Determination and risk evaluation of PAHs and PCBs in seawater samples collected at north and south of the Vila do Conde ornithological reserve

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INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR

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ESPECIALIZAÇÃO EM BIOLOGIA E ECOLOGIA MARINHA

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Dissertação de Mestrado em Ciências do Mar – Recursos Marinhos

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Dissertação de Candidatura ao grau de Mestre em Ciências do Mar – Recursos Marinhos submetida ao Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto.

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Acronyms & Abbreviations

A – anthracene

AcP – acenaphthene

AcP-d_{10} – deuterated acenaphthene

AcpY – acenaphthylene

ATSDR – Agency for Toxic Substances and Disease Registry

BaA – benzo[a]anthracene

BaP – benzo[a]pyrene

BbFL – benzo[b]fluoranthene

BkFL – benzo[k]fluoranthene

BP – benzo[g,h,i]perylene

BZ – benzene rings

CE – collision energy

Chr – chrysene

Chr-d_{12} – deuterated chrysene

DAF – dissolved in the aqueous fraction

DBA – dibenz[a,h]anthracene

DL-PCB – dioxin-like PCB

EPA – Environmental Protection Agency

FL – fluoranthene

Flu – fluorene

GC/MS – gas chromatography-mass spectrometry

GC-MS/MS – gas chromatography-tandem mass spectrometry

HMW – high molecular weight

IARC – International Agency for Research on Cancer

ICES – International Commission for the Exploration of the Sea

Ind – indeno[1,2,3-c,d]pyrene

ISTD – internal standards

K_{ow} – octanol-water partition coefficient

LMW – low molecular weight

LODs – limits of detection

LOQs – limits of quantification

m/z – mass-to-charge ratio

N – naphthalene

N-d_8 – deuterated naphthalene

NDL-PCB – non-dioxin-like PCB

PAHs – polycyclic aromatic hydrocarbons

PCBs – polychlorinated biphenyls

Per-d_{12} – deuterated perylene

Phe – phenanthrene

Phe-d_{10} – deuterated phenanthrene

Pyr – pyrene

r^2 – correlation coefficient

RSD – residual standard deviation

SD – standard deviation

SSM – solid suspend matter

SPE – solid-phase extraction

TCDD – 2,3,7,8-tetrachlorodibenzo-p-dioxin

TEF – toxic equivalent factor

TEQ – toxic equivalent quotient

t_R – retention time

USEPA – United States Environmental Protection Agency

V – Volt

VP – vapour pressure

WHO – World Health Organization

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Resumo

Este estudo foi realizado com o objetivo principal de avaliar a possível presença de hidrocarbonetos aromáticos policíclicos (PAHs) e bifenilos policlorados (PCBs) na zona da costa de Vila do Conde, onde se encontra uma área de reserva protegida designada por "*Reserva Ornitológica de Mindelo*".

Esta zona, que foi uma das primeiras em Portugal a receber o estatuto de área protegida, é detentora de uma grande biodiversidade principalmente de aves.

No entanto, apesar deste estatuto, esta Reserva esta encontra-se inserida a escassos km da foz do Rio Ave que além de banhar uma zona muito urbanizada e reconhecidamente industrializada tem também o porto de Vila do Conde.

Neste sentido, procedeu-se à análise em amostras de água do mar de 16 PAHs (compostos considerados como prioritários pela sua toxicidade, potencial de exposição e frequência) e de 7 PCBs sugeridos como indicadores ambientais de contaminação pela Comissão Internacional para a Exploração do Mar (CIEM).

As amostras (2 L) foram recolhidas em oito pontos distanciados entre si cerca de 500 m ao longo de aproximadamente 5 km de costa. Nestas colheitas, realizadas no inverno e na primavera de 2018, foram abrangidas quatro praias, incluindo na Reserva natural, de modo a cobrir os locais a norte e a sul da mesma.

A presença dos micropoluentes supracitados foi quantificada após a sua extração em fase sólida (SPE). Este procedimento envolveu o uso de cartuchos OASIS HLB (Waters) seguido de cromatografia gasosa com deteção de massas (GC-MS/MS).

Os resultados demonstraram a presença de PAHs e PCBs em todos os pontos de colheita. De um modo muito sucinto, observou-se que concentração média obtida para ∑16 PAHs foi de ≈ 79 ng/L e para os ∑7 PCBs foi de ≈ 50 ng/L, o que em comparação com outros locais a nível mundial nos deixa sérias preocupações sobre os riscos potenciais destes compostos em zonas de reserva e também usadas para lazer, não só dos habitantes locais mas também de turistas.

Estes resultados são reportados em primeira mão nesta área e pode constituir um começo para construção de um melhor controlo destes poluentes, como também enriquecer o conhecimento geral sobre estes nas águas marinhas europeias.

Palavras-chave: PAHs, PCBs, SPE, GC-MS/MS**,** TEQs

Abstract

This study was carried out with the main objective of evaluating the possible presence of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in the coastal zone of Vila do Conde, where exists the natural reserve designated by "Reserva Ornitológica de Mindelo".

This zone, which was one of the first in Portugal to receive the *status* of protected area, holds a great biodiversity, mainly of birds.

However, despite this status, this reserve is located just a few km away from the mouth of Rio Ave, which besides bathing a very urbanized and recognized industrial zone also holds the Vila do Conde harbour.

In this sense, 16 PAHs (compounds considered as priority because of their toxicity, exposure potential and frequency) and 7 PCBs suggested as environmental indicators of contamination by the International Commission for the Exploration of the Sea (ICES) were analysed in seawater samples.

The samples (2 L) were collected at eight points spaced from each other about 500 m along approximately 8 km of seacoast. In these harvests, held in the winter and spring of 2018, four beaches were included, including in the Natural Reserve, to cover the sites to the north and south of the same.

The presence of the aforementioned micropollutants was quantified after extraction in solid phase (SPE). This procedure involved the use of OASIS HLB (Waters) cartridges followed by gas chromatography tandem mass spectrometry (GC-MS/MS).

The results demonstrated the presence of PAHs and PCBs at all harvest points. Briefly, it was observed that the mean concentration obtained for Σ16 PAHs was ≈ 79 ng/L and for Σ7 PCBs ≈ 50 ng/L, which compared with other sites worldwide leaves us serious concerns about the potential risks of these compounds in reserve areas and also in beaches used for leisure, not only for the locals but also for tourists.

These results are the first reported in this area and can be a start to building better control of these pollutants as well as enriching the general knowledge about these pollutants in European marine waters.

Keywords: PAHs, PCBs, SPE, GC-MS/MS, TEQs

1. Introduction

It is of common knowledge that persistent pollutants are being released into the environment for many decades. This observation has originated many studies to prove that at least several compounds are having a negative influence in several species health, including in humans.

In this sense, compounds as Polycyclic Aromatic Hydrocarbons (PAHs) and Polychlorinated Biphenyls (PCBs) were investigated since their presence in the aquatic environment has been linked as the surge of toxic influences that, in the most severe situations, can conduct to endocrine disorders and even cancer (Rojo-Nieto *et al.*, 2013; Sinaei & Mashinchian, 2014). In spite of this knowledge, unfortunately, the environmental levels of these compounds are still very high, mainly in highly inhabited/industrial districts (Nwaichi & Ntorgbo, 2016).

So, it is safe to say that biomonitoring the levels of both PAHs and PCBs is an important task (Srogi, 2007).

In Portugal exists few studies concerning the presence of PAHs and PCBs in surface waters (Rocha *et al.*, 2017). In this vein, studies concerning compounds belonging to these two groups of organic pollutants are urgent.

Therefore, this particular study had its main focus in studying the presence of 16 European Protection Agency (EPA) priority PAHs and seven PCBs in Vila do Conde coastal line, an area never before investigated for the presence of these pollutants.

1.1 Polycyclic Aromatic Hydrocarbons (PAHs)

1.1.1 PAHs physicochemical properties

PAHs are a group of organic compounds that are constituted majorly by hydrogen and carbon with two or more fused benzene/aromatic rings (IARC, 2010).

They are frequently colourless, white or pale yellow solids with high melting and boiling points, low vapor pressures and low aqueous solubility (Masih *et al.*, 2012) (**Figure 1**).

Naphthalene
(**N**)
log K_{ow} = 3.30
VP = 0.085 mm Hg

Acenaphthene
(**AcP**)
log K_{ow} = 3.92
VP = 0.0022 mm Hg

Acenaphthylene
(**AcpY**)
log K_{ow} = 3.93
VP = 0.0048 mm Hg

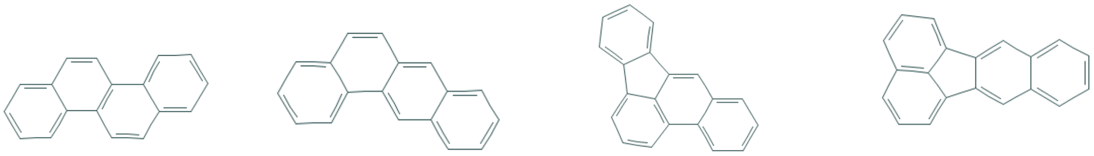
Fluorene
(**Flu**)
log K_{ow} = 4.18
VP = 6.0x10^{-4} mm Hg

Phenanthrene
(**Phe**)
log K_{ow} = 4.46
VP = 1.2 x10^{-4} mm Hg

Anthracene
(**A**)
log K_{ow} = 4.45
VP = 6.56x10^{-6} mm Hg

Fluoranthene
(**FL**)
log K_{ow} = 5.16
VP = 9.22x10^{-6} mm Hg

Pyrene
(**Pyr**)
log K_{ow} = 4.88
VP = 4.5 x10^{-6} mm Hg

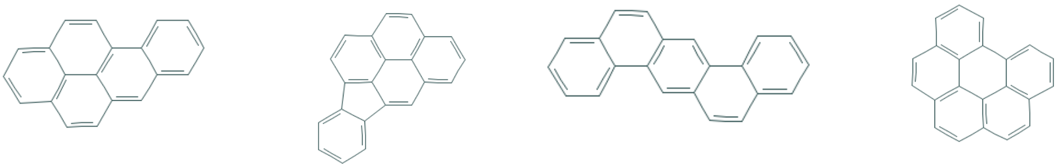


Chrysene
(**Chr**)
log K_{ow} = 5.73
VP = 6.23x10^{-9} mm Hg

Benz[a]anthracene
(**BaA**)
log K_{ow} = 5.76
VP = 2.1x10^{-7} mm Hg

Benzo[b]fluoranthene
(**BbFL**)
log K_{ow} = 5.78
VP = 5.0x10^{-7} mm Hg

Benzo[k]fluoranthene
(**BkFL**)
log K_{ow} = 6.11
VP = 9.65x10^{-10} mm Hg



Benzo[a]pyrene
(**BaP**)
log K_{ow} = 6.13
VP = 5.49x10^{-9} mm Hg

Indeno[1,2,3-cd]pyrene
(**Ind**)
log K_{ow} = 6.70
VP = 1.25x10^{-10} mm Hg

Dibenz[a,h]anthracene
(**DBA**)
log K_{ow} = 6.50
VP = 9.55x10^{-10} mm Hg

Benzo[g,h,i]perylene
(**BP**)
log K_{ow} = 6.63
VP = 1.0x10^{-10} mm Hg

K_{ow} - n-octanol-water partition coefficient; VP – Vapour pressure at 25 ºC.

Figure 1 – Chemical structure and some physicochemical properties of the 16 EPA priority PAHs which will be studied herein. Accessed on September 15th, 2018 (pubchem.ncbi.nlm.nih.gov).

It is important to refer that PAHs can differentiate in their interaction behaviour with organic matter mainly because of their physicochemical properties, namely log K_{ow} and vapor pressure (**Figure 1**). Generally, PAHs are divided in two categories which are light PAHs that contain up to four rings, and heavy PAHs, that have more than four rings, making them more stable and determine their degree of toxicity (Lawal, 2017).

1.1.2 PAHs origins

The PAHs can appear from natural or anthropologic processes (Lung & Liu, 2015). The anthropologic origins remain mainly in the production or combustion of fossil fuels (Abdel-Shafy & Mansour, 2016; Abrajano *et al.*, 2003). They are classified accordingly to their origin as pyrogenic, petrogenic or diagenic (Abdel-Shafy & Mansour, 2016; Abrajano *et al.*, 2003).

The pyrogenic PAHs are the result of a short-duration incomplete combustion at high temperatures of petroleum derivate or biomass, e.g., forest fires. On the contrary, the ones with petrogenic cause aren't formed by the combustion, but instead are the result of the low temperature maturation from crude oil or others similar processes from coal and the by-products of both. The diagenic ones are the result of biogenic precursors, such as plant terpenes (Bastami *et al.*, 2013).

Although these compounds aren't synthetized for the industry, they can be used as intermediates for several processes. For this reason they are present in pigments, plastic, preserving diluents, paints and others.

1.1.3 PAHs toxicity

The PAHs physicochemical properties, as referred before, allow them to be transported over large territorial expanses and, furthermore reach the atmosphere (Malik *et al*., 2011). In this way their presence occurs in all terrestrial environments, even in the most remote and uninhabited areas, like the Artic (Ma *et al*., 2013).

The ecological risks of these contaminants in aquatic environments are associated with their intrinsic toxicity, environmental persistence, bioaccumulation and trophic transfer (Bastami *et al*., 2013). In general, the toxic effects of contaminants are listed as acute and chronic, mutagenic, carcinogenic and immune suppressors (Rajendran *et al*., 2013).

Of all the PAHs, the **BaP**, is classified as carcinogenic for humans, but other seven, **BaA**, **Chr**, **BbFL**, **BkFL**, **DBA**, **Ind** and **BP**, are classified as possible carcinogens (IARC, 2010). It's already proved that PAHs are hydrophobic and tend to bond with organic matter. When this happens they normally bond with sediments and in contact with animals they accumulate in their fate tissue (IARC, 2010; Qiu *et al.*, 2009).

So, in consequence they tend to accumulate in the food chain and for that reason are a risk to aquatic ecosystems and latter to human health (Malik *et al.*, 2011).

Humans are exposed to this contaminants in several ways such as, daily and occupational activities, tobacco smoke, some pharmaceutical products and even food (Abdel-Shafy & Mansour, 2016; IARC, 2010). For example, since there are result from incomplete combustion they can be present in meat when it suffers grilling process (Malik *et al.*, 2011) and can also be present in species with a shell since they are in permanent contact with water and sediments because they filtered them (Nwaichi & Ntorgbo, 2016).

1.2 Polychlorinated biphenyls (PCBs)

1.2.1 PCBs physicochemical properties

PCBs are made by the chlorination of biphenyl and compress a list of 209 individual chemicals, but is important to refer that each one can have different interaction behaviours with organic matter. In this case, the less-chlorinated is the congener, more soluble will be in water, as well as more volatile and easier to biodegrade (Beyer & Biziuk, 2009).

In this list of 209 congeners, the molecular configuration can variate (**Figure 2**), given them different toxicity and behaviour depending upon the chlorine position in the phenyl ring (WHO, 2016).

In terms of chemical structure they all possess the same two rings configuration and are differentiated by congeners because of the position and number of the chlorine atoms. In each congener a "BZ number" was attributed and because of that there nominated from **PCB_1** to **PCB_{209}** (IARC, 2016) (**Table 1**).

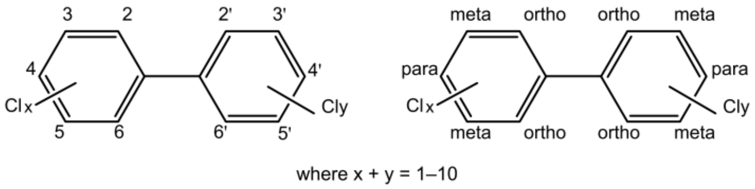
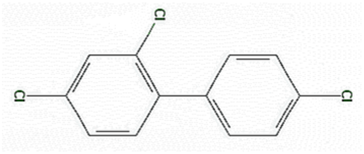
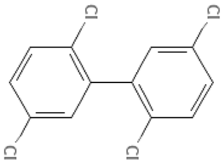


Figure 2 – General structure of PCBs, explaining the number and denomination of the possible substitutions in the phenyl rings. Image extracted from WHO (2016).

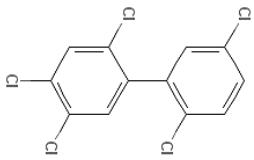
In this study will be considered seven PCBs (**Figure 3**), which taken together with the above referred 16 PAHs will give precious informations about the water quality of marine environments (Elia *et al.*, 2005; Keith, 2015).



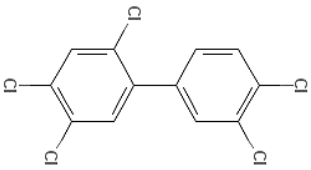
PCB$_{28}$
log K_{ow} = 5.67
VP = 4.1x10^{-4} mm Hg



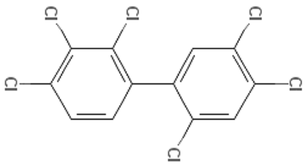
PCB$_{52}$
log K_{ow} = 5.84
VP = 9.0x10^{-5} mm Hg



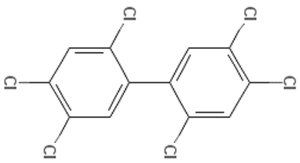
PCB$_{101}$
log K_{ow} = 6.38
VP = 2.0x10^{-5} mm Hg



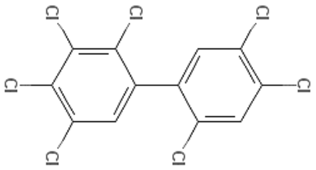
PCB$_{118}$
log K_{ow} = 6.74
VP = 2.0x10^{-5} mm Hg



PCB$_{138}$
log K_{ow} = 6.83
VP = 4.4x10^{-6} mm Hg



PCB$_{153}$
log K_{ow} = 6.92
VP = 4.4x10^{-6} mm Hg



PCB$_{180}$
log K_{ow} = 7.36
VP = 9.8x10^{-7} mm Hg

K_{ow} - n-octanol-water partition coefficient; VP – Vapour pressure at 25 ºC

Figure 3 – Chemical structure and some physicochemical properties of the seven PCBs which will be studied herein (IARC, 2016).

This seven PCBs tagged as regulation-relevant congeners are the **28**, **52**, **101**, **118**, **138**, **153** and **180** PCBs and constitute the non-dioxin-like PCBs (NDL-PCBs) group (Elia *et al.*, 2005).

Table 1 – Correspondence between the number of benzene rings (BZ) and position of chlorine atoms on each phenyl ring of the PCBs. Adapted from IARC (2016).

Position of chlorine atom on each ring	2	3	4	2,3	2,4	2,5	2,6	3,4	3,5	2,3,4	2,3,5	2,3,6	2,4,5	2,4,6	3,4,5	2,3,4,5	2,3,4,6	2,3,5,6	2,3,4,5,6
None	1	2	3	5	7	9	10	12	14	21	23	24	29	30	38	61	62	65	116
2'	4	6	8	16	17	18	19	33	34	41	43	45	48	50	76	86	88	93	142
3'		11	13	20	25	26	27	35	36	55	57	59	67	69	78	106	108	112	160
4'			15	22	28	31	32	37	39	60	63	64	74	75	81	114	115	117	166
2',3'				40	42	44	46	56	58	82	83	84	97	98	122	129	131	134	173
2',4'					47	49	51	66	68	85	90	91	99	100	123	137	139	147	181
2',5'						52	53	70	72	87	92	95	101	103	124	141	144	151	185
2',6'							54	71	73	89	94	96	102	104	125	143	145	152	186
3',4'								77	79	105	109	110	118	119	126	156	158	163	190
3',5'									80	107	111	113	120	121	127	159	161	165	192
2',3',4'										128	130	132	138	140	157	170	171	177	195
2',3',5'											133	135	146	148	162	172	175	178	198
2',3',6'												136	149	150	164	174	176	179	200
2',4',5'													153	154	167	180	183	187	203
2',4',6'														155	168	182	184	188	204
3',4',5'															169	189	191	193	205
2',3',4',5'																194	196	199	206
2',3',4',6'																	197	201	207
2',3',5',6'																		202	208
2',3',4',5',6'																			209

Note: Dioxin-like PCBs are highlighted.

This seven indicators are recommended to characterize the contamination of PCBs because they are found in high concentrations in the environment, in food and consequently in humans (WHO, 2016). In **Table 2** are highlighted the homologue groups where the above referred PCBs are included.

Table 2 – Physical and chemical properties of PCBs according to homologue group. Adapted from IARC (2016).

Homologue group	Nº of isomers	BZ Nº	Chlorine (% w/w)	Vapour pressure (Pa at 25 °C)	Melting point (°C)	Boiling point (°C)
Monochlorobiphenyl	3	1–3	18.79	1.1	25 – 77.9	285
Dichlorobiphenyl	12	4–15	31.77	0.24	24.4 – 149	312
Trichlorobiphenyl	24	16–39	41.30	0.054	28 – 87	337
Tetrachlorobiphenyl	42	40–81	48.65	0.012	47 – 180	360
Pentachlorobiphenyl	46	82–127	54.30	$2.6.10^{-3}$	76.5 – 124	381
Hexachlorobiphenyl	42	128–169	58.93	$5.8.10^{-4}$	77 – 200	400
Heptachlorobiphenyl	24	170–193	62.77	$1.3.10^{-4}$	83 – 149	417
Octachlorobiphenyl	12	194–205	65.98	$2.8.10^{-5}$	159 – 162	432
Nonachlorobiphenyl	3	206–208	68.73	$6.3.10^{-6}$	182.8 – 206	445
Decachlorobiphenyl	1	209	71.10	$1.4.10^{-6}$	305.9	456

1.2.2 PCBs origins

After the end of World War II, PCBs were intentionally produced because they were considered stable molecules with low chemical reactivity (Beyer & Biziuk, 2009; EPA, 2009). These were used as fluid insulators, in high-voltage electric transformers, in high-capacity condensers, as heat exchangers, pesticide extenders, and adhesives, dedusting agents, components of cutting oils, flame retardants, hydraulic lubricants, and components of plasticizers in paints, inks, toners, and printing inks (Henry *et al.*, 2003).

This compounds were banned in 1985, making their use and production very restricted in Europe (EC, 2001).

1.2.3 PCBs toxicity

PCBs major structure is based in carbon atoms, fact that allow them to easily interact and bound with organic matter present in the soil, water and air.

Once in the environment, PCBs are of great concern because of their persistence, easiness of transportation, bioaccumulation capacity in the trophic chain, and high toxicity (Huang *et al.*, 2014).

Actually it is known that can cause damage such as cancer, allergies and hypersensitivity, damage to the central and peripheral nervous systems, reproductive disorders, and disruption of the immune system (Stockholm Convention, 2009).

1.3 Presence of PAHs and PCBs in marine environment

Presently, the coastal marine environments are being a target of major concern in terms of ecological risk from these compounds (Wu *et al.*, 2011).

Until now, few measures have been taken in order to control the presence and release of both PAHs and PCBs in the environment, namely in the aquatic compartment, as it can be shown in **Tables 3** and **4**.

It is observed that PAHs are found in surface waters in higher concentrations (**Table 3**) when compared to PCBs. This can be explained because PAHs are continuously released from several sources, as reported above (*item 1.1.2*).

Briefly, the PAHs presence in aquatic systems is mainly due to anthropogenic actions such as industrial wastewaters, atmospheric emissions, oil spills and pyrolytic sources (incomplete combustion of fossil fuels or organic matter) (Rojo-Nieto *et al.*, 2013).

Thereafter, since PAHs are highly hydrophobic, they are rapidly adsorbed by the particulate matter present in the surface water, which makes them able to easily propagate to worldwide (Tiwari *et al.*, 2017).

Table 3 – Environmental concentrations (minimum – maximum) of PAHs in superficial marine waters worldwide (*continues in the next pages*). DL – detection limit; ND – not detected.

PAHs	Localization	Concentration (ng/L)	Reference
	Leça e Matosinhos (Portugal)	4.2 - 4.7	Rocha *et al.* (2017)
	Bay of Algeciras (Spain)	<DL - 183	Rojo-Nieto *et al.* (2013)
	Persian Gulf (Iraq)	ND	Sinaei & Mashinchian (2014)
	Persian Gulf (Iran)	4.3 - 7.2	Agah *et al.* (2016)
N	Thane Creek (India)	34.0 - 170	Tiwari *et al.* (2017)
	Bohai Bay (China)	8.8 - 437	Jia & Tian (2017)
	East Asia (China)	0.001 - 0.03	Ma *et al.* (2013)
	North Pacific (China)	0.003 - 0.01	Ma *et al.* (2013)
	High Artic (Artic)	ND - 0.004	Ma *et al.* (2013)
	Leça e Matosinhos (Portugal)	< 0.2 - 0.5	Rocha *et al.* (2017)
	Bay of Algeciras (Spain)	0.8 - 30.9	Rojo-Nieto *et al.* (2013)
	Persian Gulf (Iraq)	810 - 2634	Sinaei & Mashinchian (2014)
	Persian Gulf (Iran)	4.2 - 5.3	Agah *et al.* (2016)
AcpY	Thane Creek (India)	4.1 - 12.4	Tiwari *et al.* (2017)
	Bohai Bay (China)	0.1 - 2.2	Jia & Tian (2017)
	East Asia (China)	0.00001 - 0.003	Ma *et al.* (2013)
	North Pacific (China)	0.0001 - 0.002	Ma *et al.* (2013)
	High Artic (Artic)	0.0001 0.001	Ma *et al.* (2013)
	Leça e Matosinhos (Portugal)	< 0.7 - 0.9	Rocha *et al.* (2017)
	Bay of Algeciras (Spain)	<DL - 3.1	Rojo-Nieto *et al.* (2013)
	Persian Gulf (Iraq)	ND	Sinaei & Mashinchian (2014)
	Persian Gulf (Iran)	2.9 - 3.0	Agah *et al.* (2016)
AcP	Thane Creek (India)	2.5 - 5.6	Tiwari *et al.* (2017)
	Bohai Bay (China)	0.7 - 25.0	Jia & Tian (2017)
	East Asia (China)	0.0003 - 0.05	Ma *et al.* (2013)
	North Pacific (China)	0.0009 - 0.01	Ma *et al.* (2013)
	High Artic (Artic)	0.002 - 0.01	Ma *et al.* (2013)
	Leça e Matosinhos (Portugal)	< 0.3 - < 0.9	Rocha *et al.* (2017)
	Bay of Algeciras (Spain)	<DL	Rojo-Nieto *et al.* (2013)
	Persian Gulf (Iraq)	286 - 1123	Sinaei & Mashinchian (2014)
	Persian Gulf (Iran)	3.7 - 3.8	Agah *et al.* (2016)
Flu	Thane Creek (India)	21.3 - 50.0	Tiwari *et al.* (2017)
	Bohai Bay (China)	2.7 - 53.2	Jia & Tian (2017)
	East Asia (China)	0.003 - 0.06	Ma *et al.* (2013)
	North Pacific (China)	0.004 - 0.01	Ma *et al.* (2013)
	High Artic (Artic)	0.002 - 0.007	Ma *et al.* (2013)

PAHs	Localization	Concentration (ng/L)	Reference
Phe	Leça e Matosinhos (Portugal)	1.9 - 8.9	Rocha *et al.* (2017)
	Bay of Algeciras (Spain)	<DL	Rojo-Nieto *et al.* (2013)
	Persian Gulf (Iraq)	373 - 11104	Sinaei & Mashinchian (2014)
	Persian Gulf (Iran)	2.9 - 17.5	Agah *et al.* (2016)
	Thane Creek (India)	100.1 - 250.0	Tiwari *et al.* (2017)
	Bohai Bay (China)	5.2 - 108	Jia & Tian(2017)
	East Asia (China)	0.004 - 0.09	Ma *et al.* (2013)
	North Pacific (China)	0.004 - 0.02	Ma *et al.* (2013)
	High Artic (Artic)	0.002 - 0.01	Ma *et al.* (2013)
A	Leça e Matosinhos (Portugal)	< 2.6 - 6.5	Rocha *et al.* (2017)
	Bay of Algeciras (Spain)	<DL	Rojo-Nieto *et al.* (2013)
	Persian Gulf (Iraq)	ND	Sinaei & Mashinchian (2014)
	Persian Gulf (Iran)	11.1 - 14.0	Agah *et al.* (2016)
	Thane Creek (India)	20.1 - 40.2	Tiwari *et al.* (2017)
	Bohai Bay (China)	0.9 - 11.5	Jia & Tian (2017)
	East Asia (China)	0.0008 - 0.01	Ma *et al.* (2013)
	North Pacific (China)	0.0008 - 0.002	Ma *et al.* (2013)
	High Artic (Artic)	0.0003 - 0.001	Ma *et al.* (2013)
FL	Leça e Matosinhos (Portugal)	< 0.5 - < 1.6	Rocha *et al.* (2017)
	Bay of Algeciras (Spain)	<DL	Rojo-Nieto *et al.* (2013)
	Persian Gulf (Iraq)	734 - 2818	Sinaei & Mashinchian (2014)
	Persian Gulf (Iran)	16.5 - 23.9	Agah *et al.* (2016)
	Thane Creek (India)	18.2 - 21.0	Tiwari *et al.* (2017)
	Bohai Bay (China)	2.1 - 27.6	Jia & Tian (2017)
	East Asia (China)	0.003 - 0.06	Ma *et al.* (2013)
	North Pacific (China)	0.002 - 0.02	Ma *et al.* (2013)
	High Artic (Artic)	0.0002 - 0.004	Ma *et al.* (2013)
Pyr	Leça e Matosinhos (Portugal)	3.1 - 4.8	Rocha *et al.* (2017)
	Bay of Algeciras (Spain)	<DL	Rojo-Nieto *et al.* (2013)
	Persian Gulf (Iraq)	125 - 348	Sinaei & Mashinchian (2014)
	Persian Gulf (Iran)	7.7 - 35.3	Agah *et al.* (2016)
	Thane Creek (India)	90.2 - 107	Tiwari *et al.* (2017)
	Bohai Bay (China)	5.1 - 45.0	Jia & Tian (2017)
	East Asia (China)	0.002 - 0.1	Ma *et al.* (2013)
	North Pacific (China)	0.004 - 0.02	Ma *et al.* (2013)
	High Artic (Artic)	0.001 - 0.01	Ma *et al.* (2013)

PAHs	Localization	Concentration (ng/L)	Reference
BaA	Leça e Matosinhos (Portugal)	3.6 - 7.2	Rocha *et al.* (2017)
	Bay of Algeciras (Spain)	<DL	Rojo-Nieto *et al.* (2013)
	Persian Gulf (Iraq)	308 - 565	Sinaei & Mashinchian (2014)
	Persian Gulf (Iran)	22.6	Agah *et al.* (2016)
	Thane Creek (India)	10.2 - 16.5	Tiwari *et al.* (2017)
	Bohai Bay (China)	0.1 - 1.8	Jia & Tian (2017)
	East Asia (China)	0.0004 - 0.01	Ma *et al.* (2013)
	North Pacific (China)	0.0003 - 0.002	Ma *et al.* (2013)
	High Artic (Artic)	0.0001 - 0.0009	Ma *et al.* (2013)
Chr	Leça e Matosinhos (Portugal)	3.1 - 5.2	Rocha *et al.* (2017)
	Bay of Algeciras (Spain)	<DL - 202.8	Rojo-Nieto *et al.* (2013)
	Persian Gulf (Iraq)	26360	Sinaei & Mashinchian (2014)
	Persian Gulf (Iran)	3.8	Agah *et al.* (2016)
	Thane Creek (India)	9.6 - 10.5	Tiwari *et al.* (2017)
	Bohai Bay (China)	0.2 - 4.4	Jia & Tian (2017)
	East Asia (China)	0.0005 - 0.05	Ma *et al.* (2013)
	North Pacific (China)	0.001 - 0.01	Ma *et al.* (2013)
	High Artic (Artic)	0.0003 - 0.002	Ma *et al.* (2013)
BbFL	Leça e Matosinhos (Portugal)	10.0 - 10.2	Rocha *et al.* (2017)
	Bay of Algeciras (Spain)	<DL	Rojo-Nieto *et al.* (2013)
	Persian Gulf (Iraq)	ND	Sinaei & Mashinchian (2014)
	Persian Gulf (Iran)	4.1 - 7.0	Agah *et al.* (2016)
	Thane Creek (India)	1.4 - 4.2	Tiwari *et al.* (2017)
	Bohai Bay (China)	0.5 - 2.1	Jia & Tian (2017)
	East Asia (China)	0.0002 - 0.01	Ma *et al.* (2013)
	North Pacific (China)	0.0001 - 0.001	Ma *et al.* (2013)
	High Artic (Artic)	0.0001 - 0.0011	Ma *et al.* (2013)
BkFL	Leça e Matosinhos (Portugal)	6.6 - 6.8	Rocha *et al.* (2017)
	Bay of Algeciras (Spain)	<DL - 154.1	Rojo-Nieto *et al.* (2013)
	Persian Gulf (Iraq)	ND	Sinaei & Mashinchian (2014)
	Persian Gulf (Iran)	23.8 - 107	Agah *et al.* (2016)
	Thane Creek (India)	10.2 - 10.8	Tiwari *et al.* (2017)
	Bohai Bay (China)	< 0.24 - 1.1	Jia & Tian (2017)
	East Asia (China)	0.0002 - 0.01	Ma *et al.* (2013)
	North Pacific (China)	0.0001 - 0.001	Ma *et al.* (2013)
	High Artic (Artic)	0.0001 - 0.001	Ma *et al.* (2013)

PAH	Localization	Concentration (ng/L)			Reference
BaP	Leça e Matosinhos (Portugal)	2.3	-	2.9	Rocha *et al.* (2017)
	Bay of Algeciras (Spain)	<DL	-	122	Rojo-Nieto *et al.* (2013)
	Persian Gulf (Iraq)	741			Sinaei & Mashinchian (2014)
	Persian Gulf (Iran)	2.6			Agah *et al.* (2016)
	Thane Creek (India)	5.1	-	6.2	Tiwari *et al.* (2017)
	Bohai Bay (China)	0.2	-	1.5	Jia & Tian (2017)
	East Asia (China)	0.0001	-	0.002	Ma *et al.* (2013)
	North Pacific (China)	0.000004	-	0.0004	Ma *et al.* (2013)
	High Artic (Artic)	0.000003	-	0.0002	Ma *et al.* (2013)
Ind	Leça e Matosinhos (Portugal)	< 1.8	-	1.9	Rocha *et al.* (2017)
	Bay of Algeciras (Spain)	<DL			Rojo-Nieto *et al.* (2013)
	Persian Gulf (Iraq)	ND			Sinaei & Mashinchian (2014)
	Persian Gulf (Iran)	875			Agah *et al.* (2016)
	Thane Creek (India)	1.7	-	1.8	Tiwari *et al.* (2017)
	Bohai Bay (China)	< 0.15	-	1.6	Jia & Tian (2017)
	East Asia (China)	0.0001	-	0.001	Ma *et al.* (2013)
	North Pacific (China)	0.00003	-	0.0005	Ma *et al.* (2013)
	High Artic (Artic)	ND	-	0.0002	Ma *et al.* (2013)
DBA	Leça e Matosinhos (Portugal)	1.7	-	2.3	Rocha *et al.* (2017)
	Bay of Algeciras (Spain)	<DL	-	85.3	Rojo-Nieto *et al.* (2013)
	Persian Gulf (Iraq)	ND			Sinaei & Mashinchian (2014)
	Persian Gulf (Iran)	0.2			Agah *et al.* (2016)
	Thane Creek (India)	1.2	-	1.9	Tiwari *et al.* (2017)
	Bohai Bay (China)	<0.03	-	1.1	Jia & Tian (2017)
	East Asia (China)	0.0001	-	0.001	Ma *et al.* (2013)
	North Pacific (China)	ND	-	0.001	Ma *et al.* (2013)
	High Artic (Artic)	ND	-	0.0003	Ma *et al.* (2013)
BP	Leça e Matosinhos (Portugal)	4.8			Rocha *et al.* (2017)
	Bay of Algeciras (Spain)	<DL	-	101	Rojo-Nieto *et al.* (2013)
	Persian Gulf (Iraq)	ND			Sinaei & Mashinchian (2014)
	Persian Gulf (Iran)	4.2			Agah *et al.* (2016)
	Thane Creek (India)	2.1	-	3.1	Tiwari *et al.* (2017)
	Bohai Bay (China)	<0.14	-	1.3	Jia & Tian (2017)
	East Asia (China)	0.00004		0.001	Ma *et al.* (2013)
	North Pacific (China)	0.00002	-	0.0004	Ma *et al.* (2013)
	High Artic (Artic)	0.00001	-	0.001	Ma *et al.* (2013)

In the case of PCBs, one of the major reason for their persistency in the environment is in part because they possess chlorine atoms (Beyer & Biziuk, 2009). As mentioned above (*item 1.2.2.*), PCBs were banned from the industry many years ago and continue to persist in the environment, even though in lower levels than before (**Table 4**).

The possible present releases in the environment of this pollutants could be explain by illegal disposal of PCBs waste or poorly maintenance of waste hazardous (municipal or other), that could still contain electrical transformers that leaks PCBs (EPA, 2018). Therefore, this can also explain their transportation into the marine environments.

Table 4 – Environmental concentrations (minimum – maximum) of PCBs in superficial marine waters worldwide (*continues in the next pages*). DL – detection limit; ND – not detected.

PCBs	Localization	Concentration (ng/L)	Reference
PCB$_{28}$	North Atlantic (Azores region)	0.004 - 0.005	Lammel *et al.* (2017)
	North Atlantic, (between Azores and Jamaica)	0.003 - 0.004	Lammel *et al.* (2017)
	Barcelona (Spain)	0.03 - 0.1	García-Flor *et al.* (2009)
	Banyuls-sur-Mer (France)	0.06 - 0.1	García-Flor *et al.* (2009)
	Aegean Sea (Greece)	< 0.005	Lammel *et al.* (2015)
	Atlantic Ocean (Germany to South Africa)	0.0001 - 0.0004	Gioia *et al.* (2008)
	Equatorial Indian Ocean (between India and Indonesia)	0.001 - 0.01	Huang *et al.* (2014)
PCB$_{101}$	North Atlantic (Azores region)	0.003 - 0.005	Lammel *et al.* (2017)
	North Atlantic, (between Azores and Jamaica)	0.003 - 0.004	Lammel *et al.* (2017)
	Barcelona (Spain)	0.28 - 0.32	García-Flor *et al.* (2009)
	Banyuls-sur-Mer (France)	0.25 - 0.52	García-Flor *et al.* (2009)
	Aegean Sea (Greece)	< 0.0008	Lammel *et al.* (2015)
	Atlantic Ocean (Germany to South Africa)	ND - 0.0004	Gioia *et al.* (2008)
	Equatorial Indian Ocean (between India and Indonesia)	ND - 0.0007	Huang *et al.* (2014)

PCBs	Localization	Concentration (ng/L)	Reference
PCB_{52}	North Atlantic (Azores region)	0.004 - 0.006	Lammel *et al.* (2017)
	North Atlantic, (between Azores and Jamaica)	0.0026 - 0.0031	Lammel *et al.* (2017)
	Barcelona (Spain)	0.28 - 0.32	García-Flor *et al.* (2009)
	Banyuls-sur-Mer (France)	0.61 - 0.96	García-Flor *et al.* (2009)
	Aegean Sea (Greece)	0.0007	Lammel *et al.* (2015)
	Atlantic Ocean (Germany to South Africa)	0.00002 - 0.0004	Gioia *et al.* (2008)
	Equatorial Indian Ocean (between India and Indonesia)	ND - 0.002	Huang *et al.* (2014)
PCB_{138}	North Atlantic (Azores region)	0.003 - 0.004	Lammel *et al.* (2017)
	North Atlantic, (between Azores and Jamaica)	0.001 - 0.004	Lammel *et al.* (2017)
	Barcelona (Spain)	0.07 - 0.1	García-Flor *et al.* (2009)
	Banyuls-sur-Mer (France)	0.33 - 0.67	García-Flor *et al.* (2009)
	Aegean Sea (Greece)	< 0.001	Lammel *et al.* (2015)
	Atlantic Ocean (Germany to South Africa)	ND - 0.0002	Gioia *et al.* (2008)
	Equatorial Indian Ocean (between India and Indonesia)	ND - 0.0001	Huang *et al.* (2014)
PCB_{118}	North Atlantic (Azores region)	0.001 - 0.002	Lammel *et al.* (2017)
	North Atlantic, (between Azores and Jamaica)	0.0007 - 0.002	Lammel *et al.* (2017)
	Barcelona (Spain)	0.07 - 0.12	García-Flor *et al.* (2009)
	Banyuls-sur-Mer (France)	0.22 - 0.55	García-Flor *et al.* (2009)
	Aegean Sea (Greece)	< 0.0006	Lammel *et al.* (2015)
	Atlantic Ocean (Germany to South Africa)	ND - 0.0001	Gioia *et al.* (2008)
	Equatorial Indian Ocean (between India and Indonesia)	ND - 0.0006	Huang *et al.* (2014)

PCBs	Localization	Concentration (ng/L)	Reference
PCB_{153}	North Atlantic (Azores region)	0.0040 - 0.0041	Lammel *et al.* (2017)
	North Atlantic, (between Azores and Jamaica)	0.001 - 0.005	Lammel *et al.* (2017)
	Barcelona (Spain)	0.07 - 0.118	García-Flor *et al.* (2009)
	Banyuls-sur-Mer (France)	0.25 - 0.52	García-Flor *et al.* (2009)
	Aegean Sea (Greece)	< 0.001	Lammel *et al.* (2015)
	Atlantic Ocean (Germany to South Africa)	ND - 0.0002	Gioia *et al.* (2008)
	Equatorial Indian Ocean (between India and Indonesia)	ND - 0.0005	Huang *et al.* (2014)
PCB_{180}	North Atlantic (Azores region)	< 0.002 - 0.001	Lammel *et al.* (2017)
	North Atlantic (between Azores and Jamaica)	< 0.004 - 0.0003	Lammel *et al.* (2017)
	Barcelona (Spain)	0.01 - 0.008	García-Flor *et al.* (2009)
	Banyuls-sur-Mer (France)	0.035 - 0.09	García-Flor *et al.* (2009)
	Aegean Sea (Greece)	< 0.0042	Lammel *et al.* (2015)
	Atlantic Ocean (Germany to South Africa)	ND - 0.0001	Gioia *et al.* (2008)
	Equatorial Indian Ocean (between India and Indonesia)	ND	Huang *et al.* (2014)

1.4 Gas chromatography as a form of PAHs and PCBs quantification

In the examples shown in the **Tables 3** and **4** noticed that these two groups of micropollutants are in seawaters in extremely small amounts, in the order of the ng/L. This means that it is important to use a analysis technique that allow to detect accurately the low amounts of these compounds and provide a solid evidence of their identity and magnitude (Pitarch *et al.*, 2007).

Since the marine matrix shows large complexity, this can set difficulties in the detection and quantification of the target compounds (Sánchez-Avila *et al.*, 2011).

Therefore, since both PAHs and PCBs are volatile compounds, the analytic method that is usually used is the Gas Chromatography (GC) (Dewulf *et al.*, 2002). This method provides high selectivity and resolution, good accuracy and precision, wide dynamic concentration range and high sensitivity and is still innovating in the past years in order to satisfy the wide variety of compounds present in the environment (Santos & Galceran, 2002).

In fact, GC coupled to mass spectrometry (MS) has proven that is a very advantageous methodology in the study of organic pollutants, however now there is a higher tendency to use tandem mass spectrometry (MS/MS) as a more sensitive approach quantification and confirmation of the analysis (Pitarch *et al.*, 2007).

This GC-MS/MS methodology is more selective because it uses a two stage of mass analysis and more sensitive by showing the precursor and product ions, which provides good identification points and a one run analysis of a wide range of compounds with a reduction of chemical noise (Sánchez-Avila *et al.*, 2011).

In this sense, all analysis for the quantification of PAHs and PCBs in the seawater samples collected from Vila do Conde seacoast were analysed by GC-MS/MS.

1.5 Present study objectives

Our study area located at the seacoast of Vila do Conde was considered a region of great interest because it contains high frequented beaches, has protected sites such as the Regional Protected Landscape of the Coast of Vila do Conde and the Ornithological Reserve of Mindelo. Nonetheless, the proximity of high urbanized and industrialized centre (Ave River valley) is a serious concern and no data exists about the presence of organic pollutants in this area.

In this context the main objectives of this study were:

1. To investigate the presence of PAHs and PCBs dissolved in seawater samples collected from eight sites along the above referred protected areas;
2. Verify if there was a toxic risk promoted by these compounds at the protected zones;
3. Conclude about the seawater quality of this area;
4. Improve the GC-MS protocol, already in usage by our group for the analysis of PAHs and PCBs, into a more robust technique the GC-MS/MS.

2. Materials and Methods

2.1. Sampling area

The sampling area covers the north of the Greater Porto city area seacoast (Portugal, **Figure 4**), in Vila do Conde. This zone comprises a longshore course (≈ 8 km) that started in Azurara, a beach at Vila do Conde and near the Ave river estuary, and ended in Areia beach.

This particular area has a Regional Protected Coastal Landscape *status* and also a birdwatching reserve known as the Ornithological Reserve of Mindelo (DR, 2009). This course presents a wide variety of flora and fauna species, manly avifauna, and has a unique variety of landscapes such as dunes, cliffs and wetlands.

2.2. Water collection and physicochemical measurements

Seawater samples from eight sampling sites (S_1 to S_8) from four beaches (**A** to **D**) (**Figure 4**), were collected in 2018 during ebb tide, in winter (18th January) and spring (19th April) and collected into amber glass bottles (2 L), pre-rinsed with local water. The samples were collected with an 500 m distance from each other, which means that each site had two sampling points and was discriminated as the follow: Azurara (S_1 - 41°20'23.0"N 8°44'45.7"W; S_2 - 41°20'11.5"N 8°44'28.7"W), Árvore (S_3 - 41°19'59.9"N 8°44'24.0"W ; S_4 - 41°19'40.2"N 8°44'21.9"W), Reserva (S_5 - 41°19'24.3"N 8°44'21.8"W; S_6 - 41°19'04.4"N 8°44'23.9"W), Areia (S_7 - 41°18'43.2"N 8°44'24.8"W; S_8 - 41°18'39.3"N 8°44'26.8"W).

Temperature was measured on site. During transport to the laboratory, the flasks were stored at ca. 4 ºC. Immediately after arrival at the laboratory, the levels of pH were measured using a Basic Meter PB-11 (Sartorius, Germany) with a pH glass electrode (Hach Comp., USA). Nitrites (NO_2^-) (Merck KgaA, Germany), nitrates (NO_3^-) (Merck KgaA, Germany), ammonium (NH_4^+) (Xylem Analytics, Germany) and phosphates (PO_4^-) (Xylem Analytics, Germany) were measured using the Palintest 7000 Interface Photometer and the adequate kits for evaluations in seawater. Then the remaining waters were prepared for further chemical analysis.

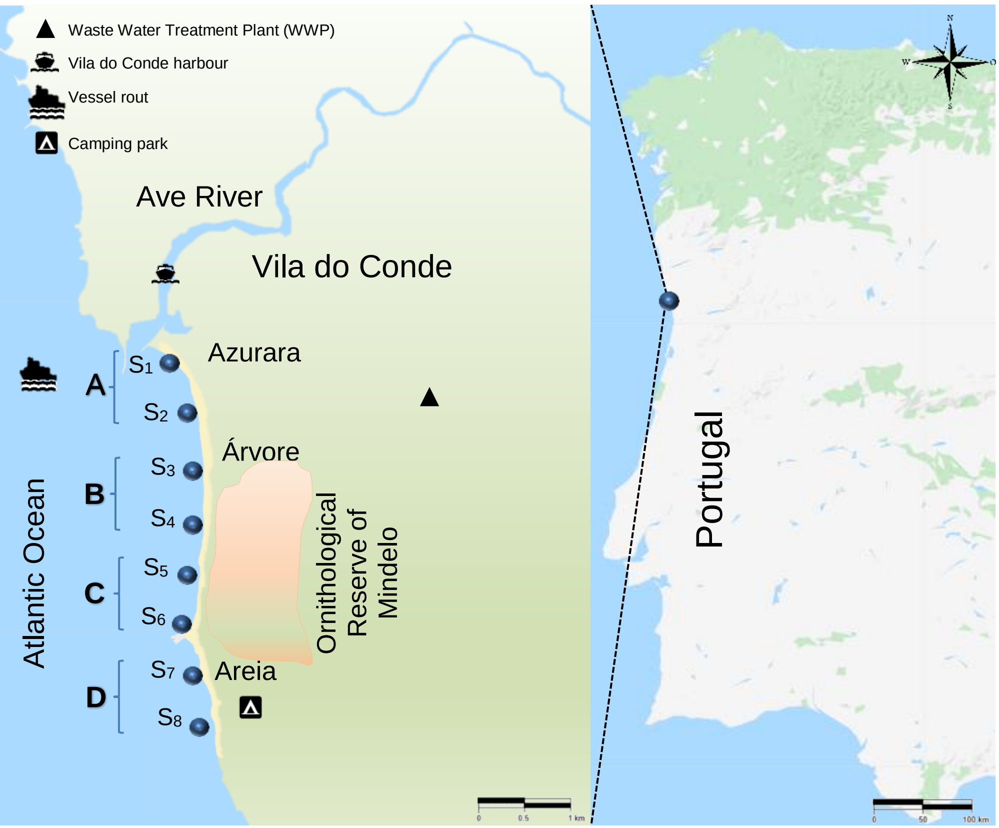


Figure 4 – Map of sampling sites. S_1 to S_4 and S_7 to S_8 are Atlantic public beaches at Vila do Conde Coast and S_5 and S_6 are located at the Ornithological Reserve beach of Mindelo. The sources that may contribute to higher amounts of PAHs and PCBs are referred in this figure. Letters **A**, **B**, **C**, and **D** refer to each of the four sampled areas.

2.3. Reagents, solutions and standards

PAHs standards (EPA TCL Polynuclear Aromatic Hydrocarbons mix) were purchased from Supelco (Bellefonte, PA). This stock mixture contained the 16 EPA priority PAHs, each at 2000 µg/mL in dichloromethane: benzene (1:1, v/v). The surrogate internal standard (IS) was a mixture containing naphthalene-d_8 (**N-d_8**), acenaphthene-d_{10} (**AcP-d_{10}**), phenanthrene-d_{10} (**Phe-d_{10}**), chrysene-d_{12} (**Chr-d_{12}**), and perylene-d_{12} (**Per-d_{12}**), which was added to the water samples before the PAHs extraction. This standard mix solution, 2000 µg/mL in dichloromethane, was purchased from Supelco (Bellefonte, PA). Both stock solutions were kept in the dark at –20ºC to minimize their potential decay. All standard solutions were stable for one year and evidence of decomposing was never observed. Stock solutions were used to prepare working standard solutions for calibration and spiking samples. From stock solutions were prepared seven nominal standard mixtures.

PCBs standards (**PCB_{28}**, **PCB_{52}**, **PCB_{101}**, **PCB_{118}**, **PCB_{138}**, **PCB_{153}**, and **PCB_{180}**) were purchased from Dr. Ehrenstorfer (Augsburg, Germany) with a 99.5% purity and a 10 ng/mL concentration. The internal standard (IS), 4.4′-difluorobiphenyl (**4.4'-DFB**) was from Sigma-Aldrich (Steinheim, Germany). All reference standards were of >96% purity.

For IS, an individual stock solution of 5000 µg/L was prepared in n-hexane. For all the PCBs included in this work, a 500 µg/L mix solution of each component in n-hexane was prepared. These solutions were stored in the dark at –20°C in amber bottles to avoid degradation, which was never observed during the study period.

The solvents n-hexane with 99.0% purity, methanol (99.8%) and acetone (99.5%) were purchased from Sigma-Aldrich (Germany). Dichloromethane (99.9%) was acquired from Romil (Cambridge, UK). Ultrapure water was supplied by a Milli-Q water system (conductivity = 0.054 µS cm^{-1}, at 25ºC).

2.4. Solid-phase extraction (SPE) of both PAHs and PCBs

Within 24 h after collection, the seawater samples (1 L) were filtrated. The PAHs and PCBs dissolved in the aqueous fraction (DAF) were separated from those adsorbed to solid suspend matter (SSM) as shown in **Figure 5** and summarized below. The final extracts were analyzed by Gas Chromatography with tandem mass spectroscopy (GC-MS/MS).

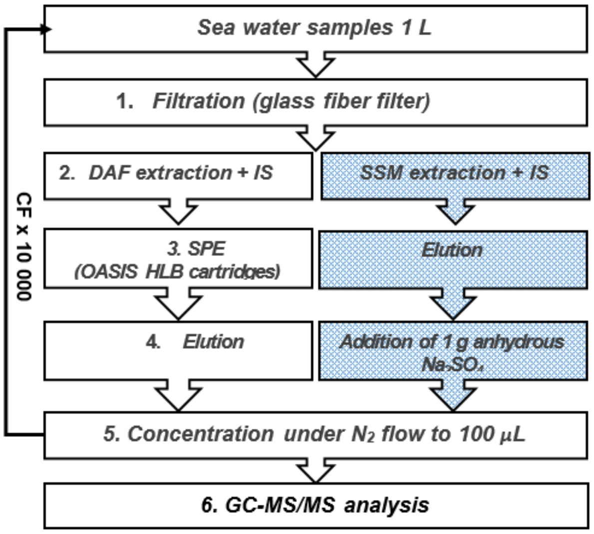


Figure 5 – Diagram of the extraction procedures for DAF and SSM. Note: Herein it is shown data concerning the DAF.

- ***Seawater filtration (glass fiber filter)***

Briefly, water samples (1 L) were filtrated to eliminate particulate matter and other suspended solids, through a 0.45 µm glass fiber filter with 47 mm in diameter. The filter was placed in a Büchner funnel and then, using a vacuum bomb connected to a kitasato flask, each sample was filter in order to separate the DAF and the SSM.

- ***PAHs and PCBs extraction from DAF and SPE protocol***

The protocol used for the extraction of PAHs and PCBs from DAF fraction, followed a previous validated SPE method specific for the extraction of these organics from water samples (Sánchez-Avila *et al.*, 2011). The recovery rates of the SPE protocol were for both the 16 analyzed PAHs and 7 PCBs above 78%. Firstly, the Oasis HLB cartridges (200 mg), were conditioned with 10 mL of hexane, 10 mL of dichloromethane, 10 mL of methanol and with 15 mL of Milli-Q ultrapure water using a manifold vacuum system device (Waters, Milford, Massachusetts, USA).

Thereafter, 1 L of seawater samples + IS were loaded into the SPE cartridges at a constant flow rate of 5 mL mL/min, which were later rinsed three times with 5 mL of ultrapure water and then dried under vacuum for 30 min.

- ***Elution***

Finally, both PAHs and PCBs were eluted with 10 mL of dichloromethane:hexane (1:1, v/v) followed by 10 mL of dichloromethane:acetone (1:1, v/v).

- ***Final step for the concentration of PAHs and PCBs***

The extracts were concentrated into 100 µL of hexane and kept in vials at –20°C until analysis.

- ***GC-MS/MS analysis***

The GC-MS/MS analytical protocol based in a previous validated GC-MS method (Rocha *et al.*, 2011) is herein amplified to perform MS/MS analysis for both PAHs and PCBs.

2.5. Quantification by GC-MS/MS

The targeted compounds were measured by GC-MS/MS accordingly to the features described in **Table 5**. Other details about the GC-MS/MS can be checked in **Table 6**.

Table 5 – General conditions of the GC-MS/MS apparatus used in the present study.

GC-MS/MS Conditions			
Gas chromatograph	Trace GC ultra, Thermo Finnigan Electron Corporation		
Detector	Ion trap mass spectrometer (Thermo Scientific ITQ™ 1100 GC-MS^n)		
Auto sampler	Thermo Scientific TriPlus™		
Injector	SSL (3 mm straight liner)		
Mode	Splitless mode		
Volume (µL)	1 µL (50 mm length needle)		
Temperature (ºC)	280		
Column	Trace GOLD column TR5MS (30 m x 0.25 mm x 0.25 µm)		
Gas carrier	Helium (99.9999% purity), maintained at a constant flow rate of 1.0 mL/min		
Oven Program	*Temperature (º C)*	*Hold time (min)*	*Rate ºC/min*
	40	2	-
1st ramp	250	1	12
2nd ramp	310	-	5
	310	5	-
Solvent delay:		5 min	
Transfer line:		280ºC	
Ion source:		280ºC	

Table 6 – Quantification and diagnostic ions used in GC-MS/MS analysis for PAHs and PCBs evaluated in DAF (*continues in the next pages*).

			Qualifier ions					
Target compounds	**t_R (min)**	**Target ions (m/z)**	**Q_1 (m/z)**	**Q_2 (m/z)**	**Q_3 (m/z)**	**Segment time (min)**	**Collision Energy (CE)**	**Ion ratios**
N-d_8	10.44	136 (88.7)	108 (11.3)	-	-	8.00-11.30	-	-
N	10.48	128 (71.3)	127 (13.5)	102 (8.4)	129 (6.8)	8.00-11.30	1.30	0.6
4.4'-DFB	12.75	190 (62.7)	188 (36.6)	128 (0.7)	-	11.30-16.00	-	-
AcP	13.65	154 (15.6)	152 (48.7)	151 (29.7)	76 (6.0)	11.30-16.00	1.03	0.6
AcpY	14.09	152 (55.5)	151 (24.5)	150 (17.6)	76 (2.4)	11.30-16.00	1.65	0.7
AcP-d_{10}	14.16	164 (57.5)	162 (41.2)	160 (1.3)	-	11.30-16.00	-	-
Flu	15.02	166 (24.2)	165 (47.6)	163 (25.9)	82 (2.37)	11.30-16.00	1.04	0.5
Phe-d10	16.91	188 (62.6)	184 (22.3)	160 (15.1)	-	16.00-18.00	-	-
Phe	16.92	178 (51.7)	176 (28.8)	152 (12.4)	179 (7.1)	16.00-18.00	1.05	0.4
A	17.32	178 (53.8)	176 (30.0)	152 (12.9)	89 (3.3)	16.00-18.00	1.05	0.4
PCB$_{28}$	17.69	256 (37.6)	258 (35.4)	150 (18.2)	220 (8.8)	16.00-18.00	1.36	0.5
PCB$_{101}$	18.58	326 (63.0)	254 (21.7)	328 (10.9)	324 (4.4)	18.00-22.30	1.18	0.5
PCB $_{52}$	18.60	292 (31.2)	255 (25.6)	290 (22.8)	220 (20.4)	18.00-22.30	1.57	0.9
PCB$_{138}$	19.30	290 (34.6)	325 (50.6)	326 (13.6)	360 (1.2)	18.00-22.30	2.28	0.3
FL	19.41	202 (44.1)	200 (31.1)	201 (23.0)	101 (1.9)	18.00-22.30	2.15	0.7
Pyr	19.53	202 (34.7)	200 (42.1)	201 (19.1)	101 (4.1)	18.00-22.30	2.15	0.5

				Qualifier ions				
Target compounds	**t$_R$ (min)**	**Target ions (m/z)**	**Q$_1$ (m/z)**	**Q$_2$ (m/z)**	**Q$_3$ (m/z)**	**Segment time (min)**	**Collision Energy (CE)**	**Ion ratios**
PCB$_{118}$	19.73	326 (58.8)	128 (20.4)	254 (14.6)	324 (6.1)	18.00-22.30	2.13	0.7
PCB$_{153}$	20.4	360 (0.14)	290 (68.3)	288 (19.9)	145 (11.6)	18.00-22.30	1.43	0.3
PCB$_{209F}$	20.4	334 (9.4)	265 (76.6)	315 (7.8)	335 (6.3)	18.00-22.30	-	-
Chr	23.05	228 (49.0)	226 (46.4)	113 (3.0)	201 (1.6)	22.30-25.00	1.96	0.1
Chr-d$_{12}$	23.12	240 (43.5)	236 (46.0)	237 (10.6)	-	22.30-25.00	-	-
BaA	23.19	228 (46.4)	226 (44.2)	229 (6.7)	114 (2.7)	22.30-25.00	1.96	0.2
PCB$_{180}$	23.65	394 (12.8)	326 (41.5)	324 (40.3)	359 (5.4)	22.30-25.00	1.12	1.0
BbFL	26.73	252 (50.1)	250 (43.8)	126 (4.1)	226 (2.0)	25.00-29.00	2.40	0.1
BkFL	26.83	252 (47.5)	250 (46.3)	126 (4.3)	226 (2.0)	25.00-29.00	2.64	0.1
BaP	27.75	252 (41.9)	250 (48.8)	126 (5.1)	226 (4.2)	25.00-29.00	2.40	0.1
Per-d$_{12}$	27.97	264 (50.9)	252 (46.7)	228 (2.4)	-	25.00-29.00	-	-
Ind	31.46	276 (47.5)	274 (43.1)	138 (6.5)	248 (2.9)	29.00-37.00	2.80	0.2
DBA	31.54	278 (3.1)	276 (83.3)	138 (11.0)	226 (2.5)	29.00-37.00	2.85	0.1
BP	32.19	276 (41.3)	274 (45.7)	138 (8.8)	248 (4.1)	29.00-37.00	2.80	0.2

Note: The relative abundance of ions (m/z) for each target compound is indicated between brackets.

Since the current PAHs and PCBs were measured in ng/L, method blanks were used to ensure the absence of contamination by laboratory material.

Replicate samples (n = 3), and procedural blanks were used as quality control procedures.

Reproducibility and recovery were high (78–142%) with relative standard deviation (RSD) ≈ 10%. Additionally, unbiased samples were both spiked with the 16 assayed PAHs and 7 assayed PCBs at an intermediate concentration of the calibration curve (30 µg/L) and submitted to usual analysis (Rocha *et al.*, 2011).

To measure the quality control, the procedural blanks were periodically analysed for each batch of 10 samples.

2.6. Samples confirmation and quantification

According to the EU Commission Decision 2002/657/EC (CEC, 2002) for the confirmation and identification of pharmaceuticals, when using GC-MS/MS as instrumental technique, a minimum of three identification points (IPs) are required. In this study, two MS transitions (**Table 6**) were monitored for all the compounds and the ion ratios were calculated as the relation between the abundances of both transitions. In addition the retention times (t_R) were used to confirm the presence of the PAHs and PCBs in the seawater samples.

The first transition was used for quantification and the second one for confirmatory purposes. Quantification was then performed on the basis of external calibration plots for all the compounds. Calibration standards were analysed at the beginning of a sample sequence. The variations in signal intensity were monitored by the analyses of three QC samples after approximately ten injections.

2.7. Calibration and detection limits

The determination of the method linearity was made by doing three independent calibrations curves in solvent and in seawater matrix. The main purpose was to verify if the results were directly propositional to the concentration of compounds of interest in a certain interval.

For each calibration curve, seven different calibration points were used with the following concentration: 5 µg/L; 10 µg/L; 20 µg/L; 40 µg/L; 60 µg/L; 80 µg/L and 100 µg/L. All this concentrations were injected in triplicated and the correlation coefficient (r^2) was determined to evaluate the linearity.

After this, the limit of detection (LOD) and the limit of quantification (LOQ) were accurate based on mathematic formulas that used a proportion with standard deviation (SD) of the response and the median slope (S) of the three calibration curves:

$$(1)\ \mathrm{LOD} = \frac{\mathrm{SD\ x\ 3}}{\mathrm{S}}$$

$$(2)\ \mathrm{LOQ} = \frac{\mathrm{SD\ x\ 10}}{\mathrm{S}}$$

Finally, the precision, accuracy and recoveries were also determined using this concentrations points and the fortified seawater matrix (**Table 7**).

2.8. Calculation of total TEQ

In order to evaluate the potential toxicological risk of all compounds, an assessment of the total TEQ was calculated for the two groups of our interest compounds, following the equation (Nekhavhambe *et al.*, 2014):

$$(3)\ \mathrm{TEQ}_{\mathrm{Total}} = \sum \mathrm{C_i\ x\ TEF_i}$$

Where TEQ = toxic equivalent quotient, C_i = concentration of individual PAHs and TEF_i = toxic equivalent factor relative to **BaP** (for PAHs) (Nekhavhamble *et al.*, 2014) or to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (**TCDD**) for PCBs (Yang *et al.*, 2010).

Table 7 – Calibration parameters of the method, including the calibration curves equation, the correlation coefficients (r^2), and the detection and quantification limits (LODs and LOQs) of the method.

Target compounds	Regression equations	r^2	LOD (ng/L)	LOQ (ng/L)	Extraction recoveries (%)
N	y = 0.006x	0.99	0.19	0.58	109.4
AcP	y = 0.00002x	0.99	0.17	0.50	112.7
AcpY	y = 0.00008x	0.99	0.01	0.04	114.8
Flu	y = 0.0005x	0.99	0.11	0.34	112.1
Phe	y = 0.09x	0.99	0.10	0.30	94.3
A	y = 0.07x	0.99	0.29	0.87	141.5
PCB_{28}	y = 0.02x	0.99	0.22	0.68	78.3
PCB_{101}	y = 0.03x	0.99	0.20	0.61	94.6
PCB_{52}	y = 0.01x	0.99	0.25	0.76	82.2
PCB_{138}	y = 0.003x	0.98	0.10	0.31	92.3
FL	y = 0.008x	0.99	0.11	0.33	107.9
Pyr	y = 0.009x	0.99	0.23	0.71	107.9
PCB_{118}	y = 0.02x	0.99	0.06	0.19	84.5
PCB_{153}	y = 0.02x	0.99	0.15	0.44	87.5
Chr	y = 0.02x	0.99	0.23	0.70	93.6
BaA	y = 0.02x	0.99	0.31	0.94	98.0
PCB_{180}	y = 0.008x	0.99	0.18	0.54	99.7
BbFL	y = 0.01x	0.99	0.32	0.97	90.5
BkFL	y = 0.002x	0.99	0.07	0.21	92.5
BaP	y = 0.005x	0.99	0.06	0.18	95.9
Ind	y = 0.003x	0.99	0.13	0.40	99.5
DBA	y = 0.002x	0.99	0.18	0.56	86.8
BP	y = 0.002x	0.99	0.07	0.20	95.8

3. Results

In this section, all data refer to mean ± SE levels provided by the measurement of physicochemical or organic contamination in seawater samples collected from Azurara (**A**, S_1-S_2), Árvore (**B**, S_3-S_4), Reserva (**C**, S_5-S_6), and Areia (**D**, S_7-S_8) beaches.

3.1. Seawater physical and chemical parameters

Table 8 shows the physicochemical parameters observed in 16 samples collected from Vila do Conde seacoast. Values of pH and temperature were, in average, ≈ 8 and ≈ 15 ºC. The other parameters were ≈ 1.3 mg/L, ≈ 0.1 mg/L, ≈ 0.7 mg/L and ≈ 0.08 mg/L for ammonia, nitrites, nitrates and phosphates. No apparent differences were observed amongst seasons or sampling sites.

Table 8 – Physicochemical parameters evaluated in the four beaches located in Vila do Conde region in January and April 2018 (mean ± SE).

Physicochemical parameters	A n = 4			B n = 4			C n = 4			D n = 4		
Temperature °C	14.6	±	2.3	15.1	±	1.7	15.0	±	1.0	15.8	±	1.0
pH	7.9	±	0.1	7.9	±	0.05	8.0	±	0.05	7.9	±	0.03
NH_4^+ mg/L	1.3	±	0.9	1.3	±	0.9	1.2	±	1.0	1.2	±	1.0
NO_2^- mg/L	0.1	±	0.01	0.1	±	0.03	0.1	±	0.1	0.1	±	0.1
NO_3^- mg/L	0.8	±	0.4	0.7	±	0.4	0.6	±	0.4	0.6	±	0.4
PO_4^- mg/L	0.1	±	0.1	0.1	±	0.1	0.1	±	0.03	0.03	±	0.01

3.2. GC-MS/MS analysis of PAHs in the seawater samples

Table 9 expresses the individual levels of the sixteen PAHs dissolved in seawater and shows that the sum of all PAHs per sampling site was ≈ 84 ng/L at **A**, ≈ 77 ng/L at **B**, ≈ 82 ng/L at **C**, and ≈ 72 ng/L at **D**. In average the total sum of the PAHs concentration in the studied area (**A** to **D**) was ≈ 79 ng/L.

Table 10 shows that, in terms of abundance (%), those PAHs with higher profusion were those containing three benzene rings (≈ 43%), followed by those containing four benzene

Table 9 – Average levels of PAHs (ng/L) dissolved in seawater samples (n = 16) collected from Atlantic seacoast located in Vila do Conde do Conde (**A** to **D**) (mean ± SE).

PAHs	**TEF values***	**IARC Groups (IARC, 2016)**	**MDL (ng/L)**	**MQL (ng/L)**	**Abundance (%)**	**PAHs (ng/L)**			
						A **n = 4**	**B** **n = 4**	**C** **n = 4**	**D** **n = 4**
2 Rings									
N	0.001	2B	0.2	0.6	100	6.1 ± 3.2	12.3 ± 5.3	8.7 ± 2.3	6.4 ± 1.0
3 Rings									
AcP	0.001	-	0.2	0.5	100	11.6 ± 5.3	7.8 ± 3.4	5.8 ± 1.7	8.8 ± 1.8
AcpY	0.001	3	0.01	0.04	100	2.8 ± 1.5	3.3 ± 0.5	4.6 ± 1.2	3.0 ± 0.5
A	0.01	3	0.3	0.9	94	2.3 ± 0.9	3.5 ± 1.8	4.0 ± 2.3	2.8 ± 1.5
Phe	0.001	3	0.1	0.3	100	10.1 ± 6.0	17.0 ± 8.9	13.2 ± 6.0	14.6 ± 7.1
Flu	0.001	3	0.1	0.3	100	5.7 ± 2.4	5.8 ± 1.7	3.8 ± 0.9	5.2 ± 1.4
4 Rings									
FL	0.001	3	0.1	0.3	100	9.1 ± 5.7	5.0 ± 2.6	10.0 ± 6.1	8.1 ± 4.2
BaA	0.1	2B	0.3	0.9	100	1.7 ± 1.0	1.0 ± 0.3	2.0 ± 1.0	1.4 ± 0.7
Chr	0.01	2B	0.2	0.7	100	2.0 ± 1.2	1.0 ± 0.2	1.7 ± 0.8	2.3 ± 1.4
Pyr	0.001	3	0.2	0.7	100	14.7 ± 9.0	6.1 ± 2.6	13.3 ± 7.4	9.1 ± 5.1
5 Rings									
BaP	1	1	0.3	0.9	100	4.0 ± 2.5	2.0 ± 0.8	2.9 ± 1.0	2.6 ± 1.0
BbFL	0.1	2B	0.3	1.0	56	1.3 ± 0.8	1.4 ± 1.0	1.4 ± 0.7	1.1 ± 0.8
BkFL	0.1	2B	0.1	0.2	100	6.5 ± 4.0	5.5 ± 3.3	5.4 ± 3.6	2.4 ± 1.7
DBA	1	2A	0.2	0.6	75	0.3 ± 0.2	1.2 ± 0.5	1.8 ± 0.6	1.3 ± 0.8
6 Rings									
BP	0.01	3	0.1	0.2	94	2.4 ± 1.4	2.6 ± 1.8	1.3 ± 0.6	2.1 ± 0.9
Ind	0.1	2B	0.1	0.4	100	3.5 ± 2.2	1.6 ± 0.8	1.6 ± 0.5	1.0 ± 0.4
					∑16PAHs per Sampling site	84.1	77.0	81.5	72.3

*TEF values as proposed by EPA (2012)

rings (≈ 28%). In terms of carcinogenicity, and accordingly to the IARC classification (IARC, 2010), this Table also shows that in average, those PAHs of Group 3 represents ≈ 59% of the total load of these compounds in the studied area. In spite of this, the other groups are also present, being their percentages of ≈ 4%, ≈ 1.5% and ≈ 25% for PAHs of Groups 1, 2A and 2B, respectively.

Table 10 – PAHs (%) dissolved in the water considering both their number rings and their potential carcinogenicity per sampling site.

% PAHs	A	B	C	D
2 Rings	7.3	16.0	10.7	8.9
3 Rings	38.6	48.5	38.5	47.6
4 Rings	32.7	17.0	33.1	28.9
5 Rings	14.4	13.1	11.9	10.2
6 Rings	7.0	5.4	5.8	4.3
% PAHs (IARC, 2016) per Group and sampling site				
Group 1	4.8	2.6	3.6	3.6
Group 2A	0.4	1.6	2.2	1.8
Group 2B	25.1	29.6	25.5	20.2
Group 3	56.0	56.2	61.6	62.2

In order to understand the possible origin of the measured PAHs, several ratios were established between target compounds (**Table 11**). The quotient between low molecular weight (**LMW**) and high molecular weight (**HMW**) PAHs was calculated following the next equation (Magi *et al.*, 2002):

$$(4)\ \frac{\mathbf{A+Phe+FL+Pyr}}{\mathbf{BaA+Chr+BaP+BbFL+BkFL+DBA+Ind}}$$

Table 11 – Relationship between PAHs in water samples as to their possible origin.

Ratios between PAHs	Petrogenic	Pyrogenic	This study			
			A	B	C	D
Phe/A	>10	<10	4.39	4.86	3.30	5.21
A/ (A + Phe)	<0.1	>0.1	0.19	0.17	0.23	0.16
BaA/ (BaA + Chr)	<0.2	>0.35	0.46	0.50	0.54	0.38
FL/ (FL + Pyr)	<0.4	>0.4	0.38	0.45	0.43	0.47
	Fuel combustion	Grass/coal/wood combustion				
FL/ (FL + Pyr)	0.4-0.5	>0.5	-	-	-	-
LMW/HMW	>1.0	<1.0	1.88	2.31	2.41	2.86

Generally, it is shown that in almost all sites the PAHs have a possible petrogenic origin. The calculation of TEQ for PAHs dissolved in water followed the EPA criteria (EPA, 2012). In **Figure 6** it is shown that the most carcinogenic PAHs, namely **BaP** (Group 1) and DBA (Group 2A) are the main contributors to the potential toxicity (carcinogenesis/ mutagenesis) of the studied waters. Individually, the sum of all TEQ values for PAHs dissolved in water at each sampling site was ≈ 6 ng/L (**A**), ≈ 4 ng/L (**B**), ≈ 6 ng/L (**C**) and ≈ 5 ng/L (**D**).

In **Figure 7** (**I** to **IV**), the cross plots show the most probable source of the studied pollutants per sampling sites. There is a predominance of petroleum and petroleum combustion PAHs at **A** and **B** and other sources, such as biomass and coal combustion at **C** and **D**. Nonetheless, petroleum contamination also occurred in this area, as it can be observed in **Figure 7**.

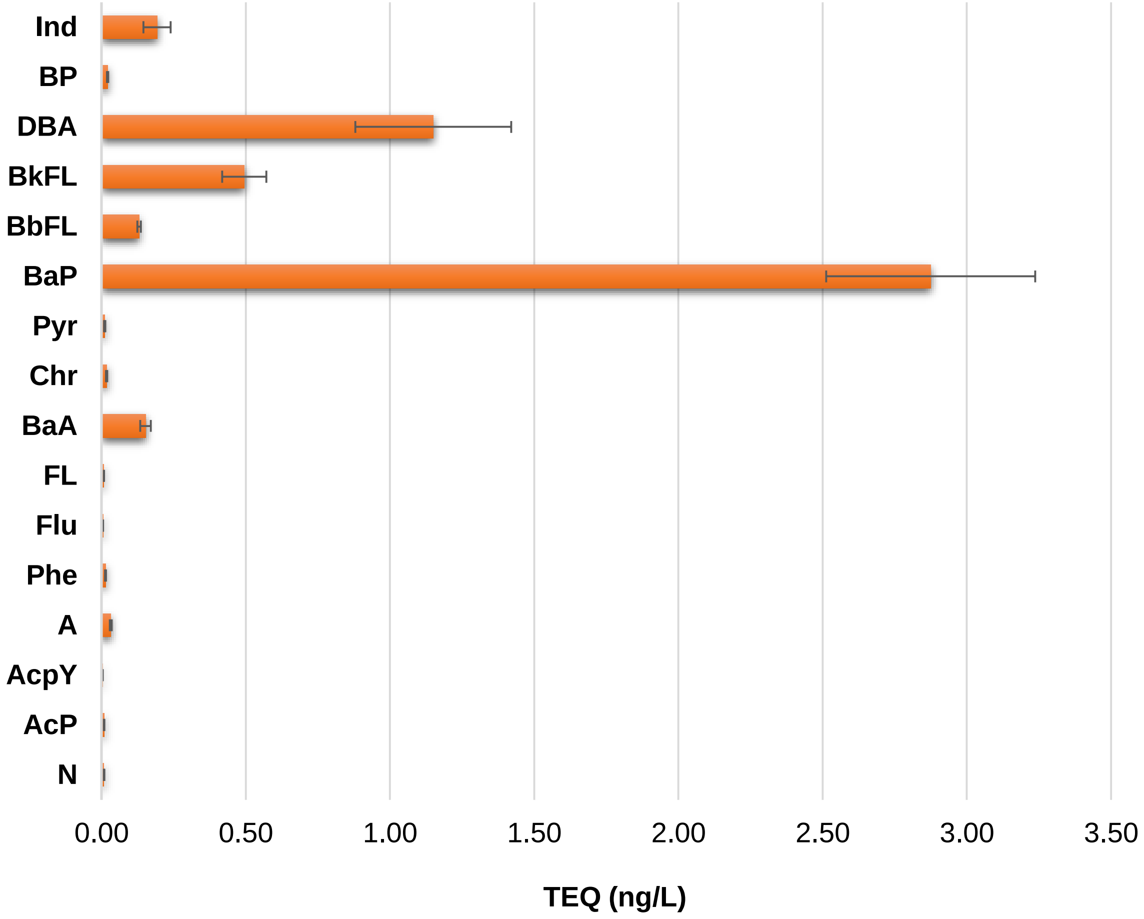


Figure 6 – Normalisation of the average concentrations of PAHs measured in seawater samples collected at Vila do Conde seacoast in terms of TEQ units of **BaP**. Data is shown as mean ± SE (n= 4).

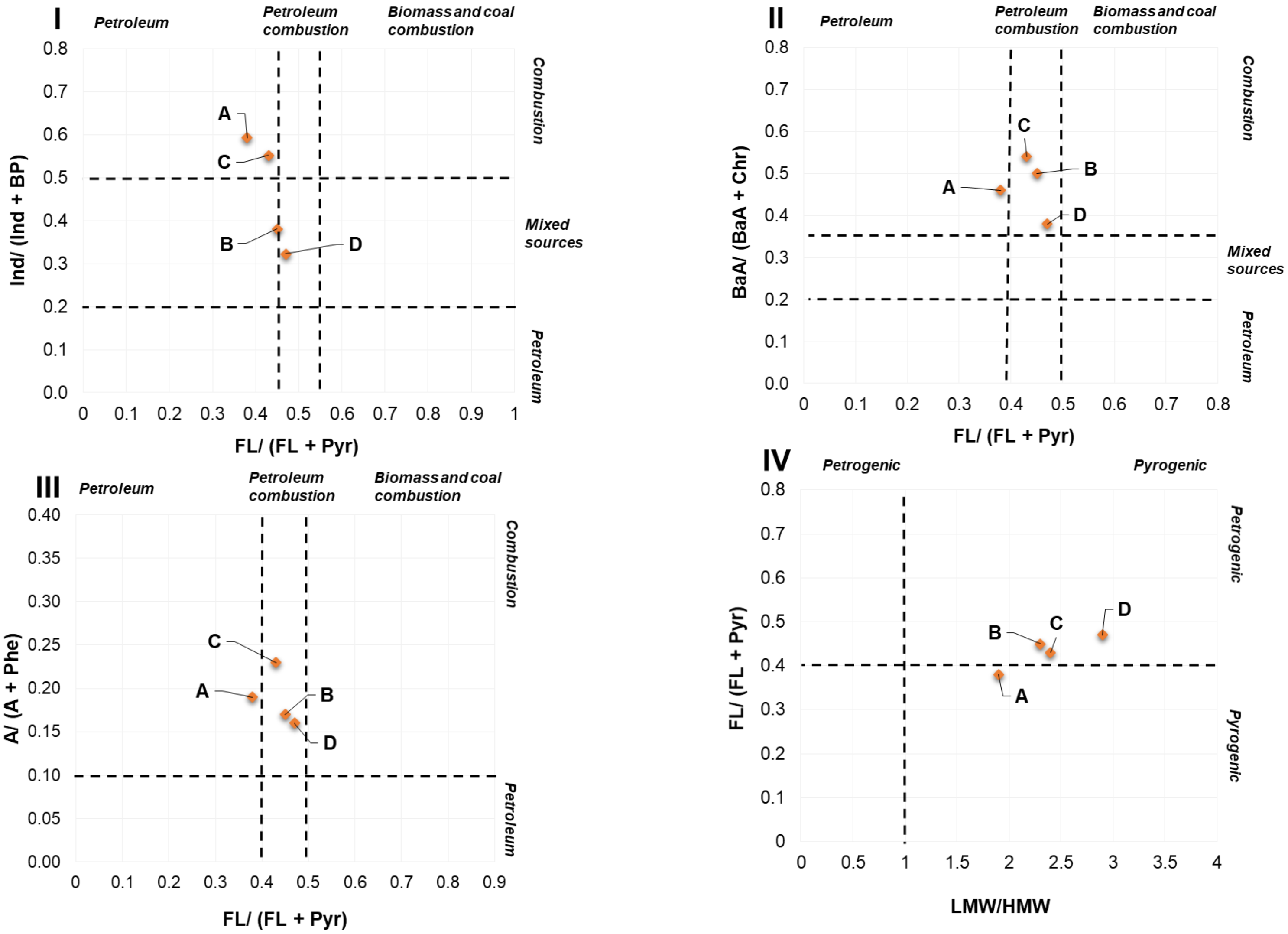


Figure 7 – PAH cross-plots (**I** – **IV**) for water samples from Vila do Conde seacoast (**A** to **D**).

3.3. GC-MS/MS analysis of PCBs in the seawater samples

In accordance with the International Council for the Exploration of the Sea (ICES), **Table 12** reports the concentrations of seven PCBs, which includes the mono-ortho congener **PCB_{118}** in addition to the six PCBs (**PCB_{28}**, **PCB_{52}**, **PCB_{101}**, **PCB_{138}**, **PCB_{153}**, and **PCB_{180}**), usually measured in higher concentrations in environmental samples (ICES, 2012; Webster *et al.*, 2013). So, herein it is observed that the global concentration of these seven PCBs are, at each sampling site ≈ 59 ng/L at **A**, ≈ 48 ng/L at **B**, ≈ 47 ng/L at **C**, and ≈ 45 ng/L at **D**.

Moreover, the six PCBs referred above are classified as possible carcinogenic (Group 2B) and **PCB_{118}** classified as a possible carcinogenic (Group 1) for humans (IARC, 2016). However, it is stressed that there is no sufficient certain about the carcinogenic effect of the **PCB_{118}**, presently exists sufficient data that say that can have a carcinogenic effect in animals (Yang *et al.*, 2010) reason why data shown in this sampling area was also converted in TEQ units of **TCDD** (**Figure 8**).

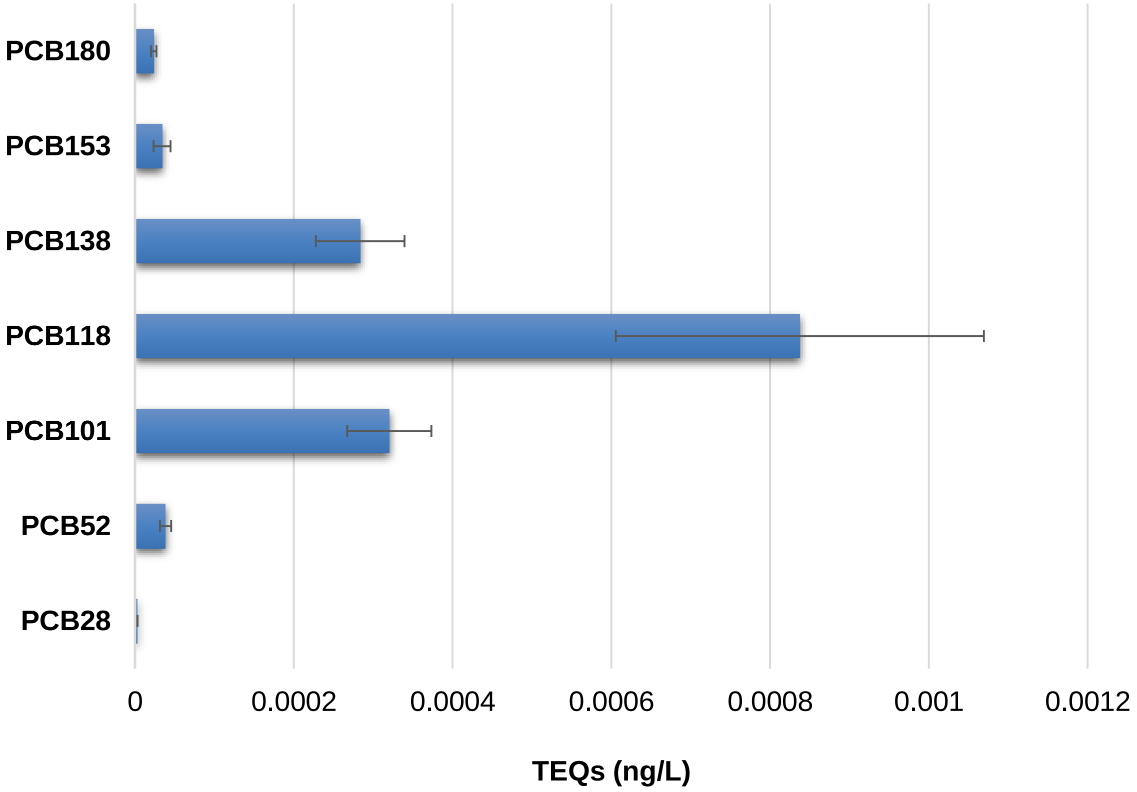


Figure 8 – Normalisation of the average concentrations of PCBs measured in water of the Vila do Conde seacoast in terms of TEQ units of **TCDD**. Data is shown as mean ± SE (n= 4).

Table 12 – Average levels of PCBs (ng/L) dissolved in seawater samples (n = 16) collected from Atlantic seacoast located in Vila do Conde (**A** to **D**) (mean ± SE).

PCBs	TEF values*	MDL (ng/L)	MQL (ng/L)	Abundance (%)	PCBs (ng/L)			
					A **n = 4**	**B** **n = 4**	**C** **n = 4**	**D** **n = 4**
Trichlorobiphenyl								
PCB$_{28}$	0.000002	0.2	0.7	94	1.4 ± 0.8	1.5 ± 0.5	1.1 ± 0.4	1.6 ± 0.4
Tetrachlorobiphenyl								
PCB$_{52}$	0.000005	0.2	0.8	94	6.0 ± 3.8	12.2 ± 5.2	4.7 ± 1.9	7.7 ± 2.7
Pentachlorobiphenyl								
PCB$_{101}$	0.00003	0.2	0.6	94	15.8 ± 9.9	9.5 ± 5.0	6.0 ± 2.4	11.4 ± 1.9
PCB$_{118}$	0.0001	0.1	0.2	88	9.4 ± 5.9	2.5 ± 1.1	15.2 ± 12.3	6.4 ± 1.8
Hexachlorobiphenyl								
PCB$_{138}$	0.00002	0.1	0.3	94	17.6 ± 11.0	18.2 ± 7.6	16.3 ± 6.7	4.6 ± 0.8
PCB$_{153}$	0.00001	0.1	0.4	94	3.4 ± 2.1	2.5 ± 0.7	0.9 ± 0.3	6.8 ± 1.8
Heptachlorobiphenyl								
PCB$_{180}$	0.000005	0.2	0.5	94	4.8 ± 3.0	1.9 ± 0.8	2.7 ± 0.5	6.5 ± 2.3
∑7PCBs per Sampling site					58.5	48.3	46.9	45.0

*TEF values as proposed by Yang *et al.* (2010).

4. Discussion

This study focus on four beaches located close to the mouth of the Ave River estuary. As it was already referred in the Introduction, these areas were chosen due to their continuous usage by animals and humans.

In fact during winter-spring this is an attractive area since here exists a natural ornithological reserve that holds lots of unique species of birds (54% are resident birds included in 81 species) present, and during summer-autumn the three beaches not included in the natural reserve, are also plenty used for leisure activities such as swimming, surf, kitesurf, and as recreational fishing places. These actions pose health risks for birds, humans and also aquatic animals that contact directly with pollutants such as PAHs and PCBs.

In the marine environment, the pollution from PAHs can result from everyday leakages or land-based sources, from river discharges, urban runoff, refineries and other industrial wastewater (Neff, 1979) or from sea-based sources, such as vessel discharges, vessel spills, operational discharges, gross atmospheric deposition, and aircraft dumping (Board & Council, 2003).

Herein the possible sources of pollution by these organic compounds include the presence of several industrial poles along the Ave River, a harbour inside the mouth of its estuary, a camping park located close to the natural reserve and the existence of some agriculture activities. Due to the industrial pollution of the Ave River this stream was considered one of most polluted rivers of Portugal, due to incorrect treatment of both industrial and domestic wastewaters (Rocha *et al.*, 2013).

Recently, other studies in the Ave River support the presence of heavy metals, as well as PAHs and PCBs in sediments dredge from Vila do Conde harbour (Santos-Ferreira *et al.*, 2015). Another study, also reports the presence of pesticides, fertilizers and endocrine disruptors in the Ave River (Ribeiro *et al.*, 2016). These observations, all taken together, suggest that when the waters of this River attain the seacoast they could transport pollutants such PAHs and PCBs.

4.1. Concentration of PAHs in the seawater samples

Comparing the concentrations of PAHs measured in this study (**Table 9**) with those of **Table 13**, is its shown that in the studied area the global concentrations of these compounds are low ranging from ≈ 72 ng/L to 84 ng/L.

From **Table 13**, it is stressed that the highest concentrations of PAHs in coastal environments were measured in petrol producer countries, such as Iraq and Iran whereas, in countries such as Portugal (Rocha *et al.*, 2017) and Spain (Rojo-Nieto *et al.*, 2013) these concentrations are almost one hundred folds lower.

According with European Commission 2008/105/EC, the PAHs that are used as a criteria for evaluating the levels of pollution are respectively **A**, **Pyr**, **BaP**, **BbFL**, **BkFL**, **Chr**, **DBA**, and **Ind** (CEC, 2008).

Concerning this aspect, it was noticed that total PAH levels measured at all sampling sites are significantly lower than the maximum admissible concentrations either by the European Union (200 ng/L) or the Environmental Quality Criteria of the United States (300 ng/L) for protection of human consumers of aquatic life (EPA, 2009).

Table 13 – PAHs concentrations (Σ16PAHs (ng/L) quantified in several coastal locations around the world.

Location	n PAHs	Mean (ng/L)	Reference
Vila do conde, Portugal	16	79	This study
Leça e Matosinhos, Portugal	16	50	Rocha *et al.* (2017)
Bay of Algeciras, Spain	16	238	Rojo-Nieto *et al.* (2013)
Persian Gulf, Iraq	16	7310	Sinaei & Mashinchian (2014)
Persian Gulf, Iran	16	1059	Agah *et al.* (2016)
Thane Creek, India	16	522	Tiwari *et al.* (2017)
Bohai Bay, China	16	275	Jia & Tian (2017)
East Asia, China	18	0.2	Ma *et al.* (2013)
North Pacific, China	18	0.1	Ma *et al.* (2013)
High Artic, Artic	18	0.04	Ma *et al.* (2013)

Nonetheless, with those shown worldwide (**Table 3**), it is possible to observe that the pattern of the 16 priority PAHs are different accordingly with their source.

For instance, the levels of **Pyr** and **DBA** in our study area are higher than those concentrations measured, respectively, in Leça and Matosinhos (Rocha *et al.*, 2017) and in

Iran (Agah *et al.*, 2016). On the contrary, other reference PAHs showed similar, as **BaP**, or much lower concentrations than those referred in the last two studies.

4.2. PAHs sources

The composition pattern of PAHs by ring size for the water samples collected from the Atlantic Ocean Sea close to Vila do Conde is shown in **Table 10**. Since the contributions of the **LMW** PAHs [i.e., those with **2 rings** (**N**) and **3 rings** (**A**, **Phe**, **AcP**, **AcpY**, and **Flu**)] represent 54% of the total load of these pollutants and those of **HMW** PAHs [i.e., those with **4 rings** (**FL**, **BaA**, **Chr**, and **Pyr**), **5 rings** (**BaP**, **BbFL**, **BkFL** and **DBA**) and **6 rings** (**BP** and **Ind**)] (Nasher *et al.*, 2013) constitute 46% of the total PAH lead us to the conclusion that these PAHs are derived from pyrogenic and petrogenic sources.

In fact, during 2017 the pyrogenic activity was intense due to forest fires occurring mainly in autumn. These observations agree with the diagnostic PAH ratios found in this study (**Table 11**), the graphics shown in **Figure 7** and with the indicator proposed by Magi *et al.* (2002).

In particular, herein it was observed that in average the ratio **Phe**/**A** was ≈ 4, i.e., pyrogenic; **A**/(**A** + **Phe**) was ≈ 0.2, i.e., pyrogenic; **BaA**/(**BaA** + **Chr**) was ≈ 0.5, i.e. pyrogenic; **FL**/(**FL** + **Pyr**) was ≈ 0.4, i.e. both petrogenic and pyrogenic and finally **LMH/HMW** was ≈ 2.4, i.e., petrogenic.

4.3. PAHs toxicity

Concerning the toxicity promoted by PAHs it is stressed that the EPA (2000) established, for water quality, the goal to protect human health from the carcinogenic effects of PAHs exposure. These criteria involved to set a non-detectable level (zero concentration for carcinogenic PAHs) in ambient water. Meanwhile, the maximum contaminant level was 0.2 ppb (200 ng/L) for **BaP**, since this compound is the most carcinogenic PAH (EPA, 2000).

In this sense, herein the levels of all measured PAHs were converted in TEQ units, which normalize the toxicity in **BaP** units (Nekhavhambe *et al.*, 2014). In average the **BaP** concentration was ≈ 2.9 ng/L but the TEQ total was ≈ 5.3 ng/L. Thus, fortunately these levels are below the one established by European Commission for environmental water and USEPA (ATSDR, 2009; CEC, 2008). Also none of the measured PAHs concentrations exceed this water concentrations limits, in which are discriminated by compound (**BaP** with 100 ng/L; **BaP**, **Pyr**, **BbFL** and **BkFL** with 200 ng/L; **Chr**, **DBA** and **A** with 300 ng/L; and **Ind** with 400 ng/L).

However, so far it hasn’t been prove long-term effects when exposure to small concentrations and for that reason, even in low levels PAHs could be harmful.

In fact, according to **Table 10**, the two most abundant groups are the PAHs of the Group 3 followed by the Group 2B (IARC, 2010). It is important to say that the **AcP** is not included in the Group 3 PAHs because there is no data that confirms its carcinogenicity.

Despite the fact, that all the concentrations are within this range, the long-term effects should not be discarded since PAHs can have adverse effects such as damage in the DNA and act in the immune systems, besides the carcinogenicity (Rajendran *et al.*, 2013).

It is also important to refer that besides humans, other species are present in the area such as mussels and birds (natural habitants of the reserve). This two species tend to be more sensitive to the pollution of their habitat because are in constant contact with it and for that reason, can be added as an important fact to consider when even minimal values are found in the environment (Abdel-Shafy & Mansour, 2016).

4.4. Concentration of PCBs in the seawater samples

The seven non-dioxin-like PCB (NDL-PCBs) analysed in this study are used as indicators of marine water quality because are the ones present with the highest concentrations in the environment, food and in human fluids and tissues (WHO, 2016).

Comparing the global values presented in **Table 14**, it is observed that the highest values of ∑PCBs occur in two European countries, i.e., France (2.6 ng/L) and Spain (1.2 ng/L) (García-Flor *et al.*, 2009).

According to data in **Tables 12** and **14**, it is noticed that all PCBs measured herein show concentrations ranging from ≈ 45 ng/L to 59 ng/L. Thus, all beaches analysed around and at the Ornithological Reserve exhibit amounts of PCBs higher than expected, being ≈ 20 folds higher than the data observed in both France and Spain (García-Flor *et al*, 2009).

Taking in consideration the last observation, although the levels of PCBs may seem extremely concerning, it is important to stress that previous studies provided by EPA (1980) reveal that acute toxicity to saltwater aquatic life probably will occur at concentrations above 10 μg/L. Nonetheless, more recent guidelines propose that global concentrations of 30 ng/L are sufficient for the occurrence of bioconcentration phenomena (EPA, 2009). Thus, since this study reveals ∑PCBs of ≈ 50 ng/L, it is hypothesized that bioconcentration by these compounds may occur in Vila do Conde seacoast.

Table 14 – The total aqueous phase PCBs concentrations (Σ7PCBs (ng/L) from several locations around the world.

Location	n PCBs	Mean (ng/L)	Reference
Vila do conde, Portugal	7	50	This study
North Atlantic, Azores	7	0.02	Lammel *et al.* (2017)
North Atlantic, between Azores and Jamaica	7	0.01	Lammel *et al.* (2017)
Barcelona, Spain	7	1.2	García-Flor *et al.* (2009)
Banyuls-sur-Mer, France	7	2.6	García-Flor *et al.* (2009)
Aegean Sea, Greece	7	0.03	Lammel *et al.* (2015)
Atlantic Ocean, Germany to South Africa	7	0.0005	Gioia *et al.* (2008)
Equatorial Indian Ocean, between India and Indonesia	7	0.004	Huang *et al.* (2014)

4.5. PCBs sources

It is difficult to identify the sources which are responsible for the concentrations measured in the study area, because so far the levels of PCBs have been decreasing since 1985 and also the use and marketing of PCBs in the European Community were heavily restricted (EC, 2001).

However, due to their physicochemical properties the PCBs are a group of contaminants able to persist in the environment either by their continuous recycling through the elements and trophic chains or due to poor treatment of antique hazardous waste sites. These infrastructures, accumulate old products able to leak these compounds in the soil which ultimately drain through the rivers until reach the ocean (ATSDR, 2014).

4.6. PCBs toxicity

In terms of pharmacokinetics PCBs have intrinsic half-life backgrounds levels of several years. To understand the rate of persistency of these pollutants in the body these values are: for **PCB**$_{28}$, 5.5 years; **PCB**$_{52}$, 2.6 years; **PCB**$_{138}$, 10.8 years; **PCB**$_{153}$, 14.4 years; and

PCB_{180}, 11.5 years (Ritter *et al.*, 2011). This observation points that their presence in the environment could reach for periods that might last almost 15 years.

Even though the NDL-PCBs aren't still confirmed as carcinogenic, they can have other adverse effects such as neurotoxicity, immunotoxicity, endocrine disruption, oxidative stress (especially in fish), among others (Brenerová *et al.*, 2016; Elia *et al.*, 2005). This observation is applicable for **PCB_{28}**, **PCB_{52}**, **PCB_{138}** and **PCB_{180}** which are suspects of promoting endocrine disruptors (Petersen *et al.*, 2007).

In spite of **PCB_{118}** is being considerate a compound that maybe belong to Group 1, the others ones evaluated in this study were included in the Group 2B for carcinogenicity since there is still not enough information about their ability to promote physiologic damages in humans (IARC, 2016).

In order to evaluate the risks of exposure for humans and animals of the environmental levels measured in the present area, these were normalized in TEQ values considering as the **TCDD** the most toxic PCB. The total TEQ obtained was $\approx$ 0.0015 ng/L.

Figure 8 shows the extrapolation of the TEQs values from each PCB. The same one reveals that of all the PCBs the one who demonstrate a higher TEQ value was **PCB_{118}** with $\approx$ 0.0008 ng/L.

Since, **PCB_{118}** can act sometimes as a dioxin-like PCB, the fact that it have a high value is concerning in terms of both animal and human exposure (Elia *et al.*, 2005).

With all this in mind, the concentrations measured herein are a matter of concern and shouldn't be ignored.

4.7. Physicochemical parameters

The physicochemical parameters measured point to a poor water quality at the seacoast. In fact, the levels measured for ammonia in all sampling sites, considering temperatures of 15ºC and pH of 8 were above 1.2 mg/L which are well above the LC_{50} recommended by EPA (EPA, 1985).

Converting the levels of nitrates and nitrites to levels nitrogen (1 mg/L as nitrate = 0.226 mg/L as nitrate-nitrogen; 1 mg/L as nitrite = 0.304 mg/L), the latter never surpass 1 mg/L at all sampling sites, which is the concentration above which the WHO considers the presence of nitrogen as a hazardous for human health (WHO, 2011).

The average levels of phosphates (1 mg/L = 0.024 mg/L of phosphorous), which sources are mainly sewage effluents and/or organophosphorus pesticides, never surpass 0.010 mg/L, which is the recommended limit for total phosphorus in flowing streams to prevent eutrophication (Daniel *et al.*, 1998). This occurrence shows that in terms of this parameter, inputs from direct sewage discharges are not problematic in this area (**Figure 4**).

All other physicochemical data, i.e., pH and temperature were within the usual characteristics of this area (Rocha *et al.*, 2012).

5. Conclusions

This study provides important and unique information about pyrogenic and petrogenic sources PAHs, and about PCBs concentrations in seawater samples collected from northern Portugal (NW Iberian Peninsula). This allowed to unveil a baseline data sets against which future chemical monitoring can be compared with coastline reserve areas, while contributing with data for building global scenarios for pollution by these organic compounds in Europe.

The data suggest that the surveyed habitat is being impacted by these compounds, at levels that have in spite of do not have potency to cancer they are still able to induce metabolic disorders in aquatic organics, birds and promote unpredicted impacts on human health.

Moreover, since the studied habitat also has a wide range of other pollutants, e.g., estrogenic endocrine disruptors (Rocha *et al.*, 2013) these organics, all together, might form a cocktail with unknown extent of toxicant effects.

The ecosystem and health impacts of such complex situations have been reported worldwide, calling for actions at affected locations (Nekhavhambe *et al.*, 2014; Viguri *et al.*, 2002).

To prevent health impacts for local biota and humans, and to ensure the zero impact by PAHs and PCBs, monitoring and depollution measures should be undertaken in this area, in order to protect humans and the Ornithological Reserve from this harmful effects.

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