

## Article

# Occurrence of Selected Known or Suspected Endocrine-Disrupting Pesticides in Portuguese Surface Waters Using SPME-GC-IT/MS

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**Abstract:** A multiresidue analytical methodology based on a solid-phase microextraction (SPME), followed by gas chromatography-ion trap mass spectrometry (GC-IT/MS), has been developed for trace analysis of 20 known or suspected endocrine-disrupting pesticides. The SPME conditions are optimized considering several key parameters to obtain the maximum sensitivity. After the optimization, the method validation is performed, and the limits of detection (ranged from 2–150 ng/L) and the coefficient of determination (above 0.990) of studied compounds are determined for all the analytes. A robust sampling of twenty sampling points of surface water samples from the north and center of Portugal is performed, and the validated methodology is applied. In total, 20 compounds from four chemical families (13 organochlorine, 1 organophosphorus, 2 dicarboximide, and 4 pyrethroids) are studied, and the pesticides most frequently detected are eight organochlorine pesticides ( $\alpha$ -,  $\beta$ -HCHs, lindane, HCB, *o,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD,  $\alpha$ -endosulfan), cypermethrin, and vinclozolin.

**Keywords:** gas chromatography; monitoring; endocrine-disrupting pesticides; Portugal; solid-phase microextraction; surface waters



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## 1. Introduction

The term endocrine-disruptors have been generally used to call compounds with characteristics in common, namely, with the capability to mimic the actions of hormones interfering with the behavior of endocrine systems in various living species [1]. The endocrine-disruptors compounds (EDCs) can be different chemical groups, such as pesticides, pharmaceuticals, and personal care products, polybrominated diphenyl ethers, among others [2]. Most of these are released to the environment by anthropogenic activities and may coexist and accumulate in crops and human bodies through food chains, posing risks to human health and the ecosystem [3,4]. Endocrine-disrupting pesticides (EDPs) are the largest group of EDCs. Despite all the benefits related to pesticide application (e.g., control of pests and diseases), they still can contaminate different environmental compartments being the aquatic environment the most vulnerable because of massive global use and its consequent degradation products [5,6]. The industry introduced several synthetic pesticides, such as organochlorine pesticides (OCs) (1940s), organophosphorus (OPs) (1960s), carbamates (1970s), and pyrethroids (PYR) (1980s). OCs, many of them classified as EDPs, were banned or restricted after the 1970s in most countries of the world, but continuously detected until today, due to their characteristics, such as high lipophilicity, bioaccumulation, high persistence, and potential of long-range transport [7]. The other chemical families of pesticides (e.g., OPs, carbamates, and PYR) are placed on the market

as alternatives to OCs, and they are described as being less persistent, but more acutely toxic to control insect pests [8,9]. Studies have reported that chronic low-level exposure to OPs in the uterus and childhood are associated with poorer cognitive and behavioral problems [10]. PYR are also reported as lipophilic compounds, accumulate in sediments, bioaccumulated in biota [11], and neurotoxic [12,13]. In fact, national and international governments established programs and legislation to assess chemical safety regarding their potential to interact with the endocrine system of humans and wildlife, and considerable efforts have been made regarding ECPs control levels in waters. European Union (EU) collected data about EDPs, and priority lists are defined [14]. In terms of water analysis, maximum allowable concentration-Environmental Quality Standards (MAC-EQS) based on the directive 2013/39/EU [15], and the drinking water 98/83/EC [16] directive are strictly consulted and discussed. Due to the low levels of pesticides in the environment, ranging from ppb to ppt, and the complexity of the environmental matrices, the preconcentration of the samples before the analysis is usually required. One of the analytical techniques reported for isolation and preconcentration of pesticides from water samples is solid-phase microextraction (SPME) [17–19]. It is based on the sorption of analytes directly from the sample in one-step extraction and preconcentration of analytes [18,20]. The SPME has demonstrated its simplicity, usefulness, selectivity, and efficiency in the analysis of a broad range of compounds in several matrixes without requiring sample pretreatment [21].

Filho et al. [22] reported a multiclass residues analysis by GC-MS after SPME in twenty-six water samples from Brazil. Recently SPME was tested successfully for sixteen water samples from the North of Lebanon and the results obtained indicate a potential risk of environmental contamination and the need to perform seasonal monitoring [23].

Contamination, due to multiclass pesticides, has been reported in different countries, indeed recently, e.g., in surface water samples [24], and sediments [25]. In Portugal, several studies about the presence of these priority pesticides, resulting from the different sources, were reported in food [26,27], soils [28], sediments [29–32], marine samples [33], rivers waters [34–38], and Small Public Water Supply Systems [39]. Despite the resulting contamination from the last decades, there are only a few studies on the quantification and monitoring of known and suspected EDPs in Portuguese surface water samples.

The present study aimed to: (i) Select sampling sites focus on areas considered susceptible to be contaminated, namely, with intense urbanization, agricultural and industrial sites; (ii) develop an SPME coupled with GC-IT/MS for analyzing known and suspected EDPs in surface waters; (iii) apply the methodology in twenty surface water samples located in the north and center of Portugal; (iv) assess the river samples quality for twenty pesticides (OCs, OPs, dicarboximide, and PYR).

## 2. Materials and Methods

### 2.1. Reagents, Solvents, and Materials

The pesticide characteristics (e.g., chemistry groups, company supplier, chemical structure, and molar mass) are presented in Table S1 (Supplementary Material). Methanol was supplied by Sigma Aldrich (Steinheim, Germany), and n-hexane was purchased from Merck (Darmstadt, Germany). Both solvents with purity over 99%. Glacial acetic acid was obtained from Carlo Erba (Val de Reuil, France), and sodium hydroxide p.a. grade was purchased from Pronolab (Saint Paul, MN, USA). Individual stock solutions of each standard ( $\alpha$ -,  $\beta$ -HCHs, lindane, HCB, *o,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, aldrin, dieldrin, endrin,  $\alpha$ - and  $\beta$ -endosulfan, methoxychlor, bifenthrin, cypermethrin, deltamethrin, diazinon, fenvalerate, iprodione, and vinclozolin) were prepared in n-hexane in the 100  $\mu\text{g/L}$  concentration and stored at  $-18\text{ }^\circ\text{C}$ . The work standard mixture containing the twenty pesticides was prepared by dilution in n-hexane of the stock solutions up to a concentration of 5  $\mu\text{g/L}$ .

Ultrapure water (resistivity of 18.2  $\text{M}\Omega\cdot\text{cm}$ ) was produced using a Simplicity 185 system (Millipore, Molsheim, France). All weight measurements were done on an analytical balance, Mettler Toledo (Columbus, OH, USA), and the pH adjustment was measured in a GLP 22 pH meter supplied by Crison (Barcelona, Spain).

## 2.2. Site Selection and Sampling

Twenty spot samples from ten rivers (Douro, Cávado, Lima, Minho, Sousa, Tâmega, Leça, Cabrum, Caima, and Ave), one lagoon (Ria de Aveiro), and one stream (Moscoso) located in the north and center of Portugal were collected from 20 different sampling points. A global position system (GPS) was used to locate the sampling points (Figure 1). Samples were collected in precleaned amber glass bottles (250 mL), previously rinsed several times with the surface water, acidified with glacial acetic acid (1%, *v/v*) immediately after collection, and stored at 4 °C. Samples were processed in 24 h. The samples were collected during the spring (April) and summer (June) seasons.

## 2.3. Description of the Surface Water Samples (Rivers, Lagoon, and Stream)

The Douro is one of the longest rivers in the Iberian Peninsula, sharing its 930 km with Spain and Portugal [40]. The Douro River samples were representative of an urban area. Sousa, Cabrum, and Tâmega Rivers are tributaries of Douro River. The Leça River has its estuary near the port of Leixões. Chemical and bacteriological quality data led to classify the Leça River water quality as “bad” or “very bad” [41]. The Leça River is from the urban and industrial areas. The Minho River extends over 300 km in a southwest direction in the northwest corner of the Iberian Peninsula. Its terminal 70 km constitutes the natural border between Portugal and Spain, including the main estuarine axis of approximately 40 km (upstream limit of springtides) [42,43]. The Lima River estuary is an urban-industrialized estuary; the input of agricultural runoff impacts this system and urban and industrial sewage, which discharges nutrients and other substances that are transported from domestic, industrial, and agricultural areas into the estuarine area [44]. The Cávado River is in north Portugal. It runs 135 km from Gouveia to its mouth next to the city of Esposende. The Ave River basin is in a very populated region of the country. River water is intensively used for agricultural and industrial purposes, namely, textile industries [45]. Ria de Aveiro coastal lagoon is an estuarine system on the northwest coast of Portugal [46]. It is a shallow system that occupies an area of 43 km<sup>2</sup> at low tide. The Moscoso Stream and Caima River are tributaries from Ria de Aveiro estuarine zone. The Caima River drains a catchment area of about 196 km<sup>2</sup>, in the north-center region of Portugal [47]. These samples were from the urban, agricultural, and industrial areas.

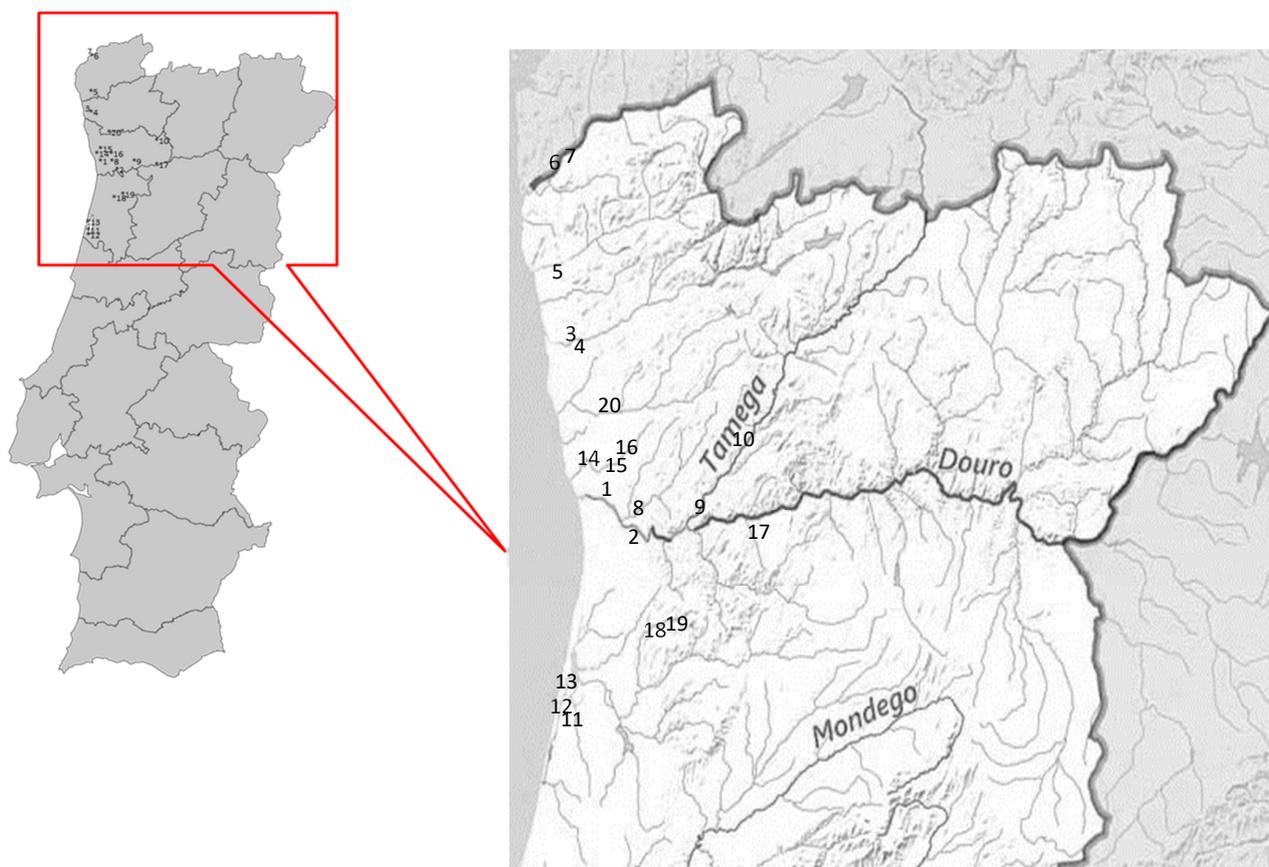
## 2.4. Extraction Procedure

### 2.4.1. Conditioning and Cleaning Procedure

The SPME (100 µm Polydimethylsiloxane (PDMS) fiber) procedure was performed with a manual fiber holder assembly supplied by Supelco (Darmstadt, Germany). A magnetic stirring and heater unit (AGIMATIC-N, Mundilab) was used during the SPME procedure. According to factory recommendations, the PDMS fiber used in the present study was conditioned at 250 °C in a current of helium for one hour in the GC-MS injector (Supelco®). Routinely, the conditioning was achieved by exposing the fibers to the injector port at 260 °C for 30 min before use. Fiber blanks were performed to ensure that no interferences from the fibers were present in GC chromatograms. After thermic desorption of the analytes in the GC, the fiber remained in the injector for several minutes for further cleaning at a high temperature. The liner in GC-MS was also replaced and cleaned every week to prevent the accumulation of analytes, and thus, unwanted peaks in the chromatograms.

### 2.4.2. Sample Enrichment

Standard water samples were prepared by spiking ultrapure water with a mixture of pesticides (5000 ng/L of the twenty pesticides) to validate the SPME step. Atrazine D-5 was used as an internal standard at 5000 ng/L. The comparison between studies with ultrapure water and a surface water sample (residue-free) was conducted to evaluate the SPME efficiency.



Sample	Type of Surface Water	Coordinates	Sampling Point, City	District
1	Douro River	41°8'36" N; 8°38'53" W	Afurada	Porto
2		41°4'20" N; 8°29'10" W	Lever, V.N. Gaia	Porto
3	Cávado River	41°30'54" N; 8°46'12" W	Braga	Braga
4		41°31'2" N; 8°46'17" W		
5	Lima River	41°41'57" N; 8°D'36" W	Viana do Castelo	Viana do Castelo
6	Minho River	41°56'28" N; 8°44'47" W	Cerveira	Viana do Castelo
7		41°56'27" N; 8°45'2" W	Goian	Galicia
8	Sousa River	41°5'26" N; 8°30'39" W	Porto	Porto
9	Tâmega River	41°5'50" N; 8°15'42" W	Amarante	Porto
10		41°16'8" N; 8°4'36" W		
11	Ria de Aveiro Lagoon	40°37'32" N; 8°44'18" W	Aveiro	Aveiro
12		40°36'52" N; 8°44'53" W	Aveiro	Aveiro
13		40°39'38" N; 8°43'50" W	Aveiro	Aveiro
14	Leça River	41°12'52" N; 8°40'5" W	Leça	Porto
15		41°13'5" N; 8°37'27" W		
16		41°12'5" N; 8°36'03" W		
17	Cabrum River	41°5'51" N; 8°2'12" W	Resende	Viseu
18	Moscoso Stream	40°49'18" N; 8°24'8" W	Aveiro	Aveiro
19	Caima River	40°49'59" N; 8°23'30" W	Vale de Cambra	Aveiro
20	Ave River	41°20'57" N; 8°31'13" W	Lousado	Braga

Figure 1. Schematic localization of the twenty sampling points of the surface water samples collected.

### 2.4.3. Optimization of the SPME Process

Before the sample's analysis, the optimization of the SPME process was carried out. Variables affecting the process of adsorption to the fiber and the extraction, namely, ionic strength (0, 0.05, 0.1 and 0.2% of NaCl), desorption temperature (250, 260 and 270 °C), organic solvent (0, 2.5, 5.0 and 7.5% methanol), extraction time (15, 30, 45 and 60 min) and extraction temperature (20 °C, 38 °C and 60 °C) were evaluated.

### 2.5. Gas Chromatography

Gas chromatograph-ion trap mass spectrometer (Thermo Trace-Ultra GC from Thermo Fisher Scientific (Waltham, MA, USA)) equipped with a fused-silica capillary column ZB-XLB (30 m × 0.25 mm ID, 0.25 µm film thickness, Phenomenex) was used for the separation of the study pesticides, using helium 99.99% as carrier gas at a 1.3 mL/min flow rate. Injector mode was split/spitless injector in the split mode (split/ratio 10) at 260 °C during the chromatographic run. The oven temperature was as followed: Initial oven temperature was held at 60 °C for 1 min, programmed with a gradient of 20 °C/min up to 200 °C where it stays for 1 min and then an increase of 5 °C/min up to 245 °C where it stays for 32 min. The mass detector conditions were: Transfer line temperature 250 °C, ion source temperature 250 °C, and ionization mode electron impact at 70 eV. The retention times were determined in the full scan mode and validation study in the selected ion monitoring (SIM) mode. In SIM mode, the main ions (Q1, Q2, and Q3) were selected for each analyte (Table 1). A matrix-matched calibration curve (using a residue-free surface water sample) was performed using nine concentration levels (10, 50, 100, 500, 1000, 1500, 2000, 2500, and 3000 ng/L). The addition of the IS, atrazine D-5, was performed at 1000 ng/L. The limits of detection (LOD) and quantification (LOQ) of the method were evaluated considering a signal-to-noise ratio of 3 and 10, respectively. Each concentration was measured twice. Precision was evaluated by calculating the relative standard deviation (RSD) from 3 replicates.

**Table 1.** GC-IT/MS parameters for each study pesticide.

Compounds *	Time Retention (min)	SIM Ions (Q1, Q2 and Q3) (m/z)	GC Segment (min)
α-HCH	9.66	181, 219, 109	8.50–9.90
HCB	9.82	284, 142, 249	8.50–9.90
diazinon	10.04	179, 199, 304	9.90–10.20
β-HCH	10.36	181, 219, 109	10.20–11.20
lindane	11.04	181, 219, 109	10.20–11.20
vinclozolin	11.38	212, 124, 187	11.20–11.85
aldrin	12.47	263, 293, 66	11.85–13.00
α-endosulfan	14.89	241, 195, 209	14.10–15.00
<i>p,p'</i> -DDE	15.54	246, 176, 318	15.00–16.00
dieldrin	15.68	79, 263, 277	15.00–16.00
endrin	16.35	244, 263, 317	16.00–17.00
<i>o,p'</i> -DDT	16.74	235, 165, 81	16.00–17.00
<i>p,p'</i> -DDD	17.21	235, 165, 199	17.00–18.00
β-endosulfan	17.30	195, 335, 339	17.00–18.00
bifenthrin	19.51	165, 166, 141	18.00–24.00
methoxychlor	20.19	227, 152, 165	18.00–24.00
iprodione	20.32	187, 244	18.00–24.00
cypermethrin	31.72, 31.98, 32.44	163, 181, 91	24.00–35.00
fenvalerate	38.59, 40.63	125, 167, 419	35.00–43.00
deltamethrin	46.62	181, 253	43.00–50.00

\* pesticides in the table organized by their retention time.

### 3. Results and Discussion

#### 3.1. Optimization of the SPME Procedure

##### 3.1.1. Ionic Strength Adjustments

The effect of the amount of salt influenced the extraction efficiency of some analytes present in water samples. All the chemical families presented a good performance without the use of NaCl (Figure 2A). A few compounds (HCH isomers and diazinon) showed higher areas with the addition of 0.2% NaCl. This may be related to the increase in the ionic strength of the matrix and consequently decreases the solubility of these compounds in water. However, the results showed that the salt aggregate does not help in most of the studied compounds.

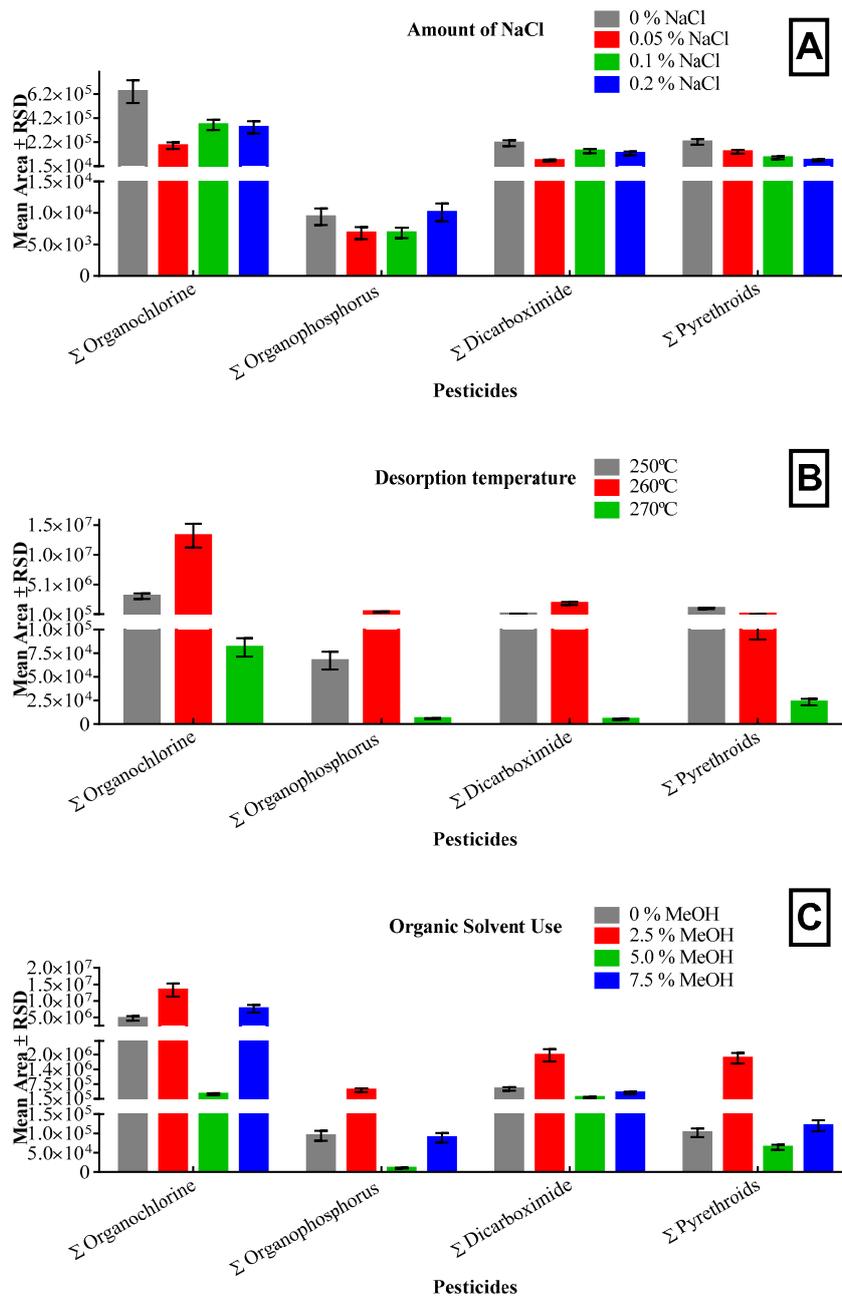
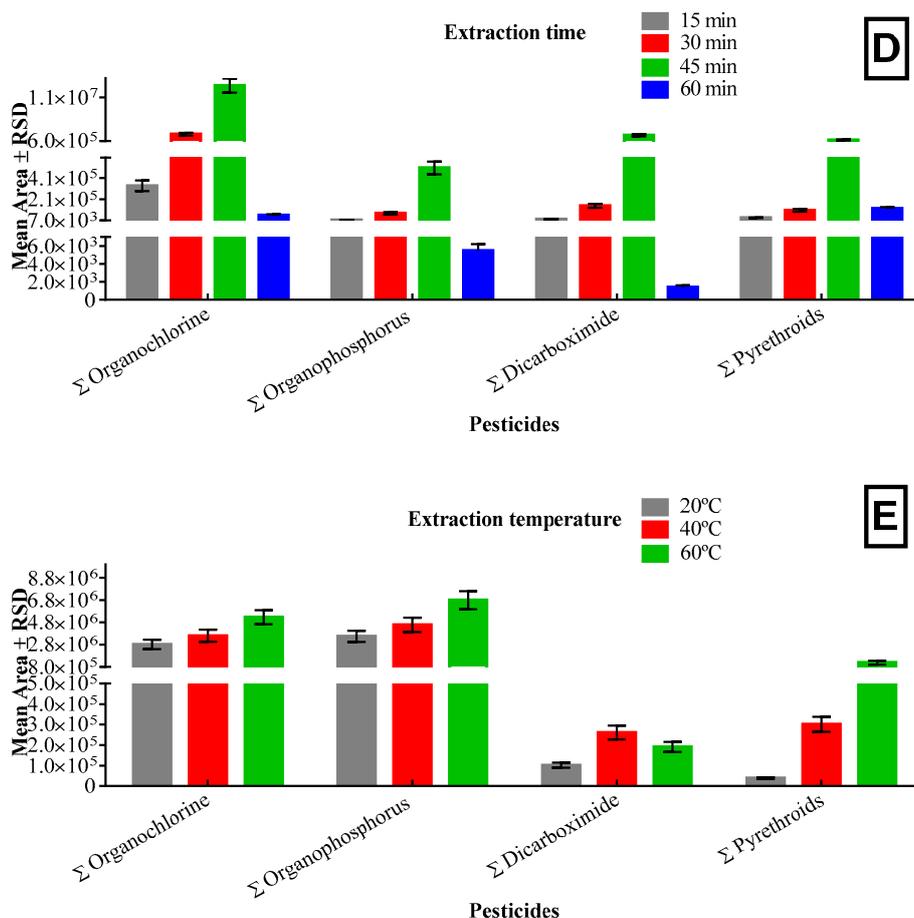


Figure 2. Cont.



**Figure 2.** Plot of the areas from each pesticide with: (A) The addition of NaCl at 0.00%, 0.05%, 0.10%, and 0.20%; (B) desorption temperature at 250 °C, 260 °C, and 270 °C; (C) the use of organic solvent (MEOH) at 0%, 2.5%, 5.0% and 7.5%; (D) extraction time of 15 min, 30 min, 45 min, and 60 min; (E) extraction temperature of 20 °C, 40 °C and 60 °C.

### 3.1.2. Desorption Temperature

Figure 2B showed the best results for the complete desorption of most pesticides with the desorption temperature at 260 °C. Bifenthrin and iprodione presented complete desorption with a temperature of 250 °C.

### 3.1.3. Organic Solvent

The effect of organic solvent was evaluated, and the addition of 2.5% methanol improved the extraction efficiency of the target compounds. The effect of this parameter is well demonstrated in Figure 2C.

### 3.1.4. Extraction Temperature and Time

In terms of extraction time, the results showed that as the time increased up to 45 min, the extraction efficiency improved. However, for longer times (60 min), the performance of the methodology decreased (Figure 2D,E). The results obtained for the extraction temperature study showed similar behavior to the extraction time. As the temperature was increased, better areas were obtained. Only the dicarboximide pesticides had an opposite behavior at 60 °C.

The best conditions were selected, and the extractions were performed in glass vials by immersing the SPME fiber (PDMS) in 10 mL of water sample with 2.5% methanol,

pH 3.5, and placed in the stir plate (700 rpm) for 45 min at 60 °C. Finally, the desorption temperature selected was 260 °C.

### 3.2. Method Validation

After the optimization of the methodology, the validation study was performed. The proposed method measured different families of pesticides (OCs, PYR, OPs, and dicarboximide). Also, the methodology showed good performance, and the analytical parameters obtained at optimized conditions were calculated and presented in Table 2. Good linear regressions ( $R^2 \geq 0.9904$  in all cases) were obtained over the tested analytical range. The method achieved LODs and LOQs between 0.2–150 ng/L and 0.6–450 ng/L, respectively. The precision was evaluated, and the relative standard deviation (RSD) was lower than 15% is acceptable for all the studied analytes. The validation parameters obtained are in the same order as those reported for other authors applying SPME [19].

**Table 2.** Coefficient of determination, LOD, and LOQ, of 20 standard pesticides obtained by GC-MS method.

Groups	Pesticides	Coefficient of Determination	LOD ng/L	LOQ ng/L
Organochlorines	$\alpha$ -HCH	0.9912	25.0	75.0
	$\beta$ -HCH	0.9991	60.0	180.0
	Lindane	0.9991	75.0	225.0
	HCB	0.9978	2.5	7.5
	aldrin	0.9983	2.4	7.2
	dieldrin	0.9976	0.7	2.1
	endrin	0.9978	12.0	36.0
	<i>p,p'</i> -DDE	0.9957	0.3	0.9
	<i>o,p'</i> -DDT	0.9904	1.8	5.4
	<i>p,p'</i> -DDD	0.9935	5.3	15.9
	$\alpha$ -endosulfan	0.9991	5.9	17.7
	$\beta$ -endosulfan	0.9977	3.0	9.0
	metoxychlor	0.9923	1.6	4.8
Pyrethroids	bifenthrin	0.9925	7.0	21.0
	cypermethrin	0.9976	30.0	900.0
	fenvalerate	0.9917	3.3	9.9
	deltamethrin	0.9939	150.0	450.0
Organophosphorus	diazinon	0.9990	3.1	9.3
Dicarboximide	Iprodione	0.9990	27.3	91.0
	vinclozolin	0.9994	0.2	0.6

### 3.3. Monitoring of 20 Known or Suspected Endocrine-Disrupting Pesticides in Portuguese Surface Water Samples

The national and international authorities and the scientific community have shown interest and concern on the topic of water pollution and its adverse implications for humans and the ecosystem.

The literature has recently reported the presence of several priority pesticides, most of them classified as EDPs in aquatic environments [24,34–36,38,48,49]. Table 3 summarizes the results obtained in the present study with 20 surface water samples in terms of frequency of detection, minimum, maximum, and mean values detected. As the values obtained are different in terms of range, the graphs below show the data distribution by samples and each compound. All the data, including the value below LOQ (estimated by the analytical value), were counted for the data analysis.

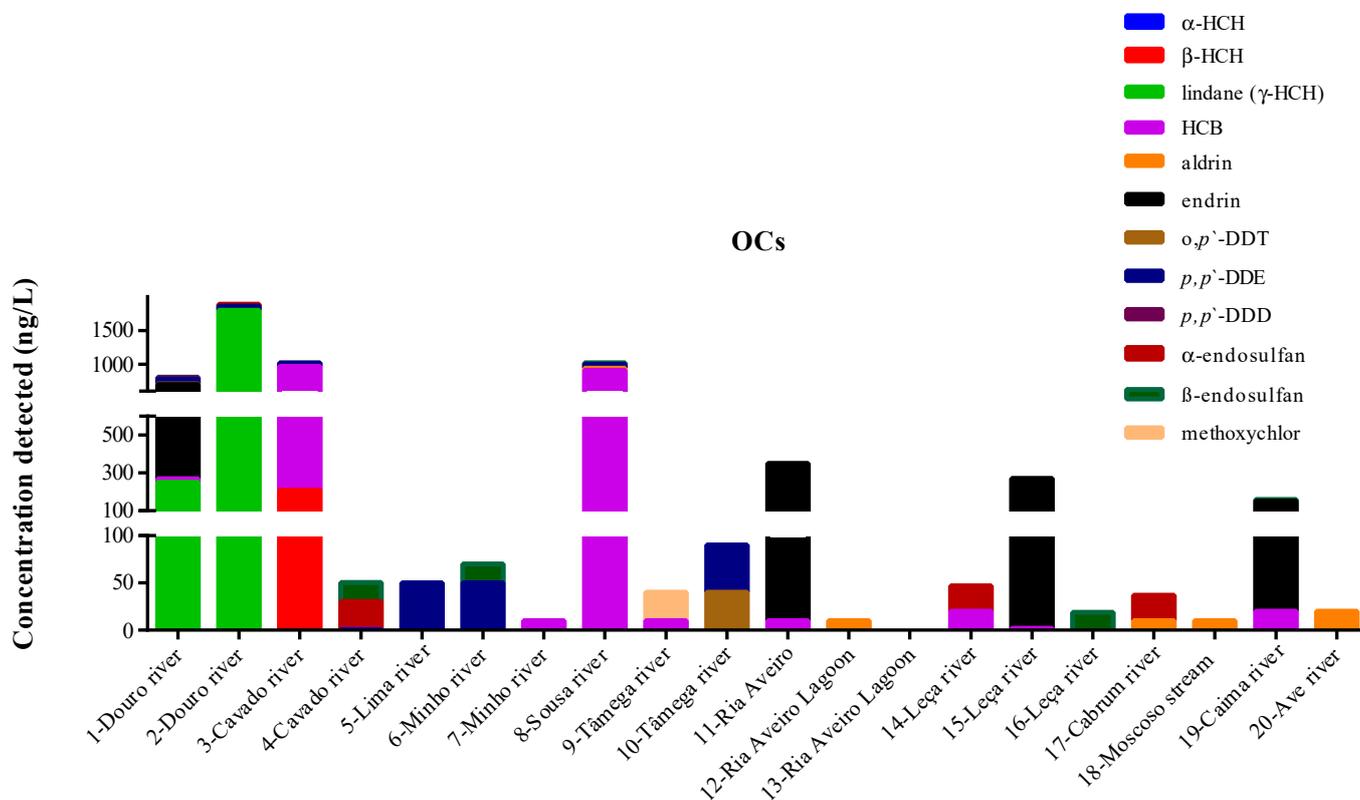
**Table 3.** A summary of the results obtained in the 20 samples.

Group	Compounds	Frequency (%)	Min ng/L	Max ng/L	Mean ng/L
Organochlorines	$\Sigma(\alpha\text{-HCH} + \beta\text{-HCH} + \text{lindane})$	30	<LOQ	1800	460
	HCB	45	<LOQ	912	201
	$\Sigma(\text{aldrin} + \text{dieldrin} + \text{endrin})$	45	10	430	142
	$\Sigma(p,p'\text{-DDE} + o,p'\text{-DDT} + p,p'\text{-DDD})$	40	<LOQ	70	63
	$\Sigma(\alpha\text{-endosulfan} + \beta\text{-endosulfan})$	75	<LOQ	30	11
	methoxychlor	5	nd	31	31
Pyrethroids	bifenthrin	10	nd	<LOQ	-
	cypermethrin	55	<LOQ	351	261
	fenvalerate	10	10	294	150
	deltamethrin	5	nd	<LOQ	-
Dicarboximide	vinclozolin	45	20	62	43

Regarding the 20 spot surface water samples, only the diazinon and iprodione were not detected in the analyzed samples. The  $\alpha$ - and  $\beta$ -endosulfan were the most detected EDPs (75%), and the methoxychlor (5%) and deltamethrin (5%) were the least detected. Although the samples were collected in different seasons, no significant differences were observed. Even so, pyrethroids were more detected in the summer samples.

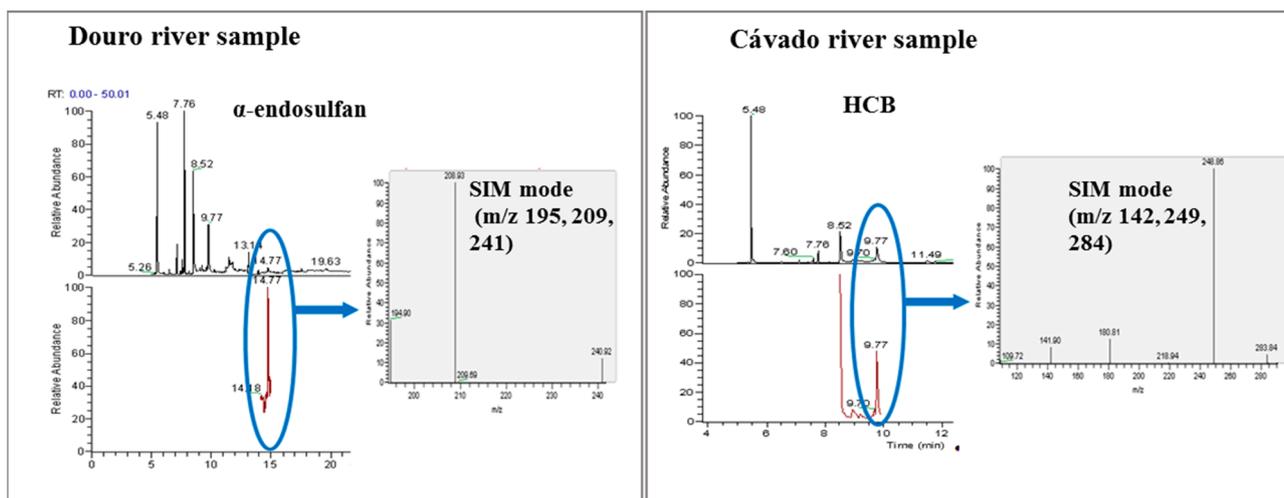
### 3.3.1. Organochlorine Pesticides in Surface Water Samples

Several decades after the OCs were banned, they are frequently detected in different environmental samples, due to their persistence and bioaccumulation. These compounds are in continuous movement between the different environmental compartments, such as water, soil, sediments, air, and/or bioconcentrate, in the living organisms [50]. Figure 3 shows a plot with the concentrations detected of each OCs by sample. The values.



**Figure 3.** Concentration in ng/L of OCs detected in the 20 surface water samples.

The highest individual mean value was found for HCHs isomers in the Douro River (Mean  $\Sigma$ HCH = 2150 ng/L) and Cávado River (Mean  $\Sigma$ HCH = 350 ng/L). Indeed, the cumulative values were always above the maximum allowable concentration-Environmental Quality Standards (MAC-EQS) established by the Directive 98/83/EC and 2013/39/EU [15,16]. HCHs isomers were also detected in the Minho and Sousa Rivers, but below the quantification limit (<LOQ). The organochlorine compound, HCB, presented concentrations 18.2-fold above the MAC-EQS (50 ng/L) in the Cávado River (760 ng/L) and Sousa River (910 ng/L) (Figure 3). The presence of HCB in other samples was quantified below the MAC-EQS (Rivers: Douro (20  $\mu$ g/L), Minho (10  $\mu$ g/L), Tâmega (10 ng/L), Leça (20 and 2 ng/L), Caima (20 ng/L); Lagoon: Ria Aveiro (10 ng/L)). Endrin was detected above the average annual amounts allowed for surface waters (AA-EQS =  $\Sigma$  0.05), in three rivers (Douro (430 ng/L), Leça (271 ng/L), and Caima (132 ng/L), and one lagoon (ria Aveiro (342 ng/L)) samples. The other cyclodiene pesticide, aldrin, was detected below the AA-EQS in the three rivers (Sousa (32 ng/L), Cabrum (10 ng/L), Ave (22 ng/L)), and one stream (Moscoso (12 ng/L)). The same behavior was observed with DDT isomers ( $\Sigma$  *p*, *p'*-DDT + *p*, *p'*-DDE + *p*, *p'*-DDD) pesticides analyzed in this work. The sum of the concentration in rivers (Douro ( $\Sigma$  = 130 ng/L), Cávado ( $\Sigma$  = 53 ng/L), Lima ( $\Sigma$  = 50 ng/L), Minho ( $\Sigma$  = 54 ng/L), Sousa ( $\Sigma$  = 62 ng/L), and Tâmega ( $\Sigma$  = 90 ng/L)) achieved amounts above the AA-EQS ( $\Sigma$  = 0.025  $\mu$ g/L). The endosulfan isomers studied were ( $\alpha$ ,  $\beta$ ), and the MAC-EQS = 0.01 was considered for endosulfan. All rivers, except the Lima River, showed the presence of endosulfan isomers. Finally, methoxychlor was observed at a concentration of 33 ng/L in the Tâmega River. In general, the rivers with the greatest OCs contamination were the Douro, Cávado, and the Sousa Rivers, as shown in Figure 3. Values above MAC-EQS were found in the seven river samples. Figure 4 presents the chromatogram obtained from the Douro and Cávado Rivers samples, demonstrating the confirmation by GC-MS of the presence of OCs.



**Figure 4.** GC-MS chromatograms were obtained in SIM mode and the MS spectrum confirming the detection of HCB and  $\alpha$ -endosulfan in the Cávado and Douro Rivers, respectively.

Comparing present data with Cruzeiro et al., [37] in Portuguese surface water samples, the 4,4'-DDT and 4,4'-DDD were detected in 100% of samples analyzed (N = 20), while in the present work, it was 40%. Indeed, the values obtained were above the restrictive European legislation (98/83/EC and 2013/39/EU). The same compound, 4,4'-DDT was quantified in another Portuguese study in concentrations 21.5 fold above the maximum (10 ng/L) level acceptable for inland and surface waters [38]. Although these authors reported HCB levels below its method quantification limit, the opposite occurred in the present work, where these values exceeded the LOQ. In 2017, 4,4'-DDT and HCB were also detected in the Douro River estuary, in 8 and 19 samples, respectively, over 2013/39/EU

levels [35]. Some studies reported pollution by these OCs in other samples in Portugal, such as, sediments samples from the Douro River [30,49,51] fruits [26], soil [28,52], and human samples [53]. This study contributes to the continuous evaluation of these compounds and emphasizes the relevance of continuous monitoring studies. Even though the usage of OCs was banned in Portugal, the recorded levels can be a consequence of their persistence or their misuse or illegal use. However, with this work, we can say that its incidence seems to be decreasing, at least for DDT.

### 3.3.2. Pyrethroids and Dicarboximide Pesticides in Portuguese Surface Water Samples

In this study, two PYR (cypermethrin and fenvalerate) and one dicarboximide pesticides (vinclozolin) were detected. Figure 5 shows a plot with the mean concentration in ng/L reported by the sample. Cypermethrin was the most frequently detected, and the Cávado, Cabrum, and Minho Rivers presented higher values. Nevertheless, all the surface water samples showed concentration values above the admissible amounts MAC-EQS. Fenvalerate was detected only in two samples (Cávado and Lima Rivers) at levels of 290 and 100 ng/L, respectively.

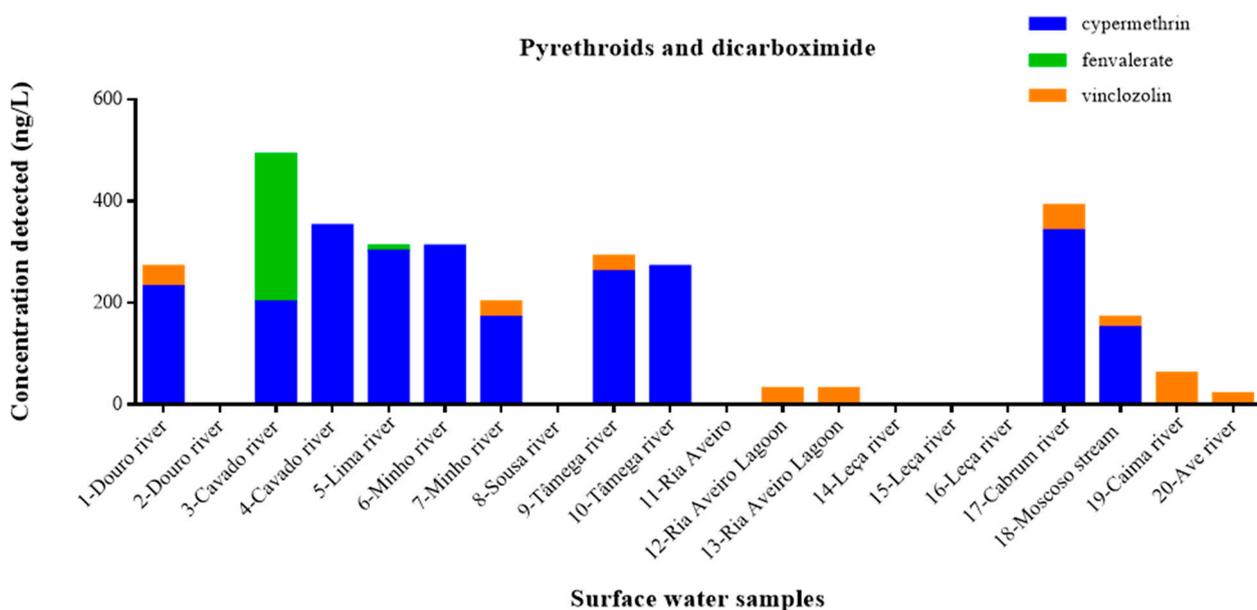


Figure 5. Pyrethroids and dicarboximide pesticides were detected in the 20 surface water samples.

Regarding other studies conducted in Portugal [38], our levels of cypermethrin were slightly lower (350 ng/L) in terms of environmental levels, compared with a concentration of 453 ng/L obtained in Ria Formosa lagoon in summer samples. The authors discussed the evidence that the higher concentration presented in samples from the summer period is related to the application of pesticides. In a study conducted in 2017 in the Douro River samples [35], nine samples (in a total of 24 sampling sites) presented concentrations over the limits established by EU, and an average concentration for cypermethrin of 35.9 ng/L. This study has already shown lower values than those detected in samples from the Douro River (230 ng/L) in the present study. The vinclozolin and iprodione were evaluated in the current work, but only vinclozolin was detected.

Although there is no legislation for this pesticide in surface waters, it was quantified in nine samples: The Douro River (41 ng/L), the Minho River (30 ng/L), the Tâmega River (33 ng/L), Ria Aveiro (32 µg/L), the Cabrum river (51 ng/L), the Moscoso Stream (25 ng/L), the Caima River (64 ng/L), and the Ave River (26 ng/L). The vinclozolin is a fungicide frequently used to control diseases in vineyards [54]. The vineyards in the north region of Portugal are in the hills on the banks of the rivers. The use of this pesticide in agriculture systems may be one of the reasons for the frequent detection.

#### 4. Conclusions

The proposed analytical method, SPME-GC-IT/MS, was firstly optimized for the simultaneous determination of 20 priority pesticides. In terms of analytical validation, the methodology showed good performance and fulfilled the requirements according to European analytical standards to determine the target compounds in water samples. We can conclude that this work stood out for the diversity of sampling sites in Portugal, despite being spot samples. In addition, the surface water samples from several sampling sites from the north end center of Portugal presented known and potential EDPs, with many of them above the established MAC-EQS. The following pesticides were detected in order of decreasing frequency of detection:  $\alpha$ - and  $\beta$ -endosulfan (75%) > cypermethrin (55%) > HCB, aldrin, dieldrin, endrin, and vinclozolin (45%) > *p,p'*-DDE + *o,p'*-DDT + *p,p'*-DDD (40%) > HCH isomers (30%), > fenvalerate (10%) > methoxychlor and deltamethrin (5%).

The persistence of OCs was again proven, as well as the presence of other pesticides. Despite the legislation established for these samples, this work also concluded that it is still insufficient when the presence of other non-legislated compounds was confirmed.

This study reinforces the data regarding the presence of priority pesticides in the environment and concluded that detected levels are still alarming. The monitoring and control of pesticides in rivers are an important issue that should continue to be explored in many areas and countries, and although the SPME performs well, more automated extraction methods should be explored to decrease the time and the cost of the monitoring analysis.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/separations8060081/s1>, Table S1: List of endocrine-disruptor compounds and their chemistry groups, company supplier, chemical structure, chemical formula, and molar mass.

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