

Potential of lost fishing gears for adsorption of PAHs

Henry van der Gracht

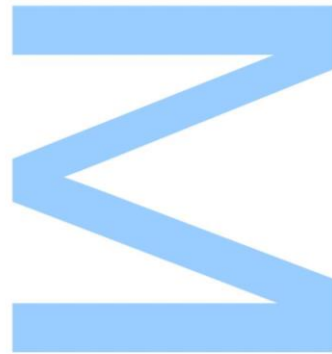
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Abstract

Extreme and unconscious consumptions patterns led to frightening results, at least 640.000 tons of fishing gears are lost each year in the marine environment and thousands tons of Polycyclic Aromatic Hydrocarbons (PAHs) enter it. PAHs are one of the most widespread organic environmental pollutants and are causing a serious threat to the marine environment due to their toxic, carcinogenic and mutagenic properties. This study investigated if plastic fishing gears can adsorb PAHs on their surface and act as a sink of 10 priority polycyclic aromatic hydrocarbons (PAHs), creating new highly polluted areas and posing even more harm to marine ecosystems. For this, 3 approaches were followed. In the first one, environmental monitoring of two lost gears hotspots along the NW Portuguese coast was achieved to evaluate the effect of lost fishing nets on PAHs levels in water and sediment. Surveyed areas (a marine protected area and an artificial reef) were classified as weakly polluted by these pollutants and no significant influence of the presence of lost nets on PAHs environmental levels was observed. In the second approach, laboratory experiments were performed to assess if pieces of plastic fishing nets can adsorb PAHs on their surface in highly polluted conditions (seawater doped with PAHs). It was found that all 10 priority PAHs were adsorbed and that adsorption increased with the molecular weight of PAHs increase. In the third approach, in-situ experiments at a seawater marina were performed to assess both the adsorption and accumulation process of PAHs to and around fishing nets. It was found that all 10 priority PAHs were retained on the nets but results did not indicate any significant influence of the nets in the accumulation of PAHs in nearby waters.

Overall, this work indicates that plastic lost fishing gears concentrate PAHs on their surface and can therefore be an hazardous to the environment.

Keywords : Polycyclic Aromatic Hydrocarbons, Adsorption, Plastic, Lost Fishing Gear, Gas-Chromatography – Mass Spectrometry, Water, Sediments, Portuguese NW coast.

Resumo

Padrões de consumo extremo e inconsciente levaram a resultados assustadores, pelo menos 640.000 toneladas de artes de pesca são perdidas todos os anos no meio marinho e milhares de toneladas de hidrocarbonetos aromáticos policíclicos (HAP) entram nele. HAP são um dos poluentes ambientais orgânicos mais difundidos e estão a causar uma séria ameaça ao meio marinho devido às suas propriedades tóxicas, cancerígenas e mutagénicas. Este estudo investigou se as artes de pesca de plástico podem adsorver HAP na sua superfície e funcionar como um reservatório de 10 HAP, criando novas áreas altamente poluídas e causando ainda mais danos aos ecossistemas marinhos. Para tal, 3 abordagens foram seguidas. Na primeira abordagem, foi efectuada uma monitorização ambiental de dois hotspots de redes perdidas, na costa NW portuguesa para avaliar o efeito das redes de pesca nos níveis de HAP na água e sedimentos. As áreas seleccionadas foram classificadas como fracamente poluídas por estes poluentes e os resultados não indicaram qualquer influência significativa da presença de redes perdidas nos níveis ambientais de HAP. Na segunda abordagem, foram realizadas experiências laboratoriais para avaliar se pequenos pedaços de redes de plástico podem adsorver HAP na sua superfície em águas significativamente poluídas (água do mar dopada com HPAs). Verificou-se que todos os 10 HAP prioritários foram adsorvidos e que o processo de adsorção aumentou com o aumento do peso molecular dos HAP. Na terceira abordagem, foram realizadas experiências in situ numa marina de água salina para avaliar tanto o processo de adsorção como o processo de acumulação de HAP devido à presença de redes de pesca. Verificou-se que todos os 10 HAP prioritários foram retidos nas redes, mas os resultados não indicaram qualquer influência significativa das redes na acumulação de HAP nas águas mais próximas. Globalmente, este trabalho indica que as redes de pesca de plástico perdidas no oceano concentram os HAP na sua superfície e podem, por conseguinte, ser nefastas para o meio ambiente.

Palavras-chave: Hidrocarbonetos aromáticos policíclicos, Adsorção, Plástico, Artes de Pesca Perdidas, Cromatografia Gasosa – Espectrometria de Massa, Água, Sedimentos, Costa NW portuguesa.

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List of acronyms and abbreviations

Ace	Acenaphthene
Acn	Acenaphthylene
ALDFG	Abandoned, lost or discarded fishing gears
Ant	Anthracene
As	Arsenic
Ba	Barium
BaA	Benzo(a)anthracene
C	Concentration
CC	Calibration Curve
Cd	Cadmium
Ch	Chrysene
Cu	Copper
EC	European Commission
ECBC	European Cetacean Bycatch Campaign
EDC	Endocrine Disrupting Chemical
EMFF	European Maritime and Fisheries Fund
FAO	Food and Agriculture Organization of the United Nations
Fe	Iron
Fl	Fluorene
Fluo	Fluoranthene
g	Gram
GC	Gas Chromatography
GESAMP	Joint Group of Experts on the Scientific Aspects of Marine Pollution
h	Hour
HDPE	High-Density Polyethylene
Hg	Mercury
IS	Internal Standard
kg	Kilogram
km	Kilometre

L	Litter
LoD	Limit of detection
LoQ	Limit of quantification
m	Metter
mg	Milligram
min	Minute
mL	Millilitre
mm	Millimetre
Mn	Manganese
MS	Mass Spectrometry
MSC	Marine Stewardship Council
Nap	Naphthalene
NOAA	National Oceanic and Atmospheric Administration
PA	Polyamide
PAH	Polycyclic Aromatic Hydrocarbons
Pb	Lead
PE	Polyester
PET	Polyethylene
Phe	Phenanthrene
POP	Persistent Organic Pollutant
PP	Polypropylene
Py	Pyrene
r	Correlation coefficient
rpm	Revolutions per minute
s	Second
SPME	Solid Phase Microextraction
UNEP	United Nations Environment Program
V	Volume
Zn	Zinc

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1. Introduction

“Human being is capable of the best and the worst, but it’s in the worst he is the best”, G. Lacroix

In 1870, Hyatt brothers invented the first plastic matter; the celluloid (Rossell, 2000). Since then, multiple innovations, accomplishments and developments have brought plastics as an essential material for countless aspect of humanity’s evolution and improvement. Plastics have replaced natural materials such as stone, bronze, iron, wood, leather, linen, wool and continue to replace many others and enhance properties in order to respond to a continuous growth. Banishing plastics would send humanity back to the 19TH century’s way of living (Gilbert, 2017).

Extreme and unconscious consumptions patterns led to frightening results with annual worldwide plastics production evolved from 1.5 million metric tons in 1950 to 359 million metric tons in 2018, with no sign of decrease in the future (PlasticsEurope, 2019). The United Nations Environment Program estimates that about 13 million tons of plastics enter our oceans each year, causing a serious threat to biodiversity, ecosystems, economy and human health (UNEP, 2018).

Since the dawn of time, men took advantage of the marine environment and their associated communities to feed themselves. Fishing methods and materials have evolved over time in the perpetual research of sustainability and efficiency. With the blow up of the plastic industry after World War II, natural fibres-made gears used until then were replaced by synthetic fibres-made gears. They have the advantage to resist moisture and to be designed into almost any kind of product (Oxvig & Hansen, 2007).

Ocean plastic pollution includes not only consumer’s waste but also abandoned, lost or discarded fishing gears (ALDFG) - another undeniable threat that ultimately become the so-called ‘ghost gears’. The Food and

Agriculture Organization of the United Nations estimates that at least 640.000 tons of fishing gears enter our oceans every year (FAO, 2018). Besides plastics, whether it is via atmospheric transport, run-off into waterways or direct disposal, every day a growing cocktail of chemicals enter and threaten our oceans. It includes, among many others, Polycyclic Aromatic Hydrocarbons (PAHs) (Lloyd-Smith & Immig, 2018). Several studies have proven that plastic materials showed a great adsorption level for PAHs (Bouhroum et al., 2019; Fisner et al., 2013; Hirai et al., 2011; Rios* et al., 2010). However, none of them has looked into the adsorption possibilities of plastic fishing gears. The following thesis will focus on the evaluation of the potential of lost fishing gears for adsorption of PAHs.

1.1 Context of this thesis

In 2017, the European Maritime and Fisheries Fund (EMFF) from the European Commission launched a call for projects to promote a “Sustainable Blue Economy” across Europe and in the Mediterranean with a total budget of 15.1 million euros (Fig.1.1).



Fig.1.1. Resume of the sustainable blue economy call, associated strands and attributions (European Commission, 2017).

The NetTag project - for which the present thesis is achieved - is part of the second strand consisting to fight marine litter. The project - who

started on 1st of January 2019 and will end the 31st December 2020 - was granted with a budget of 394.449,00 euros from the entire EC budget.

It involves CIIMAR (Interdisciplinary Centre of Marine and Environmental Research, Porto, Portugal), INESC TEC (Institute for Systems and Computer Engineering, Technology and Science, Lisbon, Portugal), UNEW (University of Newcastle, Engineering School, Newcastle, United Kingdom), UAVR (University of Aveiro, Aveiro, Portugal), USC (University of Santiago de Compostela, Coruna, Spain), APMSHM (Biggest Security Association of Men of the Sea, Póvoa de Varzim, Portugal), ARVI (Cooperative of Fishing Shipowners of Vigo, Vigo, Spain), Lankhorst EURONETE Portugal (world's leading supplier of fishing nets, Viana do Castelo, Portugal).

The purpose of the project is to reduce and prevent marine litter derived from fisheries. To achieve it, eight work packages were established with their associated responsible entity (Fig.1.2). The success of the project relies on two different types of preventive measures (NetTag, 2020).

The first type consists in the development of low cost, miniature and environmental-friendly acoustic tags and transceivers. Attached on gears, they allow to track them in case of loss. Automated robotic systems are also developed for afterwards recovery.

The second type involves awareness actions to promote better fishermen practices on-board in order to decrease marine litter derived from fishing vessels.

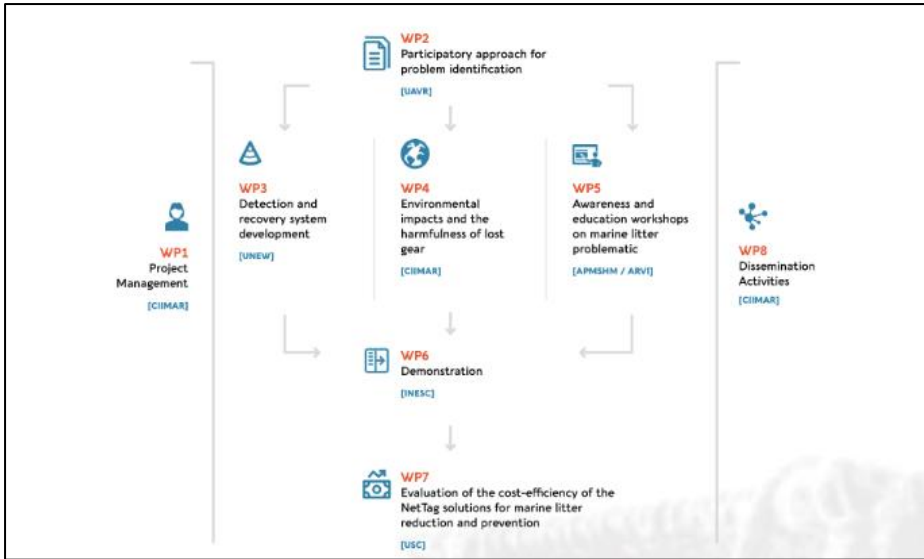


Fig.1.2. Different work packages of the NetTag project and corresponding operators (NetTag, 2020).

In parallel, environmental impact assessment of lost gears as a new pollutant and cost-efficiency evaluation of developed technologies are achieved.

The present thesis enters the framework of the fourth work package for which CIIMAR is responsible, namely ‘environmental impacts and the harmfulness of lost gear’. The main goal of this work package is to investigate the harmfulness of lost fishing gear as a new pollutant with environmental impacts assessments by in situ monitoring and ecosystem-based approach. In parallel, laboratory manipulative experiments are achieved to evaluate three fishing gears’ phenomena: the potential for the release of materials, the potential for adsorption of inorganic pollutants and the potential for adsorption of organic pollutants. This thesis will focus on the potential of fishing nets for adsorption of organic pollutants with PAHs being chosen as the organic pollutants for investigation.

1.2 Overview of the thesis

It investigated the potential of lost gears for adsorption of PAHs in a multi-scale perspective: environmental monitoring of two lost gear hotspots, adsorption laboratory experiments and in situ experiments (Fig.1.3).

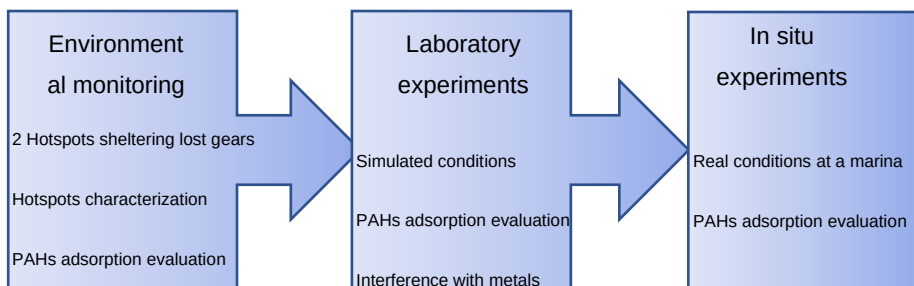


Fig.1.3. General scheme of the work described in the thesis.

1.3 Goals and originality of the research

On one hand, thousands tons of PAHs enter aquatic environments each year (Yim et al., 2007). They are one of the most widespread organic environmental pollutants and are causing a serious threat to the marine environment due to their toxic, carcinogenic and mutagenic properties (Abdel-Shafy & Mansour, 2016)

On the other hand, microplastics have become a major concern in aquatic pollution over the last years (Andrady, 2011). It consists of small pieces of plastic (less than 2 mm) and classified under two types.

Primary microplastics include microbeads, plastic pellets and plastic fibres. Secondary microplastics result from the breakdown of larger plastics and occur when these plastics undergo weathering through wave action, wind action and ultraviolet radiation (Rogers, 2020). These microplastics are ingested by marine species and enter the marine food web, turning into a threat for both aquatic life and human health (Andrady, 2011).

Several studies proved that microplastics act as a carrier of toxic contaminant, including PAHs, by adsorbing them on their surface (Fisner et al., 2013; Hirai et al., 2011). Another study showed that the adsorption process and efficiency is not limited to small pieces of plastic but also occur for larger objects such as plastic ropes, buoys, caps and many other larger fragments (Rios* et al., 2010).

Each year, millions of tons of plastic fishing gears are lost in our oceans with all their potential physical threats to marine environment and human health. PAHs adsorption to plastic materials in water, namely those that are part of marine litter, has been observed (Rios* et al., 2010). However, to the best of our knowledge, PAHs adsorption on fishing gears has never been investigated in previous researches. Therefore, the aim of this thesis is to increase the scientific knowledge about PAHs adsorption on plastics fishing gears. Besides participating in tackling one of the biggest threats of the marine environment, the author found a tremendous interest in conducting a study never done before with all the associated challenges.

Regarding these phenomena, the author conducted the study with the following hypothesis: If PAHs are adsorbed, lost gears in the ocean could concentrate PAHs in surrounding zones, creating a new highly polluted area and therefore lost gear can become a new pollutant posing even more harm to marine ecosystems.

The three different approaches were developed to evaluate previous assumptions.

2. Literature review

2.1 Fishing overview

Fishing activities are regrouped in two applications, namely fisheries and aquaculture. Fisheries exist since the dawn of fishing and have remained at the same level for the past years, while aquaculture was developed later and has since then faced a tremendous growth (Fig.2.1). Although they are similar within several aspects, it is necessary to differentiate them. The FAO established this difference as “Aquaculture is the farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants. Farming implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc. Farming also implies individual or corporate ownership of the stock being cultivated. For statistical purposes, aquatic organisms which are harvested by an individual or corporate body which has owned them throughout their rearing period contribute to aquaculture, while aquatic organisms which are exploitable by the public as a common property resources, with or without appropriate licenses, are the harvest of fisheries” (FAO, 1992)

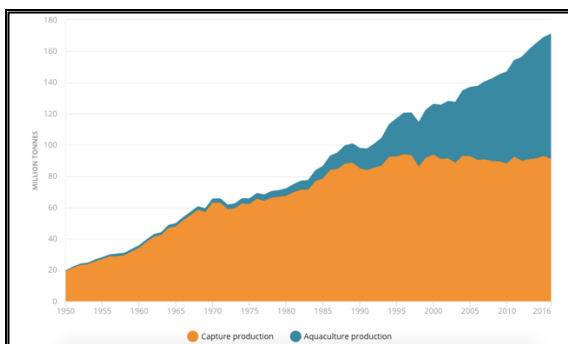


Fig.2.1. World capture fisheries and aquaculture production (FAO, 2018).

Because it is possible to apply a more effective control on aquaculture equipment (essentially statics), ALDFG levels related to aquaculture are extremely low compared to fisheries and difficult to estimate. Therefore, following of this thesis ALDFG is focused on fisheries.

2.1.1 Fisheries

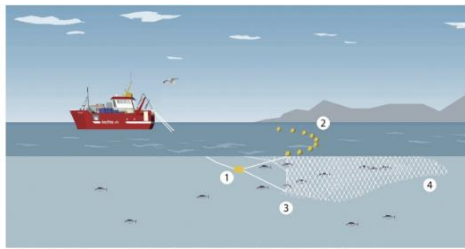
Fisheries can be divided in four categories (Zinabu, 2006):

- Inshore fisheries: target species found in waters close to shore, and in depths down to 200 meters.
- Pelagic fisheries: target species that swim in schools in surface waters, and in depths down to 200 meters. These species range over wide areas of open ocean.
- Mid-Water fisheries: target species found in depths higher than 200 meters. Therefore, specialist equipment is required.
- Deep-water fisheries: target species found in depths higher than 600 meters. Therefore, large boats and highly specialized equipment is required.

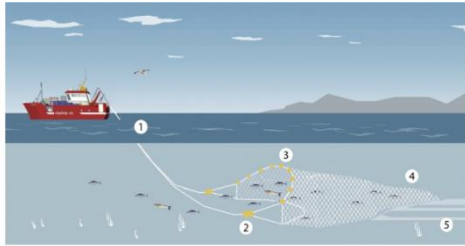
Capturing fish can be done actively or passively.

2.1.2 Active fishing methods

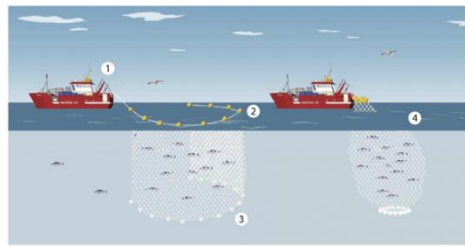
Active capture techniques involve the capture of fishes or other aquatic animals by trapping or encirclement gears that are actively moved by humans or machines while the organisms are being captured. It is based on the aimed chase of the species (Hubert et al., 2012). Principal active methods include mid-water and bottom trawling, purse seining and dredging (Fig.2.2).



1. Gates keep the net open 2. Floats 3. Weights 4. Fish collect in the cod end



1. Tow ropes 2. Gates keep the mouth of the net open 3. Floats 4. The cod end where the fish collect 5. Trawl marks left from the weights dragging over the sea floor



1. The fishing vessel surrounds a school of fish 2. Floats 3. Weights 4. The bottom of the net is brought together and then hauled on-board



1. Scallops and clams are harvested using dredges 2. Steel teeth comb through the sea bed 3. Fins direct the dredge 4. Dredges can cause damage to sea floor habitats.

Mid-water trawling

Schooling pelagic species, squid, shrimp*

No impact on bottom habitats and bottom structures

Risks to sea turtles and some marine mammals

*Can be categorized as coastal and oceanic and coastal. Coastal pelagic species include for example forage fish such as anchovies, sardines, shad and menhaden, oceanic pelagic species include for example larger fish such as swordfish, tuna, mackerel and sharks

Bottom trawling

Bottom and demersal species such as sole, turbot, plaice, halibut, whiting, red hake, dogfish, crab, shrimp, flounder

Removal or damage of sedentary living organisms (e.g. seaweed and coral). Creation of suspended sediments.

Capture and removal from the ecosystem of small sized organisms.

Purse seining

Schooling pelagic species, squid

No impact on the bottom habitat

Bycatch of dolphins, risks to sea turtles and some marine mammals.

Dredging

Shellfish and molluscs such as oysters, scallops, clams, mussels, crabs, sea urchins, sea cucumbers, conch.

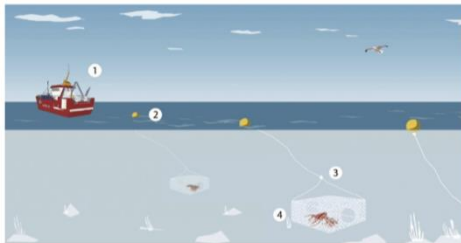
Removal or damage of sedentary living organisms (e.g. seaweed and coral). Creation of suspended sediments.

Impacts on benthic organisms.

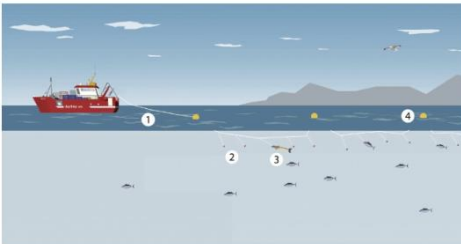
Fig.2.2. Principal active fishing methods and related target species, environmental and species impact (GoodFishBadFish, 2012; NOAA, 2020).

2.1.3 Passive fishing methods

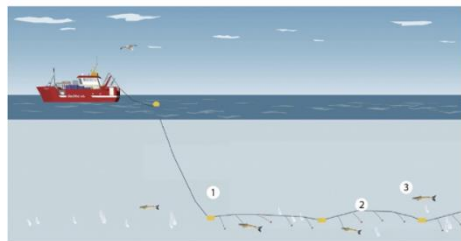
Passive capture techniques involve the capture of fishes or other aquatic animals by entanglement (fishes are ensnared or tangled in the gear), entrapment (fishes enter in an enclosed area through one or more funnel- or V-shaped openings that hinder escape after entrance), or angling gears (fishes are captured with a baited hook and a line) that are not actively moved by humans or machines while the organisms are being captured. The behaviour and movements of the animals themselves result in their capture (Hubert et al., 2012). Principal passive methods include traps and pots, longlining and gillnetting (Fig.2.3).



1. The fishing vessel sets a number of traps 2. Floats indicate the positions of traps 3. The traps are baited with fish scraps 4. Rock-lobsters are among the species captured using traps



1. The main line 2. Baited hooks on branching lines also known as snoods 3. Bycatch can be a problem with longlining 4. Floats



1. Weights 2. Small branching lines with baited hooks known as snoods 3. Species targeted using this method include sharks, halibut and Patagonian toothfish

Traps and Pots

Crustaceans and molluscs such as lobster, crabs, shrimps, octopus, whelk, and all kinds of reef fish (eels, scup, black sea bass) and euryhaline species.

Low negative environmental and seabed impact caught juveniles or undersized species can be released alive.

Longlining

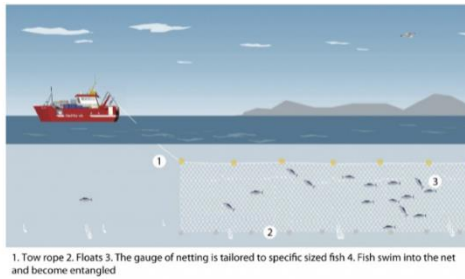
Apex predators (tuna, billfish, sharks and swordfish).

*No impact on the bottom habitat
Bycatch of endangered species and seabirds.*

Bottom longlining

Demersal species such as sharks, halibut, flounder, sole and other groundfish.

*No impact on the bottom habitat
Bycatch of endangered species*



Gillnetting

Pelagic, demersal and benthic species such as salmon, groundfish (cod, haddock, pollock, flounder, hake), barracuda, herring, mullet, rockfish, seabass, shad, sharks, sturgeon, swordfish

Risks to sea turtles and marine mammals.

No impact on the bottom habitat

Fig.2.3. Principal passive fishing methods and related target species, environmental and species impact (GoodFishBadFish, 2012; NOAA, 2020).

2.1.4 Materials science

Until World War II, fishing gears were made of natural fibres such as hemp, cotton, sisal or even wood. The introduction of synthetic fibres at this time and the expansion of the plastic industry led to today's gears all made from synthetic fibres. They present the advantages to resist moisture and can be designed into almost any kind of properties (Oxvig & Hansen, 2007).

Synthetic fibres are made from raw materials based on chemicals or petrochemicals: oil, coal, lime and various salts. Monomers are produced from these products and have the ability in further chemical and physical processes to polymerize in a mass of synthetic materials who will be transformed in fibres in a extrusion process (Oxvig & Hansen, 2007). Synthetic fibres currently dominant in European fishing tools are Polyamide (PA), Polyester (PE), Polyethylene (PET), Polypropylene (PP) and High-density polyethylene (HDPE)(Oxvig & Hansen, 2007).

Their properties are shown in Table 2.1.

Table 2.1. Material properties for synthetic fibres (Oxvig & Hansen, 2007).

**Poor*
***Acceptable*
****Good*
*****Excellent*

Fibres are then joined in large or small numbers and

	Polyethylene	Polypropylene	Polyester	Polyamide	Aramid	HDPE
Shock load	*	***	**	****	*	***
Handling	***	***	****	****	****	****
Durability	**	***	****	****	****	****
Rot, fungal rot, moisture	****	****	****	****	****	****
UV radiation	**	*	****	**	**	**
Acid resistance	****	****	****	***	***	****
Alkaline impact	****	****	****	****	***	****
Wearing resistance	**	**	****	****	*	****
Storage	wet/dry	wet/dry	wet/dry	wet/dry	wet/dry	wet/dry
Buoyancy (density)	barely floats (0.95)	floats (0.90)	sinks (1.38)	sinks (1.14)	sinks (1.44)	floats (0.97)
Melting point	approx. 128 °C	approx. 150 °C	approx. 245 °C	approx. 250 °C	approx. 427 °C	approx. 147 °C

spun into a yarn. These thin yarns are further spun and braided or twisted, one or more times, into thicker lines and ropes who are knotted and form fishing nets (Fig.2.4) (Oxvig & Hansen, 2007).

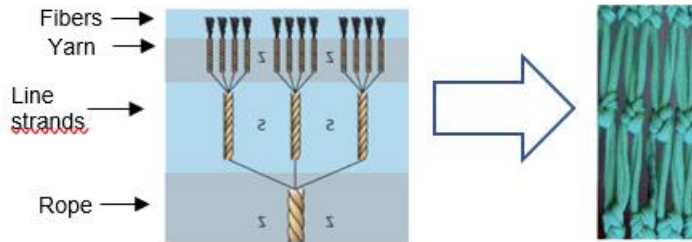


Fig.2.4. Creation of fishing nets (Oxvig & Hansen, 2007).

2.2 Ghost gears

Ghost gear refers to any abandoned, lost or otherwise discarded fishing gear (ALDFG) in the marine environment. It is difficult to measure the exact proportion of ALDFG in marine litter, nevertheless some large-scale quantification attempts could show an estimation. According to the FAO, at least 640.000 tons of fishing gears are lost each year and account for 10 % of all marine debris (FAO, 2018). Land-based sources are the predominant cause of marine debris in coastal zones while merchant chipping is responsible offshore (Macfadyen et al., 2009). ALDFG phenomenon is not new, it has existed since the start of fishing and undergoes a perpetual growth because of exponential levels of fishing capacities and activities in our oceans. This was not a problem when gears were made of biodegradable materials. Today, most of them are made out of plastics, taking more than 600 years before they dissolve (MSC, 2018).

2.2.1 Sources of ALDFG

Ghost gears are the result of three actions; the abandon (deliberate non-retrieval), the discard (deliberate disposal at sea) and the loss (accidental). Non-exhaustive potential sources for each of these three phenomena are listed in Fig.2.5.

Fig.2.5. Sources of ALDFG (Macfadyen et al., 2009).

Abandoned	IUU fishing (Illegal, Unregulated, Uncontrolled). <i>Gears abandoned from illegal fishermen when the risks of detection appear.</i> Illegal Gears <i>Abandon of illegal gears when risks of detection appear.</i> Too much gears and lack of time <i>Gears abandoned when it would need too much time or difficulties for fishermen to remove from waters.</i>
Discarded	Too much gears for space <i>If too much gears are placed in the water, there may be not enough place on the ship to take them all and some are deliberately discarded (ex: space intended for gears is filled with fresh fishes).</i> Chosen over onshore disposal <i>Most of the gears have limited lifetime. Fishermen discard them when it is more practical or economical than onshore disposal.</i> Damaged gear <i>Fishermen discard damaged gears for practical and economic reasons.</i>
Lost	Gear conflict <i>Gears enter in contact with passive vessels (merchant shipping) and get lost or active gears enter zones where passive gears are placed.</i> Defective gears <i>Misconceived gears or used gears (old gears) increase the risk of breakage and tear which lead to losing the product.</i> Poor and extreme weather <i>Bad weather conditions or strong currents cause a high loss of gears.</i> Neglected fishing methods <i>Errors and unconsidered methods from fishermen such as introducing passive gears in high snagging-risk zones, leaving gears anchored for too long making them to move and get lost.</i> Snagging & damage <i>Gears hang on marine elements and organisms who break gears and hold them back.</i>

2.2.2 Impacts of ALDFG

The impacts of ALDFG is dependent on the vulnerability and the sensitivity of the specific environment, therefore correlating an impact to a specific gear is difficult. (Macfadyen, Huntington & Cappell, 2009). Principal impacts described below remain global and cannot be attributed to a specific type of gear.

Ghost fishing, a major concern related to ALDFG, consists of the capture or entanglement of organisms in gears after they have been lost, discarded or abandoned (Fig.2.6) (Macfadyen et al., 2009). Those unmonitored captures lead to the death of sea creatures indiscriminately. It represents a serious threat for a variety of non-target, especially protected species. Non endangered species are also affected by a depletion of the population and a reduction of fisheries' sustainability and economic viability (Macfadyen et al., 2009). Ghost fishing will continue perpetually until the gear loses its functionality. Decomposition of captured marine organisms serves as bait and attracts other organisms hereby also trapped, and the cycle continues. It is a phenomenon of automated re-bait and increasing ghost fishing. Furthermore, certain species are attracted by living in the gear, creating an increasing abundance of organisms, adding even more to the vicious circle and its efficiency (Baeta et al., 2009; Matsuoka et al., 2005; Scheld et al., 2016; Stelfox et al., 2016).

The capacity of ALDFG for ghost fishing is highly specific to gear type and conditions under which it was abandoned, lost or discarded. It also depends on the nature of the local environment, especially in terms of currents, depth and location (Macfadyen, Huntington & Cappell, 2009).

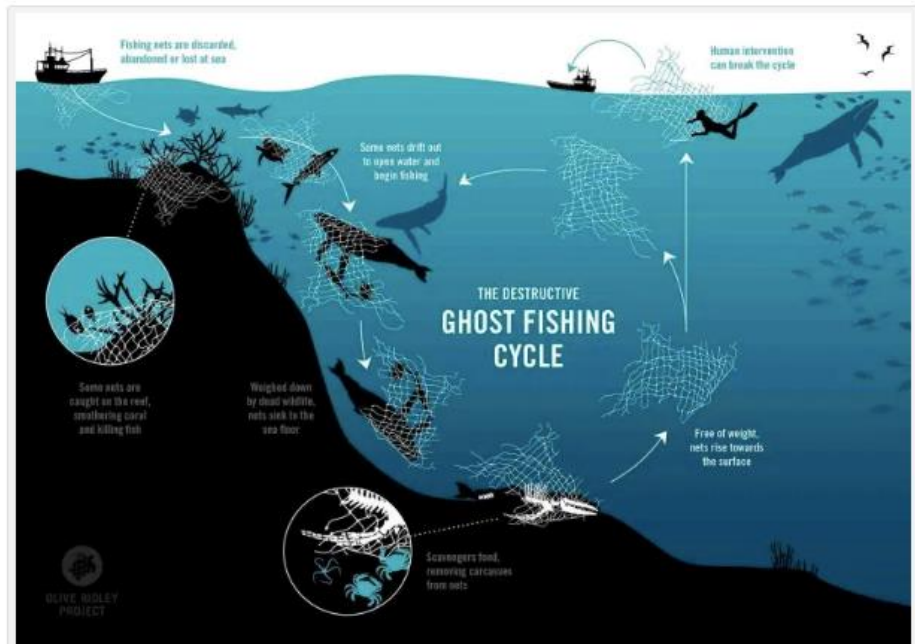


Fig.2.6. Ghost fishing cycle (Stelfox et al., 2016).

Other serious ghost gears threat include:

- Damaging habitat. When gears are at the bottom and dragged by currents, they can scour and abrade the seabed and associated communities, including sensitive habitats like coastal seagrass beds, coral reefs, benthic habitats and sponge fields. Besides damaging the critical habitat itself, the gear may be located in places where it poses a hazard to wildlife, including in foraging areas, fish spawning grounds, turtle nesting areas and migration routes (FAO, 2015).
- Navigational hazards (Macfadyen et al., 2009).
- Vector for invasive species. ALDFG and other marine debris transport invasive alien species, which can disrupt community structure and processes (FAO, 2015)
- Introduction of synthetic material into the marine food web. Plastic-made fishing nets can release microplastics that fishes will

ingest due to their continuous uptake of water, contributing to their introduction in the food chain (Macfadyen et al., 2009).

- Loss of amenity and disruption to enjoyment of beaches and coastal areas (Macfadyen et al., 2009).
- Additional costs resulting from fouling vessels and other gear (Macfadyen et al., 2009).
- Entrap fine sediment, smothering benthic communities, and can obstruct waterflow, creating anoxic areas, which can cause substantial mortalities (FAO, 2015).
- Accumulation as mass concentrations in pelagic ecosystems at ocean convergence zones for extended periods of time, potentially altering community structure and processes (FAO, 2015).

2.3 Ocean pollutants

The United Nations Joint Group of Experts on the Scientific Aspects of Marine Pollution defines marine pollution as the: "Introduction by man, directly or indirectly, of substances or energy into the marine environment (including estuaries) resulting in such deleterious effects as harm to living resources, hazards to human health, hindrance to marine activities including fishing, impairment of quality for use of sea water and reduction of amenities." (GESAMP, 1991)

2.3.1 Overview of pollutants

Whether it is via atmospheric transport, runoff into waterways or direct disposal, a variety of chemical releases reaches and threatens our oceans every single day (Lloyd-Smith & Immig, 2018). Ocean pollutants include persistent organic pollutants (POPs), endocrine disrupting chemicals (EDCs), metals, pesticides, pharmaceuticals, oil as well as other industrial and agricultural pollutants (Table 2.2).

Table 2.2. Global marine pollutants and related impacts on marine environment (Lloyd-Smith & Immig, 2018).

POPs – Persistent Organic Pollutants.

EDCs – Endocrine Disrupting Chemicals.

Pollutants	Description	Impacts on marine environment
POPs	Toxic carbon-based compounds. Persistent. Long-range transport capacity.	Health problems including certain cancers, birth defects, dysfunctional immune and reproductive systems, greater susceptibility to disease, diminished intelligence and death.
EDCs	Exogenous substance interfering with the endocrine system.	Affect reproduction, metabolism, development and immune function of wildlife. Role in the worldwide loss of species and reduced population numbers of amphibians, mammals, birds, reptiles, freshwater and marine fishes and invertebrates.
Metals	Ubiquitous endocrine disrupting contaminant. Pb, Zn, Cd, Zn, Cu, Mn, Fe, Hg, As, Ba.	Similar characteristics as POPs in terms of toxicity, persistence, bioaccumulation and capacity for long-range transport. Poisoning.
Pesticides	Ingredients in pesticides used today are toxic to marine organisms, including the active constituents; the formulating chemicals like surfactants; and the impurities and metabolites.	Cause death, cancers, tumours and lesions, reproductive inhibition and failure, suppression of the immune system, disruption of the endocrine system, and cellular and DNA damage. Behavioural changes that can alter animal survivability, and changes in population dynamics and/or ecosystem imbalance.
Pharmaceuticals	Wastewater treatment systems are not designed to remove pharmaceutical residues, many of these are hereby released into the marine environment. Different pharmaceutical agents are in the marine environment, including antibiotics, nonsteroidal anti-inflammatory drugs, analgesics, lipid lowering drugs, estrogens, and drugs from other therapeutic groups.	Adverse impacts on the physiology and behaviour of organisms even at low concentrations, exacerbated by chronic, long-term exposure.
Oil	Most conspicuous forms of marine pollution.	Oil spills cause mass mortality and contamination of fish and wildlife. Disruption of the marine food chain and species population decline.

Although it is never easy to determine with certainty the specific origin of every pollutant, some activities and phenomenon are obviously contributing to ocean pollution and toxicity.

Industries such as manufacturing, waste incineration, coal fired power stations and fossil fuel production release tons of hazardous emissions into the atmosphere every year (Lloyd-Smith & Immig, 2018). Many of these pollutants potentially end up in our oceans through atmospheric deposition.

Fuels combustion (transport sector, factories, smelters,...) produce/release hydrocarbons that potentially end up in our oceans through atmospheric deposition (Lloyd-Smith & Immig, 2018).

Rivers watersheds facilitate the transport of terrestrial sediments, organic carbon, nitrogen, metals, oil, pesticides, sewage, plastic wastes and various other industrial waste and contaminants into the oceans (Lloyd-Smith & Immig, 2018).

Tanker disasters and operation, urban-based runoff, natural oil seeps and operational discharge of fuel from boating traffic and port operations contribute to oil pollution (Lloyd-Smith & Immig, 2018).

2.3.2 Polycyclic Aromatic Hydrocarbons

Crude oil is a fossil fuel made naturally from the slow transformation remains of prehistoric plants and animals. It is a complex mixture of thousands of compounds, predominantly hydrocarbons (compounds consisting of only carbon and hydrogen, between 65 and 95% of its composition) (Marchand, 2017). Main compounds of crude oil are shown in Fig.2.7.

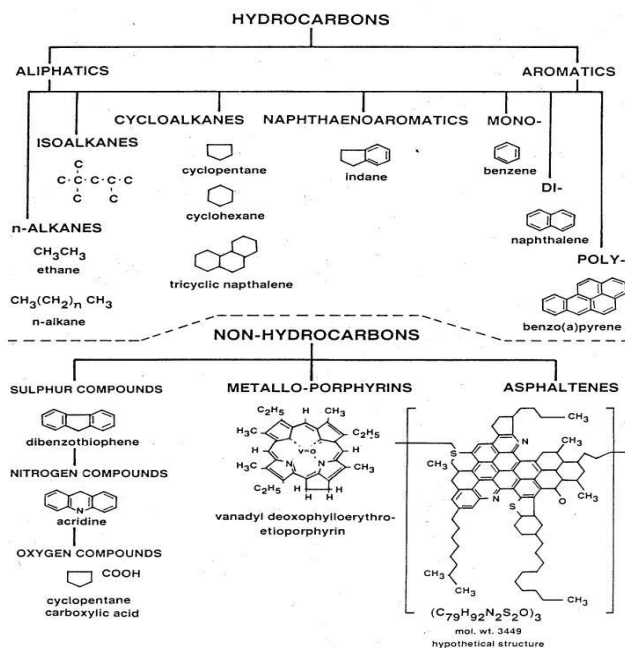


Fig.2.7. Main compounds of crude oil (Marchand, 2017).

One group of compounds found in crude oil are PAHs. They are composed of two or more fused aromatics (benzene rings) and a hydrophobic and lipophilic double bonds system throughout their hydrocarbon rings (Alegbeleye et al., 2017). In the environment, they can be of pyrogenic and petrogenic origin (Wang et al., 2008). Pyrogenic PAHs are formed by incomplete combustion of organic material such as the burning of fossil fuel or municipal solid waste incineration (anthropogenic sources), volcanic eruptions, forest fires, or exudates from trees (natural sources) and can end up in our oceans through atmospheric deposition (Xu et al., 2006). Petrogenic PAHs are present in oil and some oil products and can be found in the marine environment due to accidental release (oil spills, pipeline leaks and tanker accidents) and industrial activities and agriculture activities runoff (Tavakoly Sany et al., 2014).

PAHs present a serious threat because of their toxicity (mutagenic, tumorigenic and carcinogenic properties) and tendency to bioaccumulate because they are poorly soluble in water but very soluble in oil and fat. The solubility of PAHs in water decreases with an increase of molecular weight and therefore they tend to be adsorbed to the surface of the sediment (Abdel-Shafy & Mansour, 2016; Honda & Suzuki, 2020). The Environment Protection Agency (EPA) evaluated 16 priority PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, benzo(ghi)perylene, dibenzo(a,h)anthracene) among the 126 priority pollutants considering them priority PAHs (Table 2.3) (Pazos et al., 2010).

Table 2.3. Properties and chemical structures of the 16 EPA PAHs (Pazos et al., 2010).

Name	CAS	Chemical structure	Molecular weight (g/mol)	Boiling point (°C)	Melting point (°C)	Water solubility (mg/L)	Vapor pressure (mmHg)	Partition coefficient, logK _{ow}	Henry's law constant, H (atm·m ³ /mol)
Acenaphthene	82-32-9		154.21	277	95	1.93	4.47 × 10 ⁻³	3.98	7.91 × 10 ⁻⁵
Acenaphthylene	208-96-8		152.2	265-275	92-93	3.93	0.029	4.07	1.45 × 10 ⁻³
Anthracene	120-12-7		178.2	342-340	218	0.0076	1.7 × 10 ⁻⁵	4.45	1.77 × 10 ⁻⁵
Benzo(a)anthracene	56-55-3		228.29	435	162	0.010	2.2 × 10 ⁻⁸	5.61	1 × 10 ⁻⁶
Benzo(a)pyrene	50-32-8		252.3	495	179-179.3	0.0023	5.6 × 10 ⁻⁹	6.06	4.9 × 10 ⁻⁷
Benzo(b)fluoranthene	205-99-2		252.3	481	168.3	0.00125 × 10 ⁻⁷	6.04	1.22 × 10 ⁻⁵	
Benzo(ghi)perylene	191-24-2		276.34	550	273	0.00026	1.3 × 10 ⁻¹⁰	6.50	1.44 × 10 ⁻⁷
Benzo(k)fluoranthene ^a	207-08-9		252.31	481	215-217	-	-	-	-
Chrysene	218-01-9		228.3	448	255-256	0.0028	6.3 × 10 ⁻⁷	5.16	1.05 × 10 ⁻⁶
Dibenzo(a,h)anthracene	53-70-3		278.35	524	262	0.0005 × 10 ⁻¹⁰	6.84	7.3 × 10 ⁻⁸	

Name	CAS	Chemical structure	Molecular weight (g/mol)	Boiling point (°C)	Melting point (°C)	Water solubility (mg/L)	Vapor pressure (mmHg)	Partition coefficient, logK _{ow}	Henry's law constant, H (atm·m ³ /mol)
Fluoranthene	206-44-0		202.36	375	111	0.20-0.26	5.0 × 10 ⁻⁶	4.90	6.5 × 10 ⁻⁶
Fluorene	86-73-7		166.2	295	116-117	1.68-1.98	5.0 × 10 ⁻⁶	4.90	6.5 × 10 ⁻⁶
Indeno(1,2,3-cd)pyrene	193-39-5		276.3	530	163.6	0.062	10 ⁻¹¹ -10 ⁻⁶	6.58	6.95 × 10 ⁻⁸
Naphthalene ^a	91-20-3		128.17	218	80.5	-	-	3.37	-
Phenanthrene	85-01-8		178.2	340	100	1.20	6.8 × 10 ⁻⁶	4.45	2.56 × 10 ⁻⁵
Pyrene	129-00-0		202.3	393	156	0.077	2.5 × 10 ⁻⁶	4.88	1.14 × 10 ⁻⁵

2.4 PAHs analysis

The three different approaches of this study have required the analysis for afterwards quantification of PAHs in a large number of samples. These analyses were achieved by gas chromatography – mass spectrometry (GC-MS) with solid-phase microextraction (SPME) (Fig.2.8).

Gas chromatography separates the components of a mixture, and mass spectroscopy characterizes each of the components individually, through their mass. By combining the two techniques, qualitative and quantitative evaluation of chemicals in a solution are achieved.

Before GC analysis, samples need to be prepared (by SPME in this study) in order to isolate volatile and semi-volatile substances and prevent the presence of ionic or high molecular weight species to be injected into the GC. The outgoing extracts are volatile mixtures suitable to GC-MS analysis (Stashenko & Martínez, 2014).

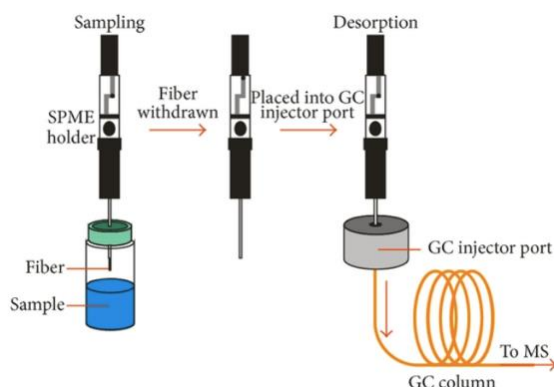


Fig.2.8. Diagram of analysis with solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) (Schmidt & Podmore, 2015).

2.4.1 Solid-Phase Microextraction

In SPME, a fused-silica fibre is coated with a polymer. This polymer will extract the analytes from the sample. The fibre may be placed above the sample (liquid or solid) or immersed in the sample (liquid). The extraction will continue until the chemical potential of each substance in all phases is equal. The fibre is then placed into the GC injector port where analytes

in gaseous form are injected for further analysis (Stashenko & Martínez, 2014).

2.4.2 Gas Chromatography

Analytes extracted from the fibres are injected into a mobile phase in the GC, which is an inert gas such as helium. This gas transports analytes through a stationary phase contained in a tube (column) where compounds in the mobile phase interact with that stationary phase each at a different rate. The fastest interacting compounds elute first from the column, the slowest exit last from it. With this process, different mixtures of chemicals can be separated by changing characteristics of the mobile and stationary phase (SRIF, 1998)

Exiting compounds enter a detector, creating a signal that increases with increasing concentrations and decreases with lower concentrations. In the meantime, a software generates a chromatogram. X-axis shows the retention time (time from when injection is made to when compounds are exiting the column) and Y-axis shows the intensity (abundance). All compounds of the sample are hereby separated and will be identified with MS (Fig.2.9)(SRIF, 1998).

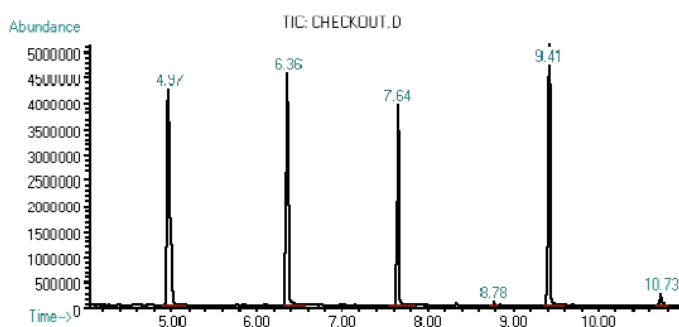


Fig.2.9. Example of a chromatogram generated by a GC (SRIF, 1998).

2.4.3 Mass Spectrometry

Each compound exits the GC column and enters the electron ionization detector (MS) where a stream of electrons breaks them into fragments. The mass to charge ratio (m/z) is obtained for each compound. It corresponds of the mass of the fragment divided by the charge. Since fragments have a charge of +1, the ratio represents the molecular weight of the fragment. Ions created are concentrated into the ion trap where they undergo manipulations to be finally ejected from the trap and detected. The detector creates a signal while the computer generates a mass spectrum, the x-axis shows the mass to charge ratio, and the y-axis shows the abundance (Fig.2.10) (SRIF, 1998).

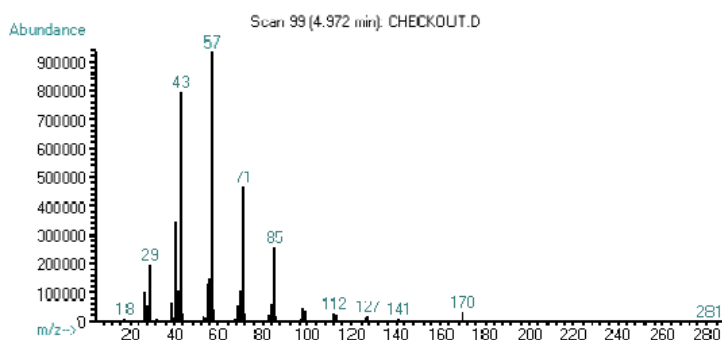


Fig.2.10. Example of a mass spectrum generated by a MS (SRIF, 1998).

This mass spectrum corresponds to a fingerprint for a molecule, it is every time the same for a given chemical compound. The computer contains a library of spectra and is able to identify the chemical from the sample mixture (SRIF, 1998).

The combination SPME, GC and MS allows to take an organic solution, inject it in the machine, separate the individual components and identify each of them. Calibration curves and standard addition methods are subsequently achieved to determine the concentrations of each compound (Stashenko & Martínez, 2014).

3. Material and Methods

3.1. Materials

Table 3.1 describes all equipments, materials and solutions/compounds used during the study for PAHs analysis and their related function.

Table 3.1. Equipments, materials and solutions/compounds used in the study.

Equipment	Function	Type
Oven	Ovens for high-forced volume thermal convection applications. These ovens generally provide uniform temperatures throughout to dry solid samples.	Manufacturer: HERAEUS Model: D-6450 HANAU
Vortex mixer	Device allowing to mix small vials of liquid.	Manufacturer: VWR Model: Analog Vortex mixer 444-2790
Ultrasound bath	Metallic container filled with water through which ultrasound is generated. It allows to extract PAHs from sediment samples by acoustic cavitation phenomenon.	Manufacturer: ELMA Model: Transsonic analogous ultrasonic unit T 460/H
Shaking bath	Equipment to regularly shake and mix samples while maintaining a constant temperature. Temperature and speed range are programmable.	Manufacturer: J.P. SELECTA Model: Unitronic reciprocating shaking bath
Centrifuge	Laboratory device driven by a motor and working by the sedimentation principle used for the separation of solids, fluids, gases or liquids, based on the density.	Manufacturer: J.P. SELECTA Model: Centrifuge Electronic digital control "MIXTASEL-BL"
Lyophilizer	Equipment used to remove water from liquid, solid or pasty samples without disrupting its structure, also known as freeze-drying.	Manufacturer: CHRIST Model: ALPHA 1-4
Gas Chromatograph – Mass spectrometer	See Point 2.4	Manufacturer: Varian Model: Saturn 2000 GC / MS / MS
Material	Description	
Micropipettes	Volumes: 2-20 µL, 20-100 µL, 50-200 µL, 200-1000 µL, 1000-5000 µL, 1-10 mL	

Vials	20 mL Precision Thread Headspace-Vial, 75,5 x 22,5 mm, amber glass, 1st hydrolytic class, rounded bottom (for magnetic screw caps)
Caps	18 mm Magnetic Universal Screw Cap, silver, centre hole, Silicone transparent blue/PTFE white, 45° shore A, 1,3 mm.
Analytical balance	Precision: 0,01 g
Solution/Compound	Description
Florisil	Magnesia-loaded silica powder used to trap polar impurities within non-polar matrices.
Internal standard	Chemical substance added in a constant amount to samples, the blank and calibration standards. A mixture of 5 deuterated internal standards composed of: Naphthalene d8, Acenaphthene d10, Phenanthrene d10, Chrysene d12 and Perylene d12 with a concentration of 2 mg/L
External standard	Chemical substance added to calibration standards. A mixture of the 16 priority PAHs with a concentration of 10 mg/L
Deionized water	Water whose ions were removed and with no charge.
Methanol	Solvent for extraction of PAHs from sediments.

3.2 Analytical procedures

3.2.1 Extraction of PAHs from sediments

To determine PAHs in sediments, they were first extracted with the following methodology.

One day before the procedure, the flask of Florisil is left in the oven at 200 °C. Right before the procedure, the flask is cooled, weighted and 2% of water is added.

1. Weighing 1g of sediment previously dried by lyophilization in a 20 mL vial.
2. Adding 5 mL of methanol.
3. Vortex for 30 s and extracting in the ultrasound for 30 min.
4. Centrifuging samples during 5 min at 2500 rpm.

5. Transferring the obtained extract with a pipette to a similar vial containing 0.3 g of Florisil (careful not to transfer any sediment).
6. Agitating during 10 min at 50 u.
7. Centrifuging samples during 5 min at 2500 rpm.
8. Transferring the obtained extract with a pipette to a new vial (careful not to transfer any Florisil) and kept in the freezer until analysis.

3.2.2 Calibration curve

To successfully quantify PAHs concentration in samples, the external calibration curve method was used.

The CC method involves the creation of a curve (line) with the corresponding equation:

$$y = mx + b$$

(x-axis: concentration, y-axis: equipment response, m : slope, b : intercept).

The aqueous PAHs standard solutions for this calibration curve were prepared using PAHs standard stock solution of 10 and 100 µg/L

Abundance signals obtained for specific PAHs in the study samples with an unknown concentration are subsequently implemented in the curve, making it possible to calculate concentrations.

The addition of internal standards was used to improve the precision of the analysis and to determine the concentration of PAHs of interest by calculating the response factor. Calibration and analysis were always performed with the addition of 5 internal standards (deuterated PAHs) according to the following grouping:

- Naphthalene d8 → Naphthalene
- Acenaphthene d10 → Acenaphthylene, Acenaphthene, Fluorene
- Phenanthrene d10 → Phenanthrene, Anthracene
- Chrysene d12 → Chrysene, Benzo(a)anthracene, Pyrene, Fluoranthene

This grouping was chosen as mass to charge ratio of internal standards is similar to the one of the corresponding PAH (Table 3.2).

Table 3.2. Mass to charge ratios of 10 priority PAHs and their related IS.

Compound	m/z	Compound	m/z
Phenanthrene d10	188	Naphthalene d8	136
Phenanthrene	178	Naphthalene	128
Anthracene	178	Chrysene d12	240
Acenaphthene d10	164	Chrysene	228
Acenaphthylene	152	Benz(a)anthracene	228
Acenaphthene	154	Pyrene	202
Fluorene	166	Fluoranthene	202

Table 3.3 shows volumes of water, internal standard solution and external PAHs standard solution added to each vial to elaborate a calibration curve.

Table 3.3. Procedure for elaboration of calibration curves.

It shows volumes of water (V. of water), internal standard solution (I.S.) and external PAHs standard solution (P10 and P100) added in vials to obtain the desired PAHs concentration (C.).
I.S. is a mixture of 5 deuterated internal standards composed of: Naphthalene d8, Acenaphthene d10, Phenanthrene d10, Chrysene d12 and Perylene d12 with a concentration of 2 mg/L.
P10 is a mixture of the 16 priority PAHs with a concentration of 10 µg/L.
P100 is a mixture of the 16 priority PAHs with a concentration of 100 µg/L.

C. (mg/L)	V. of water (mL)	I.S. (µL)	P10 (µL)	P100 (µL)
0	10	20	/	
10	10	20	10	
20	10	20	20	
50	10	20	50	
100	10	20	100	
200	10	20		20

The principle remains the same for higher PAHs concentrations.

3.2.3 Samples preparation for analysis

Table 3.4 resumes the volumes of sample, water, internal standard solution and external PAHs standard solution to add in each vial to prepare the samples for GC-MS analysis.

Doped samples with a known concentration of PAHs, by adding a known amount of an external PAHs standard solution, were used for an afterward precision evaluation of GC-MS analysis.

Table 3.4. Procedure of samples preparation for analysis.

It shows volumes of sample (V. of sample, volume of extract solution), water (V. of water), internal standard solution (I.S.) and external PAHs standard solution (P10) added in vials to prepare the samples for GC-MS analysis.

I.S. is a mixture of 5 deuterated internal standards composed of: Naphthalene d8, Acenaphthene d10, Phenanthrene d10, Chrysene d12 and Perylene d12 with a concentration of 2 mg/L.

P10 is a mixture of the 16 priority PAHs with a concentration of 10 µg/L.

Doped samples with a known concentration of PAHs, by adding a known amount of an external PAHs standard solution, were used for an afterward precision evaluation of GC-MS analysis.

	V. of sample	V of water (mL)	I.S. (µL)	P10 (µL)
Sediment samples	180µL	10	20	/
Doped sediment samples	180µL	10	20	20
Water samples	10mL	/	20	/
Doped water samples	10mL	/	20	20

3.2.4 Analysis and Calculations

3.2.4.1 Calibration curve

First step: calculation of the areas

After analysis with GC-MS, areas (of signals) for each internal standard and for each PAH were obtained as showed as an example in Table 3.5.

Table 3.5. Example of areas of the signals obtained for each internal standard and for each PAH with GC-MS analysis.

	PAHs concentrations (ng/L)						
	0	10	20	50	100	200	500
Compounds	Area of the signal						
Naphthalene d8	972087	792001	970487	953294	958836	1057000	1039000
Naphthalene	874	1818	3093	6987	12963	32560	90461
Acenaphthene d10	2021000	1714000	1939000	1683000	1958000	2095000	2045000
Acenaphthylene	0	2141	3084	6127	16889	45126	136582
Acenaphthene	0	2342	5006	11309	28880	71666	187363
Fluorene	0	3994	5590	12735	42364	103781	276664
Phenanthrene d10	6243000	5770000	6246000	4419000	6483000	6139000	6380000
Phenanthrene	2870	6225	11046	17572	68194	165020	501514
Anthracene	0	5049	10455	14542	74621	173343	579346
Chrysene d12	1284000	2255000	1752000	594186	1520000	1194000	1106000
Chrysene	955	2241	1798	1976	4471	13382	34387
Benz(a)anthracene	1628	2594	3562	2165	4359	8670	45463
Pyrene	1005	3938	6620	9324	41370	72214	272155
Fluoranthene	2751	7199	9489	12302	60758	107406	377103

Areas for individual PAHs were then corrected through response factor of its related internal standard: $\text{Corrected}_{\text{Area}} = \text{Area}_{(\text{PAH})} / \text{Area}_{(\text{corresp.I.S})}$

Second step: Calculation of curves

For each PAH, a curve was elaborated with the concentration as x-axis and areas of the individual signal as y-axis, calculated as mentioned above. An example is shown in Fig.3.1.

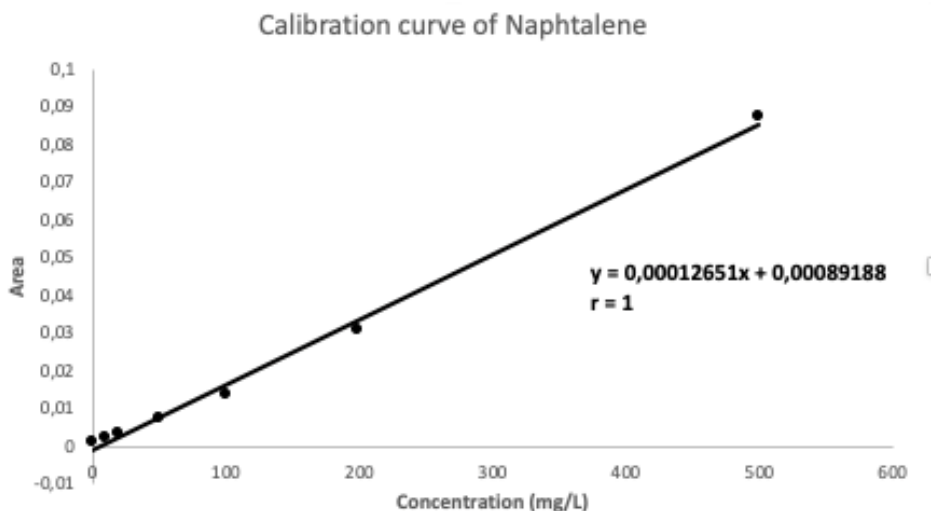


Fig.3.1. Example of a calibration curve for Naphtalene.

Third step: calculation of detection and quantification limits

Detection limit (LoD) is the lowest analyte concentration at which detection is feasible.

Quantification limit (LoQ) is the lowest analyte concentration at which quantification is feasible.

These limits were calculated with the signal to noise ratio (S/N) given by GC-MS as shown in Fig.3.2.

$$LoD_{(ng/L)} = \left(\frac{\text{Lowest Conc. detected}}{\text{Height of detected peak}} \right) \times (3 \times S/N \text{ ratio})$$

$$LoQ_{(ng/L)} = DOL \times 3,3$$

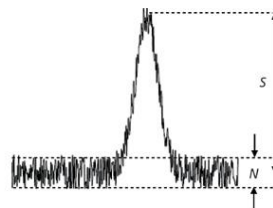


Fig.3.2. Representation of peak height (S) and noise (N) from a chromatogram.

3.2.4.2 Concentration of PAHs in water samples

After analysis with GC-MS, areas for internal standards and each PAH were obtained and corrected as explained previously. With calibration

curves (lines) previously determined for each PAH, concentrations of individual PAHs in samples were calculated with:

$$C_{\left(\frac{ng}{L}\right)} = \frac{Corrected\ area_{PAH} - b_{PAH}}{m_{PAH}}$$

b = Intercept

m = slope

3.2.4.3 Concentration of PAHs in sediment samples

The same principle was applied to determine PAHs concentration in sediment samples. The following conversion was done to obtain the concentration in terms of mass of sediments:

$$C_{\left(\frac{ng\ of\ PAH}{g\ of\ sediment}\right)} = \frac{(C_{(ng/L)} \times V_{vial}) \times \left(\frac{V_{sample}}{V_{methanol}}\right)}{m_{sediments}}$$

3.2.5 Quality control

Following requirements were established to ensure a quality analysis:

- 1) Before starting a worklist, detector was calibrated in the mass range.
- 2) Calibration curves were adjusted with an $r > 0.995$.
- 3) A blank was analysed after calibration and after each series of 10 samples. In case of very contaminated samples, a blank was inserted to avoid the effect of carryover on fibre.
- 4) Replicate samples (n=3) were achieved for each sample.
- 5) One sample per series of 10 was doped to calculate and verify recoveries. Necessary corrections were made if the range 75-125 % was not achieved.

3.3 First approach: Environmental monitoring of lost gear hotspots

3.3.1 Goal and methodology

The first approach consisted in the study of two hotspots on the North West Portuguese coast where ALDFG can be found. Water and sediment samples were collected at different locations and distances from the hotspots and analysed in the lab in terms of PAHs concentration. The main objective was to evaluate if ALDFG could act as a sink of PAHs in the closest zones. Samples were also collected at different times of the year to take into account environmental seasonality.

3.3.2 Study sites and sampling

3.3.2.1 Matosinhos hotspot

About 3 km and 30 m deep offshore from the Matosinhos coast (district of Porto, Portugal), lies the wreck of the German submarine U1277 of World War II (Fig. 3.3). The crew of the war vessel decided to open the valves and sink it on 3rd of June 1945 to prevent it from landing into allies' hands after Germany's capitulation.

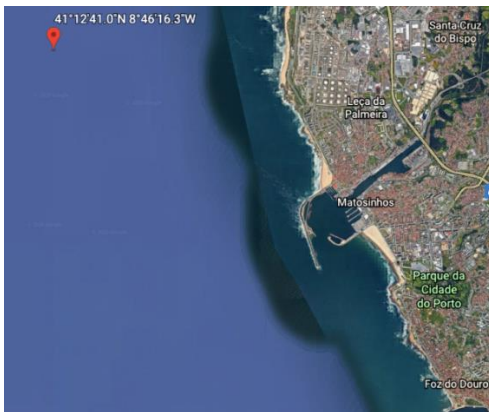


Fig.3.3. Location of the submarine. Latitude: 41.211396 °, Longitude: -8.771197°, depth :30 m (Google Earth).

The wreck remained quite intact after all this time, colonized by different marine species, and became a spot for biodiversity as well as a hotspot for lost fishing gears that get stuck in the metal frame. Some of the gears are in contact with sediments (Fig. 3.4).



Fig.3.4. Submarine wreck and fishing gears stuck in the metal frame (photos from Submersus diving school).

Figure 3.5 shows water and sediments collection zones and number of replicates by sample types. Three zones were studied, two near the submarine wreck (A: torpedo zone, B: periscope zone) and one 50 m far from the submarine, used as control zone (C). It was intended to have three replicates but only Two replicates for water and sediment were collected in zone A , the third one was lost due to constrains in the collection. Three replicates were collected in zone B and C for both sediment and water. Sampling was done by the recreational Submersus diving school divers in spring (16/06/2019) and summer (19/09/2019) in the same conditions. However, sediment was not collected in the summer sampling campaign due to logistic constrains. Water samples (ca. 50 mL)

were collected into glass flasks and kept at -20°C until analysis as described in point 3.2. Sediment samples were collected into plastic bags. In the lab, aliquots of sediment not in contact with the plastic bags were stored in aluminium foil and kept at -20°C.

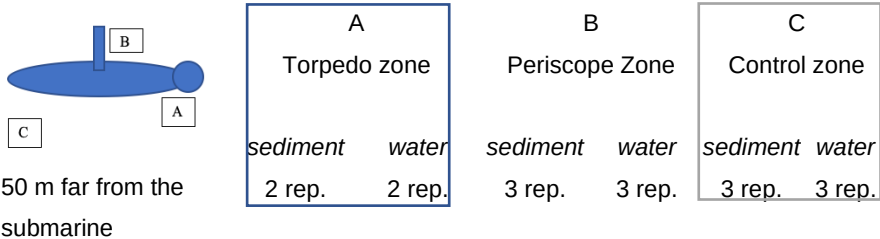


Fig.3.5. Sampling conditions for Matosinhos hotspot.

3.3.2.2 Esposende hotspot

This hotspot is located in a coastal marine protected area, Parque Natural do Litoral Norte, along the Esposende coast (district of Braga, Portugal) that contains a natural rocky bottom area with several reefs, Cavalos de Fão. Lost fishing gears are found in this area. Fishing nets are either floating or are attached to the rocky reefs, without contact with sediments. Figure 3.6 shows the location of the ALDFG hotspot, water and sediments collection zones and dates. One replicate for each sampling point was collected. Water samples were collected at 6 locations in the area where lost gears are present (sites W1 to W6) while sediment samples were collected at one locations in this area (sites S1 to S3). An area without lost gears was selected as control, and water samples were collected at 3 locations (sites W7 to W9) while sediment samples were collected at one location (sites S4 to S6 in this area). Water samples (ca. 50 mL) were collected into glass flasks and kept at -20°C until analysis as described in point 3.2. Sediment samples were collected through a Van Veen grab and kept in plastic bags. In the lab, aliquots of sediment not in contact with the plastic bags were stored in aluminium foil and kept at -20°C. Three sampling campaigns were carried out in Winter (04/01/2019), Spring

(21/03/2019) and Summer (26/06/2019). The lack of good weather and navigability conditions did not allowed doing an Autumn sampling campaign. Sediment samples were only collected during winter and summer campaigns, as sediments tend to have less seasonal variations. Sampling was carried out by researchers of CIIMAR.

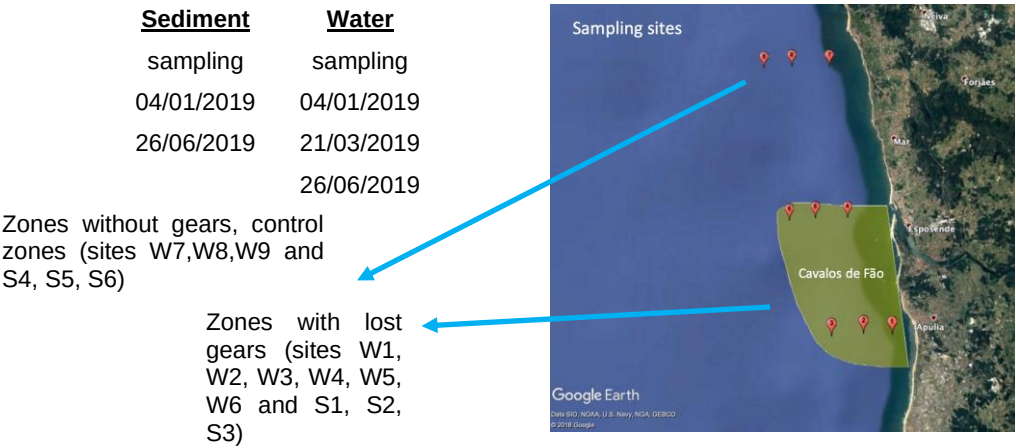


Fig.3.6. Sampling sites at the Esposende hotspot. Sampling sites at the Esposende hotspot. Sites at Cavalos de Fão with lost gears (6 sites for water collection and 3 sites for sediments) and sites at the control zone without lost gears (3 sites for water and 3 sites for sediments).

3.4 Second approach: PAHs adsorption experiments

The second approach consisted in controlled laboratory experiments to evaluate the potential adsorption of PAHs on new plastic fishing nets. Experiments were carried out in seawater doped with a selected concentration of PAHs, under agitation and in the dark.

The ocean is divided into three zones based on depth and light level; euphotic (0-200 m), dysphotic (200-1000m) and aphotic (beyond 1000 m) zone (NOAA, 2020). The penetration of sunlight through the ocean decreases rapidly with depth and penetrates rarely beyond euphotic zone (NOAA, 2020). Photodegradation for some PAHs can occur through UV-B radiation from the sunlight effect (Nadal et al., 2006) .As these experiments focused on the adsorption potential of fishing nets, all

samples were placed under a dark cover to avoid degradation of PAHs by light.

Surface currents can reach 250 cm/s maximum, but the speeds of deep currents vary from 2 to 10 cm/s or less (Sanitkov, 2002). As we placed the situation in the dysphotic zone (or beyond) by absence of light, agitation was defined to agree with deep currents speeds. Agitator was calibrated at 80 oscillations/minute which correspond to a speed 4.3 cm/s (stroke length of 32 mm).

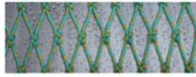
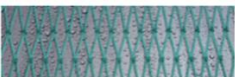
Seawater was doped with a PAHs solution containing the 16 PAHs considered a priority by EPA, resulting in a concentration of 1 µg/L of each PAH (15µL of a 10 mg/L solution were used). This concentration of PAHs was chosen taking in consideration the levels found in contaminated seawater (Bakker et al., 2010).

Additionally, an experiment was conducted to evaluate gears could afterwards release the adsorbed PAHs.

3.4.1 Nets selected

Fishing nets used and their characteristics are described in Table 3.6. Experiments were carried out with four fishing nets with two different polymer compositions and two thicknesses. The fishing nets were supplied by Euronete, a manufacturer of fishing gear, and partner of the NetTAG project.

Table 3.6. Fishing nets and their characteristics.

Characteristic	Net			
	1	2	3	4
Mesh				
Material	High Tenacity Polyethylene fibres (HTPE)	Polyethylene fibres (PE)	Nylon fibres	Nylon fibres
Rope assembly	Braided	Twisted	Twisted	Monofilament
Diameter (mm)	3	1.8	2.3	0.3
Mesh size (mm)	150	100	53	200

3.4.2 Methodology

Adsorption

The same methodology, described below, was applied for the four different nets with only one replicate per net type. The net presenting highest adsorption rate (see Results section) was afterward selected for a second experiment in triplicate using also the same methodology.

Figure 3.7 shows the experimental scheme. Seawater was collected at Matosinhos beach. Pieces of net per flask included 2 plugs and 1 knot. Weights of each net in each flask are shown in Table 3.7. Flasks were placed on the agitator at a speed of 80 oscillations/minute under a dark cover and 10 mL of sample were taken over time at define periods in corresponding flasks (so that no significant volume changes occurred). Collected samples were kept at -20°C before analysis as described in point 3.2. The difference between Flask 6 with no nets and Flask 1-5 in terms of PAHs concentration allowed to establish the adsorption percentage over time. Adsorption percentage at a given time period was calculated using the following equation:

$$\text{Adsorption percentage} = \left(\frac{C_{\text{solution without net}} - C_{\text{solution with net}}}{C_{\text{solution without net}}} - C_{\text{blank}} \right) \times 100$$

- $C_{\text{solution with net}}$ = PAH concentration found in water of corresponding flasks with net (Flask 1 – 5)
- $C_{\text{solution without net}}$ = PAH concentration found in water of corresponding flask without net (Flask 6)
- C_{blank} = PAH concentration found in water of corresponding flask with net, without PAHs solution added (Flask 7)

This calculation takes in consideration the natural degradation of PAHs in water over time, allowing to obtain the percentage of PAHs adsorbed on the nets.

In parallel, experiments with one metal (Cu or Pb) and PAHs were carried out with one of the net (Net 1) to understand whether the presence of both compounds (organic and inorganic) alters PAHs adsorption behaviour. The metal concentration was $50 \mu\text{g L}^{-1}$.



Flask 1	Flask 2	Flask 3	Flask 4	Flask 5	Flask 6	Flask 7
150 mL SW	150 mL SW	150 mL SW	150 mL SW	150 mL SW	150 mL SW	150 mL SW
PN	PN	PN	PN	PN	15 μL SOL	PN
15 μL SOL	15 μL SOL	15 μL SOL	15 μL SOL	15 μL SOL	Each time	Each time
T1	T2	T3	T4	T5		
T6	T7	T8	T9			

Fig.3.7. Experiment scheme with flasks compounds and related sample times.

SW – seawater

PN – piece of net

SOL - solution containing the 16 priority PAHs at a concentration of 10 mg/L each used to dope the seawater. Final concentration in seawater of 1 $\mu\text{g/L}$ for each PAH

T1 (30 min), T2 (1 h), T3 (2h), T4 (4h,) T5 (8h), T6 (24h,) T7 (48h,) T8, (1 week) T9 (2 weeks) are sampling times in corresponding flasks

Flask 6 and 7 – Control flasks

Table 3.7. Weights of nets (g) placed in corresponding flasks.

Flask	Net			
	1	2	3	4
1	0.74	0.48	2.93	0.04
2	0.71	0.49	2.78	0.03
3	0.76	0.51	2.77	0.04
4	0.75	0.50	2.86	0.02
5	0.72	0.48	2.86	0.02
6	No net	No net	No net	No net
7	0.73	0.52	2.92	0.03

Release

An experiment was carried out with the net pieces collected at the end of the adsorption experiment to evaluate if net pieces could afterwards release adsorbed PAHs when placed in a PAHs free medium.

Seawater collected at Matosinhos beach, was placed in new flasks adding the pieces of net from the triplicate adsorption experiment. Flasks were again placed on the agitator at a speed of 80 oscillations/minute under a dark cover and 10 mL of sample were taken over time at the same periods as before in corresponding flasks. Collected samples were kept at -20°C until analysis as described in point 3.2. PAHs analysis in these samples allowed to determine the potential PAHs release. Results are expressed in percentage of PAH released, derived from the adsorbed amount in the adsorption experiments and were calculated using the following equation:

$$PAH \text{ release} = \frac{C_{\text{adsorbed after stabilization}}}{C_{\text{released}}}$$

3.5 Third approach: in situ experiments

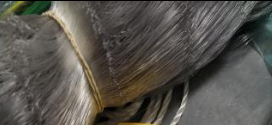
The third approach consisted in the evaluation of the potential adsorption of PAHs on new fishing nets in quasi-real conditions. Leixões marina (Matosinhos, Portugal) was chosen as the site to place the new fishing nets in the water.

The main purpose of this experiment was to monitor a possible increase of PAHs concentration over time in the water where nets were placed and evaluate if plastic fishing nets could potentially adsorb water PAHs, acting as a sink of PAHs in the area where nets were placed.

3.5.1 Nets selected

Three fishing nets used in the previous laboratory adsorption experiments were selected. Their characteristics are described in Table 3.8.

Table 3.8. Fishing nets and their characteristics.

Characteristic	Net		
	A	B	C
Visual			
Material	Polyethylene fibres (PE)	Nylon fibres	Nylon fibres
Rope Assembly	Twisted	Twisted	Monofilament
Diameter (mm)	1.8	2.3	0.3
Mesh size (mm)	55	53	200

3.5.2 Methodology

Experiment preparation

Fig.3.8 shows the experimental assay. A rope of about 9 m was attached on the ground to the wooden dock of the marina. Large pieces of net were cut and kept transversally open with a rope on the top, and connected with a stone at the bottom, allowing a longitudinal stretching. Top ropes of each net were attached to the fixed dock rope and nets were placed in the water. Fig.3.9 illustrates the experiment assembly in situ.

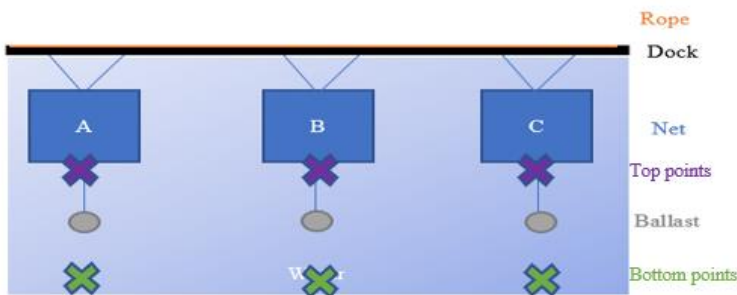


Fig.3.8. Schematic of in-Situ experiment.

A = Twisted Polyethylene fibres net

B = Twisted Nylon fibres net

C = Monofilament Nylon fibres net



Fig.3.9. Preparation of in situ experiment.

Sampling

The assembly was carried out by researchers of CIIMAR on 04/02/2020. Four monthly sampling campaigns were carried out: in February (07/02/2020), March (09/03/2020), May (18/05/2020) and July (22/07/2020). In each sampling campaign, water was collected with a water bottle and 100 mL was transfer into glass flasks. Water was collect near each net. At each location water was collected at surface and bottom

(below the net), in a total of 6 samples per sampling campaign (see Fig. 3.8). Bottom samples were used as control. In the last sampling campaign water was also collected at surface and bottom in the marina at a site without nets and used for supplementary control. As the February sampling campaign occurred 3 days after nets were placed, this sampling is stated in the results as showing initial PAHs levels at the different locations (T0). Water samples were kept at -20°C before analysis as described in section 3.2. Pieces of each net were cut in each sampling campaign and maintained at -20°C until analysis to evaluate possible PAHs adsorption. To evaluate PAHs adsorbed to the fishing nets, the collected pieces of nets were placed in flask with methanol (ca. 10 ml), vortex 1 min and subjected to a 15 min ultrasonic extraction, similarly to sediment extraction. Each extract was analysed following sediment analysis protocol. A similar extraction procedure was done to pieces of net not exposed to marina water to be used as a blank. Results are expressed in level of PAH adsorbed and were calculated using the following equation:

$$PAH_{\text{adsorbed}} = C_{\text{net marina}} - C_{\text{blank net}}$$

3.6 Data analysis

The three different approaches generated several datasets that were analysed for various purposes. Results were presented by the mean and respective standard deviation.

In the first approach, two hotspots of lost gears were chosen as sampling sites with sub-samples being collected at various distances from these hotspots. For each sampling location, three replicates were analysed in the lab in terms of PAHs concentration for both water and sediments. Mean was calculated to obtain the overall trend of each location. Standard deviation was calculated to identify the possible presence of outliers. Mean levels were compared to quantification and detection limits

of the GC-MS and to Maximum Permissible concentrations (MPCs). MPC refers to the concentration in an environmental compartment at which no effect to be rated as negative is to be expected for ecosystems (Verbruggen, 2012). Unpaired statistical t-tests were used to determine if the difference between control zones and zones with lost gears was significant and if differences among sampling campaigns were significant. The null hypothesis (H_0) stated if there were no statistically significant differences between zones or campaigns for each PAH. P-values were calculated for a confidence level of 95 %.

In the second approach, 4 nets were chosen as sample for the determination of adsorption percentage of individual PAHs. Only one replicate was preliminary achieved for the selection of the net with higher adsorption percentage. Three replicates were achieved for selected net for both adsorption and release evaluation. Mean was calculated to obtain the overall trend over time. Standard deviation was calculated to identify the possible presence of outliers, who were rejected.

In the third approach, 3 nets were chosen as sample for the evaluation of adsorption of individual PAHs. Only one replicate was achieved.

4. Results and discussion

4.1 First approach – Environmental monitoring of lost gear hotspots

4.1.1 Water from Matosinhos lost gear hotspot

Levels of PAHs for each water sample from Matosinhos Hotspot are shown in Table 4.1. Results showed that:

- 1) Levels of Acenaphthene, Acenaphthylene, Fluorene, Anthracene, Fluoranthene, Pyrene and Chrysene were always below LoD or LoQ.
- 2) Levels of Naphthalene and Phenanthrene quantified were low, being lower than the maximum permissible concentrations (Table 4.5).
- 3) Levels of Benzo(a)anthracene quantified were high, being almost 10 times higher than the maximum permissible concentrations (Table 4.5) at each detected zone. It was detected in similar levels at locations with lost gears (A1-B3) and control locations (C1-C3), therefore the nets showed no significant influence on Benzo(a)anthracene levels.
- 4) For other PAHs, locations with lost fishing nets (A1-B3) showed no significant differences with zones without lost fishing nets (C1-C3).
- 5) Globally, there was no significant difference between sampling campaigns.

Table 4.1. Levels of polycyclic aromatic hydrocarbons (PAHs (ng/L), mean $\pm$ standard deviation, $n = 3$) in water collected in Matosinhos lost gear hotspot.

*LoD, **LoQ

Analysis being done at different times results in different values for LoD and LoQ.

Sampling		Compounds									
Sites		Nap	Ace	Acn	Fl	Phe	Ant	Fluo	Py	BaA	Ch
A1	First sampling	<1*	<3*	<4*	<2*	<2**	<1*	<3**	<3*	<3*	<2*
	Second sampling	<5**	<3*	<4*	<2*	2.0 $\pm$ 0.6	<1*	<1*	<3*	<9**	<2*
A2	First sampling	<1*	<3*	<4*	<2*	<2**	<1*	<1*	<3*	<9**	<2*
	Second sampling	<5**	<3*	<4*	<2*	3.6 $\pm$ 0.4	<1*	<1*	<3*	11 $\pm$ 3	<2*
B1	First sampling	18 $\pm$ 4	<3*	<4*	<2*	2.1 $\pm$ 0.4	<1*	<1*	<3*	<9**	<2*
	Second sampling	<5**	<3*	<4*	<2*	2.7 $\pm$ 0.3	<1*	<1*	<3*	11 $\pm$ 2	<2*
B2	First sampling	17 $\pm$ 2	<3*	<4*	<2*	3.1 $\pm$ 0.2	<1*	<1*	<3*	<3*	<2*
	Second sampling	<5**	<3*	<4*	<2*	<2**	<1*	<1*	<3*	<9**	<2*
B3	First sampling	11 $\pm$ 5	<3*	<4*	<2*	2.4 $\pm$ 0.4	<1*	<3**	<3*	<3*	<2*
	Second sampling	<5**	<3*	<4*	<2*	2.6 $\pm$ 0.3	<1*	<1*	<3*	<9**	<2*
C1	First sampling	<5**	<3*	<4*	<2*	<2**	<1*	<3**	<3*	<9**	<2*
	Second sampling	<5**	<3*	<4*	<2*	3.2 $\pm$ 0.7	<1*	<1*	<3*	<9**	<2*
C2	First sampling	7.1 $\pm$ 0.6	<3*	<4*	<2*	<2**	<1*	<3**	<3*	<3*	<2*
	Second sampling	<5**	<3*	<4*	<2*	4 $\pm$ 1	<1*	<1*	<3*	11 $\pm$ 3	<2*
C3	First sampling	<1*	<3*	<4*	<2*	<2**	<1*	<3**	<3*	14.9 $\pm$ 0.1	<2*
	Second sampling	5 $\pm$ 2	<3*	<4*	<2*	2.7 $\pm$ 0.3	<1*	<1*	<3*	<9**	<2*

4.1.2 Sediments from Matosinhos lost gear hotspot

Levels of PAHs for each sediment sample from Matosinhos hotspot are shown in Table 4.2. No sediments were collected in the second sampling campaign due to logistic constrains.

Results showed that:

- 1) Levels of Acenaphthene, Acenaphthylene, Fluorene, Anthracene, Fluoranthene, Pyrene, Benzo(a)anthracene and Chrysene were always below LoQ or LoD.
- 2) Levels of Naphthalene and Phenanthrene quantified were low, being lower than the maximum permissible concentrations (Table 4.5).
- 3) Locations with lost fishing nets (A1-B3) showed no significant differences with zones without lost fishing nets (C1-C3).

*Table 4.2. Levels of polycyclic aromatic hydrocarbons (PAHs (ng/g), mean $\pm$ standard deviation, n = 3) in sediments collected in Matosinhos lost gear hotspot.
*LoD, **LoQ*

Sampling Sites	Compounds									
	Nap	Ace	Acn	Fl	Phe	Ant	Fluo	Py	BaA	Ch
A1	<0.6*	<1*	<1*	<0.8*	<0.8*	<0.7*	<2*	<0.8*	<2*	<2*
A2	<0.6*	<1*	<1*	<0.8*	<0.8*	<0.7*	<2*	<0.8*	<2*	<2*
B1	<0.6*	<1*	<1*	<0.8*	<0.8*	<0.7*	<2*	<0.8*	<2*	<2*
B2	2.4 $\pm$ 0.3	<1*	<1*	<0.8*	<0.8*	<0.7*	<2*	<0.8*	<2*	<2*
B3	2.6 $\pm$ 0.6	<1*	<1*	<0.8*	<0.8*	<0.7*	<2*	<0.8*	<2*	<2*

C1	<2**	<1*	<1*	<0.8*	<0.8*	<0.7*	<2*	<0.8*	<2*	<2*
C2	2.4 ± 0.2	<1*	<1*	<0.8*	2 ± 1	<0.7*	<2*	<3**	<2*	<2*

4.1.3 Water from Esposende lost gear hotspot

Levels of PAHs for each water sample collected at Esposende lost gear hotspot are shown in Table 4.3.

Results showed that:

- 1) Levels of Acenaphthene, Acenaphthylene, Fluorene, Anthracene, Pyrene, Benzo(a)anthracene and Chrysene were always below LoQ or LoD.
- 2) Levels of Naphthalene, Phenanthrene and Fluoranthene quantified were low, being lower than the maximum permissible concentrations (Table 4.5).
- 3) Locations with lost fishing nets (W1-W6) showed no significant differences with zones without lost fishing nets (W7-W9).
- 4) There was no significant difference between sampling campaigns.

Table 4.3. Levels of polycyclic aromatic hydrocarbons (PAHs (ng/L), mean ± standard deviation, n = 3) in water collected in Esposende lost gear hotspot.

*LoD , **LoQ

Analysis being done at different times results in different values for LoD and LoQ.

Zones with lost fishing nets (sites W1, W2, W3, W4, W5, W6)

Zones without lost fishing nets (sites W7, W8, W9).

Zones without fish fishing nets (sites W1, W2, W3):											
Sampling		Compounds									
Sites		Nap	Ace	Acn	Fl	Phe	Ant	Fluo	Py	BaA	Ch
W1	First sampling	<1.2**	<0.3*	<0.5*	<0.4*	<0.2*	<0.2*	<0.5*	<0.6*	<0.3*	<0.4*
	Second sampling	<0.4*	<0.3*	<0.5*	<0.4*	<0.2*	<0.2*	<0.5*	<0.6*	<0.3*	<0.4*
	Third sampling	<2.1*	<4.3*	<3.7*	<2.7*	<2.7*	<2.2*	<6*	<3*	<6*	<8.6*

W2	First sampling	<1.2**	<0.3*	<0.5*	<0.4*	<0.2*	<0.2*	<0.5*	<0.6*	<0.3*	<0.4*
	Second sampling	<0.4*	<0.3*	<0.5*	<0.4*	<0.2*	<0.2*	<0.5*	<0.6*	<0.3*	<0.4*
	Third sampling	<0.7*	<0.9*	<1.1*	<0.7*	2.1 ± 0.2	<0.3*	<1*	<1*	<9.1**	<1.9*
W3	First sampling	<1.2**	<0.3*	<0.5*	<0.4*	<0.2*	<0.2*	<0.5*	<0.6*	<0.3*	<0.4*
	Second sampling	<0.4*	<0.3*	<0.5*	<0.4*	5 ± 1	<0.2*	<0.5*	<0.6*	<0.3*	<0.4*
	Third sampling	<2.4**	<0.9*	<1.1*	<0.7*	1.9 ± 0.3	<0.3*	<1*	<1*	<9.1**	<1.9*
W4	First sampling	<1.2**	<0.3*	<0.5*	<0.4*	<0.7**	<0.2*	<1.6**	<0.6*	<0.3*	<0.4*
	Second sampling	<0.4*	<0.3*	<0.5*	<0.4*	<0.2*	<0.2*	<0.5*	<0.6*	<0.3*	<0.4*
	Third sampling	<2.1*	<4.3*	<3.7*	<2.7*	<2.7*	<2.3*	<6*	<3*	<6*	<8.6*
W5	First sampling	<1.2**	<0.3*	<0.5*	<0.4*	<0.2*	<0.2*	1.9 ± 0.9	<0.6*	<0.3*	<0.4*
	Second sampling	<0.4*	<0.3*	<0.5*	<0.4*	<0.2*	<0.2*	<0.5*	<0.6*	<0.3*	<0.4*
	Third sampling	<2.1*	<4.3*	<3.7*	<2.7*	<2.7*	<2.3*	<6*	<3*	<6*	<8.6*
W6	First sampling	<1.2**	<0.3*	<0.5*	<0.4*	<0.2*	<0.2*	<1.6**	<0.6*	<0.3*	<0.4*
	Second sampling	<0.4*	<0.3*	<0.5*	<0.4*	<0.2*	<0.2*	<0.5*	<0.6*	<0.3*	<0.4*
	Third sampling	<2.1*	<4.3*	<3.7*	<2.7*	<2.7*	<2.3*	<6*	<3*	<6*	<8.6*
W7	First sampling	2 ± 2	<0.3*	<0.5*	<0.4*	<0.2*	<0.2*	<1.6**	<0.6*	<0.3*	<0.4*
	Second sampling	<0.4*	<0.3*	<0.5*	<0.4*	<0.2*	<0.2*	<0.5*	<0.6*	<0.3*	<0.4*
	Third sampling	2.8 ± 0.7	<0.9*	<1.1*	<0.7*	2.1 ± 0.4	<0.3*	<1*	<1*	<9.1**	<1.9*
W8	First sampling	<0.4*	<0.3*	<0.5*	<0.4*	<0.2*	<0.2*	<1.6**	<0.6*	<0.3*	<0.4*
	Second sampling	<0.4*	<0.3*	<0.5*	<0.4*	<0.2*	<0.2*	<0.5*	<0.6*	<0.3*	<0.4*
	Third sampling	2 ± 1	<0.9*	<1.1*	<0.7*	<1.6**	<0.3*	<1*	<1*	<9.1**	<1.9*
W9	First sampling	<1.2**	<0.3*	<0.5*	<0.4*	<0.2*	<0.2*	2.2 ± 0.7	<0.6*	<0.3*	<0.4*
	Second sampling	<0.4*	<0.3*	<0.5*	<0.4*	<0.2*	<0.2*	<0.5*	<0.6*	<0.3*	<0.4*
	Third sampling	3 ± 3	<0.9*	<1.1*	<0.7*	<1.6**	<0.3*	<1*	<1*	<9.1**	<1.9*

4.1.4 Sediments from Esposende lost gear hotspot

Levels of PAHs for each sediment sample collected in Esposende lost gear hotspot are shown in Table 4.4.

Results showed that:

- 1) Levels of Acenaphthene, Acenaphthylene, Fluorene, Anthracene, Benzo(a)anthracene) and Chrysene were always below LoQ or LoD.
- 2) Levels of Naphthalene, Phenanthrene, Fluoranthene and Pyrene quantified were low, being lower than the maximum permissible concentrations (Table 4.5).
- 3) Locations with lost lost fishing nets (S1-S3) showed no significant differences with zones without lost fishing nets (S4-S6).
- 4) There was no significant difference between sampling campaigns.

Table 4.4. Levels of polycyclic aromatic hydrocarbons (PAHs (ng/g), mean $\pm$ standard deviation, n = 3) in sediments collected in Esposende lost gear hotspot.

*LoD, **LoQ

Analysis being done at different times resulted in different values for LoD and LoQ.

Zones with lost gears (sites S1, S2, S3).

Zones without gears, control zones (sites S4, S5, S6).

Sampling		Compounds									
Sites		Nap	Ace	Acn	Fl	Phe	Ant	Fluo	Py	BaA	Ch
S1	First sampling	2.1 $\pm$ 0.2	<0.3*	<0.3*	<0.2*	3.4 $\pm$ 0.9	<0.1*	1.9 $\pm$ 0.8	2.3 $\pm$ 0.9	<2.5**	<1.8**
	Second sampling	<0.6*	<1.2*	<1*	<0.8*	<2.5**	<0.6*	<1.7*	<0.8*	<1.7*	<2.4*
S2	First sampling	2.2 $\pm$ 0.6	<0.3*	<0.3*	<0.2*	2.7 $\pm$ 0.6	<0.1*	<0.3*	1.4 $\pm$ 0.7	<0.8*	<0.5*
	Second sampling	<0.6*	<1.2*	<1*	<0.8*	<0.8*	<0.6*	<1.7*	<0.8*	<1.7*	<2.4*
S3	First sampling	1.2 $\pm$ 0.3	<0.1*	<0.1*	<0.1*	<0.1*	<0.1*	<0.1*	<0.2*	<0.1*	<0.1*
	Second sampling	<0.6*	<1.2*	<1*	<0.8*	<0.8*	<0.6*	<1.7*	<0.8*	<1.7*	<2.4*
S4	First sampling	2 $\pm$ 1	<0.1*	<0.1*	<0.1*	5 $\pm$ 3	<0.1*	7 $\pm$ 1	5 $\pm$ 3	<0.1*	<0.1*
	Second sampling	3.6 $\pm$ 0.5	<1.2*	<1*	<0.8*	1.8 $\pm$ 0.7	<0.6*	<1.7*	<0.8*	<1.7*	<2.4*

S5	First sampling	1.3 ± 0.6	<0.1*	<0.1*	<0.1*	<0.1*	<0.1*	<0.1*	<0.2*	<0.1*	<0.1*
	Second sampling	3.0 ± 0.1	<1.2*	<1*	<0.8*	<2.5**	<0.6*	<1.7*	<0.8*	<1.7*	<2.4*
S6	First sampling	1.8 ± 0.3	<0.3*	<0.3*	<0.2*	5 ± 1	<0.1*	3 ± 1	4 ± 1	<0.8*	<0.5*
	Second sampling	<1.9**	<1.2*	<1*	<0.8*	<0.8*	<0.6*	<1.7*	<0.8*	<1.7*	<2.4*

4.1.5 General discussion

In both hotspots, levels of PAHs, except Benzo(a)anthracene in water of Matosinhos lost gears hotspot, were low, being in general below detection and quantification limits, and substantially lower than Maximum Permissible Concentrations in marine water and sediment, according to the study done by Verbruggen, for those PAHs quantified (Table 4.5).

Table 4.5. Maximum Permissible Concentration for each PAH (Verbruggen, 2012).

Compounds	MPC _{marine water} (µg/L)	MPC _{marine sediment} (µg/g)
Naphthalene	2.0	0.16
Acenaphthene	0.13	0.017
Acenaphthylene	0.38	0.10
Fluorene	0.30	0.17
Phenanthrene	1.1	0.78
Anthracene	0.10	0.0047
Fluoranthene	0.12	4.11
Pyrene	0.023	0.84
Benzo(a)anthracene	0.0012	0.04
Chrysene	0.0070	0.16

Accordingly, both hotspots were classified as weakly polluted by these pollutants, except waters of Matosinhos lost gears hotspot that were highly polluted by Benzo(a)anthracene at some sampling locations and campaigns. As our site is located 3km offshore and 30 m depth, the source and irregularity of Benzo(a)anthracene levels throughout campaigns and at different sites are unknown. Although levels were lower, this accounts also for Naphthalene and Phenanthrene levels detected in water of Matosinhos hotspot.

Water from Esposende hotspot is close to the coast and more exposed to surface and wind-driven currents, carrying hereby potential contaminants with them, which could explain the irregularity in levels detected throughout campaigns and locations.

Levels in sediments, which tend to be more stable, could have been a better indicator of areas contamination. Considering levels were extremely low in sediments of both hotspots, they can be classified as weakly polluted.

Globally, even for detected PAHs in the study, there was no difference between zones with lost gears and zones without gears. Therefore, it can be concluded that the presence of lost nets showed no significant influence on PAHs levels, probably due to the low levels of these pollutants in the two hotspots.

As the influence of ghost gears could not be proved in those areas, attention must be paid on the weak hotspots contamination. A previous study revealed that sediments and water of two Atlantic beaches (Fig. 4.1, S1 and S2) from Porto city, a coastal area few km south of our study area, were contaminated by PAHs (Rocha et al., 2017).

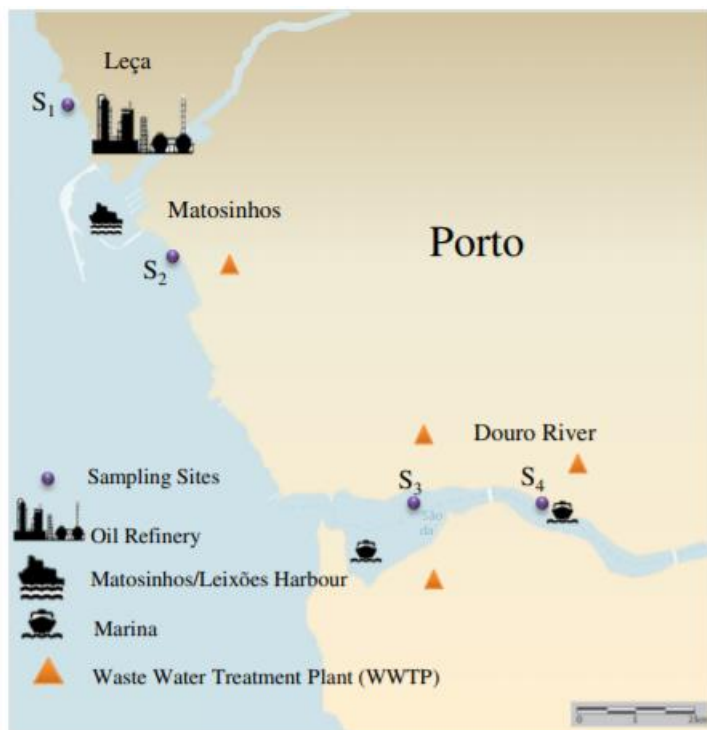


Fig.4.1. Map of study sites. S1 (41° 13' 06.8" N 8° 42' 57.9" W) and S2 (41° 10' 23.5" N 8° 41' 25.9" W) (figure from Rocha et al., 2017).

In Rocha et al (2017) study, Naphthalene, Phenanthrene, Pyrene, Benzo(a)anthracene and Chrysene were detected in water at S1 and all 16 PAHs, with the exception of Fluorene, were detected in water at S2. Levels of Benzo(a)anthracene in both sites were also above MPCs for marine water. All PAHs were detected in sediments of both sites with levels generally above MPCs for marine sediment. Accordingly, both beaches can be classified as highly polluted by PAHs. Such contamination was associated with the intense maritime traffic associated with the Leixões Harbour, the oil refinery and to the important urban centres nearby these beaches.

Our site is located 3 km offshore and 30 m deep, so probably no impact by the coastal contamination sources (Leixões harbour and oil refinery) occurred.

Esposende hotspot could have showed higher concentrations of PAHs since it was closer to the coast and can be more exposed to anthropogenic derived contamination. However, the low maritime traffic associated with the low industry and density area probably resulted in the observed poor contamination levels. Such low levels of contamination support the fact that this hotspot is located in a marine protected area. In fact, Esposende beaches hold blue flags and detected PAHs levels in this study agree with this.

4.2 Second approach – laboratory PAHs adsorption experiments

4.2.1 Preliminary experiments

Tables 4.6 to 4.9 show PAHs adsorption over time to four different fishing nets. Only one replicate was prepared in those studies to select the net with higher adsorption potential.

Levels of PAHs in control vessels (flasks without PAHs, with a piece of net) were always either not detected or below quantification limit.

Results for the first net (braided polyethylene fibres net) showed that PAHs adsorption in general increased until 48h, although there was only a small increase after 24h. For the heavier hydrocarbons adsorption percentages stabilised after 48h, but for lighter PAHs it was not possible to evaluate adsorptions as PAHs degradation occurred over time. In fact, after 48h light PAHs concentrations in flasks without nets were very low. Therefore, following adsorption experiments were only done until 48h of contact.

Net 2 (Twisted Polyethylene fibres net) showed also PAHs adsorption but rates at first sampling did not increase regularly with PAHs molecular weight, with lower and more irregular adsorption rates after stabilization

for heaviest hydrocarbons compared to net 1. Net 3 (Twisted Nylon Fibres net) showed an adsorption behaviour similar to that of net 1 (Braided Polyethylene fibres net), with high adsorption percentages for each PAH since first sampling until stabilization. Net 4 (Monofilament Nylon fibre net) showed the lower adsorption percentages, with extremely variable adsorption evolution over time for each PAH.

Table 4.10 compares the PAHs adsorption behaviour of the nets tested. Globally, it shows that adsorption occurred for all nets and that adsorption percentages increased with molecular weight. Heavier hydrocarbons showed higher adsorption percentage since the first sampling and until stabilization.

The two first nets are made of the same polymer (Polyethylene). The other two nets were made of Nylon. Nylon showed higher adsorption rates but one parameter should also be taken into account, the diameter and therefore the volume of the net fibres used for our experiments. Net 4 fibres have a diameter of only 0.3 mm, the weight of net placed in vials was much lower than that of other nets, adsorption being lower. Net 2 fibres have a diameter smaller than those of net 1 (respectively 1.8 mm and 3 mm) and the adsorption percentages were lower. Net 1 fibres have a bigger diameter than those of net 3 but showed lower PAHs adsorptions. Results indicate that adsorption efficiency is dependent of two parameters, the net polymer and the thickness of the net fibres, that has an impact on the volume of net used for the experiment.

Table 4.6. PAHs adsorption percentage over time (% , n = 1) to Net 1 (Braided Polyethylene fibres net).

PAH	Collection times								
	0.5 h	1 h	2 h	4 h	8 h	24 h	48 h	1 w	2 w
Nap.	0%	6%	10%	20%	11%	23%	25%	0%	0%
Ace.	1%	10%	11%	25%	23%	43%	40%	0%	3%
Acn.	3%	11%	12%	32%	32%	54%	57%	64%	66%
Fl.	12%	21%	23%	42%	49%	70%	63%	0%	21%
Phe.	13%	27%	32%	53%	67%	81%	89%	0%	0%
Ant.	18%	36%	41%	63%	78%	88%	91%	1%	14%
Fluo.	19%	33%	46%	69%	84%	95%	100%	100%	100%
Py.	17%	32%	48%	72%	87%	96%	100%	100%	100%
BaA.	31%	52%	61%	80%	92%	95%	97%	96%	71%
Ch.	32%	50%	59%	82%	96%	98%	100%	99%	96%

Table 4.7. PAHs adsorption percentage over time (% , n = 1) to Net 2 (Twisted Polyethylene fibres net).

PAH	Collection times						
	0.5 h	1 h	2 h	4 h	8 h	24 h	48 h
Nap.	11%	6%	0%	18%	36%	37%	63%
Ace.	0%	0%	3%	12%	17%	36%	54%
Acn.	0%	2%	10%	17%	21%	43%	60%
Fl.	0%	10%	14%	21%	34%	60%	77%
Phe.	19%	25%	16%	27%	61%	79%	92%
Ant.	22%	27%	26%	39%	70%	84%	89%
Fluo.	14%	33%	37%	44%	67%	83%	82%
Py.	17%	35%	39%	47%	70%	84%	88%
BaA.	12%	28%	47%	65%	54%	76%	75%
Ch.	20%	23%	45%	69%	57%	74%	74%

Table 4.8. PAHs adsorption percentage over time (% , n = 1) to Net 3 (Twisted Nylon fibres net).

PAH	Collection times						
	0.5 h	1 h	2 h	4 h	8 h	24 h	48 h
Nap.	25%	30%	13%	43%	50%	62%	64%
Ace.	17%	40%	31%	52%	69%	78%	80%
Acn.	21%	39%	35%	55%	72%	78%	77%
Fl.	36%	47%	50%	63%	82%	84%	80%
Phe.	48%	64%	72%	84%	91%	92%	91%
Ant.	45%	54%	62%	77%	87%	87%	84%
Fluo.	69%	73%	84%	91%	98%	99%	99%
Py.	67%	74%	83%	91%	97%	98%	99%
BaA.	58%	65%	80%	86%	81%	88%	89%
Ch.	55%	68%	78%	87%	93%	95%	96%

Table 4.9. PAHs adsorption percentage over time (% , n = 1) to Net 4 (Monofilament Nylon fibre net).

PAH	Collection times						
	0.5 h	1 h	2 h	4 h	8 h	24 h	48 h
Nap.	17%	0%	0%	0%	0%	4%	0%
Ace.	0%	6%	0%	0%	49%	0%	0%
Acn.	3%	3%	1%	0%	12%	4%	0%
Fl.	19%	0%	5%	0%	15%	0%	0%
Phe.	14%	2%	4%	3%	26%	23%	27%
Ant.	16%	21%	9%	25%	29%	25%	27%
Fluo.	41%	8%	20%	30%	50%	65%	73%
Py.	40%	35%	44%	31%	51%	70%	77%
BaA.	49%	33%	25%	31%	50%	62%	78%
Ch.	41%	56%	40%	55%	63%	65%	86%

Table 4.10. Comparison of PAHs adsorption behaviour for the 4 different nets tested.

A = adsorption rate at T1 (0.5h).

Low : rate < 15%; Middle : 15 % < rate < 30 %; High : rate > 45%

B = Evolution over time ; +/- : variable ; + : always increasing until stabilization

C = adsorption percentage after stabilisation

PAH	Net 1 Braided Polyethylene fibres			Net 2 Twisted Polyethylene fibres			Net 3 Twisted Nylon fibres			Net 4 Monofilament Nylon fibres		
	A	B	C	A	B	C	A	B	C	A	B	C
Nap.	Low	+/-	25%	Low	+/-	63%	Middle	+/-	64%	Low	+/-	0%
Ace.	Low	+/-	40%	Low	+	54%	Middle	+/-	80%	Low	+/-	0%
Acn.	Low	+	57%	Low	+	60%	Middle	+/-	77%	Low	+/-	0%
Fl.	Low	+/-	63%	Low	+	77%	High	+	80%	Middle	+/-	0%
Phe.	Low	+	89%	Middle	+/-	92%	High	+	91%	Middle	+/-	27%
Ant.	Middle	+	91%	Middle	+	89%	High	+	84%	Middle	+/-	27%
Fluo.	Middle	+	100%	Low	+	82%	High	+	99%	High	+/-	73%
Py.	Middle	+	100%	Middle	+	88%	High	+	99%	High	+/-	77%
BaA.	High	+	97%	Low	+	75%	High	+	89%	High	+/-	78%
Ch.	High	+	100%	Middle	+	74%	High	+	96%	High	+/-	86%

4.2.2 Possible metals interference on PAHs adsorption

Tables 4.11 and 4.12 show PAHs adsorption over time to Net 1 (Braided Polyethylene fibres net) in the presence of Cu (Table 4.11) or Pb (Table 4.12). Table 4.13 compares the PAHs adsorption behaviour in the presence and absence of metals.

Table 4.11. PAHs adsorption percentage over time (% , n = 1) to Net 1 (Braided Polyethylene fibres net) in the presence of Cu.

PAH	Collection times							
	0.5 h	1 h	2 h	4 h	8 h	24 h	48 h	1 w
Nap.	0%	13%	0%	27%	19%	37%	47%	0%
Ace.	0%	0%	0%	24%	30%	50%	90%	19%
Acn.	0%	0%	0%	30%	37%	58%	92%	84%
Fl.	1%	4%	8%	47%	51%	70%	94%	82%
Phe.	9%	28%	34%	67%	70%	86%	90%	-7%
Ant.	16%	32%	37%	75%	75%	88%	90%	76%
Fluo.	19%	35%	63%	77%	73%	86%	91%	90%
Py.	21%	38%	65%	78%	76%	88%	92%	91%
BaA.	35%	51%	75%	84%	88%	89%	91%	94%
Ch.	16%	41%	59%	71%	72%	76%	88%	100%

Table 4.12. PAHs adsorption percentage over time (% , n = 1) to Net 1 (Braided Polyethylene fibres net) in the presence of Pb.

PAH	Collection times							
	0,5 h	1 h	2 h	4 h	8 h	24 h	48 h	1 w
Nap.	9%	6%	22%	-1%	-3%	45%	55%	0%
Ace.	8%	19%	47%	39%	50%	68%	79%	49%
Acn.	9%	20%	50%	43%	55%	72%	83%	75%
Fl.	10%	22%	55%	52%	68%	81%	88%	74%
Phe.	11%	24%	45%	53%	67%	87%	91%	-11%
Ant.	15%	35%	56%	67%	77%	89%	92%	70%
Fluo.	24%	52%	53%	71%	76%	87%	90%	87%
Py.	24%	54%	53%	72%	78%	88%	89%	88%
BaA.	29%	54%	74%	82%	88%	84%	89%	86%
Ch.	21%	53%	71%	82%	85%	83%	87%	84%

Table 4.13. Comparison of PAHs adsorption behaviour for net 1(Braided Polyethylene fibres net) in the presence or absence of Pb or Cu.

A = adsorption rate at T1 (0.5h).

Low : rate < 15%; Middle : 15 % < rate < 30 %; High : rate > 45%

B = Evolution over time

+/- : variable ; + : always increasing until stabilization

C = adsorption percentage after stabilization

PAH	Net 1 Braided Polyethylene fibres			Net 1 + Cu			Net 1 + Pb		
	A	B	C	A	B	C	A	B	C
Nap.	Low	+/-	25%	Low	+/-	47%	Low	+/-	55%
Ace.	Low	+/-	40%	Low	+/-	90%	Low	+/-	79%
Acn.	Low	+	57%	Low	+/-	92%	Low	+/-	83%
Fl.	Low	+/-	63%	Low	+/-	94%	Low	+	88%
Phe.	Low	+	89%	Low	+	90%	Low	+	91%
Ant.	Middle	+	91%	Middle	+	90%	Middle	+	92%
Fluo.	Middle	+	100%	Middle	+	91%	Middle	+	90%
Py.	Middle	+	100%	Middle	+	92%	Middle	+	89%
BaA.	High	+	97%	High	+	91%	Middle	+	89%
Ch.	High	+	100%	Middle	+	88%	Middle	+	87%

Globally, the behaviour of the net in terms of adsorption was the same when Cu and Pb were present. Adsorption percentages were higher for heavier hydrocarbons at first sampling and increased in a similar way to that observed in the absence of metals over time. However, adsorption percentages after stabilization were different in the presence of metals and depended on the PAHs.

Adsorption percentages after stabilization were:

- Higher in the presence of metals for naphthalene, Acenaphthene, Acenaphthylene, Fluorene
- Identical for Phenanthrene and Anthracene

- Slightly lower in the presence of metals for Fluoranthene, Pyrene, Benzo(a)anthracene and Chrysene

Nevertheless, results are only informative in this case and further experiments with additional samples and different pollutant concentrations are needed.

4.2.3 Triplicate adsorption experiment

Adsorption

Twisted Nylon net with 2.3 mm diameter fibres (net 3) showed to be the one with higher PAHs adsorption. A second experiment was then carried out with triplicates to confirm results observed in preliminary experiments (section 4.2.1). The same methodology was applied and results are shown in Table 4.14.

Table 4.14. PAHs adsorption percentage (% , mean $\pm$ standard deviation, $n = 3$) to Net 3 (Twisted Nylon fibres net).

PAH	Collection times						
	0.5 h	1 h	2 h	4 h	8 h	24 h	48 h
Nap.	33 $\pm$ 5%	33 $\pm$ 8%	32 $\pm$ 2%	37 $\pm$ 6%	45 $\pm$ 3%	74 $\pm$ 2%	66 $\pm$ 2%
Ace.	38 $\pm$ 4%	13 $\pm$ 8%	34 $\pm$ 5%	56 $\pm$ 7%	64 $\pm$ 2%	87 $\pm$ 1%	77 $\pm$ 2%
Acn.	40 $\pm$ 3%	13 $\pm$ 6%	33 $\pm$ 5%	54 $\pm$ 8%	60 $\pm$ 1%	87 $\pm$ 1%	71 $\pm$ 3%
Fl.	44 $\pm$ 5%	22 $\pm$ 6%	56 $\pm$ 3%	68 $\pm$ 3%	76 $\pm$ 2%	90 $\pm$ 1%	81 $\pm$ 1%
Phe.	61 $\pm$ 6%	69 $\pm$ 3%	77 $\pm$ 1%	84 $\pm$ 1%	87 $\pm$ 1%	89 $\pm$ 1%	88 $\pm$ 1%
Ant.	53 $\pm$ 6%	67 $\pm$ 3%	80 $\pm$ 1%	88 $\pm$ 1%	89 $\pm$ 1%	90 $\pm$ 1%	89 $\pm$ 1%
Fluo.	70 $\pm$ 12%	77 $\pm$ 1%	89 $\pm$ 1%	95 $\pm$ 2%	98 $\pm$ 1%	99 $\pm$ 1%	98 $\pm$ 1%
Py.	72 $\pm$ 12%	80 $\pm$ 2%	91 $\pm$ 1%	96 $\pm$ 2%	97 $\pm$ 1%	99 $\pm$ 1%	99 $\pm$ 1%
BaA.	70 $\pm$ 4%	72 $\pm$ 5%	89 $\pm$ 3%	98 $\pm$ 1%	97 $\pm$ 8%	96 $\pm$ 1%	98 $\pm$ 2%
Ch.	65 $\pm$ 4%	82 $\pm$ 6%	91 $\pm$ 1%	97 $\pm$ 3%	97 $\pm$ 1%	97 $\pm$ 1%	97 $\pm$ 2%

Results show that:

- Adsorption at T1 (0.5 h) was already high (33-70%) and globally increasing with PAH molecular weight.
- Adsorption percentages increased over time until stabilization (24h), with higher values and faster for heavier hydrocarbons.
- After stabilization, adsorption for Fluoranthene, Pyrene, Benzo(a)anthracene and chrysene was total.
- Phenanthrene and anthracene adsorption rates were stabilized close to 90 %.
- Acenaphthylene, Acenaphthene and Fluorene adsorption rates were stabilized also close to 90 %, but these rates undergo a significant decrease in the following 24h. Besides, acenaphthylene and Acenaphthene adsorption rates showed a significant decrease between first and second sampling time.
- Naphthalene adsorption percentage increased only slightly until 4h, increasing afterwards until 74% at 24h and showing a significant decrease in the following 24h.

Results showed that adsorption was occurring for all PAHs and that adsorption rates were higher for heavier hydrocarbons throughout all sampling times. Moreover, stabilization rates edify another phenomenon, rates evolve similarly in group. Chrysene, Benzo(a)anthracene, Pyrene and Fluoranthene have four benzene rings and present the highest adsorption percentages in the same range at stabilization (100 %). Phenanthrene, Anthracene, Acenaphthylene, Acenaphthene and Fluorene have three benzene rings and presented lower adsorption percentages in the same range of stabilization (90 %). Naphthalene has two benzene rings and presented the lowest adsorption percentage (74%).

Potential release of PAHs adsorbed to fishing net

Pieces of net used in previous experiment, with PAHs adsorbed after 48h, were placed in flasks containing new and not doped seawater. Water was sampled following times presented in Table 4.15 and analysed in terms of PAHs concentration as before.

Table 4.15. PAHs release rates (% , mean $\pm$ standard deviation, $n = 3$). Net 3 has been used (Twisted Nylon fibres net).
n.d. = PAH not detected, no released occurred

PAH	Collection times						
	0.5 h	1 h	2 h	4 h	8 h	24 h	48 h
Nap.	n.d.	15.0 $\pm$ 0.6%	19 $\pm$ 1%	20 $\pm$ 2%	25.0 $\pm$ 0.7%	24 $\pm$ 2%	24 $\pm$ 1%
Ace.	n.d.	9 $\pm$ 2%	10.0 $\pm$ 0.5%	14 $\pm$ 1%	14.0 $\pm$ 0.6%	14 $\pm$ 1%	15 $\pm$ 2%
Acn.	9.0 $\pm$ 0.5%	9 $\pm$ 2%	13.0 $\pm$ 0.6%	14 $\pm$ 2%	15 $\pm$ 2%	14.0 $\pm$ 0.4%	15 $\pm$ 2%
Fl.	10.0 $\pm$ 0.3%	11 $\pm$ 2%	12.0 $\pm$ 0.6%	14 $\pm$ 1%	14 $\pm$ 1%	13.0 $\pm$ 0.7%	16.0 $\pm$ 0.4%
Phe.	15.0 $\pm$ 0.1%	15.0 $\pm$ 0.1%	15.0 $\pm$ 0.2%	16.0 $\pm$ 0.1%	14.0 $\pm$ 0.2%	16.0 $\pm$ 0.1%	16.0 $\pm$ 0.3%
Ant.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fluo.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Py.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BaA.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ch.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Results indicate that:

- Anthracene, Fluoranthene, Pyrene, Benzo(a)anthracene and Chrysene were not released.
- Phenanthrene, Fluorene, Acenaphthene, Acenaphthylene release rates were stabilized close to 15%.
- Naphthalene release rate was stabilized close to 24%.

- Naphthalene release rate underwent the highest evolution with a majoring of 9% between the first release and stabilization time while others PAHs were 6% majored (except phenanthrene; +1%).

As mentioned previously, heavier hydrocarbons are adsorbed easily and more effectively. But their release is more hard than for lighter hydrocarbons. As for adsorption, release rates evolve quite similarly in group. Heavier PAHs with four rings were not released. Phenanthrene, Fluorene, Acenaphthene, and Acenaphthylene have 3 benzene rings and presented low release rates in the same range after stabilization (15%). Naphthalene has two benzene rings and presented the highest release rate (24%).

Another phenomenon to consider is the decrease in rates for some PAHs between following sampling times, which happened for lighter hydrocarbons. This may be explained by the fact that plastic polymers have the potential to adsorb PAHs, but also to release a part of them. Lighter PAHs are more difficult to adsorb, but apparently they are released more easily. For heavier hydrocarbons, the inverse phenomena occurs, since they are more easily adsorbed but apparently hardly released.

4.2.4 General discussion

This approach showed two phenomena: fishing net polymers have the potential to adsorb PAHs on their surface, with no exception, and adsorption is correlated to PAH molecular weight.

Table 4.16 shows several studies highlighting the potential of plastic to adsorb PAHs. Studies 1, 2, 3 and 5 showed that all individual PAHs were adsorbed (except Ant and BaA in study 3) on microplastics. Studies 1 and 4 showed that the adsorption process and efficiency was not limited to small pieces of plastic but also occur for large plastic pieces (study 4) and larger plastic objects such as plastic ropes, buoys, caps and many other larger fragments (study 1). Plastic types found in those studies were mainly Polyethylene (PE) and Polypropylene (PP). Study 1 collected several PE rope-lines which are theoretically used to make fishing gears. High PAHs concentrations (up to 14459 ng/g) were detected at the surface of some rope-lines, which agree with our study. As adsorption occurred for all PE samples, regardless of the size, our study agrees with all these researches when stating that Polyethylene fishing nets (Net 1 and 2 in our study) have the potential to adsorb PAHs on their surface. To our knowledge, no studies until now evaluate the potential PAHs adsorption on nylon and more specifically on nylon fishing gears. A high variability in PAHs concentrations was observed in plastic debris from studies in Table 4.16, probably due to the availability of the contaminants in the water. Moreover, it is difficult to determine the length of time that these debris were exposed in the environment. These two important parameters can affect adsorption process and do not allow to evaluate any specific behaviour for individual PAHs or correlate it with our laboratorial study.

Table 4.16. Studies about PAHs adsorption on plastic debris.

PE – Polyethylene

PP – Polypropylene

PET – Polyethylene terephthalate

PS – Polystyrene

Rate = percentage of samples for which adsorption occurred

PAHs = PAHs adsorbed

Study	Location	samples	Type	Rate	PAHs	Reference
1	North Pacific Central Gyre – between San Francisco and Hawai	-29 macro plastic debris (>5mm) -8 micro plastic debris (<5mm)	PE PP	77 %	All Nap Phe, Fluo and Py principals.	(Rios* et al., 2010)
2	Santos Bay, Brazil	-1 g of plastic pellets (packages of micro plastics less than 5 mm) -30 samples	Unknown	100 %	All Highest concentrations for Ch, Py, Nap, Fluo, Ant and Phe	(Fisner et al., 2013)
3	Indonesian Cilacap coast	-146 micro plastic debris (<5 mm)	PE PP	100 %	All except Ant and BaA Nap,Fl,Py,Fluo systematically evidenced	(Bouhroum et al., 2019)
4	North Atlantic Gyre	-15 macro plastic debris (>1 cm)	PE PET	100 %	All Nap,Ace,Fl,Phe systematically present for PET Phe,Py systematically present for PE	(Bouhroum et al., 2019)
5	Cresmina and Fonte da Telha beaches, Portugal	-1 g of plastic pellets (packages of micro plastics less than 5 mm) -33 samples	PE PP PS	100 %	All Highest concentrations for Fl, Phe, Py and Ch	(Frias et al., 2010)

4.3 Third approach - In situ experiments

4.3.1 Gears as a sink of PAHs

In the marina experiment, the analysis of water near the nets over time was used to evaluate if plastic fishing nets could act as a sink of PAHs in the marine environment and create new polluted areas.

Table 4.17 shows individual PAHs levels in water at the different net locations and sampling campaigns. Surface water samples were collected at surface close to the net and bottom water samples below the net, close to ground, being used as control (no net location). Surface water sample close to Net A and the samples from the control site from July campaign were not analysed due to laboratorial constraints.

Results showed that Acenaphthylene, Acenaphthene, Fluorene and Anthracene were always below detection or quantification limits. The presence of the nets showed no significant influence on these PAHs levels, probably due to their low levels. Fluoranthene, Pyrene and Chrysene were detected at Net C surface water in February (T0). But they were not detected further at the same location in following campaigns, indicating they could potentially have been adsorbed on net C. However, the presence of the nets showed no significant influence on those PAHs levels, as they were not detected in following sampling campaigns. Phenanthrene and Benzo(a)anthracene were detected irregularly at the bottom and surface water of all nets and control location, but there was no difference between bottom, surface and control zones. Moreover, levels were similar throughout all sampling campaigns and did not change through time. Therefore, the presence of the nets showed no significant influence on those PAHs levels. Naphthalene was detected only in July in all locations. Therefore, the presence of the nets showed no significant influence on Naphthalene levels. So, nets do not seem to increase or decrease PAHs levels in their surrounding waters.

Table 4.17. Levels of polycyclic aromatic hydrocarbons (PAHs (ng/l); n = 1) in water collected at the marina with presence of nets.

Analysis being done at different times resulted in different values for LoD and LoQ.

*LoD, **LoQ

Net A = Twisted Polyethylene fibres net

Net B = Twisted Nylon fibres net

Net C = Monofilament Nylon net

Net	Sampling campaign	Sampling point	PAHs									
			Nap	Ace	Acn	Fl	Phe	Ant	Fluo	Py	BaA	Ch
A	Feb. (T0)	Bottom	<2.0*	<1.8*	<3.0*	<2.8*	<1.0*	<1.0*	<2.7*	<2.5*	11	<1.9*
		Top	<2.0*	<1.8*	<3.0*	<2.8*	14	<1.0*	<2.7*	<2.5*	13	<1.9*
	Mar.	Bottom	<2.0*	<1.8*	<3.0*	<2.8*	13	<1.0*	<2.7*	<2.5*	19	<1.9*
		Top	<2.0*	<1.8*	<3.0*	<2.8*	13	<1.0*	<2.7*	<2.5*	<1.9*	<1.9*
	May	Bottom	<2.0*	<1.8*	<3.0*	<2.8*	<1.0*	<1.0*	<2.7*	<2.5*	<1.9*	<1.9*
		Top	<2.0*	<1.8*	<3.0*	<2.8*	14	<1.0*	<2.7*	<2.5*	8.4	<1.9*
	July	Bottom	2.5	<1.6*	<2.1*	<0.7*	17	<0.3*	<0.7*	<0.6*	24	<1.0*
		Top	No analysis									
B	Feb.(T0)	Bottom	<2.0*	<1.8*	<3.0*	<2.8*	13	<1.0*	<2.7*	<2.5*	19	<1.9*
		Top	<2.0*	<1.8*	<3.0*	<2.8*	13	<1.0*	<2.7*	<2.5*	<6.2**	<1.9*
	Mar.	Bottom	<2.0*	<1.8*	<3.0*	<2.8*	13	<1.0*	<2.7*	<2.5*	13	<1.9*
		Top	<2.0*	<1.8*	<3.0*	<2.8*	13	<1.0*	<2.7*	<2.5*	<1.9*	<1.9*
	May	Bottom	<2.0*	<1.8*	<3.0*	<2.8*	<1.0*	<1.0*	<2.7*	<2.5*	9.2	<1.9*
		Top	<2.0*	<1.8*	<3.0*	<2.8*	15	<1.0*	<2.7*	<2.5*	<1.9*	<1.9*
	July	Bottom	11	<1.6*	<2.1*	<0.7*	19	<0.3*	<0.7*	<0.6*	23	<1.0*
		Top	5.2	<1.6*	<2.1*	<0.7*	18	<0.3*	<0.7*	<0.6*	21	<1.0*
C	Feb. (T0)	Bottom	<2.0*	<1.8*	<3.0*	<2.8*	<1.0*	<1.0*	<2.7*	<2.5*	28	<1.9*
		Top	<2.0*	<1.8*	<3.0*	<2.8*	18	<1.0*	27	14	<6.2**	16
	Mar.	Bottom	<2.0*	<1.8*	<3.0*	<2.8*	14	<1.0*	<2.7*	<2.5*	<6.2**	<1.9*
		Top	<2.0*	<1.8*	<3.0*	<2.8*	14	<1.0*	<2.7*	<2.5*	<6.2**	<1.9*
	May	Bottom	<2.0*	<1.8*	<3.0*	<2.8*	<1.0*	<1.0*	<2.7*	<2.5*	11	<1.9*
		Top	<2.0*	<1.8*	<3.0*	<2.8*	13	<1.0*	<2.7*	<2.5*	10	<1.9*
	July	Bottom	19	<1.6*	<2.1*	<0.7*	11	<0.3*	<0.7*	<0.6*	23	<1.0*
		Top	24	<1.6*	<2.1*	<0.7*	14	<0.3*	<0.7*	<0.6*	24	<1.0*
Control site	July	Bottom	7.7	<1.6*	<2.1*	<0.7*	23	<0.3*	<0.7*	<0.6*	18	<3.3**
		Top	2.6	<1.6*	<2.1*	<0.7*	12	<0.3*	<0.7*	<0.6*	18	<1.0*

4.3.2 Gears as a carrier of PAHs

The analysis of PAHs retained on the nets over time was carried out to evaluate if nets could act as carrier of PAHs in the marine environment. Table 4.18 shows individual PAHs levels retained on the nets for each sampling campaign. Overall, results showed that retention occurred for almost all nets and for several PAHs, however with high variability and irregularity. Only Anthracene was not retained after the second sampling campaign (March) on net A, being retained again in July. Only Naphthalene and Acenaphthylene were retained in March on net B. Fluoranthene and Pyrene were retained in July, Chrysene in May. Acenaphthene, Fluorene, Phenanthrene, Anthracene and Benzo(a)anthracene were never detected on net B. Naphthalene, Acenaphthene, Fluorene, Anthracene and Chrysene were retained in March on net C. Fluoranthene was retained in May, Acenaphthylene, Pyrene and Benzo(a)anthracene in July on net C. Phenanthrene was never detected on net C.

It appears also that all retained PAH levels in March on all nets either decreased or increased over time, there was no stabilization observed. Net A was the only net showing to have retained all PAHs throughout all campaigns. Variations in the concentrations observed do not allow to highlight any significant difference between the different nets in the retention process.

Table 4.18. Levels of polycyclic aromatic hydrocarbons (PAHs (ng/g); n = 1) retained on different nets for each sampling campaign.

Net A = Twisted Polyethylene fibres net

Net B = Twisted Nylon fibres net

Net C = Monofilament Nylon net

Na = not adsorbed

PAH	Sampling campaign								
	March			May			July		
	A	B	C	A	B	C	A	B	C
Nap	24	12	16	25	1,5	26	0.7	NA	6.8
Ace	9.1	2.0	NA	NA	NA	NA	11	7.7	10
Acn	3.9	NA	5.1	2.2	NA	3.3	NA	NA	NA
Fl	2.4	NA	9.4	4.8	NA	5.5	NA	NA	NA
Phe	1.4	NA	NA	NA	NA	NA	NA	NA	NA
Ant	NA	NA	9.0	NA	NA	8.9	2.1	NA	NA
Fluo	5.9	NA	NA	4.2	NA	12	13	11	2.3
Py	12	NA	NA	0.7	NA	NA	6.7	10	4.3
BaA	2.3	NA	NA	NA	NA	NA	NA	NA	8.7
Ch	7.8	NA	14	10	3.4	13	NA	NA	3.4

4.3.3 General discussion

The third approach was established for two purposes. First, to evaluate if fishing nets could concentrate PAHs in the surrounding water. Only Phenanthrene and Benzo(a)anthracene were detected at sampling locations of the marina throughout the time. Naphthalene, Fluoranthene, Pyrene and Chrysene were detected but showed high variability. The other PAHs were always below detection or quantification limits. No difference in Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene and Benzo(a)anthracene levels occurred over time between control and nets zone. Therefore, the three nets did not show any significant influence on these PAHs levels in water nearby. Fluoranthene, Pyrene and Chrysene were detected when the nets were

placed at Net C location, but no difference occurred over time between control and nets zone, therefore, net C did not show any significant influence on PAHs levels in the closest zone. However, they were never detected anymore in following samplings, so net C could potentially have totally retained them.

As the influence of gears could not be proved in the area in terms of PAHs accumulation in the surrounding water, attention must be paid on the marina contamination. A previous study (described in point 4.1.5 – Fig.4.1) revealed that water of two Atlantic beaches from Porto metropolis, next to our study site, were contaminated by PAHs (Rocha et al., 2017). Table 4.19 resumes individual PAHs levels found in water at these two sites of the study.

Table 4.19. Levels of polycyclic aromatic hydrocarbons (PAHs (ng/L), mean $\pm$ standard deviation, n = 6) in water collected from Atlantic seacoast .

**LoD, **LoQ*

S1 = 41° 13' 06.8" N 8° 42' 57.9" W

S2 = 41° 10' 23.5" N 8° 41' 25.9" W

Taken from study done by Rocha et al., 2017

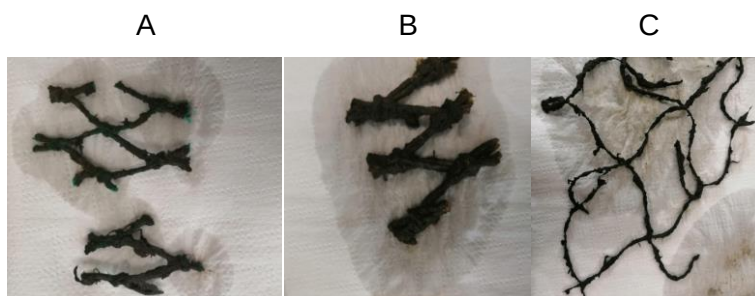
Site	PAHs									
	Nap	Acn	Ace	Fl	Phe	Ant	Fluo	Py	BaA	Ch
S1	4.7 $\pm$ 0.5	<0.7**	<0.2*	<0.3*	1.9 $\pm$ 0.1	<2.6**	<0.5*	3.1 $\pm$ 1.2	7.2 $\pm$ 2.2	5.2 $\pm$ 1.3
S2	4.2 $\pm$ 0.6	0.9 $\pm$ 0.4	0.5 $\pm$ 0.1	<0.9**	8.9 $\pm$ 0.9	6.5 $\pm$ 0.8	<1.6**	4. $\pm$ 0.5	3.6 $\pm$ 0.7	3.1 $\pm$ 0.5

According to Rocha et al., 2017, levels of Acenaphthene, Acenaphthylene, Fluorene, Fluoranthene, Anthracene (only S1) were low (below our detection limits) or below detection limits, which agree with levels founds at the marina. In contrast, Naphthalene, Phenanthrene, Pyrene, Benzo(a)anthracene and chrysene were detected at both sites of the study, which agree with our results, however in lower concentrations. Indeed, samples from our study were taken inside the marina being a confine zone with high traffic zone associated with the Leixões Harbour, and higher concentrations can be expected.

Secondly, results showed that nets can retain PAHs on their surfaces. It was observed for almost all PAHs and for the three nets, however with high variability. This variability could be related with the availability of the contaminant in the surrounding water, which could be highly variable considering water movements and natural degradation, among others. But variability could be associated also with biofilms formation on the nets over time. Indeed, throughout all the experiment, biofilms formed on all nets (Fig.4.2). PAHs tend to adsorb on organic matter, including biofilms, whose amount on the nets generally increased over time. One must say that the nets were not clean prior to PAHs extraction, so PAHs levels detected can be adsorbed on the net itself or in the biofilm or even both.

Globally, results did not show any correlation between PAHs levels found in the water and levels adsorbed on the nets over time.

Fig.4.2. Pieces of net collected in July at the marina and biofilms formed.



4.4 Global discussion

The three different approaches in this study highlight an important question that all scientists encounter, are laboratory behaviors representative and a good indicator of real and outside laboratory conditions behaviors?

Our second approach was achieved within laboratory conditions to evaluate if several plastic fishing nets could adsorb PAHs on their surface. All involved parameters were fixed to mimic as close as possible real-life conditions, with a clear control over all of them. Globally, adsorption occurred for all PAHs on all nets tested, furthermore speed and efficiency of the process increased with PAHs molecular weight.

Our first approach was achieved within complete real-life conditions to evaluate if lost fishing gears could concentrate PAHs in the closest zones and create new polluted areas. None of the involved parameters were under control. Globally, the results did not show any influence of lost nets, probably due to the low contamination status of the studied hotspots.

Our third approach was achieved within simulated conditions in a quasi-real environment to evaluate if several plastic fishing nets could adsorb PAHs on their surface and concentrate PAHs in the water nearby. None of the involved parameters, except the type, amount and location of net, were under control. Globally, the results did not show any influence of the nets in terms of PAHs accumulation in the surrounding waters, but PAHs were retained on the nets however with strong variability.

Considering this, laboratory experiments achieved in this study were only a rough approximation of real-life situations for two reasons.

First, of all the possible independent variables that influence behaviors in any practical situation, a laboratory experiment selects only a few for test (Chapanis, 2007). The potential of plastic fishing gears for adsorption of PAHs on their surface and accumulation in the closest zones in real-life situation include many variables, namely:

- Type of plastic
- Amount of net
- Availability and amount of PAHs in surrounding zones
- Effect of oceanic and wind-driven currents
- Interaction with other organic and inorganic pollutants
- Effects of light and its alternance
- Presence of organic matter in water column and presence of sediment
- Exposure time

This list is non exhaustive and it is highly probable that a large amount of other variables influence the process. Our second approach took consideration of all above parameters, however, hidden variables that occurred in the first and third approach could have easily altered taken conclusions of this approach.

Second, variables always change when they are brought into the laboratory and are variables of convenience. Furthermore, the methods used to present variables in the laboratory are sometimes artificial and unrealistic (Chapanis, 2007). Although our second approach took in consideration the most evident variables in the process, their influence and action were established in the frame of technical limitations, involving unrealistic situations. Indeed, except the type of plastic, all above listed variables were fixed to a define and unique value in the second approach. All of these variables are constantly changing, differing and uncontrolled in real life situation which could alter conclusions of this approach.

The present discussion highlights the precaution that should be taken when generalizing the results from the laboratory approach of this study to in-situ approaches. Adsorption of PAHs on plastic fishing nets has been evidently evaluated in controlled conditions in the laboratory in our second approach. PAHs were retained on the nets at some period in our

third approach which make these two approaches agree. However, the amount of PAHs available, the amount of net, the exposure time, the presence of organic matter (creation of biofilms on the net in the third approach), the alternance of sunlight, the currents, the interaction with other pollutants and many other variables were differing between both approaches. Therefore, although none of the results and conclusions taken along these studies should be neglected, their interpretation and correlation needs to be generalized with extreme caution. In our first approach, the potential adsorption of PAHs on lost nets has not been achieved. Evaluating if PAHs are retained on those nets could be an interesting further step of the study. This could highlight if PAHs were adsorbed without any control on the exposure time variable of gears in the environment, as the time since these gears were lost is unknown. Unfortunately that was not possible due to the very low amount of net collected.

None of the approaches allowed to highlight the potential of lost fishing nets to accumulate PAHs in the nearby environment. For in-situ approaches, this is probably due to the low contamination at the hotspots and at the marine site and the fact that water is constantly moving due to currents and other actions. Again, too many parameters were uncontrolled, changing and unknown to take any conclusions. But these approaches showed the potential that plastic fishing nets have to adsorb PAHs, an adsorption that in a quasi-real environmental scenario also occurred, indicating that adsorption can occur due to physical-chemical and biological (biofilm) phenomena. Concentrating PAHs on fishing nets can therefore be an hazardous to the environment, namely for organisms that can feed on biofilms.

5. Conclusion

The present thesis focussed on evaluating the potential of plastic fishing gears for adsorption of PAHs. First, to assess if PAHs could stick on these nets which could affect and cause potential threats to associated microbial communities. Second, to observe if this phenomenon could cause the accumulation of PAHs in the closest zones, creating new highly polluted areas and posing threats to associated ecosystems. To achieve this goal, three approaches were developed: environmental monitoring of two lost gears hotspots, in-situ experiments and laboratory experiments. Major results and finding are presented below:

- 1) Water and sediment from a hotspot located about 3 km and 30 m deep offshore from the Matosinhos coast (an artificial reef) and a hotspot located along the Esposende coast (in a marine protect area) were weakly polluted by PAHs.
- 2) Four different fishing nets adsorbed 10 priority PAHs on their surface in laboratory controlled conditions. Adsorption rate and percentage was correlated with the molecular weight of concerned PAHs. Twisted nylon fibre nets showed to be the most effective in the adsorption process.
- 3) PAHs levels in water of the marina of Leixões harbour were evaluated over time and at several locations of the marina, showing low levels.
- 4) Three different fishing nets retained the 10 priority PAHs when placed in seawater at a marina of Leixões harbour over time.

These research results are useful for further studies about PAHs contamination evaluation in studied hotspots and PAHs adsorption evaluation on plastic fishing gears.

6. References

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