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Occurrence and Risk Assessment of Steroidal Hormones and Phenolic Endocrine Disrupting Compounds in Surface Water in Cuautla River, Mexico

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Abstract: In this study, two hormones 17β-estradiol (E_2) and 17α-ethynylestradiol (EE_2), and three phenolic compounds, bisphenol A (BPA), 4-*N*-nonylphenol (4-NP) and 4-tert-octylphenol (4-t-OP), were determined in surface water in the Cuautla River at the State of Morelos during the dry-season in Mexico. The endocrine disrupting compounds (EDCs) were extracted from water samples using solid-phase extraction (SPE) with end-capped C_{18}, and then the extracts were chemically derivatized to TMS (trimethylsylane)-compounds and analyzed by gas chromatography coupled to mass spectrometry (GCMS). The most abundant compound was BPA (22.46 ± 30.17 ng L^{-1}), followed by 4-t-OP (11.24 ± 11.76 ng L^{-1}), 4-NP (7.53 ± 14.88 ng L^{-1}), EE_2 (2.37 ± 4.36 ng L^{-1}) and E_2 (0.97 ± 1.82 ng L^{-1}). The residual amounts of target compounds could either reach stream surface water from direct domestic wastewater discharges, conventional wastewater treatment plant or can be a result from the use of agrochemicals in crop areas. The EDCs in Cuautla River exerted a high pressure on the aquatic ecosystem because their presences in surface water caused medium and high potential ecological risk. Besides, it was found that aquatic organisms were exposed to estrogenic activity.

Keywords: hormones; steroids; phenolic; disrupting compound; surface water; estrogenicity

1. Introduction

Nowadays, there is a growing interest in the possible health and environmental threats posed by endocrine disrupting compounds [1]. The exposure to endocrine disrupting compounds (EDCs) is particular concern, as they have the capacity to modify the hormonal activity [2] and could cause serious eco-toxicological [3] and human health affectations [4]. The EPA has defined them as an "exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and development process" [5]. Results obtained by animal models, human clinical test and epidemiological studies have shown that EDCs have a strong association with several health effects [6]. The EDCs are a broad class of molecules, including pharmaceutical, organochlorinated compounds; components of personal care products with different sources and widespread uses and applications within industrial processes, and farming and agricultural activities; and household items [7]. Accordingly, their occurrence in the environment could be a potential risk for the human health, the survival of populations, the sustainability of the ecosystems and the economy of the countries [8].

In aquatic environments, 17α-estradiol (E_2) and man-made estrogens estrone (E_1) and 17β-ethynylestradiol (EE_2) are the most potent hormones, and the major contributors of estrogenic activity [9,10]. Currently, it is known that their endocrine disrupting effects can occur even at environmental concentrations as low as 0.1 ng L^{-1} [11], and as it was shown with fish exposure at aqueous concentrations ranging from 0.1 to 10 ng L^{-1} [12–14]. While both humans and other organisms [15] naturally eliminate E_2, EE_2 is a drug widely used in hormonal treatments such as birth control [16]. The phenolic compounds bisphenol A (BPA), 4-nonylphenol (4-NP) and 4-tert-octylphenol (4-t-OP) are also EDCs widely found in surface water samples. Although their estrogenic effects are moderate, they may still cause detrimental effects on aquatic organisms and human health. This is because those phenolic compounds own more stable chemical structures and lipophilic properties [17]; thus, they can undergo persistence through different environmental compartments [18] and reach higher trophic levels [19,20], favoring their bioaccumulation into fat tissues in aquatic organisms and humans [1]. Furthermore, high production and frequent use has resulted in their sustained release and widespread distribution in aquatic environment compartments. BPA is one of the world's most high-volume produced and commercialized chemicals and is annually growing [21]; it is extensively applied in industry to produce resins and polymers, such as polycarbonate, epoxy and polyacrylate, which are widely used as raw materials in making industrial products such as food packing, medical items and electronic products [22]. In addition, there are several epidemiologic and laboratory research animal studies based on low-dose effects, which have suggested a consistent link of BPA exposure to adverse health effects, including incremented rates of breast and prostate cancers, neurobehavioral problems, obesity, reproductive abnormalities and metabolic disorders. It has also been shown to elicit slight to moderate affectations on growth and development of plants [23]. The phenolic compounds 4-NP and 4-t-OP are important intermediates in the production and degradation of alkylphenol ethoxylates, which are widely used as nonionic surfactants in industrial, agricultural and household applications [24]. Thus, phenolic compounds might become key EDCs to evaluate the impacts of anthropogenic activities on aquatic environment and human health.

The EDCs can be incorporated in surface water by direct discharge of industrial and domestic wastewaters, agricultural drains to streams and rivers, overflow after rainfall events or discharge of conventional wastewater treatment plants' (WWTPs') effluents [25]. The occurrence and fate of those five EDCs have been widely investigated [9,25–28]. However, the information of the estrogenic potency of surface water is still scarce in several parts around the world, limiting a more comprehensive understanding and an improved management of risk [29]. In Mexico, few studies, to our knowledge, have simultaneously investigated the occurrence, estrogenic activity and toxicological risk of surface water; because of the presence of those five EDCs, most of the studies have mainly focused on the determination of those compounds in wastewater, spring water [30], wetlands [31] and groundwater [32]. Recently, the occurrence of two hormones and two phenolic EDCs, and the evaluation of health risk exposure based on synthetic hormone EE_2 were reported in surface water samples collected from Apatlaco River in Morelos State, Mexico [33].

The Cuautla River is the Morelos' second largest basin; it covers a territorial extension of about 85 km, where about 304,704 inhabitants are settled on the riverbank mainly in Cuautla, Ayala and Yecapixtla urban areas (88%). That led us to suppose an anthropic impact, which can be risky to human health, and the sustainability of the ecosystems of those natural protected areas located downstream. In addition to human settlements, there are also industrial activities, growing areas and animal grazing and breeding.

Hence, this work aimed to link EDCs with routes—how they reach river water—and simultaneously assess estrogenic activity and ecological risk. This was carried out by determining environmental levels of two hormones—E_2 and EE_2—and three phenolic compounds—BPA, 4-NP and 4-t-OP—in surface water samples, obtained from nine distinct sampling points along the Cuautla River section as the river flowed through Morelos State, Mexico.

2. Materials and Methods

2.1. Chemicals and Materials

The standards and solvents used were of analytical grade or HPLC grade (purity > 98%). The 17β-estradiol (E_2); 17α-ethynylestradiol (EE_2); bisphenol A (BPA); chrysene-d_{12} (Chry-d_{12})—used as an internal standard; and 1,1-Dichloro-2,2-bis(p-chlorophenyl) ethylene (DDE)—used as a surrogate—were purchased from Sigma-Aldrich, while 4-nonylphenol (4-NP) and 4-tert-octylphenol (4-t-OP) were from Supelco. Derivatization of EDCs was performed using N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) from Supelco (Bellefonte, PA, USA). Solid phase extraction (SPE), tube packed of 0.5 g end-capped octadecyl (C_{18}), were purchased from Macherey-Nagel (Torrance, CA, USA). HPLC grade acetone, methanol and pyridine were acquired from Meyer.

2.2. Preparation of EDCs, Surrogate and Chrysene-d_{12} Stock Standards and Working Solutions

The individual standards of EDCs were prepared by accurately weighing 1.0 mg of powdered E_2, EE_2, BPA, 4-NP and 4-t-OP in a microanalytical balance, Citizen CX265, and by dissolving to those powders in 1.0 mL of acetone each up to obtain stock solutions all at concentrations of 1000 μg mL^{-1}. Similarly, the stock standard solution of DDE was prepared by diluting 1.0 mg in 1.0 mL of acetone. A standard solution of chrysene-d_{12} at concentration of 2000 μg mL^{-1} was used as a stock solution. The working solutions were prepared by suitably diluting the stock solutions in acetone afresh before use.

2.3. Sampling Sites

The surface water samples were obtained from nine distinct sites located along a section of the Cuautla River from the Barranca Grande (18°52′59.75″ N, 98°53′4.18″ W) to Curtidora site (18°47′33.01″ N, 98°58′20.34″ W), which crosses through Morelos state in México (Figure 1). Samples were collected during the cold dry-season (February), when the caudal of Cuautla River reaches the lowest levels (0.097–1.189 m^3 s^{-1}). Briefly, samples of surface water were obtained in duplicate in a glass container and were transported at 4 °C.

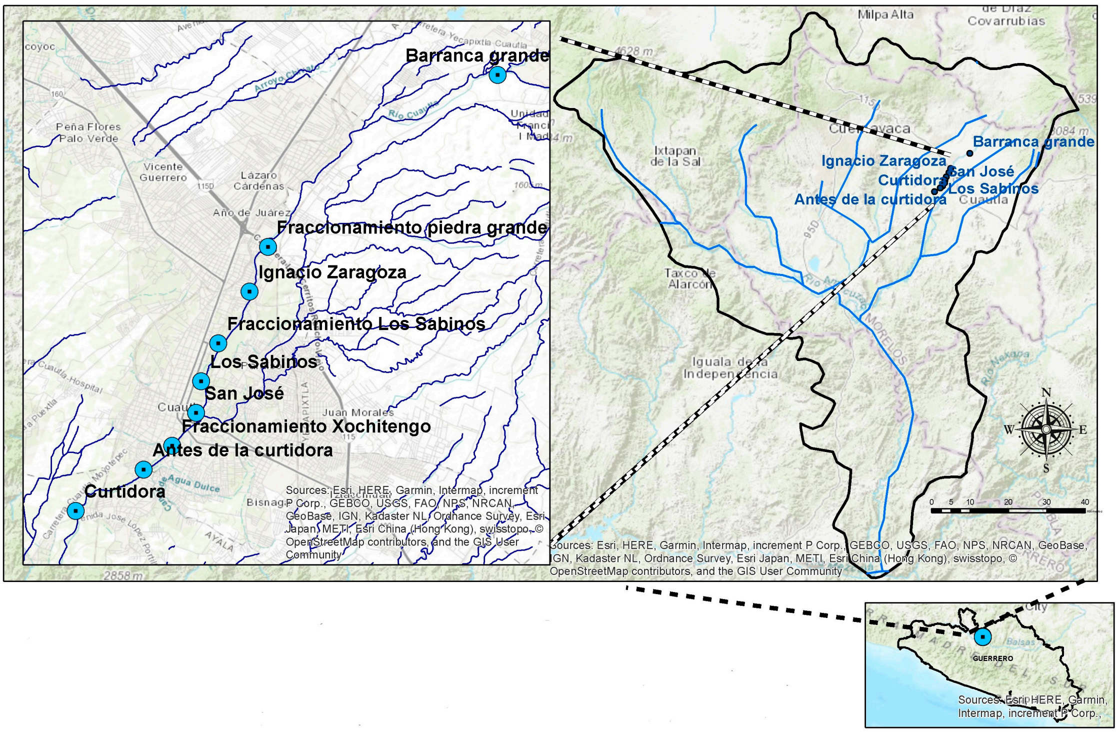

Figure 1. Location and spot sampling on Cuautla River, Morelos, México. BG: Barranca Grande (18°52′59.75″ N, 98°53′4.18″ W), PG: Fraccionamiento Piedra Grande (18°50′50.78″ N, 98°55′56.34″ W), IZ: Ignacio Zaragoza (18°50′17.40″ N, 98°56′10.01″ W), FS: Fraccionamiento Los Sabinos (18°49′38.54″ N, 98°56′33.50″ W), LS: Los Sabinos (18°49′10.26″ N, 98°56′46.29″ W), SJ: San José (18°48′46.61″ N, 98°56′50.14″ W), XT: Fraccionamiento Xochitengo (18°48′21.92″ N, 98°57′7.86″ W), T1: Antes de la curtidora (18°48′3.85″ N, 98°57′29.28″ W) and T2: Curtidora (18°47′33.01″N, 98°58′20.34″ W).

2.4. Sample Preparation

The procedure was adapted and modified from the studies carried out by Sánchez-Torres [34], Vallejo-Rodriguez [35] and Ronderos-Lara [33]. Briefly, the samples were quantitatively transferred to graduated cylinders at room temperature, the sampling volume was accurately measured, and immediately, 100 ng of DDE was added. The samples were individually filtered through a 47 mm glass fiber filter (Whatman), and then they were collected in an amber bottle. The filtered and spiked samples were passed through 0.5 g of C_{18} SPE by applying a vacuum, which was previously conditioned with 6 mL of methanol and acetone (3:2) followed by 6 mL of methanol and 6 mL of ultrapure water by gravity. The retained compounds were eluted from C_{18} by 10 mL of methanol and acetone (3:2), collected in a round-bottomed flask, and then that was concentrated with a rotatory evaporator (Buchi) and the remaining organic solvent was dried under a gentle stream of nitrogen. In order to obtain derivatized EDCs, the dry extract was added with pyridine: BSTFA-TMCS (1:1) and it was kept in a water bath at 65 °C for 60 min. Once the reaction elapsed, the vials at room temperature and derivatized EDCs were spiked with 180 ng of chrysene-d_{12} and were stored in dry conditions to chromatographic analysis.

2.5. Analysis of EDCs and Quality Control

The calibration standards and samples were both analyzed by gas chromatography (GC) and mass spectrometry (MS) 7000 D (Agilent Technologies, Santa Clara, CA, USA). An aliquot of 2 µL was automatically injected at 280 °C, and then separation was carried out on a capillary column HP5MS 30 m × 25 mm internally coated with a film thickness of 25 µm, and ultra-high purity helium was used as carrier gas at 1 mL min^{-1}. The oven program was set up to start at 40 °C; ramped at 20 °C min^{-1} to

110 °C; then up to 300 °C; and immediately to 310 °C after 10 min. The mass spectra were obtained by electron impact (70 EV) with a quadrupole mass analyzer. The SCAN mode was used to define retention times and select m/z of each compound; the most abundant molecular fragments were used to quantify and other two ones were used as qualifiers to confirm structures of derivatised EDCs. The SIM mode was used for quantification by calibration curves prepared by dilution of standards, which ranged from 10 to 200 ng mL^{-1} with correlations coefficients (r) higher than 0.99; this was based on internal standard method using chrysene-d_{12} at 1800 ng mL^{-1}. The detection limit of the method was obtained as 3 × signal/noise, which individually ranged from 0.13 ng L^{-1} (TMS-4t-OP) to 1.20 ng L^{-1} (TMS-BPA). Blanks of the instrumental method and sample preparation were monitored for signals corresponding with target compounds. Recoveries ± standard deviations of EDCs analyzed ranged from 31% ± 0.08 % for 4-NP to 90% ± 3 % for BPA based on the surrogate procedure.

2.6. Equivalent Estrogenicity

The total estrogenic activity was estimated by comparing it with the activity of the natural estrogen 17β-estradiol (E_2), and then it was expressed as equivalents to estradiol (EEQ). It should be noted that estrogenicity was only calculated in those groups of compounds for which the study by Céspedes [36] provides experimental estrogenicity values by means of the following equation.

$$EEQ = C_i E_{2equiv}, \tag{1}$$

where, C_i is the concentration in water of a given estrogenic compound and E_{2equiv} is its respective estradiol equivalent factor, 1.0, 0.17, 2.13×10^{-4}, 5.05×10^{-4} and 2.43×10^{-5} for E_2, EE_2, OP, NP and BPA, respectively.

2.7. Ecological Risk

The ecological risk associated with the target compound in Cuautla River was assessed according to risk quotient (RQ), which was obtained as the ratio of measured environmental concentration (MEC) of the target compound to the predicted no-effect concentration (PNEC) of 4NP, 4-t-OP, BPA, E_2 and EE_2, using the following equation.

$$RQ = \frac{MEC}{PNEC} \tag{2}$$

The risk level was classified as low, medium or high if the RQ value was < 0.1, 0.1 to 1.0 or ≥ 1.0, respectively. The PNEC values were obtained as HC5/AF, where HC5 is the pollutant's hazardous concentration at which 95% of the species can be protected. The values of HC5 for 4NP, 4-t-OP, BPA, E_2 and EE_2 were 1.43 μg L^{-1}, 35 μg L^{-1}, 6.82 ng L^{-1}, 4.91 μg L^{-1} and 0.1 ng L^{-1}, respectively, and the AF is the assessment value, which was set to 3 [37–40].

3. Results and Discussion

3.1. Environmental Levels of EDCs

In this study all the EDCs analyzed were detected in Cuautla River. The averaged concentrations ± standard deviations of five EDCs ranged from 0.97 ± 1.82 for E_2 to 22.46 ± 30.17 for BPA (Table 1), while the compound most often determined was 4-t-OP (100%), followed by E_2 (88%), EE_2 (66%), BPA (66%) and 4-NP (44%). In the most sites, BPA was the predominant compound, except in Xochitengo spot; there, 4-t-OP had the highest concentration (Table 1). It was found that the compounds were all in water samples of Ignacio Zaragoza. The highest concentrations of BAP, E_2 and EE_2 were also found in Ignacio Zaragoza, while 4-NP and 4-t-OP were highest in Barranca Grande. Among the five EDCs determined, 4-NP had the highest variation among sites; its variation coefficient was 197 %, and was followed by E_2 (187%), EE_2 (183%), BPA (134%) and 4-t-OP (104%). The differences of individual concentrations and strong variation between sampling sites in Cuautla River could be a consequence of several factors, such as point discharges [25]; non-point sources, such as surface runoff from agricultural activity and sewage from a scattered residential area without a treatment plant [41]; and inconsistent and deficient efficiencies of treatment in WWTPs [42].

Table 1. Individual concentrations of endocrine disrupting compounds (EDCs) in surface water along Cuautla River (ng L^{-1}).

Sampling Point	4NP	4-t-OP	BPA	E_2	EE_2
BG	44.74	27.84	<LOD	0.07	0.14
PG	<LOD	1.42	<LOD	<LOD	<LOD
IZ	1.23	2.17	97.81	5.77	4.80
FS	<LOD	0.57	21.85	0.24	0.39
LS	<LOD	0.30	15.07	0.32	0.85
SJ	<LOD	10.17	26.98	0.81	0.72
XT	<LOD	28.10	17.12	0.77	1.30
T1	1.46	8.26	23.01	0.38	<LOD
T2	16.52	22.40	<LOD	0.34	<LOD
Detection Frequency (%)	4 (44)	9 (100)	6 (66)	8 (88)	6 (66)
Mean ± SD	7.53 ± 14.88	11.24 ± 11.76	22.46 ± 30.17	0.97 ± 1.82	2.37 ± 4.36
Min-Max	1.23–44.77	0.30–28.10	17.12–97.81	0.07–5.77	0.14–4.80

LOD: limit of detection; BG: Barranca Grande; PG: Fraccionamiento Piedra Grande; IZ: Ignacio Zaragoza; FS: Fraccionamiento Los Sabinos; LS: Los Sabinos; SJ: San José; XT: Fraccionamiento Xochitengo; T1: Antes de la Curtidora; T2: Curtidora.

In addition to E_2, EE_2, BPA and 4-NP previously analyzed by Ronderos-Lara [33], in this study, we determined 4-t-OP. The individual concentrations of E_2 and EE_2 found in Cuautla River were lower than those reported in Apatlaco river basin locate also in Morelos, México, while those of BPA and 4-NP had similar orders of magnitude (Table 2). Since E_2 and EE_2 come mainly from municipal wastewater, this shows the prevalent effect of urban effluents on Apatlaco river. In comparison with surface water in different countries, the environmental levels in Cuautla River were lower than those found in more industrialized countries, such as China, but had similar values to those with comparable economic development and higher than those reported in countries with tighter control on EDCs in effluents (Table 2).

Table 2. Environmental levels of EDCs in Cuautla River 2016 and in other countries (ng L^{-1}).

River (Country)	E_2	EE_2	BPA	4-NP	4-t-OP	Ref.
México (Cuaultla River)	0.07–5.77	0.14–4.80	15.07–97.81	1.23–44.74	0.30–28.08	This study
México (Apatlaco basin)	37.3–103.6	31.2–624.3	39.1–174.6	<26.6–85.5	-	[33]
Southern Korea (Yeongsan and Seomjin rivers)	3.5–4.3	<LOD	2.5–4.3	95.7–163.5	0.1–0.2	[9]
UK (River Ray)	<1.2–7.6	<0.4–1.9	-	-	-	[27]
Vietnam (Sai Gon and Dong Nai river)	6.4–54	-	4.5–30.5	14.8–17.6	3.6–17.5	[41]
China (Luoma Lake)	2.52–21.82	2.85–4.25	N.D–49.38	N.D–769.96	ND–4.67	[25]
China (rivers of southern Jiangsu)	ND–52.71	-	48.24–725–94	-	-	[39]
China (Yangtze River-Nanjing section)	-	-	ND–563(119)	1.4–8.58(199)	ND–100(21.2)	[25]
China (Pearl River Estuary)	-	-	12.41–62.78	233.04–3352.86	1.20–3.99	[43]
Spain Manzanares River	N.D	N.D	12–71	185–1483	N.D	[26]
	N.D	N.D	6–126	96–869	50–474	[26]

N.D: not detected, LOD: limit of detection.

3.2. Surface Water Pollution Sources

It must be considered that the human settlements located on Cuautla basin have connections to sewer networks at a rate lower that 55%.The WWTPs work below of their original capacity, and they are mainly based on conventional treatment processes, such as active sludge, wetland, reactor anaerobes of flow hybrids and sprayed filters removing mainly colloidal and dissolved organic materials.

The higher levels of BPA concentration, with individual values from 15.07 ng L^{-1} to 97.81 ng L^{-1} (Table 1), could be a result its solubility value, which is 120 mg L^{-1}, while 4-NP, 4-OP, E_2 and EE_2 have lower values 1.57, 12.6, 13 and 4.8 mg L^{-1}, respectively [44–47]. However, strong correlations of BPA were found with E_2 (r = 0.83, $p < 0.05$) and EE_2 (r = 0.78, $p < 0.05$), indicating that those three compounds may have a similar route to reaching the stream surface water of Cuautla river. The correlation between BPA and steroids mainly suggests a possible contribution by spilling of effluents simultaneously containing those three compounds. It must also be considered that different types of effluents are often mixed into the domestic sewerage/wastewater network in Mexican urban areas. It was consistent with measurements obtained in Ignacio Zaragoza, where BPA, EE_2 and E_2 peaked with the highest concentrations of whole study (Table 1). The river could be receiving wastewater from commercial activity in Ignacio Zaragoza as a consequence of both the supermarket and cinema mall settled there; they could have contributed to predominant residual amount of BPA and other substances migrating toward commercial wastewater effluents coming likely from products made of polymeric materials containing BPA as food packaging and items households. In addition, the treatments applied to effluents from those facilities are unknown. Ignacio Zaragoza is also a residential area (Figure 2C); therefore, the correlation with steroids E_2 and EE_2 indicates domestic discharges as they both are released to the environment through human excretion of urine and feces [48]. Thus, their determination in surface water can be attributed to municipal effluents being spilt either from direct domestic wastewater discharges or due to wastewater treatment plants (WWTPs).

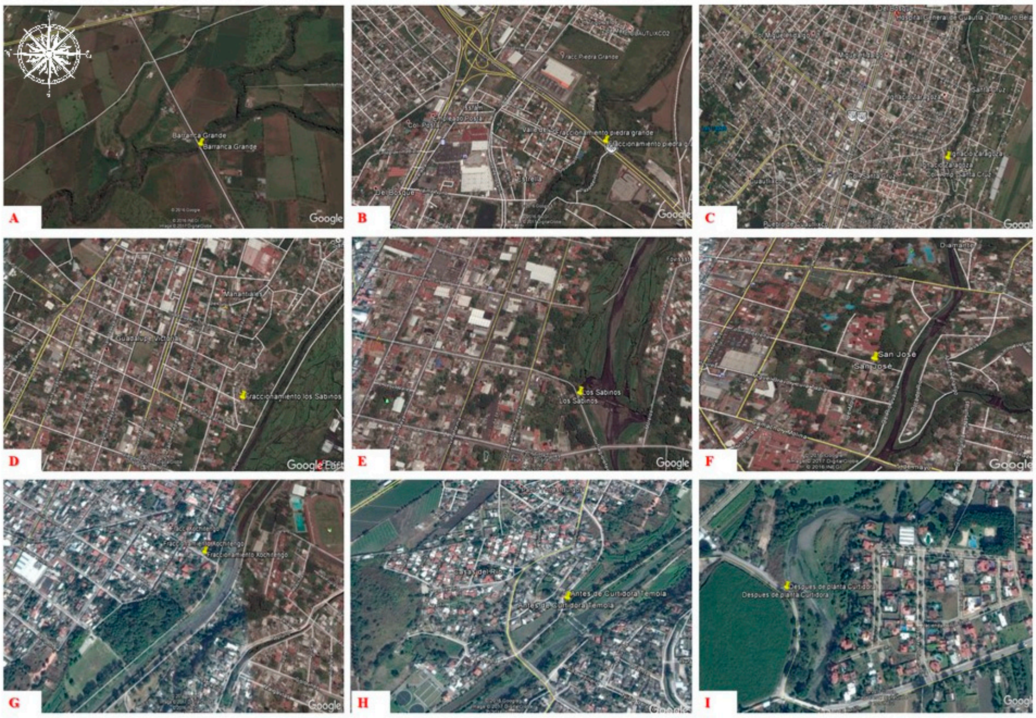

Figure 2. Satellite view of sampling locations along Cuautla River: (**A**) BG: Barranca Grande, (**B**) PG: Fraccionamiento Piedra Grande, (**C**) IZ: Ignacio Zaragoza, (**D**) FS: Fraccionamiento Los Sabinos, (**E**) LS: Los Sabinos, (**F**) SJ: San José, (**G**) XT: Fraccionamiento Xochitengo, (**H**) T1: Antes de la Curtidora and (**I**) T2: Curtidora.

The highest concentrations of 4-NP and 4-t-OP in Barranca Grande suggest direct discharge, likely coming from domestic wastewater as they are used in the production of surfactants for household goods [49] In addition, abundant foam was observed when a sample was obtained, resulting from its surfactant effect. In most sites, the concentration of 4-t-OP was higher that 4-NP, except in the Barranca Grande (Table 1). Therefore, the activities in those crop areas near Barranca Grande (Figure 2A) could also be contributing to 4-NP resulting from its use as constituent of formulations of pesticide products improving the spraying of vegetation [44]. It has been found that the nonylphenol polyethoxylate precursor enters the environment as an inert ingredient in pesticide sprays, potentially traveling great distances from its application site; then, it biodegrades into nonyphenols in the environment [50,51].

3.3. Estrogenic Activity

The estimation of the total estrogenic activity showed an average value of 1.2 ± 2.1 ng L^{-1} (Table 3). The highest estrogenicity was found in Ignacio Zaragoza (6.6 ng L^{-1}), which was followed by Xochitengo (1.0 ng L^{-1}) and San José (0.9 ng L^{-1}). In these sites, the estrogenicity values found were close to toxic effect level of 1.0 ng L^{-1}, which is a recommended limit value for aquatic ecosystems [52].

Table 3. Estrogenic activity along the Cuautla River basin.

Spot	Estrogenic Activity (ngL^{-1})
BG	0.4
PG	0.02
IZ	6.6
FS	0.3
LS	0.5
SJ	0.9
XT	1.0
T1	0.6
T2	0.1

BG: Barranca Grande, PG: Fraccionamiento piedra grande, IZ: Ignacio Zaragoza, FS: Fraccionamiento Los Sabinos, LS: Los Sabinos, SJ: San José, XT: Fraccionamiento Xochitengo, T1: Antes de la Curtidora and T2: Curtidora.

The greatest contribution to estrogenicity was mainly due to the occurrence of E_2, followed by EE_2, Ignacio Zaragoza being the place with the most (Figure 3). In spite of the fact that natural and synthetic steroids were the EDCs least abundant, their high estrogenic potency is responsible for most of the estrogenic effects to organisms in aquatic ecosystems [53]. It was found that both compounds were at concentrations ranging from 0.14 to 5.77 ng L^{-1}, and it has been shown with fish that endocrine effects can occur within 0.1–10 ng L^{-1} [11,12,14]. In addition to commercial activities in Ignacio Zaragoza, effluents from the near hospital facilities should be considered likely contributors.

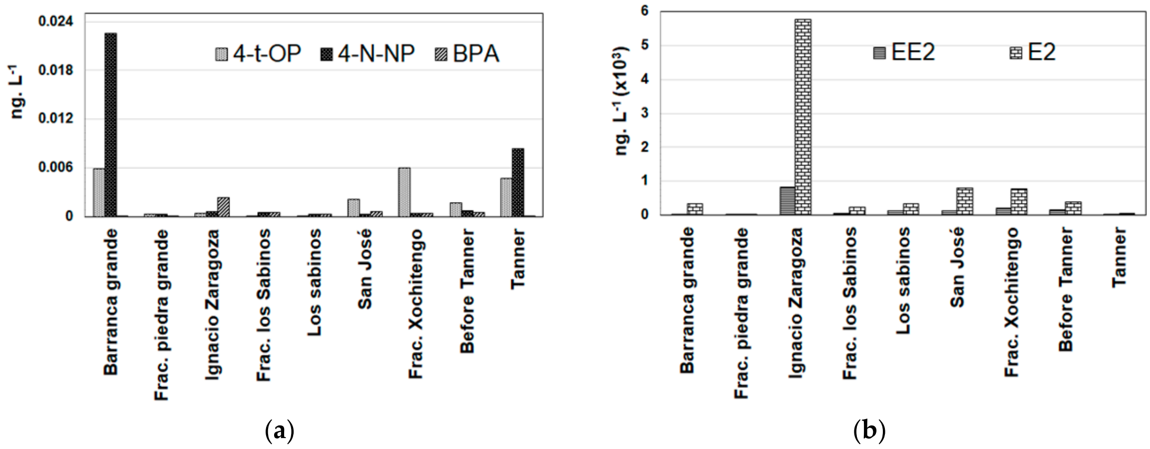

Figure 3. Estrogenicity measured in samples from the Cuautla River. Phenolic compounds (**a**) and Hormones (**b**).

The averaged estrogenicity estimated in the Cuautla River (1.2 ng L^{-1}) was similar to that found in the Liaohe River in China [54], but lower than the levels found in surface water bodies such as Paraiba do Soul in Brazil, the YundangLagoon in China and the Yeongsan River in Korea (Table 4). Since Cuautla basin within Morelos hosts 80 birds, 16 mammals and amphibians, 13 reptiles and five fish species, these pollutants represent a risk for the existing exposed biota in the basin [55].

Table 4. Averaged estrogenicity in water samples from Cuautla River and other locations.

System	Country	EEQ E_2 (ngL^{-1})	Reference
Cuautla River	México	1.2 (0.1–6.6)	This study
Río Liaohe	China	1.06	[53]
Laguna Yundang	China	14	[56]
Río Paraiba do Sul	Brazil	17	[49]
Río Yeongsan	Korea	5.9	[9]

3.4. Ecological Risk

In the surface water of Cuautla River, the individually calculated RQs of EDCs had values within 0.1–1.0 and the total exceeded 1.0 at most of the sampling sites, indicating aquatic organisms were exposed to medium and high ecological risks associated with EDCs (Figure 4).

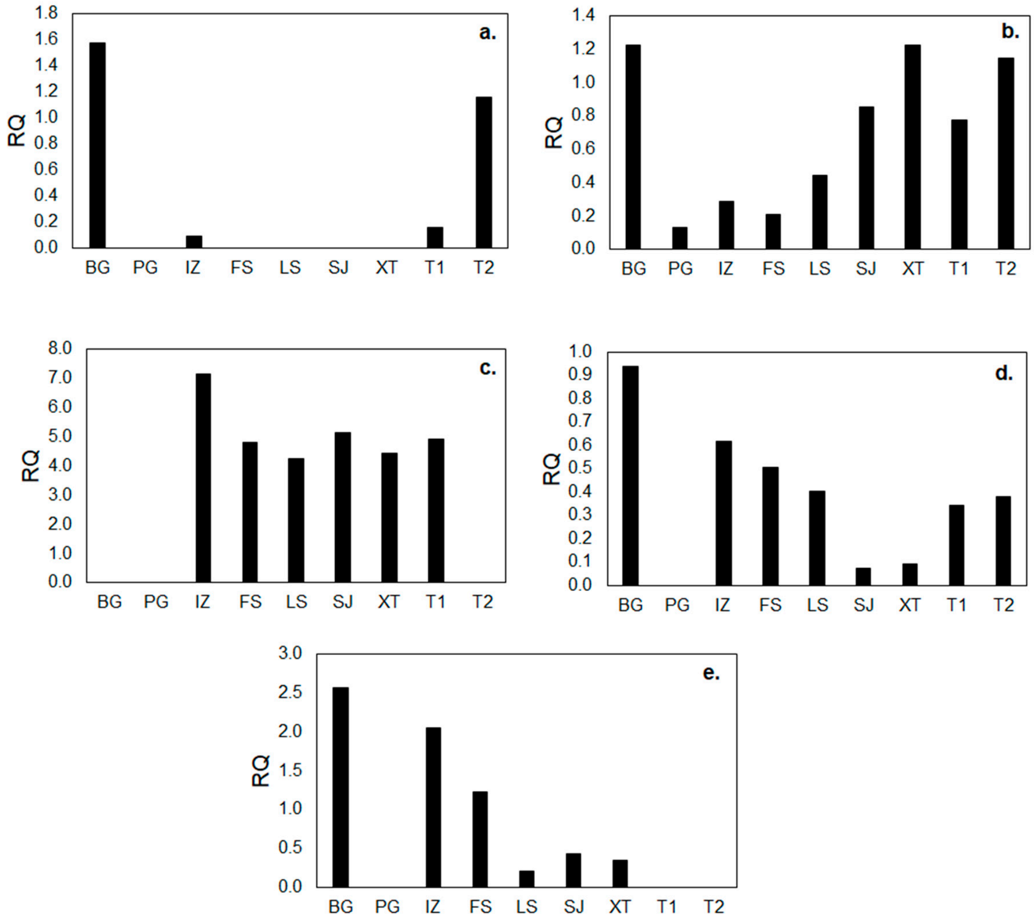

Figure 4. Risk quotient values of the target EDCs in surface water for 4NP (**a**), 4-t-OP (**b**), BPA (**c**), E_2 (**d**), EE_2 (**e**).

The RQs of alkilphenolic compounds ranged from 0.09 to 1.57, 0.13 to 1.23 and 4.24 to 7.16 for 4NP, 4-t-OP and BPA, respectively (Figure 4). The risk probabilities of 4NP, 4-t-OP and BPA were 50%, 33% and 100%, respectively, with RQs exceeding 1.0 (Figure 5). The RQs of steroids ranged from 0.07 to 0.94 and 0.21 to 2.56 for E_2 and EE_2, respectively (Figure 5). The sampling sites for the RQs greater than 1 accounted for EE_2 (50%). Therefore, they could be causing ecological damage, especially considering the combined interactions and exposed biota of the Cuautla River [55].

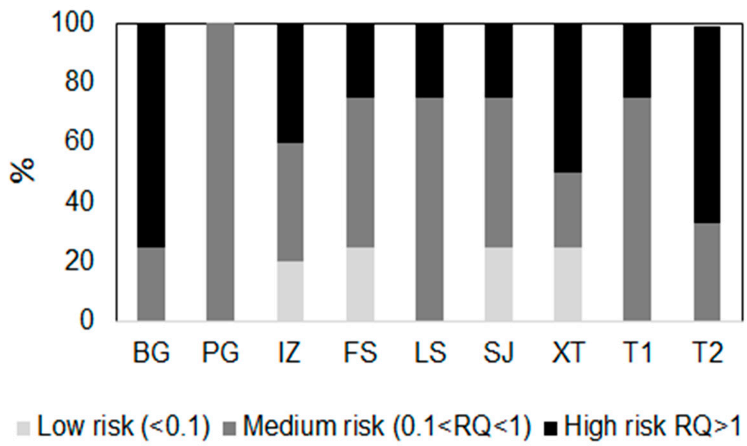

Figure 5. Proportions of different risk levels for distinct spots in Cuautla River.

4. Conclusions

The study showed that occurrence of EE_2, E_2, 4-t-OP, 4NP and BPA in Cuautla River is mainly a result of pressures exerted by distinct activities performed along the Cuautla River. High variability of EDCs concentrations was also found, which suggests that there are several routes through which those compounds could enter the surface water of Cuautla River. The values of estrogenic activity and environmental risk were high in spite of the fact that Cuautla River had only trace concentrations of EDCs, which could have serious effects on sustainability and the diversity of organisms, particularly fish species. Therefore, actions must be implemented which are focused on reducing the presence of EDCs in aquatic systems. In addition, the sampling and analysis other possible sources should be considered in further work.

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