Emerging pollutants and antibiotics removed by conventional activated sludge followed by ultraviolet radiation in a municipal wastewater treatment plant in Mexico

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ABSTRACT

Different groups of emerging pollutants (EPs) were identified (drugs, pesticides, hormones, illicit drugs, and fire retardant), and the removal of four antibiotics was determined (sulfamethoxazole 62.2–94.4%, ciprofloxacin 71–83.1%, norfloxacin 82–89%, and ofloxacin 78–97.9%) in a municipal wastewater treatment plant (WWTP) in Acapulco, Gro. Mexico. The compounds were extracted from influent and effluent samples by solid phase extraction (SPE). The identification of non-target EPs was performed by liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS). The antibiotic quantification was performed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Most of the non-target compounds were detected only in the influent samples. Antibiotics levofloxacin and nalidixic acid, the anticonvulsants carbamazepine and oxycarbamazepine, the local anesthetic lidocaine, and the pesticide tridemorph were the main EPs not removed by the WWTP. In this study, it was shown that the Aguas Blancas WWTP does not manage to remove 100% of the various EPs identified in the effluent, although the elimination degree is high in most cases, despite being one of the model plants in Mexico.

Key words: antibiotics, emerging pollutants, non-target, wastewater

HIGHLIGHTS

- Different groups of emerging pollutants (EPs) were identified.
- Detection of non-target compounds.
- Removal of four antibiotics (sulfamethoxazole, ciprofloxacin, norfloxacin, and ofloxacin).
- The study was carried out in a model plant in Mexico.
- The non-target analysis was able to detect pesticides included in the highly dangerous pesticides (PAP) list.

GRAPHICAL ABSTRACT
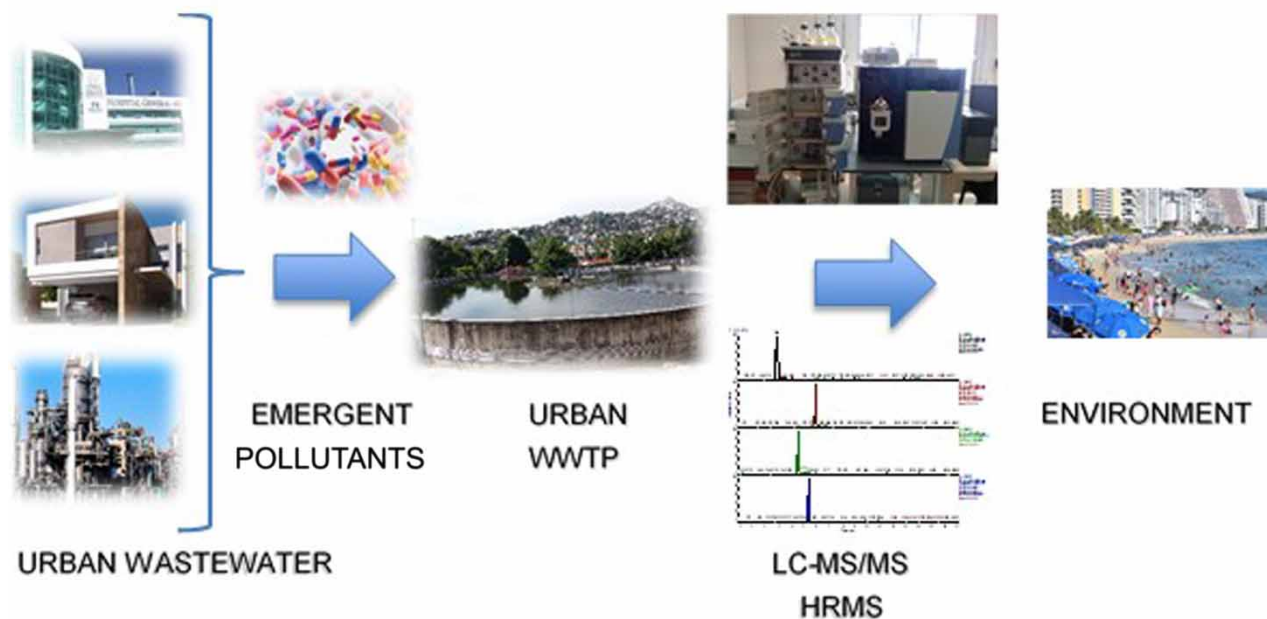
INTRODUCTION

There is great scientific interest worldwide due to the presence and effects of emerging pollutants (EPs) in aquatic ecosystems. EPs are compounds of different origin and chemical nature, which are recently introduced into the environment or which have been introduced into the environment for a long period of time and, however, they have only recently been detected (Barceló & López de Alda 2008). Most EPs have been studied around the world and are currently regulated and commonly monitored; however, in Latin America, the lack of information related to EPs in the aquatic environment has led to their limited regulation (Peña-Guzmán et al. 2019). EPs have the potential to cause known or suspected adverse ecological effects and represent a great risk for human health (Geissen et al. 2015). There is a great variety of EPs, among which pharmaceutical products, illicit drugs, personal care products, pesticides, surfactants, steroids, and hormones stand out. EPs have been identified in wastewater (WW; Tran et al. 2018), surface water (Peng et al. 2018), groundwater (Félix-Cañedo et al. 2013), and even in drinking water (Glassmeyer et al. 2017). Conventional wastewater treatment plants (WWTPs) are not designed for the removal of EPs and are considered as one of the main sources of entry of EPs into the aquatic environment (Tran et al. 2018; Zhou et al. 2019).

Pharmaceutical products are the EPs that cause the greatest concern, due to their variety, high consumption, degree of persistence, transformation into more toxic molecules, and their incidence in the aquatic environment (Rivera-Jaimes et al. 2018; López-Velázquez et al. 2021). The most studied drugs for their possible effects on aquatic organisms and their return to drinking water systems are antibiotics. A large number of these molecules and their metabolites are released into the aquatic environment. Their introduction is through excreta or urine (not completely metabolized within the body) and by the elimination of unused or expired products (Kümmerer 2009b). Antibiotics have a high degree of resistance to degradation in the environment some even tend to bioaccumulate or biotransform (Kümmerer 2009a). The development of new technological advances and more sensitive and precise analytical methods such as the use of liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) have allowed the detection and quantification of EPs in the aquatic environment at concentrations from ng L^{-1} to μg L^{-1} (Tran et al. 2018).

Fluoroquinolones (FQs) and sulfonamides (SAs) are two important groups of antibiotics that are continuously consumed in large quantities in the world. FQs and SAs are incompletely metabolized by organisms, and their resistance to microbiological degradation and negative effects in the aquatic environment make them the object of priority study in WWTPs (García-Galán

et al. 2012; Teglia *et al.* 2019). Removal efficiencies of FQs and SAs during wastewater treatment are still quite scarce, and elimination rates reported so far are usually low (García-Galán *et al.* 2012).

In Mexico, studies of EPs in aquatic environments are scarce (Félix-Cañedo *et al.* 2013; Estrada-Arriaga *et al.* 2016; Robledo-Zacarías *et al.* 2017; Rivera-Jaimes *et al.* 2018; Hernández-Tenorio 2021; López-Velázquez *et al.* 2021). Besides, only 57% of WW generated and collected in municipal sewage systems receive some type of treatment before being discharged to water bodies CONAGUA (2016). Taking into account that the technologies for the treatment of WW have not been fully developed and a large part of the WW only receives primary treatment or is carried out by means of stabilization ponds, exacerbates the problem of the discharge of EPs into the aquatic environment. Therefore, it is advisable to know the presence and removal of these types of contaminant in WWTPs including the secondary treatment with disinfection by UV radiation, as it is the case of Aguas Blancas WWTP. The objective of this study was to identify EPs and quantify FQs and SAs antibiotics present in the influent and effluent, as well as to determine the removal efficiency of a WWTP.

METHODS

Description of the study area

Acapulco, Guerrero, is one of the most important tourist destinations in Mexico. Its population growth in recent years has promoted the development of the urban area in a significant way, generating a challenge in the supply of public services, especially for the treatment of WW. The study was carried out at the WWTP that treats the WW generated by an estimated population of 800,000 inhabitants. The WWTP treats 60% of the WW by discharging the treated water to the sea, at the point known as Playa Olvidada. The WWTP has an installed capacity of 1,350 L s^{-1} of flow in the effluent. The preliminary treatment consists of the elimination of floating matter, garbage, and coarse solids by means of mechanical treatments of coarse and fine slabs, mechanical desanding with aeration and with mechanical extraction of the granular material. The secondary treatment consists of the biological aerobic process by activated sludge in the following modality: carbonaceous oxidation, with a BOD_5 removal efficiency of 90.7%, hydraulic retention time of 5.8 h, cell retention time of 3.10 days, retention time in the 9-day aerobic digester, BOD_5/BOD ratio is 0.68, TSS removal efficiency 0.52%, effluent organic nitrogen 12.0 mg/L, total effluent Kjelldal nitrogen 36.0 mg/L, and total phosphorus concentration 11.0 mg/L. Finally, it also has tertiary treatment based on disinfection by UV radiation, the type of lamps is low pressure/high intensity, the transmittance of 65% of 253.7 nm. The plant receives WW collected from the Bahía de Santa Lucía amphitheater in the influent, including 42 hospitals, some of which are first-class (e.g. General public sector hospital, Cancer hospital), as well as private hospitals and clinics in the city of Acapulco (Figure 1). The WWTP is considered a model plant for removal of organic pollutants by the Comisión Nacional del Agua en México, based on its results for physico-chemical parameters, since it was rehabilitated in 2010 (CONAGUA 2016).

Sampling

Three sampling campaigns were carried out taking influent and effluent samples of the WWTP at different times of the year September 2017, February, and July 2018. Simple samples of 250 mL were collected every 4 h over a 24-h period to complete composite samples of 1 L in amber glass bottles. Each sample was acidified (pH 3) with hydrochloric acid to reduce microbial activity and improve solid phase extraction (SPE). Hereafter, this composite sample will be referred to as sample (S1, S2, S3; September 2017, February, and July 2018 sampling, respectively). The samples were transported and stored at 4 °C in the dark until extraction within the next 48 h. In the laboratory, the samples were filtered (0.45 μm, GF/C, Whatman).

Reagents

Antibiotics such as sulfamethoxazole, sulfamethazine, sulfadiazine, sulfamerazine, ciprofloxacin, enrofloxacin, norfloxacin and ofloxacin were purchased from Sigma-Aldrich (St Louis, MO, USA). The isotopically labeled antibiotics such as enrofloxacin-d5 hydrochloride and sulfamethoxazole-d4 were purchased from Sigma-Aldrich and Toronto Research Chemicals, respectively. All antibiotics were analytical grade (purity > 98%). Methanol (MeOH), MS grade (Merck), ≥99% ammonium formate (Sigma-Aldrich), formic acid (Honeywell, Germany), LS/MS grade ultrapure water (Praxair, Carlo Erba), 37% hydrochloric acid (Merck).
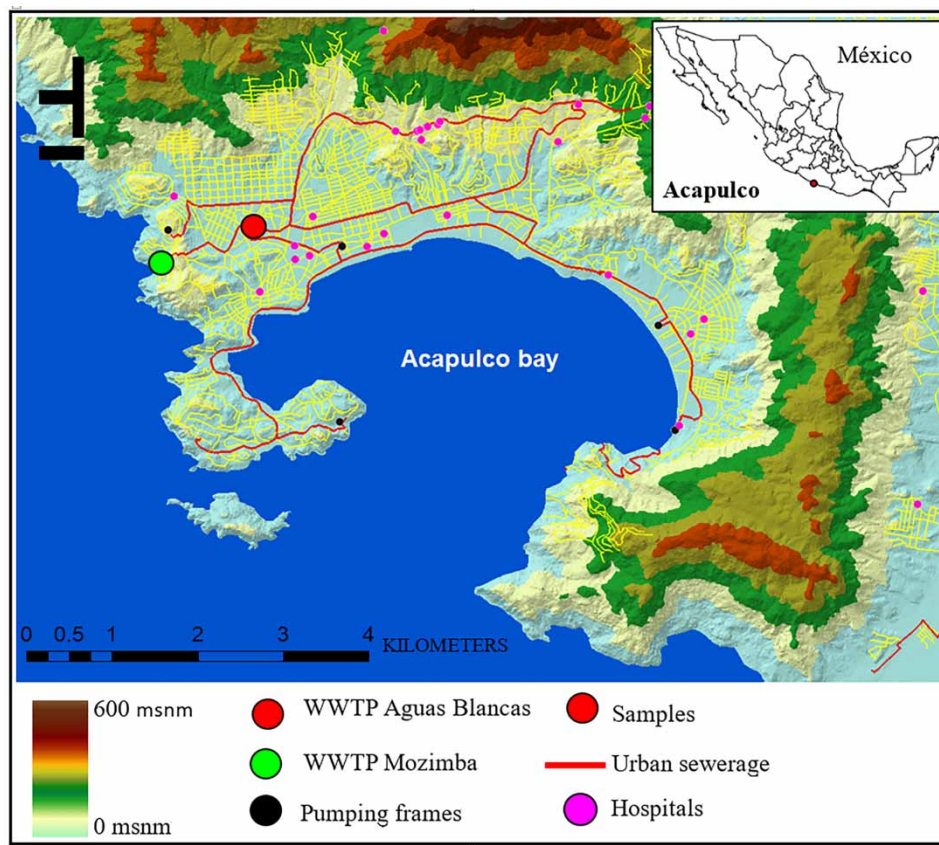
Figure 1 | Location of the Wastewater Treatment Plant (WWTP) in the city of Acapulco, the altitude change that surrounds the city, the sewer lines, and the main streets and avenues that delimit the urban area are also presented. Samples from WWTP Aguas Blancas.

Preparation of standard solutions

Deuterated antibiotic solutions were prepared as internal standards, to compensate for random and systematic errors. The internal standard for sulfa drugs (sulfamethoxazole-d4) was prepared with Methanol (MeOH), and the internal standard for fluoroquinolones (enrofloxacin-d5 hydrochloride) was dissolved in a water-methanol (H_2O/MeOH) mixture (1:1) that contains 0.2% (v/v) of hydrochloric acid (Golet *et al.* 2001), after preparation they were stored at -20 °C. The individual standard solutions of each antibiotic were prepared and dissolved in methanol, except for the fluoroquinolones, which were dissolved in a mixture of water–methanol (H_2O/MeOH) (1:1) with 0.2% (v/v) hydrochloric acid. All mixed working standards were prepared prior to analysis.

Solid phase extraction

A known amount of sulfamethoxazole-d4 and enrofloxacin-d5 were added to each influent and effluent sample, prior to extraction with internal standards, to quantify antibiotics from two of the most common antibiotic groups used in Mexico. The SPE method was adapted from Ngumba *et al.* (2016). SPE was carried out using a Phenomenex brand Strata X SPE column (500 mg/6 mL). The phases were conditioned with 6 mL of MeOH followed by 6 mL of ultrapure water and 6 mL of acidified water at pH 3. Subsequently, 500 mL of the effluent and 250 mL of the influent from each sample were passed through the columns at a speed flow rate of approximately 10 mL min^{-1} using vacuum. The cartridges were kept at a temperature of 4 °C until elution. Elution was carried out with methanol at a flow rate of 6 mL min^{-1}. Subsequently, the samples were evaporated to dryness under a stream of nitrogen and then reconstituted to 1 mL with 90:10 (H_2O:MeOH 3 mM ammonium formate, 0.1% formic acid solution). The samples were later stored at -20 °C before analysis. Two reagent blanks were run with samples to evaluate analytical quality and the purity of reagents.

Liquid chromatography coupled to mass spectrometry

For the quantification of antibiotics, a liquid chromatography equipment (Accela 1250 HPLC) was used, coupled to a triple quadrupole mass spectrometer equipped with a heated electrospray ionization source (TSQ Quantum Ultra HESI-II; Thermo Scientific, San José, CA, USA).

Chromatographic separation was performed with a Phenomenex Kinelex C18 column (2.6 μm, 100 × 2.1 mm). The column oven temperature was maintained at 40 °C and the injection volume was 10 μL. The mobile phases consisted of water (aqueous phase A) and methanol (organic phase B), both with 0.1% (v/v) formic acid and 3 mM ammonium formate at a flow of 300 μL min^{-1}. An elution gradient was applied, it being of: 0–2 min: 10% B; 2–7: 10–60% B; 7–9 min: 60–90%; 9–10: 90% B; 10–15 min: 90–10% B (returning to initial conditions); 15–18 min: 10% B (column balance).

In the selected reaction monitoring (SRM) acquisition mode, transitions and collision energies were selected for each compound, which were optimized by direct infusion into the source in a positive mode (Table 1).

The optimal working conditions for the ESI source were: source voltage, 3,500 V; source gas pressure, 28 ua (arbitrary units); desolvation gas pressure, 2 ua; auxiliary gas pressure, 5 ua; vaporization temperature, 300 °C and capillary temperature, 350 °C and collision gas pressure, 1.5 mTorr. For the control of the equipment, the acquisition, and processing of the data, the Xcalibur 2.2 and Trace Finder TM 3.1 software was used. This analysis was carried out at the University of Santiago de Compostela, Spain, in the 'Laboratory of Research and Development of Analytical Solutions (LIDSA)' in the Department of Analytical Chemistry, Nutrition and Food Science of the Faculty of Chemistry.

The method exhibited suitable performance in terms of linearity with R^2 from 0.992 to 0.999, with the exception of ofloxacin, which resulted in the order of 0.987. The instrumental precision showed relative standard deviation (RSD) between 0.4 and 12%. SPE Recoveries were carried out loading fortified ultrapure water (1 μg L^{-1}) at three different pHs between 3 and 5.5, obtaining acceptable values about 60% for the fluoroquinolones and higher than 83% for the SAs. The method precision was also satisfactory, with deviation values lower than 11%. In general, non-significant differences were observed and a pH value of 3 was selected. LODs of the overall SPE-LC-MS/MS were calculated giving a signal-to-noise ratio of three (S/N = 3). They were between 0.7 and 7.1 ng L^{-1} allowing the detection of trace levels for the target antibiotics (see Supplementary Material, Table S1).

Liquid chromatography coupled to high-resolution mass spectrometry

For the identification of unknown EPs present in the WW samples, an HPLC system (Shimadzu Nexera X2) consisting of a low-pressure pump and two high-pressure pumps (LC-20AD and LC 30AD) and an automatic injector (SIL-30AC) was used. HRMS detection was performed using a TripleTOF 5600 + SCIEX (Ontario, Canada) equipped with a DuoSpray ion source and electrospray ionization (ESI).

Chromatographic separation was performed with a Phenomenex Kinetex ECO C18 column (100 × 2.1 mm, 2.6 μm). The column oven temperature was maintained at 40 °C and the injection volume was 10 μL. The mobile phase consisted of water (aqueous phase A) and methanol (organic phase B), both buffered with 3 mM ammonium formate at a flow of 180 μL min^{-1}. An elution gradient of 0–8 min: 10–100% B was applied, with a total time of 15 min.

The conditions for HRMS were: source temperature of 550 °C, ion source gas at 50 ua, sweep gas at 30 ua, and ionization voltage at 5,500 V (4,500 V negative). The HRMS workflow consisted of a complete scan, using 250 ms as the accumulation

Table 1 | List of antibiotics and HESI-II-MS/MS conditions optimized for analysis

Antibiotic	Ionization mode	Precursor ion, m/z	Product ions, m/z (Collision) Energy (eV)	Retention time (min)
Ciprofloxacin	+	332.1	314.1 (21), 288.1 (17), 245.0 (23), 231.0 (35)	5.75
Enrofloxacin	+	360.2	316.0 (20), 245.0 (30)	5.86
Norfloxacin	+	320.1	302.1 (21), 276.1 (16), 233.1 (24), 203.0 (40)	5.61
Ofloxacin	+	362.1	318.2 (18), 261.1 (27), 221.0 (36), 205.0 (40)	5.48
Sulfadiazine	+	251.0	156.0 (17), 108.0 (26), 92.0 (28)	2.71
Sulfadimidine	+	279.1	186.0 (18), 124.1 (25), 108.1 (29), 92.1 (31)	5.30
Sulfamerazine	+	265.0	156.0 (18), 172.0 (17)	4.41
Sulfamethoxazole	+	254.0	156.0 (16), 108,0 (24), 92.1 (28), 65.1 (41)	5.73

time and 80 V (−80 V in negative mode) as the decomposition potential in the ESI. Simultaneously, an independent acquisition approach to the data was performed based on SWATH (Sequential Window Acquisition of all Ion Mass Spectra of Theoretical Fragments). A wide mass range (80–850 Da) was divided into 30 mass windows with an accumulation time of 35 ms for each. The decay potential was adjusted to 80 V (−80 V in negative mode) and the collision energy was 40 V (−40 V in negative mode), with a power distribution of 20 V. It was operated with Analyst 1.7.1 control software, PeakView 2.2 processing software, and MultiQuant 3.0 quantification software. This study was carried out at the Centro de Investigaciones Agrarias (AGACAL-CIAM), Unidad de Contaminantes Orgánicos, in A Coruña, Spain.

Antibiotic removal efficiency

The antibiotic removal efficiency was used for the concentration of the target antibiotics in the influent and the effluent in each of the samplings, the equation proposed by Kovalova *et al.* (2012) was calculated.

$$\%\mathrm{Removal} = \left(\frac{(C_i - C_e)}{C_i}\right) \times (100) \tag{1}$$

where C_i is the concentration of the antibiotic in the influent and C_e is the concentration of the antibiotic in the effluent.

Quality control

Procedural blanks were included in all analyses in order to avoid false-positive findings.

The linear relationship between target compounds contents and peak areas was daily checked as well as the intraday precision. Samples were analyzed in triplicate ($n = 3$), with each replicate being produced from a subsample of the original sample.

Firstly, the quality criteria for potential compound identification were based on the exact masses of the unknown compounds, accepting only mass error below 5 ppm. However, for the unambiguous identification of these compounds, additional criteria besides exact mass were needed. Therefore, isotope profile scores and high-resolution MS/MS spectra were also taken into account for confirmation. Spectral libraries containing more than 2,000 high-resolution mass spectra were available.

The combination of the three above-mentioned criteria provided a final combined score. All results showing a combined score below 50 were excluded from the final list of identified compounds.

RESULTS AND DISCUSSION

Antibiotics quantification by LC-MS/MS

This research provides pertinent data on the identification and quantification of various EPs present in effluent samples in an urban WWTP. Three samples were analyzed at different times of the year, and the concentrations in (ng L^{-1}) of two groups of widely used antibiotics were determined sulfonamide drugs (sulfamethoxazole) and fluoroquinolones (ciprofloxacin, norfloxacin, and ofloxacin), as well as their respective removal percentage, is shown in Table 2, demonstrating that the WWTP fails to eliminate 100% of various types of contaminants, although the degree of elimination can be considered acceptable since the compounds were removed in more than 80% in most cases.

Sulfamethoxazole was detected in concentrations of 231.6–153.6 ng L^{-1} in the influent and 87.5–8.6 ng L^{-1} in the effluent with a removal efficiency of 62–94%. A similar concentration in the influent but lower removal efficiency (17%) was reported by Rosal *et al.* (2010), who studied an urban WWTP, located in Madrid, Spain, operated under A2O multistage configuration with nitrification–denitrification and enhanced phosphorous-accumulating microorganisms. In contrast, Estrada-Arriaga *et al.* (2016) found results with different concentrations (1,190 and 2,330 ng L^{-1}) and determined that sulfamethoxazole was not removed, both in the rainy and the dry season, from an urban WWTP located in Guanajuato, Mexico. The WWTP is equipped with oxidation ditches in simultaneous nitrification–denitrification, designed for the removal of organic matter and nitrogen with disinfection module through UV. Estrada-Arriaga *et al.* (2016) also analyzed an activated sludge plant with simultaneous nitrification–denitrification, and phosphorus removal, with disinfection with chlorine dioxide and UV located in Valle de Bravo, Mexico, detecting high percentages of removal rates of sulfamethoxazole (99–96%). Another study by Rivera-Jaimes *et al.* (2018) showed a removal percentage of sulfamethoxazole with 36 and 53% in a WWTP of

Table 2 | Antibiotic results with their respective concentrations (ng L^{-1}) and their removal percentages

Sample	Influent	Effluent	% Removal
Sulfamethoxazole			
Sampling 1	231.63	87.47	62.2
Sampling 2	153.63	9.27	94.0
Sampling 3	154.13	8.63	94.4
Ciprofloxacin			
Sampling 1	2,227.33	444.70	80.0
Sampling 2	1,717.10	494.60	71.2
Sampling 3	2,733.33	461.43	83.1
Norfloxacin			
Sampling 1	45.8	8.3	81.9
Sampling 2	_	31.7	_
Sampling 3	42.8	4.6	81.9
Ofloxacin			
Sampling 1	291.47	52.47	82.0
Sampling 2	299.57	64.43	78.5
Sampling 3	338.93	7.07	97.9

Cuernavaca, Mexico. The WWTP is equipped with a conventional activated sludge (CAS) treatment after aerobic and anaerobic digestion, and a tertiary treatment based on UV oxidation.

Fluoroquinolone ciprofloxacin was detected in concentrations of 2,733–1,717 ng L^{-1} in the influent and 494.6–444.7 ng L^{-1} in the effluent with a removal efficiency of 71–83%. The concentrations determined in this study are lower than those reported by Rosal et al. (2010), who archived removal of 57%. Estrada-Arriaga et al. (2016) obtained different results (21 and 89%), in the WWTP of Guanajuato and 85% to complete removal, in the WWTP of Valle de Bravo. Norfloxacin was detected in concentrations of 45.8–42.8 ng L^{-1} in the influent and 8.3–4.6 ng L^{-1} in the effluent with a removal efficiency of 82–89%. Norfloxacin was not detected in the two plants studied by Estrada-Arriaga et al. (2016). Ofloxacin was detected in concentrations of 338.9–291.5 ng L^{-1} in the influent and 64.4–7.1 ng L^{-1} in the effluent with a removal efficiency of 78–98%. The concentrations determined in this study are lower than those reported by Rosal et al. (2010), who archived removal of (64%). The percentages have different removal rates and effluent concentrations compared with the study by Estrada-Arriaga et al. (2016) that determined different removals in the rainy than the dry season, as well as different concentrations, they reported ofloxacin removal of (0 and 63%) in the WWTP of Guanajuato and complete removal in the WWTP of Valle de Bravo.

The difference in the concentration of the antibiotics studied with respect to other WWTPs in other regions shows the variation in the amount of antibiotics used in the different regions. Removal efficiencies may be mainly due to variations in activated sludge treatment. The Acapulco WWTP shows acceptable performance compared with the Madrid and Guanajuato plants, but is lower than the performance of the Valle de Bravo plant.

Identification of emerging contaminants by LC-HRMS

A non-target study of the samples was carried out to identify unknown compounds in the influent and effluent of the WWTP (Tables 3 and 4). These compounds were identified by LC-HRMS (SWATH), using libraries containing more than 2,000 high-resolution mass spectra. Isotope profiles and mass accuracies were also taken into account and the combination of the three criteria provided a final combined score. All results showing a combined score below 50 were excluded from the final list of identified compounds.

The intensity of the responses of the different types of drugs, such as antibiotics, illicit drugs, antipsychotics, β-blockers, anti-inflammatories, analgesics, anticonvulsants, dewormers, stimulants, hormones, muscle relaxants, and antihistamines, was used to compare the levels of compounds in the samples. Different pesticides were also identified including insecticides,

Table 3 | Identification of the different groups of non-target drugs

Drug	Category	Influent	Effluent
Caffeine	Stimulants	S1, S2, S3	
Theophylline		S1, S2, S3	
Naproxen	Anti-inflammatory	S1, S2, S3	
Ketorolac		S1, S2, S3	
Acetaminophen (paracetamol)	Analgesic	S1, S2, S3	
Albendazole	Deworming	S1, S2, S3	
Benzoylecgonine	Illicit drugs and their metabolites	S1, S2, S3	
Cocaine		S1, S2	
Norcocaine		S1	
Cimetidine	Antihistamine	S2	
Medroxyprogesterone acetate	Hormone	S3	
Olanzapine	Antipsychotic	S3	
Trimethoprim	Antibiotics	S1, S3	
Erythromycin		S2, S3	
Nalidixic acid		S1, S2, S3	S1, S2, S3
Levofloxacin		S1, S2, S3	S1, S2
Lidocaine	Local anesthetic	S1, S2, S3	S1, S2, S3
Oxcarbazepine	Anticonvulsants	S1, S2, S3	S1, S2, S3
Carbamazepine		S1, S2, S3	S1, S2, S3
Metaxalone	Muscle relaxant	S1, S2, S3	S1, S3
Metoprolol	β-blocker	S2, S3	S1, S3
Alprenolol		S2, S3	S2

S1, composite sample September 2017; S2, composite sample February 2018; S3, composite sample July 2018.

Table 4 | Pesticides detected in the influent and effluent samples through non-target analysis

Pesticides	Category	Influent	Effluent
DEET	Insecticide	S1, S2, S3	S1, S2, S3
Carbofuran	Insecticide	S2, S3	S3
Dichlorvos	Insecticide	S1, S2, S3	
Malathion	Insecticide	S1, S2, S3	
Pyrethrin I	Insecticide	S1, S3	
Promecarb	Insecticide	S1, S3	
Pirimiphos-methyl	Insecticide	S1	
Trimetacarb	Insecticide	S1	
Tridemorph	Fungicide	S1, S2, S3	S1, S2, S3
Silthiofam	Fungicide	S1, S2, S3	
Norflurazon	Herbicide	S1	
Butralin	Herbicide	S3	
Quinoclamine	Algaecide	S2	

S1, composite sample September 2017; S2, composite sample February 2018; S3, composite sample July 2018.

herbicides, and fungicides; in addition to a plasticizer and fire retardant (triphenyl phosphate). A high number of non-target compounds were identified in the effluent and especially in the influent samples, using the HRMS technique.

Despite being one of the model plants in Mexico, it fails to remove 100% of various types of EPs, although in general, the degree of elimination is high, and many of the non-target compounds were only detected in the influent samples. It should be mentioned that these types of pollutants have been widely used and therefore detected in different parts of the world, and for this reason, they remain within the main lines of research by the scientific community.

Drugs removed by the WWTP

Different groups of drugs were only detected in the influent samples. This is the case of caffeine and theophylline (stimulants), naproxen and ketorolac (anti-inflammatories), paracetamol (pain reliever), albendazole (dewormer), cocaine and its metabolites (illicit drugs), medroxyprogesterone acetate (hormone), erythromycin and trimethoprim (antibiotics), which could indicate they are removed by the WWTP (Figure 2). Removal percentages of some of these compounds in an activated sludge WWTP were reported by Rosal et al. (2010), who reported similar removal of paracetamol and caffeine (100 and 95%, respectively); different removals to naproxen and ketorolac (61 and 44%, respectively); and low removals of trimethoprim and erythromycin (5 and 4%, respectively). With the exception of ketorolac, albendazole, and the hormone, medroxyprogesterone acetate, all other drugs were reported in the two WWTPs studied by Estrada-Arriaga et al. (2016), who reported high removal rates of 96–100%, while the use of oxidation ditches without disinfection with chlorine dioxide showed a weakening in the elimination of antibiotics (erythromycin and trimethoprim) and cocaine and its metabolite (benzoylecgonine). Results of Rivera-Jaimes et al. (2018) showed a complete removal rate of paracetamol and high removal rates of 90–97%, in contrast, with incomplete removal of trimethoprim (0–50%).

Drugs not removed by the WWTP

Other drugs such as antibiotics, levofloxacin and nalidixic acid, anticonvulsants, carbamazepine and oxcarbazepine, lidocaine (local pain reliever), among others were detected in both influent and effluent samples, indicating that they are not
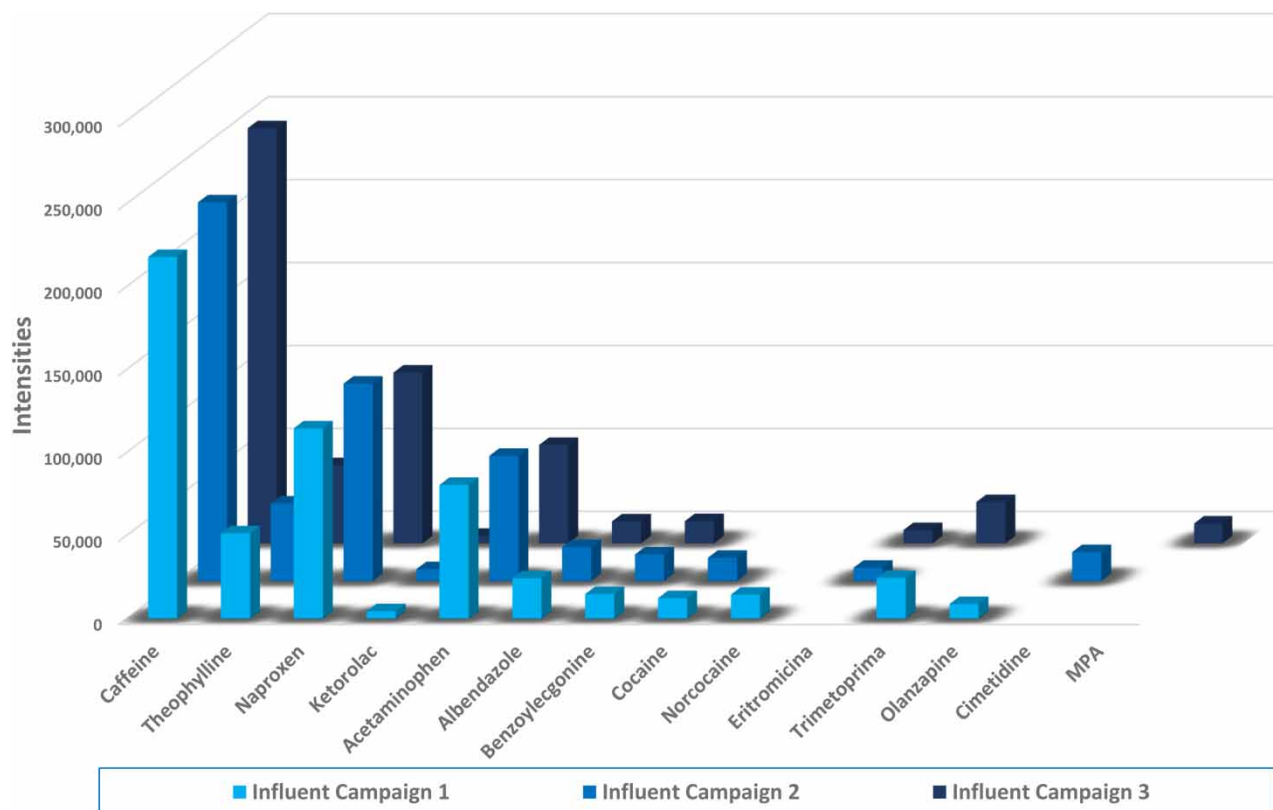

Figure 2 | Drugs removed by the municipal WWTP in Mexico. MPA, Medroxyprogesterone acetate.

removed or partially removed by the WWTP (Figure 3). Carbamazepine was persistent, which agreed with the result from Rosal *et al.* (2010), Rivera-Jaimes *et al.* (2018), and Estrada-Arriaga *et al.* (2016). Beta-blocker Metoprolol was described as persistent by Estrada-Arriaga *et al.* (2016).

Pesticide identification

A high number of pesticides were identified in the WW samples. DEET and silthiofam were the compounds with the most intense signal. With the exception of DEET, tridemorph, and carbofuran, the other pesticides were below detection in the effluent samples, which means that they are removed in very high percentage by the WWTP (Table 4). In the case of DEET, the intensity in the influent samples is much higher compared with the effluent samples in the three sampling campaigns, which means that although it is not completely eliminated, its removal is a very high percentage (>90%) (Figure 4). Tridemorph was detected in the three sampling campaigns, and the intensities in the effluent samples were lower than in the influent samples with an average removal efficiency about 60%.

Carbofuran was detected in the influent in two campaigns (S2 and S3), but only in the effluent of campaign 3. These three pesticides are not completely removed by the WWTP.

Some of the pesticides identified in this study are included in the list of highly hazardous pesticides (HHPs) by the PAN (Pesticide Action Network International) (Neumeister & Weber 2015), due to their acute toxicity, long-term effects, environmental toxicity, and/or because their uses are regulated or prohibited by international agreements in some countries. Carbofuran and dichlorvos are classified as highly dangerous pesticides (Class Ib) according to the WHO and as deadly substances if inhaled (H330), according to the Globally Harmonized System of Classification and Labeling of Chemical Products (GHS), in addition to carbofuran being on the list of pesticides stipulated in the Rotterdam Convention. Malathion is
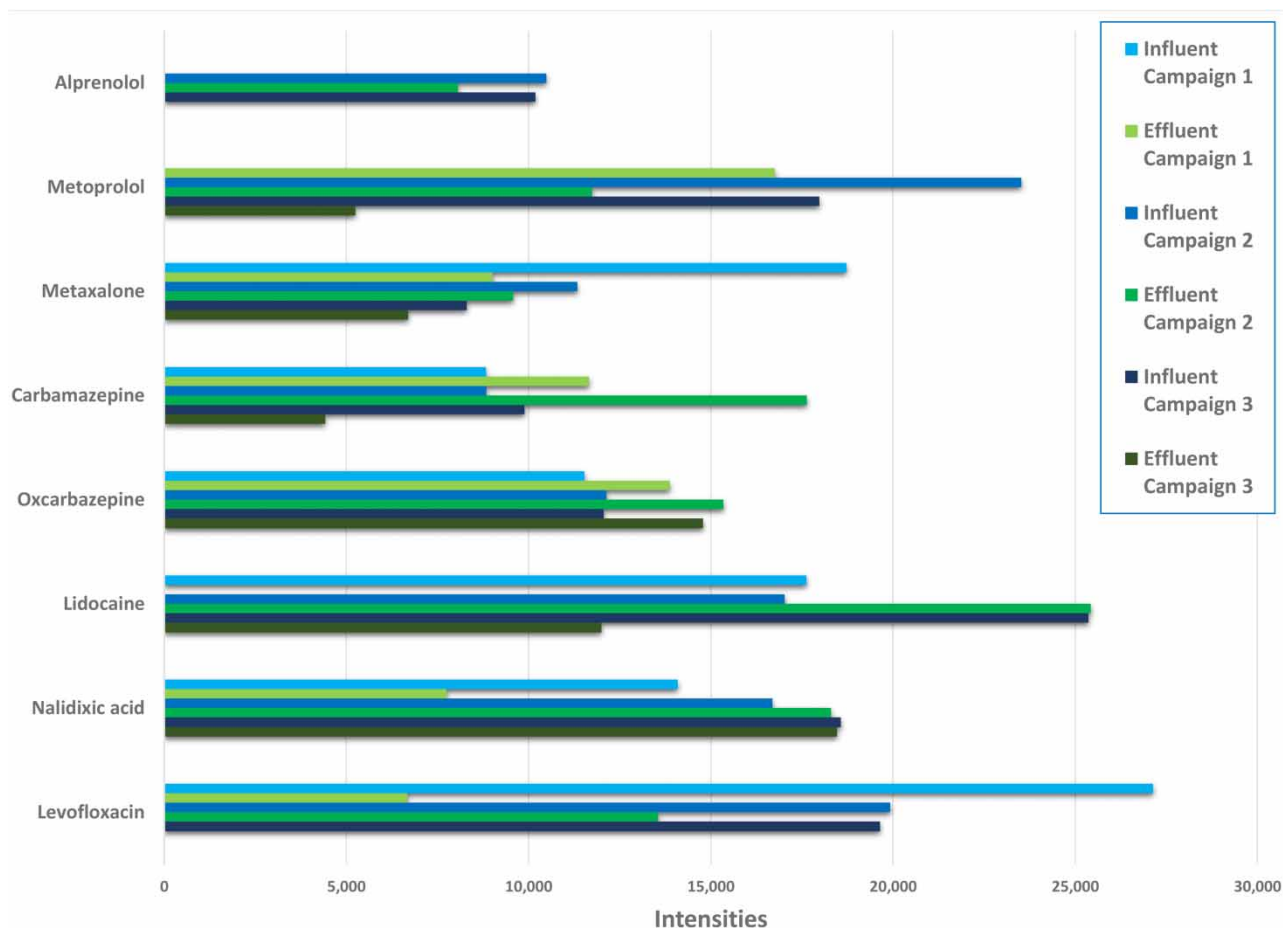

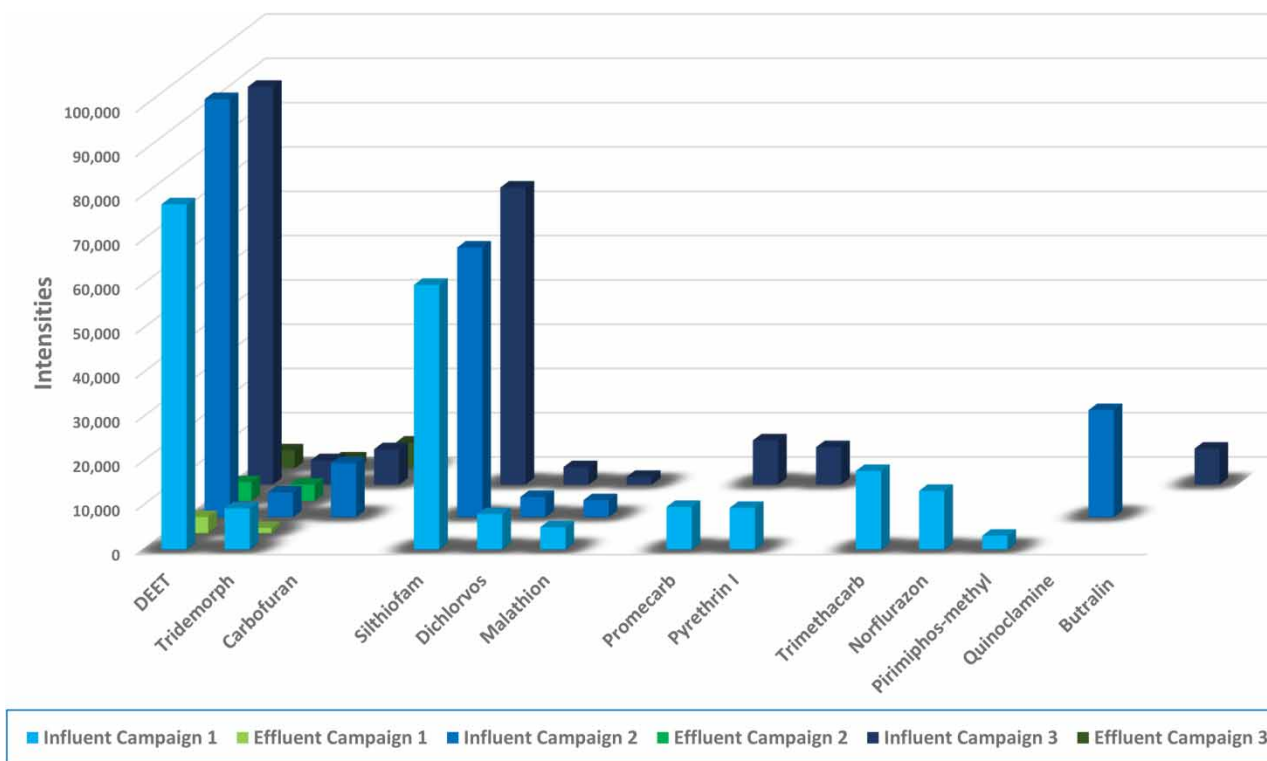**Figure 3** | Drugs not removed by the municipal WWTP in Mexico.

Figure 4 | Pesticides identified in the WWTP.

considered a probably carcinogenic substance by the International Agency for Research on Cancer IARC (2006). Tridemorph is listed as a toxic substance for human reproduction in the European Union (EC 2008). The pesticide pirimiphos-methyl is considered HHPs according to the list of PAP due to its high degree of environmental toxicity, since it is very toxic to bees, essential in pollination.

Trimetacarb (trimethacarb, landrin) is an insecticide without EU's regulatory approval for use. It is moderately soluble in water, very volatile and there is also a slight risk of leaching to groundwater. In general, it is not persistent in soil systems; however, it can degrade rapidly by hydrolysis in aquatic systems. It should be mentioned that it is moderately toxic to mammals. The carbamate pesticides trimethacarb were detected in a non-target analysis of the dryer lint samples collected from two households in the Netherlands (Ouyang *et al.* 2017).

CONCLUSION

The data presented above show the presence of EPs in the effluent of the WWTP studied, which represents a priority problem at present in Mexico, as well as worldwide. Most of the research comes from developed countries, because they have the necessary economic resources and infrastructure. However, water contamination also exists in developing countries such as Mexico, which is why they are considered to be pollutants that must be continuously monitored even though they are not regulated by Mexican Standards.

Removal rates for the target compounds (sulfamethoxazole, ciprofloxacin, norfloxacin, and ofloxacin) were high, as well as for most of the non-target compounds. However, some of the identified compounds, mainly drugs, are not effectively eliminated by the urban WWTP, being discharged into the effluent (e.g. antibiotic levofloxacin, anticonvulsant carbamazepine, beta-blocker metoprolol). On the other hand, among the non-target compounds, pesticides were identified, some of which are not eliminated by the WWTP (e.g. tridemorph).

Considering that the discharge of the studied WWTP is located in a world-renowned tourist destination, and its discharge is done near the tourist beaches, the treatment conditions must be improved by applying efficient tertiary treatments in the removal of the different contaminants detected, to improve the quality of treated water and reduce contamination by EPs, which would allow it to be safely reused for recreational activities.

ACKNOWLEDGEMENTS

FUNDING

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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