



Occurrence and seasonal variation of several endocrine disruptor compounds (pesticides, bisphenols, musks and UV-filters) in water and sediments from the estuaries of Tagus and Douro Rivers (NE Atlantic Ocean coast)



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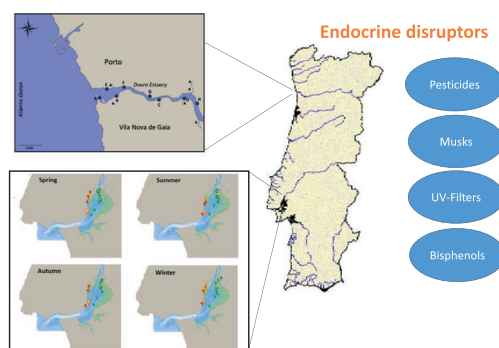
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HIGHLIGHTS

- Alachlor and BPA were commonly detected in both water and sediment samples.
- HHCB was detected in more than 96% of water samples.
- EHMC and EHS were the UV-filters more frequent in sediment and water, respectively.
- Tagus estuary samples had higher levels of EDCs than Douro estuary ones.
- Seasonal variation in EDCs levels was found for all the classes analysed.

GRAPHICAL ABSTRACT



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ABSTRACT

Exposure of aquatic environments to emerging contaminants is a global issue, special relevant in many estuaries due to impacts from anthropogenic activity. The aim of this work was to evaluate thirty-seven endocrine disruptor chemicals (EDCs) from four different classes (pesticides, bisphenols, polycyclic musks and UV-filters) in water and sediment samples collected during one-year in the estuaries of Tagus and Douro Rivers located into the NE Atlantic Ocean coast. EDCs analysis was achieved afterward validation of a gas-chromatography mass spectrometry (GC-MS) method using Dispersive Liquid-Liquid Microextraction (DLLME) as extraction procedure for water samples, and Quick, Easy, Cheap, Efficient, Rugged and Safe (QuEChERS) combined with DLLME for sediments. Tagus estuary presented higher levels of contamination with pesticide residues and bisphenols (BPs) than the Douro estuary in both water and sediment samples. Contrariwise, levels and frequency of polycyclic musks (PCMs) and UV-filters (UVF) were

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slightly higher in Douro estuary. Levels of pesticide residues in both sediment and water samples, and levels of PCMs and UVF in water samples were higher in warmer seasons (summer and spring) than in colder ones (winter and autumn). The opposite was found in what respect levels of BPs in water and sediment samples, and PCMs and UVF levels in sediment samples. Although the levels found for each contaminant are low, usually in the order of a few ng/mL(g), the presence of a high number of toxic compounds is a source of concern and requires constant monitoring.

1. Introduction

Today's environment is rapidly changing, mainly due to population growth, and consequent growing demand for goods and services. These alterations include rapid land-use change, invasion of natural habitats, and overuse of natural resources with the concomitant biodiversity loss (UN, 2021).

Anthropogenic pressures from human activities in many coastal areas has contributed to the degradation of estuaries, largely driven by the increased input of municipal, agricultural, and industrial runoff, in addition to storm water discharges and accidental water overflows, among other factors. Nearly 2.4 billion people of the worldwide population (40%) live within 100 km of the coast (UN, 2021); for example, in Portugal 85% of the population (8.773.777 inhabitants) live in coastal regions (Risc-Kit, 2021).

The hydrological basins of the Tagus and Douro Rivers are among the largest ones in the Iberian Peninsula (South Europe) and are in the vicinity of the densely populated metropolitan areas of Lisbon and Porto, respectively, thus being impacted by strong anthropogenic pressures, including inputs of many environmental contaminants (Tavares et al., 2015; Ribeiro et al., 2018). Several of them reach the estuaries of Tagus and Douro Rivers, located in the Portuguese coast (Southwest and Northwest coast, respectively) ending into the North East Atlantic Ocean (NE Atlantic Ocean). Currently, waters are being regularly controlled at the European level by directives that set environmental quality standards (EQSs) for an extensive range of chemical and biological contaminants (Directives 2000, 2008, and 2013; Decision, 2018/840; Decision (EU), 2020/1161) providing data used for management and risk assessment purposes. Notwithstanding, the information available for contaminants without maximum limits set by the European legislation or international authorities is still scarce.

Among environmental contaminants, endocrine-disrupting substances (EDCs) like bisphenols (BPs), polycyclic musks (PCMs), UV-filters (UVF) and EDC pesticide residues are of particular concern, mainly because their bioactivity and potential to decrease the reproductive success of wild animals and humans (Yilmaz et al., 2020). BPs, such as bisphenol A (BPA), bisphenol S (BPS), bisphenol F (BPF), and bisphenol AF (BPAF) are primarily used as intermediates in the production of polycarbonate plastics and epoxy resins (Cunha and Fernandes, 2021). These compounds, particularly BPA, have been commonly documented in estuaries and coastal lagoons located in different regions across the world, such as Portugal (Ribeiro et al., 2008), Spain (Salgueiro-González et al., 2015), USA (Meador et al., 2016), China (Sun et al., 2018; Lan et al., 2019; Zhao et al., 2019), Japan (Yamazaki et al., 2015) and Malaysia (Omar et al., 2018). PCMs are widely used as additives in personal care products and household cleaners to lend them a long-lasting and pleasing odour (Molins-Delgado et al., 2014). Some studies have been reported their presence in several estuaries (Sumner et al., 2010; Lee et al., 2014; Huang et al., 2016; Allinson et al., 2018) but the knowledge is still very limited. UVF, such as benzophenone-3 (BP3), ethylhexyl methoxycinnamate (EHMC), octocrylene (OC), and 4-methylbenzylidene camphor (4MBC) are mainly used in sunscreens and cosmetic products to protect from UV radiation (Molins-Delgado et al., 2014; Cunha et al., 2015a; Cunha et al., 2018). These EDCs have been found in some estuaries located in different regions (e.g. Victorian estuary, Australia, Allinson et al., 2018; Pearl River estuary, China, Huang et al., 2016) but the knowledge is also still limited. Pesticide residues are frequently detected in estuarine environments as result of massive use in agriculture to control pest and diseases. Among the different pesticide residues present in the estuarine environment, lipophilic EDCs have a strong tendency to present high stability, and high persistency, causing serious health and eco-toxicological concerns (Huang et al., 2016; Sousa et al., 2020; Peris

and Eljarrat, 2021). For example, dichlorodiphenyldichloroethane (DDTs), hexachlorobenzene (HCB), mirex, and chlordane are still found in the estuaries and rivers, despite being banned in Europe for many years (Cruzeiro et al., 2017; Sousa et al., 2020; Peris and Eljarrat, 2021).

Water and sediment contamination in estuaries can be conditioned by temporal variations of anthropogenic activities, as well as seasonal natural fluctuations (ca. rainfall and tides). Therefore, some studies have evaluated seasonal variation of EDCs in aquatic environments (Carafa et al., 2007; Barbosa et al., 2018; Tham et al., 2019; Ashfaq et al., 2019; Yang et al., 2020).

Analysis of emerging contaminants in environmental matrices is particularly challenging due to the low levels generally present and the complex nature of the samples (Pintado-Herrera et al., 2016; Peris and Eljarrat, 2021). The most efficient approach involves the use of chromatographic techniques coupled to mass spectrometry detectors preceded by multistep sample preparation. In fact, despite the high sensitivity and selectivity of such instrumental techniques, sample preparation including extraction, cleanup and concentration is crucial to achieve low limits of detection and quantification.

Nowadays, there is a search for "Green Chemistry" methods that use less volume of organic solvents, are simpler and faster (Cunha and Fernandes, 2018). One of the approaches that follow this trend is Quick, easy, cheap, effective, rugged, and safe (QuEChERS) procedure. This technique involves microscale extraction using organic solvents usually acetonitrile in presence of magnesium salts followed by dispersive solid-phase extraction (d-SPE) cleanup. In d-SPE, depending on the matrices and interferences present, several sorbents can be used such as PSA (primary secondary amine), C18 (octadecylsilane), GCB (graphitized carbon black), Z-Sep (zirconium dioxide-based sorbent), EMR-lipid (enhanced matrix removal-lipid), among others. Also, dispersive liquid-liquid microextraction (DLLME) follows the principle of Green Chemistry. In this approach, the extractant is mixed with an organic solvent miscible with water, called the disperser, and is then injected into the aqueous phase to form a cloudy solution that has a wide contact surface between the sample and the extractor. Then, after a fast centrifugation, a drop of the water-immiscible solvent that contains the analytes of interest is collected. This technique allows a huge enrichment factor when compared to QuEChERS. The combination of both techniques has been successfully applied in the extraction of pesticide residues (Andraščíková et al., 2013), BPs (Cunha et al., 2020), PCMs and UVF (Cunha et al., 2018) in different food matrices. To the best of our knowledge, the combination of both techniques in extraction and concentration of a wide range of contaminants classes in environmental samples was not yet documented. Gas-chromatography coupled to mass spectrometry (GC-MS) or tandem spectrometry (MS/MS) are the techniques commonly used in determination of the emerging contaminants above reported (Cunha et al., 2018; Cunha et al., 2020; Peris and Eljarrat, 2021).

The main aim of this work was to simultaneously analyse thirty-seven EDCs in water and sediment samples collected seasonally along one year in the estuaries of two important Rivers (Tagus and Douro) of the Iberian Peninsula (South Europe) located in the Portuguese coast and ending into the NE Atlantic Ocean. To achieve this aim a GC-MS method preceded by a QuEChERS and DLLME extraction was validated for the matrices under study.

2. Experimental

2.1. Chemicals and standard solutions

Analytical standards of pesticides were supplied by Sigma-Aldrich (Saint Louis, MO, USA) and were > 98% pure. The polycyclic musk fragrances cashmeran (DPMI), celestolide (ADBI), galaxolide (HHCB), and

tonalide (AHTN) were purchased from Promochem Iberia (Barcelona, Spain) and were 98% pure. Standards of UV-filters including 2-ethylhexyl-4-methoxycinnamate (EHMC) and 2-Ethylhexyl salicylate (EHS) both acquired from Sigma-Aldrich (Steinheim, Germany), 2-hydroxy-4-methoxybenzophenone (BP3) acquired from Alfa Aesar (Heysham, Lancashire, UK), and isoamyl-4-methoxycinnamate (IMC) acquired from TCI (Haven, Zwijndrecht, Belgium) all were >95%. 2,2-bis(4-hydroxyphenyl)propane (BPA), 4,4'-hexafluoroisopropylidene diphenol (BPAF), 4,4'-(1-phenylethylidene)bisphenol (BPAP), 2,2-bis(4-hydroxyphenyl)butane (BPB), 4,4'-ethylidenebisphenol (BPE), 4,4'-methylenediphenol (BPF), and 4,4'-cyclohexylidenebisphenol (BPZ) were supplied by Sigma-Aldrich (West Chester, PA, USA) and were >98% pure. The internal standards such as d16-bisphenol A (BPAd16) and d8-4,4'-DDT (DDT d8) were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA), d10-benzophenone (BPd10) from Sigma-Aldrich (Steinheim, Germany), d3-tonalide (AHTN d3) from Dr. Ehrenstorfer GmbH (Augsburg, Germany), and triphenyl phosphate (TPP) from Sigma-Aldrich (Saint Louis, MO, USA) and were >98% pure. The cleanup sorbents such as primary secondary amine (PSA) were purchased from Supelco (Bellefonte, PA, USA); Z-Sep bulk Supel™ QuE (14 nm pore size) from Sigma-Aldrich (Saint Louis, MO, USA); graphitized carbon black from Merck (Darmstadt, Germany); and QuEChERS d-SPE EMR-lipid from Agilent Technologies, Inc. (Santa Clara, CA, USA). The ultrapure water ($18.2 \text{ m}\Omega \text{ cm}^{-1}$) was purified by a Milli-Q gradient system from Millipore (Milford, MA, USA).

Individual standard solutions were prepared in acetonitrile, acetone, methanol or toluene depending on analyte solubility at a concentration of $1000 \mu\text{g/mL}$ and were stored in amber screw-capped glass flasks in the dark at -20°C . Working standard solutions were obtained by diluting the stock solutions with acetonitrile to final concentrations of $100 \mu\text{g/mL}$ (pesticides and UVF) and $10 \mu\text{g/mL}$ (polycyclic musk and bisphenols), which were maintained in the dark at -20°C .

Acetonitrile, methanol, acetone and toluene, for HPLC analysis, were acquired from Honeywell/ Riedel-de-Haën™ (Muskegon, MI, USA). Carbon tetrachloride (CTC, 99% purity) was purchased from Panreac Quimica SA (Barcelona, Spain); and the derivatization reagent acetic anhydride (AA, 99% purity) from Sigma-Aldrich, Co. (Saint Louis, MO, USA). Anhydrous magnesium sulfate and sodium acetate, all reagent grade, were supplied by Honeywell/ Fluka (Muskegon, MI, USA); and anhydrous potassium carbonate by Fluka Chemie GmbH

(Buchs, France). Previously the anhydrous magnesium sulfate was treated for at least 3 h and at 500°C in a muffle furnace.

2.2. Estuaries and sampling

The estuary of the Tagus River, hereafter indicated as Tagus estuary, is the largest estuarine system in the Iberian Peninsula with a coastal area of about 320 km^2 (Tavares et al., 2015; Vaz et al., 2019). This estuary of coastal plain, is surrounded by the large metropolitan area of Lisbon, one of the most populated areas of Portugal, with approximately 3 million inhabitants (INE, 2021). The Tagus estuary is a “mesotidal” estuary, having a complex geomorphology consisting of a deep and long system connecting the Atlantic Ocean to a broad shallow inner basin with extensive tidal flats and salt marsh areas (Vaz et al., 2019; Table S1). Considering the large coastal area of the Tagus estuary, two sampling areas were selected (Fig. 1a): a less contaminated area approximately in the middle of the estuary, more far from industrial and dense population zones, and near the protected Tagus Estuary Natural Reserve; and a more contaminated area, near to Lisbon city coastline and several pollution sources (e.g., large urban center with domestic, industrial, hospital and livestock effluent discharges, presence of chemical and petrochemical industries, pollution from marinas, ports and boats, intensive agriculture, commerce and transport).

The Douro River estuary is located on the west coast of Portugal and has approximately 21.6 km long. It has a natural semidiurnal tidal range of 2–4 m and an average depth of approximately 8 m, being the water level dependent on both flood and ebb tides and freshwater flow controlled by the Crestuma-Lever dam (Bordalo and Vieira, 2005; Ribeiro et al., 2018). This estuary is highly impacted, due to the high population density and related anthropogenic activities, receiving effluents from diffuse industries, sewages, municipal wastewater and from eight Wastewater Treatment Plants (WWTPs), being five located in the medium and lower estuary stretch (Fig. 1b) (Ribeiro et al., 2018; Rocha et al., 2015).

For the present study, five sampling sites were selected along the Douro River estuary, in both south and north margins, bordering a densely inhabited region: A (41.141692° , -8.664811°); B (41.141079° , -8.651925°); C (41.138803° , -8.610529°); D (41.139792° , -8.584460°); E (41.146870° , -8.657213°); F (41.147787° , -8.650225°); G (41.140331° , -8.617067°) and H (41.137051° , -8.571945°) (Fig. 1a). Sampling sites A and B are sandy beaches, located

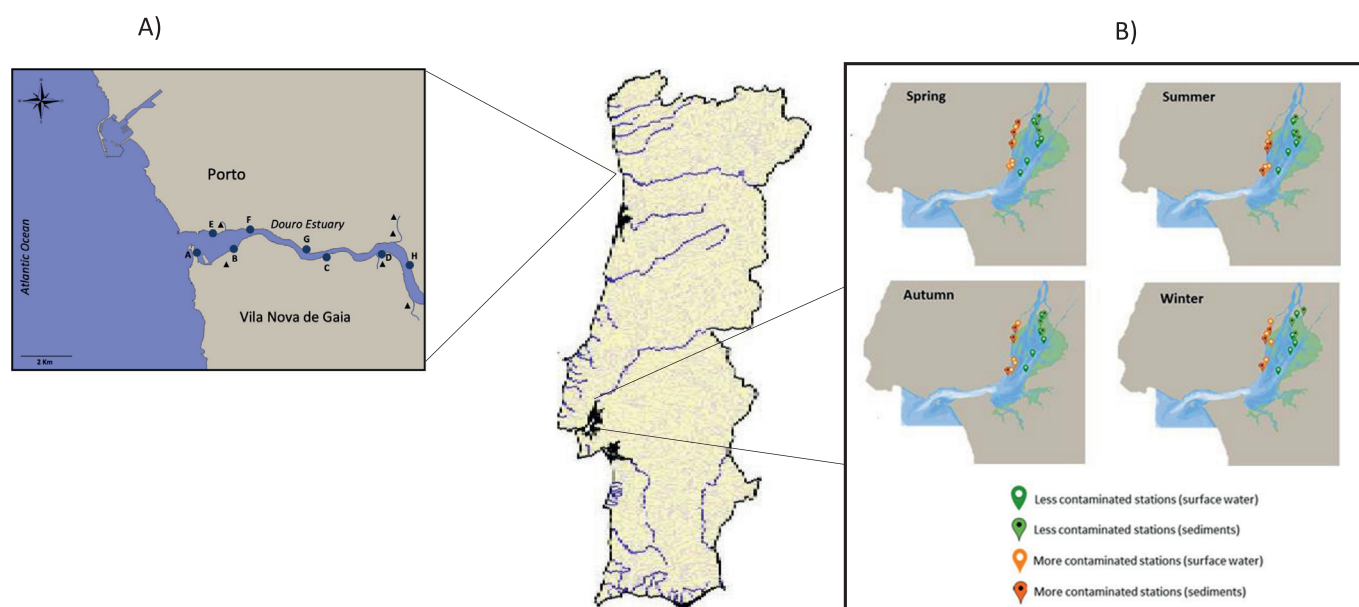


Fig. 1. A) Sampling stations of water and sediments in Douro estuary (site A; B, D, E, F and H collection water and sediments and site C and G collection of water) and B) Sampling stations of surface water and sediments in Tagus estuary.

near the estuary mouth, and in the area of the Nature Reserve of the River Douro Estuary- less contaminated. Sites G and C are distributed near the Porto wine cellars- medium contaminated. The sampling areas D, E, F and H were selected near effluents that receive WWTPs discharges- most contaminated.

The GPS coordinates of the sampling sites in Tagus and Douro estuaries are indicated in Supplementary materials (Table S2-S4). Sampling was carry out seasonally along one year: spring (March–April 2019), summer (June of 2019), autumn (October–November of 2019) and winter (February 2020). A total of 56 water samples (collected in the surface layer of the water column) and 46 sediment samples (collected in the surface layer of sediment) were collected, with three replicate samples per sampling site.

In the Tagus estuary, all samples were collected by local fishermen under the supervision of Portuguese Institute for the Sea and Atmosphere (IPMA, I.P.) team. All the water samples were collected in amber glass bottles and kept at 4 °C until they arrived at the laboratory where they were stored at –20 °C until further chemical analysis. Sediment samples were collected in aluminum trays and were kept at 4 °C until they arrived the laboratory where they were stored at –80 °C and then lyophilized (Model Cryodos 80 Telstar, CA, USA). Once lyophilized, they were stored at 4 °C until further chemical analysis.

2.3. Sample preparation

The extraction of the EDCs from sediment samples was performed accordingly to the procedures of Berlioz-Barbier et al. (2014) and Cunha et al. (2015a, 2018) with some modifications. Briefly, homogenized sediments (10 g) were accurately weighed into a 50 mL amber glass vials. These were spiked with a 100 µL internal standard mixture (TPP, BPAd16, BPd10 at 5 mg/L), and 10 mL of ultra-pure water plus 10 mL of acetonitrile were added for overnight extraction, using a platform mixer at 420 rpm (D-72379 Hechingen, Edmund Bühler GmbH, Germany). After that, 2 g of magnesium sulphate and 0.5 g of sodium acetate were added, the vials were shaken again for 1 min and centrifuged 5 min. The supernatant (1.1 mL) was cleaned-up using EMR-Lipid (200 mg), previously activated with 1.5 mL of ultra-pure water. Then, the cleaned extract was transferred to another vial and mixed with 125 µL of anhydride acetic (derivatization reagent) and 85 µL of carbon tetrachloride (extraction solvent). Subsequently, this mixture was rapidly injected into a conical glass tube containing 3 mL of water (pH > 7 adjusted with a 23% aqueous potassium carbonate solution). Then, after centrifugation at 1690 × g during 5 min, 75 µL of bottom layer was collected and 15 µL of internal standard mixture (DDT d8 and AHTN d3 at 1 µg/mL) were added before the GC–MS analysis.

The extraction of the EDCs from water samples was carried out accordingly to previous works of the authors (Cunha et al., 2015b, 2020) with some modifications. For DLLME with in situ acetylation of bisphenols, 1 mL of acetonitrile (as dispersion solvent), 125 µL of anhydride acetic (derivatization reagent), and 85 µL of carbon tetrachloride (extraction solvent) were added to 4 mL screw-capped glass flask and gently shaken by hand. Then, the mixture was rapidly injected into 5 mL homogenized sample (pH > 7 adjusted with a 23% aqueous potassium carbonate solution) placed into a 15 mL conical centrifuge glass tube containing 50 µL of internal standard mixture (TPP, BPAd16, BPd10 at 2.5 µg/mL) followed by fast vortex agitation for 1 min. Then, after centrifugation at 1690 × g during 5 min, 75 µL of bottom layer was collected and 15 µL internal standard mixture (DDT d8 and AHTN d3 at 1 µg/mL) were added before the GC–MS analysis.

2.4. Gas chromatography-mass spectrometry analysis

A Hewlett Packard HP6890 gas chromatography system (Agilent Technologies, USA) equipped with a PAL LSI autosampler (CTC Analytics AG, Switzerland) and interfaced to an Agilent 5973 single quadrupole mass selective detector with electron ionization (EI) source (Agilent Technologies, USA) was used in the present work. The GC–MS analyses were established on a Zebron ZB-5HT column (30 m × 250 µm × 0.1 µm; Phenomenex,

Folom, CA, USA), whose injector temperature was kept at 250 °C and 1.2 µL of extract was injected in pulsed splitless mode (pressure 40 psi until 0.85 min; purge flow to split vent of 100 mL/min). The initial oven temperature was 95 °C, which was held for 1.5 min; ramped to 180 °C at 20 °C/min; then, increased to 230 °C at 5 °C/min; and finally ramped to 290 °C at 25 °C/min and held for 15.85 min, with additional 1 min for post-run at 95 °C; totaling 35 min of run. A solvent delay of 4.5 min was applied. Ultra-high purity helium (99.999%) was employed as carrier gas at constant flow of 1 mL/min. The EI energy was 70 eV and the temperature of transfer line, EI source, and quadrupole mass analyzer was set at 280 °C, 230 °C, and 150 °C, respectively. The GC–MS system was operated under ChemStation software, and the data acquisition was in selective ion monitoring (SIM) mode (Table S5).

2.5. Quality control/quality assurance

Plastic material was avoided and all the glassware was previously washed with acetone. Before each batch of samples, a blank prepared using the same chemical reagents and volumes was analysed.

Linearity, linear range, sensitivity, precision, and accuracy were evaluated using samples (water and sediments free of compounds) spiked with both surrogate and internal standards before the analytical procedure, according to the International Conference on Harmonization (Guideline I.C.H., 2005) recommendations, and EU guidelines (SANTE/12682/2019, 2019). For each substance, calibration curves were set-up through eight matrix-matched calibration levels in the presence of specific internal standards for each chemical class.

Detection and quantification limits were calculated using low level points for sediments and water to achieve a ratio signal noise of 3 and 10, respectively.

Both precision (repeatability and intermediate precision) and accuracy were measured in three different concentration levels (six replicates per level per day), in two non-consecutive days: low level (1.25 ng/mL water and 20 ng/g sediments), medium level (5 ng/mL water and 80 ng/g sediments) and high level (20 ng/mL water and 200 ng/g sediments).

2.6. Statistical analysis

Data were first tested for normality and homoscedasticity through Kolmogorov–Smirnov and Levene tests, respectively. A normal distribution of the residuals was evaluated through the Shapiro–Wilks test (sample size <50). The Kruskal–Wallis H test was used to compare samples from different sampling sites in each estuary. Additionally, if a statistically significant difference was verified, Mann–Whitney U test was applied for means comparison of more than two independent samples. Kruskal–Wallis test, with posterior Dunn's multiple comparison test, was applied to compare the studied estuaries considering their EDCs bulk. The significance level was 0.05. All the statistical analyses were done using the SPSS statistical package, version 27.0 (IBM Corporation, New York, USA).

3. Results and discussion

3.1. Optimization of the sample preparation method

3.1.1. Water samples

The chemical composition of water is much less complex than that of sediments, so a DLLME procedure was selected because lower sample amounts and extraction solvents are needed. The extraction and dispersive solvents were selected based on our previous works in BPs and PCMs (Cunha et al., 2018) where carbon tetrachloride demonstrated to give good results. Optimal DLLME conditions, i.e., acetonitrile (1 mL) used as a dispersive solvent, carbon tetrachloride (85 µL) as extraction solvent, and acetic anhydride (125 µL) as derivatization reagent were selected because they showed results with higher extraction yields of target substances.

3.1.2. Sediment samples

3.1.2.1. Extraction. The development of the protocol performed in sediment samples (each analyte with 200 ng/g) was based on the selection of solvent type (acetonitrile or acetonitrile:toluene, 4:1 (v/v)) in order to achieve higher extraction yields. The mixture of acetonitrile:toluene (4:1 v/v) provided dirtiest extracts when compared with the use of acetonitrile alone resulting in lower extraction yields (between 35 and 65%, on average), particularly for the organochloride pesticides. Therefore, acetonitrile combined with magnesium sulphate and acetate sodium was chosen, providing good extraction yields for all analytes, despite their naturally different partition coefficients.

3.1.2.2. Cleanup and derivatization. Different sorbents (PSA, GCB, Z-Sep⁺, isolated or combined, and EMR-lipid) were tested as cleanup agents in the dSPE procedure. High recoveries (>70%) of target analytes were obtained for combined Z-Sep⁺/GCB (95/5 mg per mL of extract) and EMR-lipid (200 mg per mL of extract). EMR-lipid was finally selected as cleanup agent due its low cost and easiness to use in comparison to Z-Sep⁺/GCB.

DLLME allowed a rapid and efficient concentration of the analytes; moreover, when combined with acetic anhydride derivatization, it allows the transformation of BPs into acetylated derivatives with excellent chromatographic behaviour, easy to separate and quantify. It is worth noting that acetylation does not cause any disturbance in the separation and resolution of the remaining studied analytes. The evaluation of DLLME with and without derivatization showed no loss in analytical signal for PCMs, UVF

and pesticide residues (Fig. S1). Acetic anhydride has been used to derivatize phenols and amines but not alcohols under both aqueous and anhydrous conditions. Despite the derivatization of compounds such as HCB are possible, in this study probable due to the small amount of acetic anhydride and very short reaction time the derivatization was not verified.

3.2. Analytical performance

3.2.1. Linearity

Matrix-matched calibration curves were used in order to overcome the effect of the matrix that possibly resulted from the adsorption of target analytes to active sites or from thermal degradation that led to a lower analytical response. Linearity was verified for a large working range in water and sediments from 0.01 to 40 ng/mL and from 0.025 to 200 ng/g dry weight (dw), respectively, with correlation coefficients generally higher than 0.99 (See Tables 1 and 2).

3.2.2. Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) (Table 1) in water ranged between 0.005 and 2.5 ng/mL and between 0.01 and 2.5 ng/mL, respectively. The LODs values are identical to those published using DLLME procedure (0.002 to 30 µg/L for UVF, Cunha et al., 2015a; 0.001 to 0.054 ng/mL for musks, Homem et al., 2016; 0.029 to 0.035 ng/mL for bisphenols and 0.029 to 0.377 ng/mL pesticide residues, Asati et al., 2017), liquid-liquid extraction (LLE) (up to 0.73 ng/L for organochloride pesticides, Peris and Eljarrat, 2021) and solid-phase

Table 1

Analytical parameters for water, linearity range (r^2 -coefficient of determination), recoveries (%) and precision intra and inter-day (RSD- relative standard deviation).

Analytes	Linearity range		Limite of detection (ng/mL)	% Recoveries (precision intra-day, RSD)			Precision inter-day (RSD, %)
	(ng/mL)	r^2		1.25 ng/mL	5 ng/mL	20 ng/mL	
Pesticides							
Lindane (γ HCH)	0.01–40	0.9992	0.005	110 (18)	84 (6)	110 (6)	7
HCB	0.01–40	0.9979	0.005	105 (13)	84 (6)	96 (4)	4
Atrazine	0.025–40	0.9936	0.010	63 (13)	71 (13)	75 (11)	9
α HCH	0.01–40	0.9994	0.005	80 (13)	91 (6)	116 (5)	8
Vinclozolin	1.25–40	0.9927	0.750	65 (15)	78 (5)	89 (16)	7
Chlorpyrifos-methyl	0.025–40	0.9979	0.017	69 (3)	88 (2)	79 (3)	6
Alachlor	0.01–40	0.9998	0.005	61 (10)	80 (6)	110 (4)	4
Fenitrothion	0.025–40	0.9990	0.010	74 (11)	79 (5)	110 (13)	10
Aldrin	0.01–40	0.9888	0.005	74 (7)	65 (4)	107 (8)	8
Clorpyrifos	0.01–40	0.9983	0.005	77 (9)	71 (6)	115 (2)	6
Fipronil	0.01–40	0.9987	0.005	74 (6)	75 (7)	105 (2)	6
α -Chlordane	0.01–40	0.9997	0.005	64 (15)	62 (5)	101 (5)	9
γ -Chlordane	0.01–40	0.9910	0.005	66 (18)	75 (6)	107 (3)	5
Ethion	0.025–40	0.9956	0.018	63 (4)	76 (8)	101 (3)	8
P, P-DDT	0.01–40	0.9983	0.005	64 (6)	77 (11)	86 (9)	8
Bifenthrin	0.025–40	0.9869	0.020	73 (14)	82 (10)	105 (2)	8
Mirex	0.025–40	0.9911	0.020	74 (9)	86 (10)	84 (11)	9
Permethrin	0.01–40	0.9898	0.008	108 (12)	77 (15)	102 (12)	10
Prochloraz	5–40	0.9892	2.500	–	62 (12)	76 (4)	6
Cypermethrin	5–40	0.9864	2.000	–	60 (10)	68 (12)	9
Deltamethrin	5–40	0.9984	2.000	–	70 (20)	87 (5)	9
Bisphenols							
BPAF	0.025–40	0.9953	0.0100	68 (1)	75 (2)	79 (9)	5
BPF (4,4F)	0.025–40	0.9925	0.0100	66 (16)	99 (6)	98 (8)	9
BPE	0.01–40	0.9950	0.0050	71 (13)	91 (11)	87 (6)	3
BPA	0.01–40	0.9970	0.0050	67 (14)	85 (6)	78 (16)	4
BPB	0.025–40	0.9886	0.0100	66 (17)	80 (5)	76 (10)	15
BPS	0.025–40	0.9801	0.0100	63 (17)	70 (9)	97 (5)	9
BPZ	0.01–40	0.9975	0.0050	105 (13)	86 (5)	94 (9)	9
BPAP	0.025–40	0.9983	0.0100	106 (10)	119 (15)	91 (4)	8
Musks							
DPMI	0.025–40	0.9991	0.0200	110 (5)	94 (10)	86 (6)	10
ADBI	0.025–40	0.9998	0.0200	78 (4)	85 (1)	115 (8)	8
AHTN	0.025–40	0.9989	0.0200	88 (9)	78 (5)	101 (8)	6
HHCB	0.025–40	0.9979	0.0200	73 (5)	82 (1)	121 (5)	9
UV- Filters							
EHS	0.025–40	0.9921	0.0150	70 (1)	85 (15)	97 (7)	7
IMC	0.025–40	0.9983	0.0150	71 (14)	70 (10)	110 (9)	8
EHMC	0.025–40	0.9952	0.0150	70 (5)	74 (2)	104 (8)	9
BP3	0.01–40	0.9917	0.0050	79 (20)	75 (13)	110 (4)	12

Table 2Analytical parameters for sediments, linearity range (r^2 -coefficient of determination), recoveries (%) and precision intra and inter-day (RSD- relative standard deviation).

Analytes	Linearity range		Limite of detection (ng/g dw)	% Recoveries (precision intra-day, RSD)			Precision inter-day (RSD, %)
	(ng/g dw)	r^2		20 ng/g	80 ng/g	200 ng/g	
Pesticides							
<i>Lindane (γ HCH)</i>	1.25–200	0.9998	0.4	120 (9)	101 (16)	82 (12)	11
<i>HCB</i>	5–200	0.9901	2.0	108 (10)	109 (19)	101 (15)	11
<i>Atrazine</i>	5–200	0.9987	2.0	90 (6)	84 (10)	81 (8)	9
α HCH	5–200	0.9907	2.0	108 (10)	106 (19)	101 (15)	12
<i>Vinclozolin</i>	5–200	0.9980	1.8	62 (6)	112 (12)	120 (9)	9
<i>Chlorpyrifos-methyl</i>	5–200	0.9935	1.5	102 (5)	91 (5)	118 (3)	18
<i>Alachlor</i>	5–200	0.9899	1.5	103 (4)	113 (10)	105 (4)	7
<i>Fenitrothion</i>	5–200	0.9939	1.5	74 (10)	100 (13)	97 (12)	12
<i>Aldrin</i>	5–200	0.9906	1.5	106 (11)	86 (10)	91 (4)	6
<i>Clorpyrifos</i>	5–200	0.9962	1.5	95 (8)	99 (10)	103 (7)	9
<i>Fipronil</i>	5–200	0.9977	1.5	101 (16)	100 (16)	103 (11)	8
α -Chlordane	5–200	0.9982	1.8	104 (8)	71 (12)	87 (6)	6
γ -Chlordane	5–200	0.9964	1.8	94 (8)	88 (12)	92 (5)	9
<i>Ethion</i>	5–200	0.9985	1.5	101 (2)	90 (6)	95 (5)	9
<i>P, P-DDT</i>	5–200	0.9998	2.0	85 (4)	100 (2)	91 (4)	8
<i>Bifenthrin</i>	5–200	0.9920	1.5	80 (2)	108 (6)	101 (4)	5
<i>Mirex</i>	5–200	0.9989	2.0	91 (8)	81 (10)	88 (2)	9
<i>Permethrin</i>	5–200	0.9892	1.5	76 (10)	92 (8)	97 (7)	6
<i>Prochloraz</i>	5–200	0.9867	1.5	79 (14)	78 (5)	84 (6)	10
<i>Cypermethrin</i>	5–200	0.9897	2.0	117 (6)	99 (10)	91 (7)	10
<i>Deltamethrin</i>	5–200	0.9888	2.5	78 (18)	88 (9)	92 (10)	11
Bisphenols							
<i>BPAF</i>	1.25–200	0.9968	0.35	85 (12)	81 (10)	92 (9)	9
<i>BPF (4,4F)</i>	1.25–200	0.9982	0.25	70 (9)	77 (8)	93 (6)	6
<i>BPE</i>	1.25–200	0.9987	0.25	83 (9)	109 (12)	90 (13)	8
<i>BPA</i>	1.25–200	0.9907	0.25	85 (8)	82 (4)	90 (5)	6
<i>BPB</i>	1.25–200	0.9900	0.25	80 (11)	79 (7)	95 (8)	7
<i>BPS</i>	1.25–200	0.9891	1.00	75 (8)	81 (5)	93 (6)	6
<i>BPZ</i>	1.25–200	0.9940	1.00	72 (8)	85 (10)	89 (5)	9
<i>BPAP</i>	1.25–200	0.9871	0.50	74 (14)	76 (19)	80 (8)	7
Musks							
<i>DPMI</i>	1.25–40	0.9966	0.500	102 (11)	94 (16)	73 (20)	6
<i>ADB1</i>	1.25–40	0.9986	0.300	109 (8)	96 (8)	102 (13)	9
<i>AHTN</i>	1.25–40	0.9903	0.300	107 (6)	105 (6)	100 (8)	6
<i>HHCB</i>	1.25–40	0.9981	0.300	103 (9)	86 (16)	93 (12)	11
UV-Filters							
<i>EHS</i>	1.25–40	0.9849	0.200	92 (16)	86 (14)	75 (11)	6
<i>IMC</i>	1.25–40	0.9949	0.300	98 (8)	92 (8)	86 (6)	8
<i>EHMC</i>	1.25–40	0.9839	0.300	99 (9)	98 (10)	101 (10)	9
<i>BP3</i>	1.25–40	0.9965	0.300	86 (10)	84 (14)	103 (12)	8

extraction (SPE) (0.002 to 2.7 ng/mL for organochloride pesticides, Almeida Azevedo et al., 2000).

Regarding sediments, LODs ranged from 0.01 to 2.5 ng/g dw and LOQs from 0.025 to 200 ng/g dw (Table 2). To date, QuEChERS/DLLME procedure for the analysis of multi-class EDCs in sediments is not been previously studied, to the best of authors' knowledge. Nevertheless, LODs obtained herein for sediments are lower than those reported by Berlioz-Barbier et al. (2014) using QuEChERS (8.5 ng/g for BPA). Moreover, LODs values were similar to those obtained using solid-liquid extraction (SLE) (0.11 to 0.28 ng/g for UVF, Sánchez-Brunete et al., 2011; 0.02 to 0.54 ng/g dw for non-polar pesticides, Peris and Eljarrat, 2021) and Soxhlet-SPE-LC-MS (0.29 ng/g for BPA, Omar et al., 2018); however they were higher than those obtained by pressurized liquid extraction (PLE) (0.013 to 2.4 ng/g dw for polycyclic musk, 0.009 to 0.065 ng/g dw for UVF and 0.012 to 0.401 ng/g dw for organochloride pesticides, Pintado-Herrera et al., 2016).

3.2.3. Precision and accuracy

Repeatability, expressed by the coefficients of variation for all target analytes were, on average, 15% for sediments and 17% for water samples at the intra-day assays, and 8% for sediments and 8% for water samples at the inter-day assays. The results shown in Tables 1 and 2 are similar to those reported in the literature (2–30%, Almeida Azevedo et al., 2000; 5–27%, Berlioz-Barbier et al., 2014; 2–15%, Homem et al., 2016; 0.2–17%, Pintado-Herrera et al., 2016; 3.7–15.2%, Asati et al., 2017; up to 97%, Omar et al., 2018; and up to 173%, Peris and Eljarrat, 2021).

The accuracy was assessed through recovery tests using three levels of calibration (Tables 1 and 2). The recoveries for all substances ranged from 61 to 116% for water samples and from 71 to 117% for sediments. The majority of the results (93%) were within the reference limits established by SANTE 2019 (recoveries should be between 70% and 120%, with RSD \leq 20%). For water, our DLLME method provided comparable results with those reported using DLLME-GC-MS (79–103% for musks, Homem et al., 2016; and 95–104% for BPS and pesticides, Asati et al., 2017) and SPE-GC-MS (63–161% for pesticide, Mansilha et al., 2010). In general, the presented method provides better results than those obtained by SLE-GC-MS/MS and LLE-GC-MS/MS (45–121% and 18–81% for organochloride pesticide residues in sediments and water, respectively, Peris and Eljarrat, 2021) for sediments and water, respectively. The obtained recoveries in sediments were comparable to the previously reported data using SPE-GC-MS (63–147% for organochloride pesticide residues, Almeida Azevedo et al., 2000; and 89–105% for UV-filters, Sánchez-Brunete et al., 2011), DLLME-GC-MS (74–76% for BPA, Yuan et al., 2015) and PLE-GC-MS/MS (71–134% for polycyclic musks, 61–134% for UVFs, and 68–117% for organochloride pesticide residues, Pintado-Herrera et al., 2016).

3.3. General occurrence of EDCs in water samples

The results of the water samples from Tagus and Douro estuaries along one year (Table S6) showed the presence of 33 out of the 37 target substances. HHCB was the substance most commonly detected (frequency >

96%). HCB, prochloraz, BPAP and BP3 were not detected in any of the water samples analysed.

Among the pesticide residues studied, alachlor was the most frequently detected (34%) with quantified levels ranging from 0.013 (Douro estuary) to 1.292 ng/mL (Tagus estuary). This compound had been previously detected in water samples from the Douro estuary, with levels ranging from 0.036 to 0.627 ng/mL (Cruzeiro et al., 2017). Similar levels and frequencies of those found in the present study were also documented in estuarine and marine water samples from several other locations in Portugal (Sousa et al., 2020). α -HCH was the second pesticide residue most frequently detected (27%) in the waters analysed, although above half of the positive samples were below the LOQ. Overall, the highest concentration of pesticide residues was found for bifenthrin (3.043 ng/mL, Tagus estuary). This pyrethroid was only found in 4 samples, all with significantly high values (average = 1.415 ng/mL). Its presence was previously reported in waters collected in rivers from the North of Portugal (Vera et al., 2021), but at lower levels (up to 0.021 ng/mL) than those found in the present study.

In 25 of the 56 samples analysed, residues of at least 1 pesticide were found (frequency = 44.6%, see Table S6). Considering the sum of all residues found, the values in the positive samples varied between 0.020 and 21.549 ng/mL with an average of 2.221 ng/mL.

Comparing the results of total pesticide residues in water with those reported previously in literature, it is possible to verify lower levels of pesticide residues in Cachapoal River basin in Chile (0.4 to 7.5 ng/mL; Climent et al., 2019), in Catalonia River in Spain (from 0.018 to 0.190 ng/mL; Peris and Eljarrat, 2021) and in Rufiji river Delta in Tanzania (from 0.292 to 0.870 ng/mL; Mwevura et al., 2021).

Among BPs, BPA was detected in 38% of the samples analysed with quantified levels ranging from 0.054 to 4.424 ng/mL (Table S6). BPA levels found were higher than those previously documented in water samples from the Tagus estuary (up to 0.190 ng/mL in the same sampling areas of the present study, Rocha et al., 2015), and in water samples from the Mondego River estuary, also located in the Portuguese Atlantic coast (up to 0.880 ng/mL, Ribeiro et al., 2008). The amounts of BPA in the present study (average of 0.0154 ng/mL in Douro estuary and 0.940 ng/mL in Tagus estuary) are in agreement with those found in Korsch River in Germany (up to 0.272 ng/mL; Körner et al., 2001), in Ningbo city River in China (0.0134 to 3.34 ng/mL; Wang et al., 2015), in Iberian Rivers in Spain (up to 0.280 ng/mL; Gorga et al., 2015) and in Romagna area in Italy (up to 0.171.3 ng/mL; Pignotti et al., 2017). BPB, BPE, and BPS were frequently detected below the LOQ. Their presence in water samples may be due to the replacement of BPA by other BPs in many products and consequently their release for the environment (Cunha et al., 2020). The presence of these substances in the analysed water samples is in agreement with findings from the Pearl estuary (South China), where analogues of BPA, such as BPS, BPF, BPAF, BPB and BPP, were found at comparable levels (0.0017 to 0.282 ng/mL, Zhao et al., 2019).

Among PCMs, the presence of HHCB stood out, with a frequency of 96.4% (54 out of 56 water samples) with quantified levels ranging from 0.028 to 0.597 ng/mL. ADBI and AHTN were detected in few samples always below the LOQ, while DPMI was not detected in any sample (Table S6). PCMs had already been documented in water samples from the Leça River in Portugal (Homem et al., 2016) with levels of HHCB and AHTN up to 0.828 and 0.462 ng/mL, respectively, therefore higher than those reported herein. The predominance of HHCB in both estuaries and the amounts found are in accordance with findings reported in river waters from other countries. For example, HHCB was found in levels up to 0.395 ng/mL in the North canal River watershed in China (Zhang et al., 2020), in levels up to 0.037 ng/mL in Songhua River also in China (Lu et al., 2015), and in levels up to 0.090 ng/mL in Sava River in Serbia (Relić et al., 2017).

EHS was the most frequently UVF detected (38%) with quantified levels ranging from 0.058 to 0.470 ng/mL (Table S6). Other UVF, EHMC and IMC, were detected in 21% and 14% of the water samples with quantified levels ranging from 0.061 to 0.539 ng/mL and from 0.165 to 0.321 ng/mL, respectively. In Portugal, EHMC was previously detected in water

of several Portuguese rivers as reported by Barbosa et al. (2018) with a maximum level of 0.562 ng/mL, similar to those reported in this study. From the few references elsewhere about UVF, only EHMC was found in seawater in levels up to 0.756 ng/mL in Canaria, Spain (Rodríguez et al., 2015).

3.4. Comparison of water contamination in Tagus and Douro estuaries

Total pesticide residue in water of Tagus estuary was higher than those detected in Douro estuary with average levels of 2.50 and 0.39 ng/mL, respectively (Fig. 2). Furthermore, significant differences ($p < 0.05$) were verified among the estuaries in what concerns the total pesticide residues found. The higher presence of organochloride pesticide residues in water of Tagus estuary, such as alachlor, compared to the Douro estuary, may be related to the runoff of agricultural land, in particular rice production that covers large areas that drain into the Tagus. Other causes may arise from regional differences in pesticide use or the use of counterfeit and illegal pesticides, a problem that are known for long and that continue to occur, including in Iberia (Europol, 2021).

Regarding BPs in water (Fig. 2), Tagus estuary presented total higher amounts (average of 2.10 ng/mL) than those found in Douro estuary (average of 0.47 ng/mL), however this difference was not statistically significant ($p > 0.05$). The high anthropogenic activity found in Tagus estuary compared to Douro estuary could be related with the difference verified. Indeed, Tagus estuary, one of the largest in Europe (320 km² total area) suffers from contamination mainly by two industrial areas located in the north and south margins and by domestic effluents from the metropolitan area of Lisbon.

Total PCMs were very similar in the both studied estuaries (Douro estuary with an total average level of 0.20 ng/mL and Tagus estuary with 0.14 ng/mL) (Fig. 2), with no significant differences ($p > 0.05$) between them.

The total UVF average level was 0.18 ng/mL found in water from Douro estuary, while in Tagus estuary was 0.37 ng/mL (Fig. 2), with significant differences among the estuaries found ($p > 0.05$). The higher frequency of IMC and EHMC in Tagus estuary compared to those verified in Douro estuary justify statistically differences observed. The presence of these compounds is of utmost relevance for the environment and human health. For example, EHMC has been included since 2014 into the CoRAP list for evaluation as suspected persistent, bioaccumulative and toxic and it is potential for endocrine activity in amphibians and fish (ECHA (European Chemicals Agency), 2013). On this basis, EHMC was placed on the European watch list of substances that may pose a significant risk to the aquatic environment (Directive 2008/105/EC). However, in 2018, it was decided to remove EHMC from the watch list and its re-inclusion remains under study.

3.5. General occurrence of emerging contaminants in sediments

The levels of pesticide residues, PMCs, UV-filters, and BPs in sediments from Douro and Tagus estuaries are shown in Tables S5 and S6. Thirty-one out of the 37 substances have been detected in sediments. Exceptions were permethrin, prochloraz, BPF, BPZ, BPAP and BP3. The majority of these substances have a restricted use in the EU; for instances, the use of permethrin as plant protection product was banned (Regulation (EC) No 1107/2009), while BP3 has a maximum level of 0.5% in cosmetic products, with the exception of sunscreen cosmetic products 6.0% (Commission Regulation (EU) 2017/238). For their part, the BPA analogues in question are still in very limited use.

Alachlor was the most frequent (52%) pesticide residue detected in the analysed sediments followed by α -HCH (32%) and bifenthrin (26%). The highest level observed was from lindane (γ -HCH) with 50 ng/g dw (Tagus estuary) followed by alachlor with 42.6 ng/g dw (Tagus estuary). In a previous study with sediment samples from the Douro River lindane was detected at 392 ng/g dw (Villaverde et al., 2008). These differences can be related with different analytical approaches and perhaps the changes in pesticides uses in the last 10 years.

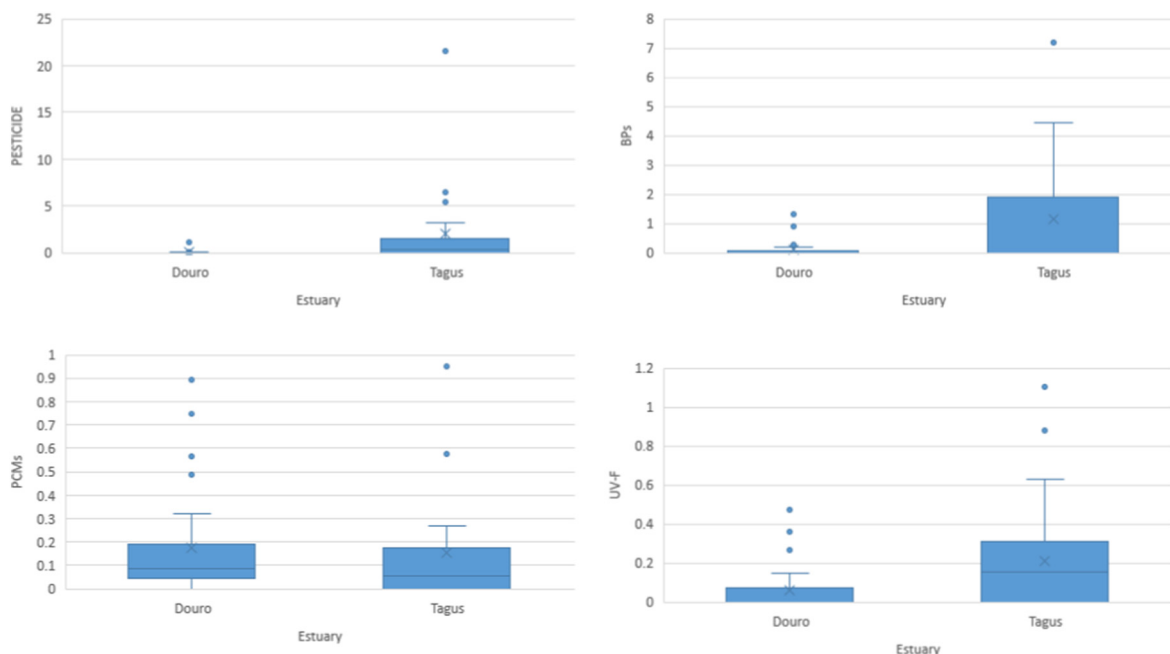


Fig. 2. Levels pesticide residues, bisphenols (BPs), polycyclic musks (PCMs) and UV-filters (UVF) in water: Douro estuary versus Tagus estuary. The lines from bottom to top in box plot were minimum, lower quartile, median, upper quartile and maximum, respectively. The X was the mean and the asterisks out the box were extreme outliers.

Although alachlor, a chloroacetanilide herbicide, was banned in 2006 by the EU (Regulatory Decision excluding the substance from Annex I of Directive 91/414) due to ubiquitous and unavoidable contamination of water, its presence is still reported in Nordic countries (Norway, Sweden, Finland and Denmark) (Carafa et al., 2007; Isen et al., 2019). Also, atrazine and isomers of HCH (γ and α) which use is prohibited, were widely detected in sediments of both estuaries, with quantified levels ranging from 7.17 to 18.96 ng/g dw and from 5.93 to 50 ng/g dw, respectively. The presence of atrazine in marine sediments has been previously reported in Ebro delta in Spain and Northern Adriatic in Italy with levels ranging from no detectable to 39.2 ng/g dw (Ferrer et al., 1997) and from 30 to 980 ng/ng dw (Carafa et al., 2007), respectively. HCH isomers are widely distributed in the environment due to their persistence; they have been previously found in sediment of Baltic Sea (0.0022 to 0.36 ng/ng dw; Pikkarainen, 2007), Mediterranean Sea (0.04 to 0.80 ng/ng dw; Gómez-Gutiérrez et al., 2007), Pearl river estuary in China (n.d. to 10.8 ng/ng dw; Pintado-Herrera et al., 2016), Catalonia river (0.1 to 1.3 ng/g dw; Peris and Eljarrat, 2021) and Rufiji river Delta in Tanzania (up to 2.9 ng/g dw; Mwevura et al., 2021).

Comparing the results of total pesticide residues in sediments (ranging from <LOD to 186.69 ng/g dw, average of 13,91 ng/g dw) with those reported previously in literature, it is possible to verify higher levels of pesticide residues in Catalonia River (from 1 to 860 ng/g dw; Peris and Eljarrat, 2021) while lower levels were reported in Rufiji River Delta (from 16 to 38 ng/g dw; Mwevura et al., 2021).

Regarding BPs, BPA was the most frequent (35%) with the highest amount of 16 ng/g dw, in a sample from Tagus estuary. BPAF, BPS and BPE were detected in 33%, 15%, and 15% of the sediment samples, respectively, but frequently showing levels below the LOQ. In Portugal, BPA was previously monitored in sediments collected from several sites along the country (Ponte Nova Barcelos, Formariz, Ponte Moreira, Ria Aveiro, Ponte Ribeira Pernes, Monte da Vinha, Ponte do Sacavém) (Céspedes et al., 2003), but no quantified levels were observed. BPA was one of the most frequent emerging contaminants in sediments from Klang River estuary in Malaysia (Omar et al., 2018) and from Xiaoqing River estuary in China (Lan et al., 2019), with levels ranging from 8.5 to 16.8 ng/g dw and 1.0 to 5.4 ng/g dw, respectively.

All PCMs studied were detected in sediments of both estuaries. HHCB was detected in all the samples analysed with quantified levels

ranging from 1.37 to 7.76 ng/g dw (Douro estuary). AHTN was detected in about 43% of the samples analysed with quantified levels ranging from 2.59 to 9.92 ng/g dw (Douro estuary). Detectable levels of DPPI and ADBI were always below the LOQ. PCMs, as far we know, were not reported in sediments from Portuguese rivers, although high amounts of PCMs have already been confirmed in the water of Leça river (Homem et al., 2016). Comparing with other countries, it can be observed that HHCB and AHTN levels are similar to those reported in Nakdong River in South Korea (Lee et al., 2014) with levels up to 6.3 ng/g dw and 2.3 ng/g dw, respectively. On the other hand, the levels here observed were slightly lower than those found in North Canal River watershed in Beijing (China), with levels of 8.18 ng/g dw for HHC and 7.31 ng/g dw for AHTN (Zhang et al., 2020). Despite HHCB and AHTN were widely reported in rivers' sediments around the world (Zhang et al., 2020), few studies have been reported in estuaries in particular. Huang et al. (2016) found levels of 4.6–27.1 and 1.9–13.6 ng/g dw in Pearl River estuary in China for HHCB and AHTN, respectively.

Among the UVFs, EHMC, EHS, and IMC were detected in 46%, 39% and 28% of the sediment samples, with quantified levels ranging from 1.03 to 24.30 ng/g dw, 2.73 to 24.70 ng/g dw, and 1.69 to 23.73 ng/g dw, respectively. In Portugal, other UVFs, in particular octocrylene and ethylhexyltriazone, were previously found in sediments from several sites in Ribeira dos Covões, in the outskirts of Coimbra (Ferreira et al., 2019), but no data as far we know have been reported for the UVF studied in this work. The data herein, particularly for EHMC, are similar with those reported in Colombia and Chile (Magdalena River and Biobio region, levels up 47 ng/g dw; Barón et al., 2013), in China (Pearl river estuary with levels up 22 ng/g dw; Pintado-Herrera et al., 2017) and in Australia (Victorian estuary with levels up to 18 ng/g dw; Allinson et al., 2018).

3.6. Comparison of sediments contamination in Douro and Tagus estuaries

Data distribution of pesticide residues, BPs, PCMs and UVF in sediments collected in Douro and Tagus estuaries is presented in Fig. 3. From the 4 classes of contaminants, pesticide residues were the ones found in higher levels in both estuaries.

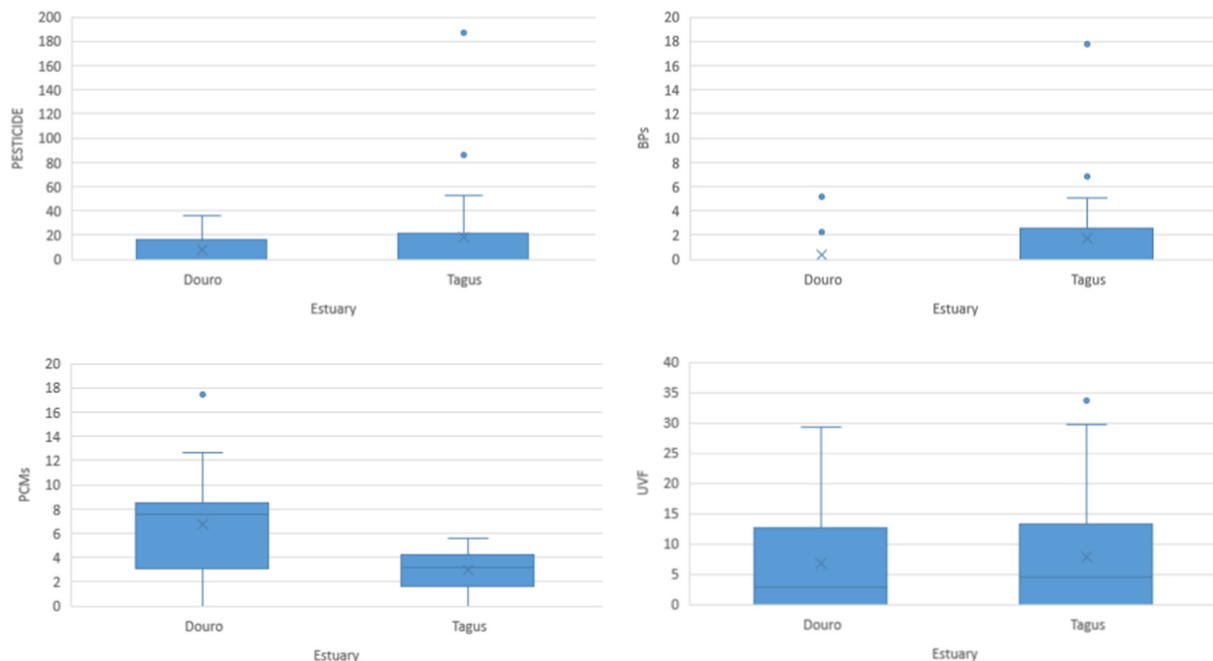


Fig. 3. Levels pesticide residues, bisphenols (BPs), polycyclic musks (PCMs) and UV-filters (UVF) in sediments: Douro estuary versus Tagus estuary. The lines from bottom to top in box plot were minimum, lower quartile, median, upper quartile and maximum, respectively. The X was the mean and the asterisks out the box were extreme outliers.

Total pesticide residue levels in sediments were higher in Tagus estuary than in Douro estuary with an average of 18.78 ng/g dw and 7.56 ng/g dw, respectively (Fig. 3). Higher levels in Tagus estuary can be associated with a greater extension of agricultural areas that drain into the river, mainly rice, wheat, and grapes crops (Rocha et al., 2015).

Total BPs levels in sediments of Tagus estuary were also higher (average of 1.08 ng/g dw) than those found in Douro estuary (average of 0.37 ng/g dw) (Fig. 3). As mentioned above BPA was the most frequent BP, with levels from 1.6 to 16.2 ng/ng dw. Curiously, BPA was not detected above the LOQ in any sample from Douro

River, where only two samples showed quantifiable amounts of BPAF and BPS.

Sediments from Douro estuary presented higher levels of total PCMs (average 6.71 ng/g dw) than those found in Tagus estuary (3.01 ng/g dw) (Fig. 3). The higher frequency of AHTN in sediments from Douro estuary compared to those reported in Tagus estuary is the main difference between both estuaries.

Total UVFs levels were higher in the sediments of Douro estuary with an average level of 13.51 ng/g against 7.79 ng/g for Tagus estuary. The main difference came from the presence of higher levels of EHS.

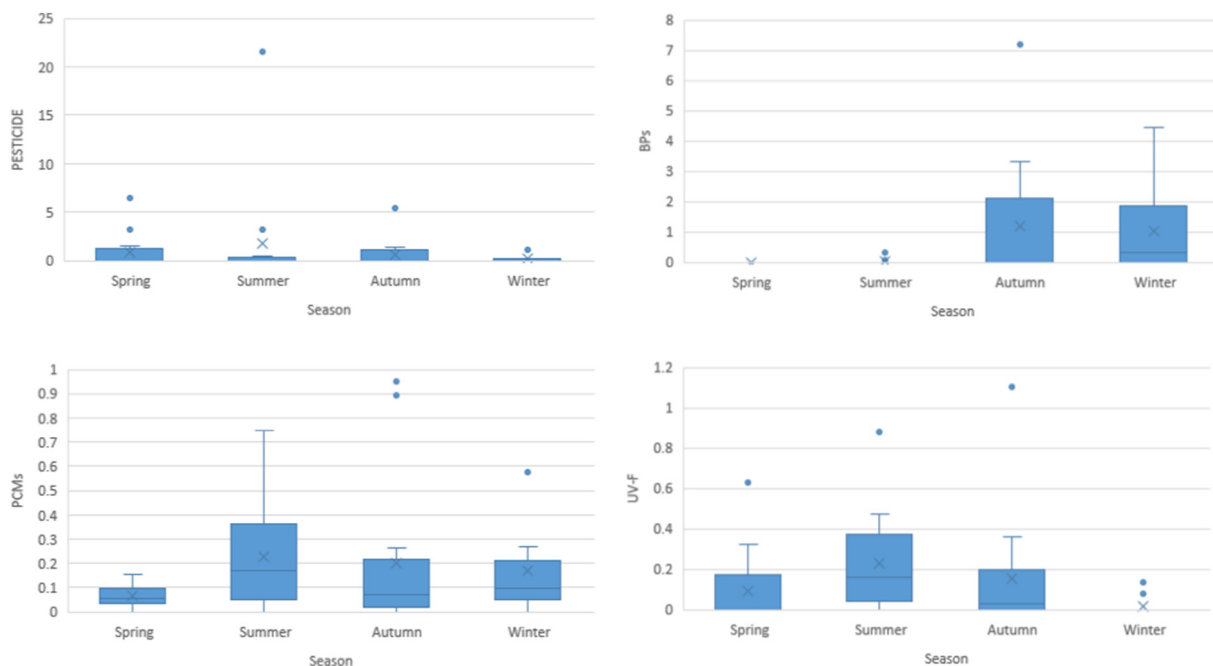


Fig. 4. Levels pesticide residues, bisphenols (BPs), polycyclic musks (PCMs) and UV-filters (UVF) in water along the year of collection. The lines from bottom to top in box plot were minimum, lower quartile, median, upper quartile and maximum, respectively. The X was the mean and the asterisks out the box were extreme outliers.

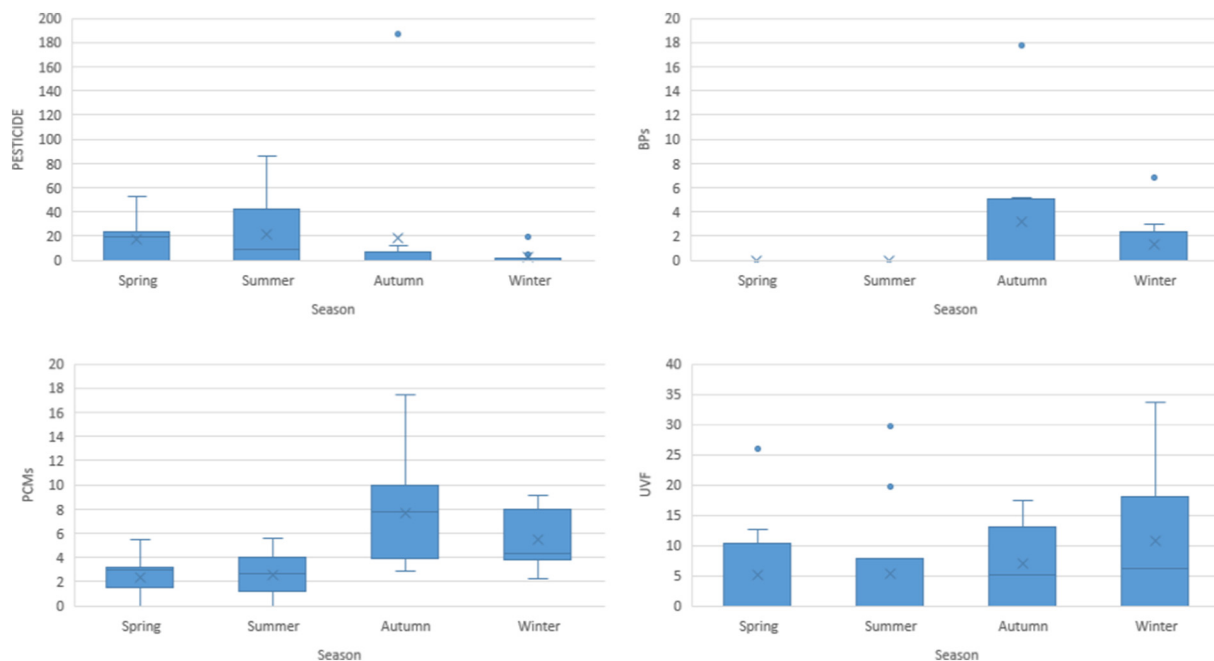


Fig. 5. Levels pesticide residues, bisphenols (BPs), polycyclic musks (PCMs) and UV-filters (UVF) in sediments along the year of collection. The lines from bottom to top in box plot were minimum, lower quartile, median, upper quartile and maximum, respectively. The X was the mean and the asterisks out the box were extreme outliers.

The statistic correlation from the results of both estuaries showed only significant differences ($p < 0.05$) for the presence of total of PCMs.

3.7. Temporal variation

In water, the highest levels of pesticide residues were verified in spring and autumn (Fig. 4). This can be explained by the fact that some pesticides whose formulation contains the substances studied herein are applied in maize and rice cultivation during that seasons, coinciding with the date of samplings (Rocha et al., 2015). The levels of pesticide residues in sediments were higher in summer and spring than in autumn and winter, which may be due to the decrease of discharge of freshwater in these seasons (Fig. 4).

The highest levels of BPs found in both water and sediment samples were detected in autumn and winter (Figs. 4 and 5). These data are in accordance with those previously reported by Ribeiro et al. (2008) for water in Mondego estuary in Portugal.

PCMs presented the highest levels in sediments during autumn for both estuaries; while in water the highest levels were observed in the summer period, particularly in Douro estuary (Figs. 4 and 5). Despite several studies reporting the presence of PCMs in sediments and water (Lee et al., 2014; Huang et al., 2016; Zhang et al., 2020), none of them had evaluate their seasonality. The presence of higher amounts of PCMs in waters in summer can be related with the increased use of personal care products, including skin protectors containing PCMs during the that period (Cunha et al., 2015b; Cunha et al., 2018).

The presence of UVF for both estuaries was higher during winter in sediments for both estuaries, while in water was higher in summer for both estuaries. As PCMs, UVF are widely used in various personal care products in particular skin protectors, which are frequently used in summer and possibly explain their presence in water (Molins-Delgado et al., 2014; Cunha et al., 2015b; Cunha et al., 2018). Concerning sediments, a significant number of studies concerning UVFs have been performed in sediments (Ferreira et al., 2019; Barón et al., 2013; Pintado-Herrera et al., 2017; Allinson et al., 2018), but few have screened the range of substances comparable to those herein and none has screened the levels seasonally.

To understand the variability of emerging contamination among samples, the results were statistically correlated with the seasons. Significant differences ($p < 0.05$) for water were observed in what concerns the

presence of BPs and UVFs, whereas for sediments a significant difference was only observed for PCMs ($p < 0.05$; Table S8).

4. Conclusions

New analytical methodologies were developed and validated targeting the simultaneous extraction, identification, and quantification of 37 endocrine disruptors at trace levels, in water and sediments. The analytical performance showed adequate precision, accuracy, and sensitivity for all analytes, in line with the suggested standard guidelines. The developed methods were applied in the analysis of 56 water samples and 46 sediment samples collected in selected areas of the Tagus and Douro estuaries (NE Atlantic Ocean coast). Thirty-three and thirty-one out of 37 substances under study were detected in water and sediment samples, respectively. The polycyclic musk HHCB was the residue most found in both water and sediments, with frequencies of 96% and 100%, respectively, and maximum levels of 0.90 ng/mL and 7.64 ng/g dw, respectively. Alachlor was the pesticide residue most detected in both water and sediments, with frequencies of 34% and 52%, respectively, while BPA was the most representative bisphenol in either water and sediments. EHS was the UVF more frequent in water samples while EHMC was prevalent in sediments. Tagus estuary presented higher level of contamination than Douro estuary mostly regarding pesticide residues and BPs. The levels of PCMs and UVFs were slightly higher in Douro estuary in particular for sediments. A seasonal variation for all the classes of compounds studied was verified along one year of collection. The highest levels of pesticide residues in both sediments and water were found in warm seasons (summer and spring), while BPs were more prevalent in cold seasons (autumn and winter). For PCMs and UVF, the highest levels in water were also found in the warm seasons, whereas in sediments were found in the cold seasons.

The results obtained in this study showed relatively low levels of contamination in both estuaries, but comprehending a large number of compounds, which is cause of concern and implies the need for constant monitoring.

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CRedit authorship contribution statement

Sara C. Cunha: Original Draft, Validation; Formal analysis; Methodology – planning; Writing – review & editing, Supervision and Funding;

Ricardo Ferreira: Conceptualization; Methodology – planning; Investigation; Validation; Formal analysis;

Isa Marmelo, Ana Maulvault, Patrícia Anacleto, António Marques, Luís Vieira: Collection of the samples, Methodology – planning and Writing - review & editing;

Lúcia Guilhermino: Methodology – planning, Writing - review & editing;

José O. Fernandes: Formal analysis; Methodology – planning, Writing - review & editing.

Compliance with ethical standards

The authors declare no conflict of interest.

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.

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