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Seasonal occurrence and risk assessment of endocrine-disrupting compounds in Tagus estuary biota (*NE Atlantic Ocean coast)*

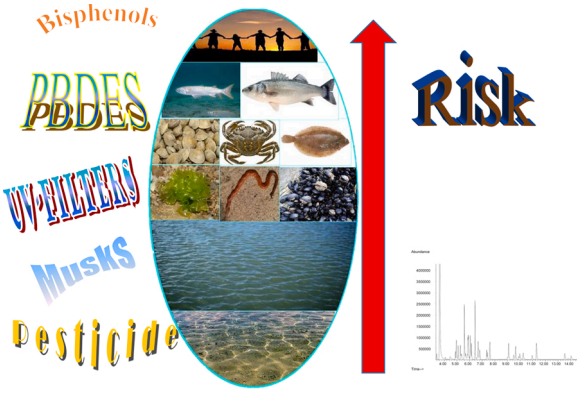
Antía Lestido-Cardama [a,b], Mateus Petrarca [b], Carolina Monteiro [b], Ricardo Ferreira [b], Isa Marmelo [c,d,e], Ana Luísa Maulvault [c,e], Patrícia Anacleto [c,d,f], António Marques [c,d], José O. Fernandes [b,*], Sara C. Cunha [b,*]

[a] *Department of Analytical Chemistry, Nutrition and Food Science, Faculty of Pharmacy, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain*
[b] *LAQV-REQUIMTE, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Rua Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal*
[c] *IPMA, Portuguese Institute for the Sea and Atmosphere, I.P., Division of Aquaculture, Upgrading and Bioprospection (DivAV), Av. Portuguese Institute for the Sea and Atmosphere (IPMA, I.P.), Av. Doutor Alfredo Magalhães Ramalho 6, 1495-165 Lisboa, Portugal*
[d] *CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal*
[e] *UCIBIO-REQUIMTE, Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, NOVA University of Lisbon, 2829-516 Caparica, Portugal*
[f] *MARE – Marine and Environmental Sciences Centre, Guia Marine Laboratory, Faculty of Sciences of the University of Lisbon (FCUL), Av. Nossa Senhora do Cabo, 939, 2750–374 Cascais, Portugal*

HIGHLIGHTS

- Twenty-one of 50 endocrine disruptor contaminants studied were detected.
- Mullet and sole collected in Tagus estuary were the most contaminated fish species.
- First assessment of bisphenols in earthworm specimens.
- Seasonal variation in EDC was found in Tagus estuary biota.
- Low risk to human health through estuarine fish consumption.

GRAPHICAL ABSTRACT



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ABSTRACT

Estuaries are continually threatened by anthropogenic pressures, consequently, a large group of contaminants harmful to human health affects the aquatic biota; therefore, it is necessary to monitor their quality. This study deals with the determination of a large group of compounds representing different endocrine-disrupting compounds (EDCs) classes [21 pesticides, 4 polycyclic musk fragrances, 4 UV-filters, 7 bisphenols, 6 polybrominated diphenyl ethers (PBDEs) and 8 of their methoxylated (MeO-BDEs)] in several estuarine species (fish, bivalves, crustaceans, earthworm, and macroalgae) collected seasonally along one year in two distinct areas of Tagus River

* Corresponding authors.
E-mail addresses: josefer@ff.up.pt (J.O. Fernandes), sara.cunha@ff.up.pt (S.C. Cunha).

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Estuarine seafood
Macroalgae

estuary ("contaminated" vs. "clean" areas). The most abundant compounds found were galaxolide (HHCB) (81% positive samples; 0.04–74 ng/g ww), isoamyl 4-methoxycinnamate (IMC) (64%; 1.13–251 ng/g ww), alachlor (44%; 0.08–16 ng/g ww), and BDE-47 (36%; 0.06–2.26 ng/g ww). Polycyclic musks were the most frequent contaminants in fish (seabass, barbus, mullet, and sole) and macroalgae samples, while UV-filters were predominant in bivalves and crustaceans, and bisphenols in earthworms. Seasonal variation was verified for Σpesticides and Σmusks, with significantly higher levels in summer and autumn, whereas ΣUV-filters highest levels were found in spring and summer, and for ΣPBDEs statistically higher levels were registered in cold seasons (autumn and winter). Σbisphenols were significantly lower in spring than in the other seasons. In general, considering all species analysed in both areas, no statistically significant differences ($p > 0.05$) were verified between the two collection areas. Based on the estimated daily intake data, consumption of fish from this estuary is unlikely to be a human health concern, since the levels of contamination were below the toxicological threshold values. Overall, the data obtained in this study will allow regulatory authorities to identify and prioritize contaminants monitoring programs in estuaries, such as the case of bisphenol A, which was found, for the first time, in earthworm and clam species.

1. Introduction

Estuaries correspond to transitional waters with distinctive characteristics, in which different complex ecosystems can coexist, contributing to fisheries and social goods (Bhattacharya et al., 2003; Leadprathom et al., 2009). Unfortunately, most estuaries around the world are under threat from many anthropogenic pressures and different chemical/biological hazards that directly affect aquatic biota (Ribeiro et al., 2016; Peng et al., 2017; Aminot et al., 2021).

Tagus estuary, located at the central western coast of Portugal, North-East Atlantic coast, South Europe (38°44′N; 09°08′W), represents a huge source of commercial seafood, covering an area of approximately 320 km^2 which ranks it among the largest estuaries in Europe (Moura et al., 2017). Tagus estuary is located in the center of the most populated region of Portugal (Lisbon metropolitan area) and has been subjected for a long time to intensive anthropogenic pressures (urban expansion, industrial development, agriculture, port infrastructures, and fishing activities) that lead to increased pollution (Moura et al., 2017). Therefore, estuarine seafood is not only an important human food source of nutrients, but may also represent a source of harmful environmental contaminants including endocrine-disrupting compounds (EDC) (Pironti et al., 2021).

These contaminants include bisphenols and polybrominated diphenyl ethers (PBDEs) from industrial chemicals/plastics, pesticides from crops, or personal care products (PCPs), such as musk fragrances and ultraviolet (UV) filters frequently used in cosmetic and hygienic products, among others. The massive production and use of these compounds can result in their persistence in the environment, namely, in water, sediments, sludge, and living species, with negative effects in all trophic levels (Vandermeersch et al., 2015; Cunha et al., 2022). Additionally, most compounds are associated with potential multi-organ toxicity and disruptive endocrine activity (Wee and Aris, 2017). Exposure to EDCs causes endocrine system disruption in humans, at a wide scope, which ranges from acute to chronic diseases, namely reproductive abnormalities, cardiovascular diseases, metabolic effects, immune effects, diabetes, behavioural changes, neurological disorders, obesity, disrupted fetal development, and growth, and a wide range of cancers (Pironti et al., 2021; Wee and Aris, 2017).

Societal concerns about combined exposure to EDCs through different ways such as diet, and ingestion/inhalation of indoor dust and/or indoor air are growing. Among these, the dietary route seems to play a considerable role on the overall human exposure, but so far there are still no legal maximum permissible limits for many foods, including fish.

Bisphenol A (BPA), the best-known bisphenol, has strict limits on its use in food containers around the world. The maximum acceptable amount of BPA released from a container or article into food is 0.05 mg/kg, while no BPA is allowed to be found on materials and articles intended for infants and young children (European Commission, 2011). The tolerable Daily Intake (TDI) of BPA was set in 2015 at 4 μg/kg body weight (bw), but is currently being revised with a proposal of 4 ng/kg/bw (EFSA, 2021).

These restrictions have caused the growing use of BPA structural analogues (such as bisphenol B, S, F,) that are already increasingly found in seafood samples (Wong et al., 2017, 2022).

Nowadays, organochlorine pesticides (OCPs) application in agriculture to control pests and diseases has been banned or strongly restricted due to their acute and chronic toxicity, leading to potential negative impacts on humans and wildlife (Tsygankov, 2019). Despite that, OCPs can still be detected in the environment and several aquatic species (e.g., *Alpinia oxyphylla, Alestes baremoze,* and *Synodontis bastiani)* due to their high persistence and bioaccumulation capacity (Zhao et al., 2016; Tongo et al., 2019; Shin et al., 2021). Human exposure to OCPs occurs mainly through the consumption of contaminated food, especially fish with a relatively high lipid content (Solaesa et al., 2019). Other group of pesticides usually reported in aquatic environments are pyrethoids (Gajendiran and Abraham et al., 2018) and organophosphorus pesticides that still are one of the most used insecticides in Europe (Erurostat, 2022).

UV filters are frequently used in PCPs and thus are regularly released into the environment (Brausch and Rand, 2011). Some studies have demonstrated its ability to bioaccumulate in marine organisms and biomagnify through the marine food chain (e.g., corals, crabs, shrimps, prawns, squids, fish, dolphins, and seabirds) (Wang et al., 2022). Although no limits have been established for UV-filters in the aquatic environment, ethylhexyl methoxycinnamate (EHMC) had been included on the Watch List as a priority pollutant in surface waters under the Environmental Quality Standards Directive (EC, 2013). However, in 2022, it was decided to remove EHMC from the Watch List since the monitoring dataset was good enough to perform a risk assessment (EC, 2022).

Musks, particularly polycyclic musks (such as cashmeran-DPMI, celestolide-ADBI, galaxolide-HHCB, and tonalide-AHTN), are found in a wide diversity of products like household chemicals, soaps, and detergents, with high concentrations in PCPs (Cunha et al., 2022). Due to their high lipophilicity, when released into the environment they tend to bioaccumulate, affecting the biota regardless of the trophic level. Therefore, musk contamination has been reported in aquatic biota from several estuaries worldwide, such as Douro, Mondego, and Sado in Portugal (Ribeiro et al., 2016), Pearl River in China (Peng et al., 2017), Seine, Loire, Gironde, and Rhône in France (Aminot et al., 2021), Hugli in India (Bhattacharya et al., 2003) and Chanthaburi in Thailand (Leadprathom et al., 2009).

Polybrominated diphenyl ethers (PBDEs) are additive brominated flame retardants widely used in plastics, textiles, and electronic casting products (Cruz et al., 2015). Owing to their environmental persistence, bioaccumulation, and biomagnification, numerous studies have been published concerning their presence in biotic and abiotic matrices, including seafood (Domingo, 2012). Despite a TDI has not been established for PBDEs, EFSA (2011)) applied the margin of exposure (MOE) approach to characterize the risk of BDE47, BDE99, BDE153, and

BDE209 through the daily intake, concluding that in Europe only BDE99 can be considered a potential health concern.

The presence of EDCs in the environment and its consequent exposure risk has been confirmed in the last two decades thanks to the development of appropriate and sensitive methods for its detection, such as advanced chromatographic and mass spectrometric technologies that have allowed the determination of minute amounts of a wide range of compounds, so a more comprehensive assessment of environmental contamination (Pironti et al., 2021). However, studies reporting the simultaneous occurrence of this cocktail of EDCs in several biological matrices from estuarine environments are scarce, and only very few studies have specifically evaluated spatial and temporal contamination features, that are crucial for a comprehensive insight into their fate and transport in coastal environments. In this context, this study intended to evaluate the presence of six different types of EDCs (21 pesticides, 4 polycyclic musk fragrances, 4 UV-filters, 7 bisphenols, 6 PBDEs and 8 MeO-BDEs) in fish, bivalves, crustaceans, earthworms, and macroalgae samples collected seasonally along one year in two distinct areas of Tagus estuary ("contaminated" vs. "clean" areas). EDcs were selected based on the following criteria: i) regular occurrence in the aquatic environment according to the available literature; ii) persistence in the environment; and iii) potential for bioaccumulation.

2. Materials and methods

2.1. Chemicals and analytical standards

High purity analytical standards of pesticides, polycyclic musk fragrances, UV-filters, and bisphenols used in this study were previous described by Petrarca et al. (2022).

All PBDE (congeners 28, 47, 99, 153, 154, 183 – including internal standards 37, 77) and MeO-BDE (congeners 2-MeO-BDE-68, 6-MeO-BDE-47, 5-MeO-BDE-47, 4-MeO-BDE-49, 5-MeO-BDE-100, 4-MeO-BDE-103, 5′-MeO-BDE-99, 4′-MeO-BDE-101 - including internal standard ^{13}C-6-MeO-BDE-47) standards used in this study were obtained from Wellington Laboratories, Inc. (Guelph, Ontario, Canada) and were > 99% pure. 5′ fluoro 3,3′,4,4′,5 pentabromodiphenyl ether (FBDE-126) was acquired from AccuStandard, Inc. (New Haven, USA) and also were > 99% pure.

Individual standard solutions of 1 g/L of pesticides, UV-filters, and bisphenols were prepared in acetonitrile (ACN) and stored at − 20 °C, while the individual standard solutions of PBDEs and MeO-BDE were prepared in hexane and stored at 5 °C.

Internal standards were purchased from Cambridge Isotope Laboratories (bisphenol A-d16 and *p,p′*-DDT-d8), Toronto Research Chemicals (bisphenol F-d10), Sigma-Aldrich (benzophenone-d10 and triphenyl phosphate), and from Dr. Ehrenstorfer GmbH (AHTN-d3). Toluene 99.7% and ACN for HPLC ≥ 99.9% were obtained from Honeywell (Riedel-de Haën, Seetze, Germany). Acetic anhydride ≥ 99% was acquired from Sigma-Aldrich (Schnelldorf, Germany). Carbon tetrachloride and potassium carbonate (K_2CO_3) were purchased from Panreac (Barcelona, Spain). Anhydrous magnesium sulphate and sodium chloride, ≥ 99.5%, from Honeywell/ Fluka (Muskegon, MI, USA). Both salts were 99.5% pure and baked at 350 °C for 2 h, in a muffle furnace (P Selecta), before use. Clean-up sorbent, QuEChERS dSPE Enhanced Matrix Removal - Lipid (EMR-Lipid), was purchased from Agilent Technologies (USA), while the activated carbon was from Sigma-Aldrich (Saint Louis, MO, USA). Ultra-pure water was obtained from a "Seralpur Pro 90 CN" water purifying system (Seral, Ransbach-Baumbach, Germany). pH indicator strips were obtained from Merck (Darmstadt, Germany). Helium gas with a purity of 99.999% was used for the chromatographic analysis (Gasin, Porto).

2.2. Samples

A total of 296 samples were collected during four annual seasons: spring (April 2019), summer (June 2019), autumn (October 2019), and winter (February 2020). Considering the large area of Tagus estuary, two sampling areas were defined (Supplementary Fig. S1 and Table S1): i) a likely less contaminated zone ("clean"), far from industries and from most densely populated areas, and near to the protected area of the Tagus Estuary Natural Reserve; and ii) a likely more contaminated zone ("contaminated"), near to Lisbon city coastline, subject to several points of diffuse pollution sources (e.g., domestic, hospital, chemical and petrochemical industries, agricultural, and livestock effluent discharges, to which is added the presence of pollution from marinas, ports and boats, commerce, and transport).

All samples were collected by local fishermen under the supervision of the Portuguese Institute for the Sea and Atmosphere (IPMA, I.P.) team. In each sampling area, several specimens for each species were collected. Samples of 9 species of biota were collected including fish, bivalves, crustaceans, earthworms, and macroalgae. The fish species collected were seabass (*Dicentrarchus labrax*), barbus (*Barbus barbus*), mullet (*Mugil cephalus*), and sole (*Solea solea*). In addition to fish, the other biota groups investigated were macroalgae (*Ulva* sp.), mussels (*Mytilus galloprovincialis*), clams (*Ruditapes philippinarum*), green crabs (*Carcinus maenas*), and earthworms (*Lumbricus terrestris*). The selection of the studied seafood species was based on those most consumed among the Portuguese population and those more suitable to reflect the environmental contamination of the estuary. Despite the efforts of the fishermen, it was not possible to capture some of the fish species in all seasons. In these cases, priority was given to the capture of similar species, to always have the representation of the entire trophic chain. The number of samples included in each collection is specified in Supplementary Table S1.

The biometric data (generally total weight and total length for fish; shell width and height in the case of bivalves) was registered. For fish species, the edible portion (muscle) and the liver were dissected and collected separately (Supplementary Table S1).

Regarding fish samples, 34 were collected in spring (including seabass, barbus, and mullet), 50 in the summer (including seabass, barbus, mullet, and sole), 28 in autumn (including seabass, barbus, and mullet), and 42 in winter (including seabass, barbus, and mullet).

Concerning mussels and clams, pools of 150 individuals were collected in each season. After the separation of the digestive glands, the edible part was used for analysis (Supplementary Table S1). In crabs, hepatopancreas and gonads were previously separated, and then 50 muscle samples were pooled and used for analysis (Supplementary Table S1). For earthworms and macroalgae, the whole organism was used for analysis (Supplementary Table S1). All samples were homogenized and freeze-dried at − 80 ºC (Telstar Cryodos-80, Spain) before analysis.

2.3. Sample extraction

Sample preparation performed in this study was based on previous validated methods (Menezes-Sousa et al., 2021; Petrarca et al., 2022). Briefly, 0.5 g of homogenised sample was weight into a 15 mL amber screw-capped glass flask and added with 50 µL of internal standards solution in ACN (TPP, bisphenol A-d16, bisphenol F-d10, and benzophenone-d10 at concentration of 2.5 µg/mL and BDE-37, BDE-77, ^{13}C-6-MeO-BDE-47, and FBDE-126 at concentration of 75 µg/L). After 30 min, 5 mL of ACN and 4.5 mL of Milli-Q water were added and the mixture was shaken overnight on an orbital shaker-incubator ES-20 (BioSan, Riga, Latvia) at 240 rpm and 25 °C. Afterward, the tubes were stirred for 30 s in a vortex and 2 g of $MgSO_4$ and 0.5 g of NaCl were added, followed by shaking and centrifugation at 479 x *g* for 10 min. Then, one mL of the supernatant (ACN phase) was cleaned-up with 0.2 g of activated EMR-lipid. For macroalgae samples, 5 mg of activated carbon was used in the clean-up step instead of EMR-lipid. For all the analytes except PBDEs and MeO-BDEs, one mL of the cleaned ACN extract was mixed with 125 µL of acetic anhydride and 85 µL of carbon

tetrachloride and the mixture transferred to a tube containing 4 mL of Milli-Q water with 500 μL of K_2CO_3 23%. After centrifugation at 809 x *g* for 5 min, an aliquot of 75 μL of the bottom layer was transferred to 2 mL amber vial with 100 μL insert, and 15 μL of the internal standard solution (AHTN-D3 and *p,p'*-DDT at a concentration of 1 μg/mL in toluene) was added, for subsequent GC-MS analysis of 1 μL. For PBDEs and MeO-BDEs analysis, after the clean-up step, 1 mL of supernatant was evaporated under a gentle nitrogen stream at 40 °C, then, recovered to a total volume of 70 μL in trichloroethylene. The extract was then transferred to 2 mL amber vial and 1 μL was analysed by GC-MS/MS.

2.4. Instrumental analysis

2.4.1. Analysis of pesticide residues, bisphenols, polycyclic musks and UV-filters

Analysis was performed in an Agilent HP 6890 Series gas chromatograph equipped with an Agilent 5973 N single quadrupole mass spectrometer (Agilent Technologies, Santa Clara, USA) and a Pal-LSI autosampler (CTC Analytics, Switzerland). The chromatographic capillary column was a Zebron 5HT (30 m × 0.25 mm×0.25 μm, Phenomenex, CA, USA). The injector temperature was set at 250 °C, the injection was made in pulsed splitless mode at 40 psi for 0.85 min, and helium was used as carrier gas at a constant flow rate of 1 mL/min. The oven program was initially set at 95 °C for 1.5 min, then increased at a rate of 20 °C/min until 180 °C, followed by another ramp of temperature at a rate of 5 °C/min until 230 °C, and finally ramped to 290 °C at 25 °C/min and held for 15.85 min, the total run was 36 min. The transfer line and source temperature were set at 280 °C and 230 °C, respectively, while the MS quadrupole temperature was 150 °C. ChemStation software was used for data acquisition and processing. Data were acquired in the selected ion monitoring (SIM) mode.

2.4.2. Analysis of PBDEs and MeO-BDEs

For PBDEs and MeO-BDEs analysis, a gas chromatograph Agilent 7890B (Agilent Technologies, USA) equipped with an auto-sampler 7683 (Agilent Technologies, USA) and coupled to an Agilent 7000 C triple quadrupole mass spectrometer (Agilent Technologies, USA) was used, in electron ionization mode (EI). Separation was achieved with a capillary column, 30 m × 0.25 mm × 0.25 μm DB-5 MS (Agilent Technologies, USA). One μL of extract (in trichloroethylene) was injected at 300 °C in pulsed splitless mode (pulse pressure of 32 psi for 1 min and purge flow of 50 mL) with the following oven programmed: 150 °C, hold for 1.5 min, ramp at 40 °C/min to 250 °C, ramp again at 7 °C/min to 320 °C and hold for 3 min. The flow rate was 1 mL/min using helium as the carrier gas. The temperatures of the transfer line, ion source, 1st, and 2nd quadrupole were 250, 320, 150, and 150 °C, respectively. The collision cell gases were nitrogen (1.5 mL/min) and helium (2.25 mL/min). The triple quadrupole MS was operated in multiple reaction monitoring (MRM) mode, detecting two transitions for each analyte (Cruz et al., 2019). The MassHunter quantitative analysis software (v. B.02.03) was used for data processing.

2.5. Quality assurance and Quality control

All detailed QA/QC information for fat and lean fish muscle, macroalgae, and mussels analysis, including recovery, precision, and limits of detection (LOD) and quantification (LOQ), have been described in our previous studies (Cruz et al., 2019; Menezes-Sousa et al., 2021; Petrarca et al., 2022). Briefly, to reduce contamination during the experimental procedure, glassware was baked at 450 °C overnight and pre-cleaned with acetone. In the procedural blanks, target analytes were always below the detection limits.

For all analytes, except PBDEs and MeO-BDEs, matrix-matched calibration with five calibration levels using the internal standard method was performed for each type of matrix, except for liver. The determination coefficients for each analyte were > 0.99. The recoveries ranged from 25% to 118% with a relative standard deviation lower than < 20%. LOD ranged from 0.5 to 50 μg/kg dw and LOQ ranged from 1 to 50 μg/kg dw (for more details see Petrarca et al., 2022). For PBDEs and MeO-BDEs, a matrix-matched calibration with eight calibration levels was used in each matrix: macroalgae, mussel, crab, lean fish, and fatty fish (0–20 ng/g). The determination coefficients for each analyte were > 0.90. Details on method detection and quantification limits, native recoveries of the analytes (mussel, fish muscle, macroalgae, and crabs), and sample preparation can be seen in the previous publications of our group, which included Certified Reference Material (CRM) (Cruz et al., 2019; Menezes-Sousa et al., 2021).

Regarding liver analysis, validation data are reported in the supplementary Table S2. The matrix-matched calibration curves were performed using a blank European bass (*Dicentrarchus labrax*) liver. Calibration was performed with five calibration levels ranging from 1 to 1000 μg/g. The calculated determination coefficients were, in all cases, higher than 0.990 for all target analytes, showing very good linearity of calibration function in the concentration range under study. Recovery studies were carried out at three concentration levels (100, 250, and 500 μg/g), performing 5 replicates at each level. The recovery values ranged from 71% to 114%, which are acceptable according to the guidelines (70–120%) established by the EU (SANTE/12682/2019). Repeatability was evaluated at the three concentration levels of the recovery studies, and the repeatability values (expressed as a percentage of relative standard deviation, RSD%) were always lower than 20%.

2.6. Dietary intake and risk assessment

The dietary exposure of the Portuguese population was estimated using a deterministic method. The estimated dietary intake (EDI) was calculated following the recommendations of the World Health Organization (WHO, 2006) taking into account the obtained concentration of the selected analytes in each biota sample and the estimated weakly consumption rate of seafood, through the following equation:

$$EDI_{p,j} = C_{p,I} \text{ x } W_{i,j}$$

where C is the concentration of the compound *p* in each specie *i* (ng/g wet weight), W is the estimated weakly consumption rate of seafood *i* per individual *j* (μg/kg bw/day) and EDI is the estimated dietary intake expressed as ng/g body weight (bw) per week. The minimum, maximum, and median Portuguese consumption rate (g/week) was based on data collected by Jacobs et al. (2017). For results reported to be below the LOD (not detected), the value was zero.

A risk assessment associated with human dietary exposure was evaluated. Since no established health-based guidance value is available for the selected contaminants, the obtained chemical intake values were compared with the NOAEL (No Observed Adverse Effect Level), the acceptable daily intake (ADI) values in the case of chronic toxicity or the accurate reference dose (ARfD) in the case of acute toxicity, when the same were available.

Particularly for PBDEs, a margin of exposure (MOE) approach was used for the risk characterization of exposure based on the lower benchmark dose limit (BMDL) and the estimated daily intake (EDI) (EFSA, 2011). The MOE was calculated as follows: $MOE = BMDL_{10}/EDI$; where $BMDL_{10}$ values (corresponding lower 95% confidence limit for a benchmark response of 10% incidence) were 309 μg/kg bw/day and 12 μg/kg bw/day for BDE-47 and BDE-99, respectively, considering effects on neurodevelopment as the critical endpoint (EFSA, 2011).

2.7. Statistical analysis

Data were firstly tested for normality and homoscedasticity through Kolmogorov–Smirnov and Levene tests, respectively. A normal distribution of the residuals was evaluated through the Shapiro–Wilk's test (sample size < 50). The Kruskal–Wallis H test was used to compare

samples from different sampling sites. Additionally, if a statistically significant difference was verified, the Mann–Whitney U test was applied for means comparison of more than two independent samples. The significance level was set at 0.05. All the statistical analyses were done using the SPSS statistical package, version 28.0 (IBM Corporation, New York, USA).

3. Results and discussion

3.1. Levels of contaminants in biota

Twenty out of the 50 analytes under study were identified and quantified in the biota collected during one year in the Tagus River estuary, including 5 pesticide residues, 3 UV-filters, 2 polycyclic musks, 3 bisphenols, 3 PBDEs and 4 MeO-BDEs. A summary of the average levels of the studied contaminants in the different species analysed, expressed as wet weight (ww), is reported in Tables 1, 2, and 3. The percentage contribution of each contaminant in each species in the different seasons is shown in Fig. 1.

Among the 21 pesticide residues analysed, alachlor, ethion, *p,p′*-DDT, bifenthrin, and γ-chlordane were quantified in biota (Table 1). The highest levels of alachlor, bifenthrin *p,p′*-DDT, and ethion were found in fish, while the highest value of γ-chlordane was found in macroalgae. Overall, the total amount of pesticide residues was higher in fish samples with a mean value of 4.43 μg/kg ww, followed by crustaceans (1.25 μg/kg ww), bivalves (1.22 μg/kg ww), and macroalgae (0.30 μg/kg ww). Mullet and sole were the fish most contaminated with pesticide residues (average of 7.42 and 5.49 μg/kg ww, respectively).

Regarding the 4 UV-filters analysed, 3 analytes were detected in the samples analysed, the exception was BP3 (Table 2). In general, IMC was the UV-filter most frequent and abundant (Fig. 1). Fish samples showed the highest levels of ΣUV-filters with a mean value for of 13.02 μg/kg ww, followed by macroalgae (10.96 μg/kg ww), bivalves (7.76 μg/kg ww), earthworms (6.47 μg/kg ww), and crustaceans (3.93 μg/kg ww). Similarly to the pesticide residues, sole (average of 10.17 μg/kg ww), and mullet (average of 13.76 μg/kg ww) were the most UV-filters contaminated species.

Two out of 4 polycyclic musk fragrances evaluated, HHCB and AHTN, were found in the samples analysed (Table 1). The sum of musks was higher in earthworm samples with a mean value of 54.06 μg/kg ww, followed by fish (34.55 μg/kg ww), macroalgae (16.25 μg/kg ww), bivalves (6.69 μg/kg ww), and crustaceans (3.28 μg/kg ww). As can be seen in Fig. 1, HHCB was the most prevalent and abundant polycyclic musk, representing in most samples more than 20% of all contaminants detected, except for seabass, earthworm, and sole where AHTN levels were higher.

Concerning bisphenols, only BPF, BPA, and BPB were found (Table 2). BPF was the most abundant in most species, except earthworms (in all seasons), clams (in spring and autumn), and macroalgae (in winter) (Table 2). Higher levels of Σ bisphenols were found in earthworms with a mean value of 81.32 μg/kg ww, followed by macroalgae (7.48 μg/kg ww), fish (5.28 μg/kg ww), crustaceans (1.42 μg/kg ww) and bivalves (0.76 μg/kg ww).

Among the six PBDEs investigated, the congeners 28, 47, and 99 were detected in the muscle of all fish species, with the highest contents of ΣPBDEs found in seabass (1.45 μg/kg ww) and barbus (0.94 μg/kg ww) (Table 3). Regarding the other biota studied, the BDE-47 was detected in mussels, crabs, earthworms and macroalgae samples, while BDE-99 was only found in earthworms and macroalgae. In general, the highest levels of ΣPBDEs, including both congeners 47 and 99, were found in earthworm samples (2.46–10.17 μg/kg ww; Table 3). 4 PBDE metabolites were detected in fish muscle at least in one season for each species, with seabass presenting the highest incidence. Specifically, 5-MB-47 was found in the muscle tissue of all fish species studied. In mussels, the metabolites 2′-MB-68 and 6-MB-47 were more prevalent, whereas 6-M-47 was also detected in some samples of crabs and earthworms (Table 3).

3.1.1. Species and seasonal differences

The evaluation of temporal variability of EDCs in the aquatic environment is extremely scarce so far, despite the strong influence of seasons on contamination levels found by some authors (Aminot et al., 2021). Fig. 2 shows the percentage of total pesticide residues, UV-filters, polycyclic musks, bisphenols, PBDEs, and MeO-BDEs for each species during the four seasons.

In macroalgae, musks and UV-filters, particularly HHCB and IMC, were the analytes more representative throughout the year, with higher levels found in winter followed by autumn. The presence of HHCB was previously reported in macroalgae, but at lower levels (Cunha et al., 2015), while the IMC levels were higher than those reported in macroalgae (*Eisenia bicyclis*) commercialized in Portugal (Petrarca et al., 2021). BPA and BPB were the bisphenols detected in macroalgae, with the highest levels found in summer. Macroalgae (*Ulva pertusa*) can bioaccumulate BPA as reported by Zhang et al. (2021), but there are no previous reports to the best of the author's knowledge concerning bisphenol levels in macroalgae. Pesticide residues, namely alachlor and γ-chlordane, were detected in macroalgae with the highest levels found in spring (Figs. 1 and 2). Pesticide residues were previously detected in macroalgae samples collected from Óbidos Lagoon (Portugal) in winter and summer, but not γ-chlordane (Pinto et al., 2014). Alachlor was quantified in green macroalgae (*Halimeda*) from Society Islands (Tahiti, French Polynesia) at levels ranging from 20 to 40 ng/g dw (Salvat et al., 2016), slightly lower than those here quantified. Among the studied PBDEs, macroalgae samples showed quantifiable levels of BDE-47 and BDE-99, particularly in autumn and winter, while MeO-BDE was not observed in any of them. A higher level (0.093 μg/kg ww) of BDE-99 was reported in sea lettuce (*Ulva* sp.) collected in Douro estuary, Portugal (Menezes-Sousa et al., 2021).

BPA and BDE-47 were the main contaminants in earthworms, followed by musks (AHTN and HHCB) (Fig. 1), especially in winter and spring (Fig. 2). In addition, 6-MB-47 was only found in one sample of earthworms in autumn. The presence of these contaminants in earthworms is reported for the first time, to the best of the author's knowledge.

Crab samples presented high levels of UV-filters in autumn, winter, and spring (Figs. 1 and 2), namely IMC and EHMC. Additionally, pesticide residues were commonly found in crabs, being bifenthrin the most frequently detected with higher levels found in summer and spring. The UV-filters and pesticide analytes were also observed in sediment and water samples from Tagus estuary (Cunha et al., 2022). HHCB was the only polycyclic musk found, presenting the highest levels in summer. In a previous study carried out with crabs commercialized in Europe, HHCB was the most abundant polycyclic musk detected with levels similar to those found here (Cunha et al., 2018). Among bisphenols, BPF and BPB were found in crabs either in summer or spring (Figs. 1 and 2). These bisphenols were detected for the first time in crabs to the best of the author's knowledge. In all seasons, crabs presented BDE-47, with the highest levels being detected in winter. In addition, 6-MB-47 was found in two crab samples, one in spring and another in autumn. Similarly, BDE-47 was the most abundant congener in green crabs (*Carcinus maenas*) from Douro river (Portugal), with concentrations ranging between 0.94 μg/kg ww (spring) and 2.15 μg/kg ww (summer) (Menezes-Sousa et al., 2021). Among the studied biota, the feeding habits of crabs have been associated with the high levels of PBDEs to species, because they are detritivores with an omnivorous diet, feeding on organisms from higher trophic levels (Bernárdez et al., 2000; Barros et al., 2008; Menezes-Sousa et al., 2021).

In bivalve samples (mussels and clams) the most abundant contaminants were HHCB and EHS, especially found in warmer seasons (summer and spring; Fig. 2). HHCB and EHS levels in mussels were higher than those reported in a previous study performed in commercial seafood samples (Cunha et al., 2018), with average levels of 22.30 and

Table 1
Overview of the musk fragrances and pesticide residues levels (average, minimum-maximum, ng/g ww) in each species collected in Tagus Estuary during the four seasons.

Species (n samples analysed)	Season (n samples analysed)	Site (n samples analysed)	HHCB	AHTN	Σmusks	Alachor	Ethion	*p,p′* DDT	Bifenthrin	γ- Chlordane	Σpesticides
Seabass (n = 47)	**Spring (n = 4)**	Contam. (n = 0)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Clean (n = 4)	n.d.	0.13 (n.d.–0.52)	0.13	2.85 (2.16–3.75)	n.d.	n.d.	n.d.	n.d.	2.85
	Summer (n = 5)	Contam. (n = 2)	8.52 (3.80–13.24)	1.01 (n.d.–2.02)	9.53	2.52 (2.26–2.76)	n.d.	n.d.	n.d.	n.d.	2.52
		Clean (n = 3)	7.38 (2.95–15.26)	n.d.	7.38	3.08 (2.75–3.69)	n.d.	n.d.	n.d.	n.d.	3.08
	Autumn (n = 26)	Contam. (n = 12)	9.96 (0.92–44.41)	29.60 (8.83–169.06)	39.56	4.13 (n. d.–15.73)	1.26 (n. d.–3.67)	1.36 (n. d.–16.06)	n.d.	n.d.	2.62
		Clean (n = 13)	3.01 (n.d.–9.44)	8.28 (n.d.–18.33)	11.29	3.31 (1.92–4.66)	n.d.	n.d.	n.d.	n.d.	3.31
	Winter (n = 12)	Contam. (n = 6)	8.76 (6.12–12.01)	0.89 (n.d.–2.38)	9.65	1.17 (n. d.–2.51)	n.d.	n.d.	n.d.	n.d.	1.17
		Clean (n = 6)	5.22 (3.84–5.77)	n.d.	5.22	1.80 (n. d.–2.76)	n.d.	n.d.	n.d.	n.d.	n.d.
Barbus (n = 27)	**Spring (n = 6)**	Contam. (n = 0)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Clean (n = 6)	9.25 (2.77–25.71)	n.d.	9.25	4.32 (3.37–5.96)	n.d.	n.d.	n.d.	n.d.	4.32
	Summer (n = 5)	Contam. (n = 2)	33.20 (22.63–43.78)	n.d.	33.20	4.81 (4.63–4.98)	n.d.	n.d.	n.d.	n.d.	4.81
		Clean (n = 2)	116.31 (n. d.–139.67)	n.d.	116.31	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Autumn (n = 8)	Contam. (n = 4)	7.38 (4.14–8.91)	n.d.	7.38	2.30 (n. d.–3.63)	n.d.	n.d.	n.d.	n.d.	2.30
		Clean (n = 4)	5.28 (3.76–6.37)	n.d.	5.28	0.74 (n. d.–2.95)	n.d.	n.d.	n.d.	n.d.	0.74
	Winter (n = 8)	Contam. (n = 4)	17.95 (5.65–31.85)	n.d.	17.95	2.70 (n. d.–3.63)	n.d.	n.d.	n.d.	n.d.	2.70
		Clean (n = 4)	28.16 (9.27–38.19)	n.d.	28.16	0.81 (n. d.–3.25)	n.d.	n.d.	n.d.	n.d.	0.81
Mullet (n = 58)	**Spring (n = 8)**	Contam. (n = 4)	3.01 (1.49–7.21)	n.d.	3.01	1.85 (n. d.–2.96)	n.d.	n.d.	n.d.	n.d.	1.85
		Clean (n = 3)	3.80 (n.d.–15.19)	n.d.	3.80	4.01 (3.36–4.40)	n.d.	n.d.	n.d.	n.d.	4.01
	Summer (n = 28)	Contam. (n = 8)	0.82 (n.d.–3.81)	n.d.	0.82	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Clean (n = 13)	126.21 (n. d.–147.29)	12.03 (n. d.–125.07)	138.24	3.71 (n. d.–7.97)	0.34 (n. d.–4.48)	8.99 (n. d.–77.93)	0.89 (n. d.–6.17)	n.d.	13.93
	Autumn (n = 8)	Contam. (n = 4)	11.60 (1.56–20.80)	1.44 (n.d.–5.75)	13.04	4.42 (2.36–6.91)	n.d.	n.d.	n.d.	n.d.	4.42
		Clean (n = 4)	11.70 (4.36–23.73)	n.d.	11.79	3.38 (2.66–4.36)	3.20 (n. d.–12.81)	1.94 (n. d.–7.74)	23.98 (n. d.–95.91)	n.d.	32.50
	Winter (n = 14)	Contam. (n = 7)	8.31 (1.75–20.10)	0.29 (n.d.–2.06)	8.60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Clean (n = 7)	10.79 (2.76–26.92)	n.d.	10.79	0.37 (n. d.–2.57)	0.69 (n. d.–4.87)	n.d.	1.55 (n. d.–9.59)	n.d.	2.61
Sole (n = 39)	**Summer (n = 15)**	Clean (n = 15)	4.42 (0.33–9.38)	0.97 (n.d.–2.95)	5.39	4.33 (n. d.–6.78)	n.d.	n.d.	1.47 (n. d.–3.56)	n.d.	5.80
	Autumn (n = 24)	Contam. (n = 12)	3.71 (0.04–14.85)	1.96 (0.51–3.40)	5.67	1.59 (n. d.–4.01)	0.45 (n. d.–5.41)	6.75 (n. d.–8.95)	1.12 (n. d.–3.06)	n.d.	9.91
		Clean (n = 12)	21.12 (n. d.–171.58)	146.52 (n. d.–899.17)	167.64	n.d.	n.d.	0.76 (n. d.–9.14)	n.d.	n.d.	0.76
Mussels (n = 24)	**Spring (n = 6)**	Contam. (n = 3)	25.67 (20.82–28.63)	2.01 (n.d.–6.02)	27.68	0.25 (n. d.–0.62)	n.d.	n.d.	2.53 (n. d.–6.32)	n.d.	2.78
		Clean (n = 3)		n.d.	12.76		n.d.	n.d.		n.d.	1.95

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Table 1 (*continued*)

Species (n samples analysed)	Season (n samples analysed)	Site (n samples analysed)	HHCB	AHTN	Σmusks	Alachor	Ethion	*p,p′* DDT	Bifenthrin	γ- Chlordane	Σpesticides
			12.76 (8.11–17.50)			0.22 (n.d.–0.65)			1.73 (1.41–2.15)		
	Summer (n = 6)	Contam. (n = 3)	26.80 (11.27–40.32)	n.d.	26.80	0.23 (n.d.–0.41)	n.d.	n.d.	1.95 (1.77–2.12)	n.d.	2.18
		Clean (n = 3)	7.62 (4.56–12.60)	n.d.	7.62	0.17 (n.d.–0.38)	n.d.	n.d.	0.49 (0.28–0.79)	n.d.	0.66
	Autumn (n = 6)	Contam. (n = 3)	7.79 (6.39–9.89)	n.d.	7.79	n.d.	n.d.	n.d.	0.63 (n.d.–1.22)	n.d.	0.63
		Clean (n = 3)	6.38 (2.85–11.86)	2.33 (n.d.–6.98)	8.71	0.03 (n.d.–0.08)	n.d.	n.d.	0.37 (n.d.–0.65)	n.d.	0.40
	Winter (n = 6)	Contam. (n = 3)	9.67 (4.73–15.70)	n.d.	9.67	0.18 (n.d.–0.53)	n.d.	n.d.	1.48 (0.92–2.31)	n.d.	1.66
		Clean (n = 3)	4.27 (2.03–8.33)	n.d.	4.27	0.17 (n.d.–0.50)	n.d.	n.d.	2.07 (1.25–2.85)	n.d.	2.24
Clams (n = 24)	**Spring (n = 6)**	Contam. (n = 3)	8.85 (5.46–12.27)	n.d.	8.85	0.61 (0.32–0.77)	n.d.	n.d.	0.36 (n.d.–1.09)	n.d.	0.97
		Clean (n = 3)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.36 (n.d.–1.07)	n.d.	0.36
	Summer (n = 6)	Contam. (n = 3)	1.81 (n.d.–5.18)	n.d.	1.81	n.d.	n.d.	n.d.	1.64 (1.27–2.18)	n.d.	1.64
		Clean (n = 3)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.92 (0.73–1.08)	n.d.	0.92
	Autumn (n = 6)	Contam. (n = 2)	3.23 (2.97–3.49)	n.d.	3.23	n.d.	n.d.	n.d.	0.19 (n.d.–0.39)	n.d.	0.19
		Clean (n = 2)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.17 (n.d.–0.34)	n.d.	0.17
	Winter (n = 6)	Contam. (n = 3)	2.88 (1.55–4.87)	n.d.	2.88	n.d.	n.d.	n.d.	1.17 (n.d.–2.79)	n.d.	1.17
		Clean (n = 3)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.34 (n.d.–7.03)	n.d.	2.34
Crabs (n = 24)	**Spring (n = 6)**	Contam. (n = 3)	1.93 (1.36–2.37)	n.d.	1.93	n.d.	n.d.	n.d.	0.85 (n.d.–1.34)	n.d.	0.85
		Clean (n = 3)	0.29 (n.d.–0.88)	n.d.	0.29	n.d.	n.d.	n.d.	2.15 (1.32–3.31)	n.d.	2.15
	Summer (n = 6)	Contam. (n = 3)	16.50 (n.d.–49.51)	n.d.	16.50	0.39 (n.d.–1.16)	n.d.	2.73 (n.d.–8.20)	0.61 (n.d.–1.82)	n.d.	3.73
		Clean (n = 3)	0.28 (n.d.–0.83)	n.d.	0.28	n.d.	n.d.	n.d.	0.86 (n.d.–2.59)	n.d.	0.86
	Autumn (n = 6)	Contam. (n = 3)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Winter (n = 6)	Contam. (n = 3)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.53 (n.d.–1.60)	n.d.	0.53
		Clean (n = 3)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.57 (n.d.–1.08)	n.d.	0.57
Earthworms (n = 5)	**Spring (n = 1)**	Contam. (n = 1)	7.25	n.d.	7.25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Autumn (n = 2)	Contam. (n = 1)	23.57	10.10	33.67	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Clean (n = 1)	15.41	13.00	28.41	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Winter (n = 2)	Contam. (n = 1)	32.84	78.44	111.28	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Clean (n = 1)	24.56	49.53	74.09	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Macroalgae (n = 48)	**Spring (n = 12)**	Contam. (n = 6)	21.33 (2.64–88.51)	n.d.	21.33	n.d.	n.d.	n.d.	n.d.	1.15 (n.d.–6.89)	1.15
		Clean (n = 6)	3.67 (1.32–7.26)	n.d.	3.67	0.37 (n.d.–2.21)	n.d.	n.d.	n.d.	0.34 (n.d.–2.02)	0.71
	Summer (n = 12)	Contam. (n = 6)	12.34 (1.73–38.85)	n.d.	12.34	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

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Table 1 (*continued*)

Species (n samples analysed)	Season (n samples analysed)	Site (n samples analysed)	HHCB	AHTN	Σmusks	Alachor	Ethion	*p,p′* DDT	Bifenthrin	γ- Chlordane	Σpesticides
		Clean (n = 6)	9.89 (n.d.–20.49)	n.d.	9.89	1.41 (n.d.–6.07)	n.d.	n.d.	n.d.	n.d.	1.41
	Autumn (n = 12)	Contam. (n = 6)	22.92 (11.65–34.10)	n.d.	22.92	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Clean (n = 6)	7.63 (4.01–13.49)	n.d.	7.63	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Winter (n = 12)	Contam. (n = 6)	28.06 (7.36–73.90)	3.65 (n.d.–21.92)	31.71	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Clean (n = 6)	15.54 (8.80–22.98)	n.d.	15.54	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not detected.

14.7 ng/g dw, respectively. Concerning pesticide residues, bifenthrin (mussels and clams) and alachlor (mussels) were prevalent (Fig. 1). The presence of bifenthrin (pyrethroid insecticide) in aquatic organisms was already detected in several aquatic rivers in Spain (Pico et al., 2019), despite bifenthrin have been banned for European agriculture use in 2019 (Regulation 2022/643). Regarding bisphenols, higher amounts of BPF, BPB, and BPA were quantified in clams during winter, while in mussels only BPF was detected. The differences between species can be related to their feeding habits, despite both species are filter feeders, mussels generally filter particles out of the water, depending on waves and currents, while clams live buried in surface sediments. The levels of BPF in bivalves were slightly higher than those reported in a recent study with mussels (*Perna perna*) collected in different sites of South Africa, ranging from not detected to 25.6 ng/g dw (Castro et al., 2021). Similar to that observed in crabs, BDE-47 was the PBDE congener that was also prevalent in mussel samples, being present in all seasons. A similar average content of ΣPBDEs (0.14 μg/kg ww) was quantified in mussels from the Netherlands (Gebbink et al., 2019) and Douro estuary (Portugal) (Menezes-Sousa et al., 2021). The bioaccumulation of PBDEs might be expected as a result of the slower growth rate of these filter-feeding organisms, which will accumulate persistent organic compounds for a long period (Beyer et al., 2017; Menezes-Sousa et al., 2021). MeO-BDEs, 2′-MB-68 and 6-MB-47 were found in mussels caught in all seasons. ΣMeO-BDE levels varied between 0.33 μg/kg ww (spring) and 0.84 μg/kg ww (autumn) in mussel samples (Table 3). However, in clam samples, only 2′-MB-68 was detected, specifically in autumn. Higher levels of 2′-MB-68 and 6-MB-47 from 1.34 μg/kg ww (spring) to 4.97 μg/kg ww (summer) were reported in mussels from Douro estuary (Menezes-Sousa et al., 2021).

In European seabass (*Dicentrarchus labrax*), as well as in other fish species, pesticide residues were present in all seasons, especially in spring and summer. The pesticide residue mainly found in European seabass and barbus was alachlor, while in mullet and sole the most relevant compounds were alachlor, ethion, *p,p′* DDT, and bifenthrin. The presence of these pesticide residues was coincident with those reported in water and sediments collected in the same timeframe in Tagus estuary (Cunha et al., 2022). The use of pesticides in agriculture, particularly in the intensive production of rice, corn, and wine, with highly intensive farming in the areas surrounding the Tagus estuary could contribute to the pesticide found in fish species. Other classes of contaminants widely found were polycyclic musks (HHCB and AHTN), particularly in mullet samples, with the highest levels found in summer. In seabass, musk represented more than 50% of the contribution in most seasons except spring (Fig. 2), while in sole samples (only collected in autumn and summer) these musk contaminants were more abundant in autumn. This is in accordance with our previous study performed on seafood collected in different European markets, with HHCB reaching 414.4 μg/kg d.w. in plaice/sole (Cunha et al., 2018). UV-filters (IMC, EHMC, and EHS) were one of the most prevalent contaminants in European seabass and mullet, particularly in winter, while for sole higher prevalence was found in the summer (Fig. 1). The levels of IMC were particularly higher compared with EHS levels, which is in accordance with the results obtained in our previous study (Cunha et al., 2018). Regarding bisphenols, BPB and BPF were also found in mullet, with autumn achieving the highest levels. The presence of analogues of BPA, such as BPB and BPE, was previously reported in *Dicentrarchus labrax, Trachurus trachurus* and *Scomber colias* obtained from Portuguese coastal waters (continental shelf) of the North East (NE) Atlantic Ocean (Barboza et al., 2020). The PBDEs 28, 47, and 99, the most frequent PBDEs found in fish samples from Portuguese and Netherlands rivers (Gama et al., 2006; Cruz et al., 2019, 2020; Gebbink et al., 2019; Menezes-Sousa et al., 2021), were present in all fish species analysed, with higher prevalence found in autumn for sole and mullet samples. Regarding the PBDE metabolites, 4-MB-49 was detected in seabass in all seasons, while 5-MB-47 was found in mullet and sole in autumn.

Regarding seasonal variation, statistically significant differences

Table 2
Overview of the UV-filters and bisphenols levels (average, minimum-maximum, ng/g ww) in each species collected in Tagus Estuary during four seasons.

Species (n samples analysed)	Season (n samples analysed)	Site (n samples analysed)	EHS	IMC	BP3	EHMC	ΣUV-filters	BPF	BPA	BPB	Σbisphenols
Seabass (n = 47)	**Spring (n = 4)**	Contam. (n = 0)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Clean (n = 4)	0.81 (n. d.–1.62)	2.01 (n. d.–3.13)	n.d.	2.35 (n. d.–9.42)	5.17	n.d.	n.d.	n.d.	n.d.
	Summer (n = 5)	Contam. (n = 2)	n.d.	2.48 (n. d.–4.97)	n.d.	n.d.	2.48	n.d.	n.d.	n.d.	n.d.
		Clean (n = 3)	5.94 (n. d.–12.35)	0.37 (n. d.–1.13)	n.d.	n.d.	6.31	n.d.	n.d.	n.d.	n.d.
	Autumn (n = 26)	Contam. (n = 12)	5.21 (n. d.–19.23)	3.01 (n. d.–22.83)	n.d.	n.d.	8.22	1.87 (n. d.–6.49)	n.d.	5.92 (n. d.–28.48)	7.79
		Clean (n = 13)	0.63 (n. d.–6.42)	4.07 (n. d.–31.16)	n.d.	0.10 (n. d.–1.28)	4.80	0.30 (n. d.–1.73)	n.d.	n.d.	0.30
	Winter (n = 12)	Contam. (n = 6)	4.24 (n. d.–17.6)	0.58 (n. d.–2.47)	n.d.	n.d.	4.82	0.02 (n. d.–0,12)	n.d.	n.d.	0.02
		Clean (n = 6)	0.69 (n. d.–4.16)	0.54 (n. d.–2.17)	n.d.	n.d.	1.23	n.d.	n.d.	n.d.	n.d.
Barbus (n = 27)	**Spring (n = 6)**	Contam. (n = 0)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Clean (n = 6)	n.d.	2.92 (1.86–4.43)	n.d.	n.d.	2.92	n.d.	n.d.	n.d.	n.d.
	Summer (n = 5)	Contam. (n = 2)	n.d.	23.38 (19.53–27.23)	n.d.	n.d.	23.38	n.d.	n.d.	n.d.	n.d.
		Clean (n = 2)	41.15 (n. d.–82.30)	n.d.	n.d.	n.d.	41.15	n.d.	n.d.	n.d.	n.d.
	Autumn (n = 8)	Contam. (n = 4)	n.d.	3.42 (n. d.–8.88)	n.d.	n.d.	3.42	n.d.	n.d.	n.d.	n.d.
		Clean (n = 4)	n.d.	6.59 (n. d.–14.84)	n.d.	n.d.	6.59	n.d.	n.d.	n.d.	n.d.
	Winter (n = 8)	Contam. (n = 4)	n.d.	0.78 (n. d.–2.02)	n.d.	n.d.	0.78	n.d.	n.d.	n.d.	n.d.
		Clean (n = 4)	n.d.	2.26 (n. d.–5.49)	n.d.	n.d.	2.26	n.d.	n.d.	n.d.	n.d.
Mullet (n = 58)	**Spring (n = 8)**	Contam. (n = 4)	n.d.	3.10 (n. d.–5.95)	n.d.	n.d.	3.10	n.d.	n.d.	n.d.	n.d.
		Clean (n = 3)	0.14 (n. d.–0.43)	5.51 (n. d.–12.33)	n.d.	n.d.	5.65	n.d.	n.d.	n.d.	n.d.
	Summer (n = 28)	Contam. (n = 8)	n.d.	48.55 (n. d.–135.54)	n.d.	n.d.	48.55	18.84 (n. d.–124.59)	n.d.	n.d.	18.84
		Clean (n = 13)	3.30 (n. d.–13.77)	29.67 (n. d.–250.57)	n.d.	n.d.	32.97	0.64 (n. d.–8.36)	n.d.	n.d.	0.64
	Autumn (n = 8)	Contam. (n = 4)	0.94 (n. d.–2.61)	4.32 (n. d.–9.77)	n.d.	0.17 (n. d.–0.68)	5.43	0.16 (n. d.–0.66)	n.d.	0.77 (n. d.–3.08)	0.93
		Clean (n = 4)	0.25 (n. d.–0.98)	9.87 (2.25–27.34)	n.d.	0.39 (n. d.–1.58)	10.51	17.02 (n. d.–68.09)	n.d.	80.73 (n. d.–322.92)	97.75
	Winter (n = 14)	Contam. (n = 7)	n.d.	2.21 (n. d.–3.89)	n.d.	n.d.	2.21	n.d.	n.d.	n.d.	n.d.
		Clean (n = 7)	n.d.	1.62 (n. d.–3.35)	n.d.	n.d.	1.62	1.61 (n. d.–9.44)	n.d.	n.d.	1.61
Sole (n = 39)	**Summer (n = 15)**	Clean (n = 15)	n.d.	7.42 (n. d.–11.27)	n.d.	1.68 (0.26–3.64)	9.10	n.d.	n.d.	n.d.	n.d.
	Autumn (n = 24)	Contam. (n = 12)	2.86 (0.43–11.09)	8.00 (n. d.–16.53)	n.d.	1.92 (1.06–4.68)	12.78	n.d.	n.d.	0.08 (n. d.–0.90)	0.08
		Clean (n = 12)	n.d.	8.62 (n. d.–33.31)	n.d.	n.d.	8.62	2.77 (n. d.–24.24)	n.d.	0.53 (n. d.–3.17)	3.30
Mussels (n = 24)	**Spring (n = 6)**	Contam. (n = 3)	15.05 (10.70–22.01)	47.93 (n. d.–143.79)	n.d.	4.04 (2.50–6.57)	67.02	6.52 (n. d.–19.57)	n.d.	n.d.	6.52
		Clean (n = 3)	7.13 (4.28–9.45)	1.13 (n. d.–3.40)	n.d.	4.98 (2.74–7.36)	13.24	0.14 (n. d.–0.41)	n.d.	n.d.	0.14
	Summer (n = 6)	Contam. (n = 3)	1.86 (n. d.–2.82)	2.92 (2.55–3.46)	n.d.	1.32 (n. d.–2.73)	6.10	0.12 (n. d.–0.35)	n.d.	n.d.	0.12
		Clean (n = 3)	4.42 (3.20–6.86)	0.57 (n. d.–0.98)	n.d.	1.34 (1.15–1.68)	6.33	n.d.	n.d.	n.d.	n.d.
	Autumn (n = 6)	Contam. (n = 3)	5.39 (4.74–6.39)	3.77 (1.83–5.24)	n.d.	2.14 (1.61–2.67)	11.30	n.d.	n.d.	n.d.	n.d.
		Clean (n = 3)	7.04 (0.60–17.73)	2.32 (n. d.–6.08)	n.d.	1.87 (0.37–4.28)	11.23	0.16 (n. d.–0.41)	n.d.	n.d.	0.16
	Winter (n = 6)	Contam. (n = 3)	3.48 (1.35–4,67)	2.18 (1.44–3.24)	n.d.	1.48 (0.92–2.31)	7.14	n.d.	n.d.	n.d.	n.d.
		Clean (n = 3)	3.40 (n. d.–9.00)	4.91 (2.17–6.78)	n.d.	n.d.	8.31	0.01 (n. d.–0.02)	n.d.	n.d.	0.01
Clams (n = 24)	**Spring (n = 6)**	Contam. (n = 3)	2.73 (n. d.–4.72)	0.78 (n. d.–2.35)	n.d.	1.84 (1.21–2.85)	5.35	n.d.	n.d.	n.d.	n.d.

(*continued on next page*)

Table 2 (*continued*)

Species (n samples analysed)	Season (n samples analysed)	Site (n samples analysed)	EHS	IMC	BP3	EHMC	ΣUV-filters	BPF	BPA	BPB	Σbisphenols
		Clean (n = 3)	0.96 (n.d.–1.44)	n.d.	n.d.	n.d.	0.96	n.d.	1.63 (n.d.–4.90)	n.d.	1.63
	Summer (n = 6)	Contam. (n = 3)	1.10 (n.d.–3.29)	4.60 (2.31–8.80)	n.d.	1.52 (0.82–2.52)	7.22	0.13 (n.d.–0.39)	n.d.	n.d.	0.13
		Clean (n = 3)	5.12 (n.d.–14.92)	n.d.	n.d.	n.d.	5.12	n.d.	n.d.	n.d.	n.d.
	Autumn (n = 6)	Contam. (n = 2)	3.66 (1.08–9.90)	n.d.	n.d.	0.17 (n.d.–0.33)	3.83	n.d.	n.d.	n.d.	n.d.
		Clean (n = 2)	1.34 (0.73–1.96)	n.d.	n.d.	n.d.	1.34	n.d.	n.d.	n.d.	n.d.
	Winter (n = 6)	Contam. (n = 3)	3.79 (n.d.–11.37)	0.55 (n.d.–1.64)	n.d.	0.11 (n.d.–0.21)	4.45	0.35 (n.d.–1.04)	n.d.	n.d.	0.35
		Clean (n = 3)	2.31 (1.14–2.98)	n.d.	n.d.	n.d.	2.31	1.52 (n.d.–4.57)	n.d.	6.62 (n.d.–19.86)	6.62
Crabs (n = 24)	**Spring (n = 6)**	Contam. (n = 3)	0.26 (n.d.–0.58)	6.61 (3.20–12.83)	n.d.	1.90 (1.50–2.47)	8.77	n.d.	n.d.	n.d.	n.d.
		Clean (n = 3)	n.d.	5.58 (3.94–8.73)	n.d.	n.d.	5.58	0.46 (n.d.–1.37)	n.d.	n.d.	0.46
	Summer (n = 6)	Contam. (n = 3)	0.49 (n.d.–1.47)	2.00 (n.d.–3.54)	n.d.	2.00 (0.44–3.66)	4.49	4.47 (n.d.–13.40)	n.d.	2.67 (n.d.–8.01)	7.14
		Clean (n = 3)	n.d.	3.71 (2.19–5.27)	n.d.	n.d.	3.71	0.27 (n.d.–0.81)	n.d.	n.d.	0.27
	Autumn (n = 6)	Contam. (n = 3)	n.d.	0.86 (n.d.–2.58)	n.d.	0.51 (0.37–0.72)	1.37	n.d.	n.d.	n.d.	n.d.
	Winter (n = 6)	Contam. (n = 3)	n.d.	3.24 (2.51–4.47)	n.d.	0.69 (0.56–0.78)	3.93	n.d.	n.d.	n.d.	n.d.
		Clean (n = 3)	n.d.	3.15 (2.88–3.29)	n.d.	n.d.	3.15	n.d.	n.d.	n.d.	n.d.
Earthworms (n = 5)	**Spring (n = 1)**	Contam. (n = 1)	0.74	n.d.	n.d.	n.d.	0.74	n.d.	7.21	n.d.	7.21
	Autumn (n = 2)	Contam. (n = 1)	0.18	3.58	n.d.	0.46	4.22	n.d.	88.93	n.d.	88.93
		Clean (n = 1)	1.96	3.44	n.d.	n.d.	5.40	n.d.	97.31	n.d.	97.31
	Winter (n = 2)	Contam. (n = 1)	1.27	13.62	n.d.	n.d.	14.89	n.d.	106.75	n.d.	106.75
		Clean (n = 1)	0.55	4.66	n.d.	n.d.	5.21	n.d.	81.68	n.d.	81.68
Macroalgae (n = 48)	**Spring (n = 12)**	Contam. (n = 6)	n.d.	12.33 (2.26–30.32)	n.d.	1.64 (0.07–5.17)	13.97	0.19 (n.d.–0.62)	1.23 (n.d.–5.38)	n.d.	1.42
		Clean (n = 6)	n.d.	10.09 (4.29–15.08)	n.d.	n.d.	10.09	n.d.	n.d.	0.23 (n.d.–1.37)	0.23
	Summer (n = 12)	Contam. (n = 6)	n.d.	3.34 (n.d.–6.19)	n.d.	n.d.	3.34	6.54 (n.d.–39.26)	0.80 (n.d.–4.83)	38.69 (n.d.–232.17)	46.03
		Clean (n = 6)	n.d.	11.46 (3.60–26.39)	n.d.	n.d.	11.46	n.d.	n.d.	n.d.	n.d.
	Autumn (n = 12)	Contam. (n = 6)	n.d.	13.58 (2.02–51.91)	n.d.	n.d.	13.58	n.d.	n.d.	n.d.	n.d.
		Clean (n = 6)	n.d.	7.55 (3.22–17.83)	n.d.	n.d.	7.55	0.12 (n.d.–0.70)	0.94 (n.d.–5.65)	0.79 (n.d.–4.76)	1.85
	Winter (n = 12)	Contam. (n = 6)	n.d.	14.13 (4.75–42.42)	n.d.	0.21 (n.d.–1.19)	14.34	n.d.	n.d.	0.26 (n.d.–1.54)	0.26
		Clean (n = 6)	n.d.	14.49 (2.56–48.42)	n.d.	0.32 (n.d.–1.07)	14.81	n.d.	1.18 (n.d.–7.06)	n.d.	1.18

n.d.: not detected.

were found for both ∑pesticide residues and ∑musks, with summer and autumn revealing higher levels than winter and spring. The levels of ∑bisphenols were lower in spring than in the other seasons (see Supplementary Table S5). As previously described by Bachelot et al. (2012), EHMC levels increase during months when the air temperature rises. The average concentrations of ΣPBDEs were statistically higher ($p < 0.05$) in autumn and winter compared to spring or summer. The ΣPBDE levels did not significantly differ ($p > 0.05$) between autumn and winter, as well as between spring and summer. Particularly, the BDE-99 content was statistically higher ($p < 0.05$) in autumn. Regarding PBDE metabolites (MeO-BDE), no statistical difference ($p > 0.05$) was registered between seasons. On the other hand, the average concentration of ΣMeO-BDE was statistically higher ($p < 0.05$) in mussels, when compared with other species. Besides, BDE-28 and BDE-99 contents were statistically higher ($p < 0.05$) in seabass and sole, which did not differ significantly ($p > 0.05$) from each other. The BDE-47 contents were statistically higher ($p < 0.05$) in seabass, sole, mussels, and crabs, which did not differ significantly ($p > 0.05$) from each other.

3.1.2. *"Clean" area versus "contaminated" area*

For a better comparison of results, some data obtained for seabass and barbus in spring, sole in summer, and earthworms in spring were excluded to guarantee an equivalent analysis in both areas.

Among fish species, seabass collected in the "contaminated" area presented higher levels of contamination than those from the "clean" area. In contrast, mullet, barbus, and sole, particularly in certain seasons, presented higher levels of analytes in the "clean" area than in the "contaminated" area.

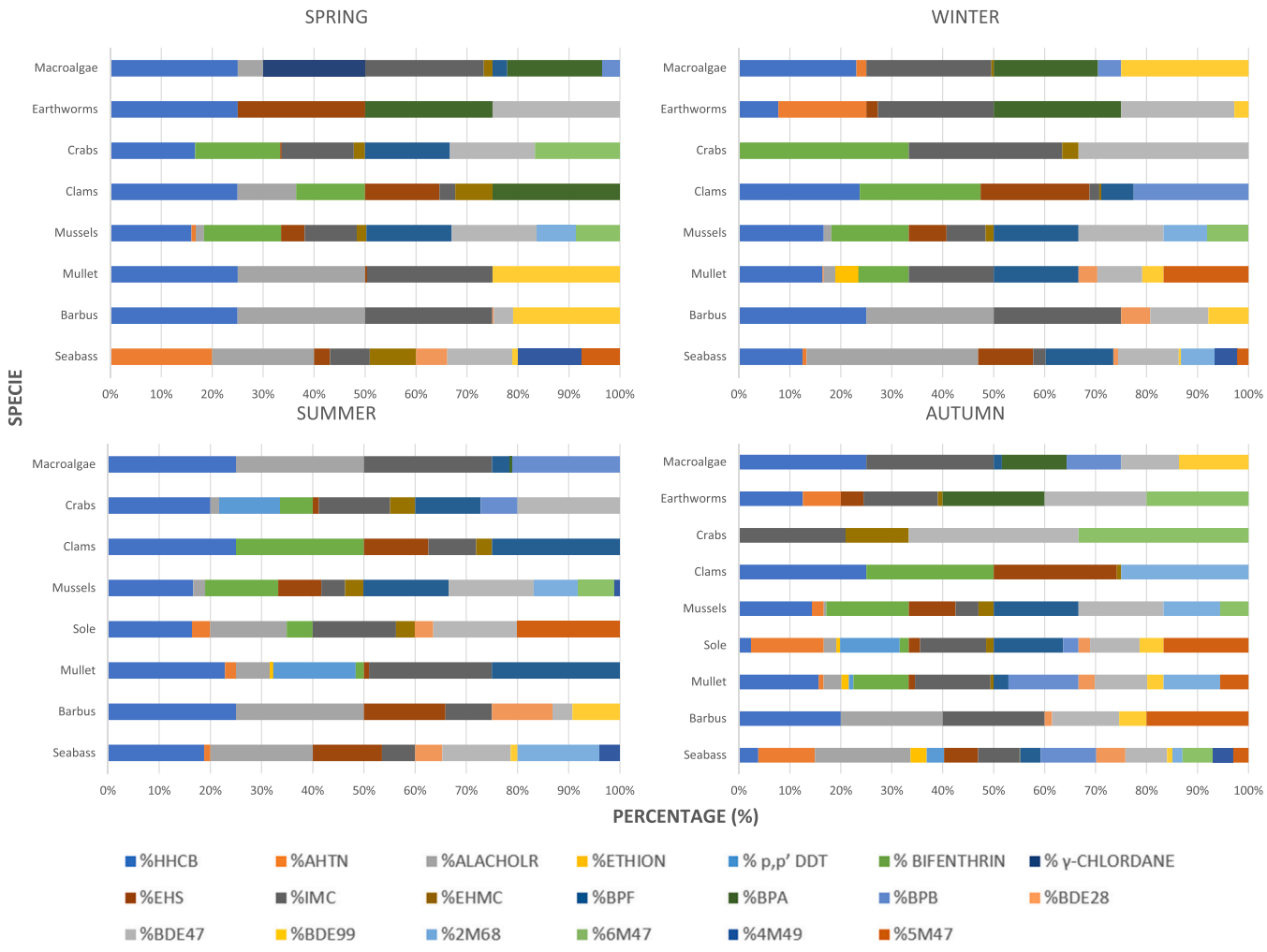

Fig. 1. Percentage of the analytes quantified in each specie collected in Tagus estuary in the different seasons. Specie vs percentage (%).

Regarding the other organisms included in this study (mussels, clams, green crabs, earthworms and macroalgae), the levels of analytes in samples collected in the "contaminated" area were higher compared with those collected in the "clean" area, as expected since they are sedentary or slow-moving animals. Concerning the contaminants found in these species, the number of analytes detected increased in "contaminated" areas compared to "clean" areas, particularly for pesticide residues (biphentrin, *p,p′-DDT,* and alachlor), UV-filters (EHMC), and BDE-47 (Tables 1 to 3).

These results were somehow expected since the samples collected in "contaminated" areas of Tagus estuary are subjected to high anthropogenic activities, having a high population density around, including Loures (population density of 1.226.1 inhab./km^2), Odivelas (population density of 11.884.9 inhab./km^2) and Vila Franca de Xira (population density of 429.7 inhab./km^2) (Moura et al., 2017). These areas are also subject to different sources of point and diffuse pollution, such as large urban wastewater with domestic effluents, discharges of treated and untreated effluents from industrial, domestic, hospital, and livestock origins, chemical and petrochemical industries, pollution from marinas, ports, and boats (cruises, ferryboats, catamarans, fishing boats, and freighters) and intensive agriculture (fertilizers and pesticides). However, considering all species analysed in both areas ("clean" and "contaminated"), no significant differences were found between them.

3.2. Seafood consumption and risk assessment

Estimated dietary intakes (EDI) for polycyclic musks, pesticide residues, UV-filters, bisphenols, PBDEs, and their metabolites through fish and clams consumption are shown in Tables 4 and 5. Calculations were based on raw concentrations determined for all analytes combined with the consumption data of the Portuguese population available in Jacobs et al. (2017). In general, Portuguese adult consumers revealed the highest exposure to these contaminants through seafood consumption compared with other European countries, since it is reported that more than 70% of the Portuguese population eat seafood more than once a week, reaching a consumption of 56 kg/person/year (Almeida et al., 2015; Oceans and fisheries, 2019).

The average EDI of musks ranged between 0.1 and 16.0 μg/kg bw/day for clams and soles, respectively. Due to the very limited data, concerning the safety levels of polycyclic musks, the risk assessment was carried out by dividing the NOAEL by 100 as an uncertainty factor (to account for species differences and human variability). The European Chemical Agency (ECHA) established a NOAEL of 150 and 5 mg/kg bw/day for HHCB and AHTN, respectively (ECHA, 2008a,b). The values obtained in this study were well below the reference values, thus is unlikely that a consumers are affected by these contaminants through seafood consumption. Regarding other musks, such as DPMI or ADBI, due to the lack of toxicological information, threshold values for risk characterization have not yet been established.

In the case of pesticide residues, the average EDI ranged between 0.1 and 2.0 μg/kg bw/day for clams and mullet, respectively. Since the levels detected for *p,p′-DDT* revealed a human health risk of developing chronic diseases, the value of ADI (0.01 mg/kg bw/day) was considered instead of ArfD (FAO/WHO, 2000). Regarding the other pesticide

Table 3
Overview of the PBDE and MeO-BDE levels (average, minimum-maximum, ng/g ww) in each species collected in Tagus Estuary during for seasons.

Specie (n samples analysed)	Season (n samples analysed)	Site (n samples analysed)	BDE28	BDE47	BDE99	∑BDE	2´-MB-68	6-MB-47	4-MB-49	5-MB-47	∑MeO-BDE
Seabass (n = 44)	**Spring (n = 5)**	Clean (n = 4)	0.11 (0.03–0.22)	0.23 (n. d.−0.80)	0.02 (n. d.−0.05)	0.36	n.d.	n.d.	0.05 (n. d.−0.15)	0.03 (n. d.−0.10)	0.08
	Summer (n = 4)	Clean (n = 4)	0.08 (n. d.−0.21)	0.20 (n. d.−0.79)	0.02 (n. d.−0.04)	0.30	0.04 (n. d.−0.17)	n.d.	0.01 (n. d.−0.03)	n.d.	0.05
	Autumn (n = 24)	Contam. (n = 12)	0.33 (n. d.−2.51)	0.53 (n. d.−1.34)	0.02 (n. d.−0.10)	0.88	0.02 (n. d.−0.18)	0.06 (n. d.−0.74)	0.01 (n. d.−0.04)	0.01 (n. d.−0.04)	0.10
		Clean (n = 12)	0.16 (n. d.−0.53)	0.17 (n. d.−0.86)	0.07 (n. d.−0.64)	0.40	n.d.	n.d.	0.03 (n. d.−0.25)	0.02 (n. d.−0.21	0.05
	Winter (n = 11)	Contam. (n = 4)	0.09 (n. d.−0.34)	0.45 (n. d.−1.54)	0.01 (n. d.−0.03)	0.55	0.03 (n. d.−0.17)	n.d.	0.02 (n. d.−0.05)	0.01 (n. d.−0.04)	0.06
		Clean (n = 5)	0.05 (n. d.−0.12)	1.35 (n. d.−2.17)	0.05 (n. d.−0.10)	1.45	n.d.	n.d.	n.d.	n.d.	n.d.
Barbus (n = 26)	**Spring (n = 5)**	Clean (n = 2)	0.01 (n. d.−0.02)	0.14 (0.10–0.17)	0.78 (0.57–0.98)	0.93	n.d.	n.d.	n.d.	n.d.	n.d.
	Summer (n = 6)	Contam. (n = 1)	n.d.	n.d.	0.03	0.03	n.d.	n.d.	n.d.	n.d.	n.d.
		Clean (n = 2)	0.46 (0.03–0.89)	0.15 (n. d.−0.30)	0.33 (0.03–0.63)	0.94	n.d.	n.d.	n.d.	n.d.	n.d.
	Autumn (n = 8)	Clean (n = 2)	0.03 (0.01–0.05)	0.27 (n. d.−0.53)	0.11 (0.04–0.19)	0.41	n.d.	n.d.	n.d.	0.02 (n. d.−0.05)	0.02
	Winter (n = 7)	Contam. (n = 1)	n.d.	n.d.	0.03	0.03	n.d.	n.d.	n.d.	n.d.	n.d.
		Clean (n = 1)	0.08	0.16	0.08	0.32	n.d.	n.d.	n.d.	n.d.	n.d.
Mullet (n = 49)	**Spring (n = 7)**	Clean (n = 1)	n.d.	n.d.	0.09	0.09	n.d.	n.d.	n.d.	n.d.	n.d.
	Autumn (n = 8)	Contam. (n = 3)	0.05 (n. d.−0.14)	0.16 (n. d.−0.49)	0.05 (n. d.−0.07)	0.26	0.06 (n. d.−0.18)	n.d.	n.d.	0.03 (n. d.−0.09)	0.09
	Winter (n = 12)	Contam. (n = 2)	0.04 (n. d.−0.08)	0.06 (0.06–0.06)	0.06 (0.05–0,08)	0.16	n.d.	n.d.	n.d.	n.d.	n.d.
		Clean (n = 2)	0.19 (0.15–0.23)	0.49 (0.48–0.50)	0.20 (0.18–0.22)	0.88	n.d.	n.d.	n.d.	0.01 (n. d.−0.03)	0.01
Sole (n = 36)	**Summer (n = 14)**	Clean (n = 14)	0.10 (n. d.−0.55)	0.46 (n. d.−1.13)	0.002 (n. d.−0.01)	0.56	n.d.	n.d.	n.d.	0.01 (n. d.−0.06)	0.01
	Autumn (n = 22)	Contam. (n = 12)	0.10 (n. d.−0.42)	0.36 (n. d.−1.07)	0.22 (n. d.−0.48)	0.68	n.d.	n.d.	n.d.	0.01 (n. d.−0.07)	0.01
		Clean (n = 10)	0.04 (n. d.−0.13)	0.24 (n. d.−1.00)	0.07 (n. d.−0.17)	0.35	n.d.	n.d.	n.d.	n.d.	n.d.
Mussels (n = 24)	**Spring (n = 6)**	Contam. (n = 2)	n.d.	0.34 (n. d.−0.69)	n.d.	0.34	0.15 (0.13–0.17)	0.11 (0.09–0.14)	n.d.	n.d.	0.27
		Clean (n = 2)	n.d.	0.21 (n. d.−0.43)	n.d.	0.21	n.d.	0.06 (n. d.−0.12)	n.d.	n.d.	0.06
	Summer (n = 6)	Contam. (n = 3)	n.d.	1.50 (0.98–2.06)	n.d.	1.50	0.11 (n. d.−0.18)	0.14 (0.07–0.19)	n.d.	n.d.	0.24
		Clean (n = 3)	n.d.	0.19 (n. d.−0.56)	n.d.	0.19	0.28 (0.21–0.37)	0.18 (0.15–0.21)	0.05 (n. d.−0.15)	n.d.	0.51
	Autumn (n = 6)	Contam. (n = 3)	n.d.	n.d.	n.d.	n.d.	0.26 (0.17–0.35)	0.13 (0.08–0.17)	n.d.	n.d.	0.39
		Clean (n = 3)	n.d.	0.15 (n. d.−0.46)	n.d.	0.15	0.30 (0.24–0.38)	0.15 (n. d.−0.25)	n.d.	n.d.	0.45
	Winter (n = 6)	Contam. (n = 3)	n.d.	1.10 (n. d.−1.67)	n.d.	1.10	0.17 (0.13–0.20)	0.17 (0.13–0.20)	n.d.	n.d.	0.34
		Clean (n = 2)	n.d.	0.36 (n. d.−0.72)	n.d.	0.36	0.23 (0.21–0.26)	0.20 (0.19–0.20)	n.d.	n.d.	0.43
Clams (n = 24)	**Autumn (n = 6)**	Contam. (n = 1)	n.d.	n.d.	n.d.	n.d.	0.06	n.d.	n.d.	n.d.	0.06
Crabs (n = 23)	**Spring (n = 6)**	Contam. (n = 3)	n.d.	0.33 (n. d.−0.55)	n.d.	0.33	n.d.	0.01 (n. d.−0.04)	n.d.	n.d.	0.01
		Clean (n = 1)	n.d.	0.86	n.d.	0.86	n.d.	n.d.	n.d.	n.d.	n.d.
	Summer (n = 6)	Contam. (n = 1)	n.d.	0.08	n.d.	0.08	n.d.	n.d.	n.d.	n.d.	n.d.
		Clean (n = 1)	n.d.	0.59	n.d.	0.59	n.d.	n.d.	n.d.	n.d.	n.d.
	Autumn (n = 6)	Contam. (n = 3)	n.d.	0.61 (n. d.−0.93)	n.d.	0.61	n.d.	0.01 (n. d.−0.04)	n.d.	n.d.	0.01
	Winter (n = 5)	Clean (n = 3)	n.d.	1.79 (1.51–2.26)	n.d.	1.79	n.d.	n.d.	n.d.	n.d.	n.d.
Earthworm (n = 5)	**Spring (n = 1)**	Contam. (n = 1)	n.d.	2.46	n.d.	2.46	n.d.	n.d.	n.d.	n.d.	n.d.
	Autumn (n = 2)	Contam. (n = 1)	n.d.	4.74	n.d.	4.74	n.d.	0.04	n.d.	n.d.	0.04

(*continued on next page*)

Table 3 (*continued*)

Specie (n samples analysed)	Season (n samples analysed)	Site (n samples analysed)	BDE28	BDE47	BDE99	∑BDE	2´-MB-68	6-MB-47	4-MB-49	5-MB-47	∑MeO-BDE
		Clean (n = 1)	n.d.	3.94	n.d.	3.94	n.d.	n.d.	n.d.	n.d.	n.d.
	Winter (n = 2)	Contam. (n = 1)	n.d.	n.d.	0.79	0.79	n.d.	n.d.	n.d.	n.d.	n.d.
		Clean (n = 1)	n.d.	9.74	0.43	10.17	n.d.	n.d.	n.d.	n.d.	n.d.
Macroalgae (n = 48)	**Autumn (n = 12)**	Contam. (n = 2)	n.d.	0.05 (n. d.–0.11)	0.01 (n. d.–0.02)	0.06	n.d.	n.d.	n.d.	n.d.	n.d.
		Clean (n = 2)	n.d.	n.d.	0.05 (0.04–0.07)	0.05	n.d.	n.d.	n.d.	n.d.	n.d.
	Winter (n = 12)	Contam. (n = 1)	n.d.	n.d.	0.02	0.02	n.d.	n.d.	n.d.	n.d.	n.d.
		Clean (n = 1)	n.d.	n.d.	0.06	0.06	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not detected.

Fig. 2. Percentage of contribution of pesticide residues, UV-filters, polycyclic musks, bisphenols, PBDEs and MeO-BDEs in the different seasons and species. Specie vs percentage (%).

residues, ethion ArfD established by WHO was considered (0.02 mg/kg bw; JECFA, 2017), as well as alachlor NOAEL (0.5 mg/kg bw/day; FAO-UNEP, 2016), and bifenthrin ADI (0.01 mg/kg bw; WHO, 2012). As shown in Table 4, the established limits for these analytes were not exceeded, indicating no risk of exposure to these pesticide residues. For UV-filters, the NOAEL was considered for EHS (25 mg/kg bw/day), EHMC and IMC (450 mg/kg bw/day in both cases) (ECHA, 2008). All of them are higher than the EDI calculated here (4.9 μg/kg bw/day for sole) (Table 4), therefore revealing a limited risk of UV-filters exposure for seafood consumers. Concerning BPA, the calculated EDI ranged between 0.1 μg/kg bw/day for clams and 4.3 μg/kg bw/day for mullet, where the latter exceeded the temporary tolerable day intake (t-TDI) of 4 μg/kg bw/day (EFSA, 2015). Recently, EFSA's expert Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) has re-evaluated the risk of BPA and a lower TDI of 0.04 ng/kg bw/day has been published in a draft (EFSA, 2021), making that all the species analysed constitute a risk. Regarding the other bisphenol analogues, the risk assessment was not possible due to the absence of international regulatory levels.

The highest average EDI for the ΣPBDEs and their metabolites was

Table 4
Estimated daily intakes (μg/kg bw/day) of polycyclic musks (HHCB and AHTN), pesticide residues (alachlor, ethion, *p,p'-DDT*, bifenthrin, chlordane), UV-filters (EHS, IMC, BP3, and EHMC) and bisphenols (BPA, BPB and BPF) through consumption of seabass, barbus, mullet, sole and clams collected in Tagus Estuary. (STD, standard deviation).

EDI	Musks	Pesticide residues	UV-filters	Bisphenols
Seabass				
Average (±STD)	3.0 (5.0)	0.5 (0.8)	1.1 (1.8)	0.3 (0.5)
Median (min-max)	0.3 (0.0011–14.2)	0.1 (0.0002–2.3)	0.1 (0.0004–5.1)	0.031 (0.0001–1.4)
Barbus				
Average (±STD)	7.7 (12.7)	0.5 (0.8)	2.8 (4.7)	n.d.
Median (min-max)	0.8 (0.0027–36.3)	0.05 (0.0002–2.2)	0.3 (0.001–13.5)	n.d.
Mullet				
Average (±STD)	6.7 (11.2)	2.0 (3.3)	3.8 (6.3)	4.3 (7.2)
Median (min-max)	0.7 (0.0024–31.9)	0.2 (0.0007–9.5)	0.4 (0.001–17.9)	0.46 (0.0015–20.6)
Sole				
Average (± STD)	16.0 (26.7)	1.3 (2.2)	4.9 (8.1)	0.5 (0.8)
Median (min-max)	1.7 (0.0057–76.0)	0.1 (0.0005–6.2)	0.5 (0.0017–23.2)	0.05 (0.00017–2.2)
Clams				
Average (± STD)	0.1 (0.2)	0.1 (0.1)	0.3 (0.5)	0.1 (0.1)
Median (min-max)	0.04 (0.0002–0.6)	0.02 (0.0001–0.4)	0.1 (0.0004–1.5)	0.03 (0.0001–0.4)

n.d.: not detected.

obtained considering the consumption of seabass (Table 5). Interestingly, the lowest average EDI values were observed for BDE-28, BDE-47, and BDE-99 taking into account the consumption of mullet (Table 5). The average EDI values for BDE-47 and BDE-99 are in agreement with those reported in seabass and sole collected in Douro estuary; however, higher average EDI values were reported for the ΣPBDEs (0.2–0.49 ng/kg bw/day) and ΣMeO-BDEs (5×10^{-2}–0.51 ng/kg bw/day) (Menezes-Sousa et al., 2021). Comparing the results here obtained with the results of Pardo et al. (2014) (Region of Valencia - 0.137 ng/kg bw/day) and of Trabalón et al. (2015) (Tarragona County - 0.45 ng/kg bw/day) the daily intake of ΣPBDEs was lower (Table 5). Considering EDI values, the MOE was calculated for 47 and 99 congeners, which varied from 1×10^5 (BDE-99 in barbus) to 1.1×10^{11} (BDE-47 in mullet) (Table 5). Therefore, MOE values were higher than 2.5 indicating that there is no human health concern for the estimated levels of exposure through seafood consumption (EFSA, 2011). Overall, considering the results of the risk assessment performed, consumption of seafood from Tagus estuary does not seem to represent a risk to Portuguese population.

3.3. Contamination in liver

Table 6 shows the mean levels of analytes found in fish liver samples (ng/g ww). Among the 155 fish liver samples analysed, only BPF, ethylhexyl salicylate, HHCB, AHTN, and DPMI were detected. Among the species analysed, only in sole no analytes addressed in this study were detected.

No pesticide residues were detected in fish liver samples. The bioaccumulation rate of pesticides in fish depends on several factors including species, life stage, amount of fat reserves in the different fish tissues, fish diet, chemical and physical properties of analytes, and the rate of water pollution (Banaee, 2012). In addition, cytochrome P450 enzymes were identified in the liver of some freshwater fish, which are involved in the detoxification mechanisms of certain contaminants like organophosphate and carbamate insecticides (Banaee, 2012). In a study performed with fish from two rivers in the Biebrza National Park (Poland), residues of 340 pesticides were investigated in muscle and liver samples, of which only 6 analytes, including atrazine, *p-p*'-DDT, heptachlor, methoxychlor, and S-metolachor) were detected in 48% liver samples (Kaczyński et al., 2017). However, not all analytes found in the liver were detected in fish muscle and vice versa. In another study with fish (*Micropogonias furnieri*) caught in Patos Lagoon estuary (Brazil), among 7 pesticides assessed (atrazine, clomazone, dimethoate, fenitrothion, fipronil, malathion and tebuconazole), only the herbicide clomazone was detected in 10% liver samples (Caldas et al., 2013).

Regarding bisphenols, the only one detected in liver was BPF, being found in a mullet sample collected in spring that did not present BPF in the muscle. EHS was found in the liver of seabass, barbus, and mullet at higher levels than those reported for muscle. Similar behaviour was observed by Molins-Delgado et al. (2018), where the mean UV-filter concentrations of fish liver were 11 times higher than those measured here in the mullet muscle. Since liver is the organ where detoxifying metabolism takes place, this could explain the higher levels observed.

Three polycyclic musks were detected in fish liver samples, namely AHTN, HHCB, and DPMI. Two of them, AHTN and DPMI, were never found in muscle samples, while HHCB was found in fish muscle at higher levels than those found in the liver. The highest level of AHTN was found in barbus. HHCB was detected in seabass and mullet, reaching the highest levels in mullet. DPMI was only detected in spring in mullet. A previous study showed that PCPs accumulate more in fish liver than in fish muscle (Vimalkumar et al., 2021). Among the seasons analysed, summer revealed, in general, a higher number of analytes detected in the species analysed.

4. Conclusions

This study evaluated the levels and distribution of 50 EDCs in nine seafood species including fish, bivalves, crustaceans, macroalgae, and oligochaete collected in four seasons in two distinct areas of Tagus estuary. The prevalent contaminants were HHCB (81% positives; 0.04–74 ng/g ww), followed by IMC (64% positives; 1.13–251 ng/g ww), alachlor (44% positives; 0.08–16 ng/g ww), and BDE-47 (36% positives; 0.06–2.26 ng/g ww). In fish (seabass, barbus, mullet, and sole) and macroalgae samples, polycyclic musks were the most prevalent contaminants, while bivalves and crustaceans were mainly contaminated with UV-filters, and earthworms with bisphenols, particularly BPA. Among the different fish species, mullet and sole presented the highest levels of contamination for all classes of EDCs investigated, except for PBDEs, which were higher in seabass. Mussels presented higher levels of contamination than clams.

Seasonal variations were registered for all classes of analytes, with the highest levels of ΣUV-filters found in spring and summer, while for both Σpesticides and Σmusks significantly higher levels were found in summer and autumn. Σbisphenols was significantly lower in spring than in other seasons, while ΣPBDEs were statistically higher in autumn and winter. Regarding the collection areas, no statistically significant differences ($p > 0.05$) were observed between them, considering all species analysed in each area. Low potential human health risk through the consumption of fish muscle from Tagus estuary was revealed using the margin of exposure proposed by EFSA. Nevertheless, given the contamination levels verified in this study, there is an urgent need to continue the monitoring and enlarge the number of analytes to analyse.

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Table 5
Estimated daily intakes (μg/kg bw/day) of PBDEs through consumption of seabass, barbus, mullet, and sole collected in Tagus Estuary. (STD, standard deviation).

EDI	BDE-28	BDE-47	BDE-99	ΣPBDEs	ΣMeO-BDEs
Seabass					
Average (± STD)	3.9×10^{-5} (6.6×10^{-5})	9.7×10^{-5} (1.6×10^{-4})	8.4×10^{-6} (1.4×10^{-5})	1.4×10^{-4} (2.4×10^{-4})	1.4×10^{-5} (2.3×10^{-5})
Median (min–max)	4.2×10^{-6} (1.4×10^{-8} – 1.9×10^{-4})	1×10^{-5} (3.4×10^{-8} – 4.6×10^{-4})	8.9×10^{-7} (2.9×10^{-9} – 3.9×10^{-5})	1.5×10^{-5} (5.1×10^{-8} – 6.9×10^{-4})	1.5×10^{-6} (4.9×10^{-9} – 6.6×10^{-5})
Barbus					
Average (± STD)	9.9×10^{-6} (1.6×10^{-5})	1.2×10^{-5} (1.9×10^{-5})	2.4×10^{-5} (4×10^{-5})	4.6×10^{-5} (7.6×10^{-5})	4.2×10^{-7} (7×10^{-7})
Median (min–max)	1×10^{-6} (3.5×10^{-9} – 4.7×10^{-5})	1.3×10^{-6} (4.2×10^{-9} – 5.6×10^{-5})	2.6×10^{-6} (8.5×10^{-9} – 1.1×10^{-4})	4.9×10^{-6} (1.6×10^{-8} – 2.2×10^{-4})	4.5×10^{-8} (1.5×10^{-10} – 2×10^{-6})
Mullet					
Average (± STD)	2.9×10^{-6} (4.9×10^{-6})	7.9×10^{-6} (1.3×10^{-5})	3.8×10^{-6} (6.3×10^{-6})	1.5×10^{-5} (2.4×10^{-5})	1.4×10^{-6} (2.4×10^{-6})
Median (min–max)	3.2×10^{-7} (1×10^{-9} – 1.4×10^{-5})	8.4×10^{-7} (2.8×10^{-9} – 3.7×10^{-5})	4×10^{-7} (1.3×10^{-9} – 1.8×10^{-5})	1.6×10^{-6} (5.2×10^{-9} – 6.9×10^{-5})	1.5×10^{-7} (5.1×10^{-10} – 6.8×10^{-6})
Sole					
Average (± STD)	1.5×10^{-5} (2.5×10^{-5})	6.5×10^{-5} (1.1×10^{-4})	1.9×10^{-5} (3.1×10^{-5})	9.9×10^{-5} (1.6×10^{-4})	8.6×10^{-7} (1.4×10^{-6})
Median (min–max)	1.6×10^{-6} (5.3×10^{-9} – 7.1×10^{-5})	6.9×10^{-6} (2.3×10^{-8} – 3.1×10^{-4})	2×10^{-6} (6.7×10^{-9} – 8.9×10^{-5})	1 × 10–5 (3.5×10^{-8} – 4.7×10^{-4})	9.2×10^{-8} (3×10^{-10} – 4.1×10^{-6})
MOE					
Seabass					
Average (± STD)		1.7×10^{6} (3.1×10^{6})	7.8×10^{5} (1.4×10^{6})		
Median (min–max)		2.9×10^{4} (6.7×10^{2} – 8.9×10^{6})	1.3×10^{4} (3×10^{2} – 4×10^{6})		
Barbus					
Average (± STD)		1.4×10^{7} (2.5×10^{7})	2.7×10^{5} (4.8×10^{5})		
Median (min–max)		2.4×10^{5} (5.5×10^{3} – 7.4×10^{7})	4.7×10^{3} (1×10^{2} – 1.4×10^{6})		
Mullet					
Average (± STD)		2.1×10^{7} (3.8×10^{7})	1.7×10^{6} (3.1×10^{6})		
Median (min–max)		3.7×10^{5} (8.3×10^{3} – 1.1×10^{9})	2.9×10^{4} (6.7×10^{2} – 8.9×10^{6})		
Sole					
Average (± STD)		2.6×10^{6} (4.7×10^{6})	3.5×10^{5} (6.2×10^{5})		
Median (min–max)		4.5×10^{4} (1×10^{3} – 1.3×10^{7})	6×10^{3} (1.3×10^{2} – 1.8×10^{6})		

Table 6
Overview of the musk, UV-filters and bisphenols levels (ng/g ww) detected in the fish liver samples of several species collected in Tagus Estuary. (STD, standard deviation).

Species/Seasons		Musks ± STD (n = positive samples)			UV-Filters ± STD (n = positive samples)	Bisphenol ± STD (n = positive samples)
		HHCB	AHTN	DPMI	Ethylhexyl salicylate	BPF
Seabass (n = 44)	**Spring (n = 5)**	n.d.	n.d.	n.d.	68.60 ± 23.45 (n = 3)	n.d.
	Summer (n = 10)	16.05 ± 10.68 (n = 2)	24.8 (n = 1)	n.d.	34.2 (n = 1)	n.d.
	Autumn (n = 15)	68.1 (n = 1)	n.d.	n.d.	20.40 ± 23.90 (n = 2)	n.d.
	Winter (n = 14)	n.d.	n.d.	n.d.	n.d.	n.d.
Barbus (n = 36)	**Spring (n = 10)**	n.d.	n.d.	n.d.	66.66 ± 22.43 (n = 5)	n.d.
	Summer (n = 10)	n.d.	n.d.	n.d.	n.d.	n.d.
	Autumn (n = 7)	n.d.	178.60 ± 77.78 (n = 3)	n.d.	n.d.	n.d.
	Winter (n = 9)	n.d.	n.d.	n.d.	n.d.	n.d.
Mullet (n = 73)	**Spring (n = 19)**	n.d.	n.d.	n.d.	n.d.	5.6 (n = 1)
	Summer (n = 29)	57.0 ± 19.71 (n = 3)	n.d.	76.67 (n = 1)	8.89 (n = 1)	n.d.
	Autumn (n = 6)	n.d.	n.d.	n.d.	n.d.	n.d.
	Winter (n = 19)	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not detected

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CRediT authorship contribution statement

Antía Lestido- Methodology; Formal analysis; Original Draft, Mateus Petrarca- Methodology; Validation and Formal analysis; Carolina Monteiro, Ricardo Ferreira- Sample preparation, Isa Marmelo, Ana Maulvault, Patrícia Anacleto, António Marques, - Collection of the samples and Writing – review & editing; José O. Fernandes- Writing – review &

editing and Supervision and Funding, Sara C. Cunha - Conceptualization; Original Draft, Validation; Formal analysis; Writing – review & editing, Supervision and Funding.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2022.130387.

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