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DISSERTAÇÃO DE MESTRADO

TOXICOLOGIA E CONTAMINAÇÃO AMBIENTAIS

Potential of lost fishing gears for adsorption of metals

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INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR

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POTENTIAL OF LOST FISHING GEARS FOR ADSORPTION OF METALS

Dissertation for the Master degree in Environmental Toxicology and Contamination submitted to the Abel Salazar Biomedical Sciences - Institute from the University of Porto.

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RESUMO

As artes de pesca perdidas são essencialmente compostas por polímeros plásticos. Embora as redes de pesca sejam consideradas inertes à dinâmica ambiental, estas podem induzir modificações na sua superfície, tornando as redes mais ou menos suscetíveis à adsorção de poluentes e a degradação em fragmentos plásticos menores.

A poluição química associada diretamente à presença destas redes de pesca perdidas ainda não está suficientemente estudada nem clarificada. O trabalho desenvolvido visa avaliar a nocividade ambiental das artes de pesca perdidas, avaliando as redes de pescas como um novo poluente. Para tal, inicialmente foi realizada uma monitorização *in-situ* de contaminantes químicos inorgânicos (metais) em zonas de acumulação de redes de pesca perdidas. O primeiro local, um submarino naufragado localizado ao largo da praia de Angeiras, é um local considerado um recife artificial. O segundo local, em Cavalos de Fão, Esposende, é uma área marinha natural protegida, de fundo rochoso e com diversos recifes. Ambos os locais selecionados apresentam intensa atividade pesqueira. No geral, os níveis de metais no material particulado em suspensão e nos sedimentos mostram-se baixos não permitindo avaliar uma clara influência das redes de pesca perdidas.

Experiências laboratoriais foram posteriormente realizadas de modo a avaliar o potencial de adsorção de metais pelas redes de pesca, nomeadamente de Cu e Pb, dois dos metais encontrados nas áreas estudadas. Para tal, foram avaliados quatro tipos de redes de pesca, nomeadamente *Euroline®* (Polietileno), *Braided PE* (Polietileno), *Thin Nylon* (Nylon), *Twisted Nylon* (Nylon). De um modo geral, a rede *Twisted Nylon* mostrou maiores valores de adsorção para ambos os metais (até 53% para Cu e até 39% para Pb). Experiências de libertação de metais indicam que Cu, mas não Pb, previamente adsorvido às redes de pesca pode ser libertado (até 15% do total de metal adsorvido).

Posteriormente, foi efetuada uma experiência em cenário quase real, na Marina de Leixões, para investigar a adsorção de metais a redes de pesca. Os resultados mostram que a adsorção foi dependente não só de processos físico-químicos, como demonstrado na experiência laboratorial, mas também de processos biológicos, devido à formação de biofilme na superfície das redes de pesca.

Os resultados obtidos sugerem que as redes de pesca efetivamente apresentam potencial para adsorção de metais, sendo este um tema inovador que carece de mais pesquisas.

No futuro, um estudo mais profundo sobre a temática é necessário para esclarecer alguns fatores e processos que parecem influenciar os valores de adsorção.

Este trabalho contribuiu para uma primeira abordagem sobre o tema. Seria também,

por exemplo, interessante um estudo para avaliar o potencial das redes de pesca (virgens e envelhecidas) em locais conhecidos como ambientalmente contaminados. Em geral, os resultados obtidos nesta tese apresentam um contributo significativo para a escassa informação disponível na literatura sobre a interação de compostos inorgânicos (metais) e as redes de pesca, que são frequentemente detetadas nos nossos rios e oceanos.

Palavras-chave: Lixo marinho, poluentes inorgânicos, metais, redes de pesca perdidas, plásticos

ABTRACT

Lost fishing gears are mainly composed by plastic polymers. Although fishing nets are considered inert to environmental dynamics, they can suffer changes in their surface, making them susceptible to the adsorption of pollutants and degradation in smaller plastics fragments.

Chemical pollution directly associated with the presence of these lost fishing nets is still not studied or clarified. The work developed aims to evaluate the environmental harmfulness of lost fishing nets as a new pollutant.

For this purpose, initially an in-situ monitoring of inorganic chemical contaminants (metals) was carried out in two known hotspots of lost gear. The first site was a submarine wreck located on the coastline of Angeiras beach, Matosinhos, this site being considered an artificial reef. The second site, in Cavalos de Fão, Esposende, is a marine protected natural area, with a rocky bottom and the presence of reefs. Both selected locations have intense fishing activity. In general, the levels of metals in water particulate matter and sediment showed low metal levels preventing to assess a clear influence of the lost fishing nets.

Laboratory experiments were carried to evaluate fishing nets potential to adsorbed metals, namely Cu and Pb, two metals found in the environmental areas studied. For that, four types of fishing nets were tested, namely Euroline® (Polyethylene), Braided PE (Polyethylene), Thin Nylon (Nylon), and Twisted Nylon (Nylon). In general, the Twisted Nylon fishing net showed higher adsorption values for both metals (up to 53% for Cu and up to 39% for Pb). Metals release experiments indicated that Cu, but not Pb, previously adsorbed to the fishing nets could be release (up to 15% of total adsorbed metal).

Subsequently, an experience in a quasi-real scenario, at the Marina of Leixões was assembled to investigate metal adsorption. Results showed that adsorption of metals to fishing nets was dependent not only on physical-chemical processes, as demonstrated in the laboratory experiments, but also on biological processes, due to the formation of biofilm on fishing net surfaces.

The results obtained suggested that fishing nets effectively have potential for adsorption of metals, being this an innovative topic that needs further research.

In the future, a deeper study is needed to clarify some factors and processes that seem to influence the sorption values.

For instance, it would be interesting to assess the potential of fishing nets (virgin and aged) in sites known to be environmentally contaminated.

In general, the results obtained in this thesis significantly contribute to the scarce information available in the literature on the interaction of inorganic compounds (metals)

and fishing nets, which are frequently found in our rivers and oceans.

Keywords: Marine litter, inorganic pollutants, metals, lost fishing nets, plastics

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ABBREVIATIONS

ALDFG	Abandoned, lost, or discarded fishing gears
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cu	Copper
FAO	Food and Agriculture Organization
Fe	Iron
HNO_3	Nitric acid
HTPE	High Tenacity Polyethylene Fibers
LOD	Detection limit
Mn	Manganese
MPs	Microplastics
$(NH_4)H_2PO_4$	Ammonium Dihydrogen Phosphate
NH_4NO_3	Ammonium Nitrate
Ni	Nickel
NW	Northwest
PA	Polyamide
PAHs	Polycyclic Aromatic Hydrocarbons
Pb	Lead
PE	Polyethylene
PNLN	North Coast Natural Park
POPs	Persistent Organic Pollutants
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl Chloride
UV	Ultraviolet
WWTPs	Wastewater Treatment Plants
Zn	Zinc

CHAPTER 1

INTRODUCTION

1. Introduction

1.1. Fishing

1.1.1. Fishing in Portugal

The fishing sector in Portugal has a long history and tradition, assuming high social, regional, and local relevance (European Commission, 2013). Fisheries contribute to the local development of coastal communities, employment, maintenance of local traditions, and also to the creation and maintenance of economic activities (Ferraz de Arruda *et al.,* 2011). In addition, the fishing sector has strategic importance for the country's socioeconomic situation (PNP, 2015), and is also one of the most important coastal activities (DGRM, 2020).

The professional fishing in Portugal is regulated by seven ordinances that establish the characteristics of the gear that can be used as well as their dimensions, areas of operation, and species to be collected, among other characteristics (DGRM, 2020).

Currently, fishing activity has been described as direct and major contributors to oceanic pollution by plastics and microplastics (MPs) (Dowarah & Devipriya, 2019), since gear and/or fishing nets have been commonly detected in the oceans and sometimes make up more than half the total mass of plastics found, as observed at the Great Pacific Ocean (Lebreton *et al.,* 2018).

1.1.2. Types of fishing gear

Fishing gear can be generally classified in two categories: i) active and ii) passive. Active fishing is based, in general on the exclusive pursuit of target species (e.g,. through trawl nets, purse seine), while in passive fishing the catch is made using the movement of target species towards the fishing gear (e.g., traps, longlines) (Bjordal, 2002).

There are several classifications for fishing methods, however, in Portugal majority the most common types are multipurpose fishing (66.5%), purse seine (13.5%) and trawling (9.7%) (INE/DGRM, 2019).

Multipurpose fishing consists of the use of various gear such as trammel nets, traps, longlines fishing gear and gillnets (DGRM, 2020).

The purse siene fishing method consists of using a long high-starting fishing net, which is maneuvered in order to surround the prey and close, thus reducing its escape capacity. It is the method use to capture pelagic species (DGRM, 2020).

As for the trawling method, although there are more than one type of trawling, it is essential a fishing method that uses fishing nets towed by one or two vessels composed of a large bag than can be extended sideways by relatively small "wings" (DGRM, 2020).

1.2. Fishing gear composition

Before the 1960s, fishing nets were composed of natural fibers such as *Gossypium spp.* (cotton) and *Musa textilis* (Manila hemp) (Kim *et al.,* 2020). However, with the end of World War II, rapid evolution and industrialization also emerged, which led to the exploitation of synthetics fibers and later to their use to produce fishing gear. These synthetic fibers have some characteristics that make them competitive and attractive to fishers, namely, flexibility, durability (have great resistance to breakage), and good handling performance, in addition to the low cost associated (Kim *et al.,* 2020; SICOR, 2020). Therefore, these plastic fishing nets very quickly replaced the old ones (Kim *et al.,* 2020).

Currently, there are several synthetic fibers used in the production of fishing nets, which are essentially made up of polyethylene (PE), polypropylene (PP) and Polyamide (PA), generically knows as nylon (nylon 6/6), replacing the prior art made using natural fibers. One of the polymers used in the composition of fishing nets, PE is used in the manufacture of oceanic, semi-oceanic, trawl and demersal fishing gear (DGRM, 2020; SICOR, 2020). This polymer has as main characteristics its long-life cycle, good resistance to rupture, good elasticity, and floatability (density 0.91-0.92) (FAO, 1990). Another polymer is PP, which is also resistant to rupture, also presenting a long-life cycle, being light and easy to handle. PE and PP float in seawater and can travel long distances, moving easily away from their source of entry into the aquatic ecosystem (Zhang *et al.*, 2018; SICOR, 2020). Nylon (PA) is also widely used, essentially in pelagic trawls and is a material that is very resistant to abrasion and extra resistant with low floatability (FAO, 1990; DGMR, 2020).

1.3. Pollution from lost, abandoned, or discarded fishing nets

The inadequate discharge of various types of waste presents currently a serious, severe, and global problem. The residues that are drifting in the seas, rivers and oceans pose a great threat to the ecosystem from an ecological and socioeconomic point of the view (Link *et al.,* 2019).

Currently, a type of waste frequently found in the oceans, which has a global concern in recent decades, are gear and fishing items (Lebreton *et al*., 2018; Link *et al.,* 2019), being that the fishing industry contributes to marine litter with more than 45% of biomass plastic debris found in the ocean (Lebreton *et al.,* 2018). On the European coast, plastic represents about 80-85% of the marine litter of which 50% are disposable plastic items and 27% result from fishing, such as nets, lines, pans, traps, among others (European Commission, 2018).

Abandoned, lost, or discarded fishing gear (ALDFG) has been classified as a global problem since the 1980s by the Food and Agriculture Organization (FAO). Since then, several strategies have been implemented by countries all over the world, with the objective

of controlling and reducing losses of fishing nets and consequently ghost fishing (Kim *et al.*, 2020). Over the past decade, the widespread concern about ALDFG has grown, as well as the concern associated with the impacts that these ALDFG can have on the marine environment (Macfadyen *et al.,* 2009; Huntington, 2019).

Fishing gear is included in the European Commission's proposal to reduce the impact of plastic waste on the environment (European Commission, 2018). The causes behind these losses are extremely important and relevant and vary between and within the fishing sectors (Brown *et al.,* 2005). In the 1990s, little research existed on what is currently classified as ghost fishing by seines, gill, and bottom trammel nets, both globally and in European waters (Brown *et al.,* 2005). However, ALDFGs comprise a significant amount of the marine plastic pollution worldwide, with an estimated 640.000 tons of ALDFGs discarded in the marine environment each year (Richardson *et al.,* 2018), and about 20% of fishing gear in the EU is lost or discarded at sea, due to several reasons ranging from accidents, storms and entanglements to intentional abandonment (European Commission, 2020).

Synthetic fishing gear was designed to withstand the adverse environmental conditions commonly observed in the seas and oceans, and it is estimated that monofilament fishing nets can take up to 600 years to degrade (European Commission, 2018). Due to this characteristic, fishing gears are difficult to degrade in the marine ecosystem and their function can be maintained for a long period of time (Richardson *et al.,* 2018; European Commission, 2018), causing impacts on the ecosystem as entanglement, habitat destruction and ghost fishing that can last for days, months or even several years (Breen 1987; Link *et al.,* 2019; Lively & Good, 2019).

The presence of ADLFGs are one the main problems for marine fishing and conservation, since their presence in the marine ecosystem can have a significant impact on commercial fishing and sea-dependent industry due to ghost fishing (Lusher *et al.,* 2017). Along with impacts on marine organisms and marine conservation, ghost fishing is classified as the most serious consequence of fishing by the FAO (FAO, 1995; Link *et al.,* 2019; Lively & Good, 2019; Kim *et al.,* 2020). In addition, the potential impact of MPs fibers resulting from fishing ropes and nets on marine life is also worrying (Ramos *et al.,* 2012; Rodriguez *et al.,* 2012).

In a recent study, carried out in the Great Pacific Garbage Patch (an area of plastic accumulation), it was estimated that there were about 42.000 tons of mega plastics (over 50 cm in size), of which 86% would come from the fishing sector, namely fishing nets (Lebreton *et al.,* 2018).

The loss of biodiversity that is also observed today is strongly related to the presence of marine waste, not only because of the high persistence of the material of fishing

equipment, but also due to the fact that ADLFG continues to fish and extract biomass from the ocean (Eerkes-Medrano *et al.,* 2015).

1.4. Microplastics

Coastal and marine areas are increasingly subject to continuous anthropogenic pressures (Auta *et al.,* 2017). Plastic waste has been identified in marine environments for several decades, but recently it has become an area of increasing and worrying interest (Ng & Obbard, 2006; Holmes *et al.,* 2012; Holmes *et al.,* 2014; Cózar *et al.,* 2014; Jasna *et al.,* 2018; Vedolin *et al.,* 2018; Dowarah & Divipriya, 2019).

Plastic fragments can easily enter the marine ecosystem either through direct or indirect mechanisms, either through drainage systems, debris from the coast and beaches, and even by direct deposition of humans, which can easily reach different habitats through sea currents (Ahechti *et al.,* 2020).

ALDFG residues present in the oceans and even in freshwater ecosystems may result in smaller fragments – the so called microplastics (MPs) (Oz *et al*., 2019), resulting from slow degradation through chemical and physical processes in waters, such as wave abrasion and ultraviolet (UV) aging (Graham & Thompson, 2009; Wang *et al.,* 2017; Huntington, 2019; Ahechti *et al.,* 2020).

MPs are commonly classified as plastic particles smaller than ≤5 mm and are currently considered a major omnipresent environmental problem (Andrady, 2015; European Commission, 2011; UNEP, 2016; Almeida *et al.,* 2020), result of their ability to spread easily in the ocean and freshwater environments (Ng & Obbard, 2006).

When fishing gears degrades and consequently result in smaller fragments, they share the same problems associated with plastic pollution, namely, entanglement, chemical risk and loss of biodiversity that is currently observed (Eerkes-Medrano *et al.*, 2015; Wang *et al.,* 2017). It is estimated that population decline of some species may also be related to the ADLFG that continue to fish (ghost fishing) (Eerkes-Medrano *et al.,* 2015), which may result in the potential ingestion of plastic fragments by aquatic organisms (Ahechti *et al.,* 2020). Consequently, MPs can compromise human health, since the presence of MPs has been detected in aquatic organisms consumable by human and which can have adverse effects on human health and well-being, resulting from the long-term consumption of these foods (Oliveira *et al.,* 2018; Peixoto *et al.,* 2019).

The presence of these plastics debris in the waters leads to a growing awareness of the risks of pollution associated with these items lost, abandoned, or discarded in some way in the seas and oceans (Prunier *et al.,* 2019). It is estimated that 88% of ocean pollution is associated with plastic debris resulting from primary or secondary sources (Cózar *et al.,*

2014). Primary sources include those in personal care products, including facial and hand cleaning, cosmetic preparations, synthetic clothing (released during washing) and waste from plant production or plastic processing. Secondary sources include the fragmentation of larger plastics as a result of the environmental hydrodynamics of the ocean (Cole *et al.,* 2011) and UV (Graham & Thompson, 2009; Wang *et al.,* 2017; Huntington, 2019; Ahechti *et al.,* 2020).

Fragment plastics resulting from fishing activity, such as fishing nets and other plastic debris, occurs globally both in terrestrial and marine habitats, being more frequent in the latter (Brennecke *et al.,* 2016). Currently, it is estimated that there are about 5.25 trillion of MPs in the seas, and it is believed that 10% of these plastics originate from fishing activity (Oz *et al.,* 2019).

Hereupon, assessing the contamination of coastal and marine environments presents one of the most complex current issues in ecotoxicology and environmental management (Auta *et al.,* 2017). Marine litter has become, in recent decades, a global environmental problem that effects all aquatic ecosystems (Shim &Thompson, 2015; Ahechti *et al.,* 2020).

1.5. Interactions between plastic fragments and metals

Environmental pollution associated with inorganic compounds, namely metals, occurs in general in rivers, water reservoirs, lakes, estuaries, and oceans, due to the significant anthropogenic activity occurring these days, as a result of the rapid and continuous industrialization that is still taking place (Buyang *et al.,* 2019).

Several studies indicate that MPs can adsorb different types of contaminants, such as persistent organic pollutants (POPs), xenoestrogens, metals, among others (Bakir *et al.,* 2014; Holmes *et al.,* 2014; Koelmans *et al.,* 2016; Brennecke *et al.,* 2016; Wang *et al.,* 2018; Wang *et al.,* 2019; Frias *et al*., 2010). However, there are more studies, reporting adsorption of organic compounds by plastic fragments, since they can be more easily adsorbed to MPs (Yu *et al.,* 2019). In fact, studies of adsorption of metals to plastic particles were scarce until a decade ago (Ahechti *et al.,* 2020).

According to studies available in the literature (Godoy *et al.,* 2019; Brennecke *et al*., 2016; Xu *et al.,* 2020), metals, including Cd, Cu, Cr, Ni, Pb, and Zn are often found in wastewater. Although the metals Cu, Fe, Ni, and Zn are nutritious for microorganisms, they become inhibitors or even toxic, when their concentration in a given environmental compartment is much higher than needed levels (Xu *et al.,* 2020).

Metals and plastic fragments are included in two distinct classes of pollutants, and the interaction between these two environmental stressors is poorly understood (Brennecke *et al.,* 2016), highlighting the need for more studies in this topic. Although it was thought that

plastic polymers would be completely inert to aqueous metal ions (Plastic Europe, 2011; Oz *et al.,* 2019), some recent studies have shown that interactions can effectively occur as a result of plastic surface changes in the marine environment (Vedolin *et al.,* 2018; Holmes *et al*., 2012; Holmes *et al.,* 2014; Koelmans *et al.,* 2016; Brennecke *et al.,* 2016; Duarte *et al.,* 2010; Dobaradaran *et al.,* 2018; Jasna *et al*., 2018; Bakir *et al.,* 2014; Bayo *et al.,* 2017; Yu *et al.,* 2019; Li *et al.,* 2018; Llorca *et al.,* 2018; Godoy *et al.,* 2019). Consequently, after studies reporting that effectively the interactions between metal and plastic could occur, plastic fragments were considered an important intermediary for the transport of metals in aquatic environments, although most studies are reported for freshwater environments (Holmes *et al.,* 2012). Adsorption of some metals such as Cu, Cr, Ni, Pb, and Zn have been studied (Brennecke *et al.,* 2016; Dobaradaran *et al.,* 2018; Hodson *et al.,* 2017; Holmes *et al.,* 2012; Saeedi *et al.,* 2018; Godoy *et al.,* 2019).

Based on the available literature, Ahston *et al.* (2010), observed interactions between metals and plastic production pellets, sampled from four beaches in SW England, with plastic pellets adsorbing metals like Cd and Pb. Later, a study by Holmes *et al.* (2012) concluded that plastic showed the capacity for adsorption of metals such as Cd, Cu, Pb, Ni, and Zn. Holmes *et al.* (2014) studied the adsorption behaviors of Cd, Cr, Cu, Co, Ni and Pb in plastic fragments composed of PE (in estuarine conditions) and according to the results obtained, aged pellets have a greater capacity for adsorption of metals than virgin PEs. In a subsequent study by Turner & Holmes (2015), the authors found an increase in the adsorption of trace metals (Cd, Pb, Ni, and Zn) to virgin pellets, emphasizing that increase in adsorption would be depending on the increase in pH and contacting time. Turner & Holmes (2015) also described that the oxidation and weathering that the plastic fragments undergo lead to changes in the surface of the fragments, thus facilitating the obtaining of an electric charge and consequently the adsorption of metals ions in order to obtain the balance of the charge. The authors thus explain the fact that higher adsorptions are reported in the literature for aged plastic fragments (Turner & Homes, 2015).

Brennecke *et al.* (2016), carried out laboratory experiments with different polymers such as polystyrene (PS) and polyvinyl chloride (PVC), to investigate if, they could adsorbed metals (Cu and Pb) from seawater, with concentrations up to 800 times higher in plastic fragments when compared to surrounding seawater. However, it has also been proven that plastic resin pellets can have an important influence on the transport of metals in the marine environment (Holmes *et al*., 2012).

In the same year, Turner *et al.* (2016) verified the presence of some metals such as Cd, Cu, Cr, Ni, Pb and Zn in fragments of fishing nets that were collected from beaches in SW English coast. The authors found that the adsorption would be depended on three factors, exposure time, pH, and the salinity of the surrounding environment, complementing

the information already reported by Turner & Holmes (2015).

Gao *et al.* (2019) showed that although, adsorption occurs between metals and plastic fragments certain polymers had distinct absorbability taking into account the type of metal ions concentration, particle size and exposure time. The authors also mention that combined adsorption between metals and polycyclic aromatic hydrocarbons (PAHs) on PP particles were similar in order of magnitude.

Ahechti *et al.* (2020), found that virgin PE and PP pellets were able to adsorb metals (Cd, Cu, Pb, Zn) in the aquatic environment although in low rates (<10%) in most cases, being the adsorption maximum for Pb (20%). The authors emphasized that the "exposure time" has an important effect on adsorption capacities, the adsorption of metals in plastic fragments increasing in a directly proportional way to the exposure time. However, they did not report differences of adsorption to the different types of plastic used. On the other hand, these authors verified that adsorption time also vary with the type of metal, in the case of Zn the increase in adsorption occurred in the first four hours while for Cd the adsorption was only significant after a long period of time. Generally, metals showed similar behavior for parameters such as pH and salinity, decreasing adsorption as salinity increases and pH decreases when salinity decreases.

Overall, it is known that plastic fragments tend to adsorb and accumulate pollutants that are available in the surrounding water, due to their small size, with the concentrations of pollutants in plastic fragments dependent on the proximity to the possible sources of pollution and pollutants concentrations at that site (Guo & Wang, 2019). However, it is also known that pollutants adsorption can also be influenced when the surface of MPs is altered, as a result of environmental dynamics (Brennecke *et al.,* 2016; Koelmans *et al.,* 2016; Holmes *et al.,* 2014; Kedzierski *et al.,* 2018; Almeida *et al.,* 2020).

Most studies of interactions between plastic fragments and metals are directed towards bioaccumulation and toxicity in aquatic organisms (Khan *et al.,* 2015; Barboza *et al.,* 2018), with studies on the environmental impacts of both stressors still being scarcer. There are also a few studies found in literature that address the adsorption of metals to plastic fragments under controlled conditions in the laboratory (Holmes *et al.,* 2012; Holmes *et al.,* 2014; Turner & Holmes 2015; Brennecke *et al.,* 2016; Hodson *et al.,* 2017; Llorca *et al.,* 2018; Zhang *et al.,* 2018).

No previous study on metallic adsorption to plastic fragments of fishing nets under controlled conditions has been found in the literature, being necessary to assess if they can in fact adsorb metals. Moreover, if plastic fragments of ALDFG adsorb metals, they can become a new pollutant by concentrating metals in that particular area surrounding the ALDFG, becoming a new pollutant itself, which can have a negative impact in the environment, a subject that deserves investigation.

1.6. Aims of the work

To evaluate the environmental harmfulness of lost fishing gear as a new pollutant, this work aimed to evaluate the potential of fishing nets for the adsorption of inorganic compounds. Three different methodological approaches were used. One consisted in the environmental characterization of two lost fishing nets hotspots localized on the NW Portugal coast marine environment. For that water and sediments were collected seasonally in locations with and without lost fishing nets, analyzing metals such as Cd, Cu, Fe, Mn, Ni, Pb and Zn. The second methodological approach included controlled laboratory experiments investigating the possible adsorption of metals (Cu and Pb) to fishing nets made of different polymers (PE and nylon). For that, small pieces of fishing nets were exposed to seawater contaminated with Cu or Pb. The third methodological approach included experiences in a quasi-real environment, in which fishing nets of different polymers (PE and nylon) were placed in seawater water in a marina for a specific period of time (ca. 6 months). In this case, metals in surrounding water and adsorbed to the fishing nets were evaluated.

This work is part of the NetTag project, a project aiming to reduce and prevent marine litter from fisheries.

CHAPTER 2

MATERIALS AND METHODS

2. Materials and Methods

2.1. Lost fishing gears hotspots - Area of study and sampling

Sampling took place at two locations where lost fishing nets are concentrated offshore the NW coast of Portugal (Fig. 1), namely: Matosinhos submarine wreck and Cavalos de Fão (Esposende). The first site is an artificial reef, while the second site is a protected natural area with a rocky bottom, with several reefs. Both sites have intensive fishing activity. The coastal zone between the two selected lost gear hotspots is threatened by anthropogenic pressure, e.g. spillage of chemicals, loss of fishing nets due to its proximity to major shipping lanes and the presence of industry (Gouveia *et al.,* 2018).

Both sites were analyzed in terms of metal concentration in the nearby water particulate matter and sediments.

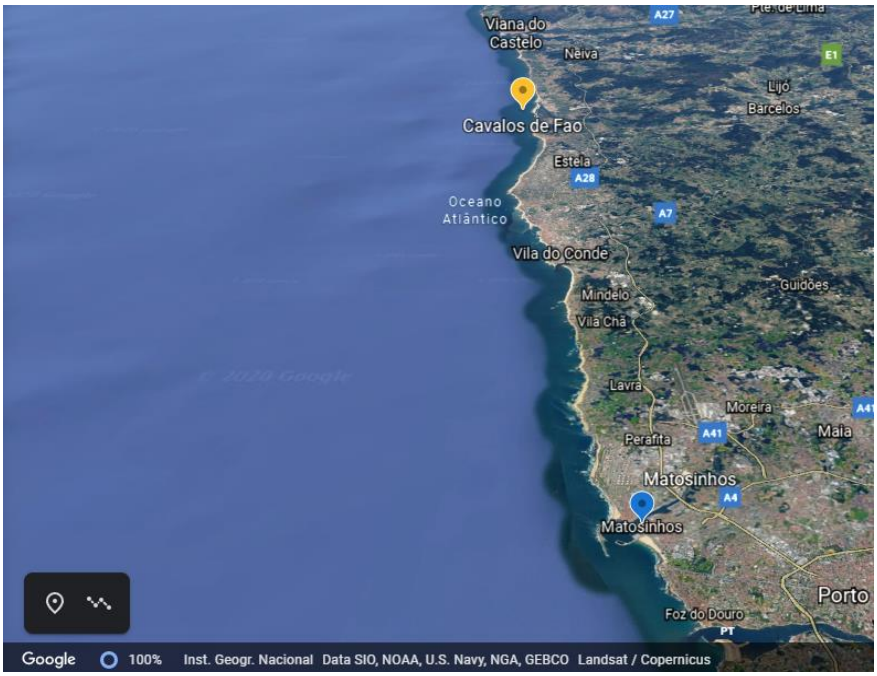

Figure 1. Location of the two hotspots: Cavalos de Fão (yellow location pin) and the Matosinhos submarine wreck (blue location pin) of lost fishing gears.

2.1.1. Matosinhos submarine wreck hotspot of fishing gears

The Matosinhos area is a highly urbanized and modified location, being subject to domestic wastewater discharges as well as industrial effluents (Ramos *et al.,* 2015). The north coast of Portugal is subject to several urban pressures, some of which are habitat changes, fishing, marine traffic, among others (Ramos *et al.,* 2015).

Since the end of the World War II the wreckage of the submarine (U-1277) has been sunk off. This submarine is located at 2.485 miles from the coast, more or less in front of the beach of Angeiras, Matosinhos, at a depth of 30 meters with the aft completely silted

and tipped to port to about 45 degrees. The wreck is the only structure and in this area is where the pieces of lost fishing nets are trapped and concentrated.

For this hotspot, two sampling sites were selected around the submarine (Fig. 2), one close to the torpedoes exit (Site A) and another close to the periscope of the submarine (Site B). At these two sites, pieces of fishing nets are trapped and attached to the submarine structure (Fig. 3A). A third site, 50 meters apart from the submarine was selected as control (Site C) (Fig. 1). The samples from the Matosinhos hotspot were collected by divers from the Submersus school.

At the Matosinhos hotspot, water and sediment samples were collected in two sampling campaigns, one campaign at spring (June 2019) and another at summer (September 2019). In general, at each site, three water samples and three sediment samples were collected. The exception was for site A, for which only two water samples were collected and for sites A and C for which only two sediment samples were collected in the spring sampling campaign. Several factors constrained the number of collected samples, namely the weather and navigability conditions, boat availability, and the difficulty to properly collect and store water and sediment samples at 30 meters depth (several samples were lost).

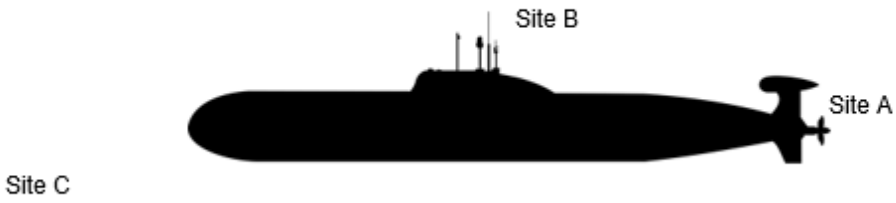

Figure 2. Location of the three sampling sites around the Matosinhos submarine wreck. Sites A and B were close to the lost fishing nets attached to the metallic structure of the submarine; site C was used as a control.

Figure 3. A - Fishing gear attached submarine; B - Fishing net pieces found in Matosinhos submarine wreck.

2.1.2. Cavalos de Fão (Esposende) hotspot of fishing gears

The Esposende hotspot is in a region with strong industrial activity, receiving impact from industrial and urban effluents as well as impacts resulting from small port infrastructure, shipbuilding, and fishing (Machado *et al.,* 2012). For several decades this area received leachate from mining activity, which is currently deactivated (Almeida *et al.*, 2008).

The rocky reefs of Cavalos de Fão are located in a marine protected area, Parque Natural do Litoral Norte (ICNF, 2020).

At the Esposende hotspot, three sampling campaigns were carried out, one in winter (March 2019), one in spring (June 2019) and another in summer (August 2019). Due to lack of good weather and navigability conditions, it was not possible to perform the autumn campaign. For this lost fishing gear hotspot (Fig. 4), a total of nine sampling sites were selected for water collection, with six sites located within the area were lost fishing nets are present: site 1-3 at the southern limit and sites 4-6 at the northern limit of the Cavalos de Fão area. Three other sites (7-9) located outside the area (without lost fishing nets) were selected as control sampling sites. For sediment, only three sites within the hotspot area (Cavalos de Fão) were sampled (S1-S3), due to the presence of many rocky reefs that prevent the collection of sediment samples, and three sampling sites (S4-S6) were surveyed in the control zone. Sediment samples were only collected during winter and summer campaigns, as sediments tend to have less seasonal variations.

Some lost fishing gear pieces found are shown in Fig. 5. In this region are essentially composed of small pieces of lost fishing nets and ropes (all less than 1 meter long) that get attached to the rocky reefs or are floating. The samples from the Esposende hotspot were collected by CIIMAR researchers.

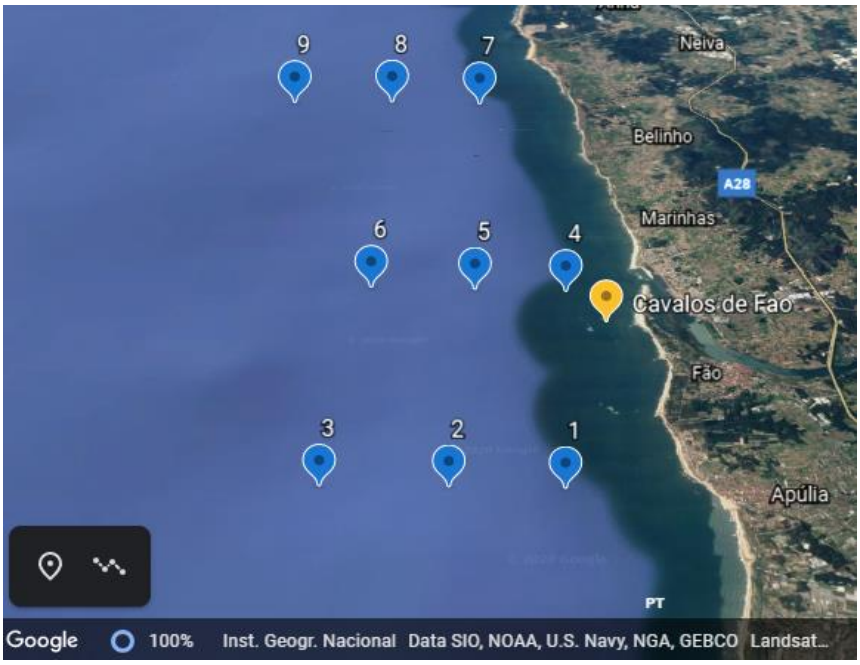

Figure 4. Location of water sampling sites (1-9) of lost gear hotspot Cavalos de Fão (Esposende). Site 1-6 were close to lost fishing nets hotspots; Site 7-9 were used a control.

Figure 5. Examples of lost fishing gear found in Cavalos de Fão hotspot.

2.2. Metal adsorption laboratorial experiments

To investigate if the fishing nets have the potential for adsorption of metals, laboratory experiments (Fig. 6) were carried out in controlled conditions by exposing fishing nets to contaminated seawater. The fishing nets used in the laboratory experiments on the adsorption of metals were supplied by Euronete, a manufacturer of fishing gear. The seawater was collected in Matosinhos beach (41.178816; -8.697282).

Two metals were selected for the laboratory experiments, Cu and Pb. Both metals were found at the two lost fishing net hotspots surveyed. Cu is a micronutrient that can be toxic when present at high concentrations. Pb is a toxic metal with no known function in organisms. For the experiments with either metal, concentrations of 50 µg L^{-1} were selected.

The concentration selected took in consideration the values reported for environmentally polluted sites (Bakke *et al.,* 2010).

Different treatments were prepared, as shown in Fig. 6, with a volume of seawater of 150 mL for all treatments. Seawater doped with the respective metal (Cu or Pb) to evaluate metal behavior over time (metal control); a control treatment with fishing net pieces in seawater, to evaluate possible metal release of the fishing nets (as nets have inks in their composition that can release metals) (net control); and seawater doped with the respective metal and the selected net. Solutions were sampled at different times: 30 minutes, 1 hour (60 min), 2 hours (120 min), 4 hour (240 min), 8 hour (480 min), 24 hour (1440 min), 48 hour (2880 min) and 7 days from different flasks prepared in simultaneous so that no significant volume changes occurred. Only one replicate was collected at each time. The flasks were in constant agitation at 80 rpm to simulate seawater movement and in the dark to prevent light degradation.

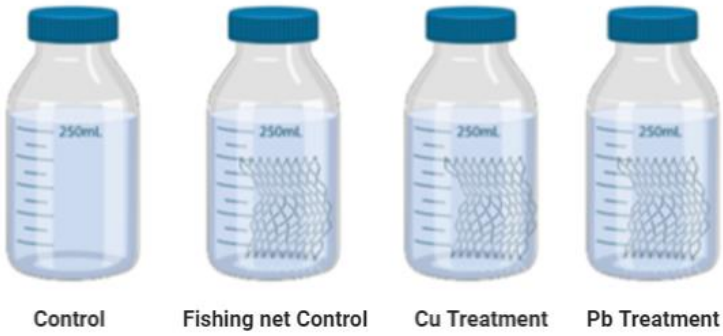

Figure 6. Experimental design for metal adsorption experiments.

Experiments were carried out with four fishing nets with two different polymer compositions. Initially, PE polymer net was used namely, the **Euroline® PE** (Euronete) which is made with Hight Tenacity Polyethylene fibers (HTPE). Its compact construction and thermofixed knots make the fishing net 30% more resistant than single PE often used in fishing nets. The fishing nets have the characteristics, according to the supplier: “Euroline® 3 mm, mesh 150 mm”. Another fishing net used was **Braided Polyethylene** (Euronete), made of PE, as the name indicates. Compared with the previous one, its mesh is finer (1.5 mm), and its tenacity is lower, according to the supplier. Two other types of nets were also used for laboratory experiments, being both made of nylon. The **Twisted Nylon** (Euronete) fishing net, as the name implies, is made with 6 high tenacity nylon braided threads, and is normally used in pelagic trawling. According to the supplier its designation is “Twisted-Nylon 2.3 mm, mesh 53 IM”. The other nylon net was thinner than the previous one presenting as characteristics, according to the supplier, 0.30 mm, and mesh 100 FM, forward called, **Thin Nylon** (Euronete). All pieces of fishing nets were previously weight

(Tables 1, 2 and 3).

Table 1. Weight (in grams) of fishing net **Euroline®** pieces used in Cu and Pb adsorption laboratory experiments for all flasks with fishing nets.

Weight (grams)						
			Euroline®		Euroline®	
			Cu	Pb	Cu + PAHs	Pb + PAHs
Treatment	Net control flaks		0.6125	0.5079	0.7470	0.7204
	Adsorption flasks	30 min.	0.6369	0.7662	0.8213	0.8537
		60 min.	0.7959	0.8049	0.7556	0.8103
		120 min.	0.7908	0.5469	0.7626	0.8216
		240 min.	0.7735	0.7937	0.8031	0.8332
		480 min.	0.7964	0.7449	0.8213	0.8537
		1440 min.	0.6369	0.7662	0.7556	0.8103
		2880 min.	0.7959	0.8049	0.7626	0.8216
		10080 min.	0.7908	0.5469	0.8031	0.8332

Table 2. Weight (in grams) of fishing net **Braided PE** pieces used in Cu and Pb adsorption laboratory experiments for all flasks with fishing nets.

Weight (grams)				
			Braided-PE	
			Cu	Pb
Treatment	Net Control Flasks		0.4685	0.4885
	Adsorption flasks	30 min.	0.4757	0.3488
		60 min.	0.4802	0.4815
		120 min.	0.4757	0.3488
		240 min.	0.4802	0.4815
		480 min.	0.4757	0.3488
		1440 min.	0.4802	0.4815
		2880 min.	0.4757	0.3488
		10080 min.	0.4802	0.4815

Table 3. Weight (in grams) of fishing net **Thin Nylon and Twisted Nylon** net pieces used in Cu and Pb adsorption laboratory experiments for all flasks with fishing nets.

Weight (grams)						
			Thin Nylon		Twisted Nylon	
			Cu	Pb	Cu	Pb
Treatment	Net Control Flasks		0.0481	0.0407	2.7390	2.7654
	Adsorption flasks	30 min.	0.0275	0.0331	2.8378	2.7214
		60 min.	0.0445	0.0330	2.7788	2.7613
		120 min.	0.0275	0.0331	2.8378	2.7214
		240 min.	0.0445	0.0330	2.7788	2.7613
		480 min.	0.0275	0.0331	2.8378	2.7214
		1440 min.	0.0445	0.0330	2.7788	2.7613
		2880 min.	0.0275	0.0331	2.8378	2.7214
		10080 min.	0.0445	0.0330	2.7788	2.7613

Table 4. Weight (in grams) of fishing net **Twisted Nylon** pieces used in the triplicate Cu and Pb adsorption laboratory experiments for all flasks with fishing nets.

			Weight (grams)					
			Cu			Pb		
			1.1.	1.2.	1.3.	1.1.	1.2.	1.3.
Treatment		Net Control Flasks	2.8439	2.8439	2.8439	2.8578	2.8578	2.8578
	Adsorption flasks	30 min.	2.6961	2.8372	2.8009	2.7954	2.8087	2.8086
		60 min.	2.8126	2.8419	2.9271	2.8561	2.8410	2.8525
		120 min.	2.6961	2.8372	2.8009	2.7954	2.8087	2.8086
		240 min.	2.8126	2.8419	2.9271	2.8561	2.8410	2.8525
		480 min.	2.6961	2.8372	2.8009	2.7954	2.8087	2.8086
		1440 min.	2.8126	2.8419	2.9271	2.8561	2.8410	2.8525
		2880 min.	2.6961	2.8372	2.8009	2.7954	2.8087	2.8086

In parallel, experiments with one metal and PAHs (the 16 PAHs considered a priority by the United States Environmental Protection Agency) were carried out with **Euroline® PE** net to understand whether the presence of both compounds (organic and inorganic) alters metal adsorption behavior. The concentration of metals remained the same as in the previous experiment (50 µg L^{-1}) and the concentration of each PAH was 1 µg L^{-1}. Experiments were identical to those previously describe. Fishing net pieces weights are shown in Table 2.

Considering all results obtained, the fishing net that showed the higher potential for metal adsorption (see results section) was **Twisted Nylon** and for this reason it was selected to repeat the experiment, preparing each treatment in triplicate. The nets were duly cut with weights identical to that of the initial experiment (Table 4; Cu, mean weight 2.812g; Pb mean weight 2.807g), and the experiments prepared in the same way as the previous experience with the same fishing net. Exposition was carried out until 48 h. After this time period metal adsorption to fishing net pieces were in general identical or lower (see results section).

At the end of the adsorption experiments (48 h), the fishing nets were transferred to new flasks and new seawater was added. The objective was to verify if, eventually, the metals adsorbed to the nets would be release over time when placed in a metal free medium. Samples of seawater were collected over time (same time period as before) until 48 h.

At each time, 5 mL of seawater were collected from each flask and 1% HNO_3 was added. Subsequently, the samples were analyzed by atomic absorption spectrometry with electrothermal atomization analysis as described in metal analysis section. Small adaptations of the analytical protocol had to be made due to the matrix effect cause by salinity. For that the matrix modifiers $(NH_4)H_2PO_4$ and NH_4NO_3 were used for Pb and Cu

analysis, respectively.

Adsorption percentages were calculated considering the metal concentrations observed in the metal control solutions and net exposing solutions collected at same time, according to equation:

$$1 - \left(\frac{Treatment\ solution}{Metal\ control\ solution}\right) \times 100$$

where treatment solution corresponds to metal concentration measured in seawater containing fishing net and metal (Cu or Pb) and Metal control solution corresponds to metal concentration in seawater doped with the metal (Cu or Pb).

Desorption percentages were calculated considering metal concentration in treatment solution after 48 h net exposure from the triplicate experiments and metal concentration in seawater during desorption time, according to equation:

$$\frac{Desorption\ seawater - Fishing\ net\ control}{Adsorbed\ value} \times 100$$

where desorption seawater corresponds to metal concentration in seawater with the fishing net from the triplicate experience, Fishing net control corresponded to metal concentration possibly released from virgin fishing net, and adsorbed value corresponds to the estimated metal concentration adsorbed taking in consideration metal concentration in treatment solution after 48 h exposing.

2.3. In-situ experiments in a marina environment and sampling

In-situ experiments were carried out to investigate fishing nets potential to adsorb metals, concentrating them in their surrounding and becoming a new pollutant. For that, fishing nets were exposed to **quasi-real** environmental conditions (Fig. 7). The experience was carried out at Marina of Leixões, Matosinhos. Three different fishing nets placed at 50 cm between each other, were placed in the water, namely **Braided PE** (Site A), **Twisted Nylon** (Site B) and **Thin Nylon** (Site C) (Fig. 7). The in-situ experiment started in February 2020 and sampling of water and pieces of fishing net occurred in February, March, May, and July 2020.

Water was collected at the bottom (below each net) corresponding to control samples (no net) and on the surface (near each net) corresponding to fishing net samples. A total of 6 samples per sampling campaign were collected. Sampling took place 3 hours after low tide, where 500 mL of water was collected at each net location with a water sample bottle and transfer into a plastic container. The processing of water samples followed the same

process adopted for the samples of the environmental characterization (filtration for metal determination in water particulate matter) and the same procedure was applied in their analysis.

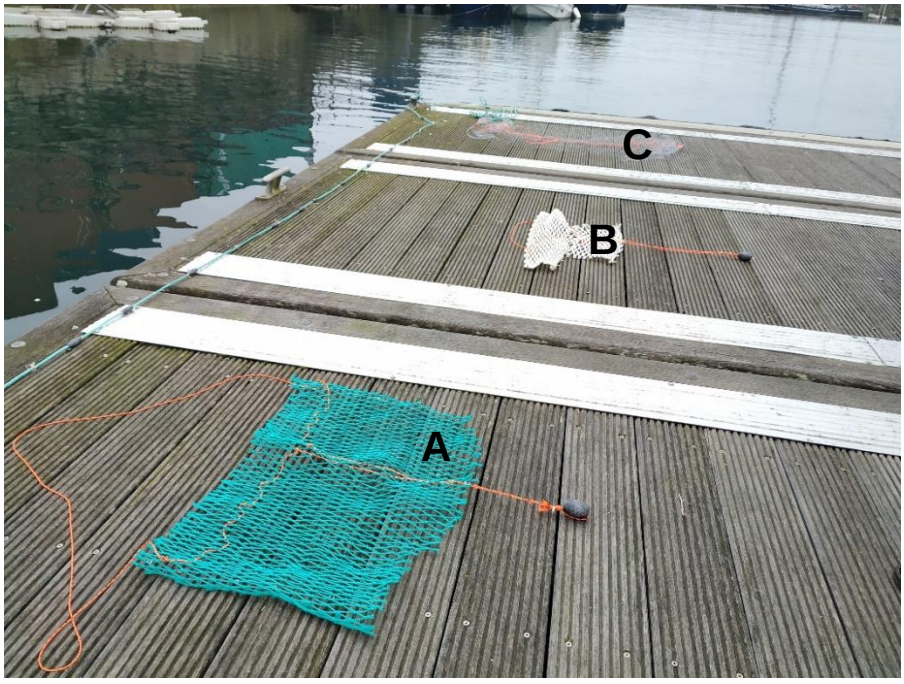

Figure 7. Experimental design of quasi-real experiments at Marina of Leixões, Matosinhos. Site A (**Braided PE**), Site B (**Twisted Nylon**) and Site C (**Thin Nylon**).

Fishing nets pieces collected each time were stored at -20 ºC until analysis.

To evaluate metals adsorbed to the fishing nets, net pieces collected were thawed, cut to the appropriate size (Table 5), so that it was possible to place them at the bottom of the glass flasks and be submersed by extraction solution, and weight. Virgin fishing nets were also subjected to the same procedure, to check if, eventually, some metal was released from the net. To the flasks containing the fishing nets pieces, 20 mL of a 10% solution of HNO_3 was added. Flasks were vortex for 1 minute and then placed in an ultrasound device (Elma, Transsonic 460/H) for 15 minutes. The solution was transferred to 15 mL falcons and the metal concentrations measured in solution as before.

Table 5. Length (in cm) and weight (in grams) of fishing nets pieces collected from the Marina experiment that were used in the laboratory desorption experiments. Same parameters were measured for virgin fishing nets (length and weight, n=3).

	Braided PE (A)		**Twisted Nylon (B)**		**Thin Nylon (C)**	
	Length	**Weight**	**Length**	**Weight**	**Length**	**Weight**
Virgin net	5.7	0.3982	6.0	2.2045	6.4	0.0379
March	5.7	0.6467	6.7	2.6862	6.0; 6.3 *	0.0290
May	5.4	0.7364	5.7	2.4045	8.0; 6.5; 8.0 *	0.1053
June	7.5	0.8930	6.4	2.5718	14.0; 5.0*	0.3019

*For this net more than one fraction of the fishing net was placed in the bottom flasks.

2.4. Sample preparation for metals analysis

For analysis of metals in water suspended particulate matter, water samples were filtered (ca. 500 mL of each sample) through nitrate cellulose filters (0.45 µm porosity) (Fig. 8). Filters were then dried at room temperature and afterwards digested in a high-pressure microwave system (Ethos, Milestone), using a previously optimized temperature program of 25 minute (Almeida *et al.,* 2004). For that, filters were placed in teflon vessels and 5 mL of 65% concentrated HNO_3 (Merck) were added. After digestion, the entire sample was transferred to a 15 mL plastic flask and the volume was made up at 12 mL with deionized water. No metal analysis was done in water as metal levels in dissolved phase are in general below detection limits.

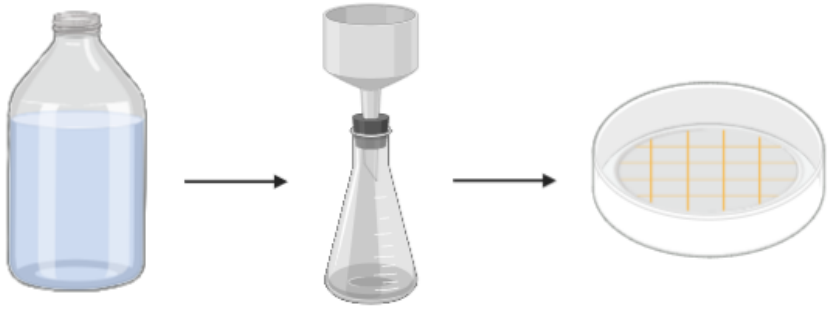

Figure 8. Sampling preparation for water suspended particulate matter.

Sediments were dried at room temperature until constant weight, being afterwards digested at high pressure in the same microwave system. For that, 0.25 g of dry sediment was weighed and then, 5 mL of 65% concentrated HNO_3 were added. The same procedure used for water suspended particulate matter filters was used for the sediment samples.

2.5. Metal analysis

Metals concentrations were determined by atomic absorption spectrometry with flame (Perkin Elmer AAnalyst 200) or with electrothermal atomization (Perkin Elmer 4100ZL) depending on metal levels. For metals quantification, aqueous standard solutions of each metal were prepared and used to perform a calibration line for each measured metal.

The metals selected were those commonly detected on the NW Portuguese coast (Vasconcelos *et al*., 2011; Gouveia *et al.,* 2018; Almeida *et al*., 2008).

2.6. Data analysis

Sediments and water particulate matter samples were analyzed for the respective metals. The mean and standard deviation of the three repetitions of the hotspot characterization samples and triplicate laboratory experiments were calculated. The obtained data were analyzed using the IBM SPSS Statistics 23 statistical program. For metal concentration, significant ($P<0.05$) differences among samples were evaluated applying the unilateral parametric variance analysis (ANOVA) followed by Turkey pair wise comparisons test.

CHAPTER 3

RESULTS

3. Results

3.1. Monitoring in-situ - hotspots characterization

Metals (Zn, Pb, Cr, Cu, Fe, Mn, Cd, Ni) levels in water particulate matter and sediment in both lost fishing net hotspots sites selected were measured.

3.1.1. Matosinhos submarine wreck hotspot of fishing gears

a) Sediment samples

Metal concentrations in sediments are shown in Table 6.

In Matosinhos hotspot, sites C1, C2 and C3 were used as controls, as they were further away from the submarine and without lost fishing nets. Locations A1, A2, B1, B2 and B3 were close to the lost fishing nets.

In general, the measured metal values in sediments were below or very close to the detection limits for all sampling points, including those in the control area (Table 6). Only Fe was detected in all sediment samples, since this element is naturally found in the earth's crust, being part of it.

Some metals, Zn, Mn, Fe, Pb and Cr showed significant differences ($P<0.05$) between control sites and sites where the fishing nets are present, with higher levels in control sites. Comparing sampling campaigns, significant differences ($P<0.05$) were observed between spring and summer campaigns for Pb, Mn and Cr, with higher metal levels in the spring campaign.

Table 6. Metal concentration (mean and standard derivation n=3, µg g_{sed}^{-1}, except Fe mg g_{sed}^{-1} for each collected sample) observed in sediment of Matosinhos submarine wreck hotspot collected in spring and summer. Average metal concentration at each site is also shown. Sites A and B were close to the lost gears attached to the structure of the submarine, site C was 50 m apart and was used as a Control. In spring sampling campaign only two samples were collected at sites A and C due to logistics constraints.

		A1	A2	A3	Average	B1	B2	B3	Average	C1	C2	C3	Average
Cu	**Spring**	<5*	<5*	n.c.	**<5***	6.5 ± 0.9	<5*	<5*	**5.5 ± 0.9**	<5*	9.8 ± 0.3	n.c.	**7 ± 3**
	Summer	<5*	<5*	<5*	**<5***	<5*	<5*	<5*	**<5***	<5*	<5*	<5*	**<5***
Zn	**Spring**	<2*	<2*	n.c.	**<2***	<2*	<2*	<2*	**<2***	11 ± 8	22 ± 5	n.c.	**16 ± 8**
	Summer	4 ± 2	3 ± 2	<2*	**3 ± 1**	2.2 ± 0.4	2.0 ± 0.3	3.1 ± 0.8	**2.4 ± 0.6**	<2*	<2*	<2*	**<2***
Fe	**Spring**	1.4 ± 0.4	1.6 ± 0.3	n.c.	**1.5 ± 0.3**	1 ± 1	1 ± 1	0.8 ± 0.8	**0.7 ± 0.9**	2.9 ± 0.6	4.3 ± 0.5	n.c.	**3.6 ± 0.9**
	Summer	1.4 ± 0.5	1.1 ± 0.2	1.34±0.05	**1.3 ± 0.3**	1.2 ± 0.2	1.3 ± 0.2	1.4 ± 0.2	**1.3 ± 0.2**	1.3 ± 0.3	1.3 ± 0.3	0.6 ± 0.6	**1.1 ± 0.5**
Pb	**Spring**	3 ± 1	2.4 ± 0.4	n.c.	**2.7 ± 0.4**	4.5 ± 0.5	4 ± 2	5 ± 2	**3 ± 2**	5 ± 2	6.1 ± 0.4	n.c.	**5.6 ± 0.7**
	Summer	<2*	<2*	4 ± 2	**2.5 ± 0.8**	<2*	<2*	<2*	**<2***	3 ± 1	<2*	3 ± 2	**2.6 ± 0.6**
Cd	**Spring**	<0.2*	<0.2*	n.c.	**<0.2***	<0.2*	<0.2*	<0.2*	**<0.2***	<0.2*	<0.2*	n.c.	**<0.2***
	Summer	<0.2*	<0.2*	<0.2*	**<0.2***	<0.2*	<0.2*	<0.2*	**<0.2***	<0.2*	<0.2*	<0.2*	**<0.2***
Ni	**Spring**	<2*	<2*	n.d.	**<2***	<2*	<2*	<2*	**<2***	<2*	4.9 ± 0.9	n.c.	**3.4 ± 2**
	Summer	<2*	<2*	<2*	**<2***	<2*	<2*	<2*	**<2***	<2*	<2*	<2*	**<2***
Mn	**Spring**	<25*	27 ± 15	n.c.	**26 ± 1**	<25*	58 ± 16	29 ± 2	**37 ± 18**	57 ± 32	56 ± 3	n.c.	**56.5 ± 0.9**
	Summer	<25*	<25*	<25*	**<25***	<25*	<25*	26 ± 15	**25.5 ± 0.8**	33 ± 20	41 ± 33	44.2±12.4	**39 ± 6**
Cr	**Spring**	1.9 ± 0.9	10.4 ± 0.8	n.c.	**6 ± 6**	5.7**	4.5**	4.1**	**4.8 ± 0.8****	10.8 ± 0.5	11 ± 2	n.c.	**11.0 ± 0.3**
	Summer	<0.4*	<0.4*	<0.4*	**<0.4***	<0.4*	<0.4*	<0.4*	**<0.4***	0.72±0.05	<0.4*	<0.4*	**<0.4***

Detection limit**; * Only one replicate analyzed due to technical problems; **n.c.-** Not collected due to logistical constrains.

b) Water particulate matter samples

Metal concentrations in water particulate matter are shown in Table 7. Metal levels were in general below the detection limits.

The only metal detected in all water samples was Fe, which presented a high variability between spring and summer. In the summer sampling campaign, Fe values were significantly higher ($P<0.05$). Fe concentration did not vary significantly ($P>0.05$) between the control site and the other two sites (considering the average values per location, Table 7).

Table 7. Metal concentration (mean and standard derivation n=3, µg L_{water}^{-1} for each collected sample) observed in water particulate matter of Matosinhos submarine wreck hotspot collected in spring and summer. Average metal concentration at each site is also shown. Sites A and B were close to the lost gears attached to the structure of the submarine, site C was 50 m apart and was used as a Control. At site A only two samples were collected due to logistics constraints.

		A1	A2	Average	B1	B2	B3	Average	C1	C2	C3	Average
Cu	**Spring**	<0.60*	<0.60*	**<0.60***	<0.60*	<0.60*	<0.60*	**<0.60***	<0.60*	<0.60*	<0.60*	**<0.60***
	Summer	<0.60*	<0.60*	**<0.60***	<0.60*	<0.60*	<0.60*	**<0.60***	<0.60*	<0.60*	<0.60*	**<0.60***
Zn	**Spring**	<0.60*	<0.60*	**<0.60***	<0.60*	<0.60*	<0.60*	**<0.60***	<0.60*	<0.60*	<0.60*	**<0.60***
	Summer	<0.60*	<0.60*	**<0.60***	<0.60*	<0.60*	<0.60*	**<0.60***	<0.60*	<0.60*	<0.60*	**<0.60***
Fe	**Spring**	8.4	6.9	**8 ± 1**	6.9	7.8	8.9	**8 ± 1**	9.2	8.9	8.1	**8.7 ± 0.6**
	Summer	15	67	**41 ± 37**	165	64	73	**101 ± 56**	10	44	41	**32 ± 19**
Pb	**Spring**	<1.2*	<1.2*	**<1.2***	<1.2*	<1.2*	<1.2*	**<1.2***	<1.2*	<1.2*	<1.2*	**<1.2***
	Summer	<1.2*	<1.2*	**<1.2***	<1.2*	<1.2*	<1.2*	**<1.2***	<1.2*	<1.2*	<1.2*	**<1.2***
Cd	**Spring**	<0.12*	<0.12*	**<0.12***	<0.12*	<0.12*	<0.12*	**<0.12***	<0.12*	<0.12*	<0.12*	**<0.12***
	Summer	<0.12*	<0.12*	**<0.12***	<0.12*	<0.12*	<0.12*	**<0.12***	<0.12*	<0.12*	<0.12*	**<0.12***
Ni	**Spring**	<0.60*	<0.60*	**<0.60***	<0.60*	<0.60*	<0.60*	**<0.60***	<0.60*	<0.60*	<0.60*	**<0.60***
	Summer	<0.60*	<0.60*	**<0.60***	<0.60*	<0.60*	<0.60*	**<0.60***	<0.60*	<0.60*	<0.60*	**<0.60***
Mn	**Spring**	<1.2*	<1.2*	**<1.2***	<1.2*	<1.2*	<1.2*	**<1.2***	<1.2*	<1.2*	<1.2*	**<1.2***
	Summer	<1.2*	<1.2*	**<1.2***	<1.2*	<1.2*	<1.2*	**<1.2***	<1.2*	<1.2*	<1.2*	**<1.2***
Cr	**Spring**	<0.48*	<0.48*	**<0.48***	<0.48*	<0.48*	<0.48*	**<0.48***	<0.48*	<0.48*	<0.48*	**<0.48***
	Summer	<0.48*	<0.48*	**<0.48***	<0.48*	<0.48*	<0.48*	**<0.48***	<0.48*	<0.48*	<0.48*	**<0.48***

***Detection limit**

3.1.2. Cavalos de Fão (Esposende) hotspot of fishing gears

a) Sediment samples

Metal concentrations in sediment samples are shown in Table 8. In Cavalos de Fão (Esposende) hotspot, in general, all metals quantified were above the detection limit, except Cd and Ni.

Considering the average values per location (Table 8), Cu, Fe, Pb, Cd and Cr levels were in general similar among sites. For the other metals there was some variation.

However, in general, no significant differences (P>0.05) were observed between the control and the site where the fishing nets were present, except for Mn in the summer sampling campaign, where significant differences (P<0.05) were observed.

Comparing samples campaigns, in the summer the metal values were lower than in winter. However, significant differences (P<0.05) were observed only for Cu.

Table 8. Metal concentration (mean and standard derivation n=3, µg g^{-1} except Fe mg g^{-1} for each collected sample) observed in sediments of Cavalos de Fão (Esposende) hotspot collected in winter and summer. Average metal concentration at each site is also shown. Sites S1-S3 are within lost fishing net hotspot area and sites S4-S6 are outside.

		S1	S2	S3	Average	S4	S5	S6	Average
Cu	**Winter**	13 ± 5	16.6 ± 0.8	14 ± 2	**14 ± 2**	19.4 ± 0.5	12 ± 2	20 ± 2	**17 ± 4**
	Summer	<5*	<5*	<5*	**<5***	<5*	<5*	7.0 ± 0.4	**6 ± 1**
Zn	**Winter**	29 ± 4	33 ± 2	5 ± 4	**22 ± 15**	43 ± 4	6 ± 1	38 ± 3	**29 ± 20**
	Summer	4 ± 2	16 ± 10	4 ± 2	**8 ± 7**	28 ± 15	4 ± 3	30 ± 2	**21 ± 15**
Fe	**Winter**	8.0 ± 0.3	9.2 ± 0.8	7.6 ± 0.4	**8.2 ± 0.8**	9.9 ± 0.3	8 ± 2	9.6 ± 0.3	**9.3 ± 0.9**
	Summer	3.0 ± 0.4	6 ± 3	2.8 ± 0.8	**4 ± 2**	10 ± 4	7 ± 2	10.3 ± 0.2	**9 ± 2**
Pb	**Winter**	7 ± 1	8.2 ± 0.4	6 ± 2	**7 ± 2**	11 ± 1	7 ± 2	9 ± 2	**9 ± 2**
	Summer	4.2 ± 0.7	6 ± 2	3.0 ± 0.4	**4 ± 1**	8 ± 4	7 ± 1	8.7 ± 0.8	**8 ± 1**
Ni	**Winter**	10 ± 3	13.5 ± 0.3	<2*	**9 ± 6**	18 ± 2	<2*	18 ± 1	**13 ± 9**
	Summer	<2*	5 ± 1	<2*	**3 ± 2**	8 ± 2	<2*	7 ± 2	**6 ± 3**
Mn	**Winter**	77 ± 9	97 ± 9	**	**87 ± 14**	123 ± 28	153 ± 61	129 ± 26	**135 ± 16**
	Summer	34 ± 7	54 ± 17	44 ± 24	**44 ± 10**	113 ± 43	142 ± 22	90 ± 6	**115 ± 26**
Cr	**Winter**	5 ± 1	5.6 ± 0.6	2.0 ± 0.4	**4 ± 2**	6.6 ± 0.7	3 ± 1	6.7 ± 0.8	**5 ± 2**
	Summer	2.4 ± 0.1	6 ± 3	2.7 ± 0.3	**4 ± 2**	7 ± 4	3.2 ± 0.1	9.2 ± 0.5	**7 ± 3**
Cd	**Winter**	<0.2*	<0.2*	<0.2*	**<0.2***	<0.2*	<0.2*	<0.2*	**<0.2***
	Summer	<0.2*	<0.2*	<0.2*	**<0.2***	<0.2*	<0.2*	<0.2*	**<0.2***

***Detection limit**; **considered an outliner

b) Water particulate matter samples

Metal concentrations in water particulate matter are shown in Table 9. Only levels of Cu, Fe, Ni and Zn in water suspended particulate matter were in general, above the detection limit. All metals detected did not present significant differences ($P>0.05$) between sites, namely between the control sites (W7-W9) and the sites where the fishing nets are present (W1-W6) (considering the average values per location, Table 9). Among sampling campaigns, metals also did not significantly present differences ($P>0.05$).

Table 9. Metal concentration (mean n=3 µg L_{water}^{-1} for each collected sample) observed in water particulate matter of Cavalos de Fão (Esposende) hotspot collected in spring, summer, and winter. Average metal concentration at each site is also shown. Sites 1-6 are within hotspot area and sites 7-9 are outside.

		W1	W2	W3	Average	W4	W5	W6	Average	W7	W8	W9	Average
Cu	**Winter**	0.67	1.8	1.1	**1.2 ± 0.6**	0.92	0.83	0.93	**0.9 ± 0.1**	1.0	2.5	1.3	**1.6 ± 0.8**
	Spring	2.0	1.4	1.7	**1.7 ± 0.3**	2.5	1.3	0.88	**1.6 ± 0.8**	6.2	1.0	0.69	**3 ± 3**
	Summer	1.9	1.5	0.87	**1.4 ± 0.5**	1.4	1.3	0.88	**1.2 ± 0.3**	2.7	1.3	11.4	**5 ± 5**
Zn	**Winter**	0.53	1.1	6.6	**3 ± 3**	1.5	2.1	1.4	**1.7 ± 0.4**	2.4	9.5	6.9	**6 ± 4**
	Spring	5.0	11	9.5	**9 ± 3**	12	<0.60*	5.2	**6 ± 6**	14	7.9	0.70	**8 ± 7**
	Summer	2.8	1.9	1.6	**2.1 ± 0.6**	3.0	1.3	2.3	**2.2 ± 0.9**	16	3.7	23	**14 ± 10**
Fe	**Winter**	<1.2*	<1.2*	3.7	**2 ± 1**	8.6	7.5	2.2	**6 ± 3**	9.8	10.9	3.4	**8 ± 4**
	Spring	44	3.8	0.93	**16 ± 24**	31	3.0	2.4	**12 ± 16**	67	12	0.40	**26 ± 36**
	Summer	48	50	12	**37 ± 21**	15	15	11	**14 ± 2**	47	11	20.0	**26 ± 19**
Pb	**Winter**	<1.2*	<1.2*	<1.2*	**<1.2***	<1.2*	<1.2*	<1.2*	**<1.2***	<1.2*	<1.2*	<1.2*	**<1.2***
	Spring	<1.2*	<1.2*	<1.2*	**<1.2***	1.4	<1.2*	<1.2*	**1.3 ± 0.1**	2.0	<1.2*	<1.2*	**1.5 ± 0.5**
	Summer	<1.2*	<1.2*	<1.2*	**<1.2***	<1.2*	<1.2*	<1.2*	**<1.2***	<1.2*	<1.2*	1.8	**1.4 ± 0.3**
Cd	**Winter**	<0.12*	<0.12*	<0.12*	**<0.12***	1.2	0.30	<0.12*	**0.5 ± 0.6**	1.4	<0.12*	<0.12*	**0.5 ± 0.9**
	Spring	<0.12*	<0.12*	<0.12*	**<0.12***	<0.12*	<0.12*	<0.12*	**<0.12***	2.7	<0.12*	<0.12*	**1 ± 1**
	Summer	<0.12*	<0.12*	<0.12*	**<0.12***	<0.12*	<0.12*	<0.12*	**<0.12***	<0.12	<0.12*	<0.12*	**<0.12***
Ni	**Winter**	1.3	0.62	4.3	**2 ± 2**	2.7	0.80	1.0	**2 ± 1**	2.8	13	4.3	**7 ± 6**
	Spring	1.7	2.8	3.3	**2.6 ± 0.8**	4.3	<0.60*	1.2	**2 ± 2**	0.90	3.0	<0.60*	**2 ± 1**
	Summer	3.8	1.5	0.86	**2 ± 2**	1.8	0.80	1.7	**1.4 ± 0.6**	11	7.5	28	**16 ± 11**
Mn	**Winter**	<1.2*	<1.2*	<1.2*	**<1.2***	<1.2*	<1.2*	<1.2*	**<1.2***	<1.2*	2.0	<1.2*	**1.5 ± 0.5**
	Spring	3.6	<1.2*	<1.2*	**2 ± 1**	2.3	<1.2*	<1.2*	**1.6 ± 0.6**	5.9	<1.2*	<1.2*	**3 ± 3**
	Summer	<1.2*	<1.2*	<1.2*	**<1.2***	<1.2*	<1.2*	<1.2*	**<1.2***	<1.2*	<1.2*	<1.2*	**<1.2***
Cr	**Winter**	<0.48*	<0.48*	<0.48*	**<0.48***	<0.48*	<0.48*	<0.48*	**<0.48***	<0.48*	<0.48*	<0.48*	**<0.48***
	Spring	0.54	<0.48*	<0.48*	**0.5**	1.0	<0.48*	<0.48*	**0.7 ± 0.3**	0.77	<0.48*	<0.48*	**0.6 ± 0.2**
	Summer	<0.48*	<0.48*	<0.48*	**<0.48***	1.4	<0.48*	<0.48*	**0.8 ± 0.5**	<0.48*	<0.48*	<0.48*	**<0.48***

***Detection limit**

3.2. Laboratory experiments

Net control solutions (only seawater with pieces of net) indicated that no significant ($P>0.05$) metal amount was released from the net itself, with values in solutions, in general, below detection limit for both Cu and Pb.

Metal concentrations in metal control solutions (seawater doped with either metal) showed in some cases a slight decrease over time, always lower than 10%. Nevertheless, to compensate this decrease, metal adsorption percentages were always calculated taking in consideration metal concentrations in metal control solutions collected at the same time.

3.2.1. Copper adsorption experiments

The first laboratory experiment (experiment 1) was carried out with a braided net with the characteristics: **Euroline®** (Euronete) 3 mm, mesh 150 mm. The polymer of the fishing net was PE.

In this first experiment, low Cu adsorption values were generally observed, varying between 0-15% (Fig. 9). Although with a slight variation in the initial time period, over time there was an increasing trend of metal adsorption. The highest adsorption value was recorded for the 10080 minutes, which corresponds to the seven days after the beginning of the experiment, with the adsorption values reaching 15% for this time.

In the next experiment (experiment 2), a different type of fishing net was used, **Braided Polyethylene**, a net thinner than **Euroline®** used in the previous experience. However, the constituent polymer was also PE. This fishing net had as characteristics: Braided Polyethylene (Euronete) 1.5 mm, mesh 55 mm.

For this net, the adsorption percentage observed for Cu vary also between 0-14% (Fig. 9), similar to that observed in the first experiment, which was also expected since the type of polymer was the same. The highest adsorption value was again recorded for the 10080 minutes (Fig. 10), which corresponds to the seven days after the beginning of the experiment, with the adsorption value reaching 14%. In general, the values obtained for this type of fishing net were similar to those observed for the first experiment, indicating that the thickness of the net line was not influencing Cu adsorption.

New experiments were carried out, using fishing nets but with a different polymer in their composition, nylon.

Initially nylon experiments (experiment 3) were carried out with a **Thin Nylon** (Euronete) net with the characteristics: 0.30 mm, mesh 100 mm.

The adsorption values observed for Cu were similar to those observed for PE fishing net experiments, ranging between 0-17%, with an increasing trend over time after an initial

high adsorption variability. The highest value was once again recorded for the time 10080 minutes (7 days), being 17%.

Another experiment with nylon fishing net was carried out (experiment 4). The fishing net was a twisted net with the characteristics: **Twisted Nylon** (Euronete) 2.3 mm, mesh 53 mm.

In this experiment Cu adsorption values were slightly higher than the values observed for the remaining fishing nets, varying between 0-53% (Fig. 9). At the time 30 minutes, after the beginning of the experiment, the percentage of Cu adsorption in the fishing net was 5%. In the 60 minutes time, the adsorption percentage increased to 23%. Afterwards, adsorption decreased, reaching 0% at time 480 minutes (8 h). After that there was an increasing adsorption trend. The highest values of percentage of adsorption were observed for the times 2880 minutes (48 h) and 10080 minutes (7 days), reaching 47% and 53% respectively.

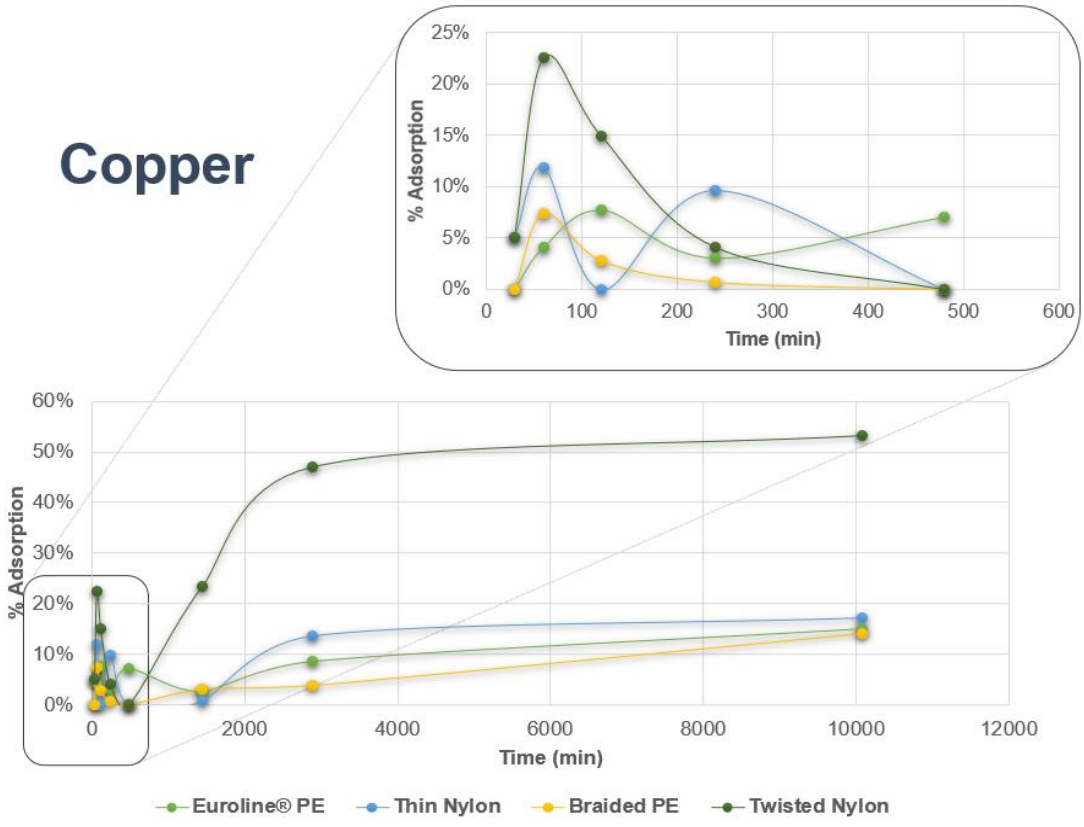

Figure 9. Cu adsorption to different fishing nets over time. Samples collected at times 30, 60, 120, 240, 480, 1440, 2880 and 10080 minutes.

Therefore, **Twisted Nylon**, although it presented a similar behavior to the other tested fishing nets until 480 minutes, higher adsorption percentage were observed after that, stabilizing between 48 h and 7 days at ca. 50%. So, polymer type and net thickness had a different adsorption behavior.

3.2.2. Lead adsorption experiments

In the initial experiment with **Euroline®** (experiment 1), Pb adsorption percentages vary between 0% and 47% (Fig. 10), with a high variability over time. Between 30 minutes and 1440 minutes (24 h), in general, there was no adsorption. The highest percentage of adsorption was recorded for the time 2880 minutes (48 h), ca. 47%, decreasing to 18% in the next sampling time at 10080 minutes (7 days).

In the next experiment (with **Braided PE**) (experiment 2), Pb adsorption percentages varied between 0-24% (Fig. 10). The highest adsorption value was observed at the time 1440 minutes, ca. 24%, decreasing to 18% at the time 2880 minutes and to 7% at the time corresponding to the seven days after the beginning of the experiment. Compared with the first experiment (experiment 1), adsorption variability was similar, being slightly lower in the experience with **Braided PE** in the last three sampling times.

The adsorption values observed for the **Thin Nylon** net showed lower values compared to the PE experiment, adsorption varying between 0-7%. The highest adsorption value was recorded for the time 30 minutes and 2880 minutes.

In the experiment with the **Twisted Nylon**, Pb adsorption percentages varied between 0-39%. Adsorption percentages varied from 15% in the time 30 minutes to 21% for the time 480 minutes and to the highest value obtained, 39% for the time 2880 minutes. Afterwards, adsorption percentage decreased, reaching 17% after 10080 minutes of the beginning of the experiment. Comparing the values obtained in these two experiments with nylon polymer fishing nets there was a higher Pb adsorption to the **Twisted Nylon** fishing net compared to the **Thin Nylon**, for which the adsorption values were quite low.

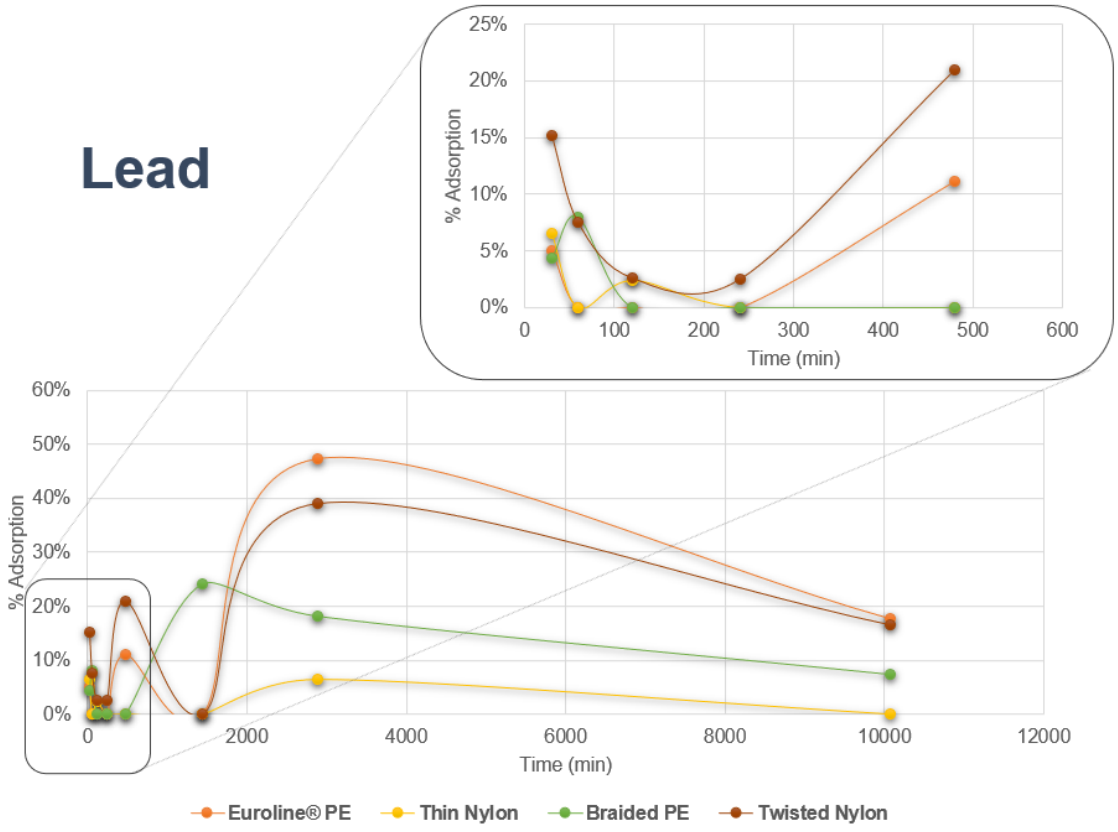

Figure 10. Pb adsorption to different fishing nets over time. Samples collected at times 30, 60, 120, 240, 480, 1440, 2880 and 10080 minutes.

The behavior of the **Twisted Nylon** fishing net and the **Euroline® PE** fishing net was similar, presenting high adsorption percentages when compared with the other two fishing nets. Results clearly indicate that net thickness influenced Pb adsorption.

3.2.3. Metals and PAHs adsorption experiment

A new laboratory experiment was carried out with the fishing net used in the first experiment (**Euroline®**). In this experiment, PAHs were added to seawater along with the metals, to investigate the adsorption behavior of metals in the presence of organic compounds.

Results obtained in the Cu + PAHs experience are shown in Fig. 11. Generally, the adsorption values obtained in this experiment were slightly lower than the values obtained in the experiment without PAHs (experiment 1).

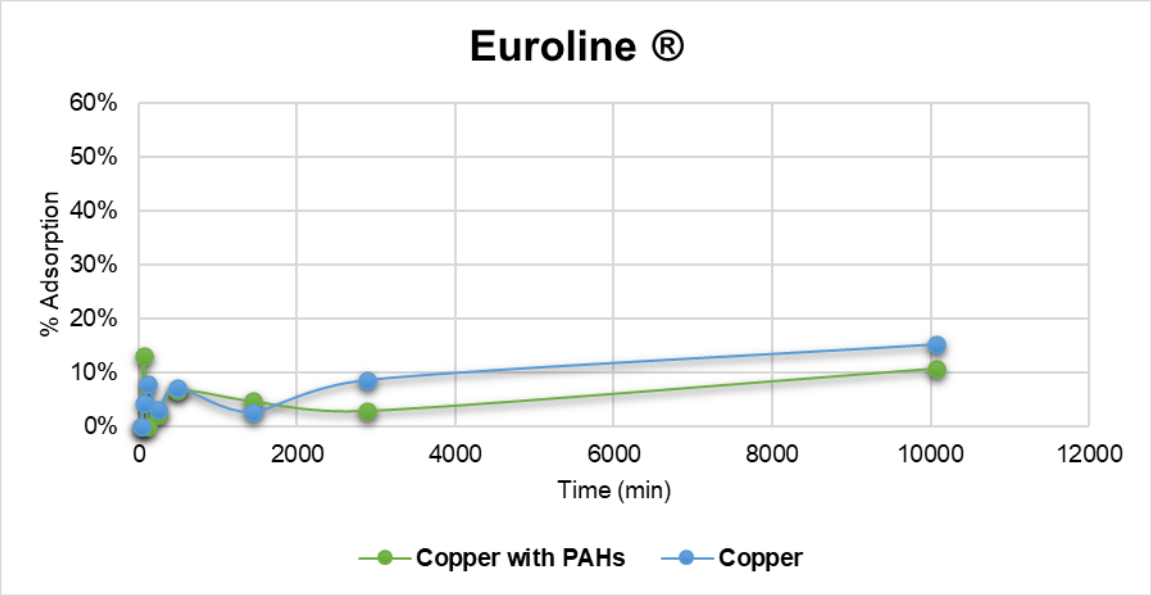

Figure 11. Cu, and Cu with PAHs adsorption to Euroline® net over time. Samples collected at times 30, 60, 120, 240, 480, 1440, 2880 and 10080 minutes.

For Pb, in the presence of PAHs (Fig 12), the adsorption values vary between 0% and 29%. In general, adsorption percentages observed in this experiment were also slightly lower than those observed in the experiment without PAHs.

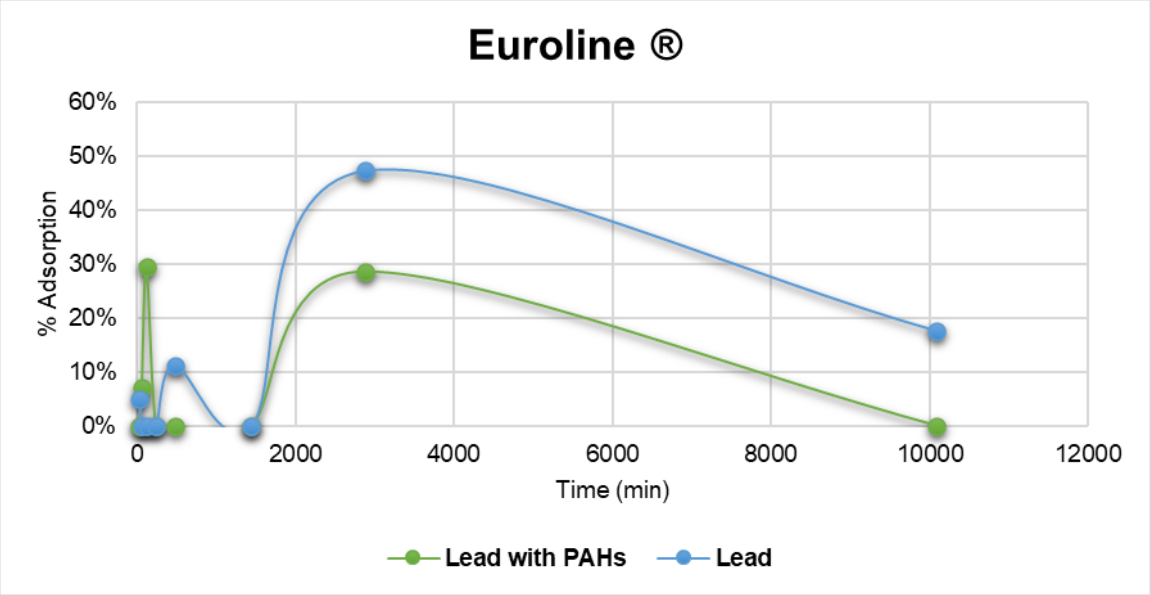

Figure 12. Pb, and Pb with PAHs adsorption to Euroline® net over time. Samples collected at times 30, 60, 120, 240, 480, 1440, 2880 and 10080 minutes.

3.2.4. Metal adsorption triplicate experiment

Twisted Nylon net was selected to confirm adsorption results observed as well to evaluate possible metal desorption phenomena, as this net showed the high adsorption potential for both metals. The experiment was carried out in similar conditions, this time in triplicates, to confirm the values obtained previously and to better understand what occurs over time with this fishing net.

In this experiment, for Cu (Fig. 13), the observed results, in general showed increasing adsorption values over time, in contrast to what was observed previously, where the adsorption percentage varied over time, even in the initial exposure time. For Pb (Fig. 13), the behavior was similar to that recorded for Cu. The triplicates had a very low variability, generally <1% for Cu and <4% for Pb.

For Cu, up to 120 minutes, no adsorption was observed, contrasting with what was observed previously for the fishing net under study. However, afterwards, and up to 2880 minutes it is noticeable an increase in the adsorption percentage reaching ca. 30%, a value slightly lower than in the previously experiment (ca. 47%).

For Pb (Fig. 13), adsorption percentages were in accordance with the previous experiment, with an increase in the percentage of adsorption as the time of exposure of fishing nets to Pb increased. Again, the highest adsorption obtained in this experiment (28%) was observed at 2880 minutes.

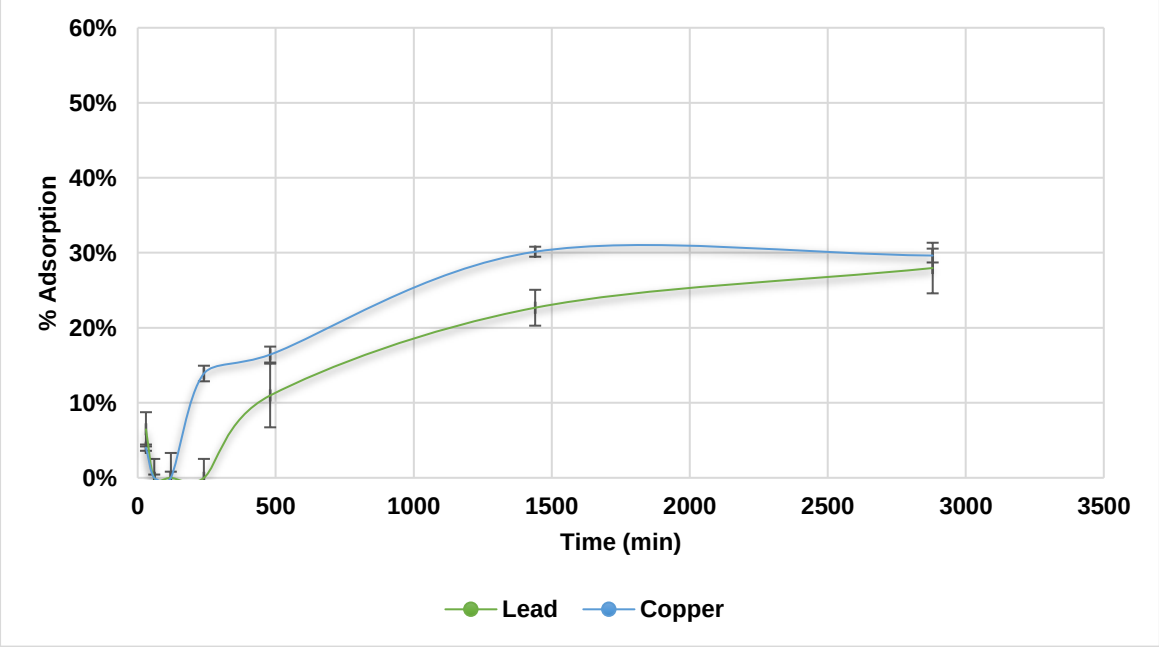

Figure 13. Cu and Pb adsorption (triplicate experiment, n=3) **Twisted Nylon** net over time. Samples collected at times 30, 60, 120, 240, 480, 1440 and 2880 minutes.

3.2.4.1. Metal desorption from fishing nets

At the end of the triplicate experiments, the fishing nets were transferred to new flasks and new seawater was added. The objective was to verify if, eventually, the metals adsorbed to the nets would be release over time when placed in a metal free medium.

For Cu, small amounts of the metal were released from the fishing net into the surrounding seawater, but only, at times 1440 and 2880 minutes (desorption percentages ca. 17% and 27%, respectively, Table 10). For Pb, no metal was released into the water.

Table 10. Cu desorption percentages (% mean and standard derivation n=3) obtained for **Twisted Nylon** over time, Samples collected at times 30, 60, 120, 240, 480, 1440 and 2880 minutes.

Time (min)	Cu
30	0
60	0
120	0
240	0
480	7 ± 7
1440	17 ± 2
2880	27 ± 2

3.3. In-situ experiments at marina of Leixões

Metal (Zn, Pb, Cr, Cu, Fe, Mn, Cd, Ni) levels present in the water particulate matter and adsorbed to fishing gears collected from the marina were measured in all collected samples.

A different type of fishing nets was placed in each location, at the marina pier, namely: Site A – **Braided PE**; B – **Twisted Nylon**; C – **Thin Nylon**. Samples of water were collected on the surface corresponding to Fishing Nets Site, as they are close to the fishing nets, and at the bottom corresponding to the Control Site (sites without nets).

For Mn and Ni, metals in water particulate matter were not detected, being below the detection limit (detection limit: 25 µg L^{-1} for Mn and 10 µg L^{-1} for Ni). Cd was only detected in some samples in the last sampling campaign, varying between 8 to 12 µg L^{-1} (Cd detection limit: 2.5 µg L^{-1}). In general, Cu was not detected in sites with nets (Cu detection limit: 25 µg L^{-1}) whereas for control site Cu was detected only when assembling the experiment (T0) and after the first sampling campaign (T1), varying between 25 and 53 µg L^{-1}.

All other metals, Cr, Fe, Pb and Zn were, in general, always detected and in lower amounts in surface water particulate matter samples than in bottom samples (no nets). Generally, these levels were higher in T1 sampling campaign (Fig. 14).

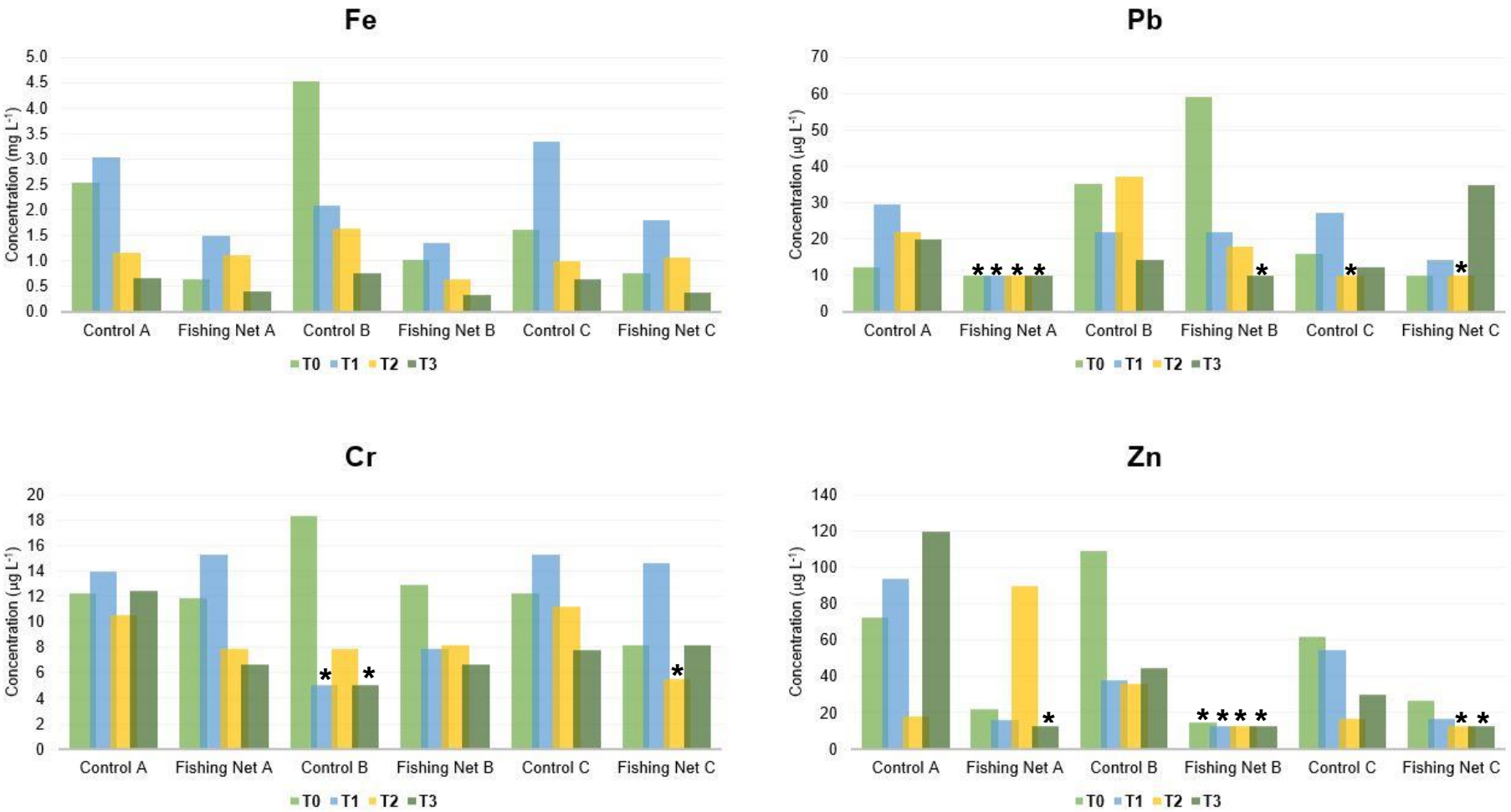

Figure 14. Metal concentration (µg L^{-1}), except for Fe (mg L^{-1}) determined in water particulate matter of Marina Leixões, collected in February (beginning of the experiment, T0); March (T1); May (T2) and July (T3) 2020. Control corresponds to bottom water samples, without fishing nets, and Fishing net corresponds to surface water samples, where fishing nets are present. ***Detection limit**

Regarding metal adsorbed to fishing net pieces, in general, all metals were detected (Table 11). Results are presented per liter of extraction solution as the weight of the net pieces was very much influenced by biofilm formation. In general, metals released by virgin nets were below detection limit. When detected, levels in general were 10 times lower than values measured in extraction solution with net pieces from the Marina experiment, except for Ni, Mn, and Cd.

Generally, the retention of metals in fishing nets decreased as Fishing net B (**Twisted Nylon**) > Fishing net A (**Braided PE**) > Fishing net C (**Thin Nylon**) in March and May sampling campaigns, whereas in July sampling campaign it was Fishing net B (**Twisted Nylon**) > Fishing net C (**Thin Nylon**) > Fishing net A (**Braided PE**). In fact, Fishing net C showed an increased metal retention over time particularly relevant in the last sampling campaign. On the other hand, Fishing net B (**Twisted Nylon**) show a tendency to slightly decrease metal retention, whereas for Fishing net A (**Braided PE**) metal retention either increased (Cu, Pb) decreased (Fe, Ni) or increased and then decreased (Zn, Cd, Mn). Metal retention amounts varied not only among nets but also among metals, with Cd and Ni showing very low retention.

Table 11. Metal concentration (µg L^{-1}, except Fe in mg L^{-1}) adsorbed in fishing nets exposed in Marina Leixões. Samples collected in March, May, and July 2020. Metal released by virgin net are also included. Results are presented per liter of extraction solution.

		Cu	Zn	Fe	Pb	Cd	Ni	Mn	Cr
Fishing Net A (Braided PE)	**Virgin net**	<50*	<25*	0.3	20	<0.50*	7.5	<50*	1.9
	March	354	800	24.4	204	0.8	15.4	745	61
	May	424	915	22.3	210	1.6	15.8	1454	50
	July	513	866	21.2	386	1.1	7.8	1050	70
Fishing Net B (Twisted Nylon)	**Virgin net**	57	0.104	0.4	32	0.7	7.7	229	2.2
	March	1014	1609	62.2	480	3.0	49.5	1243	185
	May	504	1145	33.9	256	2.5	21.9	1159	82.5
	July	623	1087	37.2	361	1.4	19.8	1111	102
Fishing Net C (Thin Nylon)	**Virgin net**	<50*	<25*	<0.10*	20	<0.50*	4.0	<50*	1.4
	March	<50*	85	2.1	49	<0.50*	6.2	72	19
	May	124	372	10.7	124	<0.50*	6.8	140	31
	July	543	1014	34.7	411	1.2	8.9	773	91

***Detection limit**

CHAPTER 4

DISCUSSION & CONCLUSIONS

4. Discussion

4.1. Monitoring in-situ - hotspots characterization

4.1.1. Matosinhos submarine wreck hotspot of lost fishing gears

Metal levels observed in Matosinhos hotspot were generally low, and in some case even below the detection limits.

In water samples, only Fe was detected in particulate matter samples collected.

Regarding sediments, for some metals (Zn, Mn, Fe, Pb and Cr) significant differences between the control site (Site C) and the sites with fishing nets (Site A and B) were observed, differences that were also influenced by the sampling campaign. Pb, Mn and Cr levels were significantly higher in the spring campaign.

Metal levels can be influenced by hydrodynamics of the area as well as by the type of sediment. Besides, each metal can behave differently because it is involved in different biogeochemical processes, which can also influence metal levels. However, the levels of metals observed either in water particulate matter or in sediment were, in general, lower than the values reported for NW Portuguese coast nearby (APA, 2019). Some of these metals have been detected in the area (Fernandes *et al.,* 2020), however, the sampling sites is located offshore and at a depth of 30 meters, that can explain the low metal levels observed.

The lost fishing nets did not seem to have a clear influence on the accumulation of metals around the submarine wreck. Some variability among sites was observed, and some metals, Zn, Mn, Fe, Pb and Cr, showed slightly higher levels in control sites, indicating that lost fishing nets could be removing metals from the water preventing their deposition in sediment. But the observed variability may be also due to several natural or anthropogenic factors, such as biological activity, the formation of biofilms, the physical-chemical changes associated with environmental dynamics, geological characteristics of sediments, namely organic matter contents, and anthropogenic pressures (Rocha *et al.,* 2019).

4.1.2. Cavalos de Fão (Esposende) hotspot of lost fishing gears

Metal levels observed in Cavalos de Fão (Esposende) hotspot were generally low and, in some cases, also below the detection limits.

For water particulate matter, levels of Cd, Cr, Pb and Mn were in general below the detection limits. On the other land, levels of Cu, Fe, Ni and Zn were detected in almost all samples. Their levels varied between locations and among sampling campaigns, without a clear pattern of variation.

For sediment samples, in general, all metals were quantified, except Cu, Cd and Ni,

for which levels were below the detection limit (in most samples). Significant differences between the control site and the location with lost fishing nets were only observed for Mn, differences that were also influenced by the sampling campaign. Higher concentrations of Mn were observed for control site. The observed differences were higher in the summer sampling campaign. Significant differences between sampling campaigns were only observed for Cu, with higher values being observed in winter.

As previously mentioned for Matosinhos hotspot, levels of metals observed can be influenced by several factors, such as biological activity, physical-chemical changes resulting from hydrodynamics, geology of sediments or anthropogenic pressures (Rocha *et al.,* 2019). Besides, each metal can present a different behavior as it is involved in different biogeochemical processes. However, the metal levels observed were in line with what has been reported for NW coast of Portugal for seawaters and sediments (APA, 2019). Although the area is close to the Cávado River which is subject to a strong industrial and anthropogenic activity along its course that can increase metal levels (Gredilla *et al.,* 2015), this study showed relative low values for metal concentrations. Such low metal contamination observed support the good environmental quality of Cavalos de Fão that is integrated in a marine protected area (PNLN).

Similarly, to what was observed in Matosinhos hotspot, no clear influence of lost fishing nets on the accumulation of metals in lost fishing net hotspot was identified, as the levels were similar between sites with and without lost fishing nets.

4.2. Laboratory experiments

To investigate if fishing nets have the potential to adsorb metals, some adsorption experiments were made in controlled laboratory conditions by exposing different fishing nets to seawater contaminated with either Cu or Pb.

The fishing net that presented the higher level of Cu adsorption was the **Twisted Nylon**. The highest adsorption value was observed after 7 days, with approximately 53% metal adsorption. However, after 2880 minutes (48 h) the adsorption was already high, being ca. 47%. But the values varied over time with no clear tendency in the first exposing hours. The fishing net with lower adsorption potential was, in general, **Braided PE**, with the maximum adsorption values of 14% observed after 7 days. The observed adsorption values also vary over time, without a clear tendency. **Euroline® PE** showed a similar behavior to that of the **Braided PE** fishing net, with variable adsorption rates over time. These differences between **Twisted Nylon** and the other two fishing nets can be due to their polymer composition, which can influence metal adsorption behavior. Still, the adsorption of Cu in **Thin Nylon** varied over time, also showing low adsorption values (0-17%). So,

metal adsorption was also influenced by nets structure, namely braided, twisted, and thermosetting knots.

For Pb, the fishing net with the highest adsorption value was **Euroline ® PE** with ca. 47% adsorption. **Twisted Nylon** also had a high adsorption value, ca. 39%. In the first exposure times (up to 480 minutes), **Twisted Nylon** showed higher adsorption values compared to **Euroline® PE** net. However, after that time **Euroline® PE** showed higher adsorption values. **Braided PE** also showed some variability over time of exposure, without a clear tendency throughout the experience. So, results indicate that net polymer influenced also Pb adsorption. The fishing net with the lowest Pb adsorption values was **Thin Nylon**, with very low or inexistent adsorption, indicating again that net structure has a clear influence of metal adsorption. Generally, adsorption values observed for Pb varied over time, increasing essentially between 48 h and 7 days. There are several factors that can influenced Pb behavior, among which, metal itself, hydrodynamics of water, contact surface and/or the structure and the polymer of the fishing net.

Metal adsorption experiments in the presence of PAHs, showed slightly lower adsorption percentages comparing to the absence of PAHs. So, PAHs seem to influence the adsorption of Cu and Pb, decreasing the adsorption values.

After laboratory experiments with the various fishing nets, the fishing net that showed, generally, higher metal adsorption (**Twisted Nylon**) was selected to confirm the results obtained previously.

In general, the maximum adsorption values of Cu and Pb were slightly lower than those before. Cu adsorption was only observed after 120 minutes (2 h), contrasting with what was previously observed for this fishing net. After 240 minutes (4 h), the adsorption values showed a consistent and stable growth until the final time, 2880 minutes (48 h). At this time, the percentage adsorption reached ca. 30%, a value slightly lower than the obtained in the previous experiment for the same fishing net (ca. 47%).

The percentage of Pb adsorbed was within the expected, with the percentage of adsorption increasing with time of exposure of fishing nets to Pb. Only after 480 minutes (8 h) adsorption values were registered, showing a consistent and stable growth until the final time. The highest adsorption values obtained for Pb (ca. 28%) was observed at 2880 minutes, this value being lower than that reported in the previous experiment for the same net (ca. 39%), at the same time.

In general, there were increasing adsorption values over time, contrary to what was previously obtained, where the values increased and decrease over the first timings of contact. This difference in behavior between the two experiences with **Twisted Nylon** could be related with several factors, namely with the seawater used in the laboratory experiments, as this seawater was collected at different times, and may be under the

influence of seasonality, pH values and biological activity at the sampling time. These factors may explain the differences in behavior compared to the initial experience. However, the triplicate experiment allowed to confirm metals adsorption potential observed previously and to verify that exposure time influences the adsorption of Cu and Pb to fishing nets, namely, to **Twisted Nylon**.

Subsequently, metal desorption experiments showed that Cu started to be release after 480 minutes. In the last time (2880 minutes), a desorption value of 27% was obtained. These desorption values confirm that, effectively, after exposure of **Twisted Nylon** to Cu, it can grab the metal for a period of time and then release it again in the surrounding water where the net is present. For Pb, no desorption was observed indicating that the fishing net may not allow Pb to be released into the surrounding water, or the behavior of the metal itself may present characteristics that do not allow release in a short period of time (only 48 h).

Pb adsorption values were slightly higher (maximum adsorption value ca. 47%) than the values reported by Ahechti *et al.* (2020) when investigating Pb adsorption to virgin plastic pellets made of PE with a maximum adsorption of ca. 20%. The observed behavior of **Euroline® PE** fishing net may be due to the fact it is made of HTPE, which makes the net more resistant than the usual ones made of common PE, a characteristic that might increase metal adsorption. On the other hand, Cu adsorption percentage were lower than those observed for Pb (with a maximum adsorption of 15%), but generally identical to those reported by Ahechti *et al*. (2020).

Regarding nylon fishing nets, the adsorption percentages observed for Cu were generally higher when compared with those of Pb. These results are in accordance with the study developed by Gao *et al.* (2019) which observed different metal adsorption percentages for the same polymer type. But, in relation to adsorption percentages of Pb to nylon nets, values showed a significant variability, maximum adsorption values of ca. 39% and 7% for **Twisted Nylon** and **Thin Nylon**, respectively.

Hence, all experiments results showed that PE an PA (nylon) fishing nets adsorbed Cu and Pb. These results are important, because although PE and PA (nylon) are among the most studied polymers in the laboratory context, there are still few studies on the adsorption of inorganic compounds (metals, namely) as most studies concern adsorption of organic compounds (Guo & Wang, 2019).

4.3. In-situ experiments at marina of Leixões

In water suspended particulate matter metals were detected, in general, in at least one of the sampling sites, with the exception of Ni and Mn, which were always below the

detection limit in all samples at all sampling times.

Cu was only detected when the experiment was assembled (T0) and in the first sampling (T1), in general in control sites. Cd was only detected in the last sampling time (T3) in samples corresponding to control (without nets).

In general, Fe, Pb, Zn and Cr, also showed higher levels in water particulate matter of the control site (without nets). So, the presence of fishing nets seems to influence the levels of metals observed, since results indicate that lost fishing nets could be removing metals from the water. However, variability could also be related with seasonality as also in control sites metal values varied along time.

Metal levels in Marina water particulate matter were in general higher than those observed at the two lost fishing nets hotspot, Cavalos de Fão (Esposende) or Matosinhos submarine wreck. This difference could be due to the fact that the marina of Leixões is a confined place, not being in the open sea as it happens for the hotspots, which can facilitate the retention of metals in suspended particulate matter. This area is also subject to several anthropogenic factors that can affect metal levels. In fact, the Leça river drains at this marina and along the course of Leça river there are several points of discharge of effluents, sometimes untreated. Downstream from Leça river the area is characterized by high industrial and urban density (Gomes *et al.,* 2014; Rocha *et al.,* 2014; Rodrigues *et al.,* 2019) with two important wastewater treatment plants (WWTPs) located in its course, discharging effluents as well as with industry of different types, such as stamping and dyeing, metallurgic, mechanical and agri-food (Gomes *et al.,* 2014). All these anthropogenic pressures can influence the values of metals present in the marina of Leixões water. However, observed metal levels were in accordance with the values reported for the area (APA, 2019; Gomes *et al.,* 2014; Fernandes *et al.,* 2020). Biological activity, physical and chemical changes resulting from hydrodynamics and the individual behavior of each metal can also influence the metal levels at the site.

Regarding metal retained in fishing nets in Marina of Leixões, results showed that **Twisted Nylon** had notorious higher retention metal values than the other nets. **Braided PE** also showed high concentrations and, in some cases, close to **Twisted Nylon**. **Thin Nylon** showed low metal retention concentrations than the other two fishing nets, although only in the first two sampling campaign. Compared to the laboratory experiment, the levels of metals observed in the Marina nets are considerably higher. In fact, **Thin Nylon** showed practically no adsorption in the laboratory experiments and in the quasi-real environment, metal retention occurred. However, these results may be influenced by the presence of microorganisms, including bacteria, protozoa, algae, archaea, fungi, and protists, due to their capacity to form biofilms on the surface of plastics (Zettler *et al.*, 2013; Flemming,

2020). These biofilms were recorded in the fishing nets of the marina experience (Fig. 15).

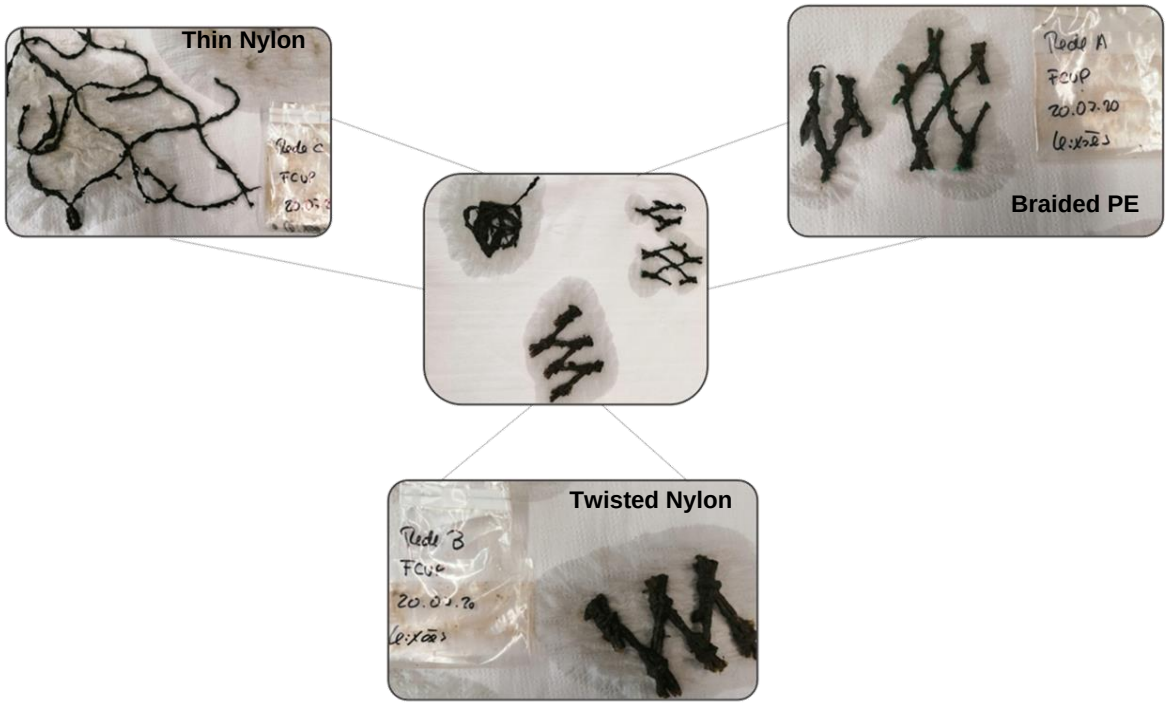

Figure 15. All fishing nets collected of Marina experiment. **Thin Nylon**, **Braided PE** and **Twisted Nylon**.

Apparently, **Twisted Nylon** and **Thin Nylon** were the fishing net where the presence of these biofilms was more intense compared to **Braided PE** fishing nets under study.

Although the levels of retained metals are higher in some cases, the presence of these biological communities (biofilms) can influence these levels either directly or indirectly. That is, directly, the sorption processes may be taking place in the biofilm itself (Gunaalan *et al.,* 2020), or indirectly, since the formation and development of biofilms may alter the morphology and physicochemical properties of plastics, and consequently the adsorption capacity of metal into biofilms can be increased (Guan *et al.,* 2020). However, it was not possible to distinguish if the processes of metal sorption in quasi-real environment was occurring in the fishing net or in the biofilm present.

4.4. Environmental Harmfulness of fishing nets

The results obtained in the environmental characterization of the hotspots of Matosinhos and Cavalos de Fão (Esposende), showed low metal concentration preventing to infer a clear effect of lost fishing nets on those levels.

Adsorption laboratory experiments showed the potential for adsorption of metals to fishing nets. The absence of a clear tendency for adsorption in the first exposure times showed that the exposure time can effectively influence the adsorption values, since after the initial times of exposure, the adsorption values seem to vary less. This trend was verified

in general for all fishing nets. Another important point was that different metals respond differently to different fishing nets, being able to be influenced both by the polymer and by its structure. In fact, these sorption processes under laboratory-controlled conditions seemed to be dependent on physical-chemical processes. In addition, laboratory experiment also indicated that some metals adsorbed could be released again into the medium.

That said, the sorption processes observed in laboratory experiences lead to the environmental implications of two possible scenarios (Fig. 16).

Fishing nets have metal sorption capacity, concentrating metals in their surface and functioning as sinks of pollutants.

Fishing nets can adsorbed and desadsorbed into the surrounding water, concentrating them in the environment around them, but a significant effect on metal levels in net surrounding water was not observed.

Figure 16. Environmental implications raised in laboratory experiments.

Subsequently, the behavioral assessment of the nets was carried out in a quasi-real environment, the in-situ experience of Marina of Leixões. Results obtained showed metal retention an order of magnitude higher than that recorded in the adsorption laboratory experiment. Namely, for **Thin Nylon**, in general, metal concentrations were recorded in the fishing nets in increasing order over the sampling time, contrary to what was observed in the laboratory experiment where this net showed no significant metal adsorption. These results suggest a different behavior to what was observed in the laboratory experience, indicating that, in addition to physical-chemical processes, the biological component (biofilm) present in the fishing nets can influence and increase metal retention on fishing nets. In general, **Twisted Nylon** and **Braided PE**, showed a decrease in metal levels present in fishing nets over time. Compared to the results obtained in laboratory experiments, these fishing nets also showed higher values in a quasi-real environment, indicating that effectively, biological processes such as the presence of biofilms influence metal retention.

The behavior observed in the in-situ experience is very similar to the behavior that may occur in the environment. This means that the removal of contaminants from the

surrounding water, concentrating them in fishing nets, is dependent not only on all the previously mentioned processes, but also on the presence and/or absence of UV light, exposure time, aging of nets, among other, as well as the physical-chemical and biological processes of the environmental compartment under study. Taking in consideration the results observed in this study, we may conclude that lost fishing net can promote the accumulation of metals in the area where they are located.

The presence of fishing nets in the marine environment present an environmental risk, being dangerous for marine life, since several aquatic species are easily entangled in these fishing gears and may even ingest smaller fragments resulting from these. It is known that a wide range of aquatic organisms easily confuse natural food with plastic fragments, accidentally ingesting them and, consequently, the upper trophic levels, can accumulate these plastic fragments indirectly, by biomagnification (Gunaalan *et al.,* 2020). The present study showed that fishing nets can have another, environmental risk, associated with their capacity to adsorb metals and act like a pollutant sink can cause harm to these aquatic organisms.

4.5. Conclusion and future perspectives

Pollution of coastal waters is a reality that has been reported as well as the presence of lost fishing nets in our oceans, essentially in areas of intense fishing activity. Adsorption of metals to fishing nets is a recent and innovative theme regarding water pollution and deserves further research.

In fact, potential for adsorption of metals to fishing nets was recorded, under controlled conditions in laboratory. In quasi-real scenario, this potential was also observed, but it was not possible to prove whether the adsorption actually occurs in the fishing nets or if it is being influenced by the presence of attached biofilms. The metal retention process on fishing nets has a physical-chemical dependent component, as shown by the controlled adsorption laboratory experimental part, and a biological dependent component, as shown in the Marina experiment, in which the presence of biofilms probably contributed for metal retention on fishing nets. More research on this topic is needed to fully elucidate these processes. In addition, it would be interesting to test the potential for adsorption of fishing nets in places that are known, a prior, that there are higher levels of contamination. The deeper investigation in a quasi-real environment, with aged fishing nets would also be an interesting approach, as ageing can change fishing net surface and structure. Laboratory investigation, changing some factors that are known to influence the adsorption values will also be an interesting approach, for example, to carry out experiments in the presence of direct UV light, since the presence of UV light cause change the surface of polymers. Other polymers that are used in the manufacture of nets can and should also be analyzed for their metal adsorption/retention behavior.

Further investigation into the interactions between plastic fragments and metal is needed. Models that explain how these adsorptions occur and what the exact factors that make these adsorptions vary need to be clarified. Various types of plastic polymers, whether they are higher or low density, should also be studied for sorption capacities. So, a deeper study of this matter is also necessary to clarify these sorption processes.

About fishing nets, studies are also needed to characterize this marine litter over time, since today it is easily found in our oceans and even on our beaches. Fishing nets in conjunction with other pollutants, whether organic or inorganic, may constitute a new pollutant and consequently a new threat to the marine ecosystem and beyond.

This work contributed to a first approach on this topic, and future research is necessary to solve the questions that remained unclear.

CHAPTER 5

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5. REFERENCES

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