Margarida Nunes Cardoso

PERSISTENT ORGANIC POLLUTANTS IN PORTUGUESE ESTUARIES

Occurrence and distribution of PCDD/Fs and dioxin-like PCBs

Tese de Doutoramento em Biologia, na especialidade de Ecologia, orientada pelo Professor Doutor Miguel Ângelo Pardal e pelo Professor Doutor Fernando Ramos e apresentada à Faculdade de Ciências e Tecnologia da Universidade de Coimbra

Setembro 2013

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Universidade de Coimbra

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Doctoral Thesis in Biology (scientific area of Ecology) under the supervision of
Professor Doutor Miguel Ângelo Pardal and Professor Doutor Fernando Ramos, presented to the
Faculty of Sciences and Technology of the University of Coimbra

2013

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Faculdade de Ciências e Tecnologia
Universidade de Coimbra

This thesis was financially supported by

FCT – Fundação para a Ciência e Tecnologia through a PhD grant attributed to Margarida Cardoso (SFRH/BD/46969/2008), funded by Programa Operacional Potencial Humano of QREN framework within the European Social Fund and by the Portuguese budget through the Ministry of Science Technology and Education

“This is an adventure.”

Steve Zissou in *The Life Aquatic with Steve Zissou*

Abbreviations

AhR	Aryl hydrocarbon receptor
ANOVA	Analysis of variance
dl-PCB	Dioxin-like polychlorinated biphenyl
dw	Dry weight
EQS	Environmental quality standards
GC-HRMS	Gas chromatography coupled to high-resolution mass spectrometry
HpCDD	Hexachlorodibenzo-*p*-dioxin
HpCDF	Hexachlorodibenzofuran
HxCDD	Heptachlorodibenzo-*p*-dioxin
HxCDF	Heptachlorodibenzofuran
ISO	International Organization for Standardization
K_H	Henry's law constant
K_{OC}	Organic carbon-water partition coefficient
K_{OW}	Octanol-water partition coefficient
lw	Lipid weight
LOD	Limit of detection
OCDD	Octachlorodibenzo-*p*-dioxin
OCDF	Octachlorodibenzofuran
PCA	Principal component analysis
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo-*p*-dioxin
PCDF	Polychlorinated dibenzofuran
PeCDD	Pentachlorodibenzo-*p*-dioxin

PeCDF	Pentachlorodibenzofuran
POP	Persistent organic pollutant
RCF	Root concentration factor
SQG	Sediment quality guideline
TCDD	Tetrachlorodibenzo-*p*-dioxin
TCDF	Tetrachlorodibenzofuran
TEF	Toxic equivalency factor
TEQ	Toxic equivalent
TOC	Total organic carbon
WFD	Water Framework Directive
WHO	World Health Organization
ww	Wet weight

Publications

The work presented in this thesis resulted in the publication/submission in peer-reviewed international scientific journals of the following manuscripts:

Nunes M, Marchand P, Vernisseau A, Le Bizec B, Ramos, F Pardal MA (submitted) Occurrence of PCDD/Fs and dioxin-like PCBs in superficial sediment of Portuguese estuaries. Environmental Monitoring and Assessment

Nunes M, Marchand P, Vernisseau A, Le Bizec B, Ramos, F Pardal MA (2013) Distribution of PCDD/Fs and dioxin-like PCBs in sediment and plants from a contaminated salt marsh (Tejo estuary, Portugal). Environmental Science and Pollution Research *in press*

Nunes M, Martinho F, Marchand P, Vernisseau A, Le Bizec B, Ramos F, van der Veer HW, Cabral HN Pardal MA (submitted) Early contamination of European flounder (*Platichthys flesus*) by PCDD/Fs and dioxin-like PCBs in European waters. Marine Pollution Bulletin

Nunes M, Marchand P, Vernisseau A, Le Bizec B, Ramos, F Pardal MA (2011) PCDD/Fs and dioxin-like PCBs in sediment and biota from the Mondego estuary (Portugal). Chemosphere 83, 1345–1352

Abstract

Despite their ecological and economical importance, estuaries receive and retain numerous chemical contaminants. Persistent organic pollutants (POPs) such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (dl-PCBs) are widely recognized by the scientific community as being a risk to wildlife and human health due to their high toxicity and ability to bioaccumulate in biota and biomagnify in food webs. In this regard, the occurrence and distribution of 2,3,7,8-substituted PCDD/Fs and dl-PCBs was investigated in the Portuguese estuarine environment, through four different studies.

The initial study provided a general overview of the occurrence of PCDD/Fs and dl-PCBs in superficial sediments from several estuaries along the Portuguese coast (Lima, Ria de Aveiro, Mondego, Tejo, Sado, Mira and Ria Formosa). In general, the higher concentrations were detected near large populated regions and industrial complexes (e.g. Tejo estuary), while the lowest PCDD/F and dl-PCB values were measured in less impacted areas (e.g. Ria Formosa). The different PCDD/F profiles detected among the Portuguese estuaries suggest the existence of different contamination sources. Furthermore, this work showed that the most abundant PCDD/F congeners existing in superficial sediments are those with more chlorine substitutions. In contrast, the dl-PCB profiles were fairly similar among the estuaries. The contamination levels found in the collected sediments were lower than those found in highly impacted areas from different parts of the globe. Nevertheless, comparison with guidelines and quality standards from other countries indicated that some Portuguese estuarine areas with a high industrialization level present PCDD/F and dl-PCB concentrations in superficial sediment that may constitute a risk to aquatic organisms.

The following study focused on the salt marsh plants *Sarcocornia perennis* and *Halimione portulacoides* capacity to accumulate PCDD/Fs and dl-PCB. According to this work carried

out in the Tejo estuary (Portugal), the selected species retain PCDD/Fs and dl-PCBs in their organs. However, the major fraction of these contaminants remains associated with sediment. In addition, both plant species accumulate significantly higher concentrations of PCDD/Fs and dl-PCBs in its roots in comparison with its aboveground tissues, suggesting that despite part of the sediment contaminants being incorporated in the roots, they are not substantially translocated to the aboveground vegetation. This study also showed that *S. perennis* accumulate lower quantities of dioxin-like compounds in comparison with *H. portulacoides*, confirming the diversity in uptake and translocation of organic contaminants among plant species. It was also possible to verify that salt marsh sediments without vegetation show higher PCDD/F and dl-PCB concentrations, suggesting that these plant species may contribute to reduce dioxin-like compound concentrations in contaminated sediments, although in a minor extent. Moreover, congener profiles changed between sediments and plant tissues, reflecting a selective accumulation of low chlorinated PCDD/Fs and non-*ortho* dl-PCBs in the studied plants.

The third study addressed the contamination of juvenile European flounder (*Platichthys flesus*) contamination by PCDD/Fs and dl-PCBs in a number of nursery areas along the species' geographical distribution in the northeastern Atlantic Ocean. According with results obtained, the lowest tissue residue levels were detected in juveniles caught in the Sørfjord (Norway), whereas the highest value was found in *P. flesus* captured in the Wadden Sea (Netherlands), in agreement with the long history of pollution reported in this area. The PCDD/F and dl-PCB concentrations detected in the muscle of juvenile flounder are not expected to adversely affect fish.

Finally, a preliminary survey was carried out in the Mondego estuary (Portugal) to investigate the occurrence of PCDD/Fs and dl-PCBs in sediment and biota. The contamination levels found in the study area were lower when compared with PCDD/F and dl-PCB concentrations reported in impacted estuarine and coastal systems around the world. This study suggests different behaviors of PCDD/Fs and PCBs along the food web. While concentrations of PCDD/Fs were lower in higher trophic-level organisms, higher dl-PCB values were generally found in fish. In addition, our results showed differences between PCDD/F and dl-PCB profiles. In the Mondego estuary, macroalgae, plants and benthic invertebrates maintained the sediment PCDD/F profile, whereas organisms at higher levels of the food web (i.e., fish) tend to selectively accumulate lower chlorinated PCDD/F homologues. On the other hand, quite similar dl-PCB profiles were observed in the different species from the Mondego estuary (except algae and plants). From a human health perspective, the concentrations detected in edible aquatic organisms collected in the Mondego estuary were below the maximum permissible levels established by the European legislation and, therefore, are safe for human consumption.

Resumo

Os sistemas estuarinos são de extrema importância, quer do ponto de vista ecológico quer económico, mas são também ameaçados pela presença de diversos contaminantes. Os poluentes orgânicos persistentes (POPs) tais como as dibenzo–*p*–dioxinas policloradas (PCDDs), os dibenzofuranos policlorados (PCDFs) e os bifenilos policlorados sob a forma de dioxinas (dl-PCBs), são conhecidos pelos seus efeitos adversos para a saúde humana e animal devido à sua elevada toxicidade e capacidade de se bioacumularem e biomagnificarem ao longo da cadeia trófica. Neste sentido, a presente tese teve como objectivo estudar a ocorrência e a distribuição de PCDD/Fs e dl-PCBs em sistemas estuarinos portugueses.

O primeiro estudo apresentado avalia os níveis de contaminação por PCDD/Fs e dl-PCBs em sedimentos superficiais de vários estuários portugueses (Lima, Ria de Aveiro, Mondego, Tejo, Sado, Mira e Ria Formosa). No geral, as concentrações mais elevadas foram detectadas na proximidade de grandes centros urbanos e zonas industriais (e.g. Tejo), enquanto os valores mais baixos foram encontrados em sistemas menos perturbados (e.g. Ria Formosa). Verificou-se ainda a existência de diferentes perfis de PCDD/Fs entre os sistemas estuarinos em estudo, apesar dos congéneres mais clorados se encontrarem sempre em maior proporção. Por outro lado, os perfis de dl-PCBs são bastante semelhantes entre os locais amostrados. Os níveis de contaminação dos sistemas estuarinos portugueses são inferiores aos reportados em várias zonas costeiras e estuarinas sujeitas a grandes pressões antropogénicas a nível mundial. No entanto, alguns dos locais de estudo apresentam sedimentos superficiais com concentrações de PCDD/Fs e dl-PCBs superiores aos valores definidos em directrizes de outros países, podendo, por isso, representar algum perigo para a vida aquática.

No segundo estudo foi avaliada a capacidade das plantas de sapal, nomeadamente *Sarcocornia perennis* e *Halimione portulacoides*, acumularem PCDD/Fs e dl-PCBs. De

acordo com este trabalho, desenvolvido no estuário do Tejo, ambas as espécies têm a capacidade de reter PCDD/Fs e dl-PCBs nos seus órgãos. No entanto, a maior fracção destes contaminantes permanece associada aos sedimentos. Os resultados obtidos indicam que ambas as espécies acumulam maior quantidade de PCDD/Fs e dl-PCBs nas raízes do que nos caules ou nas folhas, sugerindo que, apesar de parte dos contaminantes presentes nos sedimentos ser acumulada nas raízes, eles não são translocados significativamente para a parte aérea das plantas. Este estudo também demonstrou que *S. perennis* acumula menores quantidades de PCDD/Fs e dl-PCBs em comparação com *Halimione portulacoides*, confirmando a existência de diferentes capacidades de captação e translocação de contaminantes orgânicos dependendo das espécies. Também se verificou que sedimentos sem coberto vegetal apresentam maior concentração de PCDD/Fs e dl-PCBs. Para além disso, foi possível observar uma alteração entre os perfis de contaminação dos sedimentos e dos tecidos vegetais, reflectindo uma acumulação selectiva de PCDD/Fs menos clorados e não-*ortho* dl-PCBs nas plantas de sapal estudadas.

O terceiro estudo centra-se na contaminação de juvenis de solha (*Platichthys flesus*) por PCDD/Fs e dl-PCBs em diferentes áreas estuarinas e costeiras, utilizadas pela espécie como zonas de viveiro, ao longo da sua distribuição geográfica no nordeste do Oceano Atlântico. Os resultados obtidos indicam que os indivíduos capturados em Sørfjord (Noruega) apresentam os menores níveis de contaminação, enquanto os juvenis do Wadden Sea (Holanda) são os mais contaminados. Tendo em consideração as concentrações de PCDD/Fs e dl-PCBs detectadas, não são esperados efeitos adversos nos juvenis de *P. flesus* em nenhum dos locais de estudo.

Por fim, são ainda apresentados os resultados de uma investigação realizada no estuário do Mondego com o objectivo de estudar a ocorrência de PCDD/Fs e dl-PCBs em sedimento e em várias espécies típicas de sistemas temperados. O presente estudo sugere que os PCDD/Fs se comportam de uma forma particular relativamente aos dl-PCBs. Os PCDD/Fs foram encontrados em menor concentração em organismos de maior nível trófico, ao passo que foi nestes que se registaram valores mais elevados de dl-PCBs. Além demais, os resultados obtidos mostram diferenças entre os perfis de PCDD/fs e dl-PCBs. Enquanto as macroalgas, plantas e organismos bentónicos recolhidos no estuário do Mondego apresentam um perfil de PCDD/Fs semelhante ao dos sedimentos, os peixes tendem a acumular selectivamente PCDD/F menos clorados. Por outro lado, observou-se perfis semelhantes de dl-PCBs na maioria das espécies. Em termos de saúde pública, as concentrações reportadas em organismos aquáticos capturados no estuário de Mondego, são inferiores ao valor máximo de PCDD/Fs e dl-PCBs estabelecido pela legislação da União Europeia, e deste modo, considera-se que o consumo destas espécies não representa perigo para o homem.

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CHAPTER I

General Introduction

During the 20th century, as a consequence of the rapid technical development, the environment became increasingly contaminated with numerous organic chemicals. Many of them were either produced as undesirable industrial by-products or used in several applications. Over the years, scientists began to recognize that certain chemicals were able to persist for long periods of time in the environment and to bioaccumulate, reaching levels that could adversely affect wildlife and human health (UNEP 2001). These chemical contaminants are commonly known as persistent organic pollutants (POPs). With the awareness of the potential hazards of POPs, numerous countries imposed a strict control on their use and release, leading to a substantial reduction of primary sources (UNEP 2001). Nevertheless, human and wildlife exposure to these chemicals continues to be a concern, since significant concentrations persist in the global environment.

Estuaries are characterized by high biological productivity and have been considered to be among the most valuable ecosystems on earth in terms of services and functions that support human society (Costanza et al. 1997; Able 2005; Barbier et al. 2011). Although estuarine systems represent important nursery and recruitment areas for many species (McLusky and Elliott 2004; Martinho et al. 2009; van der Veer et al. 2011), due to their fine-grained sediments high in organic matter content, they are also sinks for POPs such

as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs).

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)

The PCDDs and PCDFs are two families of chlorinated aromatic compounds with very similar chemical properties (Fig. 1). The number of chlorine substitutions on the benzene rings may range from one to eight, which means that 75 PCDDs and 135 PCDFs congeners are theoretically possible. The most toxic and thoroughly researched PCDD/F congener is the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (van den Berg et al. 1998). PCDD/Fs can be categorized by their degree of chlorination. The term homologue is hence used for all compounds with the same number of chlorines, e.g., PCDD congeners with eight chlorines attached are termed octachlorodibenzo-*p*-dioxins (OCDDs).

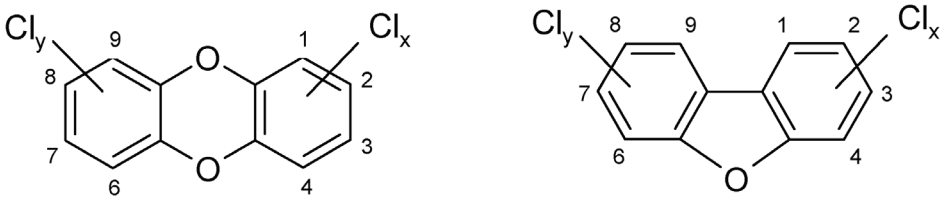

Figure 1 Structural formula of **(a)** polychlorinated dibenzo-*p*-dioxins (PCDDs) and **(b)** polychlorinated dibenzofurans (PCDFs) and numbering of the carbon atoms (Cl_X + Cl_Y = 1 to 8).

Despite the global distribution of PCDD/Fs, they have never been intentionally produced other than for scientific purposes (Altarawneh et al. 2009). However, PCDD/Fs are formed in a multitude of industrial and non-industrial processes, through homogeneous and heterogeneous pathways (Altarawneh et al. 2009). The homogeneous pathway involves the formation of PCDD/Fs in the gas phase from structurally related precursors, at temperatures ranging from 400 to 800 °C. The heterogeneous pathways comprise two different routes: a catalytic formation of PCDD/Fs from precursors, at temperature between 200 and 400 °C, and the so-called *de novo* mechanism, which consists on breakdown of a carbon matrix in a series of oxidation and chlorination reactions, also between 200 and 400 °C (Altarawneh et al. 2009). Chemical processes known to produce significant amounts of PCDD/Fs include

the bleaching of paper pulp (Rappe et al. 1990), the production of chlorine-alkali using graphite electrodes (Xu et al. 2000) and the chemical manufacture of chlorinated chemicals such as phenoxyacetic acid, a major constituent of defoliant Agent Orange used in the Vietnam War, the pesticide pentachlorophenol (PCP) and PCBs (Quaß et al. 2004; Young et al. 2004; Fiedler 2007). PCDD/Fs are also formed in combustion processes such as waste incineration, ferrous and non-ferrous metal production, transports (diesel and heavy oil-fired engines), manufacture of mineral products (cement, lime and bricks) and power generation and heating using fossil fuels and biomass (Fiedler 2007). In Portugal, the main industrial sources of atmospheric emissions of these organic chemicals are linked to the burning of fossil fuels, municipal and hospital waste incineration plants, thermal processes in metallurgical industry and cement production industry burning non-hazardous waste (Pereira et al. 2009). In addition to anthropogenic activities, PCDD/F formation may also occur in forest fires and other natural processes (Ingersoll et al. 1997; Rappe et al. 2001; Kim et al. 2003).

Due to process changes and improvements in the cleansing technology, the industrial emissions of PCDD/Fs have been globally reduced (Quaß et al. 2004). For example, Portuguese pulp industry have made investments in order to eliminate the use of elemental chlorine for the purpose of bleaching thus, reducing pollutant loads in liquid effluents (APA 2010). However, emissions from non-industrial sources hardly decreased (Quaß et al. 2004). In the near future, PCDD/F emissions from non-industrial sources are thus likely to exceed those from industrial installations, dominating the overall annual emissions in Europe (Quaß et al. 2004).

Polychlorinated biphenyls (PCBs)

PCBs are a group of chemical compounds synthesized by catalyzed chlorination of biphenyl (IPCS 2003). The theoretically possible PCB 209 congeners differ in the number of chlorine atoms (1 to 10) and their position in the molecule (Fig. 2). Positions 2, 2', 6, 6' are called *ortho*, positions 3, 3', 5, 5' are named *meta* and positions 4, 4' are called *para*. An important characteristic of PCBs is that their benzene rings can rotate around the bond connecting them. The rings are forced towards either the same plane (called coplanar PCBs) or perpendicular planes (named non-planar PCBs) by the electrostatic repulsion of the highly electronegative chlorine atoms (Andersson et al. 1997). The degree of planarity of a PCB

congener depends on the number of chlorine substitutions in the *ortho* positions. Thus, all non-*ortho*-substituted PCBs and some mono-*ortho*-substituted PCBs are considered to be coplanar, whereas a non-planar orientation is produced by multiple substitutions in the *ortho* positions (IPCS 2003).

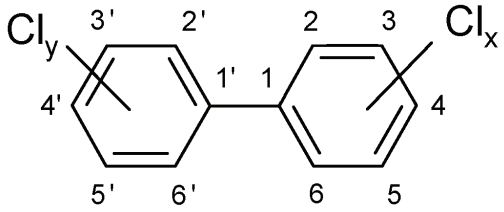

Figure 2 Structural formula of polychlorinated biphenyls (PCBs) and numbering of the carbon atoms (Cl_x + Cl_y = 1 to 10).

PCBs began to be produced commercially in 1929 as technical products comprising complex mixtures of congeners and were sold under various trade names such as Aroclor, Pyranol (USA), Phenochlor, Pyralene (France), Clophen, Elaol (Germany), Sovol (USSR), Kanechlor and Santotherm (Japan) (Frame et al. 1996; IPCS 2003). Due to their physical and chemical stability, non-flammability, high boiling point, low heat conductivity and high dielectric constants, these compounds were produced on a large scale to be used in a wide range of industrial and commercial applications (de Voogt and Brinkman 1989). For example, PCBs were used in electric, hydraulic and heat transfer equipment (e.g. transformers, capacitors and hydraulic fluids) (Erickson 1997). They were also used as flame retardants and additives in pesticides, plastics and paints (Erickson 1997).

Manufacture and importation of PCBs were forbidden in the European Community in 1985, when prohibition of their commercial use was approved due to their high toxicity, suspected carcinogenicity and environmental persistence (EC 1985). The cumulative global production of PCBs has been estimated to be over 1.3 million tons from 1930 to 1993 (Breivik et al. 2002), when Sovol manufacture ceased in Russia, presumably ending worldwide manufacture of PCBs (AMAP 2000). Monsanto Industrial Chemicals Co. was the responsible for almost 50% of the known reported historical production (Breivik et al. 2002). Although the commercial manufacture and use of PCBs was banned, leakage from old equipment, building materials, stockpiles and landfill sites continues to constitute a threat of PCB emissions (Bignert et al. 1998). For instance, according to the national Portuguese inventory, these compounds can

still be found in old equipment in use, such as air conditioning, capacitors, transformers, induction coils and rectifiers (APA 2010). Furthermore, PCBs may also be unintentionally formed as by-products of a variety of chemical processes (Erdal et al. 2008; Mansour 2009).

Physicochemical properties of PCDD/Fs and PCBs

The knowledge on physical and chemical properties of compounds is essential for understanding and predicting their mobility, degradation and accumulation potential in the environment. Vapor pressure, water solubility, octanol-water partition coefficient (K_{OW}) and organic carbon-water partition coefficient (K_{OC}) are some of the key properties that affect PCDD/F and PCB environmental behavior (Li et al. 2003; Åberg et al. 2008).

PCDD/Fs and PCBs comprise a large number of congeners that differ substantially in their physicochemical characteristics according to the degree and position of chlorine substitutes. Moreover, since different methodologies are used to determine PCDD/F and PCB characteristics, the measured values reported in the literature can vary widely (Mackay et al. 2006). As an illustration, a compilation of key physicochemical properties of 2,3,7,8-TCDD, the most potent congener within PCDD/Fs and PCBs, is given in Table 1.

In general, PCDD/Fs and PCBs have low vapor pressure and very low solubility in water, and their volatility and aqueous solubility change with molecular size, namely, they tend to decrease with an increase in the number of chlorine substituents (Li et al. 2003). Moreover, values for PCB compounds are typically one to two orders of magnitude greater than the equivalent chlorinated PCDD/F congeners (Mackay et al. 2006). Additionally, within PCB homologue groups, congeners with chlorine substitutions in the *ortho* positions have higher vapor pressure, i.e., the more planar PCB congeners have a lower volatility (IPCS 2003; Li et al. 2003). The Henry's law constant (K_H) combines the properties of vapor pressure and water solubility of an individual compound and estimates its distribution between gaseous and aqueous phase (Åberg et al. 2008). PCDD/Fs and PCBs generally have quite low K_H values, reflecting their low volatility. Nevertheless, as the number of *ortho*-chlorine substitutions increases in PCB congeners, decreasing their planarity, the K_H increases (Li et al. 2003).

The octanol-water partition coefficient (K_{OW}) provides information on how an organic chemical will partition between aqueous and organic phases. Considering the extensive range of values found for this parameter, K_{OW} is normally expressed as its logarithm (van

Table 1 Physicochemical properties of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD).

Physicochemical property		Reference
Molecular weight (g mol^{-1})	**321.971**	Mackay et al. 2006
Melting point (°C)	**295**	Lide 2003
Boiling point (°C)	**421.2**	Schroy et al. 1985
Vapor pressure (Pa, 25 °C or as indicated)	**8.14×10^{-8} – 6.17×10^{-4}**	
	4.50×10^{-6} (gas saturation GC)	Rordorf 1985a
	4.61×10^{-7} (30.1°C, gas saturation GC/MS)	Schroy et al. 1985
	9.87×10^{-8} (^{14}C-gas saturation method)	Podoll et al. 1986
	8.14×10^{-8} (20 °C, solid vapor pressure)	Bidleman and Foreman 1987
	6.00×10^{-5} (20 °C, supercooled liquid pressure)	Bidleman and Foreman 1987
	6.17×10^{-4} (supercooled liquid pressure)	Passivirta et al. 1999
	5.25×10^{-4} (supercooled liquid pressure)	Harner et al. 2000
	2.57×10^{-5} (GC-RI correlation)	Wang and Wong 2002
	5.75×10^{-5} (SOFA method)	Wang and Wong 2002
	5.56×10^{-7} (gas saturation GC/MS)	Mader and Pankow 2003
Water solubility (mg l^{-1}, 25 °C or as indicated)	**1.29×10^{-5} – 1.58×10^{-2}**	
	2.0×10^{-4} (shake flask-GC/ECD)	Crummett and Stehl 1973
	3.17×10^{-4} (generator column HPLC/LSC)	Webster et al. 1983
	1.93×10^{-5} (22 °C, shake flask GC/MS)	Marple et al. 1986a
	1.29×10^{-5} (4.3 °C, generator column GC/MS)	Lodge 1989
	4.83×10^{-4} (17.3 °C, generator column GC/MS)	Lodge 1989
	1.58×10^{-2} (GC-RI correlation)	Wang and Wong 2002
Henry's law constant (Pa m^3 mol^{-1}, 25 °C or as indicated)	**0.12 – 1.64**	
	1.64 (calculated)	Podoll et al. 1986
	1.62 (SOFA method)	Govers and Krop 1998
	1.12 (GC-RI correlation)	Wang and Wong 2002
	1.62 (SOFA method)	Wang and Wong 2002
Octanol-water partition coefficient, express as log K_{OW}	**6.42 – 7.06**	
	6.64	Marple et al. 1986b
	6.42	Sijm et al. 1989a
	6.80	Mackay et al. 1992a
	7.02	Sangster 1993
	6.53	Hansch et al. 1995
	7.06	Wang and Wong 2002
	6.67	Åberg et al. 2008
Organic carbon-water partition coefficient, express as log K_{OC}	**6.14 – 7.59**	
	7.39 – 7.58	Jackson et al. 1986
	6.60	Walters and Guiseppi-Elie 1988
	7.25 – 7.59	Lodge and Cook 1989
	6.14	Jury et al. 1990
	6.80	Broman et al. 1991

Leeuwen and Vermeire 2007). This partition coefficient is widely used in risk assessment to predict the distribution of hydrophobic organic chemicals between aqueous and organic media due to its relation with water solubility, soil/sediment adsorption coefficients and bioconcentration factors for aquatic life (Kenaga 1980; Meylan et al. 1996). The log K_{OW} values are generally inversely related to aqueous solubility and increase with chlorine content (Chen et al. 2001; Wang and Wong 2002). PCDD/Fs and PCBs are referred to as highly hydrophobic because of their great log K_{OW} values, i.e., greater than 6. Behavior and bioavailability of PCDD/Fs and PCBs in the environment are also determined

by their distribution between solid and liquid phases. The organic carbon-water partition coefficient (K_{OC}) may be used, for example, to estimate the adsorption partition coefficient that describes the distribution of contaminants between suspended sediment and the water column (Dueri et al. 2008). Compounds with low water solubility and high adsorption coefficient such PCDD/Fs and PCBs occur in aquatic systems predominantly adsorb to particulate matter (Gotz et al. 1994).

Toxicity and effects of PCDD/Fs and PCBs

The toxicity of each PCDD/F congener is highly dependent on the position of the chlorine substituents (van den Berg et al. 1994). Their toxic action is mediated through an interaction between the congeners and the intracellular aryl hydrocarbon receptor (AhR), which is present in most vertebrate tissue (Okey 2007). The AhR has high affinity for 2,3,7,8-substituted PCDD/Fs, and thus, once in cells, these congeners bind to the receptor and activate it. As a result, the abnormal AhR activation may disrupt the cell function by changing the transcription of genes, causing an extensive range of toxic effects (Poland et al. 1985). Of the 210 possible PCDD/F congeners, there are 7 PCDDs and 10 PCDFs with chlorine atoms in the 2, 3, 7 and 8 positions and hence those are the 17 PCDD/Fs considered of toxicological concern (Poland et al. 1985). Furthermore, due to their chlorine substitution pattern, 12 PCBs can easily adopt a planar conformation similar to PCDD/Fs and consequently bind to the AhR (Safe et al. 1985). These 12 coplanar congeners are called dioxin-like PCBs (dl-PCBs) because they exhibit the same mode of toxicological action as PCDD/Fs (Poland et al. 1985). PCDD/Fs and PCBs are found in the environment as complex congener mixtures, complicating the risk evaluation for animals and humans (Safe 1990). Therefore, the concept of toxic equivalency factors (TEFs) was developed and introduced to facilitate the risk assessment and regulatory control of exposure to these mixtures (e.g Barnes 1991; Ahlborg et al. 1992; Safe 1990; van den Berg et al. 1998). Different TEF systems were established for PCDD/Fs and dl-PCBs, but since the early 90's, the World Health Organization (WHO) has organized expert meetings with the purpose of harmonize the TEFs on the international level. It was proposed that to be included in the WHO-TEF scheme a compound should: 1) show structural relationship to PCDD/Fs; 2) bind to the AhR; 3) elicit AhR-mediated biochemical and toxic responses; and 4) be persistent and accumulate in the food chain (van den Berg et al. 1998, 2006). The WHO scheme assigned individual TEFs to each 2,3,7,8-substituted

PCDD/F and dl-PCB congener using the 2,3,7,8-TCDD as a reference (TEF = 1) (van den Berg et al. 1998, 2006).

The sensitivity of species that possess the AhR to dioxin-like toxicity varies greatly between taxonomic classes (van den Berg et al. 1998). Further, the relative sensitivity of organisms is not constant across chemical groups. For instance, fish are generally quite sensitive to 2,3,7,8-substituted PCDD/Fs, as are birds and mammals (Clemons et al. 1994; Richter et al. 1997). However they are very insensitive to mono-*ortho* PCBs, while these PCBs are toxic to birds and mammals (van der Weiden et al. 1994; Newsted et al. 1995). Therefore, there was a need to develop separated sets of TEFs for each class. In 1998, the WHO derived consensus TEFs for fish, wildlife and human risk assessment (van den Berg et al. 1998) (Table 2). The WHO-TEFs for fish and birds were determined based essentially on concentrations of chemicals measured in organism tissues. In contrast, the mammalian WHO-TEFs are largely based on data associated with the administered dose. For that reason, in order to obtain a more accurate estimation of toxicological risks, mammalian TEFs should be applied to dietary exposures rather than to concentration in tissues (van den Berg et al. 2006). In the latest re-evaluation of the WHO-TEF system, in 2005, the human/mammalian TEFs established in 1998 were modified (van den Berg et al. 2006) (Table 2). Presently, there is not definitive evidence of the presence of AhR in amphibians and reptiles to propose TEFs for these groups (Hahn et al. 1998; van den Berg et al. 1998). Moreover, since invertebrates are considered to be insensitive to 2,3,7,8-TCDD-induced toxicity, the TEF methodology is not applicable to them (West et al. 1997; Hahn et al. 1998; Barber et al. 1998; van den Berg et al. 1998).

Additivity is a fundamental assumption of the TEF concept (van den Berg et al. 1998, 2006). The total toxic equivalent (TEQ) of a given mixture of PCDD/F and dl-PCB congeners express its dioxin-like toxicity and can be calculated as the sum of the products of the concentration of each compound multiplied by its individual TEF, as shown by the following equation:

$$\mathrm{TEQ}=\sum_{n=1}^{7}(PCDD_n \times TEF_n)+\sum_{p=1}^{10}(PCDF_p \times TEF_p)+\sum_{q=1}^{12}(PCB_q \times TEF_q)$$

where, $PCDD_n$, $PCDF_p$ and PCB_q represent the concentration of *n* PCDD, *p* PCDF and *q* dl-PCB congeners present in the mixture under analysis, and $TEF_{n,p,q}$ are the TEF for each individual PCDD, PCDF, and dl-PCB congener, respectively.

Table 2 Toxicity equivalency factors (TEFs) assigned for 2,3,7,8-substituted PCDD/F and dioxin-like PCB congeners (van den Berg et al. 1998, 2006).

Compound		WHO-TEF$_{1998}$			WHO-TEF$_{2005}$
		Fish	Birds	Humans/mammals	Humans/mammals
Polychlorinated dibenzo-*p*-dioxins					
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	2,3,7,8-TCDD	1	1	1	1
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin	1,2,3,7,8-PeCDD	1	1	1	1
1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin	1,2,3,4,7,8-HxCDD	0.5	0.05	0.1	0.1
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin	1,2,3,6,7,8-HxCDD	0.01	0.01	0.1	0.1
1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin	1,2,3,7,8,9-HxCDD	0.01	0.1	0.1	0.1
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin	1,2,3,4,6,7,8-HpCDD	0.001	<0.001	0.01	0.01
1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin	OCDD	<0.0001	0.0001	0.0001	0.0003
Polychlorinated dibenzofurans					
2,3,7,8-Tetrachlorodibenzofuran	2,3,7,8-TCDF	0.05	1	0.1	0.1
1,2,3,7,8-Pentachlorodibenzofuran	1,2,3,7,8-PeCDF	0.05	0.1	0.05	0.03
2,3,4,7,8-Pentachlorodibenzofuran	2,3,4,7,8-PeCDF	0.5	1	0.5	0.3
1,2,3,4,7,8-Hexachlorodibenzofuran	1,2,3,4,7,8-HxCDF	0.1	0.1	0.1	0.1
1,2,3,6,7,8-Hexachlorodibenzofuran	1,2,3,6,7,8-HxCDF	0.1	0.1	0.1	0.1
1,2,3,7,8,9-Hexachlorodibenzofuran	1,2,3,7,8,9-HxCDF	0.1	0.1	0.1	0.1
2,3,4,6,7,8-Hexachlorodibenzofuran	2,3,4,6,7,8-HxCDF	0.1	0.1	0.1	0.1
1,2,3,4,6,7,8-Heptachlorodibenzofuran	1,2,3,4,6,7,8-HpCDF	0.01	0.01	0.01	0.01
1,2,3,4,7,8,9-Heptachlorodibenzofuran	1,2,3,4,7,8,9-HpCDF	0.01	0.01	0.01	0.01
1,2,3,4,6,7,8,9-Octachlorodibenzofuran	OCDF	<0.0001	0.0001	0.0001	0.0003
Non-*ortho* polychlorinated biphenys					
3,3',4,4'-Tetrachlorobiphenyl	PCB 77	0.0001	0.05	0.0001	0.0001
3,3',4,5-Tetrachlorobiphenyl	PCB 81	0.0005	0.1	0.0001	0.0003
3,3',4,4',5-Pentachlorobiphenyl	PCB 126	0.005	0.1	0.1	0.1
3,3',4,4',5,5'-Hexachlorobiphenyl	PCB 169	0.00005	0.001	0.01	0.03
Mono-*ortho* polychlorinated biphenys					
2,3,3',4,4-Pentachlorobiphenyl	PCB 105	<0.000005	0.0001	0.0001	0.00003
2,3,4,4',5-Pentachlorobiphenyl	PCB 114	<0.000005	0.0001	0.0005	0.00003
2,3',4,4',5-Pentachlorobiphenyl	PCB 118	<0.000005	0.00001	0.0001	0.00003
2',3,4,4',5-Pentachlorobiphenyl	PCB 123	<0.000005	0.00001	0.0001	0.00003
2,3,3',4,4',5-Hexachlorobiphenyl	PCB 156	<0.000005	0.0001	0.0005	0.00003
2,3,3',4,4',5'-Hexachlorobiphenyl	PCB 157	<0.000005	0.0001	0.0005	0.00003
2,3',4,4',5,5'-Hexachlorobiphenyl	PCB 167	<0.000005	0.00001	0.00001	0.00003
2,3,3',4,4',5,5'-Heptachlorobiphenyl	PCB 189	<0.000005	0.00001	0.0001	0.00003

The TEF concept was primarily developed to calculate the potential toxic effects associated with the exposure to dioxin-like compounds using concentrations in either the tissues of organisms being assessed or their food (van den Berg et al. 1998). However, the WHO expert panel recognized that is currently common practice to use TEQ concentration directly to characterize contamination of abiotic environmental samples by 2,3,7,8-substituted PCDD/Fs and dl-PCBs, and to evaluate their toxicity to biota (van den Berg et al. 1998). In fact, the TEF scheme was even adopted in national and international legislation. For example, the Canadian Council of Ministers of the Environment (CCME) established sediment quality guidelines for PCDD/Fs and PCBs expressed as WHO-TEQ $_{fish}$ (CCME 2001).

PCDD/Fs and PCBs have been classified as human carcinogens (IARC 1997; Lauby-Secretan et al. 2013). Besides being carcinogenic, a broad spectrum of adverse effects has also been reported in animals and humans, including endocrine disruption actions (Longnecker and

Michalek 2000; Pavuk et al. 2003), neurological dysfunctions (Guo et al. 2003; Kakeyama and Tohyama, 2003), immunosuppression (Weisglas-Kuperus et al. 2000; Ten Tusscher et al. 2003), dermal toxicity (Guo et al. 1999; Schecter et al. 2006), teratogenicity and reproductive disorders (Guo et al. 2003; Miettinen et al. 2004; Balabanič et al. 2011). Nevertheless, toxicological effects are not only congener dependent, as mentioned before, but may also differ according to species and gender, in part as a result of differences in body fat composition and metabolism (Geyer et al. 1990; Roeder et al. 1998).

Fate of PCDD/Fs and PCBs in the aquatic environment

Once released in the environment, POPs can be transported over short and long distances, and consequently are distributed in different media and biological systems (air, water, sediment, soil, plants, animals and humans) (Mackay and Paterson 1991). Their entry in the aquatic environment may occur through various pathways. For instance, direct industrial and municipal effluent discharges, riverine and groundwater transport, runoff from land and atmospheric deposition have been regarded as possible sources of PCDD/Fs and PCBs to estuarine systems (Schwarzbauer 2006; Castro-Jiménez et al. 2008; Addison et al. 2005). In the aquatic environment, 2,3,7,8-substituted PCDD/Fs and dl-PCBs adsorb to organic matter due to their very low water solubility and high degree of hydrophobicity (i.e., log K_{ow} > 6), becoming readily associated with suspended material and ultimately settling in bottom sediment (Lohmann and Jones 1998; Dueri et al. 2008). Therefore, measurement of their concentrations in water is very difficult, in particular for PCDD/Fs because they are present in the environment in much smaller amounts than PCBs (Rose et al. 1994). Since strong regulations on the use and release of these chemicals have been imposed worldwide, their input into the environment has decreased significantly. Nevertheless, large amounts of PCDD/Fs and PCBs are still accumulated in aquatic sediment and are continuously dispersed to organisms living in constant association with this sediment. This contamination can continue for extended time periods, and it is believed that the secondary sources, such as contaminated sediment, can become the major source of PCDD/Fs and PCBs as the releases from primary sources decrease (Weber et al. 2008).

PCDD/Fs and PCBs retained in sediment may be taken up by benthic organisms through absorption from pore-water via dermal surfaces or through dietary absorption after ingestion of contaminated particles (Shaw and Connell 1987; Forbes et al. 1998). In the

case of hydrophobic compounds the uptake from ingestion is the more significant route of contamination (Thomann et al. 1992). Differences in feeding strategies (e.g. deposit-feeding or suspension-feeding) may also affect the bioaccumulation of these chemicals (McLeod et al. 2008). The 2,3,7,8-substituted PCDD/Fs and coplanar PCBs have the potential to bioaccumulate and biomagnify along the food web because of their high lipophilic nature and resistance to metabolism (Fisk et al. 2003; Wan et al. 2005).

Estuaries are crucial habitats in the life history of many species providing food, shelter and nursery areas. Therefore, through bioaccumulation and biomagnification processes, the presence of PCDD/Fs and dl-PCBs in the estuarine environment can represent a threat not only to biodiversity and ecosystem function but also to human health. In fact, with the exception of specific cases of accidental or occupational exposure, general population is exposed to POPs mainly through diet, being fish and fishery products the main contributors to total dietary intake of PCDD/Fs and dl-PCBs (EFSA 2012; Caspersen et al. 2013). In order to protect the consumers' health and reduce human intake to levels below the tolerable weekly intake of 14 pg WHO-TEQ kg^{-1} body weight established by the European Union Scientific Committee on Food (2001), new maximum levels of PCDD/Fs and dl-PCBs in fish and fishery products (EC 2011) were settled.

General objectives

Despite their ecological and economical importance, estuaries receive and retain numerous chemical contaminants. For that reason, the characterization of levels and distribution of toxic compounds in these ecosystems is essential to identify possible risks to human and ecological health. Nevertheless, the presence of PCDD/Fs and PCBs in estuarine ecosystems has been poorly studied in Portugal. In this context, the purpose of the thesis was to gain an insight into the contamination of Portuguese estuaries by POPs. The occurrence and distribution of 2,3,7,8-substituted PCDD/Fs and dl-PCBs was investigated in different estuarine compartments and the results obtained are described in four chapters (Chapter II – Chapter V).

Chapter II reports the contamination profiles of the target compounds in superficial sediments collected from seven estuaries along the Portuguese coast (Lima, Ria de Aveiro, Mondego, Tejo, Sado, Mira e Ria Formosa). The results are compared with available sediment guidelines and quality standards, together with concentrations found in sediments from

estuarine systems around the world.

Chapter III focused on two plant species growing in a contaminated salt marsh from the Tejo estuary (Portugal). Accordingly, potential differences in PCDD/F and dl-PCB concentrations between plant tissues (roots, stems and roots) are explored, and the accumulation capability of *Sarcocornia perennis* and *Halimione portulacoides* is compared. In addition, the relation between congener/homologue profiles of sediments and plant tissues is investigated in order to understand the incorporation mechanism of these compounds in *S. perennis* and *H. portulacoides.*

Chapter IV investigates the early contamination of different populations of European flounder (*Platichthys flesus*) to persistent organic pollutants. A comparison is made between PCDD/F and PCB levels and profiles in juveniles along the species' geographical distribution range in the northeastern Atlantic.

Chapter V presents an overview of the PCDD/F and dl-PCB contamination in the Mondego estuary (Portugal). Levels and profiles are described in sediment and key-species representative of different trophic levels of temperate estuarine systems. Moreover, in the context of human consumption, concentrations detected in fish and bivalves are compared with maximum limit values established by the European Commission (EC).

At last, chapter VI summarizes the main outcomes of this thesis and identifies future research needs.

CHAPTER II

Occurrence of PCDD/Fs and dioxin-like PCBs in superficial sediment of Portuguese estuaries

Abstract

Superficial sediments collected from seven estuarine systems located along the Portuguese coast were analyzed for 7 polychlorinated dibenzo-*p*-dioxins (PCDDs), 10 polychlorinated dibenzofurans (PCDFs) and 12 dioxin-like polychlorinated biphenyls (dl-PCBs). Total PCDD/F concentration ranged from 4.6 to 463.9 pg g^{-1} dry weight (dw), while those of dl-PCBs varied from 26.6 to 8698.2 pg g^{-1} dw. In general, the highest PCDD/F and dl-PCB concentrations were associated with densely populated and industrially impacted areas. Additionally, PCDD/F and dl-PCB profiles revealed a predominance of OCDD to total PCDD/Fs, while PCB 118 was the major contributor to total dl-PCBs.

This study provided a global perspective of the contamination status of Portuguese estuaries by dioxin-like compounds and allowed a comparison between the investigated systems and others worldwide. PCDD/F and dl-PCB levels found in the collected sediments were lower than those of highly impacted areas from different parts of the globe. Nevertheless, comparison with guidelines and quality standards from other countries indicated that some Portuguese estuarine areas with a high industrialization level present PCDD/F and dl-PCB concentrations in superficial sediment that may constitute a risk to aquatic organisms.

Keywords

PCDD/Fs; PCBs; persistent organic pollutants; sediment; estuary; Portugal

Introduction

Dioxin-like compounds such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) were classified as priority persistent organic pollutants (POPs) at the Stockholm Convention (UNEP 2001). Due to their chemical stability and hydrophobicity, these compounds have been detected globally in various compartments of the environment including air, water, sediment and biota (He et al. 2006; Castro-Jiménez et al. 2008). PCDD/Fs have never been intentionally manufactured, and are generally released in the environment as unwanted by-products resulting from thermal processes (Weber et al. 2008). As for PCBs, they were synthesized and produced for commercial and industrial purposes but, despite an almost worldwide ban on PCBs production and usage, these compounds continue to be released from old equipment, landfills and contaminated soil and sediment (Breivik et al. 2007; Davis et al. 2007).

The ecological and socioeconomic value of estuarine systems is unquestionable. These ecosystems are highly productive and play an important role in the life history of many species, serving as nursery grounds, feeding and migration routes (Doi et al. 2005; Dolbeth et al. 2008; Martinho et al. 2009). Nevertheless, the presence of dioxin-like contaminants in estuarine systems has been highlighted in several reports, particularly in sediments (Davis et al. 2007; Castro-Jiménez et al. 2008). Due to their low solubility in water and high octanol–water partition coefficients (K_{OW}), in aquatic environments PCDD/Fs and PCBs quickly become associated with particulate matter, and eventually end up in bottom sediments (Dueri et al. 2008). Consequently, estuarine sediments constitute a major repository for PCDD/Fs and PCBs, and a source of potential exposure to organisms living in or having direct contact with them (Guerzoni et al. 2007). Once present in the food web, these compounds can bioaccumulate and be transferred to higher trophic levels, thus representing a potentially significant hazard to aquatic ecosystems and ultimately affect human health (van der Oost et al. 2003; Weber et al. 2008).

The occurrence of PCDD/Fs and dl-PCBs in estuaries has been poorly studied in Portugal. Therefore, it is important to assess sediment contamination by dioxin-like compounds for better management and protection of these valuable coastal ecosystems. The present study investigated the PCDD/F and dl-PCB concentrations and profiles in superficial sediments to ascertain the contamination status in estuarine systems along the Portuguese coast. In addition, a comparison of PCDD/F and dl-PCB values with different sediment quality guidelines (SQG) is included to evaluate the potential risk posed by the dioxin-like

contaminants in the studied sediments to aquatic life.

Material and methods

Sampling and preparation of samples

Surface sediments (0-10 cm) were collected between January and February 2011 in seven estuarine systems distributed along the Portuguese coast: (1) Lima estuary, (2) Ria de Aveiro, (3) Mondego estuary, (4) Tejo estuary, (5) Sado estuary, (6) Mira estuary and (7) Ria Formosa (Fig. 1).

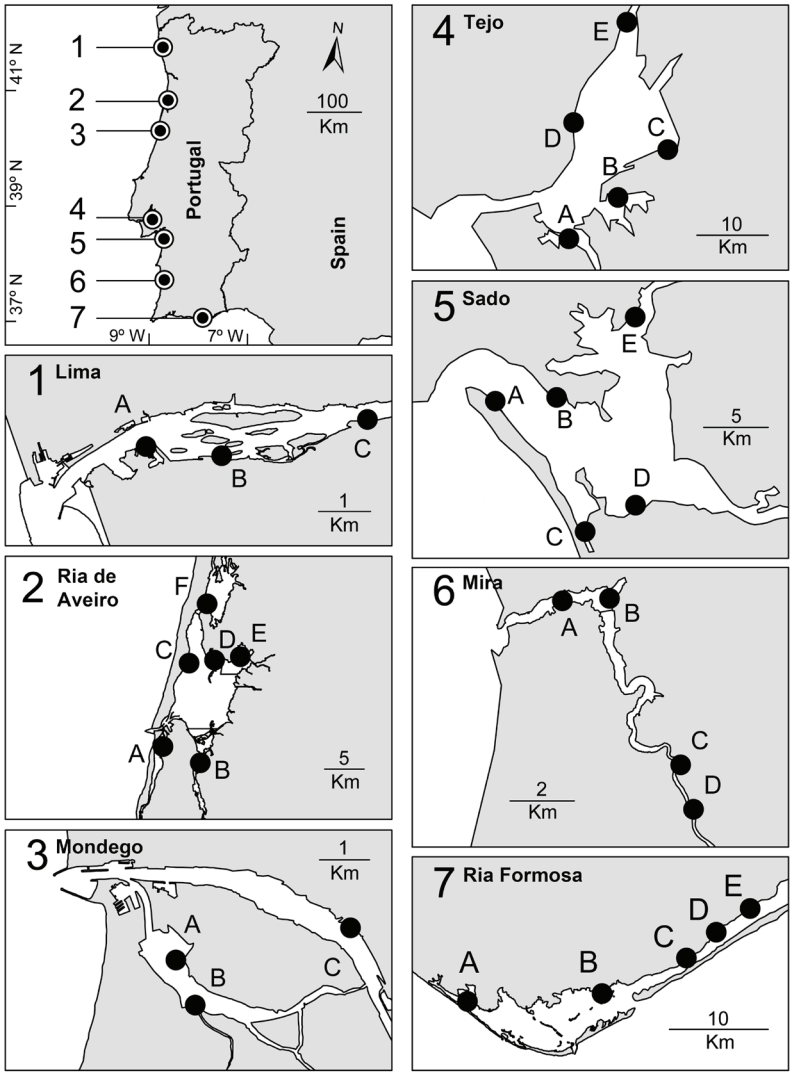

Figure 1 Location of the Portuguese estuarine systems studied and respective sampling sites.

Sampling was conducted in the intertidal mudflats during low tide. To obtain more representative samples of the sediment contamination within a site, six individual samples were collected using a stainless steel scoop at each location and pooled into two to five composite samples. An additional composite sample was also taken for determination of total organic carbon (TOC) and fine fraction (< 63 μm). Prior to PCDD/F and dl-PCB analysis, samples were thoroughly homogenized after removing pebbles, shells and twigs, oven-dried and ground. TOC content in sediments was quantified using a CHN analyzer (Carlo Erba Instruments) and grain size analysis was performed according to Brown and McLachland (1990) classification method.

PCDD/Fs and dl-PCBs analysis

The toxicity of the 210 possible PCDD/F and 209 PCB congeners varies widely, depending on the number and position of chlorine atoms within the molecules. Of these, only 17 PCDD/Fs and 12 PCBs that were assigned toxic equivalency factors (TEFs) by the World Health Organization (WHO) (van den Berg et al. 1998, 2006) were analyzed.

Detailed descriptions of extraction and cleanup procedures can be found elsewhere (e.g. Costera et al. 2006). Briefly, sediments were extracted in a Pressurized Liquid Extraction system (ASE, Dionex, Sunnyvale, CA, USA) followed by three successive static extraction cycles using a mixture of toluene/acetone 70:30 (v/v) at 100 bar and 120 °C. Finally, extracts were purified by sequential multilayered silica gel, Florisil and carbon chromatographic columns.

Identification and quantification of PCDD/Fs and dl-PCBs were performed by gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS) using a Hewlett–Packard 6890 gas chromatograph (Palo Alto, CA, USA) equipped with a DB-5MS column and coupled to a JEOL JMS-800D double sector mass spectrometer (Tokyo, Japan). The HRMS was operated in electron ionization mode at 38-40 eV and the ion source temperature was set to 280 °C. All target compounds were quantified using the isotope-dilution method.

All profile determinations were undertaken at LABERCA, the French National Reference Laboratory in charge of PCDD/Fs and PCBs analysis in food and feed. The procedure integrated the quality assurance parameters to fulfill the requirements of the European legislation laying down sampling procedures and the method of analysis for determination of PCDD/Fs and dl-PCBs (EC 2006). Procedural blanks were included in every series of samples and did not contain quantifiable amounts of any target compounds. The chromatographic separation

was checked (<25% peak to peak between 1,2,3,4,7,8-HxCDF and 1,2,2,6,7,8-HxCDF) and recoveries of individual congeners were within 30-140% as required by the EC regulation 1883/2006. The limits of detection (LOD) ranged from 0.001 to 0.020 $pg.g^{-1}$ of dry weight (dw) for PCDD/Fs and 0.036 to 0.080 $pg.g^{-1}$ dw for dl-PCBs.

Data analysis

For comparison with other studies, concentrations of PCDD/Fs and dl-PCBs in sediments are reported on a dry weight basis. Pearson's correlation analysis was performed to assess the correlations between TOC content and fine particles (< 63 m) with PCDD/Fs and dl-PCBs in sediment samples. These statistical analyses were performed using SigmaStat (Systat Software Inc., California, USA).

To evaluate the potential risk posed to aquatic organisms exposed to PCDD/Fs and dl-PCBs present in the sediment, toxic equivalent (TEQ) concentrations were calculated based on TEFs values for fish derived by the WHO in 1998 ($WHO\text{-}TEQ_{fish}$; van den Berg et al. 1998). In addition, since some SQGs available are expressed in TEQ based on human TEFs ($WHO\text{-}TEQ_{2005}$), those values are also presented (van den Berg et al. 2006).

Results and Discussion

Sediment characteristics

Properties such as grain size and TOC may play a significant role in controlling hydrophobic organic contaminants levels in sediments (Hung et al. 2010). As expected from sampling in natural deposition areas, silt and clay (< 63 µm) accounted for 22 to 58% of superficial sediment samples (data available in Appendix A). The TOC content of the analyzed sediments ranged from 1.4 to 6.1% (data available in Appendix A). The significant positive correlation ($r = 0.80$, $p < 0.001$) found between percentage of fine particles and TOC content reflects the larger surface area of fine-grained sediments, and thus the greater amount of organic carbon that can be adsorbed (Lee et al. 2006). Nevertheless, in the present study no significant correlation was observed between sediment characteristics and dioxin-like contaminants, contrarily to the expectation (fine content and PCDD/Fs: $r = 0.31$, $p > 0.05$; fine content and dl-PCBs: $r = 0.35$, $p > 0.05$; TOC and PCDD/Fs: $r = 0.37$, $p > 0.05$; TOC and dl-PCBs: $r = 0.39$, $p > 0.05$). The existence of different contamination levels among sampling sites with similar sediment characteristics can explain the absence of correlation between

both variables.

PCDD/F and dl-PCB concentrations

All collected samples exhibited detectable concentrations of the 17 PCDD/F and 12 dl-PCB congeners analyzed (data available in Appendix A), indicating their ubiquity in the Portuguese estuaries. Our sediment samples showed lower PCDD/F levels than those of dl-PCBs, except in site A from Ria Formosa (Fig. 2).

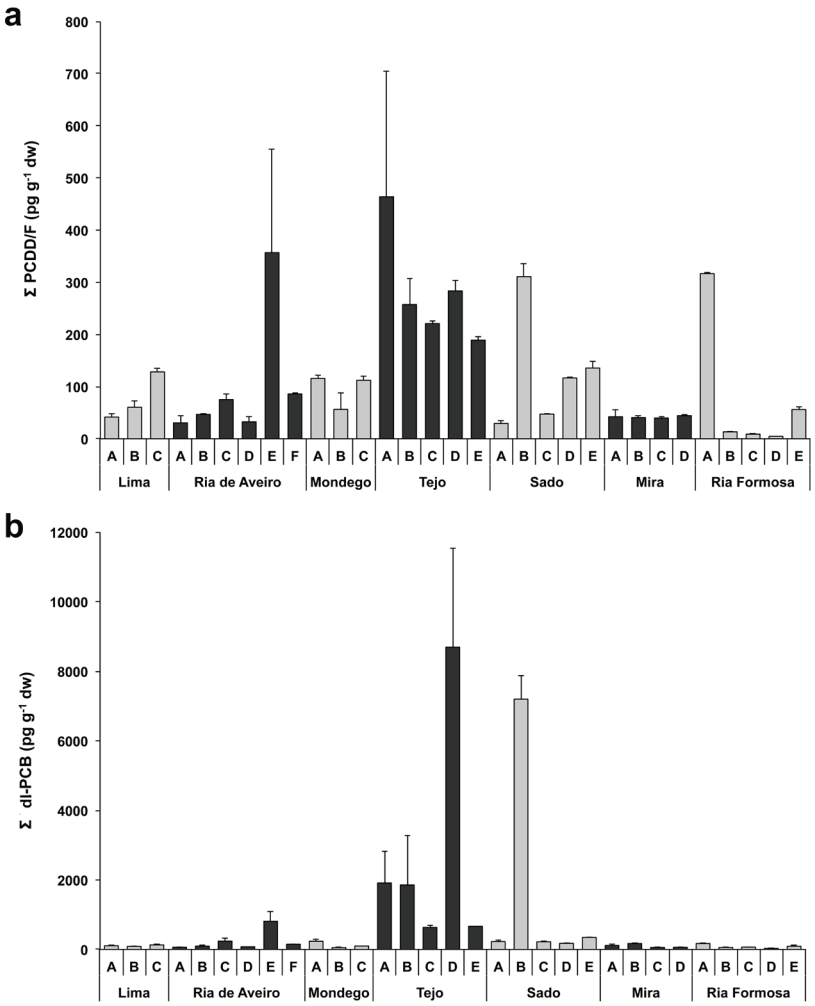

Figure 2 Total concentration of (**a**) 2,3,7,8-substituted PCDD/Fs and (**b**) dioxin-like PCBs in superficial sediments from seven Portuguese estuarine systems (pg g^{-1} dw). Results are expressed as the mean + standard deviation.

In general, dioxin-like compounds were found at higher concentrations in points near large populated areas and industrial complexes, whereas their lowest values were

measured in less impacted areas (Table 1). Accordingly, the lowest PCDD/F and dl-PCB total concentrations were found in Ria Formosa, a natural protected area with status of Ramsar site. On the contrary, Tejo estuary, one of the largest in Europe, displayed the most elevated ΣPCDD/F and Σdl-PCB values in this study (Fig. 2). The widespread distribution of these contaminants within Tejo is most likely due to domestic effluents from the metropolitan

Table 1 Total concentration of 2,3,7,8-substituted PCDD/Fs and dioxin-like PCBs (pg g^{-1} dw) and WHO-TEQ concentration based on TEFs for fish (WHO-TEQ_{fish}; pg TEQ g^{-1} dw) and for humans (WHO-TEQ_{2005}; pg TEQ g^{-1} dw) in superficial sediment from seven Portuguese estuarine systems. Results are expressed as the mean ± standard deviation.

Location		ΣPCDD/Fs [a]	Σdl-PCBs [b]	WHO-TEQ $_{fish}$	WHO-TEQ $_{2005}$
1. Lima	**A**	41.74 ± 5.92	105.60 ± 22.71	0.30 ± 0.05	0.43 ± 0.06
	B	60.56 ± 11.29	88.71 ± 13.60	0.42 ± 0.04	0.63 ± 0.07
	C	128.18 ± 6.89	129.48 ± 30.14	0.74 ± 0.19	1.14 ± 0.25
2. Ria de Aveiro	**A**	30.59 ± 13.07	62.93 ± 16.75	0.28 ± 0.16	0.35 ± 0.20
	B	46.74 ± 1.94	97.88 ± 14.47	0.37 ± 0.04	0.53 ± 0.06
	C	75.22 ± 10.06	238.59 ± 98.37	0.87 ± 0.10 [c]	1.17 ± 0.001
	D	32.45 ± 10.51	75.92 ± 3.97	0.39 ± 0.11	0.54 ± 0.14
	E	356.81 ± 197.70	811.66 ± 287.58	3.71 ± 1.27 [c]	4.37 ± 1.25 [d]
	F	86.10 ± 1.87	150.34 ± 5.79	0.75 ± 0.01	1.05 ± 0.04
3. Mondego	**A**	115.77 ± 5.39	234.98 ± 55.35	0.74 ± 0.03	1.07 ± 0.09
	B	56.34 ± 31.87	51.69 ± 29.36	0.32 ± 0.17	0.49 ± 0.15
	C	112.22 ± 7.08	99.99 ± 5.75	0.58 ± 0.03	0.85 ± 0.01
4. Tejo	**A**	463.93 ± 241.40	1911.97 ± 898.25	4.06 ± 1.13 [c]	4.74 ± 0.280 [d]
	B	257.34 ± 49.47	1856.13 ± 1406.65	3.57 ± 2.00 [c]	4.23 ± 1.094 [d]
	C	220.98 ± 5.26	632.95 ± 53.00	3.62 ± 0.21 [c]	4.62. ± 0.251 [d]
	D	283.52 ± 19.77	8692.76 ± 2854.38	3.61 ± 0.68 [c]	5.57 ± 1.014 [d]
	E	189.04 ± 6.51	666.29 ± 10.29	2.17 ± 0.01 [c]	2.54 ± 0.021 [d]
5. Sado	**A**	29.35 ± 6.36	224.82 ± 29.97	0.70 ± 0.02	1.10 ± 0.11
	B	310.87 ± 24.43	7197.41 ± 671.31	3.49 ± 0.04 [c]	11.07 ± 0.72 [d, e]
	C	47.39 ± 0.75	219.52 ± 15.80	1.14 ± 0.12 [c]	1.59 ± 0.21
	D	116.59 ± 2.01	175.71 ± 3.01	3.03 ± 0.02 [c]	3.38 ± 0.002 [d]
	E	135.74 ± 12.12	347.27 ± 19.23	4.74 ± 0.46 [c]	4.99 ± 0.45 [d]
6. Mira	**A**	42.29 ± 12.77	115.12 ± 39.43	1.01 ± 0.36 [c]	1.16 ± 0.33
	B	40.62 ± 3.37	171.57 ± 6.88	0.58 ± 0.09	0.79 ± 0.10
	C	39.77 ± 2.86	56.80 ± 4.44	0.37 ± 0.09	0.51 ± 0.10
	D	44.45 ± 2.32	61.43 ± 10.71	0.38 ± 0.04	0.54 ± 0.04
7. Ria Formosa	**A**	316.57 ± 2.93	170.37 ± 13.85	1.27 ± 0.11 [c]	1.84 ± 0.45
	B	13.31 ± 0.13	54.61 ± 19.03	0.17 ± 0.02	0.31 ± 0.04
	C	8.83 ± 1.00	66.40 ± 7.61	0.10 ± 0.01	0.15 ± 0.02
	D	4.64 ± 0.05	26.59 ± 15.85	0.09 ± 0.01	0.14 ± 0.02
	E	56.15 ± 4.97	92.08 ± 45.91	0.27 ± 0.01	0.46 ± 0.07

[a] PCDD/Fs = 2,3,7,8-TCDD + 1,2,3,7,8-PeCDD + 1,2,3,4,7,8-HxCDD + 1,2,3,6,7,8-HxCDD + 1,2,3,7,8,9-HxCDD + 1,2,3,4,6,7,8-HpCDD + OCDD + 2,3,7,8-TCDF + 1,2,3,7,8-PeCDF + 2,3,4,7,8-PeCDF + 1,2,3,4,7,8-HxCDF + 1,2,3,6,7,8-HxCDF + 2,3,4,6,7,8-HxCDF + 1,2,3,7,8,9-HxCDF + 1,2,3,4,6,7,8-HpCDF + 1,2,3,4,7,8,9-HpCDF + OCDF

[b] dl-PCBs = PCB 77 + PCB 81 + PCB 126 + PCB 189 + PCB 105 + PCB 114 + PCB 118 + PCB 123 + PCB 156 + PCB 157+ PCB 167+ PCB 189

[c] Value above the threshold effect level proposed for Canada (0.85 pg TEQ_{fish} g^{-1} dw; CCME 2001)

[d] Value above the Italian EQS (2 pg TEQ_{2005} g^{-1} dw; Decreto Legislativo 219/2010)

[e] Value above the background level established for Norway (10 pg TEQ_{2005} g^{-1} dw; NEA 2011)

area of Lisbon and to the several industries scattered all over the region (e.g. chemicals, petrochemicals, metallurgic industries, shipyards and cement manufacturing) (Canário et al. 2007). In particular, the prominent Σdl-PCB concentrations found in site D can be the result of former uncontrolled industrial activities such as paper and chemical manufacture (Pinheiro et al. 1999). The second most PCDD/F contaminated site was located in Ria de Aveiro (site E). Elevated concentrations of toxic metals have already been identified in sediments from this area (Monterroso et al. 2007; Nunes et al. 2008; Cardoso et al. 2013) as a consequence of indiscriminate discharges of industrial effluents from a chemical complex for several decades. Considering that conventional chlor-alkali procedure released elevated levels of PCDD/Fs from the electrolysis process (Xu et al. 2000), the concentrations found there may be related to the past discharges from the chlor-alkali plant. In Ria de Aveiro, the heterogeneous concentrations of dioxin-like compounds within the system may hence be indicative of local sources of PCDD/Fs and dl-PCBs. The same pattern was observed in other estuaries. In the Sado estuary, site B is in the vicinity of a pulp mill and is characterized by high anthropogenic pressure combined with low hydrodynamics (Caeiro et al. 2005), which can explain the high dioxin-like compound concentrations. The elevated ΣPCDD/F values detected in sediments from site A of Ria Formosa also suggest that a significant source exists (or existed) in the area, although no anthropogenic cause was found nearby.

PCDD/F and dl-PCB profiles

Different PCDD/F homologue profiles can be found among the studied estuaries (Fig. 3a). OCDD was always the major contributor, ranging from 44 to 92%, in agreement with most results of sediment investigation in other parts of the world (Ren et al. 2009; Naile et al. 2011). This typical predominance of OCDD may be a result of its higher stability in the environment compared to other congeners (Sinkkonen and Paasivirta 2000). Its lower water solubility and greater affinity to fine particles may lead to long-term accumulation, particularly in organic rich sediment (Sinkkonen and Paasivirta 2000). The hepta-CDDs, and octa- and hepta-CDFs are the following most abundant homologues.

The remarkable high contribution of OCDD in samples from site A of Ria Formosa together with its high concentration in these sediments, show strong similarities to other specific sites worldwide (e.g. Müller et al. 2002). Natural formation processes have been previously considered a possible source resulting in high OCDD concentrations found in American ball clay and in kaolin clay from Germany and Spain (Rappe et al. 2001), in sediments from Queensland area (Gaus et al. 2001) and in mudflats of the Mai Po Marshes Nature Reserve

in Hong Kong (Müller et al. 2002). To the best of our knowledge, there is no anthropogenic source of OCDD near the site A of Ria Formosa, suggesting that its geological history and environmental conditions may favor processes that result or have resulted in the formation of OCDD.

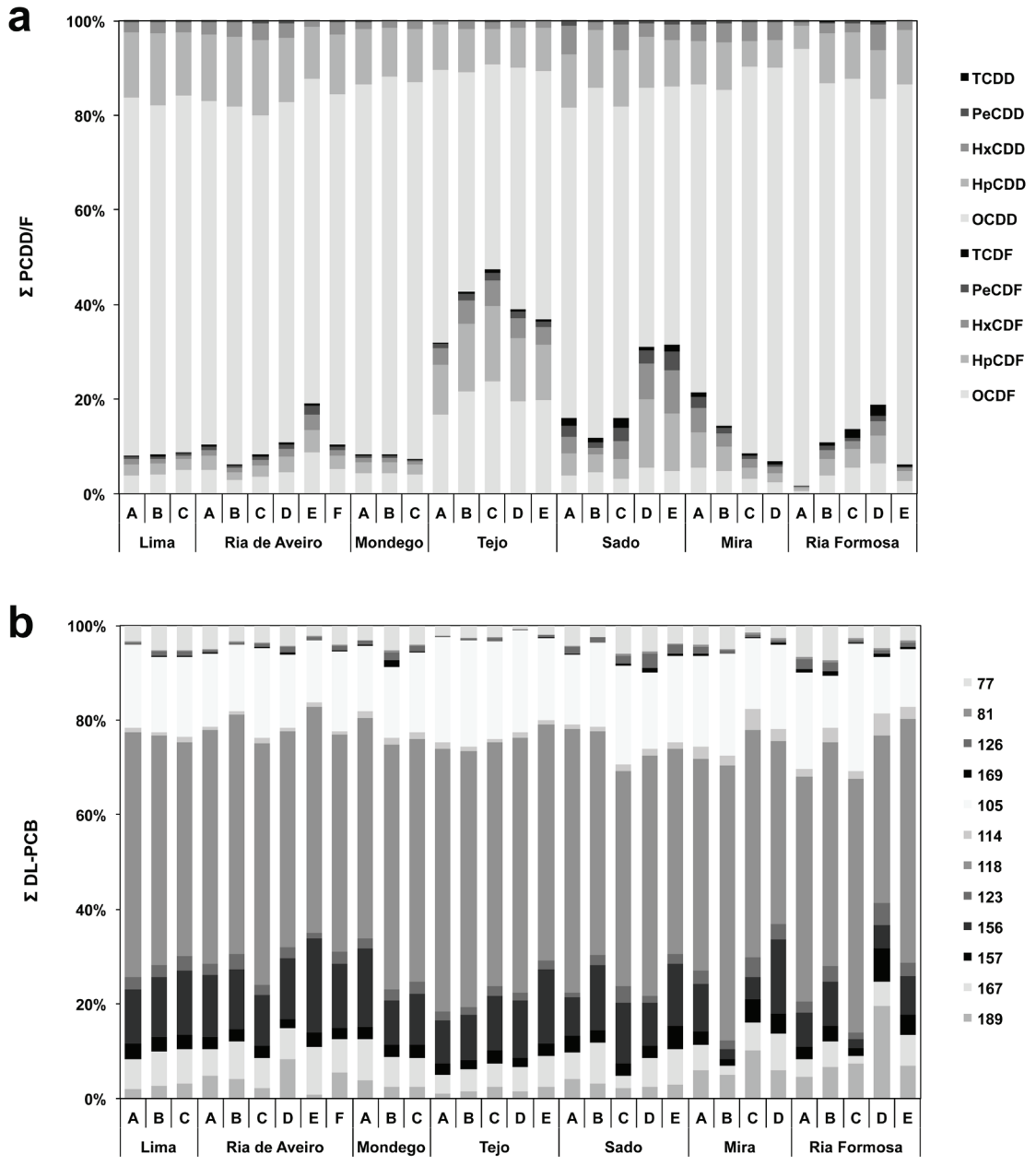

Figure 3 Homologue and congener profiles of (**a**) 2,3,7,8-substituted PCDD/Fs and (**b**) dioxin-like PCBs in superficial sediments from seven Portuguese estuarine systems. Results are expressed as percentage of tota§l concentration of 2,3,7,8-substituted PCDD/Fs or dioxin-like PCBs.

A global view of Fig. 3a shows that, in general, estuaries north of Tejo have similar PCDD/F profiles. This spatial homogeneity of the profiles indicates possible similar PCDD/F origins between estuaries and underlines the absence of relevant local sources (with exception of site E of Ria de Aveiro). Furthermore, considerably higher PCDF percentages, in particular higher chlorinated PCDFs, were detected in samples collected in the Tejo estuary. This estuarine system was also the one that showed the highest overall PCDF concentrations. Higher proportion of PCDFs in sediments has been described in other regions affected by anthropogenic contamination from industrial and urban areas (Terauchi et al. 2009; Antunes et al. 2012). The remaining estuarine systems show slightly different PCDD/F homologue profiles among sampling sites. This heterogeneity between profiles suggests that sites located in the same estuary have either different sources of PCDD/Fs or that they have similar origins but underwent advanced differential decomposition.

As for dl-PCBs, PCB 118, followed by 105 and 156 were the most abundant congeners (Fig. 3b). The predominance of PCB 118, which in the analyzed sediments represented 35 to 58% of Σdl-PCB, has been reported in various matrices in the environment (El-Kady et al. 2007; Okay et al. 2009). Although dl-PCB concentrations differed from site to site, the congener profiles for the superficial sediments were fairly similar among the studied estuaries.

WHO-TEQ concentration and ecotoxicological concern

WHO-TEQ_{fish} concentration in estuarine sediments ranged from 0.09 ± 0.01 to 4.74 ± 0.46 pg TEQ_{fish} g^{-1} dw (Fig. 4). The maximum WHO-TEQ_{fish} values were recorded at site E of Sado estuary, while the lowest were found in Ria Formosa. The low contribution of PCBs to total WHO-TEQ_{fish} concentration is explained by the low PCB TEFs established for fish. According to van den Berg et al. (1998), fish are generally quite sensitive to PCDD/F toxicity, as are birds and mammals, but are very insensitive to mono-*ortho* PCBs.

Contaminated sediments may constitute a particular threat for aquatic organisms. Therefore, sediment quality guidelines (SQGs) have been developed and implemented by regulatory authorities in order to evaluate ecotoxicological risks and predict adverse biological effects of sediment-associated pollutants on aquatic organisms. In the absence of environmental assessment criteria for these contaminants in Portugal, the data obtained in the present study were compared to SQGs proposed for Canada, Italy and Norway (CCME 2001; Decreto Legislativo 219/2010; NEA 2011). Based on Canadian guidelines, adverse biological effects would rarely be observed at WHO-TEQ_{fish} concentrations below the threshold effect level (TEL; 0.85 pg TEQ_{fish} g^{-1} dw) whereas concentrations above the probable effect level (PEL;

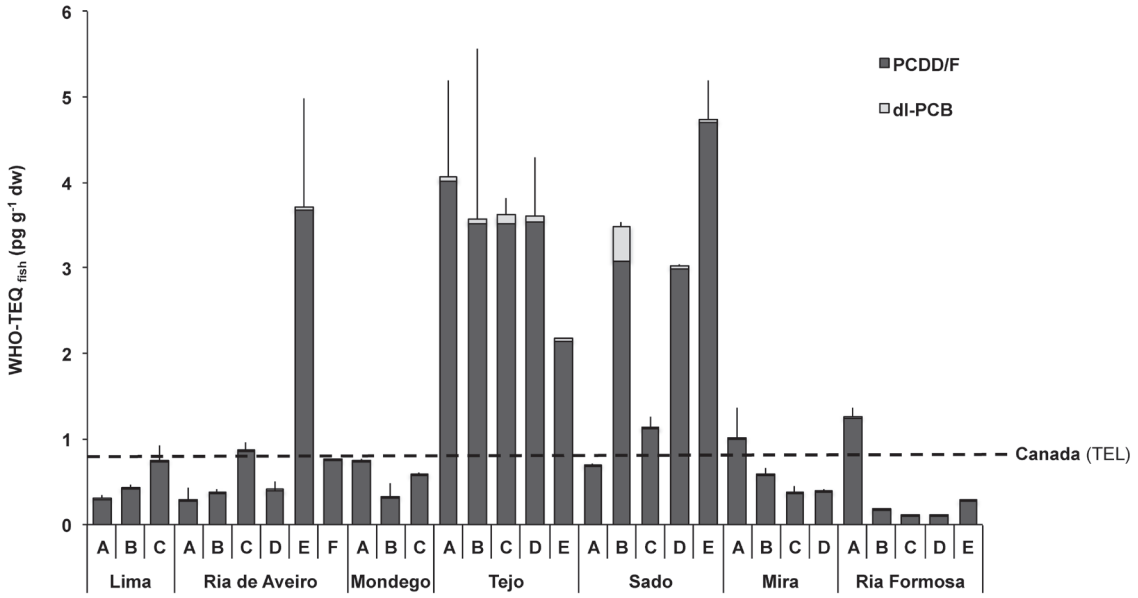

Figure 4 WHO-TEQ_{fish} concentration (pg TEQ g^{-1} dw) in superficial sediments from seven Portuguese estuarine systems based on TEFs for fish. Results are expressed as the mean + standard deviation. Sediment quality guideline proposed for Canada is also represented (- - -) (CCME 2001).

21.5 pg TEQ_{fish} g^{-1} dw) are expected to cause adverse effects on aquatic biota (CCME 2001). The Italian legislation establishes an environmental quality standard with a recommended exposure limit of 2 pg TEQ_{2005} g^{-1} dw in order to assure a good chemical status of the aquatic environment (based on human TEFs; Decreto Legislativo 219/2010).

In Norway, a national guideline for classification of environmental quality in coastal waters and fiords divides sediment quality into five classes, from the background level to very bad quality (background level < 10 pg TEQ_{2005} g^{-1} dw; NEA 2011). Although the TEQ concept is not directly applicable to abiotic matrices, it has been very useful to evaluate the toxicity of environmental samples, including sediment (van den Berg et al. 2006; Yang et al. 2009).

The comparison of the dioxin-like compound concentrations obtained in this study against the previously mentioned guidelines showed that more than 40% of the sampling sites exceeded the strictest guideline from Canada (TEL) (Fig. 4). This percentage decreased to 29% when comparing the WHO-TEQ_{2005} values with the Italian quality standard (Table 1). Most of these sites are mainly located in Tejo and Sado estuaries, systems clearly impacted by several anthropogenic activities (Table 1). Nevertheless, only site B of Sado estuary showed WHO-TEQ_{2005} concentration higher than the upper limit of the background class established for Norway (10 pg TEQ_{2005} g^{-1} dw), and none of the studied sites exceed the PEL value of the Canadian guidelines (21.5 pg TEQ_{fish} g^{-1} dw) (Table 1). Although many of the sampling sites

had low WHO-TEQ concentrations in superficial sediments, the highest values found in the studied Portuguese estuaries exceeded some of the available thresholds, indicating that adverse biological effects on aquatic organisms may occur due to the presence of PCDD/Fs and dl-PCBs in sediments.

Global comparison of PCDD/Fs and dl-PCBs in sediments

The characterization of sediment contamination by dioxin-like compounds presented in this study allows a comparison between Portuguese estuaries and coastal ecosystems from other parts of the globe. Although data on PCDD/F and PCB levels in sediment are quite numerous, different sampling and analytical procedures together with different ways of reporting data make the comparison difficult. Moreover, dl-PCBs are not so frequently analyzed due to their low concentration and ease of co-elution with other congeners. In spite of these difficulties, levels of PCDD/Fs and dl-PCBs in sediments from the studied Portuguese estuaries were found to be lower than those observed in various highly anthropogenic impacted locations as for example, Venice lagoon in Italy (Bellucci et al. 2000), Houston Ship Channel in the USA (Suarez et al. 2006), Haihe estuary in China (Liu et al. 2007) and Port Jackson in Australia (Birch et al. 2007) (Table 2). Additionally, concentrations observed in part of our studied sites (e.g. Ria Formosa) are comparable to less disturbed estuarine and coastal areas including Santoña estuary in Spain (Gómez-Lavín et al. 2011), St. Lawrence estuary in Canada (Brochu et al. 1995), coastal lagoons of Nador and Moulay Bousselham in Morocco (Piazza et al. 2009), Changjiang estuary in China (Wen et al. 2008) and Torrens estuary in Australia (Birch et al. 2007) (Table 2).

Conclusions

In the light of our findings, the following conclusions can be drawn. PCDD/Fs and dl-PCBs were detected in all the analyzed samples, showing their ubiquity in sediments from Portuguese estuaries. Both ΣPCDD/F and Σdl-PCB concentrations were found to be variable not only among estuaries, reflecting the different degrees of urbanization and industrialization of the studied estuarine systems, but also within each estuary, suggesting the existence of local contamination sources. Furthermore, samples collected in the most highly contaminated system, the Tejo estuary, revealed a different PCDD/F homologue profile.

The data obtained in this study provide a global perspective of contamination of Portuguese

Table 2 Total and WHO-TEQ concentrations (pg g^{-1} dw) of PCDD/Fs and dioxin-like PCBs in superficial sediment from various locations around the world.

Location		ΣPCDD/Fs	ΣPCBs	WHO-TEQ$_{1988}$			WHO-TEQ$_{2005}$			Reference
				PCDD/Fs	PCBs	Total	PCDD/Fs	PCBs	Total	
Europe										
Portugal, estuarine systems (Lima, Ria de Aveiro, Mondego, Tejo, Sado, Mira and Ria Formosa)		4.6 - 634.6	16.0 - 11278.8	0.1 - 6.1	<0.1 - 9.0	0.1 - 12.7	0.1 - 5.3	< 0.1 - 8.2	0.1 - 11.6	This study
Spain, coastal area	Industrial outflow	1696 - 3831	3225 - 9877	5.3 - 21.1	1.0 - 62.5	10.7 - 74.9	5.3 - 20.6	0.3 - 61.6	10.2 - 74.0	Eljarrat et al. 2005
	Harbor	22 - 187	1773 - 8703	3.1 - 12.2	0.2 - 3.7	3.6 - 14.1	3.0 - 11.9	0.1 - 2.9	3.3 - 12.9	
	Rivers' mouth	72 - 30007	125 - 12058	0.1 - 48.3	0.2 - 5.5	0.3 - 48.3	0.1 - 50.5	0.1 - 5.4	0.2 - 51.1	
Spain, Cantabria region	Tina Menor	1.19 - 3.99	309.6 - 467.6	0.04 - 0.16	0.15 - 0.30	0.19 - 0.46	0.03 - 0.14	0.12 - 0.27	0.16 - 0.40	Gómez-Lavín et al. 2011
	Mogro	0.15 - 1.52	595.1 - 691.4	<0.001 - 0.09	0.08 - 0.21	0.08 - 0.30	<0.001 - 0.08	0.03 - 0.14	0.03 - 0.22	
	Santoña	1.22 - 2.28	138.6 - 172.1	0.04 - 0.10	0.09 - 0.13	0.17 – 0.19	0.04 - 0.09	0.08 - 0.12	0.16 – 0.17	
Italy, Venice Lagoon	Salt marshes	$65x10^3$ - $18x10^4$	-	500 – 4400	-	-	-	-	-	Bellucci et al. 2000
	Central lagoon	$24x10^4$ - $23x10^5$	-	1900 – $34x10^3$	-	-	-	-	-	
	Industrial canals	$66x10^4$ - $14x10^7$	-	$23x10^2$ - $29x10^5$	-	-	-	-	-	
France, Thau Lagoon		153.3 - 1656.1	-	2.9 - 13.8	-	-	2.5 - 12.7	-	-	Castro-Jiménez et al. 2008
English Channel and southern **North Sea**		0.1 - 3.2	-	0.1 - 1.2	-	-	0.1 – 1.0	-	-	Danis et al. 2006
UK, estuarine systems: Tees, Thames and Firth of Forth Estuary		-	-	0.41 - 18.3	4.64 - 4.84	3.42 - 18.3	-	-	-	Hurst et al. 2004
Finland, Kymijoki River Estuary and Gulf of Finland		577 - 35500	-	14 - 214	-	-	13 - 216	-	-	Salo et al. 2008
America										
Canada, St. Lawrence Estuary		68.8 - 94.9	-	0.3 - 4.9	-	-	0.3 - 4.2	-	-	Brochu et al. 1995
USA, Pensacola Bay System		19.8 - 60.83	21.3 - 23.54	-	-	-	0.2 - 129.3	< 0.1 - 21.7	0.3 - 145.5	Liebens et al. 2011
USA, Florida Panhandle Bay System		-	-	0.5 - 77.5	-	-	-	-	-	Hemming et al. 2002
USA, Houston Ship Channel		2737 - 6502	-	17.5 - 32.5	-	-	17.6 - 32.6	-	-	Suarez et al. 2006
Trinidad and Tobago, Sea Lots	Coastal area	32	-	0.5	-	-	0.4	-	-	Mohammed et al. 2009
	Harbor	230 - 389	-	2.4 - 7.0	-	-	2.2 - 6.3	-	-	
Africa										
Morocco, Nador lagoon and Moulay Bousselham lagoon		-	-	-	0.004 - 0.04	-	-	-	-	Piazza et al. 2009
Morocco, Tangier Port, Larache Port and Kenitra Port		-	-	-	0.04 - 2.7	-	-	-	-	Piazza et al. 2009
Kuwait	Reference area	0.4 - 4	-	-	-	-	0.2	-	-	Gevao et al. 2009
	Industrial area	13 - 314	-	-	-	-	0.2 - 3.7	-	-	

Table 2 (*Continued*)

Location		ΣPCDD/Fs	ΣPCBs	WHO-TEQ$_{1988}$			WHO-TEQ$_{2005}$			Reference
				PCDD/Fs	PCBs	Total	PCDD/Fs	PCBs	Total	
Asia										
Japan, Toyano Lagoon		370 - 54000	-	0.5 – 76.0	-	-	-	-	-	Sakai et al. 2008
Japan, Tokyo Bay				3.1 - 49	0.2 - 3.0	3.3 - 52.0	-	-	-	Hosomi et al. 2003
South Korea, industrialized bays		-	-	-	-	-	1.2 - 7.2	0.1 - 5.4	1.3 - 10.8	Moon et al. 2008
South Korea, Masan Bay		720 - 4684	-	18.7 - 248.4	-	-	16.8 - 222	-	-	Hong et al. 2009
South Korea, Gwangyang Bay		1178 - 384	14 - 220	1.0 - 4.2	0.1 - 0.2	1.1 - 4.4	1.0 - 4.0	0.1	1.1 - 4.1	Kim et al. 2008
South Korea	Industrial areas	-	-	3.0 - 90	0.7 - 2.9	4.6 - 92.9	-	-	-	Terauchi et al. 2009
	Non-industrial areas	-	-	2.3 - 2.8	0.05 - 0.07	2.4 - 2.9	-	-	-	
China and **South Korea**, Yellow Sea		35 - 81	-	0.1 – 4.1	-	-	0.1 – 4.0	-	-	Naile et al. 2011
China, Nanpaiwu River Estuary		$12x10^4$ - $33x10^5$	$49x10^2$ - $19x10^4$	549 - 21361	21 - 1135	569 - 22496	559 - $21x10^3$	30 - 1619	589 - $23x10^3$	Hu et al. 2005
China, Bohai Bay		448	31	2.5	0.1	2.6	2.5	0.2	2.7	Hu et al. 2005
China, Haihe River Estuary and Dagu Drainage River		$11x10^3$ - $53x10^4$	69 - 3154	19 - 893	0.5 - 21	19.5 - 914	20 - 975	0.5 - 21	21 - 996	Liu et al. 2007
China, Kaifaqu and Yongdingxin River Estuary		177 - 317.6	13 - 24	1.7 - 8.2	0.08 - 0.09	1.8 - 8.3	1.7 - 2.7	0.10 - 0.11	1.8 - 2.8	Liu et al. 2007
China, Changjiang River Estuary		26 - 343	-	0.4 - 1.4	-	-	0.4 - 1.4	-	-	Wen et al. 2008
Hong Kong, Pearl River Estuary	Mai Po Marshes Nature Reserve	4439 - 9404	-	6.9 - 9.0	-	-	7.8 - 10.4	-	-	Müller et al. 2002
	Victoria Harbor	7642 - 10997	-	16.2 - 27.7	-	-	16.7 - 27.5	-	-	
Vietnam, Saigon River Estuary	Estuarine area	410 - 688	287- 294	1.0 - 3.1	0.1 - 0.2	1.1 - 3.3	1.1 - 3.2	0.1 - 0.1	1.1 - 3.3	Shiozaki et al. 2009
	Coastal area	182 - 397	21 - 394	0.8 - 2.2	0.1	0.9 - 2.3	0.8 - 2.3	0.06 - 0.12	0.9 - 2.4	
Vietnam, coastal lagoons (Dam Nai, Lang Co, Nuoc Man, Nuoc Ngot, O Loan, Truong Giang, Thuy Trieu, Cam Ranh Bay, Thi Nai, and Tam Giang-Cau Hai)		-	-	-	-	-	0.3 - 5.2	-	-	Piazza et al. 2010
Oceania										
Australia, Port Jackson	Undeveloped areas	$45x10^3$ - $40x10^5$	-	26.1 - 29.6	-	-	41.2 - 48.9	-	-	Birch et al. 2007
	Industrial and urban areas	$60x10^3$ - $11x10^4$	-	46.9 - 3035	-	-	53.6 - 3785	-	-	
Australia, Darwin Port		-	-	0.9	0.001	0.9	-	-	-	Müeller et al. 2004
Australia, Brisbane Port		-	-	0.1 - 0.3	0.01 - 0.01	0.1 - 0.3	-	-	-	Müeller et al. 2004
Australia, Torrens River Estuary (Adelaide)		-	-	0.3	0.2	0.5	-	-	-	Müeller et al. 2004
Australia, Botany Bay (Sidney)		-	-	20 - 31	2.2 - 3.9	22 - 35	-	-	-	Müeller et al. 2004
Australia, Parramatta River Estuary (Sidney)		-	-	100 - 510	2.9 - 14	100 - 520	-	-	-	Müeller et al. 2004

estuaries by dioxin-like compounds and allow comparison with studies made in other countries. The sediments analyzed show PCDD/F and dl-PCB concentrations lower than those found in highly impacted areas from other parts of the world. However, some guidelines and quality standards defined for other countries are exceeded at sites located essentially in Tejo and Sado estuaries. Thus, in some Portuguese estuarine areas the PCDD/F and dl-PCB concentrations in superficial sediment may eventually constitute a risk to aquatic organisms. Nevertheless, because of their different values and definitions, the available guidelines and quality standards allowed just a rough evaluation whether the PCDD/F and dl-PCB concentrations detected in Portuguese estuarine sediments may be considered as safe or may constitute a risk to aquatic organisms. Hence, site-relevant or national SQG should be developed for Portuguese estuaries in order to take into account site-specific conditions (e.g. bioavailability, sensitivity of indigenous organisms, exposure pathways).

Acknowledgements

The authors would like to express their gratitude to Sandra Beilvert, Séverine Bouchaud, Aline Brosseaud, Frédéric Larvor, Emilie Noël-Chéry, Vincent Vaccher, Cyrille Vallée, Solenn Vincent and Zita Zendong for helping with the PCDD/F and PCB analysis. The manuscript was greatly improved by the comments of three anonymous reviewers and the handling editor. This study was supported by the FCT – Fundação para a Ciência e Tecnologia through a PhD grant attributed to MN Cardoso (SFRH/BD/46969/2008), co-funded by the POPH/FSE.

CHAPTER

III

Distribution of PCDD/Fs and dioxin-like PCBs in sediment and plants from a contaminated salt marsh (Tejo estuary, Portugal)

Abstract

Concentrations and profiles of 2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) were investigated in sediment and plants collected from a salt marsh in the Tejo estuary, Portugal. The highest PCDD/F and dl-PCB concentrations were detected in uncolonized sediments, averaging 325.2 ± 57.6 pg g^{-1} dry weight (dw) and 8146.3 ± 2142.1 pg g^{-1} dw, respectively. The plants *Sarcocornia perennis* and *Halimione portulacoides* growing in PCDD/F and dl-PCB contaminated sediments accumulated contaminants in both roots, stems and leaves. PCDD/F and dl-PCB concentrations in aboveground tissues were significantly lower in comparison with roots, suggesting that these contaminants are not substantially transported inside the studied plants. In general, concentration of ΣPCDD/Fs and Σdl-PCBs in *H. portulacoides* tissues were found to be two fold higher than those in *S. perennis*. Therefore, the results indicate that there is a difference in the accumulation capability of both species. Furthermore, congener profiles changed between sediments and plant tissues, reflecting a selective accumulation of low chlorinated PCDD/Fs and *non-ortho* dl-PCBs in plants.

Keywords

Persistent organic pollutants; PCDD/Fs; PCBs; sediment; halophytes; salt marsh; estuary

Introduction

Assessment of dioxin-like compound contamination in estuarine systems continues to be of concern all over the world due to their deleterious effects on ecosystem functions and human health (UNEP 2001). Chemical contaminants such as polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs) have the propensity to bioaccumulate in biota and biomagnify in food webs. They are also known to cause adverse effects including carcinogenicity, reproductive impairment and endocrine disruption in wildlife and humans (van den Berg et al. 2006; Wenning et al. 2011). Salt marshes are deposition areas of suspended particulate matter transported by tidal currents, and consequently act as sinks for sediment bound contaminants (Barra et al. 2004; Hwang et al. 2006). Since salt marsh plants make significant contributions to the detrital estuarine food web (Masters and Inman 2000; Wall et al. 2001; Sousa et al. 2010), the uptake of dioxin-like compounds accumulated in sediments may be responsible for a further transfer of pollutants into the food web.

Several studies have evaluated the ability of marsh vegetation to accumulate heavy metals (Válega et al. 2008; Castro et al. 2009), polycyclic aromatic hydrocarbons (Watts et al. 2006), organochlorine pesticides (Liu et al. 2006) and tributyltin (Carvalho et al. 2010). However, little information is available on PCDD/F and PCB contamination in salt marsh plants (Mrozek and Leidy 1981; Masters and Inman 2000). Moreover, plant uptake of PCDD/Fs and PCBs has been a controversial topic, with numerous authors considering that the hydrophobic nature of such compounds and the consequent strong adsorption on sediment particles rendered them less available to be up taken by roots (Wu et al. 2002; Schuhmacher et al. 2006; Uegaki et al. 2006). On the contrary, other researchers state that the primary pathway of PCDD/F and PCB contamination in plants is uptake from roots and possible translocation to aboveground parts (Engwall and Hjelm 2000; Zeeb et al. 2006; Greenwood et al. 2011). Thus, the objectives of this work were (1) to determine the concentration of 2,3,7,8-substituted PCDD/Fs and dl-PCBs in two plant species commonly found in temperate estuaries and associated sediments; (2) to compare the dioxin-like compound contents between plant species and between tissues (root, stems and leaves); and (3) to explore the relationship between PCDD/F and dl-PCB profiles of sediments and plant tissues.

Material and methods

Study site and sampling

Tejo estuary is the largest in the western European coast. It covers an area of about 320 km^2 and is considered the most impacted estuarine system in Portugal, as a result of different anthropogenic pressures arising from heavy industrialization and urbanization. The estuary has extensive intertidal mudflats with about 15% of the area covered by salt marshes, where *Sarcocornia perennis* (Caryophyllalles, Chenopodiaceae) and *Halimione portulacoides* (Caryophyllalles, Chenopodiaceae) are two of the most representative halophyte species found (Caçador and Duarte 2012). This study was carried out in a salt marsh located at the mouth of a polluted tributary, the Trancão River (38°79'N 9°09'W), that discharges directly into Tejo estuary (Fig. 1).

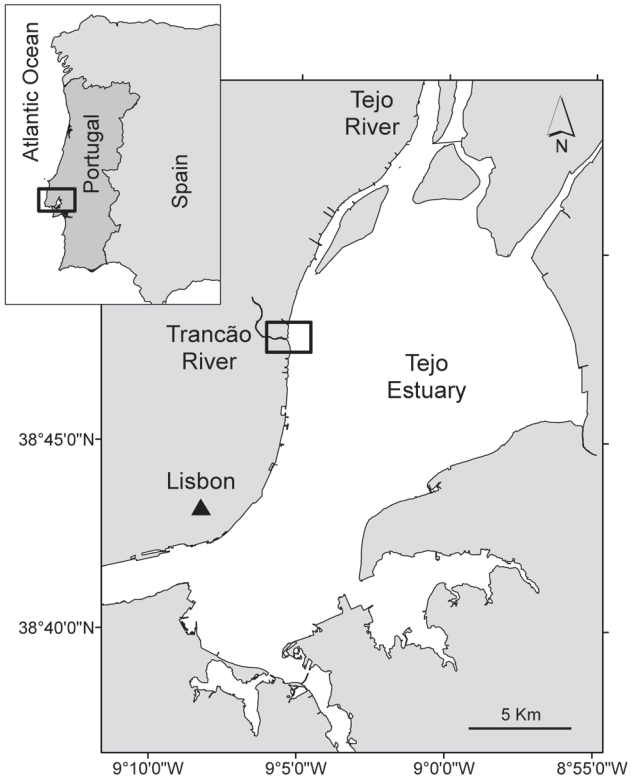

Figure 1 Tejo estuary and location of the sampling area.

Samples were randomly collected over an area of 500 m^2 in August 2011 during low tide. Five replicates of leaves, stems and belowground tissues were taken from each species (*S. perennis* and *H. portulacoides*), together with rhizosediment samples (sediment

surrounding plant roots and rhizomes). Since *S. perennis* does not have a true shoot system with leaves and stems, the swollen photosynthetic stems (referred to as leaves hereafter) were differentiated from the dry perennial shoots (referred to as stems hereafter). Aboveground material was harvested, and belowground material and rhizosediment were taken with a spade from the same place to a depth of 15 cm, where most roots and rhizome are present. Five composite sediment samples from the lower uncolonized intertidal area were also collected. Each sample consisted of sediment from 0 to 15 cm depth taken in three random points within 1 m^2. All samples were stored in aluminium foil and transported to the laboratory where they where kept in a fridge at 4 °C.

Sample preparation

Belowground plant parts were carefully separated from sediment under a flux of water using a 500 µm mesh size. Roots, stems and leaves were carefully rinsed with ultra-pure Milli-Q water to remove any adhering particles. Prior to PCDD/F and dl-PCB analysis, plant tissues were oven-dried at 60 °C until a constant weight was reached. The dry sample was then ground to a power using a grinding mill.

Similarly, sediment samples were oven-dried, cleaned of roots and debris, homogenized and ground using a pestle and mortar. Sediment samples were also analyzed for total organic carbon (TOC) and fine fraction (< 63 µm) content. TOC was determined using a Carlo Erba CHN analyzer and grain size analysis was performed according to the classification method of Brown and McLachland (1990).

PCDD/F and PCB analysis

The 17 PCDD/F congeners with chlorine substitution in the 2, 3, 7 and 8 positions and the 12 PCBs with dioxin-like activity (non-*ortho* PCBs 77, 81, 126 and 169, and mono-*ortho* PCBs 105, 114, 118, 123, 156, 157, 167 and 189) were considered for this purpose.

The analysis was performed as described by Costera et al. (2006). Samples were extracted in an Accelerated Solvent Extraction device (ASE 300, Dionex, Sunnyvale, USA), using as solvent a mixture composed of toluene and acetone at 70:30 (v/v) and with pressure and temperature set to 100 bar and 120 °C, respectively. The resultant extract were purified and fractionated in three successive chromatographic steps involving multilayered silica gel, Florisil and carbon columns. Analysis of clean extracts was conducted using a Hewlett–Packard 6890 gas chromatograph (Palo Alto, CA, USA) equipped with a high-resolution mass spectrometer. All target compounds were quantified using the isotope dilution method.

Samples were analyzed according to a validated and accredited method (ISO 17025:2005 standard). The procedure integrated the quality assurance and quality control criteria to fulfill the requirements of the European legislation laying down sampling procedures and the analysis methods for determination of PCDD/Fs and dl-PCBs (EC 2006). To ensure quality of our data, blanks were included in every series of samples to check for interference and cross-contamination. The recoveries of individual congeners were within 30 to 140% as required by the EC regulation 1883/2006. The limits of detection (LOD) ranged from 0.003 to 0.010 pg g^{-1} of dry weight (dw) for PCDD/Fs and from 0.009 to 0.062 pg g^{-1} dw for dl-PCBs.

Data analysis

The relationship between PCDD/F and dl-PCB concentrations and physical characteristics of sediment (TOC and fine fraction content) were tested using Pearson's correlation. Analysis of variance (one-way ANOVA) was performed to determine differences in sediment characteristics between colonized and uncolonized sediments, and to evaluate differences in PCDD/F and dl-PCB concentrations between sediments, and between tissues of the same plant species. Student's t-test was used to compare PCDD/F and dl-PCB concentrations between similar tissues of *S. perennis* and *H. portulacoides*. All statistical tests were performed using the software Statistica 8.0 (StatSoft Inc., USA). Principal component analysis (PCA) was used to further explore differences in the PCDD/F and PCB congener profiles between sediments and tissues of both plant species. Multivariate analysis was carried out using the software package CANOCO 4.5 (Microcomputer Power, USA).

Results and discussion

PCDD/F and dl-PCB concentrations in sediments

The highest PCDD/F and dl-PCB concentrations were detected in uncolonized sediments, averaging 325.2 ± 57.6 pg g^{-1} dw and 8146.3 ± 2142.1 pg g^{-1} dw, respectively (data available in Appendix B). *S. perennis* rhizosediments were found to have a total PCDD/F concentration (ΣPCDD/Fs) of 293.1 ± 39.0 pg g^{-1} dw and those of *H. portulacoides* had 294.9 ± 75.9 pg g^{-1} dw. Whereas, the total dl-PCB concentration (Σdl-PCBs) was 6404.6 ± 3781.6 pg g^{-1} dw in *S. perennis* rhizosediments and 5874.2 ± 3255.2 pg g^{-1} dw in those of *H. portulacoides*. The lower ΣPCDD/F and Σdl-PCB levels found in rhizosediments may be a result of the presence of salt marsh plants. However, the difference of ΣPCDD/F and Σdl-PCB concentrations

between colonized and uncolonized sediments was not statistically significant (H = 1.860 for PCDD/Fs; F = 0.512 for dl-PCBs, always with $p > 0.05$). Nevertheless, this result suggests that salt marsh plants may play some role in reducing dioxin-like compound concentration from contaminated sediments. Highly hydrophobic contaminants (octanol-water partition coefficient > 6) bind strongly to sediment particles and therefore, are not expected to be susceptible to plant uptake (Wu et al. 2002). However, a few studies reported uptake of PCDD/Fs and dl-PCBs by plants, considering that root exudates may be involved in a mechanism of solubilization of hydrophobic compounds, and consequently in enhanced desorption from soil and increased root uptake (Campanella and Paul 2000). Moreover, even when compounds are poorly taken up by plants, roots can interact with the surrounding sediment by different processes, stimulating the microbial activity in the rhizosphere and increasing compound degradation and/or removal. For example, plant exudates contain molecules that can be used by microorganisms as substrate or as factors inducing dehalogenation of chlorinated compounds (Chaudhry et al. 2005). Plants can also secrete enzymes into the rhizosphere that can initiate transformation of organic compounds and facilitate further microbial metabolism (Alkorta and Garbisu 2001). Furthermore, plants increase oxygen diffusion in the rhizosphere, which potentially enhances microbial oxidative transformation (Chaudhry et al. 2005).

Sediments were constituted mainly by silt and clay (< 63 µm), independently of the presence of plants. Nevertheless, uncolonized sediment had lower fined grain content compared with rhizosediments, which had more than 60% of total particle size inferior to 63 µm (data available in Appendix B). TOC content ranged from 3.0 ± 0.28% to 3.7 ± 0.42%. Rhizosediments of *S. perennis* and *H. portulacoides* contained higher percentage of fine particles (one-way ANOVA, F = 13.334, $p < 0.001$) and TOC than uncolonized sediments (one-way ANOVA, F = 4.158, $p < 0.05$). Since fine particles exhibit a large surface area available for adsorption of organic carbon (Lee et al. 2006), it is not surprising that a significant correlation (r = 0.869, $p < 0.001$) was found between fine fraction and TOC content in the analyzed sediments. However, no significant correlation between the referred sediment characteristics and dioxin-like contaminants was observed in the present study (fines and PCDD/Fs: r = 0.122; fines and dl-PCBs: r = 0.119; TOC and PCDD/Fs: r = 0.083; TOC and dl-PCBs: r = 0.479; always with $p > 0.05$).

PCDD/F and dl-PCB distribution in plant tissues

All target analytes were detected in the analyzed plant tissues (data available in Appendix

B). Mean ΣPCDD/F concentrations in *S. perennis* varied from 17.3 ± 1.6 pg g^{-1} dw in roots to 1.7 ± 0.1 pg g^{-1} dw in leaves. In *H. portulacoides* values ranged between 30.9 ± 11.9 pg g^{-1} dw in roots and 2.3 ± 0.5 pg g^{-1} dw in leaves (Fig. 2a). Mean Σdl-PCB concentrations in *S. perennis* varied between 971.6 ± 139.6 pg g^{-1} dw in roots and 159.1 ± 45.7 pg g^{-1} dw in stems; whereas in *H. portulacoides*, it ranged from 2253.7 ± 822.2 pg g^{-1} dw in roots to 247.7 ± 54.3 pg g^{-1} dw in stems (Fig. 2b). Due to the strong adsorption of PCDD/Fs and PCBs to fine-grained sediments rich in organic matter, a low bioavailability and subsequent low uptake by roots was expected for these highly hydrophobic compounds (Liu and Schnoor 2008). As a matter of fact, *S. perennis* root tissue showed only 6% of ΣPCDD/F concentration of the corresponding rhizosediment, and 15% of Σdl-PCBs. The ΣPCDD/F concentration on *H. portulacoides* roots was found to be 11% of the respective rhizosediment, while Σdl-PCB concentration was 38%. Moreover, the results indicate a minor root accumulation of PCDD/Fs compared to dl-PCBs.

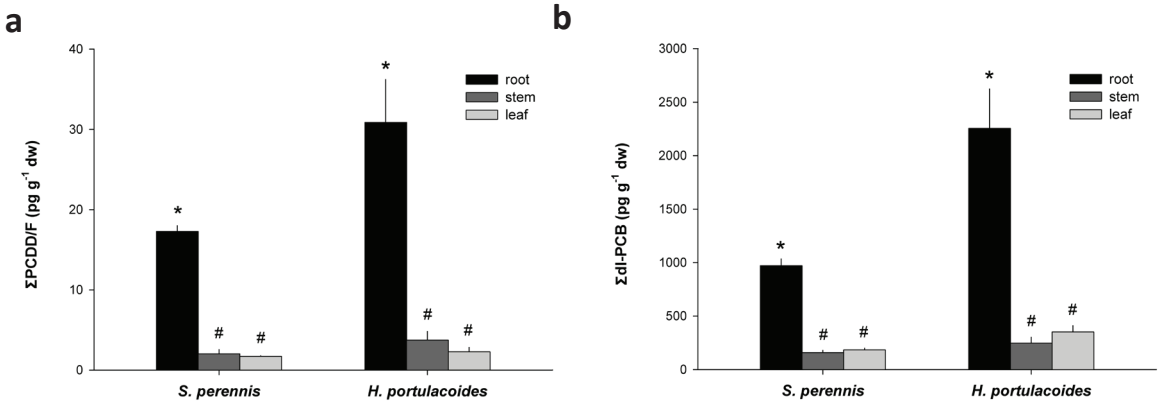

Figure 2 Total concentration of (**a**) 2,3,7,8-substituted PCDD/Fs and (**b**) dioxin-like PCBs in roots, stems and leaves of two plant species (*Sarcocornia perennis* and *Halimione portulacoides*) from the Tejo estuary (pg g^{-1} dw). Results are expressed as the mean + standard deviation (n=5). Different symbols above bars indicate statistically significant differences between tissues of the same species (one-way ANOVA, $p < 0.05$).

A similar partition of PCDD/F and PCB content was observed in *S. perennis* and *H. portulacoides* (Fig. 2a, b). In general, PCDD/F concentrations in tissues showed a declining gradient from roots >> stems > leaves, while dl-PCB values decreased in the order roots >> leaves > stems. In addition, both species accumulated significantly lower (one-way ANOVA, $F = 325.21$ for PCDD/Fs in *S. perennis*; $F = 139.83$ for PCBs in *S. perennis*; $F = 89.66$ for PCDD/Fs in *H. portulacoides*; $F = 108.23$ for PCBs in *H. portulacoides;* always with $p < 0.001$)

concentrations in the aboveground tissues in comparison with roots, suggesting that PCDD/Fs and dl-PCBs are not substantially transported inside *S. perennis* and *H. portulacoides*. In fact, dioxin-like compounds with a log octanol-water partition coefficient (log K_{OW}) higher than 5 have been reported to be insignificantly translocated within plants (Hülster and Marschner 1993; Liu and Schnoor 2008), with the exception of the species *Cucurbita pepo* (Zeeb et al. 2006; Greenwood et al. 2011).

The interpretation of PCDD/F and dl-PCB concentrations in stems and leaves is difficult since accumulation of contaminants in aboveground tissues may be attributed to a combination of translocation after root uptake, foliar uptake from air or by direct contact between sediment particles and plant surfaces (Smith and Jones 2000). Moreover, possible losses/transformation of compounds due to metabolism within plants cannot be excluded (Wild et al. 2005; Liu et al. 2009).

Previous studies showed that there is a remarkable diversity in uptake and translocation of organic contaminants, dependent not only on the specific properties of each compound but also on the characteristics of the plant species (Liu and Schnoor 2008; Matsuo et al. 2011). In the present study, the two species showed significantly different (t-test, $p < 0.05$) concentrations of dioxin-like compounds in analogous tissues (the only exception occurred for PCDD/Fs in leaves). ΣPCDD/F and Σdl-PCB concentrations in *H. portulacoides* tissues was found to be roughly twice as much as that in *S. perennis*, with exception of PCDD/Fs in leaves where values are only slightly higher in *H. portulacoides* (Fig. 2a, b). The different concentration levels found in belowground tissues may be caused by distinct type or amount of organic exudates from the roots of the studied species (Ryan et al. 2001). Also, Dettenmaier et al. (2009) suggested that differences in root lipid contents might influence plants uptake of hydrophobic compounds from sediment, since these chemicals can be predicted to adsorb to lipids present in root endodermis and not actually taken up. Similarly, the foliage area, lipid composition and roughness of the leaves can be species-specific properties that influence the retention and accumulation of pollutants in vegetation (Wagrowski and Hites 1998).

Root concentration factors

To compare the ability of *S. perennis* and *H. portulacoides* roots to accumulate dioxin-like compounds, the root concentration factor (RCF), defined as the ratio between concentration of a given chemical in roots and in the surrounding medium, was calculated (Fig. 3). RCFs in *S. perennis* were significantly lower (t-test, $p < 0.05$) than in *H. portulacoides* for most of

the PCDD/F homologues (except HpCDD, OCDD and TCDF) and dl-PCB congeners (except mono-*ortho* PCBs 104, 115 and 123). These results indicate that there is a difference in accumulation capability between the two plant species, as mentioned above.

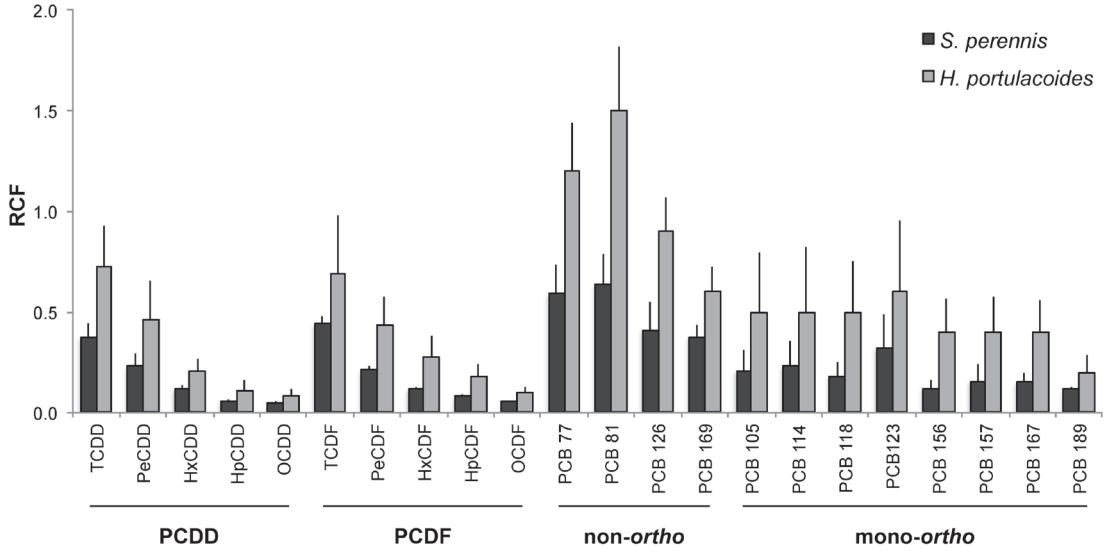

Figure 3 Root concentration factors (RCFs) for 2,3,7,8-substituted PCDD/F homologues and dioxin-like PCB congeners in *Sarcocornia perennis* and *Halimione portulacoides* roots. Results are expressed as the mean + standard deviation (n=5).

The log K_{ow} is used to predict and model the migration of hydrophobic organic compounds in water and sediment/soil. For PCDD/F and PCB congeners, log K_{ow} rises with increasing chlorine content (Chen et al. 2001; Yeh and Hong 2002). This means that higher chlorinated forms are less soluble and in consequence are expected to be less bioavailable to plants (Zeeb et al. 2006). In the present study, both plant species showed lower RCFs for PCDD/Fs than for dl-PCBs with corresponding number of chlorine substitutions (Fig. 3). The superior log K_{ow} of PCDD/Fs in comparison with the coefficient of dl-PCBs with identical chlorine content might explain why dl-PCBs seem to be more susceptible to accumulate in roots than PCDD/Fs. In addition, a reduction of RCFs with increase in chlorine substitutions in PCDD/F was observed for both plants (Fig. 3). The significant negative correlation (r = -0.639, $p < 0.05$ for *S. perennis*; r = -0.738, $p < 0.001$ for *H. portulacoides*) found between RCFs and log K_{ow} confirms that PCDD/F accumulation by roots can be influenced by the number of chlorines in the molecules and, thus, by their solubility (Satchivi et al. 2001). Inui et al. (2008) also observed this decrease in accumulation of PCDD/Fs with increasing hydrophobicity.

In contrast, dl-PCB accumulation in both studied plants seems to be affected not only by

chlorine content, and hence log K_{ow} and molecular weight, but also by the presence of chlorine atoms in *ortho*-positions; congeners with no substitution in the *ortho*-positions had higher RCFs in comparison with mono-*ortho* congeners with an identical number of chlorines (Fig. 3). In agreement, RCFs for dl-PCB congeners were not significantly correlated with log K_{ow}. Structure-selective accumulation was documented before for dioxin-like compounds (Inui et al. 2008; Matsuo et al. 2011) in some particular zucchini cultivars. However, in opposition to our findings, they reported bioconcentration factors for mono-*ortho*-chlorinated biphenyls several times as high as those for PCBs without chlorine at the *ortho*-positions. Since only *ortho*-substituted congeners may be polar molecules with the ability to form hydrogen bonds and, thus, show higher solubility in water (IPCS, 2003), the results found in the present study are contrary to our expectations. Further studies are necessary to understand the mechanism underlying the preferential uptake of non-*ortho* by these salt marsh plants.

PCDD/F and dl-PCB profiles in sediment and plant tissues

Both sediment and plant tissues presented PCDD/F profiles with greater contributions of higher chlorinated homologues, namely octa-CDD/F and hepta-CDF (Fig. 4a). However, plant tissues showed greater percentages of tetra-, penta- and hexa-CDD/F than sediments. Regarding dl-PCB congener contributions, PCB 118 and 105 were the most abundant in all analyzed samples (Fig. 4b). Further, non-*ortho* PCBs (congeners 77, 81, 126 and 169) had higher contributions in tissues of *S. perennis* and *H. portulacoides* in comparison with the surrounding sediments, again suggesting the existence of selective accumulation of dl-PCBs with no chlorine at the *ortho*-positions in plants.

PCA results confirmed the distinct PCDD/F and dl-PCB profiles of sediments and plant tissues (Fig. 5). For PCDD/Fs, the first principal component (PC1) explained 98.2% of data variability. PC1 showed that sediment samples are clearly associated with higher contributions of octa-CDD/F, hepta-CDF and hexa-CDF homologue groups, whereas plant tissues are mostly related with less chlorinated PCDD/Fs (Fig. 5a). These differences may reflect an easier adsorption/absorption of congeners with a low degree of chlorination, due to their lower log K_{ow}, higher solubility and smaller molecular weight (Zeeb et al. 2006; Inui et al. 2008). Although *S. perennis* and *H. portulacoides* showed significantly different PCDD/F concentrations (Fig. 3a), according to the PCA no distinction was found between their tissue profiles (Fig. 5a). The second principal component (PC2) described a very small fraction of total variance (0.9%), and allowed a slight separation of root samples from stems

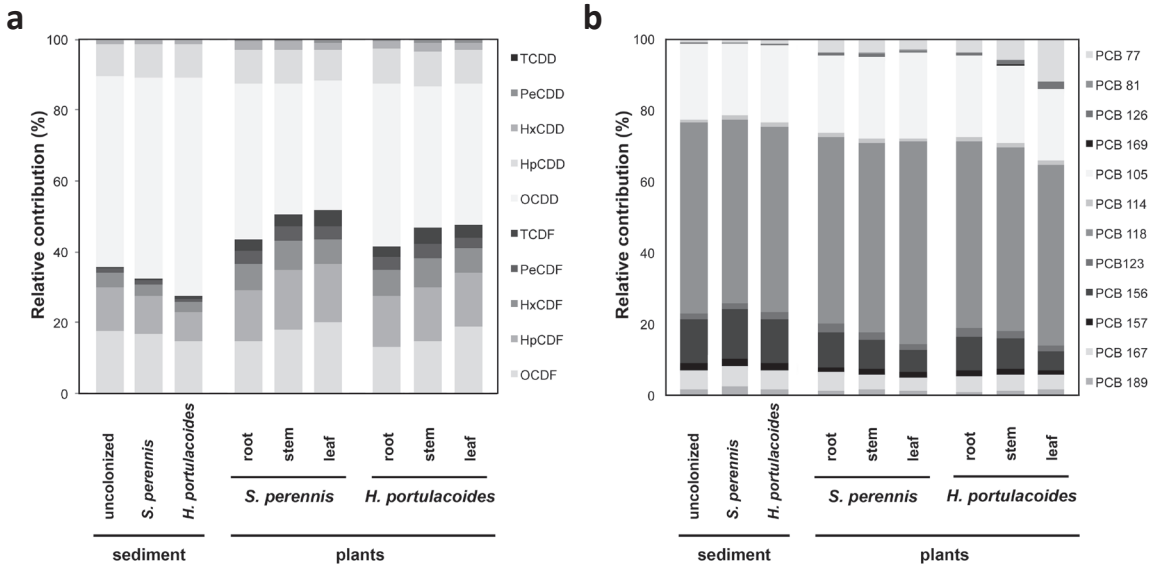

Figure 4. Mean relative contribution of **(a)** 2,3,7,8-substituted PCDD/F homologues and **(b)** dioxin-like PCB congeners in sediments and tissues of salt marsh plants *Sarcocornia perennis* and *Halimione portulacoides* collected from the Tejo estuary (n=5).

and leaves, independently of the plant species (Fig. 5a). The profile found in roots illustrates the transition between sediments and aboveground plant tissues. The structure of root epidermis, particularly the thickness of waxes on its surface, determines the root capacity for adsorbing PCDD/Fs and consequently the diffusion into the root core and vascular tissues (Müller et al. 1994; Meneses et al. 2002). Thus, the lipid nature of root epidermis may result in higher chlorinated congeners adsorbed to root surface, while less chlorinated PCDD/Fs are preferentially taken up. Sediment samples also showed a gradient along PC2 (Fig. 5a). This profile variation demonstrates that the presence of salt marsh plants may in fact influence the PCDD/F content of sediments.

As for dl-PCBs, the PCA showed that the profiles of all three sediment types were similar, but differed considerably in comparison with plant samples (Fig. 5b). The two principal components accounted for 90.8% of total variance (84.0% for PC1 and 6.8% for PC2). According to PC1, sediment samples showed higher contributions of mono-*ortho* PCBs, while plant tissues were mainly associated with non-*ortho* PCBs. Thus, the absence of chlorine atoms in the *ortho*-positions seems to facilitate the accumulation of dl-PCB congeners in plant tissues, despite their lower water solubility. Similarly to PCDD/Fs, root samples revealed an intermediary dl-PCB profile. In addition, PC2 highlighted the dissimilarities between aboveground tissues of *S. perennis* and *H. portulacoides*: all samples are associated with non-*ortho* dl-PCBs, but *H. portulacoides* tissues, and especially their leaves, displayed

a higher contribution of PCB 77 and PCB 126. Contrary to the observed for PCDD/Fs, the

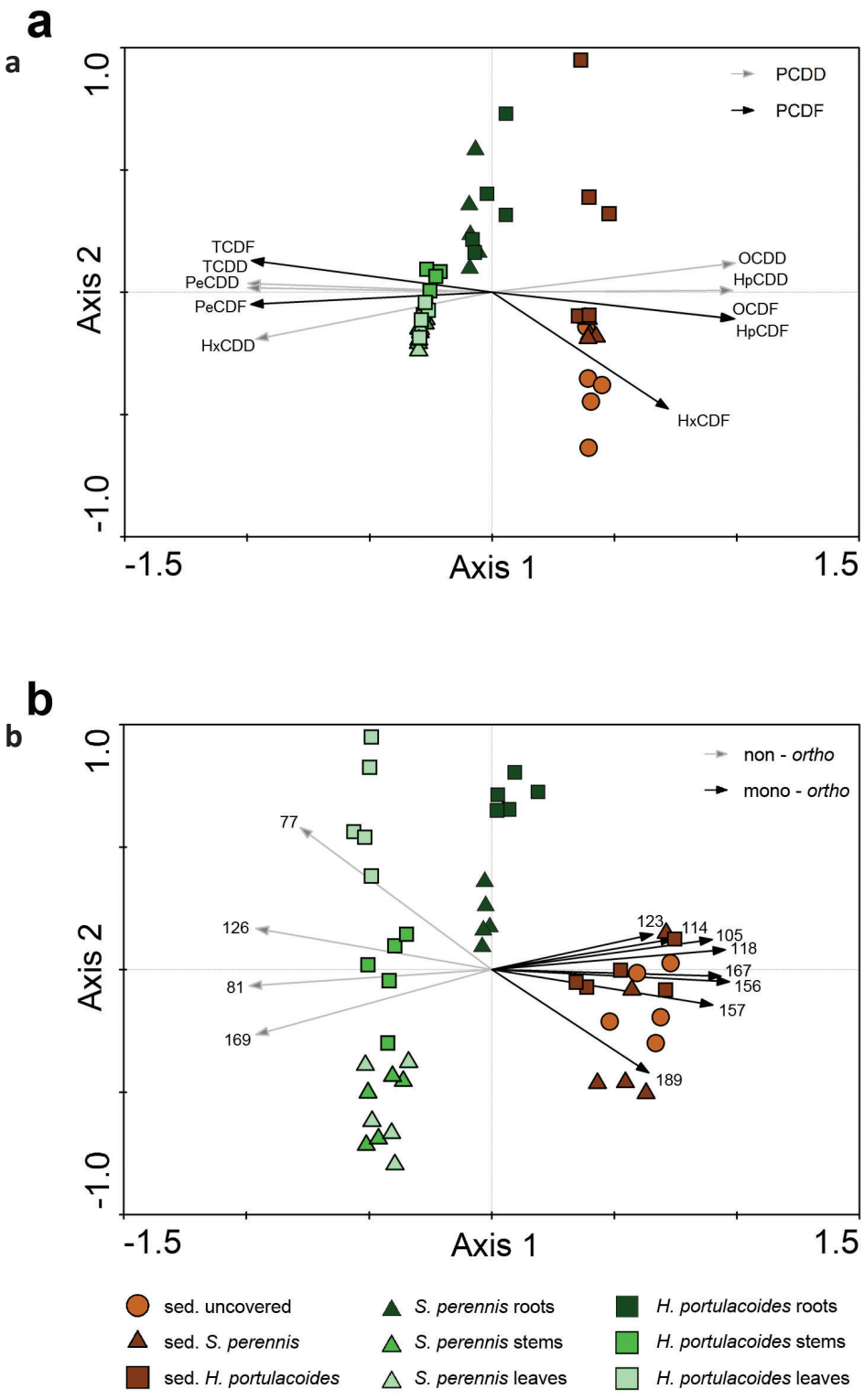

Figure 5 Principal component analysis (PCA) biplots of **(a)** 2,3,7,8-substituted PCDD/F homologues and **(b)** dioxin-like PCB congeners in tissues of salt marsh plants *Sarcocornia perennis* and *Halimione portulacoides* and surrounding sediments collected from the Tejo estuary.

two plant species not only accumulated different dl-PCB concentrations (Fig. 3b), but also showed distinct dl-PCB profiles (Fig. 5b).

As mentioned before, contamination of aboveground vegetation by dioxin-like compounds may occur from sediment through a variety of mechanisms as follows: root uptake and consequent translocation, volatilization from the sediment surface following by adsorption onto the plant surface, contamination of the plant's foliage by direct contact with sediment particles and direct deposition from atmosphere (Trapp and Matthies 1997; Wagrowski and Hites 1998; Smith and Jones 2000). Although volatilization followed by plant adsorption may be a major PCDD/F and PCB pathway in greenhouses, in the field, this mechanism is thought to be of minor importance (Trapp and Matthies 1997). In the present study there are no indications regarding sediment deposition constituting a pathway of foliage contamination. The differences found between PCDD/F and dl-PCB profiles in the aboveground parts of *S. perennis* and *H. portulacoides* may be a result of each plant's characteristics. Plants may also be impacted by atmospheric contamination from sources such as municipal and hospital waste incineration plants (Wagrowski and Hites 1998; Schuhmacher et al. 2000). Yet, according to Coutinho et al. (2007), PCDD/F concentrations measured in ambient air from Lisbon region are comparable to those found in low contaminated urban areas. Moreover, similar locations in the Tejo estuary showed inferior dioxin-like concentrations in sediment (Nunes et al. submitted). Thus, atmospheric inputs of dioxin-like compounds in the studied salt marsh are assumed to be insignificant compared to the inputs from the Trancão River. Therefore, in this study, root uptake and transportation within plants might be a primary mechanism of PCDD/F and dl-PCB contamination of aboveground tissues, although only very small amounts can reach stems and leaves.

Conclusions

The presence of plants was associated with lower levels of dioxin-like compounds in salt marsh sediments. Furthermore, differences in homologue profiles of uncolonized sediment and rhizosediment samples suggests that vegetation may influence their PCDD/F composition.

Both plant species incorporated lower concentrations in the aboveground tissues in comparison with its roots, suggesting that despite the contaminant transfer occurred from sediments to the roots, PCDD/Fs and dl-PCBs are not significantly translocated inside

plants. For instance, PCDD/F levels in rhizosediments are one to two orders of magnitude higher than concentrations in roots and aboveground parts, respectively. Nevertheless, root uptake and transportation within plants might be a main mechanism of PCDD/F and dl-PCB contamination of aboveground vegetation in the study area, although involving reduced amounts of compounds.

This work also revealed that *S. perennis* accumulated lower quantities of dioxin-like compounds in comparison with *H. portulacoides*, meaning that one species may have a higher contribution in PCDD/F and PCB transfer from sediment to the food web. PCDD/F concentrations varied between *S. perennis* and *H. portulacoides* but their homologue compositions were similar, suggesting that the mechanisms controlling the distribution of PCDD/Fs were similar in the two species. On the other hand, *S. perennis* and *H. portulacoides* not only accumulated different dl-PCB concentrations, but also showed distinct dl-PCB profiles. These results reveal that accumulation capability and eventual metabolism mechanisms of salt marsh plants vary, depending on the specific properties of each compound and on the characteristics of the plant species. Moreover, congener profiles shifted between sediments and plant, reflecting a selective accumulation of low chlorinated PCDD/Fs and non-*ortho* PCBs in plants.

Acknowledgements

The authors would like to express their gratitude to Xavier Marques for the valuable help in the field campaign. The manuscript was greatly improved by the comments of three anonymous reviewers and the handling editor. This work was financially supported by the FCT – Fundação para a Ciência e Tecnologia through a PhD grant attributed to MN Cardoso (SFRH/BD/46969/2008) co-funded by the POPH/FSE.

CHAPTER

IV Early contamination of European flounder (*Platichthys flesus*) by PCDD/Fs and dioxin-like PCBs in European waters

Abstract

Estuarine and coastal habitats constitute nursery areas for the European flounder (*Platichthys flesus*) during its critical juvenile period. However, these systems are also known to accumulate various chemical contaminants. Therefore, the present work aimed to investigate the contamination levels and profiles of 7 polychlorinated-*p*-dioxins, 10 polychlorinated furans (PCDD/Fs) and 12 dioxin-like polychlorinated biphenyls (dl-PCBs) in juvenile *P. flesus* captured in different nursery areas in the northeastern Atlantic coast across its geographical distribution range. The toxic equivalency concentrations (WHO-TEQ_{fish}) were also determined in order to evaluate which *P. flesus* population was more exposed to dioxin-like toxicity. Juveniles caught in the Sørfjord (Norway) showed the lowest WHO-TEQ_{fish} concentration (0.052 pg g^{-1} wet weight) whereas the highest value was observed in fish from the Wadden Sea (Netherlands, 0.291 pg g^{-1} ww), mainly due to the greater contribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, the most toxic congener. Nonetheless, the PCDD/F and dl-PCB concentrations detected in the muscle of juvenile flounder are not expected to adversely affect fish.

Keywords

PCDD/Fs; PCBs; fish; flounder; juvenile; nursery; latitude

Introduction

The European flounder *Platichthys flesus* (Linnaeus 1758) is an abundant flatfish species commonly found in coastal waters of the northeastern Atlantic Ocean, with a latitudinal distribution range from 40 to 72°N (Wheeler 1978; Martinho et al. 2013). This species supports an important commercial fishery in the northeast Atlantic with an estimated global annual catch above 18,000 tons between 2000 and 2011 (FAO 2011). Regarding life cycle, *P. flesus* spawn in marine waters and the pelagic larvae migrate to inshore to settle in shallow coastal and estuarine habitats, where they remain during the juvenile stage (van der Veer et al. 1991; Grioche et al. 1997; Martinho et al. 2008, 2013). These important nursery habitats are essential for fish population renewal, being characterized by high food availability and providing refuge against predators and favorable conditions for a rapid growth (Gibson 1994; Able et al. 2005; Cabral et al. 2007; Vasconcelos et al. 2011).

Because of their benthic lifestyle and bottom-feeding behavior, the European flounder is particularly exposed to sediment-associated pollutants such as polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) (Shelepchikov et al. 2008). In addition, early life stages are more susceptible to dioxin-like toxicity compared to adult fish (Lanham et al. 2012), requiring considerably lower body burdens to elicit adverse effects (Peterson et al. 1993; Lanham et al. 2012). Growth retardation, cutaneous hemorrhage, craniofacial malformations, histopathologic lesions in several tissues or lethality have been described in early life stages of different fish species exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Henry et al. 1997; Elonen et al. 1998; Lanham et al. 2012). The exposure of juvenile *P. flesus* might, thus, induce disruption in their growth and survival, and consequently affect the viability of adult population (Courrat et al. 2009). Due to their physicochemical properties, PCDD/Fs and dl-PCBs can also bioaccumulate and biomagnify in the food web, reaching levels that could adversely affect wildlife and human health (UNEP 2001; Wan et al. 2005). In addition, the consumption of fish and seafood is the main pathway of human exposure to these contaminants (Domingo and Bocio 2007; EFSA 2012).

The present study investigated the PCDD/F and dl-PCB concentrations and congeners profiles in flounder juveniles in a number of nurseries along the species' geographical distribution in the northeastern Atlantic. The objective was to report the early contamination of different populations of *P. flesus* and to explore the possibility of adverse effects associated with dioxin-like compounds (i.e., 2,3,7,8-substituted PCDD/Fs and dl-PCB congeners).

Material and methods

Flounder juveniles (0-group) were collected at several estuarine and shallow coastal nurseries: Mondego estuary (Portugal), Vilaine estuary (France), Slack estuary (France), western Wadden Sea (Netherlands) and Sørfjord (Norway) (Fig. 1). These sampling sites were selected in order to cover most of the geographical distribution range of *P. flesus* in European waters, comprising a range of 20° in latitude. Juvenile flounders were collected during the estuarine colonization stage between mid-June to mid-July 2010, using a beam trawl in all sampling sites. After collection, fish were transported on iceboxes back to the laboratory and frozen for later analysis.

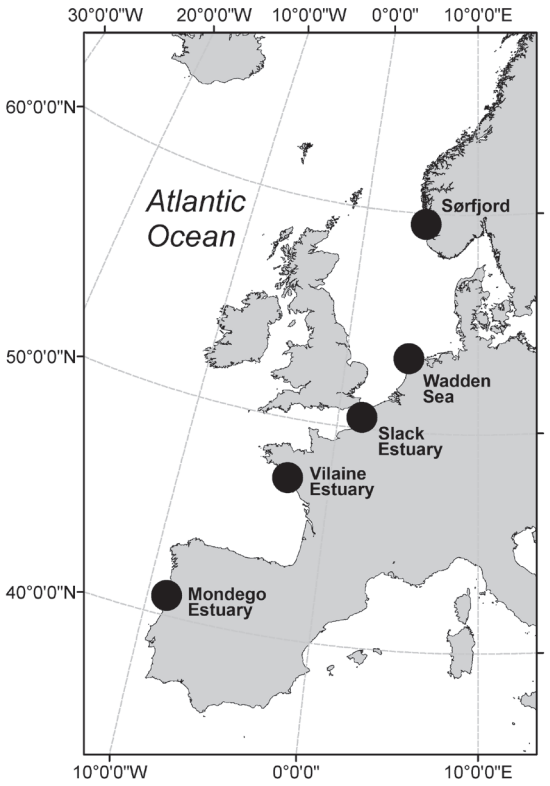

Figure 1 Location of the sampling sites across the main geographical distribution of *Platichthys flesus* in the northeastern Atlantic.

At the laboratory, fish were measured (total length, mm) and weighed (wet weight, mg). Muscle samples were removed from each individual and pooled together in order to prepare composite samples, after which they were freeze-dried and stored for later analysis (Table 1).

Table 1 Location of *Platichtys flesus* nursery areas selected across the geographical distribution in the northeastern Atlantic, respective sampling date, number of juveniles collected, total length (average ± standard deviation), total weight (average ± standard deviation) and number of composite samples analyzed.

Nursery area		Sampling date	Number of individuals	Total length (mm)	Total weight (mm)	Number of samples
Mondego Estuary, Portugal	40°08'N, 8°52'W	17.06.2010	23	75.09 ± 16.49	3.51 ± 1.59	2
Vilaine Estuary, France	47°30'N, 2°30'W	02.07.2010	32	76.44 ± 7.47	4.59 ± 1.48	3
Slack Estuary, France	50°48'N, 1°36'E	01.07.2010	84	51.83 ± 6.42	1.35 ± 0.62	2
Wadden Sea, Netherlands	53°04'N, 5°03'E	17.07.2010	44	53.55 ± 6.27	1.60 ± 0.71	1
Sørfjord, Norway	60°30'N, 5°24'E	17.07.2010	58	56.67 ± 7.48	1.81 ± 1.05	2

Samples were analyzed for the 17 2,3,7,8-substituted PCDD/Fs and the 12 PCBs that have dioxin-like activity (non-*ortho* PCBs 77, 81, 126 and 169, and mono-*ortho* PCBs 105, 114, 118, 123, 156, 157, 167 and 189). Those are the 29 congeners with toxic equivalency factors (TEFs) assigned by the World Health Organization (WHO) (van den Berg et al. 1998, 2006).

Detailed descriptions of extraction and cleanup procedures can be found elsewhere (e.g. Costera et al. 2006). Briefly, samples were extracted automatically in an Accelerated Solvent Extraction device (ASE 300, Dionex, Sunnyvale, CA, USA), followed by purification and fractionation in three successive chromatographic steps involving multilayered silica gel, Florisil and carbon columns. Analysis of PCDD/Fs and dl-PCBs was performed by gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS) using a Hewlett–Packard 6890 gas chromatograph (Palo Alto, CA, USA), equipped with a DB-5MS column and coupled to a JEOL JMS-800D double sector mass spectrometer (Tokyo, Japan). All target compounds were quantified using the isotope dilution method.

Samples were analyzed at LABERCA, the French National Reference Laboratory in charge of PCDD/F and PCB analysis in food and feed, according to a validated and accredited method (ISO 17025:2005 standard). The procedure integrated the quality assurance and quality control criteria according to European legislation laying down sampling procedures and the analysis methods for determination of PCDD/Fs and dl-PCBs (EC 2006). Blanks were included in every series of samples to check for interference and cross-contamination. Chromatographic separation was checked (<25% peak to peak between 1,2,3,4,7,8-HxCDF and 1,2,2,6,7,8-HxCDF) and recoveries of individual congeners were within 30 to 140% as required by the EC regulation 1883/2006. Limits of detection (LOD) were 0.007 pg g^{-1} of wet weight (ww) for PCDD/Fs and 0.05 pg g^{-1} ww for dl-PCBs.

Since the size of the individuals caught for this study was well below the minimum length for capture (Table 1), it was not considered that they were suitable for human consumption. Thus, instead of using the toxic equivalency factors (TEFs) derived for human assessment risk, the toxic equivalency (WHO-TEQ) concentrations were calculated based on TEFs specific for fish in order to compare the dioxin-like toxicity affecting the *P. flesus* juveniles (van den Berg et al. 1998).

Results

The 17 PCDD/F and 12 dl-PCB congeners were detected in all samples, indicating a global contamination by these compounds in *P. flesus* captured in the different nursery areas (data available in Appendix C). Total 2,3,7,8-substituted PCDD/F concentrations (ΣPCDD/Fs) ranged from 0.39 to 1.30 pg g^{-1} ww. The lowest value was detected in fish from the Sørfjord (Norway) whereas the highest was found in juveniles from the Wadden Sea (Netherlands) (Fig. 2a). Total dl-PCB concentrations (ΣPCBs) were at least two orders of magnitude higher than ΣPCDD/Fs. Fish caught in the Mondego estuary (Portugal) showed the lowest Σdl-PCB concentration (170.34 pg g^{-1} ww). The highest ΣPCB value (1002.09 pg g^{-1} ww) was recorded in *P. flesus* from the Slack estuary (France), although it was comparable to levels found in the Wadden Sea and Vilaine estuary (France) (Fig. 2b).

Since PCDD/Fs and PCBs are highly lipophilic compounds, the fat content is an important factor that influences individual body burden (Pastor et al. 1996). Results on a lipid basis clearly showed higher PCDD/F and dl-PCB contamination levels in the muscle of juveniles from the Wadden Sea when compared with samples from other nursery areas (data available in Appendix C). Regarding ΣPCDD/Fs, fish from the Wadden Sea were up to five times more contaminated than those from the Sørfjord (233.48 and 49.76 pg g^{-1} lipid weight (lw), respectively). Similarly, *P. flesus* from the Wadden Sea showed also the highest ΣPCB concentration (166.91 ng g^{-1} lw). As seen for ΣPCB concentration expressed on wet weight, the fish captured in the Mondego estuary exhibited the lowest PCB contamination (22.44 ng g^{-1} lw).

WHO-TEQ_{fish} concentrations ranging from 0.05 and 0.29 pg g^{-1} ww were found in the analyzed samples. The lowest value was observed in the Sørfjord while the highest WHO-TEQ_{fish} concentration, which stands out from the rest, was found in the Wadden Sea. The range for WHO-TEQ_{fish} concentrations on a lipid basis was between 6.90 pg g^{-1} lw (Sørfjord) and 52.21 pg g^{-1} lw (Wadden Sea). In all samples, PCDD/Fs constituted more than 70% of

WHO-TEQ$_{fish}$ concentration.

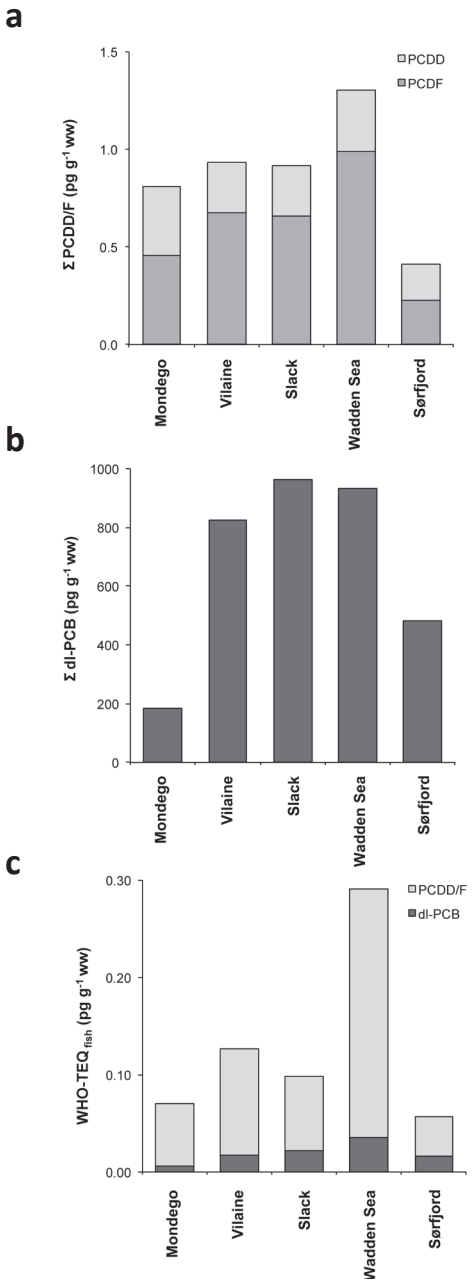

Figure 2 Total concentration of (**a**) 2,3,7,8-substituted PCDD/Fs, (**b**) dioxin-like PCBs and (**c**) WHO-TEQ$_{fish}$ in juvenile *Platichtys flesus* from five nursery areas across the northeastern Atlantic.

The relative contribution of each PCDD/F homologue group to the total concentration varied among the studied nursery grounds (Fig. 3). The two areas where the least contaminated juveniles were caught (Sørfjord and Mondego estuary) showed comparable PCDD/F profiles, with OCDD being the most abundant congener. In the Wadden Sea, Vilaine estuary and Slack estuary, PCDFs displayed higher contributions to ΣPCDD/F concentration (> 70%), mainly

due to the higher percentage of TCDF congener. An evident higher contribution of TCDD congener was reported in muscle of *P. flesus* from the Wadden Sea. In contrast to PCDD/Fs, juveniles from different areas showed rather similar profiles of dl-PCB congeners, although PCB 156 had a slightly higher percentage in the Sørfjord. PCB 118 was the most abundant congener in all *P. flesus* samples, while PCB 81 showed an insignificant contribution to Σdl-PCB concentration (< 0.007%).

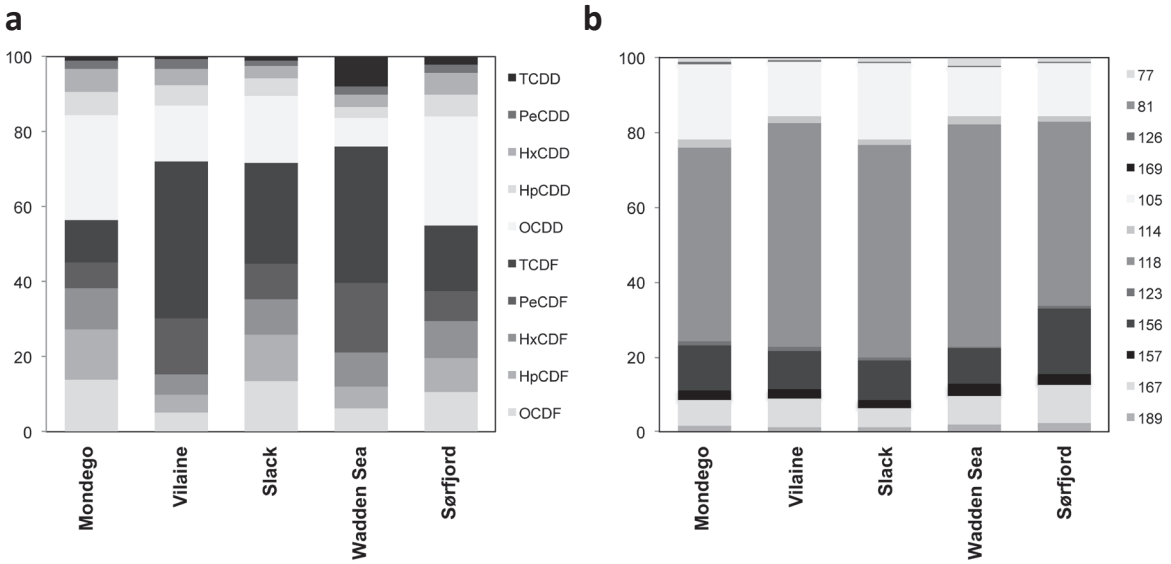

Figure 3 Profiles of (**a**) 2,3,7,8-substituted PCDD/F homologues and (**b**) dioxin-like PCB congeners in juvenile *Platichtys flesus* from five nursery areas across the northeastern Atlantic. Results are expressed as percentage of total concentration of 2,3,7,8-substituted PCDD/Fs or dioxin-like PCBs.

Discussion

This work provided reference values on juvenile flounder contamination by PCDD/Fs and dl-PCBs in nursery areas along the main geographical distribution in the northeastern Atlantic. Most information available is focused on adult *P. flesus* samples from the coastal Baltic Sea and Grenlandfjords due to the very high amounts of dioxin-like compounds found in these regions (Knutzen et al. 2003; Pandelova et al. 2008; Shelepchikov et al. 2008; Isosaari et al. 2006; Ruus et al. 2006b). The ΣPCDD/F concentration in flounder caught in Grenlandfjords and Baltic Sea has been reported to reach 184 pg g^{-1} ww (Knutzen et al. 2003) and 8140 pg g^{-1} lw (Ruus et al. 2006), respectively. These values are much higher than those obtained in the samples analyzed in this study.

The fish tissue residue data indicated that *P. flesus* juveniles captured in the Wadden Sea

were more subjected to PCDD/Fs and dl-PCBs than those caught in the other nursery areas. The greater WHO-TEQ_{fish} concentrations were a result of higher accumulation levels of TCDD, which is considered the most toxic congener. The Wadden Sea has a long history of pollution, and has been monitored for several decades (Duinker, 1986; Vethaak and Jol 1996; Becker et al. 2001; Vethaak et al. 2009; Laane et al. 2013**).** The massive decline of the Dutch harbor seal population in the sixties and seventies was demonstrated to have been caused by accumulation of PCBs in their tissues, impairing its reproductive capacity (Reijnders 1980, 1986). Moreover, it has been suggested that the relatively high prevalence of neoplastic liver lesions observed in European flounder from the Dutch Wadden Sea in the eighties was also due to persistent organic pollutants (Vethaak and Jol, 1996; Vethaak and Wester, 1996). Nevertheless, in the last years the input and concentration of chemical contaminants have significantly decreased in water, sediments and biota from the Wadden Sea, and consequently, the adverse effects on organisms have also declined (Vethaak et al. 2009; Laane et al. 2013).

The exposure to contaminants, including PCDD/Fs, may lead to altered energy balance and depletion of lipid content in fish (van der Weiden et al. 1994; Meador et al. 2006). Although the fat content determined in juvenile flounder from the Wadden Sea was lower than the remaining estuaries, the few samples analyzed do not allow a sound statistical evaluation of the data, even though the number of individuals in each composite sample was high (Table 1). Thus, it is not possible to conclude that the PCDD/F and dl-PCB levels found in the Wadden Sea were directly related with the lower lipid content in fish.

Bioaccumulation of highly hydrophobic compounds in adult fish occurs manly through diet (Ruus et al. 2006a), while in early life stages maternal transfer to eggs is considered the most significant source of contaminants (Tietge et al. 1998; Loizeau et al. 2001). For example, Tietge et al. (1998) verified that approximately 39% of maternal tissue residues of TCDD were transferred to eggs of brook trout (*Salvelinus fontinalis*) during development. This fact, together with the possible reduction in concentration by growth dilution (Buckman et al. 2006), could explain the higher PCDD/F and dl-PCB values found in flounder juveniles from the Mondego estuary in comparison with adults caught in the same location (Baptista et al. 2013). The migration of adult *P. flesus* to marine waters, a less contaminated environment (Gómez-Gutiérrez et al. 2007; Parnell et al. 2008), may also be associated with a decrease of PCDD/F and dl-PCB levels in fish (Baptista et al. 2013).

Literature data on concentrations of PCDD/Fs and dl-PCBs in juvenile *P. flesus* and associated toxicity could not be found. Therefore, in the present study tissue residues determined on

flounder were compared with benchmark values proposed by Steevens et al. (2005). These tissue residue-based toxicity benchmarks were estimated based on information concerning early life stage toxicity for several fish species (mainly freshwater salmonids). The WHO-TEQ_{fish} concentrations obtained in this study for European flounder juveniles were below the residue effect concentration protecting 90 and 99% of the fish community of 0.699 and 0.057 pg g^{-1} lw (expressed as WHO-TEQ_{fish}), respectively (Steevens et al. 2005). In general, no adverse effects are expected in *P. flesus* at the detected concentrations, although differences in species sensitivity introduce uncertainty into this comparison. Nevertheless, salmonids are among the most sensitive species to contaminants having a dioxin-like mechanism of toxic action (Elonen et al. 1998; Kannan et al. 2000), whereas *P. flesus* has been reported as relatively insensitive to TCDD exposure (Grinwis et al. 2000). In addition, it should be noted that some fish populations with long-term exposure to contaminated conditions could develop resistance to toxicity of PCDD/Fs and PCBs (Nacci et al. 2002; Marchand et al. 2004).

Acknowledgements

The authors would like to express their gratitude to Daniel Crespo, Dániel Nytrai, João Rito, Cláudia Moreira, Rita Guillot and Hans Witte for the valuable help in sampling in the Mondego estuary, Wadden Sea and in the Sørfjord, and to Louis Quiniou and Rachid Amara for providing flounder samples form the French estuaries. This work was supported by FCT – Fundação para a Ciência e Tecnologia through a PhD grant attributed to Margarida Cardoso (SFRH/BD/46969/2008) and a postdoctoral grant attributed to Filipe Martinho (SFRH/BPD/63527/2009).

CHAPTER

V PCDD/Fs and dioxin-like PCBs in sediment and biota from the Mondego estuary (Portugal)

Abstract

The concentrations of 17 polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs), and 12 dioxin-like polychlorinated biphenyls (dl-PCBs) were measured in sediment and key species as an initial investigation on PCDD/F and dl-PCB contamination in the Mondego estuary (Portugal). The results demonstrated that total PCDD/F concentrations were considerably lower than those of dl-PCBs in all the studied samples. Regarding the contribution of individual congeners, OCDD was the predominant PCDD/F and the mono-ortho PCB 118 and PCB 105 were the dominant PCBs in the majority of the samples. Our results suggest that PCDD/Fs and PCBs behave quite differently along the aquatic food web: total PCDD/F concentrations were lower in higher trophic-level organisms with fish presenting a distinct PCDD/F profiles; on the contrary, the higher total dl-PCB values were found in upper-level biota, although not exclusively, and quite similar dl-PCB congener profiles were observed in nearly all the studied species.

Keywords

PCDD/Fs; PCBs; profile; Mondego estuary; Portugal

Introduction

Coastal and estuarine environments are subjected to numerous disturbances, among which chemical pollution is a major concern. Over the last decades, increasing attention has been paid to the occurrence of persistent organic pollutants (POPs) in these ecosystems. POPs such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are widely recognized by the scientific community as being a risk to wildlife and human health due to their high toxicity and ability to bioaccumulate (Mandal 2005). These pollutants enter the coastal and estuarine systems via atmospheric deposition, river input and point sources along the coast, leading to relatively high concentrations in water, sediment and biota adjacent to major coastal and riverine industrial and urban zones (Suarez et al. 2005; Wu et al. 2001). Due to their hydrophobic nature, PCDD/Fs and PCBs strongly adsorb to suspended and bottom sediments in the aquatic environment. For that reason sediments represent both a sink and a source of contamination to benthic organisms as a result of their intimate and constant relationship with sediment. Once present in food webs, these compounds bioaccumulate and biomagnify, reaching higher concentrations in species at upper trophic levels (Binelli and Provini 2003; Naso et al. 2005). Moreover, the dietary intake, especially the consumption of aquatic organisms, is considered to be the main pathway of human exposure to these pollutants (Darnerud et al. 2006; Domingo and Bocio 2007). Numerous contamination episodes have demonstrated how PCDD/Fs and PCBs can easily reach the top of food webs, including humans (Bernard et al. 2002; Malish 2000). Thus, the presence of these contaminants in food webs and its consequences to food safety has raised awareness and concern of researchers and policy makers.

Mondego River flows along 227 km, draining a hydrological basin of approximately 6670 km^2, the largest entirely comprised in the Portuguese territory. During its course, it runs through rural as well as highly urbanized and industrialized areas before reaching its 1,600 ha estuary. The Mondego River basin supports over half a million inhabitants and as a result the estuary has been subjected to a strong anthropogenic pressure. Its main pollution sources are wastewaters, most resulting from high population density through domestic sewage (partially untreated) and high industrial activities through industrial sewage. The system also receives agricultural runoff from 15,000 ha of agricultural land, which requires the use of great amounts of fertilizers and pesticides. Additionally, the port of Figueira da Foz is also responsible for industrial pressure in the estuarine area.

Over the last 15 years, applied research has been conducted in the Mondego estuary, providing a comprehensive dataset on several areas (e.g. Baeta et al. 2008; Coelho et al. 2004; Dolbeth et al. 2007; Marques et al. 2007; Martinho et al. 2007; Verdelhos et al. 2005) and an insight into the pollution levels of the estuary (e.g. Ferreira et al. 2004; Pereira et al. 2005; Ribeiro et al. 2009). However, regarding PCDD/F levels there is no published data, and the information available on PCB contamination is scarce and limited to sediment samples. Based on this, a preliminary survey was carried out in order to investigate the occurrence of 2,3,7,8-substituted PCDD/Fs and dioxin-like PCBs (dl-PCBs) in sediment and selected biota collected from the Mondego estuary (Portugal).

Material and methods

Study site

Mondego estuary is a relatively small, warm-temperate, polyhaline intertidal system located in the Atlantic coast of Portugal (40°08' N, 8°50' W, Fig. 1). It consists of two arms separated by an alluvium-formed island. The northern arm is deeper and constitutes the main navigation channel and the location of the Figueira da Foz harbor. The southern arm is shallower and is characterized by large areas of intertidal mudflats (almost 75% of the area) exposed during low tide.

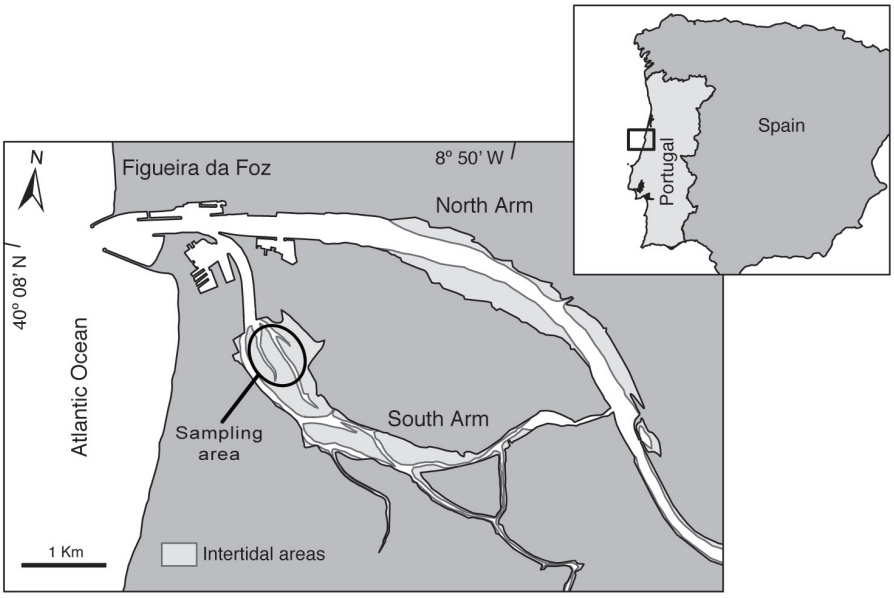

Figure 1 Mondego estuary and location of the sampling area.

Sample collection and preparation

The sampling campaign was carried out in a well-documented part of the south arm in November 2009 (Fig. 1). The study area corresponds to a deposition zone, and sediment grain-size distribution does not differ within this part of the estuary, mainly constituted by fine particles (silt and clay) (Cunha and Dinis 2002).

Sediment samples from the upper 5 cm were collected manually during low tide in the intertidal area, together with green macroalgae *Ulva* sp., red macroalgae *Gracilaria* sp. and macrophyta *Spartina maritima*. Polychaete *Hediste diversicolor* and bivalves *Scrobicularia plana* and *Cerastoderma edule* were simultaneously taken from the corresponding area. Common shrimp *Crangon crangon* and fish, specifically sea bass (*Dicentrarchus labrax*), sole (*Solea solea*) and European eel (*Anguilla anguilla*) were sampled by beam-trawl surveys.

At the laboratory, the 5 sediment samples collected randomly were homogenized and combined into a single composite, and then sub-sampled for analysis of contaminants and grain size. Sediment organic matter content was quantified by weight difference between sediment oven dried at 60 °C for 72 h and combusted at 450 °C for 8 h. Grain size analysis was carried out by mechanical separation through a column of sieves with different mesh sizes. *S. maritima* specimens were divided into roots and aerial part. The ragworms *N. diversicolor* and shellfish were depurated in water from the sampling site for 24 h. Next, bivalves were shelled, the head and tail were cut-off from shrimp, and muscle was taken from fish. Specimens belonging to the same species were pooled to prepare a composite sample for chemical analysis (180 *C. edule*, 168 *S. plana*, 155 *N. diversicolor*, 171 *C. crangon*, 2 undifferentiated sex *D. labrax*, 2 females *S. solea* and 2 undifferentiated sex *A. Anguilla*).

PCDD/F and PCB analysis

The 17 PCDD/Fs with chlorine substitution in the 2,3,7,8 positions and the 12 dl-PCBs that were assigned toxic equivalency factors (TEFs) by the World Health Organization (WHO) (van den Berg et al. 1998, 2006) were analyzed as described by Costera et al. (2006). Briefly, extraction was performed in an Accelerated Solvent Extraction device (Dionex ASE 300), using as solvent a mixture composed of toluene and acetone at 70:30 (v/v). Purification included three successive chromatographic steps with silica, Florisil and celite/carbon columns. Detection and identification of PCDD/Fs and PCBs was carried out using a Hewlett–Packard 6890 gas chromatograph, equipped with a DB-5MS column and coupled to a Jeol JMS-800D double sector mass spectrometer set at a resolution of 10,000. All target compounds were quantified using the isotope dilution method.

Quality assurance/quality control

All profile determinations were undertaken at LABERCA, the French National Reference Laboratory in charge of PCDD/F and PCB analysis in food and feed. The procedure integrated the quality assurance parameters to fulfill the requirements of the European legislation laying down sampling methods and the methods of analysis for determination of PCDD/Fs and dl-PCBs (EC 2006b) such as: (1) the method has been validated and accredited (ISO 17025:2005); (2) the laboratory has successfully participated in several interlaboratory studies to test the method; (3) a blank control and a sample from a certified reference material were run with each series of samples; (4) recoveries of individual congeners were within 30-140%; (5) chromatographic separation was checked (< 25% peak to peak between 1,2,3,4,7,8-HxCDF and 1,2,2,6,7,8-HxCDF). Limits of detection (LOD) for sediment were 0.0009 $pg.g^{-1}$ of dry weight for PCDD/Fs and 0.036 $pg.g^{-1}$ dw for dl-PCBs, and for biota matrices were 0.0005 $pg.g^{-1}$ of wet weight (ww) for PCDD/Fs and 0.02 $pg.g^{-1}$ ww for dl-PCBs.

Results and discussion

PCDD/F and dl-PCB concentrations

In general, ΣPCDD/F concentrations were lower than those of Σdl-PCBs (data available in Appendix D). In addition, results demonstrated differences in ΣPCDD/F and Σdl-PCB levels depending on the type of matrices (Figs. 2, 3). Sediment was characterized by fine sediments, composed essentially by fine sand (73%) and silt (20%), and higher organic matter content (mean 6.2% ± 1.8). The concentration on a dry weight basis was 109.68 $pg.g^{-1}$ dw for ΣPCDD/Fs and 199.23 $pg.g^{-1}$ dw for Σdl-PCBs (data available in Appendix D).

Concerning biota samples, concentration of ΣPCDD/Fs in green macroalgae *Ulva* sp. and red macroalgae *Gracilaria* sp. was low, however they were higher than those reported in Sendai bay, Japan (Okumura et al. 2004) (Fig. 2). *S. maritima* showed higher ΣPCDD/F concentration in roots than in leaves, suggesting that these contaminants are not transported inside the plants extensively. As regards bivalves, ΣPCDD/F values are higher in clam *S. plana* than in cockle *C. edule*. The discrepancy observed between the two bivalves could be explained taking into account its different feeding mode (McLeod et al. 2008). While *S. plana* is predominantly a surface deposit feeder, especially at low tide, *C. edule* is mainly a filter feeder, though it may occasionally use its siphons to collect nutritional particles from the mud flats. Considering concentration on a wet weight basis, the fish *S. solea*, followed by

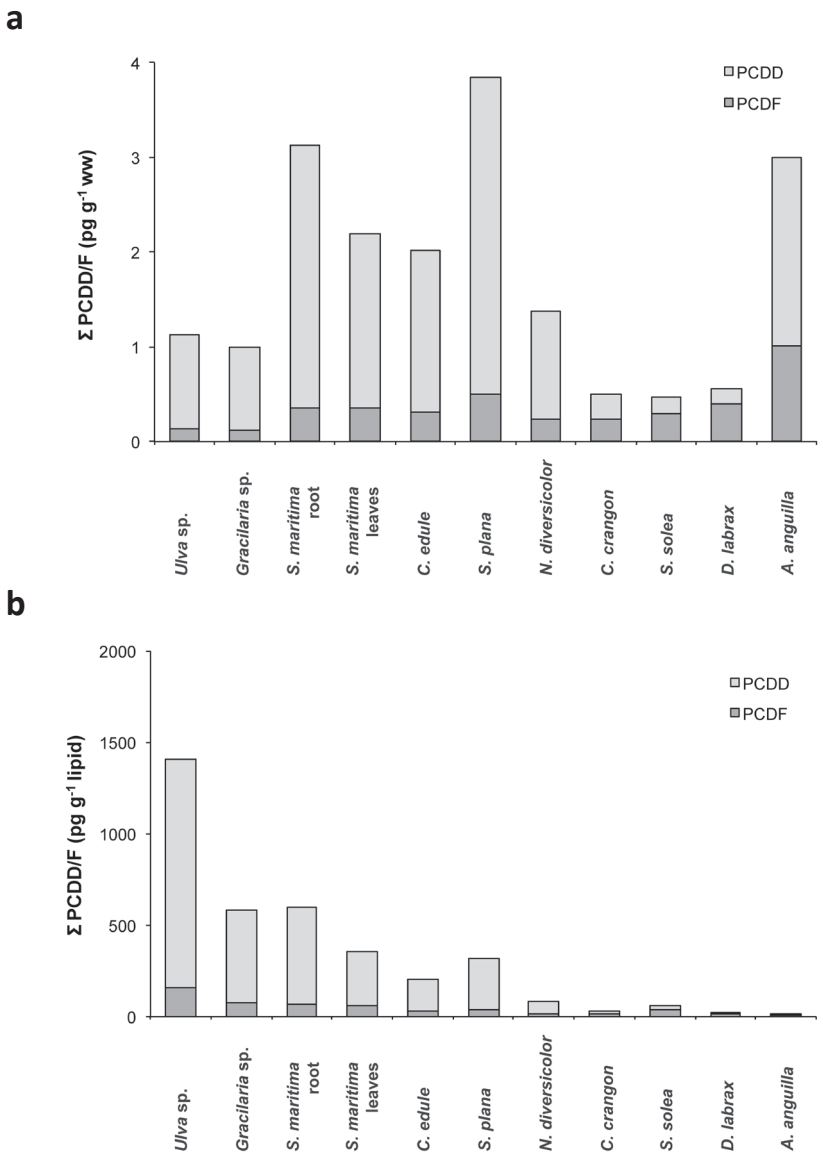

Figure 2 Total concentration of 2,3,7,8-substituted PCDD/Fs in biota from the Mondego estuary expressed in (**a**) pg g^{-1} of wet weight and in (**b**) pg g^{-1} of lipid weight.

common shrimp and sea bass, presented the lowest PCDD/F values (Fig. 2a). The first one is a top predator consuming small fish and a large variety of invertebrates, whereas common shrimp feeds on fish larvae and invertebrates living close to the bottom such as polychaetes and bivalves, and the third species is a voracious predator of polychaetes. Results on a lipid basis (Fig. 2b) showed clearly that in the study area concentrations of PCDD/Fs declined with increasing trophic level, assessed based on isotopic information reported in Baeta et al. (2008). These results are consistent with findings of others (Wan et al. 2005; Ruus

et al. 2006). As Ruus et al. (2006) suggested in a study conducted in the Grenland fjord system, Norway, two mechanisms may explain the lower concentrations of total PCDD/Fs in the food web in upper trophic levels. There may be a less effective uptake of 2,3,7,8-PCDD/Fs in organisms at higher trophic levels than in organisms at lower trophic levels, and on the other hand, there may be a higher metabolization and/or elimination of the same compounds by organisms higher in the food chain.

Contribution of ΣPCDDs to ΣPCDD/F concentration varied among samples (data available in Appendix D). While sediment, algae, cordgrass *S. maritima* and benthic invertebrates presented a greater percentage of PCDDs (> 82%), in sole and sea bass PCDDs contribute to less than 37%. Okay et al. (2009) described the dominance of PCDDs in sediment samples from Turkey coast, and Hashimoto and Morita (1995) reported higher levels of these compounds in macroalgae from the Japanese coast. The present results were also comparable to those reported by Bordajandi et al. (2006), who found a predominance of the PCDDs in shellfish species, and a similar presence of both compounds or a slightly predominance of the PCDFs in fish species.

As seen for PCDD/Fs, Σdl-PCB concentration varied largely between the matrices analyzed with values ranging from 0.015 ng g^{-1} ww in green algae to 2.808 ng g^{-1} ww in eel, and from 1.24 ng g^{-1} lipid weight (lw) in common shrimp to 29.46 ng g^{-1} lw in red algae (Fig. 3). Total dl-PCB concentrations were considerably higher than those of the PCDD/Fs in all the studied samples, which may be due to the greater bioaccumulative properties of PCBs compared with PCDD/Fs (Niimi 1996). Furthermore, Σdl-PCB showed different contributions to the sum of PCDD/Fs and dl-PCBs, depending on the studied samples. Whereas in sediment Σdl-PCBs accounted for approximately 65%, in biota samples they represented a higher fraction ranging between 88.0 and 99.9%. Concentrations of Σdl-PCBs detected in macroalgae are similar with those obtained in other studies. Pavoni et al. (2003) considered the life cycle as the most meaningful factor in determining the PCB concentration in seaweeds to explain the higher levels of PCBs in *Gracilaria*, which is a perennial macroalgae, compared to *Ulva*, which has a short seasonal life cycle. Concentrations of Σdl-PCBs in cordgrass samples were quite low and roots and leaves showed similar values.

On a wet weight basis (Fig. 3a), Σdl-PCB concentration increased from primary producers, to benthic invertebrates and ultimately to fish, with eel presenting the greatest value (2.808 ng g^{-1} ww), followed by sea bass (0.598 ng g^{-1} ww) and sole (0.143 ng g^{-1} ww). Lipid-normalised concentrations were higher on both fish and macroalgae (Fig. 3b). Common shrimp showed quite low values of dl-PCB contamination, as well as of PCDD/Fs.

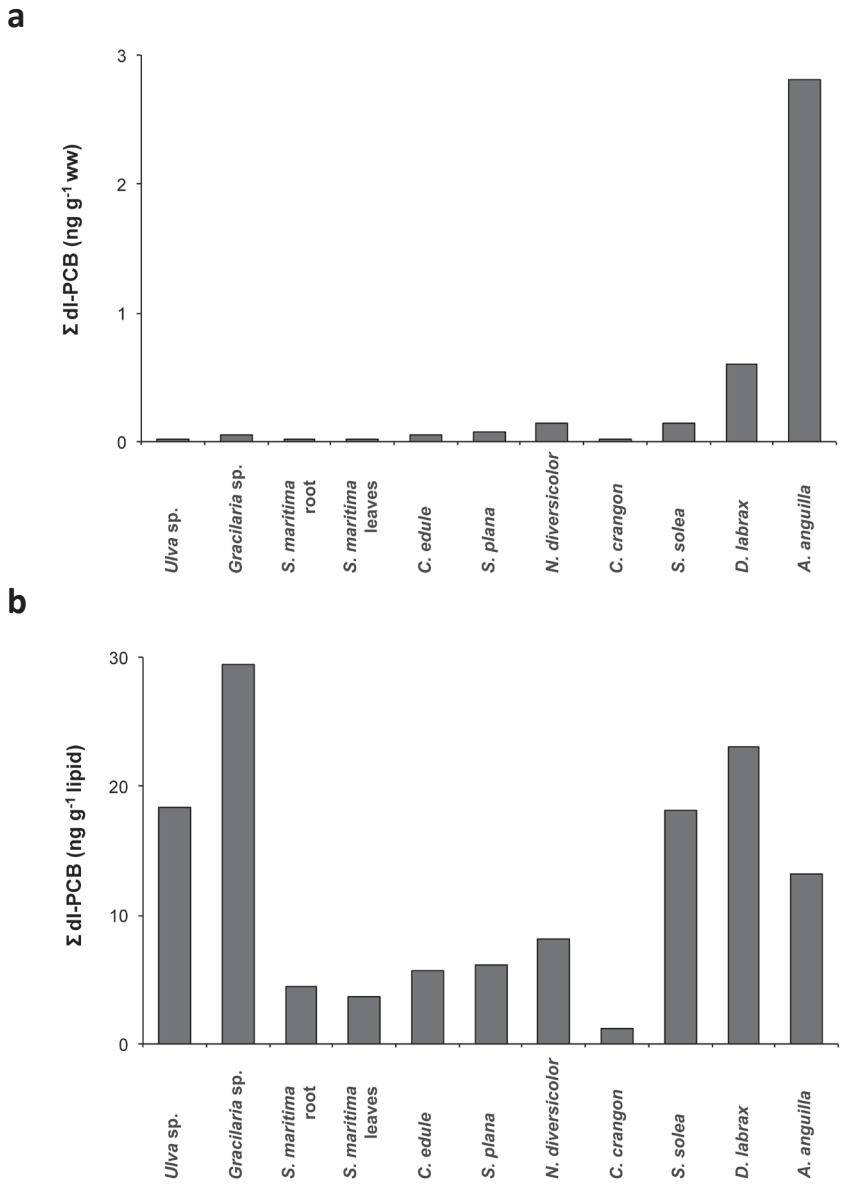

Figure 3 Total concentration of dioxin-like PCBs in biota from the Mondego estuary expressed in (**a**) pg g^{-1} of wet weight and in (**b**) pg g^{-1} of lipid weight.

The contribution of mono-*ortho* PCBs to the total dl-PCB concentration ranged between 92.10% (sediment) and 99.94% (eel), while non-*ortho* PCBs represented a very small percentage (0.35–7.90%). The very lower contribution of non-*ortho* substituted congeners is in accordance, for example, with findings of Okumura et al. (2003) for sediments, and of Bordajandi et al. (2006) for seafood and fish from the Spanish Atlantic southwest coast.

Numerous factors can influence the contamination levels of aquatic animals, for instance, biological factors such as age or size class, reproductive status, trophic level, and

environmental factors such as season or habitat location (Matthews et al. 2008; Niimi 1996; Pandelova et al. 2007). While this study attempted to analyze a wide range of species from the Mondego estuary, the number of samples is limited and does not allow further conclusions on PCDD/F and PCB contamination.

PCDD/F and dl-PCB profiles

PCDD/F homologue profiles differed among the samples collected in the Mondego estuary (Fig. 4a). Wu et al. (2001) reported that in the food web of Ya-Er Lake area (China) some aquatic plants and benthic invertebrate animals can accumulate PCDD/Fs from sediment or suspended particulate matter and maintained the sediment pattern, whereas organisms at higher levels of the food web (i.e. fish) tended to selectively accumulate some 2,3,7,8-substituted congeners. A similar picture could also be observed in the present survey, being possible to recognize two main profiles. The first (for sediment, algae, cordgrass, bivalves and ragworm) was mainly characterized by the presence of OCDD and 1,2,3,4,6,7,8-HpCDD, which together accounted for more than 78% of total PCDD/F concentration. In contrast, fish samples contained a wide range of congeners. Furthermore, there is no distinct predominance of one particular congener, and OCDD and 1,2,3,4,6,7,8-HpCDD combined accounted for no more than 35%. The common shrimp presented an intermediate pattern, in which these two compounds together contributed to 50% of the ΣPCDD/Fs. The higher percentage contribution of OCDD in invertebrates in comparison with fish was already reported in other studies (e.g. Matthews et al. 2008). This congener is known to be present in high proportions in sewage sludge (Baker and Hites 2000). Geyer et al. (2000) also reports that OCDD is often the most prevalent PCDD found in pentachlorophenol (PCP). PCP has primarily been utilized in the timber processing industry to protect against fungal rot or wood-boring insects, but may also be used as a general pre-emergence herbicide and as a biocide in industrial water systems. However, the authors are not aware of information related to the application of PCP within the Mondego drainage basin. It should be noted that the high contribution of OCDF observed in fish samples is unexpected, since compounds with higher levels of chlorination and high K_{OW} values are usually reported to accumulate in organisms to a lesser extent and at slower rates because of steric constraints and high hydrophobicity (Marvin et al. 2002; Ruus et al. 2006).

In general, dl-PCB profiles showed a predominance of the mono-*ortho* PCB 118 and PCB 105, with exception of *S. maritima* roots were PCB 189 prevailed (Fig. 4b). Profiles were very similar for all matrices and the two prevalent congeners together accounted for 64%

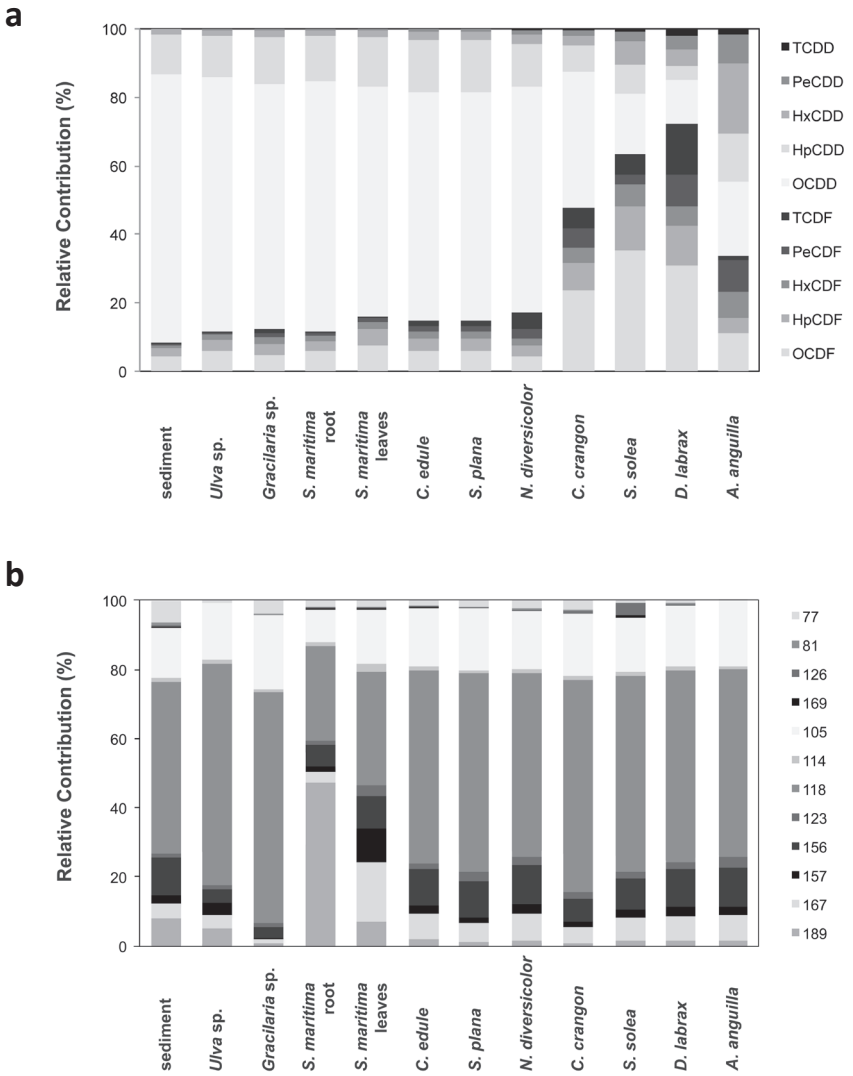

Figure 4 Relative contribution of (**a**) 2,3,7,8-substituted PCDD/F homologues and (**b**) dioxin-like PCB congeners in sediments and biota from the Mondego estuary.

to 88% of total dl-PCB concentration. The exceptions to the common pattern were *S. maritima* samples, where these compounds represented only 37% and 48%, in roots and leaves, respectively. In agreement with our data, the predominance of PCB 118 and PCB 105 congeners has been reported in various matrices in the environment (Bayarri et al. 2001; Bodin et al. 2007; Okay et al. 2009). Results suggest that congener profiles may not be altered by differential metabolism and other toxicokinetic differences of the various species. If the individual congeners were differently metabolized and taken up by organisms (Kay et al. 2005), it would be expected that the proportion of each congener could change along the food web and the profile in higher trophic animals (i.e., fish) might be quite different

from that of the source. However, our data showed similarities in dl-PCB profiles among the studied samples, apart from cordgrass.

WHO-TEQ values

WHO-TEQ_{2005} concentration based on current TEFs for humans ranged from 0.01 and 0.59 pg g^{-1} ww. The highest WHO-TEQ levels corresponded to fish, with eel exhibiting the highest value (data available in Appendix D). Differences between the relative contribution of WHO-PCDD/F-TEQ and WHO-PCB-TEQ to the total WHO-TEQ were observed. Eel samples presented a clearly higher percentage of PCDD/Fs (> 75%) of the total WHO-TEQ, while in other matrices PCDD/F-TEQ and PCB-TEQ accounted for similar contributions. On contrary, in sea bass and sole samples, the WHO-TEQ of PCDD/Fs was very low (< 20 %), with dl-PCBs contributing to the majority of the TEQ values.

The European Commission established maximum permissible levels of 4 pg WHO-TEQ_{1998} g^{-1} ww for PCDD/Fs and 8 pg WHO-TEQ_{1998} g^{-1} ww for the sum of PCDD/Fs and dl-PCBs in the muscle meat of fish, excluding eel, and fishery products for human consumption (EC 2006a). The maximum levels defined for eel are 4 WHO-TEQ_{1998} g^{-1} ww for PCDD/Fs and 12 WHO-TEQ_{1998} g^{-1} ww for the sum of PCDD/Fs and dl-PCBs. The bivalves, common shrimp, sole, sea bass and eel samples analyzed in the current survey presented levels well below those established by European legislation (data available in Appendix D).

Conclusions

Based upon this study, contamination levels found in the study area were quite lower compared with levels reported in impacted ecosystems around the world (e.g., Eljarrat et al. 2001; Kim et al. 2009; Schelepchikov et al. 2008; Suarez et al. 2005; Verta et al. 2007; Wu et al. 2001). However, only a part of the Mondego estuary was contemplated in the present survey, and future research on PCDD/Fs and PCBs in all extension of the Mondego estuary should be considered.

This initial investigation into contamination of the Mondego estuary suggests different behaviors of PCDD/Fs and PCBs along the food web. While concentrations of PCDD/Fs were lower in higher trophic-level organisms, higher Σdl-PCB values were found in upper-level biota, although not exclusively. However, it should be referred that concentrations of pollutants were measured only in muscle tissue and not in whole fish, whereas it is

well known that these compounds are also accumulated in other body parts, as liver and gonads. In addition, our results showed differences between PCDD/F and dl-PCB profiles. Regarding PCDD/F homologues, macroalgae, aquatic plants and benthic invertebrate animals maintained the sediment pattern, whereas organisms at higher levels of the food web (i.e., fish) tended to selectively accumulate PCDD/F congeners. On the contrary, quite similar dl-PCB congener profiles were observed in the different trophic levels (with the exception of cordgrass).

Acknowledgements

The authors would like to thank to the colleagues that have contributed for this study, namely through field and laboratory work. This study was supported by the FCT – Fundação para a Ciência e Tecnologia through a PhD grant attributed to MN Cardoso (SFRH/BD/46969/2008) co-funded by the POPH/FSE.

CHAPTER

VI General conclusions and final remarks

The environmental consequences of POPs and their threats to human health, due to their persistence and high toxicity, led to the establishment of the Stockholm Convention on Persistent Organic Pollutants in 2001 (UNEP 2001). This treaty, which entered into force on May 2004, establishes a strong international framework for promoting global action on POPs and focuses on eliminating or reducing their environmental releases (Stockholm Convention 2008). In this context, several regulatory measures were adopted, resulting in a significant decrease of environmental emissions of contaminants such as PCDD/Fs and PCBs (EEA 2013). However, due to their high stability and resistance to degradation, these compounds released from former sources, can persist in soils, sediments and waste reservoirs over decades or even centuries (Weber et al. 2008). Therefore, both PCDD/Fs and dl-PCBs continue to be detected at dangerous levels in a multitude of matrices all over the globe (Sundqvist et al. 2009; Chan et al. 2013; Hu et al. 2013; Miller et al. 2013). Special attention has been paid to the estuarine environment since they receive and retain large quantities of organic pollutants (Bellucci et al. 2000; Müller et al. 2002; Hu et al. 2005; Salo et al. 2008). The lack of data regarding PCDD/F and dl-PCB concentrations in Portuguese estuaries prompted a survey to obtain relevant information about the national contamination status of this environment. This work confirmed the ubiquitous presence

of these organic pollutants in Portuguese estuaries, as has been reported in other similar systems worldwide (Brochu et al. 1995; Hurst et al. 2004; Müeller et al. 2004; Gómez-Lavín et al. 2011; Piazza et al. 2010).

Pollutants with low water solubility and high particle affinity have the tendency to settle with particles and accumulate in the bottom sediment (Dueri et al. 2008). Regarding PCDD/F and dl-PCB contamination, in the aquatic environment, sediment is considered a better descriptor than water (Rose et al. 1994). Moreover, sediments comprise an important component of the aquatic ecosystem, providing habitat for a wide range of benthic organisms and consequently, representing an established route of entry of pollutants into the aquatic trophic web (Forbes et al. 1998; Wan et al. 2005). In this regard, it is important to gain a better knowledge of the sediment chemical contamination and consequent risk to biota. Chapter II provided a general idea of the occurrence of PCDD/Fs and dl-PCBs in sediments from several estuaries along the Portuguese coast. The studied estuarine systems showed a wide range of PCDD/F and dl-PCB levels. In general, the higher concentrations were detected near large populated regions and industrial complexes (e.g. Tejo estuary), while the lowest PCDD/F and dl-PCB values were measured in less impacted areas (e.g. Ria Formosa). Similar results were observed in previous studies, with concentrations decreasing with increasing distance from industrialized areas (Bazzanti et al. 1997; Gómez-Gutiérrez et al. 2007; Moon et al. 2012; Grilo et al. 2013). Since both PCDD/Fs and dl-PCBs are historically associated with industrial activities (Erickson 1997; Altarawneh et al. 2009; Weber et al. 2008), the results are as expected, even though the environmental releases have declined significantly over the years (EEA 2013).

The different PCDD/F profiles detected among the Portuguese estuaries suggest the existence of different contamination sources. For instance, the profiles found in sediment from the Tejo estuary showed higher proportion of PCDFs, which have been related with emissions from industrial and urban areas in past studies (Terauchi et al. 2009; Antunes et al. 2012). Furthermore, this work showed that the most abundant PCDD/F congeners existing in superficial sediments are those with more chlorine substitutions. Their lower water solubility and greater affinity to fine particles could result in relatively long term accumulation, especially in organic rich sediment, explaining the greater proportion of the congener OCDD in estuarine sediment (Sinkkonen and Paasivirta 2000; Hu et al. 2005; Wan et al. 2005). In contrast, the dl-PCB profiles were fairly similar among the estuaries and less variable when compared with those of PCDD/Fs. Moreover, the predominance of PCB 118 observed in sediment is in agreement with prior research (El-Kady et al. 2007; Okay et al.

2009).

The data presented in Chapter II provides a general insight into the sediment contamination of several Portuguese estuaries by dioxin-like compounds, allowing a comparison with PCDD/F and dl-PCB levels reported in other countries. In addition, the work developed may also establish a basis for future monitoring programs aimed at evaluating if the environmental PCDD/F and PCB concentrations in Portuguese estuaries reflect the effectiveness of the existing policies and legislation implemented to reduce the emissions of POPs.

The chemical contaminants existent in aquatic ecosystems have the potential to cause adverse effects on biota. Thus, the need for appropriate legislation to ensure that such impairments are minimal or non-existent has long been recognized. In Europe, the Water Framework Directive (WFD; EC 2000) was introduced to protect and improve the ecological status of European aquatic environments, including transitional waters such as estuaries. The recent Directive 2013/39/EU added the PCDD/Fs and dl-PCBs to the priority substances list (EC 2013). However, despite good chemical quality of a water body being also dependent on sediment, no environmental quality standards (EQS) were defined for these dioxin-like compounds in sediment (Dueri et al. 2008). Nonetheless, the development of EQS values for this aquatic compartment is particularly important for hydrophobic contaminants, since these may be taken up by organisms in close contact with sediment (Forbes et al. 1998; Wan et al. 2005). In addition, no EQS exist for PCDD/Fs and dl-PCBs concentrations in sediment at a national level and therefore, no threshold is available to evaluate the risk Portuguese estuarine sediments may pose to aquatic organisms. The present work showed that guideline values and quality standards defined for other countries were exceeded in some locations. Nevertheless, a proper assessment of their applicability regarding Portuguese estuaries should be done to assure a correct evaluation of the hazard these sediments represent to aquatic organisms.

Given the PCDD/F and dl-PCB contamination of salt marshes sediments and the significant contribution of plants to the detrital estuarine food web (Wall et al. 2001; Sousa et al. 2010), Chapter III focuses on *Sarcocornia perennis* and *Halimione portulacoides* capacity to accumulate these chemical compounds. According to this work carried out in the Tejo estuary (Portugal), the selected salt marsh plants retain PCDD/Fs and dl-PCBs in their organs. However, the major fraction of these contaminants remains associated with sediment. In addition, both plant species accumulate significantly higher concentrations of PCDD/Fs and dl-PCBs in its roots in comparison with its aboveground tissues, suggesting that despite part of the sediment contaminants being incorporated in the roots, they are not substantially

translocated to the aboveground vegetation. These results are consistent with the existing literature regarding the limited translocation of these highly hydrophobic compounds (Hülster and Marschner 1993; Liu and Schnoor 2008).

Once contaminants are incorporated in plant tissues, they can enter the estuarine trophic web. Although salt marsh plants are not a usual direct food source for organisms, as the halophytes decay, they become reduced to organic detritus that detritivores and juvenile fish can consume. However, according to this work, the amounts of PCDD/Fs and dl-PCBs accumulated in *S. perennis* and *H. portulacoides* are minimal when compared with the amounts existing in sediment. Therefore, the consumption of detrital plant material by estuarine organisms and consequent toxicological risk resulting from faunal uptake is probably of no consequence in the studied system.

This study showed that *S. perennis* accumulate lower quantities of dioxin-like compounds in comparison with *H. portulacoides*, confirming the diversity in uptake and translocation of organic contaminants among plant species (Liu and Schnoor 2008; Matsuo et al. 2011). The disparities found between concentrations in belowground tissues of *S. perennis* and *H. portulacoides* may be caused by a distinct type or amount of organic exudates released from the roots of the studied species and/or may be a result of differences in root lipid contents (Ryan et al. 2001; Dettenmaier et al. 2009). A plant with higher capability for taking up organic contaminants from sediment can have higher potential for food web contamination but can also have a higher potential to remove the target compound from contaminated environments in phytoremediation applications. However, despite *H. portulacoides* having a higher accumulation capability for PCDD/Fs and dl-PCBs, the amount of contaminants involved is so low that, independently of the plant species, the trophic web contamination and the phytoremediation efficacy are likely to be negligible.

It was also possible to verify that salt marsh sediments without vegetation show higher PCDD/F and dl-PCB concentrations, suggesting that salt marsh plants may contribute to reduce dioxin-like compound concentrations in contaminated sediments, although in a small extent. Even when compounds are slightly incorporated by plants, as is the case with *S. perennis* and *H. portulacoides*, roots can interact with the surrounding sediment by different processes, stimulating the microbial activity in the rhizosphere and increasing compound degradation and/or removal (Alkorta and Garbisu 2001; Chaudhry et al. 2005). Moreover, PCDD/F and dl-PCB profiles changed between sediments and plant tissues, reflecting a selective accumulation of low chlorinated PCDD/Fs and non-*ortho* dl-PCBs in the studied plants.

Chapter IV presented, for the first time, values on juvenile flounder (*Platichthys flesus*) contamination by PCDD/Fs and dl-PCBs in a number of nursery areas along the species' geographical distribution in the northeastern Atlantic Ocean. Although the determination of dioxin-like compounds in fish is important from the human health point of view, it also essential to evaluate the risk posed by PCDD/Fs and dl-PCBs to fish populations. The exposure of juvenile fish to these contaminants might induce disruption in their growth and survival, compromising the viability of adult population (Courrat et al. 2009). According with results obtained, the highest tissue residue levels were detected in juveniles caught in the Wadden Sea (Netherlands), in agreement with the long history of pollution reported in this area (Duinker 1986; Vethaak and Jol 1996; Becker et al. 2001; Vethaak et al. 2009; Laane et al. 2013). The dl-PCB concentrations found in *P. flesus* from the Mondego estuary (Portugal) were noticeable low when compared with the other studied areas. Nevertheless, these dl-PCB values are higher than those found in adults caught in the same location (Baptista et al. 2013). The migration of adult *P. flesus* to marine waters (Martinho et al. 2013), a less contaminated environment (Gómez-Gutiérrez et al. 2007; Parnell et al. 2008), may be related with the decreasing contamination levels throughout *P. flesus* lifespan in the Mondego estuary. Growth dilution, defined as the reduction of biomass-specific concentration of pollutants due to accumulation of new biomass, may also explain the decrease of dioxin-like compound levels in adult *P. flesus* (Buckman et al. 2006). In the present study, the PCDD/F and dl-PCB concentrations detected in European flounder juveniles were below the residue effect concentration established by Steevens et al. (2005) and, thus, no adverse effects caused by dioxin-like compounds are expected in fish.

A preliminary survey was carried out to investigate the occurrence of PCDD/Fs and dl-PCBs in sediment and biota from the Mondego estuary. The contamination levels found in the study area, whether in sediment or in organisms, were lower when compared with PCDD/F and dl-PCB concentrations reported in impacted estuarine and coastal systems around the world. Results presented in Chapter V also revealed that higher PCDD/F values were detected in lower trophic-level organisms. According to Wu et al. 2005, it can be related with a less effective uptake of PCDD/Fs in organisms at higher trophic levels and in addition, biota at a higher position in the food web may have a higher PCDD/F metabolism. Contrary to PCDD/Fs, the highest dl-PCB levels were generally found in fish.

Contaminated sediments can act as a source of pollutants to the aquatic trophic web (Forbes et al. 1998; Wan et al. 2005). However, the specific properties of each PCDD/F and dl-PCB congener affect its bioavailability, bioaccumulation and metabolism in biota, together with

its biomagnification along the food web (Wan et al. 2005). As a consequence, the relative concentration of individual chemicals compounds in organisms usually does not reflect the profile observed in the sediment, and varies with species and trophic level. In the Mondego estuary, macroalgae, plants and benthic invertebrates maintained the sediment PCDD/F profile, whereas organisms at higher levels of the food web (i.e., fish) tend to selectively accumulate lower chlorinated PCDD/F homologues. Ruus et al. (2006) also verified a similar change in the relative contribution of PCDD/F homologues with trophic level. On the other hand, quite similar dl-PCB profiles were observed in the different species from the Mondego estuary (except algae and plants).

In the context of human health, the concentrations found in edible aquatic organisms collected in the Mondego estuary were below the maximum permissible levels established by the European legislation (EC 2006) and therefore, are safe for human consumption.

During the course of this work, aspects about the occurrence of PCDD/Fs and dl-PCBs in Portuguese estuaries have been clarified. Nevertheless, some uncertainties remained, namely, the risks PCDD/Fs and dl-PCBs present in estuarine sediments could pose to aquatic organisms. The determination of the ecological risk of contaminated sediments has long been recognized as a key issue to assess the effects of anthropogenic pressure onto the natural environments. Therefore, future research efforts should be directed towards the development of national EQS for estuarine systems to allow an assessment of the potential of PCDD/F and dl-PCB sediment contamination to adversely affect aquatic organisms.

Appendix A

Mean values of total organic carbon (TOC), fine particles content (< 63 µm) and concentration (pg g^{-1} dw) of 2,3,7,8-substituted PCDD/Fs and dioxin-like PCBs in superficial sediments from seven Portuguese estuarine systems.

Location		TOC (%)	Fine particles (%)	2.3.7.8-TCDD	1.2.3.7.8-PeCDD	1.2.3.4.7.8-HxCDD	1.2.3.6.7.8-HxCDD	1.2.3.7.8.9-HxCDD	1.2.3.4.6.7.8-HpCDD	OCDD	2.3.7.8-TCDF	1.2.3.7.8-PeCDF	2.3.4.7.8-PeCDF	1.2.3.4.7.8-HxCDF	1.2.3.6.7.8-HxCDF	1.2.3.7.8.9-HxCDF	2.3.4.6.7.8-HxCDF	1.2.3.4.6.7.8-HpCDF	1.2.3.4.7.8.9-HpCDF	OCDF	PCB 77	PCB 81	PCB 126	PCB 169	PCB 105	PCB 114	PCB 118	PCB 123	PCB 156	PCB 157	PCB 167	PCB 189
Lima	A	1.7	23	0.02	0.09	0.10	0.41	0.38	5.81	31.53	0.13	0.10	0.12	0.17	0.13	0.02	0.10	1.01	0.06	1.57	3.51	0.17	0.56	0.14	18.49	0.93	54.75	2.63	12.13	3.51	6.60	2.18
	B	2.3	33	0.01	0.13	0.14	0.64	0.72	9.19	44.70	0.20	0.14	0.16	0.22	0.17	0.03	0.13	1.34	0.12	2.49	4.63	0.25	0.74	0.30	14.14	0.52	43.04	2.35	11.22	2.58	6.51	2.42
	C	2.4	32	0.03	0.25	0.24	1.33	1.36	16.93	96.88	0.26	0.20	0.25	0.35	0.30	0.09	0.25	2.84	0.22	6.39	6.88	0.32	1.22	0.26	21.84	1.28	58.79	3.70	17.85	3.86	9.50	3.97
Ria de Aveiro	A	1.6	28	0.01	0.08	0.10	0.36	0.35	4.26	22.26	0.12	0.09	0.11	0.19	0.10	0.04	0.08	0.84	0.08	1.53	3.09	0.17	0.31	0.15	9.69	0.51	31.02	1.59	8.26	1.52	3.61	3.00
	B	2.0	33	0.02	0.13	0.14	0.63	0.61	6.91	35.35	0.14	0.09	0.12	0.19	0.11	0.03	0.09	0.78	0.08	1.32	3.19	0.20	0.47	0.16	13.41	0.62	48.81	3.11	12.23	2.57	7.61	4.00
	C	3.9	50	0.04	0.29	0.43	1.16	1.19	11.96	53.86	0.30	0.25	0.29	0.36	0.26	0.06	0.20	1.63	0.14	2.78	8.23	0.53	1.74	0.48	45.22	2.41	120.83	4.82	25.40	6.22	15.22	5.00
	D	2.7	44	0.02	0.13	0.13	0.40	0.47	4.39	23.36	0.15	0.15	0.16	0.23	0.16	0.04	0.12	0.96	0.07	1.52	3.03	0.18	0.94	0.30	11.13	0.61	33.02	1.79	9.36	1.30	4.75	6.00
	E	2.5	42	0.08	0.37	0.40	2.06	1.51	39.08	244.88	2.33	3.47	2.45	8.11	2.50	0.57	1.31	13.13	3.21	31.33	18.06	0.91	4.99	1.27	103.69	8.27	380.56	9.98	159.10	23.75	79.77	7.00
	F	2.7	46	0.04	0.24	0.20	1.02	0.99	10.87	63.71	0.37	0.34	0.32	0.56	0.27	0.07	0.21	1.95	0.33	4.62	5.77	0.36	1.32	0.31	24.10	1.03	65.76	3.51	19.43	3.38	9.88	8.00
Mondego	A	2.3	36	0.03	0.23	0.25	0.81	0.74	13.53	90.56	0.29	0.21	0.31	0.39	0.29	0.07	0.30	2.44	0.28	5.04	7.84	0.33	2.27	0.22	35.27	3.21	117.86	5.33	42.10	6.44	22.44	9.54
	B	1.4	22	0.02	0.09	0.10	0.36	0.33	5.70	45.08	0.16	0.14	0.13	0.23	0.15	0.04	0.11	1.09	0.14	2.48	2.73	0.27	0.81	0.75	7.68	0.75	26.75	1.17	4.97	1.33	3.20	1.28
	C	2.0	30	0.03	0.17	0.21	0.73	0.73	12.65	89.36	0.28	0.16	0.21	0.26	0.25	0.07	0.22	2.07	0.22	4.59	4.12	0.21	1.07	0.33	16.72	1.50	51.42	2.43	10.78	2.80	6.04	2.57
Tejo	A	1.7	30	0.05	0.34	0.51	1.50	1.27	44.33	267.97	0.89	1.92	1.97	6.29	5.33	1.39	3.40	42.86	6.17	77.74	39.86	1.80	5.04	1.11	425.72	23.39	1062.50	35.68	176.32	43.35	76.67	20.53
	B	2.6	43	0.07	0.37	0.53	1.73	1.63	23.32	119.72	1.20	1.83	1.89	5.99	3.95	0.77	2.05	31.58	4.84	55.87	48.30	2.15	6.26	1.44	418.02	18.75	1000.92	32.30	176.42	37.75	86.47	27.35
	C	1.9	29	0.10	0.40	0.48	1.25	1.33	16.68	96.10	1.27	1.44	2.05	4.47	3.70	1.52	2.48	30.84	4.20	52.66	15.73	0.89	4.05	1.09	130.17	3.57	326.22	14.24	72.46	17.39	31.83	15.30
	D	3.1	49	0.08	0.36	0.47	1.51	1.45	24.02	145.29	1.39	1.45	2.07	4.89	3.69	0.79	2.99	32.82	4.79	55.46	62.72	2.62	15.67	1.58	1879.03	108.85	4672.08	149.44	1049.73	165.57	455.99	129.47
	E	2.7	41	0.05	0.26	0.30	1.05	1.24	17.26	99.01	1.04	0.97	1.20	2.68	2.17	0.77	1.56	19.26	2.69	37.54	12.83	0.59	2.84	0.75	116.56	5.19	332.72	12.75	105.39	17.00	43.68	15.99
Sado	A	2.9	31	0.03	0.23	0.13	0.66	1.05	3.25	19.32	0.45	0.32	0.36	0.33	0.34	0.10	0.26	1.27	0.11	1.14	9.54	0.55	3.01	0.71	33.29	2.13	125.20	2.11	18.69	7.56	12.62	9.42
	B	5.8	52	0.14	0.60	0.63	2.35	2.34	37.78	230.30	2.97	1.15	2.23	1.83	1.47	0.37	1.22	10.02	1.04	14.45	171.88	6.22	72.08	10.48	1274.34	65.54	3420.85	140.73	1001.44	186.66	612.59	234.60
	C	2.2	42	0.06	0.32	0.19	0.90	1.46	5.67	31.23	0.98	0.63	0.66	0.59	0.51	0.27	0.43	1.82	0.17	1.51	13.27	0.97	3.39	0.90	45.85	3.09	99.72	7.63	28.53	5.54	5.52	5.11
	D	4.3	50	0.07	0.47	0.46	1.37	1.46	12.59	64.02	0.73	1.15	2.05	2.73	2.61	0.59	2.98	15.71	1.03	6.56	9.52	1.00	5.28	1.67	28.45	2.33	89.44	2.34	16.13	4.73	10.65	4.19
	E	2.1	38	0.15	0.80	0.66	1.88	2.15	13.18	74.30	1.81	1.87	3.57	3.48	3.56	1.36	3.85	15.53	1.09	6.51	12.99	0.87	6.44	1.96	63.75	4.70	150.32	7.63	45.33	16.82	26.30	10.16
Mira	A	2.4	31	0.07	0.23	0.17	0.66	0.71	3.88	27.49	0.42	0.40	0.60	0.69	0.70	0.20	0.53	2.92	0.31	2.31	4.69	0.57	1.54	0.54	22.10	3.12	51.44	3.11	11.77	3.20	6.30	6.75
	B	2.6	48	0.02	0.19	0.14	0.68	0.82	4.07	28.80	0.23	0.24	0.20	0.42	0.39	0.18	0.17	2.02	0.13	1.91	8.73	0.15	1.20	0.12	37.09	3.46	99.83	2.97	3.85	2.36	3.30	8.51
	C	3.1	52	0.02	0.12	0.07	0.62	0.89	2.12	32.47	0.21	0.14	0.12	0.18	0.25	0.22	0.10	0.90	0.07	1.25	0.86	0.24	0.24	0.24	8.45	2.50	27.36	2.44	2.60	2.73	3.43	5.75
	D	1.9	29	0.03	0.13	0.09	0.66	0.92	2.55	37.01	0.25	0.12	0.12	0.16	0.25	0.19	0.07	0.70	0.07	1.13	1.68	0.18	0.37	0.20	10.98	1.52	23.71	2.07	9.51	2.69	4.69	3.64
Ria Formosa	A	6.1	57	0.15	0.50	0.41	1.05	1.43	15.18	291.95	0.43	0.27	0.34	0.34	0.36	0.08	0.25	1.64	0.13	2.06	11.25	0.87	3.52	1.09	34.95	2.57	81.15	4.17	12.24	4.45	6.28	7.83
	B	1.5	24	0.03	0.05	0.03	0.11	0.14	1.40	10.09	0.10	0.05	0.06	0.10	0.08	0.02	0.06	0.44	0.03	0.51	3.97	0.24	1.13	0.50	5.91	1.68	25.92	1.69	5.16	1.79	3.02	3.59
	C	2.0	40	0.01	0.02	0.03	0.07	0.08	0.87	6.54	0.15	0.04	0.04	0.06	0.04	0.02	0.02	0.33	0.03	0.48	1.79	0.27	0.27	0.27	17.88	0.98	35.60	0.98	1.20	1.09	1.09	4.97
	D	2.4	36	0.01	0.03	0.02	0.09	0.14	0.48	3.00	0.10	0.03	0.03	0.06	0.04	0.03	0.02	0.26	0.02	0.29	1.26	0.12	0.22	0.19	3.14	1.25	9.41	1.25	1.32	1.87	1.35	5.21
	E	1.8	30	0.01	0.07	0.09	0.45	0.45	6.53	45.07	0.19	0.09	0.10	0.14	0.16	0.06	0.09	0.99	0.08	1.57	2.92	0.44	0.75	0.46	11.31	2.40	47.46	2.42	7.65	3.76	6.16	6.34

Appendix B

Concentration of 2,3,7,8-substituted PCDD/Fs and dioxin-like PCBs in sediment (pg g^{-1} dw) and plants (pg g^{-1} dw) collected in a salt marsh from the Tejo estuary. Fine particle (< 63 µm) content and percentage of total organic carbon (TOC) of uncovered sediment and rhizosediment of *Sarcocornia perennis* and *Halimione portulacoides* are also presented. Results are expressed as the mean ± standard deviation (n=5).

	Sediment			Plants					
				S. perennis			*H. portulacoides*		
	Uncovered	*S. perennis*	*H. portulacoides*	Roots	Stems	Leaves	Roots	Stems	Leaves
2,3,7,8-TCDD	0.097 ± 0.015	0.074 ± 0.007	0.071 ± 0.014	0.028 ± 0.005	0.004 ± 0.002	0.006 ± 0.002	0.050 ± 0.009	0.009 ± 0.002	0.007 ± 0.002
1,2,3,7,8-PeCDD	0.415 ± 0.076	0.349 ± 0.062	0.319 ± 0.095	0.080 ± 0.017	0.010 ± 0.004	0.010 ± 0.001	0.138 ± 0.043	0.024 ± 0.005	0.015 ± 0.003
1,2,3,4,7,8-HxCDD	0.535 ± 0.073	0.428 ± 0.078	0.430 ± 0.157	0.053 ± 0.005	0.008 ± 0.003	0.007 ± 0.003	0.081 ± 0.019	0.016 ± 0.005	0.009 ± 0.003
1,2,3,6,7,8-HxCDD	1.793 ± 0.248	1.484 ± 0.201	1.293 ± 0.215	0.187 ± 0.021	0.025 ± 0.008	0.016 ± 0.004	0.295 ± 0.080	0.041 ± 0.010	0.020 ± 0.005
1,2,3,7,8,9-HxCDD	1.677 ± 0.223	1.362 ± 0.217	1.279 ± 0.371	0.146 ± 0.006	0.018 ± 0.007	0.011 ± 0.004	0.217 ± 0.048	0.034 ± 0.006	0.020 ± 0.003
1,2,3,4,6,7,8-HpCDD	29.602 ± 5.145	28.298 ± 3.334	27.331 ± 1.873	1.647 ± 0.216	0.188 ± 0.064	0.148 ± 0.023	3.036 ± 1.230	0.367 ± 0.102	0.208 ± 0.041
OCDD	175.132 ± 35.783	166.145 ± 25.320	184.020 ± 60.284	7.636 ± 1.205	0.753 ± 0.210	0.635 ± 0.057	14.645 ± 7.233	1.507 ± 0.497	0.917 ± 0.218
ΣPCDDs	209.252 ± 41.084	198.140 ± 28.828	214.744 ± 61.534	9.776 ± 1.412	1.006 ± 0.293	0.833 ± 0.077	18.464 ± 8.478	1.998 ± 0.613	1.196 ± 0.267
2,3,7,8-TCDF	1.503 ± 0.158	1.254 ± 0.144	1.507 ± 0.771	0.556 ± 0.099	0.068 ± 0.017	0.074 ± 0.029	0.885 ± 0.195	0.172 ± 0.124	0.081 ± 0.041
1,2,3,7,8-PeCDF	1.627 ± 0.245	1.320 ± 0.202	1.030 ± 0.196	0.256 ± 0.026	0.038 ± 0.011	0.036 ± 0.002	0.400 ± 0.114	0.059 ± 0.015	0.031 ± 0.008
2,3,4,7,8-PeCDF	2.264 ± 0.540	1.791 ± 0.216	1.556 ± 0.523	0.407 ± 0.044	0.042 ± 0.013	0.032 ± 0.006	0.681 ± 0.176	0.091 ± 0.026	0.038 ± 0.009
1,2,3,4,7,8-HxCDF	5.094 ± 0.733	4.210 ± 0.568	3.158 ± 0.819	0.468 ± 0.044	0.073 ± 0.017	0.059 ± 0.011	0.871 ± 0.257	0.119 ± 0.029	0.078 ± 0.023
1,2,3,6,7,8-HxCDF	3.962 ± 0.702	3.240 ± 0.535	2.425 ± 0.755	0.363 ± 0.036	0.049 ± 0.014	0.034 ± 0.003	0.630 ± 0.169	0.093 ± 0.027	0.042 ± 0.013
1,2,3,7,8,9-HxCDF	0.576 ± 0.259	0.376 ± 0.085	0.299 ± 0.106	0.062 ± 0.014	0.006 ± 0.003	0.004 ± 0.001	0.087 ± 0.020	0.011 ± 0.006	0.006 ± 0.002
2,3,4,6,7,8-HxCDF	3.462 ± 1.017	2.697 ± 0.336	2.202 ± 0.660	0.345 ± 0.026	0.038 ± 0.012	0.024 ± 0.001	0.539 ± 0.155	0.076 ± 0.023	0.034 ± 0.007
1,2,3,4,6,7,8-HpCDF	34.637 ± 6.107	26.968 ± 2.789	21.443 ± 5.047	2.205 ± 0.335	0.299 ± 0.065	0.256 ± 0.010	3.849 ± 1.341	0.524 ± 0.147	0.314 ± 0.081
1,2,3,4,7,8,9-HpCDF	5.156 ± 0.811	4.126 ± 0.624	3.181 ± 0.827	0.278 ± 0.042	0.036 ± 0.009	0.027 ± 0.003	0.448 ± 0.156	0.055 ± 0.018	0.034 ± 0.010
OCDF	57.717 ± 9.704	48.967 ± 4.950	43.341 ± 14.784	2.574 ± 0.472	0.366 ± 0.093	0.344 ± 0.029	4.013 ± 1.402	0.549 ± 0.177	0.426 ± 0.097
ΣPCDFs	115.998 ± 18.294	94.950 ± 10.289	80.142 ± 22.057	7.515 ± 0.972	1.015 ± 0.242	0.890 ± 0.066	12.403 ± 3.826	1.749 ± 0.469	1.084 ± 0.276
ΣPCDD/Fs	325.250 ± 57.547	293.090 ± 39.029	294.895 ± 75.878	17.291 ± 1.585	2.021 ± 0.530	1.723 ± 0.131	30.867 ± 11.933	3.747 ± 1.070	2.280 ± 0.536

(Continued)

	Sediment			Plants					
	Uncovered	*S. perennis*	*H. portulacoides*	*S. perennis*			*H. portulacoides*		
				Roots	Stems	Leaves	Roots	Stems	Leaves
PCB 77	72.540 ± 8.410	66.681 ± 23.496	70.276 ± 12.327	37.268 ± 6.325	6.400 ± 1.980	5.528 ± 0.937	82.100 ± 16.966	14.461 ± 3.287	41.677 ± 7.317
PCB 81	3.117 ± 0.363	2.542 ± 0.711	2.288 ± 0.358	1.557 ± 0.280	0.253 ± 0.096	0.308 ± 0.061	3.432 ± 0.817	0.494 ± 0.100	0.990 ± 0.235
PCB 126	19.095 ± 2.587	15.444 ± 7.449	14.141 ± 4.188	5.605 ± 0.513	1.377 ± 0.437	1.257 ± 0.241	12.169 ± 3.503	3.030 ± 0.880	6.238 ± 1.382
PCB 169	1.815 ± 0.291	1.548 ± 0.391	1.515 ± 0.198	0.565 ± 0.038	0.158 ± 0.073	0.157 ± 0.025	0.905 ± 0.249	0.262 ± 0.072	0.303 ± 0.046
Σnon-*ortho* PCBs	96.567 ± 9.757	86.215 ± 32.014	88.219 ± 16.907	44.995 ± 7.041	8.188 ± 2.549	7.250 ± 1.216	98.606 ± 21.135	18.247 ± 4.336	49.207 ± 8.749
PCB 105	1744.190 ± 592.358	1332.957 ± 894.434	1322.160 ± 857.730	212.009 ± 30.887	36.778 ± 12.277	44.072 ± 8.668	519.213 ± 192.372	54.971 ± 15.267	71.821 ± 19.973
PCB 114	96.775 ± 35.519	65.347 ± 43.099	72.441 ± 42.473	11.486 ± 2.098	1.569 ± 0.731	1.860 ± 0.820	28.079 ± 10.164	2.513 ± 0.860	3.754 ± 0.968
PCB 118	4370.483 ± 1259.265	3378.731 ± 2192.630	3053.328 ± 1701.945	506.939 ± 83.273	84.172 ± 22.364	104.238 ± 21.601	1180.135 ± 440.659	126.892 ± 22.835	177.063 ± 25.940
PCB 123	141.193 ± 62.069	107.821 ± 64.463	112.502 ± 63.002	26.125 ± 9.000	3.173 ± 1.002	3.491 ± 1.262	51.549 ± 15.532	5.026 ± 2.114	6.372 ± 2.321
PCB 156	992.135 ± 214.150	836.692 ± 360.446	714.379 ± 367.807	92.277 ± 14.902	13.347 ± 4.148	11.329 ± 2.650	218.924 ± 87.182	21.243 ± 6.735	18.467 ± 4.336
PCB 157	151.858 ± 35.147	128.125 ± 79.212	121.686 ± 61.943	15.144 ± 2.287	2.734 ± 0.775	2.718 ± 1.057	38.402 ± 10.311	4.006 ± 1.383	4.350 ± 0.821
PCB 167	425.788 ± 91.181	344.918 ± 148.600	300.883 ± 133.011	48.494 ± 10.397	6.647 ± 2.694	6.954 ± 2.122	100.230 ± 47.153	11.771 ± 3.648	13.840 ± 2.844
PCB 189	127.339 ± 34.846	125.834 ± 28.580	88.571 ± 31.871	14.157 ± 2.335	2.462 ± 1.153	2.271 ± 1.184	18.561 ± 5.432	2.997 ± 0.904	5.869 ± 2.495
Σmono-*ortho* PCBs	8049.762 ± 2138.07	6320.427 ± 3749.79	5785.950 ± 3238.59	926.630 ± 132.762	150.881 ± 43.393	176.932 ± 35.691	2155.094 ± 802.007	229.420 ± 50.00	301.537 ± 51.59
ΣPCBs	8146.329 ± 2142.141	6406.642 ± 3781.595	5874.169 ± 3255.148	971.624 ± 139.604	159.069 ± 45.703	184.182 ± 36.374	2253.700 ± 822.243	247.666 ± 54.249	350.744 ± 59.794
Fine particles (%)	46.9 ± 3.2	60.7 ± 5.3	61.3 ± 6.0	-	-	-	-	-	-
TOC (%)	3.0 ± 0.3	3.6 ± 0.4	3.7 ± 0.4	-	-	-	-	-	-

Appendix C

Concentration of 2,3,7,8-substituted PCDD/Fs and dioxin-like PCBs (pg g^{-1} wet weight; pg g^{-1} lipid weight) in juvenile *Platichthys flesus* from five nursery areas across the northeastern Atlantic. Percentage of lipid content is also presented. Results are expressed as the mean ± standard deviation.

Compound	Mondego Estuary (0.79 ± 0.12 % lipid)		Vilaine Estuary (0.78 ± 0.11 % lipid)		Slack Estuary (1.06 ± 0.30 % lipid)		Wadden Sea (0.56 % lipid)		Sørfjord (0.74 ± 0.18 % lipid)	
	pg g^{-1} ww	pg g^{-1} lw	pg g^{-1} ww	pg g^{-1} lw	pg g^{-1} ww	pg g^{-1} lw	pg g^{-1} ww	pg g^{-1} lw	pg g^{-1} ww	pg g^{-1} lw
2,3,7,8-TCDD	0.007 ± 0.001	0.95 ± 0.24	0.007 ± 0.001	0.87 ± 0.11	0.009 ± 0.001	0.95 ± 0.36	0.104	18.700	0.009 ± 0.003	1.25 ± 0.15
1,2,3,7,8-PeCDD	0.019 ± 0.003	2.41 ± 0.02	0.023 ± 0.010	2.11 ± 1.04	0.013 ± 0.003	1.31 ± 0.70	0.027	4.800	0.008 ± 0.000	1.11 ± 0.26
1,2,3,4,7,8-HxCDD	0.008 ± 0.004	1.03 ± 0.72	0.004 ± 0.003	0.63 ± 0.38	0.006 ± 0.005	0.71 ± 0.63	0.005	0.967	0.006 ± 0.002	0.84 ± 0.08
1,2,3,6,7,8-HxCDD	0.030 ± 0.004	3.85 ± 0.09	0.029 ± 0.003	3.59 ± 0.21	0.018 ± 0.003	1.79 ± 0.81	0.029	5.233	0.011 ± 0.001	1.52 ± 0.28
1,2,3,7,8,9-HxCDD	0.010 ± 0.006	1.37 ± 0.92	0.007 ± 0.003	0.77 ± 0.38	0.007 ± 0.003	0.78 ± 0.53	0.010	1.850	0.006 ± 0.002	0.81 ± 0.13
1,2,3,4,6,7,8-HpCDD	0.050 ± 0.003	6.37 ± 0.56	0.053 ± 0.008	6.50 ± 1.10	0.044 ± 0.011	4.48 ± 2.34	0.036	6.500	0.024 ± 0.004	3.29 ± 0.23
OCDD	0.228 ± 0.018	29.47 ± 6.91	0.138 ± 0.005	16.42 ± 0.82	0.160 ± 0.016	15.60 ± 2.98	0.099	17.800	0.120 ± 0.006	16.56 ± 3.30
ΣPCDDs	0.353 ± 0.019	45.46 ± 9.42	0.260 ± 0.008	33.65 ± 4.87	0.258 ± 0.011	25.60 ± 8.35	0.312	55.850	0.185 ± 0.019	25.39 ± 3.72
2,3,7,8-TCDF	0.089 ± 0.001	11.38 ± 1.63	0.390 ± 0.045	44.87 ± 6.90	0.249 ± 0.023	24.89 ± 9.35	0.473	84.800	0.071 ± 0.002	9.88 ± 2.24
1,2,3,7,8-PeCDF	0.024 ± 0.003	3.16 ± 0.91	0.040 ± 0.005	4.56 ± 0.85	0.033 ± 0.003	3.28 ± 1.20	0.080	14.417	0.011 ± 0.001	1.55 ± 0.49
2,3,4,7,8-PeCDF	0.034 ± 0.003	4.44 ± 1.08	0.102 ± 0.024	10.73 ± 2.49	0.054 ± 0.008	5.44 ± 2.32	0.162	29.067	0.021 ± 0.001	2.97 ± 0.58
1,2,3,4,7,8-HxCDF	0.050 ± 0.011	6.54 ± 2.38	0.023 ± 0.002	2.60 ± 0.22	0.050 ± 0.006	4.98 ± 2.03	0.061	10.917	0.022 ± 0.004	3.05 ± 0.21
1,2,3,6,7,8-HxCDF	0.018 ± 0.002	2.32 ± 0.62	0.014 ± 0.002	1.56 ± 0.14	0.021 ± 0.002	2.09 ± 0.79	0.034	6.017	0.006 ± 0.000	0.88 ± 0.26
1,2,3,7,8,9-HxCDF	0.008 ± 0.005	1.08 ± 0.85	0.004 ± 0.002	0.36 ± 0.02	0.006 ± 0.004	0.62 ± 0.54	0.003	0.600	0.005 ± 0.001	0.74 ± 0.30
2,3,4,6,7,8-HxCDF	0.011 ± 0.001	1.34 ± 0.06	0.010 ± 0.000	1.12 ± 0.01	0.009 ± 0.002	0.92 ± 0.12	0.020	3.617	0.005 ± 0.001	0.74 ± 0.30
1,2,3,4,6,7,8-HpCDF	0.101 ± 0.011	13.09 ± 3.38	0.037 ± 0.012	4.67 ± 1.84	0.096 ± 0.007	9.55 ± 3.39	0.064	11.517	0.031 ± 0.009	4.16 ± 0.24
1,2,3,4,7,8,9-HpCDF	0.008 ± 0.006	1.08 ± 0.90	0.007 ± 0.001	0.76 ± 0.02	0.018 ± 0.005	1.88 ± 1.02	0.013	2.383	0.007 ± 0.001	1.02 ± 0.16
OCDF	0.113 ± 0.001	14.47 ± 2.36	0.048 ± 0.011	5.20 ± 1.57	0.124 ± 0.011	12.39 ± 4.63	0.080	14.300	0.043 ± 0.001	5.98 ± 1.30
ΣPCDFs	0.456 ± 0.040	58.90 ± 14.18	0.674 ± 0.061	87.86 ± 20.36	0.660 ± 0.068	66.04 ± 25.40	0.991	177.633	0.225 ± 0.015	30.97 ± 5.61
ΣPCDD/Fs	0.809 ± 0.058	104.36 ± 23.60	0.935 ± 0.055	121.51 ± 24.88	0.918 ± 0.079	91.64 ± 33.75	1.303	233.483	0.409 ± 0.034	56.35 ± 9.33
PCB 77	2.122 ± 0.090	273.16 ± 53.85	5.724 ± 0.324	664.64 ± 46.92	11.084 ± 1.277	1111.46 ± 439.40	19.380	3472.333	4.545 ± 0.352	625.94 ± 107.23
PCB 81	0.127 ± 0.048	15.83 ± 3.60	0.233 ± 0.062	30.13 ± 8.33	0.507 ± 0.018	50.30 ± 16.12	0.281	50.267	0.205 ± 0.071	27.31 ± 2.79
PCB 126	0.908 ± 0.118	115.38 ± 2.97	2.020 ± 0.389	238.61 ± 65.48	2.266 ± 0.248	227.07 ± 88.52	4.281	767.100	2.368 ± 0.034	329.88 ± 86.06
PCB 169	0.199 ± 0.034	25.24 ± 0.39	0.437 ± 0.047	51.30 ± 7.87	0.363 ± 0.145	33.79 ± 4.04	0.937	167.967	0.320 ± 0.054	45.38 ± 18.56
Σnon-*ortho* PCBs	3.356 ± 0.110	429.61 ± 52.82	8.414 ± 0.772	1090.48 ± 204.56	14.220 ± 1.398	1422.62 ± 540.01	24.880	4457.667	7.438 ± 0.335	1028.51 ± 209.05
PCB 105	36.824 ± 1.243	4713.39 ± 575.03	120.229 ± 9.475	14113.08 ± 1591.50	195.134 ± 15.449	19468.43 ± 7041.35	121.538	21775.500	68.498 ± 8.977	9370.46 ± 1105.86
PCB 114	3.396 ± 0.017	435.68 ± 65.51	14.042 ± 2.857	1725.19 ± 453.37	14.018 ± 0.643	1373.76 ± 332.92	18.491	3312.917	7.006 ± 0.428	966.90 ± 181.28
PCB 118	95.356 ± 11.630	12121.58 ±409.73	493.635 ± 49.900	58522.81 ± 8439.12	548.626 ± 30.720	54556.53 ± 18542.78	553.172	99109.917	238.015 ± 34.484	32503.58 ± 3384.93
PCB 123	1.828 ± 0.579	228.87 ± 37.86	8.184 ± 1.114	1006.64 ± 161.34	8.793 ± 0.971	881.13 ± 344.41	4.838	866.833	2.990 ± 0.121	417.89 ± 119.54
PCB 156	21.888 ± 1.470	2794.30 ± 247.91	85.910 ± 11.399	9988.92 ± 1879.61	99.361 ± 7.987	9914.96 ± 3597.35	89.935	16113.333	83.254 ± 0.943	11560.39 ± 2730.18
PCB 157	5.367 ± 1.939	669.37 ± 141.81	18.722 ± 2.923	2085.39 ± 378.93	20.835 ± 1.888	2082.09 ± 775.33	28.974	5191.250	14.472 ± 1.142	1992.68 ± 338.56
PCB 167	12.273 ± 0.464	1570.46 ± 185.31	63.326 ± 9.549	7394.44 ± 1593.90	50.299 ± 2.367	4928.57 ± 1188.67	71.785	12861.417	49.078 ± 1.1448	6799.40 ± 1485.29
PCB 189	3.098 ± 0.997	387.61 ± 66.21	11.595 ± 0.865	1332.86 ± 113.88	12.030 ± 0.566	1178.75 ± 284.26	17.988	3222.833	11.415 ± 1.389	1611.07 ± 585.38
Σmono-*ortho* PCBs	180.030 ± 18.340	22921.27 ± 1237.62	815.643 ± 86.423	105583.14 ± 19303.09	949.095 ± 53.438	94384.22 ± 32107.06	906.720	162454.000	474.728 ± 45.913	65222.37 ± 9931.03
ΣPCBs	183.386 ± 18.450	23350.88 ± 1290.44	824.056 ± 87.187	106673.62 ± 19506.56	963.316 ± 54.837	95806.84 ± 32647.06	931.600	166911.67	482.166 ± 46.248	66250.88 ± 10140.08
WHO-TEQ $_{fish}$	0.070 ± 0.003	8.99 ± 1.73	0.127 ± 0.025	16.65 ± 5.34	0.098 ± 0.015	9.91 ± 4.30	0.291	52.21	0.057 ± 0.006	7.76 ± 1.15
WHO-TEQ $_{1998}$	0.191 ± 0.014	24.37. ± 2.05	0.462 ± 0.074	60.00 ± 14.60	0.465 ± 0.074	46.48 ± 17.67	0.860	154.04	0.368 ± 0.007	51.09 ± 11.73
WHO-TEQ $_{2005}$	0.164 ± 0.012	22.30 ± 2.62	0.350 ± 0.060	38.75 ± 8.73	0.345 ± 0.034	33.03 ± 12.82	0.733	102.31	0.299 ± 0.001	41.96 ± 9.93

Appendix D

Concentration of PCDD/Fs and dl-PCBs in sediment (pg g^{-1} dw) and biota (pg g^{-1} ww; pg g^{-1} lipid in brackets) from the Mondego estuary. Lipid content (%) of biota is also presented.

	Sediment	*Ulva* sp.	*Gracilaria* sp.	*S. maritima* root	*S. maritima* leaves	*C. edule*	*S. plana*	*N. diversicolor*	*C. crangon*	*S. solea*	*D. labrax*	*A. anguilla*
PCB 77	12.07	0.08 (100.00)	1.94 (1141.18)	0.39 (75.00)	0.37 (60.66)	0.78 (79.59)	1.36 (113.33)	3.13 (184.12)	0.49 (33.56)	0.89 (112.66)	5.02 (193.82)	0.92 (4.32)
PCB 81	1.48	0.03 (37.50)	0.10 (58.82)	0.05 (9.62)	0.04 (6.56)	0.15 (15.31)	0.07 (5.83)	0.36 (21.18)	0.05 (3.42)	0.13 (16.46)	0.32 (12.36)	0.07 (0.33)
PCB 126	1.28	0.02 (25.00)	0.10 (58.82)	0.12 (23.08)	0.09 (14.75)	0.20 (20.41)	0.27 (22.50)	0.60 (35.29)	0.15 (10.27)	5.10 (645.57)	2.69 (103.86)	0.47 (2.21)
PCB 169	0.90	<LOD	<LOD	0.08 (15.38)	0.06 (9.84)	0.12 (12.24)	0.04 (3.33)	0.21 (12.35)	0.02 (1.37)	1.16 (146.84)	0.32 (12.36)	0.17 (0.80)
Σ non-*ortho* PCBs	15.74	0.13 (162.50)	2.15 (1264.71)	0.64 (123.08)	0.56 (91.80)	1.25 (127.55)	1.74 (145.00)	4.30 (252.94)	0.71 (48.63)	7.28 (921.52)	8.35 (322.39)	1.63 (7.65)
PCB 105	28.72	2.36 (2950.00)	10.68 (6282.35)	2.14 (411.54)	3.48 (570.49)	9.39 (958.16)	13.06 (1088.33)	23.03 (1354.71)	3.24 (221.92)	22.38 (2832.91)	105.25 (4063.71)	528.62 (2481.78)
PCB 114	2.14	0.21 (262.50)	0.51 (300.00)	0.26 (50.00)	0.55 (90.16)	0.64 (65.31)	0.84 (70.00)	1.77 (104.12)	0.20 (13.70)	1.39 (175.95)	7.18 (277.22)	31.76 (149.11)
PCB 118	98.85	9.36 (11700.00)	33.31 (19594.12)	6.28 (1207.69)	7.22 (1183.61)	31.45 (3209.18)	42.27 (3522.50)	74.03 (4354.71)	11.07 (758.22)	81.55 (10322.78)	331.50 (12799.23)	1520.51 (7138.54)
PCB 123	2.35	0.17 (2.12.50)	0.63 (370.59)	0.26 (50.00)	0.68 (111.48)	0.91 (92.86)	1.89 (157.50)	3.13 (184.12)	0.39 (26.71)	2.72 (344.30)	12.29 (474.52)	90.23 (423.62)
PCB 156	21.69	0.57 (712.50)	1.64 (964.71)	1.41 (271.15)	2.08 (340.98)	5.75 (586.73)	7.82 (651.67)	15.74 (925.88)	1.21 (82.88)	12.58 (1592.41)	66.51 (2567.95)	320.40 (1504.23)
PCB 157	4.76	0.55 (687.50)	0.17 (100.00)	0.35 (67.31)	2.15 (352.46)	1.49 (152.04)	1.35 (112.50)	3.66 (215.29)	0.26 (17.81)	3.33 (421.52)	14.81 (571.81)	61.58 (289.11)
PCB 167	8.23	0.55 (687.50)	0.63 (370.59)	0.77 (148.08)	3.85 (631.15)	4.16 (424.49)	4.01 (334.17)	10.76 (632.94)	0.87 (59.59)	9.92 (1255.70)	44.12 (1703.47)	208.08 (976.90)
PCB 189	16.41	0.74 (925.00)	0.36 (211.76)	10.86 (2088.46)	1.52 (249.18)	1.04 (106.12)	0.81 (67.50)	2.26 (132.94)	0.11 (7.53)	2.12 (268.35)	8.33 (321.62)	45.16 (212.02)
Σ mono-*ortho* PCBs	183.49	14.51 (18137.50)	47.93 (28194.12)	22.33 (4294.23)	21.53 (3529.51)	54.83 (5594.90)	72.05 (6004.17)	134.38 (7904.71)	17.35 (1188.36)	135.99 (17213.92)	589.99 (22779.54)	2806.34 (13175.31)
ΣPCBs	199.23	14.64 (18300.00)	50.08 (29458.82)	22.97 (4417.31)	22.09 (3621.31)	56.08 (5722.45)	73.79 (6149.17)	138.68 (8157.65)	18.06 (1236.99)	143.27 (18135.44)	598.34 (23101.93)	2807.97 (13182.96)
Ratio ΣPCDD/Fs : ΣPCBs	0.551	0.077	0.020	0.136	0.099	0.036	0.052	0.010	0.028	0.003	0.001	0.001
WHO-TEQ$_{1998}$	–	–	–	–	–	0.07 (7.41)	0.11 (8.90)	–	0.04 (3.07)	0.57 (72.41)	0.43 (16.55)	1.00 (4.68)
WHO-TEQ$_{2005}$	–	–	–	–	–	0.07 (7.03)	0.07 (5.52)	–	0.04 (2.61)	0.58 (72.92)	0.35 (13.65)	0.59 (2.78)
Lipid content	–	0.08	0.17	0.52	0.61	0.98	1.20	–	1.46	0.79	2.59	21.30

(Continued)

	Sediment	*Ulva* sp.	*Gracilaria* sp.	*S. maritima* root	*S. maritima* leaves	*C. edule*	*S. plana*	*N. diversicolor*	*C. crangon*	*S. solea*	*D. labrax*	*A. anguilla*
PCB 77	12.07	0.08 (100.00)	1.94 (1141.18)	0.39 (75.00)	0.37 (60.66)	0.78 (79.59)	1.36 (113.33)	3.13 (184.12)	0.49 (33.56)	0.89 (112.66)	5.02 (193.82)	0.92 (4.32)
PCB 81	1.48	0.03 (37.50)	0.10 (58.82)	0.05 (9.62)	0.04 (6.56)	0.15 (15.31)	0.07 (5.83)	0.36 (21.18)	0.05 (3.42)	0.13 (16.46)	0.32 (12.36)	0.07 (0.33)
PCB 126	1.28	0.02 (25.00)	0.10 (58.82)	0.12 (23.08)	0.09 (14.75)	0.20 (20.41)	0.27 (22.50)	0.60 (35.29)	0.15 (10.27)	5.10 (645.57)	2.69 (103.86)	0.47 (2.21)
PCB 169	0.90	<LOD	<LOD	0.08 (15.38)	0.06 (9.84)	0.12 (12.24)	0.04 (3.33)	0.21 (12.35)	0.02 (1.37)	1.16 (146.84)	0.32 (12.36)	0.17 (0.80)
Σ non-*ortho* PCBs	15.74	0.13 (162.50)	2.15 (1264.71)	0.64 (123.08)	0.56 (91.80)	1.25 (127.55)	1.74 (145.00)	4.30 (252.94)	0.71 (48.63)	7.28 (921.52)	8.35 (322.39)	1.63 (7.65)
PCB 105	28.72	2.36 (2950.00)	10.68 (6282.35)	2.14 (411.54)	3.48 (570.49)	9.39 (958.16)	13.06 (1088.33)	23.03 (1354.71)	3.24 (221.92)	22.38 (2832.91)	105.25 (4063.71)	528.62 (2481.78)
PCB 114	2.14	0.21 (262.50)	0.51 (300.00)	0.26 (50.00)	0.55 (90.16)	0.64 (65.31)	0.84 (70.00)	1.77 (104.12)	0.20 (13.70)	1.39 (175.95)	7.18 (277.22)	31.76 (149.11)
PCB 118	98.85	9.36 (11700.00)	33.31 (19594.12)	6.28 (1207.69)	7.22 (1183.61)	31.45 (3209.18)	42.27 (3522.50)	74.03 (4354.71)	11.07 (758.22)	81.55 (10322.78)	331.50 (12799.23)	1520.51 (7138.54)
PCB 123	2.35	0.17 (2.12.50)	0.63 (370.59)	0.26 (50.00)	0.68 (111.48)	0.91 (92.86)	1.89 (157.50)	3.13 (184.12)	0.39 (26.71)	2.72 (344.30)	12.29 (474.52)	90.23 (423.62)
PCB 156	21.69	0.57 (712.50)	1.64 (964.71)	1.41 (271.15)	2.08 (340.98)	5.75 (586.73)	7.82 (651.67)	15.74 (925.88)	1.21 (82.88)	12.58 (1592.41)	66.51 (2567.95)	320.40 (1504.23)
PCB 157	4.76	0.55 (687.50)	0.17 (100.00)	0.35 (67.31)	2.15 (352.46)	1.49 (152.04)	1.35 (112.50)	3.66 (215.29)	0.26 (17.81)	3.33 (421.52)	14.81 (571.81)	61.58 (289.11)
PCB 167	8.23	0.55 (687.50)	0.63 (370.59)	0.77 (148.08)	3.85 (631.15)	4.16 (424.49)	4.01 (334.17)	10.76 (632.94)	0.87 (59.59)	9.92 (1255.70)	44.12 (1703.47)	208.08 (976.90)
PCB 189	16.41	0.74 (925.00)	0.36 (211.76)	10.86 (2088.46)	1.52 (249.18)	1.04 (106.12)	0.81 (67.50)	2.26 (132.94)	0.11 (7.53)	2.12 (268.35)	8.33 (321.62)	45.16 (212.02)
Σ mono-*ortho* PCBs	183.49	14.51 (18137.50)	47.93 (28194.12)	22.33 (4294.23)	21.53 (3529.51)	54.83 (5594.90)	72.05 (6004.17)	134.38 (7904.71)	17.35 (1188.36)	135.99 (17213.92)	589.99 (22779.54)	2806.34 (13175.31)
ΣPCBs	199.23	14.64 (18300.00)	50.08 (29458.82)	22.97 (4417.31)	22.09 (3621.31)	56.08 (5722.45)	73.79 (6149.17)	138.68 (8157.65)	18.06 (1236.99)	143.27 (18135.44)	598.34 (23101.93)	2807.97 (13182.96)
Ratio ΣPCDD/Fs : ΣPCBs	0.551	0.077	0.020	0.136	0.099	0.036	0.052	0.010	0.028	0.003	0.001	0.001
WHO-TEQ$_{1998}$	–	–	–	–	–	0.07 (7.41)	0.11 (8.90)	–	0.04 (3.07)	0.57 (72.41)	0.43 (16.55)	1.00 (4.68)
WHO-TEQ$_{2005}$	–	–	–	–	–	0.07 (7.03)	0.07 (5.52)	–	0.04 (2.61)	0.58 (72.92)	0.35 (13.65)	0.59 (2.78)
Lipid content	–	0.08	0.17	0.52	0.61	0.98	1.20	–	1.46	0.79	2.59	21.30

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Agradecimentos

Aos meus orientadores, Professor Miguel Pardal e Professor Fernando Ramos, por terem acreditado neste projecto e nas minhas capacidades. Muito obrigada pelo apoio, paciência e conselhos dados ao longo destes anos.

Ao Doutor Bruno Le Bizec, que tornou possível a realização dos trabalhos conducentes à execução da dissertação aqui apresentada. Ao Philippe Marchand e à Anaïs Vernisseau pela ajuda fundamental neste trabalho.

A todos os colegas do CEF pela companhia nas horas de trabalho, pelos passeios (congressos, digo eu!) e pelos lanches sempre tão bons: Filipe, Cláudia, Daniel Crespo, Rito, Masha, Lígia, Marina, Sónia, Ivan, Mariaelena, Sónia, Joana Oliveira, Sílvia, Tiago, Joana Baptista, Joana Falcão, Eva, Daniel Nyitrai, Elsa, Patrícia, Tito, João Neves e Thony. João, quando voltamos a Nantes?

À fantástica equipa do LABERCA que tão bem me recebeu e fez sentir em casa. Merci pour tout Séverine, Cyrille, Sophie, Emilie, Sandra, Zita, Marie, Fred, Vincent et Florence !

Merci Lili !

À Sara, colega, companheira de equipa, e acima de tudo amiga.

Aos meus amigos de sempre e para sempre, estejam perto ou no outro lado do mundo. Obrigada pelos desvarios, jantares, dormidas, saídas, desabafos e muito muito mais.

Ao Xavier, por todas as horas passadas como biólogo honorário, e pelo número de vezes que me ouviste dizer PCDD/Fs, OCDD, PCBs, HPLC, FCT, TCDD. Esta tese também é tua!

À minha família adoptiva Bustuense.

À minha família. Pai, mãe, vocês são os melhores do mundo! Muito obrigada por tantas vezes fazerem o possível e o impossível. À Inês e às minhas queridas avozinhas.