

Ecological Risk Assessment of Five Endocrine-Disrupting Compounds in Wastewater Treatment Plants from Monterrey, Mexico

Khirbet López-Velázquez

Universidad Autonoma de Nuevo Leon

Jorge L. Guzmán-Mar

Universidad Autonoma de Nuevo Leon

Hugo A. Saldarriaga-Noreña

Universidad Autonoma del Estado de Morelos

Mario A. Murillo-Tovar

Universidad Autonoma del Estado de Morelos

Minerva Villanueva-Rodríguez (✉ minerva.villanuevardr@uanl.edu.mx)

Universidad Autonoma de Nuevo Leon <https://orcid.org/0000-0001-7481-2499>

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Abstract

The potential ecological risk of five residual endocrine-disrupting compounds (EDCs) in four wastewater treatment plants (WWTPs) was studied. The wastewater samples were collected in WWTPs of the Metropolitan Area of Monterrey, Mexico (designed as Monterrey City hereinafter) and 17 β -estradiol (E2), 17 α -ethinylestradiol (EE2), bisphenol A (BPA), 4-nonylphenol (4NP), and 4-tert-octylphenol (4TOP) were studied by SPE/GC-MS method. Results showed that all EDCs are widely distributed in WWTPs, finding high concentrations of BPA (450 ng/L) and EE2 (407.5 ng/L) in influents, while EE2 and 4TOP were the most abundant in effluents at levels from 1.6–26.8 ng/L (EE2) and < LOQ – 5.0 ng/L (4TOP), which corroborate that the wastewater discharges represent critical sources of EDCs to the aquatic environments. The potential ecological risk of residual EDCs was evaluated through risk quotients (RQs), and results indicated that the effluents of the WWTPs represent a high risk to exposed aquatic species, mainly due to the effect of residual estrogens E2 and EE2 which were considered as the most hazardous compounds among the studied EDCs, with RQ values up to 49.1 and 1165.2, respectively.

Introduction

Endocrine-disrupting compounds (EDCs) belong to a subclass of so-called emerging organic pollutants and their presence in the environment represents a potential risk to public health (Sauvé and Desrosiers 2014). According to the United States Environmental Protection Agency (USEPA) and the European Chemicals Agency (ECHA), EDCs are exogenous substances or a mixture of substances that alter the structure or function(s) of the endocrine system and cause adverse effects at the level of the organism, its progeny, populations or subpopulations of organisms (EPA 1998; ECHA and EFSA 2018). These compounds are widely distributed in the environment, mainly in aquatic ecosystems, and have attracted special attention due to their potential risk to the health of aquatic organisms and humans, even at very low concentrations such as ng/L (Adeel et al. 2017). Several studies have reported that EDCs may generate different adverse effects on exposed organisms, including feminization and masculinization, deficiencies in the sexual, prostate, brain, and immune development, gonadal atrophy, infertility, precocious puberty, and different types of cancer (prostate, testicles, breast, ovaries, among others) (Roby 2013; Kabir et al. 2015; Wang et al. 2019; Jackson and Klerks 2020). Moreover, it has been reported that EDCs may be associated with a high incidence of obesity and the development of diseases such as diabetes in humans (Hatch et al. 2010).

Among the different EDCs, the estrogens E2 and EE2, the alkylphenols 4NP and 4TOP, as well as the plasticizer BPA, are the most frequently EDCs detected in various aquatic environments such as rivers, lakes, and coastal areas (Ronderos-Lara et al. 2018; Calderón-Moreno et al. 2019; Inam et al. 2019; Salueiro-González et al. 2019; Jiang et al. 2020; Čelić et al. 2020); due to their high estrogenic potential, these substances are being regulated by the European legislation through the European Water Framework Directive (2000/60/EC). In this sense, 2.0 μ g/L has been defined as the maximum admissible concentration for 4NP and 4TOP in surface water (European Parliament 2008). In addition, BPA and E2 were recently proposed for inclusion as priority hazardous substances, recommending 0.01 μ g/L and 0.001 μ g/L as the maximum allowable concentration in surface water, respectively (European Parliament 2018). Regarding EE2, has been included in the watch list to monitor it and evaluate its possible incorporation as a priority hazardous substance for human health (European Parliament 2013).

In another way, it is known that effluents from WWTPs are one of the main sources of EDCs emissions to aquatic environments, and several studies have reported the detection of EDCs in WWTP effluents around the world, revealing that these substances are partially removed during wastewater treatment processes (Manickum and John 2014; Wee et al. 2019; Jiang et al. 2020; Čelić et al. 2020). For example, Jiang et al. (2020) detected E2 (< LOQ –

50.03 ng/L) and EE2 (4.72–35.03 ng/L) in effluents from 38 WWTPs in China. Similarly, Manickum and John (2014) reported the detection of E2 (4.0–107.0 ng/L) and EE2 (1.0–8.0 ng/L) in the effluent of a WWTP in South Africa. On the other hand, BPA (3.31 ng/L), 4NP (4.62 ng/L), and 4TOP (4.44 ng/L) were detected in a WWTP effluent in Portugal (Carvalho et al. 2016). Furthermore, 4NP (420–2120 ng/L) and 4TOP (5.3–54.8 ng/L) were detected in effluents of nine WWTPs in Iran (Bina et al. 2018). Therefore, the incomplete removal of EDCs in WWTPs contributes to their frequent detection in surface water, which may subsequently impact on the essential activities such as agriculture, livestock, fisheries, drinking water, and domestic and recreational activities (Čelić et al. 2020).

In Mexico, some studies have reported the detection of E2, EE2, and BPA in several WWTPs in a wide concentration range from < LOD – 16,600 ng/L (Díaz-Torres et al. 2013; Estrada-Arriaga et al. 2016; López-Velázquez et al. 2020; Villarreal-Morales et al. 2020). Likewise, other authors such as Calderón-Moreno et al. (2019), and Ronderos-Lara et al. (2018) detected E2, EE2, BPA, 4NP and 4TOP in river water (Morelos, Mexico) at levels from < LOD – 624.3 ng/L, emphasizing EE2 and BPA as the most abundant EDCs.

In particular, the study of EDCs in Monterrey city is of great interest due to it is the second most populated region in Mexico (5,341,171 inhabitants; INEGI 2021), and stands out as one of the most industrialized zone in the country (SEDATU 2015) contributing with the emission of a large number of pollutants into the environment, including some EDCs as was informed by Cruz-López et al. (2020), and Villarreal-Morales et al. (2020). Moreover, is of great interest to evaluate the ecological risk on the aquatic systems due to their high estrogenic potential and the continuous emission of EDCs towards the environment, which may impact directly on the ecosystems of the receiving water bodies such as the urban rivers Pesqueria, Santa Catarina, La Silla, Sabinal, and Topo Chico (Fig. 1), which are tributaries to the Rio San Juan that flows into the Rio Bravo (natural border between Mexico and United States) and the Gulf of Mexico.

Recently, some studies have been focused on evaluating the potential environmental risk through hazard index associated with E2, EE2, BPA, 4NP, and 4TOP in the dissolved phase of surface water (Stasinakis et al. 2012; Peng et al. 2017; Calderón-Moreno et al. 2019) as well as influents and effluents of WWTPs (Yu et al. 2013; Manickum and John 2014; Patrolecco et al. 2015; Ben et al. 2018; Jiang et al. 2020). In these studies, the risk quotients (RQ) have been calculated for several aquatic organisms (i.e. algae, crustaceans, and fish), and it is considered a useful strategy for the evaluation of water resources, which could contribute to improve the wastewater treatment processes, implementing effective strategies of management and control of wastewater discharges, surface, and drinking water, for the health protection of human and aquatic organisms (Xu et al. 2016). Therefore, this study aims to determine the potential environmental risks associated with five dissolved EDCs in four WWTPs from Monterrey City, which to our knowledge, constitutes the first report of this type in WWTPs from Mexico.

Materials And Methods

Chemical and reagents

17 β -estradiol ($\geq 98.0\%$), 17 α -ethinylestradiol ($\geq 98.0\%$), bisphenol A ($\geq 99.0\%$), 4-nonylphenol ($\geq 99.0\%$), 4-tert-octylphenol ($\geq 99.0\%$), chrysene-d12 ($\geq 99.0\%$), N,O-bis(trimethylsilyl)trifluoroacetamide + trimethylchlorosilane (BSTFA + TMCS, 99:1), pyridine ($\geq 99.0\%$), hexane, methanol and dichloromethane HPLC grade were purchased from Sigma-Aldrich (St. Louis, MO, USA). Water HPLC grade was acquired from Tedia Company, and acetone HPLC grade was acquired from Meyer. The Oasis HLB solid-phase extraction cartridges (500 mg, 6 mL) were purchased from Waters (Milford, USA).

Standard stock solutions (1000 µg/mL) of individual EDCs and Chrysene-d12 used as internal standard (1500 µg/mL) were prepared in acetone. These solutions were stored in amber vials at 4°C until use.

Sample collection and instrumental analysis

In this work, four WWTPs that receive urban and industrial wastewater were studied, the geographical location of WWTPs is shown in Fig. 1, and detailed information of each WWTP and the main quality parameters of the wastewater samples were included in Table 1.

Table 1
Main characteristics of the studied WWTPs and wastewater samples.

WWTP	Capacity (m ³ /day)	Population served	Average discharge (m ³ /day)	Physicochemical characteristics of the wastewater samples						
				Stage	T (°C)	pH	DO (mg/L)	TSS (mg/L)	COD (mg/L)	BOD ₅ (mg/L)
A	17280	377480	15322.5	I	24.3	7.4	1.9	267.7	593.7	218.9
				E	24.1	7.1	7.2	17.3	57.5	13.9
B	345600	1931229	232465.6	I	22.9	7.2	3.0	385.3	660.7	255.6
				E	25.9	7.2	6.0	21.3	54.0	14.3
C	162000	444006	97436.5	I	23.6	7.1	1.7	257.7	607.2	209.9
				E	24.4	7.3	6.0	17.0	62.3	8.2
D	648000	796337	589671.4	I	22.8	7.4	1.9	286.4	726.2	230.2
				E	24.2	7.2	6.6	23.2	57.1	12.4

I, influent; E, effluent; DO, dissolved oxygen; TSS, total suspended solids; COD, chemical oxygen demand; BOD₅, biological oxygen demand at the five days.

Two sampling campaigns were carried out in January and August 2019, and composite wastewater samples were collected in influents and disinfected effluents of each WWTP (2 L, made up of three simple samples). The samples were stored in amber glass bottles, transported at 4°C, and preserved at -20°C until their analysis, according to Method 1698 from the USEPA (2007). Subsequently, the solid-phase extraction and gas chromatography coupled to the mass spectrometer (SPE/GC-MS) were employed for the EDCs analysis as was described in previous work (López-Velázquez et al. 2021) and strict quality control procedures were adopted. Briefly, based on the internal standard method, good correlation coefficients ($R^2 > 0.982$) were obtained for all EDCs; limits of detection (LOD) ranged from 0.21 ng/L (E2) to 1.1 ng/L (EE2) and limits of quantification (LOQ) ranged from 0.71 ng/L (E2) to 3.01 ng/L (EE2). The precision of the method (expressed as relative standard deviation) was < 4.9% at 25 ng/L, and < 6.4% at 230 ng/L for each EDC in both concentration levels. Moreover, recoveries of EDCs ranged from 74.6% (EE2) to 103.4% (BPA), and all instrumental and procedural blanks were below LOD.

Data analysis

Daily mass

Using data of EDCs levels detected in the WWTPs, the daily mass of each EDC in the influents and effluents ($Mass_{inf}$ or eff) was calculated by Eq. 1.

$$Mass_{inf\ or\ eff} = (C_{inf\ or\ C_{eff}}) * Q_{water} \quad (Eq. 1)$$

Where C_{inf} and C_{eff} are the concentrations of the selected EDCs in the influents and effluents of the WWTPs (mg/m^3). Q_{water} is the daily wastewater flow in each WWTP (m^3/day) which were considered equals in influents and effluents since the loss of wastewater during the treatments and the effect of the dilution by rainfall was considered small and negligible in the sampling dates (20–30 mm/month; CONAGUA 2019). Moreover, the approximate remotion (AR) for each EDC were estimated following Eq. 2:

$$AR(\%) = \left(1 - \frac{Mass_{eff}}{Mass_{inf}}\right) * 100 \quad (Eq. 2)$$

Ecological risk assessment

In this study, the potential environmental risk of the EDCs detected in the WWTPs was assessed employing the risk quotients (RQ), which is an internationally accepted parameter for environmental risk assessment (European Commission 2003; EMEA 2006). RQ values were calculated as follows (Eq. 3):

$$RQ = \frac{MEC}{PNEC} \quad (Eq. 3)$$

Where MEC is the measured concentration in influents and effluents of WWTPs (ng/L), and PNEC is the predicted no-effect concentration. PNEC values for each EDCs in wastewater were calculated by Eq. 4:

$$PNEC = \frac{HC5}{AF} \quad (Eq. 4)$$

Where HC5 is the hazardous concentration for 5% of the exposed species, and AF is the assessment factor which was set to 3 in order to protect other more sensitive organisms no considered in the toxicological studies (Tamis and Jongbloed 2019). HC5 values for E2, EE2, BPA, 4NP, and 4TOP were taken from scientific literature and are shown in Table 5. Further, the criteria for establishing the potential ecological risk were the following: $RQ \geq 1$, high risk; $0.1 \leq RQ < 1$, medium risk, and $RQ < 0.1$, low risk (Huang et al. 2018). In addition, regarding RQs for surface water, a dilution factor of 10 was used for MEC values (Jiang et al. 2020), assuming that effluents of WWTP would be diluted after being discharged.

Results And Discussion

Concentrations of EDCs in influents

The frequency of detection of the five EDCs in the influents was variable, ranging from 62.5% (4NP) to 100% (EE2) as shown in Table 2. Notably, EE2 was detected in all samples collected during the two sampling campaigns reaching high levels up to 407.5 ng/L (Fig. 2). These EE2 levels were higher than those detected in 38 WWTPs in China, ranging from 4.96 to 51.06 ng/L (Jiang et al. 2020), and those detected in South Africa (one WWTP) ranging from 10 to 95 ng/L (Manickum and John 2014). However, EE2 concentrations are comparable to that reported by Belhaj et al. (2016), who detected concentrations between 199 and 600 ng/L in the influent of one WWTP (Tunisian). Furthermore, E2 levels in influents ranged from $< LOD - 15.1\ ng/L$ (frequency of 75%) and were lower than those

detected in 38 WWTPs from China (< LOQ – 62.92 ng/L) (Jiang et al. 2020), and those detected in one urban WWTP from Tunisian, between 45.0–75.0 ng/L (Belhaj et al. 2016). According to previous studies, it is known that the occurrence of estrogens such as E2 and EE2 is related to the extensive use of these substances as therapeutic or contraceptive agents, and their subsequent excretion mainly in urine, which is considered one of the main sources of estrogens to the environment (Manickum and John 2014).

Table 2
Concentrations (ng/L) of selected EDCs in four WWTPs.

	Influents				Effluents			
	Range	mean	median	DF ^a (%)	Range	mean	median	DF (%)
E2	<LOQ ^b – 15.1	4.3	2.9	75.0	<LOQ – 9.5	1.5	<LOQ	37.5
EE2	<LOQ ^c – 407.5	73.2	14.2	100	1.6–26.8	9.7	7.4	100
BPA	<LOQ ^d – 450	72.1	7.1	87.5	<LOQ – 3.0	0.43	<LOQ	25.0
4NP	<LOQ ^e – 18.9	3.3	0.5	62.5	<LOQ – 2.3	0.35	<LOQ	37.5
4TOP	<LOQ ^f – 58.8	8.8	1.2	87.5	<LOQ – 5.0	1.2	0.5	62.5
^a DF = detection frequency; LOQ = ^b 0.71 ng/L, ^c 3.0 ng/L, ^d 1.7 ng/L, ^e 0.86 ng/L, ^f 0.91 ng/L.								

The frequency of detection of BPA and 4TOP in the influents was also high (87.5% for each one) and the range of concentration was < LOQ – 450 ng/L for BPA and < LOQ – 58.8 ng/L for 4TOP in the four WWTPs. These concentrations of BPA and 4TOP are lower than those detected in five WWTPs from the USA, between 60–600 ng/L for BPA, and 80–3900 ng/L for 4TOP (Yu et al. 2013), and to those reported in six WWTPs from Mexico-USA, ranging between 175.5–1476.8 ng/L for BPA, and 259.3–3693.7 ng/L for 4TOP (De la Torre 2011). Similarly, 4NP was detected in the influents of the four WWTPs but with lower frequency (62.5%) at levels from < LOQ – 18.9 ng/L, much lower than reported by Bina et al. (2018) in nine WWTPs of Iran (ranging from 1250 to 17020 ng/L) and Jiang et al. (2020) in 38 WWTPs from China (ranging between 1519.5 and 27736.7 ng/L).

As shown in Fig. 2, EE2 as well as BPA, were the most abundant EDCs in the influents of WWTPs, and its wide concentration range reflects the high consumption rates and its constant emission in urban and industrial wastewater discharges from Monterrey City. Furthermore, in this study, low levels of 4NP and 4TOP compared to those detected levels in other research suggests a moderate use of these alkylphenols contained in surfactants and detergents, mainly used in domestic and commercial activities (Bina et al. 2018). In addition, Table 3 shows a summarized comparison between EDC levels detected in this research and those reported in other regions, where the EDCs amounts are often widely detected of the order of ng/L.

Table 3
Comparisons of EDCs levels detected in WWTPs from other countries.

EDC	Country	N° of studied WWTP	Influents (ng/L)	Effluents (ng/L)	References
E2	Mexico	4	<LOQ – 15.1	<LOQ – 9.5	This study
	China	38	<LOQ – 62.92	<LOQ – 50.03	(Jiang et al. 2020)
	South Africa	1	20–199	4–107	(Manickum and John 2014)
	USA	6	<LOD – 106.5	<LOD – 42.2	(De La Torre 2011)
	Mexico	2	ND	<LOD – 0.7	(Díaz-Torres et al. 2013)
	Mexico	2	20–31.5	ND	(Estrada-Arriaga et al. 2016)
	Tunisian	1	45–78	10.0–46.0	(Belhaj et al. 2016)
EE2	Mexico	4	<LOQ – 407.5	1.6–26.8	This study
	China	38	4.96–51.06	4.72–35.03	(Jiang et al. 2020)
	South Africa	1	10–95	1–8	(Manickum and John 2014)
	Tunisian	1	199–600	179–294	(Belhaj et al. 2016)
BPA	Mexico	4	<LOQ – 450	<LOQ – 3.0	This study
	Greece	1	ND	151–790	(Stasinakis et al. 2012)
	USA	5	60–600	<LOQ – 44	(Yu et al. 2013)
	USA	6	175.5–1,476.8	11.1–685.6	(De La Torre 2011)
	México	1	5,900–16,600	ND	(Villarreal-Morales et al. 2020)
	Mexico	2	ND	<LOD – 30	(Díaz-Torres et al. 2013)
	Portugal	1	10.01 ± 1.89	3.31 ± 0.09	(Carvalho et al. 2016)
4NP	Mexico	4	<LOQ – 18.9	<LOQ – 2.3	This study
	China	38	1,519.51–27,736.71	1,346.75–7,513.57	(Jiang et al. 2020)
	Greece	1	ND	2,581–4,552	(Stasinakis et al. 2012)
	USA	5	220–870	<LOQ – 50	(Yu et al. 2013)
	USA	6	2,412.8–66,457.7	71.6–7,899.5	(De La Torre 2011)
	Iran	9	1,250–17,020	420–2,120	(Bina et al. 2018)
	Portugal	1	4.62 ± 0.32	4.44 ± 0.19	(Carvalho et al. 2016)
4TOP	Mexico	4	<LOQ – 58.8	<LOQ – 5.0	This study

ND = not detected, LOQ = limit of quantification, LOD = limit of detection.

EDC	Country	N° of studied WWTP	Influents (ng/L)	Effluents (ng/L)	References
	USA	5	80–3,900	<LOQ – 100	(Yu et al. 2013)
	USA	6	259.3–3,693.7	7.2–416.1	(De La Torre 2011)
	Iran	9	35–718	5.3–54.8	(Bina et al. 2018)
	Portugal	1	ND	ND	(Carvalho et al. 2016)

ND = not detected, LOQ = limit of quantification, LOD = limit of detection.

Concentrations of EDCs in effluents

The detection frequency and concentrations of the five selected EDCs in the effluents were lower than those detected in the influents (Table 2 and Fig. 2), indicating that the treatment processes in WWTPs have a notable effect on the removal of these substances. In effluents, levels of estrogens E2 and EE2 ranged from < LOQ – 9.5 and 1.6–26.8 ng/L, respectively. The synthetic estrogen EE2 standing out for its frequent detection (100%) in all WWTPs effluents, which reveal its high persistence to treatment processes, which is associated with the ethinyl functional group that makes it highly resistant to biodegradation (Ting and Praveena 2017), this characteristic could also explain its frequent detection in the effluents of WWTPs around the world (Manickum and John 2014; Belhaj et al. 2016; Jiang et al. 2020; López-Velázquez et al. 2020).

BPA was detected in only 25% of the samples collected in effluents, ranging in low levels from < LOQ to 3.0 ng/L; these results suggest that the conditions of all WWTPs allowed the high removal of BPA (> 96.9%). This is consistent with those reported by Villarreal-Morales et al. (2020), who reported high BPA concentrations in the influent of one WWTP in Monterrey City (5,900–16,600 ng/L), and not detected levels in the effluent; however, it is worth note that they used SPE/LC-UV technique, and likely levels of BPA in the effluents could be underestimated.

For 4NP and 4TOP, the detected concentrations in the effluents ranged from < LOQ to 2.3 ng/L and < LOQ to 5.0 ng/L, respectively, and were considered low compared with those quantified in other WWTPs effluents in ranges from 1,346.7 to 7,513.5 ng/L, and 420 to 2,120 ng/L for 4NP (Bina et al. 2018; Jiang et al. 2020); and from 7.2 to 416.1 ng/L for 4TOP (De la Torre 2011). These authors related the levels of 4NP and 4TOP in WWTPs with the extensive use of polyethoxylated alkylphenols (active substances of detergents and surfactants) in domestic, urban and hospital clean activities. Moreover, the detected levels of these alkylphenols in the wastewater may be regulated to some extent by microbial activity in the environment by degrading the polyethoxylated alkylphenols to give rise to large amounts of 4NP and 4TOP (Lee et al. 2013; Santhi et al. 2015).

Mass balance of EDCs dissolved in WWTPs

The mass balance for the five EDCs in the soluble phase was determined based on the concentrations detected in the WWTPs and the volume of water treated per day (Table 1). The results are shown in Table 4 and it was observed that the total loading mass of the EDCs in the influents of WWTPs equals 118,687.0 mg/day and are distributed as follows: BPA (61,282.6 mg/day) > EE2 (43,559.5 mg/day) > 4TOP (8,751.4 mg/day) > E2 (3,411.2 mg/day) > 4NP (1,682.3 mg/day). In the WWTPs, 93.4% of the total mass of the EDCs was removed by treatment processes, and the

remaining mass in effluents was 7,792.9 mg/day distributed as follows: EE2 (5,741.3 mg/day) > 4TOP (905.7 mg/day) > E2 (658.1 mg/day) > 4NP (283.5 mg/day) > BPA (204.3 mg/day); these results are similar to estimates by Ashfaq et al. (2018) who reported 951 mg/day of 4NP, 669 mg/day of E2, and 127 mg/day of 4NP in the effluents from nine WWTPs (China). However, the emission rate for 4NP and BPA were much lower than those estimated by Stasinakis et al. (2012) in a WWTP in Greece ($\sim 5 \times 10^4$ mg/day for 4NP and $\sim 5 \times 10^3$ mg/day for BPA), which was associated with intense urban and industrial activities. Additionally, Fig. 3 shows the daily mass of EDCs in the effluents of the WWTPs, and it was observed that the emission rate of EDCs is directly related to the number of the population served and the volume of wastewater treated (see Table 1). Also, it is observed that EE2 is the major constituent (73.7%) of the dissolved EDCs in the four effluents, which are directly discharged to La Silla, Sabinal, Santa Catarina, and Pesquería rivers (Fig. 1), suggesting a potential health risk to exposed aquatic ecosystems and human health.

Table 4
Daily mass load (mg/day) and approximate remotion of EDCs in the WWTPs.

		WWTP A	WWTP B	WWTP C	WWTP D	Σ mass load (mg/day)
E2	mass _{inf}	3.1	685.8	1130.3	1592.1	3411.3
	mass _{eff}	18.4	0	462.8	176.9	658.1
	AR (%)	-493.5	100.0	59.0	88.9	
EE2	mass _{inf}	37.6	14680.2	20822.2	8019.5	43559.5
	mass _{eff}	211.5	2301.4	1135.1	2093.3	5741.3
	AR (%)	-462.5	84.3	94.5	73.9	
BPA	mass _{inf}	90.4	52816.2	4808.5	3567.5	61282.6
	mass _{eff}	0	58.1	146.2	ND	204.3
	AR (%)	100.0	99.9	96.9	100.0	
4NP	mass _{inf}	4.6	674.2	1003.6	ND	1682.4
	mass _{eff}	1.5	267.3	14.6	ND	283.4
	AR (%)	67.4	60.3	98.5	-	
4TOP	mass _{inf}	14.6	7287.8	63.3	1385.7	8751.4
	mass _{eff}	38.3	116.2	73.1	678.1	905.7
	AR (%)	-162.3	98.4	-15.5	51.0	
Σ EDCs in influents (mg/day)						118687.2
Σ EDCs in effluents (mg/day)						7792.8
AR = approximate remotion, ND = not detected.						

Based on the mass balance of EDCs in influents and effluents of the WWTPs, the approximate remotion of EDCs were estimated, and results are shown in Table 4. The remotion values for E2 and EE2 in WWTP B, C, and D, ranged between 59–100% (E2), and 73.9–94.5% (EE2), these values were higher than those reported by Jiang et al. (2020) from 6.3 to 29.64% for E2, and from - 57.6 to 18.51% for EE2. In the case of BPA, the four WWTPs showed high remotion values, between 96.9 and 100% (Table 4), which agrees well with those observed by Ben et al. (2018) in 14 WWTPs from China. In addition, the remotion of 4NP ranged from 60.3 to 98.5% in WWTP A, B, and C; and for 4TOP, the remotion was 98.4 and 51.0% in WWTP B and D, respectively. These results agree with those reported by Bina et

al. (2018) who studied 9 WWTPs and estimated remotion values between 61.9 and 93.1% for 4NP, and between 71.5 and 99.2% for 4TOP; also, similar values were reported by Ben et al. (2018) and Jiang et al. (2020).

It is worth noting that some negative values were observed for E2, EE2, and 4TOP in WWTP A (-493.5, -462.5, and -162.3%, respectively), and this phenomenon was also observed for 4TOP in WWTP C (-15.5%). These negative values indicate that levels of these EDCs were higher in effluents than influents, and can be attributed to the likely transformation of conjugated estrogens to their active forms, and the biodegradation of polyethoxylated alkylphenols to give rise to metabolites such as 4TOP by the action of the microbial consortia in the WWTPs, which increase their concentration in the effluents, and it is consistent with that described by Yu et al. (2013) and Zuo et al. (2006).

Risk assessment

The occurrence of EDCs caused for the discharge of treated and untreated wastewater may represent a potential ecological risk to both aquatic species and humans. Therefore, the risk quotients (RQ) were calculated from the influent and effluent data of the four WWTPs using the MEC values, and the PNEC values for each EDC were taken from the scientific literature. The results are shown in Table 5 and Fig. 4, and it is observed that the highest RQ values for E2, EE2, and BPA were 78.1, 17,442.8 and 10.4, indicating that the untreated wastewater represent a high risk for aquatic species. While, the highest RQ values for 4NP and 4TOP in untreated wastewater were 0.2 and 0.5, suggesting a medium risk for exposed species. Likewise, the treated water in WWTPs effluents were also considered as a high risk for aquatic organisms, mainly attributed to the abundance of E2 (RQ = 49.1) and EE2 (RQ = 1,165.2), which are considered as potent endocrine disruptors due to their high estrogenic potential, even at trace levels such as pg/L (European Commission 2012; Brion et al. 2019). In addition, as can be seen in Fig. 4, BPA, 4NP, and 4TOP represent a low risk in WWTPs effluents (RQs < 0.1 for each); however, this was not significant because they constitute a minimal part of the remaining EDCs in the effluents (Fig. 3). Finally, the possible ecological risk was estimated for the receiving waterbodies impacted by the effluents of WWTPs (Fig. 4), and results suggest that the organisms in these aquatic ecosystems may be exposed to high risk, attributed to the abundance of remaining E2 (RQ = 4.9) and EE2 (RQ = 116.5) in the effluents of WWTPs (Fig. 3).

Table 5
HC5 values for the protection of 95% of the species, risk quotient (RQ) calculated from maximum EDCs levels detected in the four WWTPs.

EDCs	HC5 (ng/L)	PNEC (ng/L)	RQ = MEC/PNEC					
			Influents		Effluents		Surface water ^d	
E2	0.58 ^a	0.19	78.1	High risk	49.1	High risk	4.9	High risk
EE2	0.07 ^b	0.023	17442.8	High risk	1165.2	High risk	116.5	High risk
BPA	129 ^a	43.00	10.4	High risk	0.07	Low risk	0.007	Low risk
4NP	280 ^a	93.33	0.2	Medium risk	0.02	Low risk	0.002	Low risk
4TOP	360 ^c	120.00	0.5	Medium risk	0.04	Low risk	0.004	Low risk

^a(Huang et al. 2019), ^b(Brion et al. 2019), ^c(Tamis and Jongbloed 2019), ^dDilution of 10 times was assumed in receiving waterbody.

Additionally, in the worst-case scenario, the results obtained in this study could underestimate the potential ecological risk related to EDCs since in this study only two sampling campaigns were carried out. Moreover, the suspended phase of EDCs in the WWTPs and the fraction of untreated wastewater directly discharged to the environment were not considered. In addition, the emission of pollutants such as EDCs may be a function of population consumption and excretion rates, as well as urban and industrial activities which vary over time. Also, in the environment, EDCs are regulated to some extent by environmental factors such as temperature, precipitation, solar radiation, microbial activity, among others.

Conclusions

This study revealed that EE2 and BPA were the most frequently detected EDCs in the influents of WWTPs, likely associated to the extensive use of EE2 as a contraceptive method and the use of BPA as a plasticizer in industrial activities. In addition, the detected levels of 4NP and 4TOP suggest their moderate use in urban activities in Monterrey City. Furthermore, EE2 was detected in all WWTP effluents ranging from 1.6 to 26.8 ng/L being the most recalcitrant compound in the treatment processes. Also, the mass balance in the dissolved phase revealed that the WWTP effluents constitute critical sources of EDCs emission to the environment, despite that these compounds present high removal percentages in some WWTPs. Moreover, through the RQ values, it was estimated that the wastewater in influents and effluents of the studied WWTPs represent a high risk to exposed aquatic species, due to the abundance of dissolved E2 and EE2. Also, a preliminary assessment of the risk quotient in receiving water bodies indicated a potential environmental risk for La Silla, Santa Catarina, Sabinal, Topo Chico, and Pesqueria rivers. However, ecotoxicological studies and extensive monitoring are needed to protect the biodiversity of these aquatic environments. Additionally, it is necessary pay more attention to the possible public health risk associated with the occurrence of EDCs in WWTPs in densely populated and industrialized zones such as Monterrey City.

Declarations

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Author contribution KLV: Investigation, conceptualization, writing and original draft. JLGM: Supervision, resources, writing-review and editing. HASN and MAMT: Methodology, data curation, writing-review, and editing. MVR: Project administration, supervision, funding acquisition, conceptualization, writing-review, and editing.

References

1. Adeel M, Song X, Wang Y, Francis D, Yang Y. 2017. Environmental impact of estrogens on human, animal and plant life: A critical review. *Environ. Int.* 99:107–119. doi:10.1016/j.envint.2016.12.010.
2. Ashfaq M, Li Y, Wang Y, Qin D, Rehman MSU, Rashid A, Yu CP, Sun Q. 2018. Monitoring and mass balance analysis of endocrine disrupting compounds and their transformation products in an anaerobic-anoxic-oxic wastewater treatment system in Xiamen, China. *Chemosphere.* doi:10.1016/j.chemosphere.2018.04.028.
3. Belhaj D, Athmouni K, Jerbi B, Kallel M, Ayadi H, Zhou JL. 2016. Estrogenic compounds in Tunisian urban sewage treatment plant: occurrence, removal and ecotoxicological impact of sewage discharge and sludge disposal. *Ecotoxicology* 25:1849–1857. doi:10.1007/s10646-016-1733-8.
4. Ben W, Zhu B, Yuan X, Zhang Y, Yang M, Qiang Z. 2018. Occurrence, removal and risk of organic micropollutants in wastewater treatment plants across China: Comparison of wastewater treatment processes. *Water Res.* doi:10.1016/j.watres.2017.11.057.
5. Bina B, Mohammadi F, Amin MM, Pourzamani HR, Yavari Z. 2018. Determination of 4-nonylphenol and 4-tert-octylphenol compounds in various types of wastewater and their removal rates in different treatment processes in nine wastewater treatment plants of Iran. *Chinese J. Chem. Eng.* 26:183–190. doi:10.1016/j.cjche.2017.04.009.
6. Brion F, De Gussem V, Buchinger S, Hollert H, Carere M, Porcher JM, Piccini B, Féray C, Dulio V, Könemann S, et al. 2019. Monitoring estrogenic activities of waste and surface waters using a novel in vivo zebrafish embryonic (EASZY) assay: Comparison with in vitro cell-based assays and determination of effect-based trigger values. *Environ. Int.* doi:10.1016/j.envint.2019.06.006.
7. Calderón-Moreno GM, Vergara-Sánchez J, Saldarriaga-Noreña H, García-Betancourt ML, Domínguez-Patiño ML, Moeller-Chávez GE, Ronderos-Lara JG, Arias-Montoya MI, Montoya-Balbas IJ, Murillo-Tovar MA. 2019. Occurrence and risk assessment of steroidal hormones and phenolic endocrine disrupting compounds in surface water in Cuautla River, Mexico. *Water (Switzerland)* 11:2628. doi:10.3390/W11122628.
8. Carvalho AR, Cardoso V, Rodrigues A, Benoliel MJ, Duarte E. 2016. Fate and Analysis of Endocrine-Disrupting Compounds in a Wastewater Treatment Plant in Portugal. *Water. Air. Soil Pollut.* 227:202. doi:10.1007/s11270-016-2910-3.
9. Čelić M, Škrbić BD, Insa S, Živančev J, Gros M, Petrović M. 2020. Occurrence and assessment of environmental risks of endocrine disrupting compounds in drinking, surface and wastewaters in Serbia. *Environ. Pollut.* doi:10.1016/j.envpol.2020.114344.
10. CONAGUA. 2019. Comisión Nacional del Agua. Precipitación por Entid. Fed. y Nac. 2019. [accessed 2020 Dec 6]. <https://smn.conagua.gob.mx/tools/DATA/Climatología/Pronóstico climático/Temperatura y Lluvia/PREC/2019.pdf>.
11. Cruz-López A, Dávila-Pórcel RA, de León-Gómez H, Rodríguez-Martínez JM, Suárez-Vázquez SI, Cardona-Benavides A, Castro-Larragoitia GJ, Boreselli L, de Lourdes Villalba M, Pinales-Munguía A, et al. 2020. Exploratory study on the presence of bisphenol A and bis(2-ethylhexyl) phthalate in the Santa Catarina River in Monterrey, N.L., Mexico. *Environ. Monit. Assess.* 192:1–13. doi:10.1007/s10661-020-08446-4. [accessed 2020 Jul 19]. <https://link.springer.com/article/10.1007/s10661-020-08446-4>.
12. Díaz-Torres E, Gibson R, González-Farías F, Zarco-Arista AE, Mazari-Hiriart M. 2013. Endocrine disruptors in the Xochimilco Wetland, Mexico City. *Water. Air. Soil Pollut.* 224:1586-1594. doi:10.1007/s11270-013-1586-1.

13. ECHA and EFSA. 2018. Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. (Pre-publication version; June 2018) 16:1–135. doi:10.2903/j.efsa.2018.5311.
14. EMEA. 2006. Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use. Eur. Med. Agency.
15. EPA. 1998. Endocrine Disruptor Screening and Testing Advisory Committee Final Report. EDSTAC Final Rep.:1–17. doi:10.1097/01.AOG.0000445580.65983.d2.
16. EPA. 2007. Method 1698: Steroids and hormones in water, soil, sediment, and biosolids by HRGC / HRMS. EPA Method:1–69.
17. Estrada-Arriaga EB, Cortés-Muñoz JE, González-Herrera A, Calderón-Mólgora CG, de Lourdes Rivera-Huerta M, Ramírez-Camperos E, Montellano-Palacios L, Gelover-Santiago SL, Pérez-Castrejón S, Cardoso-Vigueros L, et al. 2016. Assessment of full-scale biological nutrient removal systems upgraded with physico-chemical processes for the removal of emerging pollutants present in wastewaters from Mexico. *Sci. Total Environ.* 571:1172-1182. doi:10.1016/j.scitotenv.2016.07.118.
18. European Commission. 2003. Technical Guidance Document on Risk Assessment. *Sci. Tech. Res. Reports*:337.
19. European Commission. 2012. Proposal for a DIRECTIVE OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. Brussels.
20. European Parliament. 2008. DIRECTIVE 2008/105/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC,.
21. European Parliament. 2013. DIRECTIVE 2013/39/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy.
22. European Parliament. 2018. Proposal for a DIRECTIVE OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on the quality of water intended for human consumption (recast). Brussels.
23. Hatch EE, Nelson JW, Stahlhut RW, Webster TF. 2010. Association of endocrine disruptors and obesity: Perspectives from epidemiological studies. In: *International Journal of Andrology*.
24. Huang H, Wu J, Ye J, Ye T, Deng J, Liang Y, Liu W. 2018. Occurrence, removal, and environmental risks of pharmaceuticals in wastewater treatment plants in south China. *Front. Environ. Sci. Eng.* doi:10.1007/s11783-018-1053-8.
25. Huang Y, Xie X, Zhou LJ, Ji X, Gao B, Xu GZ, Li A. 2019. Multi-phase distribution and risk assessment of endocrine disrupting chemicals in the surface water of the Shaying River, -Huai River Basin, China. *Ecotoxicol. Environ. Saf.* doi:10.1016/j.ecoenv.2019.02.016.
26. Inam EJ, Nwoke IB, Udosen ED, Offiong NAO. 2019. Ecological risks of phenolic endocrine disrupting compounds in an urban tropical river. *Environ. Sci. Pollut. Res.* doi:10.1007/s11356-019-05458-7.
27. INEGI. 2021. Instituto Nacional de Estadística, Geografía e Informática. Censo Población y Vivienda 2020. [accessed 2021 May 20]. <https://censo2020.mx/>.
28. Jackson L, Klerks P. 2020. Effects of the synthetic estrogen 17 α -ethinylestradiol on *Heterandria formosa* populations: Does matrotrophy circumvent population collapse? *Aquat. Toxicol.* doi:10.1016/j.aquatox.2020.105659.

29. Jiang R, Liu J, Huang B, Wang X, Luan T, Yuan K. 2020. Assessment of the potential ecological risk of residual endocrine-disrupting chemicals from wastewater treatment plants. *Sci. Total Environ.* 714:136689. doi:10.1016/j.scitotenv.2020.136689.
30. Kabir ER, Rahman MS, Rahman I. 2015. A review on endocrine disruptors and their possible impacts on human health. *Environ. Toxicol. Pharmacol.* doi:10.1016/j.etap.2015.06.009.
31. De La Torre RJ. 2011. Analysis of Endocrine Disrupting Compounds in Wastewater Treatment Plants: A Perspective of Trans-Boundary Waterborne Pollution. University of Texas at El Paso. [accessed 2020 Jul 27]. https://scholarworks.utep.edu/cgi/viewcontent.cgi?article=3465&context=open_etd.
32. Lee CC, Jiang LY, Kuo YL, Hsieh CY, Chen CS, Tien CJ. 2013. The potential role of water quality parameters on occurrence of nonylphenol and bisphenol A and identification of their discharge sources in the river ecosystems. *Chemosphere* 91:904–911. doi:10.1016/j.chemosphere.2013.02.006.
33. López-Velázquez K, Guzmán-Mar JL, Saldarriaga-Noreña HA, Murillo-Tovar MA, Hinojosa-Reyes L, Villanueva-Rodríguez M. 2021. Occurrence and seasonal distribution of five selected endocrine-disrupting compounds in wastewater treatment plants of the Metropolitan Area of Monterrey, Mexico: The role of water quality parameters. *Environ. Pollut.* doi:10.1016/j.envpol.2020.116223.
34. López-Velázquez K, Villanueva-Rodríguez M, Mejía-González G, Herrera-López D. 2020. Removal of 17 α -ethinylestradiol and caffeine from wastewater by UASB-Fenton coupled system. *Environ. Technol. (United Kingdom)*:1–12. doi:10.1080/09593330.2020.1740799.
35. Manickum T, John W. 2014. Occurrence, fate and environmental risk assessment of endocrine disrupting compounds at the wastewater treatment works in Pietermaritzburg (South Africa). *Sci. Total Environ.* 468:584–597. doi:10.1016/j.scitotenv.2013.08.041.
36. Patrolecco L, Capri S, Ademollo N. 2015. Occurrence of selected pharmaceuticals in the principal sewage treatment plants in Rome (Italy) and in the receiving surface waters. *Environ. Sci. Pollut. Res.* 22:5864–5876. doi:10.1007/s11356-014-3765-z.
37. Peng FJ, Pan CG, Zhang M, Zhang NS, Windfeld R, Salvito D, Selck H, Van den Brink PJ, Ying GG. 2017. Occurrence and ecological risk assessment of emerging organic chemicals in urban rivers: Guangzhou as a case study in China. *Sci. Total Environ.* doi:10.1016/j.scitotenv.2017.02.200.
38. Roby KF. 2013. Endocrine disruptors. In: Hoyer PB, editor. *Ovarian Toxicology, Second Edition*. 2nd editio. CRC Press. p. 387.
39. Ronderos-Lara J, Saldarriaga-Noreña H, Murillo-Tovar M, Vergara-Sánchez J. 2018. Optimization and Application of a GC-MS Method for the Determination of Endocrine Disruptor Compounds in Natural Water. *Separations* 5:33. doi:10.3390/separations5020033.
40. Salgueiro-González N, Campillo JA, Viñas L, Beiras R, López-Mahía P, Muniategui-Lorenzo S. 2019. Occurrence of selected endocrine disrupting compounds in Iberian coastal areas and assessment of the environmental risk. *Environ. Pollut.* doi:10.1016/j.envpol.2019.03.107.
41. Santhi VA, Juahir H, Retnam A, Mustafa AM. 2015. Chemometric Interpretation on the Occurrence of Endocrine Disruptors in Source Water from Malaysia. *Clean - Soil, Air, Water* 43:804–810. doi:10.1002/clen.201300777.
42. Sauvé and Desrosiers M. 2014. A review of what is an emerging contaminant. *Chem. Cent. J.*:8(1), 15. doi:10.1186/1752-153X-8-15.
43. SEDATU, CONAPO, INEGI. 2015. Delimitación de las zonas metropolitanas de México 2015 Delimitación de las zonas metropolitanas de México 2015. Ciudad de México.

44. Stasinakis AS, Mermigka S, Samaras VG, Farmaki E, Thomaidis NS. 2012. Occurrence of endocrine disrupters and selected pharmaceuticals in Aisonas River (Greece) and environmental risk assessment using hazard indexes. *Environ. Sci. Pollut. Res.* 19:1574–1583. doi:10.1007/s11356-011-0661-7.
45. Tamis J, Jongbloed R. 2019. MICROPROOF Micropollutants in Road RunOff: Environmental risk assessment. Wageningen Marine Research. [accessed 2021 Feb 21]. <https://research.wur.nl/en/publications/e265872d-7b74-486b-b109-dbd3459dcde6>.
46. Ting YF, Praveena SM. 2017. Sources, mechanisms, and fate of steroid estrogens in wastewater treatment plants: a mini review. *Environ. Monit. Assess.* 189:178. doi:10.1007/s10661-017-5890-x.
47. Villarreal-Morales R, Hinojosa-Reyes L, Hernández-Ramírez A, Ruíz-Ruíz E, Maya Treviño M de L, Guzmán-Mar JL. 2020. Automated SPE-HPLC-UV methodology for the on-line determination of plasticisers in wastewater samples. *Int. J. Environ. Anal. Chem.*:1–14. doi:10.1080/03067319.2020.1742891. [accessed 2020 Jul 19]. <https://www.tandfonline.com/doi/full/10.1080/03067319.2020.1742891>.
48. Wang Q, Yang H, Yang M, Yu Y, Yan M, Zhou L, Liu X, Xiao S, Yang Y, Wang Y, et al. 2019. Toxic effects of bisphenol A on goldfish gonad development and the possible pathway of BPA disturbance in female and male fish reproduction. *Chemosphere*. doi:10.1016/j.chemosphere.2019.01.033.
49. Wee SY, Aris AZ, Yusoff FM, Praveena SM. 2019. Occurrence and risk assessment of multiclass endocrine disrupting compounds in an urban tropical river and a proposed risk management and monitoring framework. *Sci. Total Environ.* doi:10.1016/j.scitotenv.2019.03.243.
50. Yu Y, Wu L, Chang AC. 2013. Seasonal variation of endocrine disrupting compounds, pharmaceuticals and personal care products in wastewater treatment plants. *Sci. Total Environ.* 442:310–316. doi:10.1016/j.scitotenv.2012.10.001.
51. Zuo Y, Zhang K, Deng Y. 2006. Occurrence and photochemical degradation of 17 α -ethinylestradiol in Acushnet River Estuary. 63:1583–1590. doi:10.1016/j.chemosphere.2005.08.063.

Figures

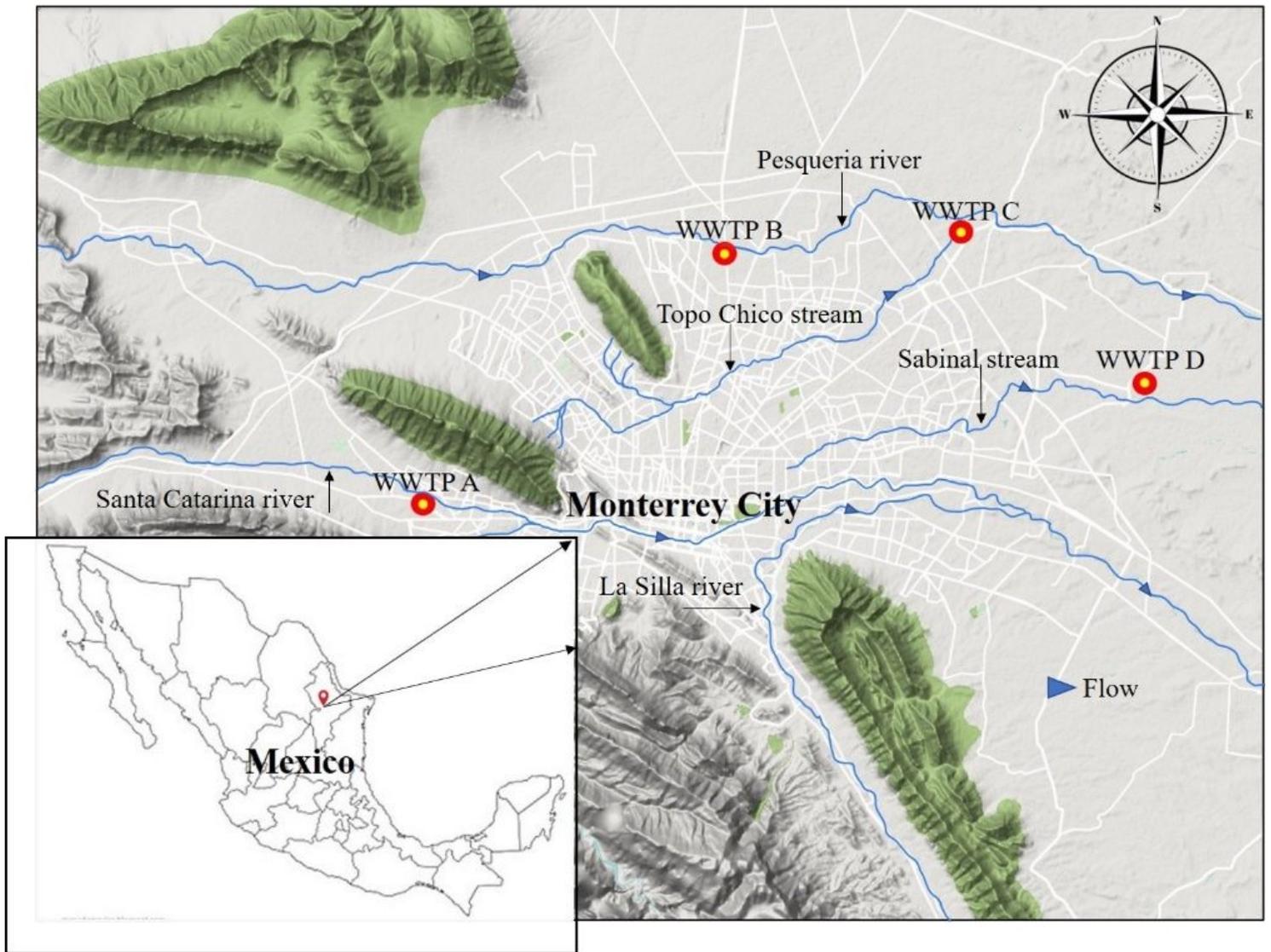


Figure 1

Map showing the location of the Monterrey City, and the four studied WWTPs, as well as the main receiving waterbodies.

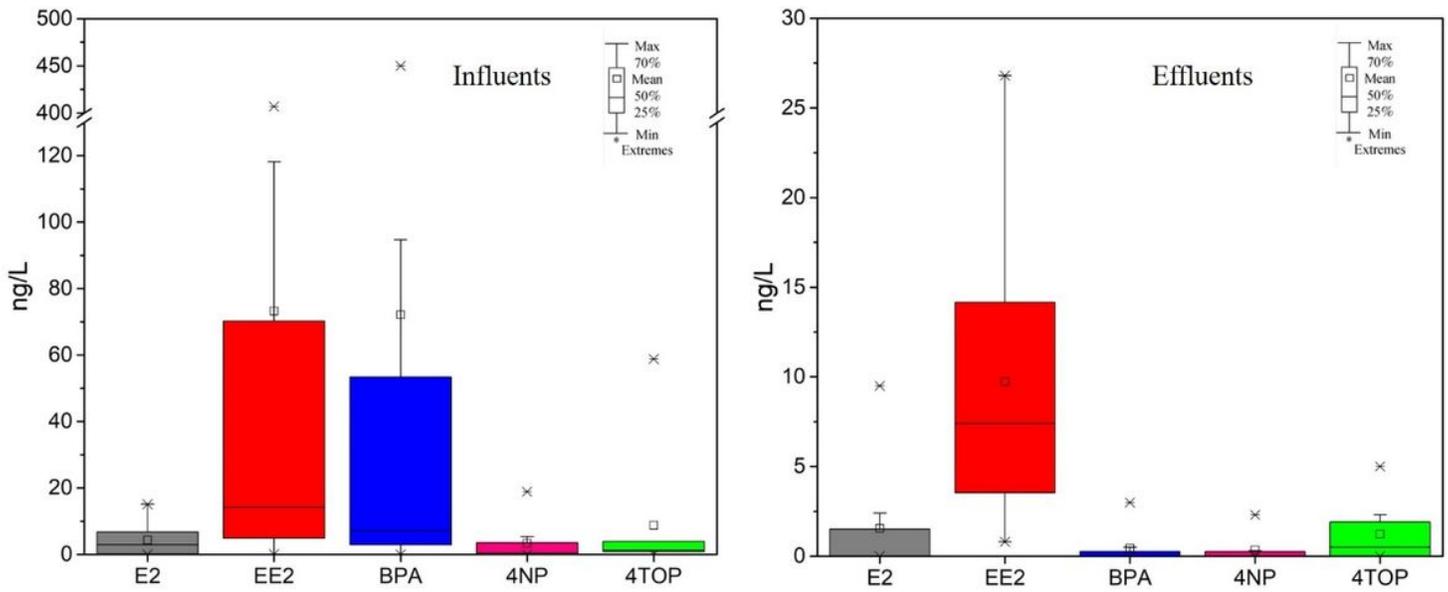


Figure 2

Concentration ranges of five EDCs detected in four WWTPs of Monterrey City.

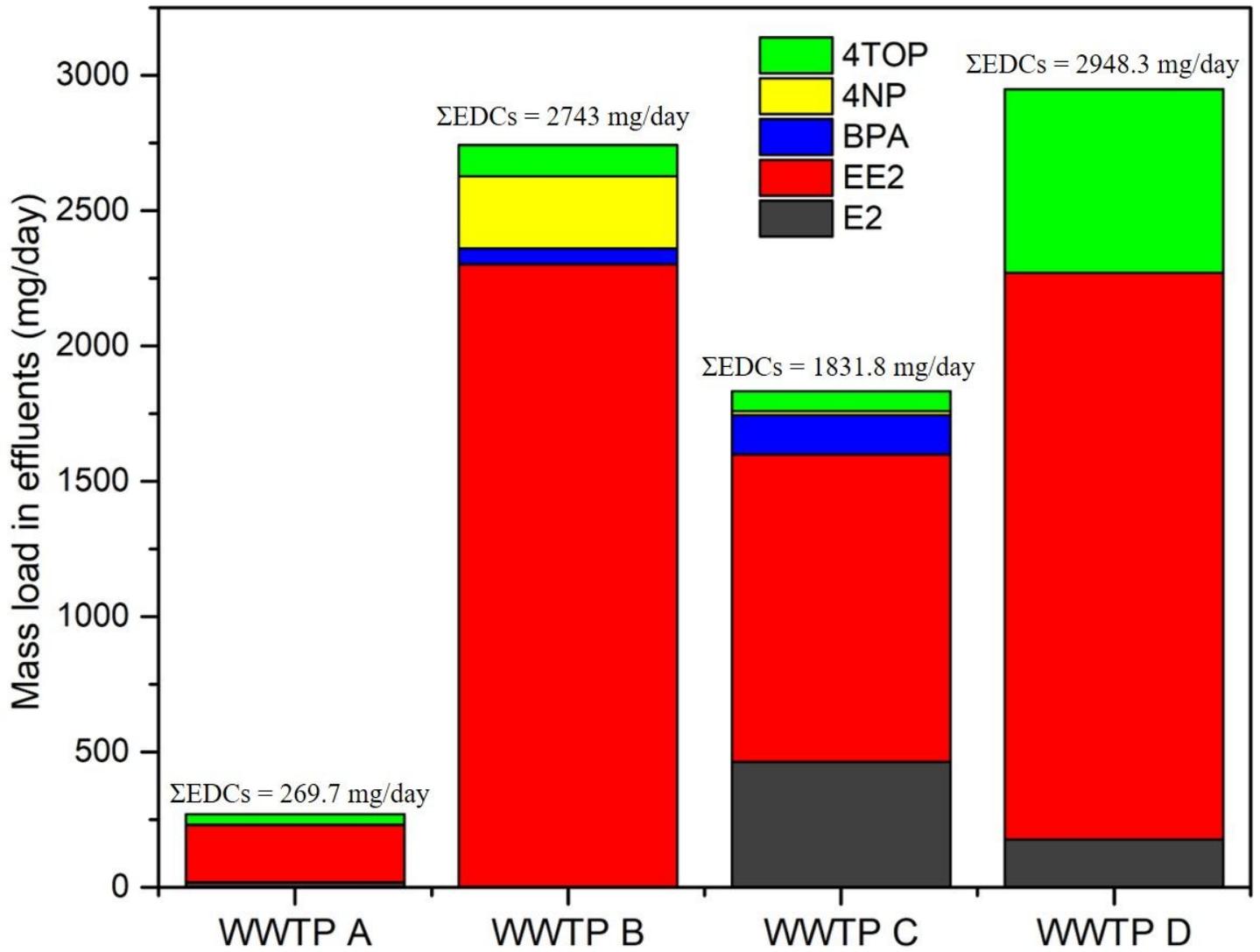


Figure 3

Daily mass load of selected EDCs in effluents of four WWTPs in Monterrey City.

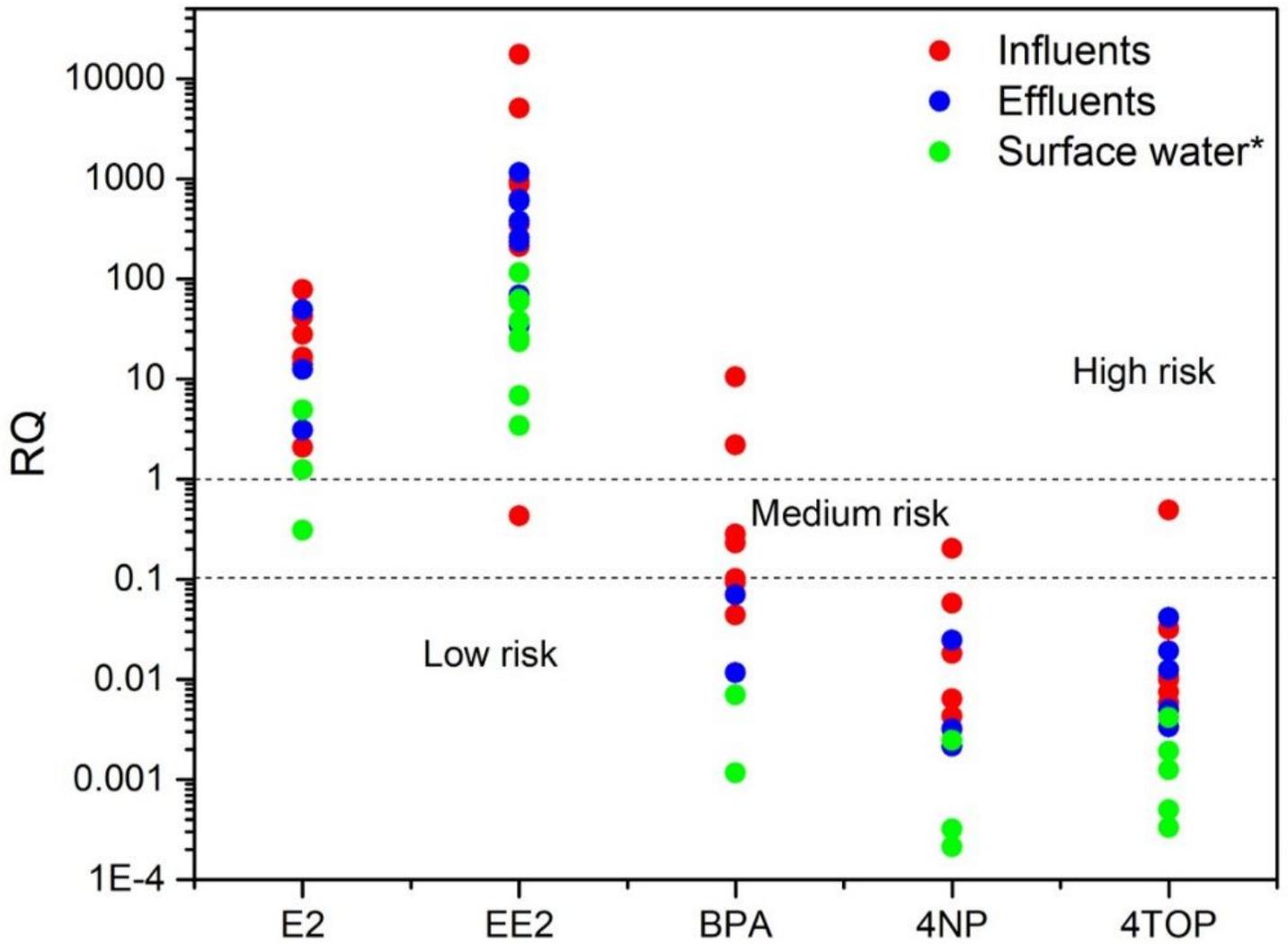


Figure 4

Risk quotient values calculated from EDCs levels detected in four WWTPs. *Dilution of 10 times was assumed for surface water.