusing on them as proven not to be the best solution for the problem (Williams & Buitrago, 2022). As the entire production and usage of plastic is anthropogenic, human solutions for plastics are both mandatory and feasible. Hence, various societal actors/sectors are necessary: consumers, producers, policy makers, industries, law makers and state agents (Heidbreder *et al.*, 2019). To gain a deeper insight into addressing this issue, it is highly recommended to read the previously mentioned article, which elucidates each phase of the process comprehensively and offers statistics and practical, real-life examples.

Furthermore, it is also worth exploring the Life Cycle Approach (LCA) documented by UNEP. Some globally appliable measures presented by the UN (United Nations), which follow the whole plastic pollution process from extraction to disposal, respect a thorough system consisting of 5 complex steps (UNEP, 2017):

1. Follow a scientific basis, respecting the scientific method and conduct quantitative analysis.
2. Structure Action plans according to geographic and demographic factors.
3. Target hotspots accordingly respecting the social scientific studies.
4. Elaborate Guides, Training opportunities, and multiple financial studies which present Finance Sustainable Solutions.
5. Build from Existing Initiatives, take advantage of already successful measures and ideas strengthening them and scale them up globally.

As an introduction to potential solutions, UNEP created **Figure 7,** which offers some ideas regarding the Government, Investors, Material Producers and Products/Services Producers. In **Table 1 and 2**, in attachments, Sharma *et al.* (2023), compiled Plans of Action, policies/laws, as well as challenges that different polluting countries throughout the world have been trying to fight.

As of right now, the production and utilization of plastics haver reached such heights that they have become, a mandatory asset in the global marketplace. It is clear that they pose a significant hazard to not only humans but also the multitude of organisms on which we depend, but it also ends up impacting tourism and recreation, just as shown in **Figure 8**, it threatens rivers, coasts, the ocean life and in the end of the day, serve as a sobering reflection of human selfishness, actions, attitudes, and behaviour (Williams & Buitrago, 2022).

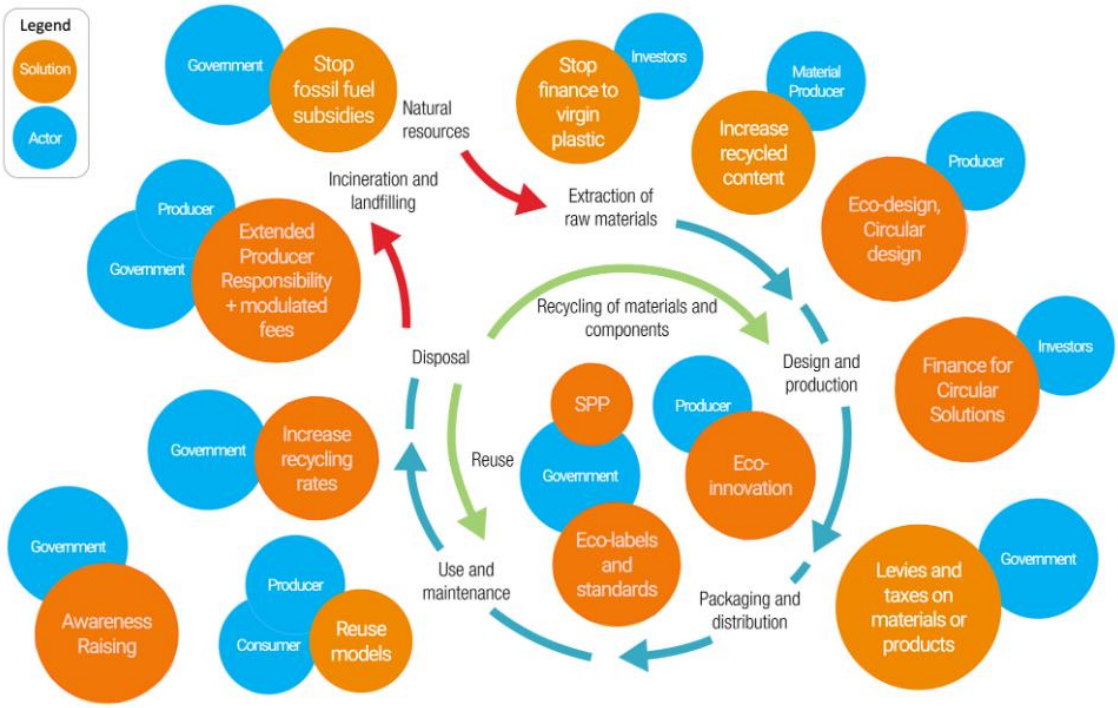

Figure 7 - Hotspots, Solutions and Actors. Obtained from Life Cycle Initiative by UN environment programme in October 2023. Retrieved from Life Cycle Initiative, 2023.

Figure 8 - Pictures taken in Azurara Beach showing piles of plastic debris scattered around the beach and groyne, Portugal on the 5th of April 2023.

There are numerous documents and records of plastic litter pollution in diverse environments. These range from high-altitude mountains like the Everest (Napper *et al.*, 2020), deep ocean structures and floors like the Mariana trench, and bays like the Great Australian Bight (Jamieson *et al.*, 2017; Barrett *et al.*, 2020), to rivers (Williams & Simmons, 1997; Kunz *et al.*2023) and even the atmosphere, through the "atmospheric rivers" (Brahney *et al.*, 2021; Zhang *et al.*, 2020). Additionally, there are abundant records and studies of plastic pollution in common and anthropogenically affected environments such as coastal and fluvial beaches, sub-tropical gyres, water columns, dunes, mangroves, and many others (Williams & Buitrago, 2022).

We can easily understand the direct impacts of plastic pollution on multiple organisms. For instance, there are multiple examples shared across social media platforms, like straws stuck in turtle's noses, the substantial number of plastics found in the gastrointestinal tracts of birds and fishes, or the entanglement of seals in plastic nets. These are just a few examples of this alarming issue. We have all witnessed the distressing images and videos of children and adults scavenging plastic bottles from colossal 'mountains of plastic' in exchange for a meagre compensation, barely enough for their survival, while this might not directly affect their health, it is the socio-economic condition that leads to such exploitation.

In addition, plastics have substantial economic impacts on consumers and society as a whole. The entire lifecycle of plastics, from extraction to disposal, is governed by the interests of various companies and actors. A notable example is the recent COVID-19 pandemic, which highlighted humanity's remarkable ability to adapt and mass-produce affordable single-use plastic products that were crucial for our survival. However, this was often done at the expense of the environment and proper waste management (Adyel, 2020; Benson *et al.*, 2021; Wang *et al.*, 2023). Here are some journalistic articles related to the subject, which may provide additional insights into the impact of plastic pollution:

https://www.nytimes.com/2021/09/18/world/covid-trash-recycling.html,
https://newseu.cgtn.com/news/2020-05-22/Is-the-pandemic-triggering-a-spike-in-plastic-pollution–QBobeagfok/index.html

https://www.reuters.com/world/asia-pacific/hong-kong-zero-covid-policies-create-mountains-plastic-waste-2022-04-19/

This pandemic led to a temporary decrease in gas emissions during quarantine periods However, as we started lifting the restrictions, the exploration, extraction, and production of products came back in full force, reaching pollution levels never seen before. Notably, plastics were one of the primary contributors to these increased pollution levels, as illustrated in **Figures 3**, **4**, and **5**.

This has been a concise general introduction to the issue of plastic pollution. While it provides a valuable overview, the subject is profoundly complex. Therefore, I highly recommend diving into the articles and documents mentioned and explore each aspect of the topic comprehensively.

3. Microplastics

3.1. Formation

As it is evident, plastic pollution is a significant issue, and its gravity becomes more apparent when we delve into finer particle sizes (Ukaogo, 2020). It is also common knowledge that plastics, when exposed to the elements, will be broken down into microplastics by an array of physical, chemical, and biological processes. Thermal degradation, physical degradation, photodegradation, thermo-oxidative degradation, and hydrolysis are all examples of non-biodegradation originating what we call of Micro - and Nanoplastics (Van Cauwenberghe *et al.*, 2013; Yee *et al.*, 2021). Physical deterioration through weathering causes bigger polymers to break down into smaller pieces naturally, but thermal degradation, or heat degradation, is an artificial, industrial process. Contrarily, two naturally occurring chemical processes — hydrolysis and photodegradation — use water molecules and UV-Visible light, respectively, to dissolve the chemical bonds in plastics and transform them into monomeric forms. Plastic structures are broken down by non-biodegradation processes, changing their mechanical characteristics and surface area, which enhances physical-chemical reactions and interactions with microbes as we will be able to further understand in this document (Lucas *et al.*, 2008). **Figure 9** summarizes and explains graphically the basic plastic particle formation and transportation system. Microplastics (MP) are defined as polymer particles with a length under 5mm (Betts, 2008), sourcing from 2 different origins:

- Primary microplastics, voluntarily created micro- and nanoplastics for commercial purposes, mainly consumer and industrial uses (e.g.: clothes or rags, cosmetics, cleansers, drug delivery particles or even industrial air blasting).
- Secondary microplastics, are particles resulting from the fragmentation of larger pre-existing plastic items such as bottles, plastic bags, basins, single-use utilities, and others (Prata *et al.*, 2019; Besley, 2016).

Most plastics are extremely durable and can persist for decades, or even centuries, in their original form (Hopewell *et al.*2009). Due to their physical properties, plastics can contaminate the environment on a global scale (Doyen *et al.*, 2019) and thus, will bring consequences when consumed by various living beings (Besley *et al.*, 2016), gaining the title of one of the most growing problems of the 21st century (Thompson *et al.*, 2004). To get around this problem, attempts have been made to reach a consensus and establish bases in the analysis of microplastics, starting with their origin, that is, whether they are primary or

secondary; their shape, if they present as fragments, pellets (spheres, discs, cylinders), filaments/fibers, irregular shapes, thin films, plasticized foams, or granules. And one of the parameters that brought more disagreement among authors, dimension: Macroplastics > 2.5cm, Mesoplastics 0.5 - ≤ 2.5cm, Large microplastics 1 - ≤ 5mm, Small microplastics 1µm - ≤ 1000µm and Nanoplastics 1nm – ≤ 1µm (Van Cauwenberghe *et al.*, 2015; Gigault *et al.*, 2018).

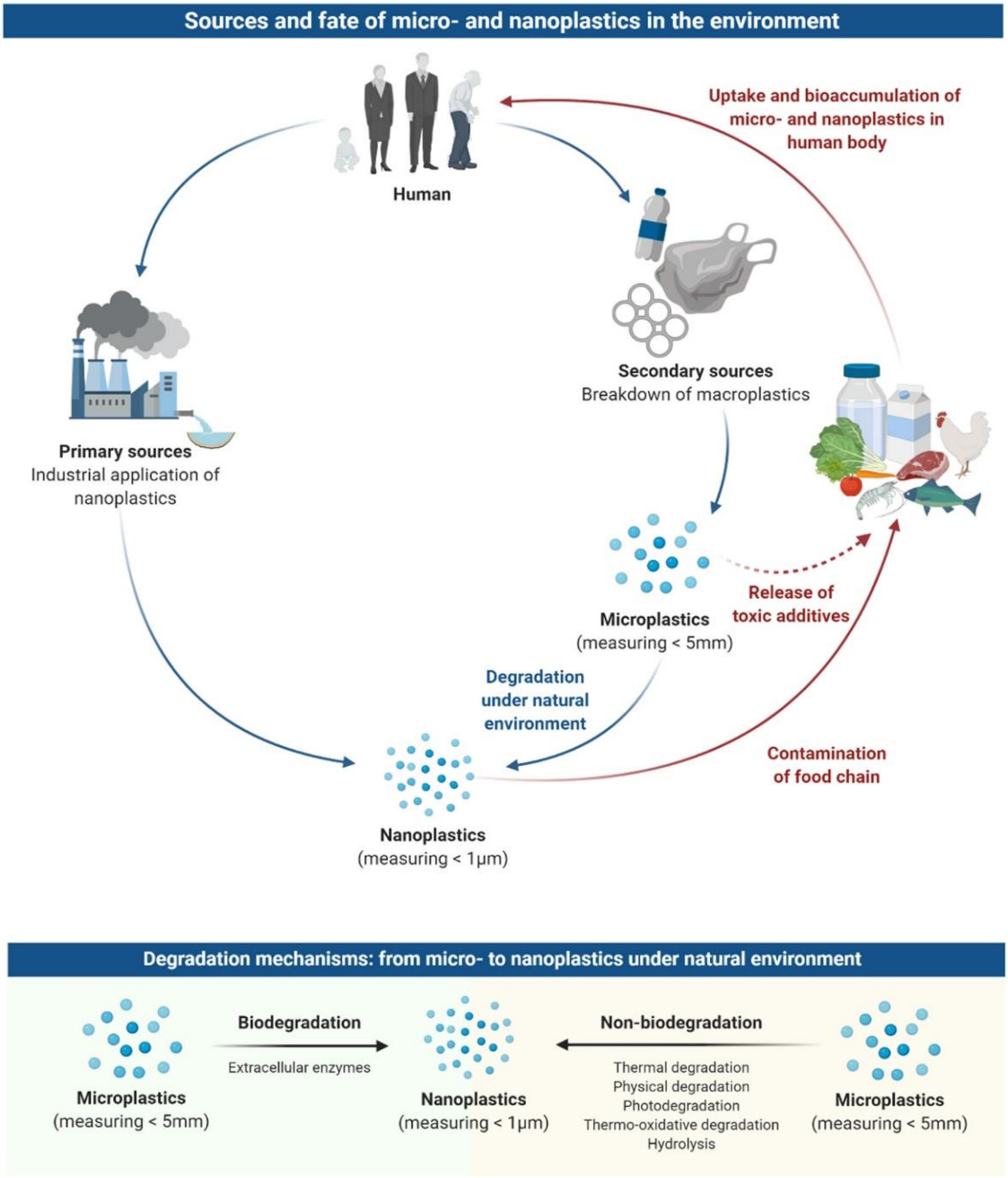

Figure 9 - Micro- and nanoplastics formation and transportation system. Retrieved from Yee *et al.*, 2021.

3.2. Detection and Quantification Methods

As mentioned, it is not easy to achieve a consensus in the characterisation of these particles, so to fight it, the scientific community has been making the effort to create a "standardized methodology" regarding different physical and chemical aspect to ease the identification and characterisation.

3.2.1. Density

Plastics present very diversified densities, **Table 3**, in attachments, ranging from less dense ones such as XPS (0.028 – 0.045 g/cm^3) until the denser ones such as PVC (1.35 – 1.39 g/cm^3) e o PET (1.38 – 1.41 g/cm^3). Considering this property, it is a good option to perform density separation, for example, using a saline solution. Some of the recommended solutions would be concentrated NaCl solutions (density of 1.2 g/cm^3), NaI (1.6 – 1.8 g/cm^3), ZnCl2 (1.5 – 1.7 g/cm^3) and $CaCl_2$ (1.345 – 2.16 g/cm^3; OXY, 2021; Rodríguez-Seijo & Pereira, 2017; Schröder *et al.*, 2021).

3.2.2. Visual Identification

The most common method for identifying plastics involves visual inspection, using the naked eye for larger microplastics or magnification tools such as a microscope for smaller microplastics. This examination focuses on assessing the physical properties of the microplastics. There is a consensus that visual identification should not be followed as protocol to particles with less than 500 µm since the probability of misidentification is very high (Xu *et al.*, 2019). Additionally, observational errors are usually vast due to observers' subjectivity. For example, Fries *et al.* (2013), reported that three different people produced results differing 1 – 4MP particles in the same sediment sample with the support of a microscope (Xu *et al.*, 2019).

Another particularly important factor is colour, serving as a preliminary indicator of their possible composition, since, for example, transparent materials are described as PP, white plastics as PE and opaque plastics as LDPE (Hidalgo-Ruz *et al.*, 2012). Not only it gives us an insight of the possible composition, but it also allows us to understand the period of weathering and/or "presence" on the surface of the water, since the darkening/yellowing of these is due to the increase in the carbonyl index, resulting from photo-oxidation or even from "aging" (Stolte *et al.*, 2015), whereas pigmented plastics tend to dechlorinate. Some authors such as Stolte *et al.*(2015), Acosta-Coley & Olívero-Verbel (2015), Martins & Sobral (2011) established and distinguished 2 groups of colours: (1) blue, green, red, yellow, orange, among others; and (2) white, black, aged and translucent, however, Castro *et al.*(2016) demonstrated, through an artificially controlled study, that the colour influences the identification and quantification, when performed visually, obtaining large percentages of blue and green particles, and only a small percentage of yellow/orange particles (Rodríguez-Seijo & Pereira, 2017), as we will be able to see further in this work, most of the particles identified were different shades of red, blue or green. Nevertheless, visual identification is easily

fallacious and faulty. Hence, prior purification is needed, to reduce the challenge of discriminating MPs and other particles, whether they are of organic (branches, leaves, among others) or of crystalline origin (shells, micas, quartz, among others; Xu *et al.*, 2019). Therefore, it is helpful to include organic matter digestion, the use of a polarized lens and proceed with chemical analysis to assure trustworthy results (Lavers *et al.*, 2016).

3.2.3. Chemical Analysis and Characterization

There are multiple chemical analysis and characterization techniques which are viable for microplastics as we will see in the state of art further in this document. However, there is no perfect technique but rather a combination, for example the most efficient and common one is the combination of Raman spectroscopy and Fourier Transform Infrared Spectroscopy (FTIR). Why? Because they complement each other! Both vibrational spectroscopy methods are non-destructible and highly accurate, producing a spectrum based on the interaction of light with molecules. But what differs? FTIR produces an infrared spectrum resulting from the change in dipole moment, whereas Raman produces a molecular fingerprint spectrum based on the polarizability of chemical bonds (Prata *et al.*, 2019). Also, there are multiple FTIR techniques which can be used for the analysis, such as: Attenuated Total Reflection FTIR (ATR-FTIR) which improves the irregular MP information since it is applicable to thick and opaque particles, contrasting transmission FTIR, and Micro-FTIR which allows the production of a high-resolution map of the sample (Harrison *et al.*, 2012; Löder *et al.*, 2015). FTIR overall has a lower resolution capacity, since it allows the detection of particles between 10-20μm and presents difficulties to detect the absorbance of particles smaller than 5μm. In addition, the medium surrounding the particles will affect the absorbance spectrum resulting from the FTIR, which is strongly affected, for example, by water, however, this allows the facilitated detection of transparent particles, unlike Raman (Xu *et al.*, 2019). Raman allows a better size resolution and consequently characterization of microplastics <20μm, down to 1μm, it also allows the detection of black or opaque particles. A disadvantage is it may be limited to weak signals, caused by the strong fluorescence exhibition from microbiological entities.

A way to overcome this barrier is by extending the measurement duration, and fluorescence interference, relying on material characteristics such as colour, biofouling, and degradation of the particles (Prata *et al.*, 2019; Xu *et al.*, 2019). Still in respect of the Raman possible techniques, Stimulated Raman Scattering (SRS) has been used to identify microplastics on low Raman background filter membranes without pre-selection, allowing to characterize 1cm^2 under 4.5h. Nevertheless, the background filters used in SRS are extremely expensive, reaching 6 - 7 times the price of cellulose filters commonly used.

In conclusion, these two methods are very effective when combined, however, vibrational spectroscopy is limited by the hight cost, material and equipment availability, time and effort required in analysing and

processing samples requires complex data treatment, and the need for skilled personnel for the detection, specifically when weathered or in contaminated MP samples (Dümichen *et al.*, 2017). A get around to ease the particles characterization is by pre-selecting the MP particles by human visual identification, risking inducing a bias (Qiu et al, 2016).

Other methods, such as Thermo-analytical ones, have shown promising results in MP analysis, especially when Thermogravimetry was coupled to Differential Scanning Calorimetry (TGA-DSC), Pyrolysis-Gas Chromatography – Mass Spectroscopy (Pyr-GC/MS), and Thermal Extraction Desorption Gas Chromatography-Mass Spectrometry (TED-GC/MS). The principle behind TGA-DSC is the changing heat capacities during the solid-liquid phase transition of a polymer, whereas Pyr-GC/MS and TED-GC/MS are based on the pyrolyzation, or thermal decomposition by other words, for identification and quantification of MPs. In pyrolyzation, MPs are thermally decomposed under inert conditions and as a result a gas is formed, cryo trapped and separated on a chromatographic column (GC) (Shim *et al.*, 2017). In the case of Pyr-GC/MS, the preferred one, the material is pyrolyzed in the absence of oxygen, the resulting combustion or pyrolysis products are then identified by GC/MS. For pyrolysis, a single MP particle is introduced into a tube. Beforehand, it is customary to start by elucidating polymer pyrolysis products, sometimes referred to as polymer markers. With the aid of a cold injection system, the released gaseous chemicals are captured and delivered to a GC column that is connected to a mass spectrometer. The obtained spectra of the pyrolysis products are compared to a database of popular plastic kinds as the last stage.

Although Pyr-GC/MS can provide chemical characterization of a single or multiple MPs, it affects the particles and does not provide information about the number, size, or shape (Fries *et al.*, 2013). Thermoextraction and desorption combined with Gas Chromatography-Mass Spectroscopy (TED-GC/MS) combines thermogravimetric analysis (TGA) for thermal degradation and solid phase extraction of plastic degradation products. Thermal desorption in GC-MS is then used to further examine the results. The use of relatively large sample masses and the ability to analyse complicated heterogeneous matrices provide this technology an edge over Pyro-GC-MS and enable the identification and quantification of polymers in environmental materials without preselection. Large volumes of material are needed for liquid chromatography, which employs a suitable solvent for the characterisation of the polymer type and size exclusion chromatography (Elert *et al.*, 2017). These techniques have the benefit of examining high masses, increasing representativeness, but they are destructive and only offer information on the chemical makeup of the sample. (Prata *et al.*, 2019)

Some other authors like used other methods such as portable X-ray Fluorescence (XRF) and Scanning Electron Microscope (SEM) with an Energy-Dispersive X-ray Microanalyzer to collect information of morphology and chemical composition of microplastics.

3.2.4. Plastic Degradation and Biological Colonization

Once understood the process of chemical analysis we progress into comprehending one of the most worrying properties of MPs, which is its sorption capability. In the realm of plastic pollution, an increasing body of literature, as exemplified by Binda *et al.* (2021) and Crossman *et al.* (2020), highlights the pervasive presence of plastics in water, sediment, and soil. However, despite this growing awareness, the full extent of its ecological and human health consequences remains largely uncharted territory. In the meanwhile, additives are often used in the production of plastics to achieve desired qualities/properties such as plasticisers, antioxidants, light quenching compounds, flame retardants and dyes. However, according to Bejgarn *et al.* (2015), these chemicals frequently exhibit estrogenic and endocrine-disrupting effects. Therefore, they operate as a vector for contaminants in addition to their inherent biological harm. The harmful effects they carry can also be due to contaminants adsorbed on the surface of MPs (Liu *et al.*, 2019). Their large surface area and hydrophobic characteristics, allow the accumulation of high concentrations of POPs, such as polychlorinated biphenyls (PCBs) (Mato *et al.*, 2001), dichlorodiphenyltrichloroethane (DDT) (Frias *et al.*, 2010), hydrocarbons (HCs) (Hirai *et al.*, 2011; Van *et al.*, 2012; Lo *et al.*, 2019) and heavy metals (Ashton *et al.*, 2010; Holmes *et al.*, 2012) like Cr, Co, Ni, Cu, Zn, Cd, Pb, Ag and Hg (Cao *et al.*, 2021). Plastics have long been recognized for their capacity to adsorb compounds, having been detected pesticides and antibiotics, heavy metals, pigments, phthalate acid esters, poly brominated diphenyl esters and other biotoxic additives (Wang *et al.*, 2022), predominantly driven by hydrophobic interactions, van der Waals force, hydrogen bonding and electrostatic attraction (Cao *et al.*, 2021). Plastic polymers have traditionally been perceived as "inert" concerning aqueous metal ions. Nevertheless, recent laboratory studies have cast doubt on this established paradigm, revealing a significant interplay between metal ions and microplastics. These metal ions, commonly seen as essential micronutrients, can become environmental hazards when present in excessive concentrations (Johnson *et al.*, 2017).

The ability of these particles to migrate and absorb contaminants will both dramatically increase as they age, as they vary in type, concentration, size, total surface area and present functional groups or with the change of the levels of certain chemical characteristics of heavy metals and environmental factors such as pH, salinity, humidity, and total dissolved solids, just as shown in **figure 10** presented by Cao *et al.* (2021) (Menicagli *et al.,* 2019; Qin *et al.*, 2021; Lan *et al.*, 2021).

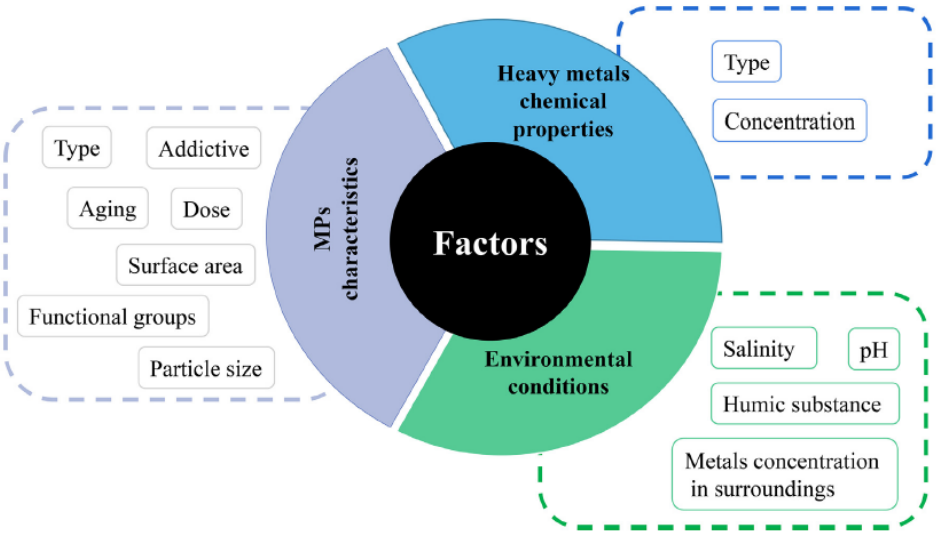

Figure 10 - Factors influencing the adsorption behaviour of heavy metals by MPs. Retrieved from Cao *et al.*, 2021.

Plastic aging emerges as a pivotal factor influencing metal sorption, with two primary processes altering plastic properties in environmental settings: physicochemical deterioration and biological colonization.

The impact of polymer aging on metal sorption has garnered extensive attention in recent studies, as evidenced by works like those by Bellasi *et al.* (2020), Liu *et al.* (2020), Lv *et al.* (2015), and Wang *et al.* (2019). **Figure 11b** provides a schematic representation of the two primary processes affecting the aging of plastic particles dispersed in the environment. These processes include physicochemical alterations, which modify plastic surfaces by changing their surface charge and inducing oxidation, and biological colonization, wherein microorganisms transform plastic surfaces, potentially

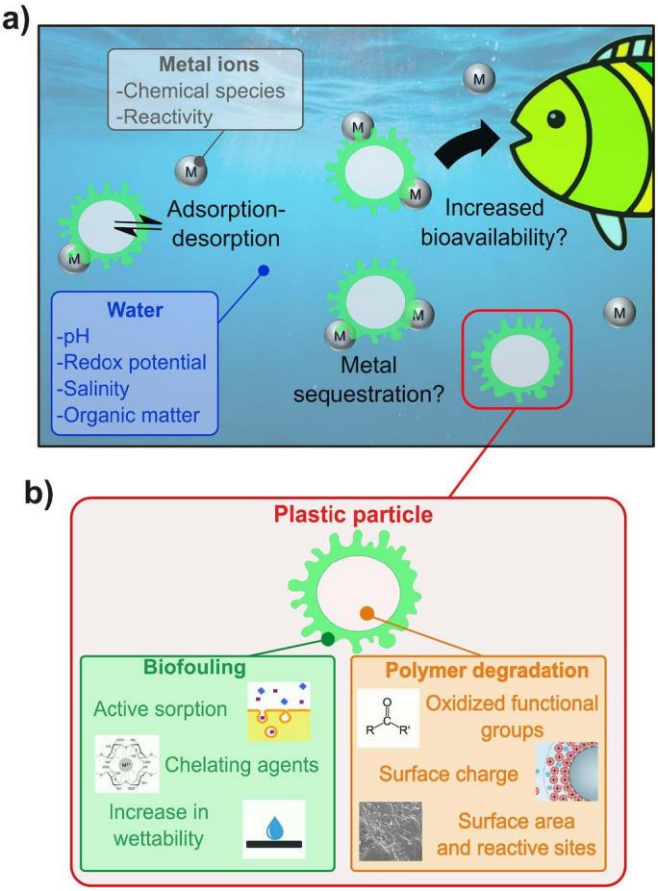

Figure 11 - (a) Graphic representation of the main factors affecting the Metal - MPs interaction in the water environment. (b) Small focus on the phenomena affecting polymer degradation and enhancing Plastic–Metal interaction, more specifically the effects on the plastic particle surface and colonization of the particles by microorganisms. Retrieved from Binda *et al.*, 2021.

enhancing interactions with trace elements by increasing wettability and exposing charged groups carried by extracellular polymeric substances characteristic of biofilm-forming microorganisms, **Figure 11a**. While chemical oxidation processes, particularly those involving UV radiation, have received considerable attention, the role of biological colonization in affecting metal ion sorption has been somewhat overlooked.

Nevertheless, it is essential to acknowledge that chemical and biological aging processes co-occur in environmental settings and can mutually influence each other. Consequently, simplistic experimental approaches may not suffice to fully elucidate system behaviour. Thus, a multi-tiered experimental approach, encompassing increasing levels of system complexity, is imperative.

The nature and underlying mechanisms of this interaction between metal ions and MPs remain shrouded in uncertainty. However, laboratory experiments have demonstrated remarkable sorption capacities for metals, with more than 40% of certain dissolved metals being adsorbed in current research, as illustrated by Holmes *et al.* (2012) and Lang *et al.* (2020). Furthermore, observations of coexisting metals adsorbed onto plastic particles within environmental contexts, as discussed by Kutralam-Muniasamy *et al.* (2021), raise significant concerns regarding the ecological ramifications of MP-metal interactions.

To further understand the sorption properties of MPs, we need to understand the 3 present mechanisms that explain adsorption, a mass transfer process from the liquid phase to the solid adsorbents, the direct interaction between MPs and heavy metals, mainly refers to the interaction under free contact conditions, which needly occurs in liquid medium (Cao *et al.*, 2021):

1. **Single electrostatic interaction or electrostatic interaction together with surface complexation**. Heavy metal ion adsorption is one of the primary processes. Through coulombic contact, heavy metals can interact with charged or polar MPs (polar areas that have developed on the surface). The presence of charged impurities and additives, such as the common brominated flame retardant hexabromocyclododecane (HBCD), or even flaws in the plastic surface can cause it to be polar. Additionally, UV weathering generates new absorption bands which enhance polarity and induce charged surfaces.
2. **New complexes via sorption and/or bioaccumulation by biofilms and natural organic matter (NOM).** Results in a change of the surface area and the surface properties of plastic particles, allowing heavy metal accumulation which is enhanced via complexation with functional groups contained in the respective biofilms including -COOH, -NH_2, and phenyl-OH.
3. **Interactions involving precipitation/coprecipitation.** By adsorbing hydrous oxides of Fe and Mn, heavy metal ions or their complexes precipitate with the hydrous oxides of Fe and Mn. Some pellets have presented decent concentrations of Fe and Mn. However, it is not that

common to verify this phenomenon since for coprecipitation to occur it is needed a high concentration of heavy metal ions in the system.

3.2.5. Pathways, Risks and Effects on Organisms and the Environment. From Soil to Soul.

3.2.5.1. General Context

As multiple studies have shown both MPs and heavy metals individually can exhibit toxic effects, but when combined they may lead to 3 kinds of effects, the first 2 being the most common ones in aquatic systems: synergistic, antagonistic, or potentiating effect where (Bhagat *et al.*, 2020) defined as:

Synergistic – Combined effect of two chemicals is much greater than the sum of each chemical.

Antagonistic – MPs sorption capacity to carry heavy metals allow a reduction of their concentration in the medium, thus reducing the environmental biological toxicity them.

Potentiating effect – The combination of a certain non-toxic chemical with a toxic one increases the second one's toxicity greatly.

These potential implications encompass enhanced metal bioavailability to biota through vector effects, as detailed by Bradney *et al.* (2019) and Naqash *et al.* (2020), along with potential alterations to the cycling of elements within ecosystems (Seeley *et al.*, 2020).

The burgeoning interest in these environmental issues is evident, reflected in the growing number of studies dedicated to unravelling the complex dynamics of MP-metal interactions. This expanding body of research underscores the urgency of comprehending the ecological and environmental consequences of this evolving facet of plastic pollution. The ecological risk further rises by releasing the contaminants into organisms after ingestion. Multiple studies have shown that different kinds of organisms, such as, zooplankton, benthic invertebrates, bivalves, fish, and large marine mammals, seabirds may ingest these particles as food (Li *et al.*, 2020), resulting in the blockage of food passages, inducing a false sense of satiety, and consequently reducing energy and nutrient levels, and leading to death. Here, just by ingestion we verify negative effects on organisms' physiology, reproduction, and survivability (Cao *et al.*, 2021).

Additionally, the heavy metals contaminants carried by these particles may end in the GI tract of many animals leading to behaviour changes, tumour formation, vital organ stress, negative impact on development and hatching rates as well as malformations (Chouchene *et al.*, 2023).

To further comprehend the impacts, a summary of general effects found in multiple studies from soil to humans will be made.

3.2.5.2. Soils

Both Zhang *et al.* (2019) and De Souza Machado *et al.* (2018) were able to conclude that PE fibers can cause soil degradation in some way or another. De Souza Machado *et al.* (2018) found that there was a reduction of bulk density due to the PE particles which on normal conditions, a bulk density reduction reflects a negative effect on root growth. However, contrarily to De Souza Machado *et al.* (2018), Zhang *et al.* (2019), found no significant alteration in the bulk density, thus having concluded that microfibers cannot affect this soil characteristic. Similarly, to the bulk density, there were no detectable changes in the soil aggregate size distribution and saturated hydraulic conductivity. De Souza Machado *et al.* (2018) also found a significant decrease in water stable aggregates, which are often regarded as impoverishment of the soil structure and the diversity of microenvironments.

Both research teams found that microplastic occurrence and accumulation in soils lead to considerable structural, physical, and biological damages, resulting in the formation of soil clumps/clods, as shown in **Figure 12**, reduction of pores volume, thus increasing water repellence, difficulted percolation and consequent decrease in water storage. As stated, the degradation of soil integrity also affects the biological properties, namely the microbial activity, due to the decreased soil microbiota diversity. Nevertheless, it is mandatory that we further study the damages and effects different types, sizes and shape particles may have in the soil physical, chemical, and biological characteristics and properties, since there is still a lot of disagreement and lack of knowledge about MPs behaviour in soils.

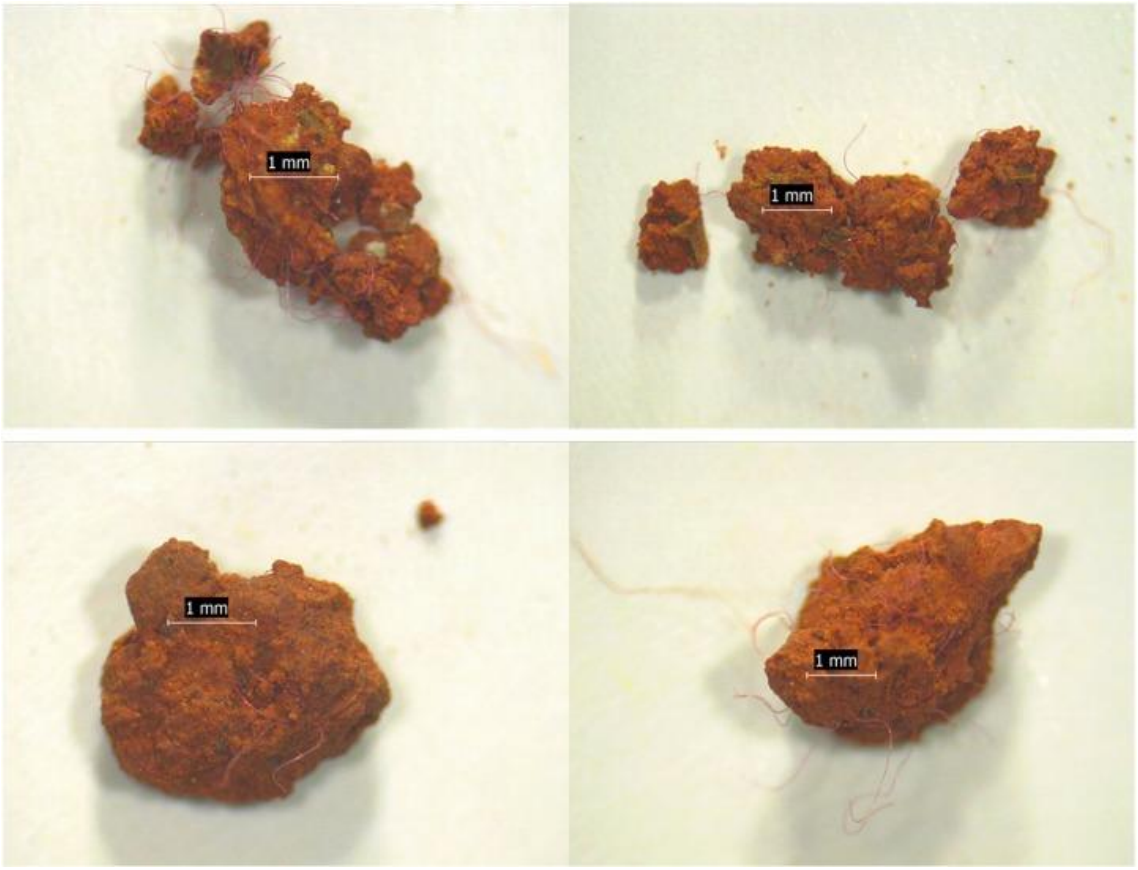

Figure 12 - Clods of polyester microfibers. Obtained and adapted from Zhang *et al.*, 2019.

3.2.5.3. Plants

With soil degradation there is a mandatory underlying problem, plants. Green plants when exposed to MPs and NPs see their physiological and biochemical processes affected such as seed germination, plant growth, photosynthesis and antioxidative systems, and can suffer genotoxicity (Bosker *et al.*, 2019; Jiang *et al.*, 2019; Zhou *et al.*, 2021), as represented in **Figure 13**. Ullah *et al.* (2022), reviewed 250 articles and compiled the impacts MPs can have on plants, from their roots to the leaves. Additionally, they pointed that Chen *et al.* (2022) highlighted that 44 articles were published about the interaction between MPs/NPs and higher plants until June of 2021. It was found MPs brought negative effects on biometric parameters, phytotoxicity and MPs/NPs accumulation in plant tissues in multiple plants including many day-to-day vegetables and cereals we commonly eat such as wheat, rice, barley, spring onion, onion, carrot, lettuce, cucumber (Ullah *et al.*, 2022). Several studies have reported multiple negative effects namely growth inhibition under PS exposure and root decline (Maity *et al.*, 2020), while Lian *et al.* (2020) found an increase wheat root elongation as well as Zhang *et al.* (2021), which additionally found a reduction in antioxidants enzyme activity, enhanced plant biomass, carbon, and nitrogen, and nitrates content and decline of some micronutrients.

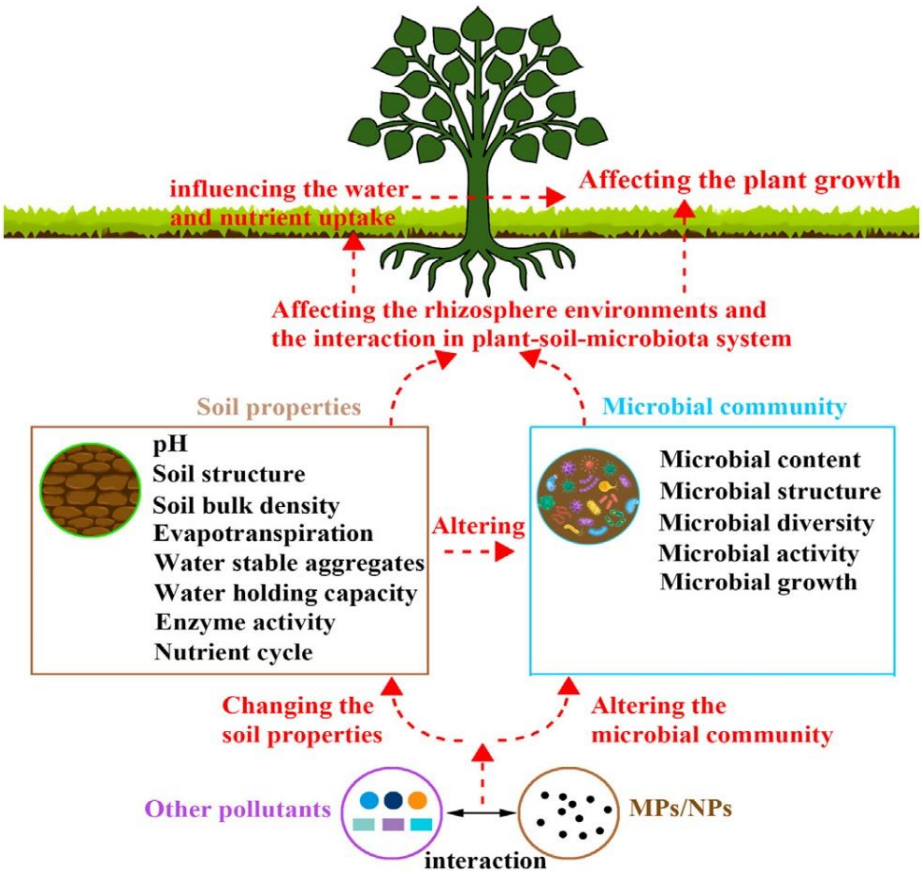

Figure 13 - Graphical summary of the effects of MPs/NPs interaction on the environment. Obtained from Chen *et al.*, 2022.

Furthermore, it was found that root tips are capable of NPs uptake and translocating them along the xylem and it was concluded that roots are more affected by plastic particles due to direct contact and higher accumulation with the MPs. It was also observed a decline in photosynthetic parameters and chlorophyll content in response to PS particles, also the combination of these with di-n-butyl phthalate (DBP) have been reported to cause severe oxidative damage by inducing Malon-di-aldehyde (MDA) and H_2O_2 content (Gao *et al.*, 2021). It was also reported high concentrations of H_2O_2 and O_2 in plant roots which were responsible for remarkable increase in glutathione reductase, dehydroascorbate reductase, ADP-Glucose pyrophosphorylase, fructokinase, and phosphofructokinase, whereas the activities of cell wall peroxidase, sucrose synthase, vacuolar invertase, phosphoglucoisomerase, and glucose-6-phosphate dehydrogenase suffered a decrease (Chen *et al.*, 2022). The adsorption capacity of MPs particles allows high concentrations of heavy metals such as As^{III} which in this case resulted in an enhancement of PS particles uptake into the cells due to deformation and distortion facilitating the circulation of these particles along the plant to stems and leaves. There are multiple reports of negative impacts not only in plant growth but also seed germination, since these particles end up blocking seed pores and inhibit shoot and root growth by adhering to root hairs and decreasing water uptake (Bhattacharya *et al.*, 2010; Jiang *et al.*, 2019; Prata *et al.*, 2018; Bosker *et al.*, 2019). Additionally, on a study realized in *Ceratopteris pteridoides*, PS NPs shown capable of inducing cytotoxicity, genotoxicity, and oxidative damage and reducing spore size and

their adhesion to spore surface, prevented germination from 10 to 88% simply by damaging the spores physically (Yuan *et al.*, 2019). PS beads of different sizes have demonstrated capable to modify the correlations between the microbial metabolism and photosynthetic activity, which in turn has been proven to affect plant growth. Although there is a lot of remarkable literature regarding MPs and NPs effects on plants there is still a huge gap in knowledge, since it is evident that interaction of MPs and phytotoxic effects on plants are diverse and based on MP type, age, size, shape, surface charge, plant growth media, plant species, and its growth stage, moreover most of these studies were conducted under hydroponic settings, while just a few publications were based on pot tests. Therefore, the results are still debatable, and further research is required to understand clearly how MPs/NPs interact/behave with plants to obtain reliable results for various plant species.

3.2.5.4. Aquatic Organisms

As mentioned before, lower trophic organisms, specifically invertebrates, can ingest and accumulate microplastic particles, thus, it is likely that microplastics will be introduced to the food web through different pathways, as shown in **Figure 14**. Hence, it is not out of line to infer that the microplastics are passed all around through different kinds of organisms and environments. As Wright *et al.* (2013) documented, the occurrence of MPs in myctophid fish and Hooker's see lion and fur seals scats suggest there is an actual transference of MPs along the food chain and consequent transference of contaminants. Other studies like (Chouchene *et al.*, 2023) confirmed this in the marine environment as well as the impacts caused in said organisms due to MPs ingestion.

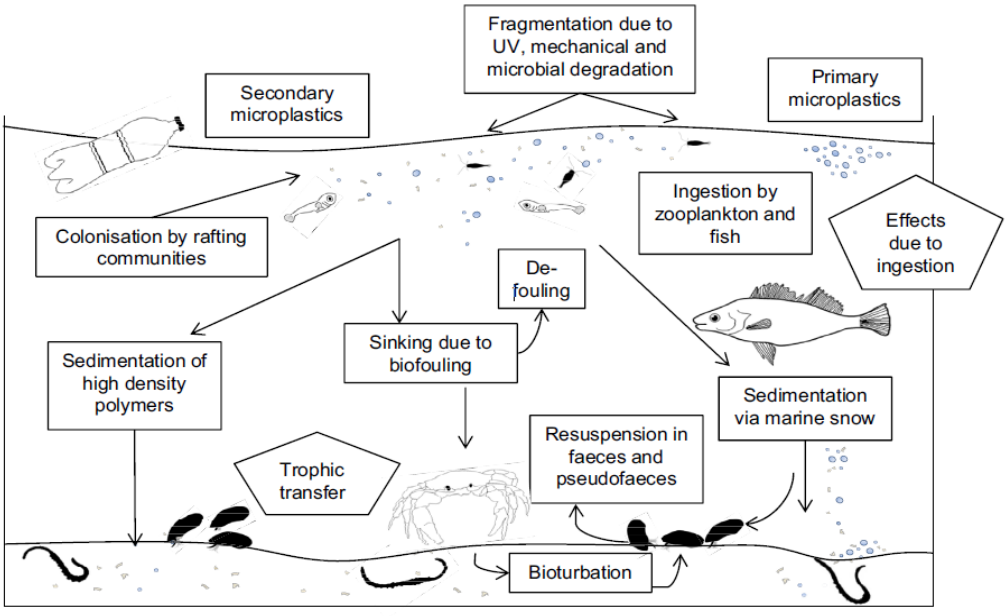

Figure 14 - Potential pathways for the transport of microplastics and its biological interactions. Obtained from Wright *et al.*, 2013.

Chouchene *et al.* (2023) were able to collect and summarize relevant information about the effects on marine organisms in different trophic levels, from a collection of 512 papers from the period 2013 – 2022, covering different impact "pathways" or "sources" ("pollutant", "additives", "metals", "pharmaceuticals", "pesticides", "toxic", "ingestion", "plasticizers", "bioavailable" and "adsorb") from these ingestion ones were the focus, for further comprehension of the effects it is suggested the reading of their review. Starting with fish it was found that MPs ingestion led to mortality (Yu *et al.*, 2019), bio accumulation, liver stress and tumour formation (Miloloža *et al.*, 2020), toxic effects on feeding, fecundity, and survival (Cássio *et al.*, 2022), impacts on development, disparities among pro- and antioxidant metabolic activities, deterioration of liver metabolism, generated oxidative stress and caused the deposition of particles in the liver, gills and gut (Kleinteich *et al.*, 2018), caused damages to the intestines as well as morphological deformations (Vagi et al,. 2021), neurotoxicity in locomotor activity (Cássio *et al.*, 2022), accumulation of MPs in the stomach and intestines (Galafassi *et al.*, 2021). In crustaceans the found uptake, accumulation, and immobilization (Rist *et al.*, 2019; Arp *et al.*, 2021; Công & Pham., 2021), gut retention (Kokalj *et al.*, 2022), significant increase in corticosterone levels, and negative effects in survival and the feeding of offspring (Kokalj *et al.*, 2022). Finally, in molluscs they found malformation development defects (Fonte *et al.*, 2016), changes in shell length growth rate (Rist *et al.*, 2019), increase of the respiration rates as well as changes in benthic assemblage (Jakubowska *et al.*, 2022; Cássio *et al.*, 2022). The authors further explore the impacts focusing on the pollutants and their direct effects on specific organisms.

3.2.5.5. Vertebrates

From another literature review done by Puskic *et al.* (2020) out of 290 documents, 82 made significant remarks about MPs ingestion from different types of animals and environments around the world. From these, they were able to conclude the presence of negative effects in birds, mammals, and reptiles. It was shown that the MPs can cause the formation of reproductive cysts and delays in chick growth and sexual maturity (Roman *et al.*, 2019), they also verified the presence of blockage, obstruction, perforation in the birds GI tracts (Roman *et al.*, 2019b), the plastics – contaminants (namely POPs) interaction (Herzke *et al.*, 2016), increased trace elements in 2 different species (Lavers *et al.*, 2016), punctured stomachs (Carey, 2011; Brandão *et al.*, 2011), alteration of the blood chemistry and negative impacts on morphometric as well as a general deterioration of birds condition (Lavers *et al.*, 2014).

Mammals presented various significant negative effects from plastic particles ingestion such as lesions, suppurative ulcerative dermatitis, perforation of the digestive tract, abscessation, suppurative peritonitis, septicaemia, physical blockage by plastic, reduced mucin, changed microbiome, hepatic stress, and obstruction of the GI tract (Unger *et al.*, 2017; Lusher *et al.*, 2015; De Stephanis *et al.*, 2013; Liang *et al.*, 2018; Attademo *et al.*, 2015).

From the reptile species it was found that the ingestion of these particles resulted in Obstruction and different pathologies, emaciation, and a consequent reduction in the animals' conditions (Ryan *et al.*, 2016; Santos *et al.*, 2015; Campani *et al.*, 2013)

3.2.5.6. Humans

There are already multiple records and sighting of micro- and nano plastics throughout our whole body such as placenta (Ragusa *et al.*, 2021), digestive, reproductive, and nervous systems (Yin *et al.*, 2021; Hua *et al.*, 2022) and muscles (S. Wang *et al.*, 2021), thus, representing a worrying subject of research.

The presence of micro- and nanoplastics in the food chain poses a risk to human health as plastic waste grows. It is remarkable that micro- and nanoplastics are found in numerous food items due to their widespread bioavailability and ubiquity in both aquatic and terrestrial environments, as we seen they are already established in most environments and are affecting many organisms which serve as food sources. Either through animals which consume other animals or plants, thus becoming contaminated with the plastic particles, or during food production processes and/or leaching/fragmentation of plastic food packaging, the sources of plastic ingestion for humans are countless (Santillo *et al.*, 2017; Karami *et al.*, 2017; Mason *et al.*, 2018). Just as we seen MPs and NPs have been found multiple foods like rice, wheat, lettuce, cucumbers, carrots, onions, spring onions, barley, honey, beer, salt, sugar, fish, shrimps, bivalves, and even tap and bottled water (Yee *et al.*, 2021; Ullah *et al.*, 2022).

Yee *et al.* (2021) compiled some articles which studied the presence/occurrence of microplastics particles in tap and bottled water. From this study it was concluded that the average human is consuming around 39,000 to 52,000 MP particles/year, variating with age and gender. If we consider inhalation the numbers rise to 74,000 to 121,000 MP particles/year.

Curiously, an individual who only consumes bottled water consumes an extra 90,000 particles in comparison to those who only drink tap water. So, it is more than clear that MPs are all over the place and are strongly embedded in our food chain. There are 3 main paths for MPs and NPs to enter our system: Inhalation, ingestion, and skin contact, just as Yee *et al.* (2021) represented in the following graphic presentation, **Figure 15**.

GI Exposure

As recent studies present, humans mostly consume plastics through ingestion, and whilst there are no studies pointing micro- and nanoplastic toxicity in humans, there are still research showing their presence in the food and drinks we consume, as previously stated. There are still no studies point the impact these particles have on our GI tract, so its pertinent we further investigate their route, whether they remain in the gut lumen or if they translocate across the gut epithelia, as Yee *et al.* 2021, stated, it is unlikely that these particles permeate at a paracellular level due to their size. However, it is possible that they enter through

lymphatic tissue, and particularly by phagocytosis or endocytosis and further infiltrate the microfold (M) cells in Peyer's patches.

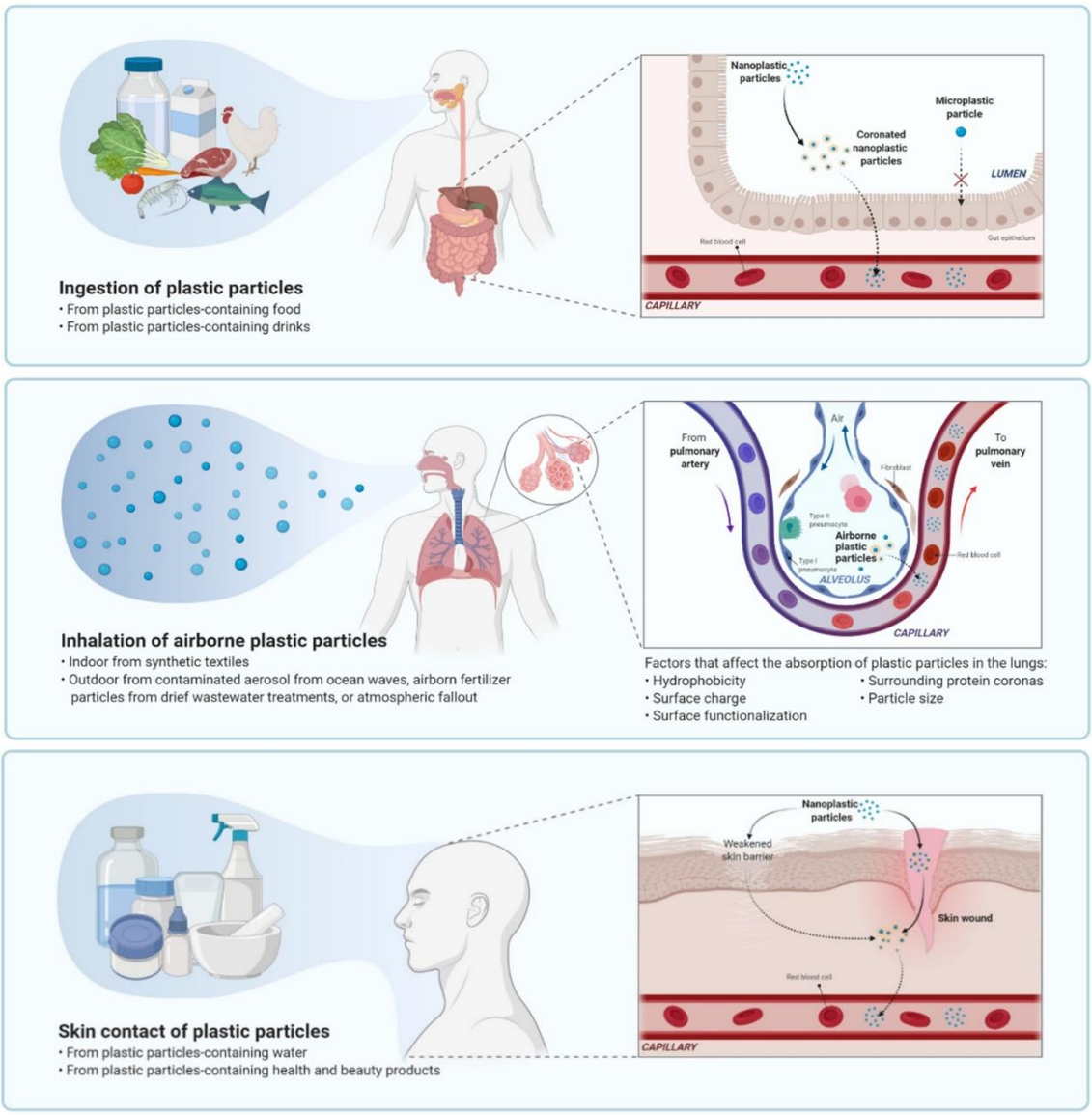

Figure 15 - MPs and NPs entry pathways to the human system. Obtained from Yee *et al.*, 2021.

From a study on mice, it was observed PMMA and PE particles entering the peritoneal macrophages through phagocytosis, however, they only presented an adsorption in intestinal tracts of 0.04 – 0.3%. So, there is a significant probability that MPs can enter, circulate, and remain in our system by permeating the gut epithelium. Like MPs, NPs do not present an actual direct threat independently of their adsorption, size, and structure. Researching the rates of nanoplastic absorption represents a challenge to the lumen of the GI tract. Nanoparticles change after being ingested, which affects absorption capacity and rates. Nanoparticles may interact with a variety of substances in the GI tract, including proteins, lipids, carbohydrates, nucleic acids, ions, and water, leading to the encompassing of these particles by a collection

of proteins known as “corona” permeabilizing the nanoparticles translocation, and aggravating their accumulation and deposition.

Pulmonary Exposure

Inhalation takes the second place in key pathways. Alongside $PM_{2.5}$ particles, microplastic circulate through air, primarily from synthetic textiles, aerosols, airborne fertilizer particles, industrial emissions, and even particles from dried wastewater treatments. The lungs' alveolar surface area is substantial – about $150m^2$ – and their tissue barrier is very thin – less than $1\mu m$. Because this barrier is permeable to nanoparticles and allows them to enter the capillary blood stream, they can disseminate freely throughout the whole human system. Knowing they can circulate easily and these particles toxicity, chemical toxicity, and capacity to introduce pathogens and parasites, it is important that we further study this subject. Given these particles size range, they can easily aggregate and deposit deep in the lung and remain on the alveolar surface or even translocate to other parts of the body. Along their size, certain factors as hydrophobicity, surface charge, surface functionalization, and surrounding protein coronas may affect adsorption and expelling of these particles from the lungs. Additionally, from the studies on animals, we can positively correlate occupational exposures with higher rates of pulmonary inflammation and cancer. Synthetic fiber particles constitute as the principal microplastic of atmospheric fallout both in urban and suburban areas of Paris, 29% being of petrochemical origin. Considering the average atmospheric flux of fibers, fiber dimensions and densities, it is estimated that 3 – 10 tons of MPs are deposited every year by atmospheric fallout. Urban areas duplicate this number in relation to suburban, with rainfall as an aggravating factor (Dris *et al.*, 2016).

From a study of Dris *et al.* (2017) we can observe that indoor environments present a significantly larger number of particles per m^3 than outdoor environments, where in this last one, most of the particles found are of natural origin.

Dermal Exposure

As pointed out before cosmetics constitute one of the main sources of micro- and nanoplastics, particularly in cleanser and exfoliating products, nanocarriers for drug delivery also present as a very important exposure route, although there is no evidence of direct impacts from this last route, small particle size and stressed skin conditions are critical factors to skin penetration. Our skin is protected by the stratum corneum, the outer layer of epidermis, which forms a barrier against injuries, chemicals, and microbial agents. Even if MPs or NPs contact with our skin through multiple cosmetics or medicines, or plastic contaminated products, it is unlikely that they we will be able to penetrate it or even be absorbed by it, however, plastic particle could see their way in through sweat glands, skin wounds or even hair follicles. In a study published, they found that 20nm – 200nm particles aggregated around the hair follicles of a pig’s skin, nevertheless, there were no records of them being capable of penetrating deeper skin tissue, having concluded that

particles with a diameter around 20nm – 200nm can only infiltrate de top skin layers down to a depth of 2 – 3μm. In other studies, a different outcome was verified, the authors observed a deeper reach by the plastic nanoparticles. Knowing that exposure to UV radiation causes skin damage, the use of "invasive" or "destructive" cosmetics and medicines can be weakening the skin even more, since many contain chemicals that permeate the skin barrier such as short chain- and long chain-alcohols, cyclic amides, esters, fatty acids, glycols, pyrrolidones, sulphoxides, surfactants and terpenes, that are used to enhance the chemical permeation of drugs and others as urea, glycerol and α–hydroxyl acids, which are very common in body lotions. Nonetheless, it is not all bad news since through the analysis of various compositions of lipid lamellae in stratum corneum samples taken from human and porcine sources, it was presented a three-layer "sandwich model", which prevent the infiltration of the plastic particles in undamaged tissue. (Jatana *et al.*, 2016; Lane, 2013; Bouwstra *et al.*, 2001; Campbell *et al.*, 2012; Som *et al.*, 2011; Schneider *et al.*, 2009; Hernandez *et al.*, 2017).

On the cellular level, it was found that there are several cellular absorption pathways and intracellular localization plastic nanoparticles that depend on their physicochemical characteristics. The interaction of plastic particles with human cells is also influenced by their size. The interaction between nanoparticles and cells is significantly different from that of bigger particles because of their high specific surface areas. Additionally, the particle's charge may have an impact on how it interacts with the cell and its structure.

In terms of direct health impacts and toxicity to the human body, there is still little to no information, however in some in vitro studies, many research teams were able to make worrying discoveries, it was stated that both micro- and nanoplastics can cause serious negative impacts e.g.: physical stress and damage, apoptosis, necrosis, inflammation, oxidative stress, and immune responses, **Table 4**, in attachments (Yee *et al.*, 2021).

As mentioned along this document, plastics tend to carry contaminants with them, chemical, heavy metals, and even POPs as well as additives used to produce them. Naturally, open to the different weathering agents, these particles are subject to leaching, resulting in increasing probability of a leakage of these chemicals out of the polymer and into the surrounding environment. For instance, polycyclic aromatic hydrocarbons (PAHs) are commonly absorbed by MPs and are known to have great consequences when consumed (K. Sun *et al.*, 2021). As a result, there is a chance that these chemicals will leak out of the polymer and into the environment. For instance, it has been demonstrated that polycyclic aromatic hydrocarbons (PAHs) are absorbed by microplastics and have a variety of harmful consequences when consumed by diverse animals (K. Sun *et al.*, 2021). Even though these chemicals are easily degraded by our system, these plastic particles serve as very resistant and durable reservoirs for the constant chemical leaching into tissues and body fluids (Engler, 2012). Some usual additives and chemical present in plastics

known to affect human health are bisphenol A (BPA), phthalates, triclosan, bisphenone, organotins and brominated flame retardants (Galloway, 2015). But what are these negative effects that are so worrying?

Well, as graphically represented by Yee *et al.* (2021) in **Figure 16**, many studies have shown that BPA commonly found in food and drink packing can cause a series of diseases such as obesity, cardiovascular diseases and can act as a hormonal disruptor, imitating or blocking the production, action, and function of hormones in the human body. Additionally, BPA is vastly known for affecting brain development in the womb, causing direct damage to the foetus. Also, the human exposure to phthalate esters, very usual in PVC products, such as butyl benzyl phthalate (BBP) and di-2-ethylhexyl phthalate (DEHP) can significantly increase tumour incidence in human, representing a carcinogenic threat.

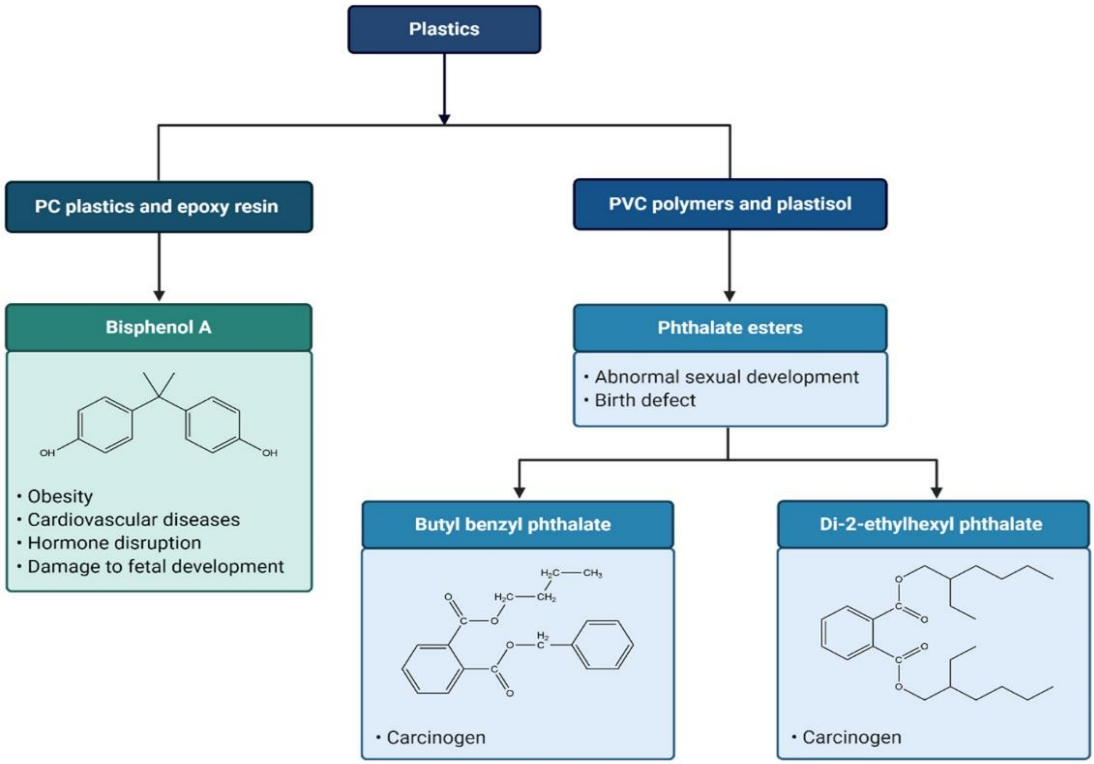

Figure 16 - Overview of the toxic effects of chemicals leaching from plastics. Obtained from Yee *et al.*, 2021.

Although the effects of microplastics and nanoplastics on the marine environment have received extensive study, we have only lately become aware of the possible routes for human exposure. After exposure, absorption by ingestion or inhalation is conceivable. Thus, is needed further study of this inevitable problem we may be facing. Unfortunately, assessing human exposure to both micro- and nanoplastic is far from being granted as the lack of scientific knowledge, validated methods, certified reference materials and standardization across the analytical procedures represent a challenge to advancements.

Inevitably and very unfortunately the hazardous effects of different types of micro- and nanoplastics to human health remain largely unknown.

3.2.6. From Start to Finish: The origin, path and destiny of these particles

As we have noticed, plastics are everywhere and there are many ways these can reach the different environments and there are many factors which can affect their concentrations. Therefore, it is increasingly important to link sources, particle behaviours and transport mechanisms, to better understand how and where microplastics will accumulate. Although the most commonly found plastic particles are fragments resulting from the weathering of larger plastic items (secondary microplastics), there are multiple sources of direct plastic pollution such as direct littering and inefficient waste management (primary microplastics) involving products like nurdles, granulates, powders, microbeads, synthetic clothing, toothpaste, scrubs, facial cleansers and nanoparticles made of plastic (Leslie *et al.*, 2017; Auta *et al.*, 2017; Horton & Dixon, 2017) which can come from either point sources, when we can pinpoint the potential origin of the pollution (e.g.: Wastewater Treatment Plants, industrial spillages, or others) WWTPs or diffuse sources, when there are multiple potential sources of pollution becoming impossible to pinpoint (e.g.: a domestic sewage net) (Leslie *et al.*, 2017).

Every microplastic has its origin on land so, a way or the other, there is where it all begins. As mentioned above, we can have multiple direct pollution sources and in land some are loss of content during the waste disposal chain, industrial spillages, release from landfill sites, improper disposal of domestic litter agricultural malpractices which use plastic in a variety of ways as mulches, bale twine and even wrapping (Nizzetto *et al.*, 2016). In addition, we can have an (in)direct input of secondary MPs using fertilizers like sewage sludge trivially collected from WWTPs, which unfortunately are a granted source of microplastics (125 and 850t of microplastics/million habitants are added annually to plantations just from sewage sludge), since it is already known that WWTPs are very effective at removing these particles, with an often success rate of 99% (Horton & Dixon, 2017; Nizzetto *et al.*, 2016; Carr *et al.*, 2016; Murphy *et al.*, 2016). Although these particles are commonly transported to freshwater systems, it is not granted that they will and this means that we sometimes may have a great accumulation and maybe increase the negative effects of these particles, a study found that sludge fertilized agriculture soils can retain MPs up to 15 years, and that the hotspots resulting by these accumulations can reach depths up to 25cm when in presence of high downward drainage flow. Additionally, the retention of MPs will be facilitated by processes such as bioturbation, drawing MPs from the surface into deeper layers of soil. Soils with higher permeability and lower rates of overland flow such as agricultural and forest one's are more likely to retain due to higher osmotic forces and easier percolation through the soil (Nizzeto *et al.*, 2016b).

When transported, they enter the most complex system regarding MPs transport and retention, freshwater systems, since they are polluted from the terrestrial environment, guide the particles to the marine environment, breakdown larger plastics into smaller particles and can contain retaining "sinks" throughout the whole system. Additionally, freshwater systems present a higher complexity just by themselves, there are rivers, streams, ditches, lakes, and ponds, all very different in many ways. But how do these particles get there?

Inadequate waste disposal, littering, wind, surface runoff, agricultural drainage, and runoff, resulting from the use of plastics and sewage-sludge, storm drainage and urban runoff which can contain from large plastic items, degraded road paint and even wear from vehicles, the late one having shown to be the main source of microplastic particles in recent studies (Reddy & Lau, 2020; Horton & Dixon, 2017). Although WWTPs are great at removing these particles they can suffer unfortunate consequence from storm events, the high flow conditions combined with combined sewage overflows, very common during autumn and winter heavy rains, are design to drain untreated sewage into the closest river or freshwater system to pressure on drainage systems and assure its stability and integrity, thus, releasing great amounts of MPs particles into different systems (Horton & Dixon, 2017).

River wise, MPs will be subject to the exact same processes as other sediments like sand and silt, and we will verify that the faster the river flow the more energy it has. Consequently, it can easily entrain and transport a higher number of particles (Knighton, 2014). Being supply-limited, rivers can transport all particles entering them. Despite plastics buoyancy there is a handful of factors which may affect the aggregation or occurrence of MPs, namely slow-moving sections of the river with high sediment deposition, population density, levels of urbanization and industrialization within catchment areas, rainfall rates and the presence of artificial barriers like weirs and dams (Lebreton *et al.*, 2017). It is factual that many MPs are indeed buoyant and float, however, density and shape play a big role in the transportation. Depending on the particle density in may naturally sink, but it is not linear since particles are subject to biofouling where the particle is dominated by microalgae and can retain contaminants thus affecting the particle's density and leading it to sink and deposit. In addition, size and shape also affect the retention, as stated by Bridge & Bennett (1992), irregularly shaped particles have higher complexity settling mechanics compared to spherical particles. In the case of riverbed sediments, larger MPs shown to be more likely to be retained (Nizzetto *et al.*, 2016b) but when compared to previous studies we see that shape may play a bigger role than size, with larger particles being more likely to be mobilized rather than fine, spherical ones (Prager *et al.*, 1996). This uncertainty shows how difficult and how little we understand these particles, given their complexity. As we will further see sediment transport and deposition in rivers also has a great degree of temporal and spatial variability (Horton & Dixon, 2017; Eo *et al.*, 2018). At a smaller scale, changes in turbulence can enforce energy to a certain area of the riverbed and affect previously deposited particles. At

larger scales, high energy, storm like, flows from floods may lead to resuspension of denser MPs along with the remaining sediment particles. Also, changes in the river's channel morphology can induce erosion of the river bars or banks, causing a reorganization of previously deposited MPs in the floodplains as documented with heavy metals (Horton & Dixon, 2017).

As previously stated, rivers are not the only freshwater system, lakes and ponds also suffer from wind-transported particles and debris, and land runoff. However, contrarily to rivers, due to their enclosed nature these environments tend to deposit the particles and accumulate at higher rates, the fact that there is retention and incorporation of MPs it is likely that there will be a burial of these and that they will be preserved for a long time (Vaughan *et al.*, 2017).

After entering the fluvial systems, they can have 2 ends, either the marine environment or get retained somewhere along the way. The first ones have already been widely studies and are seen as huge sinks of MPs. The matter is transported along the river until it disembogues into the ocean where it encounters a "barrier" caused by the greater energy of the waves. The material is transported parallel to the coast and due to the action of the wind on the waves, these adopt an oblique movement. The combination of these two factors generates the "coastal drift" or "longshore drift" mechanism responsible for the transport and deposition of sediments along the beach. (Nichols, 2009). Oceans receive this astronomic input of particles from rivers but there is also a great number of direct inputs such as mismanagement and improper disposal of maritime or fishing waste, abandoned fishing gear, cargo loss, spillage/ illegal dumping, sea recycling ports and landfills and, through zooplankton or other animals, however, according to Andrady (2011), these marine events only account for 20% for marine pollution whereas land based activities account for the remaining 80% . Once present in oceans, these particles can, very rapidly, be transported throughout the world in currents. When we take in account the vertical transport affecting factors like biofouling, egestion in faecal pellets and incorporation into marine snows (sinking detritus), we comprehend how they can reach such high/deep environments like the deep sea and artic cores (Horton & Dixon., 2017).

We have covered land, freshwater, and marine environments, however there is one lasting, which is the least studied regarding this subject, the atmosphere. Given the light nature and potential for widespread dispersal of MPs, they are subject to transportation through air currents, or "urban dust" constituted by PM particles, tyres, and paint particles and, synthetic textiles, which are posteriorly deposited in land or aquatic environments. The transportation of these particles through air albeit most common in highly populated areas, can be lead through currents far away from its source thus being difficult to trace and pinpoint their origin. Additionally, in the presence of heavy rainfall, the deposition of these particles is eased, aggravating its tracing. (Horton & Dixon, 2017).

In an effort to clarify the sources, transport and fate of MPs, it was conceptualized and designed a "Plastic Cycle", **Figure 17**. Regarding movement, transport, and fate of particles in these environments, there is not a singularity, they work as whole and are interlinked with indistinct and permeable boundaries. Depending on environmental factors and conditions, the connectivity of these systems can highly influence and affect the abundance and fate of MPs, and it is not easy to delineate a tendency since they are very variable in space and time.

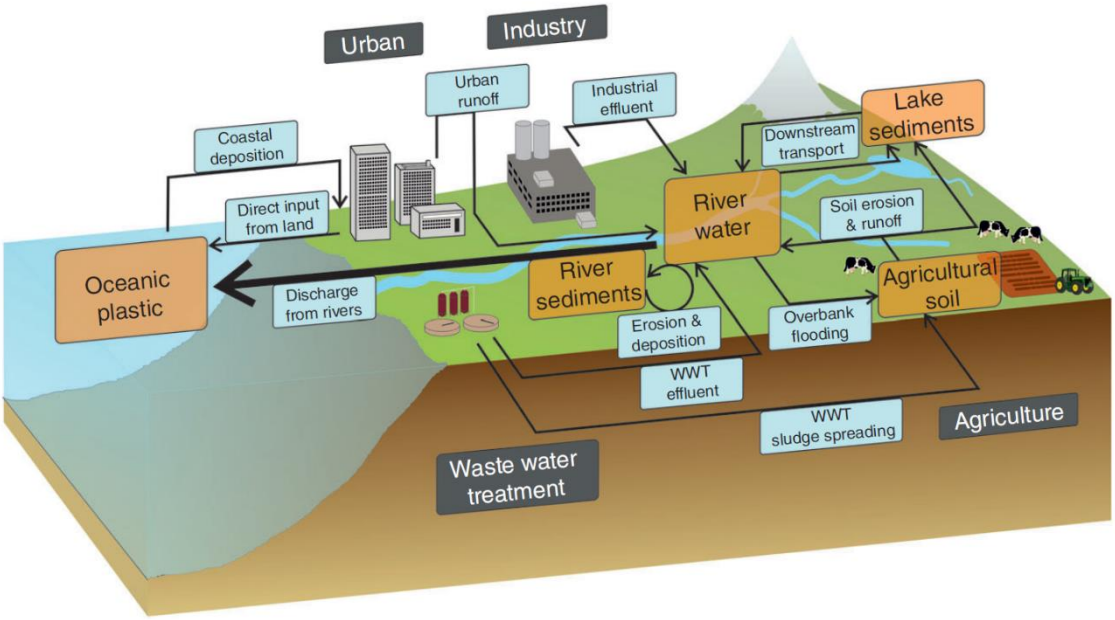

Figure 17 - Conceptual model representing the "Plastic Cycle". Retrieved from Horton & Dixon, 2017.

In conclusion, there many key pathways by which microplastics may travel along these systems. Nonetheless, the main one is from land to the marine environment, but it is not granted that once they reach the ocean, they will stay there since there are material returning events like high tides, wash ups, floodings and storm events and we can have blurry systems like estuaries, which depend of river and ocean tides, and dryland rivers which can suffer high tide variations throughout the seasons and cause "large-scale pulses" of MPs transport, due to previous deposition during dry seasons (Tooth, 2000). To further understand these particles, we should seek to consider these relations and interactions, as they may be a key to comprehend how they may inhibit, alter, or facilitate the movement, or retention of MPs (Horton & Dixon, 2017). With this said, the purpose of this work is to further understand how these particles behave in the fluvial systems and how their occurrence and concentration is affected by geographic and demographic factors like urbanization, presence of agricultural sites/fields, WWTPs and effluents.

4. State of Art

The study of micro- and nanoplastics is still a very recent subject. Hence there is not a very solid ground in terms of protocol.

It orders to compile all the information Prata *et al.* (2019) reviewed the most common methodologies from the sampling, density separation, sample processing, identification, chemical characterization, and quantification to even the mitigation of cross-contamination, in different environments. In addition, the reader, if interested, should investigate Comprehensive Analytical Chemistry by Teresa Santos and Armando Duarte, as it is a great book to understand the problematic, methods and what to expect from the study of microplastics.

There is still no consensus regarding the choice of the sampling site, however, some articles like Besley *et al.* (2016), suggested collecting along 100m stretches parallel to the coastline, covering the whole beach profile, since it gives the necessary representation taken the dynamics into account. Although this method was applied in the beach environment, it could be adapted to fluvial, agricultural, and open field sampling sites.

4.1. Sampling

4.1.1. Water

With the sampling site defined is time to collect the samples, the method of choice will depend not only on the sampling environment, but also the material available and the purpose of the study.

Prata *et al.* (2019) compared multiple sampling methods in water (Neuston and Manta nets, Plankton nets, Sieving, Pumps and Filtration or Sieving *ex* situ) as in sediment (Beach sediment collection and Seabed collection). From their review we can highlight some advantages and disadvantages of these methods regarding, cost, resource-consumption, contamination, detection and retention limitations, manipulation of the instruments, difficulty, and uncertainties.

Microplastics organize along the water column according to their physical properties, such as density, shape, size, adsorption of chemicals and state of biofouling, and environmental factors, water density, wind, currents, and waves (Prata *et al.*, 2019). Taking this into account, the chosen method is not

the only factor affecting the quantity and quality of the MPs recovered as it we will depend greatly on the location, depth, and conditions. Fortunately, both fresh and saltwater can be sampled under the same methods, facilitating the choice of the method and enables future standardization.

The use of nets, sieves and pumps allows to reduce sampling size while maintaining representativeness, here we will see characteristics of each, advantages, and disadvantages. Neuston nets or Manta nets allow a sampling closer to the surface or near-surface, they have a very intuitive use, allow the collection of large samples and between different locations, which makes them a preferred choice. However, these nets are expensive, require a boat, are very time consuming, increase potential contamination and limit detection a lot since they can only retain down to 333μm (Prata *et al.*, 2019).

Additionally, we have bongo nets which are used to gather replicate samples, nylon nets (100μm) which present concentrations of MPs 100 times higher than manta nets, and plankton nets. The last one is a great sampling instrument given its capacity to not only to sample horizontally but also vertically and obliquely, they also allow a very fast sampling – under a minute – and recovery rates 30 times higher than manta nets, in terms of fibers, 80μm plankton nets reported to reach a retention capacity 250 times higher than the 330μm manta nets. However, since it as a very small mesh size – around 100μm – it tends to be hauled at low velocities and thus can easily become clogged by organic matter and mineral material, limiting the volume of water sampled. A possible solution to this complication is to use pumps (Dris *et al.*, 2015; Dris *et al.*, 2018; Jin *et al.*, 2009)

A less techy sampling option is to collect with buckets or event glass water bottles with posterior on shore filtration or sieving, but as obvious this becomes very time consuming, limits sampling volumes, risks lack of representativeness and can promote contamination of the samples.

Some measures we can take to control representativeness and assure the least contamination possible is by using steel meshes and metal or glass materials as well as defining a minimum volume of sample to achieve the desired representativeness. Nevertheless, this still brings us to an inevitable decision, to choose between representativeness or contamination, as metal/glass materials are normally associated with limited volumes and plastic nets and pumps add up some plastic particles to our samples.

Although all these methods have proven to be great choices, NOAA, and other authors, prefer manta nets followed by sieving, as they allow the sampling of large volumes of water and are already commonly used, allowing some standardization of methods (Prata *et al.*, 2019).

4.1.2. Sediment

As stated previously, MPs distribution is widely affected by vast range of factors, making it uneven and dependent on their properties and the environmental conditions, thus the results will depend on the specific area where we are sampling (e.g.: high tide line, intertidal regions, and others) as well as depth.

Having this into account it is important to choose the right sampling site and instrument. For sediments we have two main sources, inland or in river/seabed. Inland it is very direct and intuitive, we can either follow methods like Schröder *et al.* (2021), where it is drawn a 50x50cm square on the ground and removed the first 1-2cm to assure more trustworthy results, as the top layer is more affected by dynamics, and we might risk compromising representativeness. Then, it is not only important to remove a good depth of sediment since the 10cm present a higher concentration of MPs compared with deeper layers but also grant a good number of replicates, where Besley *et al.* (2016), suggest that 11 is the perfect number, having shown a confidence level of 90%. In addition, NOAA recommends the use of 400g/replicate. For this process we can use either a metal shovel or similar instrument, or a forceps device with a defined extraction volume.

River and Sea wise, we can either collect with the assistance of a boat or from inland depending on our desired region of the substrate. Either way we can collect the sample with a grab sampler, a box corer or for example a metal bucket tied to a rope.

4.2. Separation

4.2.1. Filtration or Sieving

Once we have our samples ready, we need to find a way of obtaining the MPs to further quantify and characterize, for that we need to separate them from the rest of the sample.

For this process we have 2 steps:

- Reduction of sample volume
- Density separation.

Both sediments and water samples can and should be passed through sieves. The first ones are ought to be larger and followed by the separation and filtration of the supernatant with the help of filters, whereas the water samples can use a smaller mesh size – 0.45 to 55500µm – and reduce the sample directly *in situ* through, for example, nets, followed by filtration or sieving. Additionally, for river and ocean samples there are other not so common separation techniques such as magnetic separation and the use of biotic organisms (Nabi *et al.*, 2022)

4.2.2. Density Separation

Notably, compared to sediment, which possesses a density within the range of 2.7 g/cm^3, microplastics have a significantly smaller density range (0.8-1.6 g/cm^3). The fundamental idea behind successful separation is this differential density property.

The process consists of carefully combining the sediment with a salt solution. The residual solution is then given time to settle, during which a supernatant containing the microplastics is removed from it. It is crucial to remember that the density of microplastics may be affected by several variables, including the additive concentration, the kind of polymer, and chemicals or organisms adsorbed onto them. This highlights the complex nature of density-based separation, making careful evaluation of these factors necessary.

Is important to note that the selection of the salt solution is an essential aspect of microplastics research and should be considered in addition to the previously described approach (Tirkey & Upadhyay, 2021). For this purpose, sodium chloride (NaCl) solution, which has a density of 1.2 g/cm^3, has been used in several research since it is both affordable and recommended by reputable organizations like the MSFD Technical Subgroup of Marine Litter and NOAA. It is important to keep in mind that high-density polymers like polyvinyl chloride and polyethylene terephthalate may not be efficiently separated by NaCl because of its comparatively low density (Frias *et al.*, 2018). On the other hand, NaCl can easily separate lower-density polymers like polypropylene or polyamide (Imhof *et al.*, 2012). Researchers have looked at the usage of other high-density salts, such as sodium iodide (NaI), which has a density of 1.8 g/cm^3, to solve the constraints of NaCl while being more costly (Stock *et al.*, 2019). This novel two-step density separation method has been developed where the sample is initially fluidized in NaCl solution to reduce the sample size by 80%, and then it is floated in NaI solution, demanding less NaI due to the smaller sample size (Nuelle *et al.*, 2014).

The study conducted by Quinn *et al.* (2017) revealed that both NaCl (1.2 g/cm^3) and NaBr (1.4 g/cm^3) exhibited low recovery rates (<90%) and relatively larger error margins. In contrast, NaI (1.6 g/cm^3) and ZnBr2 (1.7 g/cm^3) demonstrated the ability to effectively separate heavier polymers with impressive recovery rates of 99% and minimal error variations.

Notably, the separation process using NaI and $ZnBr_2$ is advantageous as it necessitates only a single washing step for sediment, as opposed to the three required when using NaCl. However, it is essential to consider some caveats. NaI can react with cellulose filters, leading to discoloration, which can complicate visual identification. On the other hand, $ZnBr_2$ is considered hazardous to the environment and is relatively expensive. These issues can be mitigated by adopting sustainable practices, including careful filtration and density adjustment for $ZnBr_2$, rendering it more environmentally friendly and cost-effective.

Furthermore, NaI has demonstrated a remarkable ability to recover oleophobic fibers (93.3%) compared to other substances like $CaCl_2$ (69%). When combined with MeOH, NaI can effectively recover the majority of microplastics from marine snow (90-98%). Additionally, following the protocol established by Kedzierski *et al.* (2017), NaI can be recycled for up to 10 cycles through processes involving rising and evaporation steps. This recycling approach results in costs like those associated with NaCl. Therefore, the utilization of NaI is recommended, primarily due to its environmentally safe attributes and the potential for multiple cycles of recycling, with the caveat that cellulose filters should be avoided in the process.

Table 5, in attachments, adapted from Frias *et al.* (2018), provides a summarized overview of the characteristics of different salts employed in density separation methodologies, aiding in the selection of the most suitable salt solution for specific microplastics research objectives. Tirkey & Upadhyay (2020), also compilated the different advantages and disadvantage of the saline solutions.

The Sediment-Microplastic Isolation unit, also known as the MPs isolation unit, is an apparatus consisting of two interconnected tubes with a valve that enables the separation of supernatant and sediment. It is regarded as a dependable method for the safe removal of plastic particles, custom-built to extract microplastics from sediments in a single phase, with an average efficacy of 95.8%. This method employs $ZnCl_2$ solution with a density of 1.5 g/cm^3, enabling the removal of plastics across a range of sizes, from large to small particles. It is worth noting that this unit can also be used with other salt-saturated solutions like NaI. It allows sediments to settle while simultaneously facilitating the flotation of dense microplastics. Remarkably, it achieved a 100% recovery rate for large plastic particles, with a slightly lower removal rate of 95.5% for smaller plastics (Imhof *et al.*, 2012). While this system is efficient and reliable, it is worth noting that it can be relatively expensive to construct, making it a valuable tool for the identification and quantification of plastics in environmental samples.

There are other explored methods such as, elutriation which is a separation method that involves injecting a liquid, typically water, into a column to isolate buoyant microplastics from settling organic matter and sediment. Microplastics are then collected in a mesh within the column and subsequently separated using dense solutions like NaI. Elutriation offers cost-effective and efficient microplastic separation from large sediment volumes, enhancing environmental representation and reducing the sample volume for density-based separation. However, this method is time-intensive, taking at least an hour per sample, and necessitates prior sieving by size range.

The Munich Plastic Sediment Separator (MPSS) employs a similar approach, using a dense solution of $ZnCl_2$ injected at the column's base, allowing microplastics to ascend and be collected in the supernatant. Nevertheless, this method is more time-consuming, with settling phases taking up to 1-2 hours (Imhof *et al.*, 2012).

Another technique involves the use of oil as a separation method due to the hydrophobic properties of plastics. Various oils like pine oil and canola oil have been tested, but they exhibit recovery rates that vary. Canola oil, for example, has shown a shorter sampling time (~2 hours) and good recovery rates (96.1%), making it more efficient than salt-saturated solutions. Olive oil has been added to such solutions to enhance recovery rates from 64% to 82%. While oil-based methods have some limitations and require a cleaning step, they can be combined with saturated solutions to improve recovery rates, providing an alternative approach in microplastics separation (Prata *et al.*, 2019)

To further complement the background of separation techniques **Table 6**, in attachments, gives a general framing of the mentioned above and further presents some other uncommon techniques such as JAMSTEC and Heat Assisted density separation (Nabi *et al.*, 2022). It also includes the digestion of organic matter which will be discussed further.

4.3. Sample Processing

Biomaterial can be found in environmental samples. For example, according to Crichton *et al.* (2017), silt from beaches contains between 0.5 and 7.0% of biological material. Biological material is frequently mistaken for plastics (such as darker algal pieces), which causes environmental concentrations to be overestimated and increases the number of particles undergoing further analysis. In order to properly identify and characterize the microplastic components of the water sample, it is important to perform a pre-processing procedure to get rid of other interfering organic debris. Check **Tables 6 and 7**, in attachments, for reading aid. They summarize the digestion techniques, the undergone treatment, the recovery rates, and effects both on organic matter and MPs, as well as a supplement for the density separation methods.

4.3.1. Acid Digestion

Acid digestion is a method used to degrade organic matter in environmental samples. However, it is important to note that some polymers, like nylon, PET (polyethylene terephthalate), and others, are susceptible to degradation in the presence of acids, particularly at high concentrations and elevated temperatures. Achieving an optimal balance of acid concentration and temperature is crucial for effectively removing biological material within a reasonable timeframe.

For instance, Naidoo *et al.* (2017) demonstrated that heating nitric acid (HNO_3, 55%) to 80°C significantly accelerates the digestion of fish tissues, making it 26 times faster.

Hydrochloric acid (HCl) appears to be the least effective treatment for dealing with significant amounts of biological material. However, some studies, like that of Karami *et al.* (2017) have reported digestion efficiency exceeding 95% with HCl (37%) at 25°C, although it led to the melting of PET.

Additionally, research by (Desforges *et al.*, 2014) revealed that treating a sample with HNO_3 for one hour resulted in the complete dissolution of zooplankton tissues, while a mixture of HNO_3 and HCl led to the fragmentation of zooplankton bodies into smaller pieces.

These findings underscore the superiority of HNO_3 over HCl for effective acid digestion in microplastic analysis experiments, given its ability to dissolve biogenic compounds and maintain sample integrity.

It is also known that HNO_3 can leave oily residues, tissue debris, cause the loss of nylon, and melting of various plastic types, including PS, LDPE, PET, HDPE, PVC, and others. Resistance to digestion varies among polymers and depends on factors such as the presence of organic matter in the sample, which can mitigate the degradation of polymers, and the temperature of the solution.

Given these complexities, the use of acid digestion should be approached with caution in microplastics research, as it may lead to the underestimation of microplastics in environmental samples due to potential alterations and degradation of plastic materials.

4.3.2. Alkali Digestion

The utilization of alkali digestion as an alternative to acid digestion in microplastic analysis holds substantial promise. However, it is essential to acknowledge that alkali digestion may introduce certain challenges. Alkali digestion has been observed to potentially damage or discolour plastics, leave oily residues, and cause the redeposition of tissue residues on plastic surfaces. These effects can complicate the subsequent characterization of plastics using vibrational spectroscopy.

KOH (potassium hydroxide) has emerged as a particularly effective alkali for digesting organic matter and recovering plastics. Protocols employing KOH and NaOH (sodium hydroxide), such as a 10% KOH solution at 60°C overnight or a 60°C treatment for 24 hours, have proven to be among the most effective digestive treatments (Maes *et al.*, 2017; Dehaut *et al.*, 2016; Cole *et al.*, 2014). However, it is important to note that both KOH and NaOH may lead to discoloration or degradation of various plastic types, including nylon, PE, uPVC, polyester, PC, PET, and PVC (Dehaut *et al.*, 2016).

In terms of digestion efficiency, certain hard parts, and fats, such as fish otoliths, squid beaks, paraffin, and palm fat, have been found to withstand the digestion process when exposed to KOH (1 M) for 2 days at room temperature. A noteworthy approach involves the sequential use of both acid and alkali digestion, such as a combination of NaOH and HNO_3, which has demonstrated good digestion of biologic material and recovery rates. This method allows for comprehensive digestion while addressing the potential limitations associated with each technique. Overall, the choice between alkali and acid digestion methods should be carefully considered to ensure accurate microplastic analysis while considering the specific

characteristics and potential effects on plastic materials and organic matter (Prata *et al.*, 2019; Nabi *et al.*, 2022).

4.3.3. Oxidizing Agents

In comparison to NaOH and HCl, hydrogen peroxide (H_2O_2), which is normally present in concentrations of 30–35%, is a potent oxidizing agent that may be used to break down organic waste. Notably, it often has little to no negative effects on the integrity of polymers.

Numerous plastic polymers, including PVC, PET, nylon, ABS, PC, PUR, PP, LDPE, LLDPE, and HDPE, exhibit resistance to H_2O_2 treatment (Nuelle *et al.*, 2014). The polymers normally remain intact, notwithstanding the possibility of some mild discoloration. It is important to note, nevertheless, that Karami *et al.* (2017), found that nylon degraded, and PET changed colour after being treated with H_2O_2 (35%) at 50°C for 96 hours. The efficiency of H_2O_2 digestion is strongly influenced by the incubation temperature, Cole *et al.* (2014) proves it by discovering that a seven-day incubation at ambient temperature with H_2O_2 (35%) only resulted in a 25% breakdown of organic materials. On the other hand, employing H_2O_2 (15%) at 50°C overnight, found effective organic matter removal (Avio *et al.*, 2015).

In addition, efforts have been made to mitigate the impact of H_2O_2 on the characteristic properties of microplastic contents in samples, Zhao *et al.* (2017), pointed out that a 15% concentration of H_2O_2 is preferred to a 20% concentration, and both treatments are more effective than HCl. Also, it is recommend using a reduced concentration of 10% H_2O_2 with an exposure time of 18 hours to effectively remove organic materials while maintaining the integrity of microplastics, H_2O_2 treatments have demonstrated potential. NOOA recommends the use of H_2O_2 (30%) with Fe(II) solution (0.05 M)(sulphate (Fenton's reagent) heated at 75°C to glass beaker containing the microplastics fraction for both water and sediment samples. This technique offers a trustworthy way for analysing microplastics, especially when working with intricate organic materials (Frias *et al.*, 2018).

4.3.4. Enzymatic Digestion

Enzymatic digestion has emerged as an alternative method for microplastic analysis. Since they do not distort or degrade the plastic polymers, unlike chemical digestion, enzymes have been used in many studies to degrade or hydrolyse biological tissues. However, enzymatic digestion is also a time-consuming process, and each enzyme operates at its optimal pH and temperature condition, which must be monitored and maintained throughout the experiment. Enzyme efficiency, however, depends on the type of organic material present in the sample (Maes *et al.*, 2017; Courtene-Jones *et al.*, 2017).

Enzyme protocols vary and may include pre-digestion of sediments with an industrial enzyme blend, followed by the removal of debris using H_2O_2. For fish tissues, proteinase K has been used, followed by treatment with calcium chloride and subsequent hydrogen peroxide treatment. While these methods yield high recovery rates, calcium deposition on particles may complicate further characterization (Karlsson *et al.*, 2017).

Other enzymes like Tripsin, Collagenase, and Papain have been tested, with digestion efficiencies ranging from 72% to 88% and no observed effects on polymers (Courtene-Jones *et al.*, 2017). A more comprehensive enzymatic purification protocol has been proposed, achieving 98.3% efficiency through a multi-step process involving enzymes and hydrogen peroxide treatments over 13 days.

Despite their effectiveness, enzyme use is limited by cost considerations. Industrial Corolase 7089, presented as a more cost-effective enzyme, has shown promise in microplastic sampling, outperforming chemical treatments in some cases. However, enzyme-based protocols may require additional treatment with hydrogen peroxide to remove undigested debris. Enzymatic digestion remains a valuable approach in microplastic analysis, especially when handling complex samples, but its widespread use may be constrained by cost factors (Prata *et al.*, 2019).

4.4. Identification and Characterization

There is not a perfect method for MP for chemical analysis of MPs. However, the combination of some or the aim of the study can lead to the choice of the ideal method(s). The most common method is visual identification as most of the times is enough to identify and determine the presence and certain characteristics of MPs, such as type, colour, size, and shape. Nevertheless, it is chemical characterization is advised when we intend to identify its composition, adsorbed elements, and other properties. Some of the most common techniques are FTIR and Raman Spectroscopy, followed by SEM/EDS, and Py-GC/MS, each of them with a particular purpose: determination of chemical composition of sample particles; identify MPs morphology and detect their surface characteristics; identify the compound types of MPs as well as measure absorbed organic compounds, respectively (Wang *et al.*, 2022).

Given the deep analysis on visual identification and characterization methods conducted in the sections 3.2 and 3.3 of this work, it will not be explored in this one. However, in case of doubts or seek of better comprehension, the reading of Prata *et al.* (2019) and Tirkey & Upadhyay. (2021) reviews are recommended. Huang *et al.* (2022) also summarizes the typical methods for analysis and highlights advantages and limitations.

4.5. Cross – Contamination

As we have seen, along this process we can easy influence the results by small action and given the widespread contamination of the environment with microplastics, including air, it is crucial to implement measures during sampling to minimize the introduction of these particles and fibers. To reduce cross-contamination of microplastic samples, the following five rules are recommended:

- Use glass and metal equipment instead of plastics, which can introduce contamination.
- Avoid the use of synthetic textiles during sampling or sample handling and prefer 100% cotton lab coats.
- Clean surfaces with 70% ethanol and paper towels, wash equipment with acid followed by ultrapure water, use consumables directly from their packaging, and filter all working solutions.
- Utilize open petri dishes, procedural blanks, and replicates to control for airborne contamination.
- Keep samples covered as much as possible and handle them in clean rooms with controlled air circulation, limited access (e.g., doors and windows closed), and restricted circulation. Preferably, work within a fume hood or an algae-culturing unit, or cover equipment during handling.

Implementing these measures can significantly reduce the risk of contamination during microplastic sampling and analysis. For instance, the use of a fume hood alone can reduce contamination by up to 50%, while covering samples during filtration, digestion, and visual identification can reduce contamination by more than 90%. These precautions are essential for ensuring the accuracy and reliability of microplastic research (Prata *et al.*, 2019).

4.6. The Implications of Fluvial Dynamics

Marine environments are greatly affected by plastic pollution, and thus by MPs, having been widely studied and documented all around the world. Freshwaters however have not had the same level of attention. In this section it will be documented and discussed the matter of freshwater MPs pollution, paying special attention to the methods presented in the previous sections. Posteriorly, a deeper analysis of two articles published by Eo *et al.* (2018) and Schell *et al.* (2021) was made, since they fit the best with my thesis theme and purpose.

Rivers have a major role in particle transportation to the oceans, and it has been noted that the further we move away from the river mouths the less microplastic debris we find (Lechner *et al.*, 2014; Lebreton *et al.*, 2017; Lee *et al.*, 2013; Eo *et al.*, 2018). We also know that MPs vary a lot both vertically and horizontally in the beach profiles, and it becomes even clearer once we put seasons into account. (Erkes-Medrano *et al.*, 2015) However, river wise we do not have clear answers, since the research on spatiotemporal distribution of abundance, size, and polymer composition as well as fluxes of riverine MPs has only been studied by Eerkes-Medrano *et al.* (2015) and Rochman, (2018), until 2019.

There are already multiple records of MPs in freshwater systems worldwide, in Asia, Zhao *et al.*, 2022 – reviewed and documented multiple freshwater studies in China until 2022, Europe, North America, South America, Oceania and Africa (Eo *et al.*, 2018; Li *et al.*, 2020; Li *et al.*, 2018; Z. Wang *et al.*, 2021; Erkes *et al.*, 2015). However, just as Eo *et al.* (2018) pointed out, it is important to note that most studies only sampled once or twice during the wet and dry seasons, hence there is still a major gap in knowledge regarding seasonal effects on the occurrence and distribution of MPs.

Although, there is a considerable number of studies, these reflected mostly of the top and mid layers of the water collum, lacking information on the transport of MP particles in deeper layers including the sediment which plays a big role in material transport (Morritt *et al.*, 2014). Lima *et al.*(2014) pointed out that the abundance differences between bottom and top layers are negligible as the results of their study shown no major discrepancies, however, Mani *et al.*(2015) documented not having found a single particle at a 5m depth spot in Rhine River whereas in NW England, Hurley at al., found that a flooding event brought up 70% of the total MPs stored in the river bed, showing that the sediment serves as a reservoir for these particles (Kapp & Yeatman, 2018). More researching is needed to confirm the role played by both the sediment and seasonal effects on MP abundance, as it is noticeable that climatic, environmental, and physical characteristics widely affect the retention and resuspension of the particles. A study by Lebreton *et al.* (2017), shown trough a modelling study, in Asia, that there is a potentially astronomic daily input of plastic to oceans from rivers – 1.15 to 2.41 million macro- and microplastic particles – nevertheless, the lack of information about the effects previously mentioned brings a great uncertainty referring to particle numbers.

As highlighted initially, some authors have made reviews about the study of MPs in freshwater environments and to summarize them, here follow some of the conclusions:

Most studies were conducted in rivers, some having been based on samples collected from lakes, WWTPs and tap water. Geographically most sample sites had urbanizations near them.

As of today, most freshwater studies preferred a two-method sampling, a large flow collection with buckets, pumps and glass bottles and collection with biological nets with different apertures, where the most common ones were, by order, manta (330µm mesh size), neuston and plankton nets (Eo *et al.*, 2018; Z. Wang *et al.*, 2021). In general, surface water was more sampled than sediment, assuring a sample depth between 0.1 – 1m.

A lot of studies preferred not to do digestion or purification, due to low presence/abundance of OM, but the preferred method was 30% H_2O_2, followed by 30% H_2O_2 + Fe, Enzyme + H_2O_2 and HCL treatment.

For the density separation, NaCl was the preferred solution for water samples, whereas $ZnCl_2$ and lithium metatungstate were the preferred ones for sediment. Other options adopted include sodium polytungstate (SPT), $CaCl_2$ and NaI solutions.

Concluded the separation by densities, the supernatant was retrieved and filtered, most used glass filters of 0.45, 0.7, 1.6μm glass filters and others opted for 1.2μm cellulose and, 5μm and 100μm polycarbonate filters. Some other studies preferred to run the samples through metal sieves.

Posteriorly, for the identification and characterization of MPs the most adopted method was visual inspection with the aid of microscopes and cameras, followed by FTIR, Raman, SEM/EDS, and the combination between them.

Finally, from the studies it were identified abundancies of 0.1 – 53,250 particles/m^3 (Zhao *et al.*, 2022); 817 particles – 44,435 particles/km^2 (Erkes-Medrano *et al.*, 2015); 0.00297 g/L – 2.5803 g/L and 2.5 particles/m^3 – 3.5*10^8 particles/m^3 (Li *et al.*, 2020); 0.19 particles/m^3 – 5.66*10^5 particles/m^3 and 4.44*10^4 – 1.39*10^7 particles/km^2 (Li *et al.*, 2018); 0.1 – 3,622,00 particles/m^3 (Z. Wang *et al.*, 2021).

In addition, the most common compounds found were PE (including, PP, PS, PA, PET,PU, and PVC present in (n = articles): n = 45, 45, 34, 16, 19, 4, and 11, respectively (Z. Wang *et al.*, 2021) and PE, PP, PS, PET, PA, PU, and PVC present in: n = 44, 50, 28, 21, 13, 3, and 11, respectively (Zhao *et al.*, 2022). Li *et al.* (2020), represented the results graphically in a 100 scale, following: PE = 24%, PP = 24%, PS = 13%, PET = 11%, PA = 6%, PVC = 1%, PU = 1%, Other = 20%.

The main purpose of this work is to describe the spatial distribution of microplastics and how does fluvial dynamics affect they occurrence and transport them. Regarding this theme 2 studies were found regarding freshwater systems: Eo *et al.* (2018) and Schell *et al.* (2021). In **Table 9**, in attachments, are the summary made about both articles focusing on the sampling site, sampling methods, methods used for filtering/sieving and chemical characterization, particle abundance, size and type, as well as chemical compounds constituting the MPs.

The studies were conducted in South Korea (Eo *et al.*, 2018) and Spain (Schell *et al.*, 2021), both were conducted in rivers, the first on was Nakdong River and the second on Tagus River.

Every chosen sample site was affected by Urban areas (UAs), Agricultural sites (Agro) and Wastewater Treatment Plants (WWTPs). Eo *et al.*, collected Upstream (US), Midstream (MS) and Downstream (DS). For US and MS, the samples were collected, 3m away from the shoreline, two from the western riverbank and only one from the eastern one. DS samples were collected at three stations along the axis of the river separated by 0.5m. The top layer (20cm of the water surface + surface microlayer) was

collected using a stainless-steel beaker, the mid water was collected only from DS with the aid of a submersible pump (PD-272; Wilo). After 10s of flushing, 100 L of surface and mid water was collected and poured into a 20μm mesh portable net to obtain volume-reduced samples, which was then transferred to a 1L amber glass bottle. The authors 100L as a preventive measure from clogging the net, as (Song *et al.*, 2018) demonstrated. Sediment samples were collected from the three DS stations, before the rainy season with the help of a Van Veen grab, about 10cm. The top 2cm from the soil were collected with a stainless-steel spoon and reserved in a 1L amber glass bottle.

The authors adapted the analysis method from (Masura *et al.*, 2015) and (Song *et al.*, 2017). The water samples were passed through a 20μm sieve and washed with HPLC grade water and then transferred to 250mL beakers. The samples were dried at 60ºC and for the digestion it was used the mixture of 20 mL 35% H_2O_2 and Fe(II), on a hot plate, at 75ºC and 180rpm, for 30 minutes.

To separate the samples, it was used a solution o lithium metatungstate (LMT) and HPLC water to reach the desired density. Settled particles were then drained, and floating and suspended particles were run through polycarbonate filter paper (5μm, 4 7 mm Ø) with the help of a vacuum pump.

Finally, when dried, the samples were analysed visually and identified, to further characterize the polymer type they used FTIR.

Around 945g of river sediment was collected, from that 100g aliquot by wet weight were then subsampled into a 600 mL pre-cleaned wide neck glass bottle and dried at 60°C, to determine the dry weight. Posteriorly, they were let to settle for 24H and shaken with 300mL LMT for 1 min. 10 minutes passed the content was transferred to a 1L glass beaker. After another 24H, the supernatant was then passed through a 20μm sieve and transferred into a 250mL glass beaker.

In this work the authors formulated a way to calculate spatiotemporal discharge and distribution, which might be worth exploring.

Regarding the abundance it was found a variation between 293 – 2167 particles/m^3 US, 1653 – 2613 MS, 660 – 4760 DS and 360 – 1273 DM (DS mid water). As we see in **Table 9**, in attachments, in the water surface (n1) the most common type of particles was Fragments 69%, then Fibers 30%, and Spheres and Films <1%, whereas for the sediment (n2) they found Fragments 84%, Fibers 15% and Spheres 1%. The most common compounds found in n1 were 41.8% PP, 23.1% PES, 9.4% PE, 5.8% PA, PS 2.1, 4.2% Alkyd, 3.2% Acrylic, 2.6% PEVC, 1.4% PU, 1.1% PVC, 1% PAS and in n2 were 24.8% PP, 24.5% PE, 5.5% PES, 5.4% PVC, 5.3% PS, 4.6% Acrylic, 4.5% PDS, 3.9% PU, 3.7% PAS and 3.6% PLA. Also, there was a slight variation in compound presence between Surface (Swater) and Midwaters (Mwater). In terms of size, water MPs presented a mean size of 265μm and sediments MPs 248μm.

Schell *et al.* (2021) studied the same thematic but, in the Tagus River, Spain, in which they adopted slightly different methods. Multiple samples were collected in different WWTPs, an untreated influent (UWW), a treater effluent (TWW), and raw (RSLG) and processed sludge (PSLG). Influent (20 L) and effluent (200 L) were collected at each WWTP by filtering the water through a group of nylon nets with different mesh sizes (55, 150, 300μm). Filtered, the concentrated samples were stored in glass flasks, along with the milli-Q water used for rinsing the nets. Raw sludge was collected prior to sludge treatment, while the processed sludge was sampled after, directly from the sludge hopper. Both sludge types were dried (50°C for 48–72 h) and stored in glass flasks. The same group of nets used for the wastewater was used to collect the river water samples, in which 10,000L of water were pumped into the nets with the aid of a submersible pump, after filtered, each size fraction was stored in glass flasks along with the Milli-Q water.

Sediment samples were collected from riverbed areas, constituted of sand and slick, with a core sampler and posteriorly dried (50° for 72h) and stored in glass flasks.

Water samples were filtered either directly filtered onto filter papers (Whatman GF/A; Ø 47 mm; 1.6μm pore size) or were first taken for digestion of the organic matter. These last, were let to rest overnight and then were decanted off and vacuum filtered. The remaining content was transferred and treated using Fenton's reagent. With the samples treated, they moved on to density separation, in which they used NaI to isolate the MPs.

Just like the water samples, solid samples, sludge, and sediment, were subjected to both density separation and organic matter digestion. Afterwards, the samples were left to settle, and then filtered and transferred to filter papers (Whatman GF/A Ø 47mm). It is worth noting that a significant change was the density of the NaI solution, being higher for solid samples.

Visual inspection was conducted with a Nikon SMZ 745T stereomicroscope at 20–50x magnification, and the photos were taken with an Infinity 1 camera. Particles were defined as beads (spherical particles), fragments, fibers, films, foams, granules, glitter, or pellets. To characterize the polymer composition the authors used different FTIR techniques for different particles sizes – Agilent Cary 630 ATR-FTIR (Large MPs) and Perkin Elmer Spotlight 400 μFTIR for small MPs.

From this study the authors obtained abundancies of 1.30 - 147 particles/m^3; 0.54 - 14.6mg/m^3 from the river water samples (n1), 0 - 2910 particles/kg; 0 - 44.3 mg/kg from the river sediment samples, 850 - 11,550 particles/m^3; 1.86 - 194 mg/m^3 from UWW, 45 - 535 particles/m^3; 0.28 - 48.5mg/m^3 from TWW, 2432 - 24,828 particles/kg; 5.05 - 1525mg/kg from PSLG, and 7161 - 66,260 particles/kg; 12.7 - 553 mg/kg from RSLG.

It was also possible to conclude that fragments were the most common particle in all sites (UWW, TWW, PSLG, RSLG, n1 and n2 with the percentages 42, 69, 56, 52, 81 and 87%, followed by fibers with the values

41, 19, 44, 47, 10, and 13%, respectively. The rest were granules, foams, beads, films, and glitter (12, 11, 1.6, >1, 9, and <1%).

In terms of composition the most common polymers found were PE, PP, PES, PS, Acrylic, Paint particles, Tyre particles, PVC and EPR.

Regarding size, most of the sites verified a particle size range between 55 - 5000μm with variation of occurrence.

Both studies concluded that despite WWTPs retaining many particles, they provide 15 – 50% of the river catchment discharge. It was not observable any influence in MP removal after treatments. It was also possible to conclude that concentrations vary strongly with land-use, increasing significantly downstream of urban and industrial areas, rainy seasons, as well as particle and environmental characteristics.

In sum, there is still lack of information and knowledge to firmly build a solid basis regarding the thematic of spatiotemporal distribution of MPs in riverine environments. To contribute to the advancement of these research and to standardize a methodology, this work will focus on this problematic, however, without the time factor considered.

4.7. Fluvial Morpho-dynamics and Microplastics

As a starting point it is important to highlight that only a few studies have examined MP abundance in sediments in relation to grain size and conclusions regarding variations with grain size are frequently conflicting.

Sediments in enclosed waters and low-energy environments, for example, shallow estuarine areas and bays, that often receive large inputs of material, have shown to retain more MPs than higher-energy, deeper and further from source environments (Wang *et al.*, 2020; Uddin *et al.*, 2021).

Interestingly, microplastics deposition has been positively correlated with a multitude of factors such as the increasing of the total organic carbon (TOC) content in sediments (Maes *et al.*, 2017) which have been documented to be associated with a decrease of grain size (Bergamaschi *et al.*, 1997) indicating that maybe finer grains trap more particles (Green & Johnson, 2020).

Some authors like, Strand & Tairova (2016) reinforce this hypothesis by finding a strong correlation between MPs and the %TOC of fine (<63μm) sediments. Additionally, Enders *et al.* (2019) and Blašković *et al.* (2017) examined the relationship between sediment granulometry and MPs and reported a strong correlation between MPs and the finer fraction (<63μm) of sediment. But the seconds, contrarily to Enders

et al. (2019), could not prove the existence of an actual relationship between grain size and microplastic concentrations such as other studies like Martins & Sobral (2011) and Alomar *et al.* (2016).

Additionally, some studies found that the proximity to a certain pollution source (stress factor) (e.g., WWTPS, ports, urbanizations) did not increase the concentration of micro-debris but their distribution rather can be influenced by different factors, such as volcanic eruptions, currents, sediments and aeolian processes (Martins & Sobral, 2011; Oliveira *et al.*, 2015; Alomar *et al.*, 2016). This resulting uncertainty regarding the relationship between grain size, MP abundance and distance to sources remains, which warrants further investigation (Alomar *et al.*, 2016; Blašković *et al.*, 2017; Wang *et al.*, 2020).

On a study conducted by Marques Mendes *et al.* (2021), he found little or no effect of the relative tidal position in the distribution of microliter at regional scales, he also found that higher microliter abundances were found closer to sources. Authors found no differences in microliter abundances for fine and medium sands, and due to the absence of coarse sand in the proximity of the stress factors sources, it was not possible to assess the effects with certainty. Them, Cera *et al.* (2022), Vermeiren *et al.* (2020), and Corcoran *et al.* (2020) found a clear relationship between MPs and grain size. A higher concentration of microplastics in finer sediments (<63μm) within a 2 km distance from a known source present a decreasing MP concentration with an increase in sediment grain size and/or distance from a possible source of microparticles. Particularly, MPs are significantly more abundant in very fine sand than in fine and medium sand samples. They also found that microplastics tend to be accumulated higher in muddy sediments than in the other types of sediments, more specifically, the abundance decreases exponentially from the top layer with the increasing grain size. It is also worth noting that, smaller MPs compared to larger MPs, infiltrated deeper into coarse sandy and gravelly sediments than fine sediments, which as we have seen in the soils section, depends highly on grain size and the microbiota. Hence, the authors suggest that fine sediments may act as a "MPs trap" which accumulates these particles on the surficial layer and that this emphasises the need to further investigate and monitor the microplastic content of sediments with a grain size <63μm, accounting for the distance from known sources, as the availability and impact of such small particles benthic communities can be very high.

The mechanisms governing microplastic deposition, retention, and resuspension are very complex and still poorly understood (Alimi *et al.*, 2018; Chubarenko *et al.*, 2020). However, it is known that the strength of the water currents, hydrodynamic conditions, or benthic fauna (Maes *et al.*, 2017; K. Sun *et al.*, 2021) can influence greatly the deposition of such debris.

As theorical support, a recent review by Harris (2020) stated that the dispersal of MPs is similar to natural sediments with coarse-grained and dense particles deposited close to sources, while less dense, finer particles remain in suspension and settle in low energy environments.

In conclusion, it is expected that areas with high industrialization and urbanization also have higher concentrations of microplastics, and that other factors such as exposure, wind, waves, fishing fleet activity, and riverine inputs should be considered when investigating microplastic deposition in coastal sediments as they undoubtably impact the sediment, and thus the accumulation of MPs.

Although there are already some plausible conclusions, the different authors agree that transport and deposition of MPs in fresh waters is an under-studied topic, particularly on lentic systems. Further investigations are mandatory to better assess spatial and temporal distribution of MPs in water and sediment.

5. Methodology

5.1. Geographic Framing

The watershed of the Cávado River is located in the north-western region of Portugal (**Figure 18a**), with a maximum length of 129 km, mean width of 16km and elevation of 564m and a drainage area of 1699km^2 (Oliveira *et al.*, 2021; Vieira *et al.*, 1998). This area is characterized for having for having an annual precipitation of 900–4200mm/year with an average of 1998 mm/year and a hypsometry of 0–1600 with a decreased tendency along the stream, as represented in **Figure 19a** (Oliveira *et al.*, 2021). The river flows into the Atlantic Ocean primarily in a NE-SW direction and its main tributaries are The Homem River and the Rabagão River, with drainage areas of around 246 and 257 km^2, respectively (**Figure 18b**) as its most significant affluents (Oliveira *et al.*, 2021; Vieira *et al.*, 1998). Six of the 14 local municipalities – Terras de Bouro, Amares, Vila Verde, Braga, Barcelos e Esposende – that make up the Intermunicipal Community of Cávado (ICM) are affected by the watershed region and contain the main residential and industrial areas.

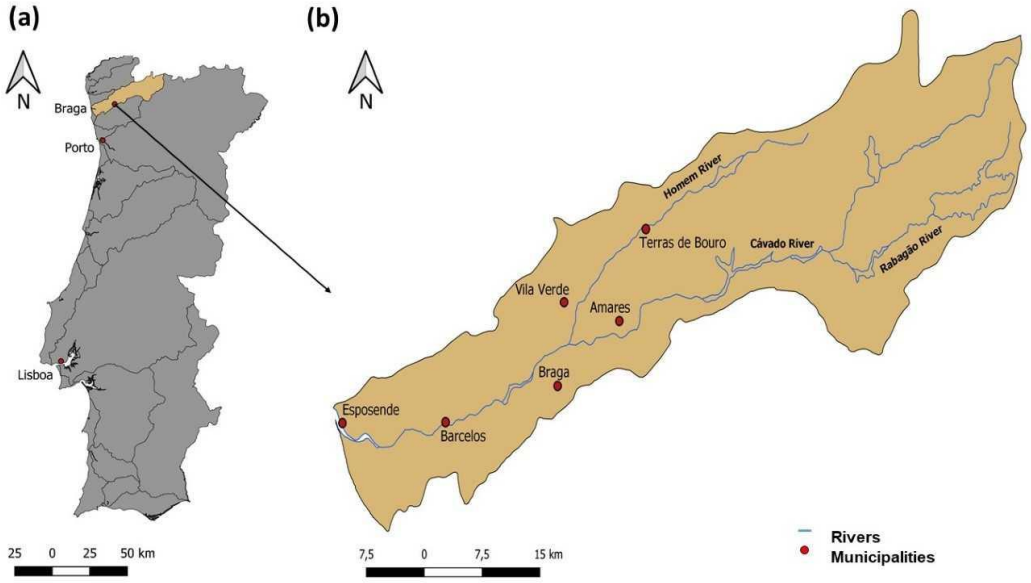

Figure 18 - (a) Geographic framing of the Cávado River. (b) Main affluents, as well as the Municipalities constituting the Cávado River area.

Figure 19b shows that 67% of the soil is occupied by forest and seminatural regions, 26% by agriculture, 5% by urbanization, and 1% by water bodies. Most of its water is widely used for hydropower generation, domestic and industrial water supply, and agricultural irrigation (Oliveira *et al.*, 2021; Vieira *et al.*, 1998).

This river's banks are home to a variety of urban and agricultural regions, including industries. Nonetheless, most of the activity is in the agricultural sector. Compared to downstream and midstream there are distinct

agricultural regions and less industrial development in the upstream portion of the Cávado River basin (Oliveira *et al.*, 2021; Vieira *et al.*, 1998). The watershed's centre has a larger population density and more industrial activity, particularly textile, around Braga and Barcelos municipalities, which are the highest populated. It is worth noting that there are two industrial parks in Braga, relatively near the Cávado River. There are also two WWTPs, Vila Verde (Cávado-Homem WWTP and Braga (Frossos WWTP). Although all 6 municipalities are equipped with WTPs and WWTPs, the WWTP of Amares has been deactivated since 2015 due to the incapacity of the regional requirements and associated discharges to Cávado River, naturally, the deactivation of the WWTP resulted in an impact in river's water quality.

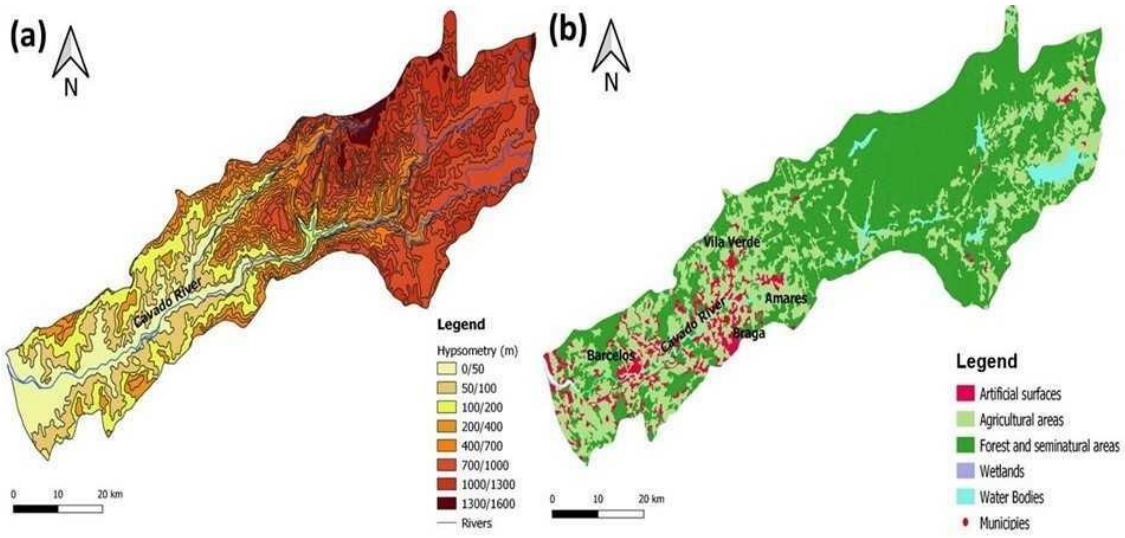

Figure 19 - (a) Hypsometry and (b) Land occupation in the Cávado River's area.

Thus, Amares and Braga WWTPs effluents are responsible for most of the wastewater inputs, whereas Barcelos is responsible for most of the untreated domestic and industrial discharges (Oliveira *et al.*, 2021; Vieira *et al.*, 1998). To facilitate the methodology and system modelling, this work considered the begging of the river study area downstream of the Caniçada dam (right after Porto Bridge) and the end near the river mouth, in Cávado River's estuary. As represented in **Figure 20**.

The climate in all sites is temperate, with dry and comfortable summers and cold, wet, and partly cloudy winters. The "summer" season occurs between June and September with an average high temperature of 26ºC and the cold season occurs between November and March with an average high temperature below 15ºC; all info available in (WeatherSpark, 2023). The sampling sites have approximately the same climatic conditions.

In this work all samples were collected from the riverbed sediment, from 14 points along the Cávado River as represented in **Figures 20 and Map 1**, last one is in Attachments and has the situational framing of each sampling site. The sampling sites were in 5 out of 6 ICM municipalities:

Figure 20 - Sampling sites with satellite framing. Retrieved from Google Earth, 2023

Figures 21, **22** and **23** depict the general method for sampling, storing of the respective and resulting trail of the sampling. Photos of each sampling site are available in the Attachments with the sampling site name, designated study ID, and coordinates. MP002 – Praia de Navarra, Braga (41°36'48.38"N, 8°23'5.76"W).

Figure 21 - Sampling in MP002 with the stainless-steel bucket.

Figure 22 - Storing of the sample inside aluminium lunch boxes.

Figure 203 - Trail resulting from the sampling.

5.2. Methods

Sampling was carried out on May 26th, 2023, with near optimal weather conditions, maximum temperature of 21°C, light winds and little to no precipitation (WeatherSpark, 2023). Sampling started from Amares to Esposende. The first sample (MP001) was collected on Ombra fluvial beach at 09:46, followed by:

MP002, on Navarra fluvial beach at 10:15;

MP003, on Autocarro Bar fluvial beach at 10:37;

MP004 and MP004B, near Mirante bar fluvial beach at 10:53;

MP005, on the backwater area near Codracheira at 11:25;

MP006, on a floating craft to the east of Areias de Vilar WTP at 11:57;

MP007, on Manhente fluvial beach at 12:30;

MP008, on the floating craft north of Barcelinhos fluvial beach, at 12:56;

MP009, on the Mariz picnic area at 14:55;

MP010, on the Perelhal parking to the north of Areal da Agra fluvial beach, at 15:13;

MP011, on the meander near the Cávado greenway, at 17:13;

MP012, MP012B and MP012C, on the fluvial beach near Fão's Sailing Club, at 15:40;

MP013, on the estuarine "beach" near the ElementFish Kite & Surf Camp, at 15:57;

MP014, around the middle of the riprap of Esposendes' Beach, at 16:22.

Sampling was carried out based on the model mentioned by Rocha-Santos & Duarte (2017), with slight adaptations to the methodology used by (Besley *et al.*, 2016; Eo *et al.*, 2018 and Schell *et al.*, 2021).

To collect the samples, it was used a personalized metallic bucket with a black rope attached to it (**Figure 24**). The sampler was launched around 3-5m into the water, in the attempt to reach the 1m depth waters, the sampler was then dragged slowly along the sediment, removing around the top 10cm layer of substrate. Following Carson *et al.* (2011), samples MP012B, MP012C, and MP013, which were collected by directly rather than by launching, as they were located inland, consisted in retrieving the first 5 to 6cm of sediment,

as most MPs are in the first layers of sand. The samples were then stored in ~1L aluminium lunch boxes with dense paper tops and taken to the lab.

Figure 24 - Sampler: a stainless-steel bucket ~ (15cm diameter x 30cm height).

Already on campus, all the samples were stored inside the laboratory oven at 45°C for 4 days and left to dry (**Figure 25**). On the 1st of June, samples were still wet, so they were left there until the 9th to assure that they were all dried. Organic matter digestion was not conducted due to lack of material to do so.

Figure 25 - Samples stored and let drying in the laboratory oven.

Meanwhile, the density separation $CaCl_2$ solutions were prepared. The solution consists of adding $CaCl_2$ and $CaCl_2$ * 2 H_2O (in the absence of $CaCl_2$) to distilled water at 20°C while stirring with a glass rod. Since we still had a lot of untouched solution left from previous research, it was only necessary to dilute the

precipitate (**Figure 26**). Afterwards, the $CaCl_2$ solution had to be filtered with paper filters and poured into Erlenmeyer's flasks, due to the presence of a lot of impurities (**Figure 27)**.

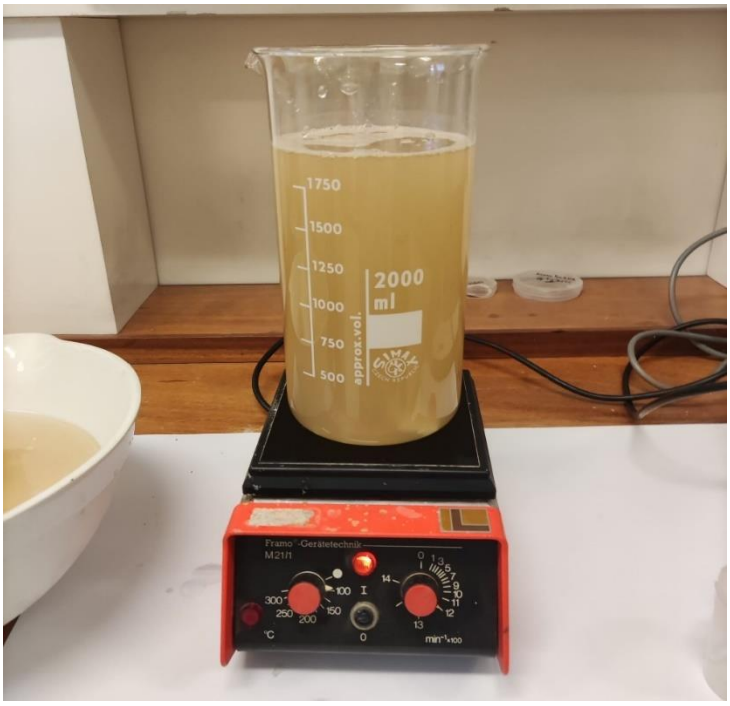

Figure 26 – Dilution of the precipitated CaCl2 solution.

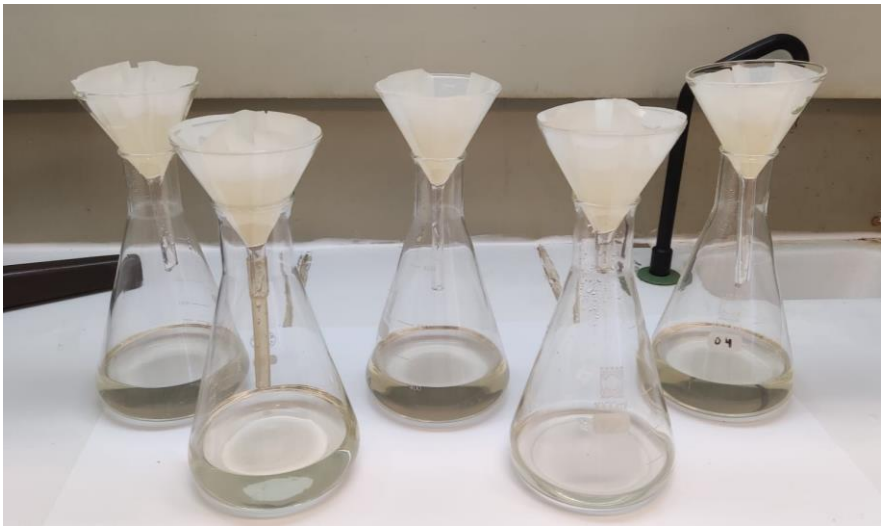

Figure 27 - Filtering of the CaCl2 solution.

After drying, the sediment samples were prepared with the aid of a small metal shovel and a metal spoon spatula. About 500g of sample, as shown in **Figure 28** was separated, and stored in a ceramic bowl (tare weight), to avoid samples contamination. The process was repeated to every sample (**Figure 29**).

Figure 28 - Weighting 500g of sample.

Figure 29 - Repeated the process for each sample.

Additionally, following the methods documented and explained by Alakangas (2015), an extra 100g of each sample was collected through quartering, to assure statistical representation.

With the sediment samples properly stored and with $CaCl_2$ solutions filtered, density separation followed. In aid of the process of identification and counting of microplastics, a pre-treatment of the samples must be carried out mandatorily, by separation of densities. In this case, as previously stated, it was used a saline solution of $CaCl_2$ with a density >1.4g/cm^3 (**figure 30**).

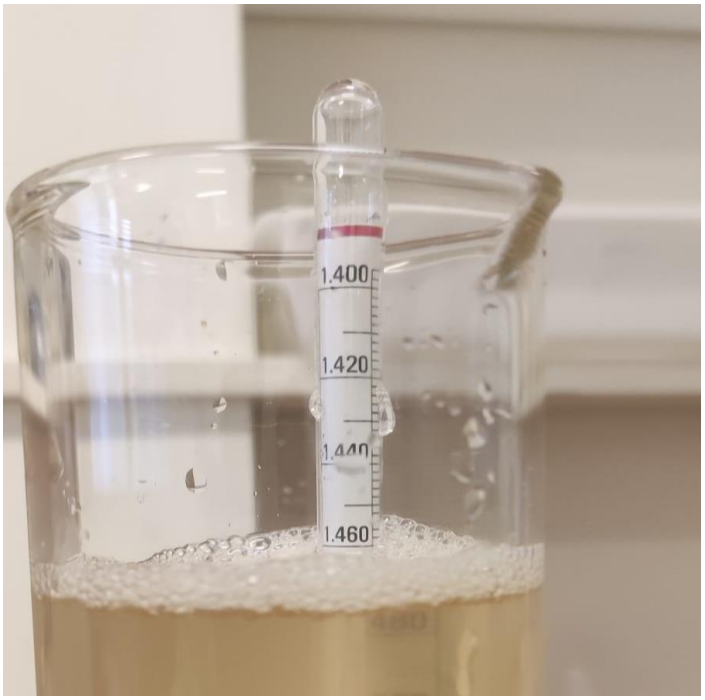

Figure 30 – Saline solution with density >1.4g/cm3

Subsequently, the samples were added to the solutions at a ratio of ½:1, that is, 500g of sample to 1000mL of solution. Following the same model as (Schröder *et al.*, 2021): (1) stirred during and after sample addition; (2) the flasks were rotated 4 times, every 10 minutes and left to settle (**Figure 31**) (3) the sample

was allowed to settle for 15 – 18 hours, before visual identification under the optical microscope (**Figure 32**), allowing the microplastics from accumulating on the surface.

Figure 31 - Stirred solutions left to settle.

Figure 32 - Settled solutions with the supernatant separated.

After the 18 hours, suspended particles were removed with a metal spoon spatula and a micropipette (**Figure 33 and 34a),** to avoid contaminating the sample. It was collected about 500µL of sample and then transferred to Petri dishes and marked with about 300µL of Nile Red to better highlight the MPs (**Figure 34b**). Sample preparation for visual inspection consisted of collecting 50µL, preparing a blade and proceed with the visualisation. This process was repeated 3 times for each sample. Visual identification was then performed with a Nikon ECLIPSE E400 POL optical microscope (**Figure 35a)** and a Leica MZ12.5 Stereo Microscope (**Figure 35b**), with x5, x10, x25 zoom lenses. Visual identification allowed a general

count of microplastics, by default, identifying microplastics with a size of less than 1 mm (small microplastics), microplastics with a size between 1 – 5 mm (large microplastics) and some macroplastics.

Figure 33 - Micropipette used for collection.

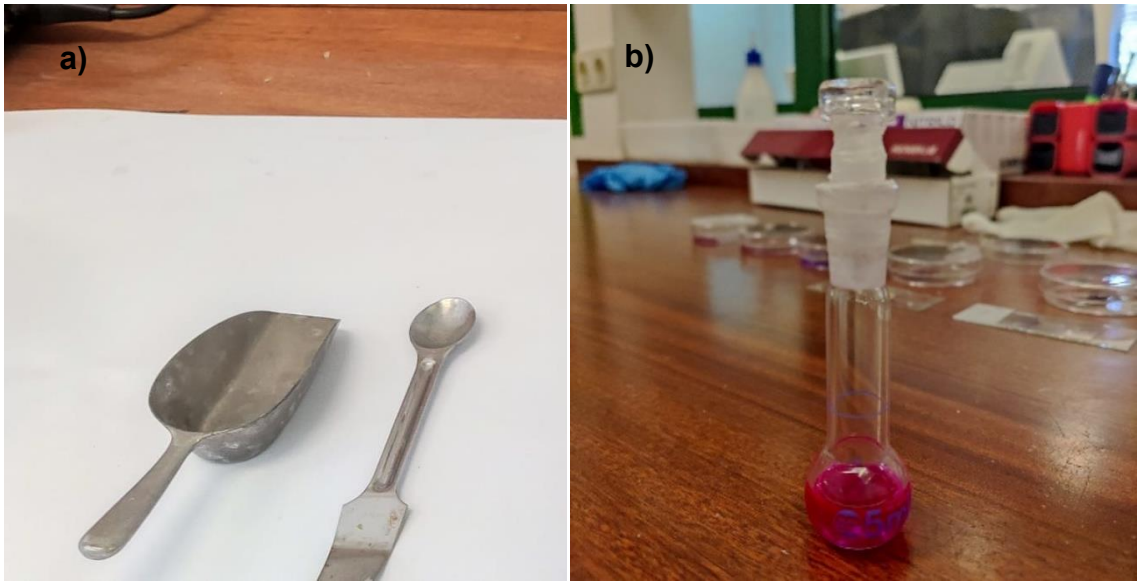

Figure 34 – (a) Spoon spatula used for collection and (b) Nile Red marker.

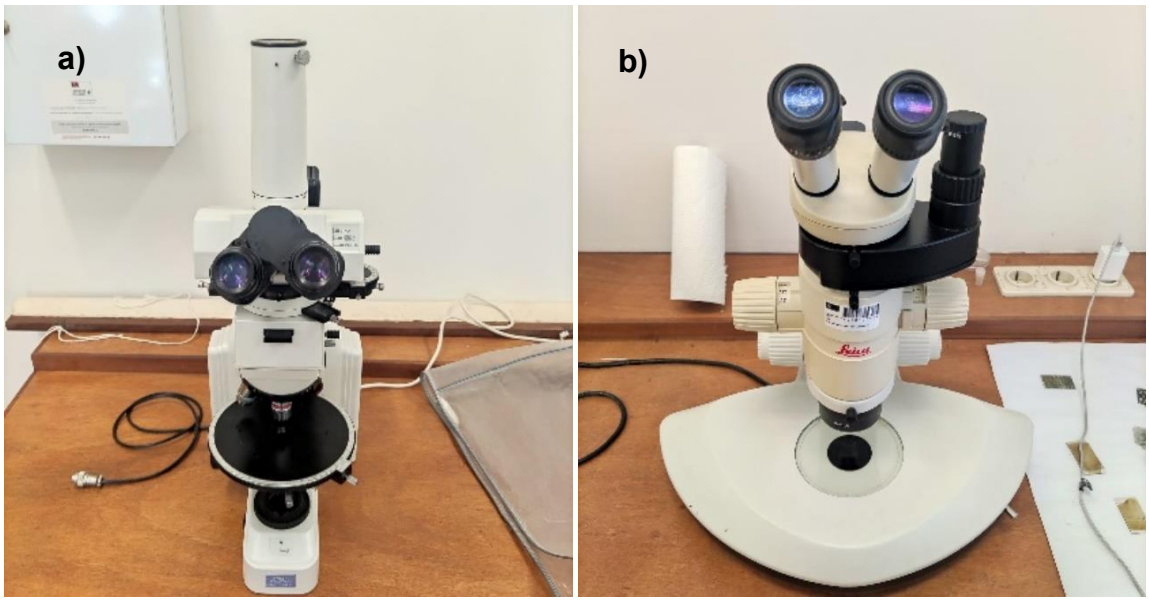

Figure 35 - (a) Nikon ECLIPSE E400 POL optical microscope and (b)Leica MZ12.5 Stereo Microscope.

5.3. Granulometry

For the characterization of the sediment, granulometry was conducted both through sieving, with multiple sized sieves, but also with the DLS Particle Size Analyser for the finer samples. After obtaining the granulometric data, the relative and cumulative frequencies were calculated. The relative frequency is obtained from the weight of the fractions obtained:

$$\frac{fraction\ weight}{total\ weight\ of\ sample\ fractions * 100}$$

Posteriorly, we calculated the cumulative frequency from the sum of the relative frequencies of each fraction. Based on the graph resulting from the cumulative frequencies and the Folk-Ward statistical parameters, it is possible to calculate the average grain size, as well as the standard deviation that will serve to characterize the sediment. (Folk, 1968; Lane *et al.*, 1947). The calculation of the average grain size (Mz) and the standard deviation (σi) is made from the phi values obtained in the cumulative frequency graph, according to the following equations:

$$Mz = \frac{\phi 16 + \phi 50 + \phi 84}{3} \qquad \sigma i = \frac{\phi 84 - \phi 16}{4} + \frac{\phi 95 - \phi 5}{6.6}$$

From the obtained mean sizes, it was possible to characterize the particle size class, shown further in this work. Granulometric analysis was carried out by the dry sieving method, according to the Rittinger scale (ratio = $\sqrt{2}$), with brass sieves. The purpose of carrying out the granulometric analysis was to determine the average size of the materials and consequently characterize the sediment (Besler, 2008).

Additionally, the distance from each sampling site to the closest known pollution source was measured with the help of Google Earth's ruler tool.

5.4. Statistical Analysis

All statistical analyses were conducted through OriginLab 2022 software (OriginPro, 2022). Normality of data distribution was evaluated with the Shapiro-Wilk and Anderson-Darling tests. In order to comprehend fluvial dynamics' influence, means were compared and differences between all groups and sample sites (MP001 to MP014; Upstream to Downstream) were assessed by a One-Way ANOVA test. Additionally, to better understand which sampling sites differ from each other, Tuskey's HSD Post-Hoc test was applied. Homoscedasticity was also studied through Levene's test. Every piece of base data is available in **Table 10**.

To assess the individual relationships between sediment grain size, distance to the pollution source, and microplastic abundance Pearson's Test was conducted, followed by the non-parametric Kruskal-Wallis ANOVA test and Dunn's post-hoc test, to effectively determine if there are statistically significant differences between the groups.

6. Results and Discussion

6.1. Microplastics Abundance

In this study a total of 571 microplastics particles was visually identified, where MP012B (n= 58) presented the highest number of particles, followed by MP009 (n= 53), MP005 (n= 45), MP003 (n= 39), and so on. **Figure 36**, in attachments, shows some of the MPs observed. In general, an average of 11.3 particles were found. **Figure 37** shows the MPs count in the 3 replicas, per sampling site. Top and bottom values represent maximum and minimum values between the replicas.

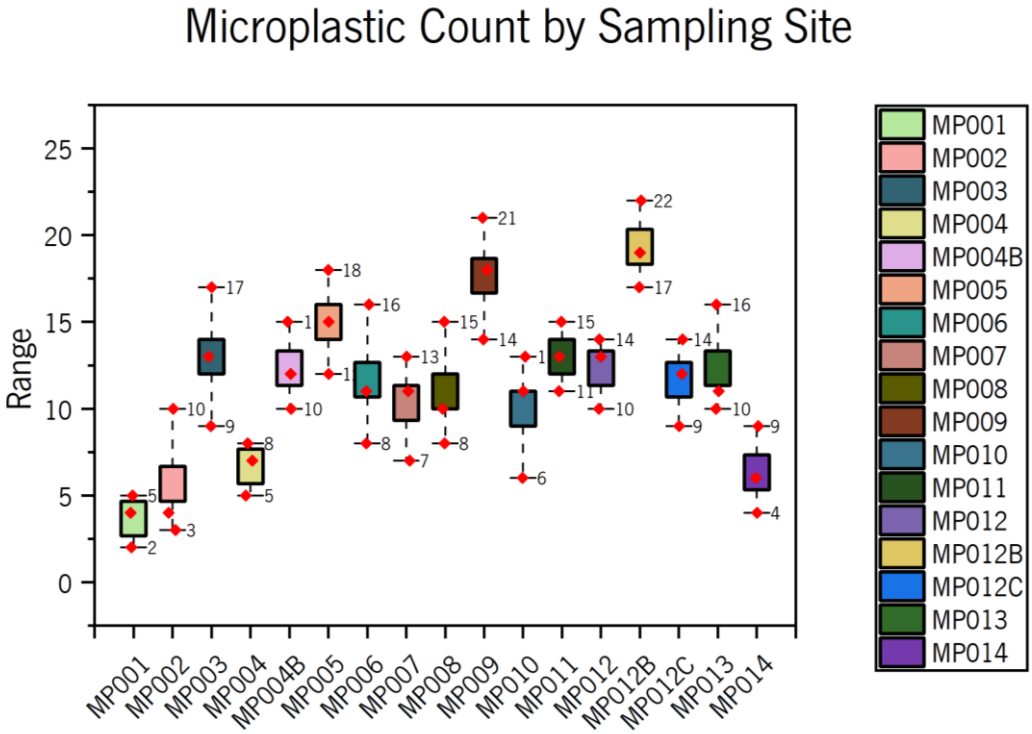

Figure 37 - Total count of MPs of the 3 replicas for each sample.

6.2. Statistical Analysis

The choice of the normality test depends on the specific characteristics of data and the number of observations within each sampling site. In this case, 3 replicas for each of the 17 sampling sites, there is a total of 51 observations. Given the relatively small sample size, both Shapiro-Wilk and the Anderson-Darling tests are very reasonable and adequate choices. Both were chosen since it is often a good practice to use both tests and consider their results collectively.

From both Shapiro-Wilk and Anderson-Darling tests it was demonstrated that the data is distributed normally as the p values > 0.05. **Figure 38**, in Attachments, represents graphically the normal distribution for every sampling site. Furthermore, Descriptive Tests were conducted to calculate the skewness, which gave information about the sample behaviour in relation to normality, in other words, it provides insights into the shape and asymmetry of each dataset aiding the understanding of the data distribution and identifying potential patterns. Also, the range between the Lower CI and Upper CI that represents the precision of the estimate, often used to report the range of plausible values for a population parameter. The wider it is, the more uncertainty.

From these tests, as we can observe in **Table 10**, MP003, 005 and 011 are symmetric. MP001, 004, 007, 009, 010, 012 and 012C present a negative value, meaning their curve is longer to the left of normality, in practical terms means they may have more lower values compared to higher ones. MP002, 006, 008, 012B, 013 and 014 present positive values meaning their curve is longer to the right of normality, in practical terms means they may have more higher values compared to lower ones. From the Lower and Upper 95% Confidence Interval, we can define a confidence interval (CI) for the population mean. From all the samples we verify the smallest interval for MP001 = 7.33333 and the highest for MP006 = 20.07905, meaning that with more replicas MP006 suggests more uncertainty, while MP001, with a narrower interval, indicates a more precise estimate of the sample mean. The initial hypothesis of this work is whether fluvial dynamics have any effect on the transport, prevalence, and consequently, abundance of microplastics. To better understand it, a one-way ANOVA test was conducted, since it is the most fitting because it allows the comparison of three or more groups of samples, that are independent and with no repeated measurements over time. The test shown that there are statistically significant differences among the sampling sites' means since the p-value < 0.05 α level – p-value = $1.27*10^{-5}$ (**Figure 39**). From this result the initial hypothesis is corroborated, so fluvial dynamics do have influence in microplastics behaviour.

Table 10 - Abundance of microplastics in water and bank sediment from the Cávado River, Portugal in 2023. R1, R2 and R3 represent the 3 replicas made for each sample (50µL*3)

Sample (50µL)	R1	R2	R3	p/150µL	p/L	Mean	MEDIAN	SD	SE of Mean	Lower 95% CI of Mean	Upper 95% CI of Mean	Skewness
MP001	4	5	2	11	73333.33	3.66667	4	1.52753	0.88192	-0.12792	7.46125	-0.93522
MP002	3	4	10	17	113333.33	5.66667	4	3.78594	2.18581	-3.73813	15.07146	1.5971
MP003	9	13	17	39	260000	13	13	4	2.3094	3.06345	22.93655	0
MP004	7	8	5	20	133333.33	6.66667	7	1.52753	0.88192	2.87208	10.46125	-0.93522
MP004B	12	10	15	37	246666.66	12.33333	12	2.51661	1.45297	6.08172	18.58494	0.58558
MP005	18	15	12	45	300000	15	15	3	1.73205	7.54759	22.45241	0
MP006	16	8	11	35	233333.33	11.66667	11	4.04145	2.33333	1.62714	21.70619	0.72211
MP007	13	11	7	31	206666.66	10.33333	11	3.05505	1.76383	2.74417	17.9225	-0.93522
MP008	8	10	15	33	220000	11	10	3.60555	2.08167	2.04331	19.95669	1.15207
MP009	21	18	14	53	353333.33	17.66667	18	3.51188	2.02759	8.94266	26.39067	-0.42327
MP010	6	11	13	30	200000	10	11	3.60555	2.08167	1.04331	18.95669	-1.15207
MP011	11	15	11	37	246666.66	13	11	2	1.1547	8.03172	17.96828	0
MP012	14	13	10	37	246666.66	12.33333	13	2.08167	1.20185	7.16219	17.50448	-1.29334
MP012B	19	22	17	58	386666.66	19.33333	19	2.51661	1.45297	13.08172	25.58494	0.58558
MP012C	14	12	9	35	233333.33	11.66667	12	2.51661	1.45297	5.41506	17.91828	-0.58558
MP013	11	16	10	37	246666.66	12.33333	11	3.21455	1.85592	4.34795	20.31872	1.54539
MP014	4	6	6	16	106666.66	6.33333	6	2.51661	1.45297	0.08172	12.58494	0.58558
Total	190	197	184	571	2826666.66	11.29411765						

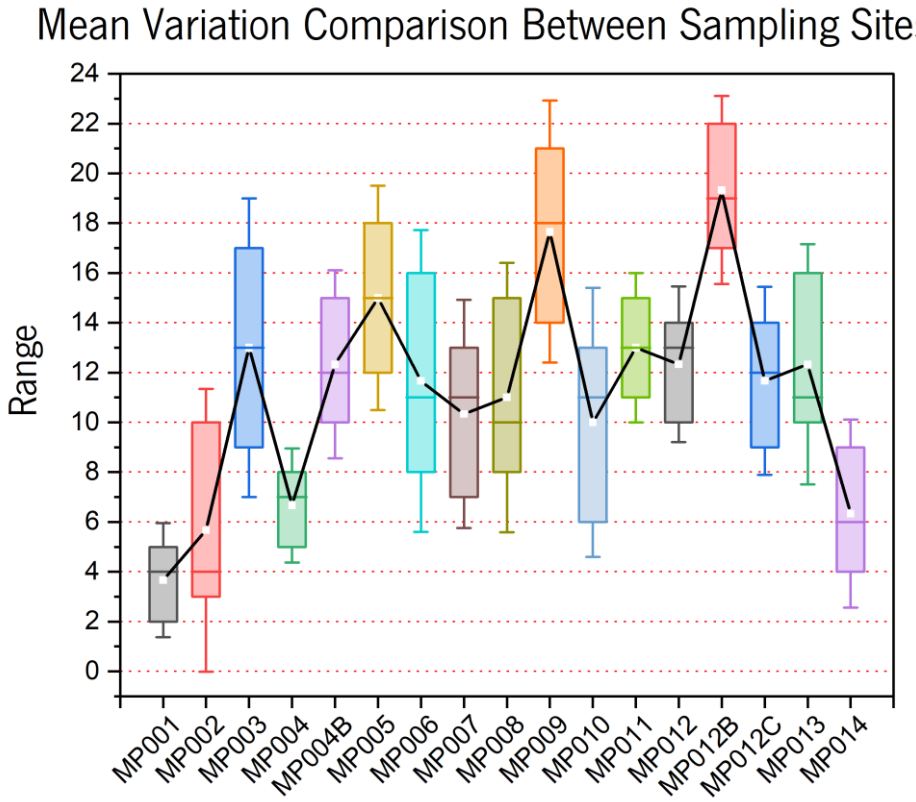

Figure 39 - The comparison between sample means. Error bars correspond to standard deviation with a coefficient of 1.

To evaluate the robustness and fitting of the chosen ANOVA model, a Levene's Test for Homogeneity of Variance was conducted as well as a fit statistics analysis. Levene's test is used to assess whether the assumption of equal variances (homoscedasticity) among groups or conditions is met in an analysis of variance (ANOVA) or other statistical tests. Since, Levene's test results revealed that the p-value = 0.91504, thus p-value >0.05, then there is no significant evidence to suggest that the variances among the sampling sites are significantly different from each other. Meaning that the homogeneity of variances assumption, a key assumption for ANOVA, is met, and that the sampling sites have roughly equal variances. Hence, the ANOVA results are reliable.

For the fitting of the chosen ANOVA model, (1) R-Square, that measures of how well the model explains the variation in the dependent variable (e.g., microplastic abundance) based on the independent variables (sampling sites), (2) Coefficient of Variation, a measure of the relative variability in your data, calculated as the ratio of the standard deviation to the mean, and (3) Root Mean Square Error, which measures the average magnitude of the residuals (the differences between the observed and predicted values) in the ANOVA model, were analysed. Based on my research, since the purpose of the thesis is proven that fluvial dynamics indeed affect the behaviour of MPs, then:

(1) A Square value of 0.72478 is relatively high, suggesting that the model accounts for a significant proportion of the variability in the dependent variable.
(2) A CV of 0.26476 suggests that the relative variation in data (standard deviation as a proportion of the mean) is relatively small, thus, there is less relative variability around the mean.
(3) A Root MSE value of 2.99018 suggests that, on average, the model's predictions are approximately 2.99018 units away from the actual data points. Given the error sources and the dimensions of both the samples and system in study, the value is considered relatively small, indicating being a good fit.

While ANOVA establishes that there are significant differences, it does not clarify the direction of those differences. Further analysis, such as post-hoc tests, will help identify which specific groups or conditions differ from each other. Since the one-way ANOVA has been proven statistically significant, a follow up post-hoc test was conducted (e.g., Tukey's HSD or Bonferroni) to identify which specific sites differ from each other. In this situation, there are 17 sampling sites, and it is needed multiple pairwise comparisons to identify which sites have significantly different means, both Tukey's HSD and the Bonferroni correction are suitable options to solve this problem, but since it is sought to find a good balance between controlling Type I errors and having reasonable power to detect true differences, Tukey's HSD was chosen. Adjusting the alpha level for multiple comparisons to control the familywise error rate, especially when conducting post-hoc tests, is very important. For tests such as Tukey's HSD, it should align with the desired balance between controlling Type I errors (false positives) and having adequate power to detect true differences. In this logic, an α Level of 0.05 (5%) was chosen.

It was possible to conclude that there are 13 pairs which present significance levels equal to 1 suggesting that there are significant differences between the means of the sampling sites being compared. However, in all 13 pairs, it was verified that the "q"-value, also known as adjusted p-value, was higher than 0.05, meaning that after adjusting for multiple comparisons, the statistical significance will become less clear. In other words, while there are apparent significant differences between the means in the individual comparisons, these differences are less clear when considering the risk of making false discoveries due to multiple comparisons. Beyond statistical significance, it is crucial to assess whether the observed differences are practically meaningful for the research. Since this study intends to comprehend whether there is an influence from the fluvial dynamics and not exactly what are they and where they occur, a q-value > 0.05 is not so meaningful.

Although, the statistical analysis reveals significant differences in microplastic abundance among different sampling sites and thus can be concluded that there are statistically significant variations in microplastic levels between these sites, it should be considered that there are more affecting factors that

should be taken into account, however, in this study specifically they do not matter from a practical standpoint. From the data analysis it is possible to comprehend that certain sampling sites are more susceptible to microplastic accumulation and that even the land use practices may have a certain impact on the behaviour of MPs in the environment. Also, as literature shows, the existence of multiple textile industries, high levels of urbanization, river beaches, (W)WTPs and other industries near the river before sampling sites like MP009 may justify its higher number of particles (Eo *et al.*, 2018; Schell *et al.*, 2021).

6.3. Granulometry

Cumulative frequencies are all displayed in a range of (0.01 – 100). As we can see, the samples present relatively the same curve behaviour, which indicates a certain homogeneity in the constitution and characteristics of the sediment (**Figure 40**). The average values of grain size do not show anomalies, MP008 was shown to have the highest mean grain size followed by MP004B, MP004 and MP003. MP013 had the lowest mean with a value of 0.73. Most of the standard deviations are negative, except for MP005 and MP006 (**Table 11**). Given the negative nature of most of the standard deviations we can conclude that the data points for grain size are primarily clustered around the mean and show limited variability or dispersion.

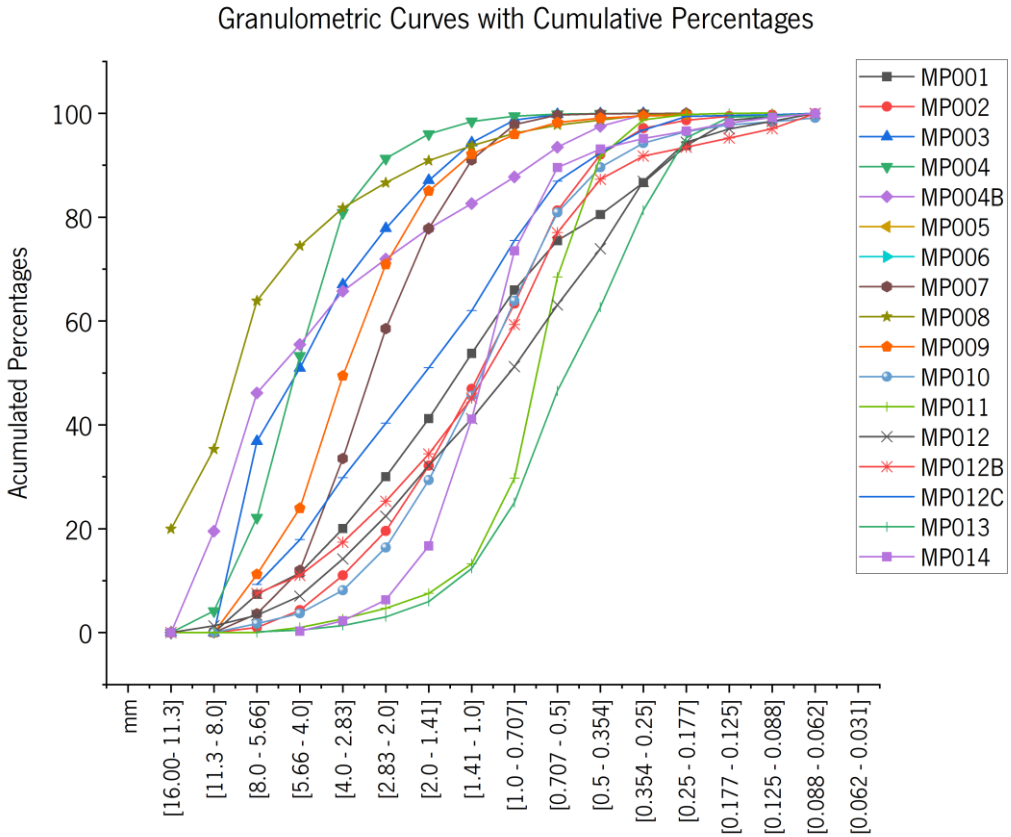

Figure 40 - Granulometric curves with the cumulative percentages. X axis represents particle sizes in mm and Y value presents the phi values.

As we can see in **Figure 40** and as previously mentioned, the samples present relatively similar behaviours. According to the terminology established by (Folk, 1954), samples MP002, MP010, MP012, MP012B and MP014 are classified as "Gravelly sand", MP001, MP003, MP004B, MP007, MP009 and MP012C as "Sandy gravel", MP005, MP006, MP011 and MP013 as "Slightly gravelly muddy sand", and MP004 and MP008 as "Gravel".

From the conducted Pearson's Test, it was inferable that there is a positive correlation between all the variables (Pearson Correlation Coefficient [r] = 1), however, due to the limitations such as lack of data and small number of samples, it is not possible to reject the null hypothesis, thus there is not sufficient evidence to conclude a statistically significant linear correlation between the variables.

Kruskal-Wallis ANOVA (Prob>Chi-Square = 0.45) and Dunn's tests resulted in a p-value > 0.05, proving no significant differences between the populations, meaning that based on these tests we cannot effectively conclude that sediment grain size has a statistically significant effect on microplastic abundance and retention.

6.4. Correlations: sources and affecting factors

As we can observe from the results, particle amount increases from upstream to downstream, being affected by some ecological and environmental factors. MP0012B, 009, 005 and 003 shown the highest particle count. As we can see in **Map 2b,** MP003 sampling site is highlighted as a highly human-altered area, with heavy daily human presence during the warmer seasons, it is also near a road which contributes with a lot of tyre fragments, it also contains a great number of urbanizations around it and it is right after a WTP, all these factors may justify its high count (Schell *et al.*, 2021).

Although MP005 does not have as much emission sources as 003, the sample was collected in an area right after an isolated urbanization containing multiple types of industries, including textile, painting, and others. It is also a backwater region, "mangrove" like, with lower water levels, higher amounts of organic matter and finer sediment grain, which promotes a higher accumulation of the MPs (De Souza Machado *et al.*, 2017)

MP009 in **Map 2g** appears right after a region full of emissions sources from the industry complex, with multiple textile industries, and the Barcelos' WWTP, this alone may be enough to justify the high count of MPs.

MP012B was one of the samples collected in Fão's region, in the wrack line, where we usually find the greatest number of particles, specifically on the top 10cm of sediment, **Map 2j**. The dissipative behaviour in this region plus the previous point, can very well explain the particle count in this area. It is also important to note that we have a lot of urbanization, roads, agricultural fields and a WWTP near this site. (Schröder *et al.*, 2021).

Additionally, the 3.º Cycle (2022-2027): PGRH Project (Hydrographic Region Management Plans) of Cávado, Ave and Leça (RH2), which is a open project being conducted, already shows multiple pressure points along the Cávado River such as water collection points for urban and agricultural water supply, water collection points for human consumption, hydroelectric stations, large riverside agricultural fields as well as manufacturing industries close to the river. For example, MP005 has two manufacturing industries (Pressure ID: QUAN_CAPTACOES_000076179 and QUAN_CAPTACOES_000072438, respectively) relatively close to it, with discharge volumes of 1,600000 and 0,085800 hm^3, which might play a role in the MPs count.

The lower microplastic counts need to be deeply look at since the methodology still has room for improvement. Apart from MP014 which is located very close to the river mouth with deeper waters, higher energy levels and deeper sediment, making the accumulation of MPs harder.

Statistical tests regarding the interaction of sediment grain size, distance to pollution and microplastics abundance, show that there is a possible positive correlation, however from these preliminary results we cannot firmly affirm that there is a linear correlation. Given the lack of information about pollution sources, current velocity, discharge volumes, and the inclusion of other factors such as runoff and direct input of litter, it is not feasible to label these results as conclusive.

Additionally, from the Kruskal-Wallis' test, it was shown that we cannot affirm the existence of a direct comparison between sediment grain size and microplastics abundance. Nevertheless, given the small number of samples, and sampling sites along the river, as well as further detailed inspection of the sediment, is not correct to affirm that there is not a direct connection between these two variables, as literature as shown that there is a certain trend for higher retention of MPs in finer grained sediment and surfaced layers (Marques Mendes *et al.*, 2021).

7. Conclusion and Future Perspectives

In conclusion, we can clearly notice an increasing tendency of MP count downstream which may be directly related with the increase of pollution sources. From the statistical analysis it was concluded that the samples presented similar behaviours, and that the river dynamics do in fact affect the transport and prevalence of MPs. MP012B, MP009, MP005 and MP003 present the higher values, probably due to all the existing pollution factors near them such as WWTPs, agricultural fields, highly concentrated urbanizations, industrial complexes, and other riverine pressure factors. The MP014 low count of microplastics could be justified thanks to the high hydrodynamic scenario and characteristics of this region, namely the depth and length of the stream, estuarine characteristics, and the interaction with both fluvial and ocean dynamics. MP001 and 002 as they presented some abnormality when submitted to stricter tests, for example when we lower the α-level or the Bonferroni test in the ANOVA model, which were not considered do to their increase of the risk of Type II errors (false negative). Additionally, since the digestion of organic matter was not done and the samples were not filtered or sieved, there might have been identification errors during the visual inspection. During the whole process, efforts were made to reduce cross-contamination, however, there are always sources like atmospheric particles, fibers from clothes and pre-existing particles in the aluminium lunch boxes and remaining materials used, that might have altered the results. In this line of thought, the laboratorial procedure should be redone, making sure to reduce all the remaining contamination factors as much as possible. It was also found that collecting more samples and improving the density separation method can generate better quality results. Following the NOAA recommendation, digestion treatment, sieving and/or filtering of the samples should also be done, to reduce the misidentification of MPs. To further complete the work/study, chemical analysis should also be conducted through FTIR and/or Raman spectroscopy to identify the compounds present in the samples, measurement and identification of sample size, type and shape are also important to document as they might help identifying potential pollution sources. An additional interesting addition to the work, would be conducting SEM analysis, to identify if there are heavy metals present in the microplastics. Regarding the sampling, methods should be further explored and developed, such as reducing cross-contamination risks, use smaller portions of samples, increase the number of sampling sites and samples, for example, a 5-point sampling model across the river (1 sample on each margin, 1 on each intermedial zone and 1 on the central axis of the river), identify every possible pollution source of MPs, conduct a study about the discharge volume from each WWTP and WTP, sludge production and use, measurement of biotic and abiotic factors, evaluate water parameters as well as a more comprehensive evaluation of organic matter abundance, overall characteristics and conditions of the sampling sites.

With this study, my objective has been fulfilled as it was to further comprehend the behaviour and develop the knowledge of these particles in the fluvial environment, particularly in the Cávado River, which

has never been studied in this sense. Furthermore, I want to proceed with the study of microplastics and I have the ambition to better understand how microplastics behave in the freshwater systems and how exactly they can affect the environment, either positive or negatively. For that I have the objective to ingress in a PhD program, developing a better and more complete method of microplastic analysis and distribution model, to help standardize a methodology for the study of these particles.

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9. Attachments

MP001 - Praia de Ombra, Amares (41°37'2.60"N, 8°21'27.97"W);

MP003 – Autocarro Bar, Vila Verde (41°36'21.26"N, 8°25'56.11"W);

MP004 & MP004B – Mirante Bar, Vila Verde (41°36'20.87"N, 8°25'59.53"W)

MP005 – Codracheira, Barcelos (41°33'58.69"N, 8°30'34.26"W)

MP006 – Areias de Vilar, Barcelos (41°32'47.32"N, 8°33'4.26"W)

MP007 – Manhente, Barcelos (41°32'16.53"N, 8°34'43.71"W)

MP008 – North Riverside of Praia de Barcelinhos, Barcelos (41°31'42.15"N, 8°37'5.70"W)

MP009 – Parque de Merendas de Mariz, Barcelos (41°31'23.48"N, 8°40'14.66"W)

MP010 – Perelhal, Barcelos (41°31'18.88"N, 8°41'16.43"W)

MP011 – Ecovia do Cávado, Barcelos (41°30'58.86"N, 8°44'35.38"W)

MP012, MP012B & MP012C – Clube Náutico de Fão Beach, Esposende (41°30'53.27"N, 8°46'27.31"W)

MP013 – Restinga de Ofir, Esposende (41°31'29.45"N, 8°47'16.00"W)

MP014 – Esposende's Beach Rip-Rap – Esposende (41°32'27.09"N, 8°47'25.12"W)

Table 1 - Legislative actions to combat (micro)plastic pollution. Retrieved from Sharma *et al.*, 2023.

Program/organization	Purpose/goal	*Plan of action*
EU circular economy (CE) action plan: European strategy for plastics	Environmental protection, reduction of marine litter, greenhouse gas emissions and reliance on fossil fuels (control of microplastics so as to accomplish circular economy)	Transformation in design, production, use and recycling of plastic products in the EU; ordinance for single-use plastics; revised legislations; actions for bio-based and biodegradable plastics
United Nations SDG's (Sustainable Development Goals)	SDG 14 (Sect. 14.1): Conserve and sustainable use the oceans, seas and marine resources	Target to reduce marine pollution by 2025, primarily through prevention of based all forms of plastics pollution through land activities, sustainable management and protection of marine and coastal ecosystems by 2020, control of illegal, and unregulated (over)fishing, prohibition of certain fisheries subsidies to stop overcapacity fishing, sustainable use of marine resources for economic benefits to Small Island developing States
f (UNEP): United Nations Environment Assembly (UNEA-5.2)	International legally binding instrument to end plastic pollution (including marine environment) with the aim to completing work by the end of year 2024	Alternative approaches to address full life cycle of plastic, plan for reusable and recyclable products, and develop international collaborations in order to assist in technological access, capacity building, and provision of scientific and technical support
UNEP IUCN and Life cycle Initiative: National Guidance for Plastic Pollution Hotspotting and Shaping Action	Nationwide guidelines intended for management of Plastic Pollution	Framework/assessment resources provision to identify plastic leakage and trace, and planning for monitoring results oriented action
ASEAN (Association of Southeast Asian Nations)	Region wise action plan for tackling of plastic pollution	Allocation of resources so as to strengthen various actions for reduction of plastic pollution, measurement and monitoring of plastic pollution, increase of plastic clean up, innovation training and investment
WHO (World Health Organization)	Call for research on effects of MPs & plastics on environment and health	Research on occurrence of microplastics, standardizations of protocols for measurement of microplastics, removal of pathogens from water that in turn can remove 90%of microplastics from marine system

Table 2 - Plan of Action of different countries and NGOs against microplastic pollution. Retrieved from Sharma *et al.*, 2023.

Country/region	*Policy/Law*	*Plan of action*	*Challenge /limitation(s)*
New Zealand	Waste minimization (microbeads) regulations	Ban on manufacture and sale of washing products that contains microbeads	
USA	Microbead-Free Waters Act (2015)	Prohibition of sale of microbeads containing cosmetic products; committee formation for response strategy	Act fails to create a suitable biodegradable alternatives that can prevent the plastic pollution
Procter & Gamble, Walmart, Toyota	Taking responsibility of their plastics	Landfilling and recycling of their plastics	
China	LPCEPSW (Law on the Prevention & Control of Environmental Pollution by Solid Wastes)	Regulation of waste dumping sites, dumping of plastic in rivers, promotion of circular energy	Challenge in law imposition in rural areas; requires ban on plastic bags and cosmetic product microbeads
Sweden	Plastic bag tax	Tax imposition on importers and producers to prevent the spread of microplastics; aimed at annual use of less than 40 plastic bags by 2025	Permission for use of less than 40 bags per year does not stop bag's use completely
Africa	Ban on plastic bags	Ban of plastic bags, tax imposition	Lack of alternatives, resistance by stakeholders
Korea	Plastic waste control plan	Reduction of plastic waste up to 50%, recycling of plastic waste (70%), aiming circular economy	Minimal recycling rate & consumer awareness; engagement of private companies in profit based recycling only
Canada	Canadian Environmental Protection Act 1999 (CEPA)	Aimed at addressing production, sale, import, utilization and disposal of plastics including single use plastics	-
Australia	Recycling and waste reduction bill	Ban of plastic export, initiative of waste management & recycling	
California, USA	Legislation of single-use plastic bags	Ban on single-use plastic, imposition of charge of 10 Cent for recycled paper	
Hawaii, USA	Honolulu plastic ordinance	Reduced single-use plastic (fossil) and its replacement with other plant based material or paper	
New York, USA	State act on plastic bag ban	Ban imposition on the use of single-use plastic by the retailers and grocery stores	There is no ban on the use of plastic at trash bags, garment bags, pharmacy bags
Other US states (Maine, Oregon, Connecticut, Vermont, Delawar)	Legislation on single-use plastic bags	Ban on plastic bags by these five states	
UK	Resource and waste strategy	Aim: plastic package must be recyclable and compostable by 2025. Imposition of tax on plastic packaging	

Continuation of table 2.

Country/region	*Policy/Law*	*Plan of action*	*Challenge /limitation(s)*
France	Circular economy law	Ban on single use plastic; promotion of economical circular models	No restriction on use of microplastics in veterinary and human medicinal products
Malaysia	Road map for zero single-use plastics	Tax imposition on single-use plastics, encouragement of research and development on alternatives to single use plastic; public awareness	Low rate of recycling; lack of awareness; poor policy implementation; high cost of plastic alternatives

Table 3 - Different polymers and respective density. Adapted from Schell *et al.*, 2021.

Polymer type	Density (g cm^{-3})
Acrylonitrile butadiene styrene (ABS)	1.05
Acrylic	1.18
Butyl rubber	1.2
Ethylene propylene diene monomer (EPDM)	1.1
Ethylene propylene rubber (EPR)	0.87
Expanded polystyrene (EPS)	0.01
Extruded polystyrene (XPS)	0.02
Ethylene-vinyl acetate (EVA) /EVA copolymer	0.94
Ethyl acrylate	0.94
Ethylene ethyl acrylate copolymer	0.93
Ethylene propylene (EPR)	0.87
Fiberglass (Polytetrafluoroethylene coated)	2.2
Hydrogenated acrylonitrile butadiene rubber (HNBR)	1.3
Hypalon	1.2
High Density Polyethylene (HDPE)	0.96
Low Density Polyethylene (LDPE)	0.91
Linear Low Density Polyethylene (LLDPE)	0.93
Nitrile rubber (NBR)	1
Neoprene	1.23
N vinylpyrrolidone vinyl acetate	1.27
Paints	1.2
Polybutylene terephthalate (PBT)	1.3
Poly 4,4-dipropoxy-2,2-diphenyl propane fumarate	0.9
Poly(1-butene)	0.86
Poly(vinyl stearate)	0.98
Poly(methyl methacrylate) (PMMA)	1.18
Polyacrylamide	1.3
Polyamide (PA)	1.24
Polyaramid	1.44
Polycarbonate (PC)	1.2
Polyester/ Polyester binder /Polyester rubber	1.39
Polyester epoxide	1.39
Polyethylene (PE)	0.92
Polyethylene terephthalate (PET)	1.38
Polypropylene (PP)	0.91
Polystyrene (PS)	1.05
Polysulfide rubber	1.27
Polyurethane (PU)	1.15
Poly(ether urethane)	1.14
Polytetrafluoroethylene (PTFE)	2.2
Polyvinyl chloride (PVC)	1.4
Polyvinyl chloride acetate (PVCA)	1.36
Silicone / Silicone rubber	1.25
Styrene-acrylonitrile resin (SAN)	1.08
Styrene acrylonitrile copolymer	1.08
Styrene-butadiene (SBR)	0.98
Styrene butyl methacrylate copolymer	1
Suspected tire	1.3

Table 4 - "Summary of potential toxic effects of micro- and nanoplastics on human health". Retrieved from Yee *et al.*, 2021.

Toxic Effects	Characteristics of Plastic Particles	Particle Size	Details
Inflammation	Polystyrene particles	202 nm and 535 nm	• Upregulation of IL-8 expression. • Induced inflammation in human A549 lung cells.
	Unaltered/Carboxylated polystyrene nanoparticles	20 nm, 44 nm, 500 nm, and 1000 nm	• Upregulation of IL-6 and IL-8 expression. • Enhanced inflammation in multiple human malignancies.
	Carboxylated and amino-modified polystyrene particles	120 nm	• Altered expression of scavenger receptors. • M2 cells increased IL-10 production. • Increased TGFβ1 (M1) and energy metabolism (M2).
	Unaltered polyethylene particles	0.3 μm, 10 μm	• Increased the secretion of IL-6, IL-1β, and TNFα in murine macrophages.
	Polyethylene particles from plastic prosthetic implants	0.2 μm and 10 μm	• Induced the expression of TNFα, IL-1, and RANKL. • Resulted in periprosthetic bone resorption. • Induced inflammatory response at the implant area.
	Polystyrene microplastics particles	5 μm and 20 μm	• Induced inflammation in the liver. • Induced adverse effects on neurotransmission.
Oxidative stress and apoptosis	Amine-modified polystyrene nanoparticles	60 nm	• Strong interaction and aggregation with mucin. • Induced apoptosis in all intestinal epithelial cells.
	Cationic polystyrene nanoparticles	60 nm	• Induced ROS generation and ER stress • Induced autophagic cell death of mouse macrophages and lung epithelial cells.
	Unaltered or functionalized polystyrene	20 nm, 40 nm, 50 nm, and 100 nm	• Induced apoptosis of several human cell types.
	polyvinyl chloride (PVC) and poly (methyl methacrylate) (PMMA)	120 nm, 140 nm	• Reduced cell viability with a reduction of ATP and increase of ROS concentrations.
Metabolic homeostasis	Pristine and fluorescent polystyrene microplastics	5 μm	• Changes in amino acid and bile acid metabolism. • Induced gut microbiota dysbiosis and intestinal barrier dysfunction.
	Anionic carboxylated polystyrene nanoparticles	20 nm	• Altered ion channel function and ionic homeostasis • Activated basolateral K^+ channels. • Induced Cl^- and HCO^{3-} ion efflux.
	Polystyrene nanoparticles	30 nm	• Blocked vesicle transport and the distribution of cytokinesis-associated proteins.
	Cationic polystyrene nanoparticles	50 nm and 200 nm	• Disrupted intestinal iron transport and cellular uptake.
	Pristine polystyrene microparticles	5 μm and 20 μm	• Reduction in hepatic ATP levels. • Impairment of energy metabolism.
	Microplastics	0.5 μm and 5 μm	• Metabolic disorder associated with gut microbiota dysbiosis and gut barrier dysfunction. • Increased the risks of metabolic disorder in the offspring.

Table 5 - Commonly used solutions for density separation. Adapted from Frias *et al.*, 2018. * ■; ■; ■, some countries may present lower costs like the CaCl2 case, depending on the grade and purpose it can cost from 6 to 60€ per 250g.

Chemical formula	Reagent name	CAS no.	(g cm-3)	Health Hazard (Toxicity)	Prince Range	(€ per 250g)	Safety-Price Index
NaCl	Sodium chloride	7647-14-5	1.0-1.2	1 (low)	€	3	■
Na2WO4·2H2O	Sodium tungstate dihydrate	10213-10-2	1.4	2(low)	€	70	■
NaBr	Sodium bromide	7647-15-6	1.37-1.40	2(low)	€ €€€€€	(3-5) * 430	■
3Na2WO4·9WO3·H2O	Sodium polytungstate	12141-67-2	1.4	2(low)	€€€€€	276	■
Li6(H2W12O40)	Lithium metatungstate	127463-01-8	1.6	1 (moderate)	€€€€€	360)‡	■
ZnCl2	Zinc chloride	7646-85-7	1.6 - 1.8	3 (high)	€	45	■
ZnBr2	Zinc bromide	7699-45-8	1.71	2 (high)	€€€	200	■
NaI	Sodium iodide	7681-82-5	1.8	2 (moderate)	€€€	130	■
CaCl2	Calcium chloride	10043-52-4	1.71 -2.24	2 (low)	€	(6 - 60)	■

Table 6 - Advantages and limitations of MPs separation methods. Retrieved from Nabi *et al.*, 2022.

Separation treatment type	MPs concentration (obtained sample before treatment)	Separation efficiency	Recovery	Advantages	Limitation
Oxidizing digestion	–	98%	98% for fibers 65.8% for PS	Provide insight about new methods and detect high-density plastics.	Polymer might get affected.
Alkaline digestion	0.23 to 0.157 items/L	100%	100% for PS	A cost-effective and efficient method for the treatment of organics and MPs.	Some polymers might be degraded.
Density separation	–	>95%	91–99%	Rapid, reproducible, low-cost single-stage method for the removal of different plastics.	Size of MPs effects on the recovery rate of plastics.
Heat assisted density separation from sediments	The average abundance was 6639 in size ranging from 0.1 to 1 mm/kg sample	93%	93%	Improves the separation performance by utilizing heat-assisted density separation.	Required further investigation for MPs pollution in offshore maricultural and also need to determine the effect of fishing.
Digestion method	700-4007 items/Kg	100%	100%	Have the ability to digest the vegetal litter and improves MPs separation.	Repeatable addition of H_2O_2 increases the cost and needs a high temperature.
JAMSTEC MPs-sediment separator	–	94–98%	92–98%	Efficient device for MPs removal with no chemical contamination.	Applied grease for lubrication may catch plastics; dilution for clay sediments is necessary.
Munich plastics sediment separator	–	95.5%	55%	Reliable, time-efficient separation, identification, and quantification method for MPs.	A standardized sampling technique for beach sediments is required.
Sieving Method	–	97.0%	97%	Easy method and first-time highlight the plastics contamination.	Dry method, and need to evaluate the Rayon separation.

Table 7 - Multiple Digestion methods for organic matter removal, highlighting particle and OM degradation. Retrieved from Prata *et al.*, 2019.

Digestion	Treatment	Recovery rate	Polymer degradation	Organic matter degradation
Acid	HNO_3 (35%), 60°C 1 h	n.a.	Fusion of PET and HDPE; destruction of PA	100%
	HNO_3 (65%), RT overnight, 60°C 2 h, dilution 80°C distilled water	n.a.	PA degradation; yellowing	n.a.
	HNO_3 (65%) and HClO4 (65%) 4:1 overnight, boiled 10 min, dilution 80°C distilled water	n.a.	PA degradation, yellowing	n.a.
	HNO_3 (5–69%), RT 96 h	<95%	Melted LDPE and PP; color change in PP, PVC, PET; decrease Raman peaks	n.a.
	HNO_3 (55%) RT 1 month	n.a.	Whitening of PVC, degradation of PA	n.a.
	HCl (5–37%), 25-60°C 96 h	n.a.	Changes in PET and PVC	>95%
Alkali	NaOH, 60°C 1 h	94%	No	100%
	NaOH (10 M), 60°C 24 h	n.a.	CA degradation	n.a.
	$K_2S_2O_8$ (0.27 M) and NaOH (0.24 M), 65°C 24 h	n.a.	CA degradation; unpredictable weight increase	n.a.
	KOH (10%), RT 3 weeks	n.a.	No	n.a.
	KOH (10%), 60°C 24 h	n.a.	CA degradation	n.a.
	KOH (10%), 50°C 96 h	n.a.	Loss of PET and PVC	n.a.
	KOH (10%), 40°C 96 h	n.a.	Loss of PET; yellowing of PA	n.a.
	KOH (1 M), RT 2 days	n.a.	Degradation of LDPE, CA, Cradonyl and PA.	Most, except otoliths, squid beaks, paraffin, palm fat
	NaOH (1 mol L^{-1}), 17.5 mL of 65% HNO_3 and 2.5 mL UP and drying	95%	Degradation of PA, PET, EPS, LDPE, PVC; color change in PVC and PET	n.a.
Oxidative	H_2O_2 (30%), 60°C for 1 h, 100°C for 7 h	n.a.	n.a.	n.a.
	H_2O_2 (35%), RT, 40°C 96 h	n.a.	Decrease in Raman peaks of PVC and PA.	n.a.
	H_2O_2 (35%), RT, 50°C 96 h	n.a.	Degradation of PA; color change of PET; foam and oxidization	n.a.
	H_2O_2 (6%) 70°C for 24 h	78% (PE)	n.a.	n.a.
	H_2O_2 (30%), 60°C until evaporation	n.a.	n.a.	n.a.
Enzymatic	Corolase 7086, 60°C 1 h	93%	No	n.a.
	Tripsin, 38-42°C 30 min	n.a.	No	88%
	Collagenase, 38-42°C 30 min	n.a.	No	76%
	Papain, 38-42°C 30 min	n.a.	No	72%
	Pepsin (0.5%) and HCL (0.063 M), 35°C 2 h	n.a.	No	Incomplete
	15 mL Tris-HCl 60°C 60 min, proteinase K (500 µg/mL) and CaCl2 50°C 2 h, shaken 20 min, incubated 60°c 2 h, 30 mL H_2O_2 (30%) overnight	97%	Calcium layer	n.a.

Table 8 - Various advantages and disadvantages of sample preparation methods. Retrieved from Stock *et al.*, 2019.

Method	Areas of applicability	Advantages	Disadvantage
Electroseparator	Sediment	Reduction of sample mass of ca. 90%	Loss of small amounts of sediment possible; not suitable for small samples
Munich Plastic Sediment Separator	Sediment (density separation, see zinc chloride)	Direct separation of entire, large sample amounts	Huge amounts of heavy solutions needed
Organic digestion			
Acidic digestion (HNO_3, HCl)	Sediment, water, biota	HNO_3: Most organics destroyed	HNO_3: Dissolution of PS and PE possible; HCl: incomplete destruction of organics
Alkaline digestion (NaOH)	Sediment, water, biota	Most organics destroyed	Some polymers degraded (e.g. PC, CA, PET; PVC)
Alkaline digestion (KOH)	Sediment, water, biota	Most organics destroyed, most polymers resistant	
Oxidizing digestion (H_2O_2)	Sediment, water, biota	Most organics destroyed	Polymers might be affected
Enzymatic degradation (cellulose, lipase, chitinase, protease, proteinase-K)	Sediment, water, biota	Most organics destroyed, not hazardous	Time-consuming, partly expensive, different enzymes for different sample
Density separation			
Sodium chloride	Sediment, water, biota	Low costs, low toxicity	Not all polymer types detectable (density of saturated solution too low)
Sodium tungstate dihydrate	Sediment, water, biota	Cost-effective, high density	
Sodium polytungstate	Sediment, water, biota	High density	Expensive
Potassium formate	Sediment, water, biota	Cost-effective, high density, not hazardous	Hygroscopic
Zinc chloride	Sediment, water, biota	Not expensive, high density	Corrosive, hazardous
Sodium iodide	Sediment, water, biota	High density	Expensive
Oil	Sediment, water, biota	Cost-effective, easy to handle	Until now, only used in few studies

Table 9 – Methodology and Results summary of Eo *et al.* and Schell *et al.* studies. UA's = Urban Areas or Urbanizations, Agro = Agricultural fields.

Country	Location	Sample	SMethod	Digestion	Methods	Density	Abundace
South Korea	Nakdong River	Water surface (20cm); Mid area (1m) and Sediment	Stainless steel beaker; Submersible pump, 20µm mesh net and 1L amber glass bottle Van Veen grab, Stainless steel spon and 1L amber glass bottle	20 mL 35% H2O2 + Fe (II) solution (75° C, 180 rpm) 30 min	20µm sieve + drying 60° FTIR	LMT	n1 = 293 - 4760 particles/m3 n2 = 1971 particles/kg; 37311 particle/m2 (top 2cm)
Spain	Tagus River	Water surface, Sediment, Raw and Processed Sludge, Untreated influent and Treated effluent	WWTPs = Nylon nets (55, 150, 300 µm) and glass flasks; River water = Submersible pump and nets Core sampler and glass flasks	Fenton's reagent	Vaccum filtered (paper filters); 38 µm stainless steel sieve Visual inpection >300µm = ATR - FTIR 55 - 300µm = µFTIR	Nal	n1 = 1.30 - 147 particles/m3; 0.54 - 14.6 mg/m3 n2 = 0 - 2910 particles/kg; 0 - 44.3 mg/kg UWW = 850 - 11,550 particles/m3; 1.86 - 194 mg/m3 TWW = 45 - 535 particles/m3; 0.28 - 48.5 mg/m3 PSLG = 2432 - 24,828 particles/kg; 5.05 - 1525 mg/kg RSLG = 7161 - 66,260 particles/kg; 12.7 - 553 mg/kg

Continuation of **Table 9**.

Country	Location	Type	ChemComp	Size	UAs	Agro	WWTPs
South Korea	Nakdong River	n1 = Fragments 69%, Fibers 30%, and Spheres and films <1% n2 = Fragments 84%, Fibers 15%, and Spheres 1%	n1 = 41.8% PP, 23.1% PES, 9.4% PE, 5.8% PA, PS 2.1, 4.2% Alkyd, 3.2% Acrylic, 2.6% PEVC, 1.4% PU, 1.1% PVC, 1% PAS n2 = 24.8% PP, 24.5% PE, 5.5% PES, 5.4% PVC, 5.3% PS, 4.6% Acrylic, 4.5% PDS, 3.9% PU, 3.7% PAS and 3.6% PLA Swater = 26% PP, 30% PES, 7% PE, 10% PA, and 6% alkyd Mwater = 34% PP, 23% PES, 8% PE, 8% PA, and 5% alkyd	n1: range = 50 - 150µm mean = 265 µm median = 154µm <300µm = 74% n2: range = 100 - 150µm mean = 248µm median = 155µm <300µm = 81%	✓	✓	✓
Spain	Tagus River	Average values UWW = 42% Fragments, 41% Fibers 12% Granules, foams, beads, films, and glitter TWW = 69% Fragments, 19% Fibers 11% Granules, foams, beads, films, and glitter PSLG = 56% Fragments, 44% Fibers 1.6% Granules, foams, beads, films, and glitter RSLG = 52% Fragments, 47% Fibers >1% Granules, foams, beads, films, and glitter n1 = 81% Fragments, 10% Fibers 9% Granules, foams, beads, films, and glitter n2 = 87% Fragments, 13% Fibers >1% Granules, foams, beads, films, and glitter	Most common UWW = PS, PE, PP, and Tyre TWW = Paint, PP, PE, Acrylic, and PS PSLG = PP, PES, PE, PS, and Acrylic RSLG = PP, PES, PE, and Acrylic n1 = PP, PES, PE, Acrylic, and Tyre n2 = PP, PES, PE, PS, EPR, PVC and Acrylic	UWW = 55 - 5000µm TWW = 55 - 5000µm PSLG = 55 - 5000µm RSLG = 55 - 5000µm n1 = 55 - 5000µm; >300µm less frequent n2 = 55 - 5000µm; most were <300µm	✓	✓	✓

Table 11 - Granulometric results. Mz - mean grain size, σi - standard deviation, p/150µm - particles per volume, Distance(km) - distance from the closest known pollution source and classification - grain size class.

Sample	Mz	σi	p/150µL	Distance (km)	Classification
MP001	2.256667	-2.41364	11	0.82	Sandy gravel
MP002	1.766667	-1.43939	17	0.49	Gravelly sand
MP003	5.853333	-3.30439	39	0.45	Sandy gravel
MP004	6.166667	-2.68583	20	1.2	Gravel
MP004B	6.866667	-4.92197	37	1.3	Sandy gravel
MP005	3.42	1.66	45	1.74	Slightly gravelly muddy sand
MP006	2.62	1.67	35	1.8	Slightly gravelly muddy sand
MP007	3.423333	-1.8697	31	0.4	Sandy gravel
MP008	9.69	-5.38742	33	1.82	Gravel
MP009	4.373333	-2.60348	53	0.61	Sandy gravel
MP010	1.6	-1.28492	30	0.26	Gravelly sand
MP011	0.92	-1.82136	37	0.3	Slightly gravelly muddy sand
MP012	2.06	-1.84818	37	1.47	Gravelly sand
MP012B	2.123333	-2.10682	58	1.47	Gravelly sand
MP012C	3.033333	-2.50152	35	1.47	Sandy gravel
MP013	0.726667	-0.52561	37	0.1	Slightly gravelly muddy sand
MP014	1.396667	-0.7528	16	1	Gravelly sand

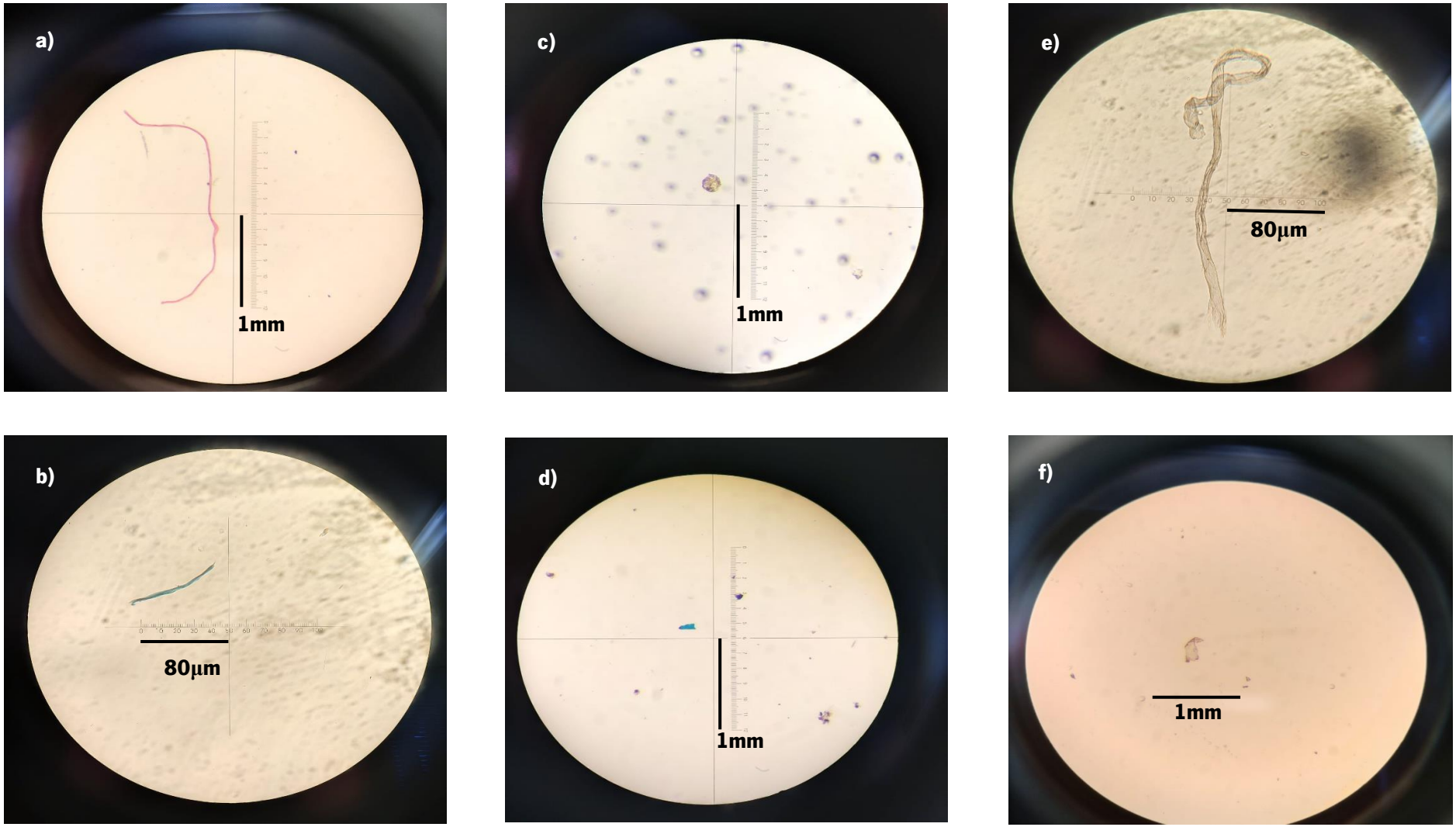


Figure 36 - Some of the visualised particles. Particles a) and b) are fibers, d), c) e), and f) are fragments.

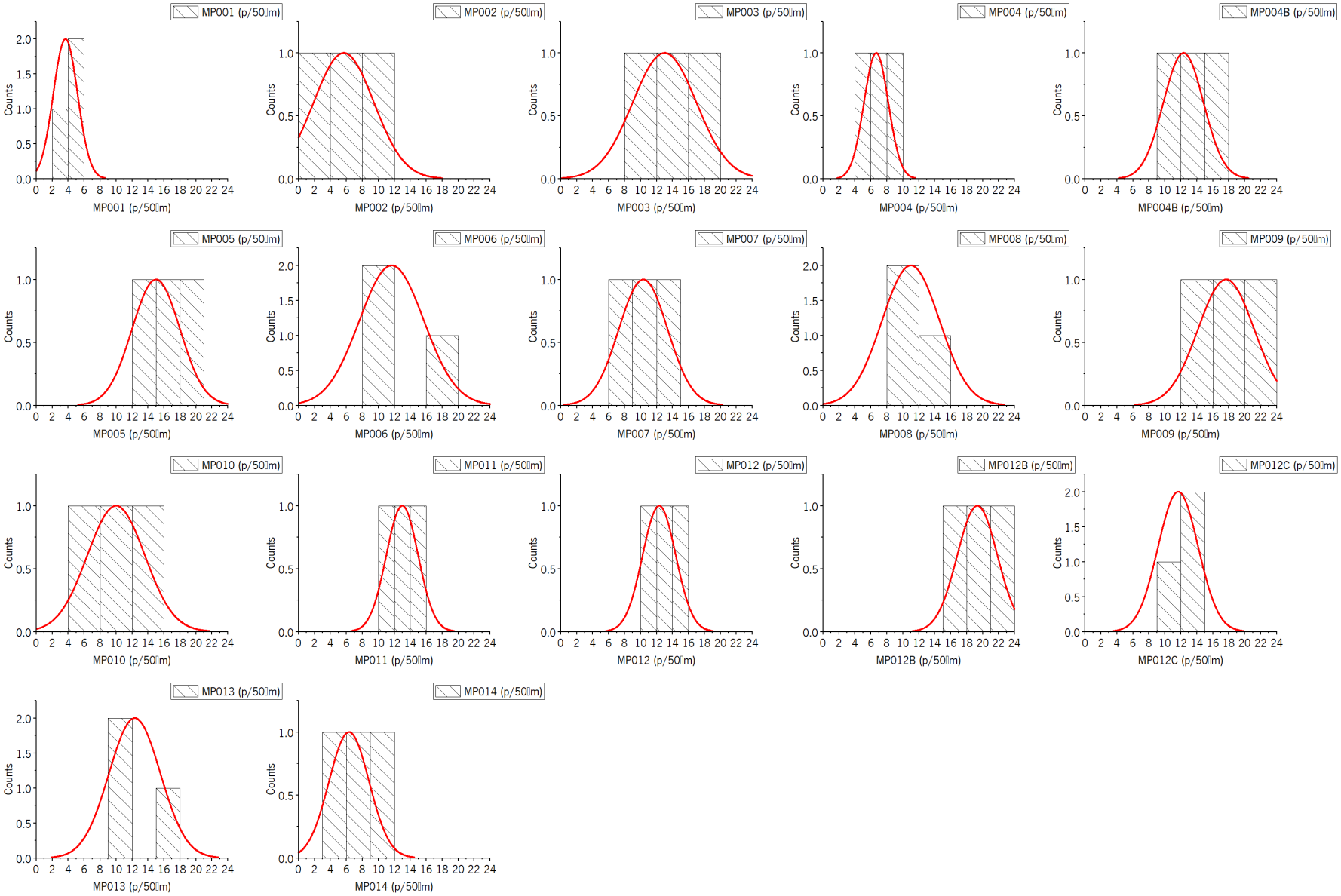
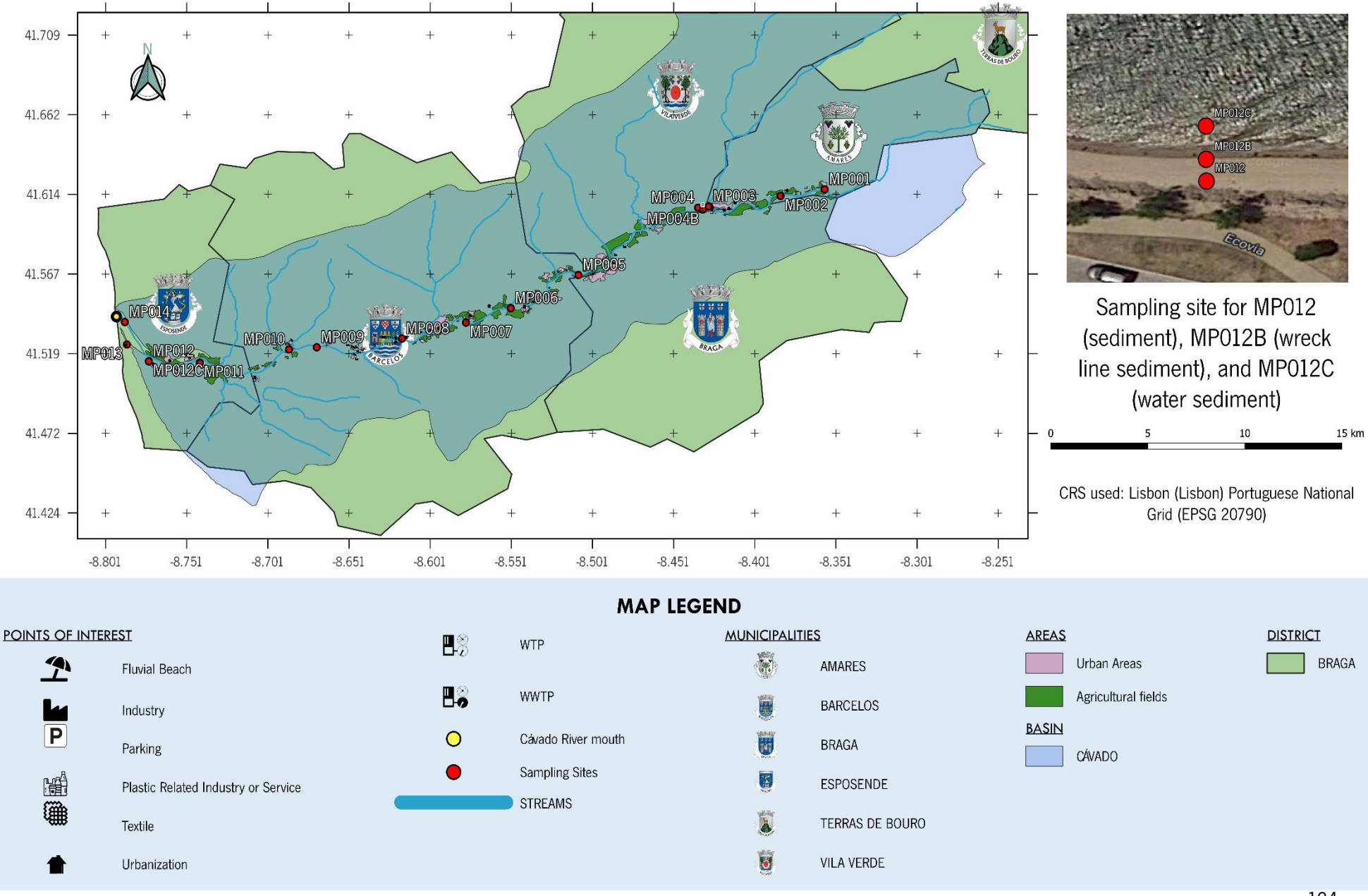
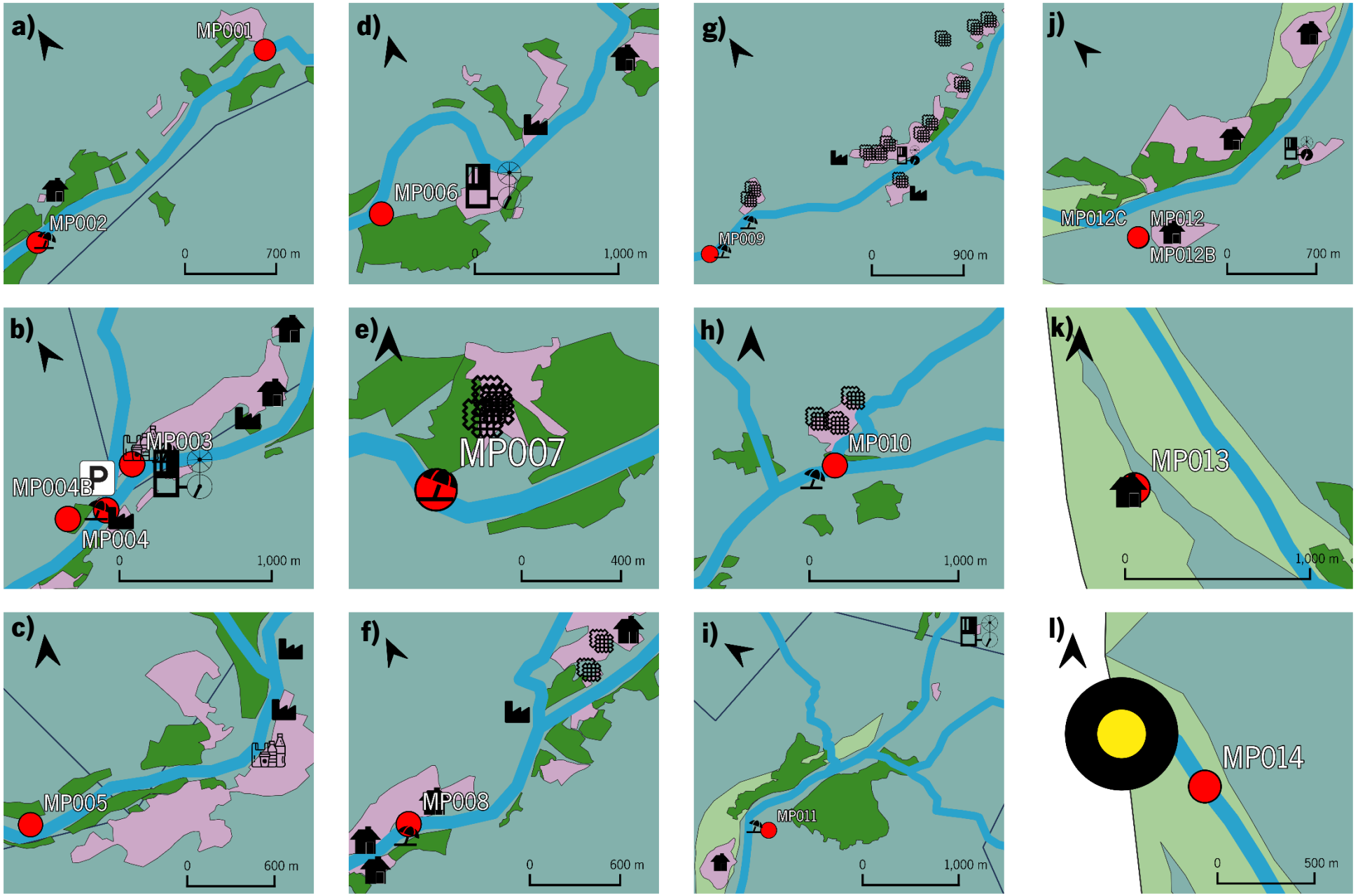


Figure 38 - Normal Distribution plots for each sampling site.

Geographical Framing of the Region and Sampling Sites



Map 1 - Graphic representation of the study area, sampling sites and riverine stress factors.



Map 2 - Sampling sites zoomed in with the possible affecting factors.