

## Article

# Distribution and Estrogenic Risk of Alkylphenolic Compounds, Hormones and Drugs Contained in Water and Natural Surface Sediments, Morelos, Mexico

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**Abstract:** This study evaluated the distribution and potential estrogenic risk of the presence of bisphenol A (BPA), 4-nonylphenol (4NP), naproxen (NPX), ibuprofen (IBU), 17- $\beta$ -estradiol (E<sub>2</sub>) and 17- $\alpha$ -ethinylestradiol (EE2) in water and sediments of the Apatlaco river micro-basin (Morelos, Mexico). The concentration of the determined compounds ranged between <LOD to 86.40 ng·L<sup>-1</sup> and <LOD to 3.97 ng g<sup>-1</sup> in water and sediments, respectively. The Log K<sub>d</sub> distribution obtained (from 1.05 to 1.91 L Kg<sup>-1</sup>) indicates that the compounds tend to be adsorbed in sediments, which is probably due to the hydrophobic interactions confirmed by the significant correlations determined mainly between the concentrations and parameters of total organic carbon (TOC), total suspended solids (TSS), biological oxygen demand (BOD<sub>5</sub>) and chemical oxygen demand (COD). Of five sites analyzed, four presented estrogenic risk due to the analyzed endocrine-disrupting compounds (EEQE<sub>2</sub> > 1 ng·L<sup>-1</sup>).

**Keywords:** endocrine disruptors; estrogenic risk; partition coefficient; surface water and sediment



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## 1. Introduction

In the last two decades, the presence of emerging compounds in the environment has raised great health concerns [1–3]. In surface water bodies, emerging compounds have been linked to feminization and other hormonal alterations in aquatic organisms [4]. It has also been found that they can cause dysfunction in the germination of vegetables [5].

Knowledge of the transport mechanisms and distribution phenomena that these pollutants experience in surface water bodies, as well as their destination in the environment, can contribute to determining the potential risk they pose to the environment and human health. However, their mobility between different environmental compartments can be affected by their properties and involve the influence of various physicochemical factors [6], such as organic-matter content, temperature, pH, conductivity, content of microorganisms and flow-rate current, among others [7,8].

The partition coefficient (Log K<sub>d</sub>) is a parameter calculated between the concentration of the compound of interest detected in water and in sediments, and it is used to determine the transport and destination of pollutants in the environment. Log K<sub>d</sub> values > 0 indicate that the compound has a tendency to be adsorbed on organic matter due to the hydrophobic interactions of the compounds. This allows the compounds retained in sediments to biomagnify their concentration through the trophic chain. The log K<sub>d</sub> value of a compound

can vary according to various physicochemical parameters of the environment. Since it depends on these, if water-compound-sediment interactions reach equilibrium [9,10], the presence in the environment of certain endocrine-disrupting compounds (EDCs) can cause different estrogenic damages in aquatic organisms. Some effects caused by these compounds are feminization in fish, as well as alteration in vitellogenin levels [11] and alterations in sexual organs [12].

The purpose of this work was to examine the distribution as a partition coefficient  $\log K_d$  and the potential estrogenic risk due to the presence of two phenolic compounds, (bisphenol A (BPA) and 4-nonylphenol (4NP)), 2 drugs, (Ibuprofen (IBU) and naproxen (NPX)) and 2 hormones (17- $\beta$  estradiol (E2), and 17- $\alpha$  ethinylestradiol (EE2)) between water and sediment from the Apatlaco river micro-basin in Morelos, Mexico.

## 2. Materials and Methods

### 2.1. Standards and Reagents

17 $\alpha$ -ethinyl estradiol (EE2  $\geq$  98%), 17 $\beta$ -estradiol (E2  $\geq$  98%), 4-nonylphenol (4NP  $\geq$  99%) and ibuprofen (IBU  $\geq$  98%) standards were obtained from Sigma-Aldrich. B = Bisphenol A standard (BPA  $\geq$  99%) was purchased from Supelco, (St. Louis, MO, USA) while the naproxen was obtained from Fluka (St. Louis, MO, USA) (NPX  $\geq$  99.9%). For its part, deuterated chrysene (Chry-D12) was obtained from Supelco (St. Louis, MO, USA). The solvents acetone ( $\geq$ 99.9%) and methanol ( $\geq$ 99.9%) used for the conditioning of material, as well as in the preparation of standards and environmental samples, were HPLC-grade (Meyer 99.9%). The solvents used were previously filtered under vacuum conditions through Pall brand nylon, 0.2  $\mu$ m and 47 mm in diameter. For the extraction of water and sediment samples, Chromabond 500 mg Solid Phase Extraction (EFS) cartridges (Macherey-Nagel, C18, encapped, Düren, Germany) were used.

For the derivatization, N, O-bis (trimethylsilyl) -trifluoroacetamide + trimethylchlorosilane (BSTFA + TMCS, 99:1, Supelco, St. Louis, MO, USA) and Pyridine (99.8%, Sigma-Aldrich, St. Louis, MO, USA) were used.

### 2.2. Description of the Sites and Sampling

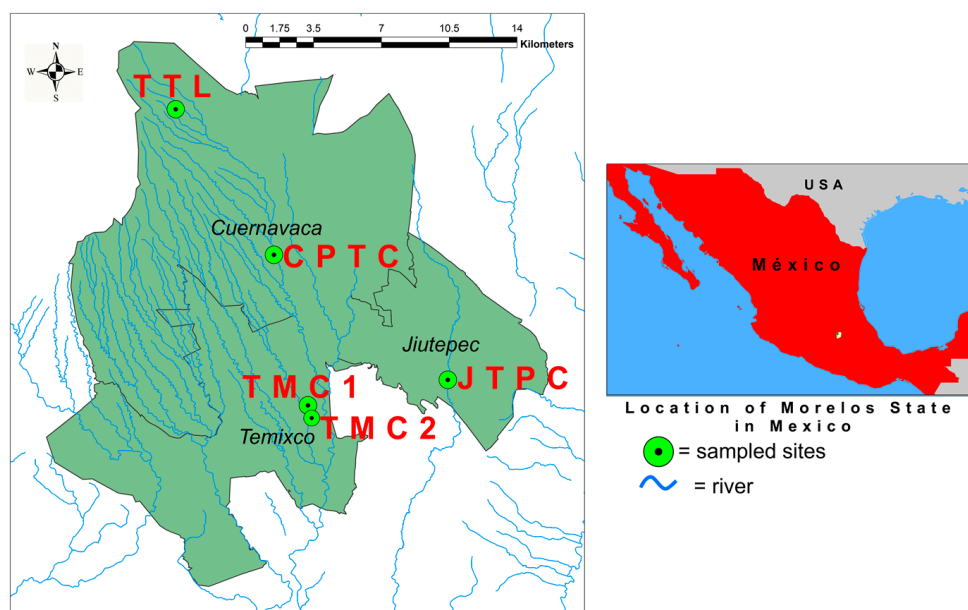
The Apatlaco river micro-basin is the most important body of surface water in the state of Morelos, Mexico. This basin crosses through 10 different municipalities before reaching the State of Guerrero and emptying into the Pacific Ocean. During its course, its waters and sediments are used in planting fields, as well as for recreational activities.

Water and sediment samples were taken from five different points of the Apatlaco river micro-basin (Figure 1). The physicochemical parameters of pH, conductivity, dissolved oxygen and temperature were measured in situ during each sampling. The samples were immediately transported to the laboratory, maintaining a temperature  $\leq$ 4 °C.

### 2.3. Sample Preparation

#### 2.3.1. Water Samples

Amounts of 50 mL of sample were passed through Solid Phase Extraction (SPE) cartridges, applying a flow of 4–6 mL min<sup>-1</sup>. Prior to extraction, the stationary phase was conditioned with 6 mL of acetone-MeOH (3:2), 6 mL of MeOH and 6 mL of deionized water. At the end of the extraction, the stationary phase was washed with 10 mL of deionized water and kept under vacuum until the complete elimination of water was observed. Compounds retained in the stationary phase were eluted with 10 mL of acetone-MeOH (3:2). The obtained eluate was evaporated to approximately 0.5 mL in a rotary evaporator. The remainder was filtered through 0.45  $\mu$ m nylon acrodisc syringe filter, brought to dryness by direct exposure to N<sub>2</sub> (99.9999%) and resuspended in 50  $\mu$ L of BSTFA + TMCS (99:1) + 50  $\mu$ L of pyridine for the derivatization of the compounds of interest. Subsequently, the vials were subjected to a water bath at 70 °C for 40 min. After this reaction time, they were left to rest until reaching room temperature, at which point 20  $\mu$ L of chry-D12 was added to each vial, for a final volume of 120  $\mu$ L (1500 ng·mL<sup>-1</sup> final concentration).



**Figure 1.** Sampling sites (Apatlaco river micro-basin); TTL (Tetela); CPTC (Chapultepec); TMC1 (Temixco 1); TMC2 (Temixco 2); JTPC (Jiutepec).

### 2.3.2. Sediment Samples

Sediment samples were placed inside a fume hood and isolated from light until dry. Subsequently, the sediments were crushed in porcelain mortar and sieved through a 2.0 mm mesh. The samples were stored in amber glass flasks under refrigeration ( $\leq 4$  °C). For extraction, 10 g of sediment was weighed and extracted by ultrasound-assisted extraction, followed by solid-phase extraction (UAE-EFS) for 20 min with 10 mL of acetone-MeOH (3:2). At the end of the extraction time, the samples were centrifuged at 4500 rpm for 5 min until complete separation of the liquid-solid phases was obtained. The liquid phase was collected, and the extraction was repeated two more times, joining the liquid phase obtained in each extraction. Subsequently, the liquid phase was reduced to approximately 0.5 mL in a rotary evaporator. The remainder obtained was resuspended in 1 mL of acetone and made up to 50 mL with deionized water. The sample was then extracted using EFS and processed as indicated in the previous section.

### 2.3.3. Evaluation of Extraction Efficiency

The efficiency of the extraction methodology was evaluated through the fortification of three types of natural surface water and sediments collected in springs and rivers that are mainly impacted by direct discharges from domestic drains and effluents from wastewater treatment. Before extraction, the water samples (50 mL) were adjusted to  $\text{pH} \approx 7$ , while the sediment samples (10 g) were dried at room temperature and sieved. The water and sediment samples were enriched with a solution containing a mixture of compounds (IBU, BPA, NX, 4NP, E2 and EE2) at two concentration levels (80 and 160  $\text{ng mL}^{-1}$ ). The repeatability was evaluated at the lowest level of the concentration and the reproducibility at the two levels. Briefly, repeatability was determined by repeating the addition of the standard concentration of 80  $\text{ng mL}^{-1}$  twice, while reproducibility was performed by adding the two concentration levels (80 and 160  $\text{ng mL}^{-1}$ ). Each level was repeated twice, and with the average of each level, the reproducibility was estimated.

### 2.3.4. Chromatographic Analysis

Sample analysis was performed on an Agilent model 6890N chromatograph coupled to an Agilent model 7000D mass spectrometer (Santa Clara, CA, USA). The injector temperature was kept at 280 °C. In each analysis, 1  $\mu\text{L}$  of sample was injected in splitless mode. The stripping gas was Helium (99.999%), using a flow of 1  $\text{mL min}^{-1}$ . Separation of

the compounds was carried out on an Agilent HP5-MS column, 30 m long × 0.25 mm in diameter, with an internal coating of 0.25 μm. The temperature of the column started at 120 °C, maintaining for 2 min. Subsequently, temperature was increased by 15 °C every minute until reaching 250 °C, with increments of 5 °C per minute until reaching 300 °C. The transfer line from the chromatograph to the mass spectrometer was kept at 310 °C. Ionization of the molecules was carried out by means of electronic impact (EI) using an ionization energy of 70 eV. The ionization source was kept at 200 °C (Table 1).

**Table 1.** Monitored ions (*m/z*) in mass spectrometry for each of the trimethylsilyl-derived (TMS) compounds of interest.

Compound	Molecular Weight (g·mol <sup>-1</sup> )	Trimethylsilyl-Derived Compound	Molecular Weight (g·mol <sup>-1</sup> )	Ion Quantitation ( <i>m/z</i> )	Ion Confirmation ( <i>m/z</i> )
IBU	206.29	TMS-IBU	278.47	160	263, 234, 278
4NP	220.36	TMS-4NP	292.54	179	292, 277
NPX	230.26	TMS-NPX	302.44	185	243, 287, 302
BPA	228.29	TMS-BPA	372.65	357	372, 207
E <sub>2</sub>	272.39	TMS-E <sub>2</sub>	416.75	285	232, 416
EE <sub>2</sub>	296.41	TMS-EE <sub>2</sub>	440.77	232	196, 425, 440

The repeatability of the injections was monitored as the variation calculated through the response obtained for Chry-D12 between injections of calibration solutions and environmental samples. In all cases, the variation obtained in the Chry-D12 response was less than 5%.

### 2.3.5. Partition Coefficient (Log K<sub>d</sub>)

The partition coefficient of drugs and EDCs between water and sediment was calculated as the Log K<sub>d</sub> of the quotient obtained between the concentration determined in sediment and water according to the following equation:

$$\text{Log } K_d = \frac{C_s}{C_w} \tag{1}$$

where C<sub>s</sub> is the average concentration of the compound determined in the sediment samples and C<sub>w</sub> is the average concentration of the compound determined in the water samples.

### 2.3.6. Estrogenicity Equivalent to E<sub>2</sub>

Estrogenic activity (EEQ<sub>E2</sub>) in water and sediments was determined by means of the estrogenicity factor equivalent to E<sub>2</sub> (EEF) and the environmental concentration measured for each compound (MEC). According to the Environmental Protection Agency (US-EPA), estrogenic risk is significant when the concentration of estrogenic compounds is greater than 1 ng·L<sup>-1</sup>. Determination of EEQ<sub>E2</sub> in both matrices was determined only for those compounds with EEF<sub>i</sub> values reported by Vega-Morales et al., 2013 [13] (4NP, BPA, E2 and EE2). The EEQ<sub>E2</sub> value in water was determined with Equation (2), while the EEQ<sub>E2</sub> value for sediments was determined with Equation (3), by converting the estrogenic activity of the selected compounds to their corresponding EEQ<sub>E2</sub> in sediments [14–16].

$$\text{EEQ}_{E2(\text{ng L}^{-1})} = \text{EEF}_i * \text{MEC}_i \tag{2}$$

$$\text{EEQ}_{E2(\text{ng L}^{-1})\text{sediment}} = \frac{1000 * \text{MEC}_{i\text{Sediment}} * \text{EEF}_i}{K_{oc,i}} * \text{TOC} (\%) \tag{3}$$

EEF<sub>i</sub> = estrogenicity factor equivalent to E<sub>2</sub>; MEC = measured environmental concentration for each compound; TOC = total organic carbon; K<sub>oc,i</sub> = normalized partition coefficient for organic carbon; MEC<sub>i Sediment</sub> = concentration measured in the environment

### 3. Results and Discussion

#### 3.1. Chromatographic Method Optimization

The chromatogram obtained for the analysis of the compounds (Figure 2) shows the correct separation of the compounds, indicating that the method is selective. The chromatographic equipment was calibrated using seven different concentration levels (0–320 ng·mL<sup>-1</sup>). The correlation coefficient (r) obtained for all compounds ranged between 0.9851 (E2) and 0.9991 (IBU). The detection limits obtained were 13.73, 0.26, 1.02, 0.41, 0.3 and 0.12 ng·mL<sup>-1</sup> for IBU, 4NP, NPX, BPA, E2 and EE2, respectively (Table 2).

#### 3.2. Extraction Efficiency

The recovery percentages obtained through the enrichment of spring samples ranged between 70 (IBU) and 83% (4NP), respectively. In the samples of water impacted by domestic drains, the recovery percentages were between 67 (BPA) and 88% (4NP). In the case of the samples impacted by discharges from wastewater treatment plants, the extraction percentages ranged between 64 (E2) and 97%, (EE2).

On the other hand, in the validation of the sediment samples taken in the same places where the water was sampled, the obtained recovery percentages ranged between 61 (4NP) and 86% (EE2) in the spring samples. For the samples impacted by domestic drains, the recovery percentages ranged between 49 (4NP) and 112% (NPX), while in the samples impacted by effluents from wastewater treatment plants, the recovery percentage ranged between 48 (BPA) and 111% (E2) (Table 3). The precision of the method in terms of the relative standard deviation (RSD) was below 20% in all cases, which indicates that the SPE method is suitable for application to surface water and sediment samples obtained from different sources (Table 3).

The recovery percentages obtained in each of the different enriched matrices were used to correct the concentration in the real samples.

#### 3.3. Concentration Levels of Drugs and ECDs in Environmental Samples of Surface-Water Sediments

All compounds of interest were detected in the analyzed water and sediment samples. In the water samples, the concentrations ranged between <LOD to 86.40 ng·L<sup>-1</sup>, while in sediment they were between <LOD to 3.97 ng g<sup>-1</sup>. NPX (50.90 ng g<sup>-1</sup>) and alkylphenol BPA (1.04 ng g<sup>-1</sup>) were the compounds with the highest average concentration in both matrices (Table 4). In water, the accumulated average concentration of the compounds at the different sites decreased in the following order: JTPC > TMC1 > TTL > TMC2 > CPTC. In sediment, the accumulated average concentration of the compounds at the different sites decreased in the following order: JTPC > TMC1 = TTL > TMC2 > CPTC. In both matrices, the site with the highest average accumulated concentration of compounds was JTPC. This behavior is probably due to the fact that this site is mainly impacted by effluents from two industrial and domestic wastewater treatment plants. Meanwhile, in the water and sediment samples corresponding to the CPTC site, only alkylphenols (4NP, BPA) and natural hormone (E2) were detected in concentrations <LOD. At this site, drugs and the synthetic hormone EE2 were not detected, probably because this site is in a recreational park and is, to some extent, under the care of government authorities.

It is well known that sewage treatment plants do not remove 100% of compounds, such as drugs and hormones [17]. The presence of various compounds in sediments depends on different physicochemical factors [8,9], so it is difficult to determine whether the analyte-sediment interactions in the environment are kept in equilibrium or if analytes are constantly undergoing adsorption or desorption processes.

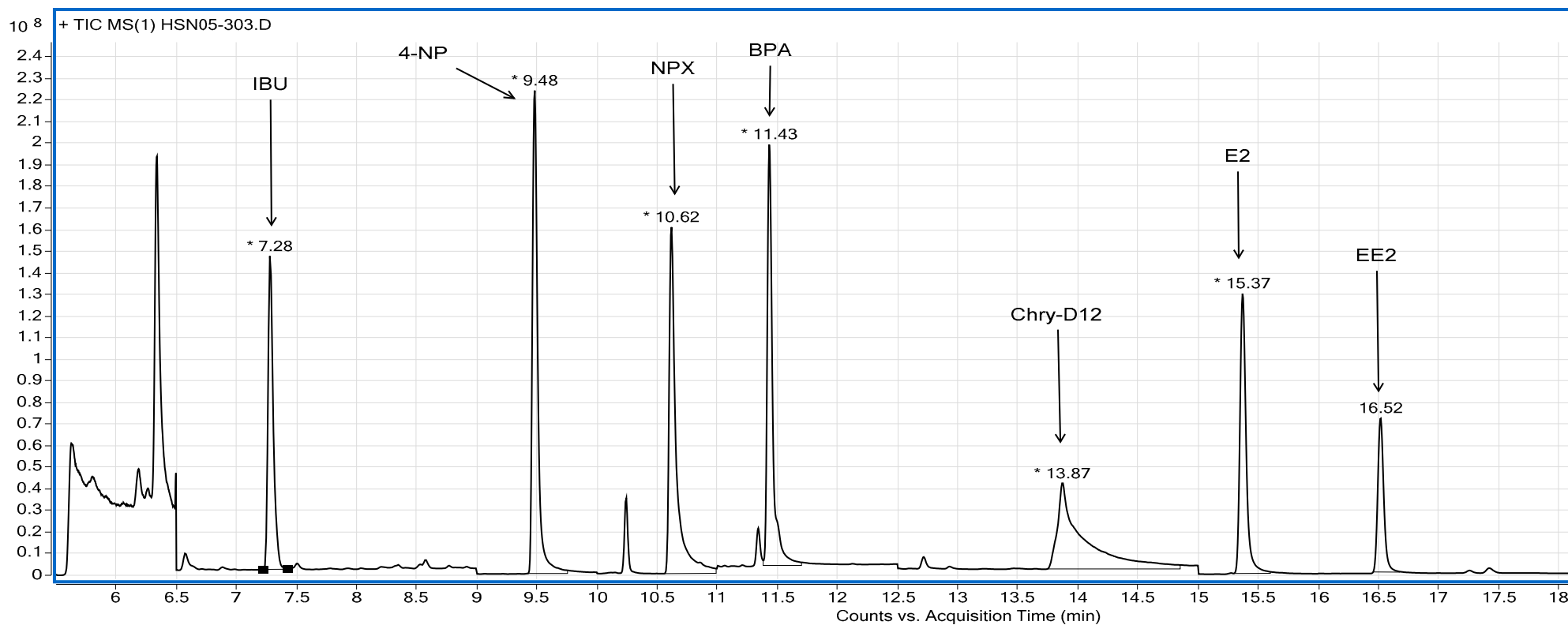


Figure 2. Chromatography that demonstrates the optimal separation of the six compounds studied by GC-MS.

**Table 2.** Summary of results obtained from the calibration for each of the compounds analyzed.

Parameter	IBU	4NP	NPX	BPA	E2	EE2
Correlation coefficient (r)	0.9991	0.9951	0.9903	0.9936	0.9851	0.9908
Regression equation	$y = 27,095X + 170,681$	$y = 68,437,723X - 54,677,536$	$y = 2,916,856 - 2,163,466$	$y = 31,517,484X - 8,421,391$	$y = 13,688,606X - 4,858,261$	$y = 7,331,801X - 1,541,923$
Linear range (ng·mL <sup>-1</sup> )	0–240	0–320	0–320	0–320	0–240	0–320
LOD (ng·mL <sup>-1</sup> )	13.73	0.26	1.02	0.41	0.03	0.12
LOQ (ng·mL <sup>-1</sup> )	45.75	0.87	3.41	1.35	0.09	0.42

LOD: limit of detection; LOQ: limit of quantification.

**Table 3.** Validation of extraction methods for water and sediment samples.

Sample Origin	Compound	Water (SPE)			Sediment (UAE–SPE)		
		Recovery ± SD (n = 2)	Repeatability (n = 2)	Reproducibility (n = 2)	Recovery ± SD (n = 2)	Repeatability (RSD)	Reproducibility (RSD)
Spring	IBU	70.35 ± 1.8	2.55	2.75	72.65 ± 1.4	1.92	7.02
	4NP	83.94 ± 0.1	0.12	1.96	61.01 ± 1.1	1.80	7.68
	NPX	79.17 ± 0.3	0.40	3.68	70.78 ± 2.5	3.53	4.63
	BPA	76.75 ± 1.4	1.82	2.30	73.86 ± 3.8	5.14	8.64
	E2	80.03 ± 1.8	2.25	2.48	68.48 ± 5.7	8.32	12.95
	EE2	75.39 ± 4.4	5.83	6.02	86.57 ± 6.3	7.27	9.63
Household drains	IBU	77.73 ± 0.1	0.13	0.70	65.23 ± 2.8	4.30	6.73
	4NP	88.18 ± 0.5	0.60	1.12	49.02 ± 2.7	5.51	7.64
	NPX	70.63 ± 0.3	0.42	0.58	112.55 ± 4.3	3.82	4.19
	BPA	67.65 ± 1.2	1.80	1.95	58.58 ± 1.7	2.90	3.09
	E2	77.70 ± 2.6	3.35	3.52	96.40 ± 6.4	6.64	12.56
	EE2	72.55 ± 2.5	3.44	3.68	83.34 ± 4.4	5.30	6.80
wastewater treatment plants	IBU	88.84 ± 0.2	0.22	1.33	51.15 ± 1.1	2.15	7.21
	4NP	68.93 ± 1.2	1.74	1.91	54.65 ± 1.8	3.30	4.61
	NPX	90.21 ± 0.7	0.80	1.38	74.86 ± 0.9	1.20	3.86
	BPA	66.25 ± 0.6	0.90	1.75	48.81 ± 8.4	17.21	17.84
	E2	64.59 ± 3.3	5.12	7.42	111.58 ± 5.7	5.11	15.12
	EE2	97.68 ± 7.9	8.10	11.13	89.34 ± 2.3	2.60	7.92

SD: standard deviation; RSD: relative standard deviation.

**Table 4.** Environmental concentrations of drugs and CDEs determined in surface water and natural sediments.

Site	Water (ng·L <sup>-1</sup> )							Average	SD
	IBU	4-NP	NPX	BPA	E2	EE2			
CPTC	ND	<LOD	ND	<LOD	<LOD	ND	0.0	0.0	
TTL	<LOD	8.45 ± 0.02	81.65 ± 0.12	19.75 ± 0.04	4.03 ± 0.04	<LOD	22.79	31.26	
TMC1	31.87 ± 0.07	9.19 ± 0.01	84.13 ± 0.11	19.81 ± 0.05	2.55 ± 0.03	<LOD	23.14	31.45	
TMC2	<LDD	<LDD	2.33 ± 0.01	10.37 ± 0.02	1.87 ± 0.01	<LDD	3.07	3.76	
JTPC	51.93 ± 0.07	11.08 ± 0.04	86.40 ± 0.03	65.21 ± 0.14	5.37 ± 0.03	16.06 ± 0.04	36.82	33.28	
Average	17.74	5.94	50.90	23.22	2.86	3.24			
SD	23.19	5.07	45.44	24.74	1.89	7.17			

Table 4. Cont.

Site	Sediment (ng g <sup>-1</sup> )						Average	SD
	IBU	4-NP	NPX	BPA	E2	EE2		
CPTC	ND	<LOD	ND	<LOD	0.01 ± 0.00	ND	0.00	0.00
TTL	0.55 ± 0	0.07 ± 0.01	0.66 ± 0.01	0.1 ± 0.03	0.85 ± 0.01	<LOD	0.37	0.36
TMC1	0.62 ± 0	0.33 ± 0.06	0.85 ± 0.01	0.26 ± 0.03	0.16 ± 0.03	<LOD	0.37	0.31
TMC2	0.37 ± 0.04	0.02 ± 0.01	0.04 ± 0	0.87 ± 0.01	0.8 ± 0.03	<LOD	0.35	0.40
JTPC	0.72 ± 0.01	0.70 ± 0.02	ND	3.97 ± 0.05	0.16 ± 0.05	0.46 ± 0.02	1.00	1.48
Average	0.45	0.22	0.31	1.04	0.40	0.09		
SD	0.28	0.30	0.41	1.67	0.40	0.21		

CPTC = Chapultepec; TTL = Tetela; TMC1 = Temixco 1; TMC2 = Temixco 2; JTPC = Jiutepec; SD = standard deviation; ND = no detected; LOD = limit of detection.

3.4. Comparisons of Concentrations of Compounds Detected with Other Studies

Table 5 shows a comparison of the concentration levels of the compounds of interest detected in water and sediments by different studies carried out in other places. The concentrations observed in the present study for IBU and NPX in water were up to two orders of magnitude below those reported by Rivera-Jaimes et al., 2018 [18] in the same basin. These differences can probably be explained by the fact that the samplings were carried out in the tributary of a wastewater treatment plant [17]. In the case of hormones E2 and EE2, Calderón-Moreno et al., 2019 [19] reported similar concentrations in the Cuautla river basin in the State of Morelos, Mexico. However, for 4NF and BPA, they found concentrations of up to one and three orders of magnitude higher than those determined in this study, which probably suggests a higher incidence of industrial discharge, mainly from the manufacture of plastics and cleaning products, respectively [20]. The results observed in the present study for IBU and NPX were similar to those reported in the Yangtze River [20], while for E2 and EE2, the concentrations were higher than those determined in the Apatlaco river basin. Meanwhile, in a study carried out in the Tagus River (Spain/Portugal), concentrations were reported to be higher than those observed in the Apatlaco river basin for IBU and NPX. Meanwhile, for E2 and EE2, similar concentrations to those determined in this study, were reported [21,22].

Table 5. Comparison of the concentration of drugs and CDEs found in the Apatlaco river basin and other places.

	Site	River	Compound					
			IBU	4NP	NPX	BPA	E2	EE2
Superficial water ng mL <sup>-1</sup>	Mexico	Apatlaco [18]	502–1106	NR	3000–4820	NR	NR	NR
		Cuautla [19]	NR	1.23–44.74	NR	15.07–97	0.07–5.77	0.14–4.8
	China	Yangtze [20]	0.4–4	1.23–104°	0.6–17	15–110	0.81–59	0.5–44
	Spain and Portugal	Tagus [21,22]	180–267	1–21	109–166	27–190	0.14–3	0.1–9
Sediment (ng g <sup>-1</sup> )	Mexico	Tula [23]	<LOD	NR	1.2–102	NR	NR	NR
	China	Three Gorges Dam [24]	NR	0.4–8	NR	0.5–41	0.08–17	0.2–37
	Italy	Different rivers [25]	NR	0.1–97	NR	0.2–23	NR	<LOD
	África	Mbokodweni [7]	0.8–3	NR	0.05–4°	NR	NR	NR

NR = not reported.

On the other hand, for sediments, the concentrations detected in the present study for IBU and NPX were lower than those reported in the Tula River, Hidalgo, Mexico [23], which suggests a greater amount of domestic discharge. Meanwhile, the concentrations observed for IBU, NPX, 4NP, E2 and EE2 in the present study are similar to those reported in sediments in the Mbokodweni River, Africa [7], in the Three Gorges Reservoir region (China) [24] and different rivers in Italy [25].



### 3.5. Log $K_d$ Distribution of Drugs and CDEs between Water and Sediment

Table 6 presents a summary of the distribution values (Log  $K_d$  partition coefficient) obtained for each of the compounds analyzed in this study, as well as comparisons with other similar studies.

**Table 6.** Distribution of the Log  $K_d$  obtained for the drugs and CDEs detected and that determined in other studies.

Parameter	BPA	E2	EE2	4NP	IBU	NPX
Log $K_d$	1.24 ± 0.59 * 2.87 [26]	1.91 ± 0.56 * 2.26 [27]	1.4 ± 0.13 * 2.45 [28]	1.35 ± 0.37 * 3.60 [29]	1.43 ± 0.26 * 1.08–1.89 [30]	1.05 ± 0.17 * 0.47 [31]

\* This study.

All the values observed for Log  $K_d$  in this work are above unity (Log  $K_d > 1$ ), which suggests that the compounds are adsorbed to a greater extent in sediments. This behavior was similar to that observed by Gong et al., 2019 [26], in different matrices from the Zhujiang and Dongjiang rivers (China) for BPA, while E2 and EE2 concentrations were similar to those reported by Gomes et al., 2011 and Murillo-Torres et al., 2012 [27,28], in treated waters from the southeast of the United Kingdom and Tula, Mexico respectively. The Log  $K_d$  value obtained for 4NP is lower than that reported by Salgueiro-González et al., 2015, in the Minho River [29]. For its part, IBU levels were similar to those reported by Agunbiade and Moodley et al. in 2016 in the Msunduzi River in southern Africa [30]. Meanwhile, NPX concentrations were lower those obtained by Mohd Amin et al., 2016 [31]. The differences observed in Log  $K_d$  values in the different studies are probably due to the differences in the physicochemical characteristics of the water and sediment samples, which affect the distribution of these compounds in both matrices.

### 3.6. Relationship between Drug and EDC Concentrations and Physicochemical Parameters

To determine the possible associations between the concentrations observed for each of the compounds and the determined physicochemical parameters, the Spearman correlation coefficient was used; the statistically significant correlations are described in Table 7.

**Table 7.** Correlations observed between the compounds detected in water and sediment with physicochemical parameters.

Parameter	Water						Sediments					
	IBU	4NP	NPX	BPA	E2	EE2	IBU	4NP	NPX	BPA	E2	EE2
Conductivity							+0.7632 *					
DO											−0.7833 *	
TOC	+0.8499 *			+0.8503 *		+0.8423 *				+0.9246		+0.8376
BOD <sub>5</sub>	+0.8369 *			+0.9272	+0.8783	+0.9027				+0.9158		+0.8990
COD	+0.8628 *			+0.9446	+0.7483 *	+0.9552		+0.8896		+0.9880		+0.9524
TSS	+0.7996 *	+0.7528 *	+0.7899 *		+0.8136 *		+0.9373					

\*  $p \geq 0.05$ ; DO: dissolved oxygen; TOC: total organic carbon; BOD: biochemical oxygen demand; COD: chemical oxygen demand; TSS: total suspended solids.

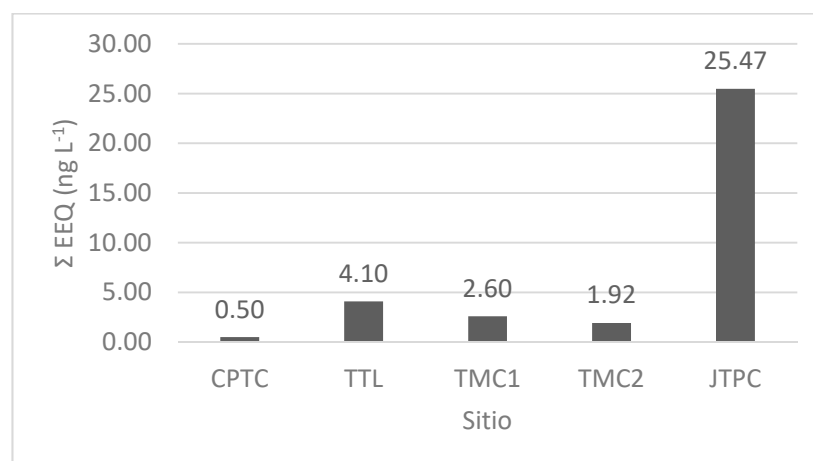
In the water samples, there were strong correlations between BOD<sub>5</sub> and COD with BPA (0.9272 and 0.9446), E2 (0.8783) and EE2 (0.9027 and 0.9552), which suggests the possible action of aquatic microorganisms in the degradation of such compounds; a similar behavior was reported by Gong et al., 2019 [26]. Likewise, significant positive correlations were observed between TOC and TS with IBU (0.8499 and 0.7996, respectively), 4NP (0.7528), NPX (0.7899), BPA (0.8503), E2 (0.8136) and EE2 (0.8423), indicating that the presence of these substances is associated with the amount of organic matter present in water and sediments.

On the other hand, in the sediment samples, at a confidence level  $p \leq 0.05$ , significant positive correlations were found between IBU and SST (0.9373), 4NF and COD (0.8896), BPA with TOC (0.9246), BOD<sub>5</sub> (0.9158) and COD (0.9880), and EE2 with TOC (0.8376),

BOD5 (0.8990) and COD (0.9524), which confirms that the content of organic matter regulates the adsorption of these compounds in aqueous media. Meanwhile, a significant negative correlation was found between EE2 and dissolved oxygen ( $-0.7833$ ), which may be indicative of increased biodegradation under aerobic conditions [26]. At this same level of probability, a significant positive correlation was found between IBU and conductivity (0.7632). According to the environment of the sampling sites, the presence of  $Al^{3+}$ ,  $Ca^{2+}$  and  $Mg^{2+}$  cations (not determined in this study) is likely, which can increase the “salting out” effect and reduce the solubility of IBU, which favors adsorption with sediments.

### 3.7. Estrogenicity and Ecological Risk

The risk of an aquatic organism being affected at the endocrine level can be determined by the risk of estrogenicity (EPA-US, 1997) [32]. The risk of estrogenicity can be studied as estrogenicity equivalent to the natural hormone Estradiol (E2) ( $EEQ = ng \cdot L^{-1}$ ). If the  $EEQ$  value  $> 1 ng \cdot L^{-1}$ , the water body presents estrogenic risk (AC01769567 1996) [33]. Meanwhile, ecological risk estimates the level of risk to which aquatic organisms are subjected due to the presence of an estrogenic compound (Figure 3).



**Figure 3.** Total EEQ value ( $\Sigma EEQ ng \cdot L^{-1}$ ) in water for each site.

The estimated average estrogenic activity obtained in water for the studied sites was  $6.92 ng \cdot L^{-1}$ . The estrogenic activity calculated for each site decreased in the following order: JTPC ( $25.47 ng \cdot L^{-1}$ )  $>$  TTL ( $4.10 ng \cdot L^{-1}$ )  $>$  TMC1 ( $2.60 ng \cdot L^{-1}$ )  $>$  TMC2 ( $1.95 ng \cdot L^{-1}$ )  $>$  CPTP ( $0.50 ng \cdot L^{-1}$ ). The total EEQ value in water for each site ( $\Sigma EEQ ng \cdot L^{-1}$ ) was calculated by taking into account 4NP, BPA, E2 and EE2. In the water samples, at four of the five analyzed sites (TTL, TMC1, TMC2 and JTPC), values of  $\Sigma EEQ > 1 ng \cdot L^{-1}$  were found. At these sites, the greatest contribution of estrogenic activity was provided by the hormones E2 and EE2, which represents a high risk of causing estrogenicity in living organisms.

Table 8 shows some studies focused on the determination of the estrogenic risk in different rivers located in different parts of the world. The estrogenic-risk range ( $EEQ ng \cdot L^{-1}$ ) determined in this work for water samples is up to an order of magnitude greater than that determined in the Cuautla river basin [19] and in the Yeongsan and Seomjin rivers, located in South Korea [34].

Meanwhile, the values observed for  $\Sigma EEQ$  in the present study were up to three orders of magnitude lower than those reported by [15,35,36] in plant-effluent wastewater treatment, which could explain these differences. On the contrary, in the Pearl River, the  $\Sigma EEQ$  calculated is up to two orders of magnitude greater than that determined for the Apatlaco River basin [37]. Meanwhile, in the Langat River, located in Malaysia,  $\Sigma EEQ$  levels were up to two orders of magnitude lower than those determined in this work [38]. The low levels of estrogenic risk are probably due to the fact that the hormones E2 and EE2 were detected in low concentrations.

On the other hand, in sediments, the range of  $\Sigma$  EEQ determined in this study was similar to the estrogenic range determined in different rivers of China [35], up to two orders of magnitude lower than the range of  $\Sigma$  EEQ determined in the Yundang Lagoon in Xiamen, China [39] and up to three orders of magnitude greater than the range of  $\Sigma$  EEQ determined in the Lhasa basin and in the Pearl River, located in China [36,37].

**Table 8.** Comparison between the estrogenicity observed in the Apatlaco River with other rivers in different parts of the world.

River/Site	Estrogenicity (EEQ ng·L <sup>-1</sup> )	Analyzed Compounds	References
<b>Agua</b>			
Apatlaco basin/Mexico	0.0–5.03	4NP, BPA, E2, EE2	This study
Cuautla basin/Mexico	0.02–6.6	4NP, BPA, E2, EE2, 4tOP	Calderón-Moreno et al., 2019 [19]
Langat/Malaysia	0.0–4.13 × 10 <sup>-2</sup>	E2, EE2, E1, E3	Praveena et al., 2016 [38]
Different rivers/China	3 × 10 <sup>-4</sup> –4.45 × 10 <sup>-3</sup>	BPA, E1, E2, EE2	Tan et al., 2018 [15]
Lhasa basin/China	5 × 10 <sup>-3</sup> –0.04	BPA, E1, E2, E3, P	Liu et al., 2020 [36]
Yeongsan and Seomjin/South Korea	3.8–5.9	4NP, BPA, OP, E2, E1, EE2	Duong et al., 2010 [34]
Different rivers/China	3.27 × 10 <sup>-3</sup> –2.24	E2, EE2, DES, BPA, 4 NP, OP	Liu et al., 2017 [35]
Pearl river/China	0.23–324	E2, DHTT, Ehrenstorfer, tamoxifen, flutamide	Zhao et al., 2011 [37]
<b>Sediments</b>			
Apatlaco basin/Mexico	0.08–28.35	4NP, BPA, E2, EE2	This study
Different rivers/China	1.87 × 10 <sup>-7</sup> –1.41	BPA, E1, E2, EE2	Tan et al., 2018 [15]
Lhasa basin/China	2–105	BPA, E1, E2, E3, P	Liu et al., 2020 [36]
Pearl river/China	0–101	E2, DHTT, Ehrenstorfer, tamoxifen, flutamide	Zhao et al., 2011 [37]
Xiamen lagoon/China	8.66–23.95	E1, E2, EE2, DES, 4NP, OP, BPA	Zhang et al., 2011 [39]

4tOP = 4 tert-octylphenol; OP = octylphenol; E1 = estrone; E3 = estriol; P = progesterone; DES = diethylstilbestrol; DHTT = dihydrotestosterone.

#### 4. Conclusions

The optimized methodology allowed for the determination of the content of different families of compounds in environmental samples with very diverse characteristics.

In both water and sediments, the accumulated average concentration of the compounds in the different sites decreases in the following order: JTTC > TMC1 > TTL > TMC2 > CPTC. This behavior is probably due to the fact that this site is mainly impacted by effluents from two industrial and domestic wastewater treatment plants.

The obtained Log K<sub>d</sub> distribution values indicate that the analyzed compounds tend to be adsorbed in sediments.

The estrogenic levels (EEQE2) determined in water and sediments and the concentrations of CDEs determined in TTL, TMC1, TCM2 and JTTC represent a potential negative risk for the health of aquatic organisms that inhabit the Apatlaco river micro-basin.

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