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Determination and Risk Assessment of PAHs and PCBs in Seawater and Blue Mussels from the Coastal Area of Vila-do-Conde, Portugal

Ana Margarida Serra Esteves

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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 e Recursos Marinhos submetida ao Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto

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Ana Esteves

Ana Margarida Serra Esteves

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ACRONYMS & ABBREVIATIONS

POPs - Persistent organic pollutants
OCPs - organochlorine pesticides
DDT - dichlorodiphenyltrichloroethane
PCBs - polychlorinated biphenyls
PBDEs - polybrominated diphenyl ethers
PFOS - perfluorooctanesulfonate
PCDDs/Fs - polychlorinated dibenzo-p-dioxins and-furans
PCDFs - polychlorinated dibenzofurans
PAHs - polycyclic aromatic hydrocarbons
EFSA - European Food Safety Authority
WHO - World Health Organization
EPA - US Environmental Protection Agency
FDA - US Food and Drug Administration
WFD - Water Framework Directive
UNEP - United Nations Environment Programme
LMW - low molecular weight
HMW - high molecular weight
K_{ow} - octanol/water partition coefficient
Cl - chlorine
Ahr - Aryl hydrocarbon Receptor
Ant - anthracene
AcP - acenaphthene
AcPY - acenaphthylene
BaA - benzo[a]anthracene
BbFL - benzo[b]fluoranthene
BkFL - benzo[k]fluoranthene (BkFL),
BP - benzo[g,h,i]perylene
BaP - benzo[a]pyrene
Chr - chrysene
DBA - dibenzo[a,h]anthracene
Flu - fluorene
FL - fluoranthene
Ind - indeno[1,2,3-c,d]pyrene
NaP - naphthalene

Phe - phenanthrene

Pyr - pyrene

US EPA - United States Environmental Protection Agency

DL-PCBs - dioxin-like PCBs

NDL-PCBs - non dioxin-like PCBs

QuEChERS - quick, easy, cheap, effective, rugged and safe

$MgSO_4$ - magnesium sulphate

NaCl - sodium chloride

PSA - Primary secondary amine

GC - gas chromatography

LC - liquid chromatography

GC/MS - gas chromatography-mass spectrometry

LC-FL - liquid chromatography with fluorescence detection

DAP - dissolved aqueous phase

SPM - suspended particulate matter

PCA - principal component analysis

TEQs - toxicity equivalents

RQs - risk quotients

NO_2^- - nitrites

NO_3^- - nitrates

NH_4^+ - ammonium

PO_4^{2-} - phosphates

SPE - solid-phase extraction

IS - internal standards

N-d8 - Naphthalene-d8

AcP-d10 - Acenaphthene-d10

Phe-d10 - Phenanthrene-d10

Chr-d12 - Chrysene-d12

Pyr-d12 - Perylene-d12

4,40-DFB - 4,40-Difluorobiphenyl

UE - Ultrasound Extraction

$RQ_{(NCs)}$ - negligible concentrations

$RQ_{(MPCs)}$ - maximum permissible concentrations

LODs - limits of detection

PCs - principal components

SD - standard deviation

DR - detection rates

TEFs – toxic equivalency factors

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RESUMO

Hidrocarbonetos aromáticos policíclicos (PAHs) e bifenilos policlorados (PCBs) são poluentes orgânicos persistentes (POP) que representam ameaças significativas para os ambientes aquáticos, uma vez que são compostos tóxicos com elevada mobilidade, persistência e resistência à degradação. Os PAHs formam-se através de processos de combustão incompleta e têm como fontes frequentes derrames de petróleo, emissões de veículos e descargas industriais. Os PCBs, por outro lado, são substâncias químicas sintéticas que foram amplamente utilizadas em equipamentos elétricos e aplicações industriais antes de serem proibidas devido aos seus efeitos nocivos. Tanto os PAHs como os PCBs possuem propriedades hidrofóbicas, resultando na sua acumulação nos sedimentos e nos tecidos adiposos dos organismos aquáticos. Esta acumulação pode perturbar o delicado equilíbrio dos ecossistemas, resultando em impactos adversos nos organismos aquáticos e colocando em risco a saúde humana através do consumo de marisco contaminado. A toxicidade destes compostos varia em função da sua estrutura química e concentração, sendo alguns compostos cancerígenos, mutagénicos e desreguladores endócrinos. Portanto, a monitorização de PAHs e PCBs em ambientes aquáticos é de extrema importância para avaliar os seus níveis de contaminação, identificar fontes e implementar medidas de controlo eficazes.

Este estudo teve como objetivo avaliar padrões de flutuação de 16 PAHs prioritários e 7 PCBs indicadores em amostras de água do mar e mexilhão selvagem, recolhidas em quatro pontos de amostragem nas proximidades de áreas industriais, pesqueiras, recreativas e de uma reserva ornitológica da Costa Atlântico-Ibérica Noroeste (Vila-do-Conde, Portugal). Os métodos de extração incluíram extração em fase sólida (SPE) para amostras de água e o método QuEChERS para amostras de mexilhão, ambos seguidos por cromatografia gasosa - espectrometria de massa (GC-MS). Foram calculados equivalentes de toxicidade (TEQ) e quociente de risco (RQ) para medir o risco ambiental, e foram também calculados a exposição por ingestão diária (EDI), quociente de risco-alvo (THQ) e risco carcinogénico (CR) para medir potenciais riscos para a saúde humana.

As concentrações médias anuais atingiram os ≈ 17 ng/L (DAP), ≈ 100 µg/kg *dw* (SPM) e ≈ 137 µg/kg *ww* (tecido de mexilhão) para os PAHs e ≈ 5 ng/L (DAP), ≈ 14 µg/kg *dw* (SPM) e ≈ 56 µg/kg *ww* (tecido de mexilhão) para os PCB. As amostras de água revelaram a presença contínua de PAHs e PCBs, classificando a região como moderadamente contaminada. Os PAHs têm origens petrogénicas e pirogénicas e

apresentam um risco ecológico de baixo a moderado, com possível potencial carcinogénico. As concentrações de PCB foram baixas e demonstraram um potencial tóxico mínimo, e as suas fontes permanecem incertas. Os mexilhões selvagens apresentaram contaminação generalizada, provavelmente devido à sua baixa metabolização e alta capacidade de acumulação. O perfil dos PAHs nos mexilhões variou ao longo do tempo, possivelmente influenciado pela interrupção das atividades poluentes durante o confinamento de COVID-19. Estes contaminantes apresentaram um risco ambiental moderado, excedendo os níveis legais, mas também revelaram um potencial carcinogénico considerável, principalmente devido a elevadas taxas de ingestão. Os PCB excederam os níveis legais num único local, mas não se verificou que induzissem efeitos tóxicos nestas concentrações.

As preocupações relativas à saúde das comunidades e ecossistemas locais são abordadas neste trabalho, uma vez que os PAHs e PCB são tipicamente encontrados em misturas de contaminantes complexas que podem apresentar riscos superiores do que os observados no presente estudo. Por conseguinte, é fundamental monitorizar e controlar os POP, a fim de proteger o ambiente e a saúde pública.

PALAVRAS-CHAVE: POPs, PAHs, PCBs, *Mytilus* sp., QuEChERS, SPE, GC-MS, poluição aquática

ABSTRACT

Polycyclic Aromatic Hydrocarbons (PAHs) and Polychlorinated Biphenyls (PCBs) are persistent organic pollutants (POPs) that pose significant threats to aquatic environments, as they are toxic compounds with high mobility, persistence and resistance to degradation. PAHs are formed through incomplete combustion processes and are commonly found in sources such as oil spills, vehicle emissions, and industrial discharges. PCBs, on the other hand, are synthetic chemicals that were widely used in electrical equipment and industrial applications before being banned due to their harmful effects. Both PAHs and PCBs exhibit hydrophobic properties, leading to their accumulation in sediments and the fatty tissues of aquatic organisms. This accumulation can disrupt the delicate balance of ecosystems, resulting in adverse impacts on aquatic organisms and posing risks to human health through the consumption of contaminated seafood. The toxicity of these compounds varies depending on their chemical structure and concentration, with some compounds being carcinogenic, mutagenic, and endocrine-disrupting. Therefore, the monitorization of PAHs and PCBs in aquatic environments is of utmost importance to assess their contamination levels, identify sources, and implement effective control measures.

This study aimed to evaluate fluctuation patterns of 16 priority PAHs and 7 indicator PCBs in seawater and wild mussel samples, collected from four sampling sites in the vicinity of industrial, fishery, recreational and ornithological reserve areas from the Atlantic Iberian Northwest Coastline (Vila-do-Conde, Portugal). Extraction methods included solid-phase extraction (SPE) for water samples and QuEChERS method for mussel samples, both followed by gas chromatography–mass spectrometry (GC-MS) analysis. Toxicity equivalents (TEQs) and risk quotient (RQ) were calculated to measure environmental risk, and exposure daily intake (EDI), target hazard quotient (THQ) and carcinogenic risk (CR) were also calculated to measure potential human health risks.

Annual average concentrations reached ≈ 17 ng/L (DAP), ≈ 100 µg/kg *dw* (SPM) and ≈ 137 µg/kg *ww* (mussel tissue) for PAHs and ≈ 5 ng/L (DAP), ≈ 14 µg/kg *dw* (SPM) and ≈ 56 µg/kg *ww* (mussel tissue) for PCBs. Seawater samples revealed the continuous presence of PAHs and PCBs, classifying the region as moderately contaminated. PAHs have both petrogenic and pyrogenic origins and exhibit a low to moderate ecological risk with possible carcinogenic potential. PCBs concentrations were low and demonstrated minimal toxic potential, and their sources remain uncertain. Wild mussels showed widespread contamination, likely due to their low metabolizing and high accumulation

capacities. The PAHs profile in mussels varied over time, possibly influenced by the interruption of polluting activities during the COVID-19 lockdown. These contaminants posed moderate environmental risk, exceeding legal levels but also revealed considerable potential carcinogenicity, mainly due to high ingestion rates. PCBs exceeded legal levels in only one location but were not found to induce toxic effects at these concentrations.

Concerns regarding the health of local communities and ecosystems are brought up in this study as PAHs and PCBs are typically found in complex mixtures of contaminants that could pose greater risks than those observed in the current study. Therefore, it is critical to monitor and control POPs in order to protect the environment and public health.

KEYWORDS: POPs, PAHs, PCBs, *Mytilus* sp., QuEChERS, SPE, GC-MS, aquatic pollution

I. INTRODUCTION

1. Environmental contamination – general approach (POPs)

Persistent Organic Pollutants (POPs) are amongst the most preoccupant environmental contaminants (EU, 2022). These once attaining the marine environment can travel long distances (Ashraf, 2017; Najam and Alam, 2023). Volatile POPs travel long distances in the atmosphere before being re-deposited. The cycle of volatilisation and deposition may be repeated many times, with the result that POPs could accumulate in an area far removed from where they were used or emitted, such as Antarctica and the Arctic area (Kosek and Ruman, 2021). Therefore, understanding POP pathways into food and the environment is crucial for public health protection, as they can easily contaminate crops, livestock, seafood, and drinking water (Christiansen and George, 1995; Guo et al., 2019; Girones et al., 2021).

It is, therefore, essential to understand POPs sources, pathways of movement, and the impacts of their presence in the environment.

Presently, it is known that the primary sources of POPs are:

(1) Pesticides, especially organochlorine pesticides (OCPs) such as dichlorodiphenyltrichloroethane (DDT) and its metabolites.

(2) Industrial and technical chemicals, including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and perfluorooctanesulfonate (PFOS);

(3) By-products of industrial processes including polychlorinated dibenzo-p-dioxins and-furans (PCDDs/Fs) and Polycyclic aromatic hydrocarbons (PAHs) (Gaur et al., 2018).

The peak release of these contaminants, mainly from the industrial and farming sectors occurred in the 1970s. Despite the current concentrations have been reduced considerably, POPs remain a concern to human health due to their long-term exposure and body accumulation (Ashraf, 2017; Girones et al., 2021).

Beyond their toxicity, POPs due to their chemical structures, also resist to degradation having extended half-lives in soils, sediments, air, or biota, with a typical half-life of years or decades in soil and sediment and several days in the atmosphere

(Sinkkonen and Paasivirta, 2000; Girones et al., 2021). Thus, soils and sediments, act as environmental reservoirs and sources of POPs (Najam and Alam, 2023). Besides, as POPs are lipophilic, they have a high affinity to lipids becoming stored in adipose tissue (López-Berenguer et al., 2023). Certain species, such as seals, other marine mammals, migratory birds, and fish, exhibit substantial variations in POP body burdens and dynamics over brief periods as the body fat deposit is re-mobilized. Thus, both the storage and release dynamics of POPs are intimately linked to fat storage and metabolism in living organisms (Tanabe et al., 1994; Dietz et al., 2019; López-Berenguer et al., 2023).

Because POPs can bioaccumulate and magnify in the foodchain, concern centres around their impact on top predator species, including humans. Exposure to high levels of these pollutants may cause numerous health problems such as endocrine disruption (Harrison et al., 1995; Zimmer et al., 2011), cardiovascular diseases, cancers, diabetes, congenital disabilities, and dysfunctional immune (Gascon et al., 2013; Kumar et al., 2014; Alharbi et al., 2018) and reproductive systems (Kogevinas, 2001; Qing Li et al., 2006).

The concerns regarding adverse health effects in humans and wildlife provide the impetus for research on their sources, environmental fate and foodchain transfer (Alharbi et al., 2018; Najam and Alam, 2023). However, because an extensive array of POPs occurs and accumulate simultaneously in biota, it is challenging to determine if an effect is due to one particular chemical, a family of chemicals, their metabolites or several families of chemicals acting synergistically (Zimmer et al., 2011; Tang et al., 2020).

Ingestion of contaminated food, especially that from animal sources, accounts for more than 90% of POP exposure in humans (Guo et al., 2019). Fishes are among the major sources of exposure to POPs (Bedi et al., 2018; Fair et al., 2018).

To protect consumers from this threat, many national and international agencies such as the *European Food Safety Authority* (EFSA), the WHO, the United States of America (USA), *Environmental Protection Agency* (EPA), and the USA *Food and Drug Administration* (FDA) have developed regulations and guidelines to reduce the exposure to POPs (Kalachova et al., 2012).

Also, the Stockholm Convention of the *United Nations Environment Program* (UNEP, 2019) pursues the global reduction or elimination of the use and production of

POPs, and it requires its parties to take action to decrease the production, use and release of the POPs on its list. The initial list was established in 2001 and included twelve POPs, known as the “dirty dozen”; by 2019, seventeen POPs were added. Additionally, the *Water Framework Directive* (WFD) of the European Commission set up a group of forty-five substances that have been prioritized and updated for action at the European Union (EU) level (EU, 2022). These international policies established the concentration limits for pollutants in water, sediment or biota that should not be exceeded to protect human health and the environment.

As a result of the regulations and legal frameworks, a general decrease was observed in POPs in recent decades (Dopico and Gómez, 2015; Stubleski et al., 2018; Najam and Alam, 2023). For instance, their presence in food and feed has generally declined due to these legislative measures and strategies for reduction from public authorities and industry (van den Berg et al., 2017; Guo et al., 2019).

In Portugal, several studies demonstrated the presence of POPs in surface waters, ground and drinking waters and wastewater as well as sediments (Cardoso, 2014; Ribeiro et al., 2016; Rocha et al., 2017).

Because this work focuses on PAHs and PCBs, more details are referred below concerning these two groups of POPs.

1.1. PAHs

PAHs can result from both human activities (*e.g.*, industrial production and mobile transports, sewage sludge, and PAH contaminated media) or natural processes (*e.g.*, vegetable decay, plant synthesis, and natural petroleum) (Baek et al., 1991; Abdel-Shafy and Mansour, 2016). Thus, PAHs have become a source of great concern due to their extensive distribution, their capacity to accumulate in the food chain, and their potential to cause mutations, cancer, and toxicity in people and other living organisms (Honda and Suzuki, 2020; Wang et al., 2021).

The Aarhus Protocol only recognised PAHs as POPs in 2003 (UNECE, 2003). Despite this, due to their lipophilicity and continuous release into the environment these compounds are one of the leading environmental concerns in urban and industrial areas as they pose a significant threat towards global pollution (Alharbi et al., 2018; Gaurav et

al., 2021). PAHs also have uniquely stable structures that allow them to persist in the environment (Cerniglia, 1993; Wilcock et al., 1996)

Chemically, PAHs comprised of two or more aromatic rings bonded in linear, cluster, or angular arrangements (Fetzer, 2000; Wilcke, 2000; Abdel-Shafy and Mansour, 2016). PAHs with 2 and 3 aromatic rings are labelled ‘low molecular weight’ (LMW), whereas those with 4 or more aromatic rings are called ‘high molecular weight’ (HMW).

PAHs generally have high melting and boiling points, low vapour pressure, and very low aqueous solubility (Masih et al., 2012; Abdel-Shafy and Mansour, 2016; WHO, 2021). The last two characteristics tend to decrease with increasing molecular weight, whilst resistance to oxidation and reduction increases (Masih et al., 2012).

PAHs are very soluble in organic solvents, as they are highly lipophilic and have a high octanol/water partition coefficient (K_{ow}). On the contrary, the aqueous solubility of these compounds decreases for each additional ring (Wilson and Jones, 1993; Masih et al., 2010). Additional characteristics of PAHs include conductivity, light sensitivity, heat resistance, corrosion resistance, and physiological action (Cerniglia, 1993; Akyüz and Çabuk, 2010).

1.2. PCBs

Industrial applications of PCBs grew during the mid-20th century, with production in the United States reaching 16.000 tonnes by 1957 and nearly 40.000 tonnes by 1980 (Hopf et al., 2009). The total amount of PCB compounds produced worldwide is estimated to be 1.3 million tonnes (Breivik et al., 2007). Following these high production rates, environmental contamination concerns increased through the end of the (20th) century as more information came to light on the toxicity of PCBs to humans and ecosystems. As a result, production in Western Europe, North America and Japan ceased in the late 1970s and in Eastern Europe and Russia in the early 1990s (Breivik et al., 2002b; Breivik et al., 2007).

Thus, before being banned, PCBs were widely used for a variety of applications due to their thermal stability, low flammability, and electric insulating properties, such as dielectric fluids in transformers and capacitors, hydraulic oils, coolants, printing ink, flame

retardants, lubricants, pesticide and wax extenders, and additives in paints, plastics, and adhesives (Stringer and Johnston, 2001; Ross, 2004; Erickson and Kaley, 2011).

Chemically, PCBs are resistant to metabolism and environmentally persistent, resulting in a food chain build-up (Ross, 2004; van den Berg et al., 2017; EFSA et al., 2018).

PCBs are up to 209 congeners that result from the variation in number and position of the chlorine (Cl) atoms connected to the biphenyl rings, and analysis is usually focused on toxic PCBs including dioxin-like PCBs (PCB-77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189) and indicator PCBs (PCB-28, 52, 101, 138, 153 and 180) (Portoles et al., 2016; Ten Dam et al., 2016).

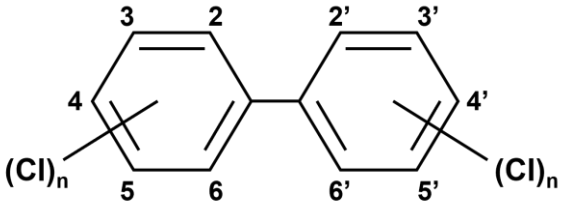


Figure 1 – General chemical structure of PCBs.

Epidemiological studies conducted over the last five decades have identified and demonstrated the toxic potential of these compounds and their metabolites on human and animal health, including systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects (Ulbrich and Stahlmann, 2004; Ruiz et al., 2008; Grimm et al., 2015; Faroon and Ruiz, 2016). Additionally, numerous studies have linked PCB occurrences to illnesses and/or anomalies in a variety of wildlife species, including fish (Incardona and Scholz, 2016; Berninger and Tillitt, 2019), molluscs (Milun et al., 2016; Santos et al., 2020), and mammals (Dedrick et al., 2012; Berghe et al., 2013).

Recent monitoring studies confirmed that, despite having been banned from open use in most countries, they continue to exist in a variety of environmental compartments, though generally at lower levels than they did a few years ago (Ross, 2004; Carro et al., 2010).

2. PAHs and PCBs in the environment

2.1. PAHs in abiotic systems

There are four types of PAHs in the aquatic environment:

(1) Derived from fossil fuels (petrogenic);
(2) Derived from incomplete combustion processes (pyrogenic);
(3) Generated by organic metabolism (biogenic);
(4) Generated by the transformation process in sediment (diagenetic) (Hylland, 2006).

Of these four types of sources, petrogenic and pyrogenic sources are mainly artificial and are important contributors to environmental PAH pollution in aquatic ecosystems.

Pyrogenic PAHs are the result of natural fires as well as from anthropogenic activities (waste incineration, combustion of fossil fuels) that give rise to complex mixtures of high molecular weight PAHs, such as pyrene, benzo[a]pyrene, dibenzo[a,h]anthracene, dibenzo[a,h]pyrene, benzo[g,h,i]perylene, benz[a]anthracene.

These PAHs are related to high mutagenicity and carcinogenicity. Petrogenic PAHs are present due to anthropogenic activities such as petroleum transportation and oil spills and also as derived from petroleum hydrocarbons. Most petrogenic PAHs have 2–4 rings (Maletic et al., 2019).

One of the critical concerns with PAHs is their omnipresence and high dwell time in the environment (Maletic et al., 2019; Billah et al., 2022), namely in the atmosphere (Dat and Chang, 2017), in water (Santana et al., 2015) and in soil (Kuppusamy et al., 2017).

PAHs tend to accumulate in sediments, traditionally seen as a sink for these contaminants in aquatic ecosystems (Page et al., 1999; Roberts, 2012). They persist and undergo very slow transformations, providing explicit records of human activities in the surrounding environments (Page et al., 1999; Sutilli et al., 2020). Buried PAHs stocks in marine and freshwater sediments could hence constitute a subsequent source for water column contamination (Chen et al., 2020; Jaglal, 2020) or bioturbation (Gonzalez et al., 2019; Tian et al., 2020), potentially threatening pelagic and benthic organisms.

PAHs, which adsorb on suspended solids and organic matter, can easily transfer to various compartments in food webs (Baumard et al., 1998), which eventually leads to the contamination of superior trophic levels (Smith et al., 2001; Grova et al., 2002).

2.2. PCBs in abiotic systems

Despite having been banned circa 50 years ago, PCBs can still be formed inadvertently during various industrial thermal processes such as waste incineration, cement production, metallurgy, and even in pigment production (Gong et al., 2017; Anh et al., 2021).

Environmental emissions of PCBs have been associated with three major processes:

(1) Intentional production, utilization, and disposal of PCB-treated products;

(2) Unintentional emission from combustion and waste processing activities;

(3) Re-emission of PCBs from landfills or environmental reservoirs (*e.g.*, soil, sediment, and water) (Breivik et al., 2002a; Martinez et al., 2010; Anh et al., 2021).

Processes (2) and (3) raise the most concerns nowadays.

Once PCB mixtures are released into the environment, processes such as volatilization, partitioning, chemical or biological transformation, and preferential bioaccumulation alter them and dictate their environmental fate, depending on the degree of chlorination of the biphenyl molecule (ATSDR, 2000).

The higher chlorinated PCB congeners adsorb strongly to sediment and soil, where they tend to persist with half-lives from months to years (Sinkkonen and Paasivirta, 2000). Highly chlorinated congeners are also more slowly metabolized, resulting in bioaccumulation and biomagnifications in the food chain (Ivanciuc et al., 2006). Similarly, to PAHs, PCBs tend to accumulate in adipose tissues because of their stability and lipophilicity (Berninger and Tillitt, 2019; Santos et al., 2020). Numerous reviews have summarized PCBs' persistence, movement, and bioaccumulation properties in the environment (Tanabe, 1988; Voogt et al., 1990).

2.3. PAHs and PCBs in Aquatic Organisms

As mentioned before, PAHs and PCBs occurrence in aquatic organisms results from bioconcentration and biomagnification processes, leading to high concentrations within the fatty tissues of top predators (*e.g.*, piscivorous birds and marine mammals). The quantity of these POPs absorbed from sources like water, sediment, and diet depends on congener properties, environmental conditions, and specie's living and feeding habits. High-trophic-level species' primary source of contamination is through diet (consumption of prey), whereas low-trophic-level species and benthic organisms absorb these compounds from other sources such as water, sediment, and benthic invertebrates (Rodenburg et al., 2015; Jafarabadi et al., 2019).

As different species respond differently to stressors and show different tolerance levels, certain organisms are sensitive to environmental changes and can reflect the overall health of an ecosystem. After a long history of extensive research and observation of these organisms and their response to environmental stressors (such as pollution), the concept of bioindicators were created. They provide valuable information about the overall condition of an ecosystem and can be used in various fields, such as environmental monitoring, ecological research, and pollution assessment. Bioindicators have become especially useful in the analysis of the impacts of human activities on ecosystems, enabling scientists and legislators to make informed decisions for conservation and management efforts.

Biomonitoring species in coastal areas require specific biological properties, such as wide distribution and settlement, easy sampling, high salinity tolerance capacity, and bioaccumulation properties for target chemicals (Monirith et al., 2003; Beyer et al., 2017). Based on these requirements, bivalves, such as oysters and mussels, are most commonly used as environmental bioindicators.

2.3.1. *Mytilus* as a bioindicator of excellence

Mussels (*Mytilus* sp.) are widely recognized as effective bioindicators due to their sensitivity to water quality changes and ability to accumulate pollutants. As filter feeders, they constantly pump water through their bodies, filtering out particles and contaminants. Due to their sessile lifestyle, they accurately reflect local environmental conditions (Jørgensen, 1990; Beyer et al., 2017), and because of their wide geographic distribution, easy sampling, tolerance to a considerable range of salinity, resistance to stress and

high accumulation of a wide range of pollutants, they are the ideal test organisms for environmental monitoring (Tanabe et al., 2000).

They filter large volumes of seawater to feed on phytoplankton, consequently accumulating lipophilic pollutants, heavy metals and microplastics from the water column (Jørgensen, 1990; Beyer et al., 2017). Additionally, ingested sediment particles may lead to further dietary exposure to these adsorbed contaminants (Baumard et al., 1999). Filter-feeding organisms, such as mussels, are recognized as time integrators, which means they embed long-term effects caused by polluting compounds in a specific study area and also allow the identification of potential sources of contamination (Hunt and Slone, 2010; Beyer et al., 2017).

Some studies show that mussel concentrations of PAHs and PCBs fluctuate along the year (Hummel et al., 1990; Webster et al., 2006), influenced by food availability, hydrological conditions, and internal factors like physiological condition, age, size, and lipid content (Widdows et al., 1979; Gilek et al., 1996; Gossiaux et al., 1996).

Mussels soft tissue lipids strongly influence the bioaccumulation of hydrophobic organic compounds like PCBs and PAHs (Livingstone, 1992; Bright et al., 1995). In these organisms, lipid content varies based on physiological conditions and reproduction cycles, increasing during gamete maturation in late winter and early spring and decreasing after spawning in late spring and summer (Zandee et al., 1980; Gabbott, 1983).

3. Toxicity of PAHs and PCBs

Organisms are frequently exposed to a complex mixture of pollutants, including parent compounds and their transformation products (Ginebreda et al., 2014). A wide range of environmental chemicals, namely the POPs, have significant toxicological weight, given their involvement in several pathological processes, including carcinogenesis and reproductive issues, and the fact that they cause harm that extends beyond the individual in present and succeeding generations (Lewis and Galloway, 2009; Devaux et al., 2011). These compounds may also produce adverse effects on the development, growth, behaviour, and reproduction (Eganhouse and Sherblom, 2001; Ginebreda et al., 2014).

PAHs and PCBs, raise the risk factors for breast cancer (Gammon et al., 2004), lung cancer (Okona-Mensah et al., 2005), and prostate cancer (Ritchie et al., 2005). So, the widespread occurrence of POPs has attracted considerable attention.

Once in the body, PAHs are usually biotransformed through toxicokinetics detoxification pathways (Penning et al., 1999; Straif et al., 2005; Xue and Warshawsky, 2005). However, the last process involves Phase I enzymes, which usually generate reactive epoxides, that may be detoxified through Phase II or bind to other cellular components, such as DNA (Xue and Warshawsky, 2005).

Generally, carcinogenic PAHs have higher molecular weight and lower vapour pressure and solubility constants than non-carcinogenic PAHs. Their toxicity, which depends on their nature and environmental factors, is increased for PAH mixtures (Ball and Truskewycz, 2013). PAHs absorption and toxic effects in different groups of aquatic organisms have been recently reviewed in several works (Honda and Suzuki, 2020).

Additionally, some studies revealed that exposure to ultraviolet or natural sunlight might increase the toxicity of PAHs (phototoxicity) (Yu, 2002; Fu et al., 2012).

The PCBs are considered human carcinogens, and congeners were observed to have different toxicities and forms of action based on the chlorination pattern on the biphenyl rings (Giesy and Kannan, 1998; White and Birnbaum, 2009). Although all PCBs are structurally similar, the number and orientation of chlorines influence toxicity empirically and mechanistically (Safe et al., 1985; Safe, 1990). A small set of the PCB congeners (18 congeners) were found to bind to the *Aryl hydrocarbon Receptor* (AhR) and elicit dioxin-like toxicity (Van den Berg et al., 1998).

All vertebrates possess homologous forms of the AhR and differ in their sensitivity to dioxins and dioxin-like PCBs (DL-PCBs). Other forms of toxicity of these compounds include neurotoxicity, endocrine disruption, and carcinogenicity (Safe et al., 1985; Safe, 1990; Safe, 1994). Over the past 20 years, toxicity thresholds for commercial PCB mixtures and individual congeners in wildlife have been developed (Giesy and Kannan, 1998; Kannan et al., 2000; Su et al., 2014).

The non-dioxin PCBs (NDL-PCBs), also induce the surge of diverse pathologies including those related to thyroid gland affecting the levels of circulating thyroid hormones, specifically T4 in laboratory animals and wildlife (Hallgren and Darnerud,

2002). Significant decreases of T3 and/or T4 were found in sea lions (Debier et al., 2005), polar bears (Haave et al., 2003) and seals (Brouwer et al., 1989).

4. Environmental levels (Legislation)

EPA has listed sixteen priority PAHs (**Figure 2**) based on toxicity, potential for human exposure, frequency of occurrence at hazardous waste sites, and the extent of information available (USEPA, 1984).

These include anthracene (Ant), acenaphthene (AcP), acenaphthylene (AcPY), benzo[a]anthracene (BaA), benzo[b]fluoranthene (BbFL), benzo[k]fluoranthene (BkFL), benzo[g,h,i]perylene (BP), benzo[a]pyrene (BaP), chrysene (Chr), dibenzo[a,h]anthracene (DBA), fluorene (Flu), fluoranthene (FL), indeno[1,2,3-c,d]pyrene (Ind), naphthalene (NaP), phenanthrene (Phe), and pyrene (Pyr). Within these, BaA, Chr, BaP, BbFL, BkFL, DBA, and Ind are probable human carcinogens (IARC, 2010).

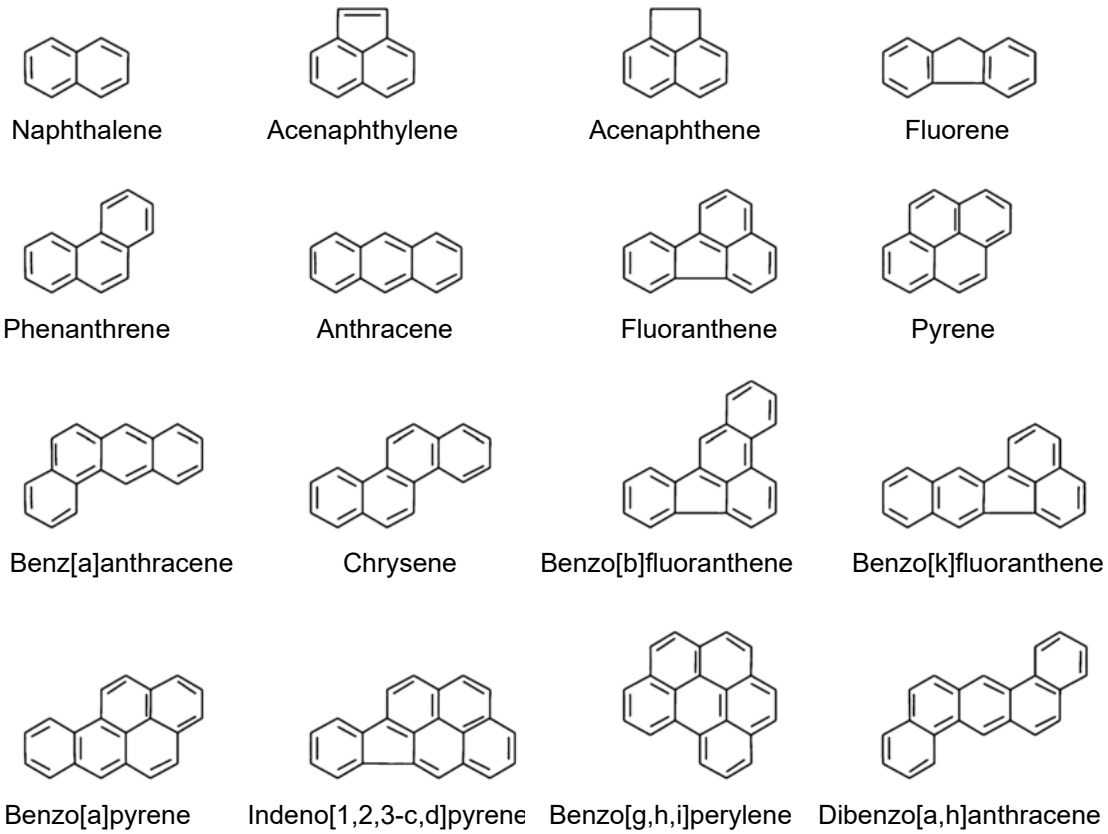


Figure 2 – Molecular structure of the 16 PAHs considered priority pollutants by the United States Environmental Protection Agency (US EPA).

Although PAHs are not among the ‘dirty dozen’ of the Stockholm convention on POPs, they were included in the convention on *Long-range Transboundary Air Pollution Protocol on Persistent Organic Pollutants* (UNECE, 2003).

Based on environmental persistence, PCBs were included in the United Nations Environmental Programme Stockholm Convention (2001) list of POPs and later in 2004 by the Organisation for Economic Co-operation and Development (Tanabe, 1988).

As mentioned, although PCBs were banned in the mid-1980s, sources of PCBs persist, *e.g.*, within waste disposal sites and PCB-containing equipment (IARC, 2016). Elevated levels of PCBs are observed primarily in coastal and estuarine marine waters, where they can severely impact resident biota, particularly on sedentary filter-feeding bivalves and bottom-dwelling polychaetes (Olenycz et al., 2015).

Fortunately, enacted laws have reduced the overall exposure of PCBs in the general population (Ross, 2004).

For environmental monitoring and evaluation of PCBs, OSPAR has determined marine monitoring to be done on a set of seven PCBs (PCB28, PCB52, PCB101, PCB118, PCB138, PCB153 and PCB180) (OSPAR, 2022). These were selected as indicators due to their relatively high concentrations in technical mixtures and their wide chlorination range (3–7 chlorine atoms per molecule). The same set of PCBs can also be used for food safety evaluation, according to EU regulation (EC) 1259/ 2011 (CEC, 2023).

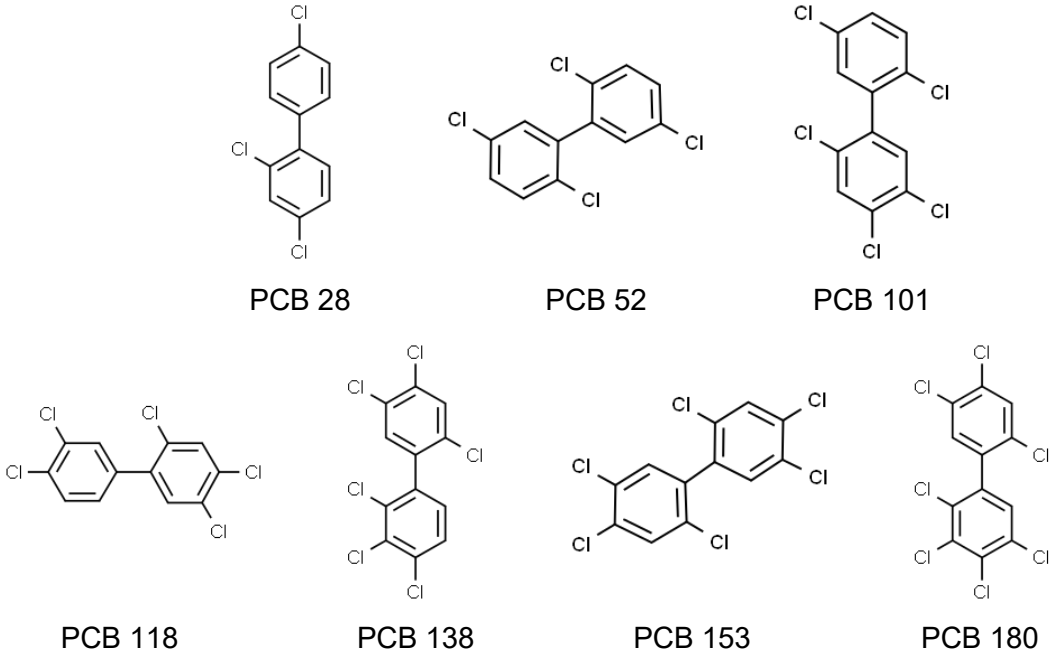


Figure 3 – Molecular of the 7 PCBs considered indicators of marine quality assessment by OSPAR (OSPAR, 2022).

The European maximum limit of dioxins and DL-PCBs is set at 6.5 pg/g *ww*, whilst the limit of NDL-PCBs is set as 75 ng/g *ww* for fishery products and bivalve molluscs (CEC, 2023). According to the mentioned regulation, BaP and the sum of the other four PAHs (BaP, BaA, BbFL and Chr) should not exceed 2.0 and 12.0 ug/kg in smoked fishery products and 5.0 and 30.0 ug/kg in bivalve molluscs, respectively (CEC, 2023).

5. Evaluation of PAHs and PCBs in environmental samples

Regarding the analytical methodologies for the detection/quantification and unequivocal identification of these organic compounds, one of the golden keys has always been the sample's pre-treatment improvement through interference reduction to reach reproducible and accurate data. For the analysis of POPs in high-complexity samples, such as the environmental ones, there has been a constant demand for new sample preparation methodologies with increasing specificity and robustness, combined with simple implementation and affordable costs, which may explain the success of the QuEChERS technique.

5.1. QuEChERS

The QuEChERS (quick, easy, cheap, effective, rugged, and safe) analysis method was introduced by Anastassiades and collaborators for the monitoring of multiclass multi-residue pesticides in various agricultural products (Anastassiades et al., 2003). The original QuEChERS approach has been continuously modified for the analysis of a wide range of pesticides (Lee et al., 2017), antibiotics (Stubbings and Bigwood, 2009), hormones, mycotoxins (Sospedra et al., 2010), PAHs (Smoker et al., 2010) and several other POPs (González-Curbelo et al., 2015; Cloutier et al., 2017; Surma et al., 2017) in food and environmental matrices.

The QuEChERS aims to simplify and streamline the extraction and purification procedures to minimize costs, and it includes miniaturization and automation to make the analysis of the pollutants easier. In contrast, conventional extraction methods require multiple steps and a considerable amount of solvent and time (Grimalt and Dehouck, 2016)

The QuEChERS procedure is based on three distinct steps: extraction, partition and clean-up.

In the extraction phase, acetonitrile is the most commonly selected extraction solvent due to its capacity to extract a wide polar range of molecules from various matrices (Anastassiades et al., 2003; Baduel et al., 2015), producing satisfactory recovery rates (80–110%) for samples with high water content (González-Curbelo et al., 2015). Acetonitrile penetrate the aqueous fraction of the sample matrices, allowing the easy separation of the two phases upon adding salt (Schenck et al., 2002). During the partitioning phase, $MgSO_4$ and NaCl are selected as drying salts because they efficiently reduce the solubility of the analytes in the liquid-liquid phase (Anastassiades et al., 2003). This step promotes the separation of the organic phase from the liquid, avoiding the dilution of the acetonitrile extract (Andersson et al., 1990).

Primary secondary amine (PSA) and $MgSO_4$ have been employed as clean-up sorbents. PSA efficiently eliminates fatty and organic acids, while $MgSO_4$ removes residual water for instrumental separation. Residual lipids or fats in extracts may be retained in gas chromatography (GC) or liquid chromatography (LC) columns. These compounds can reduce the efficiency of ion sources and detectors and shorten column

life (Lankova et al., 2013; Baduel et al., 2015), so it is important to remove them to obtain robust and reliable results.

The QuEChERS approaches were applied to analyze PAHs and PCBs in sediment, water, seafood, and meat products (Johnson, 2012; Ben Salem et al., 2016; Urban and Lesueur, 2017). Combined with the QuEChERS approach, gas chromatography-mass spectrometry (GC–MS) has replaced the traditional LC-with fluorescence detection in the analysis of PAHs due to its relatively higher selectivity (Cloutier et al., 2017; Chiesa et al., 2018).

5.2. GC-MS

Chromatographic methods have been developed and thoroughly evaluated over the past decades and are currently essential for determining organic contaminants in food and environmental samples.

GC-MS combines gas chromatography for separation and mass spectrometry to detect and identify the components of a mixture of compounds. Gas chromatography separation works best with non-polar, thermally stable, and volatile compounds (Cho et al., 2016; Urban and Lesueur, 2017).

Therefore, the most recent studies have focused on improving the separation capacity of the various PCBs and PAHs congeners, associated with a growing concern to achieve low limits of detection and quantification, even in more complex matrices. This can be achieved with GC-MS, being considered the analytical technique of excellence for the analysis of these compounds (Madureira et al., 2014a; Madureira et al., 2014b; Chiesa et al., 2018).

6. Study main objectives

This study main goals are:

(1) Provide data that could suggest seasonal fluctuation patterns of sixteen priority PAHs and seven indicator PCBs in DAP (seawater), SPM and wild mussels collected from eight sampling sites from Vila do Conde (Atlantic Iberian Northwest Coastline, Portugal) from 2018 to 2021.

(2) Investigate the potential sources of PAHs in the coastline trough the analysis of the distribution profiles, representing two-dimensional diagrams of specific diagnostic ratios for PAHs, and multivariate methods, such as principal component analysis (PCA).

(3) Investigate the potential sources of PCBs.

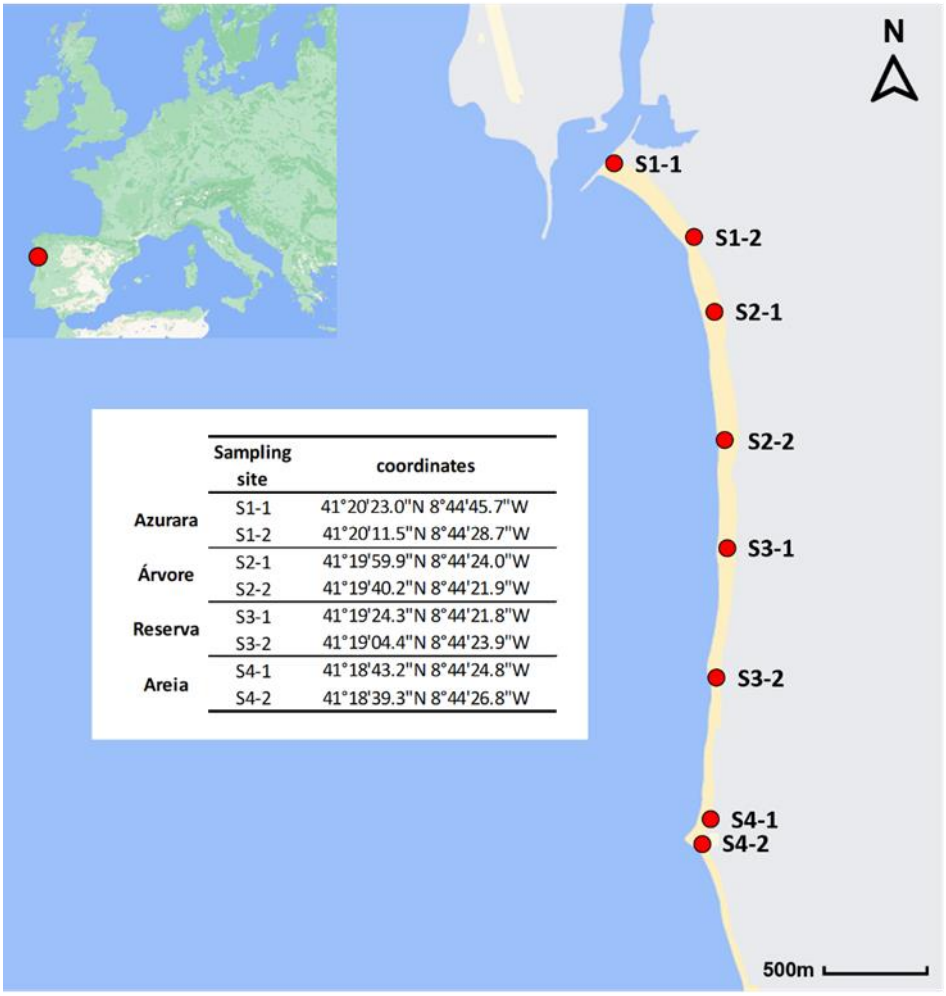
(4) Evaluate the environmental risks posed by the surveyed POPs using theoretical tools as the calculus of toxicity equivalents (TEQs) and risk quotients (RQs).

(5) Evaluate the health risks associated with the ingestion of blue mussels from the selected sites by the local population, using theoretical tools as the calculus of exposure daily intake (EDI), target hazard quotient (THQ) and carcinogenic risks (CR).

II. MATERIALS AND METHODS

1. Sampling area

The sample area (ca. 10 km) is a European Atlantic shoreline located close to a highly industrialized zone (Vila-do-Conde) in the northwest of the Iberian Peninsula (Portugal). This zone receives the Ave River waters that flow into the Atlantic Ocean after receiving anthropogenic inputs in its 97 km route from a drainage basin that covers approximately 1390 km^2 (Gonçalves et al., 1992). The sampling points are spread along the seashore, which has four beaches with rich biodiversity, primarily avifauna, and diverse landscapes, including dunes, cliffs, and wetlands. The sampling locations are indicated in **Figure 4**.



	Sampling site	coordinates
Azurara	S1-1	41°20'23.0"N 8°44'45.7"W
	S1-2	41°20'11.5"N 8°44'28.7"W
Árvore	S2-1	41°19'59.9"N 8°44'24.0"W
	S2-2	41°19'40.2"N 8°44'21.9"W
Reserva	S3-1	41°19'24.3"N 8°44'21.8"W
	S3-2	41°19'04.4"N 8°44'23.9"W
Areia	S4-1	41°18'43.2"N 8°44'24.8"W
	S4-2	41°18'39.3"N 8°44'26.8"W

Figure 4 - Location of the sampling sites in northwest Atlantic Iberian seacoast (S_1 to S_4, n=8) (adapted from KML Map).

The first sampling area, Azurara Beach, is close to the waters' entrance from the Ave River estuary into the Atlantic Ocean. Here, water samples were picked at two sites, $S_{1\text{-}1}$ (41°20'23.0"N 8°44'45.7"W) and $S_{1\text{-}2}$ (41°20'11.5"N 8°44'28.7"W). Mussels (*Mytilus* sp.) were collected at $S_{1\text{-}1}$.

The second sampling area is at Árvore Beach. This zone is widely used for recreational purposes due to its connection to a camping park (Árvore Camping Park). Here, water samples were picked at $S_{2\text{-}1}$ (41°19'59.9"N 8°44'24.0"W) and $S_{2\text{-}2}$ (41°19'40.2"N 8°44'21.9"W). Mussels were collected at $S_{2\text{-}2}$.

The third and fourth sampling areas are at Areia and Mindelo Beaches. Both areas cover a Regional Protected Coastal Landscape, home to a birdwatching reserve known as the Ornithological Reserve of Mindelo (DR 2009). Here, water sampling occurred at 4 different points, two of them in proximity to the reserve – $S_{3\text{-}1}$ (41°19'24.3"N 8°44'21.8"W) and $S_{3\text{-}2}$ (41°19'04.4"N 8°44'23.9"W) – and, further south, at the locations $S_{4\text{-}1}$ (41°18'43.2"N 8°44'24.8"W) and $S_{4\text{-}2}$ (41°18'39.3"N 8°44'26.8"W. Mussel samples were collected at $S_{3\text{-}1}$ and $S_{4\text{-}2}$.

2. Water collection and physicochemical measurements

Between November 2017 and August 2021, water samples (2 L) containing suspended surface sediments were collected, about 1 m deep, from the eight sampling sites, one harvest per season (n = 12).

These samples were stored in amber glass bottles, pre-rinsed with local water, and transported to the laboratory at ca. 4 ºC. Temperature, pH and salinity were measured immediately using portable instruments (pH 330i/Set WTW, Weilheim, Germany, and a refractometer). The levels of nitrites (NO_2^-), nitrates (NO_3^-), ammonium (NH_4^+), and phosphates (PO_4^{2-}) were measured by photometry (pHotoFlex® STD Colorimeter WTW). For the last purpose, certificated seawater evaluation kits were used, and all samples and blanks were analysed in triplicate to ensure the analytical accuracy; NO_2^- (kit 250440), NO_3^- (kit 250411), NH_4^+ (kit 252072), PO_4^{2-} (kit 252086).

3. Animal sampling

In total, 12 harvests were carried over the course of 4 years from 2017 to 2021. The collected mussels per harvest were established to produce three pools per location,

each containing a minimum of 20 g of wet soft tissue. This mass was previously determined in preliminary assays, considering the inevitable losses that arise during the preparation and homogenization of samples (Madureira et al., 2014a; Madureira et al., 2014b).

Each pooled sample was required to contain a minimum of three individuals of feral mussels hand-collected along the five locations, at low tide, and in the intertidal zone.

The bivalves were immediately transported to the laboratory in a refrigerated cooler (± 4 °C). Then, the animals were placed for 24 h in 30 L tanks with constant salinity (34 Psu ± 2) and oxygenation to release the sand and other solid particles that could interfere with the extraction protocol.

Before euthanasia, all animals were deeply anaesthetized in a magnesium chloride solution ($MgCl_2$) (60 g/L) (Butt et al., 2008). Every mussel had its length, width, and height measured (cm), and its weight (with and without shell) recorded (g). Subsequently, all the collected specimens were stored individually in aluminium foil and kept at −80 °C until further procedure.

4. Analytical methodologies

4.1. Chemicals and materials

Acetonitrile (>99.9% purity) was acquired from Sigma-Aldrich (Steinheim, Germany). The remaining solvents, including n-hexane, dichloromethane, acetone, and methanol (99.9% purity), were purchased from Romil (Cambridge, UK). Ultrapure water was supplied by a Milli-Q water system (conductivity = 0.054 μS cm^{-1}, at 25°C).

The solid-phase extraction (SPE) cartridges, 200 mg Oasis HLB (Hydrophilic-Lipophilic Balance), 6 cc, were acquired from Waters Corporation (Milford, USA). The Florisil TLC (pro-analysis grade, 30-60 mesh) was from Sigma-Aldrich (Steinheim, Germany).

QuEChERS pre-packed dSPE tubes containing 900 mg $MgSO_4$ and 150 mg Supelclean PSA and NaCl were purchased from Sigma-Aldrich (Steinheim, Germany).

The standard mixture containing the sixteen EPA PAHs (EPA TCL Polynuclear Aromatic Hydrocarbons mix) was purchased from Supelco (Bellefonte, USA).

PCBs standards (PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, and PCB180) were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). All reference standards were of >96% purity. The internal standards (IS) for PAHs were a mixture containing naphthalene-d8 (N-d8), acenaphthene-d10 (AcP-d10), phenanthrene-d10 (Phe-d10), chrysene-d12 (Chr-d12), and perylene-d12 (Pyr-d12) from Supelco (Bellefonte, PA). The IS for PCBs was the 4,4- difluoro biphenyl (4,4-DFB) from Sigma-Aldrich (Steinheim, Germany).

4.2. Extraction methods

4.2.1. SPE protocol

Water samples (1 L) were filtrated through 0.45 mm glass fibre filters (Millipore, Cork, Ireland). This procedure split the samples into two fractions, DAP and SPM, which were later prepared to analyse PAHs and PCBs.

In DAP, the last POPs were extracted by solid-phase extraction (SPE, OASIS HLB) (Sánchez-Avila et al., 2011). Briefly, cartridges were conditioned sequentially with 10 mL of hexane, followed by 10 mL of dichloromethane, 10 mL of methanol and 15 mL of ultrapure water at a 1-2 mL/min flow rate. Then, the seawater samples (1 L), added with IS, were loaded into SPE cartridges at a constant flow rate of 5 mL/min. The cartridges were rinsed thrice with 5 mL of ultrapure water and dried under vacuum for 30 min. PAHs and PCBs were eluted with 10 mL of dichloromethane:hexane (1:1, v/v) and 10 mL of dichloromethane:acetone (1:1, v/v). The extracts were concentrated into 100 mL of hexane and kept in vials at - 20 ^{o}C until analysis.

PAHs and PCBs extraction from SPM occurred by Ultrasound Extraction (UE) (Rocha et al., 2017). Briefly, the fibre filters, added with IS, were submitted to UE (AXTOR CD-4820, Lovango, Spain), following solvents/time of sonication: 10 mL of dichloromethane (10 min), 10 mL acetone (10 min), and 10 mL methanol (10 min). Also, ≈ 1 g of anhydrous Na_2SO_4 was added to remove all water. These extracts were concentrated in a Rotavapor (BÜCHI R-114, Sigma-Aldrich, Darmstadt, Germany) linked to a water bath set to 50 ^{o}C (BÜCHI B-480 Sigma-Aldrich, Darmstadt, Germany) to a final volume of ca. 2 mL, cleaned with silica gel and dried under a gentle stream of N_2.

Before GC-MS analysis, these fractions were reconstituted to a final volume of 250 mL in hexane.

4.2.2. QuEChERS protocol

The QuEChERS extraction technique performed in this study was based on the original procedure developed by Anastassiades et al. (2003) and later adapted and validated by Madureira et al. (2014) for mussels. Briefly, once defrosted, the mussel soft tissues of each pool were first transferred into a 50 mL Teflon centrifuge tube (Nalgene, Rochester, USA) and homogenized with an IKA®T10 basic Ultra-Turrax® on ice to ensure compound stability and then treated individually to avoid possible contamination between sample pools.

The resulting homogenate was mixed with 10 mL of acetonitrile. Samples were shaken for 1 min at full speed with a vortex apparatus (Vortex Mixer VX-200, Labnet International Inc.), and to ensure an efficient interaction between the chemicals and the biological matrix, they were let to stand for 2 minutes.

Then, 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were added and stirred on the vortex for 1 min. Following this, samples were centrifuged for 5 min at 3220 RFC, at 15°C (Sigma 2-16K centrifuge Montereal Blotech Inc.).

For the clean-up step, the acetonitrile layer was transferred into a Teflon tube containing 900 mg $MgSO_4$ and 150 mg PSA, capped tightly and shaken using the vortex mixer for 1 min. Finally, the extracts were centrifuged for 5 min at 3220 RFC, and 2.5 mL of the supernatant was collected for GC-MS analysis.

4.3. Gas chromatography-mass spectrometry

All analyses were carried out using a gas chromatograph (Thermo Scientific GC TRACE 1310, Thermo Finnigan Electron Corporation), coupled with an ion trap mass spectrometer (Thermo Scientific ISQ-LT GC-MS) and an autosampler (Thermo Scientific Injector Triplus 100LS™). A Trace GOLD column (TG-5MS, length—30 m, ID—0.25 mm, film thickness—0.25 µm) was used.

The analyses of the respective classes of compounds in DAP, SPM and mussel were performed by GC-MS using the equipment and the conditions referred to in **Table 1**.

Table 1 - GC-MS conditions used for adequately separating and quantifying PAHs and PCBs from environmental matrices (Madureira et al., 2014a; Madureira et al., 2014b).

GC-MS Conditions			
Gas chromatograph	Trace GC ultra, Thermo Finnigan Electron Corporation		
Detector	Ion trap mass spectrometer (Thermo Scientific ISQ-LT GC-MS)		
Auto sampler	Thermo Scientific Injector Triplus 100LS™		
Injector	SSL (3 mm straight liner)		
Mode	Splitless mode		
Volume (µL)	1 (50 mm length needle)		
Temperature (ºC)	Column Trace GOLD column TR5MS (30 m x 0.25 mm x 0.25 mm)		
Gas carrier	Helium (99.9999% purity), maintained at a constant flow rate of 1.0 mL/min		
Oven Program	Temperature (ºC)	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:	280 ºC		
Ion source:	280 ºC		

5. Risk assessment

5.1. Calculation of toxicity equivalents (TEQs) and risk quotients (RQ)

The derivation of TEQs for both PAHs and PCBs in water samples followed the equation (Nisbet and Lagoy, 1992):

$$TEQ = TEF \times PAH_{concentration} \; or \; TEF \times PCB_{concentration}$$

RQs calculation used risk quotients of negligible concentrations $RQ_{(NCs)}$ and maximum permissible concentrations $RQ_{(MPCs)}$ as suggested in the equations (1-3) ((Cao et al., 2010):

(1) $\mathrm{RQ}_{\Sigma \mathrm{PAHs}} = \sum_{\mathrm{i}=1}^{16} \mathrm{RQi}\ (\mathrm{RQi} \geq 1)$

(2) $$RQ_{\Sigma PAHs(NCs)} = \sum_{i=1}^{16} RQi\ (RQi_{(NCs)} \geq 1)$$

(3) $$RQ_{\Sigma PAHs(MPCs)} = \sum_{i=1}^{16} RQi\ (RQi_{(MPCs)} \geq 1)$$

Interpretation of the risk classification of individual PAHs and ∑PAHs followed data suggested by Cao et al. (2010). The application of TEQ-$_{TH}$ and TEQ-$_{WHO}$ simultaneously in the quantification of PCBs risks was suggested by (Yang et al., 2010).

5.1. Calculation of exposure daily intake (EDI), target hazard quotient (THQ) and carcinogenic risk (CR)

Exposure daily intake (EDI), the target hazard quotient (THQ) and carcinogenic risks (CR) were calculated to determine non-carcinogenic effects and carcinogenic effects associated with the ingestion of blue mussels. The equations are the following (Oliveira et al., 2018):

(4) $$EDI = \frac{C_b \times IR}{BW}$$

(5) $$THQ = \frac{C_b \times IR \times EF \times ED}{RfD \times BW \times AT}$$

(6) $$CR = \frac{C_b \times IR \times EF \times ED \times CSF}{BW \times AT}$$

Where C_b is the concentration of PAHs in bivalve (µg/kg *ww*); IR is the average daily ingestion rate of molluscs (Portugal: 2.17E−02 kg/*per capita*/day), provided by FAOSTAT on the “Food Balances” database (FAO, 2023); BW is the body weight of adults (70 kg) (Wang et al., 2020); EF is the exposure frequencies of 365 days/year; ED is the exposure duration of 70 years (Wang et al., 2020); RfD is the Oral reference dose (available only for AcP: 6.0E-02, Flu: 4.0E-02, FL: 4.0E-02, Ant: 3.0E−01, Pyr: 3.0E-02, Nap: 2.0E-02, mg/kg/day) (USEPA, 2023); AT is averaging time for exposure (EF × ED); CSF is the oral cancer slope factor (available only for BaA: 7.3E-01, BaP: 7.3, BbFL: 7.3E-01, BkFL: 7.3E-02, Chr: 7.3E-03, DBA: 7.3, Ind: 7.3E-01 mg/kg/day (USEPA, 2023).

6. Statistical analysis

Descriptive and inferential statistics were performed with the software's PAST 4.02 (Hammer and Harper, 2001) and GraphPad Prism (6.01, GraphPad Software, Inc., San Diego, USA). For better identification of data points and visualisation of overall variability, while in tables, the data are presented as mean followed by the standard deviation (SD), in figures, the graphs display boxplots (with median, minimum, maximum, and 1st and 3rd quartiles).

The assumptions of normality and homogeneity of variances were verified using the Shapiro-Wilk W test and the Levine test, respectively. Subsequently, comparisons between independent sites and groups of compounds were investigated through unidirectional analysis of variance (ANOVA). Tukey's test evaluated post-hoc comparisons. When parametric assumptions were invalid, and data transformation was ineffective, the non-parametric Kruskal-Wallis test, followed by Dunn's post hoc test, was used. The threshold significance level for α was set at the standardised value of 0.05. Values below the limits of detection (LODs) of the method were treated as proposed by the United States Environmental Protection Agency (EPA/ 240VB-06/003 2006), *i.e.*, $data = \frac{LOD}{\sqrt{2}}$. PCA was performed using the correlation matrix. The principal components (PCs) were extracted considering the Kaiser criterion (*i.e.* eigenvalue > 1), the Scree Plot criterion, and the %variances > 85% (Wu et al., 2020).

III. RESULTS

1. PAHs

1.1. DAP and SPM

Table 2 shows the average annual concentrations ± standard deviation (SD) of sixteen PAHs by sampling site (n = 96) in DAP (ng/L) and SPM (µg/kg dry weight, *dw*). This table also considers the IARC carcinogenic groups (WHO, 2016), the number of rings, low molecular weight (LMW) and high molecular weight (HMW) PAHs.

The sum of all PAHs (∑PAHs) in DAP and SPM was similar amongst sampling sites, *i.e.* ≈ 17 ng/L and ≈ 100 µg/kg dry weight (*dw*). In DAP, the values of BP were, for all sampling sites, below the limits of detection (LODs) of the method, and the values of Ind were below the LODs for the sampling sites S_3 and S_4. The detection rates (DR, %) range from $\frac{LOD}{\sqrt{2}}$ to 100% (**Table 2**).

The evaluation of PAHs sources by sampling site was achieved considering ratios between indicator PAHs (**Table 3**) and their distribution on bivariate cross plots (**Figure 5**). This table also exposes pyrogenic, petrogenic and vehicular emissions in DAP and SPM.

Figure 5 also confirms that pyrogenic, petrogenic and vehicular emissions occur in both DAP and SPM, alongside **Table 3**. **Figure 5** (**a**, **b**) reveals the presence of mainly petroleum combustion contamination in DAP. **Figure 5** (**c**, **d**) shows that in SPM, biomass and coal combustion PAHs occur. This figure also illustrates that in DAP, the primary sources of PAHs emissions are petrogenic, whilst in SPM, the primary sources are pyrogenic.

Table 2 - Average levels of PAHs (ng/L) in DAP and SPM (µg/kg dry weight, *dw*) in seawater samples collected at Vila-do-Conde seacoast (n = 96; mean ± SD) from 2017 to 2021. Data is organized considering low molecular (LMW) and high molecular weight PAHs (HMW), their potential carcinogenicity (IARC group) and alphabetic designation.

PAHs	LOD (ng/L)	IARC Group*	Number Rings	DR(%) DAP	DR(%) SPM	PAHs dissolved in seawater (DAP, ng/L) S_1	S_2	S_3	S_4	PAHs in suspended particulate matter (SPM, ug/kg, *dw*) S_1	S_2	S_3	S_4
NaP	0.19	2B	2	96	100	3.9 ± 2.6	2.1 ± 0.6	1.0 ± 0.2	1.9 ± 0.3	3.3 ± 0.6	5.3 ± 3.8	4.6 ± 2.7	5.1 ± 1.7
AcP	0.17	-	3	89	100	3.9 ± 1.0	7.0 ± 1.8	7.8 ± 2.5	5.3 ± 1.9	7.9 ± 6.7	9.0 ± 2.4	7.5 ± 1.3	17.0 ± 4.6
AcPY	0.10	3	3	39	100	0.4 ± 0.3	0.5 ± 0.2	0.2 ± 0.2	0.7 ± 0.3	2.2 ± 1.1	8.5 ± 6.5	3.7 ± 1.2	16.8 ± 8.4
Ant	0.29	3	3	50	100	1.5 ± 1.5	1.9 ± 1.2	0.8 ± 0.4	2.5 ± 1.5	5.0 ± 4.8	5.4 ± 3.1	9.3 ± 11.3	19.8 ± 22.1
Flu	0.11	3	3	69	100	0.5 ± 0.2	0.5 ± 0.4	1.4 ± 1.2	0.5 ± 0.3	9.9 ± 0.7	16.6 ± 5.3	18.7 ± 12.8	14.7 ± 13.0
Phe	0.10	3	3	88	100	1.2 ± 0.7	1.7 ± 1.1	1.2 ± 1.0	2.3 ± 0.4	25.4 ± 8.0	22.0 ± 9.9	39.7 ± 29.1	31.5 ± 22.2
LMW	$\sum_{LMW}$ per site					11.5 ± 1.6	13.6 ± 2.4	12.4 ± 2.8	13.2 ± 1.7	53.6 ± 8.5	66.8 ± 6.7	83.4 ± 13.7	105.0 ± 8.5
	Global LMW (average) at the 4 sampling site						**12.7 ± 2.1**				**77.2 ± 9.7**		
BaP	0.10	1	5	58	100	0.2 ± 0.0	0.4 ± 0.2	0.1 ± 0.1	0.5 ± 0.1	2.2 ± 2.3	0.5 ± 0.2	1.7 ± 0.5	0.4 ± 0.3
DBA	0.11	2A	5	39	100	0.3 ± 0.1	0.4 ± 0.4	0.2 ± 0.1	0.1 ± 0.0	2.5 ± 3.1	1.1 ± 0.4	2.0 ± 0.5	0.6 ± 0.4
BaA	0.20	2B	5	64	100	1.0 ± 0.8	1.4 ± 0.6	0.6 ± 0.3	2.0 ± 0.8	13.2 ± 6.5	10.3 ± 6.4	21.7 ± 5.5	10.3 ± 6.2
BbFL	0.22	2B	5	38	100	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	2.2 ± 2.4	1.2 ± 0.2	1.6 ± 0.4	0.3 ± 0.1
BkFL	0.10	2B	5	59	100	0.5 ± 0.3	1.0 ± 0.7	0.8 ± 1.0	1.0 ± 0.6	2.3 ± 2.2	0.8 ± 0.2	1.6 ± 0.4	0.4 ± 0.2
Chr	0.10	2B	5	97	100	4.7 ± 1.8	4.5 ± 2.1	1.6 ± 0.7	6.8 ± 1.6	11.0 ± 10.4	4.1 ± 1.5	8.8 ± 1.4	5.9 ± 4.2
Ind	0.11	2B	6	28	100	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	2.4 ± 2.9	0.2 ± 0.1	1.8 ± 0.5	0.2 ± 0.1
FL	0.10	3	4	33	100	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	3.5 ± 1.0	3.9 ± 2.1	5.8 ± 3.5	7.1 ± 5.1
Pyr	0.23	3	4	77	100	1.2 ± 0.8	2.0 ± 0.5	1.1 ± 0.6	1.9 ± 1.0	8.4 ± 5.0	11.7 ± 5.8	11.5 ± 6.9	12.8 ± 8.5
BP	0.10	3	6	1	100	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.07 ± 0.0	2.7 ± 3.2	0.8 ± 0.4	1.8 ± 0.5	0.1 ± 0.0
HMW	$\sum_{HMW}$ per site					8.5 ± 1.4	10.5 ± 1.4	4.9 ± 0.5	12.9 ± 2.1	50.4 ± 4.2	34.6 ± 4.2	58.4 ± 6.6	38.1 ± 4.8
	Global HMW (average) at the 4 sampling site						**9.2 ± 1.4**				**45.4 ± 5.0**		
	Total per site ∑16 PAHs					20.0 ± 1.5	24.1 ± 1.9	17.4 ± 1.9	26.1 ± 1.9	104.1 ± 6.2	101.4 ± 6.4	141.8 ± 10.3	143.1 ± 9.2
	Total ∑16 PAHs						**17.3 ± 1.8**				**99.9 ± 8.1**		

*(WHO 2016)

Table 3 - Diagnostic ratios of PAHs to discriminate pyrogenic and/or petrogenic sources in DAP and SPM in seawater samples from Vila-do-Conde, seacoast (n = 96, from 2017 to 2021).

Diagnostic Ratios	Literature Data			DAP				SPM			
	Ratio Value	Source	Reference	S_1	S_2	S_3	S_4	S_1	S_2	S_3	S_4
Ant / (Ant + Phe)	< 0.1 > 0.1	Pyrogenic Petrogenic (Diesel)	Sany et al. 2014	0.55	0.52	0.40	0.53	0.16	0.20	0.19	0.39
Chr / BaA	< 0.4 > 0.9	Petrogenic Pyrogenic	Zakaria et al. 2002	4.85	3.21	2.48	3.34	0.83	0.40	0.41	0.58
Flu / (Flu+Pyr)	0.2-0.5 0.4-0.5 > 0.5	Diesel, liquid fossil Fuel Wood combustion	Teixeira et al. 2012 Yunker et al. 2002 Zhang et al. 2015	0.28	0.20	0.57	0.21	0.54	0.59	0.62	0.53
Flu / Pyr	> 0.4	Combustion	Hussain et al. 2016	0.39	0.25	1.31	0.27	1.18	1.42	1.62	1.15
BaP / BP	0.3-0.4 0.46-0.81 0.9-6.6	Traffic Diesel Coal/wood combustion	Agudelo-Castañeda et al. 2014 Hanedar et al. 2014 Fu et al. 2010	2.68	6.08	1.85	7.33	0.83	0.62	0.97	3.41
BaA / (BaA + Chr)	< 0.5 > 0.5	Diesel Gasoline	Yunker et al. 2002	0.17	0.24	0.29	0.23	0.55	0.71	0.71	0.63
∑LMW / ∑HMW	> 1.0 < 1.0	Petrogenic Pyrogenic	Zhang et al. 2015	1.36	1.30	2.53	1.02	1.06	1.93	1.43	2.75

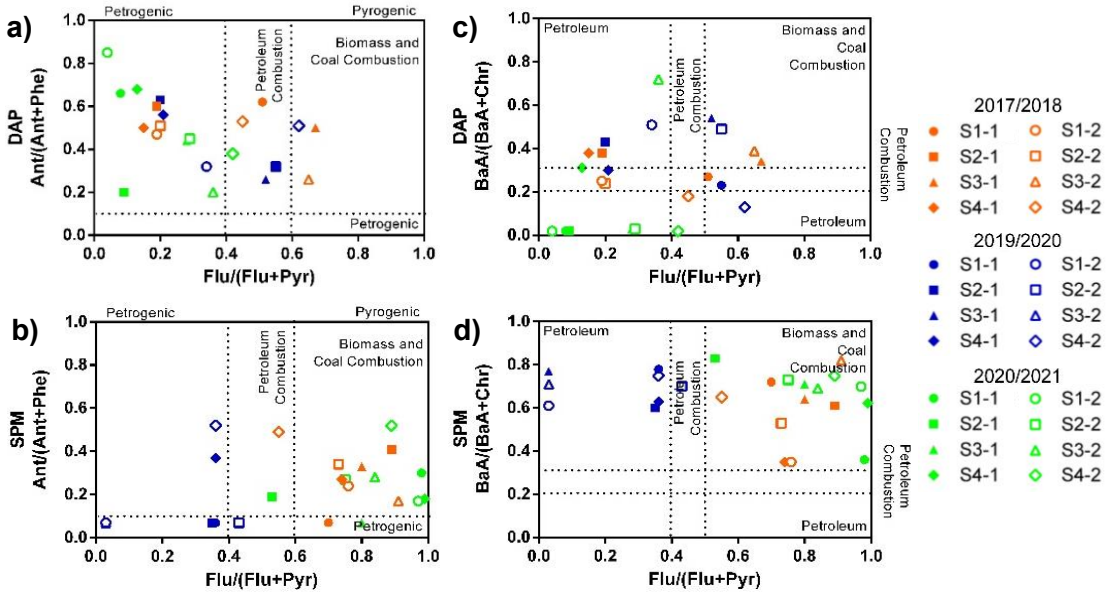


Figure 5 - Bivariate plots of molecular diagnostic ratios in DAP (a, b) and SPM (c, d). The graphics (a) and (c) refer to Ant/(Ant+Phe) *vs.* Flu/(Flu+Pyr) and those of (b) and (d) refer to BaA/(BaA+Chr) *vs.* Flu/(Flu+Pyr).

Tables 4 and 5 show the prospective carcinogenic/mutagenic PAHs in DAP (**table 4**) and SPM (**table 5**). On average, the sum of all TEQ values for PAHs in DAP and SPM were, on average, ≈ 0.9 ng/L and ≈ 4.8 µg/kg, *dw*. **Tables 4** and **5** also show the ecological risk assessment (RQ) posed by DAP and SPM. On average, in DAP, $RQ_{\sum PAHs(NCs)} \geq 1$ and $RQ_{\sum PAHs(MPCs)} \geq 1$ values were ≈ 1.4 and ≈ 0, respectively. In SPM, the values of $RQ_{\sum PAHs(NCs)} \geq 1$ and $RQ_{\sum PAHs(MPCs)} \geq 1$ were, on average, ≈ 20.5 and ≈ 1.4.

Figure 6 shows the fluctuation patterns of average ∑PAHs by season, displaying no significant differences between seasons, both in DAP and SPM.

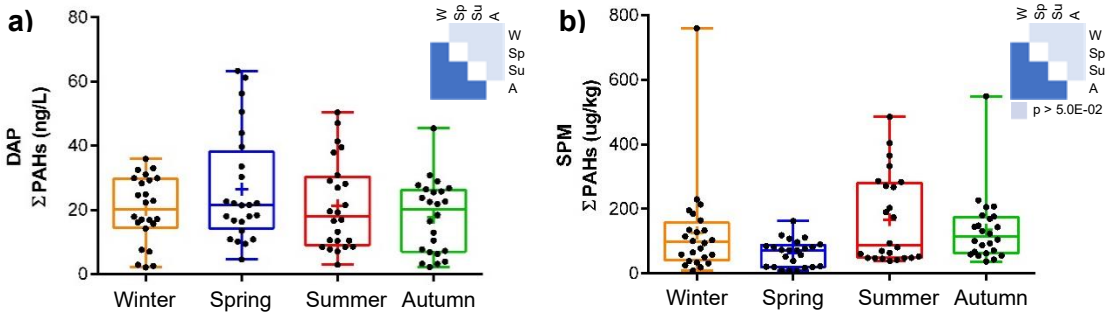


Figure 6 - Temporal distribution of PAHs in DAP (ng/L) (a) and in SPM (µg/kg, *dw*) (b). Data is expressed in boxplots with minimum, median, maximum, and interquartile range Q1-Q3. Dots represent individual values (n = 24, eight sampling sites throughout three years).

Table 4 - Toxic equivalence quantities (TEQs) and mean values of RQ(NCs) and RQ(MPCs) of PAHs in seawater samples (DAP) collected from Vila-do-Conde seacoast (S_1 to S_4), Portugal (n = 96), from 2017 to 2021.

PAHs	TEQs (ng/L)					RQs for PAHs in DAP									
	TEFs	S_1	S_2	S_3	S_4	NECs	S_1 RQ(NCs)	S_2 RQ(NCs)	S_3 RQ(NCs)	S_4 RQ(NCs)	MPCs	S_1 RQ(MECs)	S_2 RQ(MECs)	S_3 RQ(MECs)	S_4 RQ(MECs)
NaP	*0.001*	0.00	0.00	0.00	0.00	*12*	0.0	0.0	0.0	0.0	*1200*	0.0	0.0	0.0	0.0
AcP	*0.001*	0.00	0.01	0.01	0.01	*0.7*	0.0	0.0	0.0	0.0	*70*	0.1	0.1	0.1	0.1
AcPY	*0.001*	0.00	0.00	0.00	0.00	*0.7*	0.0	0.0	0.0	0.0	*70*	0.0	0.0	0.0	0.0
Ant	*0.001*	0.00	0.00	0.00	0.00	*0.7*	0.0	0.0	0.0	0.0	*70*	0.0	0.0	0.0	0.0
Flu	*0.001*	0.00	0.00	0.00	0.00	*0.7*	0.0	0.0	0.0	0.0	*70*	0.0	0.0	0.0	0.0
Phe	*0.001*	0.00	0.00	0.00	0.00	*3.0*	0.0	0.0	0.0	0.0	*300*	0.0	0.0	0.0	0.0
BaP	*1*	0.19	0.43	0.14	0.52	*0.5*	0.4	0.9	0.3	1.0	*50*	0.0	0.0	0.0	0.0
DBA	*1*	0.26	0.44	0.17	0.11	*0.5*	0.5	0.9	0.3	0.2	*50*	0.0	0.0	0.0	0.0
BaA	*0.1*	0.10	0.14	0.06	0.20	*0.1*	1.0	1.4	0.6	2.0	*10*	0.1	0.1	0.1	0.2
BbFL	*0.1*	0.03	0.03	0.02	0.03	*0.1*	0.3	0.3	0.2	0.3	*10*	0.0	0.0	0.0	0.0
BkFL	*0.1*	0.05	0.10	0.08	0.10	*0.4*	0.1	0.3	0.2	0.2	*40*	0.0	0.0	0.0	0.0
Chr	*0.01*	0.05	0.05	0.02	0.07	*3.4*	0.0	0.0	0.0	0.0	*340*	0.0	0.0	0.0	0.0
Ind	*0.1*	0.02	0.01	0.01	0.01	*0.4*	0.0	0.0	0.0	0.0	*40*	0.0	0.0	0.0	0.0
FL	*0.01*	0.00	0.00	0.00	0.00	*3.0*	0.0	0.0	0.0	0.0	*300*	0.0	0.0	0.0	0.0
Pyr	*0.001*	0.00	0.00	0.00	0.00	*0.7*	0.0	0.0	0.0	0.0	*70*	0.0	0.0	0.0	0.0
BP	*0.01*	0.00	0.00	0.00	0.00	*0.3*	0.0	0.0	0.0	0.0	*30*	0.0	0.0	0.0	0.0
$TEQs_{\sum PAHs}$		**0.7**	**1.2**	**0.5**	**1.1**	**$RQ_{\sum PAHs(NCs)} \geq 1$**	**1.0**	**1.4**	**≈ 0**	**3.1**	**$RQ_{\sum PAHs(MPCs)} \geq 1$**	**≈ 0**	**≈ 0**	**≈ 0**	**≈ 0**

Values in italics - Published data (text)

Table 5 - Toxic equivalence quantities (TEQs) and mean values of RQ(NCs) and RQ(MPCs) of PAHs in seawater samples (SPM) collected from Vila-do-Conde seacoast (S_1 to S_4), Portugal (n = 96), from 2017 to 2021.

		TEQs (ng/L)				RQs for PAHs in SPM									
	TEFs	S_1	S_2	S_3	S_4	NECs	S_1 RQ(NCs)	S_2 RQ(NCs)	S_3 RQ(NCs)	S_4 RQ(NCs)	MPCs	S_1 RQ(MECs)	S_2 RQ(MECs)	S_3 RQ(MECs)	S_4 RQ(MECs)
NaP	*0.001*	0.00	0.01	0.00	0.01	*12*	0.0	0.0	0.0	0.0	*1200*	0.0	0.0	0.0	0.0
AcP	*0.001*	0.01	0.01	0.01	0.02	*0.7*	0.0	0.0	0.0	0.0	*70*	0.1	0.1	0.1	0.2
AcPY	*0.001*	0.00	0.01	0.00	0.02	*0.7*	0.0	0.0	0.0	0.0	*70*	0.0	0.1	0.1	0.2
Ant	*0.001*	0.00	0.01	0.01	0.02	*0.7*	0.0	0.0	0.0	0.0	*70*	0.1	0.1	0.1	0.3
Flu	*0.001*	0.01	0.02	0.02	0.01	*0.7*	0.0	0.0	0.0	0.0	*70*	0.1	0.2	0.3	0.2
Phe	*0.001*	0.03	0.02	0.04	0.03	*3.0*	0.0	0.0	0.0	0.0	*300*	0.1	0.1	0.1	0.1
BaP	*1*	2.21	0.49	1.73	0.44	*0.5*	4.4	1.0	3.5	0.9	*50*	0.0	0.0	0.0	0.0
DBA	*1*	2.50	1.12	2.03	0.60	*0.5*	5.0	2.2	4.1	1.2	*50*	0.1	0.0	0.0	0.0
BaA	*0.1*	1.32	1.03	2.17	1.03	*0.1*	13.2	10.3	21.7	10.3	*10*	1.3	1.0	2.2	1.0
BbFL	*0.1*	0.22	0.12	0.16	0.03	*0.1*	2.2	1.2	1.6	0.3	*10*	0.2	0.1	0.2	0.0
BkFL	*0.1*	0.23	0.08	0.16	0.04	*0.4*	0.6	0.2	0.4	0.1	*40*	0.1	0.0	0.0	0.0
Chr	*0.01*	0.11	0.04	0.09	0.06	*3.4*	0.0	0.0	0.0	0.0	*340*	0.0	0.0	0.0	0.0
Ind	*0.1*	0.24	0.02	0.18	0.02	*0.4*	0.6	0.1	0.4	0.1	*40*	0.1	0.0	0.0	0.0
FL	*0.01*	0.03	0.04	0.06	0.07	*3.0*	0.0	0.0	0.0	0.0	*300*	0.0	0.0	0.0	0.0
Pyr	*0.001*	0.01	0.01	0.01	0.01	*0.7*	0.0	0.0	0.0	0.0	*70*	0.1	0.2	0.2	0.2
BP	*0.01*	0.03	0.01	0.02	0.00	*0.3*	0.1	0.0	0.1	0.0	*30*	0.1	0.0	0.1	0.0
$TEQs_{\sum PAHs}$		**7.0**	**3.0**	**6.7**	**2.4**	**$RQ_{\sum PAHs(NCs)} \geq 1$**	**24.8**	**14.7**	**31**	**11.5**	**$RQ_{\sum PAHs(MPCs)} \geq 1$**	**1.3**	**1.0**	**2.2**	**1.0**

Values in italics - Published data (text)

Figure 7 exhibits triangular density plots concerning the percentages of 2+3 rings, 4 rings and 5+6 rings PAHs in both DAP (**a**) and SPM (**b**), as well as their percentages by sampling site and IARC Group (**c**, **d**). **Figure 7** (**a**), shows that DAP contains higher percentages of PAHs with 2+3 rings in all sampling sites (≈ 59%) compared to those with 4 rings (≈ 32%) and 5+6 rings (≈ 9%). **Figure 7** (**b**) shows that SPM also contains higher percentages of PAHs with 2+3 rings in most sampling sites (≈ 58%) compared to those with 4 rings (≈ 36%) and 5+6 rings (≈ 6%). **Figure 7** (**c**) also shows that Group 2B PAHs, ≈ 56%, are the most abundant of these compounds in most sampling sites in DAP. In SPM (**d**), however, Group 3 PAHs, ≈ 71%, are the most abundant in all sampling sites.

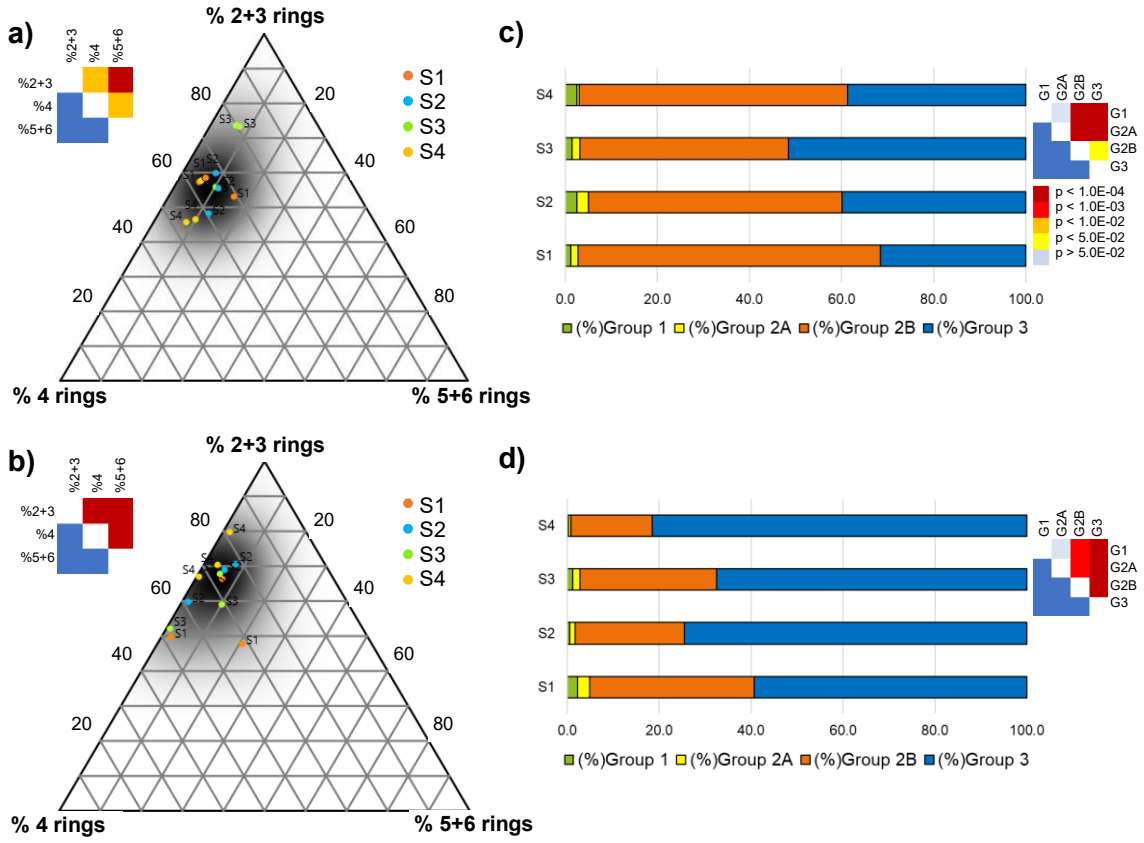


Figure 7 - PAHs ternary distribution plots (%) considering the congeners containing 2+3, 4 and 5+6 cyclic rings (**a**, **b**) and their distribution (%) by toxic/carcinogenic group (WHO, 2016) (**c**, **d**) in DAP and SPM.

Figure 8 shows PCA graphics (95% ellipses) for both DAP (**a**) and SPM (**b**), considering the content of PAHs in the different seasons and sampling sites**.** Since PCA analysis provides qualitative comparisons amongst sampling sites and allows the visualisation of both the independence and covariance of PAHs amongst the sampling

sites, this study used this tool to search veiled differences. For DAP, PCA reveals that five components (PC1, PC2, PC3, PC4 and PC5) retained most of the variation (70.4%). BaA (0.37) is the principal contributor for PC1 and BaP (0.49) for PC2. For the SPM fraction, the multivariate analysis recognised four principal components (PC1, PC2, PC3 and PC4) retaining most of the variation (81.8%). DBA (0.36) is the principal contributor for PC1 and Phe (0.46) for PC2. **Figure 8** also reveals that in both DAP and SPM, the data variability is lower in the spring and higher in the summer.

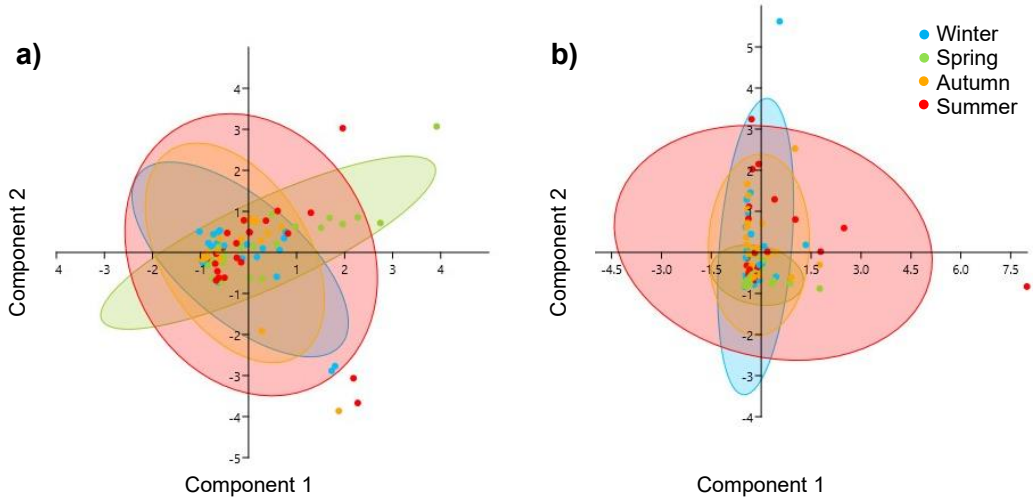


Figure 8 - Score plots of PC1 vs PC2 illustrating the distribution of individual PAHs by month and sampling sites (S_1 to S_4, n = 96) in DAP (**a**) and SPM (**b**).

1.2. Mussels

Table 6 shows the average annual concentrations ± SD of sixteen PAHs by sampling site (n = 180) in mussel tissue (µg/kg). This table also considers the IARC carcinogenic groups (WHO, 2016), the number of rings, low molecular weight (LMW) and high molecular weight (HMW) PAHs. **Table 6** also reveals that the sum of all PAHs (∑PAHs) in mussel tissue was similar amongst sampling sites, *i.e.*, ≈ 137 µg/kg. The detection rates (DR, %) of PAHs in this matrix ranged from $\frac{LOD}{\sqrt{2}}$ to 100%.

The evaluation of PAHs sources by sampling site was achieved considering ratios between indicator PAHs (**Table 7**) and their distribution on bivariate cross plots (**Figure 9**). Similarly to what was observed in water samples, this table also exposes that pyrogenic, petrogenic and vehicular emissions occur in mussel tissue.

Figure 9 also confirms that pyrogenic, petrogenic and vehicular emissions occur in mussel tissue, alongside **Table 7**, revealing the presence of mainly petroleum combustion contamination (petrogenic) in mussel tissue.

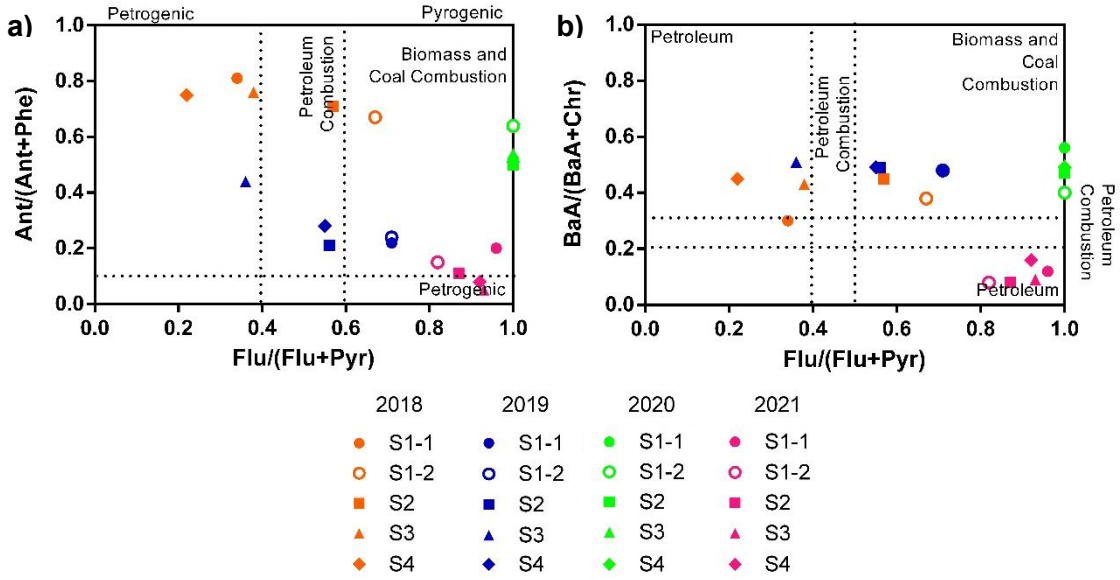


Figure 9 - Bivariate plots of molecular diagnostic ratios in mussel tissues. The graphic (a) refers to Ant/(Ant+Phe) *vs.* Flu/(Flu+Pyr) and that of (b) refers to BaA/(BaA+Chr) *vs.* Flu/(Flu+Pyr).

Table 6 - Average levels of PAHs (µg/kg) in mussel samples collected at Vila-do-Conde seacoast (n = 180; mean ± SD) from 2017 to 2021. Data is organized considering low molecular (LMW) and high molecular weight PAHs (HMW), their potential carcinogenicity (IARC group) and alphabetic designation.

PAHs	LOD (ng/L)	IARC Group*	Number Rings	DR(%)	PAHs accumulated in mussel tissue (ug/kg)			
					S_1	**S_2**	**S_3**	**S_4**
NaP	0.19	2B	2	96	57.9 ± 16.5	32.8 ± 12.4	37.1 ± 28.8	36.0 ± 19.5
AcP	0.17	-	3	89	4.6 ± 1.3	3.8 ± 1.3	4.5 ± 1.8	5.4 ± 2.6
AcPY	0.10	3	3	39	7.2 ± 2.7	8.3 ± 3.9	6.2 ± 1.0	9.9 ± 1.6
Ant	0.29	3	3	50	2.9 ± 1.2	2.4 ± 1.0	2.3 ± 0.8	1.9 ± 0.6
Flu	0.11	3	3	69	9.5 ± 4.3	12.3 ± 10.3	10.7 ± 4.8	21.4 ± 14.6
Phe	0.10	3	3	88	5.3 ± 4.2	3.9 ± 3.0	4.4 ± 4.6	7.3 ± 7.3
LMW	$\sum_{LMW}$ per site				87.5 ± 21.4	63.5 ± 11.5	65.3 ± 13.1	81.9 ± 12.8
	Global LMW (average) at the 4 sampling sites					**74.5 ± 14.3**		
BaP	0.10	1	5	58	10.9 ± 6.6	2.4 ± 0.6	2.4 ± 0.3	2.6 ± 0.9
DBA	0.11	2A	5	39	20.2 ± 7.6	35.2 ± 21.8	23.3 ± 17.8	24.6 ± 16.1
BaA	0.20	2B	5	64	27.0 ± 13.6	37.6 ± 10.4	27.3 ± 5.2	17.1 ± 6.0
BbFL	0.22	2B	5	38	1.9 ± 0.5	2.3 ± 0.6	2.1 ± 0.5	2.8 ± 1.1
BkFL	0.10	2B	5	59	0.6 ± 0.2	1.9 ± 0.9	2.0 ± 1.1	2.2 ± 0.9
Chr	0.10	2B	5	97	45.9 ± 7.8	47.8 ± 15.0	36.0 ± 13.9	22.7 ± 3.7
Ind	0.11	2B	6	28	12.3 ± 9.1	20.2 ± 6.9	20.5 ± 10.8	18.4 ± 16.7
FL	0.10	3	4	33	1.9 ± 0.7	2.8 ± 0.9	1.6 ± 0.5	1.1 ± 0.3
Pyr	0.23	3	4	77	0.7 ± 0.1	1.5 ± 0.5	1.2 ± 0.3	1.0 ± 0.7
BP	0.10	3	6	1	2.3 ± 0.7	8.0 ± 8.1	3.5 ± 0.7	2.6 ± 1.6
HMW	$\sum_{HMW}$ per site				123.7 ± 14.9	160.0 ± 17.9	119.8 ± 13.3	95.1 ± 9.9
	Global HMW (average) at the 4 sampling sites					**124.7 ± 13.9**		
	Total per site ∑16 PAHs				211.2 ± 16.9	223.5 ± 15.6	185.1 ± 12.8	177.0 ± 10.8
	Total ∑16 PAHs					**136.9 ± 14.0**		

*(WHO 2016)

Table 7 - Diagnostic ratios of PAHs to discriminate pyrogenic and/or petrogenic sources in mussel samples from Vila-do-Conde seacoast (S_1 to S_4; n = 180), from 2017 to 2021.

Diagnostic Ratios	Literature Data			Mussel tissue			
	Ratio Value	Source	Reference	S_1	S_2	S_3	S_4
Ant / (Ant + Phe)	< 0.1	Pyrogenic	Sany et al. 2014	0.35	0.38	0.34	0.21
	> 0.1	Petrogenic (Diesel)					
Chr / BaA	< 0.4	Petrogenic	Zakaria et al. 2002	1.70	1.27	1.32	1.33
	> 0.9	Pyrogenic					
Flu / (Flu+Pyr)	0.2-0.5	Diesel, liquid fossil	Teixeira et al. 2012	0.93	0.89	0.90	0.96
	0.4-0.5	Fuel	Yunker et al. 2002				
	> 0.5	Wood combustion	Zhang et al. 2015				
Flu / Pyr	> 0.4	Combustion	Hussain et al. 2016	12.83	8.34	8.64	21.42
BaP / BP	0.3-0.4	Traffic	Agudelo-Castañeda et al. 2014	4.79	0.30	0.68	1.01
	0.46-0.81	Diesel	Hanedar et al. 2014				
	0.9-6.6	Coal/wood combustion	Fu et al. 2010				
BaA / (BaA + Chr)	< 0.5	Diesel	Yunker et al. 2002	0.37	0.44	0.43	0.43
	> 0.5	Gasoline					
∑LMW / ∑HMW	> 1.0	Petrogenic	Zhang et al. 2015	0.71	0.40	0.54	0.86
	< 1.0	Pyrogenic					

Table 8 shows the calculated mussel EDIs for summed and individual PAHs in Portugal. The estimated EDIs of ΣPAHs through mussel consumption varied from 5.5E-02 and 6.9E-02 μg/kg body weight per day (average ≈ 4.25E-02 μg/kg body weight per day), and higher intakes were found for the 2+3 rings PAHs. This table also shows the calculated THQ and CR from the ingestion of bivalves by the Portuguese consumers. Among the four sampling sites (S_1-S_4), ΣTHQs ranged between 0.67 and 1.02 (mean value: 0.79) and Nap, Flu and AcP were the main contributors. The calculated ΣCR values ranged between 5.5E-02 and 9.4E-02 (mean value: 6.9E-02), and the main contributors for ΣCR were DBA and BaP.

Figure 10 shows the fluctuation patterns of average ∑PAHs by season, stressing that there were no significative differences between seasons in mussel tissues.

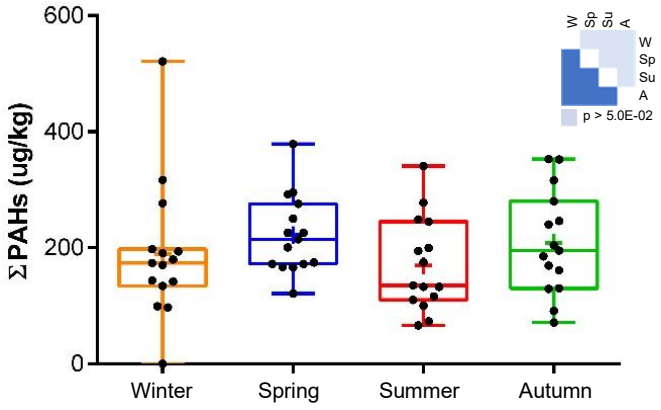


Figure 10 - Temporal distribution of PAHs in mussel tissue (μg/kg). Data is expressed in boxplots with minimum, median, maximum, and interquartile range Q1-Q3. Dots represent individual values (n = 15, five sampling sites throughout three years).

Figure 11 exhibits triangular density plots concerning the percentages of 2+3 rings, 4 rings and 5+6 rings PAHs in mussel tissue (**a**) and their percentages by sampling site and IARC Group (**b**). **Figure 11** (**a**) shows that, in general, mussels contain higher percentages of PAHs with 2+3 rings (≈ 45%), followed by those with 4 rings (≈ 32%) and 5+6 rings (≈ 23%). **Figure 11** (**b**) also shows that Group 2B PAHs, ≈ 66%, is the most abundant group of these compounds in all sampling sites.

Table 8 – Calculated exposure daily intake (EDI), target hazard quotient (THQ) and carcinogenic risks (CR) of PAHs in mussel samples collected from Vila-do-Conde seacoast (S_1 to S_4 ; n = 180), Portugal, from 2018 to 2021.

PAHs	EDI (µg/kg body weight per day)				THQ					CR				
	S_1	S_2	S_3	S_4	RfD	S_1	S_2	S_3	S_4	CSF	S_1	S_2	S_3	S_4
NaP	1.80E-02	1.02E-02	1.15E-02	1.12E-02	*0.02*	8.99E-01	5.10E-01	5.75E-01	5.58E-01					
AcP	1.44E-03	1.19E-03	1.40E-03	1.69E-03	*0.06*	2.39E-02	1.99E-02	2.33E-02	2.81E-02					
AcPY	2.24E-03	2.56E-03	1.93E-03	3.06E-03										
Ant	9.00E-04	7.40E-04	7.24E-04	5.99E-04	*0.3*	3.00E-03	2.47E-03	2.41E-03	2.00E-03					
Flu	2.95E-03	3.83E-03	3.33E-03	6.65E-03	*0.04*	7.37E-02	9.57E-02	8.34E-02	1.66E-01					
Phe	1.65E-03	1.20E-03	1.38E-03	2.26E-03										
BaP	3.40E-03	7.50E-04	7.34E-04	8.05E-04						*7.3*	2.48E-02	5.48E-03	5.36E-03	5.87E-03
DBA	6.28E-03	1.09E-02	7.24E-03	7.64E-03						*7.3*	4.58E-02	7.98E-02	5.28E-02	5.58E-02
BaA	8.39E-03	1.17E-02	8.46E-03	5.31E-03						*0.73*	6.12E-03	8.53E-03	6.18E-03	3.87E-03
BbFL	5.84E-04	7.29E-04	6.38E-04	8.68E-04						*0.73*	4.27E-04	5.32E-04	4.66E-04	6.33E-04
BkFL	1.73E-04	6.03E-04	6.35E-04	6.89E-04						*0.073*	1.27E-05	4.40E-05	4.63E-05	5.03E-05
Chr	1.42E-02	1.49E-02	1.12E-02	7.05E-03						*0.007*	1.04E-04	1.08E-04	8.15E-05	5.15E-05
Ind	3.81E-03	6.28E-03	6.37E-03	5.72E-03						*0.73*	2.78E-03	4.58E-03	4.65E-03	4.18E-03
FL	6.03E-04	8.81E-04	4.99E-04	3.34E-04	*0.04*	1.51E-02	2.20E-02	1.25E-02	8.35E-03					
Pyr	2.30E-04	4.59E-04	3.86E-04	3.11E-04	*0.03*	7.65E-03	1.53E-02	1.29E-02	1.04E-02					
BP	7.10E-04	2.50E-03	1.08E-03	7.99E-04										
∑PAHs	**6.56E-02**	**6.94E-02**	**5.75E-02**	**5.50E-02**	**∑THQ**	**1.02**	**0.67**	**0.71**	**0.77**	**∑CR**	**5.53E-02**	**9.36E-02**	**6.42E-02**	**6.46E-02**

Values in italics - Published data (text)

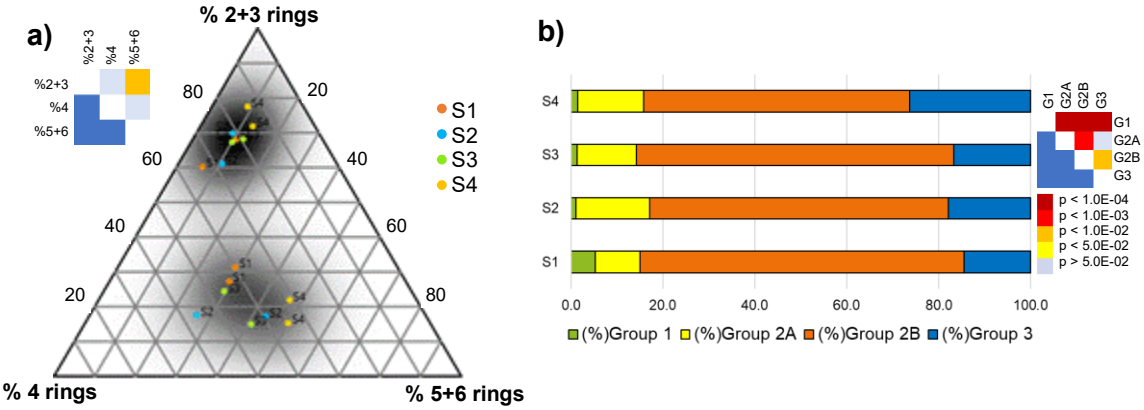


Figure 11 - PAHs ternary distribution plots (%) considering the congeners containing 2+3, 4 and 5+6 cyclic rings (a), and their distribution (%) by toxic/carcinogenic group (WHO, 2016) (b) in mussel tissue.

Figure 12 shows PCA graphics (95% ellipses) for mussel tissue, considering the content of PAHs in the different seasons and sampling sites**.** PCA reveals that six components (PC1, PC2, PC3, PC4, PC5 and PC6) retained most of the variation (76.5%). Chr (0.36) is the principal contributor for PC1 and NaP (0.42) for PC2. **Figure 12** also reveals that in mussel samples, the variability is lowest in the summer and highest in the winter.

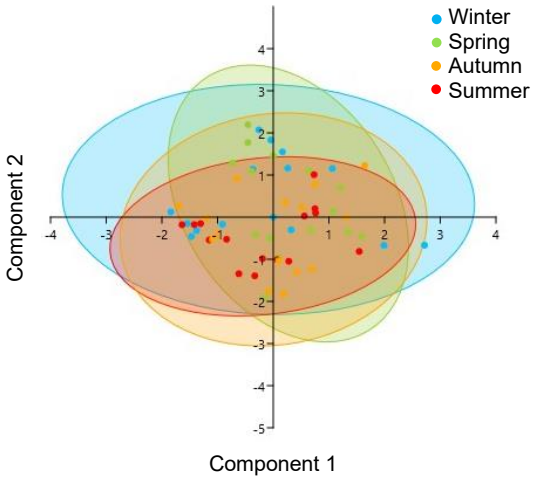


Figure 12 - Score plots of PC1 vs PC2 illustrating the distribution of individual PAHs by month and sampling sites (S_1 to S_4; n = 180) in mussel tissues.

2. PCBs

2.1. DAP and SPM

Table 9 shows the average ± SD annual concentrations of seven PCBs indicators per sampling site (n = 96) in DAP (ng/L) and SPM (µg/kg dry weight, *dw*) from 2017 to 2021. The sum of all PCBs (∑PCBs) were, on average ≈ , 6 ng/L and ≈ 14 µg/kg *dw*, respectively, and no differences were found amongst sampling sites. The detection rate (DR%) of all PCBs is expressed in this table.

Six of the evaluated PCBs were classified as possibly carcinogenic for humans (Group 2 B) and one as carcinogenic (PCB118) for humans (Group 1) (IARC 2016). Data in **Table 10** refers to thyroid toxicity equivalents (TEQ_{-TH}) and TEQ (Yang et al., 2010; WHO, 2016). On average, the sum of all TEQ_{-TH} values for PCBs in DAP and SPM was ≈ 1.4E-04 ng/L and ≈ 3.8E-04 µg/kg, *dw*. However, the sum of all $TEQ\text{-}_{WHO}$ values was ≈ 2.4E-05 ng/L and ≈ 5.4E-05 µg/kg, *dw*.

Figure 13 shows the fluctuation patterns of average ∑PCBs per season, stressing that the lowest amounts of these POPs were found in autumn for SPM. There were no significant differences in DAP.

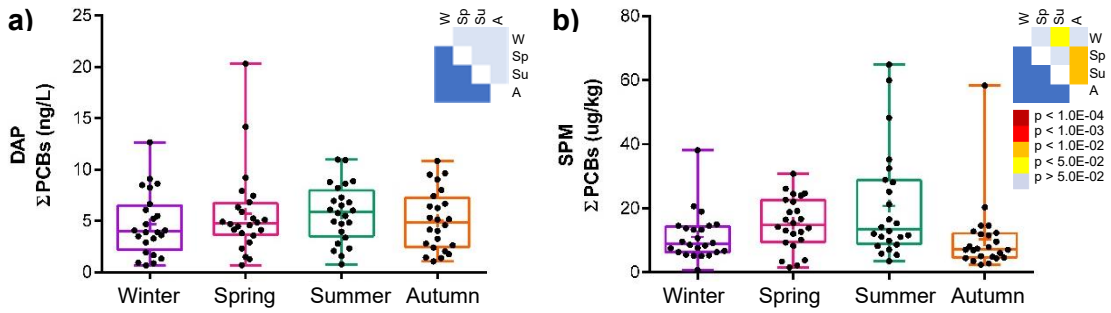


Figure 13 - Seasonal distribution of the sum of PCBs in DAP (ng/L) and SPM (µg/kg, *dw*) from 2017 to 2021. Data is expressed in boxplots with minimum, median, maximum, and interquartile range Q1-Q3. Dots represent individual values (n = 96).

Table 9 - Average levels of PCBs in DAP (ng/L) and SPM (µg/kg, *dw*) in seawater samples collected at Vila-do-Conde seacoast (n = 96; mean ± SD). Data is organized considering the number of the seven analysed congeners and their content of Cl atoms from 2017 to 2021.

PCBs	LOD (ng/L)	Number Cl atoms	DR (%)		PCBs dissolved in seawater (DAP, ng/L)				PCBs in suspended particulate matter (SPM, ug/kg, *dw*)			
			DAP	SPM	S_1	S_2	S_3	S_4	S_1	S_2	S_3	S_4
PCB28	0.09	2	68	100	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	2.0 ± 0.7	2.7 ± 1.1	2.0 ± 0.9	2.6 ± 1.4
PCB52	0.10	4	50	100	0.2 ± 0.1	0.3 ± 0.1	0.6 ± 0.2	0.4 ± 0.2	3.5 ± 0.7	2.1 ± 1.2	0.8 ± 0.3	1.5 ± 0.9
PCB101	0.08	5	97	100	1.8 ± 0.7	1.2 ± 0.1	1.3 ± 0.3	1.4 ± 0.3	5.5 ± 1.8	6.6 ± 4.9	4.4 ± 0.3	3.6 ± 2.3
PCB118	0.06	5	96	100	0.6 ± 0.1	0.4 ± 0.1	0.6 ± 0.2	1.3 ± 0.2	1.1 ± 0.3	2.4 ± 1.5	1.6 ± 0.5	2.2 ± 1.8
PCB138	0.10	6	76	100	0.6 ± 0.1	0.8 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	3.1 ± 2.6	0.2 ± 0.2	0.2 ± 0.2
PCB153	0.13	6	82	100	0.5 ± 0.0	0.5 ± 0.1	0.9 ± 0.1	0.4 ± 0.1	1.4 ± 0.8	2.0 ± 1.1	0.6 ± 0.2	0.8 ± 0.6
PCB180	0.10	7	84	100	0.7 ± 0.2	0.5 ± 0.1	1.3 ± 0.4	2.4 ± 2.5	0.5 ± 0.1	1.0 ± 0.6	0.7 ± 0.2	2.4 ± 1.8
∑PCBs per site					4.9 ± 0.5	4.0 ± 0.3	5.5 ± 0.4	6.8 ± 0.7	14.2 ± 1.9	19.7 ± 1.8	10.4 ± 1.4	13.1 ± 1.1
Global PCB (average) at the 4 sampling sites					**5.3 ± 0.5**				**14.4 ± 1.6**			

Table 10 - Toxic equivalence quantities (TEQs) in seawater samples collected from Vila-do-Conde seacoast (average values, n = 96), considering both the thyroid biomarkers (TEQs-$_{TH}$) and the carcinogenic potential of PCB118, from 2017 to 2021.

PCBs	TEF-$_{TH}$	TEF-$_{WHO}$	Annual average (S_1-S_4) × TEQ-$_{TH}$ (TEQ-$_{WHO}$)			
			TEQ-$_{TH}$ (ng/L)	TEQ-$_{WHO}$ (ng/L)	TEQ-$_{TH}$ (ng/g)	TEQ-$_{WHO}$ (ng/g)
PCB28	*2.00E-06*		9.50E-07		4.61E-06	
PCB52	*5.00E-06*		1.86E-06		9.85E-06	
PCB101	*3.00E-05*		4.23E-05		1.50E-04	
PCB118	*1.00E-04*	*3.00E-05*	7.14E-05	2.14E-05	1.81E-04	5.43E-05
PCB138	*2.00E-05*		1.11E-05		1.93E-05	
PCB153	*1.00E-05*		5.70E-06		1.18E-05	
PCB180	*5.00E-06*		6.05E-06		5.72E-06	
	∑TEQ		1.39E-04	2.14E-05	3.82E-04	5.43E-05

Values in italics - Published data (text)

Regarding abundance, **Figure 14** shows the percentages of PCBs containing 2 to 7 Cl atoms in both DAP and SPM from 2017 to 2021. On average, PCBs with 5 Cl atoms were the most abundant and different when considering DAP (40%) and SPM (48%).

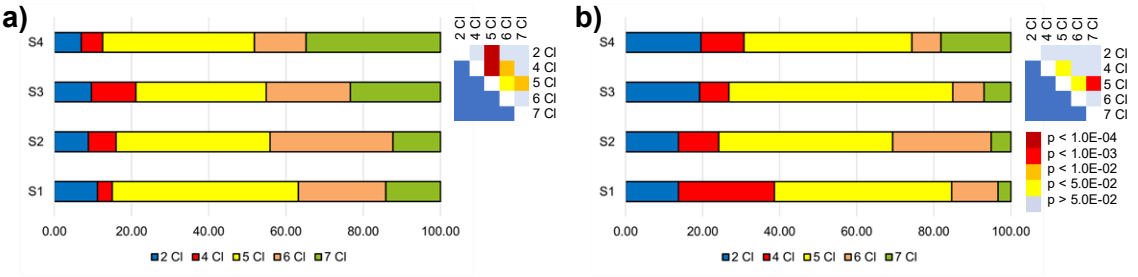


Figure 14 - PCBs distribution (%) considering the number of Cl atoms of each congener by sampling site in DAP (a) and SPM (b).

Figure 15 shows PC1 vs PC2 in DAP (**a**) and SPM (**b**). When applying PCA to PCBs data, it was found that four main components (PC1, PC2, PC3 and PC4) can be considered for DAP (**a**) and three (PC1, PC2 and PC3) for SPM (**b**). In DAP, the main contributors for PC1 and PC2 were, respectively, PCB28 (0.54) and PCB101 (0.81). In SPM, the main contributors for PC1 and PC2 were, respectively, PCB28 (0.50) and PCB153 (0.66). PCA reveals that, in DAP, the variability is fairly similar throughout the seasons. However, in SPM, the variability is lowest in the autumn and highest in the spring.

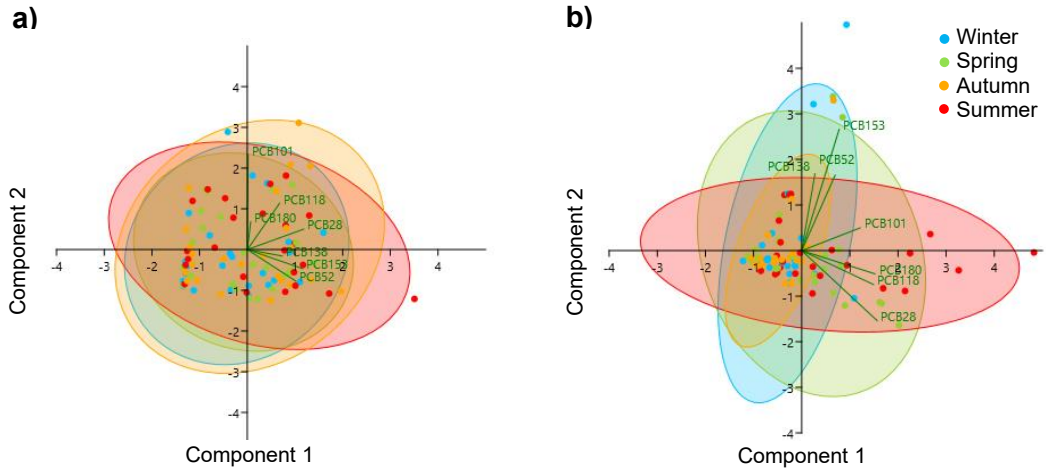


Figure 15 - Score plots of PC1 vs PC2 illustrating the distribution of individual PCBs in several months and sampling sites (S_1 to S_4; n = 96) in DAP (**a**) and SPM (**b**).

2.2. Mussels

Table 11 shows the average (± SD) annual concentrations of the seven indicator PCBs per sampling site (n = 180) in mussel tissue (µg/kg) from 2017 to 2021. The sum of all PCBs (∑PCBs) was ≈ 56 µg/kg on average, and no differences were found amongst sampling sites. The detection rate (DR%) of all PCBs is also expressed in this table.

Six of the evaluated PCBs were classified as possibly carcinogenic for humans (Group 2B) and one as carcinogenic (PCB118) for humans (Group 1) (IARC 2016). Data in **Table 12** refers to thyroid toxicity equivalents (TEQ_{-TH}) and TEQ (Yang et al., 2010; WHO, 2016). On average, the sum of all TEQ_{-TH} values for PCBs in mussel tissues was ≈ 9.1E-04 µg/kg, and that of TEQ_{-WHO} was ≈ 8.0E-05 µg/kg.

Figure 16 shows the fluctuation patterns of average $\sum_{PCBs}$ per season, revealing differences between winter and spring seasons in mussel tissue.

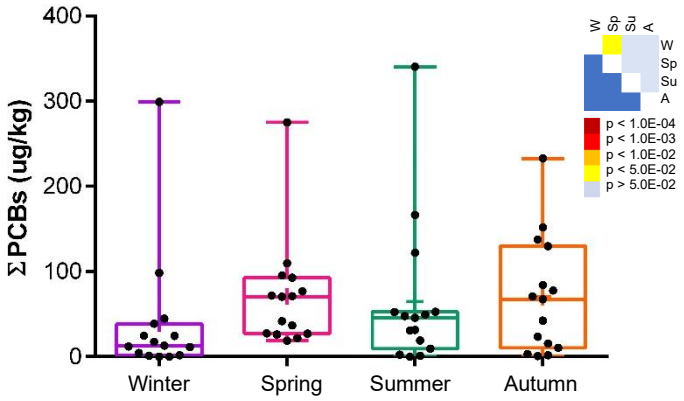


Figure 16 - Seasonal distribution of the sum of PCBs in mussel tissue (µg/kg). Data is expressed in boxplots with minimum, median, maximum, and interquartile range Q1-Q3. Dots represent individual values (n = 15, five sampling sites throughout three years).

Concerning abundance, **Figure 17** shows the percentages of PCBs containing 2 to 7 Cl atoms in mussels from 2017 to 2021. On average, PCBs with 6 Cl atoms were the most abundant and showed the most differences (43.9%).

Table 11 - Average levels of PCBs in the tissues of mussel samples collected at Vila-do-Conde seacoast (n = 180; mean ± SD). Data is organized considering the number of the seven analysed congeners and their content of Cl atoms from 2017 to 2021.

PCBs	LOD (ng/L)	Number Cl atoms	DR (%)	PCBs accumulated in mussel tissue (ug/kg)			
				S_1	**S_2**	**S_3**	**S_4**
PCB28	0.09	2	68	9.8 ± 5.7	3.1 ± 0.8	2.0 ± 1.4	6.9 ± 6.6
PCB52	0.10	4	50	28.0 ± 14.6	15.8 ± 9.9	2.0 ± 1.0	15.9 ± 10.1
PCB101	0.08	5	97	13.5 ± 1.5	2.9 ± 2.5	1.2 ± 0.5	1.3 ± 0.4
PCB118	0.06	5	96	3.4 ± 1.4	3.7 ± 4.4	1.4 ± 1.0	2.2 ± 2.0
PCB138	0.10	6	76	8.6 ± 4.3	33.5 ± 32.2	4.4 ± 3.4	17.8 ± 12.9
PCB153	0.13	6	82	12.3 ± 6.7	8.3 ± 4.4	2.5 ± 1.2	7.4 ± 4.6
PCB180	0.10	7	84	8.4 ± 5.6	3.3 ± 0.8	1.9 ± 1.3	2.8 ± 1.7
∑PCBs per site				83.9 ± 7.8	70.6 ± 11.3	15.3 ± 1.1	54.4 ± 6.7
Global PCB (average) at the 4 samplin					**56.0 ± 8.1**		

Table 12 - Toxic equivalence quantities (TEQs) in the tissues of mussel samples collected from Vila-do-Conde seacoast (average values, n = 180), considering both the thyroid biomarkers (TEQs-$_{TH}$) and the carcinogenic potential of PCB118, from 2017 to 2021.

PCBs	TEF-$_{TH}$	TEF-$_{WHO}$	Annual average (S_1-S_4) × TEQ-$_{TH}$ (TEQ-$_{WHO}$)	
			TEQ-$_{TH}$ (ng/L)	**TEQ-$_{WHO}$ (ng/L)**
PCB28	*2.00E-06*		1.09E-05	
PCB52	*5.00E-06*		7.72E-05	
PCB101	*3.00E-05*		1.41E-04	
PCB118	*1.00E-04*	*3.00E-05*	2.66E-04	7.97E-05
PCB138	*2.00E-05*		3.21E-04	
PCB153	*1.00E-05*		7.62E-05	
PCB180	*5.00E-06*		2.06E-05	
	∑TEQ		9.13E-04	7.97E-05

Values in italics - Published data (text)

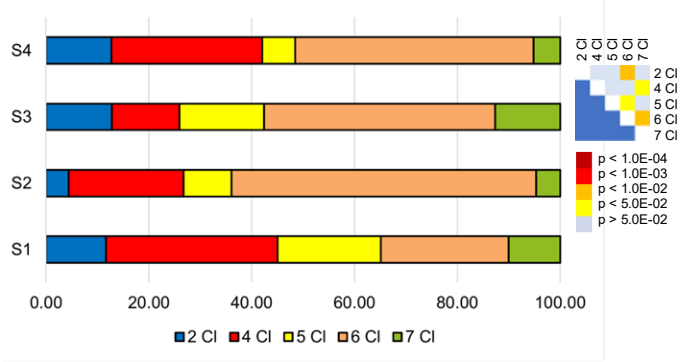


Figure 17 - PCBs distribution (%) considering the number of Cl atoms of each congener by sampling site in mussel tissue.

Figure 18 show PC1 vs PC2 in mussel samples. When applying PCA to PCBs data, it was found that three main components can be considered (PC1, PC2 and PC3). The main contributors for PC1 and PC2 were, respectively, PCB153 (0.53) and PCB52 (0.60). PCA shows that in mussel samples, the variability of data is lowest in the winter and highest in the summer.

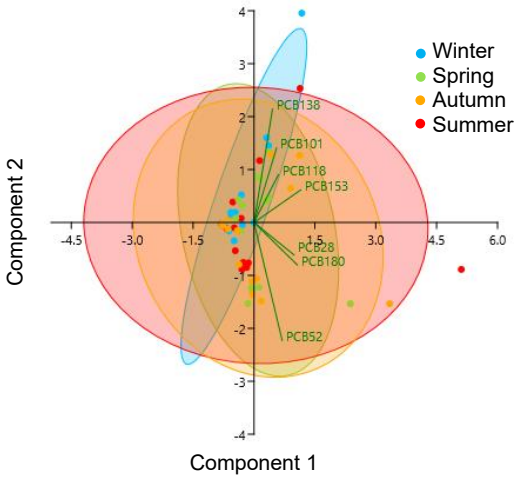


Figure 18 - Score plots of PC1 vs PC2 illustrating the distribution of individual PCBs in several months and sampling sites (S_1 to S_4; n = 180) in mussels.

3. Physicochemical parameters and biometric characterization

Table 13 shows the physicochemical parameters measured in each sampling per season. On average, temperature, pH, ammonia, phosphates, nitrates and nitrites were ≈ 15.5 ºC, ≈ 7.8, ≈ 1.7 mg/L, ≈ 0.2 mg/L, ≈ 1.1 mg/L and ≈ 0.04 mg/L.

Table 13 - Physicochemical parameters of seawater samples from Vila-do-Conde seacoast (mean ± SD; n = 96) per season.

Season	Temperature (ºC)	pH	NH_4 (mg/L)	PO_4 (mg/L)	NO_3 (mg/L)	NO_2 (mg/L)
Winter	12.84 ± 1.50	7.90 ± 0.01	0.99 ± 0.20	0.13 ± 0.13	1.37 ± 0.12	0.06 ± 0.01
Spring	15.23 ± 0.37	7.84 ± 0.12	2.35 ± 0.41	0.35 ± 0.59	1.23 ± 0.15	0.03 ± 0.03
Summer	17.60 ± 1.06	7.86 ± 0.13	2.31 ± 0.06	0.12 ± 0.03	1.14 ± 0.09	0.04 ± 0.01
Autumn	16.33 ± 1.34	7.52 ± 0.30	1.05 ± 0.03	0.09 ± 0.07	0.80 ± 0.19	0.03 ± 0.00

Wild mussels from the Vila-do-Conde seacoast showed an average length of 4.5 ± 0.54 cm, a height of 1.1 ± 0.30 cm and a width of 1.9 ± 0.20 cm. The mean weights were 9.6 ± 3.35 g with shell and 2.6 ± 1.09 g shelled. Several individuals were randomly selected from the population described to obtain 3 pools per site. In **table 14**, it is presented the biometric characterization of the pooled mussels.

Table 14 – Biometric characterization of mussel samples from Vila-do-Conde seacoast (mean ± SD; n = 180) per site.

Sites	Total no.	animals per pool	Length (cm)	Height (cm)	Width (cm)	Weight with shell (g)	Soft tissue weight (g)
S_1	605	8	4.5 ± 0.6	2.0 ± 0.3	2.1 ± 0.4	9.5 ± 4.1	13.0 ± 2.2
S_2	430	12	4.2 ± 0.8	1.7 ± 0.3	1.9 ± 0.5	7.6 ± 3.9	12.4 ± 3.3
S_3	234	7	4.8 ± 0.9	1.9 ± 0.4	2.3 ± 0.5	11.8 ± 5.9	12.6 ± 3.2
S_4	334	10	4.5 ± 0.5	1.9 ± 0.3	2.1 ± 0.3	8.1 ± 2.7	11.9 ± 2.1

IV. DISCUSSION

1. PAHs

1.1. DAP and SPM

The present data are similar to those obtained in a previous study regarding the same area and pollutants, with a slight increase in DAP (Rocha et al., 2021). However, considering studies from other aquatic ecosystems worldwide (**Table 15)**, Vila-do-Conde shows lower levels of these compounds.

Table 15 – PAHs concentrations (Σ16PAHs) quantified in several coastal locations worldwide.

Country	Site	∑PAHs		Reference
		DAP (ng/L)	SPM (ug/kg)	
Egypt	Mediterranean coast	991	-	El-Naggar et al., 2019
South Africa	Algoa Bay	74.6	4313	Adeniji et al., 2019
China	Li'an and Xincun Bays	76.3 - 522.9	929.2 - 899.8	Wang et al., 2020
Indonesia	Jakarta Bay	-	186.6 - 915.7	Aziz et al., 2021
China	Xiangshan Bay	37.9	339.1	Luo et al., 2023
Italy	Gulf of Milazzo	21.7	224.3	D'agostino et al., 2020
Japan	Northwestern Japan sea	19.6	-	Koudryashova et al., 2019
Iran	Chabahar Bay	1.7 - 2.8	ND - 92.8	Agah et al., 2017
Portugal	Atlantic Iberian NW coastline	8.6	96.7	Rocha et al., 2021
		17.3	**99.9**	**Present study**

PAHs contamination in dissolved phases are divided into four grades, “micro-polluted” (10–50 ng/L), “light-polluted” (50–250 ng/L), “moderately polluted” (250–1,000 ng/L) and “heavily polluted” (>1,000 ng/L) (Cao et al., 2010). The pollution levels of sediment are classifiable as low (0-100 ng/g), moderate (100-1,000 ng/g), high (1,000-5,000 ng/g) and very high (>5,000 ng/g) (Baumard et al., 1998).

Here, the concentrations of PAHs in DAP ranged from 17.4 to 26.1 ng/L, showing that the four sampling sites could be classified as “micro-polluted”. Despite this, the concentrations of PAHs in SPM ranged from 101.4 to 143.1 ng/g, suggesting that the four sampling sites could be classified as "moderately contaminated”.

This study shows that the origin of PAHs in DAP is petrogenic, as most PAHs have LMW. This occurrence can be justified by the proximity of the Vila-do-Conde harbour, where considerable maritime traffic exists, and the fact that LMW PAHs are the most soluble PAHs. In SPM, on the contrary, the most abundant PAHs are those HMW

PAHs that result primarily from pyrogenic contamination. This data is consistent with wildfires that significantly affect the Portuguese territory yearly and the chemical affinity of HMW PAHs to SPM.

In line with the above considerations, the evaluation of possible ecological risks posed by PAHs in seawater reveals that DAP set "Moderate-risk" to these ecosystems when considering some individual PAHs ($RQ_{(NCs)}$≥1 and $RQ_{(MPCs)}$< 1), despite as a whole they seem to pose a "Low-risk" to the targeted habitat ($RQ_{\sum PAHs(NCs)}$≥ 1; < 800 and $RQ_{\sum PAHs(MPCs)}$ ≈ 0). These data agree with higher concentrations of LMW PAHs than HMW PAHs in DAP (**Table 16**).

In SPM, several PAHs set "Moderate-risk" and BaA sets “High risk” to the evaluated ecosystem, as BaA’s RQ(MPCs) is ≥1. Moreover, the analysed mix of PAHs may cause a "Moderate-risk" to the targeted habitat ($RQ_{\sum PAHs(NCs)}$ <800 and $RQ_{\sum PAHs(MPCs)}$ are ≥1).

In summary, the observations in both fractions support that PAHs pose a moderate risk to the ecosystem.

Table 16 – Risk classification of individual PAHs and ∑PAHs (Cao et al., 2010).

Individual PAHs			∑PAHs		
	$RQ_{(NCs)}$	$RQ_{(MPCs)}$		$RQ_{\sum PAHs(NCs)}$	$RQ_{\sum PAHs(MPCs)}$
Risk-free	0		Risk-free	=0	
Moderate-risk	≥1	<1	Low-risk	≥1; <800	=0
			Moderate-risk	≥800	=0
				<800	≥1
High-risk		≥1	High-risk	≥800	≥1

Furthermore, unlike other previous studies, this study revealed no evidence of seasonal fluctuation patterns of PAHs from 2017 to 2021, either in DAP or SPM (Rocha et al., 2021). Despite this, the PCA performed for PAHs shows higher dispersion plots in the summer for both DAP and SPM, suggesting an association with an increase in recreational activities (aquatic sports, circulation of small boats, etc.) in the warmer months compared to other seasons.

Globally, PAHs are studied considering their carcinogenic potential, which depends on their concentrations and nature (Abdel-Shafy and Mansour, 2016). Considering this aspect, in this seashore, the most abundant PAHs in DAP belong to Group 2B, *i.e.*, “possibly carcinogenic to humans” (WHO, 2016), meaning that there is evidence that these compounds can cause cancer in humans but, at present, the data is far from conclusive. In SPM, however, the most abundant PAHs belong to Group 3, *i.e.*, compounds "Not classifiable as to its carcinogenicity to humans" (WHO, 2016), meaning there is no evidence at present that the compounds in question may cause cancer in humans.

The surveyed area accommodates a panoply of recreational and commercial activities that attract many people. So, in summary, these results demonstrated that the last anthropogenic activities serve as active sources of contamination by PAHs, making the surrounding seawater moderately contaminated. The environmental risk was also considered at a moderate level, which is not alarming *per se* but entails that it would be wise to keep this area under observation. Furthermore, exposition to this seacoast environment that is contaminated with “possibly carcinogenic” PAHs can lead to potential and severe risks to the population, particularly the most vulnerable groups.

1.2. Mussel tissue

The analysis of PAHs in wild mussels evidenced an almost ubiquitous contamination profile for a broad size range of these organic contaminants, demonstrating that these organisms bioaccumulate these pollutants over time (Lance et al., 2012).

When comparing the $\Sigma_{16\ PAHs}$ in mussels from Vila-do-Conde with the same sum data collected by others in different locations around the world, it is observed that our animals are neither heavily contaminated like those analysed in China or Indonesia, nor lowly contaminated, such as those analysed in Brazil and Italy (**Table 17**). However, in comparison to the results obtained in the past concerning a close coastal area (Madureira et al., 2014b), our study revealed higher concentrations of these compounds

Table 17 – PAHs concentrations (Σ16PAHs) quantified in mussels collected in various locations worldwide.

Country	Site	∑PAHs in mussels (ug/kg)	Reference
China	Li'an and Xincun Bays	2153	Wang et al., 2020
Indonesia	Jakarta Bay	11 - 1133	Aziz et al., 2021
Spain	Barcelona	212.3	Sánchez-Avila et al. 2011
Norway	Rakkfjorden	54 - 102	Storhaug et al., 2019
Italy	Campania coast	20.7	Serpe et al., 2010
Brazil	Perna Perna bay	9.5	Yoshimine et al., 2012
Portugal	Matosinhos beach	52.9	Madureira et al., 2014
	Atlantic Iberian NW coast	**136.9**	**Present study**

The approach of using the results obtained and calculating PAH ratios to infer characteristic pyrolitic/petrogenic source patterns suggests an overall fingerprint of petrogenic origin, which may be directly linked to the proximity of the Vila-do-Conde harbour, where considerable maritime traffic exists.

It is also possible to observe in **Figure 9** a sudden reallocation of the data to the "pyrogenic contamination" domain in the year 2020. The last data corresponds to the COVID-19 lockdown period, during which most activities and transportation were disrupted and could still be felt in 2021. Consequently, the input of contaminants of petrogenic origins in the environment reduced drastically, while the HMW PAHs remained in mussels.

There seems to be no evidence of seasonal fluctuation patterns of PAHs in mussel tissue since there were no significant differences between seasons, corroborating the data obtained from DAP and SPM.

However, the triangular approach of this data shows a clear distinction between two clusters concerning the abundance of congeners with 2+3, 4 or 5+6 rings in their chemical structure. The upper cluster of data, situated closer to the 2+3 rings vertex/apex, concerns samples collected in 2020 and 2021, whereas the lower cluster, situated closer to the bottom edge of the plot, concerns samples collected in 2018 and 2019. As previously mentioned, this may be related to the disruption of activities in the area during the lockdown, resulting in a contrast of accumulated PAHs in these animals throughout the years.

The prevalence of LMW PAHs indicates petrogenic origin from crude oil and petroleum spillages, shipping and shipyard activities, industrial and vehicle emissions and urban discharges. PAHs with 4 aromatic rings could be from petrogenic sources but more likely from pyrolitic inputs, as the other HMW PAHs with five rings or more originate from various combustion processes, mainly wood and coal. (Oros and Ross, 2004).

According to FAO data, the average consumption rate of bivalves in Portugal between 2018 and 2021 was 7.93 kg *per capita* per year. It should be noted that this value was the highest in the European Union, followed by France (5.62 kg/*per capita*/year) and Spain (5.30 kg/*per capita*/year). The European average (2.59kg/*per capita*/year) was three fold smaller than the Portuguese average (FAO, 2023). It is safe to say that bivalves play an important role in the portuguese diet and gastronomy.

EDI is an important tool to assess the daily ingestion of PAHs, due to several epidemiological studies showing that colorectal, gastric, and gastrointestinal tract cancers have been associated with dietary exposure to PAHs (Abdel-Shafy and Mansour, 2016). In this study, the obtained EDI values were generally lower than those found by Wang et al. (2020) in China.

THQ represents the level of exposure, below which it is improbable that even the most sensitive populations will experience potential health effects (USEPA, 2023). When $\Sigma THQ \leq 1$, the exposed population is unlikely to experience obvious adverse effects; conversely, if the ratio is above 1, value considered by the US-EPA, it assumed that there is a risk of chronic systemic effects (USEPA, 2023). In this study, this value was slightly exceeded only once, in the site S_1.

As for the carcinogenic risk, acceptable carcinogenic risks range between E−04 and E-06, *i.e.*, a risk of an individual to develop cancer, over a lifetime, is respectively 1 in 10 000 and 1 in 1 000 000 as a consequence of exposure to the potential carcinogen (USEPA, 2023). ΣCR values were all higher than the USEPA recommended safety limits, thus revealing that the ingestion of the selected mussel species may pose carcinogenic risks for consumers.

Therefore, it is presumed that the ingestion of mussels harvested from the mentioned sites may pose concerns of carcinogenic risks for consumers with higher consumption rate. Our results revealed considerably higher carcinogenic risk values than those obtained by Wang et al. (2020), Conte et al. (2016) and Ferrante et al. (2018).

Nevertheless, the risks are associated with the levels of contaminants in the food (bivalves in this study) but especially with the high daily intake rate of bivalves by the Portuguese population, leading to higher vulnerability compared to other countries.

As a last remark, when establishing a comparison between the obtained PAH concentrations and the maximum levels defined in the current European Regulation (CEC 2011) for bivalve molluscs, it is possible to get a general idea concerning the environmental health state of the environmental area in the north coast of Portugal. According to the mentioned regulation, BaP and the sum of other four PAHs (BaP, BaA, BbFL and Chr) should not exceed 5.0 and 30.0 µg/kg *ww*, respectively. This work shows that, for BaP, this value was exceeded only in one of the sampling sites (S_1), and the sum of the other four PAHs was exceeded in all the sampling sites.

Mussel analysis enabled the assessment of this habitat's condition, but it also revealed variations in contamination input throughout the years, showing that these animals are excellent bioindicators that can keep accurate chronological records of environmental contamination in their tissues. The uncovered results raise concerns about population safety because wild mussels are known to be collected from these beaches for personal consumption, without any depuration and sanitary control. This area displays a moderate-risk of exposure to PAHs, and close monitoring is recommended.

2. PCBs

2.1. DAP and SPM

Regarding PCBs, the seven analysed PCBs are used as indicators of this category of POPs in the sea (ICES, 2012). In this sense, the total sum of the quantified PCBs found in DAP and SPM was similar in all sampling sites, and their levels are very similar to the values obtained in a previous study, pointing to a continuous discharge of this contaminants in this area throughout the yearsn other works in **Table 18**.

Table 18 – PCBs concentrations (Σ7PCBs) quantified in several coastal locations worldwide.

Country	Site	∑PCBs		Reference
		DAP (ng/L)	SPM (ug/kg)	
Spain	Barcelona	5.5	305	Sánchez-Avila et al. 2011
Egypt	Mediterranean coast	ND	8.1 - 145.0	El-Naggar et al., 2019
Indonesia	Jakarta Bay	-	3 - 117	Aziz et al., 2021
China	East China Sea	0.4 - 51.8	0.8 - 45.5	Wang et al., 2022
Norway	Svalbard archipelago	0.0040-0.40	0.0020-41.2	Pouch et al., 2021
Portugal	Atlantic Iberian NW coastline	2.9	19.3	Rocha et al., 2021
		5.3	**14.4**	**Present study**

The sources of PCBs are challenging to understand, as they were banned in Portugal in 1972. However, due to their persistence and high number of congeners, they are still present in our environment (Rocha et al., 2021). These products were widely used as dielectric fluid in capacitors and transformers and, to a lesser extent, in building materials (*i.e.*, caulking, paints, and lighting ballasts). Today, exposure can come from demolition, combustion, dysfunction, or uncontrolled recycling of PCB-contaminated structures and equipment (IARC 2016). The surveyed area is highly industrialised and is known to have many private infrastructures containing deposits of old machinery/warehouses, some of which suffered from minor fires during the sampling period, possibly leaking PCBs and other undesirable pollutants into the environment ("Incêndio em edifício comercial da zona ribeirinha de Vila do Conde obriga à evacuação do prédio", 2019; "Incêndio em edifício no centro de Vila do Conde obriga ao corte de trânsito", 2020; "Antigo centro de saúde de vila do conde consumido pelas chamas", 2021).

Except for PCB118, which is involved in carcinogenesis (TEF-$_{WHO}$), the remaining PCBs (and many other congeners) are involved in thyroid endocrine disruption (TEFs-$_{TH}$) and contribute to neurotoxic effects in mammals such as rats (Yang et al., 2010). Considering these effects and assuming an identical behaviour across vertebrates, **Table 10** shows the potential impacts of these waters on the thyroid. Since the estimated TEQs-$_{TH}$ and TEQs-$_{WHO}$ are in the order of E-04 and E-05 ng/L in both DAP and SPM, they do not seem able to induce toxic effects for biota (IARC, 2016).

Regarding the number of Cl atoms of each analysed congener, it is possible to observe that PCBs with 5 Cl atoms are the most abundant. Pentachlorobiphenyls such as PCB101 and 118 are known to cause cellular genotoxic damage (Marabini et al., 2011), and the latter is a known carcinogenic.

Similarly to PAHs, there is no evidence of seasonal fluctuation patterns in PCBs in DAP. However, SPM data revealed a lower concentration of these compounds in autumn than in spring and summer, suggesting that this fraction may serve as a reservoir of these compounds due to its chemical nature.

According to the PCA analysis, in DAP, the variance of PCBs was relatively similar throughout the seasons, suggesting a continuous entrance of these contaminants into the sea. However, in SPM, the variance was lower in the autumn and highest in the spring. This occurrence might be related to precipitation rates during these seasons since autumn follows the season with the lowest precipitation rates, presenting a lower dispersion of these pollutants. Meanwhile, spring (along with winter) has high precipitation rates, thus presenting the highest dispersion of PCBs in this area.

This study demonstrated that PCBs are still present in the Vila-do-Conde coastline, but concentrations found in seawater displayed a low level of environmental risk. However, even in low concentrations, these molecules can potentially affect the most sensitive organisms, as they appear in the environment mixed with other contaminants that can reveal enhanced toxicity. Again, concerns focus on the most vulnerable groups.

2.2. Mussel tissue

PCB levels were quantified in most mussel samples, regardless of the sampling origin. This fact was not particularly surprising since molecules with log Kow > 6, as PCBs, are highly associated with the organic matter in aquatic environments (including sediments, plankton and suspended particles) and, consequently, can be taken up by filter feeders. Furthermore, bivalves have deficient activities of enzymes able to metabolize these compounds (Dailianis, 2011).

Here, the total sum of the quantified PCBs found in mussels was higher in the beaches Azurara (≈ 84 µg/kg) and Árvore (≈ 71 µg/kg), followed by Areia (≈ 54 µg/kg) and Reserva (≈ 15 µg/kg). These data suggest that Azurara is the most polluted site, probably due to its proximity to the Ave River mouth, where many pollutants are drained from the city. At the same time, Reserva is the less polluted site, which is of significant importance, as it probably means fewer pollution sources near the protected Natural Reserve.

Table 19 compares the concentrations quantified in mussels from Vila-do-Conde and those reported in other parts of the world. Similarly to what was observed previously with the PAHs, our samples revealed higher concentrations of PCBs than the values obtained by Madureira et al. (2014) in a nearby area.

Table 19 – PCBs concentrations (Σ7PCBs) quantified in several coastal locations worldwide.

Country	Site	∑PCBs in mussels (ug/kg)	Reference
Japan	Japan East coast	340 - 450	Ishiyama et al., 2019
Various	Adriatic Sea	3.7 - 114.4	Bajt et al., 2019
Italy	Taranto	65.1	Giannico et al., 2022
Spain	Galician coast	54	Rodil et al., 2019
Spain	Mediterranean coast	13 - 14	Campillo et al., 2019
Italy	Campania coast	1.5 - 6.7	Esposito et al., 2020
South Korea	South Korea coast	0.41 - 0.45	Lee et al., 2020
Portugal	Matosinhos beach	1.8 - 22.1	Madureira et al., 2014
	Atlantic Iberian NW coast	**56**	**present study**

The estimated TEQs-$_{TH}$ and TEQs-$_{WHO}$ in mussels are in the order of E-04 and E-05 ng/L, respectively. Thus, they do not seem able to induce toxic effects for biota (IARC, 2016).

The current data show a yearly pattern of lower concentrations of PCBs in the winter compared to spring. This observation might be attributed to weather patterns, such as higher precipitation rates that result in diluted concentrations of pollutants.

Regarding the number of Cl atoms of each congener, it is possible to observe that PCBs with 6 Cl atoms are the most abundant in mussel tissue, followed by PCBs with 4 Cl atoms. Highly chlorinated PCBs have long half-lives, are poorly metabolised and are known to cause genotoxicity and immuno-toxicity (IARC, 2016). This collection of features might be responsible for the long-term accumulation of these congeners in mussel tissues, and the ingestion of these animals might lead to further health complications for the consumers.

According to the PCA analysis, the variability was lower in the winter and highest in the summer in mussel tissue, possibly associated with the repetitive occurrence of small-scale fires affecting several focal points in the city mainly taking place in the summer (“Incêndio em edifício comercial da zona ribeirinha de Vila do Conde obriga à

evacuação do prédio", 2019; "Incêndio em edifício no centro de Vila do Conde obriga ao corte de trânsito", 2020).

In summary, considering PAHs and PCBs, industrialization, urbanization and tourism lead to increased pollution inputs into the surrounding marine environments of Vila-do-Conde, detrimental to the health and biodiversity of the coastal ecosystems and human health.

Seawater samples revealed a continuous input and accumulation of PAHs and PCBs in this area, resulting in the classification of this region as moderately contaminated. PAHs have both petrogenic and pyrogenic origins, their concentrations exhibit a low to moderate ecological risk, and their nature has possible carcinogenic potential. Although pentachlorobiphenyls were the most abundant congeners in water samples, the results showed that the found concentrations do not seem to induce toxic effects in biota, and their sources were impossible to say for sure.

The analysis of these POPs in wild mussels evidenced an almost ubiquitous contamination profile for a broad range of contaminants, which was expected due to these animals' contaminant accumulation and low metabolizing capacity. PAHs profile was mostly of petrogenic origin, though it revealed variations throughout the years, most likely related to the disruption of polluting activities during the COVID-19 lockdown. These contaminants pose a "moderate risk" to the targeted region, as they were found to be possibly carcinogenic, and their concentrations exceeded the maximum legal levels in all the sampling sites. PCBs maximum legal levels, however, were exceeded only in one location and risk evaluation revealed that they do not seem able to induce toxic effects at these concentrations.

This study reveals concerning findings regarding the farewell of this ecosystem in the face of PAHs and PCBs. The obtained results might not be alarming yet, and control measures might not be needed in this phase, but this could quickly become a severe concern, emphasizing the importance of monitoring the affected area. Furthermore, these compounds are present in the environment as complex mixtures of pollutants, which may pose even more significant risks than what was observed in the current study. Exposure to the most contaminated sites can negatively affect the local population, mainly through ingesting bivalves collected from these habitats, and the ecosystem may already be suffering from this problem.

V. CONCLUSIONS

PAHs and PCBs are persistent organic pollutants with significant environmental risks. These compounds have been widely used in various industrial applications, leading to their widespread environmental presence. The accumulation of PCBs and PAHs in soil, water, and air can harm ecosystems and human health. These compounds are toxic, carcinogenic, and endocrine-disrupting, posing risks to wildlife and humans.

Mussels, being filter feeders, are highly efficient at accumulating and concentrating POPs from their surrounding environment. As a result, they serve as valuable bioindicators for monitoring the levels of these contaminants in aquatic ecosystems. The presence of PCBs and PAHs in mussels can have detrimental effects on their health and survival. These compounds can disrupt their reproductive systems, impair their immune function, and even lead to mortality. Additionally, consuming contaminated mussels can pose serious health risks, including carcinogenic and toxic effects in humans.

Efforts to mitigate the environmental risks associated with PAHs and PCBs have been ongoing. Strict regulations and bans on releasing these pollutants into water bodies and monitoring programs have been implemented to reduce their levels. Additionally, research on developing remediation techniques and assessing the long-term effects of these contaminants is crucial. However, despite these efforts, these chemicals persist in the environment due to their long half-lives and resistance to degradation and continue to affect wildlife, threatening the survival of entire populations and causing imbalances in the ecosystems. Therefore, continued monitoring, research, and implementation of effective strategies are necessary to minimize their impact on ecosystems and human health.

This study aimed to assess the contamination status of the Vila-do-Conde coastline and found that the anthropogenic activities in this area pollute the surrounding marine environment, possibly impacting the health and biodiversity of coastal ecosystems and human health.

Seawater samples revealed the continuous presence of PAHs and PCBs, classifying the region as moderately contaminated. PAHs have both petrogenic and pyrogenic origins and exhibit a low to moderate ecological risk with possible carcinogenic

potential. PCBs concentrations were low and demonstrated minimal toxic potential; their sources remain uncertain.

Wild mussels showed widespread contamination, likely due to their low metabolizing and high accumulation capacities. The PAHs profile in mussels varied over time, possibly influenced by the interruption of polluting activities during the COVID-19 lockdown. These contaminants posed a moderate risk, exceeding legal levels and showing potential carcinogenicity. PCBs exceeded legal levels in only one location but were not found to induce toxic effects at these concentrations.

The consumption of mussels from these sites may pose carcinogenic risks to the local communities, not only because of the contaminants concentrations found in these animals tissues, but especially due to the high daily intake rate of bivalves, as Portugal had the highest consumption rate of bivalves in the European Union.

This study raises concerns about the well-being of local populations and ecosystems in the presence of PAHs and PCBs, as these usually appear in complex mixtures of pollutants that may pose more significant environmental risks than observed in this study. Thus, monitoring and controlling POPs is of utmost importance in safeguarding the environment and human health. By implementing effective measures to reduce and eliminate the production and release of POPs, we can mitigate their adverse effects and ensure a sustainable future for generations to come.

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