
	Dissemination Level: PU EASME/EMFF/2017/1.2.1.12/S2/02/S12.789121 - MARINE LITTER Sustainable Blue Economy	
NetTag - "Tagging fishing gears and enhancing on board best-practices to promote waste free fisheries"		

Project no.: EASME/EMFF/2017/1.2.1.12/S2/02/S12.789121
Project full title: NetTag - "Tagging fishing gears and enhancing on board best-practices to promote waste free fisheries"
Project Acronym: NetTag

Deliverable number:	D4.1
Deliverable title:	Technical report on environmental impacts of lost fishing gear
Work package:	WP4
Due date of deliverable:	31/12/2019
Start date of project:	01/01/2019
Duration:	24 Months
Author/editors:	C. Marisa R. Almeida; Sandra Ramos; Rafaela Mendes; Ana P. Mucha; Catarina Magalhães; M. Fátima Carvalho
Contributing partners:	CIIMAR

Dissemination level of this deliverable	PU
Nature of deliverable	R

The current report is part of WP4 of the research project NetTag - Tagging fishing gears and enhancing on board best-practices to promote waste free fisheries (EASME/EMFF/2017/1.2.1.12/S2/02/S12.789121), funded by the European Maritime and Fisheries Fund (EMFF) and supported by the Executive Agency for Small and Medium-sized Enterprises (EASME) delegated by the European Commission.

"The information and views set out in this presentation are those of the author(s) and do not necessarily reflect the official opinion of EASME or of the Commission. Neither EASME, nor the Commission can guarantee the accuracy of the data included in this study. Neither EASME, nor the Commission or any person acting on their behalf may be held responsible for the use which may be made of the information contained therein."

Table of Contents

Executive Summary	3
1 Introduction	4
2 Selection and sampling of hotspots of lost fishing gears	5
2.1 <i>Cavalos de Fão (Ofir/Esposende) hotspot of fishing gears</i>	6
2.2 <i>Matosinhos submarine wreck hotspot</i>	8
2.3 <i>Physico-chemical water parameters</i>	8
2.4 <i>Sample preparation</i>	10
2.5 <i>Nutrients, chlorophyll and particulate matter content</i>	11
2.6 <i>Metal analysis</i>	12
2.7 <i>PAHs analysis</i>	12
2.8 <i>Microplastics analysis</i>	12
2.9 <i>Pathogenic agents</i>	13
3 Environmental contaminants in Cavalos de Fão (Ofir/Esposende)	13
4 Environmental contaminants in Matosinhos submarine wreck hotspot	20
5 Major findings	25
References	28
Acknowledgements	31

Executive Summary

Lost fishing gears are mainly composed of plastics. Although inert, environmental dynamics can induce modifications on its surface, making it suitable to adsorb pollutants, or even promote plastics debris degradation which can result in the production of microplastics. Moreover, plastic debris provides a substrate for microorganisms, being implicated as a transportation vector not only for chemical pollutants but also for biological pollutants such as opportunistic pathogens.

Chemical and biological pollution loads in lost fishing gear (mainly composed of plastics) have not yet been sufficiently explored and are essential for obtaining a better understanding of the ecological implications of fishing-related debris. NetTag project, in the framework of WP4, aims to assess the environmental harmfulness of lost fishing gears as a new pollutant. For that, in this present project report, *in situ* monitoring of chemical (metals, polycyclic aromatic hydrocarbons (PAHs) and microplastics) and microbiological (pathogens) contaminants in lost gear hotspots was carried out.

For the present work, two hotspots of lost gears in the NW Portuguese coast were selected, based on information gathered in WP2 (technical reports D2.1 and D2.2). The first location, Cavalos de Fão (41.490N -8.796W) situated in Ofir/Esposende, is a natural rocky bottom area, with several reefs. The second location, a submarine wreck located in the coast of Matosinhos (41.209N -8.741W), is an artificial reef located on a sandy bottom without rocks nearby. Both locations have intensive fishing activity and have small pieces of lost fishing nets and ropes at some sites (all less than 1 m long). These two locations were selected as case-studies to achieve the objectives of Task 1 of WP4, namely investigate environmental impacts of lost gears as new pollutants. At each location, a site without lost fishing nets was selected to be used as control.

Overall, low levels of nutrients, metals and PAHs were found at both locations, showing that both water and/or sediments were not significantly polluted as shown by comparison with other studies in NW region of Portugal. Metals and PAHs levels were in most cases below detection limit and, in general, considerably lower than the established effect-low range (ERL) guidelines. No clear influence of lost fishing nets were observed on these contaminants levels as contaminants levels were identical between sites with and without (control) lost fishing nets. Microplastics were found at both sites. Matosinhos hotspot presented a higher

level of microplastics contamination, mainly by fibres, however further analyses are still being done to ascertain the source of those microplastics.

Studies will now proceed with laboratory and *in situ* experiments to fully address the possibility of lost gear acting as a new pollutant either by adsorption and concentration of chemical (WP4, Task 4.3, D.4.3) or biological (WP4, Task 4.4, D.4.4) contaminants or by releasing microplastics (WP4, Task 4.2, D.4.2).

1 Introduction

Fishing-related debris, including buoys, lines, nets and other fishing gear, represents more than 50 % of the weight of the floating macroplastics pollution found in marine environment (Eriksen et al. 2014). Ghost fishing is just one of the possible impacts of lost fishing gear that includes also other biological and ecological impacts such as incidental catch of non-fishery animals (e.g. marine mammals, sea turtles or seabirds), accumulation of fishing gear on seabed and beach contamination (Matsuoka et al. 2005).

Plastic is an inert material, however, environmental dynamics can induce modifications on its surface, making it suitable to adsorb pollutants (Koelmans et al. 2016). Persistent pollutants, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and nonylphenol (NP) have been detected on floating plastics, raising concern with respect to marine environmental health (Chen et al. 2018). These pollutants can be associated with ocean plastics by direct addition during production processes (e.g. flame retardants) or by sorption to plastics from the marine environment through partitioning mechanisms.

Plastics debris degradation can result in the production of microplastics (fragments <5 mm), as plastic fragments go into progressively smaller pieces through abrasion, UV photo-oxidation and biodegradation, with an increase in the risk of ingestion by different marine species as the plastic fragment size decreases. Smaller particles possess a larger surface to volume ratio and shorter intra-polymer diffusion path lengths, which under non-equilibrium conditions can lead to a dependence of pollutants concentrations on particle size (Chen et al. 2018). Nevertheless, despite the hypothesis that “microplastics will transfer hazardous chemicals to marine animals” has been central to the perceived hazard and risk of plastic in the marine environment, the effective impact of microplastics remains under investigation (Koelmans et al. 2016).

As a relatively new introduction into the marine ecosystem, plastic debris provides a substrate for microbes that lasts much longer than most natural floating substrates and these debris have been implicated as a transportation vector not only for chemical pollutants but also for biological pollutants such as opportunistic pathogens (Zettler et al. 2013). With a hydrophobic surface rapidly stimulating biofilm formation, plastics can function as an artificial “microbial reef”. Surveys of these diverse microbial communities, also referred to as the “Plastisphere”,

identified several hydrocarbon-degrading bacteria, supporting the possibility that microbes might play a role in degrading plastic marine debris (Zettler et al. 2013).

Ocean plastics possess a wide range of physical and chemical properties that influence their risks to organisms and environments. Nevertheless, chemical and biological pollution loads in lost fishing gear (mainly composed of plastics) have not yet been sufficiently explored, and are essential for obtaining a better understanding of the ecological implications of fishing-related debris.

NetTag project, in the framework of WP4, will assess the environmental harmfulness of lost fishing gears as a new pollutant through *in situ* monitoring of contaminants in lost gear hotspots. In addition, a series of laboratory manipulative experiments will be implemented to explore the potential of the lost gears to release contaminants as microplastics, and/or the potential to adsorb chemical and biological pollutants, and how the recovery of lost gears can reduce this environmental impact. The aim of this present project report is to assess *in situ* the environmental impact of lost fishing gears as a new pollutant.

2 Selection and sampling of hotspots of lost fishing gears

As reported in D2.1. and D2.2. (deliverables of WP2 of NetTag project), hotspots for lost fishing gears in Portugal (NW Coast) and Spain (Galicia coast) are located up to 5-6 NM from the coast. Most lost fishing gears can be found in irregular bottoms such as rocky bottoms, reefs or wrecks. In NW Portugal, fishers identified as common hotspots of lost gear areas of rocky bottom and/or reefs, namely Cavalos de Fão (Ofir/Esposende), south of Póvoa do Varzim and west of Viana do Castelo. In the Galician coast, most of the small-scale fishers interviewed indicated rocky bottoms of areas close to the Ría de Arousa Bay as hotspots of lost fishing gears. Researchers corroborated fishers' information, identifying fishing spots like wrecks (that work as artificial reefs), rocky bottoms and areas with high hydrodynamics as hotspots of lost fishing gears. An example is the wreck of a II World War submarine, sunk out of Matosinhos coast.

For the present work, two of these hotspots were selected, namely Cavalos de Fão (Ofir/Esposende) and Matosinhos submarine wreck (Fig. 1). The first is a natural rocky bottom area, with several reefs, while the second site is an artificial reef. Both sites have intensive fishing activity. These two sites were selected as case-studies to achieve the

objectives of Task 1 of the WP4, namely investigate environmental impacts of lost gears as new pollutants.

As previously mentioned, lost fishing gears are mostly made of plastic that have the potential to adsorb contaminants as metals, organic pollutants, pathogenic agents, and also release microplastics. Therefore, hotspots of lost fishing gear can constitute a new pollution source. To assess the environmental harmfulness of lost gear as a new pollutant, the methodological approach used consisted in analysing biological (pathogens) and chemical (metals, organic contaminants (PAHs) and microplastics) contaminants in water and sediments of the hotspots of lost gear. So, for each selected hotspot a dedicated sampling protocol was designed to monitor chemical and biological contaminants in water and sediments (when present) as the harmfulness of lost gear as a new pollutant would primarily impact the habitats.



Figure 1. Location of the two hotspots (Cavalos de Fão and the submarine wreck of Matosinhos) of lost fishing gears selected as case studies for the NetTag project.

2.1 Cavalos de Fão (Ofir/Esposende) hotspot of fishing gears

The rocky reefs of Cavalos de Fão are located in a marine protected area, Parque Natural do Litoral Norte (<http://www2.icnf.pt/portal/ap/p-nat/pnln>). According to the results of several research projects performed at the Cavalos de Fão (Ofir/Esposende) hotspot, lost gears in this

region are essentially composed of small pieces of lost fishing nets and ropes (all less than 1 m long) that get attached to the rocky reefs or are floating, being dispersed in an area of kelp algae concentrated in a rocky environment. These were the only type of fishing gear found at this location. To survey this lost gear hotspot a total of nine sampling sites were selected for water collection (Fig. 2). Six sites were located within the hotspot area: sites 1-3 at the southern limit and sites 4-6 at the northern limit of the Cavalos de Fão area. Three more sites (7-9) located outside the area were selected as control sampling sites. Only three sites within the hotspot area (Cavalos de Fão) were sampled for sediments, due to the presence of many rocky reefs that prevents the use of sediment grab, and three more sampling sites were surveyed in the control zone.

Seasonal sampling campaigns were performed to collect water and sediment samples. Planktonic trawls were also performed to investigate microplastics contamination. During the first year of NetTag project, three sampling campaigns were carried out in January (winter), March (spring) and June (summer) 2019. Due to lack of good weather and navigability conditions, it was not possible to perform the autumn campaign. Sediment samples were only collected during winter and summer campaigns, as sediments tend to have less seasonal variations.



Figure 2. Location of the sampling sites (1-9) of the lost gear hotspot Cavalos de Fão selected as a case study for the NetTag project.

At each sampling site, bottom water samples (depth ranging between 15 and 25 m) were collected with a water sample bottle and stored in decontaminated plastic or glass vessels (depending on the contaminant to be determined). Additional bottom water samples were collected at sites 2, 5 and 8, and stored in decontaminated plastic for microbiological analyses (section 2.4). Sub-surface (1-2 m depth) planktonic trawls were performed with a 500 μm mesh size net (1 m diameter and 4 m long) and the volume of water sampled was determined by a flowmeter attached to the net. After the collection, planktonic samples were immediately fixed and stored for microplastics analyses (section 2.4).

Also, scientific divers of CIIMAR working in other research projects were able to collect pieces of lost fishing nets and ropes at the Cavalos de Fão hotspot (depth ranging between 15 and 25 m) (Fig. 3) that will be used in laboratory experiments to accomplish activities from the other tasks of WP4.

Sediment samples were collected with a Van Veen grab and from each sampling site three replicates were separated into plastic bags or aluminium foil, depending on the contaminant to be determined.



Fig. 3. Examples of pieces of lost fishing nets and ropes collected from Cavalos de Fão hotspot.

2.2 *Matosinhos submarine wreck hotspot*

The wreck of the II World War German submarine (U-1277) sunk out of Matosinhos coast (near Porto city) is at ca. 30 m depth. The area of the wreck has a sandy bottom without rocks nearby. The wreck is the only structure and in this area is where the pieces of lost fishing nets are trapped and concentrated. For this hotspot two sampling sites were selected, one close to the torpedoes exit (site A) and another close to the periscope of the submarine (site B), two

sites where small pieces of lost nets and ropes (less than 1 m long) are attached to the submarine, very close to the bottom (Fig. 4 and 5). A third site, 50 m far from the submarine was selected as control (site C) (Fig. 5).

The sampling was carried out by recreational divers from the diving school *Submersus*. Two sampling campaigns were done, the first on the 16th of June 2019 and the second on the 19th of September 2019.



Fig. 4. Pieces of lost fishing nets attached to the submarine structure. The wreck of this II World War submarine is sunk out the Matosinhos coast and at ca. 30 m depth.

Water and sediment samples were collected in duplicate or triplicate from each sampling site. Several factors constrained the number of collected samples, namely the weather and navigability conditions, boat availability, and the difficulty to properly collect and store water and sediment samples at 30 m depth (several samples were lost). Hotspot characterisation was then conditioned by these issues, not being possible to retrieve samples for all parameters.

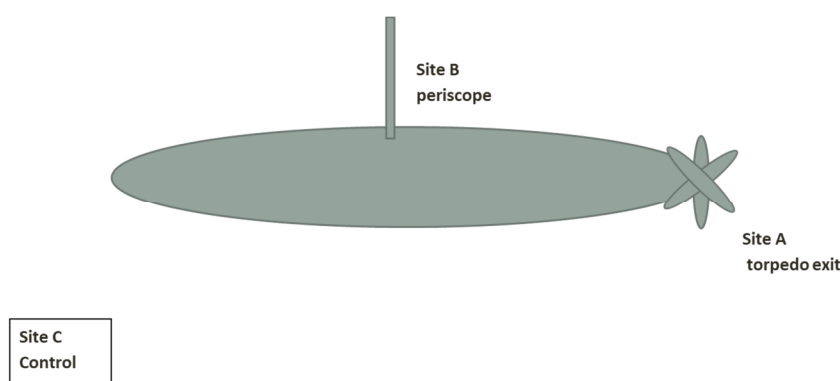


Fig. 5. Location of the three sampling sites around the Matosinhos submarine wreck. Sites A and B were close to the lost fishing nets attached to the structure of the submarine, site C was 50 m apart from the submarine and was used as a control.

Small pieces of nets and rope attached to the submarine structure were also collected (depth ca. 30 m) (Fig. 6) to be used in laboratory experiments from WP4. These were the only type

of lost fishing gear found at this location. Field sampling confirmed information provided by fishers that normally only pieces of nets are lost instead of the entire net.



Fig. 6. Pieces of nets and ropes that were attached to the submarine structure and collected by divers from *Submersus* school.

2.3 *Physico-chemical water parameters*

Physico-chemical parameters, e.g. temperature, salinity, dissolved oxygen, pH and turbidity were measured *in situ* at Cavalos de Fão (Ofir/Esposende) using a multiparametric probe (EXO I). For water samples collected in Matosinhos these parameters were not measured *in situ*, since sampling surveys were carried out by recreational divers.

2.4 *Sample preparation*

Water and sediment samples at each site of Cavalos de Fão (Ofir/Esposende) were collected into individual containers according with the specific contaminant to be analysed. For water samples collected at Matosinhos hotspot each water sample was collected into a single recipient being afterwards divided for metals, PAHs and microplastics analysis (Fig. 7). Sediment samples collected during the first campaign were also divided for the analysis of the same contaminants. For the second campaign a very low amount of sediment was collected and only metal analysis was carried out.

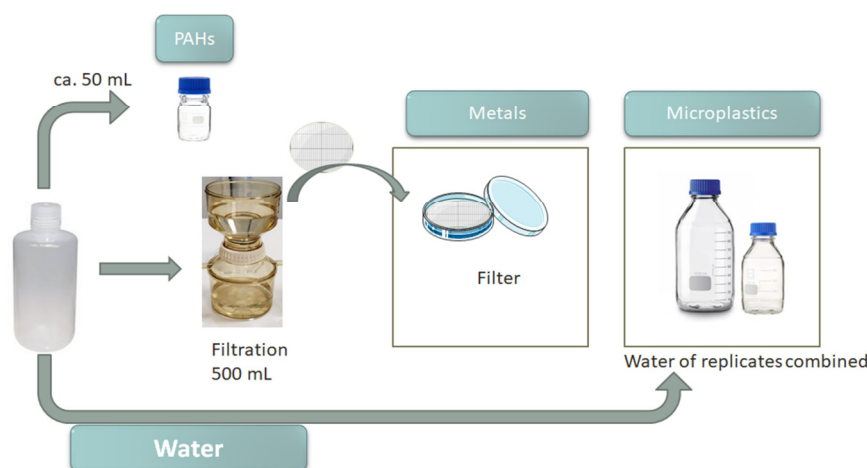


Fig. 7 - Preparation of water samples collected at Matosinhos hotspot.

For metal analysis in water suspended particulate matter, all water samples were filtered (ca. 500 mL of each sample) through nitrate cellulose filters (0.45 μm porosity). Filters were dried at room temperature until constant weight. Afterwards, filters were digested at high pressure in a microwave apparatus with concentrated nitric acid following previously optimised methodologies (Almeida et al. 2004). No metal analysis was done in water as metal levels in dissolved phase are in general below detection limits.

For PAHs analysis, water samples (ca. 50 mL) were kept at -20°C until analysis. No PAHs analysis was done in water suspended particulate matter as levels in this matrix are in general below detection limits.

For microplastics analysis, the planktonic samples collected at Cavalos de Fão were filtered through a 0.03 mm mesh size filter that was afterwards washed with deionized water and dried at 90°C . For the Matosinhos hotspot, remaining water samples collected at each site (ca. 500 mL) were combined into a single water sample (Fig. 7) that was afterwards submitted to similar procedures (filtration, washing and drying of the filter). For the latter, attending to the fact that we had no previous information about microplastics concentration and taking in consideration the studies on microplastics where the amount of microplastics per liter of water is generally very low, the three water samples collected at each site were combined to totalize a higher volume.

For microbiological analysis, 1.5 L of each water sample collected at Cavalos de Fão was filtered through a 0.2 μm sterivex™ filter attached to a manifold in a vacuum filtration system. Samples were concentrated in duplicate and sterivex filters stored at -80°C until DNA extraction for microbial community analysis.

All sediment samples were lyophilized. For metal analysis, dry sediment samples were subjected to high pressure microwave digestion as described above for filters. For PAHs, sediment samples were subjected to an ultrasonic extraction with methanol in an ultrasonic bath for 30 min following previously optimised methodology (Gonçalves et al. 2016). The extract was then clean-up with Florisil. For microplastics, dried sediment samples were sieved through a 2 mm net until further processing (section 2.7).

2.5 *Nutrients, chlorophyll and particulate matter content*

Inorganic nutrient analyses were performed in triplicate. Dissolved orthophosphate, nitrite, ammonium and silicate ions concentrations were quantified by Grasshoff et al. (1983) methods, and nitrate ion was analyzed by an adaptation of the spongy cadmium reduction technique (Jones 1984), subtracting nitrite from the total. Chlorophyll *a* was determined spectrophotometrically after extraction with 90 % acetone (Parsons et al. 1984) with cell homogenization, using the SCOR-UNESCO (1966) trichromatic equation. For total particulate matter (TPM) and particulate organic matter (POM) assessment, samples were previously filtered through pre-combusted GF/F glass-fibre filters, which were dried at 105 °C (for TPM) and then incinerated at 500 °C (for POM), according to APHA (1992).

2.6 *Metal analysis*

Metal analysis (Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn) was carried out by atomic absorption spectroscopy with either flame or electrothermal atomization, depending on metal levels. Quantification was obtained through external calibration with aqueous metal standard solutions (Almeida et al. 2004).

2.7 *PAHs analysis*

The analysis of 10 of the PAHs considered a priority by the United States Environmental Protection Agency, naphthalene (Nap), acenaphthylene (Ace), Acenaphthene (Acn), fluorene (Fl), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fluo), pyrene (Py), benz(a)anthracene (BaA) and chrysene (Ch), was carried out by headspace SPME-GC-MS (Gonçalves et al. 2016). For water samples, 10 ml of each sample were first placed in glass flasks and an internal standard solution (20 µl) was added. For sediment samples, a specific volume of deionised water was placed in a glass flask and an aliquot of the sediment extract (200 µl) and an internal standard solution (20 µl) was added. Quantification was obtained through external calibration with internal standard correction with PAHs standard solutions prepared similarly to samples.

2.8 *Microplastics analysis*

Microplastics (particles size lower than 5 mm) in water samples were determined following a previously optimised procedure (Rodrigues et al. 2019a). For that, the content of the dried filter was placed in a beaker and a H₂O₂ digestion was carried out at 75 °C to degrade all the organic matter. The procedure was repeated twice. The remaining material was then subjected to a density separation with NaCl, allowing for the collection of microplastics.

For microplastics in sediments, the NOAA protocol (Masura et al. 2015) was adapted, in which two different density separation steps and one oxidation step were carried out. First, dry sediments were mixed with a saturated NaCl solution for density separation of any microplastics (between 50-100 g of sediment for ca. 100 mL of saturated NaCl solution). The solution was left to settle until the water phase was transparent, being, afterwards, filtered through a 0.03 mm mesh size filter. The filter was then dried and subjected to the procedure described above for water samples. All microplastics collected were visually inspected and classified into particles or fibres under a stereoscope.

2.9 *Pathogenic agents*

The environmental DNA of each concentrated bottom water sample (collected in the 0.2 µm sterivex™ filter) of the sites 2, 5 and 8 from Cavalos de Fão was extracted using the PowerWater® Sterivex™ DNA Isolation Kit. The total DNA is currently being analysed at the prokaryotic (Bacteria and Archaea) taxonomic level using metabarcoding (16S rRNA) next generation sequencing technology (NGS), with special attention to the characterization of the pathogenic groups bacteria.

3 Environmental contaminants in Cavalos de Fão (Ofir/Esposende)

The environment in the Cavalos de Fão hotspot of lost gear was complemented by a general characterization of the water, including physical-chemical parameters (Table 1) and determination of the concentration of relevant compounds, as nutrients, particulate matter and chlorophyll *a* (Table 2).

Temperature ranged between 12.99 °C and 15.51 °C. Water was in general well oxygenated, with saturation over 80 % and more than 6 mg/l of dissolved oxygen. In general, water physical-chemical parameters were similar between sampling sites, including the control sites.

Table 1. Physical-chemical water parameters of the nine sampling sites (1-9) surveyed in Cavalos de Fão (Ofir/Esposende) in January (winter), March (spring) and June (summer) 2019. Sites 1-6 are within the lost gear hotspot area and sites 7-9 are outside.

Site		Depth (m)	Temperature (°C)	Salinity	Dissolved Oxygen (%)	O2 (mg/l)	pH	Turbidity (FTU)
1	January	17	14.65	35.48	86.35	6.97	8.14	0.39
	March	21	13.05	35.48	82.80	6.90	8.25	2.01
	June	*	*	*	*	*	*	*
2	January	25	14.64	35.48	85.70	6.91	8.10	0.10
	March	28	13.03	35.48	81.90	6.83	8.25	0.96
	June	*	*	*	*	*	*	*
3	January	29	14.64	35.48	90.85	7.33	7.94	0.18
	March	24	13.03	35.48	83.20	6.93	8.24	0.27
	June	19	14.28	35.32	*	*	8.26	0.01
4	January	11	14.50	35.25	91.00	7.38	8.14	0.93
	March	14	13.07	35.46	83.80	6.98	8.25	1.92
	June	20	14.36	35.37	*	*	8.29	0.00
5	January	18	14.63	35.44	90.40	7.30	8.15	0.50
	March	28	13.04	35.48	84.75	7.07	8.25	1.57
	June	15	15.51	35.18	*	*	8.31	0.04
6	January	24	14.64	35.46	89.80	7.25	8.16	0.35
	March	28	13.32	35.25	90.95	7.55	8.26	0.60
	June	26	14.11	35.32	*	*	8.25	0.01
7	January	15	14.58	35.34	88.20	7.13	8.13	0.72
	March	16	13.09	35.43	82.15	6.84	8.20	2.87
	June	19	14.11	35.32	*	*	8.22	0.17
8	January	17	14.60	35.39	87.90	7.10	8.13	0.51
	March	18	13.07	35.45	82.20	6.85	8.15	1.52
	June	*	*	*	*	*	*	*
9	January	27	14.63	35.47	89.40	7.22	8.15	0.38
	March	28	12.99	35.47	80.23	6.70	8.09	0.69
	June	*	*	*	*	*	*	*

* no data due to technical problems with the multiparametric probe

There was seasonal variability in temperature and dissolved oxygen concentration, with lower values observed during spring (Table 1). On the other hand, Salinity, pH and turbidity were seasonally stable and with typical values for the area (Ramos et al. 2017).

Nutrient concentrations in water of Cavalos de Fão area were in general low, indicating an overall good quality of the water (Table 2). There were no relevant differences between control sites (sites 7-9) and the remaining sites, indicating the absence of effect of proximity of lost gears in nutrient concentrations of the water. A similar pattern was also evident for chlorophyll *a*, TPM and POM (Table 2). Overall, these results were in accordance with other studies conducted in NW Portugal (Ramos et al., 2017; APA, 2019), and were lower in comparison with the adjacent Cávado estuary, whose river mouth is close by site 4 (Ramos et al., 2012).

Table 2. Nutrients (NO_3^- nitrate ion; NO_2^- nitrite ion; PO_4^- phosphate ion; Si- silica), chlorophyll *a* (Chl *a*), total particulate matter (TPM) and particulate organic matter (POM) concentrations in water of the Cavalos de Fão (Ofir/Esposende) area collected in January (winter), March (spring) and June (summer) 2019. Sites 1-6 are within hotspot area and sites 7-9 are outside.

		NO_3^- ($\mu\text{M/l}$)	NO_2^- ($\mu\text{M/l}$)	NH_4^+ ($\mu\text{M/l}$)	PO_4^- ($\mu\text{M/l}$)	Si ($\mu\text{M/l}$)	Chl <i>a</i> (mg/l)	TPM (mg/l)	POM (mg/l)
1	January	4.34	0.24	0.51	0.24	*	0.94	0.031	0.003
	March	7.69	0.27	1.04	0.70	4.12	0.94	0.047	0.001
	June	2.50	0.18	2.31	0.11	1.80	1.13	0.042	0.009
2	January	4.85	0.31	0.73	0.15	*	0.94	0.033	0.005
	March	7.53	0.33	0.29	0.62	3.46	1.15	0.051	0.007
	June	8.00	0.14	1.70	0.32	2.68	1.63	0.040	0.009
3	January	3.11	0.19	0.28	0.15	*	1.09	0.042	0.006
	March	9.01	0.29	0.81	0.79	3.53	0.78	0.046	0.005
	June	3.54	0.16	1.55	0.32	3.16	1.39	0.044	0.010
4	January	4.34	0.28	1.16	0.37	*	1.07	0.044	0.019
	March	9.83	0.11	2.79	0.84	4.44	0.94	0.053	0.005
	June	2.81	0.07	0.65	0.32	1.51	1.37	0.043	0.005
5	January	6.23	0.60	1.01	0.19	*	0.77	0.026	0.005
	March	9.40	0.28	1.28	0.65	3.31	0.69	0.047	0.007
	June	3.00	0.15	1.19	0.24	1.49	1.36	0.045	0.008
6	January	5.06	0.38	0.89	0.21	*	1.09	0.037	0.007
	March	8.10	0.09	0.65	0.62	3.40	1.00	0.049	0.007
	June	0.82	0.10	0.81	0.47	0.91	1.14	0.045	0.008
7	January	8.35	0.37	1.04	0.32	*	1.15	0.028	0.002
	March	5.99	0.31	0.86	0.69	2.93	1.12	0.053	0.006
	June	1.20	0.14	2.11	0.23	1.12	0.87	0.037	0.004
8	January	8.22	0.35	0.99	0.26	*	1.00	0.029	0.004
	March	8.85	0.24	0.69	0.76	3.78	0.94	0.052	0.005
	June	3.35	0.07	2.44	0.31	1.32	1.30	0.042	0.004
9	January	7.08	0.33	0.65	0.14	*	0.86	0.027	0.005
	March	9.74	0.14	1.13	0.48	4.12	1.08	0.037	0.004
	June	1.01	0.15	0.94	0.23	0.93	1.20	0.043	0.008

* under laboratory analyses

Metal concentrations in water particulate matter and sediments are shown in Tables 3 and 4, respectively.

Levels of Pb, Cd, Mn and Cr in water suspended particulate matter were in general below detection limits, whereas levels of Cu, Zn, Fe and Ni were detected in almost all samples. Levels varied significantly among sites and among sampling campaigns with no clear pattern of variation. Metal levels in water suspended particulate matter depend on water hydrodynamics that can vary with the season. Besides, each metal can present a different behaviour as they are involved in different biogeochemical processes. Overall, metal levels were similar to those reported in other studies conducted in NW Portugal for seawater (APA, 2019). No clear influence of proximity of lost fishing nets was evident on metal levels as those levels were identical between sites with and without (control) lost fishing nets.

Table 3. Concentrations (mean and standard deviation, n=3) of Cu, Zn, Fe, Pb, Cd, Ni, Mn and Cr ($\mu\text{g L}^{-1}$) in water suspended particles of the Cavalos de Fão (Ofir/Esposende) area collected in January (winter), March (spring) and June (summer) 2019. Sites 1-6 are within hotspot area and sites 7-9 are outside.

		Cu	Zn	Fe	Pb	Cd	Ni	Mn	Cr
1	January	0.67	0.53	<1.2*	<1.2*	<0.12*	1.3	<1.2*	<0.48*
	March	2.01	5.0	44	<1.2*	<0.12*	1.7	3.6	0.54
	June	1.9	2.8	48	<1.2*	<0.12*	3.8	<1.2*	<0.48*
2	January	1.8	1.1	<1.2*	<1.2*	<0.12*	0.62	<1.2*	<0.48*
	March	1.4	11	3.8	<1.2*	<0.12*	2.8	<1.2*	<0.48*
	June	1.5	1.9	50	<1.2*	<0.12*	1.5	<1.2*	<0.48*
3	January	1.1	6.6	3.7	<1.2*	<0.12*	4.3	<1.2*	<0.48*
	March	1.7	9.5	0.93	<1.2*	<0.12*	3.3	<1.2*	<0.48*
	June	0.87	1.6	12	<1.2*	<0.12*	0.86	<1.2*	<0.48*
4	January	0.92	1.5	8.6	<1.2*	1.2	2.7	<1.2*	<0.48*
	March	2.5	12	31	1.4	<0.12*	4.3	2.3	1.0
	June	1.4	3.0	15	<1.2*	<0.12*	1.8	<1.2*	1.4
5	January	0.83	2.1	7.5	<1.2*	0.30	0.80	<1.2*	<0.48*
	March	1.3	<0.60*	3.0	<1.2*	<0.12*	<0.60*	<1.2*	<0.48*
	June	1.0	1.3	15	<1.2*	<0.12*	0.80	<1.2*	<0.48*
6	January	0.93	1.4	2.2	<1.2*	<0.12*	1.0	<1.2*	<0.48*
	March	0.88	5.2	2.4	<1.2*	<0.12*	1.2	<1.2*	<0.48*
	June	1.7	2.3	11	<1.2*	<0.12*	1.7	<1.2*	<0.48*
7	January	1.0	2.4	9.8	<1.2*	1.4	2.8	<1.2*	<0.48*
	March	6.2	14	67	2.0	2.7	0.90	5.9	0.77
	June	2.7	16	47	<1.2*	<0.12*	11	<1.2*	<0.48*
8	January	2.5	9.5	10.9	<1.2*	<0.12*	13	2.0	<0.48*
	March	1.0	7.9	12	<1.2*	<0.12*	3.0	<1.2*	<0.48*
	June	1.3	3.7	11	<1.2*	<0.12*	7.5	<1.2*	<0.48*
9	January	1.3	6.9	3.4	<1.2*	<0.12*	4.3	<1.2*	<0.48*
	March	0.69	0.70	0.40	<1.2*	<0.12*	<0.60*	<1.2*	<0.48*
	June	11.4	23	20	1.8	<0.12*	28	<1.2*	<0.48*

*Limit of detection

For sediments, in general, all metals were quantified above detection limit, with the exception of Cd and Ni (for some samples) for which levels were below detection limit (Table 4). Overall, the levels observed were in accordance with levels found around this area (APA, 2019). Summer sampling showed, in general, lower levels of metals than winter sampling. For Cu, Fe, Pb, Cd and Cr, levels were in general identical among sites, but Zn, Ni and Mn showed clear variation: Zn and Ni levels were lower in S3 and S5 than in remaining sites, whereas Mn showed an opposite trend. The observed variability between sites could be associated with several factors, namely biological activity, physico-chemical changes derived from hydrodynamics, sediment geology or anthropogenic pressures (Rocha et al. 2019). Hence, no clear influence of lost fishing nets on metal levels in sediments was observed.

Table 4. Concentrations (mean and standard deviation, n=3) of Cu, Zn, Fe, Pb, Cd, Ni, Mn and Cr ($\mu\text{g g}^{-1}$ except for Fe that is in mg g^{-1}) in sediments from Cavalos de Fão (Ofir/Esposende) area, collected in January (winter) and June (summer) 2019. Sites S1-S3 are within hotspot area and sites S4-S6 are outside. *Limit of detection.

		S1	S2	S3	S4	S5	S6
Cu	<i>January</i>	13 ± 5	16.6 ± 0.8	14 ± 2	19.4 ± 0.5	12 ± 2	20 ± 2
	<i>June</i>	$<5^*$	$<5^*$	$<5^*$	$<5^*$	$<5^*$	7.0 ± 0.4
Zn	<i>January</i>	29 ± 4	33 ± 2	5 ± 3	43 ± 4	6 ± 1	38 ± 3
	<i>June</i>	4 ± 2	16 ± 10	4 ± 2	28 ± 15	4 ± 3	30 ± 2
Fe	<i>January</i>	8.0 ± 0.3	9.2 ± 0.8	7.6 ± 0.4	9.9 ± 0.3	8 ± 2	9.6 ± 0.3
	<i>June</i>	3.0 ± 0.4	6 ± 3	2.8 ± 0.8	10 ± 4	7 ± 2	10.3 ± 0.2
Pb	<i>January</i>	7 ± 1	8.2 ± 0.4	6 ± 1	11 ± 1	7 ± 2	9 ± 1
	<i>June</i>	4.2 ± 0.7	6 ± 2	3.0 ± 0.4	8 ± 4	7 ± 1	8.7 ± 0.8
Cd	<i>January</i>	$<0.2^*$	$<0.2^*$	$<0.2^*$	$<0.2^*$	$<0.2^*$	$<0.2^*$
	<i>June</i>	$<0.2^*$	$<0.2^*$	$<0.2^*$	$<0.2^*$	$<0.2^*$	$<0.2^*$
Ni	<i>January</i>	10 ± 3	13.5 ± 0.3	$<2^*$	18 ± 2	$<2^*$	18 ± 1
	<i>June</i>	$<2^*$	5 ± 1	$<2^*$	8 ± 2	$<2^*$	7 ± 2
Mn	<i>January</i>	77 ± 9	97 ± 9	484 ± 10	123 ± 28	153 ± 61	129 ± 26
	<i>June</i>	34 ± 7	54 ± 17	44 ± 24	113 ± 43	142 ± 22	90 ± 6
Cr	<i>January</i>	5 ± 1	5.6 ± 0.6	2 ± 0.4	6.6 ± 0.7	3 ± 1	6.7 ± 0.8
	<i>June</i>	2.4 ± 0.1	6 ± 3	2.7 ± 0.3	7 ± 4	3.2 ± 0.1	9.2 ± 0.5

PAHs concentrations in water and sediments are presented in Tables 5 and 6, respectively.

Table 5. Concentrations (mean and standard deviation, n=3) of ten PAHs (ng L⁻¹) in water samples from Cavalos de Fão (Ofir/Esposende) area, collected in January (winter), March (spring) and June (summer) 2019. Sites 1-6 are within hotspot area and sites 7-9 are outside.

		Nap	Ace	Acn	Fl	Phe	Ant	Fluo	Py	BaA	Ch
1	January	2 ± 1	<4*	<4*	<3*	<2*	<2*	<2*	<3*	<9*	<9*
	March	<2*	<4*	<4*	<3*	<2*	<2*	<2*	<3*	<9*	<9*
	June	2.8 ± 0.7	<4*	<4*	<3*	2.1 ± 0.4	<2*	<2*	<3*	<9*	<9*
2	January	<2*	<4*	<4*	<3*	<2*	<2*	<2*	<3*	<9*	<9*
	March	<2*	<4*	<4*	<3*	<2*	<2*	<2*	<3*	<9*	<9*
	June	2 ± 1	<4*	<4*	<3*	<2*	<2*	<2*	<3*	<9*	<9*
3	January	<2*	<4*	<4*	<3*	<2*	<2*	2.2 ±	<3*	<9*	<9*
	March	<2*	<4*	<4*	<3*	<2*	<2*	0.7	<3*	<9*	<9*
	June	3 ± 3	<4*	<4*	<3*	<2*	<2*	<2*	<3*	<9*	<9*
4	January	<2*	<4*	<4*	<3*	<2*	<2*	<2*	<3*	<9*	<9*
	March	<2*	<4*	<4*	<3*	<2*	<2*	<2*	<3*	<9*	<9*
	June	<2*	<4*	<4*	<3*	<2*	<2*	<2*	<3*	<9*	<9*
5	January	<2*	<4*	<4*	<3*	<2*	<2*	<2*	<3*	<9*	<9*
	March	<2*	<4*	<4*	<3*	<2*	<2*	<2*	<3*	<9*	<9*
	June	<2*	<4*	<4*	<3*	<2*	<2*	<2*	<3*	<9*	<9*
6	January	<2*	<4*	<4*	<3*	<2*	<2*	<2*	<3*	<9*	<9*
	March	<2*	<4*	<4*	<3*	<2*	<2*	<2*	<3*	<9*	<9*
	June	<2*	<4*	<4*	<3*	<2*	<2*	<2*	<3*	<9*	<9*
7	January	<2*	<4*	<4*	<3*	<2*	<2*	<2*	<3*	<9*	<9*
	March	<2*	<4*	<4*	<3*	<2*	<2*	<2*	<3*	<9*	<9*
	June	<2*	<4*	<4*	<3*	<2*	<2*	<2*	<3*	<9*	<9*
8	January	<2*	<4*	<4*	<3*	<2*	<2*	<2*	<3*	<9*	<9*
	March	<2*	<4*	<4*	<3*	<2*	<2*	<2*	<3*	<9*	<9*
	June	<2*	<4*	<4*	<3*	2.2 ± 0.2	<2*	<2*	<3*	<9*	<9*
9	January	<2*	<4*	<4*	<3*	<2*	<2*	<2*	<3*	<9*	<9*
	March	<2*	<4*	<4*	<3*	5 ± 1	<2*	<2*	<3*	<9*	<9*
	June	<2*	<4*	<4*	<3*	2.0 ± 0.3	<2*	<2*	<3*	<9*	<9*

*Limit of quantification

Among the 10 PAHs analysed, only naphthalene, phenanthrene and fluoranthene were detected in water samples, but at very low levels (Table 5). Overall, PAHs levels were similar to those reported in other studies conducted in NW Portugal for seawater (Rocha et al. 2018; APA, 2019). PAHs are semi volatile compounds with low water solubility and therefore, they tend to adsorb to sediments, and normally are not detected in the dissolved water phase (Iglesias et al. 2020). No clear pattern of distribution among sites was observed but, in general, levels were higher in summer. This area is located in a Marine Protected Area, with several recreational and touristic activities, with typically higher frequency during the summer what may induce an increase in anthropogenic pressure. The low values detected do not allow to identify any influence of lost fishing nets on PAHs levels in water of this hotspot.

As mentioned, PAHs tend to adsorb to sediments, thus justifying the occurrence of higher diversity and levels of PAHs found in sediment (Table 6) comparatively to water (Table 5). Four of the ten analysed PAHs were detected in several samples, the levels being in general higher in winter sampling campaign. Considering a higher anthropogenic pressure in summer, as discussed above, it can be hypothesised that PAHs adsorption to sediment occurs and that higher adsorption reflects only in the season after. Nevertheless, levels were still low, being in the same order of magnitude of that reported for this area (APA, 2019). In general, PAHs levels were identical among sites with and without (control) lost fishing nets. Therefore, no clear influence of lost fishing nets was evident on PAHs levels.

Table 6. Concentrations (mean and standard deviation, n=3) of ten PAHs (ng g⁻¹) in sediments from Cavalos de Fão (Ofir/Esposende) area, collected in January (winter) and June (summer) 2019. Sites S1-S3 are within hotspot area and sites S4-S6 are outside.

		Nap	Ace	Acn	Fl	Phe	Ant	Fluo	Py	BaA	Ch
S1	January	1.8 ± 0.2	<1*	<1*	<0.8*	3.4 ± 0.9	<0.6*	1.9 ± 0.8	2.3 ± 0.9	<1.7*	<2*
	June	<0.6*	<1*	<1*	<0.8*	<2.5**	<0.6*	<1.7*	<0.8*	<1.7*	<2*
S2	January	2.3 ± 0.6	<1*	<1*	<0.8*	2.7 ± 0.6	<0.6*	<1.7*	1.4 ± 0.9	<1.7*	<2*
	June	<0.6*	<1*	<1*	<0.8*	<0.8*	<0.6*	<1.7*	<0.8*	<1.7*	<2*
S3	January	1.2 ± 0.3	<1*	<1*	<0.8*	<0.8*	<0.6*	<1.7*	<0.8*	<1.7*	<2*
	June	<0.6*	<1*	<1*	<0.8*	<0.8*	<0.6*	<1.7*	<0.8*	<1.7*	<2*
S4	January	2 ± 1	<1*	<1*	<0.8*	5 ± 3	<0.6*	7 ± 1	5 ± 3	<1.7*	<2*
	June	3.6 ± 0.5	<1*	<1*	<0.8*	<2.5**	<0.6*	<1.7*	<0.8*	<1.7*	<2*
S5	January	1.3 ± 0.6	<1*	<1*	<0.8*	<0.8*	<0.6*	<1.7*	<0.8*	<1.7*	<2*
	June	2.98 ± 0.09	<1*	<1*	<0.8*	<2.5**	<0.6*	<1.7*	<0.8*	<1.7*	<2*
S6	January	1.8 ± 0.3	<1*	<1*	<0.8*	5 ± 1	<0.6*	3 ± 1	4 ± 1	<1.7*	<2*
	June	<0.6*	<1*	<1*	<0.8*	<0.8*	<0.6*	<1.7*	<0.8*	<1.7*	<2*

*Limit of quantification

Microplastics found in water samples are shown in Table 7. At the moment, only sites 1, 2 and 3 were processed being the remaining sites (4-9) under analysis. Microplastics found in water samples were in general higher at site 1, although contamination levels were lower than in an estuary further south (Rodrigues et al., 2019b). Also, particles microplastics concentration was much higher (between 2 to 6 times higher) in spring sampling, what may indicate that a relevant input of microplastics occurred during this season. On the other hand, the higher amount of fibres microplastics were observed in summer sampling. The polymer identification will be analysed to allow identify microplastics sources, namely fishing nets.

Table 7. Microplastics (number of MPs per 100 m³ of water, sum of fibres and particles) in water samples from Cavalos de Fão (Ofir/Esposende) collected in January, March and June 2019. Sites 4-9 are still under analysis.

Sites	1	2	3	1	2	3	1	2	3
	<i>January</i>			<i>March</i>			<i>June</i>		
MPs (<i>particles</i>)	1.1	0.3	0.4	7.6	1.6	2.6	1.7	0.3	0.0
MPs (<i>fibres</i>)	0.2	0.3	0.2	0.4	0.4	0.4	1.7	0.3	0.6

Currently the total environmental DNA extracted from the bottom water samples of the sites 2, 5 and 8 is being analysed to characterize prokaryotic communities at taxonomic level (16S rRNA metabarcoding analysis), with a special attention to the bacteria pathogenic groups.

4 Environmental contaminants in Matosinhos submarine wreck hotspot

Water and sediment samples collected were analysed for chemical contaminants (metals and PAHs) and microplastics, whereas for biologic characterization only water samples were collected. In the second sampling, for sediment samples only metal analysis was carried out due to the low amounts collected.

Metal levels in water particulate matter and in sediments are shown in Tables 8 and 9, respectively.

Metal levels in water suspended particulate matter were in general below detection limits (Table 8). The only element detected in all samples was Fe, which presented a high variability between sampling campaigns. Moreover, in the second sampling campaign, Fe levels varied also among sites, with a tendency for higher levels at site B. Water suspended particulate

matter depends on water hydrodynamics that can vary with the season. Metal levels observed were in general lower than those reported for seawater in the NW Portuguese coast nearby (APA, 2019). The very low metal levels prevented observing any clear influence of lost fishing nets on metal levels.

Table 8- Concentrations (mean and standard deviation, n=3) of Cu, Zn, Fe, Pb, Cd, Ni, Mn and Cr ($\mu\text{g L}^{-1}$) in water suspended particles of the Matosinhos area selected, collected in June and September 2019. Sites A and B were close to the lost gears attached to the structure of the submarine, site C was 50 m apart and was used as a Control. At site A only two samples were collected due to logistics constraints.

		A1	A2	B1	B2	B3	C1	C2	C3
Cu	<i>June</i>	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*
	<i>September</i>	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*
Zn	<i>June</i>	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*
	<i>September</i>	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*
Fe	<i>June</i>	8.4	6.9	6.9	7.8	8.9	9.2	8.9	8.1
	<i>September</i>	15	67	165	64	73	10	44	41
Pb	<i>June</i>	<1.2*	<1.2*	<1.2*	<1.2*	<1.2*	<1.2*	<1.2*	<1.2*
	<i>September</i>	<1.2*	<1.2*	<1.2*	<1.2*	<1.2*	<1.2*	<1.2*	<1.2*
Cd	<i>June</i>	<0.12*	<0.12*	<0.12*	<0.12*	<0.12*	<0.12*	<0.12*	<0.12*
	<i>September</i>	<0.12*	<0.12*	<0.12*	<0.12*	<0.12*	<0.12*	<0.12*	<0.12*
Ni	<i>June</i>	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*
	<i>September</i>	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*	<0.60*
Mn	<i>June</i>	<1.2*	<1.2*	<1.2*	<1.2*	<1.2*	<1.2*	<1.2*	<1.2*
	<i>September</i>	<1.2*	<1.2*	<1.2*	<1.2*	<1.2*	<1.2*	<1.2*	<1.2*
Cr	<i>June</i>	<0.48*	<0.48*	<0.48*	<0.48*	<0.48*	<0.48*	<0.48*	<0.48*
	<i>September</i>	<0.48*	<0.48*	<0.48*	<0.48*	<0.48*	<0.48*	<0.48*	<0.48*

*value below detection limits

Metal levels in sediments were in general low. In fact, most levels were below the detection limits. Only Fe, an element commonly present in the Earth crust, was detected in all collected samples, similarly to what was observed in water suspended particles. Metal levels observed were in general lower than those reported for NW Portuguese coast nearby (APA, 2019). At each site, in general, no relevant differences were observed among collected samples (two or

three per site). Comparing the selected sites, in general, there were no differences, although in some cases metal levels were slightly higher in the site selected as control, site C, which could be related to the type of sediment or the hydrodynamics of the sites. Therefore, lost fishing nets did not seem to contribute for an accumulation of metals around the wreck of the submarine. Comparing the two sampling campaigns, metal levels were similar, with the exception of Cr. Seasonal variability in environmental metal levels have been observed and can be due to several factors, such as biological activity and physico-chemical changes associated with water dynamics, sediments geology and anthropogenic pressures (Rocha et al. 2019).

Table 9. Concentrations (mean and standard deviation, n=3) of Cu, Zn, Fe, Pb, Cd, Ni, Mn and Cr ($\mu\text{g g}^{-1}$ except for Fe that is in mg g^{-1}) in sediments of the Matosinhos area selected, collected in June and September 2019. Sites A and B were close to the lost gears attached to the structure of the submarine, site C was 50 m apart and was used as a Control. In June sampling campaign only two samples were collected at sites A and C due to logistics constraints.

		A1	A2	A3	B1	B2	B3	C1	C2	C3
Cu	<i>June</i>	<5*	<5*	-	6.5	<5*	<5*	<5*	9.8 ± 0.3	--
	<i>Sept</i>	<5*	<5*	<5*	<5*	<5*	<5*	<5*	<5*	<5*
Zn	<i>June</i>	<2*	<2*	--	<2*	<2*	<2*	11 ± 7	22 ± 5	--
	<i>Sept</i>	4±2	3±1	<2*	2.2±0.4	2.0±0.3	3.0±0.8	<2*	<2*	<2*
Fe	<i>June</i>	1.4 ± 0.4	1.6 ± 0.3	--	1.6 ± 0.5	1.9 ± 0.4	1.6 ± 0.1	2.9 ± 0.6	4.3 ± 0.5	--
	<i>Sept</i>	1.4±0.5	1.1±0.2	1.34±0.05	1.2±0.2	1.3±0.2	1.4±0.2	1.3±0.3	1.3±0.3	0.6±0.6
Pb	<i>June</i>	3 ± 1	2.4 ± 0.4	--	4.5 ± 0.5	4 ± 2	5 ± 2	5 ± 2	6.1 ± 0.4	--
	<i>Sept</i>	<2*	<2*	4±2	<2*	<2*	<2*	3±1	<2*	3±2
Cd	<i>June</i>	<0.2*	<0.2*	--	<0.2*	<0.2*	<0.2*	<0.2*	<0.2*	--
	<i>Sept</i>	<0.2*	<0.2*	<0.2*	<0.2*	<0.2*	<0.2*	<0.2*	<0.2*	<0.2*
Ni	<i>June</i>	<2*	<2*	--	<2*	<2*	<2*	<2*	4.9 ± 0.9	--
	<i>Sept</i>	<2*	<2*	<2*	<2*	<2*	<2*	<2*	<2*	<2*
Mn	<i>June</i>	<25*	27 ± 15		<25*	58 ± 16	29 ± 2	57 ± 32	56 ± 3	
	<i>Sept</i>	<25*	<25*	<25*	<25*	<25*	26±15	33±20	41±33	44±12
Cr	<i>June</i>	1.9 ± 0.9	10.4 ± 0.8	--	5.7**	4.5**	4.1**	10.8 ± 0.5	11 ± 2	--
	<i>Sept</i>	<0.4*	<0.4*	<0.4*	<0.4*	<0.4*	<0.4*	0.72±0.05	<0.4*	<0.4*

*value below detection limits; **only one replicate analyzed due to technical problems

PAHs levels in water samples and sediments are shown in Tables 10 and 11 respectively.

PAHs levels in water were very low, with most PAHs levels being below detection or quantification limits, being of the same order of magnitude of levels reported in other studies in NW Portuguese coastal area nearby (APA, 2019). Among the ten PAHs measured only three were quantified: naphthalene, phenanthrene and benzoanthracene. Some differences were observed between sampling campaigns, mainly for naphthalene, but without a clear pattern. Although a few differences among sites were observed, for example, naphthalene was higher at site B in the June sampling campaign, no clear pattern could be established. So, no clear influence of lost fishing nets on PAHs levels was perceived as no clear differences between sites with and without (control) lost fishing nets were observed.

Table 10 - Concentrations (mean and standard deviation, n=3) of ten PAHs (ng L⁻¹) in water samples of the Matosinhos area selected, collected in June and September 2019. Sites A and B were close to the lost gears attached to the structure of the submarine, site C was 50 m apart and was used as a Control. At site A only two samples were collected due to logistics constraints. *Limit of detection **Limit of quantification

Site		Nap	Ace	Acn	Fl	Phe	Ant	Fluo	Py	BaA	Ch
A1	<i>June</i>	<1*	<3*	<4*	<2*	<2**	<1*	<3**	<3*	<3*	<2*
	<i>Sept</i>	<5**	<3*	<4*	<2*	2.0 ± 0.6	<1*	<1*	<3*	<9**	<2*
A2	<i>June</i>	<1*	<3*	<4*	<2*	<2**	<1*	<1*	<3*	<9**	<2*
	<i>Sept</i>	<5**	<3*	<4*	<2*	3.6 ± 0.4	<1*	<1*	<3*	11 ± 3	<2*
B1	<i>June</i>	18 ± 4	<3*	<4*	<2*	2.1 ± 0.4	<1*	<1*	<3*	<9**	<2*
	<i>Sept</i>	<5**	<3*	<4*	<2*	2.7 ± 0.3	<1*	<1*	<3*	11 ± 2	<2*
B2	<i>June</i>	17 ± 2	<3*	<4*	<2*	3.1 ± 0.2	<1*	<1*	<3*	<3*	<2*
	<i>Sept</i>	<5**	<3*	<4*	<2*	<2**	<1*	<1*	<3*	<9**	<2*
B3	<i>June</i>	11 ± 5	<3*	<4*	<2*	2.4 ± 0.4	<1*	<3**	<3*	<3*	<2*
	<i>Sept</i>	<5**	<3*	<4*	<2*	2.6 ± 0.3	<1*	<1*	<3*	<9**	<2*
C1	<i>June</i>	<5**	<3*	<4*	<2*	<2**	<1*	<3**	<3*	<9**	<2*
	<i>Sept</i>	<5**	<3*	<4*	<2*	3.2 ± 0.7	<1*	<1*	<3*	<9**	<2*
C2	<i>June</i>	7.1 ± 0.6	<3*	<4*	<2*	<2**	<1*	<3**	<3*	<3*	<2*
	<i>Sept</i>	<5**	<3*	<4*	<2*	4 ± 1	<1*	<1*	<3*	11 ± 3	<2*
C3	<i>June</i>	<1*	<3*	<4*	<2*	<2**	<1*	<3**	<3*	14.9 ± 0.1	<2*
	<i>Sept</i>	5 ± 2	<3*	<4*	<2*	2.7 ± 0.3	<1*	<1*	<3*	<9**	<2*

In general, PAHs values in sediments were below detection limits, being only naphthalene detected in several samples, with no relevant differences among sites (Table 11). Levels were of the same order of magnitude of those reported in other studies in NW Portuguese coastal area nearby (APA, 2019, Gouveia et al. 2018). As for water, no clear influence of lost fishing

nets was observed as levels were very low and no clear differences among sites with and without (control) lost fishing nets were observed.

Table 11 - Concentrations (mean and standard deviation, n=3) of ten PAHs (ng g⁻¹) in sediments of the Matosinhos area selected, collected in June 2019 (no sufficient amount of sediment was available in the sampling campaign of September 2019). Sites A and B were close to the lost gears attached to the structure of the submarine, site C was 50 m apart and was used as a Control. Only two samples were collected at sites A and C due to logistics constraints.

Site	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 ± 0.3	<1*	<1*	<0.8*	<0.8*	<0.7*	<2*	<0.8*	<2*	<2*
B3	2.6 ± 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*

*Limit of detection **Limit of quantification

Microplastics found in water and sediment samples are shown in Tables 12 and 13, respectively. In water samples, a higher concentration of microplastics particles were observed at site B, particularly in the second sampling campaign, which may be derived from the proximity of the lost nets attached to the submarine structure. Fibres microplastics are still being processed. Future microplastics analysis for polymer classification will allow to identify microplastics sources and to discriminate the influence of lost gears in microplastics contamination.

Table 12 – Microplastics (number of MPs per 100 m³ of water) in water samples from Matosinhos area selected, collected in June and September 2019. Sites A and B were close to the lost gears attached to the structure of the submarine, site C was 50 m apart and was used as a Control. For each site, water samples were combined.

Sites	A	B	C	A	B	C
	June 2019			September 2019		
MPs (<i>particles</i>)	0	1x10 ⁵	1x10 ⁵	1x10 ⁵	5x10 ⁵	3x10 ⁵
MPs (<i>fibres</i>)	*	*	*	*	*	*

*under processing.

Microplastics in sediment samples are still being processed, namely to determine fibre microplastics concentrations. Fibre microplastics looked similar to those found in water samples at this location (Table 13) and different from those observed at Cavalos de Fão. No particles microplastics were observed in these sediments despite their presence in the water. Similarly to water samples, future microplastics analysis will be carried out to identify microplastics source, as for example the lost nets attached to the submarine.

Table 13 – Microplastics in sediments (number of MPs per 100 g_{sediment}) of the Matosinhos area selected, collected in June 2019 (no sufficient amount of sediment was available in the sampling campaign of September 2019). Sites A and B were close to the lost gears attached to the structure of the submarine, site C was 50 m apart and was used as a Control. Only two samples were collected at sites A and C due to logistics constraints.

Sites	A1	A2	B1	B2	B3	C1	C2
MPs (<i>particles</i>)	0	0	0	0	0	0	0
MPs (<i>fibres</i>)	*	*	*	*	*	*	*

*under processing.

Currently the total environmental DNA extracted from the bottom water samples collected around in the submarine area is being analysed to characterize prokaryotic communities at taxonomic level (16S rRNA metabarcoding analysis), with a special attention to the bacteria pathogenic groups.

5 Major findings

The NW of Portugal is part of the Iberian Peninsula ecoregion, receiving the influence of three oceanic water masses. Its coast features rocky and sandy beaches and several estuaries, some of them under environmental protection. This area is highly urbanized, hosting the second major city of Portugal (Porto) and supporting several human activities, with tourism

exponentially growing over the last decade. There is an oil refining industry and two maritime harbours (located at *Leixões* and *Viana do Castelo*), high population density and the development of several industrial and urban activities. Moderate concentrations of metals and PAHs have been previously reported in sediments of this NW coast (Mucha et al. 2004, Guimarães et al. 2009, Gravato et al. 2010, Rocha et al. 2011, Mil-Homens et al. 2013, Reis et al. 2013). Nevertheless, some rocky shore beaches (e.g. *Carreço*, *Vila Chã*) have been used as a reference sites since they show low susceptibility to human influence and low levels of environmental contamination (Cairrão et al. 2004, Rodrigues et al. 2013). In fact, in general, the Portuguese northern rocky shores present minor contamination levels even with a high human activity and exploitation of the coastal resources (Rocha et al., 2018).

Low levels of all analysed trace metals and PAHs were found throughout the seasonal campaigns, in both hotspots. Metals and PAHs levels were, in general, considerably lower than the established effect-low range (ERL) guidelines (Bakke et al. 2010; Long et al. 1995), water being in general classified as of “good environmental conditions” and sediments in general being classified as “background” taking into consideration the two types of contaminants (Bakke et al. 2010).

At the Cavalos de Fão hotspot, the main human activities are fisheries, small-scale agriculture, small city nearby (Esposende) and maritime touristic activities. In fact, severe contamination by urban, agricultural and industrial effluents is unlikely to occur. However, the Cávado estuarine river mouth is close by and its plume can constitute a contamination source of the adjacent coastal area, where Cavalos de Fão are located. Cadmium, mercury, nickel, lead, dichloromethane, benzene, trichloroethylene can be discharged into the hydrographical network of *Cávado*, *Ave* and *Leça* rivers (PGBHb 2012), which present high industrialised areas (PGRH 2015). But in the present case contaminants levels (nutrients, metals and PAHs) were in general low, indicating a low level of contamination of the estuary as previously reported in other studies (Ramos et al., 2012; 2015; APA 2019).

The Matosinhos hotspot is located near an oil refinery and a commercial harbour. Although some oil spills have occurred in the past, at the present, contaminants levels reported had shown low levels (e.g. Gouveia et al 2018). This area can also be affected by Douro river plume. The Douro estuary is highly impacted by severe pressures (Ramos et al., 2015; Rodrigues et al., 2019b), although current studies have shown a significant decrease in contaminants levels (Iglesias et al 2020). As such, low contaminant levels were expected at this Matosinhos location.

Overall, results did not indicate any significant influence of the presence of lost fishing nets on the contamination of metals and organic pollutants, without relevant differences between control sites (without lost nets) and sampling sites with lost fishing nets, probably also due to the low levels found (mostly below detection limit).

Regarding the possibility of lost gears acting as microplastics sources, no clear inputs from lost fishing nets at each site were at the moment observed, without relevant differences between control sites and sampling sites with lost fishing nets. Microplastics concentrations observed in Cavalos de Fão were in general low, lower than those found at Douro river estuary, an estuary with high anthropogenic pressure as previously mentioned (Rodrigues et al. 2019b). At the Matosinhos hotspot, microplastics contamination observed was substantially higher in comparison with Cavalos de Fão, and even higher than in other locations known to be highly impacted (e.g. Rodrigues et al 2019b). The characterisation of the polymer of the microplastics found will clarify any possible correlation with lost fishing nets at the selected hotspots.

The environmental characterization of the two hotspots of lost fishing nets revealed several logistic sampling constraints (e.g. weather and navigability conditions, difficulties in collecting and store samples at 30 m depth) that compromised the collection of a robust dataset of each hotspot. Despite the intention to carry out the hotspot characterisation following an ecosystem based approach, namely by monitoring the benthic and pelagic habitat through the use of the MarinEye (<http://marineye.ciimar.up.pt/>), an autonomous multitrophic system for integrated marine chemical, physical and biological monitoring developed in a previous project by team members of CIIMAR and INESC, boat availability and weather conditions prevented its use. Despite all these issues, typical of environmental studies, results of this report represent the first scientific environmental characterization of hotspots of lost gears in NW Portugal, and the results obtained so far allow taking important considerations. Studies will proceed with laboratory and *in situ* experiments to fully address the possibility of lost gears acting as a new pollutant either by adsorption and concentration of contaminants in their location or by releasing microplastics. Further microplastics analyses will allow identifying the polymer and, thus, ascertaining the influence of lost gears as contamination source.

References

- Almeida, C. M. R., Mucha, A. P., & Vasconcelos, M. T. S. D. (2004). Influence of the sea rush *Juncus maritimus* on metal concentration and speciation in estuarine sediment colonized by the plant. *Environmental science & technology*, 38, 3112-3118.
- APA, 2019. EEMA project <https://apambiente.pt/?ref=16&subref=7&sub2ref=875&sub3ref=876>
- APHA. (1992). Standard methods for the examination of the water and wastewater. Washington, DC, American Public Health Association.
- Bakke, T., Källqvist, T., Ruus, A., Breedveld, G. D., & Hylland, K. (2010). Development of sediment quality criteria in Norway. *Journal of Soils and Sediments*, 10(2), 172-178
- Cairrão, E., M. Couderchet, A. M. V. M. Soares & L. Guilhermino (2004). Glutathione-S-transferase activity of *Fucus* spp. as a biomarker of environmental contamination. *Aquatic Toxicology*, 70(4), 277-286.
- Chen, Q., Reisser, J., Cunsolo, S., Kwadijk, C., Kotterman, M., Proietti, M., & Yin, D. (2018). Pollutants in plastics within the North Pacific subtropical gyre. *Environmental science & technology*, 52 (2), 446-456.
- Eriksen, M., Lebreton, L. C., Carson, H. S., Thiel, M., Moore, C. J., Borerro, J. C., & Reisser, J. (2014). Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. *PloS one*, 9(12), e111913.
- Gonçalves, C., Teixeira, C., Basto, M. C. P., & Almeida C. M. R. (2016). PAHs levels in Portuguese estuaries and lagoons: Salt marsh plants as potential agents for the containment of PAHs contamination in sediments. *Regional Studies in Marine Science*, 7, 211-221.
- Gouveia, V., C. M. R. Almeida, T. Almeida, C. Teixeira & A. P. Mucha (2018). Indigenous microbial communities along the NW Portuguese Coast: Potential for hydrocarbons degradation and relation with sediment contamination. *Marine Pollution Bulletin*, 131, 620-632.
- Grasshoff, K., Ehrhardt, M., & Kremling, K. (1983). Methods of seawater analysis. Second revised and extended edition (p. 419). Weinheim: Verlag Chemie.
- Gravato, C., L. Guimarães, J. Santos, M. Faria, A. Alves & L. Guilhermino (2010). Comparative study about the effects of pollution on glass and yellow eels (*Anguilla anguilla*) from the estuaries of Minho, Lima and Douro Rivers (NW Portugal). *Ecotoxicology and Environmental Safety*, 73(4), 524-533.
- Guimarães, L., C. Gravato, J. Santos, L. S. Monteiro & L. Guilhermino (2009). Yellow eel (*Anguilla anguilla*) development in NW Portuguese estuaries with different contamination levels. *Ecotoxicology* 18(4): 385-402.

- Iglesias, I., Almeida, C.M.R., Teixeira, C., Mucha, A.P., Magalhães, A., Bio, A., & Bastos, L. (2020) Linking contaminant distribution to hydrodynamic patterns in an urban estuary: The Douro estuary test case. *Science of the Total Environment*, 707, 135792 (14 pp).
- Jones, M. (1984). Nitrate reduction by shaking with cadmium: alternative to cadmium columns. *Water Research*, 18, 643-646.
- Koelmans, A. A., Bakir, A., Burton, G. A., & Janssen, C. R. (2016). Microplastics as a vector for chemicals in the aquatic environment: critical review and model-supported reinterpretation of empirical studies. *Environmental science & technology*, 50(7), 3315-3326.
- Long, E. R., D. D. Macdonald, S. L. Smith & F. D. Calder (1995). Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. *Environmental Management*, 19(1), 81-97.
- Masura, J., Baker, J., Foster, G., & Arthur, C. (2015). NOAA Technical Memorandum.
- Matsuoka, T., Nakashima, T., & Nagasawa, N. (2005). A review of ghost fishing: scientific approaches to evaluation and solutions. *Fisheries Science*, 71(4), 691-702.
- Mil-Homens, M., A. M. Costa, S. Fonseca, M. A. Trancoso, C. Lopes, R. Serrano & R. Sousa (2013). Characterization of Heavy-Metal Contamination in Surface Sediments of the Minho River Estuary by way of Factor Analysis. *Archives of Environmental Contamination and Toxicology*, 64(4), 617-631.
- Mucha, A. P., Bordalo, A. A., & Vasconcelos, M. T. S. D. (2004). Sediment quality in the Douro river estuary based on trace metal contents, macrobenthic community and elutriate sediment toxicity test (ESTT). *Journal of Environmental Monitoring*, 6(7), 585-592.
- Parsons, T.R., Maita, Y., & Lalli, C.M. (1984). A manual of chemical and biological methods for seawater analysis (pp. 101–112). Elmsford: Pergamon.
- PGBHb (2012). Plano de gestão da região hidrográfica do Cávado, Ave e Leça: Relatório Técnico – Comissão Europeia. Agência Portuguesa do Ambiente & Administração da Região Hidrográfica do Norte.
- PGRH (2015). Plano de gestão de região hidrográfica: Parte 2 – Caracterização e Diagnóstico. Região Hidrográfica do Minho e Lima (RH1). Agência Portuguesa do Ambiente.
- Ramos, S., Amorim, E., Elliott, M., Cabral, H. and Bordalo, A. A. (2012). Early life stages of fishes as indicators of estuarine ecosystem health. *Ecological Indicators* 19, 172-183.
- Ramos, S., Cabral, H., & Elliott, M. (2015). Do fish larvae have advantages over adults and other components for assessing estuarine ecological quality? *Ecological Indicators*, 55, 74-85.
- Ramos, S., Paris, C., Angelico, M. M. (2017). Larval fish dispersal along an estuarine-ocean gradient. *Canadian Journal of Fisheries and Aquatic Sciences*, 74, 1462-1473.

Reis, P. A., M. A. Salgado & V. Vasconcelos (2013). Seasonal variation of metal contamination in the barnacles *Pollicipes pollicipes* in northwest coast of Portugal show clear correlation with levels in the surrounding water. *Marine Pollution Bulletin*, 70(1), 155-161.

Rocha, F., Rocha, A. C., Baião, L. F., Gadelha, J., Camacho, C., Carvalho, M. L., Arenas, F., Oliveira, A., Maia, M. R. G., Cabrita, A. R., Pintado, M., Nunes, M. L., Almeida C. M. R., & Valente, L. M. P. (2019). Seasonal effect in nutritional quality and safety of the wild sea urchin *Paracentrotus lividus* harvested in the European Atlantic shores. *Food Chemistry*, 282, 84-94.

Rocha, A. C., C. Camacho, E. Eljarrat, A. Peris, Y. Aminot, J. W. Readman, V. Boti, C. Nannou, A. Marques, M. L. Nunes & C. M. Almeida (2018). Bioaccumulation of persistent and emerging pollutants in wild sea urchin *Paracentrotus lividus*. *Environmental Research* 161, 354-363.

Rocha, M. J., E. Rocha, C. Cruzeiro, P. C. Ferreira & P. A. Reis (2011). Determination of Polycyclic Aromatic Hydrocarbons in Coastal Sediments from the Porto Region (Portugal) by Microwave-Assisted Extraction, Followed by SPME and GC-MS. *Journal of Chromatographic Science*, 49(9), 695-701.

Rodrigues, S. M., Almeida, C. M. R. & Ramos, S. (2019a). Adaptation of a laboratory protocol to quantify microplastics contamination in estuarine waters. *MethodsX*, 6, 740-749.

Rodrigues, S. M., Almeida, C. M. R., Silva, D., Cunha, J., Antunes, C., Freitas, V. & Ramos, S. (2019b). Microplastics contamination in an urban estuary: abundance and distribution of microplastics and fish larvae in the Douro estuary. *Science of the Total Environment*, 659, 1071-1081.

Rodrigues, A. P., K. K. Lehtonen, L. Guilhermino & L. Guimarães (2013). Exposure of *Carcinus maenas* to waterborne fluoranthene: Accumulation and multibiomarker responses. *Science of The Total Environment*, 443, 454-463.

Zettler, E. R., Mincer, T. J., & Amaral-Zettler, L. A. (2013). Life in the “plastisphere”: microbial communities on plastic marine debris. *Environmental science & technology*, 47(13), 7137-7146.

Acknowledgments

The authors would like to thank the recreational divers of *Submersus* school in Matosinhos as well as the researchers and researchers' divers of CIIMAR that collected the samples.