UNIVERSIDAD AUTÓNOMA DE BAJA CALIFORNIA

Maestría y Doctorado en Ciencias e Ingeniería
Instituto de Ingeniería

Compounds of Emerging Concern: Occurrence, sources and environmental risk of pharmaceuticals in surface waters in Mexicali and its surrounding valley as a demonstration site in Latin America

A Dissertation Submitted to the
Universidad Autónoma de Baja California

In Partial Fulfillment to the Requirements for the Degree of
Doctor of Philosophy
In Engineering

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2019

UNIVERSIDAD AUTÓNOMA DE BAJA CALIFORNIA
Proof Of Approval

Date: 22 January 2019

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Acknowledgements

I am deeply grateful to God for giving me the opportunity to be here in this earthly world with the purpose of progressing temporally and spiritually. Thank you for your unceasing guidance and the people who accompany me on this journey. Thank you for this great opportunity that you give me to acquire and increase my knowledge. Thanks for this new achievement. I love you

And if a person gains more knowledge and intelligence in this life through his diligence and obedience than another, he will have so much the advantage in the world to come. (D&C130:19)

I want to say thanks to my mother Margarita Carrillo who has worked very hard to give me the best. I love you, Mom. Thanks for supporting and encouraging me in my university studies. Also, I want to thank my aunt Teresa Carrillo for always being by my side, really appreciate all that you do for me. Thank you both for trusting me and being part of my achievement degrees. Thanks to my entire family, who have also supported me very much. I love you all.

I also want to extend my sincerest thanks to my advisor Dr. Concepcion Carreon for accepting me once again as her student and for trusting me to carry out this doctoral project. I appreciate your observations, recommendations and great contributions to this manuscript and research. Thank you for your support, patience, advice and for contributing to my growth as a researcher. Without any doubt, I admire you.

I would like to express my gratitude to all my professors especially Dr. Jorge Ramirez and Dr. Jaime A. Reyes, who have also contributed a lot in my growth as researcher, thanks for all their valuable teachings as much of chair as of field, and laboratory. I also thank Drs. Socorro Hernandez, Fernando Solís and Leif Abrell for your comments and observations to this research. Thanks all of you for your friendship, and I think you all are extraordinary people.

I am also grateful to my project colleagues Ivan Aviña and Ramon Preciado for their help in the field and laboratory activities. To my entire fellow graduates who made these four years of study more bearable.

Thanks to the Mexican National Council for Science and Technology (CONACYT) for the scholarship granted to obtain my doctoral degree and to The Consortium for Arizona-Mexico Arid Environments (CAZMEX) for financing the research project. Finally, thanks to the Institute of Engineering for hosting me as a student and for having the Master's and Doctorate in Science and Engineering (MyDCI) program, without this nothing would have been possible. Thank you for training researchers and promoting high-level research.

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List of Abbreviations for the entire document. **It does not include the pharmaceuticals**

ACN	Acetonitrile
AF	Assessment Factor
CR	Colorado River
DF	Dilution Factor
EC	Electrical Conductivity
EC50	50% Effective concentration
ECOSAR	Ecological Structure Activity Relationship
ECs	Emerging Contaminants
EDCs	Endocrine Disrupting Compounds
EDTA	Ethylenediaminetetraacetic Acid
EMEA	European Agency Medicine
EOCs	Emerging Organic Compounds
ERA	Environmental Risk Assessment
ESI	Electrospray Ionization
EU	European Union
HR	Hardy River
LATAM	Latin America
LC50	Lethal Concentration of 50%
LCMS	Liquid Chromatography Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
MEC	Measured environmental concentration
MeOH	Methanol Solution
MRM	Multiple Reaction Monitoring
MS/MS	Tandem Mass Spectrometry
MTBE	Methyl Tert-Butyl Ether
MZMV	Metropolitan Zone of the Mexico City
NH_4OAc	Ammonium Acetate Solution
NOEC	No observed effect concentration
NR	Nuevo River
NSAIDs	Nonsteroidal anti-inflammatories drugs
PCPSs	Personal Care Products

PhACs	Pharmaceutical Active Compounds
PNEC	Predicted no affect concentration
QToF	Quadrupole Time of Flight
Qx	Average Flow
RQ	Risk Quotient
RSD	Relative Standard Deviation
SD	Standard Deviation
SPE	Solid Phase Extraction
SW	Surface Water
Tr	Retention Time
TWW	Treated Wastewater
UPLC	Ultra-performance liquid chromatography
WW	Wastewater
WWTP	Wastewater Treatment Plants

1 CHAPTER

Introduction

Abstract

Reports concerning the quantitative analysis of Pharmaceutical Active Compounds (PhACs) in the Latin American (LATAM) ecosystems are limited even though they are essential to determine the occurrence, sources and fate of pharmaceuticals in the environment and, ultimately, assess the risk of exposure of organisms and their habitats. Therefore, this dissertation includes an exploratory analysis of a decade of available data on the occurrence and sources of PhACs in LATAM. Said analysis is focused on the aquatic environment and involves a critical assessment of the current situation of PhACs in LATAM, compared with other regions of the world. References were obtained through a comprehensive revision of the state of the art in different sources of scientific information, such as academic and electronic database and search engines. The selected information was subjected to data management and statistical analyses. To elucidate the complexities of this endeavor, this investigation was strengthened with a study case on the occurrence and sources of PhACs, including an approach to the Environmental Risk Assessment (ERA) for aquatic organisms, in a specific site of LATAM, the city of Mexicali and its surrounding agricultural valley, in northwestern México. A total of 23 sites were selected in a non-parametric approach, including the influents and effluents of three Waste Water Treatment Plants (WWTPs), the three local river channels, and several irrigation canals and agricultural drains along the city and the valley. Twelve PhACs were selected taking care to consider the therapeutic classes of worldwide concern, i.e., antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), psychiatric drugs, lipid regulators, beta-blockers, and synthetic hormones. PhACs in environmental samples were determined using Solid Phase Extraction (SPE) and Liquid Chromatography tandem Mass Spectrometry (LC/MSMS) with electrospray ionization. Results from the critical analysis indicate that in LATAM there were only 66 investigation reports available along the last decade (2007-2017), whereas the quantification of PhACs in diverse environmental compartments was documented in barely ten countries of this region. Surface water was the most frequently analyzed compartment while Ibuprofen (IBF) and Diclofenac (DCF) were found to be the most investigated and detected compounds in LATAM. Wastewater, treated and untreated (no statically significant difference found between the two) proved to be the main source of PhACs in the environment. The concentration observed in LATAM were constantly higher

than those reported elsewhere for all the analyzed compounds. On the other hand, only five out of twelve PhACs were detected in the aquatic environment of Mexicali and its surrounding valley, being Sulfamethoxazole (SMX) and Carbamazepine (CBZ) the most detected (85% of the times) followed by IBF, DCF and MTP, all showing much lower frequencies of detection. Results indicate that substantial concentrations of PhACs (up to 1705 ng/L of IBF and 830 ng/L of SMX) reached the aquatic environment during the spring of 2015 through the discharges of WWTPs effluents. Once in the environment, the PhACs concentrations decreased with distance from the source, although small amounts of all five compounds dissolved in the waters of the Nuevo River were inadvertently transferred toward the Salton Sea basin (USA). This situation also seems to occur in the opposite direction, when the waters of the Colorado River enter Mexican territory carrying some dissolved compounds. In general, the local WWTPs were able to reduce the concentrations of NSAIDs in their effluents, but not those from other compounds. Nevertheless, IBP was found as far south as the estuary zone of the Gulf of California probably due to additional not-identified sources. Additionally, concentrations of SMX where constantly higher than those reported elsewhere (0.2-830.9 ng/L), marking it as the compound of maximum environmental concern in the vicinity of Mexicali. ERA for the three key aquatic organisms in the trophic chain (algae, daphnia and fish) using the three most likely scenarios (single compound, mixing of compounds and dilution factor over a mixing) showed that only SMX posed high risk for algae, which resulted the most sensitive organism under each scenario. The rest of the compounds mostly posed low to medium risk, at the worst, for the three organisms and scenarios during the spring of 2015.

1.1 Problem Definition

Currently, the continuous presence of pharmaceutical active compounds (PhACs) residues in the aquatic environment is considered of concern due to the potential threats they may pose to non-target organisms and the human health, and the possible ecological impact they may involve. Usually, these compounds are derived from the release into the environment of municipal wastewater effluents and detected in sub-therapeutic concentration levels.

PhACs are not regulated compounds, therefore, they are part of the so-called compounds of emerging concern (CEC) and share a number of their environmental issues. For instance, their recurrent presence in natural waters and the terrestrial and aquatic organisms, their so often unknown sources and fate, and the inefficiency of the removal treatments to prevent them from reaching the environment, in addition to their potential toxicological effects and ability for bioaccumulation and biotransformation, being the latter responsible for producing metabolites and transformation products even more hazardous than the parent compounds.

Nowadays, the scientific community has confronted these problems through the implementation of continuous environmental monitoring and environmental risk assessments, by using modern sensitive and selective detection techniques, such as high or ultra performance liquid chromatography in tandem with mass spectrometry, and applying predictive modeling of toxicity, such as Ecological Structure Activity Relationship (ECOSAR). Largely, continuous investigation is crucial to gain sufficient information to support priority lists that include pharmaceuticals as harmful compounds for the environment, that attract the attention of public opinion and decision-makers, and that lead to the creation and compliance of regulations.

A critical analysis is required to obtain an accurate assessment of the current state of PhACs in the aquatic environment in Latin America, a region of the world with environmental diversity but with comparable social, political and economic conditions, which entails similar environmental risks derived from the presence of these compounds.

1.2 Research Objectives

1.2.1 General Objective

This research effort was performed with the general objective of evaluating the present state of the occurrence of PhACs in the aquatic environment of Latin America, including their concentrations, spatial distribution and sources, with special emphasis in the assessment of the environmental risk that these compounds represent for the aquatic organisms in a demonstration site in México.

1.2.2 Specific Objectives

- To investigate the occurrence and concentration patterns of PhACs in the aquatic environment of Latin America, relying on previous research
- To identify the main sources of PhACs in the environment of Latin America
- To determine the environmental occurrence and concentration of PhACs as emerging contaminants in surface water bodies at a demonstration site in Latin America, the city of Mexicali and its surrounding valley.
- To assess the environmental risk associated with the presence of PhACs in treated wastewater released to the environment of the Mexicali Valley.

1.3 Explanation of dissertation format

This manuscript is divided into three major individual appended chapters; chapter one presents a general introduction of the research including, problem definition and general and specific objectives, as well as the methodological framework.

Chapter two is intended as an individual scientific article, therefore, it is composed of all the appropriate sections. It contains an overview of PhACs as compounds of emerging concern in a general context, followed by an exploratory analysis of the available scientific documents and a compilation of data on the occurrence and sources of pharmaceuticals in Latin America. This chapter also provides a critical analysis of the current situation of PhACs in the aquatic environment of Latin America compared with countries worldwide.

Chapter three is also designed as an individual scientific article. It consists of a particular study on the occurrence and source of PhACs in a specific site of Latin America. This chapter comprises a general overview of the site and background research related of PhACs as concerning emerging contaminants, describe the study area and the method and materials, as well as results and conclusion. In addition, this study includes an approach to the environmental risk assessment for aquatic organisms.

Finally, given the format of chapters two and three, each one includes its own definitions and a list of references, which were cited to support the specific research objectives. Annexes were included for detailed data and results.

1.4 Methodological framework

This section contains details of the methodology that were not contemplated in the following chapters. Most of this information was previously published in the form of a book chapter written by the defender and her research team to comply with the requirements of her doctoral program (Valdez-Carrillo et al., 2016).

1.4.1 Selection and collection of environmental samples

The sampling methods, the sites of interest and the number of samples required to characterize an environmental problem are determined both by the research approach and by the specific conditions of the site. Given the diversity of said site conditions, there is not a universal procedure, however, the experience from numerous investigations points towards the non-probabilistic sampling method. This is a well-accepted method in environmental research since the selection of a sample depends more on the objectives and requirements of the study than on the probability. The non-probabilistic samples are not necessarily representative since they have been selected to reflect the characteristics specified in the definition of the problem (Rabolini, 2009).

Grab and composite are the two types of samples most frequently used to investigate the occurrence of CEC in the environment. Grab samples are mainly applied in the determination of compounds in wastewaters (Bahlmann et al., 2014), in waters from rivers (Mendoza et al., 2014) and from other type of surface waters (Caldas et al., 2013). However, for an integrated composition of several days or even months, the composite sampling is the preferred methods (Brandt et al., 2013; Gómez et al., 2010).

Among the aspects to consider when selecting a sampling point and the number of samples to collect are the physical and environmental characteristics of the potential study site, including location, security and access, and the scope of the study with its economic limitations. All the studies mentioned in this document (Chapter 2), including the demonstration site (Chapter 3), show a detailed account of the selection of sampling points and the environmental conditions of each site under examination.

The list of the equipment and materials used to collect water samples for the determination of PhACs in the aquatic environment of the demonstration site discussed in Chapter 3 is shown in Table 1.1. In general, similar equipment has been used in the collection of grab samples for the same purpose around the world.

Table 1.1 Equipment and Materials used to collect environmental water samples

Equipment or Material	Purpose
1L Glass amber bottles	Collection and storage of samples
Gloves	Processes related to taking samples and measuring parameter
A set of glass beakers	Measurement of physicochemical parameters
GPS	Location of sampling sites
Water quality instruments	Measure pH, water temperature, electrical conductivity, and dissolved oxygen
Basic reagent kits (HACH)	Measurement of nutrients and other desired chemical parameters
MiliQ water and Ethanol	Equipment for cleaning and decontamination processes
A waste container	Storage and transport of liquid waste
Paper towels	Drying throughout the entire process
Indelible marker and tape	Label the sample bottles
Log file	Registration of sites and distances traveled
Field notebook	Registration of samples and field data
Cooler and ice	Transport of samples at 4°C

1.4.1.1 Manufacture of a manual water sampling rod

For the collection of water samples in difficult-to-reach sites, it is necessary the use of a sampling rod (Figure 1.1a). Since it was not possible to find in the market one that met the needs of the project, a water sampling rod was developed that met the following conditions: avoiding cross-contamination, adjustable to different bottle sizes (glass or plastic) (Figure 1.1b), capable of reaching sites relatively far from the edge of the water bodies (Figure 1.1c) and depths of at least 2 meters.

(a) (b) (c)

Figure 1.1 Water sampling rod; (a) collection of the sample in a site of difficult access, (b) bottle adjustment, (c) extension of the rod.

1.4.2 Selection of the PhACs of interest

The selection of PhACs of interest at the demonstration site was based on the examination of the state of the art, bearing in mind the numerous reports on the generalized occurrence of this compounds in wastewater and surface water throughout the planet, the severity of their effects, real or potential, on the ecosystems and their constant presence in the environment (e.g., Caliman and Gavrilescu, 2009; Lambropoulou and Nollet, 2014; Rodríguez-Gil et al., 2010). On the other hand, an attempt was made to consider the local consumption and disposal habits of pharmaceuticals via a pool developed and applied for this purpose, however, the results were not ready for use on time for this sampling campaign. Those results are included in this document as Appendix A and will be used in future analyses. Therefore, the selected compounds are listed Table 1.2, which includes two anti-inflammatory drugs, two antibiotics, two lipid regulators and one of each of the following groups, antidepressants, neuroactive drugs, β-blockers and hormones, for a total of ten compounds of interest. The table also includes their molecular weight, the index to express the acidity of weak acids (pK_a) and the index to state the degree of hydrophobicity, the octanol/water partition coefficient expressed in logarithmic terms (logK_{ow}).

Table 1.2 The most detected/studied PhACs in the aquatic environment. A selection used at the demonstration site discussed in Chapter 3

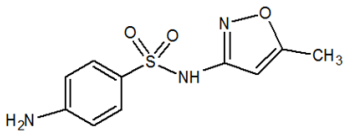
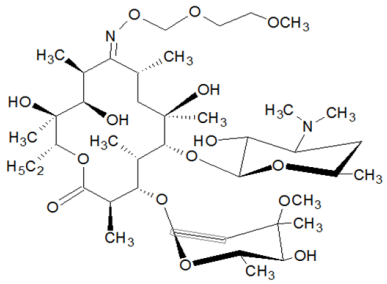
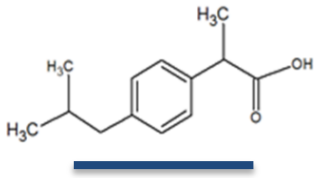
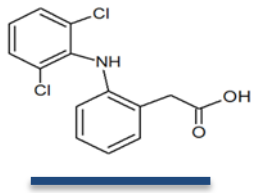
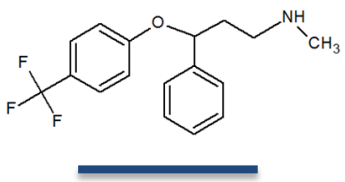
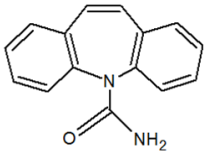
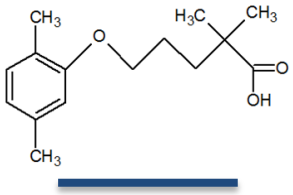
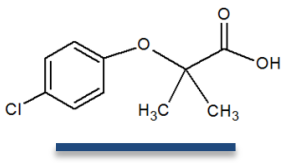
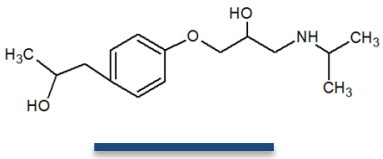
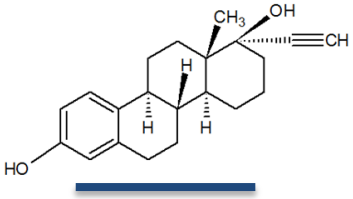
Compound/Group	Structure/Formula	Chemical Properties
Sulfamethoxazole **Antibiotics**	 **$C_{16}H_{19}N_3O_5$**	**CAS number** 723-46-6 **Molecular weight** 365.40 g/mol **pK_a** 5.8 **log K_{ow}** 0.9-2.5 (Kasprzyk-Hordern et al., 2007)
Roxithromycin **Antibiotics**	 **$C_{41}H_{76}N_2O_{15}$**	**CAS number** 80214-83-1 **Molecular weight** 836.52 g/mol **pK_a** 9.2 **log K_{ow}** -- (Göbel et al., 2004)
Ibuprofen **Analgesics and Anti-inflammatory**	 **$C_{13}H_{18}O_2$**	**CAS number** 15687-27-1 **Molecular weight** 206.29 g/mol **pK_a** 4.9 **log K_{ow}** 3.5-4.0 (Kasprzyk-Hordern et al., 2008)
Diclofenac **Analgesics and Anti-inflammatory**	 **$C_{14}H_{10}Cl_{12}N_2O$**	**CAS number** 15307-86-5 **Molecular weight** 296.15 g/mol **pK_a** 4.2 **log K_{ow}** 4.2–4.5 (Kasprzyk-Hordern et al., 2008)
Fluoxetine **Antidepressants**	 **$C_{17}H_{18}F_3NO$**	**CAS number** 54910-89-3 **Molecular weight** 309.13g/mol **pK_a** 8.7 **log K_{ow}** 3.82 (Meritxell et al., 2006)

Table 1.2 The most detected/studied PhACs in the aquatic environment. A selection used at the demonstration site discussed in Chapter 3

Compound/Group	Structure/Formula	Chemical Properties
Carbamazepine **Neuroactive drugs**	 **$C_{15}H_{12}N_2O$**	**CAS number** 298-46-4 **Molecular weight** 236.27g/mol **pK_a** 13.9 **log K_{ow}** 2.4-2.9 (Kasprzyk-Hordern et al., 2008)
Gemfibrozil **Lipid Regulators**	 **$C_{15}H_{22}O_3$**	**CAS number** 25812-30-0 **Molecular weight** 150.15 g/mol **pK_a** 4.7 **log K_{ow}** 4.77 (Meritxell et al., 2006)
Clofibric Acid **Lipid Regulators**	 **$C_{10}H_{11}ClO_3$**	**CAS number** 882-09-7 **Molecular weight** 214.0 g/mol **pK_a** 3 **log K_{ow}** 2.5/2.6 (Kasprzyk-Hordern et al., 2008; Silva, 2008)
Metoprolol **β-blocker**	 **$C_{15}H_{25}NO_3$**	**CAS number** 37350-58-6 **Molecular weight** 267.36 **pK_a** 9.7 **log K_{ow}** 1.9–2.5 (Kasprzyk-Hordern et al., 2007)
17α-Ethinylestradiol **Hormones**	 **$C_{20}H_{24}O_2$**	**CAS number** 57-63-6 **Molecular weight** 296.41 g/mol **pK_a** 10.4 **log K_{ow}** 3.67/4.15 (Daughton and Ternes, 1999a; Silva, 2008)

1.4.3 Samples preparation

Once collected and prior to the analytical procedure, the environmental samples go through the preparation process summarized in Figure 1.2.

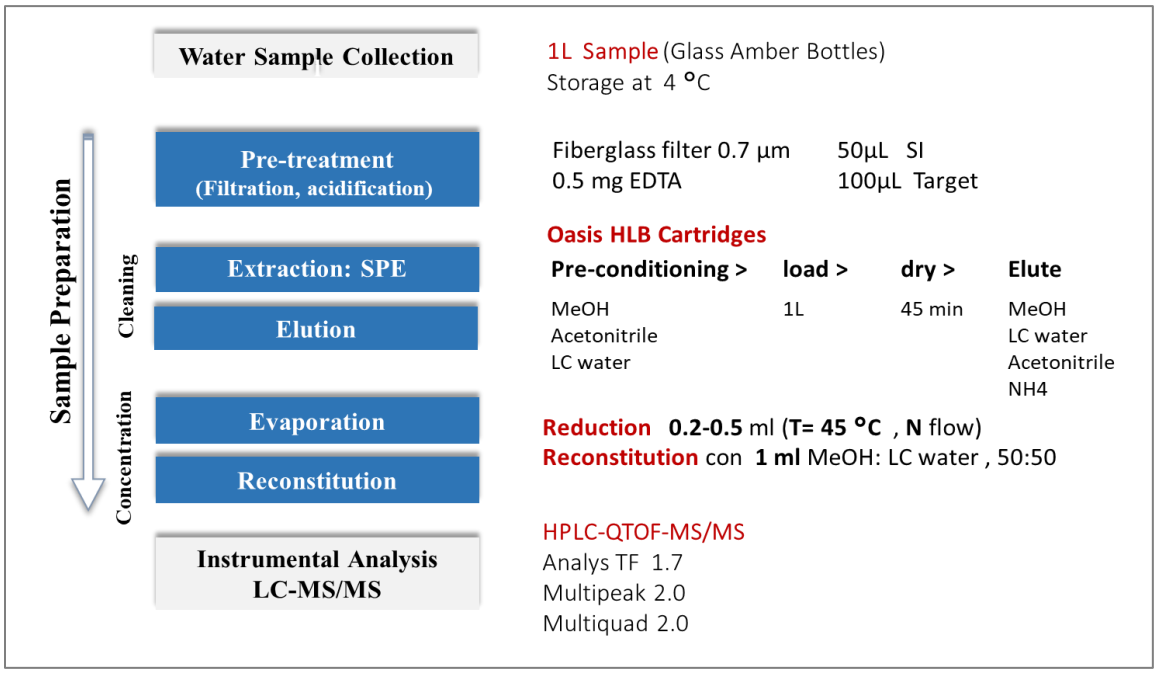


Figure 1.2 Preparation process for aqueous environmental samples.

Solid phase extraction (SPE) is the usual preparation and concentration method for the analysis of CEC in aqueous samples and is constituted by three steps: conditioning of the cartridge or column, loading of the sample, and elution and concentration. The environmental samples collected at the demonstration site discussed in Chapter 3 were treated following the protocol of the Arizona Laboratory for Emerging Contaminants (ALEC), which is here named WRF4269–July 2009 (Vanderford and Snyder, 2006) and briefly described in the following paragraphs.

Filtration is meant to minimize interferences and obstructions during the extraction phase and was performed using a 0.7 µm fiberglass filter. Filtering more than once is recommended for samples with a high load of suspended solids as was the case with several irrigation channels and ditches along the demonstration site, as well as with treated and untreated wastewater. Once filtered, 0.5 gr of ethylenediaminetetraacetic acid (EDTA) were added to remove dissolved metals and the samples were spiked with 50 µl of an isotopic standard with deuterium (D) or ^{13}C labeled versions of each one of the target compounds to be used as a control for losses and interferences.

Cartridges used for SPE contained an equilibrated proportion of n-vinylpyrrolidone (hydrophilic) y divinylbenzene (lipophilic) to optimize the retention of polar and non-polar analytes (Silva-Castro, 2008). The column conditioning (activation) was performed in a sequence of 5ml methanol (MeOH), 5 ml methyl tert-butyl ether (MTBE) and 5ml ultra-pure liquid chromatography (LC) water. In the following step, each column was loaded with an environmental sample at 3-5 ml/min and let dry for 40 min.

The elution was accomplished with a manual manifold adding a sequence of 3 ml MeOH, 3 ml NH_4OH, 3 ml de acetonitrile (ACN) and, finally, 3 ml MTBE with a 5 min pause between additions. Rinsing the column with 3 ml MeOH brought the volume to a total of 15 ml which was, in the following step, evaporated to 0.2-0.5 ml using a Turbovap with a stream of Nitrogen, 5 psi of supply pressure and a water bath at 45°C. Each extract was finally transferred to a chromatography vial and adjusted to 1.5 ml with 50:50 MeOH-LC water.

1.4.4 Analytical methods

A tandem of high performance liquid Chromatography and quadrupole time-of-flight mass spectrometry (LC-QToF-MS) with electrospray ionization (ESI) is the preferred method for the quantification of PhACs, their metabolites and the products of their transformation in the environment (e.g., Hübner et al., 2014; Lacorte and Fernandez-Alba, 2006; Lekkerkerker-Teunissen et al., 2012; Li et al., 2011), and is also the analytical method used at ALEC.

To determine the presence of the target compounds, the vials containing the concentrated samples were organized in groups, including field blanks, replicates, lab blanks and an internal control named "recovery". The last two consisted of 1.5 ml LC water that, in the case of the lab control, was spiked with 100 µl of a mix of un-labeled compounds (target) and 50 µl of an isotopic standard (labeled), which compositions are shown in Table 1.3. The mobile phase consisted on 2 mM NH_4OAC in a solution of 5% MeOH, the working temperatures were 10°C for the auto sampler and 30°C within the column with injections every 14 min. After 9 readings the equipment automatically runs an auto calibration

program with a flow of calibration liquid of 40 µl/min. Each sample was measured three times.

Quantification of the compounds of interest was completed with the assistance of three computer programs: (1) AnalystTF was used to elaborate the working method and to estimate the monoisotopic charges applying published identification parameters such as collision energies, fragment ions and charges (Table 1.4); (2) Multipeak 2.0 helped to anticipate the method functionality and to adjust the collision energies to the most convenient intensity for the signals of the fragment ions; (3) Multiquad 2.0 assisted in the inspection of chromatographs and to adjust the detection limits until obtaining the retention times.

Table 1.3 Composition of the laboratory internal control

Un-labeled compounds (target)	Concentration (ppb)	Labeled Compounds (isotopic standard)	Concentration (ppb)
Sulfamethoxazole	1576.49	Sulfamethoxazole-13C6	534.52
Roxithromycin	758.77	Roxithromycin-d7	484.34
Ibuprofen	755.60	Ibuprofen (racemic, d3)	513.08
Diclofenac	2506.32	Diclofenac-d4	500.86
Fluoxetine	9408.72	Fluoxetine-d6	519.57
Carbamazepine	15244.56	Carbamazepine-13C6	549.09
Gemfibrozil	769.87	Gemfibrozil-d6	584.43
Clofibric acid	1226.38	Clofibric acid-d4	489.94
Metoprolol	805.54	Metoprolol-d7	532.72
17a-ethynyl estradiol	788.06	17a-ethynyl estradiol-d4	440.04

Table 1.4 PhACs identification parameters integrated in the working method. Taken from (Kasprzyk-Hordern et al., 2008; Martínez Bueno et al., 2007; Meritxell et al., 2006)

Analytes	Charge (-/+)	Fragment ions (m/z)	Retention times (min)
Sulfamethoxazole	Positive	108, 156	2.02
Roxithromycin	Negative	679, 158	7.91
Ibuprofen	Negative	161	7.6
Diclofenac	Negative	250, 214	7.35
Fluoxetine	Positive	44, 148	7.58
Carbamazepine	Positive	194	6.88
Gemfibrozil	Negative	121, 127	8.62
Clofibric acid	Positive	126, 85	Not found
Metoprolol	Positive	116, 133	3.02
17a-etilnylestradiol	Negative	145	8.35

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2 CHAPTER

Pharmaceuticals as compounds of emerging concern in Latin America: an overview of their occurrence in the aquatic environment

Abstract

Pharmaceutically active compounds (PhACs) are now environmentally ubiquitous around the world, and the countries of Latin America (LATAM) are not the exception, however there is still little knowledge of the magnitude and conditions of their occurrence in LATAM and of the environmental consequences of their presence. Hence, the present work reviews 66 documents (2007-2017) on the occurrence and sources of PhACs and estrogenic hormones, as compound of emerging concern (CEC), in surface water (Swan et al.) , wastewater (WW), and treated wastewater (TWW) in LATAM, and on the circumstances of their arrival in the environment. Research efforts were carried out in only ten of these countries confirming the presence of 94 PhACs, mainly analgesics and anti-inflammatories, although extraordinarily high concentrations of 830 µg/L of carbamazepine and 6.8 µg/L of ethinylestradiol were found in Ecuador and Brazil, respectively. In general, the reported maximums far exceed the environmental concentrations detected in other parts of the world and are of concern given that much lower concentrations have the potential to adversely affect aquatic and terrestrial organisms. No statistically significant differences were found between the maximum concentrations in WW and TWW, which indicates the inadequacy of WW treatment technologies to eliminate PhACs and corroborates reports indicating that large WW volumes entering WW treatment plants (WWTP) often bypass treatment. It turns out that WW is the main source of PhACs in the environment, thus LATAM countries should direct substantial efforts to develop efficient and cost-effective treatment technologies and to plan and apply WW management strategies and regulations, besides implementing continuous environmental monitoring, creating risk evaluation guidelines and preparing lists of priority substances. This critical analysis illustrates the current situation of the occurrence and sources of PhACs in the aquatic environment of LATAM and gives a general perception of the magnitude of the environmental situation in that part of the world.

Keywords: Pharmaceuticals; Occurrence; Emerging Contaminants; Latin America; Aquatic Environment; Surface Water.

2.1 Introduction

Pharmaceutical active compounds are considered contaminants of concern (emerging contaminants, microcontaminants, etc.) due to their potentially adverse ecological impacts on wildlife and human health, and the lack of regulatory framework to protect the environment (Cruz, 2013; Daughton and Ternes, 1999b; Halling-Sorensen et al., 1998). Several therapeutic classes are included in this group of contaminants (e.g., antibiotics, analgesic and anti-inflammatories, synthetic hormones, lipid regulators, X-ray contrast media, etc.), as well as numerous metabolites and transformation products, which are molecules with diverse and complex psychochemical characteristics (Ayora et al., 2008). PhACs are prescribed to cure or prevent illnesses and, therefore, are designed with the purpose of causing biological effects (Comerton et al., 2009). Consequently, they pose traits of genotoxicity, persistency and endocrine disruption (Evgenidou et al., 2015) in addition to physicochemical proprieties similar to those of xenobiotic substances, which have a natural tendency to accumulate in the lipid compartment of organisms (Halling-Sorensen et al., 1998). For instance, it has been shown that genotoxicity of ibuprofen is responsible for altering the frequency of micronuclei in peripheral blood cells of fish (*Cnesterodon decemmaculatus* and *Oreochromis niloticus*) (Gonzalez-Nuñez et al., 2016; Ragugnetti et al., 2011) while carbamazepine and diclofenac exert a genotoxic potential over the DNA integrity of Zebrafish (*Danio rerio*) (Rocco et al., 2011).

Persistence in the case of PhACs is better defined by their continuous input into the environment (Fernández et al., 2014; Papageorgiou et al., 2016) and by their low removal rates in WWTP (e.g., carbamazepine, atenolol, diclofenac <40%) (Correia and Marcano, 2016), than by the few determinations of their half-lives ($t_{1/2}$) (Araujo et al., 2014; Araujo et al., 2011). In addition, synthetic hormones are well known endocrine disrupting compounds (EDCs) that can act on liver, kidney, brain and gonads of fish and other organisms in concentrations as low as few ng/L (Depiereux et al., 2014; Gárriz et al., 2017; Santos et al., 2010).

PhACs enter the aquatic environment through multiple pathways (Petrović et al., 2003) though WWTP have been identified as the worldwide primary sources (Correia and Marcano, 2016). They can be found in detectable concentrations of nanogramos to

micrograms per liter in compartments such as SW, WW and TWW, and less frequently in groundwater, drinking water and seawater (Jurado et al., 2012; Lacey et al., 2012; Salgado et al., 2010; Yang et al., 2017). Furthermore, they have been detected in aquatic organisms (Brooks et al., 2005; Valdés et al., 2016) and even in terrestrial species, such as carrion-feeding birds (Oaks et al., 2004; Swan et al., 2006), reinforcing the conviction that their continuous input into the aquatic environment has the potential of causing acute and chronic effects to non-targeted macro and microorganisms (Boxall et al., 2003) thus, adversely altering the ecosystem. Even so, while some authors claim that the risk is evident (e.g., Beretta et al., 2014), others report that the environmental effects of these compounds are still unclear (e.g., Gheorghe et al., 2016).

Numerous efforts have been carried out to determine the occurrence, source, fate and effects of pharmaceuticals in the environment, particularly in North America, Europe and Asia (e.g., Jurado et al., 2012; Kümmerer, 2008; Miege et al., 2009). Research on this topic is also performed in LATAM, although on a smaller scale despite the tons of pharmaceuticals that are produced, imported, used and disposed in these countries (Elorriaga et al., 2012; Voloshenko-Rossin et al., 2015). Therefore, the objective of this study is to conduct a critical analysis of the information related to the presence and sources of PhACs as compounds of emerging concern in the aquatic environment of LATAM countries, as well as to gain knowledge on the state of the art and the practice in this type of research in the regional context from a global perspective. To the best of our knowledge, this is the first document that compiles and discusses the occurrence of PhACs as EC in the aquatic system in LATAM.

2.2 Classification of PhACs

PhACs are classified into several groups according to their therapeutic use. Due to the frequency of their detection in the environment, their persistence and toxicity, the most studied are antibiotics, psychiatric drugs, hormones, analgesics and anti-inflammatories, lipid regulators, and beta-blockers (Daughton and Ternes, 1999b) with the first three as those of greatest concern for the aquatic environment (Silva and Bonora, 2014). The compounds most frequently mentioned and their acronyms can be found in Table 2.1. Although only the synthetic hormones are actual PhACs, the natural ones are well recognized EC and are usually discussed with this group. As already mentioned, persistence in the case of pharmaceuticals is more related to its continued presence in the environment than to its relatively short half-life ($t_{½}$) (e.g., naproxen with t½=10.2±0.5 to 14.6±1.3 days; gemfibrozil with t½=119.5±15.6 to 288±61.3 days) hence, it would be more appropriate to recognize them as "pseudo-persistent" compounds (Araujo et al., 2011; Rosi-Marshall et al., 2015). In addition, PhACs in the environment are frequently accompanied by their key metabolites and transformation products, whose environmental effects can be significantly different and even more dangerous than those of the parent compounds.

2.3 Pharmaceuticals in the aquatic environment

After consumption, PhACs are primarily excreted as a mix of the parent compound and its metabolites (Picó and Barceló, 2015). Some PhACs are then introduced to the environment through direct WW discharges from domestic, industrial and hospital effluents, as well as from aquaculture releases, and the resulting runoff from agriculture and livestock farming (Boxall et al., 2003; Jos et al., 2003; Petrović et al., 2003), which eventually leads to their incorporation into rivers; however, most WW and its load of contaminants are directed to WWTP. Once there, PhACs can be adsorbed to active sludge and reach the environment via the land application of residual sludge (Liu and Wong, 2013); however, the WWTP are the main source of PhACs residues in the aquatic environment because they are not designed to remove this type of compounds (Daughton and Ternes, 1999b), which often undergo the whole process with little or no change at all (Correia and Marcano, 2016)

Table 2.1 Therapeutic groups and compounds of major concern in the worldwide aquatic environment (Daughton and Ternes, 1999b; Heberer, 2002; Liu and Wong, 2013; Petrović et al., 2005; Yang et al., 2017)

Therapeutic groups	Compounds	Acronyms
Antibiotics		
Sulphonamides	Sulfamethoxazole	SMX
Tetracyclines	Tetracycline, oxytetracycline	TTC, OTC
Diaminopyridines	Trimethoprim	TMP
Macrolides	Erythromycin, clarithromycin, roxithromycin	ERT, CRT, ROX
Fluoroquinolones	Ciprofloxacin	CPF
Lincosamides	Lincomycin, clindamycin	LIN, CLN
Psychiatric drugs		
Antidepressants	Fluoxetine	FLX
Antianxietys	Diazepam	DZP
Antiepileptics	Carbamazepine	CBZ
Hormones		
Natural estrogens	Estrone	E1
	17β-estradiol	E2
	Estriol	E3
Synthetic estrogens	17α-ethinylestradiol	EE2
Analgesics and Anti-inflammatories (NSAIDs)		
Acetics	Ibuprofen, naproxen, ketoprofen	IBF, NPX, KTP
	Diclofenac	DCF
Salicylates	Acetylsalicylic acid	ASA
Metabolite	Salicylic acid	SCA
P-aminophenols	Acetaminophen	ACT
Lipid Regulators		
Fibrates	Clofibrate	CLF
	Gemfibrozil	GMZ
	Bezafibrate	BZF
Metabolite	Clofibric acid	CFA
β –blockers		
Selective	Metoprolol	MTP
	Atenolol	ATN
Non-Selective	Propranolol	PRP

2.3.1 Antibiotics

Antibiotics are defined as chemical substances that kill or inhibit the growth of microorganisms (Caracciolo et al., 2015), consequently, they have been widely used to treat, control and prevent infectious diseases in humans, animals and plants. Compounds such as sulfonamides, tetracyclines, macrolides and fluoroquinolones are utilized to prevent

viral and bacterial infections (e.g., SMX, OTC, and CPF) in aquaculture (Santiago et al., 2009), as herbicides (e.g., OTC, streptomycin) in agriculture (Vidaver, 2002), as growth promoters (e.g. TTC, ERT) in livestock farming, and in the treatment of respiratory and urinary tract infections (e.g. SMX and TMP) in humans (Jones et al., 2001). The interest in the presence of antibiotics in the aquatic environment is mostly related to their persistence as well as their participation as agents of bacterial resistance (Polar, 2007).

2.3.2 Psychiatric drugs

Psychiatric drugs are psychoactive compounds drugs that exert an effect on the internal chemistry of the brain and the nervous system. Thus, these PhACs are used to treat mental illnesses and neurologic conditions (Calisto and Esteves, 2009). Among the most detected in water bodies are antidepressants (FLX), anxiolytics (DZP) and antiepileptics (CBZ) (Fent et al., 2006). All of these are highly persistent and remain stable along the processes of WW treatment (Brodin et al., 2013; Clara et al., 2004). For instance, DZP and alprazolam are resistant to photodegradation (Calisto et al., 2011) while compounds such as oxazepam, CBZ, FLX and norfluoxetine exhibit high bioaccumulation factors (BAF) in aquatic organisms; some have been detected in algae, zooplankton, invertebrates, and organs/tissues of fish and birds both in the field and in the laboratory (Brodin et al., 2013; Valdés et al., 2016; Vernouillet et al., 2010).

2.3.3 Hormones

Hormones are EDCs and the most detected are natural estrogens (E1 and E2) and the synthetic contraceptive EE2. E2 is frequently used in livestock farming as growth promoters (e.g., Synovex: progesterone and estradiol benzoate) and in hormonal treatment for humans, cattle, and domestic animals (Ramírez-Sánchez et al., 2015). In general, compared with other therapeutic groups, hormones concentration in the aquatic environment is low (Koh et al., 2008), although high enough to, for instance, induce vitellogenin synthesis and intersex (e.g., testis-ova) in some fish species (Celiz et al., 2009; Jobling et al., 2006). Purdom et al. (1994) reported that only 0.1 ng/L of EE2 induces vitellogenin in rainbow trout males (*Oncorhynchus mykiss)*.

2.3.4 Analgesic and non-steroidal anti-inflammatory drugs

Analgesic and non-steroidal anti-inflammatory drugs (NSAID) (e.g., IBF and DCF) are among the most widely used PhACs in the world (Evgenidou et al., 2015). They are usually prescribed as pain killers in human medical care but are also often sold without prescription as so-called 'over-the counter' (OTC) drugs (Heberer, 2002). The Argentinean Pharmaceutical Confederation (CoFA from Spanish) reported that IBF is the most commonly NSAID used as OTC drug in Argentina, with more than 16.7 million units sold in 2016 (Barreiro, 2017). Meanwhile, ACT, IBF, ketorolac, DCF and mefenamic acid were the best-selling analgesic and NSAIDs in Chile, each one with figures around 3 million units sold in 2016 (IMS, 2016). Although the WWTP removal efficiency of this group is often high (>80%), it seems to be related to site-specific concentrations, the type of treatment, and the system conditions (e.g., Carmona et al., 2014; Fernández et al., 2014). Therefore, their risk is mainly related to the constant introduction of high amounts of these compounds into the environment. Among other effects, NSAID have shown to induce oxidative stress in the crustacean amphipod *H. Azteca* and the carp *C. Carpio*, the latter reported with a high incidence of liver and gill damage (Islas-Flores et al., 2013; Novoa-Luna et al., 2016).

2.3.5 Lipid regulators

Lipid regulators are divided into statins and fibrates although only the last group is of environmental significance (Santos, et al., 2010). Fibrates (GMZ, fenofibrate, BZF and CFA) are prescribed to reduce plasma triglycerides as well as low and very low-density lipoproteins (LDLs and VLDLs, bad cholesterol), and to increase high density lipoproteins (HDLs, good cholesterol), slowing the accumulation of fat in the blood vessel walls (Mimeault et al., 2005). However, reports indicate that some fibrates are not completely metabolized within the human body; for example, up to 70% of the ingested GMZ can be excreted without modifications (Mimeault, et al., 2005; Zimetbaum, Frishman, & Kahn, 1991), which adds to the environmental threat. It has been demonstrated that GMZ affects the eleutheroembryo development and the locomotor behavior of fresh water fish species (Henriques et al., 2016) and that it is capable of inhibiting the population growth of rotifers and microcrustaceans (Isidori, Nardelli, Parrella, Pascarella, & Previtera, 2006).

2.3.6 Beta-blockers

Beta-blockers (β-blockers) are used in human and veterinary treatments of various cardiovascular disorders such as hypertension, angina, and heart rhythm disturbances (Boix et al., 2014; Jacquet et al., 2012). This group includes selective blockers, such as MTP and ATN that act on cardiac β1-adrenergic receptors, and non-selective blockers, such as PRP that act on β1 and β2-adregenic receptors (Chaves Brenes, 2013; Hernando et al., 2007). These three compounds are frequently analyzed and commonly detected in aquatic ecosystems due to their widespread use and often incomplete metabolism. For instance, only 50% of the ingested ATN is absorbed by the human body (Alder et al., 2010). In addition, these compounds are highly resistant to hydrolysis and greatly bioavailable and mobile in the environment (Maszkowska et al., 2014). It has been shown that continuous exposure to environmental concentrations of PRP affects the physiology, growth and reproductive characteristics of Medaka fish (Huggett et al., 2002) and increases its bioaccumulation in Daphnia magna (Jeong et al., 2016), which does not occur at higher non-environmental concentrations.

2.4 Methodology and materials

A total of 66 studies published in English, Spanish and Portuguese were considered for this review, including peer reviewed articles, graduate thesis, and meeting proceedings on PhACs in the environment in LATAM countries, available from January 2007 to June 2017. These studies report data on 132 emerging organic compounds (EOCs), including PhACs, endocrine disrupting compounds (EDCs) and personals care products (PCPs) detected in diverse environmental matrices. Documents and data on the occurrence of PhACs were manipulated as follows:

1) Collection of documents through an exhaustive search in numerous sources of scientific information.

2) Revision and organization of documents and data according to the following criteria: i) geographical area of environmental occurrence; ii) classification of reported compounds; iii) number of reported occurrences; iv) environmental matrices involved; v) detected concentrations; and vi) date of publication of the study.

3) Development of a dynamic database to compile and handle the selected information.

4) Analysis of the information through the application of descriptive and nonparametric statistics such as box-plots of maximum concentrations and the Moods' median test.

The values presented throughout this paper are the result of the best compilation efforts of the authors. The data collected was generated through a diversity of analytical methods and under a variety of times, sampling conditions and volumes. As far as the knowledge of the authors is concerned, the data generated for graduate theses and meeting proceedings did not go through the peer review process. All of the original papers are fully cited for consultation and confirmation.

2.5 Results and discuss

2.5.1 Geographical distribution of research studies in Latin America

Compared to European, Asian and North American (USA and Canada) countries, the studies on environmental occurrence of PhACs in LATAM are scarce (Elorriaga et al., 2013a; Voloshenko-Rossin et al., 2015). This is mainly because in this part of the world environmental problems have been left behind in favor of more pressing issues, such as poverty, health and security, leaving insufficient funds for this type of research and for the acquisition of advanced analytical instruments.

On the basis of the 66 studies performed over the last decade, PhACs residues have been identified in diverse environmental matrices in ten countries of LATAM. These countries, the number of studies and the contribution (%) of each country to the total number of investigations in LATAM are indicated in Figure 2.1 along with the approximate location of each study site. Brazil and Mexico account for 64% of the studies, followed by Argentina, Colombia and Chile (23%) and, to a lesser extent, Puerto Rico (PR), Costa Rica, Venezuela, Ecuador and Uruguay (13%).

Figure 2.1 Geographical distribution of documented research, including the country of origin, the number of studies conducted in each of them (parenthesis) and the percentage they represent of the total number of investigations. Sampling sites (black dots) refer to states, cities and, occasionally, to particular locations, e.g., rivers or WWTP mentioned in the references.

Brazil stands out among these countries given that most of its states have been included in a study at least once. For instance, Machado et al. (2016) carried out the first nationwide survey on EC in 22 out of 26 Brazilian state capitals where one hundred drinking water samples and seven water sources were investigated. In addition, 50% of Brazilian studies refer to the state of São Paulo (SP) (Figure 2.1), of which 20% are focused on the Metropolitan Zone of Campinas City, making it the most studied area, while the Atibaia River, its major water supplier, is the most sampled site among the surrounding rivers (e.g., Piracicaba, Sorocaba and Cotia rivers). The SP state is a hot-spot area of concern because of its approximately 45 million inhabitants and a population density of 166 inhabitants per

km^2 (IBGE, 2017), which entails significant water quality problems since only up to 50% of the WW is properly treated before being released into the environment (Jardim et al., 2012; Montagner and Jardim, 2011).

Occurrence of PhACs in Mexico has been analyzed in 18 studies distributed throughout nine states, with the center of Mexico being the area of greatest concern. The states of Hidalgo and Mexico (Figure 2.1) together with the Mexico City (CDMX) comprise 72% of the studied sites, with the Tula Valley and/or Mezquital Valley (depending on the author), in Hidalgo, as the most studied of them all (39%). This Tula-Mezquital Valley zone hosts a large agriculture irrigated system (850 km^2) and has been the source of scientific interest due to decades of irrigation with mostly untreated WW, which has resulted in modifications in both, the hydrologic setting and the water quality (Siemens et al., 2008). On the other hand, the three canals known as Emisor Central, Interceptor Poniente and Gran Canal de Drenaje are the means to transport a mix of domestic and industrial WW, rainwater, and run off from the CDMX, passing through the Metropolitan Zone of the Mexico valley (MZMV) and discharging into the Tula-Mezquital Valley (Gibson et al., 2007; Gibson et al., 2010). Hence, these three canals are the focus of the rest of the studies in the CDMX (22%) and the MZMV (11%).

Although Argentina, Colombia and Chile account for 23% of the publications on occurrence of PhACs in LATAM, there are not reports highlighting specific sites of environmental concern. The six studies carried out in Argentina have been directed on the Pampas region, a central-eastern zone of fertile lowlands that comprises, among others, the three most important provinces of Buenos Aires, Cordoba and Santa Fe, and the Autonomous City of Buenos Aires (Figure 2.1). The sampling sites include discharging points of TWW in the Buenos Aires and Cordoba provinces (Elorriaga et al., 2013a) and several surface water bodies that eventually receive TWW, including the estuarine area of the de la Plata river and the course of the Suquía river (Elorriaga et al., 2013b; Valdés et al., 2015). The latter is part of an important endorheic basin and throughout its course it collects treated and untreated discharges from domestic, industrial and agricultural activities (Santiago et al., 2015; Valdés et al., 2014). Similarly, studies on the occurrence of PhACs in Colombia (8%) address WW and TWW (Arrubla et al., 2016; Hernández et al.,

2015; Villamizar and de Fonseca, 2011) as well as some natural and artificial water reservoirs that act as sources of drinking water (Aristizabal-Ciro et al., 2017; Gracia-Lor et al., 2012a) in the three departments of central-western Colombia, Antioquia (Medellin), Risaralda (Pereira) and Cundinamarca (Bogota). In this case, the environmental concern relates to the fact that these reservoirs are supplied by rivers and tributaries that, in turn, receive urban and agricultural WW discharges (Gracia-Lor et al., 2012a). Meanwhile, Chile's few studies (6%) have been conducted in only two south-central geopolitical zones, the Santiago Metropolitan and the Biobio Regions, the last one constituted by the Concepción province and the Biobio river basin, one of the most productive areas of the country (Henriquez, 2012).

The remaining 13% of the published studies have been carried out in Puerto Rico (3), Costa Rica (2), Ecuador (1), Uruguay (1) and Venezuela (2) where research efforts on environmental occurrence of PhACs are still in very early stages. Nevertheless, few authors have completed extensive studies and are now recognized and cited as the pioneers on this kind of research in their respective countries. In southern Puerto Rico, Colón-Ortiz (2010) analyzed five PhACs in SW in the municipalities of Lares and Yauco. Separately, the Puerto Rico Environment Quality Board (PREQB) in cooperation with the US-Geological Survey, conducted a preliminary assessment to determinate the occurrence and distribution of 14 PhACs and personal care products, among other EDCs, in seven watersheds from the east and north-central regions (PREQB, 2012; USGS, 2010). They found an important presence of PhACs in the Rio Grande de Loiza watershed, which is the longest within the island, and corroborated the occurrence in a second survey (PREQB, 2014). Later on, Rivera (2016) detected four PhACs along the Río Grande watercourse. On the other hand, in Costa Rica, Spongberg et al. (2011) implemented the first nationwide study that consisted on 86 samples of SW collected in the cities of Liberia, Quesada and Cartago, as well as in costal locations such as Puntarenas, Golfo Dulce and Limon, among others, to determine the occurrence of 34 pharmaceuticals and personal care products. Additionally, Causanilles et al. (2017) carried out a wide-scope screening of illicit drugs plus approximately 1,500 pharmaceuticals and personal care products in WW, TWW, and SW in the cities of Liberia and Puntarenas. Voloshenko-Rossin et al. (2015) are responsible for the first investigation ever on organic pollutants, including PhACs, in the San Pedro-

Guayllabamaba-Esmeraldas rivers in Ecuador while Pérez-Parada et al. (2012) performed a preliminary study in the Punta Carretas WWTP in Montevideo, Uruguay. Finally, Araujo et al. (2008) conducted a study on SW in Maracaibo, Venezuela, to measure NSAID and other PhACs while developing a new analytical method specifically designed for the determination of acidic drugs in environmental concentrations. Subsequently, WW from central Venezuela were also screened for NSAID (Correia and Marcano, 2016).

In addition to the environmental occurrence of PhACs, research in LATAM extends to the examination of the persistence of analgesics in natural waters under various environmental conditions (Araujo et al., 2014; Araujo et al., 2011) and to the estimation of the capacity of crops and soils to sorp PhACs both in the laboratory and in the irrigation fields (Carmona, 2012; Correia et al., 2017; Durán-Álvarez et al., 2012; Durán-Álvarez et al., 2014). The investigation on PhACs environmental remediation comprises the degradation of antibiotics by ozone and by photoprocesses (Santibañez, 2014; Vasconcelos et al., 2009) as well as the efficiency of wetlands to remove ibuprofen (Cervantes-Ramírez, 2015)

2.5.2 Occurrence and concentrations of PhACs in Latin America

Despite the limited number of studies, these have been sufficient to highlight the presence of PhACs and other EOCs in eight environmental matrices in LATAM, which are displayed in Figure 2.2. According to the number of reported investigations, the most examined has been SW (e.g., Henriquez, 2012; e.g., Moreira et al., 2009), which often consists of a mix of rain water, grey and black water, and even agriculture returns. In second place are WW runoffs (e.g., Peña-Álvarez and Castillo-Alanís, 2015; Pérez-Parada et al., 2012) and WWTP influents and effluents (e.g., Ferreira, 2014; Robledo-Zacarías et al., 2017), which are frequently analyzed in conjunction. A special case of TWW are the effluents from hospitals, which have been reported separately (Colón-Ortiz, 2010; Martins et al., 2008) and will remain so in this work. Drinking water (e.g., Aristizabal-Ciro et al., 2017; Silveira et al., 2013), groundwater (e.g., Félix-Cañedo et al., 2013; Metcalfe et al., 2011), and soils and sediments (e.g., Bertin et al., 2011; Siemens et al., 2008) have been the center of a much lower number of studies, while activated sludge (e.g., Vicentín and Campanella, 2013) and seawater (e.g., Pereira et al., 2016) have barely caught any attention.

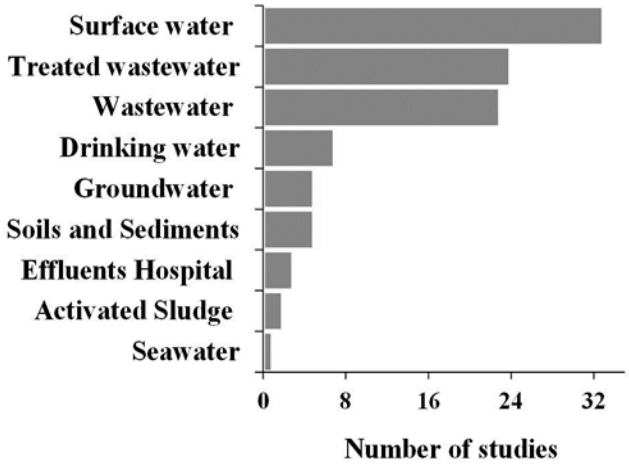

Figure 2.2 Number of studies on PhACs by environmental compartment analyzed in LATAM. Hospital effluents are a special kind of treated wastewater and are reported separately.

2.5.2.1 Occurrence of PhACs in Latin America

The 66 revised documents make mention of 132 types of EOC, including 96 PhACs and similar such as stimulants, anesthetics, X-ray contrast media and illicit drugs, 15 hormones, 13 industrial additives and personal care products such as phenols, phthalates and parabens, and 8 pesticides among which herbicides, insecticides and fungicides prevail. These documents indicate that out of 835 analytical measurements, only 75% resulted in the detection, substantial or not, of the target compound in an environmental sample. Then, the number of analytical measurements is the total number of instrumental examinations performed to determine the occurrence of certain compounds in the environmental samples, while the percentage or number of detections indicates how many of those measuring efforts confirmed the occurrence of one or more of EOC in the environment. Thus, PhACs were measured 525 times throughout the documented research with 77% detections. The therapeutic group and, in parenthesis, the percentage of measurements and the number of searched analytes are listed as follow: analgesic and NSAIDs (40%, 13), antibiotics (15%, 27), psychiatric drugs (13%, 20), β-blockers (12%, 13), lipid regulators (8%, 4), stimulants (7%, 7) and others (4%, 12). In addition, estrogenic hormones were measured 145 times with 64% of detections. From these, E2, EE2 and E1 were the most perused with individual detection levels of up to 60%.

Figure 2.3 shows the PhACs and hormones of greatest environmental concern in LATAM, i.e., those that count with five or more measurements (n≥5), and compares the number of measurements with the number of detections. Given that analgesics and NSAIDs are the most frequently analyzed, they compose the most detected therapeutic class (44%), with DCF, IBF and NPX being the most individually investigated compounds in the environment (48, 47, 38 measurements, respectively) and, consequently, the most detected (>80% in conjunction). Also in this group, SCA, the main metabolite of ASA, and ACT showed a high incidence of detection, 100 and 91%, respectively, but with a much smaller number of investigations. Reports confirm that estrogenic hormones were the second most commonly analyzed therapeutic group and that CBZ was the most prominent psychiatric drug, detected in the LATAM environment in 88% of 27 measurements, while GMZ, CFA and BZF were shown as the only lipid regulators of some environmental interest. Antibiotics are a special case given the diversity of the investigated analytes, however, out of 27 representatives that include sulfonamides, β-lactams, tetracyclines, fluoroquinolones, macrolides, nitroimidazoles and diaminopydirines, only three comply with n ≥ 5: TMP, CRT and SMX (Figure 2.3), which were detected in all the samples from WW and TWW, activated slugged, and SW. Finally, the β-blockers valsartan (VAL), irbesartan (IRB) and ATN appeared in several screenings although the first two were not frequently analyzed, all showing individual detections of 75% or more.

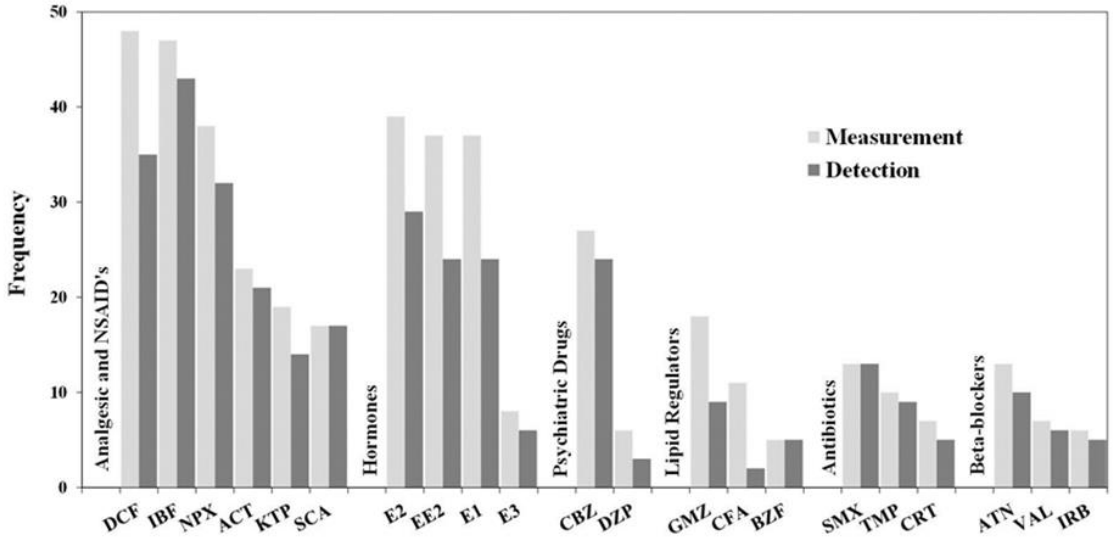

Figure 2.3 PhACs and Hormones most frequently measured (n≥5) and detected in the aquatic environment of Latin America from January 2007 to July 2017.

Although not the subject of this analysis, metabolites considered and detected in the WW systems were carbamazepine-10,11-epoxide and norfluoxetine, derived from psychiatric drugs, p-hydroxy-atorvastatin and o-hydroxy-atorvastatin, from lipid-regulators, and OM10, an omeprazole metabolite (Causanilles et al., 2017; Henriquez, 2012; Locatelli et al., 2011; Thomas et al., 2014; Voloshenko-Rossin et al., 2015). The transformation products analyzed but not found in the environment were IbB4, resulting from the transformation of ibuprofen, and ISW1b and IB5, from irbesartan (Causanilles et al., 2017). Endocrine disrupting EOC found in conjunction with PhACs were phenols, such as bisphenol-A and 4-nonylphenol, phthalates, which include diethylhexyl phthalate and butyl benzyl phthalate, and the fungicide and antibacterial triclosan (Chávez et al., 2011; Durán-Alvarez et al., 2009; Gibson et al., 2007; Melo and Brito, 2014). Chlorophenols such as pentachlorophenol and 2,4-dichlorophenoxyacetic acid were measured frequently, although they were seldom detected (Félix-Cañedo et al., 2013; Metcalfe et al., 2011; Silva-Castro, 2008). A compound rarely measured but detected in every environmental assessment that included it is the worldwide used herbicide atrazine (Henriquez, 2012; Machado et al., 2016).

2.5.2.2 WWTP as the source of PhACs in the environment

A total of 58 PhACs were detected in the WW systems of LATAM in the span of these 10 years. Table 2.2 displays their occurrence by environmental compartment (WW and TWW) and by therapeutic group, including the reported range of concentrations for each analyte, the location of the study site and the source of the information. As expected, concentrations of NSAIDs dominated over any other therapeutic group in untreated WW. Of the six analytes in this set, NPX was reported in the highest concentration of up to 235.4 µg/L in Mexico, although most of the data fell within the range of 0.4-54.3 µg/L and more often bellow 17 µg/L. KTP presented the second highest concentration found with an exceptional maximum of 93.1 µg/L, also in Mexico, though the outcomes for the rest of the sites were in the range of 0.1-1.7 µg/L. The content of DCF and SCA was quantified above 70 µg/L in Colombia and Mexico, respectively. For the former, this number represents another

atypical value given that the rest of the studies reported concentrations of less than 8.8 µg/L, while the latter was included in only three studies, therefore, there is not enough information for comparisons. IBF was the compound most frequently detected in Mexico (to up to 39 µg/L) although the second highest concentration was found in Venezuela (26.8 µg/L), whereas ACT was rarely targeted and found in maximum concentrations of 15 µg/L in Argentina and Venezuela (Table 2.2). Furthermore, of the three antibiotics reported in the aquatic environment of LATAM only SMX and TMP were found in WW compartments, both in concentrations bellow 0.3 µg/L, with the exception of a site in Ecuador, where the average concentration of SMX was of the order of 6.3±6.7 µg/L (Table 2.2). Only three types of lipid regulators were reported in México, and just one in Brazil and Argentina, the latter showing the highest content of BZF of 2.9 µg/L. Psychiatric compounds were verified in only six studies and found in concentrations of no more than 1µg/L while the presence of the β-blocker ATN was confirmed only twice and did not surpass 2 µg/L. Last but not least, steroids hormones such as progestagens, androgens and estrogens were also sought after, however, only estrogens were detected (76%). E1, E2 and EE2 were found in lower concentrations than other PhACs, mainly in the range of few ng, except in Brazil, where up to 3.1 µg/L were detected (Table 2.2).

On the other hand, data in Table 2.2 also shows that WWTP are producing treated water that will be liberated to the environment with up to 13.6 µg/L of DFC in Colombia, 47.7 µg/L of IBF in Chile, and other substantial amounts of NSAIDs in the rest of LATAM. Effluents from a hospital in Puerto Rico are reported to contain from 17 to 37 µg/L of ACT. Nonetheless, in the few cases in which the analytes were measured in both the WW and TWW, the system showed an apparent reduction (no performance calculation) of up to 80% of the NSAIDs concentrations, with the exception of DFC. Conversely, the concentrations of antibiotics, lipid regulators, psychiatric drugs and β-blockers did not show evidences of apparent reduction after treatment. Finally, the hormones were removed at a diversity of apparent rates, however, E1 and EE2 were found at 2 and 1.2 µg/L, respectively, in TWW from Brazil, concentrations high enough to cause environmental damage.

Table 2.2 Occurrence and concentrations of PhACs in the wastewater systems in Latin America

Analytes	WW (µg/L)	TWW (µg/L)	Location	References
Analgesics and Anti-inflammatories				
DCF	1.72-6.36	---	MZMV, MX	(Gibson et al., 2007)
	0.09-0.55	---	Hidalgo, MX	(Siemens et al., 2008)
	1.6±0.347	---	Hidalgo, MX	(Silva-Castro, 2008)
	2.05-4.82	---	Hidalgo, MX	(Gibson et al., 2010)
	3.76±0.258	---	MZMV, MX	(Chávez et al., 2011)
	0.105±0.081	0.036-0.241	Minas Gerais, BR	(Brandt et al., 2013)
	1.21	1.11	Santa Fe, AR	(Vicentín and Campanella, 2013)
	---	<0.03–1.2	Buenos Aires, AR	(Elorriaga et al., 2013a)
	1.5±0.1	1.3±0.4	Santiago, CL	(Manzo et al., 2014)
	1.31-2.66	0.245-0.445	Rio de Janeiro, BR	(Ferreira, 2014)
	1.1-78.7	1.1-13.6	Pereira, CO	(Arrubla et al., 2016)
	7.25-8.85	2.15-5.65	Carabobo, VE	(Correia and Marcano, 2016)
IBF	4.38-5.09	---	MZMC, MX	(Gibson et al., 2007)
	0.22-0.58	---	Hidalgo, MX	(Siemens et al., 2008)
	2.5±0.348	---	Hidalgo, MX	(Silva-Castro, 2008)
	0.57-0.89	---	Maracaibo, VE	(Araujo et al., 2008)
	0.74-1.40		Hidalgo, MX	(Gibson et al., 2010)
	4.7±0.097	---	MZMC, MX	(Chávez et al., 2011)
	23.83±15.23	6.11±0.72	Chiapas, MX	(Cruz, 2013)
	11.55	1.22	Santa Fe, AR	(Vicentín and Campanella, 2013)
	---	0.4-13.0	Buenos Aires, AR	(Elorriaga et al., 2013a)
	---	3.01-47.7	Santiago, CL	(Ascar et al., 2013)
	3.0±0.5	1.8±0.1	Santiago, CL	(Manzo et al., 2014)
	0.23-2.83	0.02-0.34	MZMC, MX	(Peña-Álvarez and Castillo-Alanís, 2015)
	0.1-0.7	0.1-2.1	Pereira, CO	(Arrubla et al., 2016)
	10.9-26.87	2-6.95	Carabobo, VE	(Correia and Marcano, 2016)
NPX	15.22-16.65	---	MZMV, MX	(Gibson et al., 2007)
	0.60-6.74	---	Hidalgo, MX	(Siemens et al., 2008)
	13.6±1.8	---	Hidalgo, MX	(Silva-Castro, 2008)
	7.27-13.60	---	Hidalgo, MX	(Gibson et al., 2010)
	16.33±1.4	---	MZMC, MX	(Chávez et al., 2011)
	142.5±92.9	<LOD	Chiapas, MX	(Cruz, 2013)
	7.20	5.06	Santa Fe, AR	(Vicentín and Campanella, 2013)
	---	0.12-2.71	Santiago, CL	(Ascar et al., 2013)
	3.9±0.3	1.44±0.007	Santiago, CL	(Manzo et al., 2014)
	2.85-54.36	0.20-0.76	MZMC, MX	(Peña-Álvarez and Castillo-Alanís, 2015)
	0.4-2.0	0.9-4.8	Pereira, CO	(Arrubla et al., 2016)
KTP	0.26-0.48	---	MZMC,MX	(Gibson et al., 2007)
	0.448±0.075	---	Hidalgo, MX	(Silva-Castro, 2008)
	0.179±0.003	---	MZVM, MX	(Chávez et al., 2011)
	67.87±25.3	5.30±1.01	Chiapas, MX	(Cruz, 2013)
	---	0.08-4.5	Santiago, CL	(Ascar et al., 2013)
	13.4±0.6	2.3±0.4	Santiago, CL	(Manzo et al., 2014)
	0.8-1.7	0.8-2.4	Pereira, CO	(Arrubla et al., 2016)
SCA	0.62-29.06	---	MZMC, MX	(Gibson et al., 2007)
	29.8±13.6	---	Hidalgo, MX	(Silva-Castro, 2008)
	72.9±3.91	---	MZMV, MX	(Chávez et al., 2011)

Table 2.2 Occurrence and concentrations of PhACs in the wastewater systems in Latin America

Analytes	WW (µg/L)	TWW (µg/L)	Location	References
ACT	---	17/37*	Lares, PR	(Colón-Ortiz, 2010)
	15.08	0.503	Santa Fe, AR	(Vicentín and Campanella, 2013)
	13-15	<LOD	Carabobo, VE	(Correia and Marcano, 2016)
	3.9-5.5	3.9-4.1	Pereira, CO	(Arrubla et al., 2016)
	0.1-4.3	0.1-1.0	Michoacán, MX	(Robledo-Zacarías et al., 2017)
Antibiotics				
SMX	0.034-0.328	0.043-0.206	Biobio, CL	(Henriquez, 2012)
	0.160	0.157	Santa Fe, AR	(Vicentín and Campanella, 2013)
	0.035±0.046	0.027±0.043	Minas Gerais, BR	(Brandt et al., 2013)
	6.3±6.7	6.0±5.7	Quito, EC	(Voloshenko-Rossin et al., 2015)
TMP	0.012-0.174	0.003-0.078	Biobio, CL	(Henriquez, 2012)
	0.064±0.028	0.004-0.039	Minas Gerais, BR	(Brandt et al., 2013)
CRT	0.08-1.40	---	Hidalgo, MX	(Siemens et al., 2008)
Lipid Regulators				
GMZ	0.64-0.68	---	MZMV, MX	(Gibson et al., 2007)
	<0.01-0.35	---	Hidalgo, MX	(Siemens et al., 2008)
	0.69±0.055	---	Hidalgo, MX	(Silva-Castro, 2008)
	0.758-1.56	0.95-0.96	Hidalgo, MX	(Sánchez et al., 2013)
CFA	0.103	---	Hidalgo, MX	(Sánchez et al., 2012)
	<LOD	0.074	Hidalgo, MX	(Sánchez et al., 2013)
BZF	0.03-0.20	---	Hidalgo, MX	(Siemens et al., 2008)
	2.96	1.20	Santa Fe, AR	(Vicentín and Campanella, 2013)
	0.095±0.075	0.022-0.099	Minas Gerais, BR	(Brandt et al., 2013)
Psychiatric Drugs				
CBZ	0.084-0.240	---	Hidalgo, MX	(Gibson et al., 2010)
	0.166-0.198	0.107-0.391	Biobío, CL	(Henriquez, 2012)
	---	0.2–2.3	Buenos Aires, AR	(Elorriaga et al., 2013a)
	0.92	0.92	Santa Fe, AR	(Vicentín and Campanella, 2013)
	0.7±0.1	0.7±0.3	Quito, EC	(Voloshenko-Rossin et al., 2015)
	0.01-0.03	0.01-0.1	BC., MX	(Preciado-Monjardin, 2017)
DZP	0.464	0.460	Santa Fe, AR	(Vicentín and Campanella, 2013)
β-blockers				
ATN	1.18-2.03	0.49-1.26	Biobio, CL	(Henriquez, 2012)
	---	0.2–1.7	Buenos Aires, AR	(Elorriaga et al., 2013a)
Hormones				
E1	0.044-0.080	---	MZMV, MX	(Gibson et al., 2007)
	0.073±0.030	---	Hidalgo, MX	(Silva-Castro, 2008)
	0.0013	---	São Paulo, BR	(Sodré et al., 2010b)
	0.077±0.006	---	MZMV, MX	(Chávez et al., 2011)
	0.009-0.034	---	México City, MX	(Estrada-Arriaga et al., 2013)
	0.408	0.004	Santa Fe, AR	(Vicentín and Campanella, 2013)
	<LOD-3.050	<LOD-2.080	Ceara, BR	(Pessoa et al., 2014)
	0.5±0.1	0.3±0.1	Quito, EC	(Voloshenko-Rossin et al., 2015)
E2	0.018-0.022	---	MZMC, MX	(Gibson et al., 2007)
	0.020±0.002	---	Hidalgo, MX	(Silva-Castro, 2008)
	---	0.0068	São Paulo, BR	(Lopes et al., 2010)
	0.0056	---	São Paulo, BR	(Sodré et al., 2010b)
	0.017±0.001	---	MZVM, MX	(Chávez et al., 2011)
	0.01-0.093	---	Mexico City, MX	(Estrada-Arriaga et al., 2013)
	<LOD-0.776	<LOD-0.397	Ceara, BR	(Pessoa et al., 2014)
	---	0.307 ± 0.198	Buenos Aires, AR	(Valdés et al., 2015)

Table 2.2 Occurrence and concentrations of PhACs in the wastewater systems in Latin America

Analytes	WW (µg/L)	TWW (µg/L)	Location	References
EE2	0.1	ND	Quito, EC	(Voloshenko-Rossin et al., 2015)
	0.0011	---	São Paulo, BR	(Sodré et al., 2010b)
	0.119	0.032	Santa Fe, AR	(Vicentín and Campanella, 2013)
	0.0001-0.082	---	Mexico City, MX	(Estrada-Arriaga et al., 2013)
	<LOD-3.180	<LOD-1.250	Ceara, BR	(Pessoa et al., 2014)
	---	0.110 ± 0.047	Buenos Aires, AR	(Valdés et al., 2015)

2.5.2.3 Occurrence of PhACs in surface water in Latin America

A total of 49 PhACs were detected in surface water in LATAM countries. Table 2.3 shows a summary of the six therapeutic classes, their concentrations, and locations of occurrence. Once again, NSAIDs were the most frequently detected with NPX at the lead of concentrations with 75.8 µg/L, followed by IBF (31.3 µg/L) and KTP (11.7 µg/L), all found in Mexican rivers impacted by WW (Cruz, 2013), and ACT (30.4 µg/L) in Brazil. Antibiotics were mostly reported bellow the detection limit (LOD; < 0.5 ng/L) but for a couple of sites, one in Brasil (< 0.5 µg/L) and the other the Machangara river in Ecuador, where SMX reached the appalling concentration of 309 µg/L. Data in Table 2.3 indicates that at a given time this watercourse contained also up to 830 µg/L of CBZ and 11.4 µg/L of E1, all related with discharges of untreated WW of diverse origins (Voloshenko-Rossin et al., 2015).

Given that the investigation on occurrence of estrogens in surface water is led by Brazil, E1, E2, EE2 and less frequently E3 were found in the Atibaia river and its tributaries in concentrations that generally did not exceed 1µg/L, though in few occasions were detected from 2 to 6.8 µg/L (Table 2.3).

Table 2.3 Occurrence and concentrations of PhACs in surface water in Latin America

Analytes	SW (µg/L)	Location	References
Analgesic and anti-inflammatories (NSAIDs)			
DCF	0.095-0.115	São Paulo, BR	(Montagner and Jardim, 2011)
	0.0285	Mexico City, MX	(Becerril, 2012)
	0.028-0.032	México City, MX	(Félix-Cañedo et al., 2013)
	0.014-0.145	Cordoba, AR	(Valdés et al., 2014)
	0.063-0.785	Amazonas, BR	(Thomas et al., 2014)
	0.0091-0.328	São Paulo, BR	(de Sousa et al., 2014)
	<LOD-0.385	São Paulo, BR	(Campanha et al., 2015)
	0.0194	Bahia, BR	(Pereira et al., 2016)

Table 2.3 Occurrence and concentrations of PhACs in surface water in Latin America

Analytes	SW (µg/L)	Location	References
IBF	0.002±0.001	MZMV, MX	(Gibson et al., 2007)
	<0.005	(5 sites), CR	(Spongberg et al., 2011)
	0.0154	Mexico City, MX	(Becerril, 2012)
	7.54-31.30	Chiapas, MX	(Cruz, 2013)
	0.001-0.045	Mexico City, MX	(Félix-Cañedo et al., 2013)
	0.389/0.548	Santiago, CL	(Ascar et al., 2013)
	9.66	Buenos Aires, AR	(Elorriaga et al., 2013b)
	0.0033-0.208	São Paulo, BR	(de Sousa et al., 2014)
	<LOD-0.743	São Paulo, BR	(Campanha et al., 2015)
	0.326-2.09	Bahia, BR	(Pereira et al., 2016)
	<0.006-0.039	Medellin, CO	(Aristizabal-Ciro et al., 2017)
	0.059-0.100	Hidalgo, MX	(Díaz and Peña-Alvarez, 2017)
NPX	0.0006-0.001	MZCD, MX	(Gibson et al., 2007)
	0.183	Mexico City, MX	(Becerril, 2012)
	0.001-0.186	Mexico City, MX	(Félix-Cañedo et al., 2013)
	17.83-75.84	Chiapas, MX	(Cruz, 2013)
	0.32-0.547	Santiago, CL	(Ascar et al., 2013)
	0.0051-0.098	São Paulo, BR	(de Sousa et al., 2014)
	<LOD-0.655	São Paulo, BR	(Campanha et al., 2015)
	0.160-0.246	Hidalgo, MX	(Díaz and Peña-Alvarez, 2017)
KTP	<0.007	(5 sites), CR	(Spongberg et al., 2011)
	0.021-0.042	Mexico City, MX	(Félix-Cañedo et al., 2013)
	5.87-11.68	Chiapas, MX	(Cruz, 2013)
	0.334-0.830	Santiago, CL	(Ascar et al., 2013)
SCA	0.009±0.012	MZMC, MX	(Gibson et al., 2007)
	0.011	(5 sites), CR	(Spongberg et al., 2011)
	0.0485	Mexico City, MX	(Becerril, 2012)
	0.001-0.309	Mexico City, MX	(Félix-Cañedo et al., 2013)
ACT	0.84	São Paulo, BR	(Sodré et al., 2007)
	0.28-13.44	São Paulo, BR	(Montagner and Jardim, 2011)
	0.015	(5 sites),CR	(Spongberg et al., 2011)
	<LOD-30.4	São Paulo, BR	(Campanha et al., 2015)
	0.017-0.034	Bahia, BR	(Pereira et al., 2016)
Antibiotics			
SMX	<0.0011	(5 sites),CR	(Spongberg et al., 2011)
	<0.0007-0.106	São Paulo, BR	(Locatelli et al., 2011)
	6.0-309	Quito, EC	(Voloshenko-Rossin et al., 2015)
TMP	<0.0005-0.484	São Paulo, BR	(Locatelli et al., 2011)
	<0.007	(5 sites), CR	(Spongberg et al., 2011)
CTR	<0.005	(5 sites), CR	(Spongberg et al., 2011)
Lipid Regulators			
GMZ	0.0094	Mexico City, MX	(Becerril, 2012)
	0.009-0.01	Mexico City, MX	(Félix-Cañedo et al., 2013)
CFA	0.001-0.009	Mexico City, MX	(Félix-Cañedo et al., 2013)
	0.0053	Hidalgo, MX	(Sánchez et al., 2013)
Psychiatric Drugs			
CBZ	0.63	Buenos Aires, AR	(Elorriaga et al., 2013b)
	0.015-0.113	Córdoba, AR	(Valdés et al., 2014)
	0.014-0.652	Amazonas, BR	(Thomas et al., 2014)
	0.005-0.659	São Paulo, BR	(de Sousa et al., 2014)
	11.6-830	Quito, EC	(Voloshenko-Rossin et al., 2015)
	<LOD-0.215	São Paulo, BR	(Campanha et al., 2015)

Table 2.3 Occurrence and concentrations of PhACs in surface water in Latin America

Analytes	SW (µg/L)	Location	References
	0.0001-0.002	B.C., MX	(Preciado-Monjardin, 2017)
β-blockers			
ATN	0.55	Buenos Aires, AR	(Elorriaga et al., 2013b)
	0.009-0.581	Cordoba, AR	(Valdés et al., 2014)
	0.005-0.412	São Paulo, BR	(de Sousa et al., 2014)
	0.080-0.543	Brazil	(Pinê Américo et al., 2015)
	<LOD-8.19	São Paulo, BR	(Campanha et al., 2015)
VAL	0.010	Bahia, BR	(Pereira et al., 2016)
Hormones			
E1	0.0001	MZMV, MX	(Gibson et al., 2007)
	<0.600	São Paulo, BR	(Lopes et al., 2010)
	0.0022-0.039	São Paulo, BR	(Sodré et al., 2010b)
	<0.023	México City, MX	(Díaz-Torres et al., 2013)
	<LOD-0.043	Hidalgo, MX	(Sánchez et al., 2013)
	0.32	São Paulo, BR	(da Silva and de Lima, 2014)
	0.004-0.008	São Paulo, BR	(de Sousa et al., 2014)
	<LOD-0.0147	São Paulo, BR	(Campanha et al., 2015)
	0.40-11.4	Quito, EC	(Voloshenko-Rossin et al., 2015)
	0.006-0.014	São Paulo, BR	(Torres et al., 2015)
E2	0.038-2.51	São Paulo, BR	(Sodré et al., 2007)
	0.001-0.036	Minas Gerais, BR	(Moreira et al., 2009)
	0.008-0.025	São Paulo, BR	(Lopes et al., 2010)
	<LOD-0.0073	São Paulo, BR	(Sodré et al., 2010b)
	0.464-6.8	São Paulo, BR	(Montagner and Jardim, 2011)
	0.0626	Minas Gerais, BR	(Moreira et al., 2011)
	<0.0027	Mexico City, MX	(Díaz-Torres et al., 2013)
E2	<LOD-0.005	Hidalgo, MX	(Sánchez et al., 2013)
	0.40-2.2	Quito, EC	(Voloshenko-Rossin et al., 2015)
	<LOD-0.0148	São Paulo, BR	(Campanha et al., 2015)
	0.041-0.087	São Paulo, BR	(Torres et al., 2015)
	0.043	Buenos Aires, AR	(Valdés et al., 2015)
E3	<LOD-0.0023	São Paulo, BR	(Sodré et al., 2010b)
	0.001/0.0077	São Paulo, BR	(Jardim et al., 2012)
	0.044/0.046	São Paulo, BR	(Torres et al., 2015)
	0.4	São Paulo, BR	(da Silva and de Lima, 2014)
EE2	0.006-0.31	São Paulo, BR	(Sodré et al., 2007)
	0.001-0.054	Minas Gerais, BR	(Moreira et al., 2009)
	0.025	São Paulo, BR	(Sodré et al., 2010a)
	0.501-4.3	São Paulo, BR	(Montagner and Jardim, 2011)
	0.056-0.063	Minas Gerais, BR	(Moreira et al., 2011)
	<LOD-0.088	Hidalgo, MX	(Sánchez et al., 2013)
	0.369	Buenos Aires, AR	(Valdés et al., 2015)
	0.30±0.2	Quito, EC	(Voloshenko-Rossin et al., 2015)
	<0.16	São Paulo, BR	(Campanha et al., 2015)
	0.026-0.150	São Paulo, BR	(Torres et al., 2015)

2.5.3 General behavior of PhACs in Latin America

Figure 2.4 shows the general behavior of the maximum concentrations of PhACs reported in the three most relevant environmental compartments in LATAM, that is, WW entering the municipal treatment systems, TWW released into the environment, and SW impacted by diverse degrees of pollution. It does not include the concentrations of natural estrogens, which leaves only the EE2 hormone and the 17 most relevant PhACs disclosed in Tables 2.2 and 2.3 (the top 18th). The box plots show medians and interquartile ranges as well as ranges of whiskers and the outliers for each type of water contemplated. Although the assembled data were generated under a diversity of environmental and analytical circumstances and the sample sizes and distributions are not equal, the non-parametric median test may provide an indication of the maximum concentrations behaviour. Then, there is an apparent trend of concentrations that goes WW>TWW>SW (Figure 2.4) however, the Moods' median test ($\alpha = 0.05$) reveals no significant difference between WW and TWW. These results are consistent with the interpretations of many authors who have indicated the limited capacity of WWTP for the elimination of PhACs, given that the removal rate depends to a large extent on the site-specific treatment processes and conditions, as well as on the concentration, number and type of compounds present in the mixture (e.g., Gros et al., 2007; Yang et al., 2017).

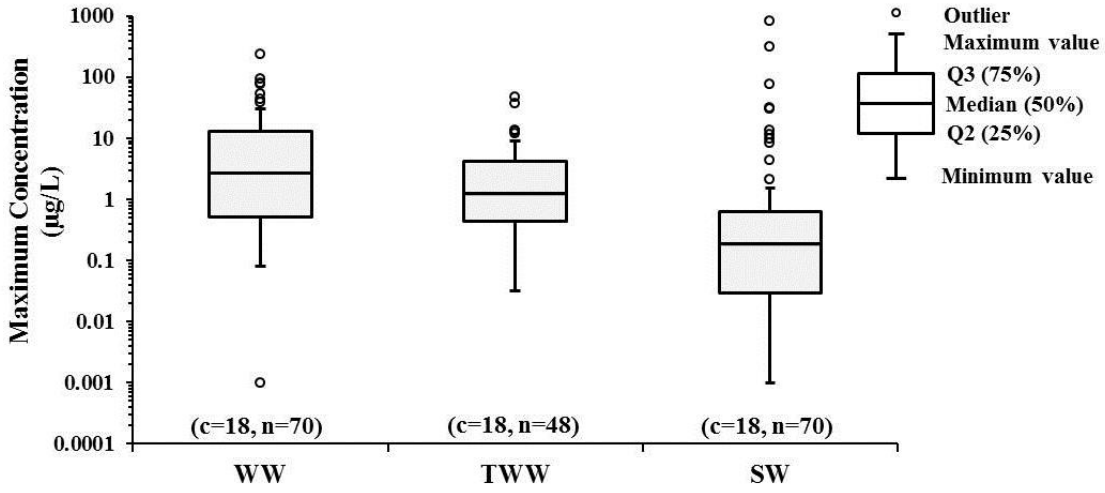

Figure 2.4 Box-plots of maximum concentrations of PhACs in WW, TWW and SW in log scale. Outliers are 25th and 75th percentile ±1.5 interquartile range. c = number of compounds and n = number of data.

In addition, although 10% of the WW data are plotted as outliers, these values correspond exclusively to NSAIDs and SCA that were reported in only two countries, Mexico and Colombia (Table 2.2). Concurrently, the TWW outliers (~10%) are also related to NSAID, this time in four other countries, and to antibiotics (SMX) in Ecuador (Table 2.2). The outstanding concentrations released into the environment are many times higher than their equivalents in Europe, Asia and North America, which report, for example, a few μg/L of NSAIDs (da Silva et al., 2011), up to 21 μg/L of antibiotics (Liu and Wong, 2013) and bellow 0.05 μg/L of E1 and E2 (Lishman et al., 2006). Therefore, these concentrations seem to be the result of the combination of the uncontrolled consumption, the limitations of the treatment technologies and the instability in the WWTP operation, in particular the unexpected failures mostly related to power outages and the inability to manage the ever increasing flow rates (Henriquez, 2012; Voloshenko-Rossin et al., 2015). Hence, it is hypothesized that WW entering WWTP often bypass treatment, intentionally or not, and reach the environment in mostly intact conditions (Ascar et al., 2013).

On the other hand, the maximum concentrations in SW are considerably lower than those in TWW, though more disperse in values and in the variety of compounds. The numerous outliers (16%) are the result of the abundant points of direct discharge of domestic, agricultural and industrial WW into the natural watercourses (Cruz, 2013; Montagner and Jardim, 2011; Voloshenko-Rossin et al., 2015) and come to equal and even surpass the source concentrations (Table 2.3). The maximums in this aquatic compartment are found in Quito, Ecuador, and correspond to CBZ and SMX in the highly polluted Machangara River, stream that receives direct contributions of untreated wastewater from several urban centers (Voloshenko-Rossin et al., 2015). The rest of the outliers are mostly related to NSAIDs in Sao Paulo, Brazil, and Chiapas, Mexico, and EE2 and ATN also in Sao Paulo (Table 2.3). This situation is generally perceived as the cost of accelerated population growth, urbanization, and industrialization combined with a deficient sanitation system and the common lack of regard for the environmental legislation in LATAM. In this respect, it is important to mention that most of the LATAM countries count with regulations that indicate that WW must undergo treatment prior to its release to the environment (DOF,

1998; RO, 2014) or, otherwise, they align with the guidelines of the World Health Organization.

However, the practice does not always comply with the law, as many authors have pointed out (e.g., Martins et al., 2008). Furthermore, there are no regulations in the worldwide legal framework related with EOC, yet, European, North American and Asian countries are making outstanding efforts in order to mitigate the presence of these and other harmful compounds in the environment. The European Union has created guidelines to evaluate the potential environmental risk posed by medical products (EMEA, 2006) and has included DCF and the hormones E2 and EE2 in the list of priority substance contained in the Water Framework Directive 2013/39/EU (EU, 2013). Similarly, Vietnam Government has set up a National Action Plan against antibiotic resistance, with the support of WHO, FAO and other organization for the period from 2013 to 2020 (Binh et al., 2018).

2.6 Concluding remarks

PhACs are ubiquitous in the environment and LATAM's continental waters are not the exemption. Results from 66 investigations available along the last decade (2007-2017) and conducted mainly in Brazil and Mexico, and to a lesser extent in Argentina, Chile and Colombia, documented the constant occurrence of PhACs in the three aquatic compartments included in this review. From this information it was possible to identify an apparent trend of maximum concentrations that goes WW>TWW>SW, although the difference between WW and TWW is not statistically significant.

It is not surprising that the most frequently sought and found PhACs in the aquatic environment of LATAM during the analyzed decade were NSAIDs (IBF and DCF) and the synthetic hormone EE2 while the natural hormone E2 was the most detected EDCs. The concentrations observed in SW were often higher than those reported in the same compartment in other parts of the world; furthermore, some exorbitant concentrations of 309 and 830 µg/L of SMX and CBZ, respectively, were detected in Ecuador, in addition to the 6.8 µg/L of E2 obtained in Brazil. These findings are of serious concern given that lower environmental concentrations have the potential to adversely affect aquatic and terrestrial organisms like fish, crustaceans, algae and birds.

The presence of PhACs and hormones in TWW, when the treatment actually occurs, reveals the inadequacy of the WW treatment technologies to eliminate these compounds, which releases a substantial amount of PhACs into the environment. It is clear then that the primary source of PhACs in the aquatic environment of LATAM is WW, whether or not it passes through the WW treatment systems. Hence it is of utmost importance for the environmental and, eventually, the human health in LATAM that the countries direct substantial efforts to (a) increment the efficiency of their WW treatment technologies and propose accessible and cost-effective methods to reduce the presence of PhACs and the products of their transformation in the TWW and, consequently, in the aquatic environment; (b) plan and implement WW management strategies capable of guaranteeing WW treatment under conditions of extraordinary volumes, such as those related to floods, and in the case of power outages; and (c) create, when necessary, and apply the proper regulations to ensure that all WW is channeled to the WWTP.

Implementing continuous environmental monitoring, creating guidelines to evaluate the potential environmental risk posed by medical products and preparing lists of priority substances are other actions that LATAM countries should include in their environmental agendas in the near future.

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3 CHAPTER

Occurrence, distribution and risk assessment of pharmaceuticals in the aquatic environment of the semiarid Mexicali, Mexico

Abstract

This study describe an investigation on the occurrence of twelve pharmaceuticals active compounds (PhACs), belonging to six different therapeutics classes: non-steroidal anti-inflammatory drugs (NSAID's), antibiotics, hormones, psychiatrics, beta-blockers and lipid regulators in surface water and WWTP's effluents, including a wetland system, in order to evaluate impact on the quality of surface water in the semiarid zone of Mexicali and its surrounding valley (northwest Mexico). In addition, an environmental risk assessment of PhACs was conducted under EMEA guidelines. These regulatory concepts are based on a set of short-term ecotoxicological studies on three different representatives trophic level species (fish, daphnid and algae) by calculating the risk quotient (RQ, the ratio between the measured environmental concentration (MEC) and the predicted no affect concentration (PNEC). A simultaneous mixture of PhACs was also calculated in surface water. Data show that only five (carbamazepine, sulfamethoxazole, ibuprofen, diclofenac and metoprolol) of out twelve compounds were detected in this study area. Carbamazepine and sulfamethoxazole were the most frequently detected compounds, followed by ibuprofen and less frequency diclofenac and metoprolol. As expected, the higher concentrations of PhACs were detected from WWTPs and the wetland system. Regarding to ERA, the compound that present the greatest threat to aquatic organisms is SMX being algae the most sensitive organism

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Keywords: Occurrence; environmental risk assessment; wastewater; pharmaceuticals

3.1 Introduction

At present, pharmaceutical active compounds (PhACs) such as antibiotics, psychiatric drugs, analgesics, anti-inflammatory drugs, beta-blockers, and lipids regulators, have become important water pollutants due to their ubiquitous occurrence, persistence and potential adverse effects on the aquatic life and humans (Domínguez et al., 2011; Evgenidou et al., 2015). The environmental concern extends to the metabolites of PhACs (e.g. carbamazepine-10-11-epoxide, norfluoxetine, salicylic acid, acetylsufamethoxazole, 4´-hydroxydiclofenac), which are generated within the organisms and subsequently released into the environment through excretions (Celiz et al., 2009; Scheurell et al., 2009), and to the transformation products (TP) (e.g., irbersartan TP as ISW1a and ISW1b), which are formed mainly throughout the wastewater treatment processes (Jelic et al., 2013). These byproducts have been detected in the aquatic environment at even higher concentrations than the parent compounds. For instance, 10,11 dihydroxycarbamazepine (CBZ-dihydroxy) metabolite was detected by Miao and Metcalfe (2003) at ca. three times higher concentrations than the parent carbamazepine in WWTPs and surface water. In addition, PhAC metabolites and TPs could be as, or even more, hazardous, persistent and mobile than the parent compound(s) (Boix et al., 2016; Godoy et al., 2015; Pereira et al., 2017). Such is the case of 4´-hydroxyphenylcarvedilol (a metabolite of carvedilol), which is thirteen times more potent than the parent beta-adrenergic blocker.

The prevalent occurrence of PhACs is reported in a variety of environmental compartments such as wastewater, WWTPs effluents, surface water, groundwater (Jurado et al., 2012; Mompelat et al., 2009; Salgado et al., 2010; Spongberg et al., 2011) and, more recently, also in seawater (Du et al., 2017; Lolić et al., 2015; Nödler et al., 2014) in concentrations ranging from nanograms to micrograms per liter. In addition, PhACs have been detected in sediments, soils and sewage sludge at a few nanograms to micrograms per kilograms (Beretta et al., 2014; Vazquez-Roig et al., 2010). As a consequence of this environmental pollution, PhACs such as diclofenac, fluoxetine, sertraline, and their metabolites have also been detected in aquatic organisms (e.g. fish tissue matrices) in concentrations >1 ng/g (Brooks et al., 2005; Islas-Flores et al., 2013; Ramirez et al., 2009) and in terrestrial species (e.g. *Gyps* vultures) in concentrations > 0.8 mg/kg (Swan et al., 2006). Clearly, the

occurrence of PhACs in the environment can lead to toxicological effects on non-target organisms (Godoy et al., 2015) as shown by Oaks et al. (2004) and Swan et al. (2006), who reported effects such as renal failure, lethargy and neck dropping behavior in *Gyps* vultures through feeding on diclofenac-treated livestock, occasionally ending in death by visceral gout illness. However, even though much information on worldwide occurrence has been generated, up to now no study has dealt with PhACs in the aquatic environment of a semiarid zone located at the very end of the transboundary Colorado River basin, the city of Mexicali and its surrounding agricultural valley, in NW Mexico. Therefore, in this study, the occurrence, concentration and spatial distribution of twelve pharmaceuticals in surface water and WWTP influents and effluents were investigated. The PhACs were selected among those more frequently reported in the worldwide aquatic environment. In addition, the ecological risk represented by PhACs was evaluated, both individually and as a mixture, for typical aquatic organisms.

3.2 Materials and methods

3.2.1 Chemicals and standards

All twelve pharmaceutical standards (purity >98%) were selected from six therapeutic classes: antibiotics (roxithromycin, sulfamethoxazole), non-steroidal anti-inflammatories (diclofenac, ibuprofen), β-blockers (Metoprolol), psychiatric drugs (carbamazepine, oxcarbazepine, diazepam, fluoxetine), Lipid Regulators (clofibric acid, gemfibrozil) and synthetic hormone (17α-ethinylestradiol). They were purchased from Sigma-Aldrich (St Louis, MO, USA), VWR International (Radnor, PA, USA) and Toronto Research Chemical (Toronto, CANADA). All solvents (MeOH, ACN, MTBE) used during the analysis were HPLC grade.

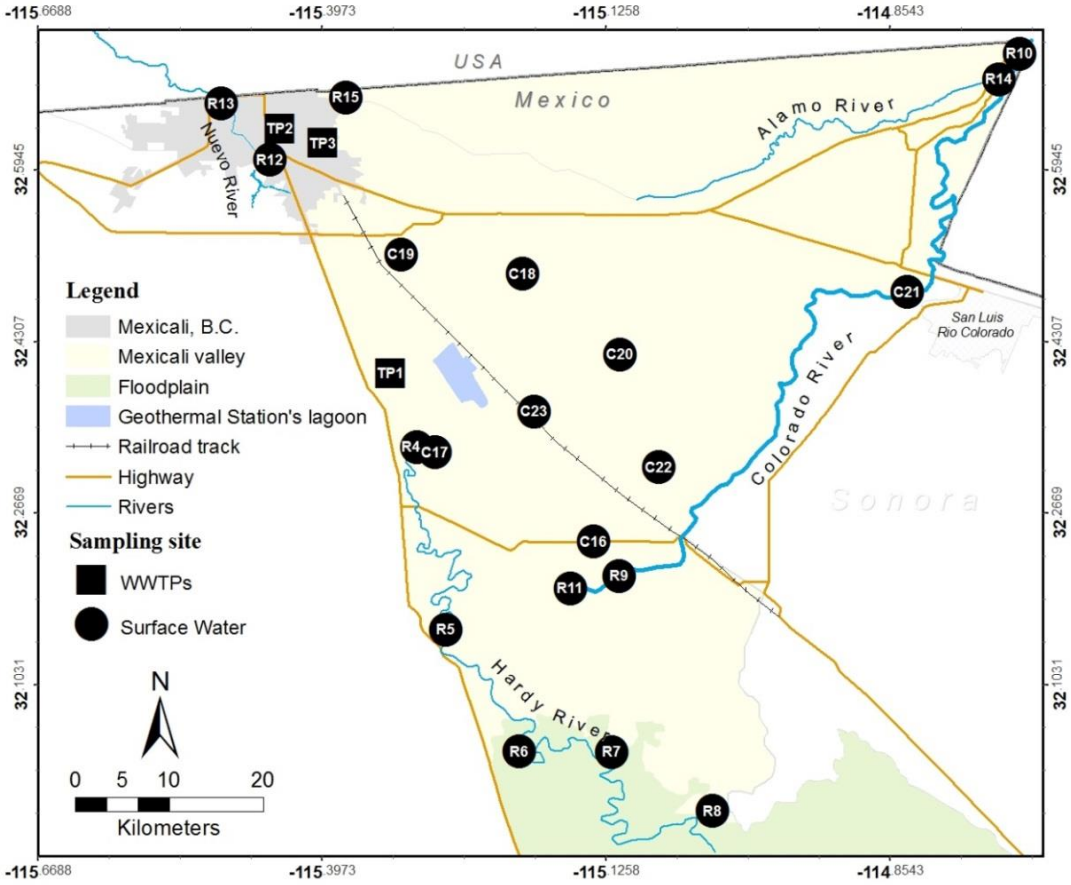
3.2.2 Site description and hydrological frame

The Mexicali Valley (Figure 3.1), which includes the city of Mexicali and its surrounding agricultural zone, extends over 13,700 km^2 on the lower portion of the Colorado River delta in northwestern Mexico. Together the city and the numerous villages distributed along the valley comprise a population of 936,826 inhabitants (INEGI, 2015). According to the Kôppen climatic classification system, Mexicali presents a very arid or very dry climate (García, 1974) with local temperatures normally exceeding 35^oC, reaching up to 50^oC in July and August, and minimum winter temperatures usually bellow 0^oC (Ramírez-Hernández, 2006). In addition, Mexicali has the record for the lowest total precipitation in the whole country with less than 50 mm per year, while potential evaporation is 2500 mm per year (INEGI, 2015).

The main surface watercourses along the Mexicali Valley are the Colorado River (CR), the Hardy River (HR) and the Nuevo River (NR). The CR is the region's water supplier and provides an annual volume of 1,850 hm^3. This water enters Mexico through the Morelos dam and is distributed throughout the valley by means of a large network of open canals to be used mainly for irrigation and to sustain all the economic sectors. In contrast, the source of water in the HR is a combination of recharge from precipitation along the skirts of the nearby mountain ranges, return flows from agriculture and, recently, certain volumes of

treated wastewater released from Las Arenitas WWTP (Sonoran Institute, 2015; Zamora-Arroyo and Santiago, 2010). This water is used for recreational purposes before joining the Colorado River channel to finally discharge in the upper Gulf of California. The NR is a watercourse that flows through the city of Mexicali receiving water from undetermined sources that mixes with treated wastewater effluents from the Mexicali-II WWTP (Ley et al., 2011). This is the only watercourse that goes north, crosses the border into the U.S.A. and discharges into the Salton Sea (Ramírez-Hernández, 2006). The present day Alamo River (AR) acts as a ditch and lagoon that collects return flows from irrigated lands and, at some points, water from industrial activities.

In addition to the rivers, the Mexicali Valley's hydrologic system includes numerous distribution canals and approximately 40 constructed agricultural ditches or drains that transport water from irrigation returns and, occasionally, undetermined volumes of urban drainage (Hernández et al., 2011) towards the surface water bodies. Flows from some of these drains have given rise to several wetlands, the largest of them known as La Cienega de Santa Clara, which formed from the sub-surface agricultural drainage of the Yuma region of Arizona (Nelson et al., 2013). The Las Arenitas wetland was recently built with the purpose of adding a biological treatment to effluents from the WWTP of the same name before release into the environment (Zamora-Arroyo and Santiago, 2010). These wetlands sustain a varied and unique wildlife, which includes protected and/or endemic species. According to the state water operator, 98% of the wastewater generated in the state receives some type of treatment. Of this, only 30% is reused in industrial activities (CEA, 2015) while the rest is sent to the aforementioned waterbodies. Amongst the municipality of Mexicali there are two aerated-lagoon WWT systems, in Mexicali, and five more elsewhere, in addition to three small WWTP based on activated sludge (CESPM, 2015).



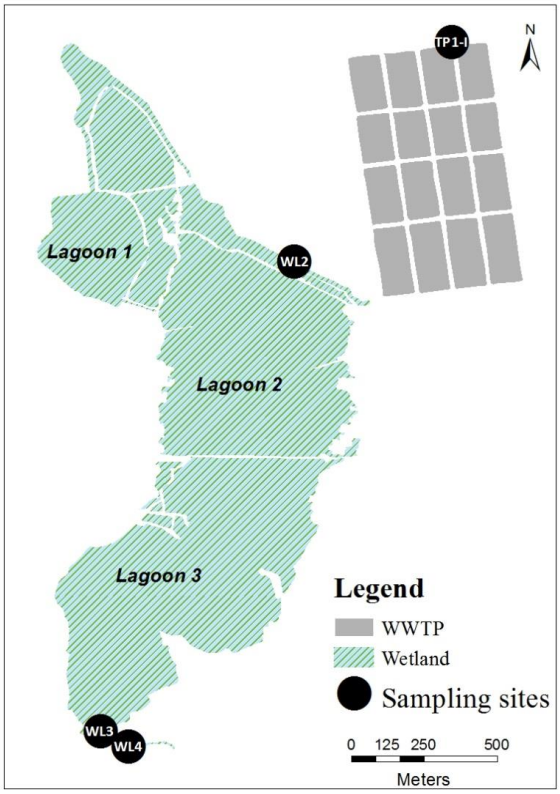
Site	WWTPs
TP1	WWTP-Wetland Las Arenitas
TP2	UABC
TP3	ITM

Site	Surface Water	Site	
R4	HR, upstream	**R14**	AR, upstream
R5	C. Mosqueda	**R15**	AR, downstream
R6	HR, downstream	**C16**	Carranza
R7	DMS15	**C17**	Sakamoto
R8	E3	**C18**	El Peligro
R9	CILA	**C19**	Guanajuato
R10	CR, upstream	**C20**	Veracruz
R11	CR, downstream	**C21**	M. Aleman
R12	NR, upstream	**C22**	Gpe. Victoria
R13	NR, downstream	**C23**	Delta

Figure 3.1 Map of the Mexicali city and valley showing the sampling sites: natural water courses and canals (circles), and from WWTPs (squares).

3.2.3 Sampling sites

To determine the occurrence of PhACs in the aquatic environment of the city of Mexicali and its surrounding valley it was decided to design a non-probabilistic sampling plan. A total of 23 sites were selected including the Colorado, Hardy, and Nuevo rivers, two small WWTPs located within higher education institutions and serving the local areas (UABC and ITM), and Las Arenitas municipal system. This survey also included irrigation canals and agricultural ditches within the Mexicali valley. Locations of the sampling sites are shown in Figure 3.1 and 3.2 and detailed information for each site is found in Table 3.1



Site	WWTP-Wetland Las Arenitas
PT1-I	WWTP Las Arenitas Influent
WL2	Wetland Influent
WL3	Wetland Lagoon 3
WL4	Wetland Effluent

Figure 3.2 Location of sampling sites within Las Arenitas WWTP-Wetland

Table 3.1 Detailed information for each sampling site

Site	Name	Description
Wastewater Treatment Plants		
TP1	**WWTP-Wetland Las Arenitas**	**WWTP-Wetland Las Arenitas System**
TP1-I	WWTP Las Arenitas Influent	Las Arenitas influent treatment plant
WL2	Wetland Influent	Las Arenitas wetland influent
WL3	Wetland lagoon 3	Las Arenitas wetland lagoon 3
WL4	Wetland Effluent	Las Arenitas wetland effluent
TP2	**WWTP UABC**	University Campus treatment plant
TP2-I	UABC Influent	University Campus influent treatment plant
TP2-E	UABC Effluent	University Campus effluent treatment plant
TP3	**WWTP ITM**	University Campus treatment plant
TP3-I	ITM influent	University Campus influent treatment plant
TP3-E	ITM effluent	University Campus effluent treatment plant
Surface Water		
R4	HR upstream	Hardy river upstream flow
R5	C. Mosqueda	Hardy river, Campo Mosqueda recreational area
R6	HR downstream	Hardy river, Ecocampo recreational area
R7	DMS15	Hardy river, restoration zone
R8	E3	Hardy river, estuarine zone
R9	CILA	Colorado river, restoration zone
R10	CR upstream	Colorado river upstream, Morelos Dam
R11	CR downstream	Colorado river downstream, Vado Carranza
R12	NR upstream	Nuevo river upstream flow
R13	NR downstream	Nuevo river downstream flow
R14	AR upstream	Alamo river upstream, Algodones
R15	AR downstream	Alamo river downstream, Border
C16	Carranza	Agricultural drain
C17	Sakamoto	Agricultural drain
C18	El peligro	Colorado river irrigation canal
C19	Guanajuato	Colorado river irrigation canal
C20	Veracruz	Colorado river irrigation canal
C21	M. Aleman	Colorado river irrigation canal
C22	Gpe. Victoria	Agricultural drain
C23	Delta	Irrigation canal

3.2.4 Samples collection

The environmental samples were collected during April and May 2015. These grab water samples were taken in 1 L amber, glass amber bottles previously washed with MeOH/Milli-Q water 50:50, rinsed with ultrapure water and baked at 475 ^{o}C for 4 hrs. Prior collection, the bottles were rinse three with the water from the site of interest (Figure 3.3 a). Samples were taken in duplicate and a field blank, consisting of a 1-L bottle of LCMS water (J.T. Baker), was opened at each site. pH, electrical conductivity (EC) and water temperature

were measured using multiparametric equipment (Figure 3.3 b and c). All the samples were transported to the laboratory in coolers and stored overnight at 4^{o}C.

(a) (b) (c)

Figure 3.3 Samples collection. (a) rinsing the bottles before taking the sample; (b) and (c) measurement of physicochemical parameters.

3.2.5 Sample extraction and UPLC-MS/MS

Once in the laboratory, the water samples were filtered through 0.7 µm glass fiber filters before the addition of 0.5 g of EDTA as the chelating agent and 50 µl of internal standard mixture. Recovery controls were spiked with 100 µl of target analyte mixture.

Off-line solid phase extraction (SPE) was carried out on a manifold (Agilent Vac Elut SPE), assisted by a vacuum pump. Oasis HLB cartridges (Waters Corp., 6 ml, 200 mg) were pre-conditioned with 5 mL methanol (MeOH), 5 mL methyl-tert-butil-eter (MTBE) and 5mL LCMS water at a flow rate of 3 mL/min. followed by sample loading and drying under vacuum for 40 min. The solutions were then extracted by SPE (Vergeynst et al., 2015) at a flow rate of 3-5 mL/min. Finally, the cartridges were eluted with 3 ml MeOH, 3 ml NH_4OH/water 95:5, 3 ml acetonitrile (ACN), 3 ml MTBE and 3 ml MeOH, and then reduced under a gentle stream of nitrogen at 40-45 ^{0}C before dilution in 1 ml of 50:50 MeOH/LCMS water and transferred to 2 ml autosampler vials.

The target analytes (pharmaceutical compounds) were measured by high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-ESI/MS^2) with multiple reaction monitoring (MRM) using a quadrupole time of flight QToF analyzer, equipped with electrospray ionization (ESI) source operated in either positive or negative mode. The chromatographic separation was performed using an Agilent C18 1.7 µm

particle size column (100 x 2.1 mm) at a flow rate of 300 µL/min. For ESI (+) the mobile phase was 0.1% formic acid in MeOH (A) and 0.1% formic acid in LCMS H_2O (B) and, for ESI(-), NH_4OAc in MeOH (A) and 2 mM NH_4OAc LCMS H_2O:MeOH (95:5) (B). The gradient program for both positive and negative modes was as follows: 25% A until 0.5 min, increasing to 85% A at 4.5 min, and to 100% A at 4.6 min, and then remaining at 100% A for 3.7 min before returning to 25% A for re-equilibration.

3.2.6 Quality controls

LCMS instrument performance and laboratory procedures were checked by running reagent lab blanks and mixed internal standard solutions at regular intervals. In all cases, the concentration of target compounds in lab and field blanks were below the limit of detection, which indicates that glassware and procedures were free of contamination. Calibration solutions of target compounds (Table 3.2) were prepared in six points within the range of 0.01 to 1000 ng/L. The mean correlation coefficients (r^2) of the calibration curves were higher than 0.98 (Table 3.2) and the relative standard deviations (RSD) were 15% and, in two cases, larger than 20%. Samples, duplicates, and controls were measured in triplicate and final concentrations were calculated by multiplying the average measured concentrations by a respective concentration factor (814 ± 81, resulting from SPE) and standardizing the result with the recovery factor obtained for each target analyte (Table 3.2).

Table 3.2 Validation of the extraction method including SPE recoveries

Classes	Compound	Acronyms	Recovery (%)	*RSD (%)*	r^2
Antibiotic	Sulfamethoxazole	SMX	71±16	22	0.992
NSAIDs	Diclofenac	DCF	106±16	15	0.997
	Ibuprofen	IBF	47±7	15	0.989
Antiepileptic	Carbamazepine	CBZ	54±12	21	0.986
β-blocker	Metoprolol	MTP	37±6	15	0.994

3.2.7 Environmental risk assessment

The European Medicines Agency (EMEA) guidelines (EMEA website) were consulted in order to estimate the potential environmental risk of each PhACs found in this study on

three different trophic levels: blue-green algae (*Selenastrum Capricornutum*), plankton (*Daphnia magna*) and fish (*Fathead minnows*). The Environmental risk assessment (ERA) was estimated for a complex treatment system (Las Arenitas wetland system) and the Hardy as the demonstrative river given that the two of them are hydrologically connected.

Individual environmental risk of each PhACs was evaluated by calculating the risk quotient (RQ) obtained from the ratio between measured environmental concentration (MEC) and the predicted no-effect concentration (PNEC), as shown in Equation (1)

$$RQ = \frac{MEC}{PNEC} \quad \text{.....Equation (1)}$$

The PNEC value is usually calculated by dividing the no observed effect concentration (NOEC) value by a default assessment factor (AF) of 1000 (EMEA, 2006). However, NOEC were substituted by the lethal concentration required to kill 50% of the population (LEC_{50}) or the half-maximal effective concentration (EC_{50}), as shown in Equation (2) (Bonvin et al., 2011) , which were obtained from the literature or using a predictive model Ecological Structure Activity Relationship (ECOSAR) to estimate acute aquatic toxicity (USEPA, 2016)

$$PNEC = \frac{NOEC}{1000} \quad \text{.....Equation (2)}$$

When RQ<1, the analyzed substance is unlikely to represent a risk to the aquatic environment but then, if RQ>1, further evaluations on the fate of the substance in the aquatic environment are needed. For risk assessment there are three levels: low risk from 0.01 to 0.1 µg/l, medium risk from 0.1 to <1, and high risk when values are ≥1 (Hernando et al., 2006).

To estimate the risk due to the simultaneous presence of PhACs, a sum of all the individual MEC divided by the sum of individual PNEC (Thomaidi et al., 2015) was calculated (Equation 3).

$$RQ_{mix} = \sum_{i=1}^{n} RQ_i = \sum_{i=1}^{n} \frac{MEC_i}{PNEC_i} \quad \text{.....Equation (3)}$$

In order to estimate the possible environmental hazard of the mixture of PhACs in the Hardy river, the $RQ_{mix,river}$ was calculated using Equation 4. Data related to water flows was taken from Carrera (2015) and from the Sonoran Institute (oral comm.) to determine dilution factors (DF) (Equation 5).

$$RQ_{mix,river} = \frac{RQ_{mix}}{DF} \quad \text{.....Equation (4)}$$

$$DF = \frac{Q_r}{Q_e} \quad \text{……..……..……..……Equation (5)}$$

Where Q_e is the average flow of treated wastewater from Las Arenitas System (m^3d^{-1}) and Q_r is the average water flow of the corresponding river (m^3d^{-1}).

Table 3.3 shows the psychochemical parameters obtained from the National Center for Biotechnology Information, U.S. National Library of Medicine public databases (Kim et al., 2015) in order to feed the ECOSAR program and obtain LEC_{50} and CE_{50} acute toxicity concentrations for the three trophic levels.

Table 3.3 Physicochemical proprieties of the PhACs obtained from PubChem

Therapeutic Classes	Compound	CAS Number	MW	Log K_{ow}	MP	S_w
			(g/mol)		°C	(mg/L)
Antibiotic	Sulfamethoxazole	723-46-6	253.27	0.89	167.0	610
Antiepileptic	Carbamazepine	298-46-4	236.27	2.45	190.2	18
Anti-inflammatory	Ibuprofen	15687-27-1	206.28	3.97	75-77	21
	Diclofenac	15307-86-5	296.14	4.51	156-158	2.37
Beta-blocker	Metoprolol	51384-51-1	267.36	1.88	120	16900

CAS: Chemical Abstract Service Registry Number, Sw: Water Solubility
PM: Molecular Weight, MP: Melting Point
Log K_{ow} : Octanol-water partition water,

3.3 Results and discussion

3.3.1 Occurrence and concentrations of PhACs

Of the twelve PhACs analyzed in the aquatic environment of Mexicali and its surrounding valley, only five were detected in surface waters. These compounds correspond to four therapeutic classes: the NSAIDs diclofenac (DCF) and ibuprofen (IBF), the antibiotic

sulfametoxazol (SMX), the psychiatric antiepileptic carbamazepine (CBZ), and the beta-blocker metoprolol (MTP). The concentrations of the five compounds for the sampling period of April-May, 2015, can be observed in Table 3.4 and location of the sampling sites is showing in Figures 3.1 and 3.2.

Table 3.4 Concentrations of PhACs detected in WWTPs and surface water in Mexicali and its surrounding valley. April-May, 2015

Site	Name	DCF	IBF	SMX	CBZ	MTP
		concentrations (ng/L)				
Wastewater Treatment Plants						
TP1	**WWTP-Wetland Las Arenitas**					
TP1-I	WWTP Las Arenitas Influent	246±3	1177±99	333.4±59	9.2±0.80	n.d
WL2	Wetland Influent	156±1	1705±161	243.0±42	12.7±0.40	0.6±0.3
WL3	Wetland lagoon 3	12.6±1	116±14	419±28	12.7±2.1	71.0±14
WL4	Wetland Effluent	21±0.7	89±10	420.6±63	14.8±1.90	122.4±65.3
TP2	**WWTP UABC**					
TP2-I	UABC Influent	220±24	1010±104	183±20	32.6±2.90	n.d
TP2-E	UABC Effluent	9.5±1	41±9.5	9.1±0.60	114.6±9.70	n.d
TP3	**WWTP ITM**					
TP3-I	ITM influent	234±5	1059±125	167.8±16	15.0±3.50	n.d
TP3-E	ITM effluent	433±28	304±23	830.9±136	18.3±1.40	n.d
Surface Water						
R4	HR upstream	24±2	75±4	145±34	8±0.5	51.1
R5	Campo Mosqueda	n.d	16.1±2.7	6.0±1.5	0.8	n.d
R6	HR downstream	n.d	29±4	4±0.8	0.6	n.d
R7	DMS15	n.d	n.d	0.3±0.2	0.6	n.d
R8	E3	n.d	17.4±4.2	n.d	n.d	n.d
R9	CILA	n.d	n.d	3.2±0.5	0.2	n.d
R10	CR upstream	n.d	n.d	12.7±1.1	0.7±0.20	1.2±0.6
R11	CR downstream	n.d	n.d	2.5±0.8	0.1	n.d
R12	NR upstream	n.d	39±5	3.8±0.4	0.6	n.d
R13	NR downstream	6.3±0.4	40±8.3	10.9±0.9	1.5	4.6±0.6
R14	AR upstream	n.d	n.d	4.4±1.0	0.3	n.d
R15	AR downstream	n.d	45.4±6	29.8±4.6	0.1	n.d
C16	Carranza	5.5±0.1	57±5	166.4±27	2.8±0.6	44.2±05.8
C17	Sakamoto	n.d	n.d	0.2	n.d	n.d
C18	El peligro	n.d	n.d	10.4±0.1	0.6±0.1	n.d
C19	Guanajuato	n.d	n.d	5.8±0.4	0.3±0.1	n.d
C20	Veracruz	n.d	n.d	11.2±1.6	0.6	0.5±0.4
C21	M. Aleman	4.7±0.5	n.d	9.7±1.5	0.4±0.1	n.d
C22	Gpe. Victoria	n.d	n.d	n.d	0.3	n.d
C23	Delta	n.d	12±0.7	n.d	0.6	0.7±0.5

Carbamazepine and sulfamethoxazole were identified in most of the sampling sites, with a detection frequency of up to 87% (21 and 20 sites, respectively). CBZ was detected in the range of 0.1-114.6 ng/L and SMX within 0.2-830.9 ng/L, with the highest concentrations associated to the effluents of the WWT systems (WL4, TP3-E and/or TP2-E). Meanwhile, the presence of both analytes was confirmed in up to 85% of the surface water samples, though at much lower concentrations. The lowest occurrence rates (75%) correspond to water from irrigation canals, which derive from the CR, with the lowest contents of 0.3-2.8 ng/L and 0.2-166.4 ng/L for CBZ and SMX, respectively, which makes it clear that some concentrations of both compounds enter the Mexicali Valley dissolved in the waters of the CR.

Ibuprofen had a frequency of detection of 58% followed by diclofenac (48%) and metoprolol with less than 38%. Concentrations of IBF ranged from 12 to 1705 ng/L, of DCF from 4.7-433 ng/L, and of MTP from 0.5-122.4 ng/L (Table 3.4). The first two were detected in all the influents and effluents from the three analyzed WWTPs but only in about 30% of de samples from irrigation canals. The highest concentrations of IBF and MTP were found within the Las Arenitas WWTP-wetland system (WL2; Figure 3.2) while site TP3-E contained the maximum values of DCF (Table 3.4).

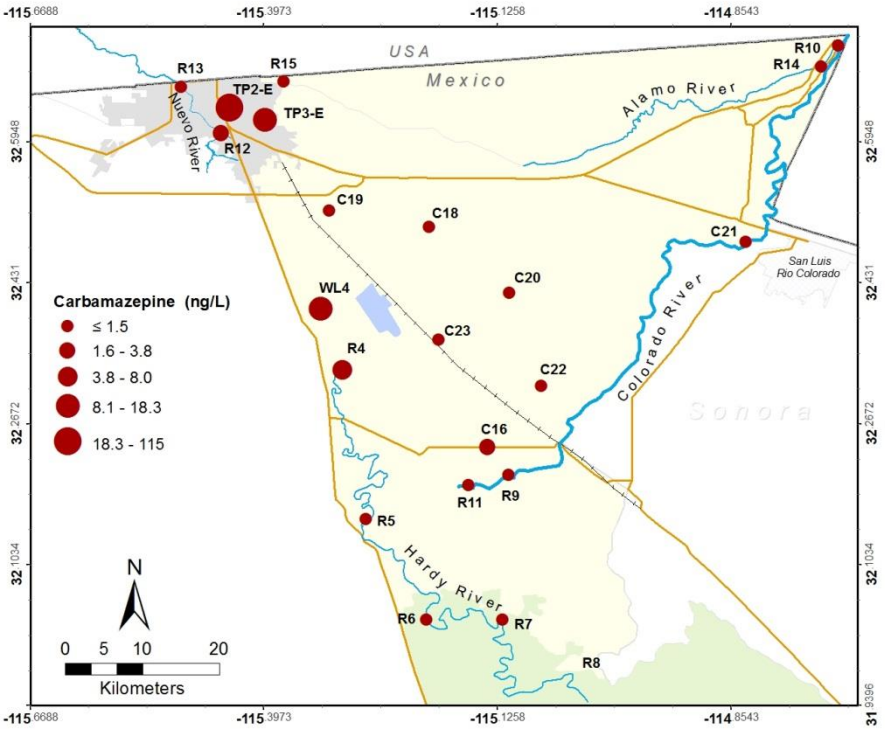
The five PhACs were detected in substantial concentrations of 2.8-166.4 ng/L in water samples from the Carranza drain (C16), an artificial ditch that receives the discharges of two small WWTPs located in the southern limit of Ciudad Guadalupe Victoria, a town with more than 20,000 inhabitants and a clear urban component. Similarly, moderate concentrations of IBF (40 ng/L), DCF (6.5 ng/L) and MTP (<4.6 ng/L), and lesser amounts of CBZ and SMX, were measured in site R13, the last downstream point of the Nuevo River before leaving the country on its way toward the Salton Sea, in the United States of America.

As expected, the average concentrations of all analytes in WWTPs were high compared with those in surface water (Table 3.4). Among them, the highest concentration of 1705 ng/L corresponds to IBF followed by SMX at 830.9 ng/L. In general, this information suggests that conventional treatments used in WWTPs are not enough to efficiently remove the compounds and prevent their arrival in the environment.

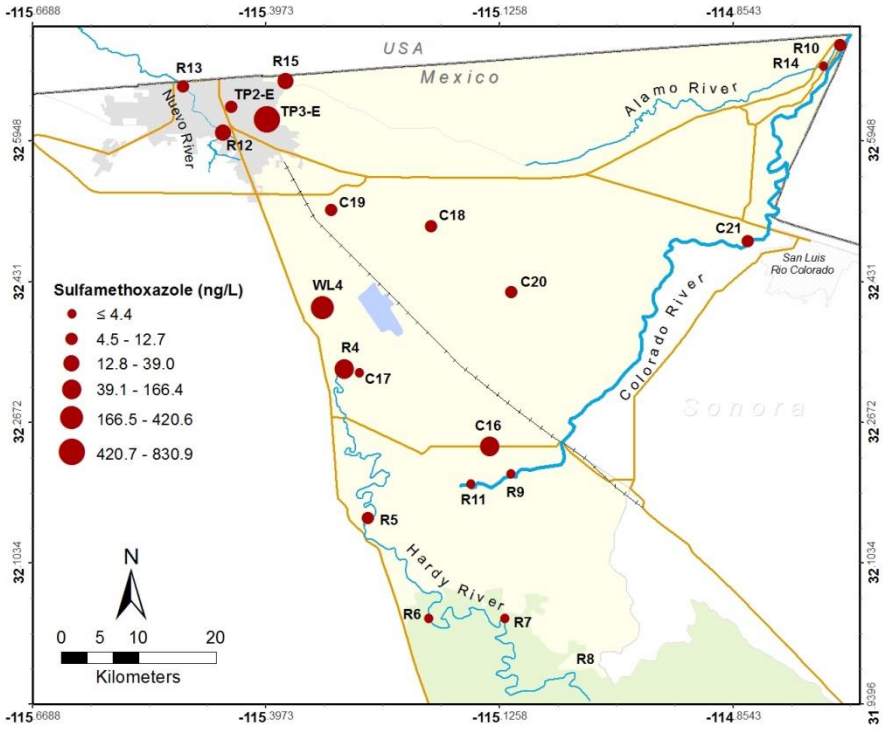
3.3.2 Spatial Distribution of PhACs in Mexicali and its surrounding valley

Figure 3.4a shows the environmental distribution of carbamazepine concentrations found along the city and the valley during the spring of 2015 while Figure 3.4b does the same for sulfamethoxazole. As stated before, CBZ was the most widely distributed PhAC in the study area followed closely by SMX. The highest concentrations of the two compounds were found in the effluents of the WWTPs (Figure 3.4), from where the treated wastewater is released to the environment or diverted to be used mainly to irrigate public and private parks, forages and pastures. Both TP2-E and TP3-E are the effluents of WWTPs located within educational institutions that treat some wastewater from their neighborhoods, including a health center in the case of TP3-E, which would explain the surprisingly high concentrations of antibiotics and other PhACs in a higher education center. However, the extraordinary concentration of CBZ in TP2-E may result from a combination of indiscriminate prescription (Zhang et al., 2008), its frequently intact form when excreted (Clara et al., 2004; Paltiel et al., 2016), and the medium-high economic status of the neighborhood.

It is noticeable that concentrations of CBZ in the Mexicali Valley are constantly lower, either in WWTPs or surface water, than those reported elsewhere with similar population and economic activities (Chen et al., 2006; Zhang and Geißen, 2010). Conversely, the contents of SMX in all of the analyzed environmental compartments are consistently higher than any of those obtained from the literature (Brandt et al., 2013; Henriquez, 2012; Spongberg et al., 2011). It is also observed a decreasing behavior of said concentrations while water moves downstream along the system composed by Las Arenitas effluent (WL4) and the Hardy River (R4 to R7, Figure 3.4; and R8, Figure 3.1). WL4 is connected to site R4 by a 9 km pipe, hence they are a similar blend of water. As CBZ and SMX mix with the aquatic environment, their apparent concentrations decrease with distance from the source. For instance, the CBZ moving downstream of the Hardy River was detected at 14.8, 8, 0.6, and 0.6 ng/L at sites R4 to R7, respectively, and was not detected (n.d) at side R8 (Figure 3.4a). Therefore, surface waters, where processes such as sorption, photolysis, and dilution may take place, contain lower concentrations of these compounds than their most likely sources. A similar behavior is observed for SMX.



(a)



(b)

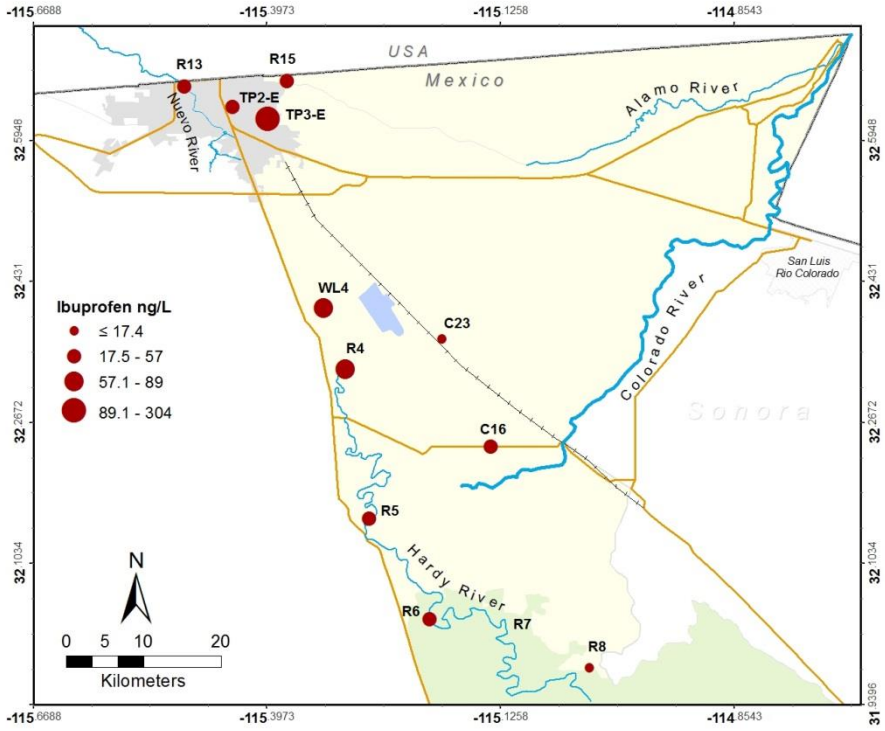
Figure 3.4 Environmental distribution of concentrations of (a) carbamazepine and (b) sulfamethoxazole in the city of Mexicali and its surrounding valley. April and May, 2015

Additionally, the NSAIDs ibuprofen and diclofenac were detected in all the WWTPs influents and effluents, including those water courses that transport mixes of wastewater (municipal, industrial and commercial), irrigation returns and treated water, such as the cases of the Nuevo River (R13) and the Carranza drain (C16) (**Figure 3.5)**. Of the two compounds, only IBF was found along the Hardy River with values that varied erratically but finally decreased from 75±4 ng/L, upstream, to n.d. in R7, downstream. Nonetheless, the site R8 shows 17.4 ng/L, which allows inferring the presence of additional sources feeding the stream. Hence, IBF is the only PhAC detected in the estuary zone of the Gulf of California (Figure 3.5a). On the contrary, DCF seems to be linked to its sources and was rarely found in watercourses (Figure 3.5b).

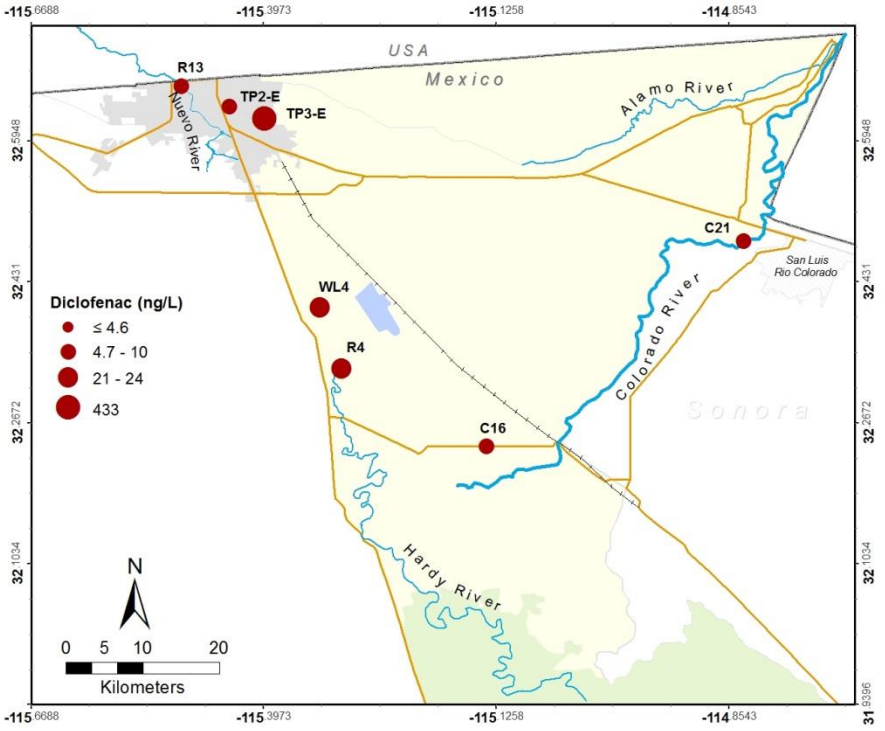
The occurrence of IBF and DCF in the present study is comparable with results obtained elsewhere in LATAM and other parts of the world. The observed concentrations of IBF in the WW influents can be related to the fact that it is an over-the-counter medication, which does not need a prescription. Though NSAIDs in the WWTPs exhibit high removal percentages and despite having short half-lives of degradations of up to 248 days, for IBF, and 60 days, for DCF, in raw and natural waters (Araujo et al., 2014), they are also consider ubiquitous and are usually present at significant concentrations in the aquatic environments. Therefore, even after going through treatment they are still detected in the effluents (Gros et al., 2010).

Metoprolol, on the other hand, was barely identified in the study area (Figure 3.6) with the highest concentrations within the Las Arenitas wetland system (WL4 and WL3, Table 3.4 and Figure 3.7). It was sporadically detected in the Colorado River and irrigation canals not exceeding 1.2 ng/L.

CBZ, SMX and IBF emerge as compounds of major environmental concern for the Mexicali area since, once released to the environment by WWTPs, they become persistent due to their bioactive capabilities. In fact, some can be transported over long distances and reach remote areas that might not seem affected by pollution, as in the case of IBF in the estuary zone (R8, Figure 3.5a).



(a)



(b)

Figure 3.5 Environmental distribution of concentrations of (a) ibuprofen and (b) diclofenac in Mexicali and its surrounding valley. April and May 2015

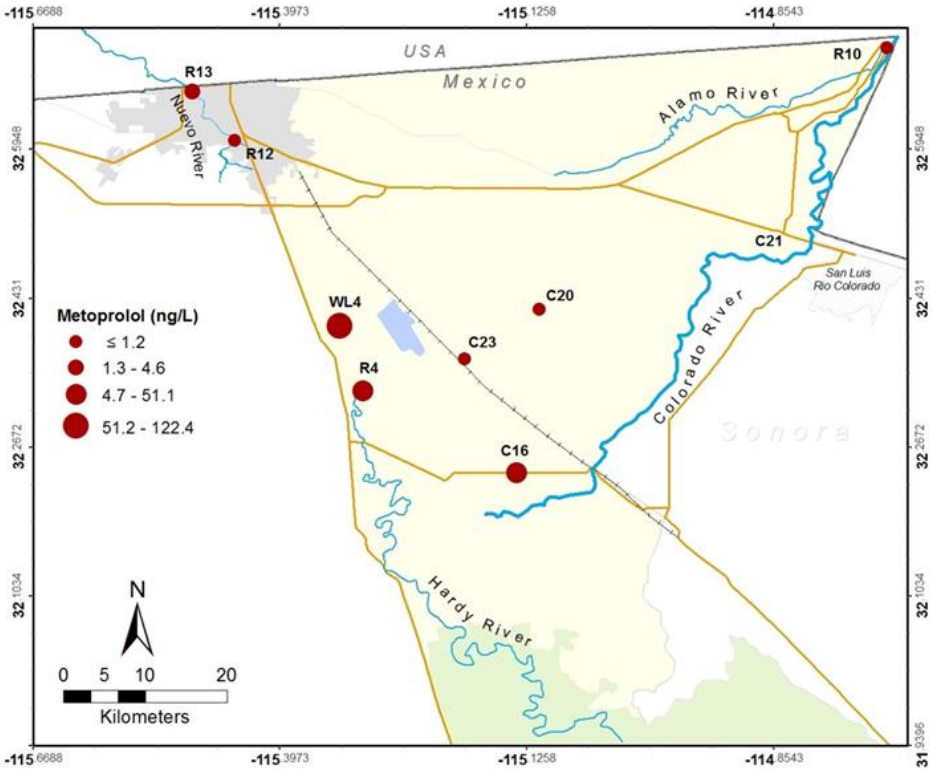


Figure 3.6 Environmental distribution of concentrations of metoprolol in Mexicali and its surrounding valley. April and May 2015

Figures 3.7 and 3.8 show the spatial distribution of the five PhACs detected throughout the Las Arenitas WWTP-Wetland system. As it has been previously mentioned, the highest concentrations of PhACs were detected in WWTP influents and effluents, nevertheless, CBZ, IBF and MTP showed much higher concentrations in the effluents of the wetland than in the influents of the WWTP (Figure 3.7a, Figure 3.7c and Figure 3.8, respectively). Even more, the latter was not detected in the influent (TP1-I) but it was found in the effluent (Figure 3.8). Numerous authors have reported higher concentrations of PhACs in many WWTPs effluents than in the corresponding influents (Gracia-Lor et al., 2012b; Jelic et al., 2011; Vieno et al., 2007). To illustrate a few examples, Thomas et al. (2007) observed this fact for DCF, MTP and trimethoprim; Lacey et al. (2008) did not find DCF in the influents of three WWTPs, but they found it in the effluents. According to several studies, this condition can be attributed to either conjugation/deconjugation of glucoronide or sulphate, to transformation of some compounds via hydrolysis, and sorption/desorption of these compounds from suspended solids/sludge (Tahar et al., 2017; Vieno et al., 2007;

Yang et al., 2017). In addition, Gracia-Lor et al. (2012b) and other authors attribute this effect to the higher complexity of the influents, which leads to strong matrix effects (ionization suppression) affecting the detection capability, the precision and the accuracy of the analyticals results.

In general, data from the April-May, 2015, screening shows that the wetland system is able to remove NSAIDs (IBF and DCF), though removal efficiency was not calculated (no retention time considered), however, the processes taking place did not seem capable of removing CBZ, SFM and MTP (Figures 3.7 and 3.8). Even worst, MTP concentrations were detected in the effluent on a day when the influent contained only trace amounts (Figure 3.8). CBZ, on the other hand, did not show evidences of being removed (Figure 3.7a). In any case, these results support the notion that variable amounts of PhACs are entering the aquatic environment of the city and valley of Mexicali despite the treatment of their wastewater.

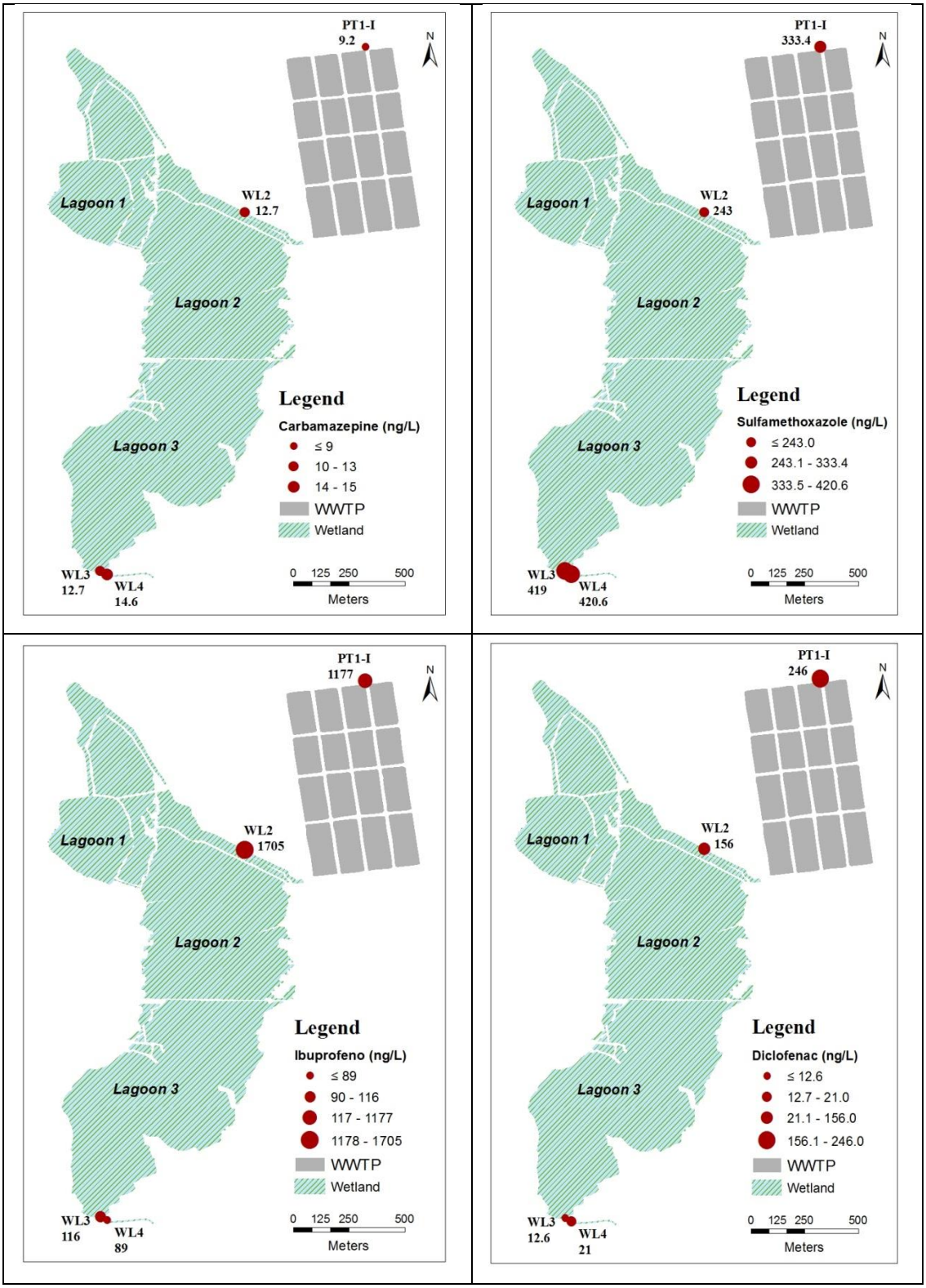


Figure 3.7 Spatial distribution of the concentrations of (a) CMZ, (b) ZMX, (c) IBF, and (d) DCF within the Las Arenitas WWTP-Wetland system.

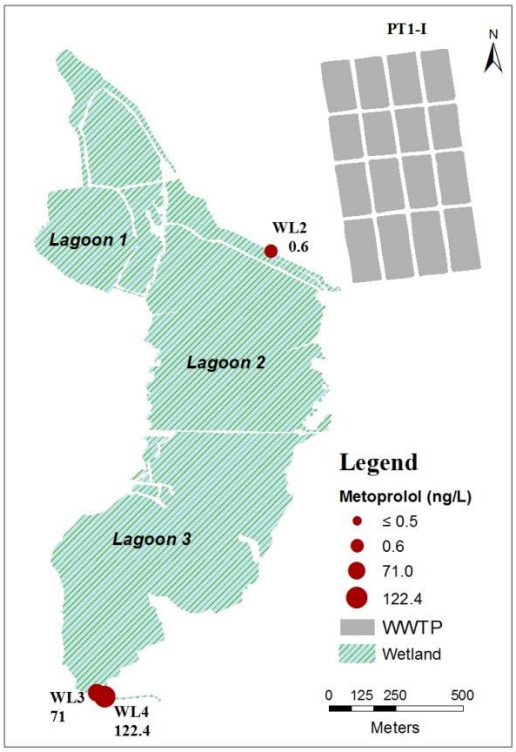


Figure 3.8 Spatial distribution of the concentration of MTP in the Las Arenitas WWTP-Wetland system

3.3.3 Environmental Risk Assessment of PhACs in surface water

Besides the quantification of PhACs in the environment, it is only prudent to evaluate the potential risk to the environment that small, environmental, concentrations may pose to non-target organism. Consequently, ERAs of CBZ, SMX, IBF, DCF and MTP are estimated in this section using three RQ approaches for three representative trophic levels of aquatic ecosystem, specifically, blue algae, the small planktonic crustacean named daphnia, and the zebra fish. Firstly, it was calculated the RQ mix of the five PhACs (Equation 3) and, finally, the estimation of $RQ_{mix,river}$ (Equation 4) when the mixing of PhACs in wastewater travels along the Las Arenitas wetland and the Hardy River.

The LEC_{50} and CE_{50} obtained with ECOSAR and used to calculate the PNEC and, in turn, the RQ values, are shown in Table 3.5. Tables with data and calculations for the two water systems are compiled in Appendix B, Tables 1-6. Results in graphical form can be observed in Figures 3.9 and 3.10.

Table 3.5 LEC_{50} and EC_{50} acute toxicity concentrations

Compuesto	Fish	Daphnid	Algae
	mg/L	mg/L	mg/L
Sulfametoxazol	214.19[c]	1.85[c]	0.146[a]
Carbamazepina	28.17[c]	9.5[c]	0.212[c]
Ibuprofeno	5[b]	9.02[b]	4[a]
Diclofenaco	13.54[c]	9.6[c]	14.5[a]
Metoprolol	61.58[c]	8.8[a]	7.9[a]

[a](Grung et al., 2008)
[b](Ginebreda et al., 2010)
[c] ECOSAR (USEPA, 2016)

The single compounds RQ acute in Las Arenitas wetland (Figure 3.9a) shows that CBZ and SMX pose at least a low risk (defined from 0.01 to 0.1) for algae in all three sampling sites, though the actual value for SMX is larger than 1, which distinguishes it as the highest risk for this organism. Additionally, DCF and MTP represent also a low acute risk for algae in at least two of the three sites. Likewise, these two compounds represent a low risk for daphnia in exactly the same sites, WL2 y WL4 for DCF and WL3 y WL4 for MTP, while SMX poses a medium risk (defined from 0.1 to <1) for daphnia in all sites. Fish is only exposed twice (WL2 and WL4) to low acute risk in the presence of DCF. Finally, IBF poses low acute risk in the effluent (WL4) and medium risk in the influent (WL2) of the wetland system for all three organisms. In contrast, the single compounds RQ acute in the Hardy River (Figure 3.10a) shows that only SMX represents a medium (R5 and R6) to high (R4) acute risk for algae. IBF, CBZ and SMX are the only compounds that pose a low risk for at least one of the three trophic levels, mainly in site R4, which receive the effluents from the wetland system.

In environmental compartments, PhACs do not occur as isolated, pure substances, thus, they are often present in the aquatic environment as a mixture of diverse compounds (Liu et al., 2015), which may be more toxic to non-target organisms than single compounds (Santos et al., 2010). For example, a mixture of fluoxetine and clofibric acid cause mortality and deformities while the combination of three to five antibiotics elicit changes in the sex ratio of daphnia (Flaherty and Dodson, 2005). Therefore, the risk quotients RQ_{mix} and $RQ_{mix,\ river}$ were calculated to estimate the potential ecological risk a mixture of PhACs in TWW and river water may pose for key organisms.

According to Figure 3.9b, algae resulted the most sensitive aquatic organism to the simultaneous presence of five PhACs, which in conjunction posed RQmix of up to 3 at the three sampling sites within the Las Arenitas wetland. The mixture also represented a medium environmental risk for daphnia and went from medium to low for fish. In contrast, the same mixture only resulted of environmental concern for the three trophic levels at the influent of the Hardy River (Figure 3.10b), where an RQ_{mix} of 1.5 posed a high risk for algae. Calculations showed SMX as the biggest contributor to the toxicity of the mixture (Appendix B).

The $RQ_{mix,wetland}$ (Figure 3.9c) resulted of interest only at sites with slow flows or in the cases when treated wastewater was the main component of the waterbody, as in the case of the stretch WL4 to R4. Nevertheless, Figure 3.9c and 3.10c signal algae at environmental risk in most conditions within the Las Arenitas wetland-Hardy River system.

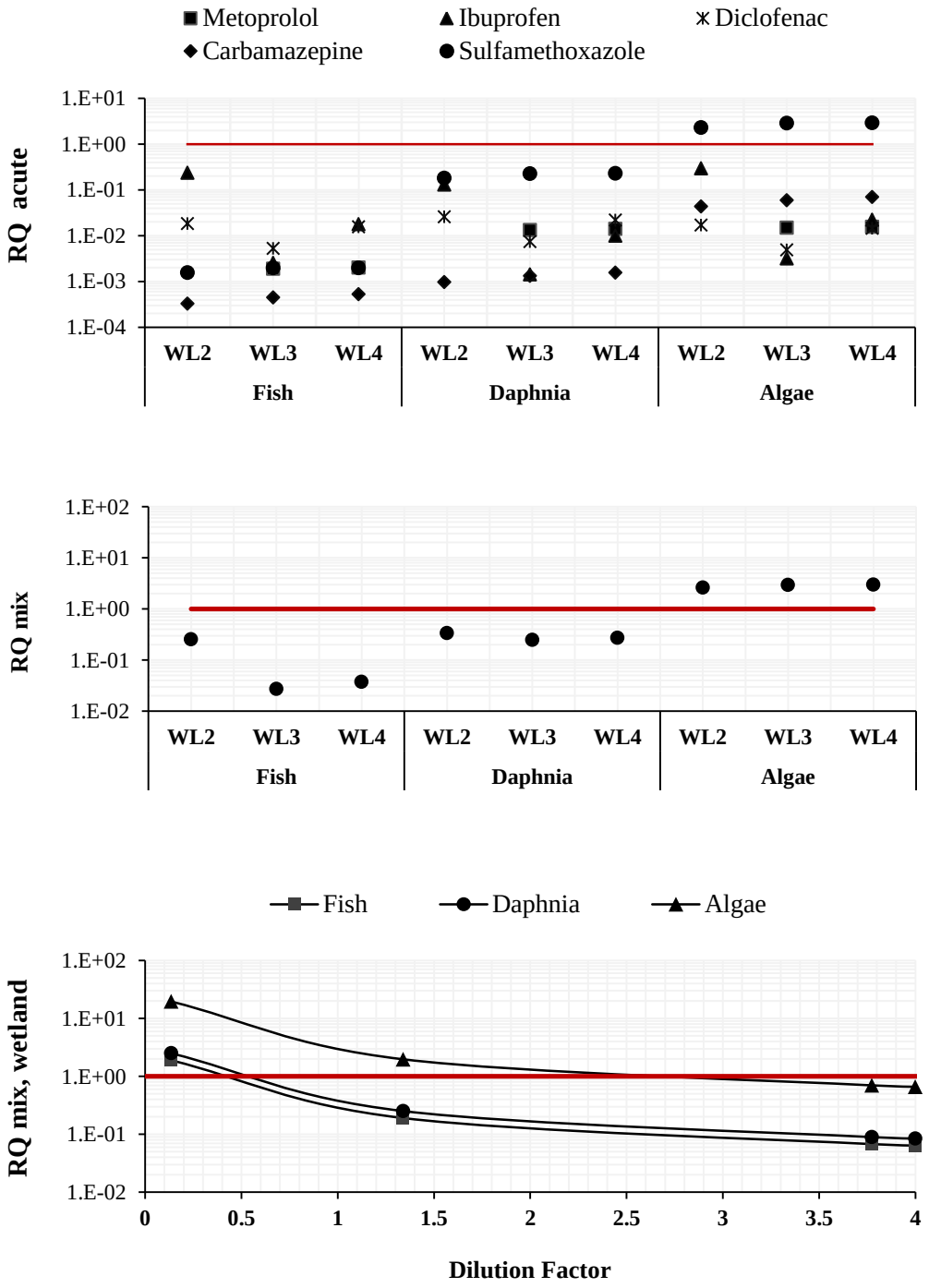


Figure 3.9 Estimation of RQ in the Las Arenitas Wetland in three different approaches for fish, daphnid, and algae: a) individual estimation (single compounds), b) given a mixture of PhACs and, c) effect to dilution factor on risk RQ_{mix} in the wetland. The red line marks the lower limit of high risk.

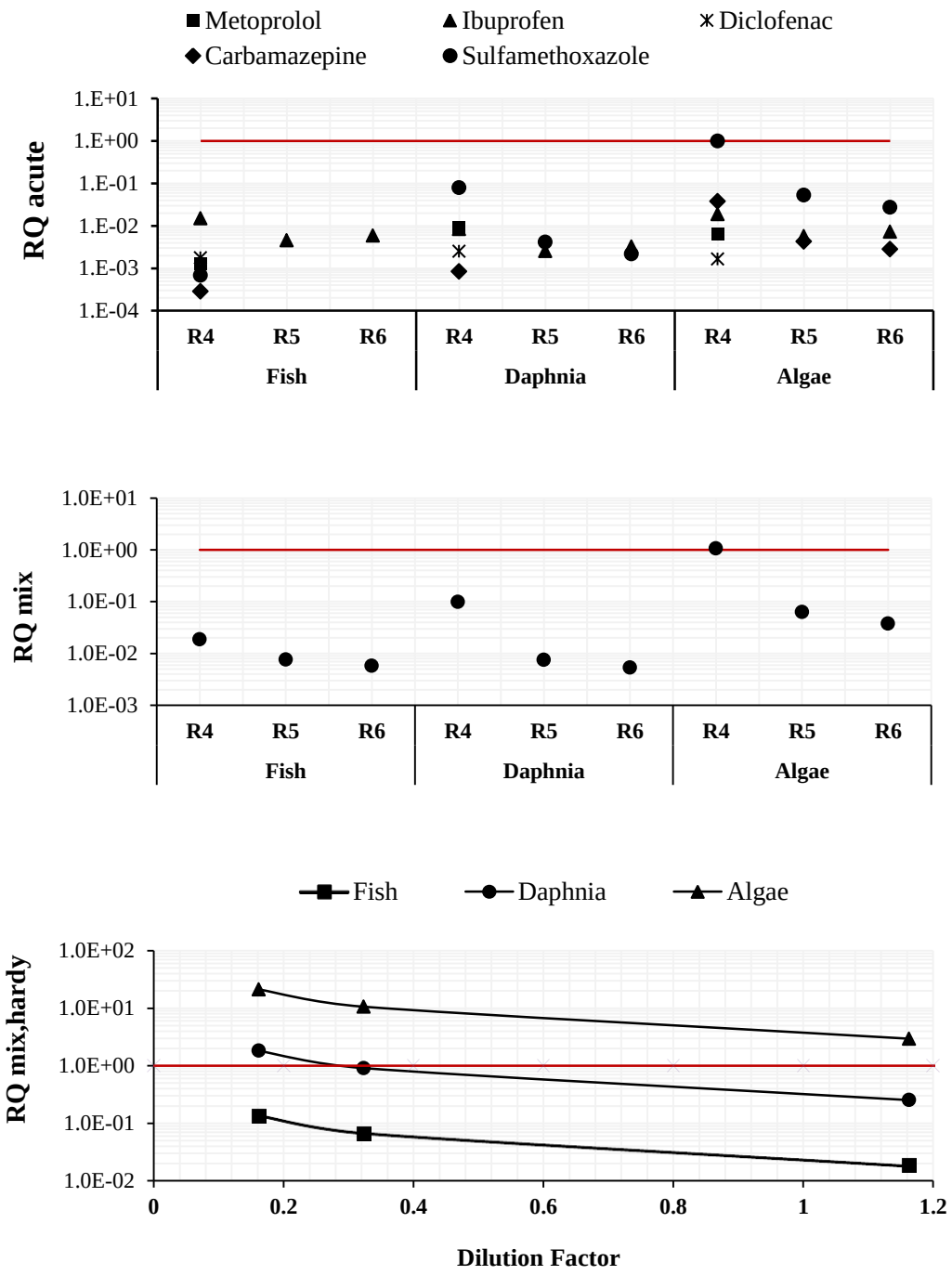


Figure 3.10 Estimation of RQ in the Hardy River in three different scenarios for fish, daphnid, and algae: a) individual estimation (single compound), b) given a mixture of PhACs and, c) effect of the dilution factor on RQmix in the river. The red line marks the lower limit of high risk.

3.4 Conclusions

This study investigates the occurrence of pharmaceuticals residues in surface water and wastewater of the Mexicali region, the capital city of the State of Baja California (Mexico) during the spring of 2015 and the environmental risk that these emerging contaminants may pose to the aquatic environment of this region. Five out of the 12 investigated pharmaceuticals were frequently detected at relevant concentrations (reached the µg/L level in a couple of cases). The detected pharmaceuticals belong to the therapeutics groups of psychiatric drugs (CBZ), antibiotics (SMX), analgesic and anti-inflammatory (DCF and IBF) and beta-blockers (MTP). These five compounds were consistently detected in the influents and effluents of three WWTPs and those sites which are used to transport irrigation water or even municipal treated water but nevertheless most of the times carry a wide mix of untreated wastewater, such as Nuevo River and Carranza Canal. In addition, DCF and MTP were rarely detected in surface water.

The total concentrations of PhACs measured in the influents and effluents of the three WWTPs and drain canals were higher than those observed in rivers and irrigation canals. The total concentrations of CBZ, SMX and IBF in surface water downstream the Las Arenitas wetland were slightly lower than those found in upstream. The occurrence of these three compounds found along of the Hardy River supports the inadequacy used treatment processes by WWTPs to eliminate these types of compounds. Also, the fact of that removal efficiency in WWTPs can vary among the different compounds and continues discharge of untreated WW.

Regard to environmental risk assessment the most critical compound was SMX which pose a high risk to algae in all RQ sceneries along to the Las Arenitas wetland and, in particular, the influent of the Hardy River

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Appendix A

Appendix A 1: Interview format. Monitoring on the consumption and disposal of medications in Mexicali, Mexico in the period of November 2014 to March 2015

FORMATO DE ENTREVISTA SOBRE USO DE FARMACOS EN MEXICALI

DIRECCIÓN_____FECHA:_____

CLAVE DE LA UBICACIÓN:_____FARMACIA:_____

1. ¿Cuáles fueron los medicamentos que compro? (Mencione)

1. _____ 4. _____
2. _____ 5. _____
3. _____ 6. _____

2. ¿Los medicamentos son comprados por recomendación médica?..... ( ) SI ( ) No

3. ¿Ha comprado medicamentos fuera del país? ..... ( ) SI ( ) No

Si la respuesta fue **SI** Mencione alguno:

_____

4. ¿Alguna vez ha consumido medicina alternativa? ..... ( ) SI ( ) No

Si la respuesta fue **SI** Mencione alguno:_____

5. ¿Cuál es aproximadamente la frecuencia con que compra fármacos durante el año?

( ) 1 a 2 veces al año ( ) 5 a 6 veces al año

( ) 3 a 4 veces al año ( ) 7 o más

6. ¿Durante que estación del año consume más fármacos?

( ) Invierno ( ) Primavera ( ) Verano ( ) Otoño

7. ¿Cuál es el fármaco que más utiliza en casa? _____

8. Al caducar un medicamento o terminar el tratamiento ¿Cómo deshecha el fármaco sobrante?

( ) Bote de la basura ( ) Taza del baño ( ) En el Suelo ( ) Los conserva

( ) Otro: _____

Appendix A 2: Matrix used to estimate the sample-size for pharmacies

Matrix of sample sizes for different margins of error and levels of confidence, when estimating a proportion in finite population

N (Population)	288
p (proportion)	0.5

Confidence level		
(α)	1-α/2	Z(1-α/2)
90%	0.05	1.64
95%	0.025	1.96
97%	0.015	2.17
99%	0.005	2.58

Formula used:

$$n = \frac{(N.p^2.Z^2)}{((N-1)e^2 + p^2.Z^2)}$$

Matrix of sample-size for a population of 117962 with a p= 0.05

Confidence Level	e[Maximum error of estimation]									
	10%	**9%**	**8%**	**7%**	**6%**	**5%**	**4%**	**3%**	**2%**	**1%**
90%	55	65	77	93	114	139	171	208	246	276
95%	72	84	99	117	139	165	195	227	257	280
97%	84	97	112	131	153	179	207	236	262	281
99%	106	120	137	156	178	201	226	249	269	283

Appendix A 3: Resulting database for 55 drugstores, which were chosen at randomly of a total of 288.

Random Numbers	#	Pharmacy	Address	ZIP	AGEB (sector)	Population by sector	Wi	ni
1	1	Similares	Los Portales 2da sección	21323	6982	1477	0.013	5
6	2	B. Santa Cruz	Valle del Pedregal	21395	6728	3402	0.029	11
7	3	F. Similares	Fracc. Valle de Puebla	21600	6499	2241	0.019	7
17	4	F. Valeria	Villa Verde	21396	6200	3282	0.028	11
18	5	F. Genéricos I.	Valle del Pedregal	21395	6198	3475	0.029	11
22	6	Centro Naturista	Villa del Rey	21377	6056	4865	0.041	16
29	7	B. Guadalupana	Fracc. del Rey	21353	5861	2986	0.025	10
31	8	Botica del Valle	Leandro Valle	21386	5804	1598	0.014	5
37	9	Farmacia de Teresa	Fracc. Villa Florida	21398	5556	3516	0.030	11
45	10	F. Similares	Fracc. Villa Verde	21399	4990	3359	0.028	11
46	11	Farmacia de La Familia	González Ortega	21397	4986	730	0.006	2
248	12	Similares	Nvo. Mexicali	21399	4929	2761	0.023	9
53	13	Farmacia Dr. Jesus	C. Urbano Esperanza	21350	4844	1850	0.016	6
61	14	F. Similares	Infonavit Virreyes	21190	4740	6271	0.053	20

Random Numbers	#	Pharmacy	Address	ZIP	AGEB (sector)	Population by sector	Wi	ni
71	15	Distribuidora Comercial	Pro Hogar	21240	4524	1627	0.014	5
75	16	B. Nueva Esperanza	Nueva Esperanza	21050	4469	1561	0.013	5
78	17	F. Benavides	Colonia Nueva	21100	4416	1605	0.014	5
79	18	F. Similares	Pueblo Nuevo	21120	437A	920	0.008	3
84	19	El Fénix de Tijuana	Colonia Alamitos	21210	4308	3077	0.026	10
102	20	F. Benavides	Colonia La Jabonera	21150	4238	1364	0.012	4
96	21	El Fénix De Tijuana	Centro	21100	4238		0.000	0
106	22	Remedio	Zona Centro	21100	4223	504	0.004	2
275	23	Paris	Zona Centro	21100	4223		0.000	0
278	24	San Andress	Primera Seccion	21100	4223		0.000	0
124	25	Farmacia de genéricos	Zona Centro	21100	4219	703	0.006	2
117	26	El Fénix de Tijuana	Zona Centro	21100	4219		0.000	0
113	27	B. San José	Zona Centro	21100	4219		0.000	0
134	28	F. Apotheke	Pueblo Nuevo	21120	4204		0.000	0
133	29	F. Alexis	Pueblo Nuevo	21120	4204	972	0.008	3
137	30	B. Guadalupana	Los Naranjos	21387	4172	1488	0.013	5
142	31	Nutrifarmacias	Col Ejidatarios	21160	3846	1583	0.013	5
145	32	F. Gabriela	Col El Robledo	21384	3140	4219	0.036	14
146	33	F. Gi	Ampliación Progreso	21610	3085	1210	0.010	4
148	34	F. Castellón	Col Hidalgo	21389	298A	4749	0.040	15
150	35	F. Similares	Col Hidalgo	21389	298A		0.000	0
155	36	Boticas Levy	Jardines De Lago	21330	2867	2494	0.021	8
157	37	Farmacia Gei	Las Flores	21300	2829	3036	0.026	10
162	38	F. Benavides	Fracc. San Marcos	21050	2782	3007	0.025	10
172	39	F. Similares	Paseos Del Sol	21399	2424	4396	0.037	14
181	40	F. Benavides	Col Constitución	21250	2299	3934	0.033	13
182	41	F. Villas del Sol	Villas Del Sol	21359	0659	3883	0.033	13
189	42	B. Guadalupana	Independencia	21359	0540	2940	0.025	10
196	43	F. Benavides	Col Vallarta	21270	0362	2433	0.021	8
201	44	F. Veronica	Industrial	21010	0324	2726	0.023	9
218	45	F.Benavides	Colonia Santa María	21240	0273	3429	0.029	11
214	46	F. Similares	Roma	21250	0273		0.000	0
226	47	F. Fredys	Col Pueblo Nuevo	21140	0165	2476	0.021	8
240	48	Mexicana	Col Revolucion	21110	5753	1525	0.013	5
241	49	Vida Y Salud	Villas del Palmar	21398	5560	4546	0.039	15
246	50	Santa Isabel	Santa Isabel	21139	5128	911	0.008	3
264	51	Mexicali	Col. Burocratas	21030	4420	1769	0.015	6
265	52	Similares De Mexicali	Col Esperanza	21140	4384	734	0.006	2
266	53	De La Salud	Fracc. Alameda	21215	4327	2408	0.020	8
271	54	Sfi	Col Nueva	21100	4242	1015	0.009	3

Random Numbers	#	Pharmacy	Address	ZIP	AGEB (sector)	Population by sector	Wi	ni
282	55	Robledo	Col El Robledo	21384	3155	2905	0.025	9
Total						**117962**	**1**	**384**

Appendix A 4: Matrix used to estimate the sample-size for interview

Matrix of sample sizes for different margins of error and levels of confidence, when estimating a proportion in finite population

N (Population)	117962
p (proportion)	0.5

Confidence level		
(α)	1-α/2	Z(1-α/2)
90%	0.05	1.64
95%	0.025	1.96
97%	0.015	2.17
99%	0.005	2.58

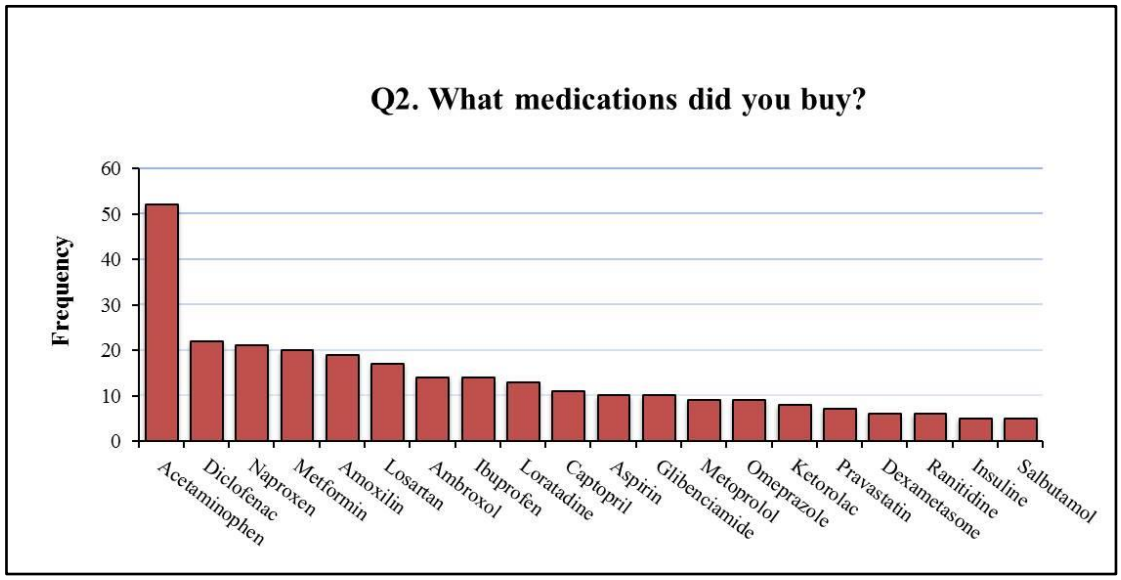
Formula used:

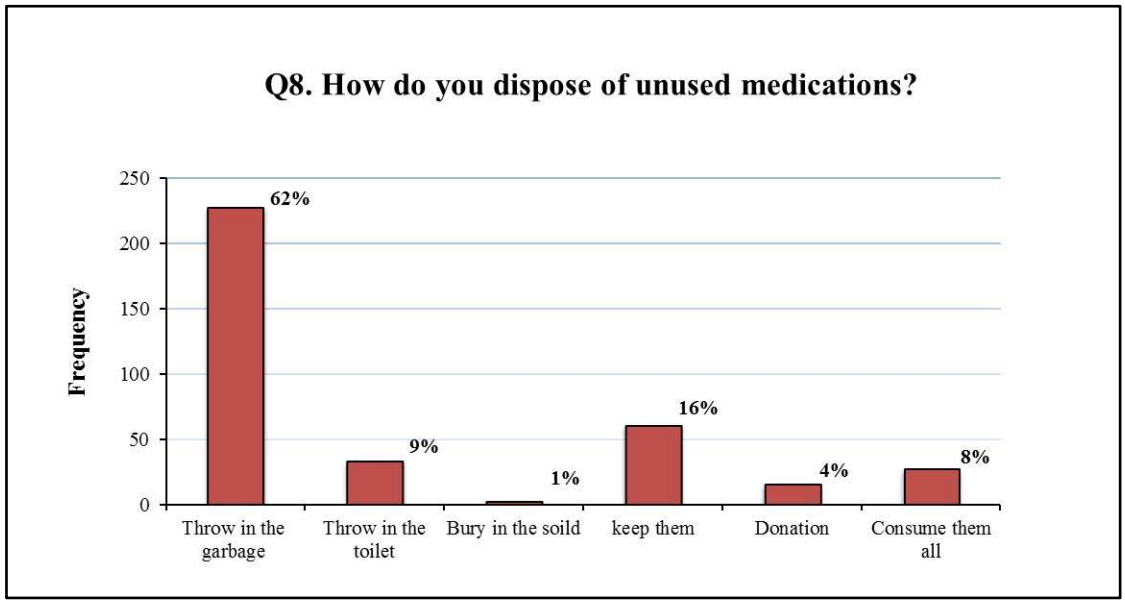
$$n = \frac{(N.p^2.Z^2)}{((N-1)e^2 + p^2.Z^2)}$$

Matrix of sample-size for a population of 117962 with a p= 0.05

Confidence Level	e[Maximum error of estimation]									
	10%	**9%**	**8%**	**7%**	**6%**	**5%**	**4%**	**3%**	**2%**	**1%**
90%	68	84	106	138	187	268	419	742	1657	6361
95%	97	120	151	197	268	383	597	1058	2353	8881
97%	119	147	185	241	328	469	731	1294	2871	10704
99%	168	207	262	341	464	662	1031	1820	4019	14584

Appendix A 5: Interview answers





Appendix B

Enviromental Risk Assessment

Appendix B 1: Risk assessment in the Las Arenitas wetland in the WL2 site.

		Fish			Daphnia			Algae		
Compound	MEC (µg/L)	PNEC (µg/L)	RQ	Risk	PNEC (µg/L)	RQ	Risk	PNEC (µg/L)	RQ	Risk
Sulfamethoxazole	0.3334	214	0.001	NR	1.85	0.180	Medium	0.146	2.283	High
Ibuprofen	1.177	5	0.235	Medium	9.02	0.130	Medium	4	0.294	Medium
Diclofenac	0.246	13.54	0.018	Low	9.6	0.025	Low	14.5	0.016	Low
Carbamazepine	0.0092	28.17	0.000	NR	9.5	0.000	NR	0.212	0.043	Low
Metoprolol	--	61.58	---	---	8.8	--	---	7.9	--	---
∑ RQ $_{mix}$			0.255	Medium		0.337	Medium		2.638	High

Appendix B 2: Risk assessment in the Las Arenitas wetland in the WL3 site.

		Fish			**Daphnia**			**Algas**		
Compound	**MEC** (µg/L)	**PNEC** (µg/L)	**RQ**	**Risk**	**PNEC** (µg/L)	**RQ**	**Riesgo**	**PNEC** (µg/L)	**RQ**	**Riesgo**
Sulfamethoxazole	0.4188	214	0.001	Low	1.85	0.226	Medium	0.146	2.868	High
Ibuprofen	0.0127	5	0.002	NR	9.02	0.001	NR	4	0.003	NR
Diclofenac	0.071	13.54	0.005	NR	9.6	0.007	Low	14.5	0.004	NR
Carbamazepine	0.0126	28.17	0.000	NR	9.5	0.001	NR	0.212	0.059	Low
Metoprolol	0.1164	61.58	0.001	NR	8.8	0.013	Medium	7.9	0.014	Low
∑ **RQ $_{mix}$**			0.027	Low		0.249	Medium		2.950	High

Appendix B 3: Risk assessment in the Las Arenitas wetland in the WL4 site.

		Fish			**Daphnia**			**Algae**		
Compound	**MEC** (µg/L)	**PNEC** (µg/L)	**RQ**	**Risk**	**PNEC** (µg/L)	**RQ**	**Riesgo**	**PNEC** (µg/L)	**RQ**	**Riesgo**
Sulfamethoxazole	0.4206	214	0.001	NR	1.85	0.227	Medium	0.146	2.880	High
Ibuprofen	0.089	5	0.019	Low	9.02	0.009	NR	4	0.022	Low
Diclofenac	0.21	13.54	0.015	Low	9.6	0.021	Low	14.5	0.014	Low
Carbamazepine	0.0148	28.17	0.000	NR	9.5	0.001	NR	0.212	0.069	Low
Metoprolol	0.1224	61.58	0.001	No Risk	8.8	0.013	Low	7.9	0.015	Low
∑ **RQ $_{mix}$**			0.037	Low		0.274	Medium		3.002	High

Appendix B 4: Risk assessment in the Hardy River: in the R4 site.

		Fish			Daphnid			Algae		
Compound	**MEC**	**PNEC**	**RQ**	**Risk**	**PNEC**	**RQ**	**Risk**	**PNEC**	**RQ**	**Risk**
	(µg/L)	(µg/L)			(µg/L)			(µg/L)		
Sulfamethoxazole	0.0077	214	3.E-05	NR	1.85	0.004	NR	0.146	0.052	Low
Ibuprofen	0.0229	5	0.004	NR	9.02	0.002	NR	4	0.005	NR
Diclofenac	n.d	13.54	---	---	9.6	---	---	14.5	---	---
Carbamazepine	0.0009	28.17	3.E-05	NR	9.5	9.4E-05	NR	0.212	0.004	NR
Metoprolol	n.d	61.58	---	---	8.8	---	---	7.9	---	---
∑ **RQ** $_{mix}$			0.004			0.006			0.062	Low

Appendix B 5: Risk assessment in the Hardy River: in the R5 site

		Fish			Daphnid			Algae		
Compound	**MEC**	**PNEC**	**RQ**	**Risk**	**PNEC**	**RQ**	**Riesgo**	**PNEC**	**RQ**	**Risk**
	(µg/L)	(µg/L)			(µg/L)			(µg/L)		
Sulfamethoxazole	0.004	214	1.E-05	NR	1.85	0.002	NR	0.146	0.027	Low
Ibuprofen	0.0293	5	0.005	NR	9.02	0.003	NR	4	0.007	NR
Diclofenac	n.d	13.54	---	---	9.6	---	---	14.5	---	---
Carbamazepine	0.0006	28.17	2.E-05	NR	9.5	0.000	NR	0.212	0.002	NR
Metoprolol	n.d	61.58	---		8.8	---	---	7.9	---	---
∑ **RQ** $_{mix}$			0.005	NR		0.005	NR		0.037	Low

Appendix B 6: Risk assessment in the Hardy River: in the R6 site

		Fish			Daphnid			Algae		
Compound	**MEC**	**PNEC**	**RQ**	**Risk**	**PNEC**	**RQ**	**Risk**	**PNEC**	**RQ**	**Risk**
	(µg/L)	(µg/L)			(µg/L)			(µg/L)		
Sulfamethoxazole	0.145	214	0.000	NR	1.85	0.078	Low	0.146	0.993	Medium
Ibuprofen	0.0754	5	0.015	Low	9.02	0.008	NR	4	0.018	Low
Diclofenac	0.024	13.54	0.001	NR	9.6	0.002	NR	14.5	0.001	NR
Carbamazepine	0.008	28.17	0.000	NR	9.5	0.000	NR	0.212	0.037	Low
Metoprolol	0.0511	61.58	0.000	NR	8.8	0.005	NR	7.9	0.006	Low
∑ **RQ** $_{mix}$			0.018	Low		0.095	Low		1.057	High

Appendix B 7: Data used to calculate RQ mix, hardy

Fish						
Site	Qe (m3/d)	Qr (m3/d)	DF	RQmix		RQ mix, hardy
R5	53481.6	62208	1.163166	0.0215		0.0185
R6	53481.6	17280	0.323102	0.0215		0.0665425
R7	53481.6	8640	0.161551	0.0215		0.133085
Daphnia						
Site	Qe (m3/d)	Qr (m3/d)	DF	RQmix		RQ mix, hardy
R5	53481.6	62208	1.163166	0.2943		0.25301625
R6	53481.6	17280	0.323102	0.2943		0.9108585
R7	53481.6	8640	0.161551	0.2943		1.821717
Algae						
Sitio	Qe (m3/d)	Qr (m3/d)	DF	RQmix		RQ mix, hardy
R5	53481.6	62208	1.163166	3.4341		2.952372
R6	53481.6	17280	0.323102	3.4341		10.628540
R7	53481.6	8640	0.161551	3.4341		21.257079

Appendix B 8: Data used to calculate RQ mix, wetland

Fish						
Sitio	Qe (m3/d)	Qr (m3/d)	DF	RQmix		RQ mix, wetland
WL2	62208	8380.8	0.1347222	0.255453		1.8961454
Lagoon 1	8380.8	11232	1.3402062	0.255453		0.1906072
Lagoon 2	16416	61948.8	3.7736842	0.255453		0.0676932
Lagoon 3	14515.2	58060.8	4	0.255453		0.0638632
Daphnia						
Sitio	Qe (m3/d)	Qr (m3/d)	DF	RQmix		RQ mix,rio
WL2	62208	8380.8	0.1347222	0.337297		2.5036511
Lagoon 1	8380.8	11232	1.3402062	0.337297		0.2516758
Lagoon 2	16416	61948.8	3.7736842	0.337297		0.0893815
Lagoon 3	14515.2	58060.8	4	0.337297		0.0843244
Algae						
Sitio	Qe **(m3/d)**	Qr **(m3/d)**	DF	RQmix		RQ mix,rio
WL2	62208	8380.8	0.1347222	2.638173		19.582318
Lagoon 1	8380.8	11232	1.3402062	2.638173		1.9684832
Lagoon 2	16416	61948.8	3.7736842	2.638173		0.6990976
Lagoon 3	14515.2	58060.8	4	2.638173		0.6595433