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Emissions and transport of pharmaceutical residues from three wastewater treatment plants in Saudi Arabia and the associated risk for the aquatic environment

Thesis presented by

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Declaration

"This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism and intellectual property."

Obaid Alharbi _____

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Abbreviations

ACE	Acetaminophen
AF	Assessment Factor
ANOVA	Analysis of Variance
AOP	Advanced Oxidation Processes
API	Active Pharmaceutical Ingredients
ARB	Antibiotic Resistant Bacteria
ARG	Antibiotic Resistant Genes
AS	Activated Sludge
ATE	Atenolol
AZI	Azithromycin
BAC	Baclofen
BLD	Below Limit of Detection
BLQ	Below Limit of Quantification
BNR	Biological Nutrient Removal
BOD	Biochemical Oxygen Demand
BQL	Below Quantification Limit
BT	Biological Treatment
CAF	Caffeine
CAS	Conventional Activated Sludge
CD	Chemical Disinfection
CEC	Chemicals of Emerging Concern
CEC	Cation Exchange Capacity
CEP	Cephalexin
CIP	Ciprofloxacin
CM	Conventional mechanical
COD	Chemical Oxygen Demand
DC	Disinfection with chlorination
DIC	Diclofenac
DL	Detection Limit
ECOSAR	Ecological Structure Activity Relationship
EDS	Endocrine Disrupting Compound
EDTA	Ethylenediaminetetraacetic acid
EMA	European Medicines Evaluation Agency
EPA	The U.S. Environmental Protection Agency
EPI	Estimation Programs Interface
EPIS	Estimation Programs Interface Suite
ERA	Environmental Risk Assessment (ERA)
ESI	Electrospray-Ionization
EU	Europe Union
FA	Formic Acid
FAO	UN Food and Agriculture Organization
FR	Flowrate
GABA	Gamma-Aminobutyric Acid
GC	Grit Chamber
GR	Grit Removal
GRE	Greece
HPLC	High Performance Liquid Chromatogram

HRT	Hydraulic Retention Time
HWWTP	Hospital Wastewater Treatment Plant
IBU	Ibuprofen
IPCS	International Program of Chemical Safety
IRA	Iran
IS	Internal Standard
K_D	The Solid-Water Distribution Coefficient
KKUH	King Khaled University Hospital
K_{OC}	Organic Carbon Partition Coefficient
K_{OW}	n-Octanol/Water Partitioning Coefficient
KSA	Kingdom of Saudi Arabia
KSU	King Saud University
LC	Liquid Chromatogram
LOD	Limits of Detection
LOQ	Limits of Quantitation
MBR	Membrane Bioreactors
MDL	Method Detection Limit
ME	Matrix Effect
MEC	Measured Environmental Concentration
MENA	Middle East and North Africa
MET	Metformin
MF	Membrane Filter
MOH	Ministry of Health
MQL	Method Quantification Limit
MRM	Multiple Reaction Monitoring
MS	Mass Spectroscopy
MW	Molecular Wight
N.D.	Not Detected
NOEC	No Observed Effect Concentration
NPDS	National Poison Data System
NR	Not Recovered
NSAID	Nonsteroidal Anti-Inflammatory Drug
NWC	National Water Company
OD	Oxidation Ditch
OFL	Ofloxacin
OTC	Over The Counter
OXY	Oxypurinol
OZ	Ozone
PE	People Equivalent
PEC	Predicted Environmental Concentration
PFC	Perfluorinated Compounds
PNEC	Predicted No Effect Concentration
P-NR	Phosphate and Nitrogen Removal
PPCP	Pharmaceuticals and Personal Care Product
PS	Primary sedimentation
QSAR	Quantitative Structural Activity Relationship
RE	Removal Efficiency
RO	Reverse Osmosis

RQ	Risk Quotient
RSD	Relative Standard Deviation
RT	Retention Time
SC	Screening
SD	Standard Deviation
SF	Sand Filter
SLV	Slovenia
SOM	Soil Organic Matter
SPA	Spain
SPE	Solid Phase Extraction
SRT	Sludge Retention Time
SS	Secondary Sedimentation
ST	Secondary Treatment
TET	Tetracycline
TF	Trickling Filter
TRC	Toronto Research Chemicals
TRI	Trimethoprim
TSS	Total Suspended Solids
TT	Tertiary Treatment
UAE	Ultrasound-Assisted Extraction
UHPLC	Ultra-High-Performance Liquid Chromatography
USEPA	US Environmental Protection Agency
UV	Ultraviolet
WHC	Water Holding Capacity
WHO	World Health Organization
WWTP	Wastewater Treatment Plant
WWTPs	Wastewater Treatment Plants

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Abstract

Pharmaceuticals are known to be poorly removed by wastewater treatment plants (WWTPs) and their wastewater-born residues could find their way to the environment and cause a potential environmental risk. This research investigates the occurrence of sixteen pharmaceuticals in three different WWTPs in Riyadh, Saudi Arabia. Removal efficiency, and the potential risk posed to the environment, together with seasonal variations of the target compounds in these different WWTPs was studied. The wastewater discharged by the studied WWTPs could be used for different purposes, like recharging groundwater and/or irrigation of crops, and therefore the leaching behaviour of the selected pharmaceuticals when applied to soils was investigated in the laboratory using soil columns. In total 144 wastewater samples over 12 months and 80 samples from the soil column experiments were collected and analysed. Samples were extracted and analyzed using either solid phase extraction (SPE) followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) or ultrasound-assisted extractions (UAE) combination with LC-MS/MS for wastewater and soil samples respectively.

Of the 16 investigated pharmaceuticals, ten and five pharmaceuticals were detected in the studied WWTP influents and effluents respectively. The highest concentrations measured in the influents of these WWTPs were for caffeine and acetaminophen (> 1000 μg L^{-1}) followed by ciprofloxacin, metformin, ofloxacin, diclofenac, atenolol, cefalexin, trimethoprim and baclofen (< 1000 μg L^{-1}). This is the first-time baclofen has been reported in the environment and was found here at concentrations ranging between 0.33 and 2.82 μg L^{-1} in WWTP influent. The two larger WWTPs (H and M) received relatively higher levels of pharmaceuticals than the smaller WWTP (KSU). The highest concentration of the five compounds detected in

the studied WWTP effluents never exceeded 34 μg L^{-1}. The investigated pharmaceuticals generally exhibited seasonal variations that were more obvious in the WWTP influents than in the effluents and were more obvious in autumn and winter.

Mass loadings in the inputs and outputs of the detected compounds in the larger WWTPs were significantly ($p \leq 0.5$) higher than for the small scale WWTP. There were very few significant correlations between the physiochemical parameters of the collected samples and the operational parameters of the WWTPs, and the observed concentrations of the studied pharmaceuticals in the studied WWTP influents and effluents.

The average removal efficiencies of the pharmaceutical compounds were estimated to be ≥70%, with caffeine and acetaminophen eliminated almost completely at 99%. Removal efficiency of studied pharmaceuticals showed no significant differences ($p \leq 0.5$) between the different studied WWTPs, regardless of the differences in their tertiary treatment processes. The removal efficiency of baclofen was found to be high between, 81% and 97%, and showed a significant correlation with the ambient air temperature and a weak correlation with TSS removal.

The environmental risk was estimated to be high to moderate for the majority of the detected compounds in WWTP influents, particularly for ofloxacin and acetaminophen. Likewise, the ecological risk was estimated as high to medium for all five compounds detected in the WWTP effluents, especially for antibiotic compounds. Ultimately, the risk assessment results support the need for urgent action on managing the release of these compounds from the studied WWTPs to decrease their environmental impact in surrounding environments.

Due to widespread use of WWTPs effluent in Saudi Arabia, the possibility of pharmaceuticals migrating from the unsaturated zone (soil) and contaminating groundwater was investigated using laboratory-scale soil column experiments. Most the investigated pharmaceuticals had a high affinity for soil particles and tended to accumulate in the top 5 cm of the soil column. Therefore, no migration of these compounds to groundwater is anticipated in the natural environment, with the possible exception of caffeine and cephalexin because they were found in the leachate at extremely low concentrations.

Overall, this research has increased knowledge on the occurrence and effects of pharmaceuticals in Saudi Arabia, highlighting their presence in three selected WWTPs and the potential risk to the environment from their presence in wastewater. The research also raises concerns surrounding the release of pharmaceutical compounds that routinely enter the environment from WWTPs in Saudi Arabia. The results obtained from this work provide new information that could contribute to the formulation and implementation of future regulations for wastewater discharges, wastewater quality and emerging contaminants.

CHAPTER ONE

General Introduction

Water quantity and quality are very important for human health and ecosystem sustainability, but human populations have shown exponential growth, while available water resources remain the same. Therefore, there has been an increasing in demand for limited fresh water supplies together with contamination of the aquatic environment (Kolpin *et al.*, 2002; Lissemore *et al.*, 2006; Kim *et al.*, 2009; Ternes, 1998; Wanda *et al.*, 2017; Tran *et al.*, 2018).

Pharmaceuticals have been produced and used for many beneficial purposes, primarily to improve health and to protect humans and animals from diseases in the modern world. They can be categorized into wide range of therapeutic classes such as nonsteroidal anti-inflammatories (NSAIDs), antibiotics, antipsychotics, antihypertensives, antidiabetics, antihistamines, lipid regulators, anticonvulsant, β-blockers, etc. However, pharmaceutical compounds also have the potential to have an adverse effect on the environment if they end up in soil, surface water and groundwater (Ahmad *et al.*, 2019).

Pharmaceutical compounds have found their way into the environment through many ways, such as wastewater treatment plants (WWTPs), septic tanks, human and animal wastes and agricultural practices (Archer *et al.*, 2017). Consequently, in recent decades, pharmaceuticals have been detected in wastewater, surface water and soil at very low concentrations (μg L^{-1} and ng L^{-1}). Moreover, the effects of pharmaceuticals in the environment are widely unknown (Li *et al.*, 2020b). The vast majority of pharmaceutical compound studies have been confined to their emissions in aqueous systems (Oppel *et al.*, 2004). The fate of a pharmaceutical compound in soil or in a

water body depends on many factors, such as physico-chemical properties (e.g., water solubility, lipophilicity, vapour pressure), environmental parameters and climate conditions (e.g., temperature, incident radiation, pH). The most important factor is the presence and activity of microorganisms that possess the ability to biodegrade them (Caracciolo *et al.*, 2015).

As pharmaceutical compounds have become an important environmental issue, more research has been conducted, which has led to the development of very advanced technology to detect these new emerging contaminants (Biel-Maeso *et al.*, 2021). These new emerging contaminants take the name of 'chemicals of emerging concern (CECs)' (Bhandari *et al.*, 2009; Tran *et al.*, 2018; Krzeminski *et al.*, 2019). CECs include pharmaceuticals and personal care products (PPCPs), endocrine disrupting compounds (EDCs), perfluorinated compounds (PFCs), surfactants, gasoline additives, disinfection by-products, algal and cyanobacterial toxins, organometallic compounds, brominated and organophosphate flame retardants, plasticizers, and nanoparticles (Gros *et al.*, 2006; Tran *et al.*, 2018).

Two thirds of the world's water scarce countries are located in the Middle East and North Africa (MENA) region, where they are among the top 20 countries (Negewo, 2012). The shortage of water in the MENA region is anticipated to increase by 290% between 2041 and 2050, from an actual shortage of 42,000 m^3/year in 2001-2010 (Droogers *et al.*, 2012). Due to the growing demand for water, reusing treated effluent has been recognized as a suitable and valuable water resource for many purposes, such as irrigation of agricultural land, golf courses and various other landscapes. Using reclaimed wastewater is therefore an attractive solution, especially for countries affected by water shortages and situated in warmer and dryer climates such as in the Middle East and Southern Europe (Carter *et al.*, 2019). Globally, many countries have

been successfully achieved a good percentage of reuse of treated water to supplement their water demand. For instance, Singapore was meeting 30% of its water need by reclaiming wastewater and is aiming to increase by 2035 to ≈ 60 per cent (Bennett, 2015).

1-1 Sources and occurrence of pharmaceuticals in the environment

In recent years there has been increasing concern about pharmaceutical compounds which are likely to leach to the environment, even though the concentrations of these chemicals in the environment are usually at trace values (typically μg L^{-1} and below). Human pharmaceuticals are classified according to their purposes, such as antibiotics and muscle relaxants. The highest usage of medicines includes antiphlogistics, antibiotics, antidiabetics, antiepileptics, beta blockers, antihistamines, calcium antagonists, psychotropics, muscle relaxants, diuretics, and decongestants (Hlavinek *et al.*, 2007; Tran *et al.*, 2018). Kummerer (2004) predicted that the total consumption of pharmaceutical compounds in urban populations was roughly 100,000 tonnes globally, with an average consumption of 15g per person every year (Kümmerer, 2004b). For example, in Canada, marketing sales of some drugs were estimated to be: paracetamol 800 t/y, aspirin 500 t/y and ibuprofen 200 t/y (Metcalfe *et al.*, 2003). Moreover, in Germany marketing data predicted that, antibiotic sales would grow by 5 t/y (Kümmerer, 2004a).

The processes by which pharmaceutical compounds enter the environment, compared with other substances, is controlled by many factors (Archer *et al.*, 2017). These factors involve the quantity of pharmaceuticals used, the fate of pharmaceuticals in the body, the behavior of the compounds during the wastewater treatment process, and their ability to interact with environmental media (Heberer *et al.*, 2002; Archer *et al.*, 2017). Pharmaceutical compounds have many major pathways to the environment,

such as via effluents from WWTPs, leakage from septic tanks or landfills, run-off, direct disposal of unused PPCPs, pharmaceutical factory waste and direct discharge to water bodies (Chiarello *et al.*, 2016; Luo *et al.*, 2014; Wiest *et al.*, 2018). Wastewater treatment plant effluents are considered as a major source of pharmaceuticals to aquatic and terrestrial systems, particularly from plants that are not designed for removing trace organic pollutants (Yang *et al.*, 2017) . Human practices, such as excretion and flushing unused drugs to sewer systems, are described as a primary cause of pharmaceutical release into the environment (Daughton, 2013). A wide range of pharmaceuticals and personal care products (PPCPs) have been detected in treated wastewater, with the potential, therefore, to be released into the environment (Arpin-Pont *et al.*, 2016; Luo *et al.*, 2014). As a result, pharmaceuticals have been observed in receiving water bodies, such as rivers (Huber *et al.*, 2016; Metcalfe *et al.*, 2003; Musolff *et al.*, 2010), as well as in groundwater (Huber *et al.*, 2016; Einsiedl *et al.*, 2010). Pharmaceutical compounds might also enter the environment from landfills containing expired and unused drugs, and from toxic gases released during their manufacture (Stackelberg *et al.*, 2004). In addition, Topp *et al.* (2008) and Arpin-Pont *et al.* (2016) found that runoff of agricultural waste could contaminate surface water with pharmaceutical residues and could leach to groundwater.

A comprehensive review reported by aus der Beek *et al*. (2016) summarized that, around 631 pharmaceuticals have been detected in 71 countries worldwide in surface water, drinking water, groundwater and tap water Figure 1-1.

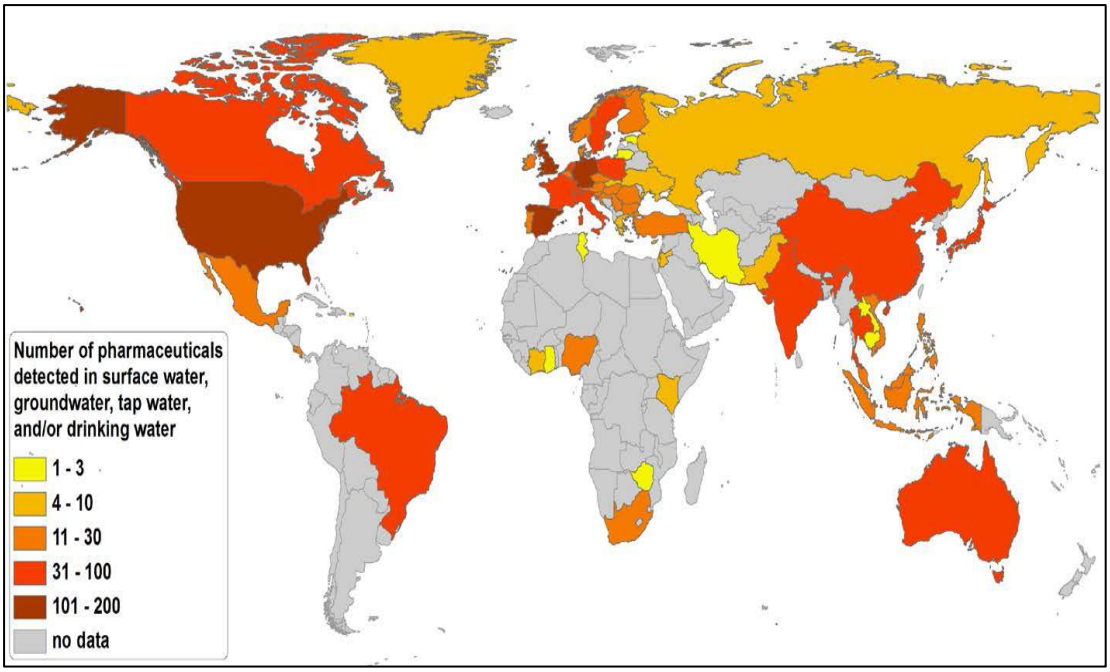


Figure 1-1: Occurrence of pharmaceuticals around the world in drinking/tap waters, groundwater, and surface waters (aus der Beek *et al.*, 2016). Copyright license secured from John Wiley and Sons and Copyright Clearance Center.

1-1-1 Pharmaceutical Manufacturing

Manufacture of pharmaceuticals is considered to be a source of pharmaceutical emissions to the environment (Larsson, 2014; Holm *et al.*, 1995; Reddersen *et al.*, 2002; Zühlke *et al.*, 2004; Kleywegt *et al.*, 2019). Approximately more than 4000 active pharmaceutical ingredients (API), such as painkillers, antibiotics, antidiabetics, beta-blockers, antidepressant, X-ray contrast media, contraceptives and lipid regulators are used worldwide (McGrane, 2016; Rasheed *et al.*, 2019).

There are many processes involved before a drug is ready to be used for humans or animals, including extraction, processing, purification, and packaging. The manufacturing of pharmaceuticals is divided into two major stages. The first phase, in which the active pharmaceutical ingredient (API) is produced, is typically referred to as primary processing or manufacture. The second stage or secondary processing results in the final medication form, where the active drugs are made into suitable products for

use, like tablets and capsules (Velagaleti *et al.*, 2002). For accurate synthesis and purification of APIs, organic solvents are usually used, which are then treated or disposed of by incineration. Williams (2005) concluded that most waste generated from pharmaceutical industry sites was solid waste (Williams, 2005).

Pharmaceutical compounds have been detected and monitored in many API factory discharges around the world, such as India (Larsson *et al.*, 2007; Larsson, 2014), Canada (Kleywegt *et al.*, 2019), Croatia (Bielen *et al.*, 2017) and Pakistan (Khan *et al.*, 2013; Ashfaq *et al.*, 2017b). Furthermore, damage due to emissions from steroid factories in France has been reported for wild fish living downstream (Li *et al.*, 2011). In Patancheru near Hyderabad, India, there are many important pharmaceutical manufacturers which produce a huge number of pharmaceuticals for worldwide use. Due to the high emissions from these pharmaceutical production facilities to nearby WWTPs, Larsson *et al.* (2007) found the concentrations of pharmaceuticals monitored in the WWTP effluent to be very high, reaching 31 mg L^{-1} for ciprofloxacin. Ciprofloxacin is one of the most used pharmaceuticals globally, and is prescribed to treat seasonal diseases like bronchitis, pneumonia, and sinusitis (Coutu et al., 2013). The concentration of ciprofloxacin reported by Larsson *et al.* (2007) was ~4500 times higher than that reported by Pal *et al.* (2010) in Australia (Larsson *et al.*, 2007; Pal *et al.*, 2010). Recently, Bielen *et al.* (2017) investigated two pharmaceutical companies in north Croatia, over a one-year monitoring period. By collecting samples from effluents and surface water nearby these factories, they observed that the concentration of azithromycin and erythromycin reached 3.8 mg L^{-1} in the spring season (Bielen *et al.*, 2017). Moreover, Khan *et al.* (2013) detected trimethoprim at a concentration of 1.7 μg L^{-1} in a river near Lahore city, Pakistan, close to the pharmaceutical factories. They observed a positive correlation between the high concentration measured in the river

and pharmaceuticals production facilities discharge (Khan *et al.*, 2013). Also, Ashfaq *et al*. (2017b) investigated 11 pharmaceuticals in different environmental matrices sampled from locations near pharmaceutical industrial units in Lahore, Pakistan, and found paracetamol and ibuprofen to have the highest detected concentrations.

High concentrations of pharmaceuticals have also been reported in Canada by Kleywegt *et al.* (2019). They observed the highest concentrations of paroxetine (3.38), sertraline (5.1), carbamazepine (575), penicillin (14.3), acetaminophen (461), codeine (49.2), ibuprofen (344), naproxen (253000), oxycodone (21), atorvastatin (0.893), metoprolol (7333.6), amlodipine (22.9), diltiazem (1160), furosemide (1200) and verapamil (7.340) μg L^{-1} in the discharges, and downstream of, five manufacturing facilities in Ontario, Canada. Lin and Tsai (2009) investigated antibiotics, estrogens, non-steroidal anti-inflammatory drugs (NSAIDs), beta-blockers and lipid regulators in the environment in Taiwan (Lin and Tsai, 2009). This study found that, due to the many factories and hospitals in this area, erythromycin-H_2O, sulfamethoxazole, gemfibrozil, and acetaminophen occurred in high concentrations, followed by NSAIDs. Larsson (2007) observed that discharges from pharmaceutical manufacturing facilities have been repeatedly shown to provide conditions where antibiotics reach concentrations that are selective for resistance enrichment (Larsson *et al.*, 2007). . Bacterial resistance to antibiotic drugs occurs when pathogens exposed to antibiotics for long period of time that could potentially lead to the development of resistance to their effects on microorganisms (Larsson et al 2007; Larsson 2014).

Emissions from pharmaceutical manufacturing are a major source of the highest concentrations of pharmaceuticals, such as antibiotics, released into the environment and, consequently, the highest abundance of antibiotic-resistance genes detected in the

environment is found in environments polluted by direct discharges from the manufacturing of antibiotics (Topp *et al.*, 2017; Larsson, 2014).

1-1-2 Wastewater treatment plants (WWTPs)

The level of occurrence and the presence of micro-pollutants in different environmental matrices, which could pose a serious environmental risk for fauna and flora, keep increasing. In addition, the increasing world population is leading to a gradual rise in the consumption of, and demand for medicines. As a result, the volume of sewage effluent which is contaminated by PPCPs is increasing. Although pharmaceuticals emerge into the environment from many pathways, wastewater treatment plants are considered as a main source and pathway to the environment (Alder *et al.*, 2001; Daughton and Ternes, 1999; Richardson and Bowron, 1985; Rosi-Marshall and Royer, 2012; Patel *et al.*, 2019; Kołecka *et al.*, 2019).

Most conventional WWTPs are not capable of removing all micro-pollutants, such as pharmaceutical contaminants, because they are not designed to treat and remove these chemical substances (Tran *et al.*, 2018). Subsequently, more and more pharmaceuticals have been observed and reported in WWTP effluents and are discharged into the environment, potentially causing toxic harm to the environment (Ferrari *et al.*, 2003; Pulicharla *et al.*, 2021). WWTPs receive wastewater from different sources like domestic, industrial and hospital waste. Typically, the treatment processes are preliminary, primary, secondary, and tertiary treatment. In the pre-treatment/preliminary treatment step, all unwanted objects that may cause damage or a failure of the plant operational system are removed. This process is typically followed by primary treatment, where heavy solids are separated from light solids by settling them. Biodegradable and trace organic constituents, colloidal solids, and nutrients present in municipal wastewaters, are then removed in the secondary treatment unit or

biological treatment step (Jelić *et al.*, 2012; Tchobanoglus *et al.*, 2003). Activated sludge (AS) is the most common type of secondary treatment. In this process, the organic matter is simply broken down by microorganisms in the presence of oxygen. In order to achieve a better effluent water quality, tertiary treatment can be added to the above processes (Jelić *et al.*, 2012). Tertiary (advanced) treatment typically uses sand filters, reverse osmosis (RO), membrane bioreactors (MBRs), nanofiltration and advanced oxidation processes (AOP), because they are more effective at to reducing the concentration of pharmaceuticals in effluents (Clara *et al.*, 2005; Huber *et al.*, 2003; Snyder *et al.*, 2007). The wastewater is then often disinfected using either chlorine gas, ultraviolet radiation (UV) or ozone prior to discharge. Final WWTP effluents are usually discharged into surface waters, reused in agriculture, industrial purposes, or collected in artificial ponds (Picó *et al.*, 2021). The effluent biosolids, known as sewage sludge, are generally used as a manure, or incinerated, depending on the policies of the country.

The fate of pharmaceutical compounds in WWTPs is controlled by many factors, such as the concentration range of the PPCPs in the WWTP inlet, the source of the influent (domestic or industry discharge), salinity, temperature, treatment method used, hydraulic retention time (HRT), population size served by the WWTP, weather conditions, rainfall, and the type of treatment implemented (Ratola *et al.*, 2012; Singer *et al.*, 2016; Larsson, 2014; Krzeminski *et al.*, 2019; Kapelewska *et al.*, 2018; Kosma *et al.*, 2014). Pharmaceutical compounds have been reported, in influents and/or effluents of WWTPs, and which subsequently could reach the environment (Funke *et al.*, 2015; Papageorgiou *et al.*, 2019; Huber *et al.*, 2016; Al Aukidy *et al.*, 2012; Oaks *et al.*, 2004). Tran *et al*. (2018) found that the concentration of most antibiotics in WWTP effluents from developing countries were higher than those located in

developed countries. Concentrations for selected pharmaceuticals in WWTP influents and effluents worldwide are given in Table 1-1, and more detail can be found in appendix Table A-3-3.

Table 1-1: The highest concentrations (ng L^{-1}) reported of selected pharmaceuticals in some WWTPs influent and effluent around the world.

Pharmaceuticals	Influent (ng L^{-1})	Country	Ref.	Effluent (ng L^{-1})	Country	Ref.
Acetaminophen	343620	South Africa	1	7400	Greece	2
Atenolol	294700	India	3	7602	UK	4
Azithromycin	330	Italy	5	245	Greece	6
Caffeine	221455	Greece	7	13900	Greece	2
Cephalexin	1318	USA	8	5624	USA	8
Ciprofloxacin	3700	Italy	5	1437	Greece	6
Diclofenac	6300	Greece	2	6500	Greece	2
Ibuprofen	25400	Greece	2	31300	Spain	9
Metformin	176417	Greece	6	4806	Greece	6
Ofloxacin	7900	Hong Kong	9	31700	Spain	10
Oxypurinol	26600	Germany	11	30000	Germany	12
Trimethoprim	6796	UK	4	3052	UK	4

[1] (Archer *et al.*, 2017), [2] (Kosma *et al.*, 2010), [3] (Mohapatra *et al.*, 2016), [4] (Kasprzyk-Hordern *et al.*, 2009), [5] (Verlicchi *et al.*, 2012a), [6] (Dasenaki and Thomaidis, 2015), [7] (Papageorgiou *et al.*, 2019), [8] (Mohapatra *et al.*, 2016), [9] (Leung *et al.*, 2012), [10] (Radjenović *et al.*, 2009), [11] (Funke *et al.*, 2015), [12] (Boulard *et al.*, 2018).

Antifungal and antimicrobial agents (i.e. miconazole, thiabendazole, triclocarban, and triclosan) which are widely used in household products, such as hair shampoos, dermal creams, soaps, toothpastes and shower gels, have been detected in WWTPs in India with triclosan having the highest concentration at 5860 ng L^{-1} (Subedi *et al.*, 2015). Emissions of pharmaceuticals from effluent treatment plant near

Hyderabad, India, have been investigated. This WWTP received wastewater from roughly 90 bulk medicine manufacturing facilities (Fick *et al.*, 2009; Kristiansson *et al.*, 2011; Larsson *et al.*, 2007). The concentration of pharmaceuticals reported in this plant was very high, and reached 31 mg L^{-1}, which is higher than concentrations typically reported in literature in the μg L^{-1} to ng L^{-1} range. Furthermore, in the effluent of the wastewater treatment plant investigated by Larsson *et al*., (2007), ciprofloxacin was the most abundant compound with a concentration of 31 mg L^{-1}, whereas ranitidine was reported at concentrations between 0.16 and 0.09 mg L^{-1}.

Al Aukidy, *et al.* (2012) reported the concentrations of 24 compounds from 27 pharmaceuticals of interest, which included nine different therapeutic classes (six analgesics/anti-inflammatories, seven antibiotics, three lipid regulators, four beta-blockers, three psychiatric drugs, one antidiabetic, one antihypertensive, one diuretic and one beta-agonist) in the liquid effluents from two WWTPs in the PoValley, northern Italy (Al Aukidy *et al.*, 2012). They concluded that diclofenac was the most dominant compound in both WWTP effluents with high concentrations ranging between 223 and 800 ng L^{-1}.

Although the above and more studies from around the world have reported pharmaceuticals in WWTPs, these compounds are usually removed by varying percentages through the WWTP processes. The removal efficiency (RE) of pharmaceuticals plays a major role in reducing their concentrations in WWTP effluents and, therefore, the potential effects of pharmaceutical compounds in the environment. This removal efficiency is affected by many factors, such as the biodegradability and physiochemical properties of the pharmaceuticals, their tendency for absorption by activated sludge or not and their tendency to volatilize (Kosma *et al.*, 2014; Couto *et al.*, 2019). Removal efficiency is estimated as a percentage (%) of the influent

concentration of pharmaceutical remaining in the effluent of the WWTP. The RE value could be positive or negative. Positive RE occurs when the concentration of the compound in the influent is higher than its concentration in the WWTP effluent. Negative removal occurs when the concentration of the pharmaceutical in the effluent of WWTP is higher than its corresponding concentration in WWTP influent. There are several possible explanations for negative removal rates like:

(1) Pharmaceuticals could remain as fecal particles and be released gradually during wastewater treatment processes (Göbel *et al.*, 2007).

(2) Negative removal of pharmaceuticals in WWTPs could be simply due to sampling schemes and/or possible instrumental errors (Verlicchi *et al.*, 2012b).

(3) Some pharmaceuticals, like ibuprofen, are transformed to other products that may later be converted back to the parent compounds (Verlicchi *et al.*, 2012b).

Even though some pharmaceuticals are not sufficiently removed using biological treatment processes, secondary treatment processes such as activated sludge, membrane bioreactor (MBR), aerobic bioreactor, anaerobic bioreactor and trickling filter have been reported as the most efficient for the removal of PPCPs (Ahmed *et al.*, 2017). In contrast, physical treatment process where suspended solids and all large objects, grit, oil and grease are reduced and removed, are not usually efficient enough to eliminate pharmaceuticals, and it has been reported that most pharmaceuticals tend to pass through primary treatment (Martín *et al.*, 2012; Radjenović *et al.*, 2009; Ternes *et al.*, 2004; Tsui *et al.*, 2014). For instance, the removal rate of triclocarban and triclosan were reported to be between 27% and 75% after primary treatment (Lozano *et al.*, 2013; Tsui *et al.*, 2014).

Adsorption into sewage sludge and biodegradation play the main role in PPCP elimination in WWTPs. During secondary treatment, pharmaceuticals have been observed to be adsorbed into the sludge (Daughton and Ternes, 1999). For instance, the major removal process of fluoroquinolones and tetracycline has been found to take place in sewage sludge (Golet *et al.*, 2003; Kim *et al.*, 2005) and activated sludge has been shown to be the most effective way to remove pharmaceuticals, compared with other types of secondary treatment (Kim *et al.*, 2008; Nakada *et al.*, 2006; Stumpf *et al.*, 1999; Ahmed *et al.*, 2017). In fact, many pharmaceuticals such as acetaminophen and caffeine are reported to be removed efficiently using aerobic activated sludge in the secondary treatment process (Gómez *et al.*, 2007; Jones *et al.*, 2007; Ternes, 1998). Nevertheless, some pharmaceuticals are not removed efficiently in this stage, such as gemfibrozil and fenofibric (Bendz *et al.*, 2005; Stumpf *et al.*, 1999; Ternes, 1998), acebutolol, sotalol, ciprofloxacin and norfloxacin (Lindberg *et al.*, 2006; Vieno *et al.*, 2006) and iopromide (Ternes *et al.*, 2007). Trans *et al.* (2018) comprehensibly reviewed the removal efficiency of different WWTPs in different geographical regions and found removal values ranging from very low (even negative) to very high (100%) for many compounds. For example, the removal rate of ciprofloxacin, tetracycline, acetaminophen, and ibuprofen were estimated between <0-100%, 34-97%, <0-100% and <0-99.8% respectively. This study aimed to examine the efficiency of WWTPs in Riyadh, Saudi Arabia, in removing 16 compounds from wastewaters (Chapter 3, section 3-5-2).

1-1-3 Soil and sewage sludge

During sewage treatment medicines can be adsorbed into sewage sludge and then end up in the environment, particularly when the sludge is used on agricultural fields as a manure (Conde-Cid *et al.*, 2020; Golet *et al.*, 2002; Oppel *et al.*, 2004; Gothwal and

Shashidhar, 2015). The land application of biosolids (treated sewage sludge) in agricultural as a fertilizer is due to their enrichment with nutrients and organic matter which positively enhance soil properties (Kinney and Heuvel, 2020; Latare *et al.*, 2014). This practice is common in many countries, such as the USA, Canada, and Europe (EU) (Angın and Yağanoğlu, 2009; Carballa *et al.*, 2009; Mantovi *et al.*, 2005; Kinney and Heuvel, 2020). It estimated that around 50% and 37% of biosolids from WWTPs are applied to land in the USA and EU respectively (Chitdeshwari *et al.*, 2001; Chang *et al.*, 2002). However, they could also be considered as a potential path for pharmaceutical residuals to enter the soil, together with the use of treated wastewater for crop irrigation (Ternes *et al.*, 2007; Caracciolo *et al.*, 2015). Reuse of wastewater is widespread globally as shown in Figure 1-2.

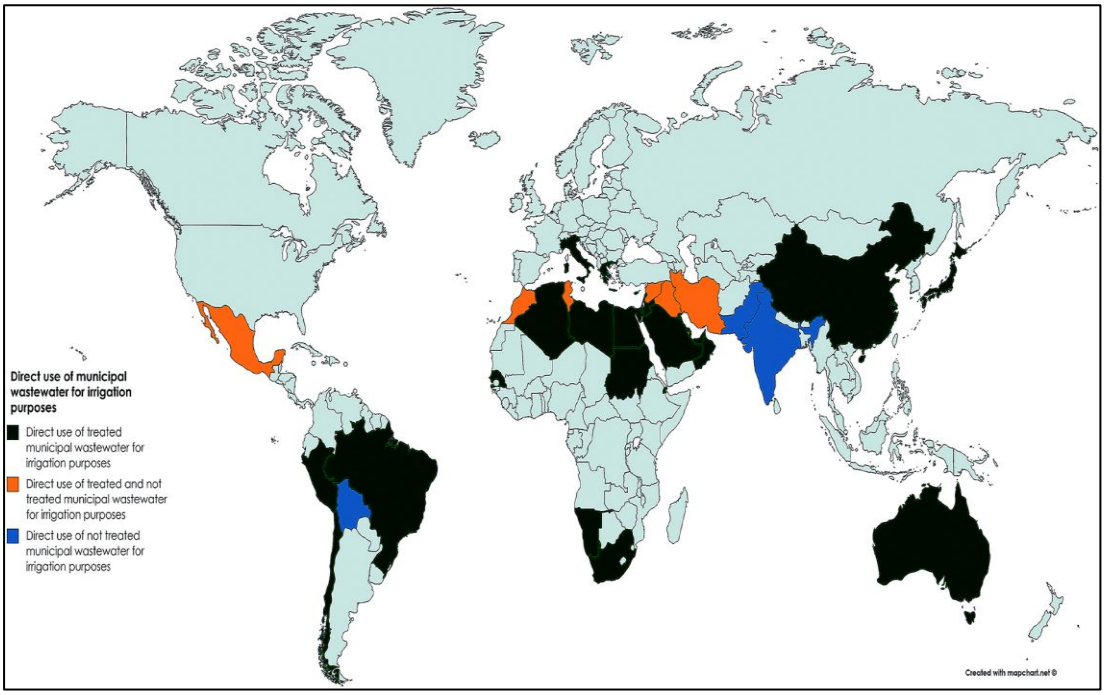


Figure 1-2: The re-use of municipal wastewater for agricultural irrigation purposes worldwide (Carter *et al.*, 2019).

A wide range of pharmaceuticals have been reported in biosolids. For instance, after screening the occurrence of 195 pharmaceutical compounds reported in 59

scientific papers, Verlicchi and Zambello (2015) reported that NSAIDs ranged between 3 and 10000 ng g^{-1}, 1 to 1000 ng g^{-1} and 4 to 1000 ng g^{-1} in primary, secondary and digested sludges respectively. Also in this study, antibiotics were observed in the ranges of 5 - 4000 ng g^{-1}, 0.1 - 70000 ng g^{-1} and 1 - 8000 ng g^{-1} in primary, secondary and digested sludges respectively (Verlicchi and Zambello, 2015).

The U.S. Environmental Protection Agency (EPA) investigated 110 biosolids samples from 94 WWTPs in USA in order to investigate 72 pharmaceuticals and personal care products (PPCPs) (McClellan and Halden, 2010). They observed that triclosan was detected at a mean concentration of 12600 ng g^{-1} and ciprofloxacin at 6900 ng g^{-1} and were among the highest concentrations detected. Hanna *et al*. (2018) conducted a study to investigate pharmaceutical residues in different environment samples collected from twelve villages in Shandong province in eastern China. They found ciprofloxacin at concentrations of 0.3 - 18.20 μg kg^{-1}, 16 - 21.74 μg kg^{-1} and 0.68 - 112.7 μg kg^{-1} in soil, household outlet sediment and river sediment respectively. Norfloxacin was measured in the range 0.04 - 4.6 μg kg^{-1} in this study (Hanna *et al.*, 2018). Concentrations of pharmaceutical compounds were reported by Huber *et al*. (2016) to be higher in sludge matrices than in samples from WWTP influents and effluents, with acetaminophen detected at higher concentration (up to 447 μg g^{-1}) than other compounds (Huber *et al.*, 2016). Martín *et al.* (2012) investigated the occurrence of 16 pharmaceuticals belonging to different therapy classes in the south of Spain, and found that primary sludge had a higher concentration than secondary sludge, with concentrations ranging from 24.9 to 4105 μg kg^{-1} (Martín *et al.*, 2012).

Ashfaq *et al*. (2017) studied the occurrence and ecological risk of 11 pharmaceuticals in industrial wastewater, sludge, solid waste, and soil located close to the drug formulation sites in Lahor, Pakistan. They reported that diclofenac, ibuprofen,

acetaminophen, ciprofloxacin and ofloxacin were detected at the highest concentrations of 4968 μg kg^{-1}, 6064 μg kg^{-1}, 483 μg kg^{-1}, 454 μg kg^{-1} and 347 μg kg^{-1} in sludge, 6632 μg kg^{-1}, 1229 μg kg^{-1}, 874 μg kg^{-1}, 161 μg kg^{-1}, and 127 μg kg^{-1} in solid waste and 257 μg kg^{-1}, 610 μg kg^{-1}, 81 μg kg^{-1}, 28 μg kg^{-1}, and 30 μg kg^{-1} in nearby soil. Singer *et al.* (2016) and Gardner *et al.* (2013) proposed that the abundancy and high concentrations of pharmaceuticals such as hydrophobic (or non-polar) antibiotic residues (tetracycline) reported in sludge can be attributed to their affinity to bind to sludge rather than sewage water (Singer *et al.*, 2016; Gardner *et al.*, 2013).

When treated wastewater or biosolids are used in agriculture there is the potential for pharmaceuticals and personal care products (PPCPs) to transfer to soil and then potentially to be taken up by the crops (Patel *et al.*, 2019).

1-1-4 Surface water

Pharmaceutical compounds are continuously introduced to surface water from human and animal use through many pathways, such as wastewater treatment plants, discharges from the pharmaceutical industry, landfill, and livestock practices. It has been reported that around 631 pharmaceutical compounds have been detected in the environment (aus der Beek *et al.*, 2016).

Due to the large quantities used and their ubiquitous occurrence, pharmaceuticals have been reported in surface water as active ingredients, with relatively few reported as metabolites and transformation products (TPs) (Rudén *et al.*, 2010). Some studies have reported pharmaceuticals in surface water include Picó *et al.*, (2020), Rivera-Jaimes *et al.*, (2018), Archana *et al.*, (2017), Agunbiade and Moodley, (2014), Al Aukidy *et al.*, (2012), Silva *et al.*, (2011), and Fick *et al.*, (2009)

Silva *et al.* (2011) detected most of the 43 pharmaceuticals they investigated in surface waters of the Ebro river basin, Spain. Among their list of compounds, the NSAIDs were detected at high concentrations; the highest concentrations were for ketoprofen, acetaminophen and ibuprofen at 1060 ng L^{-1}, 872 ng L^{-1} and 277 ng L^{-1} respectively. Atenolol was detected up to 1237 ng L^{-1}, hydrochlorothiazide up to 186 ng L^{-1} and furosemide up to 92.6 ng L^{-1}. These high concentrations were attributed to the effluent discharged to this river from a WWTP. WWTP effluents have been observed as hot spots for introducing PPCPs into the environment for at least a decade and the potential risk of environmental impacts in the receiving water bodies must be considered (Pal *et al.*, 2010).

In recent study, Picó *et al.,* (2020) reported pharmaceuticals in shallow lake in Saudi Arabia. They monitored acetaminophen, ibuprofen, diclofenac, ofloxacin, caffeine, atenolol, metformin, and trimethoprim and found them at concentrations between N.D. and 20663.5 ng L^{-1}.

Fick *et al*. (2009) studied the occurrence of 12 pharmaceutical compound in 2 lakes in Hyderabad, India. Among their list, ciprofloxacin and ofloxacin were detected at average concentrations of 3933 μg L^{-1}, and 5.8 μg L^{-1} whereas trimethoprim was not detected.

Eight acidic pharmaceuticals (four antipyretics, three antibiotics and one lipid regulator) were investigated in surface water and in a dam along the Umgeni River used for water supply in KwaZulu-Natal, South Africa, by Agunbiade and Moodley, (2014). They reported aspirin to have the highest concentration in surface water compared with other location samples. Moreover, they observed that the concentrations of these analytes in the surface water were below 10 μg L^{-1}, except for acetaminophen and

atenolol (Agunbiade and Moodley, 2014). Another study in Italy reported the presence of pharmaceuticals such as atenolol, sotalol, clarithromycin, in a canal; the highest concentrations were for sotalol, atenolol and clarithromycin at 504 ng L^{-1}, 231 ng L^{-1}, and 128 ng L^{-1} respectively (Al Aukidy *et al.*, 2012). Consequently, above studies clearly showed that surface water could be affected by pharmaceutical compounds and contaminated at even a high level of concentration (mg L^{-1}).

1-1-5 Groundwater

Despite the fact that the occurrence and fate of pharmaceuticals in the environment has become a matter of concern for human health and the aquatic ecosystem, pharmaceuticals will be used more and more due to their beneficial purpose. Concern for groundwater pollution from pharmaceuticals has increased, especially for aquifers which have been recovering from wastewater discharge (Benotti and Snyder, 2009). The occurrence and fate of pharmaceuticals underground is controlled by many factors, such as the depth to groundwater, soil porosity and permeability, flow rate, the mobility and persistence of pharmaceuticals and the density of populations (Phillips *et al.*, 2015; Schaider *et al.*, 2016; Benotti and Snyder, 2009; Bexfield *et al.*, 2019).

Pharmaceutical compounds have been reported in groundwater in many studies. For example, in a large-scale study, the occurrence of more than 100 hormone and pharmaceutical compounds from 1120 locations in 46 States across the USA were investigated by Bexfield *et al.* (2019). This study concluded that groundwaters are exposed to 34 out of more than 100 compounds because they were detected in the samples. Also, the results indicated that the shallow aquifers are more vulnerable to contamination by the detected pharmaceuticals than deep aquifers, particularly in locations recently recharged by water affected by human activities. Furthermore, high mobility compounds were likely to be detected more than others.

Eleven pharmaceutical compounds, including ciprofloxacin, ofloxacin and trimethoprim, were investigated in samples taken from wells in six villages situated near to WWTPs receiving wastewater from pharmaceutical factories in India (Fick *et al.*, 2009). The results of this study demonstrated concentrations ranging between 44 ng L^{-1} and 14000 ng L^{-1}, N.D. and 480 ng L^{-1} and N.D. and 55 ng L^{-1} for ciprofloxacin, ofloxacin and trimethoprim respectively, which was far less than their corresponding concentration found in the surface water.

1-1-6 Drinking water

Drinking water is mostly supplied from surface water (rivers and lakes) and groundwater, which are potentially exposed to contamination by pharmaceuticals and personal care products (PPCPs) (Sui *et al.*, 2015; Pulicharla *et al.*, 2021). Contamination of drinking water by pharmaceuticals could occur from different sources, such as WWTP effluents, septic tank leakage, agriculture and livestock practices, landfill leachates and pharmaceutical manufacturing effluent discharge. Discharge from WWTPs into the environment is considered as the most significant source of pharmaceuticals to drinking water (Ashfaq *et al.*, 2019; Pulicharla *et al.*, 2021).

Pharmaceuticals have been detected ubiquitously in drinking water, particularly antibiotics (sulfonamides group) and NSAIDs (acetaminophen, ibuprofen and diclofenac) which have been also among the most commonly investigated compounds in drinking water (K'oreje *et al.*, 2016). For instance, 51 pharmaceuticals were investigated in drinking water from 19 facilities across the USA (Benotti *et al.*, 2009). They reported the most frequently detected pharmaceuticals in their list were atenolol, triclosan and trimethoprim at maximum concentrations of 36 ng L^{-1}, 11 ng L^{-1} and 6.4 ng L^{-1}, whereas the highest concentration reported in this investigation was 40 ng L^{-1}

for meprobamate. Fram and Belitzp (2011), found pharmaceutical compounds in 3.1% of 1231 groundwater samples from wells in California, which are used for public drinking water. Seven of the 14 pharmaceutical compounds analysed had concentrations more than, or equal to the method detection limits (acetaminophen, caffeine, carbamazepine, codeine, paraxanthine, sulfamethoxazole, and trimethoprim) with concentration of 1.89 μg L^{-1}, 0.29 μg L^{-1}, 0.42 μg L^{-1}, 0.214 μg L^{-1}, 0.12 μg L^{-1}, 0.17 μg L^{-1}, and 0.018 μg L^{-1} respectively (Fram and Belitz, 2011).

1-2 Environmental risk of pharmaceuticals

Pharmaceutical residues have been reported in different environments like surface water (lakes and rivers), groundwater and drinking water (Agunbiade and Moodley, 2014; Al Aukidy *et al.*, 2012; Ashfaq *et al.*, 2019; Fick *et al.*, 2009; Silva *et al.*, 2011; Archana *et al.*, 2017; Rivera-Jaimes *et al.*, 2018; Picó *et al.*, 2020; Bexfield *et al.*, 2019; K'oreje *et al.*, 2016). Pharmaceuticals are introduced to the environment from a number of sources, like WWTP effluents, landfills, bathing and washing, pharmaceutical manufacturing, excreting in urine and feces, and hospitals and other medical facilities (Verlicchi *et al.*, 2013; Yi *et al.*, 2017; Niemi *et al.*, 2020; Verlicchi and Zambello, 2015; Al Aukidy *et al.*, 2012; Papageorgiou *et al.*, 2019). Most studies have reported that there is widespread occurrence of pharmaceutical residues in environmental compartments (Fent *et al.*, 2006; Papageorgiou *et al.*, 2019; Bexfield *et al.*, 2019; Carmona *et al.*, 2017; Papageorgiou *et al.*, 2016; Ebele *et al.*, 2017). This occurrence has been often associated with a potential environmental effect, such as toxicity, endocrine disruption, and antibiotic resistance in microorganisms. For instance, adverse effects were observed when the analgesic and anti-inflammatory drug diclofenac was found to cause a high death rate (85%) in oriental white-backed vultures in India and Pakistan between 2000 and 2002 (Oaks *et al.*, 2004). Diclofenac has also been found to

have an effect in liver, kidney and gills of fish (Triebskorn *et al.*, 2004). However, the effect of these chemical substances on aquatic and terrestrial systems and their environmental risk remains unclear and needs to be further investigated (Ahmed *et al.*, 2017).

By and large, the toxicity of pharmaceutical compounds has been assessed in the laboratory on non-target organisms, and long-term poisoning studies have been rare (Christen *et al.*, 2010). For assessing potential adverse risk in the environment, authorities and agencies across the world have published guidelines on how the adverse effect of these micro-pollutants can be determined. In Europe, the European Medicines Evaluation Agency (EMA) is responsible for the licensing and registration of medicinal products in EU countries (Jose *et al.*, 2020; Whomsley *et al.*, 2019). Under EU Directive 93/39/EEC (EC, 1993), an environmental risk assessment (ERA) is required prior to drug licensing, in order to determine any significant toxicological risks associated with a drug (Straub, 2002). According to EU Directive 92/18/EEC (EC, 1992), and the guideline corresponding note which was issued by the EMA, environmental risk assessment protocols for pharmaceuticals for veterinary purposes were the first to be published. In 2006, EMA issued the guideline to assist in assessing the potential environmental risks of human pharmaceuticals using a stepwise approach (Christen *et al.*, 2010; Whomsley *et al.*, 2019; Jose *et al.*, 2020). The EMA pharmaceutical environmental risk assessment protocol has been conducted worldwide to evaluate the possible negative environmental impacts from these pharmaceutical compounds in ecosystems (Ashfaq *et al.*, 2017b; Archana *et al.*, 2017; Česen *et al.*, 2018; Praveenkumarreddy *et al.*, 2021; Sadutto *et al.*, 2021).

According to EMA (2006), an ERA is carried out in two phases, Phase I and Phase II (Table 1-2). In Phase I, the Predicted Environmental Concentration (PEC) is

calculated by using consumption data. If the result of the PEC in Phase I is more or equal to 0.01 μg L^{-1}, the evaluation proceeds to Phase II (Christen *et al.*, 2010; Whomsley *et al.*, 2019; Jose *et al.*, 2020; EMA, 2006). Phase II comprises two Tiers (Tier A, Tier B): in Tier A an initial assessment of environmental fate and effect is determined, while in Tier B a refinement is applied.

Table 1-2: The phased approach in the environmental risk assessment stage in regulatory evaluation (Jose *et al.*, 2020; Whomsley *et al.*, 2019; EMA, 2006).

Stage in regulatory evaluation	Phase I	Phase II Tier A	Phase II Tier B
Stage in risk assessment	Pre-screening	Screening	Extended
Objective	Estimation of exposure	Initial prediction of risk	Substance and compartment-specific refinement and risk assessment
Method	Action limit	Risk Assessment	Risk Assessment
Test/Data and Requirement	Consumption data, logK_{ow}.	Base set aquatic toxicology and fate	Extended data set on emission, fate, and effects

1-2-1 Phase I predicted environmental concentration

Phase I is a pre-screening risk assessment exercise based on an action limit threshold of 0.01 μg L^{-1}. The PEC in this step for pharmaceuticals in surface water is conducted and calculated according to equation (1). In Phase I, PEC is calculated by using default factors according to EMA (2006) recommendations. Thus, very important parameters are not considered in equation (1), such as excretion from the human body, removal efficacy of wastewater treatment for different pharmaceuticals and temporal variability in market penetration of the pharmaceuticals.

$$\mathrm{PEC_{Surface\ water}} = \frac{\mathrm{DOSE_{ia}} \times \mathrm{F_{pen}}}{\mathrm{WASTE_{inhap}} \times \mathrm{Dilution}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where: -

$DOSE_{ia}$ is the maximum daily dose.

F_{pen} is the market penetration, which is 0.01.

$WASTE_{inhap}$ is daily wastewater per capita (200L).

Dilution is dilution of effluent in surface water (10).

1-2-2 Phase II environmental fate and effect

Phase II of the ERA is conducted if the value of $PEC_{SurfaceWater}$ for pharmaceutical substances equals or exceeds the action limit of 0.01 μg L^{-1}. In Phase II Tier A, physical and chemical properties are assessed during the WWTP process, and a Predicted No Effect Concentration (PNEC) value is calculated. The PNEC value is based on acute toxicity data generated from standardized testing on algae, *Daphnia* or fish. The PNEC is typically calculated by dividing the lowest half maximal effective concentration (EC50) or the lowest half lethal effective concentration (LC50) by the assessment factor (AF). The applied AF depends on the quality and quantity of available ecotoxicological experimental data. The AF is usually 1000 when acute toxicity data are used. If the No Observed Effect Concentration (NOEC) is available, the PNEC is estimated by dividing it by an AF value of: 100 when only one NOEC trophic value is available; 50 when two NOEC trophic levels are available; and 10 when NOECs for all three standard test organisms are available (Zhou *et al.*, 2019; Česen *et al.*, 2018). Due to the lack of ecotoxicological experimental data and NOEC values from long-term testing, quantitative structural activity relationship (QSAR) models have been used to provide environmental risk data on the chronic toxicity of pharmaceuticals in the environment (Han and Lee, 2017; Mansour *et al.*, 2016; Sanderson *et al.*, 2003; Zhou *et al.*, 2019).

The risk quotient (RQ) is calculated using the PEC and PNEC (equation 2). If the ratio is below one, there is no environmental risk, and no further action is necessary.

$$RQ = \frac{PEC}{PNEC} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

If the RQ ratio exceeds one, and the pharmaceuticals show potential for bioaccumulation or adsorption to sediment, further investigation is necessary, and Tier B is applied. In Tier B, the $PEC_{Surface\ water}$ calculated in Phase I must be re-calculated as in equation (3):

$$\text{PEC}_{\text{Surface water}} = \frac{\text{DOSE}_{\text{ia}} \times \text{F}_{\text{pen}} \times \text{CAPCITY}_{\text{stp}} \times \text{F}_{\text{stpwater}} \times \text{F}_{\text{excreta}}}{\text{WASTE}_{\text{inhap}} \times \text{CAPCITY}_{\text{stp}} \times \text{Factor} \times \text{Dilution}} \quad (3)$$

Where:

$DOSE_{ia}$ = the maximum daily dose.

F_{pen} = the market penetration which is 0.01.

$WASTE_{inhap}$ = daily wastewater per capita (200L).

Dilution = dilution of effluent in surface water (10).

$F_{excreta}$ = Fraction of pharmaceuticals excreted.

$CAPCITY_{stp}$ = Capacity of WWTP, default factor (10000 inhabitants).

$F_{stpwater}$ = Fraction of pharmaceuticals present in effluent.

Factor = fraction of pharmaceuticals likely to adsorb to sewage sludge.

It is clear to see that this formula is more comprehensive than equation (1) because many parameters are considered, such as excretion rates, removal rates by wastewater treatment and likely adsorption of the substance onto particulate matter.

Many environmental risk studies for pharmaceuticals have been conducted to estimate the potential adverse effects of pharmaceuticals on the aquatic environment using the EMA (2006) guideline such as Al Aukidy *et al*. (2012), Kosma *et al.* (2014) and Rivera-Jaimes *et al.* (2018). For instance, Al Aukidy *et al*. (2012) investigated the environmental risk of 27 pharmaceuticals in two WWTP effluents in Italy, and found that three out of 27 compounds (ciprofloxacin, clarithromycin, and sulfamethoxazole)

had a high risk, whereas the risk quotient for the other pharmaceuticals was less than one. In another study ibuprofen, acetaminophen, diclofenac, trimethoprim, and caffeine were estimated to pose low risk (RQ below 0.1), in the effluent wastewaters from different eight WWTPs in different Greek cities (Kosma *et al.*, 2014) .

In this research, the environment risk of the studied pharmaceuticals was evaluated using the RQ approach according to EMA (2006) guidelines (Chapter 4).

In 2015, the European Commission (EC) established the EU Watch List (Decision 2015/495/EU) (EC, 2015) of 16 substances for monitoring in water. This watch list included compounds from various therapy class, such as estrogenic hormones, non-steroidal anti-inflammatory compounds (NSAIDs), antibiotics, UV filters and antioxidant compounds, pesticides, and herbicides. Moreover, the European Union in the Decision 2018/840 /EU (EC, 2018) proposed a second Watch List. This watch list was updated in 2020 to include some new compounds like Azole compounds (EC, 2020). Table 1-3 shows all the compounds that are included in these watch lists.

Table 1-3: European Commission watch list for 2015 (EC, 2015), 2018 (EC, 2018) and 2020 (EC, 2020).

Name of substance	**CAS number**	**Watch list 2015**	**Watch list 2018**	**Watch list 2020**
17-Alpha-ethinylestradiol (EE2)	57-63-6	✓	✓	×
17-Beta-estradiol (E2)	50-28-2	✓	✓	×
2,6-Ditert-butyl-4-methylphenol	128-37-0	✓	×	×
2-Ethylhexyl 4-methoxycinnamate	5466-77-3	✓	×	×
Acetamiprid	135410-20-7/160430-64-8	✓	✓	×
Amoxicillin	26787-78-0	×	✓	✓
Azithromycin	83905-01-5	✓	✓	×
Azole compounds*		×	×	✓
Ciprofloxacin	85721-33-1	×	✓	✓
Clarithromycin	81103-11-9	✓	✓	×
Clothianidin	210880-92-5	✓	✓	×

Table 1-3: Continued.

Name of substance	CAS number	Watch list 2015	Watch list 2018	Watch list 2020
Diclofenac	15307-86-5	✓	×	×
Dimoxystrobin	149961-52-4	×	×	✓
Erythromycin	114-07-8	✓	✓	×
Estrone (E1)	53-16-7	✓	✓	×
Famoxadone	131807-57-3	×	×	✓
Imidacloprid	105827-78-9/138261-41-3	✓	✓	×
Metaflumizone	139968-49-3	×	✓	✓
Methiocarb	2032-65-7	✓	✓	×
O-desmethylvenlafaxine	93413-62-8	×	×	✓
Oxadiazon	19666-30-9	✓	×	×
Sulfamethoxazole	723-46-6	×	×	✓
Thiacloprid	111988-49-9	✓	✓	×
Thiamethoxam	153719-23-4	✓	✓	×
Tri-allate	2303-17-5	✓	×	×
Trimethoprim	738-70-5	×	×	✓
Venlafaxine	93413-69-5	×	×	✓

* Clotrimazole, Fluconazole, Imazalil, Ipconazole, Metconazole, Miconazole, Penconazole, Prochloraz, Tebuconazole, and Tetraconazole.

1-3 The potential for pharmaceutical compounds to reach groundwater from soil application

The main pathways of pharmaceutical compounds to enter soil and then potentially reach ground water are: 1) use of reclaimed WWTPs effluent for irrigation purposes and 2) land application of organic soil amendments, like sludge used as an agricultural fertilizer (Kinney and Heuvel, 2020; Li *et al.*, 2020a; Chefetz *et al.*, 2008). Runoff, leaching, sorption and degradation are important processes that control the mobility, fate, and availability, and therefore ecotoxicological risk, of pharmaceutical contaminants in the soil environment (Chefetz *et al.*, 2008; Li *et al.*, 2020a; Kümmerer, 2009). Moreover, the fate and mobility behaviour of pharmaceuticals in soil can be influenced by a number of soil properties like soil organic matter (SOM), water content and soil type and texture (Qin *et al.*, 2017; Dai *et al.*, 2020). Weather conditions, like temperature, have also been observed to affect the occurrence of pharmaceuticals in

soil, with pharmaceutical concentrations detected higher in cold locations than warm ones (Biel-Maeso *et al.*, 2018).

Leaching and transport of pharmaceuticals in soil have been reported in several scientific papers. For instance, potential leaching of 45 targeted pharmaceutical compounds was investigated in soil and sediment samples collected from the Guadalete River basin (SW, Spain), which was affected by WWTP effluent discharges by (Biel-Maeso *et al.*, 2017). They detected 14 compounds in the soil and sediment samples collected from this area. Diclofenac, ibuprofen, indomethacin, and caffeine were found in all sample matrices, with ibuprofen measured at the highest concentrations of 24.9 ng g^{-1} and 16.1 ng g^{-1} in sediment and soil samples respectively. This was potentially attributed to the use of WWTP effluent for irrigation and to discharges into the Torrox artificial pond located in this area. They also observed that these compounds were detected in the soil profile to depths of 25 cm, with the highest concentration measured in the top section (0-10 cm) (Biel-Maeso *et al.*, 2017). In another study a wide range of pharmaceutical compounds, including acetaminophen, diclofenac, ibuprofen, atenolol, caffeine, ciprofloxacin, ofloxacin and trimethoprim, were investigated in soil samples collected from an area irrigated by treated water from the WWTP in Jerez del Frontera city, Spain (Biel-Maeso *et al.*, 2018). This study concluded that diclofenac, acetaminophen, and caffeine were most dominant in soil samples at concentrations ranging between 1 and 14 ng g^{-1}. In addition, results from a 3 m vertical soil column collected from this area showed that the concentrations of pharmaceuticals decreased with soil depth (Biel-Maeso *et al.*, 2018).

Laboratory-scale soil column experiments can assist in evaluating the potential for contamination in the environment from chemical substances. Soil columns are recognized as one of the best tools to study the fate and behaviour of chemical

substances in the environment (Banzhaf and Hebig, 2016; Sadeghi *et al.*, 2000). Leaching experiments are usually based on soil columns designed and conducted according to the OECD 312 test guidelines (OECD, 2004). These guidelines are based on soil columns packed with disturbed soil in order to investigate the possibility of leaching of pharmaceutical compounds into soil. Understanding the mobility and behaviour of pharmaceuticals in soil plays the main role in protecting soil and groundwater from contamination by PPCPs.

Based on the OECD 312 protocol, many leaching soil column experiments have been carried out for pharmaceutical compounds. For instance, six pharmaceuticals (diazepam, ibuprofen, ivermectin, clofibric acid, iopromide and carbamazepine) were investigated by Oppel *et al.* (2004) using glass vertical leaching soil columns designed in order to study the sorption potential and transport behaviour of these PPCPs in three different soils. This study concluded that diazepam, ibuprofen, ivermectin and carbamazepine would not contaminate groundwater due to their low percolation rate, whereas aquatic systems could be polluted by clofibric acid and iopromide due to their high mobility in the selected soils. In order to study the fate of pharmaceuticals in the environment and how they affect groundwater, caffeine, methamphetamine and acetaminophen were investigated in 30 cm x 30 cm column of sand, and in undisturbed fine-grained sediments (Greenhagen *et al.*, 2014). They applied a known concentration of caffeine, methamphetamine and acetaminophen and found that around 90% of methamphetamine was removed in the undisturbed sediment column compared with the sand column. By using two undisturbed soil columns with a length of 20 cm and an internal diameter of 7.5 cm from the dairy farming regions in the North Island of New Zealand, the sorption and transport behaviour of sulfamethoxazole, sulfachloropyridazine and sulfamethazine were investigated (Srinivasan and Sarmah,

2014). They concluded that these compounds have different ways of behaving in different soils. Residual concentration of sulfamethoxazole and sulfachloropyridazine were detected in all soil sections until 18 cm depth unlike sulfamethazine. In general, this study showed that sulfonamides have high mobility in soils and weak sorption affinity.

Balogh *et al*. (2011) noticed that the pH ionic strength and soil organic matter (SOM) affected the sorption, and therefore the fate, of pharmaceuticals in soil. In their study, bezafibrate, carbamazepine, chloramphenicol and diclofenac were investigated in order to study their behaviour, such as mobility and leaching, in two different kinds of soils (medium clay loam and lighter textured more acidic soil). To summarize this study, using a leaching soil column (length 200 mm; diameter 50 mm), chloramphenicol had the highest potential to percolate and migrate through the soils, followed by carbamazepine, bezafibrate and diclofenac (Balogh *et al.*, 2011). Thus, and as pharmaceuticals have been reported in soil, it is likely that these compounds could transport through soil and reach groundwater. So, the prevention of groundwater contamination by these compounds needs greater attention (see Chapter 5).

1-4 Water resources and implementation of reused wastewater in Saudi Arabia

Saudi Arabia is located in the Middle East. It has very harsh weather conditions and an absence of permeant surface water resources, i.e., lakes and rivers. It is classified by the United Nations as water-scarce nation (Samad and Bruno, 2013) and is one of the driest countries in the world, with high evaporation rates, low and erratic rainfall, low aquifer recharge and high ambient temperature, especially during summer (El-Sheikh *et al.,* 2018; Wu *et al.,* 2014; Drewes *et al.,* 2012; Rajmohan *et al.,* 2021). The main source of clean water is predominantly non-renewable groundwater in deep confined aquifers, which have been extensively extracted during the past decades. It is reported

that 20 billion m^3 of groundwater is withdrawn, whereas the recharge is only 2.4 billion m^3 annually in Saudi Arabia, which has resulted in a 60 m drop in the water table in last two decades in the aquifers (Chandrasekharam, 2018). It has been estimated that, if the current withdrawal rate remains the same and the recharge of aquifers is less than the withdrawal, depletion could happen within 50 years (Kajenthira *et al.*, 2012). Most of the pressure on the available clean water resources in Saudi Arabia comes from the agriculture sector, because nearly 85% of groundwater is used in agricultural practices (Kajenthira *et al.*, 2012). Water treatment to enable wastewater reuse is a strategic solution to resolve the water shortage in the agriculture and industrial sectors in Saudi Arabia. The reuse of treated wastewater after appropriate treatment could therefore contribute to mitigating the pressure put on the valuable and scarce water resources of Saudi Arabia. Protecting these resources from any kind of contamination is a critical issue that needs to be addressed in Saudi Arabia.

Treated wastewater could be used in different activities, such as agricultural irrigation, groundwater recharge and industrial purposes. Alkhudhiri *et al*. (2019) reported that, in 2018, 59%, 16%, 13% and 8.50% of treated wastewaters in Saudi Arabia were used for agricultural irrigation, landscape irrigation, industrial purposes and groundwater recharge respectively. Treated wastewater used in agriculture, is expected to increase by 1.3-fold in 2035 from the 540 million m^3 per year which was reused in 2012. This figure will be increased to achieve the goal of 90% of wastewater being recycled and reused by 2040 (Enssle and Freedman, 2017). In Riyadh city, 170,000 to 200,000 m^3/d of treated effluent from WWTPs are used for different purposes, such as landscape and agricultural irrigation, whereas 15,000 to 20,000 m^3/d are used by industries, and the remainder is discharged into Wadi Al-Batha, which contributes to groundwater recharge (Al-Jasser, 2011).

Many WWTPs in Saudi Arabia use tertiary treatment and have the capability to discharge treated water achieving the standard that can be used in irrigation (Alkhudhiri *et al.*, 2019). The guidelines of major wastewater parameters for treated water used in agriculture are given in Table 1-4.

Table 1-4: Treated water standard for irrigation in different countries.

Parameters	US EPA[1]	European Union[2]	Saudi Arabia[3]
Turbidity (NTU)	≤2	≤5	5
TSS (mg/L)	≤ 30	≤10	40
BOD (mg/L)	≤ 30	≤ 6	10
COD (mg/L)	-	≤10	20
pH	6.0-9.0	6.5-8.5	6-8

[1] (USEPA, 2012), [2] (Alcalde-Sanz and Gawlik, 2017), [3] (Alkhudhiri *et al.*, 2019).

Although, the treated water is currently used for irrigation, the efficiency of WTTPs in removing contaminants that may be a major risk to human health remains a concern. The presence of waterborne micropollutants in treated wastewater that is reused has therefore raised concern due to their potential impacts on environmental and human health. More research is needed at this time to address the fate, occurrence, and environmental risk of pharmaceuticals in wastewaters in Saudi Arabia. So far, the effects of pharmaceutical residues on human health from food irrigated with treated wastewater are not well documented (Braun and Gray, 2017; Bradley *et al.*, 2018; Wu *et al.*, 2014). However, the presence of pharmaceuticals in plants irrigated with WWTP effluent has already been observed in different areas of Saudi Arabia (Picó *et al.*, 2019; Picó *et al.*, 2020; Picó *et al.*, 2021). Pico *et al.* (2019) reported the presence of 20 pharmaceuticals, including atenolol, acetaminophen, ofloxacin, trimethoprim, diclofenac and caffeine in barley, cabbages, eggplant, green beans, chili, tomatoes, and zucchini crops, and at concentrations up to 125 ng g^{-1} in cabbage, irrigated with WWTP

effluent. Pharmaceutical compounds have also been studied in wild plants (Picó *et al.*, 2020), and natural vegetation and crops (Picó *et al.*, 2021) in areas of Saudi Arabia where effluent is used for irrigation activities. Exposure to pharmaceutical-contaminated vegetables and fruits irrigated with WWTP effluent, and their associated human impacts, has been estimated in Riyadh and Al-Jubail cites, Saudi Arabia, (Picó *et al.*, 2021). The results of the study by Picó *et al.* (2021) indicated no appreciable risk to human health from the presence of trace concentrations of these compounds found in vegetables irrigated with WWTP effluent.

In addition to the risk to human health, water contaminated with pharmaceuticals and discharged to the environment could pose a significant ecological risk. This study examined the potential environmental risk, for the first time, arising from the treated wastewater from three WWTPs in Saudi Arabia (Chapter 4).

1-5 Environmental occurrence of pharmaceuticals in Saudi Arabia

The occurrence and presence of PPCPs in the environment have only just recently attracted the attention of scientific researchers in Saudi Arabia and a search of the literature revealed relatively few studies on the occurrence of pharmaceuticals in the Saudi Arabian environment. Seven studies have reported the presence of pharmaceuticals in KSA (Al Qarni *et al.*, 2016; Ali *et al.*, 2017; Alidina *et al.*, 2014; Picó *et al.*, 2019; Picó *et al.*, 2020; Shraim *et al.*, 2017; Picó *et al.*, 2021). Three studies were conducted on WWTPs influents and effluents in Riyadh, Jeddah and Almadinah Almunorah cites (Al Qarni *et al.*, 2016; Alidina *et al.*, 2014; Shraim *et al.*, 2017). The fourth investigation was carried out on the Red Sea water in Jeddah (Ali *et al.*, 2017). The fifth studied the occurrence of pharmaceuticals, personal care products, pesticides and microplastics in surface water, surface soil, plants and sediment from Al-Asfar and Al-Hubail lakes, of Al-Hassa Oasis, in Al-Hufuf city in the eastern province of Saudi

Arabia (Picó *et al.*, 2020). Samples from that study showed atenolol, caffeine, diclofenac, ibuprofen, metformin, ofloxacin, acetaminophen and trimethoprim were detected at concentrations from not detected (ND) to 20663.5 ng L^{-1}. Picó *et al.* (2019) also studied the occurrence of pharmaceuticals in soil, surface water and plants samples collected from Al Hayer area, south of Riyadh city. Moreover, Picó *et al.* (2021) investigated 131 emerging contaminants, including 34 PPCPs in water, sediment, natural vegetation and crop samples collected from South Riyadh and the Al-Jubail industrial city. These two studies reported PPCPs in the surface water samples at mean concentrations between 0.08 ng L^{-1} and 1814 ng L^{-1}, mean concentrations between 0.07 ng g^{-1} and 833 ng g^{-1} in sediment samples and between 0.01 ng g^{-1} and 893 ng g^{-1} in the different kinds of crops.

The highest concentrations reported in WWTPs influents in Saudi Arabia were 74800 and 38900 ng L^{-1} for caffeine and acetaminophen in Riyadh WWTP1 (Al Qarni *et al.*, 2016) and Almadinah WWTP (Shraim *et al.*, 2017) respectively. Atenolol was detected above its Limit of Quantification (LOQ) ranging between 329 and 2040 ng L^{-1} and between 15 and 2550 ng L^{-1} in the same WWTP influents and effluents, respectively. Bezafibrate, cyclophosphamide and erythromycin were never detected in Saudi WWTPs. The average pharmaceutical concentrations detected in WWTPs in Saudi Arabia are summarized in appendix Table A-1-1. The highest concentrations reported in surface water were for caffeine, acetaminophen and then diclofenac at concentrations of 20663.5, 3069.1 and 1390.0 ng L^{-1}, respectively. The summary of the occurrence of pharmaceuticals in surface water, soil, crops, and plants measured in the Saudi Arabia environment can be found in appendix Table A-1-2.

1-6 Hypotheses and objectives of this research

The two hypotheses addressed by this study were:

1- The occurrence of selected pharmaceuticals in wastewater in Riyadh, Saudi Arabia pose a potential environmental risk.

2- Pharmaceutical residues from wastewater used for irrigation can leach through soil layers and contaminate groundwater.

To address these hypotheses, three different WWTPs in the capital city of Riyadh in Saudi Arabia were selected to study the presence and fate of selected pharmaceuticals in wastewater before and after treatment and to determine the mass loadings and the removal efficiencies of these WWTPs. Bench-scale tests were used to study the leaching potential of the same pharmaceutical compounds.

The specific objectives of this research were:

1- To study the occurrence of, and removal efficiency for pharmaceuticals in three different Wastewater Treatment Plants (WWTPs).
2- To study the presence of some new compounds reported globally but never be studied in Saudi Arabia, in WWTPs and in the environment, like oxypurinol, allopurinol, ofloxacin, ciprofloxacin and azithromycin.
3- To investigate the occurrence of baclofen for the first time in WWTPs because it had been identified as lacking data, even though it was highly consumed (Daughton, 2014).
4- To determine whether pharmaceutical compounds reported to occur globally at high concentrations might have adverse environmental risks in Saudi Arabia.
5- To determine whether selected compounds percolate or bind to different soils and could potentially infiltrate to groundwater.

The results and discussion of this research are presented as outlined in Figure 1-3.

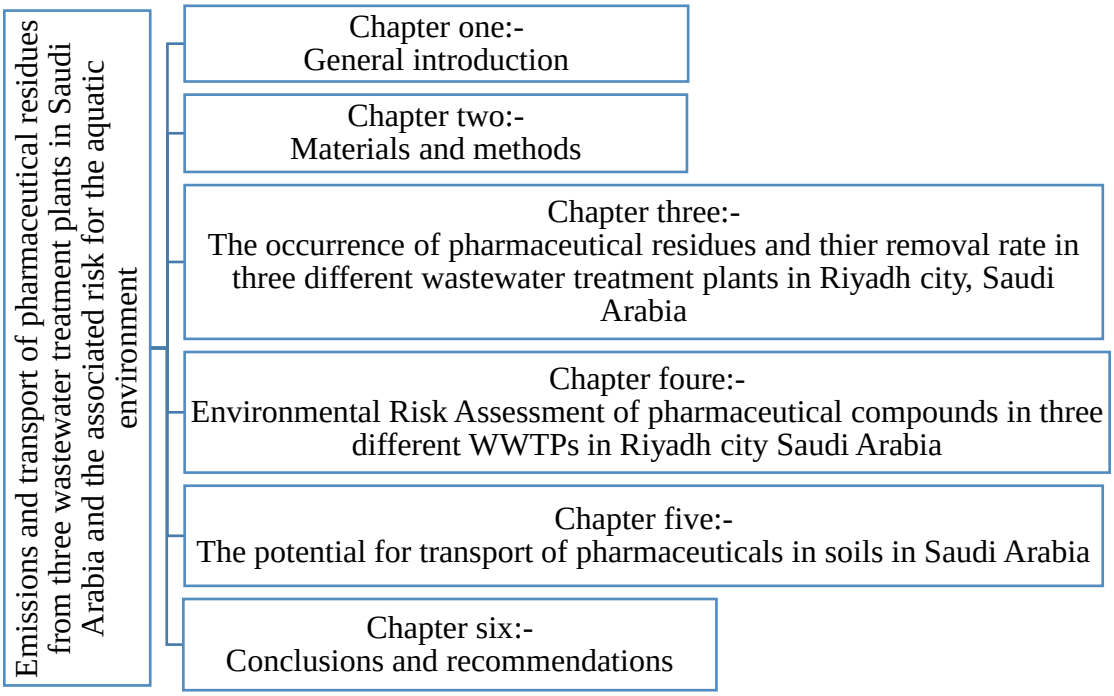


Figure 1-3: Schematic of the structure of this thesis.

CHAPTER TWO

Materials and methods

2-1 Introduction

This work is focused on the presences of 15 pharmaceutical compounds and one metabolite in three different WWTPs influent and effluent in Riyadh, KSA. In addition, this study investigated the transportation and leaching behaviour of the sixteen selected pharmaceuticals in different soils according to the OECD guideline Leaching in Soil Columns (OECD, 2004).

2-2 Selection of pharmaceutical target compounds

Knowledge of the environmental occurrence and fate of pharmaceuticals in Saudi Arabi is still scarce, although some pharmaceuticals have been reported in KSA WWTPs influent and/or effluent (Al Qarni *et al.*, 2016; Alidina *et al.*, 2014; Shraim *et al.*, 2017), sea water (Ali *et al.*, 2017), surface water, sediments, soils, crops and plants (Picó *et al.*, 2019; Picó *et al.*, 2020; Picó *et al.*, 2021). Based on the findings of these studies, and due to the difficulty to get access to data on dispensed/used pharmaceuticals, 16 pharmaceuticals were selected to study their occurrence and fate in three different WWTPs in Riyadh city, Saudi Arabia (Table 2-1). The compounds investigated in this research belong to different therapeutic classes: NSAIDs (three compounds), antibiotics (six compounds), antidiabetics, beta-blockers, muscle relaxants and simulants, antimicrobial, antigout and antigout metabolite (one compound each). These compounds were selected because of the availability of reliable analysis methods (Dasenaki and Thomaidis, 2015; Carmona *et al.*, 2017), as well as due to their occurrence in aquatic and terrestrial ecosystems in Saudi Arabia (Ali *et al.*, 2017; Al Qarni *et al.*, 2016; Alidina *et al.*, 2014; Shraim *et al.*, 2017; Picó *et al.*, 2019; Picó *et*

al., 2020; Picó *et al.*, 2021). Moreover, these compounds were selected because of their potential environmental effects. Ofloxacin and azithromycin have been reported in WWTPs (Dasenaki and Thomaidis, 2015; Petrie *et al.*, 2016) and oxypurinol has recently been investigated in an aqueous matrix (Funke *et al.*, 2015; Hermes *et al.*, 2018) and may cause potential environmental risks. However, no published studies relating to these compounds in WWTPs in Saudi Arabia were available when this study commenced and therefore, ofloxacin, azithromycin, allopurinol and oxypurinol were studied here for the first time ever in the environment of Saudi Arabia. Baclofen was included due to the lack of environmental monitoring data worldwide (Daughton, 2014). The criteria used to select these pharmaceuticals are summarized in Table 2-2.

All selected pharmaceuticals analytical standards and their corresponding internal standard (IS) were purchased from Toronto Research Chemicals (TRC), Canada. Internal standards were used in this study in order to improve the method precision, accuracy, and linearity. Internal standards were not always available or were prohibitively expensive. Consequently, only five internal standards were used for the analysis and quantification in the present study (Table 2-1). All standards and IS compounds used were of a high purity grade (>98%).

Table 2-1: Main features of the selected pharmaceuticals[1].

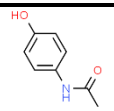
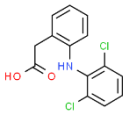
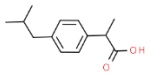
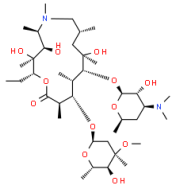
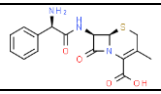
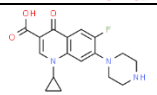
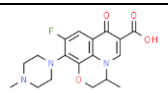
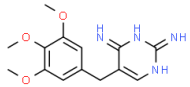
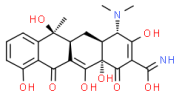
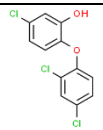
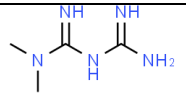
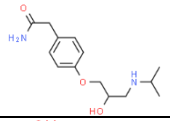
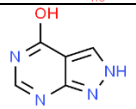
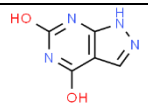
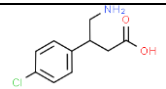
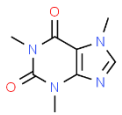
Compounds	MW	Chemical formula	Chemical structure	pK_a	logK_{ow}	W.S. (g L^{-1})	References
Acetaminophen	151.16	$C_8H_9NO_2$		9.4	0.46	14	(Kosma *et al.*, 2014)
Diclofenac	296.1	$C_{14}H_{11}Cl_2NO_2$		4.2	4.51, 0.7	50	(Golovko *et al.*, 2020; Kosma *et al.*, 2014)
Ibuprofen	206.28	$C_{13}H_{18}O_2$		4.91	3.79	0.02	(Ashfaq *et al.*, 2019)
Azithromycin	749	$C_{38}H_{72}N_2O_{12}$		8.7	4.02	0.0071	(Ofrydopoulou *et al.*, 2022)
Cephalexin	347.4	$C_{16}H_{17}N_3O_4S$		2.5, 7.6	0.65	10	(Dasenaki and Thomaidis, 2015)
Ciprofloxacin	331.34	$C_{17}H_{18}FN_3O_3$		6.38	0.4	30	(Golovko *et al.*, 2020; Verlicchi *et al.*, 2012b)
Ofloxacin	361.4	$C_{18}H_{20}FN_3O_4$		-2	5.45, 6.2	67.62	(Picó *et al.*, 2020)
Trimethoprim	290.32	$C_{14}H_{18}N_4O_3$		6.6, 7.2	1.33, 0.91	0.40	(Golovko *et al.*, 2020; Verlicchi *et al.*, 2012b)

Table 2-1: Continued.

Compounds	MW	Chemical formula	Chemical structure	pK_a	logK_{ow}	W.S. (g L^{-1})	References
Tetracycline	444.4	$C_{22}H_{24}N_2O_8$		3.3	-1.33	3.9	(Ashfaq *et al.*, 2019)
Triclosan	289.54	$C_{12}H_7Cl_3O_2$		7.9	4.76	0.01	(Ofrydopoulou *et al.*, 2022)
Metformin	129.16	$C_4H_{11}N_5$		12.47	-2.64	1×10^3	(Petrie *et al.*, 2016)
Atenolol	266.34	$C_{14}H_{22}N_2O_3$		9.6	0.23	0.3	(Verlicchi *et al.*, 2012b)
Allopurinol	136.11	$C_5H_4N_4O$		10.2	-0.55	48×10^{-5}	NIH National Library of Medicine https://www.ncbi.nlm.nih.gov/
Oxypurinol[2]	152.11	$C_5H_4N_4O_2$		2.09, 6.25	-2.17	0.005	Royal Society of Chemistry, ChemSpider database (http://www.chemspider.com/)
Baclofen	213.66	$C_{10}H_{12}ClNO_2$		9.62, 3.87	-1.32	40.5	Estimation Programs Interface (EPI) (USEPA, 2017)
Caffeine	194.19	$C_8H_{10}N_4O_2$		10.4	-0.007	0.7	(Kosma *et al.*, 2014)

[1] Internal standard were: Acetaminophen-d4, Atenolol-d7, Baclofen-d4, Caffeine-d9, Metformin-d6, [2] Metabolite of allopurinol, MW: Molecular wight, M.W.: Molecular wight, p$\boldsymbol{K_a}$: Acid dissociation constant, logK_{ow}: n-Octanol/Water Partition Coefficient, W.S.: Water solubility.

Table 2-2: Criteria applied in order to select pharmaceutical compounds for inclusion in this study.

Therapeutic class	Pharmaceuticals	Brief reasons
Analgesics and anti-inflammatories	Acetaminophen Ibuprofen Diclofenac	• Among the most pharmaceutical frequently detected in aquatic environment (aus der Beek *et al.*, 2016). • Included European Union monitoring list 2015/495/EU and/or 2018/840 /EU (EC, 2015; EC, 2018). • Potential environmental risk. • Diclofenac caused Vulture (Gyps bengalensis) death (Oaks *et al.*, 2004). • 21st WHO List of Essential Medicines (WHO, 2019).
B-blockers	Atenolol	• Up to 90% excretion unchanged. • Detected in surface water. • Persistent in WWTPs.
Antidiabetic	Metformin	• 21st WHO List of Essential Medicines(WHO, 2019). • Frist line antidiabetic medicine (Gong *et al.*, 2012). • Unmetabolized antidiabetics drug and is excreted unchanged (Gong *et al.*, 2012).
Antibiotics	Cephalexin Ofloxacin Azithromycin Ciprofloxacin Trimethoprim Tetracycline	• Among the most pharmaceutical frequently detected in aquatic environment (aus der Beek *et al.*, 2016; Tran *et al.*, 2018). • No existing monitoring data in Saudi Arabia. • Included European Union monitoring list 2015/495/EU and/or 2018/840 /EU (EC, 2015; EC, 2018). • 21st WHO List of Essential Medicines (WHO, 2019)
Disinfectants (Antimicrobials)	Triclosan	• Among the most pharmaceutical frequently detected in aquatic environment (Tran *et al.*, 2018). • Extensively has been used on the health care products.

Table 2-2: Continued.

Therapeutic class	Pharmaceuticals	Brief reasons
Antigout	Allopurinol Oxypurinol1	• No existing monitoring data in Saudi Arabia. • Frequently prescribed pharmaceutical with lacking environmental monitoring data (Daughton, 2014).
Muscle relaxant	Baclofen	• Frequently prescribed pharmaceutical with lacking monitoring data (Daughton, 2014). • No existing monitoring data in Saudi Arabia and worldwide. • Extensively excreted unchanged.
Stimulant	Caffeine	• Among the most pharmaceutical frequently detected in aquatic environment (Tran *et al.*, 2018). • 21st WHO List of Essential Medicines (WHO, 2019).

[1] Metabolite of allopurinol.

2-2-1 NSAIDs

Acetaminophen, diclofenac, and ibuprofen were selected in this study as appropriate representative compounds of this therapeutic class. NSAIDs in general are widely used and can be bought over the counter (OTC). Tran *et al.* (2018) reported that, the most used worldwide NSAIDs OTC were acetaminophen and ibuprofen. These pharmaceuticals are used due to their effective relief for pain, swelling, fever, cold, flu symptoms and headaches (Tran *et al.*, 2018). Ibuprofen has been detected in environmental matrices in 50 countries around the world at concentrations up to 21 µg L^{-1} (aus der Beek *et al.*, 2016). Oaks *et al.* (2004) attributed the catastrophic death of >95% of vultures in Pakistan, to their dietary exposure to diclofenac-treated livestock. Furthermore, diclofenac was included in the first European Union (EU) watch list of substances in Decision 2015/495/EU in the field of water policy monitoring of the European Parliament, (EC, 2015).

2-2-2 Antibiotics

Antibiotics are widely used by people (and also for animals) due to their beneficial prevention and treatment of bacterial infections. Also, antibiotics can be easily bought and sold without a prescription in over-the-counter markets in some countries and are often used indiscriminately and incorrectly (Tran *et al.*, 2018). This has led to antibiotic resistance in pathogens (Hanna *et al.*, 2018; Pallares-Vega *et al.*, 2019). Moreover, misuse of antibiotic compounds has been reported to cause bacterial resistance and also potentially pose adverse effects on aquatic organisms (Fair and Tor, 2014). Recent studies have reported very high concentrations (mg L^{-1}) of antibiotics in different environmental compartments particularly in areas affected by medicine manufacturing facility discharges (Larsson, 2014; Larsson *et al.*, 2007; Kleywegt *et al.*, 2019; Thai *et al.*, 2018). Moreover, the concentrations of the vast majority of antibiotics reported in WWTPs influents and effluents are greater than their corresponding predicted no-effect concentrations (PNECs) for ecological toxicity to aquatic organisms (Tran *et al.*, 2018). Antibiotics have been reported in WWTP influents and effluents at relatively high concentrations and they are not removed efficiently in the WWTP processes (Petrie *et al.*, 2016). Some antibiotic compounds have shown a negative removal rate where the final treated effluent concentration exceeded the measured influent wastewater concentration (Göbel *et al.*, 2007; Blair *et al.*, 2015). For instance, Blair *et al.*, (2015) reported poor elimination for ciprofloxacin, clarithromycin, and trimethoprim at removal rates between -53.1% and -88.6%. They concluded this negative removal was due to the conjugate pharmaceuticals which were not detected in the influent and could be transformed and released later in the effluent as the parent compound during biological treatment.

Ciprofloxacin, was measured in WWTP effluent at concentrations up to 31 mg L^{-1} by Larsson *et al.* (2007), which was much higher than the levels regularly found in WWTP effluents and which could potentially have adverse effects on aquatic organisms in the environment (Lindberg *et al.*, 2005). Six antibiotic compounds: azithromycin, ciprofloxacin, cephalexin, ofloxacin, tetracycline and trimethoprim, were selected to be investigated in this study from this therapy class. Interestingly, antibiotics like azithromycin, ciprofloxacin, ofloxacin, and cephalexin were found to be sold without prescription in 244 out of 327 Riyadh city pharmacies (Abdulhak *et al.*, 2011). Consequently, these compounds were selected to be investigated in this study.

2-2-3 Antidiabetics

Diabetes mellitus is a chronic illness affecting the metabolism of carbohydrates. Antidiabetics are often prescribed for those who are diagnosed with diabetes, and suffer from high blood glucose level, or hyperglycemia (high blood sugar). Metformin is an oral administration pharmaceutical and is prescribed for treatment of diabetes mellitus type II. It is reported to be amongst the most dispensed pharmaceuticals worldwide and is the first drug of choice for patients diagnosed with diabetes (Kosma *et al.*, 2015). The number of people anticipated to be diagnosed with diabetes will reach around 366 million by 2030, and therefore the use of pharmaceuticals for diabetes treatment like metformin will also increase (Wild *et al.*, 2004).

The consumption of antidiabetic drugs was shown by OECD (2019) to have increased significantly over the 17 years 2000 to 2017 by almost two-fold (Figure 2-1) due to the rising prevalence of diabetes. Metformin is excreted mainly unchanged from the human body (Straub *et al.*, 2019). Also, metformin removal rates were reported in a number of studies to be highly variable, between -103% and 99% (Straub *et al.*, 2019). Metformin has been detected in aquatic environmental samples. For example,

metformin was detected in the influent and effluent of eight wastewater treatment plants in Greece, at concentrations up to 1.2 and 0.026 µg L^{-1} respectively (Kosma *et al.*, 2015).

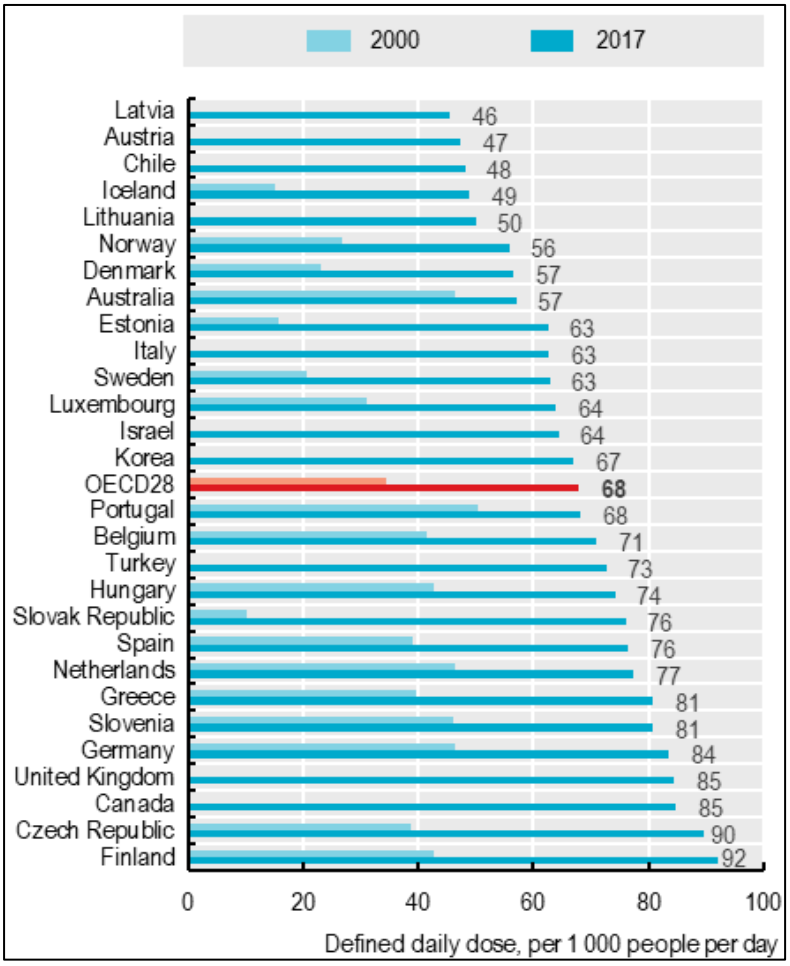


Figure 2-1: The consumption of antidiabetics drugs in 2000 and 2017 for different countries (OECD, 2019).

2-2-4 Beta-blockers

Beta-blockers like atenolol are widely used primarily for the treatment of heart disease and a variety of related conditions. These medicines are prescribed to treat high blood pressure and used during recovery from heart failure. The consumption of antihypertensive drugs - including beta blocking agents - almost doubled in 2017 from the year 2000 in OECD countries (Figure 2-2) (OECD, 2019). Atenolol is reported to be excreted after human consumption largely unchanged and, therefore, reported

frequently in WWTP inlets and outlets (Verlicchi *et al.*, 2012b). Beta-blockers have been reported to be found in the influents and effluents of WWTPs ranging between a few ng L^{-1} and several µg L^{-1} (Tran *et al.*, 2018; Dasenaki and Thomaidis, 2015). For example, atenolol was detected in WWTP influents by Carmona *et al.* (2017) and Dasenaki and Thomaidis (2015) ranging between 1.1 µg L^{-1} and 1.5 µg L^{-1}, and in the effluent up to 0.11 µg L^{-1} by (Alidina *et al.*, 2014).

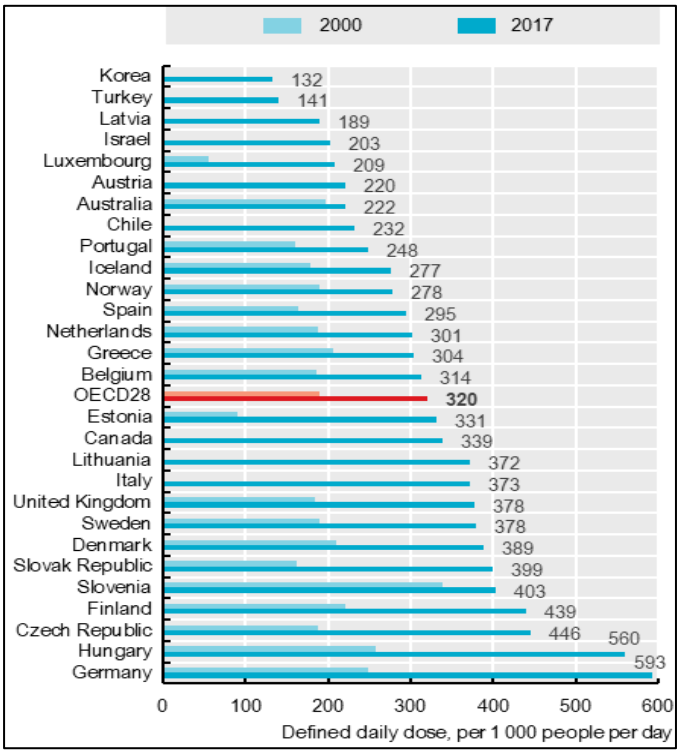


Figure 2-2: The consumption of Antihypertensive drugs - including beta blocking agents in 2000 and 2017 for different countries (OECD, 2019).

2-2-5 Antigout

Allopurinol has been extensively prescribed with limited environmental monitoring data (Daughton, 2014). Allopurinol is prescribed to reduce the production of uric acid and more than 80% is metabolized to active oxypurinol (Day *et al.*, 2007). Recently, allopurinol was included in the 21st Model List of Essential Medicines by the World Health Organization (WHO, 2019). Therefore, allopurinol and oxypurinol were selected from this therapy class for this study. Despite the fact that allopurinol is

considered as one of the most widely used pharmaceuticals, only a few studies have reported it at concentrations ranging from 0.01 µg L^{-1} to 8.234 µg L^{-1} in the raw wastewater to date (Bisceglia *et al.*, 2010; Salgado *et al.*, 2011), whereas others reported it below its LOQ or LOD, or as not detected. Before 2015, there were no published studies of environmental monitoring for oxypurinol, which is the main metabolite of allopurinol. Funke *et al.* (2015) were the first to report the occurrence and persistence of this metabolite in wastewater treatment plants, groundwater, surface waters and drinking water. Since then, some studies have investigated oxypurinol in aquatic ecosystems (Funke *et al.*, 2015; Han and Lee, 2017; Hermes *et al.*, 2018; Xu, 2017). Concentrations of oxypurinol in WWTP influents, effluents, surface water, river and groundwater have been reported in the ranges between 2.8 and 26.6 µg L^{-1}, 1 and 21.7 µg L^{-1}, 0.09 and 22.6 µg L^{-1}, 0.21 and 0.66 µg $L^{-1}$and 0.18 and 0.38 µg L^{-1} respectively (Funke *et al.*, 2015; Hermes *et al.*, 2018). There is, therefore, strong evidence of persistence of this metabolite in WWTPs and poor removal during the WWTP processes, which could potentially cause environmental risk. Han and Lee (20017) estimated the release of oxypurinol into Korean surface water up to 525.6 kg y^{-1} at predicted concentrations ranging from 1.91 to 2.77 ng L^{-1}. Based on the above information and the lack of monitoring data for this compound in the environment of Saudi Arabia, oxypurinol was included in the list of compounds to be studied here.

2-2-6 Stimulants

Caffeine is usually used to improve mental alertness and may be prescribed in combination with other medicines, such as analgesics, in order to alleviate the effects of coughs, colds and headaches (Weigel *et al.*, 2002). It also has been used as a cardiac, cerebral and respiratory stimulant and as a diuretic (Buerge *et al.*, 2003). Moreover, caffeine is consumed and used as a major ingredient in large amounts in many countries

in common drinks such as teas, coffees, energy drinks and various carbonated beverages. Caffeine has been detected in the wastewater effluents of different WWTPs in Jeddah city in Saudi Arabia ranging between 0.064 and 16.5 µg L^{-1} (Alidina *et al.*, 2014), whereas the highest concentration found in Saudi Arabia was reported in the influent of a hospital wastewater treatment plants in Riyadh between 27.5 and 74.8 µg L^{-1}, but it was not detected in its effluent (Al Qarni *et al.*, 2016). Due to above statement, caffeine was included in this study.

2-2-7 Muscle relaxants

Baclofen is a skeletal muscle relaxant and an antispasmodic agent. It is prescribed to relieve and treat muscle spasm caused by a variety of conditions like multiple sclerosis, spinal cord injury and disease. Baclofen (Lioresal) works by helping to relax the muscles improving mobility, increasing levels of independence, and facilitating both passive and active physiotherapy. Oral baclofen is one of the most common drugs used for the treatment of spasticity on a long term basis (Dario and Tomei, 2004). Only 15% of baclofen is metabolized into β-(p-chlorophenyl)-c-hydroxybutyric acid and a high proportion (~85%) is excreted as the parent compound in urine (Faigle and Keberle, 1972; Gutierrez and Queener, 2003). Baclofen is one of the most frequently prescribed drugs with an absence of environmental monitoring data (Daughton, 2014). Also, to date, no study has investigated it in the environment. For that reason, and due it is potential to enter the environment, baclofen was selected for this study, and it is the first investigation of baclofen occurrence in WWTP influents and effluents, and in environment matrices worldwide.

2-2-8 Triclosan

Triclosan is an antiseptic agent. Antiseptic compounds are commonly used to reduce and eliminate the risk of bacterial infection. These anti-microorganism substances are

used most in hospitals. Thus, the widespread use of these chemical products might cause a development of bacterial resistance and affects biological water treatment processes (McDonnell and Russell, 1999; Zhang *et al.*, 2020). Triclosan is a very effective chemical substance against a broad-spectrum of bacteria (Jones *et al.*, 2000) and has been used in health care units and in the public health sector in order to protect medical staff and public. Nowadays, triclosan is also used worldwide in wide range of health care products, such as soaps, lotions, creams, gels, mouthwashes, and toothpastes. Triclosan is considered as the most common type of micropollutant discharged from WWTP into environment and is found in raw wastewater and treated wastewater (Paxeus, 2004; Zhang *et al.*, 2020). Triclosan has been detected at WWTPs influent and effluent internationally and nationally in Jeddah, Saudi Arabia at concentration up to 23.7 μg L^{-1} and 2.27 μg L^{-1} respectively (Alidina *et al.*, 2014; Luo *et al.*, 2014). For these reasons, triclosan was investigated in this study.

2-3 Site location of wastewater treatment plants

Three WWTPs located in the capital city of Saudi Arabia were selected for this study in order to study the occurrence, presence, and environmental risk assessment of 16 pharmaceuticals belonging to different therapy classes. Figure 2-3 shows the location of these selected WWTPs. Manfuha (M-WWTP) is located southwest of Riyadh city while Hair (H-WWTP) is placed around 25 km south Riyadh city between Riyadh and Al-Kharj city. The King Saud University (KSU) campus is northwest of Riyadh and, the KSU-WWTP is located in the north-west- corner of the KSU properties (Figure 2-3). These WWTPs use secondary treatment coupled with tertiary treatment of sand filtration and disinfection of the effluent with chlorine. Table 2-3 and Figure 2-4 show the operational parameters and various treatment stages, respectively, for each WWTP. The Manfuha (M-WWTP) and Hair (H-WWTP) WWTPs had a treatment capacity of

300,000 m^3 d^{-1} and 400,000 m^3 d^{-1} of raw wastewater, respectively. Both WWTPs received sewage water from north, south, west and east Riyadh districts, but H-WWTP received more raw wastewater from east Riyadh district than other districts. King Saud University WWTP (KSU-WWTP) differed from others due to its size and the nature of the wastewater received. It is a small-scale plant and receives wastewater from King Saud University (KSU) main campus, faculty buildings, KSU staff housing and from a major hospital in Riyadh, King Khaled University Hospital (KKUH). KSU-WWTP treated around 10,000 m^3 d^{-1} during the high season when students commenced their college, but this flow rate dropped to around the half in the low season particularly when students were away on vacation.

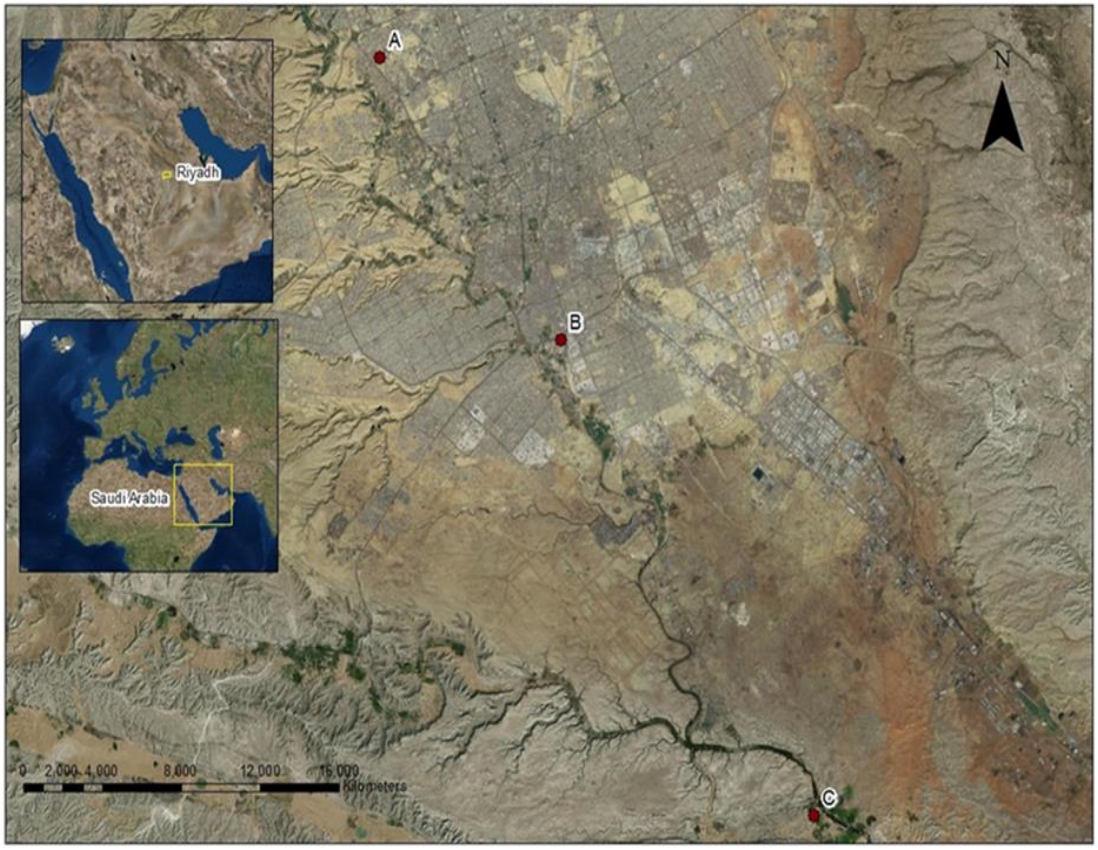


Figure 2-3: Location of sampling points of three WWTPs under investigation. (A) KSU-WWTP, (B) M-WWTP and (C) H-WWTP.

These WWTPs were chosen for the following reasons:

1. They represented the most common types of wastewater treatments plants in Saudi Arabia, including biological treatments and sand filters.
2. Two of these WWTPs were selected to represent large-scale WWTPs in Riyadh (H-WWTP and M-WWTP), receiving wastewater from domestic, industrial, and medical sources.
3. KSU-WWTP was selected to study the emissions and the presence of pharmaceuticals from and within small scale WWTP into the environment specially from main hospital nearby (KKUH).
4. These WWTPs had not previously been investigated for emissions of pharmaceutical compounds.
5. These WWTPs were the only WWTPs where it was possible to get permission from the operators to collect samples and to participate in the study.

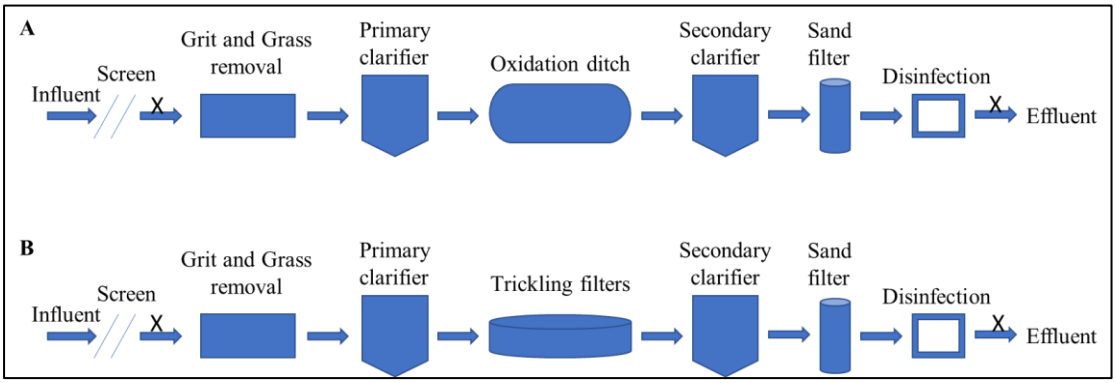


Figure 2-4: Flow diagram of treatment process lines for WWTPs under investigation. A: H-WWTP and M-WWTP, B: KSU-WWTP. (X) sample locations.

2-3-1 Manfuha and Hair WWTPs

Both plants employed the same secondary treatment processes of oxidation ditch (OD) including simultaneous configuration for nitrification (aerobic) - denitrification (anaerobic) which occurred in the anoxic (depletion or deficiency of oxygen) conditions. Oxidation ditches rely on a biological treatment process based on a modified activated sludge (Ahmed *et al.*, 2017). They comprise a single channel or multiple

channels where wastewater passes, and the organic matter is removed. These systems are used to treat wastewater because they can facilitate nitrification and denitrification process simultaneously in the same circular basin (Xu *et al.*, 2017). Subsequently, the wastewater is pumped to secondary settling tanks. This stage is followed by tertiary treatment (sand filtration). Finally, treated wastewater is passed through a disinfection unit where chlorine gas is applied (Figure 2-4A). Both WWTPs are in the southeast of Riyadh city and their population equivalent (PE) are 1,178,692 and 1,152,820 for M- and H-WWTPs, respectively. These two WWTPs received mixed sewage from domestic sources, industry, hospital, and clinic nearby. During the period of this study, M-WWTP and H-WWTP treated an average of 273,565 m^3 d^{-1} and 218,818 m^3 d^{-1} of raw sewage water, respectively.

2-3-2 KSU-WWTP

The third treatment plant monitored in this study was the KSU-WWTP, which is located inside KSU main campus in the northwest of Riyadh city (Figure 2-1). KSU-WWTP treats wastewater from KSU area and KKUH. Unlike M-WWTP and H-WWTP, KSU-WWTP used a trickling filter (TF) as the secondary (biological) treatment. The trickling filter system is another conventional aerobic biological treatment which is used for wastewater treatment technique (Van den Akker *et al.*, 2011). The trickling filters are packed with plastic media. The wastewater is sprayed over this medium and trickles downwards. Microorganisms on the medium feed on, and absorb, the organic matter in the sewage and therefore remove it from the wastewater. Afterwards, the wastewater is discharged to a secondary clarifier tank, from where it passes to the tertiary treatment step (sand filter). Sand filters consist of several layers of graded sand and are usually used in order to eliminate and reduce the concentration of micro-organisms. The effluent is finally disinfected in a chlorine gas contact chamber (Figure 2-4B).

Table 2-3: Operational parameters of the WWTPs under investigation.

Parameters	M-WWTP	H-WWTP	KSU-WWTP
Commissioning date	2005	2014	--
Design capacity ($m^3 d^{-1}$)	300000	400000	10000
Average flow rate of influent ($m^3 d^{-1}$)	273565	217401	5046
Average flow rate of effluent ($m^3 d^{-1}$)	273565	218818	5046
People equivalent capacity (PE)	1178692	1152820	45000 to 65000
Average sludge production (tonnes $year^{-1}$)	161.3	60.4	--
Hydraulic retention time (HRT) (h)	11.9	28.5	--
Sludge retention time (SRT) (days)	12	7.2	--
Average BOD influent (mg L^{-1})	297.7	271.2	159.3
Average BOD effluent (mg L^{-1})	28.2	11.7	4.4
Average COD influent (mg L^{-1})	462.8	440.9	201.9
Average COD effluent (mg L^{-1})	44.2	19.5	6.7
Preliminary and primary treatment	Coarse screens, fine screen, Grit & Grease removal tanks, primary sedimentation tanks.	Coarse screens, fine screen, Grit & Grease removal tanks, primary sedimentation tanks.	Pre-aeration commenter bar screen, grit chamber and primary sedimentation tank (2 tanks)

Table 2-3: Continued.

Parameters	M-WWTP	H-WWTP	KSU-WWTP
Secondary treatment	Activated sludge (4 aeration tanks) based on oxidation ditch biological process including nitrification (is the biological oxidation of ammonium to nitrite and nitrate) and denitrification processes (is the biological process which is used to reduce nitrate/nitrite to nitrogen gas, is carried out at anoxic (i.e. absence of oxygen) conditions) (biological nutrient removal process) and secondary clarifier (16 tanks)	Activated sludge (8 aeration tanks) based on oxidation ditch biological process. involving nitrification and denitrification processes (biological nutrient removal process) and secondary sedimentation (32 tanks)	Trickling filters (4 filters) and then settling tanks (2 tanks)
Tertiary treatment	Sand filters (20 filters)	Sand filters (60 filters)	Sand filters (4 filters)
Disinfection system	Chlorine and chlorine contact tanks		
Owner and operator	National Water Company (NWC)		KSU
Treated effluent reuse practices	Irrigation, industrial purposes, and discharged to Wadi Al-Batha.		Power plant cooling and landscape irrigation

2-4 Sample collection

2-4-1 Wastewater influent and effluent samples

One sample per month for 12 months was collected from the influent and effluent of each wastewater treatment plant. Composite samples were collected from H-WWTP and M-WWTP, while a grab sample was collected from KSU-WWTP by the plant operator for safety reason. KSU-WWTP samples were collected in the middle of the day (12:00-14:00h) in order to capture the peak inflow and effluent discharge. Samples from the raw wastewater influent after the screening unit, and effluent samples after the chlorination unit, were collected as marked by (X) in Figure 2-4 in 4L amber glass, clean, sterile bottles prerinsed with the real sample. At each WWTP, a total of 24 samples were collected from influent (12 samples) and effluent (12 samples) during the sampling campaign from March 2018 to July 2019. All samples were collected by the WWTP operators due to restricted access to these sites and for safety reason. The samples were immediately transferred to the laboratory of the College of Food and Agricultural Sciences in King Saud University in an ice box. On arrival, samples were thoroughly shaken, and one litre was obtained from 4L for vacuum-filtration through 1.2 µm pore size, 47 mm diameter, Whatman® glass microfiber, binder free, Grade GF/C filters, (Sigma-Aldrich, Ireland) and then through 0.5 µm pore size, 47 mm diameter, Advantec GC-50 Glass Fiber Filters, (STERLITECH, US) using a Fisherbrand vacuum pump, (Model FB70155). The filtered samples were then divided into 500 mL aliquots which were frozen at -20 °C. The next day, frozen samples were defrosted at room temperature and the pH was adjusted to 2.5 using hydrochloric acid (HCl) prior to enrichment by solid phase extraction (SPE) (see section 2-6-1). The sampling collection steps are illustrated in Figure 2-5. Due to financial limitations, only aqueous samples were collected and analysed in this research. It is, however,

acknowledged that samples from primary, secondary, and tertiary treatment units, as well as sludge sample, would give a more complete understanding of the presence of pharmaceutical compounds and their fate during WWTP processes.

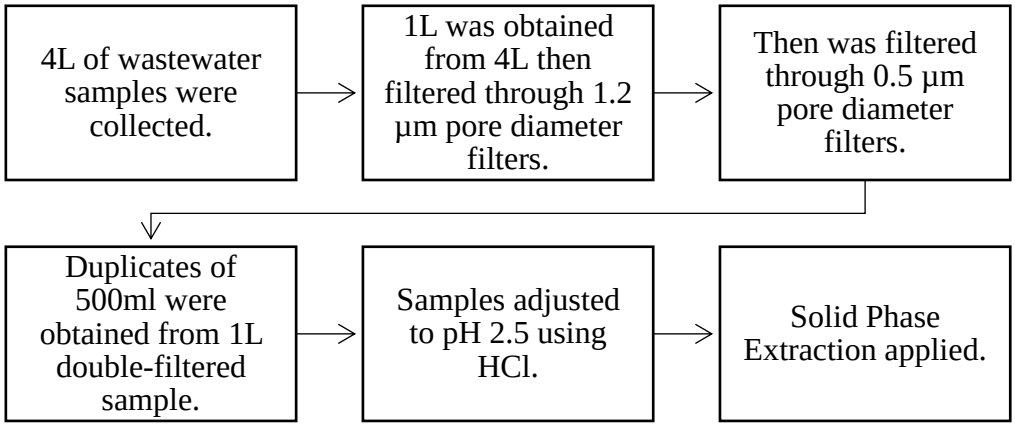


Figure 2-5: Samples collection and preparation procedures.

All apparatus and glassware used during sample collection were thoroughly rinsed with hot water and then washed with detergent. After that, apparatus and glassware were washed with methanol and rinsed with Milli-Q water to remove potential contamination.

2-4-2 Soil samples

In order to study the mobility and leachate behaver of the selected pharmaceuticals in soils in laboratory-scale experiments, four different surface soils were selected (Table 2-4). Due to the difficulty in getting soil samples sent from Saudi Arabia to Ireland, three soil samples were collected from around Cork city, Ireland and the fourth one was purchased from Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Munch, Germany (refeesol 01-A-05). This soil sample was selected due to its similar physical and chemical properties to soil found in Riyadh (Al-Hamed *et al.*, 2012; Masoud and Aal, 2019; Siham, 2007). The four selected soils differed in terms of their organic content, pH, and soil texture (Table 2-4).

Table 2-4: Soil parameters applied in leaching experiments.

Soil properties		Soil no[a]			
		1	2	3	4[b]
pH	(0.01 $CaCl_2$)	4.88	3.71	5.46	5.71
	(Deionized water)	5.88	4.64	6.21	6.45
Organic matter (%)		1.74	7.75	8.13	0.93
Water Holding Capacity (%)		8.60	27.35	15.19	29.3
Soil texture	Clay (%)	1.26	0.41	1.07	6.2
	Silt (%)	73.42	53.22	65.59	19.8
	Sand (%)	25.31	46.37	27.59	74
Soil type		Silt loam	Clay	Silty clay loam	Sandy loam

[a] Soil no 1, 2 and 3 were collected from around Cork city, while soil no 4 was purchased from Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Munch, Germany. [b] All values were obtained from the data certificate of analysis, from Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Munch, Germany.

Soils samples from Cork were collected using an auger and geological hammer. The leaves, roots and plants were cleared away from the collection site and then fresh soil samples were collected (5 to 10 cm depth). The soil samples were spread out on a piece of clean paper to dry at room temperature before removing leaves, stones, roots, and other foreign matter. Later, once the soils were dried, the whole dried soil samples were sieved through a 2 mm pore size sieve. The sieved soils were stored in a cold room at 4 °C in the dark prior to use in the experiments. Soil pH was measured using a Thermo pH meter by dissolving 1g of sieved dried samples in distilled water and 0.01 M $CaCl_2$ (1:2.5 w/v) in triplicate in order to calculate the mean (Revitt *et al.*, 2015; Revitt *et al.*, 2014; Al-Khazrajy and Abdallh, 2020). To determine total water holding capacity, 20 g subsamples of each sieved dried soil were placed in an oven at 105 ˚C for 24 hours and the moisture calculated by wight loss (Revitt *et al.*, 2014; Revitt *et al.*, 2015). Oven dried soils were then burned in a muffle furnace at 500 °C for 4 hours so as to estimate the soil organic matter by weight loss (Jensen *et al.*, 2019). Soil texture was determined using a laser granulometer (Malvern, Hydro, 2000MU, UK). Determination of pH, soil organic matter, grain size and water holding capacity was

carried out in the School of Biological, Earth, and Environmental Sciences laboratories, UCC.

2-5 Leaching of compounds from soil columns

Percolation column tests are frequently used to observe the fate and transport behaviour of organic micropollutants and their mobility in a soil environment (Banzhaf and Hebig, 2016). Column experiments are a useful and easy laboratory-scale method to study the potential transport of pharmaceuticals in soil under controlled laboratory conditions. Soil column leaching, based on the OECD 312 protocol (OECD, 2004), have been used to evaluate transport behaviour in the soil for pharmaceuticals and others chemical substances by several other research groups (Banzhaf and Hebig, 2016; Nickel *et al.*, 2015; Oppel *et al.*, 2004; Revitt *et al.*, 2015). The objective of conducting soil column leaching experiments in this study was to determine the potential leaching and mobility behaviour of the selected compounds in different soils.

Glass columns, 35 cm high and of 5 cm diameter were fabricated in the Chemistry department glassware laboratory at University College Cork (UCC) according to the design defined by the OECD 312 protocol. Superfine glass wool (11 µm) and 50 g of crystalline quartz sand (0.1-0.3 mm) were placed in the bottom of each column to prevent soil loss and to filter the leachate samples. Glass wool was purchased from SciChem (UK), and quartz sand from Kilwaughter Lime (Ireland) was generously supplied by Dr. Greg Beechinor. The dried and sieved soil samples were added to a height of approximately 30 cm in the columns. This was done gradually in small amounts and the columns were shaken gently after every portion was added to obtain uniform packing. The weight of the soils filling the columns varied between 619 and 987 g.

The artificial rain (0.01 M $CaCl_2$) was prepared by dissolving the desired weight of $CaCl_2$ powder in deionised water. The packed glass columns were pre-wetted using the artificial rain to their maximum water holding capacity by applying the artificial rain from the bottom the column upwards in order to displace the air in the soil pores, using a multi-channel peristaltic pump (Ismatec ISM827 Reglo, Cole-Parmer, UK) (Figure 2-6) as recommended by OECD 312 protocol.

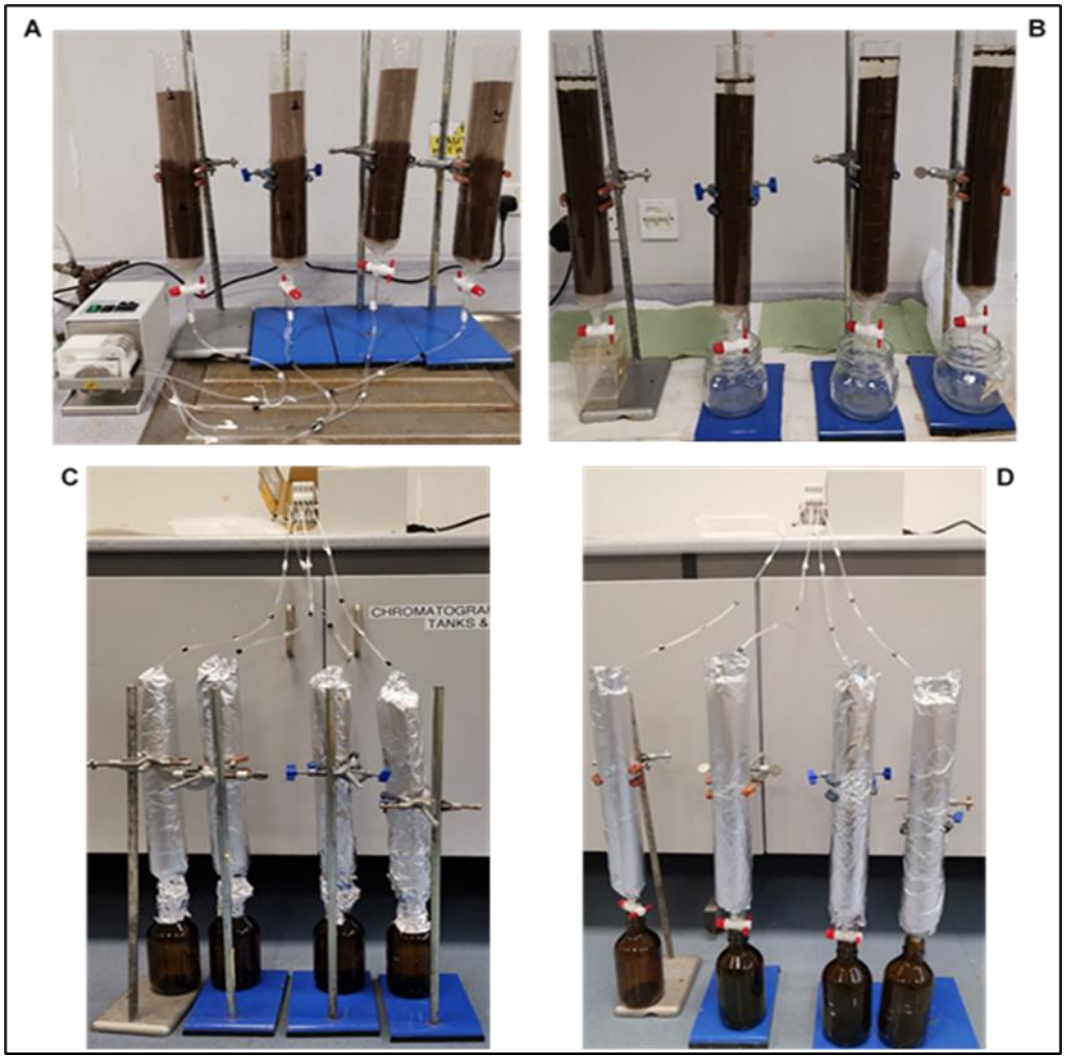


Figure 2-6: Leaching soil column experiments. A) Soil column pre-wetting process with artificial rain; B) Soil column after pre-wetting and fully saturated with $NaCl_2$ solution; C&D) Artificial rain procedure from the top of the soil column for 48 hours. One column is a control.

Subsequently, the soil columns were kept for 24 hours to equilibrate and to drain the excess water before applying the selected pharmaceuticals and their corresponding internal standard to the top of the columns at concentration of 100 ng g^{-1} dry weight as an organic mixture using a methanol solution following OECD 312 recommendations (OECD, 2004; Banzhaf and Hebig, 2016; Nickel *et al.*, 2015; Oppel *et al.*, 2004; Revitt *et al.*, 2015). The soil column was kept for 24 hours at room temperature in the dark to dry the organic solvent (MeOH) and to allow sorption of the pharmaceutical compounds into the surface soil particles (OECD, 2004; Revitt *et al.*, 2015; Nickel *et al.*, 2015).

Glass wool was then placed in the top of the column in order to distribute the artificial rain evenly. Next 251 ml, equal to 200 mm artificial rain, was applied on the top of the soil column for 48 hours using a multi-channel peristaltic pump (Ismatec ISM827 Reglo, Cole-Parmer, UK) at flow rate of 5.22 ml h^{-1} (OECD, 2004). Between 236 and 250 ml of leachate were collected from each soil column. The leachates were each made up to 500 ml using Milli-Q water and processed using the SPE methodology for the wastewater samples as described in section 2-6-1.

The soil inside the columns was then removed carefully and segmented into four vertical separate portions starting from the top (1-5, 5-10, 10-20 and 20-30 cm) and pre-treated and cleaned as described in the ultrasound-assisted extraction procedure in section 2-6-2. The leaching experiment was conducted in three columns simultaneously for each soil, together with a control column to which no pharmaceutical compounds were added. The results of the percolation behaviour studies are given in Chapter 5.

2-6 Chemical analysis

Two different extraction methods were used to clean and pre-treat water and soil samples. Solid Phase Extraction (SPE) was applied to water samples, while ultrasound-assisted extraction (UAE) was used for soil samples.

Environment samples typically have very harsh and complex matrices which could cause instrument damage or/and low instrument sensitivity during analysis. Pre-treatment of these samples is used to reduce sample matrix interference from the interested analytes and to increase the sensitivity and reliability of the instrument measurement result. To achieve low quantitation limits in environmental samples is a challenging task in analytical chemistry, so this technique helps to achieve this goal. Figure 2-7 summaries the wastewater and soil samples pre-treatment steps.

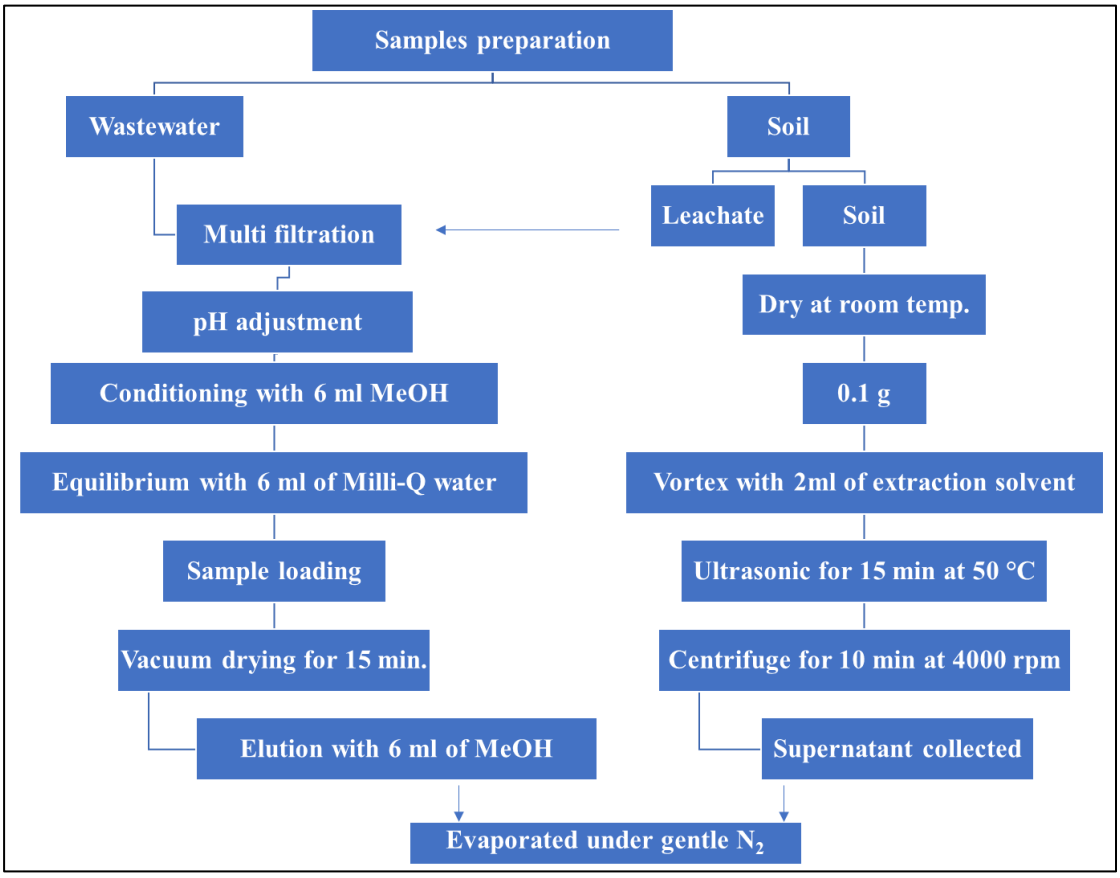


Figure 2-7: Wastewater and soil samples pre-treatment process.

2-6-1 Wastewater samples preparation

Solid phase extraction is a method and technique widely used to concentrate and separate target analytes in aquatic matrices from other unwanted analytes according to their physical and chemical properties. Also, SPE is used to purify and isolate analytes of interest and reduce or remove unwanted matrix effects like water, wastewater, urine and beverages, thereby increasing method sensitivity. The extraction process typically involves four basic steps: conditioning the sorbent, loading the sample, washing interferences, and then eluting the analyte (Figure 2-8). Conditioning is usually done by applying an organic solvent, such as methanol, and then loading the sample. Washing is used to remove impurities from the sorbent bed by applying an appropriate wash solvent that does not wash away analytes of interest. Finally, elution collects the selected compounds.

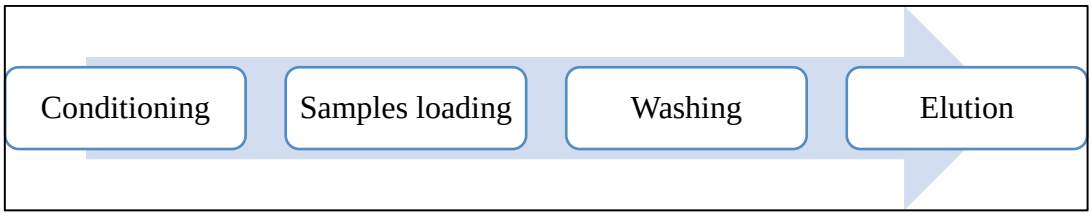


Figure 2-8: SPE procedure steps adapted from (Lucci *et al.*, 2012).

In this study, the SPE method development for extracting multiresidue analytes in the WWTP influents and effluents was mainly based on the previously-established methods (Dasenaki and Thomaidis, 2015), with minor adjustments. Duplicate wastewater samples (500 mL) from the WWTP influents and effluents were extracted in the laboratory of the College of Food and Agricultural Sciences in King Saud University (KSU). SPE was performed by using a 12 port Phenomenex manifold equipped with 12 positions (Figure 2-9) and a drying attachment in order to dry the eluate. After wastewater sample preparation and pre-treatment (see section 2-6-1), the extraction method was performed using Strata-X 33u SPE cartridges Polymeric

Reversed Phase 6 mL, 200 mg supplied by Phenomenex, UK. The SPE procedure was carried out as shown in figure 2-7. Conditioning was done by passing 6 mL of HPLC grade methanol in order to activate the cartridge sorbent, increase the porosity and to eliminate any trapped air. Afterwards, the equilibration step was performed, in which 6 mL of Milli-Q water was applied to rinse and remove excess methanol from the cartridge sorbent and to generate conditions similar to the sample matrix. Once the cartridge had emptied, 500 mL of sample spiked with 20 µg L^{-1} of internal standards was applied at a flow rate of approximately 3 mL min^{-1} using a Fisherbrand vacuum pump (Model FB70155). After the samples were passed through the columns, the cartridge was dried for 15 min under air-vacuum to remove excess water. Elution of the target compounds was performed with 6 mL of methanol (MeOH). The eluates were collected in 8 ml amber glass vials. Then the eluates were mixed using vortex and transferred in fractions of 1 ml to 2 ml to autosampler HPLC amber glass vial bottles purchased clean and sterilized from Merck. These samples were dried under a gentle nitrogen stream and frozen at -20 ^{0}C prior to HPLC-LC MS/MS analysis.

Figure 2-9: Samples undergoing SPE in the laboratory using a 12-position manifold.

For quality control and to monitor cross contamination, blank samples (Milli-Q water) were processed with every two batches (24 samples). The SPE method was validated by spiking real samples (influent, effluent) and Milli-Q water in duplicate and calculating the recovery of analytes from these samples (recovery results can be found in chapter 3 section 3-2).

2-6-2 Soil samples preparation

The extraction procedure for soil samples was based on the extraction methodology developed by Gago-Ferrero *et al.* (2015). Ultrasound refers to high-frequency sound waves, higher than the human ear can hear. There have been many studies using the UAE extraction method to extract and clean-up analytes from solid environmental matrices such as soil, sludge, and sediments (Gago-Ferrero *et al.*, 2015; Carmona *et al.*, 2017; Albero *et al.*, 2015; Albero *et al.*, 2019). It is a clean, fast, easy, and environment-friendly protocol because the amount of solvent used is less than other classical extraction techniques (Albero *et al.*, 2015). The function of the ultrasound is to enhance the ability of the solvent to penetrate through solid matrices, which leads to an increase in extraction efficiency. Sonication facilitates efficient contact between the solid and the extractant, usually resulting in good recovery of the analyte (Albero *et al.*, 2019).

All soil samples for this study were pre-treated and extracted in the School of Biological, Earth and Environmental Sciences laboratories at University College Cork. After the soil column leaching experiments had been carried out (see Chapter 5), the soil inside the columns was removed carefully and divided into four segments (1-5, 5-10, 10-20 and 20-30 cm depth). These segments were then dried at room temperature by spreading them on aluminium foil prior to the UAE procedure. Each segment was thoroughly mixed and subsampled to ensure as representative a sample as possible by using a porcelain mortar, pestle, and splitter (Riffle Box macro and nano size) to obtain

0.1 g for each segment. Each 0.1 g dried sub-soil sample was then mixed with extraction mixture of 2 mL MeOH-Milli-Q water (pH 2.5, formic acid (FA) 0.5 % and 0.1 % Ethylenediaminetetraacetic acid (EDTA)), 50:50 (v/v) by using vortex stirring (Gago-Ferrero *et al.*, 2015). Subsequently, each mixture was placed into a 15 mL plastic centrifuge tube (SARSTEDT, Germany), and subjected to ultrasound at 50 $^{\circ}$C (BANDELIN SONOREX, Germany) for 15 min. Afterwards, the extracts were centrifuged at 4000 rpm using an Eppendorf Centrifuge Model 5804 for 10 min and the supernatants were collected in new 15 mL plastic centrifuge tubes (SARSTEDT, Germany). To each tube of supernatant, 2 ml of extraction solvent was added again, and the same steps of ultrasound and centrifuge repeated, and the supernatant collected again. This procedure was repeated one more time in order to collect a total of 6 mL of supernatant. The 6 mL supernatant samples were then evaporated under a gentle stream of N_2 at 40 °C and stored at -20 $^{\circ}$C prior to HPLC- MS/MS analysis. By spiking 0.1 g of these four different soils with their corresponding internal standards for the pharmaceutical of interest, and subjecting them to the same UAE procedures, recoveries were determined.

2-6-3 LC-MS/MS analysis

High performance liquid chromatogram mass spectroscopy (HPLC-LC MS/MS) is a typical analytical method used to determine pharmaceuticals in environmental samples. Due to its high sensitivity and selectivity, it is able to detect extremely low concentrations (ng L^{-1} and ug L^{-1}) (Dasenaki and Thomaidis, 2015). Liquid chromatography mass spectroscopy analyses were carried out for the extracted samples at the Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Greece following their published method with a few adjustments (Dasenaki and Thomaidis, 2015). Instrumental analysis was carried out by

using a Thermo Scientific Accela ultra-high-performance (UHPLC) system connected to a TSQ Quantum Access triple-quadrupole (QqQ) mass spectrometer (Thermo Scientific, San Jose, CA, USA). The system was equipped with an electrospray-ionization (ESI) source (Thermo IonMAX) operated in both positive and negative mode. The separation was performed by using an Atlantis T3 C18 column (100 mm × 2.1 mm, 3 μm, Waters) protected by a guard column at a constant flow rate of 100 μL/min with 10 μL of extracted sample injected at a maintained temperature of 30 ˚C. To detect all analytes, two chromatographic runs were performed in both positive and negative ionization modes. Positive ionization mode detection was performed using the mobile phase of water containing 0.01 % (v/v) formic acid (solvent A) and methanol (solvent B). Negative mode mobile phase consisted of water containing 1 mM ammonium formate (solvent A), methanol (solvent B), and acetonitrile (solvent C) used in negative ionization mode. Both gradient elution programmes are illustrated in Table 2-5. The total time for positive mode gradient elution was 37 min while negative mode gradient was run for 27 min.

Table 2-5: Gradient elution programme in positive and negative ionization mode for HPLC-LC MS/MS analyses.

Positive mode				Negative mode				
Time (min)	Flow (μL/min)	% A	% B	Time (min)	Flow (μL/min)	% A	% B	% C
0	100	90	10	0	100	70	25	5
10	100	0	100	10	100	0	95	5
27	100	0	100	19	100	0	95	5
27.5	100	90	10	19.5	200	70	25	5
28	200	90	10	23.5	200	70	25	5
33	200	90	10	23.6	100	70	25	5
33.5	100	90	10	27	100	70	25	5
37	100	90	10					

The mass spectra and the optimum collision energy and tube lens voltage were obtained for each compound separately by direct infusion of individual standard

solutions at a concentration of 1 mg L^{-1} in formic acid: methanol (50:50, v/v) or ammonium formate: methanol (50:50, v/v), depending on whether the determination was performed in positive or negative ionization mode. The electrospray (ESI) parameters (spray voltage, sheath gas, auxiliary gas, capillary temperature) for each determination are given in Table 2-6.

Table 2-6: Electrospray parameters for both ionization modes for HPLC-LC MS/MS analyses.

ESI parameters	Positive ionization mode	Negative ionization mode
Spray Voltage	4000 V	3000 V
Capillary temperature	300 °C	270 °C
Sheath gas	25 psi	25 psi
Auxiliary (drying) gas	10 a.u.	10 a.u.

Table 2-7: Multiple reaction monitoring parameters and retention times for the investigated compounds.

ESI	Compounds	Precursor ion m/z	Product 1 m/z	Collision Energy 1 (eV)	Product 2 m/z	Collision Energy 2 (eV)	Tube lens	RT (min)
Positive	Acetaminophen	152.1	110.2	15	93.20	22	84	7.9
	Acetaminophen-d4	156.1	114.2	18	97.20	21	78	7.8
	Atenolol	267.2	145.0	26	190.00	18	94	6.2
	Atenolol-d7	274.2	145.1	28	190.10	19	78	6.1
	Azithromycin	749.7	591.1	29	157.90	37	127	8.8
	Baclofen	214.1	151.10	17	116.2	31	56	8.0
	Baclofen-d4	218.1	118.2	34	119.20	30	59	8.0
	Caffeine	195.1	138.2	18	110.20	22	87	9.3
	Caffeine-d9	204.2	144.1	21	116.20	22	92	9.5
	Cephalexin	347.8	158.0	6	106.10	30	113	8.4
	Ciprofloxacin	332.1	288.0	18	314.00	22	85	8.2
	Diclofenac	296.1	214.9	19	249.90	12	72	15.6
	Metformin	130.1	71.4	20	85.30	13	50	2.7
	Metformin-d6	136.2	77.4	23	94.30	14	53	2.4
	Ofloxacin	362.2	317.9	19	260.90	27	120	8.0
	Tetracycline	445.0	410.0	18	426.40	12	90	8.7
	Trimethoprim	290.9	230.0	25	122.90	30	87	7.5
Negative	Allopurinol	135.0	92.2	20	107.10	3.6	18	55
	Ibuprofen	205.1	161.3	10	-	-	65	14.9
	Oxypurinol	151	150.9	5	108.00	14	62	3.5
	Triclosan	287	142.2	30	-	-	60	16.3

Multiple reaction monitoring (MRM) was used, and the detailed parameters for MRM acquisition are presented in Table 2-7. Two transitions were selected for identification purposes for most of the tested analytes, but only the most intense one was used for quantification (Product 1).

2-6-4 Quality assurance and quality control

To determine the accuracy and precision of the overall HPLC-LC-MS/MS method, linearity, sensitivity (limits of detection (LOD) and limits of quantitation (LOQ)), and matrix effect (ME) were obtained. Seven points were generated for the calibration curve to determine the linearity by linear correlation coefficient (r^2). The linearity obtained for the selected compounds was $r^2 > 0.99$ in all cases. Table 2-8 represents the analytical method performance of the recovered compounds.

Table 2-8: Performance and validation data of the analytical method.

Compounds	Linearity (r^2)	Calibration range (μg L^{-1})	Average Matrix Effect		Instru-mental %RSD	LOD μg L^{-1}	LOQ μg L^{-1}
			Effluent (%)	Influent (%)			
Acetaminophen	0.999	0.5-300	-24	-45	2.6	0.004	0.01
Atenolol	0.997	1-300	-47	-72	0.2	0.08	0.23
Azithromycin	0.995	1-300	-42	-63	4.3	0.50	1.81
Baclofen	0.998	1-300	-25	-46	1.8	0.12	0.37
Caffeine	0.998	1-300	-19	-30	3.5	0.34	1.02
Cephalexin	0.995	1-300	-40	-50	2.6	0.03	0.10
Ciprofloxacin	0.991	1-300	-60	-83	3.1	0.04	0.11
Diclofenac	0.999	0.5-300	-12	-23	3.2	0.05	0.15
Metformin	0.999	1-300	-39	-46	3.1	0.06	0.17
Ofloxacin	0.998	1-300	-50	-80	2.6	0.23	0.68
Tetracycline	0.994	1-300	-24	-35	1.6	0.05	0.16
Trimethoprim	0.998	0.5-300	12	22	1.0	0.03	0.09
Allopurinol	0.998	70-30000	-60	-90	7.3	N/A	N/A
Ibuprofen	0.991	3-1000	-40	-85	3.4	0.05	0.15
Oxypurinol	0.991	60-30000	-53	-89	3.4	0.68	2.05
Triclosan	0.993	450-30000	-80	-92	5.2	N/A	N/A

N/A: Not available because no or very low recovery.

Duplicates of influent, effluent and Milli-Q water samples were spiked with low, medium, and high concentrations to assess the HPLC-LC-MS/MS method accuracy and precision. Precision was determined as the relative standard deviation (%RSD)., which was found to be between 0.2 and 4.3, indicating that the precision of the method developed was good. For method sensitivity, an LOD with signal to noise (S/N) ratio of 3 and an LOQ with S/N ratio of 10, were used. The matrix effect (ME) was estimated, and the results expressed as a percentage of suppression or enhancement.

2-6-5 Validation of solid phase extraction (SPE) method

SPE is a well-known technique used in order to preconcentrate pharmaceuticals in water samples prior to analysis (Dasenaki and Thomaidis, 2015). Optimization of the SPE method in order to achieve satisfactory recoveries of the 16 compounds from the wastewater samples was quite challenging. In the present study, extraction of the 16 pharmaceutical compounds from samples from the three selected WWTP influents and effluents, was carried out using Strata-X (200 mg, Phenomenex) cartridges following SPE method developed (Dasenaki and Thomaidis, 2015) with minor adjustments. The extraction method efficiency for the selected compounds was performed using influent, effluent and Milli-Q water spiked at three different concentrations of the studied analytes (Table 2-9). This was done at a pH value of 2.5±0.2 without the addition of EDTA, because extraction of the samples was done in Saudi Arabi and, at that time, EDTA was not available.

Except for tetracycline, recoveries were observed between 48.90% and 157% for the investigated pharmaceutical compounds. Recovery was found to be ≥70% for acetaminophen, azithromycin, baclofen, caffeine, cephalexin, ciprofloxacin, metformin, and trimethoprim and between 48.90% and 109.25% for atenolol,

diclofenac, ibuprofen, ofloxacin and oxypurinol. The remaining three compounds showed very poor recovery (tetracycline ≤15%) or no recovery (allopurinol and triclosan) (Table 2-9). In general, recoveries of detected compounds in this study are in good agreement with some previous study (Dasenaki and Thomaidis, 2015; Hermes *et al.*, 2018) and slightly better than result found by Carmona *et al*. (2017).

Table 2-9: The recovery (%) (n=2) of selected pharmaceuticals at three different level of concentrations (µg L^{-1}) for WWTP influents, effluents, and Milli-Q water.

Pharmaceuticals	Influent			Effluent			Milli-Q water		
	5	10	20	1	2	4	4	10	20
	µg L^{-1}								
Acetaminophen	71.60	72.45	71.70	71.00	72.20	70.45	71.25	72.27	71.09
Atenolol	58.05	48.90	80.55	57.65	47.75	77.95	57.85	57.80	108
Azithromycin	114.00	96.90	137.75	122.50	97.75	157.00	118.21	97.25	147.43
Baclofen	86.35	82.60	95.85	86.10	81.30	93.40	86.19	82.00	94.56
Caffeine	*	*	*	100.30	89.40	106.20	100.29	89.50	106.24
Cephalexin	115.65	98.25	76.60	117.00	98.50	71.60	116.16	98.43	74.08
Ciprofloxacin	100.85	103.75	91.35	100.40	100.70	86.15	100.61	102.19	88.78
Diclofenac	68.75	61.00	84.05	64.75	54.45	81.05	66.82	57.69	82.58
Metformin	98.75	100.50	116.10	87.50	98.40	119.10	93.12	99.51	117.62
Ofloxacin	53.40	73.75	109.25	51.90	72.70	107.10	52.66	73.19	108.25
Tetracycline	2.15	1.88	3.35	1.12	1.75	2.77	5.0	5.9	15.8
Trimethoprim	98.75	81.55	72.60	98.40	75.50	65.40	98.63	78.54	69.03
Ibuprofen	62.90	59.90	65.75	62.15	60.20	65.35	62.50	60.10	65.60
Oxypurinol	64.25	60.10	62.75	62.35	57.65	59.65	63.30	58.90	61.20
Allopurinol	NR	NR	NR	NR	NR	NR	NR	NR	NR
Triclosan	NR	NR	NR	NR	NR	NR	NR	NR	NR

*: was not possible because the concentration was too high, NR: Not recovered.

2-6-6 Recovery of UAE method

Performance of the UAE technique was evaluated through estimation of recoveries. To determine the recoveries, the four investigated soil samples were spiked with a target analyte mixture at three different concentrations (20 ng g^{-1}, 250 ng g^{-1}, and 750 ng g^{-1}) and were extracted in duplicate. Possible cross contamination was tested by including blank samples. Except metformin, oxypurinol, allopurinol and triclosan, the average recoveries were found between 16.43% and 100.80% for the investigated pharmaceutical compounds (Table 2-10). Most recovered compounds showed good overall average recoveries (≥ 64.10%). The average recoveries of the 16 studied pharmaceuticals ranged from 21.93% to 89.60%, 23.67% to 95.03%, 16.43% to 84.97% and 30.03% to 100.80% for silt loam, clay, silty clay loam and sandy loam studied soil types (Table 2-10).

Table 2-10: Average recovery as a percentage of applied pharmaceuticals in the different investigated soil types (n=2).

Pharmaceuticals	Silt loam			Clay			Silty clay loam			Sandy loam		
	50 ng g^{-1}	250 ng g^{-1}	750 ng g^{-1}	50 ng g^{-1}	250 ng g^{-1}	750 ng g^{-1}	50 ng g^{-1}	250 ng g^{-1}	750 ng g^{-1}	50 ng g^{-1}	250 ng g^{-1}	750 ng g^{-1}
Acetaminophen	71.60	75.10	73.00	96.80	88.60	82.00	52.00	70.70	64.30	43.30	49.40	44.30
Atenolol	80.40	93.30	75.90	91.50	71.10	85.20	87.60	96.00	62.00	72.10	73.70	98.40
Azithromycin	62.10	65.30	70.30	64.70	66.30	72.60	59.40	60.50	75.80	67.00	68.70	75.50
Baclofen	73.40	80.50	88.40	91.00	89.00	86.40	79.80	75.90	61.80	65.50	80.00	90.60
Caffeine	95.50	92.80	80.50	108.20	86.40	90.50	97.20	92.60	54.10	105.00	95.90	101.50
Cephalexin	19.30	23.30	23.20	20.80	24.20	26.00	12.30	16.80	20.20	30.20	28.90	31.00
Ciprofloxacin	50.00	50.60	52.60	48.90	54.30	55.60	50.30	55.50	52.30	39.00	40.60	40.50
Diclofenac	61.60	65.10	65.70	105.50	56.30	73.90	78.90	85.50	57.20	57.20	54.70	59.10
Ibuprofen	55.60	65.90	75.20	58.30	69.30	74.30	55.30	67.40	70.10	51.30	63.30	63.20
Trimethoprim	81.90	81.90	84.40	109.00	56.50	94.80	102.90	81.90	70.10	74.00	74.10	95.20
Ofloxacin	72.80	75.60	78.60	72.30	72.90	75.20	69.70	69.50	71.30	68.30	70.20	75.60
Tetracycline	≤LOD	15.6	21.5	≤LOQ	21.2	26.4	≤LOQ	20.5	22	≤LOD	26.3	18.9
Metformin	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Oxypurinol	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Allopurinol	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Triclosan	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

N/A: Not available due to no recovery observed.

2-7 Environmental risk assessment (ERA)

As many medicinal compounds are introduced into different environmental compartments at concentrations which are likely to pose a hazard for the environment, the need for risk evaluation is very important. An environmental risk assessment reflects the possibility of ecotoxicity and an effect occurring in organisms in the environment exposed to pharmaceutical residues. According to the method recommended by the European Medicines Agency (EMA) guideline (EMA, 2006), the risk quotient (RQ) is a useful protocol to estimate the potential environmental risk of pharmaceuticals in aquatic matrices in three trophic levels of the aquatic ecosystem (fish, invertebrates and algae). In order to investigate the potential adverse effects on aquatic organisms posed by each target compounds found in the WWTP influents and effluents in this study, the RQ approach was used as suggested by EMA (2006). Values for the RQ were obtained from the ratio between the highest measured environmental concentrations (MECs) of pharmaceuticals and their lowest corresponding predicted-no-effect concentrations (PNECs) Equation 1. The PNEC is the concentration of a compound at which no effects on environmental organisms are expected to occur. The PNEC values were generated from the lowest acute toxicity data EC_{50} (half maximal effective concentration) or LC_{50} (median lethal concentration) previously reported in the literature for three trophic levels: algae, *Daphnia*, and fish. The PNECs were calculated in this study using an assessment factor (AF) of 1000 as in Equation (1). This AF value was used in this study to consider the effect on other sensitive aquatic species as well (Tauxe-Wuersch *et al.*, 2005; Tsui *et al.*, 2014; Verlicchi *et al.*, 2012b; Mansour *et al.*, 2016; Al Aukidy *et al.*, 2012). The AF was applied to the lowest toxicity concentration for the most sensitive aquatic species (algae, *Daphnia* and fish) in order to obtain the PNEC. The common criteria for risk classification was applied, where RQ

≤ 0.1 poses low or negligible risk, while 0.1 ≤ RQ ≤ 1 poses moderate risk, and if the RQ is more than 1 there is a high risk to aquatic organisms (Hernando *et al.*, 2006; Tsui *et al.*, 2014; Ruan *et al.*, 2019; Al Aukidy *et al.*, 2012).

To date there have been no published environmental risk assessments of pharmaceuticals in the receiving aquatic ecosystems in Saudi Arabia. Therefore, this is the first time that an ERA of pharmaceutical residuals from WWTPs in Saudi Arabia has been conducted. The result of this study are given in Chapter 4.

$$RQ = \frac{MEC}{PNEC} = \frac{MEC}{LC50 \; or \; EC50/1000} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots . (1)$$

Where: -

MEC: Measured environmental concentration.

PNEC: Predict non-effect environmental concentration.

LC50: Median lethal concentration.

EC50: Half maximal effective concentration.

2-9 Data analysis

2-9-1 Removal efficiency

The removal efficiency of pharmaceuticals through WWTPs depends on different parameters. These parameters include biodegradability, physiochemical properties (such as water solubility), tendency for absorption by activated sludge and tendency to volatilize (Kosma *et al.*, 2014). The removal efficiency (RE%) of targeted pharmaceutical compounds and the major wastewater quality parameters (BOD5, COD and TSS) were determined as a percentage by taking the raw wastewater concentration (C_{inf}) minus the treated wastewater concentration (C_{eff}) and dividing the difference by the influent concentration, and multiplying by 100, using the equation below:

$$R\,(\%) = \frac{Cinf - Ceff}{Cinf} \times 100 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots . \dots\dots\dots\dots\dots\dots .. (1)$$

Low or negative removal occurs when R (%) < 20%, moderate removal when 20.1% < R (%) < 70% and high removal when 70.1% < R (%) < 99% (Tsui *et al.*, 2014; Papageorgiou *et al.*, 2016). LOQ/2 and LOD/2 values were used if the concentration of the compound was below its LOQ or LOD in the effluent (Beijer *et al.*, 2017; Kosma *et al.*, 2015).

2-9-2 Seasonal variation estimation

Seasonal variation of each pharmaceutical was estimated for each sampling season following equation (2) (Di Marcantonio *et al.*, 2020). The seasonal variation (%) was calculated by the following formula: -

$$Sesonal\ variation = \left(\frac{Ave.se. - Ave.sc.}{Ave.sc.}\right) \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where: -

Ave. se. is the average concentration for every single season.

Ave. sc. is the average concentration calculated for the whole sampling campaign**.**

Determination of the significant differences ($p < 0.05$) between the candidate variables in the influent and effluent of investigated WWTPs, were computed using RStudio Version 1.3.1093 (2009-2020 RStudio, PBC), one-way analysis of variance (ANOVA) using IBM SPSS Statistics 26 (SPSS Inc., Chicago, IL, USA) (Niemi *et al.*, 2020; Pallares-Vega *et al.*, 2019) and Microsoft Office Excel 365. The normality of data distribution was performed by using Tukey HSD test at a significance level of 0.05. In addition, Pearson's correlation coefficient analysis (r) was used to evaluate the relationships between flow rate (FR), air temperature (T) and physiochemical parameters of the collected wastewater samples and the pharmaceuticals' occurrence in selected WWTPs. Correlation between $-0.5 \geq r \geq 0.5$ at $p < 0.05$ was consider as

significant in this study. Targeted analytes detected below their LOQ or LOD were given value equal to LOQ/2 or LOD/2 (Kramer *et al.*, 2018; Mandaric *et al.*, 2019).

2-9-3 Pharmaceutical loading mass

Mass loadings (M.L.) of the target analytes into the WWTP (influent) and from the WWTP (effluent) to the environment were estimated by multiplying individual concentration of each pharmaceutical found in the samples (C) in mg L^{-1} by the average daily flow rate (F) in m^3 d^{-1} for the three selected WWTPs. The WWTPs loads were normalized by the population equivalent (PE) (Česen *et al.*, 2018; Kapelewska *et al.*, 2018; Kosma *et al.*, 2019; Li and Zhang, 2011; Papageorgiou *et al.*, 2016; Pereira *et al.*, 2015; Verlicchi *et al.*, 2012b). The mass load usually refers to 1000 inhabitants, as in Equation (3). M.L. is expressed in mg/day per 1000 inhabitants.

$$M.L. = \frac{C \times F}{PE} \times 1000 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots. (3)$$

A value of LOQ/2 was used if the concentration of the compound in the effluents was below its LOQ in M.L. calculation, while if the analyte concentration in the influents < LOQ, M.L. was not estimated (Česen *et al.*, 2018; Verbovšek, 2011). When PE was not available as for the KSU-WWTP, 55000 was used as its average of the range of PE given by this WWTP operational staff, which was between 45000 and 65000.

CHAPTER THREE

The occurrence of pharmaceutical residues and their removal rate in three different wastewater treatment plants in Riyadh city, Saudi Arabia

3-1 Introduction

As pharmaceutical compounds have become very important to our life in order to protect our health, hundreds of tonnes of different classes of pharmaceuticals have been used so far (Fekadu *et al.*, 2019). Hence, greater, and greater amount of these medicinal products have been discharged into the environment. They can find their way to different environment compartments through many pathways, such as wastewater treatment plants (WWTPs), human waste, livestock waste and from septic tanks (Archer *et al.*, 2017). WWTPs have been anticipated as a major and primary route for pharmaceuticals to the receiving environment. Although many studies and investigations have been conducted for pharmaceuticals in the environment, the fate and effects of these micro-pollutants is still largely unknown. Pharmaceutical residues discharged from WWTPs into the aquatic environment could be potentially toxic to aquatic life because they are ubiquitous, persistent, and biologically active (Osorio *et al.*, 2012), and lead to the development of resistance in pathogenic bacteria and cause endocrine disruption in some organisms (Taheran *et al.*, 2016).

The presence of pharmaceuticals in WWTP influents and effluents have been investigated in many countries around the world. For example, more than 80 pharmaceutical compounds were investigated in the influent and effluent of a WWTP in Athens (Greece) by Dasenaki and Thomaidis (2015) who found 40% of their selected compounds were detected in untreated and treated wastewater samples. The highest concentrations they reported were for metformin and caffeine at concentration of 176.5 $\mu g\ L^{-1}$ and 58 $\mu g\ L^{-1}$ in influent wastewater samples, respectively. The concentration of

154 pharmaceutical compounds were evaluated in three WWTPs in Germany by Hermes *et al.* (2018). They found the range of pharmaceutical concentrations detected in WWTP samples was between <0.02 μg L^{-1} and more than 1 μg L^{-1}. Therefore, reduction of the levels of these micropollutants in wastewater before their discharge into the environment from WWTPs plays a main role in protecting the environment from pharmaceutical contamination.

The removal efficiency of pharmaceuticals by WWTPs has been reported in the scientific literature in different studies worldwide, e.g. in Spain (Gros *et al.*, 2010), China (Zhang *et al.*, 2017) and Greece (Papageorgiou *et al.*, 2019). For example, ofloxacin, ciprofloxacin and trimethoprim removal efficiencies were reported between -39.7% and 98.6%, 59.1% and 100% and -40.4% and 100% respectively in 12 WWTPs using secondary treatment in Dalian, China (Zhang *et al.*, 2017). An average removal efficiency of 59% was observed for atenolol in seven secondary WWTPs operated with conventional activated sludge in the Ebro river basin (northeast of Spain) (Gros *et al.*, 2010). Table 3-1 shows the removal efficiency of detected pharmaceuticals in different WWTPs around the world. Moreover, an extensive monitoring study of 138 pharmaceutical compounds, including acetaminophen, diclofenac, ciprofloxacin, trimethoprim, metformin, atenolol and caffeine, in four different WWTPs from three different cities (Thessaloniki, Larisa and Volos) in North and Central Greece, found that average removal efficiencies were high (>90%) for atenolol, acetaminophen, ciprofloxacin, caffeine and metformin, medium (>60%) for trimethoprim, and low (>30%) for diclofenac (Papageorgiou *et al.*, 2019). The removal efficiency of a WWTP is therefore important to reduce and eliminate pharmaceuticals from the final wastewater discharge to the receiving water body.

Table 3-1: Comparison of removal efficiencies (RE%) of measured pharmaceutical compounds in various wastewater treatment processes worldwide.

Compounds	Treatment processes[1]	RE (%)	Locations	Ref.
Acetaminophen	PS+CAS+SS	87	Scotland	1
	PS+CAS	>99.9	France	2
	PS+MBR+SS	≥99	New Zealand	3
	PS+CAS+SF	95.6	Greece	4
	PS+CAS	75		
	PS+CAS+TF	94	UK	5
	GC+OD+SS+UV	>97	Mexico	6
	SC+CAS+SS+SF+DC	>98	Saudi Arabia	7
	SC+PS+CAS+SF+DC	>98		
	SC+A2/O+SS+UV	99.9	China	8
	SC+GR+PS+CAS+SS	73.4-99.3	Greece	9
Caffeine	SC+CAS+SS+SF+DC	> 9	Saudi Arabia	7
	SC+PS+CAS+SF+DC	>99		
	PS+MBR+SS	≥99	New Zealand	3
	PS+CAS	75	Greece	4
	PS+CAS+SF	89		
	GR+A/O+SS+UV+OZ	80-90	China	10
	GR+PS+A2/O+SS+SF	50-80		
	GR+PS+ A2/O+SS+MF+RO	>90		
	SC+A2/O+SS+UV	99.2		8
Ciprofloxacin	SC+A2/O+SS+UV	-5.34		
	PS+CAS+SS	76.6-92.4	Singapore	11
	PS+MBR+SS+MF	9-99.9		
	SC+CAS+SS+SF+DC	>99	Saudi Arabia	7
	SC+PS+CAS+SF+DC	>99		
Ofloxacin	PS+CAS+DC	82.8	China	12
	PS+CAS	0-66	Italy	13
	SC+A2/O+SS+UV	-8.02	China	8
	PS+CAS+SS	29	Scotland	1
	PS+MBR+SS	<50	New Zealand	3
	GR+A/O+SS+UV+OZ	>90	China	10
	GR+PS+A2/O+SS+SF	80-90		
	GR+PS+ A2/O+SS+MF+RO	>90		
Trimethoprim	GC+OD+SS+UV	< 53	Mexico	6
	PS+CAS+SS	23.8-42.2	Singapore	11
	PS+MBR+SS+MF	7-73.3	Greece	9
	SC+GR+PS+CAS+SS	21.2-98		
Diclofenac	PS+CAS+SS	47	Scotland	1
	PS+CAS	-20-50	France	2
	PS+CAS+DC	46.8	China	12
	GR+A/O+SS+UV+OZ	>90		10
	GR+PS+A2/O+SS+SF	<0		
	GR+PS+ A2/O+SS+MF+RO	>90	Mexico	6
	GC+OD+SS+UV	>46		
	SC+A2/O+SS+UV	-16.6	China	8

Table 3-1: Continued.

Compounds	Treatment processes[1]	RE (%)	Locations	Ref.
Atenolol	PS+CAS	80-90	France	2
	PS+MBR+SS	50-70	New Zealand	3
	PS+CAS+TF	78	UK	5
	GC+OD+SS+UV	<44	Mexico	6
	SC+CAS+SS+SF+DC	89	Saudi Arabia	7
	SC+PS+CAS+SF+DC	89		
Cephalexin	A2/O+SS	87-98	Iran	15
	CAS+TF	≈100		
	A2/O	67	China	16
	A2/O	100		
Metformin	CM+BT	99.7	Poland	17
	A/O+MBR (Pilot scale)	35.4	France	18
	TT+BNR+UV	79	USA	19
	ST+BNR	90		20
	TT+BNR+UV	96		
	ST+BNR	99		
	PS+CAS+P-NR	98	Netherlands	21
	MBR+CAS+SF	99		

[1] PS: Primary sedimentation, BT: Biological treatment, UV: Ultraviolet, OD: Oxidation ditch, SS: Secondary sedimentation, CD: Chemical disinfection, CAS: Conventional activated sludge, MBR: Membrane bioreactor, SF: Sand filter, TF: Trickling filter, DC: Disinfection with chlorination, A^2/O: anaerobic/anoxic/oxic, A/O: Anoxic/oxic, GR: Grit removal, MF: Membrane filter, RO: Reverse osmosis, GC: Grit chamber, SC: Screening, MF: Microfiltration membrane, CM: Conventional mechanical, BT: Biological treatment, TT: Tertiary treatment, BNR: Biological nutrient removal, ST: Secondary treatment, P-NR: Phosphate and nitrogen removal, OZ: Ozone. 1 (Niemi *et al.*, 2020), 2 (Thiebault *et al.*, 2017), 3 (Kumar *et al.*, 2019), 4 (Kosma *et al.*, 2010), 5 (Kasprzyk-Hordern *et al.*, 2009), 6 (Rivera-Jaimes *et al.*, 2018), 7 (Al Qarni *et al.*, 2016), 8 (Ashfaq *et al.*, 2017a), 9 (Papageorgiou *et al.*, 2016), 10 (Sui *et al.*, 2010), 11 (Tran *et al.*, 2016), 12 (Yan *et al.*, 2014), 13 (Castiglioni *et al.*, 2006), 14 (Inyang *et al.*, 2016), 15 (Mirzaei *et al.*, 2019), 16 (Chen *et al.*, 2012), 17 (Kot-Wasik *et al.*, 2016), 18 (Chiarello *et al.*, 2016), 19 (Oliveira *et al.*, 2015), 20 (Oliveira *et al.*, 2015), 21 (Oosterhuis *et al.*, 2013)

Although pharmaceuticals have been investigated in the environment, many of the investigations have focused on the same pharmaceutical compounds, while others have been neglected (Daughton, 2014). Thus, this study included some new compounds which have not been investigated either in Saudi Arabia WWTPs, e.g., ofloxacin,

allopurinol, azithromycin, and oxypurinol, or in the worldwide environment, i.e., baclofen. Furthermore, most research of the presence of pharmaceuticals in the environment has been focused on certain countries, such as Germany, Italy, Spain, Sweden, Switzerland, UK, the USA, Canada, and China (Petrovic *et al.*, 2004; Caliman and Gavrilescu, 2009), whereas for other countries in Africa, Asia and Middle East very little research has been conducted (Hughes *et al.*, 2013).

This study was conducted in Saudi Arabia, which is part of the Asia and Middle East region. To date very little data on the presence of pharmaceuticals in Saudi Arabia (≈ 2 million km^2 area and around 31 million population), and their removal rate have been reported in the scientific literature for aquatic matrices. Therefore, this study aimed to fill the existing gap by providing information on the occurrence, removal, and seasonal variation of 16 selected pharmaceuticals, in three different size WWTPs in the capital city of Saudi Arabia, Riyadh. The monitoring of pharmaceuticals in these three WWTPs was conducted between March 2018 and July 2019. Details of the methods used for this chapter are in Chapter 2, sections 2-4-1, 2-6-1, 2-6-3, 2-6-5, 2-9.

3-2 Factors influencing the removal efficiency of pharmaceutical compounds in WWTP

A variety of different factors have been reported to influence the removal efficiency of pharmaceuticals such as treatment technology used, physical and chemical properties of the compound, ambient temperature, and operational conditions (Verlicchi *et al.*, 2012b; Couto *et al.*, 2019; Kosma *et al.*, 2014). WWTPs can have primary, secondary, and tertiary stages, although not all treatment plants have all stages. In primary treatment large or dense matter is removed, while in secondary treatment most organic matter is removed. The tertiary treatment stage may remove phosphate and nitrate. Before discharging treated wastewater, disinfection of pathogens and viruses may also

occur. The processes have different impacts on the fate of pharmaceuticals as the effluent passes through the WWTP. Pharmaceuticals, however, differ in molecules and structure, and therefore a treatment technology which has been found to have an effect on some medicines may not necessarily affect others in the same manner. For example, acetaminophen, caffeine and ibuprofen were removed by > 80%, while ciprofloxacin and ofloxacin removal was estimated to be between 50% and 80% in seven WWTPs in Xiamen City, China (Wang *et al.*, 2018). Conventional activated sludge (CAS) was observed to remove only 23.8-42.2% of trimethoprim , but the removal increased by roughly 40% after applying membrane bioreactor (MBR) treatment (Tran *et al.*, 2016). The physiochemical properties of pharmaceuticals have also been reported to affect the removal efficiency of pharmaceuticals (Tran *et al.*, 2016). Physiochemical properties of the detected pharmaceutical compounds in this study can be found in Table 2-1 in chapter 2.

The octanol-water distribution coefficient ($\log K_{ow}$) was found to influence the removal efficiency of antibiotics in biological WWTPs. For example, ciprofloxacin ($\log K_{ow}$ = -0.24) was removed by 76.6-92.4%, whereas the removal efficiency of trimethoprim ($\log K_{ow}$ = 0.91) was observed between 23.8 and 42.2% by Tran *et al.* (2016). The highest removal efficiency was achieved for trimethoprim in summer (76%) (Kosma *et al.*, 2014), possibly due to greater microbiological activity during hot seasons (Lajeunesse *et al.*, 2012). Other operational parameters like, solid retention time (SRT), hydraulic retention time (HRT) and weather conditions, such as temperature and rain, have also been found to affect the removal efficiency of pharmaceuticals in WWTPs (Kasprzyk-Hordern *et al.*, 2009; Luo *et al.*, 2014; Verlicchi *et al.*, 2012b). A longer SRT provides more time for the growth of microorganisms which leads to effective removal of pharmaceuticals (Couto *et al.*, 2019; Clara *et al.*,

2005). Elimination of pharmaceuticals in WWTPs includes three main processes: degradation, absorption and transformation, with biodegradation being the most effective removal strategy (Wang *et al.*, 2018; Zhang *et al.*, 2008).

3-3 The occurrence of sanitary parameters and trace pharmaceuticals in the investigated WWTPs

3-3-1 Wastewater characterization parameters

In this study, major wastewater quality parameters were also analysed. These parameters included chemical oxygen demand (COD), five-day biochemical oxygen demand (BOD5), pH, and total suspended solids (TSS). These parameters are commonly considered for wastewater regulation and are used as indicators of the performance of WWTPs (Lotfi *et al.*, 2019; Mjalli *et al.*, 2007). The levels of BOD and COD indicate the amount of organic matter content in the sewage effluent by measuring the amount of oxygen consumed by aerobic microorganisms as they degrade organic matter in the sewage (Dahamsheh and Wedyan, 2017). A high BOD or COD indicates a high oxygen demand which leads to reduced oxygen concentrations in the water body, which causes stress and even death in the aquatic organisms, including fish. TSS refers to the total amount of solid particles suspended in water, which is also an indication of the amount of organic and inorganic matter present. Table 3-2 shows the range and mean level of these parameters in the WWTP influents and effluents. Results show that the range of characteristics in the influent in all investigated WWTPs were between 7.2 and 8.02, 176 mg L^{-1} and 913 mg L^{-1}, 145 mg L^{-1} and 545 mg L^{-1} and 120 mg L^{-1} and 362 mg L^{-1} for pH, COD, BOD5 and TSS respectively (Table 3-2 and appendix Table A-3-1).

Table 3-2: Characterization parameters measured in the selected WWTP influents and effluents, at the time of sample collection.

WWTP		pH		COD (mg L^{-1})		BOD5 (mg L^{-1})		TSS (mg L^{-1})	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
H	Inlet	7.2-7.7	7.36	206-913	440.92	156-545	271.67	372-156	228.59
	Outlet	7.2-8	7.76	12-41	19.5	7-24	11.67	23-5	8.17
M	Inlet	7.2-7.6	7.35	186-612	462.83	206-416	297.67	136-362	254
	Outlet	7.4-8.1	7.73	10-28	16.58	7-10	8.25	4-8	6.75
KSU	Inlet	7.35-8.02	7.65	176-226	201.92	145-175	159.33	120-130	125.08
	Outlet	7.01-8.2	7.68	6.2-7.3	6.72	3.8-5.1	4.37	5-10	9.41

A comparison of these parameters for each WWTP showed similar mean concentrations in H- and M-WWTP influents ($p \geq 0.4$), which significantly exceeded the means measured in KSU-WWTP influent ($p \leq 2.0 \times 10^{-4}$), except for pH. Similarly, one-way ANOVA test indicated some significant differences between the values of these parameters in the three different WWTP effluents. COD and BOD5 were significant higher in H- and M-WWTPs than KSU-WWTP at $p \leq 3.0 \times 10^{-4}$ and $p \leq 2.9 \times 10^{-3}$ respectively.

3-3-2 Influent concentrations for trace pharmaceuticals in the studied WWTPs

Among the selected analytes, nine compounds (acetaminophen, atenolol, caffeine, cephalexin, ciprofloxacin, diclofenac, metformin, ofloxacin and trimethoprim) were detected in the influent of H- and M-WWTPs, whereas ten compounds (acetaminophen, atenolol, baclofen, caffeine, cephalexin, ciprofloxacin, diclofenac, metformin, ofloxacin and trimethoprim) were measured in KSU-WWTP influent samples collected over 12 months between March 2018 and July 2019 (Figure 3-1). These compounds were detected in the influents of the studied WWTPs at a frequency of detection between 100% and 8% (72 and 6 out of all collected samples). Acetaminophen, atenolol, caffeine, ciprofloxacin, diclofenac, metformin and ofloxacin and trimethoprim

were found more frequently (f≥75%) than other pharmaceuticals. Details of the occurrence of the selected pharmaceuticals are provided in appendix Table A-3-2. Ibuprofen, azithromycin and oxypurinol were detected below their LOQs or LODs in all samples. This could be attributed to the fact that azithromycin and ibuprofen are not among the most frequently prescribed and used pharmaceuticals in the investigated area, or due to the quite high LODs and LOQs for these compounds. Allopurinol, triclosan and tetracycline were not measured due to their very low recovery. Thus, these six compounds are not discussed further here.

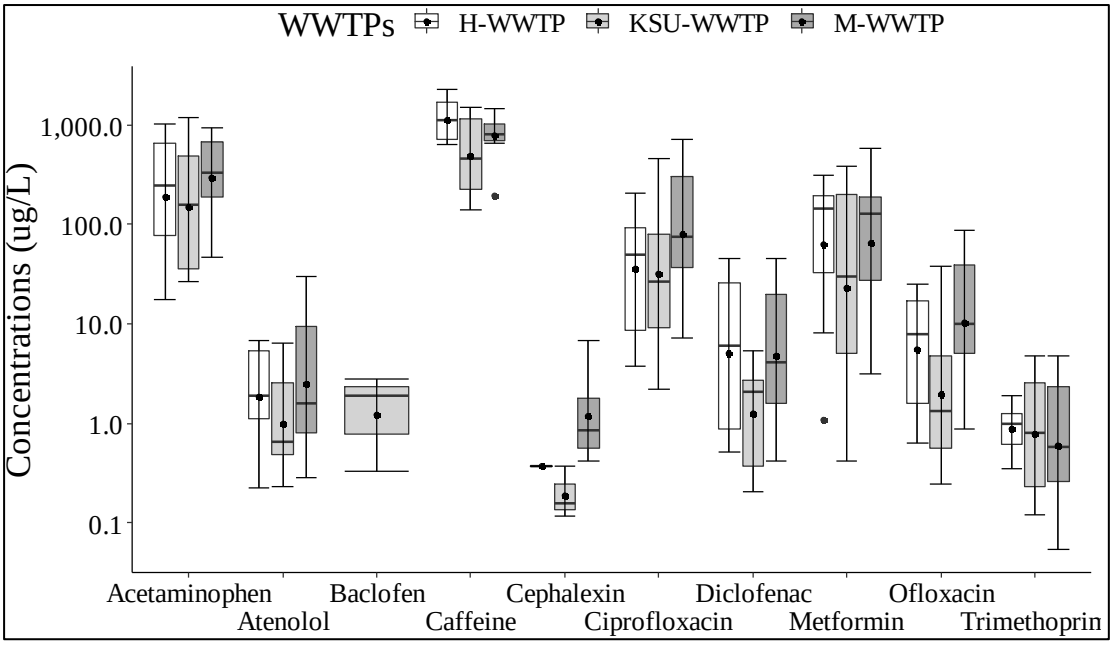


Figure 3-1: Box plot represent the median (vertical line in the box), mean is plotted as dot within the box, the quartiles one and three (bottom and top box lines), and the minimum and maximum (bottom and top whiskers) concentrations (µg L^{-1}, logarithmic scale), of detected pharmaceuticals in the studied WWTP influents. Each box plot represents 24 samples collected during sampling campaign.

The concentration of caffeine at 2281.6 µg L^{-1} (f=100%) measured in April 2018 at H-WWTP was the highest measured concentration of all compounds in the influent of these WWTPs, whereas the lowest detected concentration was 0.05 µg L^{-1} for trimethoprim in M-WWTP collected in June 2019. As expected, samples from M- and H-WWTP influents showed commonly higher average concentrations of selected

pharmaceuticals than samples from KSU-WWTP (Figure 3-1). These differences were mainly observed in acetaminophen, atenolol, caffeine, cephalexin, ciprofloxacin, diclofenac, metformin, and ofloxacin. This could be attributed to smaller size of KSU-WWTP compared with the other investigated plants.

Baclofen is reported here for the first time from Saudi Arabia and globally in WWTPs. It was detected only in three influent samples in KSU-WWTP and found to be below either its LOQ (0.37 μg L^{-1}) or LOD (0.12 μg L^{-1}) in the other investigated WWTPs (Figure 3-1). This may be because: (i) this plant received wastewater from the nearby King Khaled University Hospital (KKUH) directly without pre-treatment, and (ii) almost 80% of baclofen is excreted unchanged. It is to be expected that hospitals with a wide range of departments and facilities like KKUH will discharge a wide variety of pharmaceuticals because of the diversity of patient treatments (Oliveira *et al.*, 2017; Santos *et al.*, 2013).

Caffeine was measured at the highest concentration compared with other detected compounds in all three studied WWTPs at 2281.59, 1449.46 and 1497.60 μg L^{-1}, followed by acetaminophen at 1036.24, 942.99 and 1186.07 μg L^{-1} in H-, M- and KSU-WWTPs respectively. This is possibly due to the high consumption of coffee, tea and other soft drinks which contain caffeine (Kosma *et al.*, 2014; Kosma *et al.*, 2010). Also, another possible explanation for the high caffeine and acetaminophen concentration observed here could be due to their use in the formulation of pharmaceuticals to enhance the effects of certain analgesics, such as paracetamol treating coughs, colds, and headaches (Weigel *et al.*, 2002) Caffeine is considered as an indicator of anthropogenic contamination (Paíga and Delerue-Matos, 2017), and is often reported to have the highest concentration in many WWTP influent samples from studies worldwide. For instance, caffeine was observed at 2.28 μg L^{-1} in Greece (Kosma

et al., 2014), 62.6 μg L^{-1} in Slovenia (Česen *et al.*, 2018) and up to 63.97 μg L^{-1} in the influent of Coimbrão WWTP, Portugal (Paíga *et al.*, 2019).

Among the selected nonsteroidal anti-inflammatory drugs (NSAIDs), acetaminophen was measured at high concentrations followed by diclofenac at concentrations up to 1036.24 μg L^{-1} and 45.74 μg L^{-1}, respectively in the studied WWTP influents (Figure 3-1).

The concentration pattern of antibiotic compounds monitored in the influent of the investigated WWTPs were as follows: ciprofloxacin> ofloxacin> trimethoprim> cephalexin. Metformin was detected between 0.41 μg L^{-1} (KSU-WWTP) and 587.86 μg L^{-1} (M-WWTP) in the influent of these plants and the beta blocker agent (atenolol) was found at concentrations ranging from to 0.23 to 30.08 μg L^{-1}.

Nine pharmaceuticals out of the investigated compounds in this study have been previously reported in Saudi Arabia WWTPs (Al Qarni *et al.*, 2016; Alidina *et al.*, 2014; Shraim *et al.*, 2017). The average influent concentration of compounds detected in these plants were higher than those reported in previous studies in Saudi Arabia WWTPs (Table A-1-1) with the exception of cephalexin which was higher in the influent of KSA-WWTPs than the influent of H-WWTP.

Similarly, a global comparison between average concentrations of pharmaceuticals measured in the three WWTPs studied here and those reported in previous studies in different countries worldwide in Europe (Carmona *et al.*, 2017; Dasenaki and Thomaidis, 2015; Kasprzyk-Hordern *et al.*, 2009; Kosma *et al.*, 2010; Kosma *et al.*, 2014; Kot-Wasik *et al.*, 2016; Paíga *et al.*, 2019; Papageorgiou *et al.*, 2016; Papageorgiou *et al.*, 2019), Asia (Subedi *et al.*, 2017; Zhang *et al.*, 2018), Africa (Abdallah *et al.*, 2019; Moslah *et al.*, 2018), Middle East (Al-Mashaqbeh *et al.*, 2019; Semerjian *et al.*, 2018; Semreen *et al.*, 2019) and from America (Mohapatra *et al.*, 2016;

Rivera-Jaimes *et al.*, 2018) showed higher concentrations in this study, except for cephalexin and acetaminophen. A summary of global studies reporting the investigated pharmaceuticals in WWTP influents and effluents is available in appendix Table A-3-3. The variability in the occurrence of pharmaceuticals between different studies could be attributed to many factors such as consumption profiles, prescription regulations, climate conditions, population served and sampling schemes (Ali *et al.*, 2017; Tran *et al.*, 2018).

3-3-3 Effluent concentrations for trace pharmaceuticals in the studied WWTPs

Only five (acetaminophen, caffeine, ciprofloxacin, metformin and ofloxacin) out of 16 selected compounds were measured in the effluent of the investigated WWTPs at a frequency of detection from 58% to 11% (42 and 8 out of all collected samples). The highest concentration measured in the effluents of these WWTPs was 33.12 μg L^{-1} for ciprofloxacin in H-WWTP, while the lowest concentration was measured for acetaminophen at 0.017 μg L^{-1} in M-WWTP (Figure 3-2).

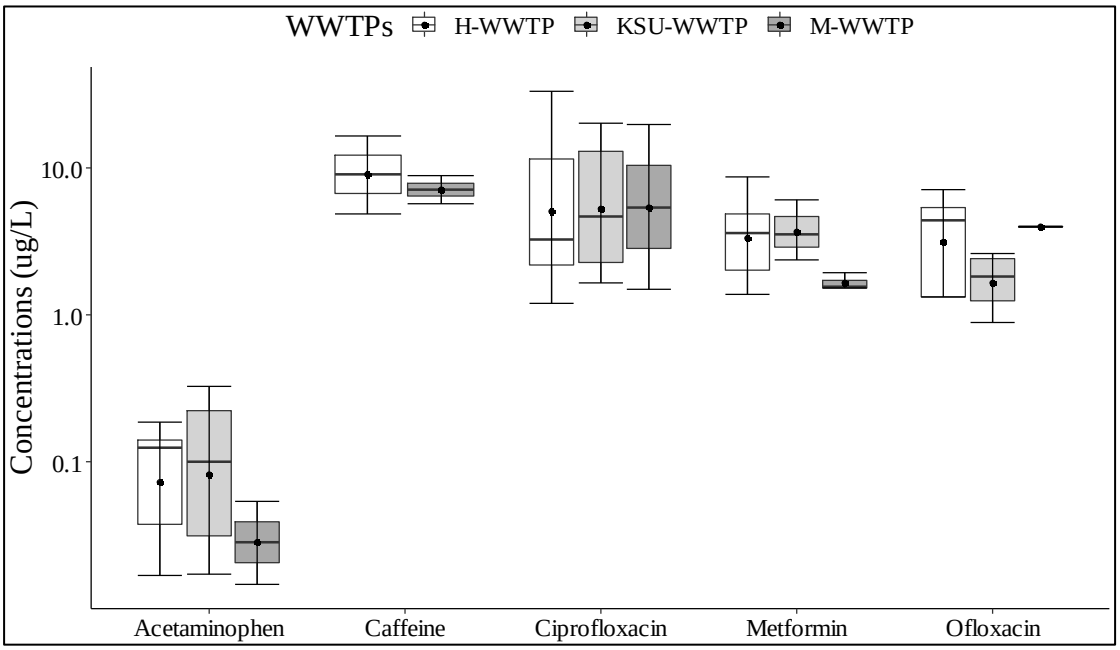


Figure 3-2: Box plot indicating the median, mean, the quartiles one and three, and the minimum and maximum concentrations (μg L^{-1}, logarithmic scale), of detected pharmaceuticals in the studied WWTP effluents.

The most predominant pharmaceutical, ciprofloxacin, was detected more frequently than other detected compounds in all studied WWTP effluent samples, particularly in H- and M-WWTPs. This might be due to its low removal rate (more detail about removal rate can be found in section 3-5-2-4). It was detected at concentrations ranging between 1.20 and 33.16, 1.49 and 19.92 and 1.64 and 20.07 μg L^{-1} in H-, M- and KSU-WWTPs respectively (Figure 3-2). In the present study, ciprofloxacin concentrations were considerably lower than the concentration reported in a WWTP effluent in India, which was between 28 and 31 mg L^{-1} (Larsson *et al.*, 2007).

The concentration of analgesics and anti-inflammatories (acetaminophen) were between 0.01 and 0.33 μg L^{-1} in the studied WWTP effluents (Figure 3-2). Other compounds were detected at concentrations between 4.92 and 16.56, 1.38 and 8.77, and 0.89 and 7.07 μg L^{-1} for caffeine, metformin and ofloxacin respectively in all three WWTP effluents.

In comparison with average pharmaceutical concentrations detected in previous studies from different WWTPs in Saudi Arabia, the average concentrations detected in this study were higher than those reported by Al Qarni *et al*. (2016), Alidina *et al*. (2014) and Shraim *et al*. (2017) except for cephalexin which was slightly higher in other studies (Table A-1-1). This potentially could be attributed to the pattern of dispensed and used pharmaceuticals in different locations, as well as the other WWTPs being smaller than those investigated here, apart from KSU-WWTP.

The detected pharmaceuticals have been previously reported worldwide in WWTP effluents. For example, 27 pharmaceuticals were monitored in two conventional WWTPs situated in the Po Valley, northern Italy by Al Aukidy *et al*. (2012) and from which they detected 20 pharmaceuticals in the wastewater effluent

samples collected. They reported that diclofenac and atenolol were detected at the highest concentrations, which were 800 ng L^{-1} and 474 ng L^{-1}, respectively. Also, they found that diclofenac, ciprofloxacin and atenolol were among the most frequently detected pharmaceuticals with a range in concentrations of 223-800 ng L^{-1}, 10-499 ng L^{-1} and 27-1168 ng L^{-1} respectively. Several other studies have also reported pharmaceuticals in WWTP effluent samples collected from Spain, Slovenia, Greece, and Iran WWTPs (Carmona *et al.*, 2017; Česen *et al.*, 2018; Dasenaki and Thomaidis, 2015; Aydin *et al.*, 2019). However, in comparison with these studies, the pharmaceutical residues measured in H-, M- and KSU-WWTP effluents were overall higher than other studies, particularly for caffeine. The exception was acetaminophen, for which the average concentration in the effluents of the WWTPs studied here was less than the average concentration found in the studies in Greece and Spain by Dasenaki and Thomaidis, (2015) and Carmona *et al*. (2017).

3-4 The influence of catchment on the occurrence of pharmaceutical compounds in the three investigated WWTPs

The two large WWTPs (H and M WWTPs) received wastewater from a large urban, industrial and hospital/clinic catchment area and discharged their final effluent into a dry canal which is mainly used for agricultural purposes and recharge of groundwater. The small KSU-WWTP treated wastewater from KSU main campus and its facilities, as well as from KKUH hospital catchment, which was a relatively small area. The final effluent was mainly used for irrigation of KSU area and for cooling power plants within KSU area.

Generally, the mean concentrations of detected compounds in these studied WWTP influents were observed to be slightly higher in the two large treatment plants (H and M WWTPs) than in the small size KSU-WWTP (Figure 3-9). The mean

concentrations varied between 0.37 μg L^{-1} and 1248.35 μg L^{-1}, 1.38 μg L^{-1} and 868.21 μg L^{-1}, and 0.22 μg L^{-1} and 656.44 μg L^{-1} for the detected compounds in H-WWTP, M-WWTP and KSU-WWTP influents, respectively. The same compounds were observed to be detected in the three WWTPs except for baclofen, which was only detected in KSU-WWTP influent possibly due to the wastewater received by this plant from KKUH. Moreover, the mean concentrations of therapy classes of the detected compounds in the three investigated WWTP influents were observed to follow the same pattern of: stimulant> NSAIDs> antidiabetics> antibiotics> b-blockers> muscle relaxants. Therefore, the catchment areas did not seem to affect the concentrations and kind of pharmaceuticals detected in these WWTPs. Also, most detected compounds showed no significant differences between their concentration in these different WWTP influents except for diclofenac, caffeine and ofloxacin concentrations in H and KSU WWTPs.

3-5 Evaluation of physical and chemical operation parameters and pharmaceuticals removal through the different WWTP prosses

The efficiency of the three WWTPs in removing pharmaceutical compounds in the influent, together with the influence of the operational parameters was evaluated by determining percentage elimination for wastewater quality parameters and pharmaceuticals using equation (1) in chapter 2 section 2-9-1.

3-5-1 Removal efficiency for sanitary parameters

The percentage reductions in COD, BOD5 and TSS were estimated to be high ≥92% in the studied WWTPs for the entire monitoring period (Figure 3-3). All three WWTPs systems had a high capability (RE ≥ 92%) for removing BOD5, COD and TSS from the wastewater, as shown in Figure 3-3 and appendix Table A-3-4. The average removal efficiencies for COD, BOD5 and TSS were between 95.26 and 96.66%, 95.30 and

97.25%, and 92.48 and 97.10%, respectively in H-, M- and KSU-WWTPs during the entire study (Figure 3-3). In general, KSU-WWTP > M-WWTP > H-WWTP for removing these parameters, except for TSS for which M-WWTP > H-WWTP > KSU-WWTP (Figure 3-3). The results obtained for the removal rate of COD, BOD5 and TSS in the studied WWTPs in this study are consistent or better than those reported in an earlier study of 11 WWTPs in Spain (Camacho-Muñoz *et al.*, 2012).

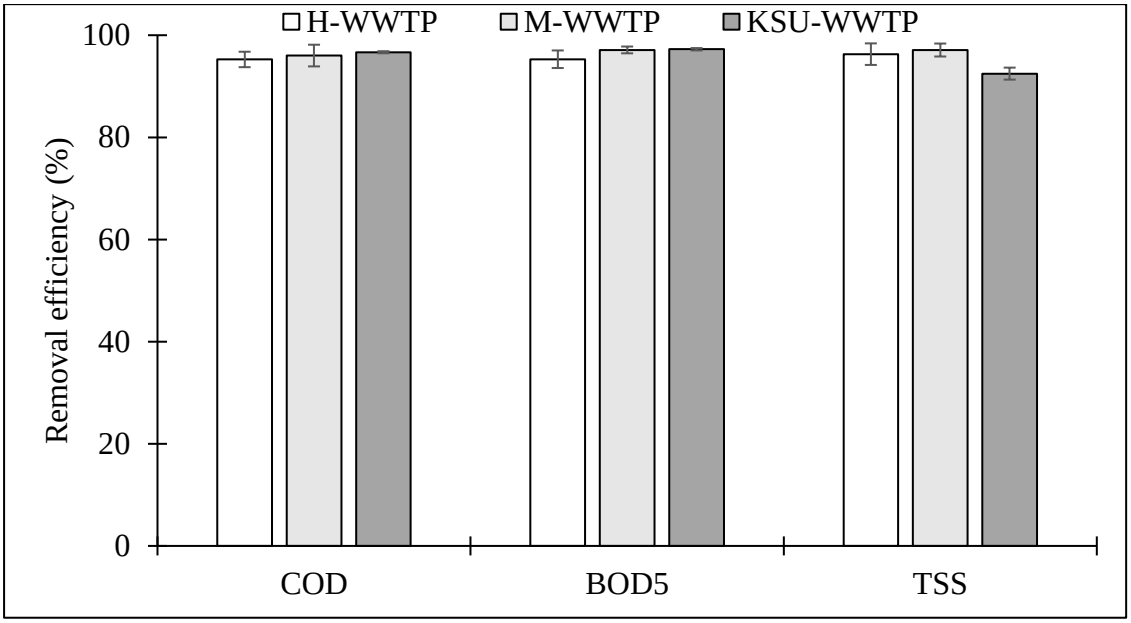


Figure 3-3: Mean removal efficiency (%, ±SD) observed for wastewater quality parameters in the studied WWTPs.

3-5-2 Removal of pharmaceutical residues by H-, M- and KSU-WWTPs

Figure 3-4 illustrates the mean removal efficiency of the measured pharmaceuticals in H-, M- and KSU-WWTPs. The average removal efficiency of the detected compounds in these WWTPs were observed to be high, between 80% and 99% (Figure 3-4). Acetaminophen had the highest mean removal efficiency followed by caffeine (≈99%) in all three WWTPs. Other studies have reported similar findings, with acetaminophen and caffeine being the most highly removed from biological treatment plants (95-98%) in China (Wang *et al.*, 2018), New Zealand (Kumar *et al.*, 2019), Saudi Arabia (Al

Qarni *et al.*, 2016), France (Thiebault *et al.*, 2017) and Greece (Kosma *et al.*, 2010). The lowest average removal efficiency observed here was for ofloxacin (65%) in KSU-WWTPs. Ciprofloxacin and ofloxacin were also reported to be removed less efficiently than other compounds by Wang *et al.* (2018), Tran *et al.* (2016) and Castiglioni *et al.* (2006). In a comprehensive study comparing the removal efficiency in different WWTPs in different geographical regions including Europe, USA and Asia, the removal efficiency of antibiotics was reported to be <40%, or even negative (Tran *et al.*, 2018). In general, KSU-WWTP showed an average removal efficiency slightly lower than the other investigated WWTPs for the detected pharmaceutical classes (Table 3-3).

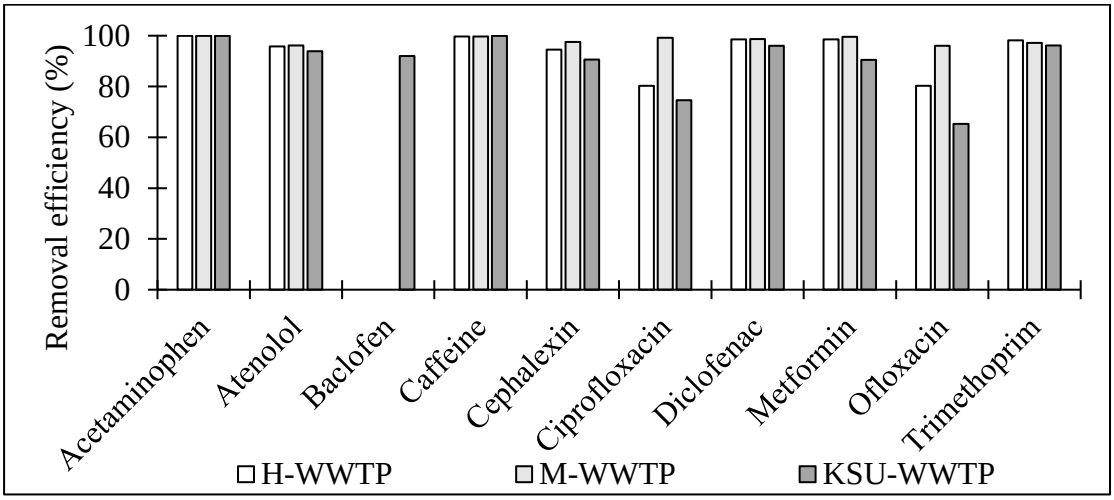


Figure 3-4: Mean removal efficiency (%) of detected compounds in three different WWTPs together with the average removal for all the WWTPs.

Table 3-3: Calculated average removal efficiency (RE%) of detected pharmaceutical classes in the investigated WWTPs.

WWTPs	NSAIDs	b-blockers	Muscle relaxant	Simulant	Anti-biotics	Anti-diabetics	Overall average (RE%)
H	99.24	95.76	N/A	99.72	88.32	98.52	**96.31**
M	99.33	96.19	N/A	99.65	97.45	99.52	**98.43**
KSU	97.96	93.83	91.96	99.88	81.68	90.45	**92.63**
Average (RE%)	**98.84**	**95.26**	**91.96**	**99.75**	**89.15**	**96.16**	**95.79**

As presented in Table 3-3, the average removal efficiency of the pharmaceutical classes in all three investigated WWTPs studied here followed the general order of: stimulant drugs > NSAIDs > antidiabetics > b-blockers > muscle relaxants > antibiotics, at average removal efficiencies of 99.75, 98.84, 96.16, 95.26, 83.48 and 80.27%, respectively. More details of the removal efficiency of the studied pharmaceuticals in the three WWTPs over the whole sampling campaign can be found in appendix Table A-3-5.

3-5-2-1 Removal efficiency for acetaminophen, diclofenac, and caffeine

The removal efficiencies of acetaminophen, caffeine and diclofenac compounds were similar in all three WWTPs. Almost complete removal (≤96%) of paracetamol, caffeine, and diclofenac was achieved (Figure 3-5).

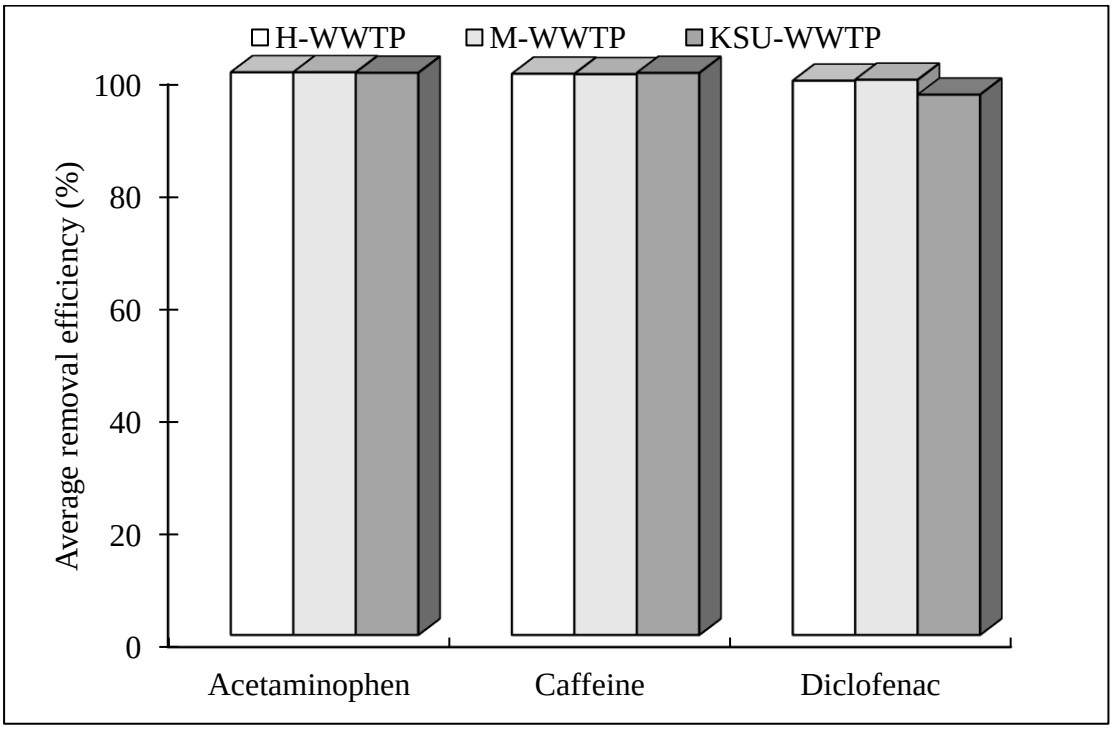


Figure 3-5: Average removal efficiency (%) of acetaminophen, caffeine, and diclofenac through studied WWTPs.

Removal efficiency for acetaminophen and caffeine have also been reported as high as 95% in eight WWTPs in Greece (Kosma *et al.*, 2014). The caffeine removal

found in this study is consistent with that reported in nine Slovene WWTPs, which was ≥95% (Česen *et al.*, 2018). In two hospital WWTPs, in Riyadh, Saudi Arabia, acetaminophen and caffeine were found to be eliminated at ≈99% (Al Qarni *et al.*, 2016). It is believed that adsorption onto sludge is the main factor controlling the removal of NSAIDs compound such as acetaminophen (Kosma *et al.*, 2014; Verlicchi *et al.*, 2012b; Stamatis and Konstantinou, 2013). Diclofenac showed low to negative removal efficiency in WWTPs in Greece (Kosma *et al.*, 2014), which is much lower than its removal efficiency estimated in this study at >90%. The variation in diclofenac removal efficiency could be attributed to its conjugated metabolites (glucuronide and sulfate) in the biological treatment (López-Serna *et al.*, 2013; Vieno and Sillanpää, 2014). It has been reported that diclofenac metabolites can undergo a rapid deconjugation during wastewater treatment and are consequently transformed to their parent compound (diclofenac) during the biological treatment process (Lee *et al.*, 2012).

Temperature has been reported to have a strong positive effect on the removal efficiency of some pharmaceuticals, possibly due to lower rates of microbial activity in cold months (Al Qarni *et al.*, 2016; Castiglioni *et al.*, 2006; Golovko *et al.*, 2020; Vieno *et al.*, 2005). For these compounds, a significant strong positive correlation was only observed between temperature and the removal efficiency of acetaminophen $r = 0.8$, $p = 4 \times 10^{-3}$ in H-WWTP.

3-5-2-2 Atenolol

Globally, atenolol removal ranges between <44% and 90% (Table 3-1). The average removal efficiency of atenolol in the three investigated WWTPs was between 96.19% and 93.82% with average of 95.76% (Figure 3-4). All the studied WWTPs achieved a high removal efficiency (≥93%) for atenolol, but the M-WWTP (oxidation ditch and sand filter) had the most efficient treatment at 96.19%, while the trickling filter and

sand filter configuration (KSU-WWTP) removed atenolol at 93.82%. The removal efficiency of atenolol has been estimated in batch bench-scale experiments, according to OECD Test No. 314 (OECD, 2008), during aerobic, anoxic and anaerobic treatments conditions by Inyang *et al*. (2016) . They concluded that aerobic degradation was the process that affected attenuation of atenolol the most. Al Qarni *et al*. (2016) found similar removal efficiency for atenolol (89%) in two biological WWTPs in Riyadh, Saudi Arabia.

Removal efficiency of atenolol showed some seasonal variation; it dropped by almost 10% in KSU-WWTP in autumn 2018 and spring 2019 (Figure 3-6). Similarly, the removal efficiency of atenolol in M-WWTP and H-WWTP decreased by 13% in spring and summer 2019, respectively.

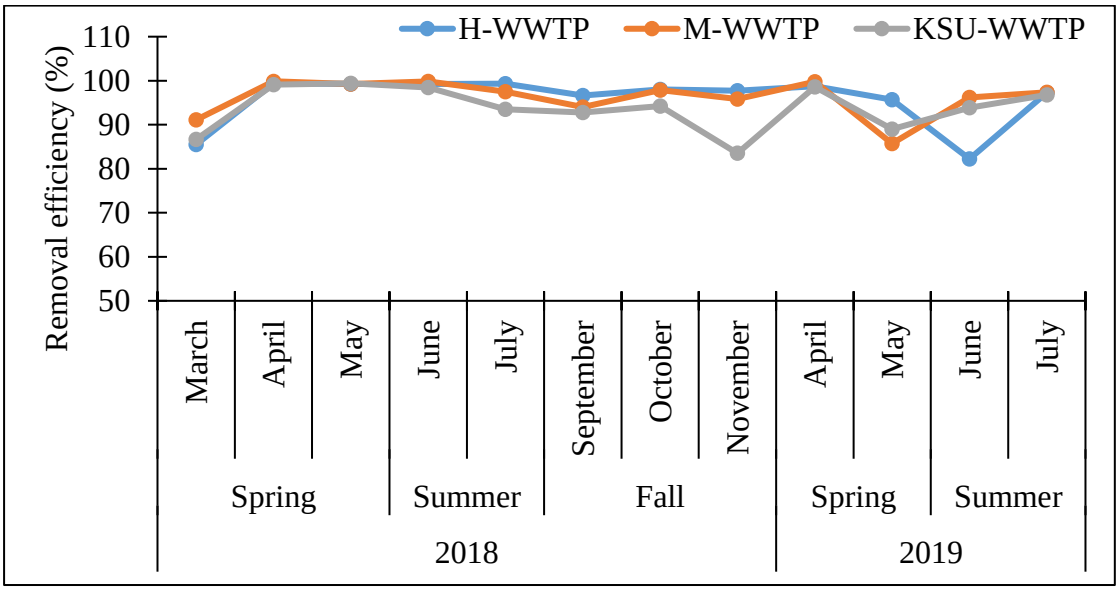


Figure 3-6: Atenolol removal efficiency (%) during different sampling seasons in three WWTPs.

3-5-2-3 Metformin

The average removal of metformin in the three investigated WWTPs during the monitoring period was 98.51% in H-WWTP and 99.51% in M-WWTP, but slightly less

in KSU-WWTP (90.45%), with an overall mean removal efficiency for the three studied WWTPs of 96.16% (Figure 3-4). The removal efficiency observed here was higher than that reported previously for some secondary WWTPs in France (Chiarello *et al.*, 2016) and USA (Oliveira *et al.*, 2015), which ranged between 35.4% and 79%. There are many factors that have been reported to play a role in the removal efficiency of pharmaceuticals from WWTPs, such as biodegradability (process where organic matter is eliminated by microorganisms like bacteria or/and fungi) physiochemical properties, and the configuration of WWTP. Biodegradation has been reported to be the main pathway for removing metformin by transforming it into guanylurea, but other reasons could also be considered such as adsorption to the sludge in biological WWTPs (Scheurer *et al.*, 2012; Kosma *et al.*, 2015). Secheurer *et al.* (2012) and Kosma *et al.* (2015) observed the removal of metformin was related to increased concentrations of its transformation product, guanylurea, in the effluents of five German WWTPs and eight WWTPs in Greece. Moreover, chlorination disinfection has been observed to be very effective at removing metformin in laboratory-scale experiments (Scheurer *et al.*, 2012). The chlorination disinfection used in the WWTPs studied here possibly played the main role in removing metformin. However, no significant difference was found in the elimination of metformin between the three WWTPs process (p-value > 0.05).

In order to examine seasonal trends in metformin removal, removal efficiency in 2018 (spring, summer and autumn) and 2019 (spring and summer) for autumn, winter, spring and summer were compared (Figure 3-7).

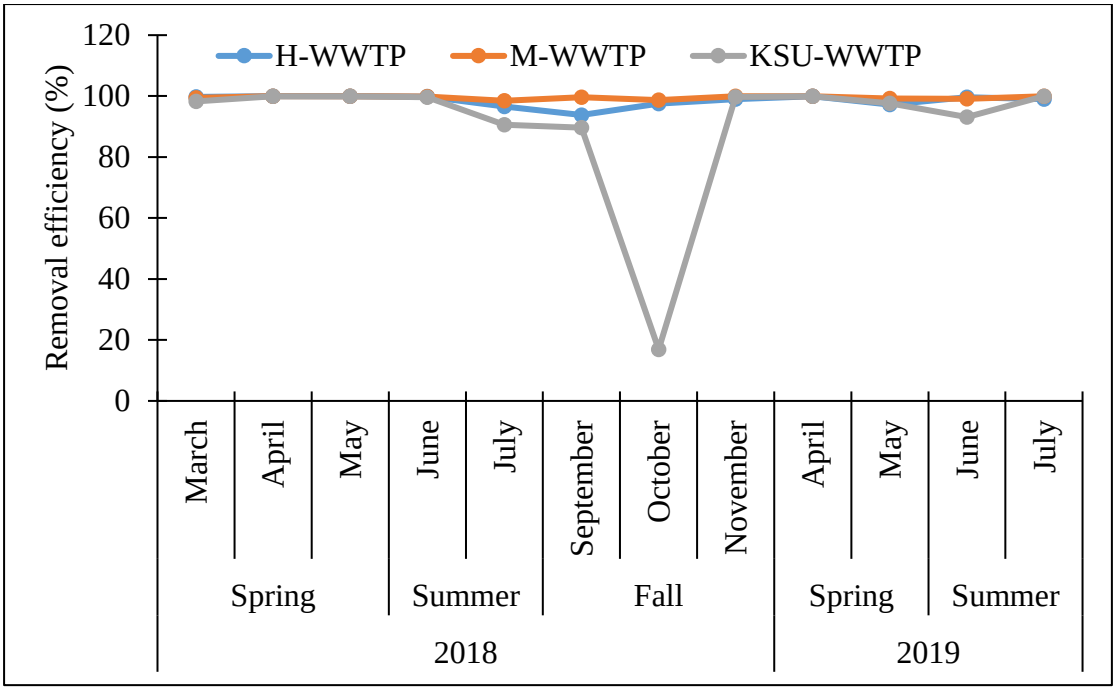


Figure 3-7: Seasonal removal efficiency (%) of metformin during 2018 and 2019 in three WWTPs.

There was no clear seasonal variation in the removal of metformin in the investigated WWTPs but, in October 2018, the removal of metformin in KSU-WWTP was notably decreased by about 80% (Figure 3-7). This was possibly due to many factors, such as:

1- A flow rate – it was the second lowest rate observed during sampling campaign (143 m^3 h^{-1}).

2- A possible mechanical fault or major maintenance activity on that date. It was not possible to confirm this after sampling because access restrictions were introduced.

3- Instrumental errors, such as column leak, tubes blook…etc.

It would have been interesting if the occurrence of guanylurea had been examined in this study, because it may have provided more information on the removal of metformin.

3-5-2-4 Antibiotic compounds

Antibiotics are considered a pseudo-persistent organic pollutant of global concern because they are consistently present in the environment (Tiwari *et al.*, 2017). Consumption and use of antibiotic pharmaceuticals were reported by Gelband *et al.* (2015) to have a grow by ≈ 20 billion standard units (SU) reaching 70 billion SU between 2000 and 2010 and will rise by 30% to 65% over the next 15 years across the globe (Gelband *et al.*, 2015). The significant spread of antibiotics into the environment from WWTP effluents are a major threat to public health due to global concerns about the development of antibiotic resistance in bacteria, leading to a potential reduction in their treatment effect against human and animal bacterial pathogens (Kumar *et al.*, 2019; Rizzo *et al.*, 2013; Kovalakova *et al.*, 2020). Conventional WWTPs using physical and biological treatment processes have variable removal efficiency for antibiotic pharmaceuticals. A high removal efficiency for these compounds could possibly play a main role in minimizing their potential impact on the environment. Kasprzyk-Hordern *et al*. (2009) reported WWTPs with trickling filters (TF), like KSU-WWTP, were not very effective at removing 55 pharmaceutical compounds including antibiotic trimethoprim compared with the conventional activated sludge (CAS) process. Antibiotic removal efficiency, including trimethoprim, was also found not to differ between membrane bioreactor (MBR) system and CAS system by (Radjenović *et al.*, 2009). It is believed that antibiotic removal efficiency not only depends on the WWTP configuration but also on other factors like physicochemical properties (Guerra *et al.*, 2014; Kosma *et al.*, 2014). According Ahmed *et al.* (2015) combining more than one treatment technology into an integrated system, such as MBR, activated sludge and an adsorptive process, possibly will remove poorly biodegradable compounds like antibiotics more efficiently (Ahmed *et al.*, 2015). The treatment configuration that is

effective in the elimination of antibiotics is reported to follow the order of: biological activated carbon > aerobic > MBR > anaerobic > constructed wetland > activated sludge (Ahmed *et al.*, 2017).

The mean removal efficiency of detected antibiotic pharmaceuticals (cephalexin, ciprofloxacin, ofloxacin and trimethoprim) in the three WWTPs under investigation have fluctuated between 65.29% and 99.13%, with average removal efficiencies between 90.65% and 97.50%, 74.63% and 99.13%, 65.29% and 96.03%, and 96.16% and 98.16% for cephalexin, ciprofloxacin, ofloxacin and trimethoprim in all three WWTPs respectively (Figure 3-8). A similar range in removal efficiency has also been reported in different countries (Tran *et al.*, 2016; Mirzaei *et al.*, 2019; Yan *et al.*, 2014). Surprisingly, the estimated removal efficiency for antibiotic compounds in M-WWTP was slightly higher than H-WWTP, although both WWTPs applied the same treatment processes, and higher than the trickling filter used in KSU-WWTPs, especially for cephalexin, ciprofloxacin and ofloxacin (Figure 3-8). The differences in the removal efficiency between M- and H-WWTPs could be due to H-WWTP being a new WWTP and was not in full operation during the sampling camping.

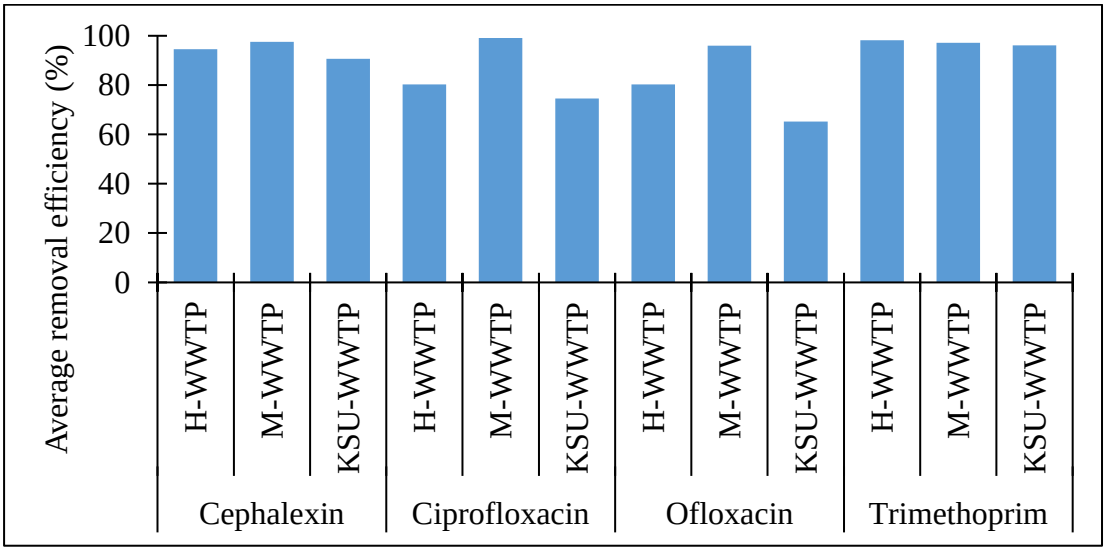


Figure 3-8: Average removal efficiency (%) of antibiotics detected in the investigated WWTPs.

The removal of trimethoprim was found to be almost the same in the three WWTPs, at average removal efficiency of 98.16%, 97.13% and 96.16% in H-, M- and KSU-WWTPs respectively (Figure 3-8). Cephalexin removal efficiencies were 97.50%, 94.57% and 90.65% in M-, H- and KSU-WWTPs respectively. These results were comparable with those reported in WWTPs equipped with CAS and TF in Iran (Mirzaei *et al.*, 2019), but much greater than the removal efficiency of 67% observed in WWTP using OD treatment process in China (Chen *et al.*, 2012). Ciprofloxacin removal was greater, by ≈20%, in M-WWTP than in the other two WWTPs studied here (Figure 3-7). Al Qarni *et al*. (2016) reported ciprofloxacin removal at 99% from two hospital WWTPs in Riyadh city, Saudi Arabia. The removal efficiency of ofloxacin in M-WWTP was higher than the removal efficiencies observed in H- and KSU-WWTPs, which were 96.03%, 80.26% and 65.29% respectively. Removal efficiency of ciprofloxacin and ofloxacin in seven WWTPs in China, applying oxidation ditch and others different configurations and technology, were reported to be at 66% and 58% respectively (Wang *et al.*, 2018). In contrast, ofloxacin and ciprofloxacin were negatively eliminated by -8.02% and -5.34% in a biological treatment plant (Ashfaq *et al.*, 2017a). The removal of trimethoprim found in this study is in good agreement with its removal efficiency in an activated sludge treatment plant in Greece, which achieved up to 98% removal (Papageorgiou *et al.*, 2016).

A significant relationship was found only between the removal efficiency of trimethoprim and temperature in M-WWTP at $r = 0.71$ and $p = 0.003$.

Seasonal variations in removal efficiency of the detected antibiotics can be seen in Figure 3-9. Variations were observed for ciprofloxacin and ofloxacin, particularly in KSU-WWTP. Decreases of 79% and 21% in removal efficiency of ciprofloxacin in summer and autumn 2018 respectively were observed in KSU-WWTP. Ofloxacin

showed a similar decline by 75% in autumn 2018 in KSU-WWTP. Similarly, the removal efficiency of H-WWTP ciprofloxacin was estimated to drop by 41% in spring 2019. In fact, ciprofloxacin showed negative removal in June 2018 with a removal efficiency of -48.04% in KSU-WWTP and ofloxacin also exhibited negative removal efficiency (-63.48%) in October 2018 for the same WWTPs (Table A-3-5).

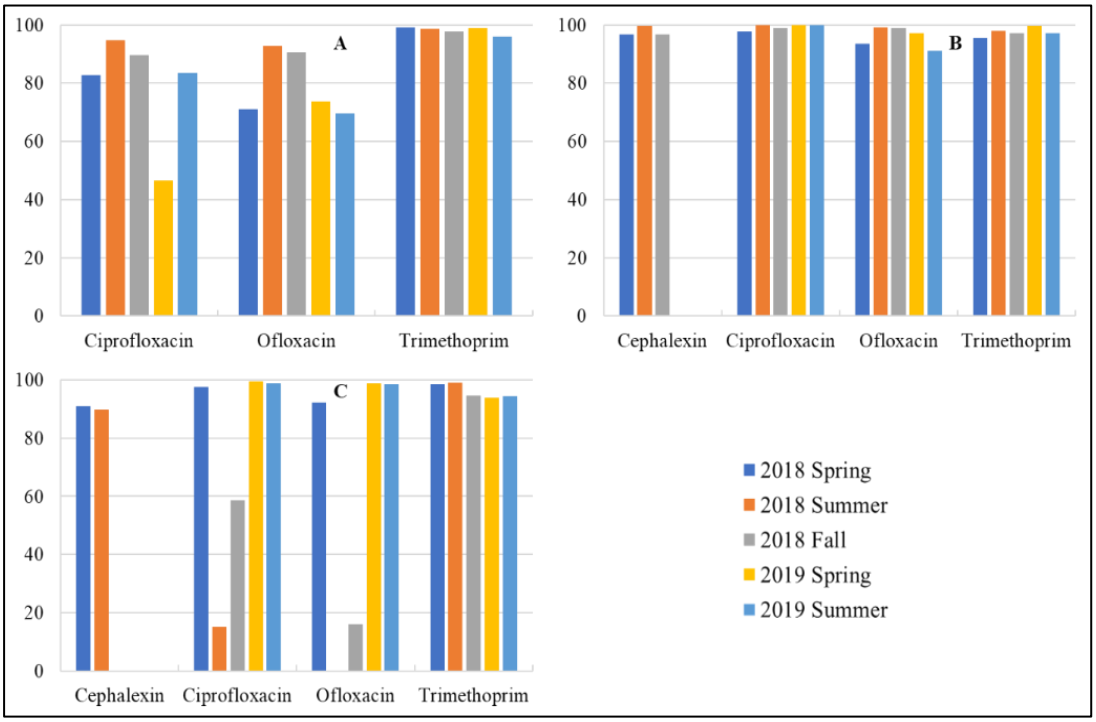


Figure 3-9: Seasonal removal efficiency (%) in (A) H-WWTP, (B) M-WWTP and (C) KSU-WWTP for the detected antibiotics.

Negative elimination has been reported elsewhere for some antibiotics. For instance, in a biological wastewater treatment process in the USA, trimethoprim, ciprofloxacin and ofloxacin were observed to have negative removal efficiency at -53, -88 and -124% respectively (Blair *et al.*, 2015). Negative removal is observed when an increase in the concentration of an analysed compound is measured in the outflow of the WWTP compared with the inflow. This phenomenon could occur due to transformation of pharmaceutical glucuronides and other conjugated metabolites, that are not detected in the influent, back into the initial parent compounds through the

biological processes applied in the WWTP (Verlicchi *et al.*, 2012b; Göbel *et al.*, 2005; Evgenidou *et al.*, 2015; Jelic *et al.*, 2011; Tran *et al.*, 2018). In addition, most antibiotics are excreted with urine and faeces and could be released gradually from faecal particles during biological treatment, leading to the phenomenon of increased concentrations in the effluent compared with the influent samples (Göbel *et al.*, 2007; Jelic *et al.*, 2011).

3-5-2-5 Baclofen

Baclofen is a gamma-aminobutyric acid (GABA) agonist prescribed as a skeletal muscle relaxant. Baclofen has been commonly used worldwide in neurology under commercial names Irex® or Lioresal® and prescribed to alleviate painful spasticity disorders (D'Orazio *et al.*, 2019). It has been particularly used for treating muscle spasticity associated with the spinal cord or cerebral injury since its introduction in 1967 (De Beaurepaire, 2018; Müller *et al.*, 2015; D'Orazio *et al.*, 2019). Recently, baclofen has been reported to be a useful treatment for reducing alcohol consumption and/or symptoms of alcohol withdrawal syndrome for alcoholic addicts by reducing alcohol cravings (De Beaurepaire, 2018; Liu and Wang, 2019). Negative effects of off-label baclofen use have been reported and the National Poison Data System (NPDS) of the USA has reported > 30% of all toxic exposures arose from baclofen misuse, and use in suicide attempts among adults in the USA (Kent *et al.*, 2020). Interestingly, around 46% of those who reported baclofen toxic exposure between 2014 and 2018 resulted from suicide attempts in USA (Reynolds *et al.*, 2020). Baclofen is rapidly adsorbed in the human body after oral administration and almost completely excreted (>80%) unchanged (Roberts *et al.*, 2015). Baclofen was only measured above its LOQ in three samples collected from the influent of KSU-WWTP, whereas for the other WWTPs (H and M) baclofen was either below its LOQ or LOD. No other reports of the presence of baclofen in WWTPs and of its removal efficiency during different

treatment processes could be found. In fact, as an absence of environmental data for baclofen was also reported by (Daughton, 2014).

The removal efficiency of baclofen was observed here to be between 81.33% and 97.82%, with an average of 91.96% (Figure 3-10, Figure 3-4). Baclofen removal increased during summer and autumn seasons, and the lowest removal occurred during the cold season (spring). Unfortunately, no previous studies have been conducted on the occurrence of baclofen in the environment with which these results can be compered. Therefore, more study is needed on the behaviour of this compound in the aquatic and terrestrial environments.

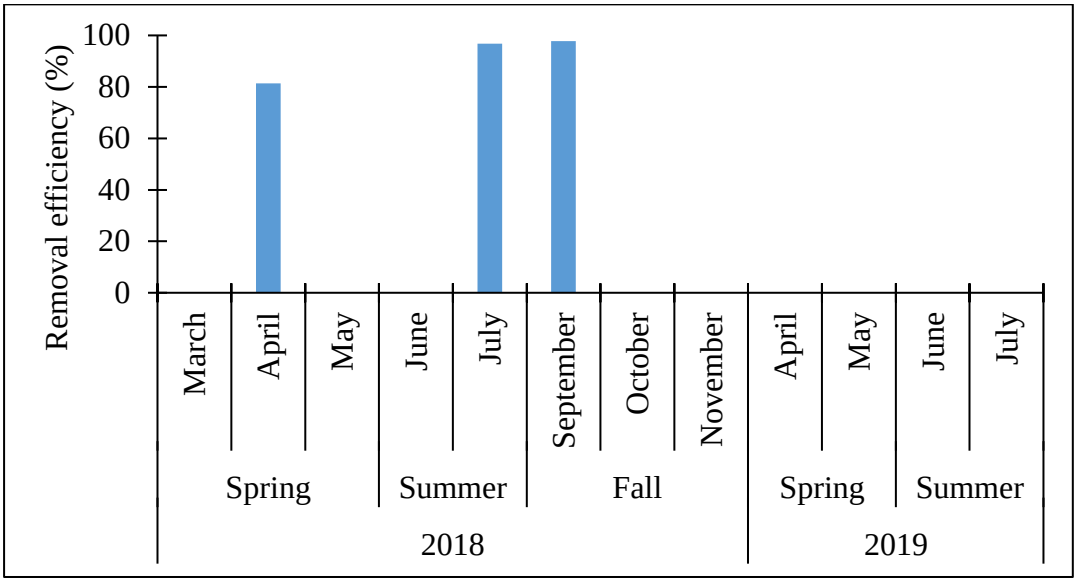


Figure 3-10: Baclofen removal efficiency (%) in KSU-WWTP during sampling period.

3-6 Mass loadings

The mass loadings of the inputs and outputs of the studied pharmaceuticals were estimated using the method described in chapter 2 section 2-9-3.

3-6-1 Comparison between influent mass loadings of detected pharmaceuticals in H-WWTP, M-WWTP and KSU-WWTP

For the three studied WWTPs the calculated mass loadings of detected pharmaceuticals in the influents were in the order of: KSU-WWTP< M-WWTP < H-WWTP. Table 3-4 shows the estimated average mass loading of the all detected compounds combined received by the studied WWTPs were 0.50, 41.43 and 41.81 mg d^{-1} 1000 inhabitants in KSU, M and H WWTPs, respectively. The range, average and total mass loading of all detected compounds in the WWTP influents are depicted in Table 3-4. H-WWTP and M-WWTP received similar total mass loadings (4513.351 and 4469.947 mg d^{-1} 1000 inhabitants respectively) for all detected pharmaceuticals, which is significantly higher than the total mass loadings estimated for KSU-WWTP influent (59.296 mg d^{-1} 1000 inhabitants), at *p*-value of 1.51×10^{-5} and 1.86×10^{-5} respectively (Figure 3-11). This is possibly due to H- and M-WWTPs serving a greater population than KSU-WWTP which are 1.15×10^{6}, 1.18×10^{6}, and 6.5×10^{4} PE, respectively. Verlicchi *et al.* (2012a) and Huber *et al.* (2016) suggest that the amount of pharmaceuticals entering WWTPs is controlled by many factors, like the number of people served by the WWTP (greater populations produce more waste, which contains more organic pollution) and the type of wastewater pumped to the WWTP. The highest mean loading was observed for caffeine at 255.19, 191.72 and 2.77 mg d^{-1} 1000 inhabitants in H, M and KSU WWTP influents, respectively.

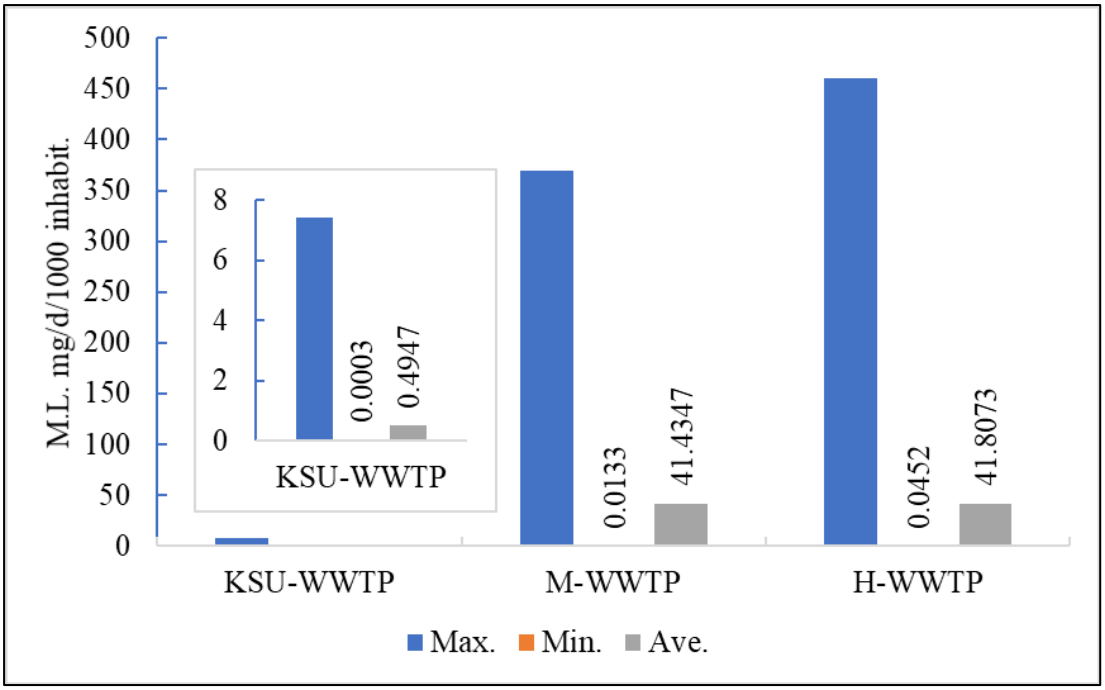


Figure 3-11: Range, average and total mass loading (mg d^{-1} 1000 inhabitants) of all detected compounds in the three investigated WWTP influents.

Table 3-4: Range, mean and total mass loading (mg d^{-1} 1000 $inhabtants^{-1}$) estimated at three selected WWTPs influent for the detected pharmaceuticals.

Compounds	H-WWTP Influent (mg d^{-1} 1000 $inhabtants^{-1}$)				M-WWTP Influent (mg d^{-1} 1000 $inhabtants^{-1}$)				KSU-WWTP Influent (mg d^{-1} 1000 $inhabtants^{-1}$)			
	Range		Ave.	Total	Range		Ave.	Total	Range		Ave.	Total
	Max.	Min.			Max.	Min.			Max.	Min.		
Acetaminophen	173.30	3.81	74.90	898.78	212.65	11.65	95.96	1151.59	5.09	0.05	1.39	16.63
Atenolol	1.08	0.05	0.56	6.68	7.66	0.07	1.65	18.11	0.03	0.0004	0.01	0.08
Baclofen	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01	0.002	0.01	0.02
Caffeine	460.60	125.47	255.19	3062.23	369.22	42.63	191.72	2300.59	7.41	0.36	2.77	33.22
Cephalexin	0.11	0.11	0.11	0.11	1.21	0.11	0.42	1.68	0.002	0.0005	0.001	0.004
Ciprofloxacin	38.39	1.09	13.46	161.51	132.18	1.76	39.79	477.44	2.29	0.01	0.36	4.37
Diclofenac	7.83	0.09	2.72	32.60	8.11	0.10	2.45	29.39	0.03	0.001	0.01	0.09
Metformin	59.77	0.32	27.12	325.47	130.11	0.77	35.62	427.43	1.34	0.002	0.37	4.48
Ofloxacin	5.37	0.18	2.02	24.23	15.80	0.22	5.01	60.09	0.19	0.0011	0.03	0.34
Trimethoprim	0.32	0.07	0.19	1.75	1.22	0.01	0.30	3.65	0.02	0.0003	0.01	0.07
Grand values	**460.60**	**0.05**	**41.81**	**4513.35**	**369.22**	**0.013**	**41.43**	**4469.95**	**7.41**	**0.0003**	**0.50**	**59.30**

The caffeine mass loading was significantly higher in H-WWTP than KSU-WWTP (p = 8.55×10^{-9}), and in M-WWTP than KSU-WWTP (p = 1.33×10^{-6}). The second highest average mass loading in these WWTP influents was acetaminophen followed by ciprofloxacin in M-WWTP and metformin in H- and KSU-WWTP. Mean mass loadings of acetaminophen were reported at 74.90, 95.96, and 1.39 H-, M- and KSU-WWTP influents respectively, whereas ciprofloxacin average mass loadings estimated at 39.79 and metformin at 27.12, and 0.37 mg d^{-1} 1000 $inhabtants^{-1}$ in M-, H- and KSU-WWTP influents respectively (Table 3-4). Acetaminophen average mass loading in the influents were significantly higher in M-WWTP than for KSU-WWTP with p-value of 0.001. In general, average mass loadings of the detected compounds in the investigated WWTP influents were observed to follow the order: caffeine> acetaminophen> ciprofloxacin> metformin> ofloxacin> diclofenac> atenolol> cephalexin> baclofen> trimethoprim. As expected, average mass loadings of detected compounds in H- and M-WWTP influents were far greater than for KSU-WWTP, except for baclofen. The lowest average mass loading in any of these WWTPs influents were for cephalexin at 0.11 and 0.001 mg d^{-1} 1000 inhabitants and trimethoprim at 0.3 mg d^{-1} 1000 inhabitants for in H, KSU and M WWTPs respectively (Table 3-4). Other compounds were estimated to have an average M.L. between 0.01 and 39.79 mg d^{-1} 1000 inhabitants in the three WWTP influents.

The average mass loading for the six selected pharmaceutical classes were also estimated for the influents as shown in Figure 3-12. They ranged between 0.56 and 255.19, 1.65 and 191.72 and 5.9×10^{-3} to 2.77 mg d^{-1} per 1000 inhabitants in H-, M- and KSU-WWTP influents, respectively. The H-WWTP and M-WWTP influent average mass loading were greater than those for the KSU-WWTP for all classes (Figure 3-12). This could be attributed to the similarity in size and the source of wastewater received

by H-WWTP and M-WWTP. The KSU-WWTP is far smaller and has a different population size than H-WWTP and M-WWTP (Česen *et al.*, 2018). Another, possible explanation of variations in mass loadings could be due to the differences in sampling protocols, i.e. (grab sampling for KSU-WWTP) and time-proportional for H- and M-WWTPs as proposed by (Česen *et al.*, 2018).

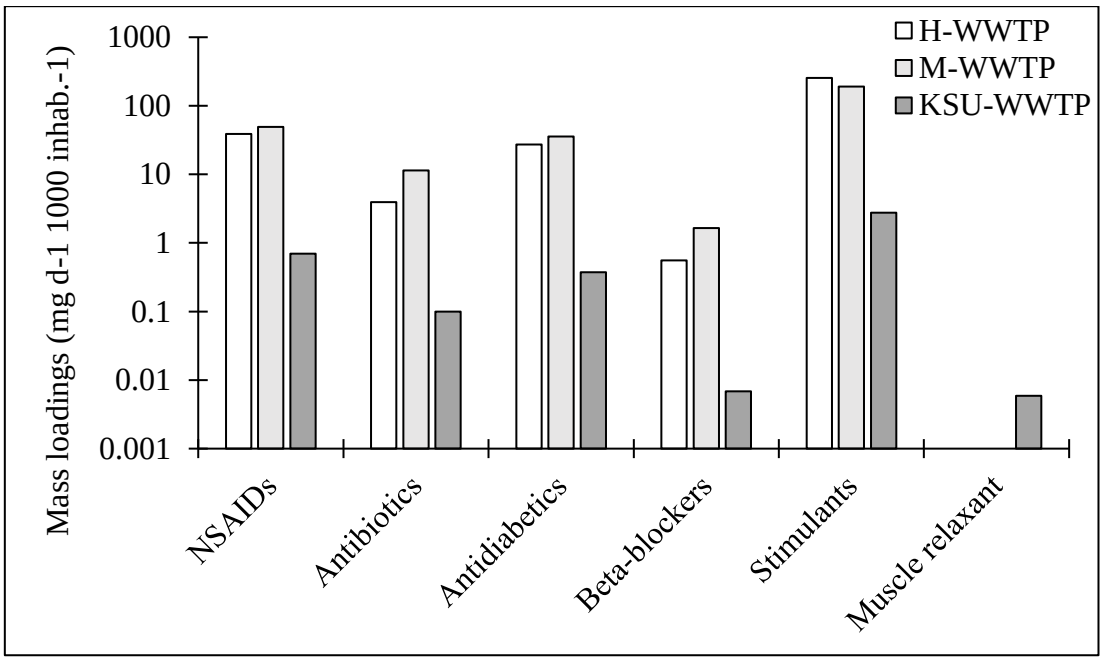


Figure 3-12: Average mass loading (mg d^{-1} 1000 $inhabitants^{-1}$) of pharmaceutical therapeutic classes in the investigated WWTP's influents (logarithmic scale).

In all WWTPs, the highest average mass loading was found for stimulants (Figure 3-12). The descending order of M.L. were the same for all three WWTPs: stimulant > analgesics/anti-inflammatories > antidiabetics > antibiotics > beta-blockers > muscle relaxant. Mass loading of baclofen was estimated between 0.002 and 0.01 with a mean of 0.01 mg d^{-1} per 1000 $inhabitants^{-1}$ in KSU-WWTP influent.

In general, the daily mass loading found in these WWTP influents are lower than, or similar to, those reported in the scientific literature (Papageorgiou *et al.*, 2016; Subedi *et al.*, 2015; Česen *et al.*, 2018; Pereira *et al.*, 2015; Pereira *et al.*, 2016) (Table 3-5). Regarding baclofen mass loadings, no reports of baclofen mass loading in WWTP nationwide or globally have been found with which to make a comparison.

Table 3-5: Range, average and total detected compound mass loading (mg d^{-1} per 1000 inhabitants) found in some studies worldwide.

Compounds	Influent			Effluent			References
	Min.	Max.	Ave.	Min.	Max.	Ave.	
Acetaminophen	12	23580.3	3223.73	11	80.4	147.98	1,3,4,5,6
Atenolol	49	551	407	53	407	187	1,5,6
Baclofen	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Caffeine	21	1323	3853	6	209	974.75	1,6,2
Ciprofloxacin	13	10439.1	454.33	N.D.	836.5	105.8	1,3,4,5
Diclofenac	2	1190	97.14	3.2	541	93.53	1,3,4,5
Ofloxacin	6.3	1292	N/A	23	42	N/A	5
Trimethoprim	8.2	48.3	26.5	3	36	10.74	1,5,6

1 (Papageorgiou et al., 2016), 2 (Česen et al., 2018), 3 (Pereira et al., 2015), 4 (Pereira et al., 2016), 5 (Santos et al., 2013), 6 (Subedi et al., 2017).

3-6-2 Comparison between mass loading discharged by H-WWTP, M-WWTP and KSU-WWTP effluent

Overall, during all sampling campaigns five out of the 16 compounds under investigation (acetaminophen, ciprofloxacin, ofloxacin, metformin and caffeine) were quantified in H, M and KSU WWTP effluents. The predominant detected compounds in the effluent of H-WWTP and KSU-WWTP were ciprofloxacin (*f*= 91.67% and 75% respectively) and metformin (*f*= 25%) in M-WWTP.

The daily mass loading discharged were estimated between 2.8×10^{-3} and 7.41, 3.7×10^{-3} and 3.83 and 1.0×10^{-4} and 0.05 mg d^{-1} 1000 inhabitants for H-, M- and KSU-WWTP effluents, respectively. The range, average and total mass loading of individual detected compounds in the effluents are given in Table 3-6. As for the influents, the total mass loading of all detected pharmaceuticals in this study in the effluents at H- and M-WWTPs were higher than that calculated for KSU-WWTP effluents during the sampling period.

H-WWTP was found to discharge a total mass loading of all detected compounds of 36.11 mg d^{-1} 1000 inhabitants, which is almost four-fold greater and

more than 120 times greater than total mass loadings for M-WWTP (9.1 mg d^{-1} 1000 inhabitants) and KSU-WWTP (0.29 mg d^{-1} 1000 inhabitants) effluents (Table 3-6).

Table 3-6: Range, average and total mass loadings (mg d^{-1} 1000 inhabitants) (n=24) discharged of measured pharmaceuticals in the effluent of three WWTPs in Saudi Arabia.

WWTPs		Acetaminophen	Caffeine	Ciprofloxacin	Metformin	Ofloxacin	Total mass loadings
H	Max.	0.05	5.13	7.41	1.65	1.41	**36.11**
	Min.	2.8×10^{-3}	0.14	0.011	0.02	0.06	
	Ave.	0.02	2.23	1.73	0.62	0.35	
	Total	0.10	6.70	20.73	4.34	4.24	
M	Max.	0.01	1.51	3.83	0.43	0.76	**9.08**
	Min.	3.7×10^{-3}	0.11	8.5×10^{-3}	0.02	0.08	
	Ave.	7.1×10^{-3}	0.97	0.60	0.16	0.42	
	Total	0.01	2.90	4.21	1.11	0.84	
KSU	Max.	1.4×10^{-3}	3.3×10^{-3}	0.05	0.02	6.8×10^{-3}	**0.29**
	Min.	1.0×10^{-4}	9.0×10^{-4}	3.0×10^{-4}	2.0×10^{-4}	9.0×10^{-4}	
	Ave.	5.0×10^{-4}	1.9×10^{-3}	0.02	4.2×10^{-3}	3.1×10^{-3}	
	Total	2.3×10^{-3}	0.02	0.20	0.04	0.02	

Notably, the highest individual total mass loading discharged into the environment from these WWTPs was ciprofloxacin at 20.73, 4.21 and 0.20 mg d^{-1} 1000 inhabitants in H-, M- and KSU-WWTP respectively (Table 3-6). This could be attributed to a low removal rate, or even negative rate observed in some samples for this compound. This is discussed in more detail in 3-5-2-4. The estimated total mass loadings were found to follow the pattern of ciprofloxacin> caffeine> metformin> ofloxacin> acetaminophen in H- and M-WWTP effluents while in the KSU-WWTP effluent it was ciprofloxacin> metformin> caffeine> ofloxacin> acetaminophen.

The daily mass loading found in the present study for some compounds like acetaminophen and caffein are lower than those reported in two WWTPs located in the State of Karnataka, southern India, where values ranging between 120 and 3030 mg d^{-1} per 1000 people were reported (Subedi *et al.*, 2017). The daily mass loading found in the present study also indicate lower levels of caffeine, acetaminophen and

ciprofloxacin than those reported in three WWTPs located in Volos, Greece by (Papageorgiou *et al.*, 2016).

Concern has been raised about the potential environmental effect of antibiotics (Larsson, 2014; Topp *et al.*, 2017). This suggests potential adverse ecological effects may be caused by ciprofloxacin persistence in the WWTP effluents studies here. Compounds detected in the influent and effluent of WWTPs could be considered as pollutants that potentially pose an environmental risk. This is discussed in more detail in chapter 5.

Table 3-7: One-way ANOVA test result of significant differences between mass loadings of detected pharmaceuticals in the effluent of three WWTPs. Significant relationships are in bold.

Pharmaceuticals	WWTPs		*p*-values
Acetaminophen	H-WWTP	M-WWTP	0.996804
		KSU-WWTP	**1.33×10^{-4}**
	KSU-WWTP	M-WWTP	**1.06×10^{-4}**
Caffeine	H-WWTP	M-WWTP	**0.01**
		KSU-WWTP	**0.001**
	KSU-WWTP	M-WWTP	0.78
Ciprofloxacin	H-WWTP	M-WWTP	0.14
		KSU-WWTP	**0.02**
	KSU-WWTP	M-WWTP	0.58
Metformin	H-WWTP	M-WWTP	**3.75×10^{-4}**
		KSU-WWTP	**5.27×10^{-6}**
	KSU-WWTP	M-WWTP	0.32
Ofloxacin	H-WWTP	M-WWTP	0.82
		KSU-WWTP	**0.01**
	KSU-WWTP	M-WWTP	**0.002**

In general, the daily mass loading of detected compounds in KSU-WWTP effluent were significantly less than the daily mass loading in H- and/or M-WWTP effluents at *p*-values between 5.27×10^{-6} and 0.02 (Table 3-7). This may reflect the small size of this plant compared with two larger WWTPs (M and H). Although H- and M-WWTPs are similar, the mass loadings discharged from these WWTPs are significantly different. For instance, the mass loadings of caffeine and metformin were significantly

higher in H-WWTP than M-WWTP effluents at *p*-values of 0.01 and 5.27×10^{-6} respectively. It could be due to H-WWTP was not fully function during sampling campaign.

3-7 Seasonal variations in the concentrations of selected pharmaceuticals in the influents and effluents of the three WWTPs

Seasonal variations in the detected pharmaceuticals in the influent and effluent of the three investigated WWTPs were estimated using the method of Di Marcantonio *et al.* (2020). More details about the methodology can be found in Chapter 2 section 2-9-2.

It is necessary to bear in mind that several factors might be responsible for the seasonal variation in pharmaceutical concentrations in WWTPs. These factors include consumption of pharmaceuticals, excretion rate, temperature (microbial activity), sunlight, dilution, flow rate, rainfall, WWTP configuration, and the influence of tourists (Pereira *et al.*, 2015; Sun *et al.*, 2014; Česen *et al.*, 2018).

3-7-1 Influent seasonal variations

Figure 3-13 illustrates seasonal variation in the influent concentration for detected pharmaceuticals in H-, M- and KSU-WWTPs. The data are given in appendix Table A-3-6. Seasonal variation was observed in the WWTP influents but was not as clear in the wastewater effluents, possibly due to levels of detection of some of the pharmaceuticals being below LOQ or LOD.

The seasonal variation in NSAIDs in the influents during the cold to moderate seasons (spring 2019) possibly reflects their increased consumption pattern in these seasons, whereas during the summer season 2019 they showed a decrease in the influent (Figure 3-13).

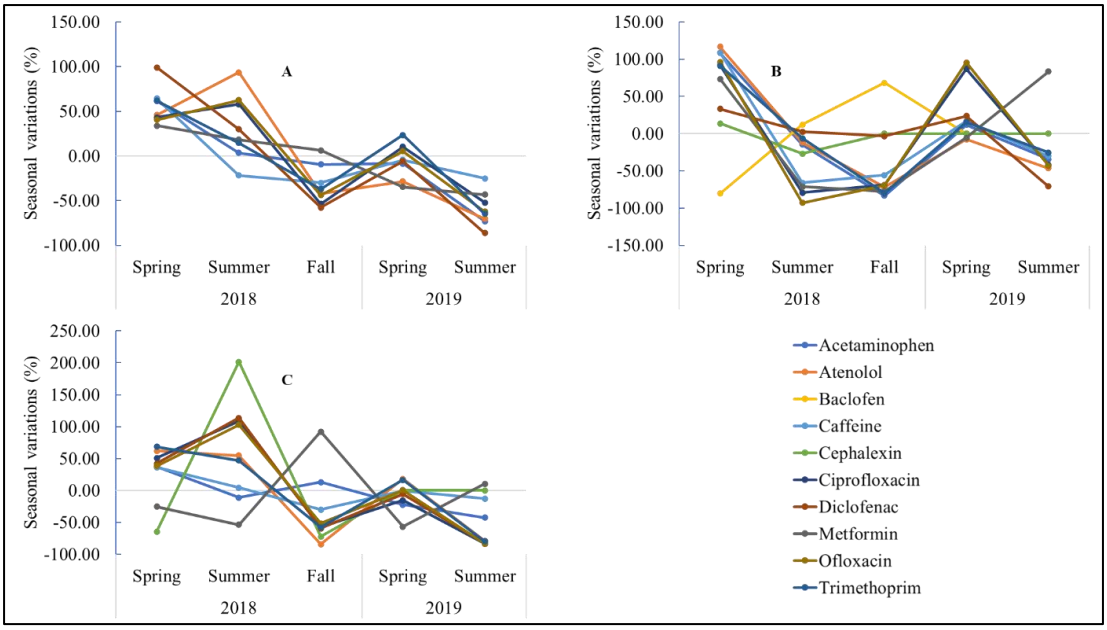


Figure 3-13: Seasonal variations as the percentage deviation of specific seasons from the average full sampling campaign (%) of target analytes in the influent of H-WWTP (A), KSU-WWTP (B) and M-WWTP (C).

It is worth noting that ofloxacin, ciprofloxacin, atenolol, diclofenac, and trimethoprim had almost the same seasonal variation pattern in H-WWTP influent except for summer 2018. Diclofenac and trimethoprim declined through summer and fall 2018 (Figure 3-14). This finding is in a good agreement with the strong significant positive correlation between ofloxacin, ciprofloxacin, atenolol, diclofenac, and trimethoprim concentrations at H-WWTP influents which was between 0.78 and 0.96 at $p \leq 0.003$ (Figure 3-15). The correlation found between concentrations of ciprofloxacin and ofloxacin in this plant were largely consistent with those observed between the same compounds ($r > 0.65$) in seven WWTPs influent, China (Wang *et al.*, 2018).

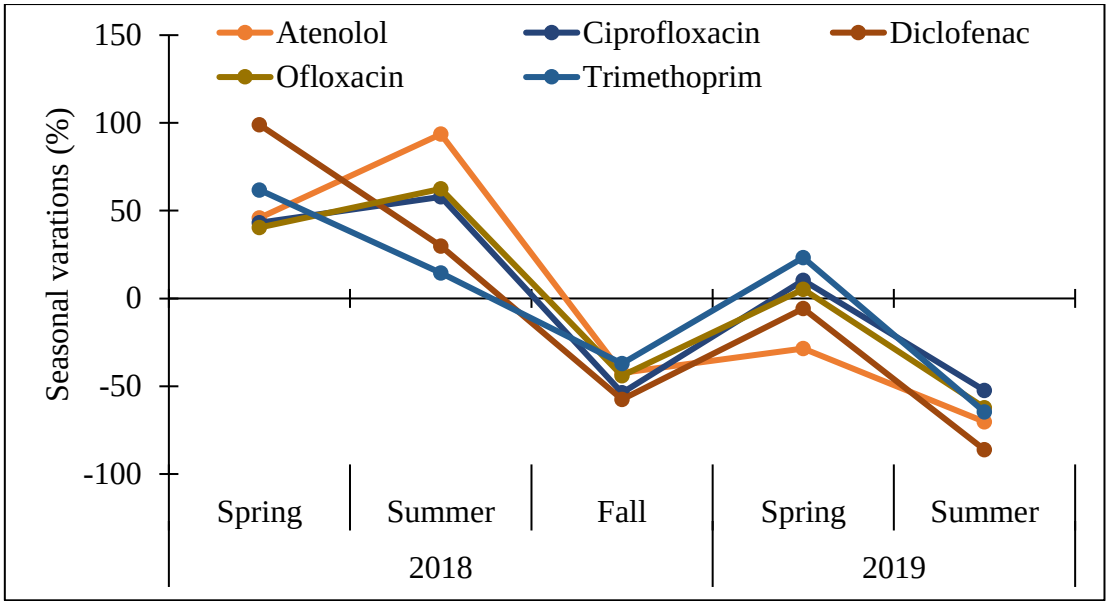


Figure 3-14: Seasonal variation as the percentage deviation of specific seasons from the average full sampling campaign (%) of atenolol, diclofenac, trimethoprim, ciprofloxacin and ofloxacin in H-WWTP influent.

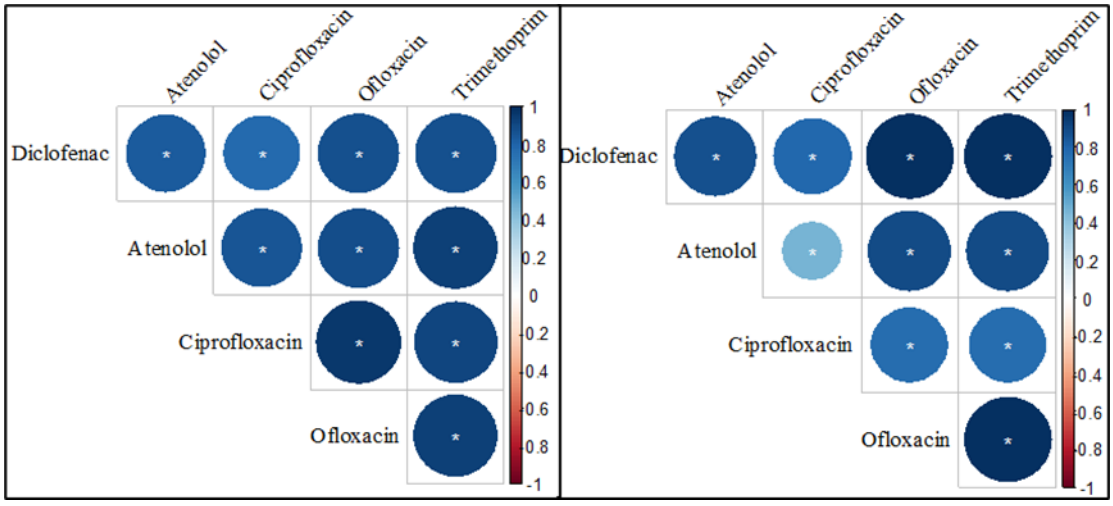


Figure 3-15: A correlation matrix between detected compound concentrations using Pearson's correlation for the H-WWTP (left) and M-WWTP (right) influents. Values significant at $p \leq 0.05$ are indicated *. Size of circle indicates strength of r value, while colours represent whether a positive or negative correlation.

The seasonal variations of ciprofloxacin, ofloxacin, diclofenac, trimethoprim, and atenolol concentrations in M-WWTP influent were found to follow the same pattern as in H-WWTP (Figure 3-16), possibly because H- and M-WWTPs received wastewater from the same sewer system. Also, the correlation obtained between the influent concentrations of these compounds in M-WWTP, were the same as correlation

coefficients for the same pharmaceutical compounds in H-WWTP which were a significant strong positive correlation (Figure 3-15).

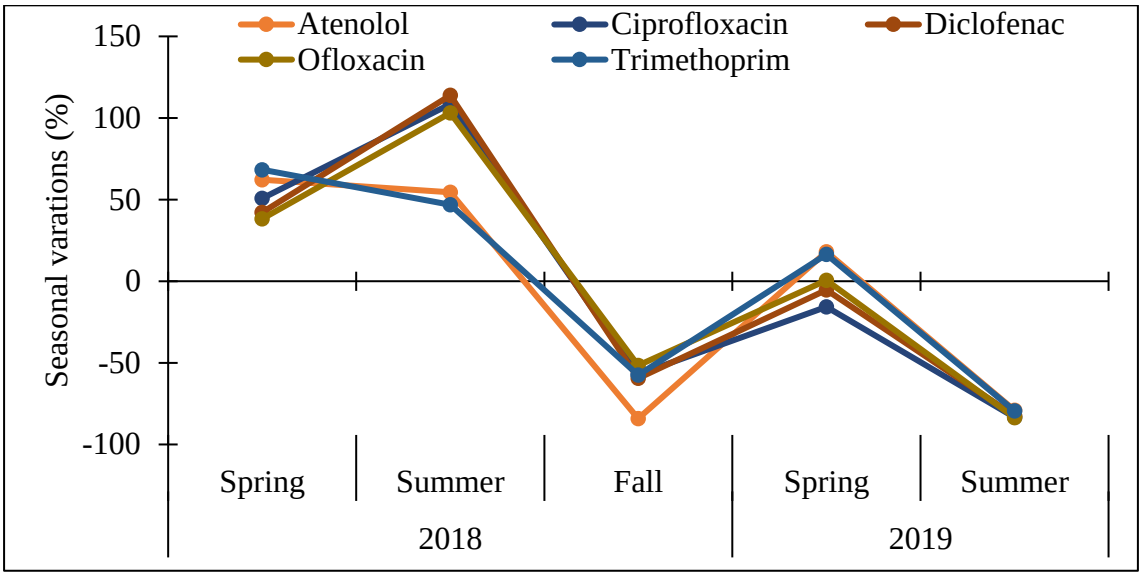


Figure 3-16: Seasonal variation as the percentage deviation of specific seasons from the average full sampling campaign (%) of atenolol, diclofenac, cephalexin, trimethoprim, ciprofloxacin and ofloxacin in M-WWTP influent.

Interestingly, descending trends in the seasonal variation of atenolol ciprofloxacin, ofloxacin diclofenac and trimethoprim are clear in Figures 3-14 and 3-16 in H- and M-WWTP influents during this sampling campaign. This could be attributed to the effect of new regulations that came into effect in March 2018 from the Ministry of Health (MOH) to keep over-the-counter medicines (OTC) under control and dispensed only by prescription by healthcare professional and pharmacy services.

Seasonal variations in the M-WWTP influent showed that antibiotic compounds (ciprofloxacin, ofloxacin) and the NSAID, diclofenac, were at their peak concentrations during summer with the highest seasonal variation percentages of 108.49%, 103.11% and 113.86%, respectively (Figure 3-16). Seasonal variation in atenolol, which is used evenly over the entire year, and trimethoprim showed a similar pattern (Figure 3-17). This variation is consistent with their strong positive significant correlation (r=0.72, p=0.002) observed between their detected concentrations in M-WWTP influent.

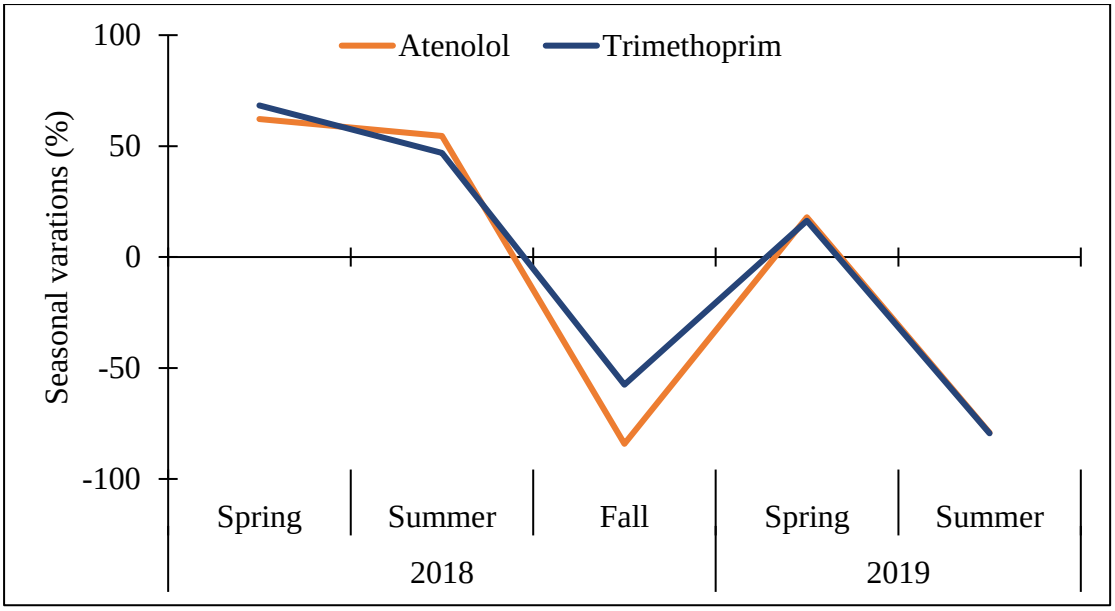


Figure 3-17: Atenolol and trimethoprim seasonal variation as the percentage deviation of specific seasons from the average full sampling campaign (%) in M-WWTP influent.

Seasonal variation in cephalexin was only estimated in M-WWTP 2018 campaign influents because its concentrations were below the LOQ in other seasons. Cephalexin showed a sharp increase and peaked (201.33%) at an average seasonal concentration of 6.75 µg L^{-1} in the summer 2018 (Table A-3-6). However, seasonal fluctuations in antibiotics and NSAIDs could be attributed to their use for contagious and bacterial infectious during cold seasons (Coutu *et al.*, 2013). It is also possible that the biodegradability of these compounds is reduced during the cold season, resulting in an increase in their concentrations in WWTP samples (Vieno *et al.*, 2005).

Metformin is required and used all year-round globally. It showed no seasonal variation in the H-WWTP influent, although its average seasonal concentration declined from 175.14 µg L^{-1} in Spring 2018 to 74.41 µg L^{-1} in Summer 2019. Metformin in the M-WWTP influent showed a fluctuating pattern during the sampling campaign. Its seasonal fluctuation percentage values were -25.40%, -53.56%, 92.13%, -56.76% and 10.23% in 2018 (spring, summer, fall) and 2019 (spring and summer), respectively (Figure 3-18).

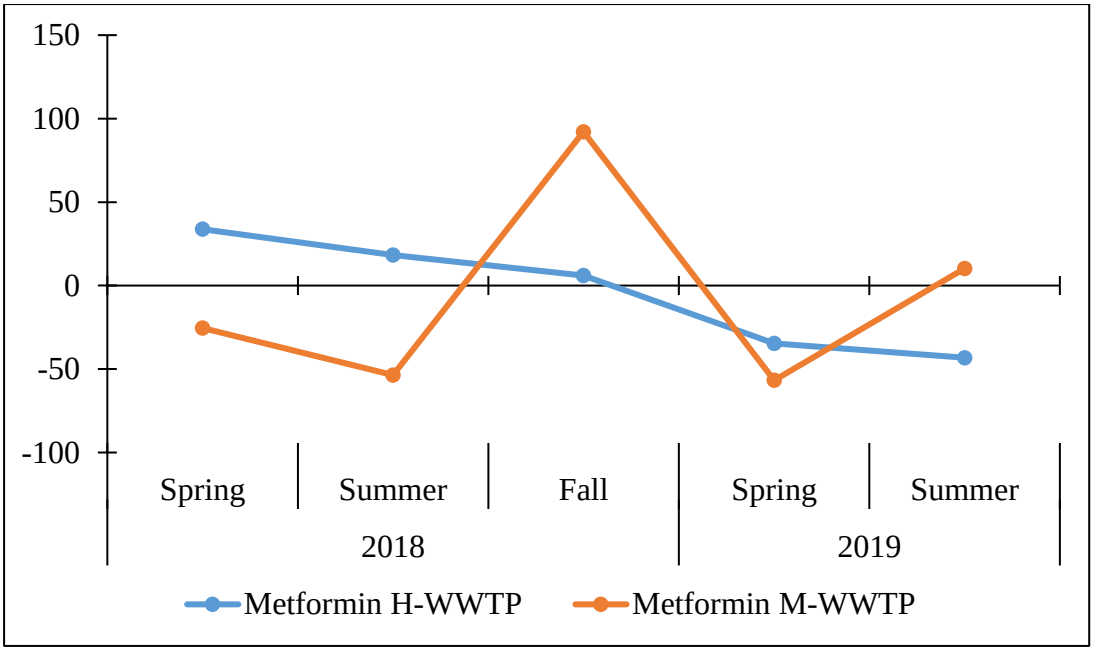


Figure 3-18: Seasonal variations in metformin expressed as the percentage deviation in each season from the average full sampling campaign (%) observed in H and M WWTP influents.

Acetaminophen and caffeine were observed to follow the same seasonal variation pattern in H-WWTP and KSU-WWTP influents (Figure 3-19). This is consistent with the correlation of their occurrence in these WWTPs (r = 0.70, p = 0.01) and (r = 0.77, p = 0.003) in H- and KSU-WWTP influents, respectively. Caffeine has been used together with pain killer medicines, particularly acetaminophen, because it can boost the effects of analgesics treating coughs, colds, and headaches (Lin *et al.*, 2010; Straube *et al.*, 2011). Therefore, the similarity in the seasonal pattern between acetaminophen and caffeine found here may be related to their use in combination.

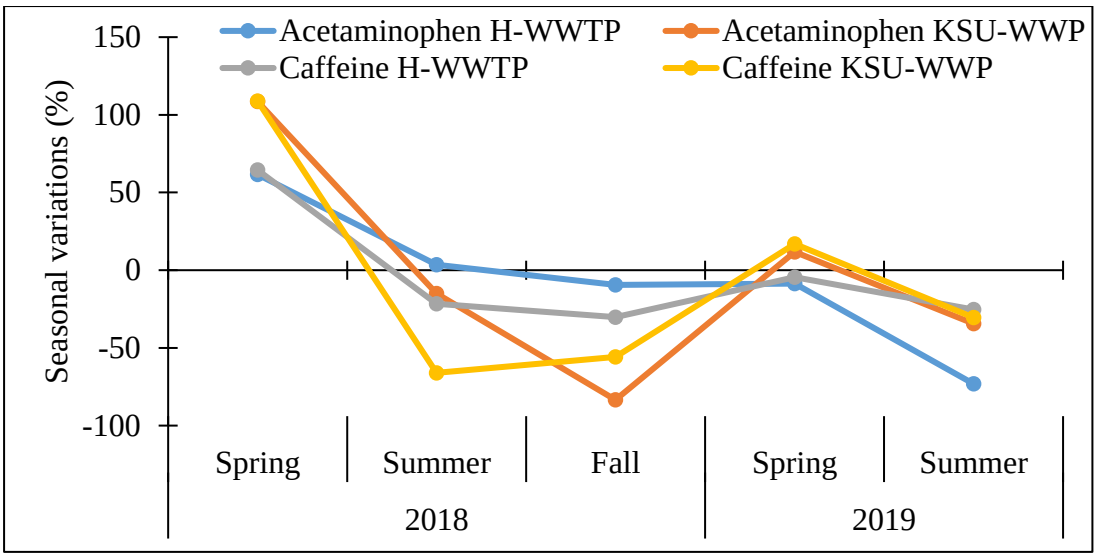


Figure 3-19: Seasonal variations as the percentage deviation of specific seasons from the average full sampling campaign (%) of acetaminophen and caffeine observed in H and KSU WWTP influents.

For the KSU-WWTP influent, seasonal variation was observed during the one-year sampling campaign (Figure 3-13B), where a higher seasonal variation percentage occurred during the spring than summer and fall, particularly for acetaminophen, atenolol, trimethoprim, ciprofloxacin, ofloxacin and caffeine (Figure 3-20). The results potentially demonstrate that the high consumption of these compounds during the spring is due to higher incidence of certain illnesses (Ruan *et al.*, 2019; Di Marcantonio *et al.*, 2020).

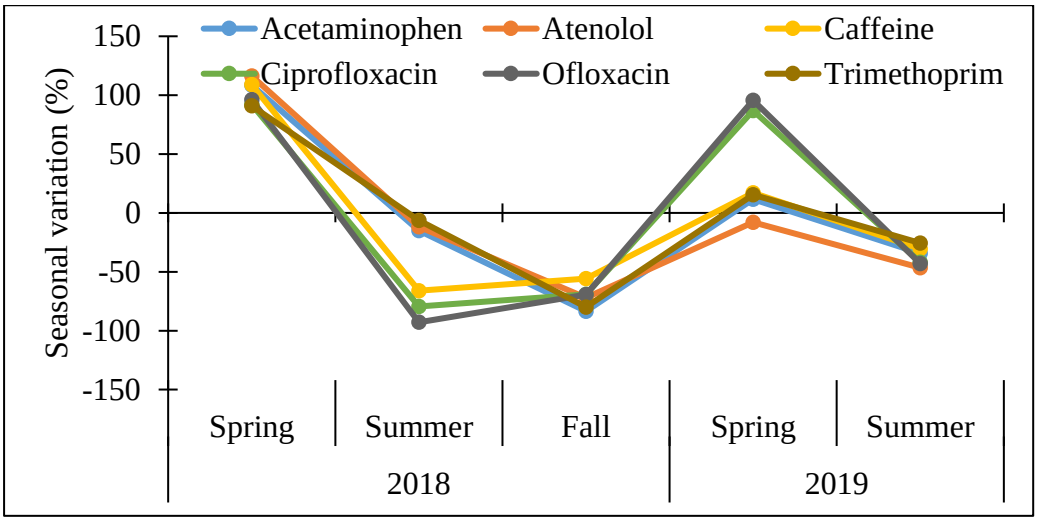


Figure 3-20: Seasonal variation as the percentage deviation of specific seasons from the average full sampling campaign (%) of acetaminophen, atenolol, caffeine, ciprofloxacin, ofloxacin and trimethoprim in KSU-WWTP influent.

Table 3-8: Detected compounds average seasonal concentrations (μg L^{-1}) and their corresponding seasonal variations (%) in KSU-WWTP influent.

Compounds	Seasonal average concentrations (μg L^{-1})				
	2018			2019	
	Spring	Summer	Fall	Spring	Summer
Cephalexin	0.24	0.16	N/A	N/A	N/A
Diclofenac	2.72	2.09	1.97	2.52	0.59
Metformin	180.25	29.77	23.23	98.22	190.35
Compounds	Seasonal variation (%)				
	2018			2019	
	Spring	Summer	Fall	Spring	Summer
Cephalexin	13.46	-26.92	N/A	N/A	N/A
Diclofenac	33.28	2.43	-3.32	23.46	-70.84
Metformin	73.44	-71.35	-77.64	-5.50	83.16

Diclofenac occurrence was highest and lowest in spring 2018 (33.28%) and summer 2019 (-70.84%) at mean seasonal concentrations of 2.72 μg L^{-1} and 0.59 μg L^{-1} respectively (Table 3-8). Conversely, metformin was observed to peak in summer 2019 and was lowest in the fall of 2018 at mean concentrations of 190.35 μg L^{-1} and 23.23 μg L^{-1} respectively (Table 3-8). As for cephalexin in H-WWTP influent, cephalexin in KSU-WWTP influent exhibited seasonal variation only in the 2018 sampling campaign, which is possibly explained by its detection being below the LOQ or LOD in other seasons.

In general, seasonal variations of diclofenac and trimethoprim observed in the influents of the WWTPs, are in good agreement with seasonal variations reported previously in the influent of WWTPs internationally (Paíga *et al.*, 2016; Papageorgiou *et al.*, 2016; Sun *et al.*, 2014; Yu *et al.*, 2013). For example, diclofenac, and trimethoprim were reported to hit their peak concentrations in spring, at mean concentrations of 833 ng L^{-1} and 170 ng L^{-1} respectively by Papageorgiou *et al.* (2016). Similarly, acetaminophen was reported to reach its highest concentration in March in a WWTP in Xiamen, China (Sun *et al.*, 2014), whereas it was observed at its highest concentration in winter in five WWTPs in southern California, USA (Yu *et al.*, 2013).

Likewise, acetaminophen was found in higher concentration in samples collected in March in two WWTPs in Portugal (Paíga *et al.*, 2016). By contrast, the seasonal variation observed in WWTP influents in this study differed from those previously reported in the influent of 15 Portuguese WWTPs by (Pereira *et al.*, 2015). They reported that, ciprofloxacin, diclofenac and acetaminophen were detected at higher concentrations during summer than during spring with mean concentrations of 8666.7 ng L^{-1}, 183 ng L^{-1} and 28685.3 ng L^{-1}, respectively. The possible explanation for this is that:

1- Some of WWTPs in Portugal are affected by seasonal population increases which affect the flow rate of the studied WWTPs.
2- The studied area in Portugal was affected by low precipitation (resulting in low dilution).

Acetaminophen and ciprofloxacin were reported to have the highest mean seasonal concentrations in autumn and winter at 406 ng L^{-1} and 1996 ng L^{-1} respectively by Paíga *et al.* (2016). Caffeine was reported by Papageorgiou *et al*. (2016) in the city of Volos, Grecce, to have the highest mean seasonal concentration in spring whereas atenolol showed little variation during their whole sampling campaign (Papageorgiou *et al.*, 2016).

The seasonal variation in baclofen observed in this study was evaluated only for spring, summer and fall in 2018 in the KSU-WWTP influent because to its concentration was below the LOQ in other seasons. The average seasonal variation of baclofen showed an upward trend from a mean of 0.33 μg L^{-1} in spring to 2.82 μg L^{-1} in fall (Figure 3-21). There are no other published studies with which results found here could be compared.

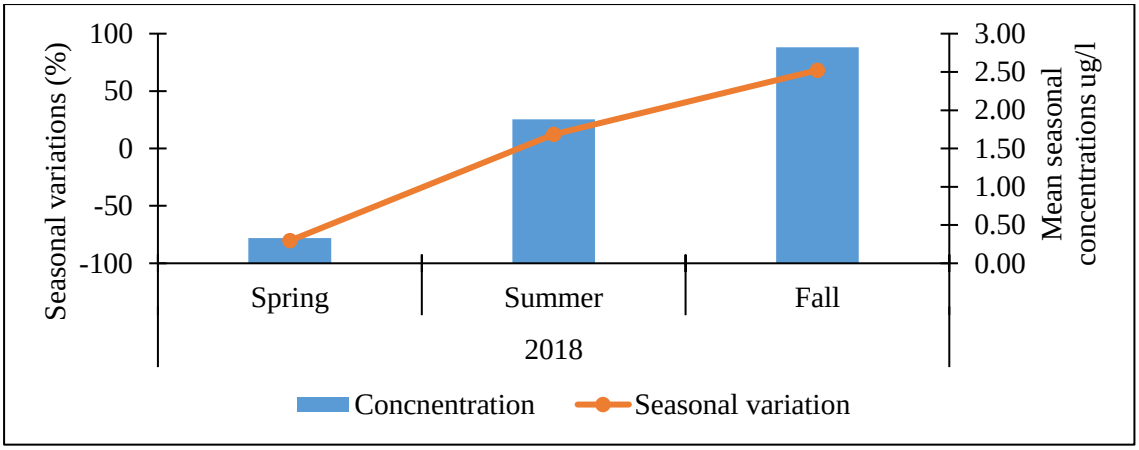


Figure 3-21: Baclofen seasonal variation (%) and mean concentrations in the influent of KSU-WWTP.

3-7-2 Effluent seasonal variations

Table 3-9 shows the average seasonal concentrations of detected compounds in the effluent of H-, M- and KSU-WWTPs. The seasonal variation of detected compounds in the effluents of the selected WWTPs were less obvious than their seasonal variation in WWTP influents because the compounds were only found in two, or even one, season and in low concentrations. This resulted in insufficient data for any seasonal comparison (Table 3-9). This was presumably due to the high removal performance of the treatment processes in these WWTPs for most of the studied pharmaceuticals (Meierjohann *et al.*, 2016; Ruan *et al.*, 2019). Seasonal variation was only observed for ciprofloxacin and ofloxacin which decreased during the hot season and increased during cold to moderate seasons in H-WWTP effluent (Table 3-10).

Table 3-9: Seasonal average concentration (μg L^{-1}) of the detected pharmaceuticals in H-, M- and KSU-WWTP effluents.

Years	Seasons	n	WWTP	Acetaminophen	Caffeine	Ciprofloxacin	Metformin	Ofloxacin
2018	Spring	3	H	0.09	N/A	16.06	N/A	5.74
			M	0.05	N/A	19.92	N/A	3.69
			KSU	0.17	N/A	4.41	N/A	N/A
	Summer	2	H	0.04	N/A	4.93	8.77	1.33
			M	N/A	N/A	N/A	1.55	N/A
			KSU	N/A	N/A	9.79	3.58	1.15
	Fall	3	H	N/A	16.56	1.85	3.49	N/A
			M	N/A	7.31	1.49	1.73	N/A
			KSU	0.12	N/A	12.28	4.22	2.48
2019	Spring	2	H	0.19	4.92	15.76	N/A	5.37
			M	0.01	N/A	N/A	N/A	N/A
			KSU	N/A	N/A	1.68	N/A	N/A
	Summer	2	H	N/A	N/A	9.70	1.38	1.34
			M	N/A	N/A	N/A	N/A	N/A
			KSU	N/A	N/A	1.64	N/A	N/A

N/A: Not available due to the detection being below LOQ or LOD.

Table 3-10: Ciprofloxacin and ofloxacin seasonal variations (%) and their mean corresponding seasonal concentrations (μg L^{-1}) in H-WWTP effluent.

Seasons		Ciprofloxacin		Ofloxacin	
		Average seasonal concentrations (μg L^{-1})	Seasonal variation (%)	Average seasonal concentrations (μg L^{-1})	Seasonal variation (%)
2018	Spring	16.06	68.49	5.74	47.02
	Summer	4.93	-48.23	1.33	-65.80
	Fall	1.85	-80.55	<LOD	N/A
2019	Spring	15.76	65.42	5.37	37.49
	Summer	9.70	1.79	1.34	-65.73

Ciprofloxacin seasonal variation was estimated between -80.55% and 68.49% at mean seasonal concentration between 16.06 μg L^{-1} and 1.85 μg L^{-1} in H-WWTP effluent. Conversely, ciprofloxacin in KSU-WWTP effluent was observed to have the highest and lowest mean seasonal concentrations during summer 2018 and summer 2019 with variation percentages of 108.50% and -83.38% respectively (Figure 3-22).

As mentioned above, the other detected compounds showed no seasonal variation in the WWTP effluents possibly due to their low concentrations or because they were detected below their LOQ or LOD. Higher seasonal concentrations of ciprofloxacin were observed during summer in 15 Portuguese WWTPs at average seasonal concentration of 9800 ng L^{-1} (Pereira *et al.*, 2015) and in winter in Volos city WWTP, Greece at 499 ng L^{-1} (Papageorgiou *et al.*, 2016). This contrasts with the seasonal variations found in H- and M-WWTP effluents but is in accordance with the KSU-WWTP effluent.

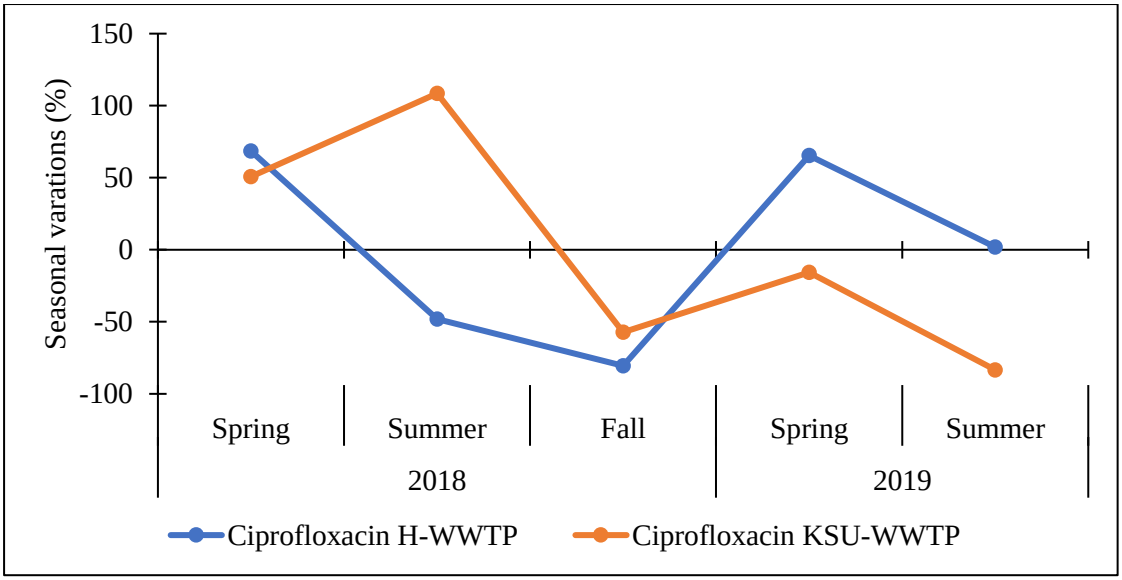


Figure 3-22: Seasonal variation as the percentage deviation of specific seasons from the average full sampling campaign (%) for ciprofloxacin in H- and KSU-WWTP effluents.

3-8 Conclusions

Ten of the targeted pharmaceutical compounds were quantified and detected in the investigated WWTPs influents at average concentrations between 0.22 µg L^{-1} and 1248.35 µg L^{-1} whereas only five compounds were measured in the WWTPs effluents at mean concentrations ranging between 0.03 µg L^{-1} and 10.74 µg L^{-1}. Caffeine, acetaminophen, and ciprofloxacin had the highest concentrations in WWTP influents,

while ciprofloxacin, caffeine and metformin had the highest concentrations in the WWTP effluents.

The removal efficiency for the studied compounds was found to be between 99% and -63% in the three WWTPs. The compounds eliminated by > 90% were acetaminophen, atenolol, caffeine, diclofenac, baclofen, cephalexin, metformin, and trimethoprim, whereas less than 90% of the ofloxacin and ciprofloxacin were generally removed. Overall, the detected compounds were eliminated more efficiently in H and M-WWTPs than the KSU-WWTP, possibly because of the different treatment technology used (see section 3-2). Antibiotics, particularly ofloxacin and ciprofloxacin, were removed at a lower efficiency in KSU- and H- than M-WWTP. Most compounds showed no difference in their removal efficiency in relation to the WWTP technology applied.

Although temperature has been reported to have strong correlation with the removal rate of pharmaceuticals in WWTPs, in this study, temperature was observed to have a strong positive and significant correlation with the trimethoprim and acetaminophen removal rates ($r \geq 0.71$ and $p \leq 4 \times 10^{-3}$) only in H-WWTP.

Daily mass loading of the selected analytes was estimated for the first time in Saudi Arabian WWTPs during this study. Mass loadings for the studied WWTPs were between 460.60 and 3×10^{-4} mg d^{-1} 1000 $inhabitants^{-1}$ for the influents whereas they were between 7.41 and 1.0×10^{-4} mg d^{-1} 1000 $inhabitants^{-1}$ in the effluents. Mass loading analysis showed that the total mass loading of 4513.35 mg d^{-1} 1000 $inhabitants^{-1}$ into H-WWTP and 36.11 mg d^{-1} 1000 $inhabitants^{-1}$ discharged with the effluent of H-WWTP was the highest total mass loading observed in these investigated WWTPs during the sampling campaign. In all three WWTPs, caffeine was estimated to have the

highest average daily mass loading in the influent of the studied WWTPs, whereas ciprofloxacin estimated to have the highest average daily mass loading discharged by these WWTPs.

The occurrence of baclofen was investigated here for the first time in WWTPs. Baclofen was found only in KSU-WWTP influent at concentrations between 0.33 μg L^{-1} and 2.82 μg L^{-1}, at average mass loading of 6.0×10^{-3} mg d^{-1} per 1000 inhabitants.

Seasonal variations in the presence of detected compounds were more obvious in the influents than effluents of these WWTPs and some pharmaceuticals were only found in some seasons. The declining trend in the concentrations of selected compounds, especially in H- and M-WWTP influents throughout the sampling campaign (from March 2018 to July 2019) possibly indicated the effect of new regulations that came into effect in March 2018 from the Ministry of Health (MOH) to keep over-the-counter medicines (OTC) under control and dispensed only as prescription medicines by healthcare professionals and pharmacy services. In contrast, this downward trend in the concentrations was not significant in KSU-WWTP, possibly due to the small size of this treatment plant, the community served and the awareness of this community regarding the appropriate use of pharmaceuticals because they are well educated.

Seasonal variations in the investigated compounds were similar in the H- and M-WWTP but not in the KSU-WWTPs. The mean concentrations of the investigated pharmaceuticals generally showed an increase during spring and fall while their concentrations were observed to decline in the summer, particularly in the 2019 sampling camping for antibiotics and NSAIDs compounds.

CHAPTER FOUR

Environmental risk assessment of pharmaceutical compounds in three different WWTPs in Riyadh city, Saudi Arabia.

4-1 Introduction

The consumption of pharmaceuticals has gradually increased in modern life due to population increase and the benefit provided to humans and animals from different illnesses (Lee and Choi, 2019). Thus, more pharmaceutical residues have been detected in different environment compartments worldwide. There are many pathways for these compounds to find their way into the environment, such as WWTP outlets, hospital wastes, excretion in urine, inappropriate disposal of unused or expired drugs, and pharmaceutical manufacturing facilities (Niemi *et al.*, 2020; Papageorgiou *et al.*, 2019; Verlicchi and Zambello, 2015). Their presence in the environment has been previously reported to have potential risks, such as toxic effects, endocrine disruption, and antibiotic resistance in microorganisms living in aquatic ecosystems (De García *et al.*, 2014; Kidd *et al.*, 2007; Oaks *et al.*, 2004; Lee and Choi, 2019; Sousa *et al.*, 2019). For instance, Gunnarsson *et al.* (2009) found that the exposure of fish (Juvenile rainbow trout) to a highly diluted (1:500) treated effluent from the manufacturing of bulk drugs in India resulted in liver enlargement. Also, acute and chronic exposure to individual and mixed pharmaceutical residues have been found to have a direct effect on oocyte development, and therefore lead to a reduction in the population of zebrafish (Galus *et al.*, 2013; Khanzada *et al.*, 2020). Recently, chronic exposure to the antidepressant drug (venlafaxine), which is prescribed for anxiety, was linked to significant adverse effects on the reproductive tissue of zebrafish (*Danio rerio*) (Ikert and Craig, 2020).

Apart from the environmental risks associated with pharmaceuticals, they have also been reported to pose potential adverse risks towards human health, particularly when WWTP effluent contaminated with micropollutants or when sludge is reused in agriculture (Prosser and Sibley, 2015; Semerjian *et al.*, 2018). Uptake and transfer of pharmaceuticals from treated wastewater or sludge amended soils to plants, vegetables or fruit could end up in the human food chain (Yang *et al.*, 2017).

To assess such potential adverse environmental risk and to address this problem, different authorities, organizations and agencies across the world have established legislation, guidelines and directives to protect the environment from pharmaceutical contamination and to estimate the adverse environmental impact (Ahmed *et al.*, 2017; Esplugas *et al.*, 2007; Lee and Choi, 2019; Jose *et al.*, 2020). These organisations include the European Medicines Agency (Committee for Medicinal Products for Human Use), USA Food and Drug Administration (Center for Drug Evaluation and Research), Health Canada & Environment and Climate Change (Canada), the World Health Organization (WHO), the International Program of Chemical Safety (IPCS), The Ministry of Health, Labour and Welfare (Japan), and The National Environment Protection and Heritage Council (Australia). The most common and useful protocol for conducting an ERA for pharmaceuticals was proposed by the European Medicines Agency (EMA) (EMA, 2006), and has been extensively used in the screening of risk posed by pharmaceuticals in the environment in different countries worldwide, such as in Spain (Gros *et al.*, 2010), China (Zhang *et al.*, 2017; Wang *et al.*, 2021), Greece (Papageorgiou *et al.*, 2019; Kosma *et al.*, 2019), Italy (Al Aukidy *et al.*, 2012), India (Archana *et al.*, 2017), Pakistan (Ashfaq *et al.*, 2019), Faroe Islands, Iceland and Greenland (Huber *et al.*, 2016), Mexico (Rivera-Jaimes *et al.*, 2018), Costa Rica

(Ramírez-Morales *et al.*, 2020) and Portugal (Sousa *et al.*, 2019). Consequently, this approach is used for conducting an ERA of detected pharmaceuticals in this study.

The EU Water Framework Directive 2000/60/EC (EC, 2000) included 45 priority compounds to be monitored and removed from WWTP effluents prior to discharge into the environment (Kanaujiya *et al.*, 2019; EC, 2000). This Directive was amended by 2008/105/EC (EC, 2008) which included a list of 33 priority compounds/substances that must be monitored at EU Community level. In 2013, the Directive 2013/39/EU (EC, 2013) was published, which updated the previous directive and included 45 priority compounds/substances and highlighted the need to develop new water treatment solutions in order to avoid or reduce their release into the environment (Sousa *et al.*, 2019; EC, 2013). Decision 2015/495/EU (EU, 2015) set out the first water watch list which comprised 17 compounds/substances (Sousa *et al.*, 2019; EC, 2015; Barbosa *et al.*, 2016). This watch list was recently updated by the European Union in Decision 2018/840/EU (EC, 2018) and then by the European Union in the Decision 1161/2020/EU (EC, 2020).

Generally, the EU ERA approach consists of two stepwise Phases (I and II). In Phase I, persistence, bioaccumulation and toxicity of pharmaceutical compounds are taken into consideration and an initial predicted environmental concentration (PEC) in surface water is calculated. The PEC is calculated in this level using default values. Subject to the outcome of the PEC value, the ERA is then terminated or carried on. If PEC < 0.01 μg L^{-1}, the chemical substance is considered to be safe (i.e. has no environmental risk) and no further assessment is necessary, while if PEC ≥ 0.01 μg L^{-1}, the compound is considered to have potential environmental risk and the next stage (Phase II) is applied (Jose *et al.*, 2020; Lee and Choi, 2019; Whomsley *et al.*, 2019; EMA, 2006). In the second Phase, an ERA is performed in two tiers (A and B). In Tier

A, an initial assessment of environmental fate and effect of the drug is conducted, whereas in Tier B further extended and sophisticated ERA analysis is performed. More detail about the ERA approach can be found in chapter 1 section 1-2.

According to European Medicines Agency (EMA, 2006) guidelines, environmental risk assessment of pharmaceuticals has been evaluated by using the risk quotient (RQ) approach in many studies (Gros *et al.*, 2010; Jose *et al.*, 2020; Whomsley *et al.*, 2019; Wang *et al.*, 2021; EMA, 2006). The RQ is derived from the ratio between the PEC or Measured Environmental Concentrations (MEC) and the Predicted No-Effect Concentrations (PNEC) in three different aquatic organisms (fish, daphnid and algae). Following this protocol, ecological risk has been assessed for micropollutants in different countries worldwide, like in Spain (Gros *et al.*, 2010), China (Zhang *et al.*, 2017; Wang *et al.*, 2021), Greece (Papageorgiou *et al.*, 2019) and Portugal (Sousa *et al.*, 2019). For example, the ecological risk arising from ofloxacin, ciprofloxacin and trimethoprim was estimated in the effluent of 12 WWTPs equipped with secondary treatment in Dalian, China by Zhang *et al.* (2017). They reported that trimethoprim and ofloxacin posed low environmental risk in at all 12 WWTP effluents, whereas ciprofloxacin posed low environmental risk except in six WWTP effluents which found to cause a potential medium environmental risk particularly against algae. Wang *et al.* (2021) also found ciprofloxacin and ofloxacin residues posed high to low ecological risk against the three levels of non-target aquatic organisms from two WWTPs and two pharmaceutical manufactories WWTPs, Jinan city, China.

Gros *et al.* (2010) studied the environmental impact of 73 pharmaceutical compounds discharged from seven WWTPs into the aquatic environment in the Ebro River basin, Northeast of Spain. They concluded that the majority of the compounds on their list, including acetaminophen, atenolol, trimethoprim, diclofenac and ofloxacin,

showed low environmental risk but atorvastatin, erythromycin, sulfamethoxazole and tetracycline posed a high ecotoxicological risk.

Papageorgiou *et al.* (2019) conducted an extensive ecotoxicological risk study of 138 pharmaceutical compounds, including acetaminophen, diclofenac, ciprofloxacin, trimethoprim, metformin, atenolol, and caffeine in four WWTPs from three different Greek cities (Thessaloniki, Larisa, and Volos) in North and Central Greece using the RQ approach. The conclusion of this study is compiled in Table 4-1.

Table 4-1: RQ of some studied pharmaceuticals for three different species in 4 WWTPs effluent in Greece (Papageorgiou *et al.*, 2019).

Pharmaceuticals	RQ		
	Algae	Fish	Invertebrate
Acetaminophen	Low	Low	Medium
Diclofenac	Medium	High	Low
Ciprofloxacin	Low	Low	Low
Trimethoprim	Medium	Low	Low
Metformin	Low	Low	Low
Atenolol	Low	Low	Low
Caffeine	High	Low	Low

The environmental impact posed by pharmaceuticals in Saudi Arabian WWTPs has never been estimated. To date, there are large knowledge gaps in terms of ERA in the Saudi Arabian environment because most studies of pharmaceuticals conducted in Saudi Arabia have been focused more on the occurrence of these compounds in the different environment matrices rather than their corresponding environmental impact (Al Qarni *et al.*, 2016; Ali *et al.*, 2017; Alidina *et al.*, 2014; Shraim *et al.*, 2017; Picó *et al.*, 2020; Picó *et al.*, 2021). Only one of these investigations (i.e., Picó *et al.*, 2020) recently discussed the environmental risk posed by pharmaceuticals in surface water

samples. Thus, this study is the first environmental risk assessment of pharmaceuticals associated with three different WWTPs (Hair, Manfuha and King Saud University) in Riyadh, Saudi Arabia, thereby allowing a better understanding of environmental risk in the Saudi Arabian context. Additionally, the results of this chapter will assist in drawing the attention of relevant Saudi Arabian authorities, researchers, and other stakeholders to the issue, with the possibility of more effort and help to establish new regulations for the protection of the environment contaminated by these micropollutants. The result could also assist in setting a priority list of pharmaceuticals for monitoring and management.

The hypothesis to be tested in this chapter was that detected pharmaceuticals in the three studied WWTPs pose a potential risk at this time to non-target aquatic organisms at three trophic levels.

4-2 Methodology

4-2-1 Risk Quotient assessment (RQ)

In this study, the potential environmental risk posed by the analyte compounds against aquatic organisms in the influent and effluent of the studied WWTPs was assessed according to the European Medicines Agency guidelines (Jose *et al.*, 2020; Lee and Choi, 2019; Sousa *et al.*, 2019; Whomsley *et al.*, 2019; EMA, 2006). In order to consider the worst-case scenario, ecotoxicological risk assessment was estimated by means of the risk quotient (RQ) values as the ratio between maximum measured environmental concentration during the sampling period and their corresponding lowest predicted no-effect concentration (PNEC) in three trophic levels of the aquatic ecosystem (algae, daphnia and fish) (Ashfaq *et al.*, 2019; Kapelewska *et al.*, 2018; Al Aukidy *et al.*, 2012; Paíga *et al.*, 2019; Bu *et al.*, 2020) (equation 1). The PNEC values

were generated from the lowest acute toxicity data EC50 (half maximal effective concentration) or LC50 (median lethal concentration) previously reported in the literature for three trophic levels: algae, *Daphnia*, and fish.

The PNEC values used for this risk analysis are presented in Table 4-2. More PNEC data can be found in appendix Table A-4-1. Out of ten compounds detected in this study, ecotoxicological data were found for nine of them in the peer reviewed literature; no data were found for baclofen. Baclofen ecotoxicological data were estimated using the US Environmental Protection Agency (USEPA) Estimation Programs Interface Suite (EPISuite) software ECOSAR v1.11 (USEPA, 2017). ECOSAR has been widely used to estimate acute and chronic toxicity data for aquatic organisms (Mendoza *et al.*, 2015; Han and Lee, 2017; Cleuvers, 2005; Fent *et al.*, 2006; Sousa *et al.*, 2019; Paíga *et al.*, 2019). The lowest ecotoxicological value for the most sensitive species assayed (among algae, *Daphnia* and fish) used to calculate the PNEC was divided by an assessment factor of 1000 (equation 1) (TGD, 2003; Kapelewska *et al.*, 2018; Al Aukidy *et al.*, 2012; Bu *et al.*, 2020). The applied risk ranking criteria in this investigation those proposed by de Souza *et al.* (2009), Hernando *et al.* (2006), Kosma *et al.* (2014) and Sousa *et al.* (2019) as follows:

1- if RQ values lower than 0.1 indicate that the compound poses minimal risk to the environment,
2- $0.1 \leq RQ < 1$, median risk
3- $RQ \geq 1$, high risk (de Souza *et al.*, 2009; Hernando *et al.*, 2006; Kosma *et al.*, 2014; Sousa *et al.*, 2019).

$$RQ = \frac{MEC}{PNEC} = \frac{MEC}{LC50 \ or \ EC50/1000} \ldots\ldots (1)$$

Where: -

MEC: Measured environmental concentration.

PNEC: Predicted no-effect environmental concentration.

LC50: Median lethal concentration.

EC50: Half maximal effective concentration.

Table 4-2: The selected PNEC values (µg L^{-1}) for the studied pharmaceutical compounds and their different corresponding assayed species in this ERA.

Compounds	Algae	Ref.	Daphnia	Ref.	Fish	Ref.
Acetaminophen	0.58	1	1	2	40	3
Diclofenac	0.71	4	0.52	5	0.2	6
Atenolol	110		30	2	1096	8
Cephalexin	45.65	9	228.73	9	19	9
Ciprofloxacin	2.97	8	12.8	10	10	4
Ofloxacin	0.6	11	1.44	12	0.53	12
Trimethoprim	2.6	3	4.8	3	0.31	9
Metformin	0.511	3	0.064	13	1.9	9
Baclofen*	8.9	This study	5.1	This study	62.6	This study
Caffeine	0.76	8	46	8	4.6	8

1 (Escher *et al.*, 2011), 2 (Verlicchi *et al.*, 2012), 3 (Sanderson *et al.*, 2003), 4 (Whitacre, 2012), 5 (Du *et al.*, 2016), 6 (Lucas *et al.*, 2016), 7 (Yamamoto *et al.*, 2007), 8 (Kuzmanović *et al.*, 2015), 9 (Bu *et al.*, 2020), 10 (Santos *et al.*, 2013), 11 (van der Grinten *et al.*, 2010), 12 (Iatrou *et al.*, 2014), 13 (Cleuvers, 2003), * The ecological structure activity relationship (ECOSAR) software v1.11 was used to estimate PNEC (USEPA, 2017).

4-3 Results and discussion

The simple, deterministic quotient method was used to calculate and evaluate the degree of risk for each target analyte in the influents and effluents of the studied WWTPs to three trophic levels (algae, *Daphnia magna* and fish) according to EMA guidelines (EMA, 2006). As can be seen in Table 4-3, the RQ was estimated to be high for the majority of the compounds detected in the influent, but values ranged between 2×10^{-3} and 9.2×10^{3} (Table 4-3). The RQ value is controlled and correlated with environmental maximum concentrations of pharmaceuticals (Wang *et al.*, 2021) or is due to their low PNEC (Ramírez-Morales *et al.*, 2020). Six of the ten detected compounds were estimated to have a high potential environmental risk while others posed medium to low risk in the WWTP influents.

Table 4-3: Environment risk assessment, presented as risk quotient (RQ) values for measured compounds in the influent and effluent of the three investigated WWTPs. Red: high risk; orange: moderate risk and green: low risk.

Compounds	WWTPs	Three trophic levels					
		Algae		Daphnia		Fish	
		Inf.	Eff.	Inf.	Eff.	Inf.	Eff.
Acetaminophen	H-WWTP	1777.43	0.32	1036.24	0.19	25.91	0.005
	M-WWTP	1617.48	0.09	942.99	0.05	23.57	0.001
	KSU-WWTP	2034.42	0.56	1186.07	0.33	29.65	0.01
Atenolol	H-WWTP	0.06	N/A	0.23	N/A	0.01	N/A
	M-WWTP	0.27	N/A	1.00	N/A	0.03	N/A
	KSU-WWTP	0.06	N/A	0.21	N/A	0.01	N/A
Baclofen	H-WWTP	N/A	N/A	N/A	N/A	N/A	N/A
	M-WWTP	N/A	N/A	N/A	N/A	N/A	N/A
	KSU-WWTP	0.31	N/A	0.56	N/A	0.05	N/A
Caffeine	H-WWTP	3002.09	21.79	49.60	0.36	496.00	3.60
	M-WWTP	1907.18	11.64	31.51	0.19	315.10	1.92
	KSU-WWTP	1970.53	N/A	32.56	N/A	325.57	N/A
Cephalexin	H-WWTP	0.01	N/A	0.002	N/A	0.02	N/A
	M-WWTP	0.15	N/A	0.03	N/A	0.36	N/A
	KSU-WWTP	0.01	N/A	0.002	N/A	0.02	N/A
Ciprofloxacin	H-WWTP	69.67	11.16	16.17	2.59	20.69	3.32
	M-WWTP	239.49	6.71	55.57	1.56	71.13	1.99
	KSU-WWTP	156.11	6.76	36.22	1.57	46.36	2.01
Diclofenac	H-WWTP	64.42	N/A	87.95	N/A	228.68	N/A
	M-WWTP	63.75	N/A	87.04	N/A	226.32	N/A
	KSU-WWTP	7.53	N/A	10.28	N/A	26.73	N/A
Metformin	H-WWTP	603.59	17.16	4819.27	137.01	162.33	4.62
	M-WWTP	1150.42	3.80	9185.38	30.32	309.40	1.02
	KSU-WWTP	744.21	11.84	5942.06	94.55	200.15	3.18
Ofloxacin	H-WWTP	41.88	11.79	17.45	4.91	47.41	13.34
	M-WWTP	147.07	6.60	61.28	2.75	166.49	7.48
	KSU-WWTP	64.17	4.39	26.74	1.83	124.20	4.97
Trimethoprim	H-WWTP	0.72	N/A	0.39	N/A	6.05	N/A
	M-WWTP	1.84	N/A	1.00	N/A	15.45	N/A
	KSU-WWTP	1.83	N/A	0.99	N/A	15.37	N/A

N/A: Not available due the concentration being below the LOQ or LOD.

Of the five detected pharmaceuticals in the investigated WWTP effluents, four were evaluated to pose high risk to all three organisms in the environment (Table 4-3). Only acetaminophen was evaluated to present low to medium level of potential

ecological risk to the three different aquatic organisms from the studied WWTP effluents.

Of the compounds posing a high risk to living organisms in the environment, one was an NSAID (diclofenac) and the other an antibiotic (ciprofloxacin). These had been highlighted for further monitoring and were included on the watch list of the European Union in 2015 and 2018/2020 respectively (EC, 2015; EC, 2018; EC, 2020).

The RQ value is controlled and correlated with environmental maximum concentrations of pharmaceuticals (Wang *et al.*, 2021) or is due to their low PNEC (Ramírez-Morales *et al.*, 2020).

4-3-1 Risk Quotients of detected compounds in the investigated WWTP influents

The results obtained in this study for the environmental risk assessment of detected compounds in the influent of the investigated WWTPs are showed in Figure 4-1A. The most detected compounds (60% of the ten detected compounds) in the selected WWTP influents were estimated to pose a high risk to all three levels of non-target aquatic organisms.

Atenolol and cephalexin in the three studied WWTPs were found to present low to medium risk towards the representatives of three trophic levels in the aquatic environment (algae, invertebrates, and fish). Trimethoprim was estimated to pose medium environmental risk for all three WWTP influents against *Daphnia*, compared with a high ecotoxicological risk for fish. Low ecological risk from atenolol, baclofen, and cephalexin in the influents was estimated for fish and algae from H-, M- and KSU-WWTPs, fish from KSU-WWTP, and fish, algae and *Daphnia* from H-, M- and KSU-WWTPs (Figure 4-1A).

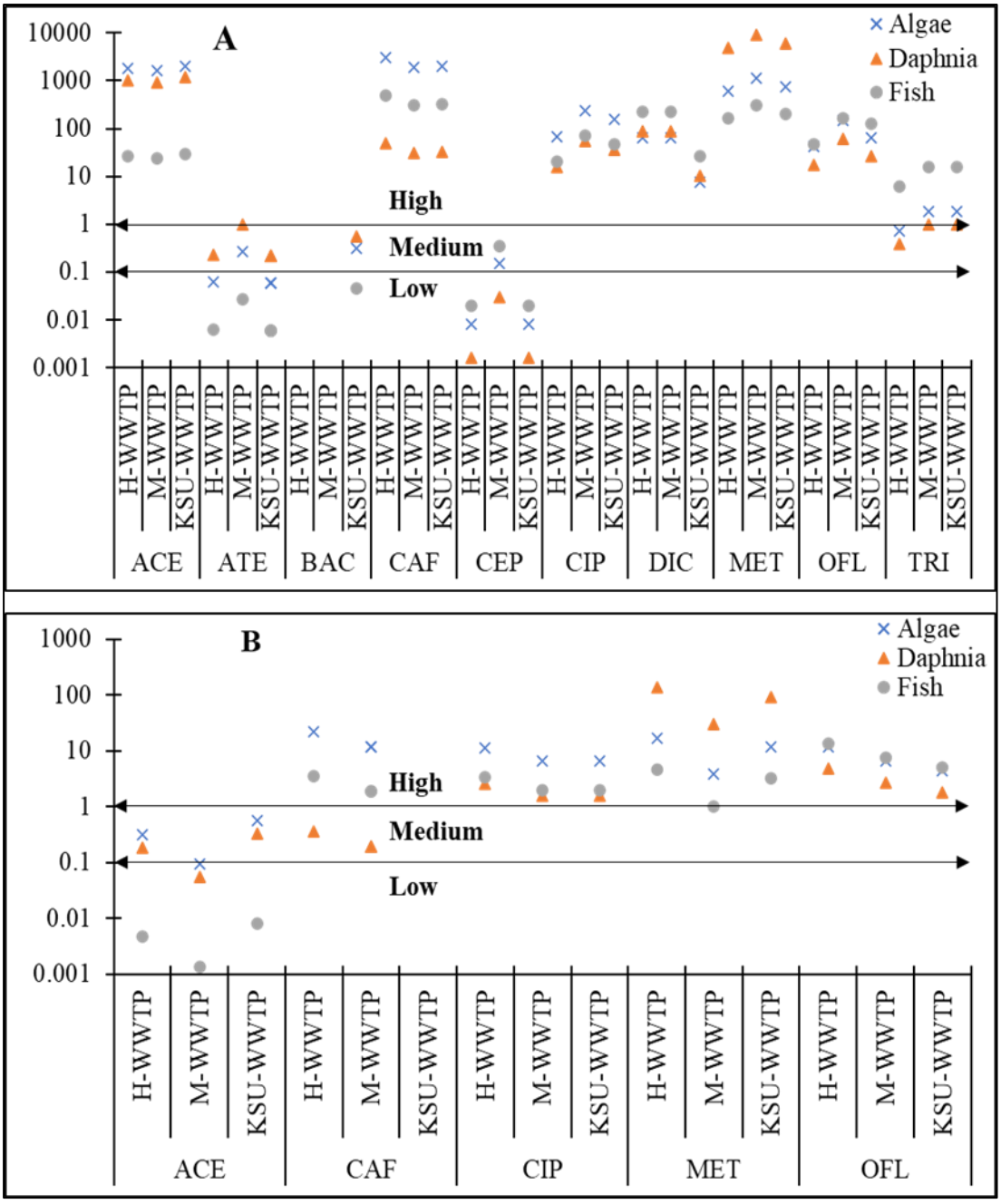


Figure 4-1: Risk quotients (RQs) for the detected compounds in studied WWTPs influents (A) and effluents (B) on a logarithmic scale. ACE: Acetaminophen, ATE: Atenolol, BAC: Baclofen, CAF: Caffeine, CEP: Cephalexin, CIP: Ciprofloxacin, DIC: Diclofenac, MET: Metformin, OFL: Ofloxacin and TRI: Trimethoprim.

The most highly ranked hazardous compounds in the WWTP influents were the antidiabetic metformin ($1.6\times10^2 \leq RQ \leq 9.2\times10^3$), the stimulate caffeine ($0.31\times10^2 \leq RQ \leq 3.0\times10^3$), the NSAID acetaminophen ($0.23\times10^2 \leq RQ \leq 2.0\times10^3$), and antibiotic drug ciprofloxacin ($0.16\times10^2 \leq RQ \leq 2.4\times10^2$) (Table 4-3 and Figure 4-1A). Algae

were observed to be the most sensitive organism to acetaminophen concentrations found in the WWTP influents, with estimated RQs ranging between 1.6×10^3 and 2.0×10^3, whereas fish were found to be the most vulnerable organism to the NSAID, diclofenac, with RQ values ranging from 26.7 to 228.7 (Table 4-3).

The antidiabetic (metformin) and the stimulant (caffeine) were also evaluated to pose high environmental risk for all three studied WWTP influents at RQ values between 31.51 and 9185.40 to all three trophic levels (Figure 4-1A and Table 4-3). Conversely, atenolol and cephalexin were observed to pose low to medium risk in studied WWTP influents at RQ values between 0.002 and 1. A high environmental risk has been previously reported for caffeine, diclofenac and acetaminophen in WWTP influents in Portugal (Paíga *et al.*, 2019), and Costa Rica (Ramírez-Morales *et al.*, 2020). On the contrary, acetaminophen and caffeine were estimated to pose low environmental risk in WWTP influents in India (Archana *et al.*, 2017).

The antibiotic compounds, ciprofloxacin and ofloxacin, were estimated to pose high ecological risk (RQ >1), while trimethoprim posed medium to high risk, in the three WWTPs influent ($0.1 \leq RQ \geq 1$) to all three trophic levels of the aquatic ecosystem (fish, invertebrates and algae). Antibiotic compounds have been reported to have potential risks for aquatic organisms in the environment by affecting their reproductive abilities and behaviours (Kovalova *et al.*, 2012; Kümmerer, 2009). Moreover, these compounds could contribute to antibiotic resistant bacteria (ARBs) and antibiotic resistant genes (ARGs), which reduce the effect of antibiotics against bacterial pathogens (Zhang *et al.*, 2015; Wang *et al.*, 2021). Antibiotic resistance in bacteria has already been reported for wastewater (Gao *et al.*, 2012; Bielen *et al.*, 2017). It is worth noting that ciprofloxacin was included recently in the latest two watch lists of the European Union 2018 and 2020 (EC, 2018; EC, 2020). The estimated RQs of

antibiotics in the WWTP influents investigated here were consistent or higher than those evaluated in some previous studies worldwide, particularly for ciprofloxacin and trimethoprim (Aydin *et al.*, 2019; Ramírez-Morales *et al.*, 2020; Paíga *et al.*, 2019; Papageorgiou *et al.*, 2016). Unfortunately, there are no available ERA for pharmaceuticals in Saudi Arabia with which to compare the results obtained here.

The environmental risk of baclofen was estimated here for the first time for WWTP influents and effluents. Baclofen was detected only in KSU-WWTP influent and was evaluated to pose a low risk (RQ = 5.6×10^{-4}) for fish and medium risk ($0.31\leq RQ\leq0.56$) for algae and *Daphnia* at the maximum measured concentration of 2.82μg L^{-1}. Unfortunately, there is no available data either nationwide or worldwide with which to compare the result found here. The high environmental risk evaluated for acetaminophen, diclofenac, caffeine, metformin, ciprofloxacin and ofloxacin in the influent of the WWTPs studied here indicate that aquatic organisms are at high risk. In addition, continuous exposure to the pharmaceuticals that are estimated to pose medium to low risk, may elevate the ecological risk to high (Archana *et al.*, 2017).

Overall, the RQ values for the detected pharmaceuticals, except atenolol and cephalexin, in the WWTP influents indicated high risk for the three different targeted aquatic organisms. These findings are in partial agreement with the hypothesis that the occurrence of selected pharmaceuticals in wastewater in Riyadh, Saudi Arabia pose a potential environmental risk.

4-3-2 Risk Quotients of detected compounds in the investigated WWTP effluents

It is particularly critical to determine potential environmental impact for effluents because they represent the risk at the final discharge point of WWTPs, after which no further treatment is applied. Except for acetaminophen and caffeine (for *Daphnia*),

three out of the five compounds detected in the sampled WWTP effluents, were found to pose high ecological risk for the three tested organisms, with estimated RQ values between 0.001 and 137.01 (Figure 4-1B). This partially supports the hypothesis of this study.

The RQ values for ciprofloxacin and ofloxacin were between 1.56 and 13.34, with algae and fish being the most sensitive organisms to the effluent concentrations respectively. Similarly, metformin was evaluated to have a high potential environmental risk to the three aquatic ecosystems for all three WWTPs with RQs between 1.02 and 137.01. This supports the hypothesis of this study.

Acetaminophen was estimated to pose low to medium potential risk to algae, *Daphnia* and fish ($0.001 \leq RQ \leq 0.56$) for the studied WWTP effluents (Figure 4-1B). Caffeine posed a high risk to algae and fish whereas it only posed a medium risk to *Daphnia*. The lower number of compounds estimated to cause high risk in effluents compared with influents could be attributed to the removal efficiency of these detected compounds through the studied WWTPs. The removal rate was discussed in more detail in chapter 3 section 3-5-2.

The environmental risk estimated for the WWTP effluents studied here were similar to those reported in research by Papageorgiou *et al.* (2016) and Pereira *et al.* (2015). Papageorgiou *et al*. (2016) reported RQ values between 1 and 0.1 for acetaminophen and caffeine, which posed medium risk in the effluents of Volos city WWTP, Greece. In the effluent of 15 Portuguese WWTPs, acetaminophen was assessed to pose medium environmental risk for aquatic organisms (Pereira *et al.*, 2015). Caffeine, acetaminophen and metformin were reported to pose high to medium environmental risk in four WWTP effluents in Greece by Patel *et al.*, (2019), whereas

ciprofloxacin and acetaminophen were found to pose high to low environmental risk in the effluents of 15 different WWTPs located in five Portuguese regions (Pereira *et al.*, 2015). Ramírez-Morales *et al*. (2020) estimated ciprofloxacin and ofloxacin to pose high ecological risk in samples collected from 11 WWTP effluents in Costa Rica.

It is worth noting that, continuous emission of these compounds evaluated to cause low potential risk, could elevate their potential risk to high when aquatic organisms are exposed in the long term (Archana *et al.*, 2017). In addition, it has been proposed that those compounds evaluated with moderate or high risk and with log K_{ow}>3 should be considered as a priority for environmental management (Chaves *et al.*, 2020) because they may have the potential to bio-concentrate in living organisms.

4-4 Conclusions

Environmental risk assessment was conducted using the worst-case scenario approach (highest MEC and lowest PNEC). The results showed that six out of ten detected compounds presented high risk for algae, *Daphnia* and fish, while three compounds exhibited low to medium risk to these organisms in the three WWTP influents. An ERA for baclofen was conducted for the first time in WWTPs in this study. Environmental risk analysis of compounds detected in the studied WWTP effluents showed that three compounds (ciprofloxacin, metformin and ofloxacin) posed a high risk to the three trophic levels, while two compounds (acetaminophen, and caffeine) exhibited low to medium ecotoxicity against at least one aquatic organism. As most of the detected antibiotic compounds, either in the inlet or the outlet of the studied WWTPs, were evaluated to cause high environmental risk, it can be concluded that this class of compound should cause the most concern. The compounds that usually present a high risk should be considered in pharmaceutical pollutant prioritization lists for further investigation in the Saudi Arabian environment. The findings of this study suggest that

more focus is also needed on their potential risk to public health associated with wastewater irrigation.

CHAPTER FIVE

The potential for transport of pharmaceuticals in soils in Saudi Arabia.

5-1 Introduction

Micropollutants such as pharmaceuticals have been frequently reported in different environmental compartments and have been of great interest to researchers (Tang *et al.*, 2019). Pharmaceutical compounds have been increasingly reported in the soil environment so far (Carmona *et al.*, 2017; Biel-Maeso *et al.*, 2018; Conde-Cid *et al.*, 2018; Conde-Cid *et al.*, 2020). There are many pathways for pharmaceuticals to contaminate the soil, such as from landfill sites, medicines administered to livestock, sludge used as a fertilizer, leaking sewers, and reuse of WWTPs effluent for irrigation.

Reusing treated wastewater for irrigation is considered as the most significant entry route of pharmaceuticals into soils (Li, 2014; Biel-Maeso *et al.*, 2017). So far, up to 100 pharmaceutical compounds have been reported in soil and manure matrices (aus der Beek *et al.*, 2016) and the six most detected compounds in the soil were trimethoprim, sulfadiazine, triclosan, ibuprofen, diclofenac and carbamazepine (Li, 2014). For example, diclofenac, caffeine and trimethoprim have been found at concentrations between 0.81 ng g^{-1} and 15.39 ng g^{-1}, 0.44 ng g^{-1}, and 2.85 ng g^{-1} and 0.02 ng g^{-1} and 0.13 ng g^{-1} respectively in 21 soil sampling points along the Guadalete River basin, Spain (Biel-Maeso *et al.*, 2017). Also, acetaminophen and trimethoprim were detected in 29 different soil sampling points along the Turia River, Spain at concentrations of 13 ng g^{-1} and 9 ng g^{-1} respectively (Carmona *et al.*, 2017). Moreover, the occurrence of 21 pharmaceutical compounds, including acetaminophen, diclofenac and ibuprofen were investigated in soil samples collected from six locations in Poland (Kumirska *et al.*, 2019). They measured acetaminophen between 4.5 ng g^{-1} and 4.8 ng

g^{-1}, whereas diclofenac and ibuprofen were detected below their method quantification limit (<MQL).

It is possible that pharmaceuticals, in particular the highly mobile compounds, migrate downwards through the soil layers until they reach the saturated and unsaturated zones and end up contaminating groundwater (Sui *et al.*, 2015; Laws *et al.*, 2011; Bexfield *et al.*, 2019). For instance, leaching of antibiotic compounds to soil was studied by Park and Huwe (2016), who observed not only an adverse effect on the maintenance of the soil function, such as nutrient cycling, as a result of changes in the structure of the microbial community, but also contaminated groundwater and plants. The latter poses a potential risk for human consumers (Park and Huwe, 2016).

Moreover, the uptake of pharmaceuticals by plants from contaminated soil could be considered as an issue of concern when people consume these plants due to the possible accumulation of these compounds in plant tissues and leaves (Al-Farsi *et al.*, 2017). For example, 15 pharmaceutical compounds, particularly pharmaceuticals with n-octanol/water partitioning coefficient ($\log K_{OW}$) < 3 and molecular wight (MW) < 300 g mol^{-1}, belonging to different classes, including antibiotic and NSAID compounds, have been reported to transfer from sewage sludge amended soils into spinach (Kodešová *et al.*, 2019).

The above findings have highlighted the significance of understanding the mobility and behaviour of pharmaceuticals in the soil in order to provide essential information on their potential for migration towards valuable groundwater aquifers, particularly in countries where water resources are scarce, and which have very low rainfall, such as Saudi Arabia Therefore, this chapter focuses on the vulnerability of groundwater to pharmaceutical pollution in areas dominated by reuse of treated

wastewater in agriculture and for groundwater recharge. Laboratory-scale column experiments have been frequently used to study the mobility behaviour of contaminants, such as pharmaceuticals, because of their simplicity and the ease with which they mimic field conditions, such as temperature and flow rate (Banzhaf and Hebig, 2016; Oppel *et al.*, 2004; Revitt *et al.*, 2015). For example, unsaturated soil columns following OECD 312 guidelines (OECD, 2004) have been used under laboratory conditions to investigate the behaviour of many pharmaceutical compounds (Oppel *et al.*, 2004; Revitt *et al.*, 2015). Leaching experiments under unsaturated conditions have been carried out to study the mobility of pharmaceuticals including ibuprofen, trimethoprim and diclofenac in soil with high clay content (57%) (Siemens *et al.*, 2010). They found that all pharmaceuticals had low mobility in this soil, particularly trimethoprim. Salvia *et al*. (2014) investigated the accumulation, transfer, and degradation of pharmaceuticals, including trimethoprim and acetaminophen in a soil leaching column (30 cm × 10 cm) packed with two different soils. They found only a few compounds in the leachate with negligible concentrations, while others were retained in the soil column at various depth layers.

The hypothesis that pharmaceutical residues from wastewater used for irrigation may leach through soil layers and contaminate groundwater was tested here using soil columns in the laboratory. The model system was constructed according to OECD 312 guidelines (OECD, 2004), which enable simulation and investigation of the influence of different field parameters. The design allowed the influence of soil characteristics such as SOM, pH, WHC and soil texture to be assessed in relation to physicochemical parameters of the pharmaceuticals and their effect on leaching. Different types of soils at constant ambient temperature received known concentrations and volumes of artificial rain, following the OECD 312 guidelines (OECD, 2004). These soil types

were chosen as they represented field soil characteristics, including those found in the study location in Saudi Arabia (see section 2-4-2, Chapter 2).

5-2 Parameters affecting the movement of pharmaceuticals in the soil

The mobility of pharmaceuticals downward through the soil profile is controlled by the physicochemical parameters of the pharmaceuticals (i.e., K_D, logK_{ow}, pK_a etc..) and soil characteristics (i.e., CEC, soil texture, SOM, pH, water holding capacity (WHC), etc.). Some studies have investigated the effect of these parameters on the downward leaching of pharmaceuticals through soil. For example, soil CEC and soil texture were reported to be the most important parameters governing the sorption of antibiotics in 13 top soils (0-20 cm) in São Paulo State, south-eastern Brazil (Leal *et al.*, 2013). The sorption and mobility of ofloxacin and diclofenac have been studied in two different types of soil: low organic carbon, high pH (6.8) and high clay content; and high organic carbon, low pH (4.3) and low clay content by Drillia *et al.* (2005). Their results showed that ofloxacin had very high adsorption with low mobility in both soil types, while diclofenac adsorption was greater in soil with low clay content. Also, soils rich with organic matter exhibit low mobility and higher sorption for pharmaceuticals than those with a low organic matter content (Revitt *et al.*, 2015), whereas water holding capacity (WHC), clay content, the volume of artificial rain applied, the duration of the experiment and the applied concentration were observed by Oppel *et al.* (2004) to be the parameters with the greatest effect on the mobility of pharmaceuticals through soil columns.

Molecules of pharmaceuticals can occur as anionic, cationic, non-ionic or zwitter-ionic forms in the environment depending on their pK_a value and the pH value of their form in solution (Schaffer and Licha, 2015). The adsorption affinities of these differently charged compounds to the soil is governed by different soil properties

(Kočárek *et al.*, 2016; Kodešová *et al.,* 2015). For example, hydrophobic partitioning to the soil organic matter and/or hydrogen bonds with the hydroxyl groups on solid surfaces were observed to play an important role for the sorption of non-ionic and ionic compounds to soil particles. Also, the negatively charged clay fraction in soil has been observed to govern the adsorption of cationic compounds, while the anionic compounds sorption process is governed mainly by the attraction to positive charges on the solid surface (Kočárek *et al.*, 2016; Kodešová *et al.*, 2015).

Wojsławski *et al.* (2019) studied the migration behaviour of tramadol, carbamazepine and two transformation products (O-desmethyltramadol and 10,11-dihydro-10-hydroxycarbamazepine) in soil columns. They found the leaching behaviour of these compounds was strongly correlated with their natural charge and their low K_D value, which allows these compounds to penetrate soil layers and to pose a potential contamination risk to the groundwater. They also reported that the high adsorption of these compounds could be attributed to the high amount of clay mineral and organic matter. For example, for the compound tramadol, the interaction between the surface positive charge and the soil type with a greater clay texture (negatively charged) could explain its adsorption and immobility in this type of soil (Wojsławski *et al.*, 2019). Soil containing more clay and less sand was observed to facilitate high mobility of pharmaceuticals by Tang *et al.* (2018) because clay has been reported as the most active mineral component in the soil (Xu *et al.*, 2020). Ion exchange was also found to inhibit the adsorption of pharmaceutical compounds, such as carbamazepine metabolites, in soil where oxygen atoms form a hydrogen bond in water molecules.

Biotic (biodegradation and metabolization) and abiotic (hydrolysis and transformations) processes were considered by Salvia *et al.* (2014) as the major controls on the fate of pharmaceuticals in the soil matrix. Also, logK_{ow}, molecular weight, solid-

water distribution coefficient (K_D), soil pH value, soil texture, soil organic matter and soil cation exchange capacity (CEC) have all been observed to play an important role in the destiny and mobility of pharmaceuticals in soil (Barron *et al.*, 2009; Kodešová *et al.*, 2019; Qin *et al.*, 2016; Xu *et al.*, 2020). The solid-water distribution coefficient (K_D) predicts the distribution of pharmaceuticals between the aqueous phase and the solid phase and is reported to be mainly responsible for whether the compound remains in the aqueous phase or is adsorbed to solid matrices. For example, the sorption and fate of 49 pharmaceuticals were studied in a solid matrix by Barron *et al.* (2009), who reported that compounds with K_D values ≤ 50 show low affinity for the soil matrix.

The adsorption process of pharmaceuticals was reported to increases significantly with the increase of the ratio of organic matter and clay content in the soil and therefore their high affinity to the soil (Rusiniak *et al.*, 2021). The sorption for the trimethoprim and atenolol were observed to positively correlate with CEC, and with clay content. Also, the correlations between sorption coefficients for trimethoprim and organic content were reported to be positive by Kodešová *et al.* (2015). Another factor potentially affecting the adsorption and mobility of pharmaceuticals in the soil is the pK_a of the investigated compound relative to the pH of the soil (Revitt *et al.*, 2015). Pharmaceutical compounds with pk_a values between 3.6 and 4.1, such as diclofenac and ibuprofen, were observed to adsorb on a solid matrix at neutral pH, and their adsorption increased at lower pH (Fent *et al.*, 2006; Gworek *et al.*, 2021).

5-2 Materials and methods

5-2-1 Selected compounds

Sixteen compounds, six antibiotics (ciprofloxacin, ofloxacin, cephalexin, azithromycin, tetracycline, trimethoprim), three NSAIDs (acetaminophen, diclofenac, ibuprofen),

caffeine, atenolol, metformin, allopurinol, triclosan, oxypurinol and baclofen, were selected to study their leaching behaviour in laboratory-scale soil columns. The complete list of analytical standards (including purity and origin) and the reasons for their selection are described in chapter 2 section 2-2.

5-2-2 Soils

Soil samples were collected from around Cork city (51.8985° N, 8.4756° W) (silt loam, clay, silty clay loam), Ireland, while a sandy loam was purchased from the Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Munch, Germany (refeesol 01-A-05). These soils differed in terms of their pH, soil organic matter (SOM), soil texture and WHC (Figure 5-1).

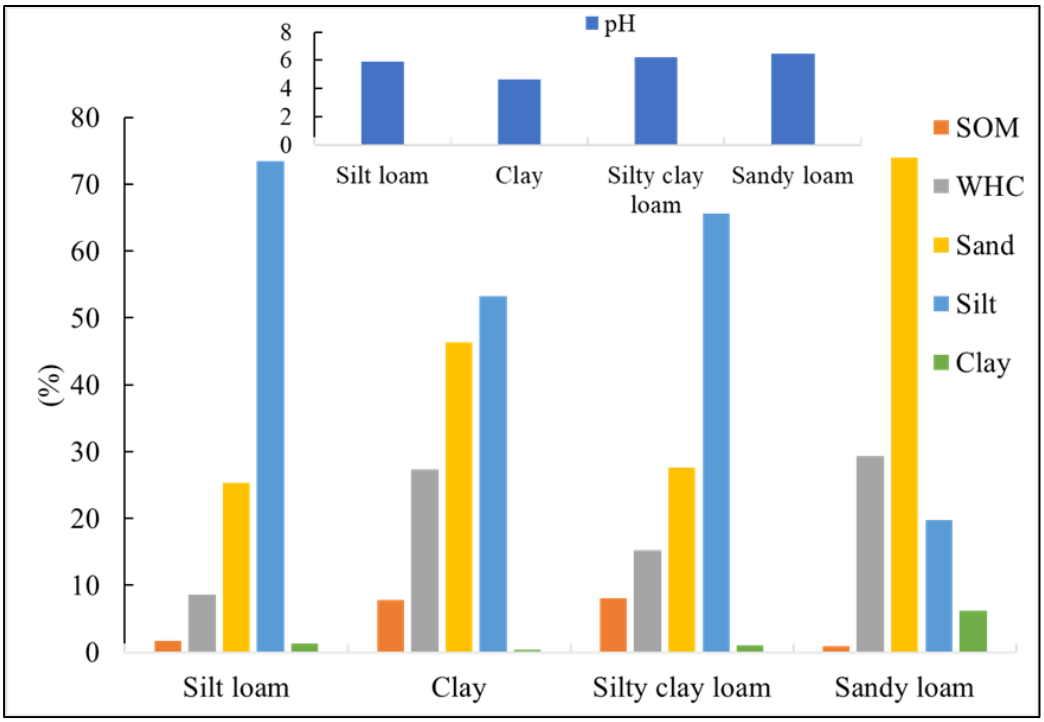


Figure 5-1: Soils characteristics for the four soils used in experimental columns. SOM: Soil organic matter, WHC: Water holding capacity.

Soil descriptions and details of collection and analysis can be found in chapter 2 sections 2-4-2, 2-6-2 and 2-6-6. Soils were air dried, homogenized and sieved (>2 mm) prior to packing into the soil leaching columns.

5-2-3 Experimental design

In order to study the mobility of the selected pharmaceuticals through the four different soil types, laboratory-scale soil columns were used. Four glass columns, each 35 cm high and 5 cm inner diameter, were fabricated in the Chemistry department glassware laboratory, University College Cork (UCC), Ireland according to the OECD 312 protocol (OECD, 2004; Oppel *et al.*, 2004; Revitt *et al.*, 2015). Glass wool and crystallized quartz sand were placed in the bottom of the columns to hold the soil particles in place before packing the four columns with one of the four soil types. Three of the columns were then spiked with the selected pharmaceuticals at a concentration of 100 ng g^{-1} and the remaining column, to which no pharmaceuticals were added, acted as a control column in order to check for any cross contamination (Revitt *et al.*, 2015). Artificial rain (0.01 M $CaCl_2$) was then applied on the top of the soil columns at flow rate around 5.2 ml h^{-1} for 48 hours for percolation to occur at room temperature (≈ 20 °C). Aluminium foil was wrapped around the columns to prevent potential photolysis. More details about the soil column experiments were given in the materials and methods chapter (chapter 2 section 2-5). The steps taken in the soil column experiments are summarized in Figure 5-2.

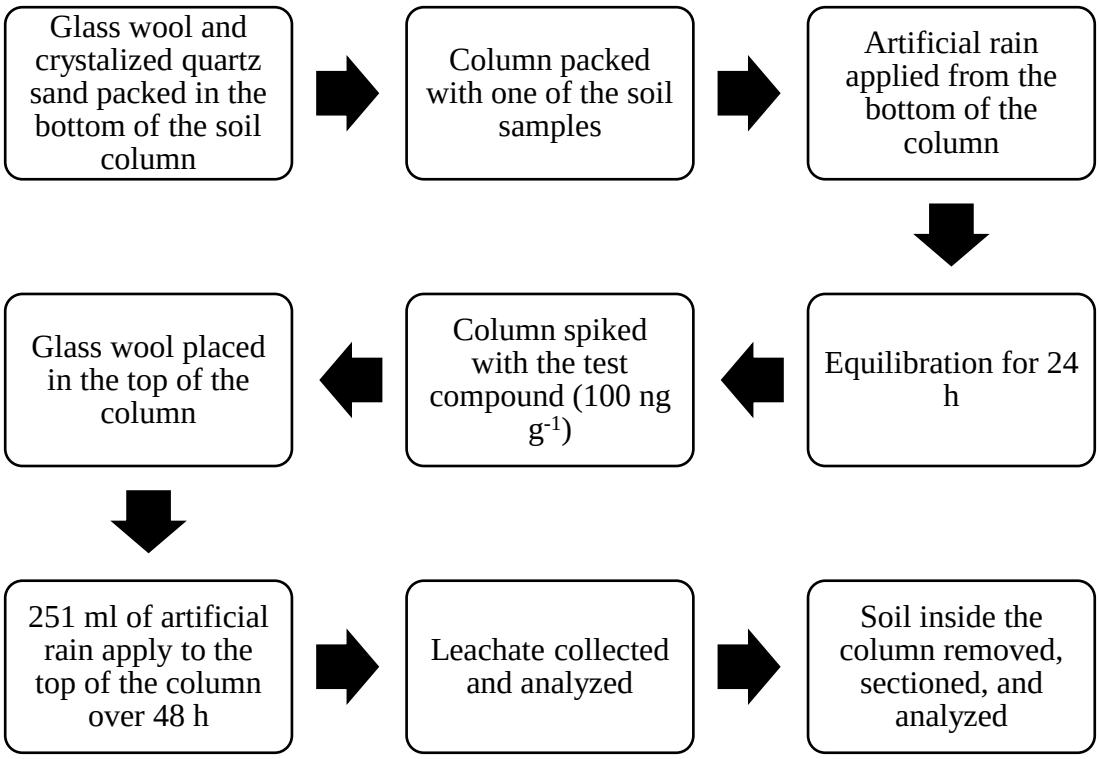


Figure 5-2: Steps taken in the soil column leaching experiments.

5-2-4 Sample extraction

Leachates that had percolated through each soil column were collected in 500 ml amber glass bottles. The leachates were immediately stored in a freezer at -20 °C and extracted the next day. Afterwards, extracted samples were sent frozen to the laboratory for HPLC-LC-MS/MS analysis. The volumes of the leachates collected from the soil columns varied between 236 and 250 ml.

5-2-4-1 Leachate

After 48 hours, at the end of the experiments, the leachates from the soil columns were extracted and cleaned by solid phase extraction (SPE) using Strata-X 33u SPE cartridges, Polymeric Reversed Phase 6 mL, 200 mg (Phenomenex, UK). The SPE method used here followed that developed by Dasenaki and Thomaidis, (2015) with some minor adjustment. Each leachate collected from the soil columns was diluted into Milli-Q water to obtain a final total volume of 500 ml. The pH was then adjusted to ≈

2.5 prior to the extraction process. The SPE cartridges were conditioned and equilibrated with 6 ml of methanol and then 6 ml of Milli-Q water prior to samples being passed through cartridges at a flow rate of around 3 ml min^{-1} using a 12-position SPE vacuum manifold Phenomenex kit. The cartridges were then dried for 15 minutes. Subsequently, the cartridges were eluted with 6 ml of methanol and the eluate dried under a gentle stream of nitrogen to dryness. Finally, the dry samples were placed in freezer at -20 °C until the LC-MS/MS analysis. More detail can be found in chapter 2 sections 2-6-1 and 2-6-5.

5-2-4-2 Soil

Ultrasound-assisted extraction (UAE) was used to extract the investigated pharmaceuticals from the soil samples following the method developed by Gago-Ferro *et al* (2015). Briefly, soils were removed carefully from the columns and divided into four sections of 1-5, 5-10, 10-20 and 20-30 cm depth and kept until dry at room temperature. A 100 mg sample of each dried soil was subsequently mixed with extraction solution (50:50 (v/v) 2 ml of MeOH and Milli-Q water (pH 2.5, FA 0.5 % and 0.1 % EDTA)) in 15 ml tubes using a vortex mixer for 1 minute. This step was followed by ultrasonication for 15 minutes at 50 °C, then centrifugation for 10 min at 4000 rpm. The supernatants were then collected. Afterwards, these steps were done two times more for the same 100 mg soil sample. A total of 6 ml was collected of supernatant and dried under a gentle stream of N2 at 40 °C. The dried samples were conserved in a freezer at -20 °C prior to LC-MS/MS analysis. Further detail can be found in chapter 2 sections 2-6-2 and 2-6-6.

5-2-5 HPLC-LC-MS/MS analysis

High performance liquid chromatogram mass spectroscopy (HPLC-LC MS/MS) was carried out on the extracted samples at the Laboratory of Analytical Chemistry,

Department of Chemistry, National and Kapodistrian University of Athens, Greece using the method published by Dasenaki and Thomaidis (2015) with few adjustments. Instrumental analysis was carried out using a Thermo Scientific Accela ultra-high-performance (UHPLC) system connected to a TSQ Quantum Access triple-quadrupole (QqQ) mass spectrometer (Thermo Scientific, San Jose, CA, USA). The system was equipped with an electrospray-ionization (ESI) source (Thermo IonMAX) operated in both positive and negative modes. The separation was performed by using an Atlantis T3 C18 column (100 mm × 2.1 mm, 3 μm, Waters) protected by a guard column at a constant flow rate of 100 μL/min and 10 μL of extracted sample was injected at a maintained temperature of 30 °C. The LC-MS/MS analysis method was outlined in detail in chapter 2 section 2-6-3 and 2-6-4.

5-3 Results and discussion

5-3-1 Leaching soil column

Eighty samples (16 leachate samples and 64 soil samples) were collected and analysed. Out of 16 compounds, 11 were detected either in the soil column sections or leachates, while five compounds (metformin, allopurinol, oxypurinol, triclosan, acetaminophen) were not detected, possibly due to their low recovery and/or high affinity to soil particles. Acetaminophen was not detected either in soil sections or leachates, possibly due to its rapid degradation (Foolad *et al.*, 2015), hence it is not discussed further here. Atenolol, caffeine, ciprofloxacin, diclofenac, ofloxacin, trimethoprim, ibuprofen, and baclofen were detected in all tested soil samples but at different percentage recoveries, ranging between 12% and 100% (Figure 5-3). This could be attributed to the different clay fractions in the soils because compounds interact with clay minerals and bind to the soil as proposed by Wojsławski *et al.* (2019). Only two compounds (caffeine and cephalexin) were detected in the percolation of the soil column. The percentage of the

applied concentrations of these compounds found in the leachate water were ranged between 0.05% and 0.42% (Table 5-1). Table A-5-1 (Appendix) shows the full results obtained from the soil leaching column experiment.

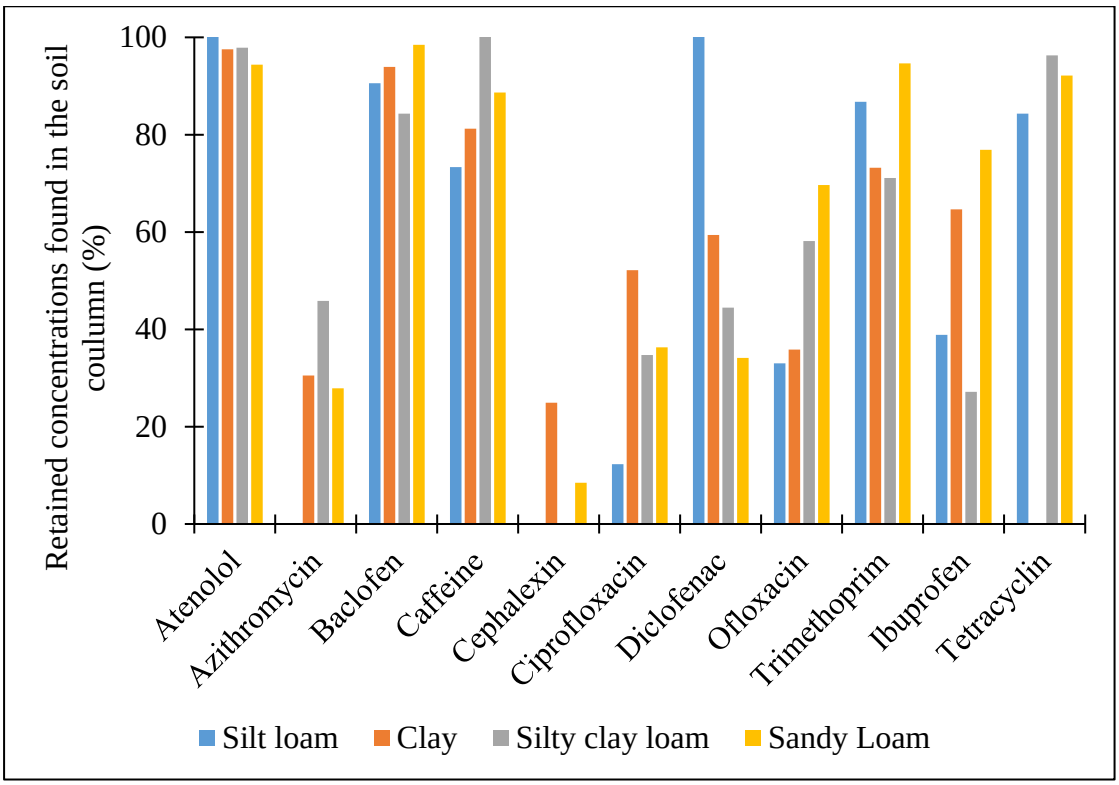


Figure 5-3: The percentage retained in different soil types from the total amount of different pharmaceutical compounds applied to the surface of the columns.

Table 5-1: Percentage recovery of pharmaceuticals after percolation through soil columns of different soil types.

Analytes	Silt loam (%)	Clay (%)	Silty clay loam (%)	Sandy loam (%)
Atenolol	<LOD	<LOD	<LOD	<LOD
Azithromycin	<LOD	<LOD	<LOD	<LOD
Baclofen	<LOD	<LOD	<LOD	<LOD
Caffeine	0.09	0.06	0.08	0.14
Cephalexin	0.05	<LOD	<LOD	0.42
Ciprofloxacin	<LOD	<LOD	<LOD	<LOD
Diclofenac	<LOD	<LOD	<LOD	<LOD
Ibuprofen	<LOD	<LOD	<LOD	<LOD
Ofloxacin	<LOD	<LOD	<LOD	<LOD
Tetracycline	<LOD	<LOD	<LOD	<LOD
Trimethoprim	<LOD	<LOD	<LOD	<LOD

5-3-2 Diclofenac and ibuprofen

Diclofenac was detected in all soil types in the segment depth of 0-5 cm at percentages ranging from 34.15% to 95.98% from its spiked concentration, and at percentage of 4.19% between 5-10 cm depth for only the loam soil sample. Similarly, ibuprofen was detected in all soil samples in the first 0-5 cm section at recoveries between 27.14% and 76.90% of the spiked concentrations (100 ng g^{-1}). Both compounds showed no mobility under experimental conditions and were detected below their LOD at all other soil column depths and in the leachate (Figure 5-4). The low mobility observed here is possibly because of the low volume of artificial rain applied in the soil columns or due to their lipophilicity, as suggested for ibuprofen (Oppel *et al.*, 2004). Silt loam soil was observed to retain almost all diclofenac (95.98%), while ibuprofen was retained more in the sandy loam soil (Figure 5-4). There was a low recovery observed for ibuprofen and diclofenac in the other soils.

It has been observed that pharmaceutical compounds with pK_a around 5 (e.g., diclofenac and ibuprofen) and their adsorption to soil is largely controlled by the high fraction of organic matter (Paz *et al.*, 2016), which was the situation for two of the soils studied here (clay and silty clay loam). The UN Food and Agriculture Organization (FAO) soil mobility classification criteria,recommended also by the US EPA, are based on K_{OC} (Soil Adsorption Coefficient), where compounds with high K_{OC} value like diclofenac (245) and very high K_{OC} like ibuprofen (3400) have moderate to very high affinity to adsorb to the soil and organic matter and will not move through the soil (FAO, 2000).

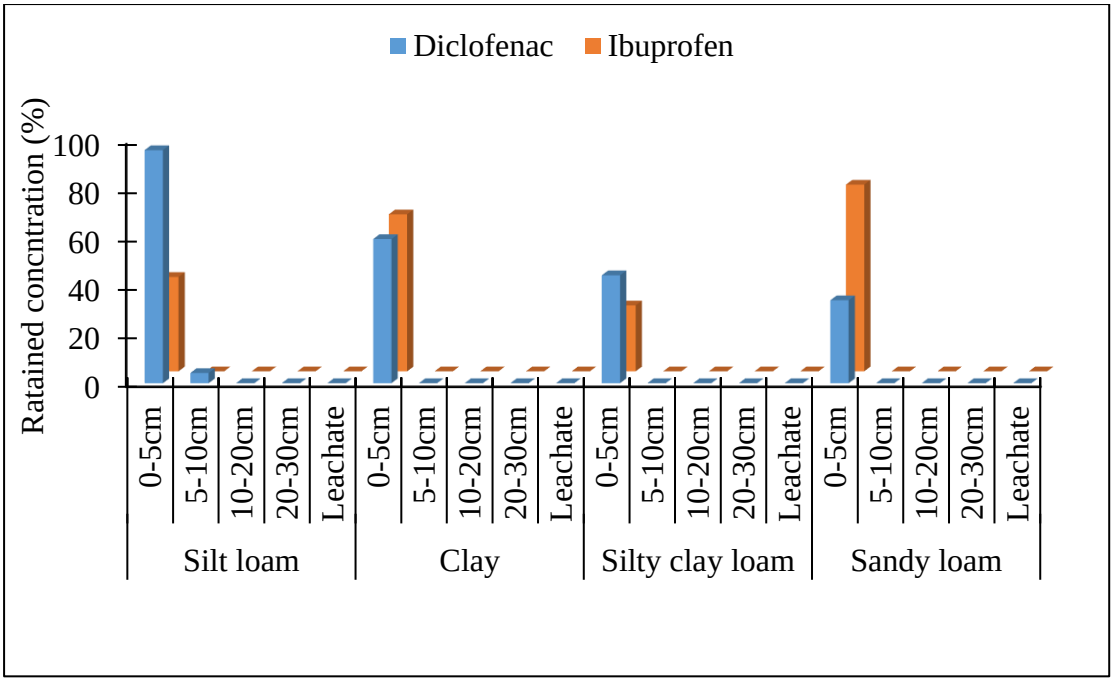


Figure 5-4: Percentage retained from the original applied concentration for diclofenac and ibuprofen for each soil column depth segment and the leachates.

The fluctuation in concentrations retained from the initial spiking concentrations of pharmaceuticals in solid matrices (soil, sludge) could be attributed to high levels of biodegradation or transformation into their metabolites, as for ibuprofen (Barron *et al.*, 2010; Oppel *et al.*, 2004). Strong affinity and low mobility of ibuprofen in two different loamy sand soils has also been reported by Oppel *et al*, (2004) using leaching soil column experiments. Diclofenac immobility has been observed in two different types of soil using soil column experiments by (Revitt *et al.*, 2015). They reported that the organic carbon partition coefficient (K_{OC}) value played a key role in the low mobility and observed retention. In a study by Williams and McLain (2012), diclofenac accumulation was investigated in groundwater basins recharged with treated wastewater. They found diclofenac in soil samples collected over three years was below its LOD (0.003 ng g^{-1}) and showed no accumulation. This was possibly due to degradation processes under aerobic conditions (Williams and McLain, 2012).

It is worth noting that ibuprofen was detected below its LOD, while diclofenac was measured at an average of 9.2 μg L^{-1} and < LOD in the three different WWTP influents and effluents respectively, which is far less than the concentrations applied to the soil column. Moreover, average concentration of 0.42 μg L^{-1} for diclofenac and ibuprofen were reported in Saudi Arabian WWTP effluents by Al Qarni *et al*. (2016), Alidina *et al*. (2014) and Shraim *et al*. (2017). In addition, the artificial rain applied to the soil column (251 ml) was higher than the average annual rainfall (<200 mm) in Riyadh city (Almajed *et al.*, 2021). Therefore, taking these factors into account, the possibility of these compounds reaching ground water in the studied area is negligible.

5-3-3 Baclofen

No previous studies of the mobility of baclofen in soil column experiments have been found in the scientific literature. Baclofen was immobile in the soils studies here and was mostly recovered in the top section (0-10cm) of the soil columns, in contrast with hypothesis of this chapter. The average percentage retention for all tested soils was 91.84%. It has been reported that compounds with logK_{ow} < 2.5, like baclofen (logK_{ow} = -1.32), have a tendency to remain in the solid matrix (Mompelat *et al.*, 2009). The percentage of the spiked concentrations (100 ng g^{-1}) of baclofen detected at 93.95% and 98.49% in clay and sandy loam were slightly higher than silt loam and silty clay loam at 90.59% and 84.31% respectively (Figure 5-3). Most of the applied concentration of baclofen was recovered from 0-5cm depth in the soil columns in all soil samples, whereas in other depth fractions it was found to be between 1.67% and 17.28% (Figure 5-5). Baclofen showed greater migration through silt loam soil, which has less SOM, than the other investigated soils and was detected down to the 10-20 cm depth fraction (Figure 5-5).

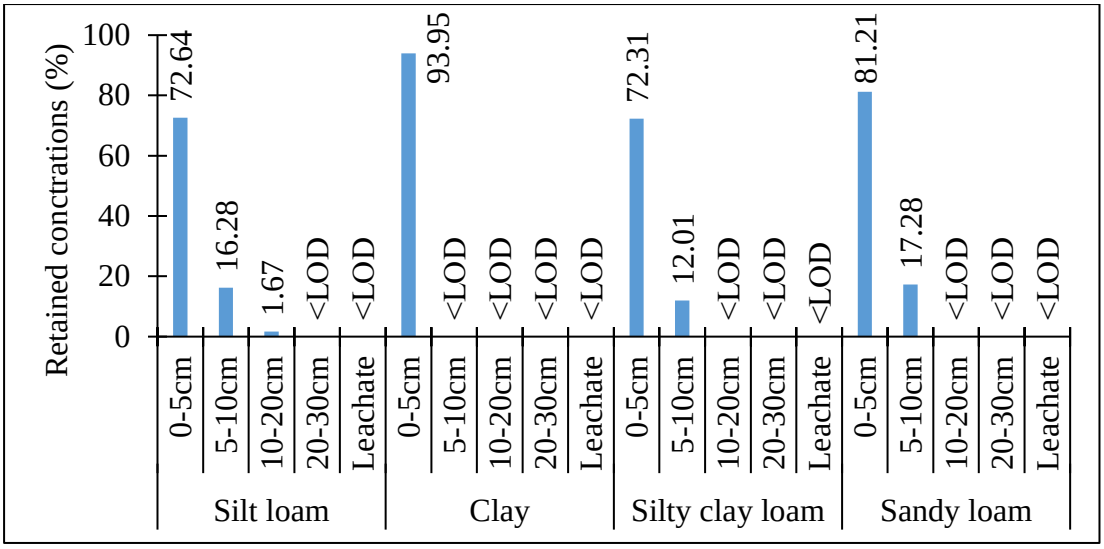


Figure 5-5: Fraction retained (%) from spiked concentration of baclofen in the different depths of the soil columns and their leachates.

It possible baclofen behave in the same way as three pharmaceutical compounds found to have high retention in soils with high organic matter using soil column (Conde-Cid *et al.*, 2019).

The result obtained here represent laboratory conditions, and considering the real harsh environmental conditions like low precipitation (<<200mm) and high evaporation rate between 67 and 497 mm in Riyadh city (Al-Arifi *et al.*, 2013; Almajed *et al.*, 2021), the transport of baclofen downwards to groundwater seems to be unlikely when the aquifer is covered with at least 20 cm of the all studied soil.

5-3-4 Atenolol

Atenolol was observed to be immobile in all four soils. A high percentage of the initial spiked concentration was detected in all the investigated soils. Almost all spiked atenolol (100 ng g^{-1}) was found in the soil depth of 0-5 cm, at percentages of 88.30%, 66.69%, 94.50%, and 84.90% in silt loam, clay, silty clay loam and sandy loam soils respectively, with a small fraction (3.35 to 30.83%) retained at 5-10 cm (Figure 5-6). This is consistent with the high average recovery rate (91%) of atenolol reported in 13

different types of soils by Kodešová *et al.* (2016). Although a high concentration of atenolol was applied to the soil column compared with its average concentration detected in the three investigated WWTP effluents (3.97 µg L^{-1}), atenolol was not migrating through the soil column and was detected below its LOD in the soil column leachates. Also, when taking in to consideration the real environmental conditions in the study area, like low rainfall and high evaporation in Riyadh city (Al-Arifi *et al.*, 2013; Almajed *et al.*, 2021), atenolol has negligible migration in soil and poses a minimal threat to groundwater. Thus, this study suggests that atenolol is unlikely to reach the deep groundwater level in Riyadh.

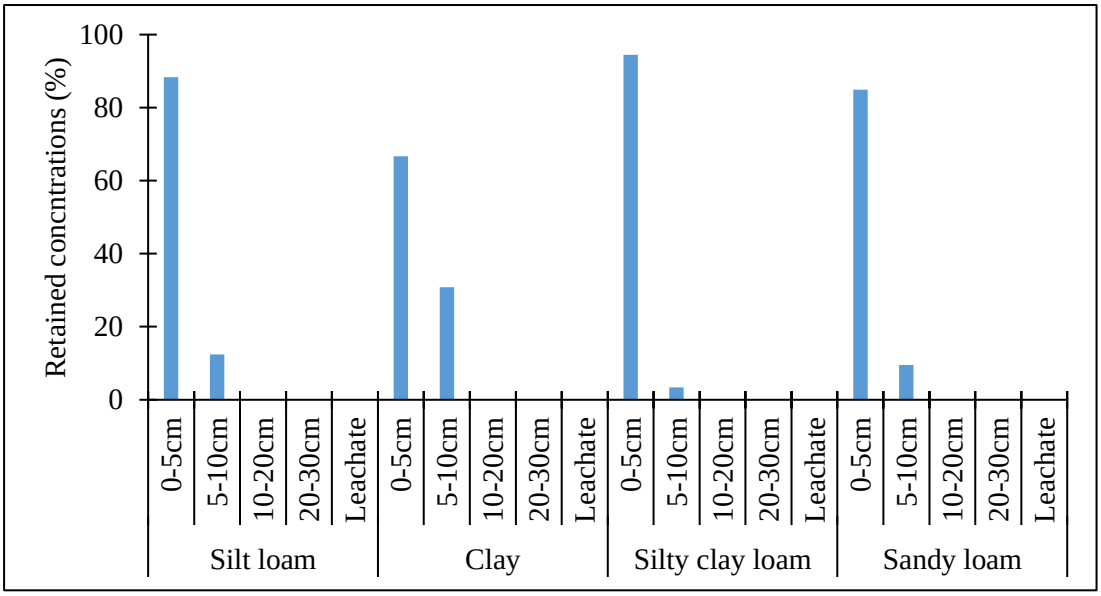


Figure 5-6: Recovery (%) from applied concentration of atenolol at different depths and in the leachates of the selected soils.

According to FAO, moderate mobility would be expected for compounds like atenolol (LogK_{OC} = 2.17) (FAO, 2000). The high water distribution coefficient (K_D) >50 of atenolol could be responsible for its tendency for adsorption in soils (Barron *et al.*, 2009). In addition, the adsorption of cationic pharmaceutical compounds (protonated bases) like atenolol (Schaffer *et al.*, 2015), has also been reported to be

enhanced by the negatively charged surfaces of soil fractions (Kočárek *et al.*, 2016; Kutzner *et al.*, 2014; Schaffer *et al.*, 2012).

The mobility of atenolol found here is in good agreement with its transport in two different soils used in laboratory column experiments, particularly in sandy loam soil (Schübl *et al.*, 2020). Consequently, atenolol could not be considered as a potential groundwater pollutant (Kodešová *et al.*, 2016).

5-3-5 Antibiotic compounds

Although the applied concentrations of the studied antibiotics to the soil columns under artificial rain were far higher than their concentrations found in the three studied WWTP effluents (≤33.16 μg L^{-1}), all selected soils intercepted the migration of these compounds and therefore no residues were detected in the soil column leachates (Figure 5-7). The exception was cephalexin, for which the percentage of 0.05% and 0.42% was found in the leachate of silt loam and sandy loam soils respectively. Silt clay loam and sandy loam soils were observed to retain most of the studied antibiotic compounds, while silt loam soil retained ciprofloxacin, ofloxacin, trimethoprim and tetracycline and clay retained cephalexin, ciprofloxacin, ofloxacin, trimethoprim and azithromycin. Trimethoprim and tetracycline showed the highest retention percentages from their applied concentrations, while cephalexin presented the lowest retained percentage from the spiked concentration of selected antibiotic compounds (Figure 5-8).

Cephalexin was retained only in clay and sandy loam soils at a low percentage of the original spiked concentration, probably due to its recovery by the UAE extraction method being very low (12.30 to 31%) compared with the other tested antibiotic compounds. Azithromycin was retained in clay, silty clay, and sandy loam soils at a low percentage of 30%, 45% and 27% respectively from its initial spiked concentration

(100 ng g^{-1}). The low recovery of the antibiotic compounds could possibly be attributed to:

1- Degradation of antibiotics in the soil (Yang *et al.*, 2012; Zhi *et al.*, 2019).

2- Hydrolysis reactions. These are considered as one of the main pathways by which antibiotics are degraded in soil, particularly macrolide antibiotics like azithromycin (Białk-Bielińska *et al.*, 2012; Conde-Cid *et al.*, 2020; Salvia *et al.*, 2014).

3- Azithromycin having a high affinity for, and immobility in, soils (Maier and Tjeerdema, 2018).

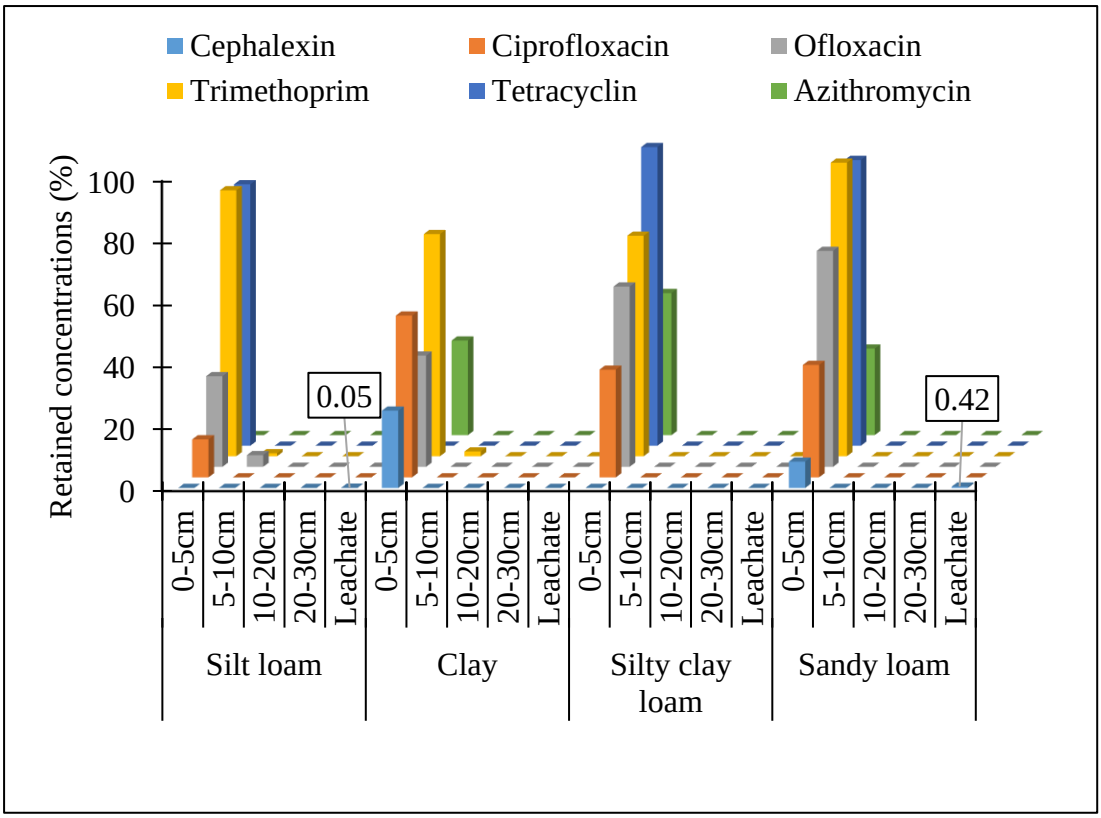


Figure 5-7: Percentage of initial applied mass of the antibiotic compounds retained in different soil column sections and in their corresponding leachates.

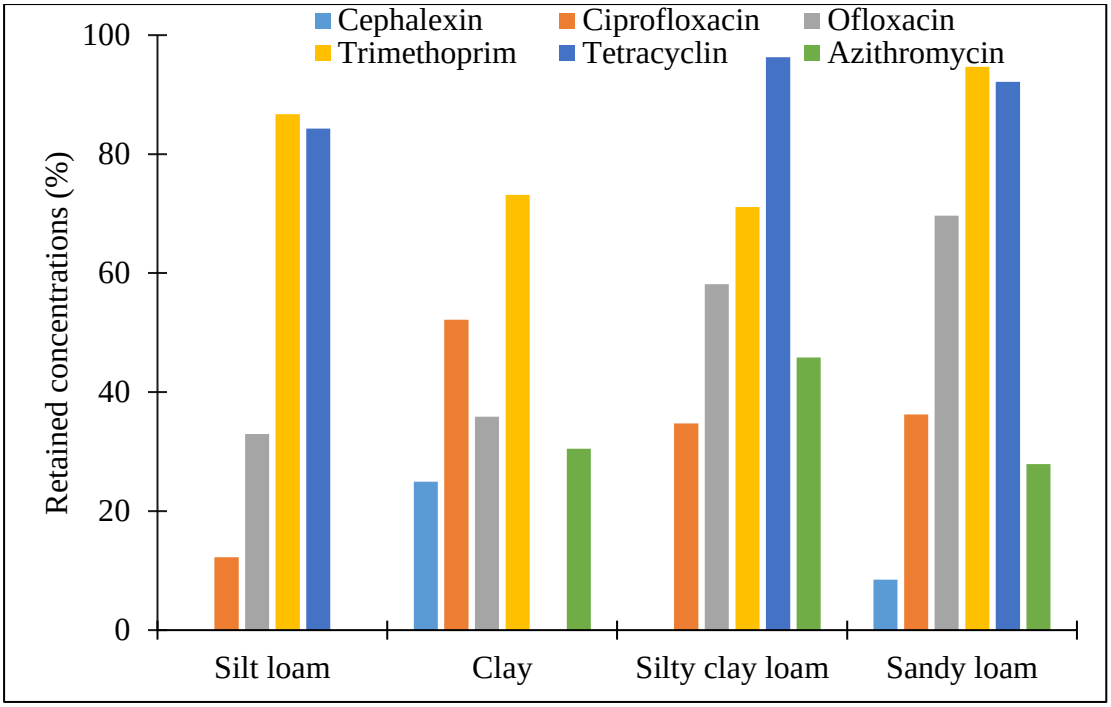


Figure 5-8: Retained concentrations (%) of antibiotic compounds in the different studied soils.

Antibiotic compounds, like ciprofloxacin and ofloxacin, have been reported to have a strong affinity for soil (Zhi *et al.*, 2019). They have been classified as having low mobility and a high affinity to the soil matrix, particularly sandy loam soil, due to their high K_D values at 91.33 and 134 L kg^{-1} respectively (Kiecak *et al.*, 2019). This is consistent with the ciprofloxacin and ofloxacin immobility observed here, especially in the sandy loam soil.

Trimethoprim showed no mobility, with up to 94% of its applied concentration recovered in the top layer (0 and 5 cm) of the soil column for all the tested soil types. This is in good agreement with some previous reports in the literature. For example, low mobility of trimethoprim was observed in soil column experiments packed with soil comprising 57% clay, 32% silt, and 11% sand by Siemens *et al.* (2010) and it was found to be immobile in two different soils (silty clay loam and sandy loam) by Salvia *et al.* (2014). The sorption affinity of positively charged trimethoprim molecules could

be explained by the negatively charged soil surfaces and their high affinity to the SOM (Kočárek *et al.*, 2016).

Therefore, and due to its immobility, trimethoprim has been detected in the soil matrix in some previous studies. For instance, trimethoprim has been measured at concentrations between 0.44 ng g^{-1} and 13 ng g^{-1} in soils collected along two river basins in Spain (Biel-Maeso *et al.*, 2017; Carmona *et al.*, 2017).

In this study, a high percentage (84% to 96%) of the applied concentrations of tetracycline was retained in the top layer of the soil columns. Tetracycline showed similar retention behaviour as trimethoprim (Figure 5-7). Soil with a high clay content was found to adsorb tetracycline better than soil with a low clay fraction when using batch sorption experiments (Teixidó *et al.*, 2012). Conde-Cid *et al*. (2018) also found tetracycline in more samples and at higher concentrations than other compounds due to its high adsorption and low mobility in soil samples with clay fractions between 15% and 31%.

It is worthwhile to note that the penetration of all the studied antibiotic compounds below the 0-5 cm layers of all four different tested soils was negligible under laboratory conditions and that the 5-cm depth represents a barrier below which compounds are unlikely to leach. It has been reported that the adsorption and strong affinity to soil for these compounds could be attributed to ion exchange processes, particularly for soil with a pH less than the pK_a of the compounds (Garduño-Jiménez *et al.*, 2022), as for the clay soil tested here.

Therefore, leaching of these compounds to groundwater under the real environmental conditions (high evaporation, low rainfall, and high temperature) in Riyadh city is unlikely, except for cephalexin for which an extremely low percentage

(≤0.42%) of its applied concentration was detected in the leachate of silt loam and sandy loam soil samples.

5-3-6 Caffeine

Caffeine was observed in all the tested soils to be slightly more mobile than the other tested compounds. It was detected in the leachate samples as well as in all soil column segments, particularly in silt loam and silt loam clay soils (Figure 5-9). Interestingly, silt loam soil and silty clay loam had less water capacity than the other tested soils. The percentage of the applied concentrations of caffeine found in the leachate of the investigated soils was in order of silt clay loam> sandy loam > clay> silt loam soils. It is worth noting that the caffeine was applied to the soil column at a concentration nine times less than its average measured concentration in the three WWTP effluents (924.34 μg L^{-1}). Therefore, it is anticipated that the actual high concentrations of caffein could reach groundwater in the studied area.

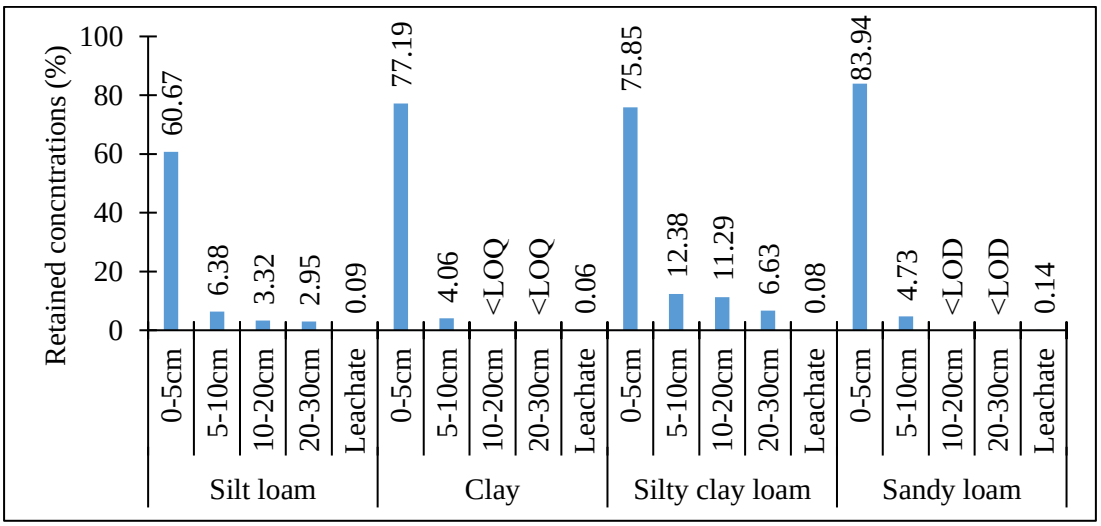


Figure 5-9: Percentage of caffeine retained at different depths and in the leachates of four soil types after application to the top of the soil column.

Caffeine can seep through soil and show low adsorption capacity, probably due to its polarity. Carbon in the environment usually adsorbs nonpolar compounds more

than polar compounds like caffeine (Sotelo *et al.*, 2014). The mobility of caffeine was investigated in the coarse-gravel unsaturated zone in the Selniška Dobrava aquifer, Slovenia (Koroša *et al.*, 2020). They reported that caffeine was very mobile and migrated towards the groundwater faster than other studied compounds at an average flow of 0.098 m day^{-1}. Also, the high water solubility, low molecular size, and low octanol-water partition coefficient of caffeine has been reported to be responsible for its high mobility behaviour in the soil matrix (Redding *et al.*, 2009; Sotelo *et al.*, 2014). This suggests that caffeine could have the potential to contaminate groundwater resources.

The accumulation and mobility of caffeine was also investigated in soils collected from groundwater recharge basins in Arizona, USA that had been recharged with treated wastewater (Williams and McLain, 2012). Caffeine was found in all soil sample depths at concentrations of 1.2 ng g^{-1}, 1.1 ng g^{-1}, and 0.6 ng g^{-1} for depths of 0-5 cm, 10-15 cm, and 25-50 cm respectively. They also found that the soil organic matter and clay content were strongly correlated with caffeine mobility.

5-4 Conclusions

Most of the investigated pharmaceutical compounds were observed to be retained in the first few centimetres of the soil columns, between 1 to 5 cm, while only a few migrated to 10 cm depth in the soil columns. Only two compounds, the stimulant caffeine, and the antibiotic cephalexin, penetrated the studied soils under laboratory conditions and were detected in the leachate. These two compounds were detected in the leachates of the investigated soils at a small fraction out of their initial spiked concentration ranging between (0.05% and 0.42%) and (0.06% and 0.14%) for cephalexin and caffeine respectively. Silt loam and sandy loam soils were penetrated

by these two compounds, possibly because these soils have the lowest SOM, whereas the other two tested soils (clay and silty clay loam) were penetrated only by caffeine.

Although, the tested soils showed almost no mobility of the most selected pharmaceuticals and, therefore, potential contamination of groundwater by the studied pharmaceuticals would be unlikely if the groundwater was covered by a sufficient depth of these soil types under real field condition. The possible exceptions to this could be caffeine and cephalexin, which were found to be slightly mobile in the selected soils. Therefore, the hypothesis that it is possible for pharmaceutical residues from wastewater used for irrigation to leach through soil layers and to contaminate groundwater was rejected.

More studies are needed to investigate the transport behaviour of these pharmaceuticals under different conditions, such as applying artificial rain in the top of the column for longer time periods. Also, core samples need to be taken from the locations where treated wastewater is discharged for use in column experiments. Moreover, using treated wastewater instead of artificial rain would reflect better the actual situation of the studied area. The influence of other environmental weather parameters such as temperature, and sunlight, together with a wide range of soil characteristics (pH, soil texture, WHC, SOM), should also be investigated. Additionally, sterile soils, where the potential for biodegradation is prevented from interfering with leaching behaviour, needs to be further explored.

CHAPTER SIX

Conclusions and recommendations

6-1 Main conclusions

To address the knowledge gap in the occurrence of pharmaceuticals in the environment of Saudi Arabia, this study evaluated the concentrations of 16 compounds in three different WWTPs, including some compounds that had never been investigated before in Saudi Arabia or globally. The results enabled a better understanding of the release of these compounds to the aquatic environment in Saudi Arabia. In addition, this study monitored the concentrations of these compounds over a 12-month period, which had not been done in previous studies. Therefore, the study has made a major contribution to reducing the knowledge gap about pharmaceuticals in the environment of Saudi Arabia, and particularly their discharge from WWTPs.

6-1-1 The occurrence of pharmaceuticals in the studied WWTPs

The results indicated that 62.5% and 31.25% of selected compounds were detected above their LOQs in the investigated WWTPs influents and effluents, respectively. Generally, this study highlighted that the concentration of pharmaceuticals found in the raw wastewater at the investigated WWTPs was higher than that reported elsewhere around the world, which might indicate a high consumption level of these compounds compared with other countries. The influent concentrations of detected compounds were between 0.23 μg L^{-1} and 2281.59 μg L^{-1}, 0.05 μg L^{-1} and 1449.46 μg L^{-1} and 0.12 μg L^{-1} and 1497.60 μg L^{-1} in H-, M- and KSU-WWTPs, respectively, whereas their concentrations in the effluents decreased dramatically and ranged between 0.02 μg L^{-1} and 33.16 μg L^{-1}, 0.01 μg L^{-1} and 19.92 μg L^{-1}, and 0.02 μg L^{-1} and 20.07 μg L^{-1} in H-, M- and KSU-WWTPs, respectively. Despite its small size, KSU-WWTP which

received a combination of hospital and municipal wastewater, was found to have similar concentrations of the pharmaceutical compounds in the influent and effluent when compared with the other two large-scale municipal wastewater treatment plants. That could, perhaps, be attributed to the wastewater which is discharged from the nearby hospital to this plant without any treatment, and which represents almost a third of the plant capacity. Baclofen was found for the first time in an aquatic matrix both nationwide and worldwide but only in KSU-WWTP influent at concentrations between 0.33 μg L^{-1} and 2.82 μg L^{-1}.

The average removal efficiency in all the investigated WWTPs was high (70%< RE <99%), with M-WWTP showing slightly better removal than the other two WWTPs. This could be due to the use of tertiary treatment technologies in all three WWTPs (trickling filter or oxidation ditch + sand filter), which eliminate pharmaceuticals from the wastewater more effectively. The ambient temperature was observed in this study to have no effect on eliminating the pharmaceutical compounds, although a positive correlation between temperature and pharmaceutical removal has been reported elsewhere. This could be because in Saudi Arabia the temperature is above 20 °C almost year-round and sampling was conducted during the moderate to hot seasons.

During the 12-month sampling campaign, the selected compound concentrations in the WWTP influents and effluents were observed to decreased from the first samples taken in April/May 2018. This decrease may be due to new legislation that came into force to minimize over-the-counter purchase of pharmaceuticals at that time.

6-1-2 Seasonal variation

Although, the occurrence of pharmaceuticals in the environment has been reported in many studies around the world, seasonal fluctuations in emissions have received little attention, especially in the Middle East and particularly in Saudi Arabia. This study examined seasonal variations in three different WWTPs over a 12-month period. Significant temporal variations in pharmaceutical concentrations were observed across different seasons, particularly in the WWTP influents. The compounds generally presented higher concentrations, particularly for antibiotics and NSAIDs, during cold months in 2019. This is possibly a result of their high consumption patterns in response to seasonal illnesses because ambient temperature showed no relationship with pharmaceutical removal for the three WWTPs. The similarity in seasonal variations antibiotics and NSAIDs is supported by their significant and strong positive correlation with each other.

Acetaminophen and caffeine followed almost the same seasonal pattern, particularly in H- and KSU-WWTP influents. This may be due to their use in combination in many medical situations, such as coughs, colds, and headaches. A lack of seasonal variation in other compounds is possibly due to their consistent use throughout the whole year. The similar seasonal variations observed in M- and H-WWTPs were probably because they received wastewater from the same sewer systems. Seasonal variability at KSU-WWTP was not significant, probably because this plant received most of its wastewater from the nearby hospital all year-round. There was no seasonal variation in the compounds detected in the WWTP effluents, probably because of their high removal rates of these compounds through the three WWTPs.

6-1-3 Environmental risk assessment

The potential environmental risk associated with the measured concentrations of the targeted pharmaceuticals in the studied WWTP influents and effluents was calculated using the RQ approach. This was the first time such an approach had been used Saudi Arabia. The results showed that the three organisms, representing three different trophic levels in the aquatic environment, would be exposed to high risk from the detected pharmaceutical residues found either in the inlets or the outlets of these WWTPs. This has highlighted, for the first time, the potential damage that could arise within the aquatic environment of Saudi Arabia, and especially from the use of treated wastewater for difference purposes, especially if the current situation remains and no action is taken to reduce the emissions of pharmaceutical compounds in Saudi Arabia.

6-1-4 Leaching behavior of selected pharmaceuticals when applied to soils

Laboratory-scale leaching studies using soil columns showed that they could contribute to the evaluation of pollution risk from pharmaceuticals percolating to groundwater. Therefore, they could guide regulators by highlighting the pharmaceuticals posing the greatest threat and informing more focused monitoring programmes. Most of the investigated compounds were adsorbed in the four different soil types and showed no mobility. Only caffeine and cephalexin were detected in leachate and could percolate towards groundwater under environmental conditions similar to those used in the laboratory experiment. It was concluded that contamination of groundwater by the 16 selected compounds, under environmental conditions experienced in Saudi Arabia, is unlikely.

6-2 Recommendations and future research

Although some studies have been conducted, knowledge about the occurrence and behaviour of pharmaceutical compounds in the environment in Saudi Arabia is still

scarce. More studies about the occurrence of pharmaceuticals in aquatic ecosystems in Saudi Arabia is needed in order to fill the gap of pharmaceuticals occurrence and support the development of relevant environmental policy in Saudi Arabia. The following are recommended to improve knowledge about the fate of pharmaceuticals in the Saudi Arabian environment:

1- In this research, the investigated compounds were limited to parent compounds and one metabolite. More research is needed that includes parent and metabolites, especially for those which release the active compound after undergoing metabolism. Also, degradation products should be considered because they are biologically active and are potentially a source of contamination.
2- Collection of solid and aqueous samples from the dry channels in which the effluents of the studied WWTPs are discharged will help to assist in further understanding the fate and potential risk arising from the presence of pharmaceuticals in the effluents.
3- Further research is required to inform strategies for the reduction in releases of pharmaceutical compounds from WWTPs nationwide in Saudi Arabia. Further surveillance programs for pharmaceuticals based on their potential environmental risk and/or consumption may assist future restrictions and regulations relating to their release directly into the environment.
4- In order to validate the findings of the soil leaching column experiments, they should be repeated using representative conditions from the study area, such as the average annual rainfall of 93.5 mm (Almazroui *et al.*, 2012; Hasanean and Almazroui, 2015) in Saudi Arabia, and the high evaporation rate which is between 497 mm in summer and 67 mm in winter in Riyadh city. The effect of

applying artificial rain for more than 48 hours, should also be investigated. Furthermore, the pharmaceutical concentrations applied on the soil columns exceeded the average real concentrations found in the three investigated WWTP effluents, so similar concentration may be worth investigating in future experiments.

5- A modelling approach could be used to estimate the likelihood of the studied compounds reaching groundwater, using the data obtained here together with other parameters like rainfall, evaporation rate, infiltration rates, depth to the water table and area receiving the effluent. Groundwater vulnerability to contaminants can be assessed using different modeling approaches, such as index models (e.g., the DRASTIC model) (Li *et al.*, 2014; Nixdorf *et al.*, 2017; Aller, 1985; Freitas *et al.*, 2019), statistical models (Nolan *et al.*, 2002; Machiwal *et al.*, 2018) and process-based models (like EuroPEARL) (Tiktak *et al.*, 2006). Such models could be used to further develop the findings from the leaching experiments using soil columns.

6- The analysis of pharmaceuticals in very complex matrices, like wastewater and soil, are a challenge. Therefore, there is a need for further research to develop more selective and accurate SPE and LC-MS/MS analytical methods in order to obtain more accurate quantification of the compounds present in the environment at concentrations below ng L^{-1} and ng g^{-1} levels.

7- Investigation of the presence of pharmaceuticals in the different wastewater treatment stages (pretreatment, primary, secondary, tertiary, disinfected) will assist understanding of the fate of pharmaceuticals in these stages and help to

determine the most effective treatment technologies for eliminating these compounds in the environmental conditions of Saudi Arabia.

8- It is generally accepted that the most significant entry route of pharmaceuticals into the environment is through WWTPs effluents, but sludge also is a potential pathway for pharmaceuticals to contaminate the environment, particularly when it is applied to farmland, incinerated, or disposed to landfill. Consequently, there is the potential for uptake by crops when sludge is used on agricultural land, with the subsequent transfer to the human food chain. Further research is therefore recommended to establish the fate of pharmaceuticals in sludge and their movement in the environment after sludge disposal.

9- It is necessary to investigate the impacts of long-term exposure to pharmaceutical compounds in environmental systems.

10- Further study is required to assess the potential ecotoxicological effects of pharmaceutical mixtures in the aquatic environment because they may present greater risk than individual pharmaceuticals.

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Appendices

Table A-1-1: The reported average concentrations of pharmaceuticals in WWTPs (ng L^{-1}) in Saudi Arabia.

Compounds	Almadinah WWTP[a]		Jeddah WWTPs[b]						Riyadh HWWTPs[7,c]			
	Inlet	Outlet	Outlet (1-1)[1]	Outlet (1-2)[2]	Outlet (2-1)[3]	Outlet (2-2)[4]	Outlet (3)[5]	Outlet (4)[6]	Inlet	Outlet	Inlet	Outlet
Acesulfame.	N/A	N/A	3390	4270	3300	4270	1430	3600	N/A	N/A	N/A	N/A
Acetaminophen	38900	31200	N/A	N/A	N/A	N/A	N/A	N/A	12400	73	12300	157
Amitriptyline	N/A	N/A	55	34	28	< 10	365	19	N/A	N/A	N/A	N/A
Atenolol	2040	545	2550	2210	15	32	1870	105	730	46	329	55
Atrazine	N/A	N/A	7	< 6	24	< 6	27	< 6	N/A	N/A	N/A	N/A
Benzophenone	N/A	N/A	825	740	5710	950	595	< 300	N/A	N/A	N/A	N/A
Bezafibrate M	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N.D.	N.D.	N.D.	N.D.
Bisphenol A	N/A	N/A	890	450	< 20	< 20	430	N.D.	N/A	N/A	N/A	N/A
Caffeine	N/A	N/A	16500	2590	< 60	< 60	975	< 60	74800	N.D.	27,500	N.D.
Carbamazepine	N/A	N/A	1200	830	57	62	865	240	151	41	73	N.D.
Cephalexin	1880	1530	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Ciprofloxacin	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	5600	N.D.	2180	N.D.
Clarithromycin	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	83	22	38	N.D.
Cyclophosphamide	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N.D.	N.D.	N.D.	N.D.
DEET	N/A	N/A	110	89	< 30	46	415	94	N/A	N/A	N/A	N/A
Diclofenac	N/A	N/A	1260	760	25	64	345	51	N/A	N/A	N/A	N/A
Dilantin.	N/A	N/A	60	46	< 20	< 20	440	38	N/A	N/A	N/A	N/A
Diphenhydramine	N/A	N/A	295	755	52	59	540	175	N/A	N/A	N/A	N/A
Erythromycin	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N.D.	N.D.	N.D.	N.D.
Fluoxetine	N/A	N/A	< 10	< 10	12	< 10	295	10	N/A	N/A	N/A	N/A

Compounds	Almadinah WWTP[a]		Jeddah WWTPs[b]						Riyadh HWWTPs[7,c]			
	Inlet	Outlet	Outlet (1-1)[1]	Outlet (1-2)[2]	Outlet (2-1)[3]	Outlet (2-2)[4]	Outlet (3)[5]	Outlet (4)[6]	Inlet	Outlet	Inlet	Outlet
Gemfibrozil	N/A	N/A	390	215	< 6	< 6	430	7	N/A	N/A	N/A	N/A
Ibuprofen	N/A	N/A	460	930	< 10	< 10	325	11	N/A	N/A	N/A	N/A
Lidocaine	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	158	114	129	<LOQ
Metformin	15200	3190	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Methylparaben	N/A	N/A	535	< 30	94	< 30	920	< 30	N/A	N/A	N/A	N/A
Nacetylsulfamethoxazol	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1200	59	506	N.D.
Naproxen	N/A	N/A	40	105	< 6	< 6	310	31	N/A	N/A	N/A	N/A
Norfluoxetine.	7070	7250	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Oxybenzone.	N/A	N/A	62	62	57	< 30	380	< 30	N/A	N/A	N/A	N/A
Primidone.	N/A	N/A	15	< 3	< 3	39	645	6	N/A	N/A	N/A	N/A
Propylparaben.	N/A	N/A	< 20	< 20	< 20	< 20	585	< 20	N/A	N/A	N/A	N/A
Sucralose	N/A	N/A	415	475	880	1410	1200	1250	N/A	N/A	N/A	N/A
Sulfamethoxazole	N/A	N/A	730	305	245	145	345	160	30	N.D.	132	N.D.
Triclocarban.	N/A	N/A	81	< 30	495	< 30	260	70	N/A	N/A	N/A	N/A
Triclosan.	N/A	N/A	2270	530	2220	< 250	845	< 250	N/A	N/A	N/A	N/A
Trimethoprim	N/A	N/A	44	41	< 10	< 10	785	< 10	N/A	N/A	N/A	N/A

[1,3] Pre-chlorination (final effluent), first sampling campaign, [2,4] Pre-chlorination (final effluent), second sampling campaign, [5] Post UV-disinfection sampling, [6] Post-chlorination sampling, N.D.: Not detected, [7] Hospital wastewater treatment plants. [a] (Shraim *et al.*, 2017), [b] (Alidina *et al.*, 2014), [c] (Al Qarni *et al.*, 2016), N/A: Not available.

Table A-1-2: Concentration of pharmaceuticals in surface water, soil, sediments, crops and plants detected in Saudi Arabia by Picó *et al.* (2019, 2020, 2021).

Compounds	Surface water ng L^{-1}		Soil ng g^{-1}		Sediments ng g^{-1}		Crops ng g^{-1}		Plants ng g^{-1}
	Al Hassa Oasis	South Riyadh and Al-Jubail industrial city[1]	Al Hayer area	Al Hassa Oasis	Al Hassa Oasis	South Riyadh and Al-Jubail industrial city[1]	Al Hayer area	South Riyadh and Al-Jubail industrial city[2]	Al Hassa Oasis
Alprazolam	N.D. - 389.4	1.28	N/A	N/A	0.00 - 87.00	2.59	N/A	3.09	N/A
Amoxicillin	N/A	22.28	N/A	N/A	N/A	9.23	N/A	89.4	N/A
Atenolol	N.D. - 327.0	465	N.D. - 39	N/A	0.00 - 13.51	1.58	N.D. - 55	N/A	N/A
Atorvastatin	23.5 - 474.7	1.24	N/A	N/A	14.00 - 84.49	8.67	N/A	N/A	N.D. - 16.70
Benzafibrate	N/A	4.04	N/A	N/A	N/A	2.02	N/A	594	N.D. - 62.06
Bisphenol A	N.D. - 484.9	854	N/A	3.87 - 45.25	0.00 - 90.85	833	N/A	1188	3.18 - 126.18
Butylparaben	N.D. - 65.2	0.08	N/A	N/A	0.00 - 11.53	0.42	N/A	808	N/A
Caffeine	230.3 - 20663.5	1814	N.D. - 96	1.74 - 25.44	7.07 - 75.96	11.5	N.D. - 125	7.47	N.D. - 5.42
Carbamazepine	N/A	N/A	N.D. - 45	N/A	N/A	N/A	N.D.	N/A	N/A
Chlofibric acid	N.D. - 1.5	5.72	N/A	N/A	N/A	1.48	N/A	779	N/A

Compounds	Surface water ng L^{-1}		Soil ng g^{-1}		Sediments ng g^{-1}		Crops ng g^{-1}		Plants ng g^{-1}
	Al Hassa Oasis	South Riyadh and Al-Jubail industrial city[1]	Al Hayer area	Al Hassa Oasis	Al Hassa Oasis	South Riyadh and Al-Jubail industrial city[1]	Al Hayer area	South Riyadh and Al-Jubail industrial city[2]	Al Hassa Oasis
Codeine	N.D. - 22.5	21.7	N/A	N/A	N/A	N/A	N/A	21.1	N/A
Diclofenac	N.D. - 1390.0	1188	N/A	0.00 - 12.46	0.00 - 21.73	24.6	N/A	49.4	N.D. - 16.04
Ethylparaben	N.D. - 6.2	N/A	N/A	0.00 - 0.20	N/A	N/A	N/A	69.1	N/A
Etoricoxib	376.7 - 474.0	0.76	N/A	N/A	0.70 - 63.95	0.81	N/A	1.05	N/A
Gemfibrozil	N/A	N/A	N.D. - 58	N/A	N/A	N/A	N.D. - 75		N/A
Ibuprofen	N.D. - 2407.0	976	N/A	0.00 - 59.57	0.00 - 23.97	N/A	N/A	313	N.D. - 135.16
Indomethacine		0.08	N/A	N/A	N/A	N/A	N/A	368	N/A
Lorazepam	415.2 - 506.7	87.7	N/A	N/A	100.58 - 126.46	1.42	N/A	N/A	N/A
Metformin	2.0 - 267.0	2.50	N/A	0.00 - 0.67	0.00 - 0.60	0.23	N/A	2.9	N.D. - 27.87
Methylparaben	N.D. - 27.4	2.65	N/A	N/A	N/A	N/A	N/A	218	N.D. - 614.34

Compounds	Surface water ng L^{-1}		Soil ng g^{-1}		Sediments ng g^{-1}		Crops ng g^{-1}		Plants ng g^{-1}
	Al Hassa Oasis	South Riyadh and Al-Jubail industrial city[1]	Al Hayer area	Al Hassa Oasis	Al Hassa Oasis	South Riyadh and Al-Jubail industrial city[1]	Al Hayer area	South Riyadh and Al-Jubail industrial city[2]	Al Hassa Oasis
Naproxen	N.D. - 142.9	4.36	N.D. - 38	N/A	N/A	6.16	N.D.	17.2	N.D. - 67.66
Norfloxacin		537.4	N/A	N/A	N/A	338	N/A	N/A	N/A
Ofloxacin	148.0 - 610.6	393	N/A	N/A	0.00 - 17.16	N/A	N/A	94.1	N.D. - 99.48
Omeprazol	N/A	13.2	N/A	N/A	N/A	8.35	N/A	330	N/A
Paracetamol	105.1 - 3069.1	29.4	N/A	N/A	11.55 - 24.98	8.60	N/A	23.1	N.D. - 28.34
Propylparaben	N.D. - 12.5	0.73	N/A	6.17 - 76.07	0.00 - 17.69	0.87	N/A	858	N/A
Salicylic acid	10.5 - 129.2	24.7	N/A	N/A	N/A	9.74	N/A	6496	90.74 - 1952.00
Simvastatin	N/A	2.20	N/A	0.00 - 0.00	38.36 - 589.27	0.55	N/A	0.12	N/A
Thiamphenicol	N/A	1.68	N/A	N/A	N/A	0.69	N/A	2571	N/A
Tramadol	289.9 - 353.5	312	N/A	0.00 - 1.76	11.30 - 107.11	7.73	N/A	18.9	N.D. - 1.16
Triclocarban	N.D. - 32.0	0.08	N/A	0.00 - 1.91	0.00 - 10.36	0.07	N/A	128	N.D. - 0.21
Triclosan	N.D. - 33.5	0.08	N/A	0.00 - 7.34	0.00 - 0.00	N/A	N/A	631	N/A

Compounds	Surface water ng L^{-1}		Soil ng g^{-1}		Sediments ng g^{-1}		Crops ng g^{-1}		Plants ng g^{-1}
	Al Hassa Oasis	South Riyadh and Al-Jubail industrial city[1]	Al Hayer area	Al Hassa Oasis	Al Hassa Oasis	South Riyadh and Al-Jubail industrial city[1]	Al Hayer area	South Riyadh and Al-Jubail industrial city[2]	Al Hassa Oasis
Trimethoprim	N.D. - 586.2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Warfarin	N/A	N/A	N/A	N/A	N/A	N/A	N/A	5685	N/A

Al Hassa Oasis (Picó *et al.*, 2019), Al Hayer area (Picó *et al.*, 2020), South Riyadh and Al-Jubail industrial city (Picó *et al.*, 2021), [1] Mean concentrations, [2] Maximum concentrations. N/A: Not available, N.D.: Not detected.

Table A-3-1: Air temperature (T), FR and physiochemical parameters measured at the time of collection of WWTP influent and effluent samples.

H-WWTP								
Sample date		T (°C)	Sample location	FR (m^3 d^{-1})	pH	COD (mg L^{-1})	BOD5 (mg L^{-1})	TSS (mg L^{-1})
2018	March	20	Influent	188232	7.5	419	236	240
			Effluent		7.5	16	9	6
	April	32	Influent	163923	7.3	206	156	251
			Effluent		7.7	14	12	5
	May	35	Influent	197268	7.4	367	210	198
			Effluent		7.9	18	9	7
	June	40	Influent	205086	7.4	913	545	203
			Effluent		7.9	12	8	8
	July	44	Influent	217188	7.4	393	230	184
			Effluent		8	23	14	6
	September	42	Influent	220881	7.4	434	375	372
			Effluent		8	18	10	5
	October	35	Influent	258456	7.4	367	210	198
			Effluent		7.8	21	12	9
	November	28	Influent	357190	7.3	684	407	238
			Effluent		7.2	41	24	23
2019	April	30	Influent	302458	7.2	465	209	219
			Effluent		7.5	29	13	10
	May	36	Influent	333618	7.2	340	192	204
			Effluent		7.8	13	7	7
	June	41	Influent	320885	7.7	380	250	156
			Effluent		7.9	14	12	5
	July	46	Influent	240177	7.9	15	10	7
			Effluent		7.2	323	240	280

M-WWTP								
Sample date		T (°C)	Sample location	FR (m^3 d^{-1})	pH	COD (mg L^{-1})	BOD5 (mg L^{-1})	TSS (mg L^{-1})
2018	March	20	Influent	291770	7.4	577	212	328
			Effluent		7.6	19	7	4
	April	32	Influent	300250	7.3	327	206	260
			Effluent		7.4	15	9	8
	May	35	Influent	226520	7.2	444	260	204
			Effluent		7.7	16	8	8
	June	40	Influent	211070	7.2	458	279	237
			Effluent		7.7	10	7	6
	July	44	Influent	266340	7.2	461	270	198
			Effluent		7.9	13	7	7
	September	42	Influent	260870	7.5	430	258	218
			Effluent		8	28	9	4
	October	35	Influent	180990	7.2	444	260	204
			Effluent	200990	7.7	16	8	8
	November	28	Influent	261310	7.4	564	335	346
			Effluent		7.6	18	9	7
2019	April	30	Influent	283654	7.3	586	358	295
			Effluent		7.5	15	10	8
	May	36	Influent	295760	7.4	612	360	362
			Effluent		7.7	17	7	8
	June	41	Influent	282960	7.5	465	416	260
			Effluent		8.1	14	8	5
	July	46	Influent	280720	7.6	186	358	136
			Effluent		7.9	18	10	8

KSU-WWTP								
Sample date		T (°C)	Sample location	FR (m^3 d^{-1})	pH	COD (mg L^{-1})	BOD5 (mg L^{-1})	TSS (mg L^{-1})
2018	March	20	Influent	8520	7.62	216	145	120
			Effluent		7.61	7.2	3.8	10
	April	32	Influent	6528	7.35	203	150	125
			Effluent		7.24	6.5	4.2	10
	May	35	Influent	5664	7.47	198	145	128
			Effluent		7.67	6.6	4.5	10
	June	40	Influent	5232	7.71	215	164	125
			Effluent		8.08	6.7	4.7	8
	July	44	Influent	4416	7.84	209	154	120
			Effluent		8.05	6.5	4.4	10
	September	42	Influent	4608	7.58	187	152	128
			Effluent		7.95	7.1	4.5	10
	October	35	Influent	3432	7.95	176	164	130
			Effluent		8.2	6.2	3.8	10
	November	28	Influent	2256	7.81	195	160	120
			Effluent		7.91	6.4	4.4	5
2019	April	30	Influent	3672	8.02	178	165	127
			Effluent		7.68	6.2	4.3	10
	May	36	Influent	6744	7.59	226	170	130
			Effluent		7.57	7.3	5.1	10
	June	41	Influent	5760	7.35	221	175	128
			Effluent		7.01	7	4.5	10
	July	46	Influent	3720	7.48	199	168	120
			Effluent		7.13	6.9	4.2	10

Table A-3-2: The concentrations of measured pharmaceuticals (μg L^{-1}) detected in investigated WWTPs influent and effluent[1].

H-WWTP Effluent												
Date			**Pharmaceutical compound concentrations**									
Years	**Seasons**	**Months**	**ACE**	**ATE**	**BAC**	**CAF**	**CEP**	**CIP**	**DIC**	**MET**	**OFL**	**TRI**
2018	**Spring**	**Mar.**	0.02	<LOD	<LOD	<LOD	<LOD	1.39	<LOD	<LOD	<LOQ	<LOD
		Apr.	0.14	<LOD	<LOD	<LOD	<LOD	33.16	<LOD	<LOD	7.07	<LOD
		May	0.13	<LOD	<LOD	<LOD	<LOD	13.62	<LOD	<LOQ	4.40	<LOD
	Summer	**June**	0.04	<LOD	<LOD	<LOD	<LOD	7.10	<LOD	<LOQ	1.33	<LOD
		July	<LOD	<LOD	<LOD	<LOD	<LOD	2.77	<LOD	8.77	<LOQ	<LOD
	Fall	**Sep.**	<LOD	<LOD	<LOD	<LOD	<LOD	2.11	<LOD	4.84	<LOQ	<LOD
		Oct.	<LOD	<LOD	<LOD	<LOD	<LOD	2.25	<LOD	3.61	<LOQ	<LOD
		Nov.	<LOD	<LOD	<LOD	16.56	<LOD	1.20	<LOD	2.03	<LOQ	<LOD
2019	**Spring**	**Apr.**	0.19	<LOD	<LOD	<LOD	<LOD	28.23	<LOD	<LOD	5.37	<LOD
		May	<LOD	<LOD	<LOD	4.92	<LOD	3.29	<LOD	<LOD	<LOQ	<LOD
	Summer	**June**	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOQ	<LOD	<LOD	<LOQ	<LOD
		July	<LOD	<LOD	<LOD	<LOD	<LOD	9.70	<LOD	1.38	1.34	<LOD
Range		**Max**	0.19	<LOD	<LOD	16.56	<LOD	33.16	<LOD	8.77	7.07	<LOD
		Min	0.02	<LOD	<LOD	4.92	<LOD	1.20	<LOD	1.38	1.33	<LOD
Average			0.10	<LOD	<LOD	10.74	<LOD	9.53	<LOD	4.12	3.90	<LOD
***f* %**			41.67	<LOD	<LOD	16.67	<LOD	91.67	<LOD	41.67	41.67	<LOD
Total			0.51	<LOD	<LOD	21.49	<LOD	104.81	<LOD	20.62	19.51	<LOD
H-WWTP Influent												
Years	**Seasons**	**Months**	**ACE**	**ATE**	**BAC**	**CAF**	**CEP**	**CIP**	**DIC**	**MET**	**OFL**	**TRI**
2018	**Spring**	**Mar.**	23.35	0.28	<LOD	1665.11	<LOD	7.52	0.57	9.67	1.08	<LOQ

H-WWTP Influent												
Date			**Pharmaceutical compound concentrations**									
Years	**Seasons**	**Months**	**ACE**	**ATE**	**BAC**	**CAF**	**CEP**	**CIP**	**DIC**	**MET**	**OFL**	**TRI**
2018	**Spring**	**Apr.**	1036.24	6.85	<LOD	2281.59	<LOD	206.93	36.65	207.30	25.13	1.88
		May	738.02	5.42	<LOD	2212.61	<LOQ	77.95	45.74	308.43	16.07	1.31
	Summer	**June**	657.99	5.36	<LOQ	1290.34	<LOQ	83.79	10.08	49.20	10.36	0.99
		July	109.65	5.74	<LOQ	666.00	<LOD	131.05	26.03	260.43	22.23	1.26
	Fall	**Sep.**	226.32	1.20	<LOD	797.49	<LOD	9.16	1.02	77.99	1.80	0.58
		Oct.	275.92	2.00	<LOD	1111.09	<LOD	42.53	3.66	145.79	5.87	0.66
		Nov.	505.71	1.76	<LOQ	705.45	0.37	42.70	13.01	192.89	9.17	0.62
2019	**Spring**	**Apr.**	660.51	3.17	<LOQ	1755.56	<LOD	146.30	25.68	169.84	20.49	1.22
		May	17.38	0.93	<LOD	628.55	<LOD	3.76	0.52	1.09	0.63	<LOQ
	Summer	**June**	26.48	0.23	<LOD	1149.35	<LOD	6.04	0.51	8.03	0.82	<LOQ
		July	172.86	1.48	<LOD	717.07	<LOQ	58.82	3.33	140.70	6.75	0.35
Range		**Max**	1036.24	6.85	<LOD	2281.59	0.37	206.93	45.74	308.43	25.13	1.88
		Min	17.38	0.23	<LOD	628.55	0.37	3.76	0.51	1.09	0.63	0.35
Average			370.87	2.87	<LOD	1248.35	0.37	68.05	13.90	130.95	10.03	0.99
***f* %**			100.00	100.00	<LOD	100.00	8.33	100.00	100.00	100.00	100.00	75.00
Total			4450.42	34.42	<LOD	14980.20	0.37	816.55	166.78	1571.38	120.40	8.87
M-WWTP Effluent												
Years	**Seasons**	**Months**	**ACE**	**ATE**	**BAC**	**CAF**	**CEP**	**CIP**	**DIC**	**MET**	**OFL**	**TRI**
2018	**Spring**	**Mar.**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD
		Apr.	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		May	0.05	<LOD	<LOD	<LOD	<LOD	19.92	<LOQ	<LOQ	3.96	<LOD
	Summer	**June**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOQ	<LOD	<LOD

M-WWTP Effluent												
Date			**Pharmaceutical compound concentrations**									
Years	**Seasons**	**Months**	**ACE**	**ATE**	**BAC**	**CAF**	**CEP**	**CIP**	**DIC**	**MET**	**OFL**	**TRI**
2018	**Summer**	**July**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.55	<LOD	<LOD
2019	**Fall**	**Sep.**	<LOD	<LOD	<LOD	5.78	<LOD	1.49	<LOD	1.94	<LOD	<LOD
		Oct.	<LOD	<LOD	<LOD	8.84	<LOD	<LOQ	<LOD	1.53	<LOD	<LOD
		Nov.	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD
	Spring	**Apr.**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		May	0.01	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD
2019	**Summer**	**June**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		July	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOQ	<LOQ	<LOD
Range		**Max**	0.05	<LOD	<LOD	8.84	<LOD	19.92	<LOD	1.94	3.96	<LOD
		Min	0.01	<LOD	<LOD	5.78	<LOD	1.49	<LOD	1.53	3.96	<LOD
Average			0.03	<LOD	<LOD	7.31	<LOD	10.70	<LOD	1.67	3.96	<LOD
***f* %**			16.67	<LOD	<LOD	16.67	<LOD	16.67	<LOD	25.00	8.33	<LOD
Total			0.07	<LOD	<LOD	14.62	0.00	21.40	<LOD	5.02	3.96	<LOD
M-WWTP Influent												
Years	**Seasons**	**Months**	**ACE**	**ATE**	**BAC**	**CAF**	**CEP**	**CIP**	**DIC**	**MET**	**OFL**	**TRI**
2018	**Spring**	**Mar.**	47.13	0.45	<LOD	722.73	<LOD	12.00	1.17	4.57	1.32	0.11
		Apr.	834.81	30.08	<LOQ	1449.46	0.42	518.91	28.91	173.46	59.99	4.79
		May	942.99	5.24	<LOQ	1374.39	1.17	319.38	19.62	182.35	36.55	2.08
	Summer	**June**	592.09	21.13	<LOQ	1050.45	6.75	711.30	45.26	48.86	88.24	3.66
		July	196.20	1.59	<LOQ	763.05	<LOQ	72.23	4.62	100.70	7.59	0.40
	Fall	**Sep.**	163.35	0.67	<LOQ	192.61	0.62	51.78	3.23	587.86	8.40	0.78
		Oct.	397.84	1.86	<LOD	945.43	<LOQ	112.74	9.19	119.33	13.68	0.64

M-WWTP Influent												
Date			**Pharmaceutical compound concentrations**									
Years	**Seasons**	**Months**	**ACE**	**ATE**	**BAC**	**CAF**	**CEP**	**CIP**	**DIC**	**MET**	**OFL**	**TRI**
2018	**Fall**	**Nov.**	941.90	0.96	<LOD	685.26	<LOD	76.96	1.78	220.98	12.12	0.34
2019	**Spring**	**Apr.**	640.12	17.07	<LOQ	1029.75	<LOD	303.45	21.48	135.65	45.40	3.15
		May	46.43	0.28	<LOD	689.99	<LOD	13.61	0.59	3.63	2.05	0.07
	Summer	**June**	281.91	<LOQ	<LOD	663.99	<LOD	7.12	0.42	3.11	0.89	0.05
		July	227.24	1.53	<LOQ	851.48	<LOQ	55.36	3.68	351.92	6.89	0.52
Range		**Max**	942.99	30.08	<LOQ	1449.46	6.75	711.30	45.26	587.86	88.24	4.79
		Min	46.43	0.28	<LOQ	192.61	0.42	7.12	0.42	3.11	0.89	0.05
Average			442.67	7.35	<LOQ	868.21	2.24	187.90	11.66	161.04	23.59	1.38
***f* %**			100.00	91.67	<LOQ	100.00	33.33	100.00	100.00	100.00	100.00	100.00
Total			5312.01	80.87	<LOQ	10418.56	8.96	2254.83	139.94	1932.44	283.09	16.60
KSU-WWTP Effluent												
2018	**Spring**	**Mar.**	0.02	<LOD	<LOD	<LOQ	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD
		Apr.	<LOD	<LOD	<LOD	<LOQ	<LOD	4.17	<LOD	<LOQ	<LOQ	<LOD
		May	0.33	<LOD	<LOD	<LOQ	<LOD	4.65	<LOQ	<LOQ	<LOQ	<LOD
	Summer	**June**	<LOD	<LOD	<LOD	<LOQ	<LOD	12.92	<LOD	<LOQ	1.41	<LOD
		July	<LOD	<LOD	<LOD	<LOQ	<LOD	6.66	<LOQ	3.58	0.89	<LOD
	Fall	**Sep.**	0.03	<LOD	<LOD	<LOQ	<LOD	2.28	<LOD	2.39	<LOQ	<LOD
		Oct.	0.10	<LOD	<LOD	<LOQ	<LOD	14.49	<LOD	6.05	2.63	<LOD
		Nov.	0.22	<LOD	<LOD	<LOQ	<LOD	20.07	<LOQ	<LOQ	2.33	<LOD
2019	**Spring**	**Apr.**	<LOD	<LOD	<LOD	<LOQ	<LOD	1.68	<LOD	<LOQ	<LOQ	<LOD
		May	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Summer	**June**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		July	<LOD	<LOD	<LOD	<LOD	<LOD	1.64	<LOD	<LOQ	<LOD	<LOD

KSU-WWTP Effluent											
			Pharmaceutical compound concentrations								
			ACE	**ATE**	**BAC**	**CAF**	**CEP**	**CIP**	**DIC**	**MET**	**OFL**	**TRI**
Range		**Max**	0.33	<LOD	<LOD	<LOD	<LOD	20.07	<LOD	6.05	2.63	<LOD
		Min	0.02	<LOD	<LOD	<LOD	<LOD	1.64	<LOD	2.39	0.89	<LOD
Average			0.14	<LOD	<LOD	<LOD	<LOD	7.62	<LOD	4.01	1.82	<LOD
***f* %**			41.67	<LOD	<LOD	<LOD	<LOD	75.00	<LOD	25.00	33.33	<LOD
Total			0.70	<LOD	<LOD	<LOD	<LOD	68.57	0.00	12.02	7.27	<LOD
KSU-WWTP Influent												
Years	**Seasons**	**Months**	**ACE**	**ATE**	**BAC**	**CAF**	**CEP**	**CIP**	**DIC**	**MET**	**OFL**	**TRI**
2018	**Spring**	**Mar.**	36.49	0.29	<LOQ	1120.62	0.37	16.44	0.32	1.62	0.81	0.37
		Apr.	989.83	4.42	0.33	1497.60	<LOD	463.64	5.35	227.66	38.50	4.76
		May	1186.07	6.42	<LOQ	1495.01	0.12	73.08	2.50	311.47	3.95	4.02
	Summer	**June**	385.20	2.43	<LOQ	211.80	0.16	8.73	2.30	21.59	0.42	1.20
		July	216.37	0.59	1.88	234.09	<LOD	30.70	1.88	37.95	0.63	1.80
	Fall	**Sep.**	114.70	0.53	2.82	142.12	<LOD	9.41	0.38	23.15	1.09	0.52
2018	**Fall**	**Oct.**	32.33	0.67	<LOQ	516.60	<LOD	22.52	2.23	7.28	1.61	0.24
		Nov.	28.49	0.23	<LOQ	211.12	<LOD	56.70	3.30	39.28	4.06	0.20
2019	**Spring**	**Apr.**	763.85	2.81	<LOQ	1189.12	<LOD	352.23	4.84	195.22	28.48	3.57
		May	26.63	0.35	<LOQ	346.24	<LOD	6.37	0.20	1.22	0.28	0.12
	Summer	**June**	37.17	0.63	<LOQ	450.70	<LOD	2.23	0.35	0.41	0.25	0.13
		July	426.67	1.20	<LOQ	462.32	<LOD	109.17	0.84	380.29	8.12	2.25
Range		**Max.**	1186.07	6.42	2.82	1497.60	0.37	463.64	5.35	380.29	38.50	4.76
		Min.	26.63	0.23	0.33	142.12	0.12	2.23	0.20	0.41	0.25	0.12
Average			353.65	1.71	1.68	656.44	0.22	95.94	2.04	103.93	7.35	1.60

***f* %**	100.00	100.00	25.00	100.00	25.00	100.00	100.00	100.00	100.00	100.00
Total	4243.79	20.56	5.03	7877.32	0.65	1151.23	24.48	1247.14	88.19	19.17

ACE: Acetaminophen, ATE: Atenolol, BAC: Baclofen, CAF: Caffeine, CEP: Cephalexin, CIP: Ciprofloxacin, DIC: Diclofenac, MET: Metformin, OFL: Ofloxacin, TRI: Trimethoprim, LOD: Limit of Detection, LOQ: Limit of Quantification, Max: Maximum, Min: Minimum, *f*: Frequency detected, [1]: Azithromycin, ibuprofen and oxypurinol were not included due to detected in all samples below of their LOQs, whereas triclosan, allopurinol and tetracycline because of low recovery and not included in the final selected tested compounds.

Table A-3-3: Studied compounds concentrations (ng L^{-1}) reported in different WWTP influent and effluent around the world.

Pharmaceuticals	Locations	Influent Range (ng L^{-1})	Mean (ng L^{-1})	Effluent Range (ng L^{-1})	Mean (ng L^{-1})	References
Acetaminophen	Greece	10555-81015	29,634	124-7419	1,925	(Dasenaki and Thomaidis, 2015)
		120.5-6421.6	2,873	BQL-207.2	96	(Kosma *et al.*, 2014)
		1139.6-4574.7	2519.6	N.D.-93.3	BQL	
		126.3-24148.8	8313.2	N.D.-532.5	195.7	
		BQL-847.1	293	BLQ-192.3	192.3	
		N.D.-358.0	139.9	N.D.-93.6	BQL	
		N.D.-5663.5	3047.2	N.D.-315.7	166	
		N.D.-7552.6	4184.9	N.D.-444.0	209.7	
		BQL-65402.8	30353.6	BQL-1060.3	368.7	
		11.09-70630	13707	<LOD-1047	63.7	(Papageorgiou *et al.*, 2019)
		<LOD-23780.3	5581.5	<LOD-214.8	28.4	
		7727.52-14546.81	12052.5	<LOD-1456	819	
		9226.5-26118.5	13868.4	<LOD-94.5	45.1	
		4700-52500	20600	500-1700	900	(Kosma *et al.*, 2010)
		3100-21200	9300	1300-7400	3600	
		62.5-2452	1422	n.d.–305	< LOQ	(Papageorgiou *et al.*, 2016)
	UK	68107-482687	211380	1826-24525	11733	(Kasprzyk-Hordern *et al.*, 2009)
		108383-246641	178116	<80-1575	353	

Pharmaceuticals	Locations	Influent Range (ng L^{-1})	Mean (ng L^{-1})	Effluent Range (ng L^{-1})	Mean (ng L^{-1})	References
Acetaminophen	Spain	20		2497		(Carmona *et al.*, 2017)
	Portugal	N.D.-728	477	N.D.-N.D.	–	(Paíga *et al.*, 2019)
	Poland	1040.0-28,254.4	9391.7	42.9-390.9	112.4	(Kot-Wasik *et al.*, 2016)
	India	5400-11000	690	330-1200	690	(Subedi *et al.*, 2017)
		2900-7500	340	<LOQ -490	340	
	US	21126-134322		N.D-58		(Mohapatra *et al.*, 2016)
		29100-113062		N.D.-33		
	Mexico	8650-12300	11600	N.D.		(Rivera-Jaimes *et al.*, 2018)
		2330-14900	7875	N.D.		
	China	N/A	6733.7	N/A	3.8	(Zhang *et al.*, 2018)
		N/A	4496.1	N/A	3.5	
		N/A	6626.7	N/A	58.4	
		N/A	8983.9	N/A	4.9	
		N/A	7310.8	N/A	2.9	
		N/A	6410.9	N/A	5.5	
		N/A	6831.3	N/A	2.9	
		N/A	739.9	N/A	3.6	
		4500–6420	5649	BLD-27.4	6.2	(Ashfaq *et al.*, 2017a)
	UAE	N/A	145250	N/A	5235	(Semreen *et al.*, 2019)

Pharmaceuticals	Locations	Influent Range (ng L^{-1})	Mean (ng L^{-1})	Effluent Range (ng L^{-1})	Mean (ng L^{-1})	References
Acetaminophen	Egypt	N/A	N/A	1509	N/A	(Abdallah *et al.*, 2019)
		N/A	N/A	978	N/A	
		N/A	N/A	15947	N/A	
		N/A	N/A	3042	N/A	
		N/A	N/A	1582	N/A	
	Tunisia	N/A	<LOD	N/A	<LOD	(Moslah *et al.*, 2018)
	Tunisia	N/A	<LOD	N/A	<LOD	(Moslah *et al.*, 2018)
		N/A	1155	N/A	<LOD	
		N/A	1208	N/A	<LOD	
		N/A	1208	N/A	409	
		N/A	<LOD	N/A	<LOD	
		N/A	<LOD	N/A	<LOQ	
	Jordan	N/A	N/A	N/A	N/A	(Al-Mashaqbeh *et al.*, 2019)
	UAE	143,905-146,596	145,250.30	3890-6581	5235.3	(Semerjian *et al.*, 2018)
Atenolol	Greece	1047-1517	1,297	484-597	540	(Dasenaki and Thomaidis, 2015)
		75.47-2313.65	321.53	38.40-362.63	75.03	(Papageorgiou *et al.*, 2019)
		127.9-899.0	247.7	<LOD-91.1	21.5	
		<LOD-771.40	197.24	<LOD-683	285	
		<LOD-266.0	125.4	<LOD-158.8	66.4	

Pharmaceuticals	Locations	Influent Range (ng L^{-1})	Mean (ng L^{-1})	Effluent Range (ng L^{-1})	Mean (ng L^{-1})	References
Atenolol	Greece	N.D.–2346	1720	N.D.–1707	970	(Papageorgiou *et al.*, 2016)
	UK	3090-33106	12913	1260-7602	2870	(Kasprzyk-Hordern *et al.*, 2009)
		8102-25146	14223	1292-3168	2123	
	Poland	107.7-503.2	282.1	51.9-169.0	94.9	(Kot-Wasik *et al.*, 2016)
	India	2100-3800	2900	<LOQ-500	1500	(Subedi *et al.*, 2017)
		1200-1900	1400	510-7100	590	
	US	537-2642	N/A	231-1035	N/A	(Mohapatra *et al.*, 2016)
		851-2340	N/A	51-924	N/A	
	Mexico	55-60	58	35-95	50	(Rivera-Jaimes *et al.*, 2018)
		60-90	80	35-50	45	
	Tunisia	2400-5900	N/A	2100-2900	N/A	(Afsa *et al.*, 2020)
		N/A	1862	N/A	1076	(Moslah *et al.*, 2018)
		N/A	2198	N/A	892	
		N/A	1104	N/A	1244	
		N/A	619	N/A	763	
		N/A	619	N/A	933	
		N/A	62	N/A	420	
		N/A	388	N/A	521	
Azithromycin	Greece	<19.3-64	<19.3	123-245	0.171	(Dasenaki and Thomaidis, 2015)

Pharmaceuticals	Locations	Influent Range (ng L^{-1})	Mean (ng L^{-1})	Effluent Range (ng L^{-1})	Mean (ng L^{-1})	References
Azithromycin	Greece	<LOD-78.80	11.59	<LOQ-114.4	17.78	(Papageorgiou *et al.*, 2019)
		<LOD-81.6	11.3	0.3-29.5	2.3	
		<LOD-43.30	26.29	<LOD-71	56	
		<LOD-28.3	22	<LOD-56.5	23.5	
	Spain	48	N/A	478	N/A	(Carmona *et al.*, 2017)
	Portugal	N.D.-453	283	207-316	257	(Paíga *et al.*, 2019)
	Tunisia	N/A	295.42	N/A	354.81	(Harrabi *et al.*, 2018)
	Iran	13.1	N/A	<DL	N/A	(Aydin *et al.*, 2019)
		2858	N/A	1815	N/A	
Caffeine	Greece	42521-58032	49,769	258-807	464	(Dasenaki and Thomaidis, 2015)
		17047.1-36238.0	26107.5	99.2-587.2	335.2	(Kosma *et al.*, 2014)
		10532.8-55698.1	28214.1	54.0-670.6	348.9	(Kosma *et al.*, 2014)
		212.9-96648.3	58087.5	N.D.-1180.5	415.8	
		N.D.-883.2	361.7	N.D.-34.0	BQL	
		N.D.-1849.1	615	N.D.-29.3	bql	
		N.D.-1927.2	642.6	N.D.-188.2	50.3	
		N.D.-64501.1	24556.5	N.D.-939.9	440.2	
		1659.8-53498.5	14928.7	N.D.-523.1	189.9	

Pharmaceuticals	Locations	Influent Range (ng L^{-1})	Mean (ng L^{-1})	Effluent Range (ng L^{-1})	Mean (ng L^{-1})	References
Caffeine	Greece	181.1-221455	27504	55.13-850.3	176.7	(Papageorgiou *et al.*, 2019)
		<LOD-71443.2	11835.2	<LOD-567.5	76.2	
		31126.24-49463.49	41395.61	<LOD-1111	603	
		<LOD-38434.0	14948.8	<LOD-335.8	189.4	
		17100-113200	74900	1900-13900	7900	(Kosma *et al.*, 2010)
		12300-42000	25800	3100-10600	6500	
		102–5597	4093	30.0–961	138	(Papageorgiou *et al.*, 2016)
	Portugal	6527-84265	55,102	N.D.	N/A	(Paíga *et al.*, 2019)
	Poland	2820.5-22,193.8	11489.9	47.2-442.1	133.5	(Kot-Wasik *et al.*, 2016)
		40,000-120,000	61000	810-1700	1100	
	US	7494-32155	N/A	N.D.-923	N/A	(Mohapatra *et al.*, 2016)
		5809-37056	N/A	N.D.-15	N/A	
	China	N/A	32638.9	N/A	93	(Zhang *et al.*, 2018)
		N/A	14454.1	N/A	35.6	
		N/A	33039.7	N/A	1790.9	
		N/A	32193.8	N/A	45.2	
	China	N/A	39665.6	N/A	102.6	(Zhang *et al.*, 2018)
		N/A	38474.6	N/A	339.4	
		N/A	28112.9	N/A	77	
		N/A	3793.6	N/A	15.8	

Pharmaceuticals	Locations	Influent Range (ng L^{-1})	Mean (ng L^{-1})	Effluent Range (ng L^{-1})	Mean (ng L^{-1})	References
Caffeine	China	2440-3160	2837	9.82-55.6	22.9	(Ashfaq *et al.*, 2017a)
	Egypt	N/A	N/A	84	N/A	(Abdallah *et al.*, 2019)
		N/A	N/A	1739	N/A	
		N/A	N/A	855	N/A	
		N/A	N/A	121	N/A	
		N/A	N/A	70	N/A	
	Tunisia	N/A	40,251	N/A	17,255	(Moslah *et al.*, 2018)
		N/A	51,069	N/A	5274	
		N/A	59,408	N/A	34,578	
		N/A	44,544	N/A	1056	
		N/A	44,544	N/A	16,806	
		N/A	2444	N/A	5825	
		N/A	25,188	N/A	10,074	
	Jordan	N/A	155,600	N/A	86	(Al-Mashaqbeh *et al.*, 2019)
Cephalexin	Greece	<7.46-<7.46	<7.46	<7.46-<7.46	<7.46	(Dasenaki and Thomaidis, 2015)
	Hong Kong	1020-1160	1080	1110-1410	1290	(Leung *et al.*, 2012)
		1270-1300	1290	171-190	179	
		1100-1150	1130	282-306	295	
		3150-4350	3750	2910-4290	3600	

Pharmaceuticals	Locations	Influent Range (ng L^{-1})	Mean (ng L^{-1})	Effluent Range (ng L^{-1})	Mean (ng L^{-1})	References
Cephalexin	Hong Kong	4430-5640	5040	4700-5070	4890	(Leung *et al.*, 2012)
		4120-4950	4540	2930-3050	2990	
		4610-4890	4750	4980-5020	5000	
	US	N.D.-13818		N.D.-5624		(Mohapatra *et al.*, 2016)
		N.D.-11842		N.D.-2330		
	Tunisia	N/A	71.47	N/A	46.62	(Harrabi *et al.*, 2018)
Ciprofloxacin	Greece	1057-2881	1.588	523-1437	1.116	(Dasenaki and Thomaidis, 2015)
		LOD-2791.9	258.95	LOD-116.4	17.1	(Papageorgiou *et al.*, 2019)
		<LOD-584.9	35	<LOD-<LOD	<LOD	
		<LOD-424.90	181.76	<LOD-302	269	
		<LOD-322.1	46	<LOD-<LOD	<LOD	
	Spain	N.D.-591	152	N.D.–591	199	(Papageorgiou *et al.*, 2016)
		143	N/A	180	N/A	(Carmona *et al.*, 2017)
	Portugal	N.D.-684	579	N.D.-285	250	(Paíga *et al.*, 2019)
	US	315-5451	N/A	130-919	N/A	(Mohapatra *et al.*, 2016)
		409-6441	N/A	23-328	N/A	
	China	16.6-42.2	27.2	6.78-11.5	8.71	(Ashfaq *et al.*, 2017a)

Pharmaceuticals	Locations	Influent Range (ng L^{-1})	Mean (ng L^{-1})	Effluent Range (ng L^{-1})	Mean (ng L^{-1})	References
Ciprofloxacin	Tunisia	N/A	727.33	N/A	394.86	(Harrabi *et al.*, 2018)
		N/A	<LOQ	N/A	<LOQ	(Moslah *et al.*, 2018)
		N/A	<LOQ	N/A	<LOQ	
		N/A	207	N/A	<LOQ	
		N/A	<LOQ	N/A	<LOQ	
		N/A	<LOQ	N/A	<LOQ	
		N/A	<LOQ	N/A	<LOD	
		N/A	<LOD	N/A	<LOD	
	UAE	697-1028	862.7	378-709	543.4	(Semerjian *et al.*, 2018)
	Iran	59.4	N/A	6.53	N/A	(Aydin *et al.*, 2019)
		937	N/A	632	N/A	
Diclofenac	Greece	514-1001	738	761-987	874	(Dasenaki and Thomaidis, 2015)
		81.1-143.0	100.8	BLQ-170.0	98	(Kosma *et al.*, 2014)
		N.D.-530.3	180.7	N.D.-188.1	63.2	
		BQL-216.5	106.9	N.D.-382.5	162.5	
		BQL-5164.0	1346	N.D.-121.5	56.1	
		BQL-238.1	98.8	N.D.-325.5	154.9	
		BQL-120.2	78.3	N.D.-69.2	BQL	
		N.D.-142.0	83.1	N.D.-250.7	97.1	
		BQL-84.0	N/A	N.D.-336.8	145.6	

Pharmaceuticals	Locations	Influent Range (ng L^{-1})	Mean (ng L^{-1})	Effluent Range (ng L^{-1})	Mean (ng L^{-1})	References
Diclofenac	Greece	54.9-3263	691.9	19.85 -2128	263.6	(Papageorgiou *et al.*, 2019)
		17.3-336.9	132.5	<LOD-408.1	78	
		248.00-2615.19	1530.4	170-1567	410	
		46.5-807.1	140.2	76.5-1186.2	993.5	
		464-6796	N/A	625-3052	N/A	
		N.D.-3900	2000	N.D.–2600	1300	(Kosma *et al.*, 2010)
		N.D.-4869	413	N.D.–2668	961	(Papageorgiou *et al.*, 2016)
	UK	26-257	69	33-142	98	(Kasprzyk-Hordern *et al.*, 2009)
		57-1161	260	6-496	179	
	Spain	331	N/A	173	N/A	(Carmona *et al.*, 2017)
	Portugal	41-2778	373	84-2922	1412	(Paíga *et al.*, 2019)
	Poland	556.2-4001.4	2138	742.7-5401.5	3018.4	(Kot-Wasik *et al.*, 2016)
	US	41-110	N/A	13-75	N/A	(Mohapatra *et al.*, 2016)
		N.D.-109	N/A	3-104	N/A	
	Mexico	2325-2470	2363	1865-2180	2030	
		60-1440	1090	466-690	593	
	US	86–580	277	N.D.–120	12	(Yu *et al.*, 2013)
	China	4.04-26.0	21.8	5.68-31.6	26	(Ashfaq *et al.*, 2017a)

Pharmaceuticals	Locations	Influent Range (ng L^{-1})	Mean (ng L^{-1})	Effluent Range (ng L^{-1})	Mean (ng L^{-1})	References
Diclofenac	Tunisia	N.D.-11	N/A	32-70	N/A	(Afsa *et al.*, 2020)
	UAE	N/A	863	N/A	543	(Semreen *et al.*, 2019)
	Algeria	N/A	2318.5	N/A	1615.7	(Kermia *et al.*, 2016)
		N/A	990.5	N/A	2710.7	
Ibuprofen	Greece	526-1928	1,269	<15.5-<15.5	<15.5	(Dasenaki and Thomaidis, 2015)
		418.2-8890.1	2633.4	N.D.–301.2	BQL	(Kosma *et al.*, 2014)
		378.9-6023.9	2048.5	N.D.–289.0	BQL	
		N.D.-425.6	177	N.D.	N/A	
		N.D.-524.2	279.9	N.D.–BQL	N/A	
		N.D.-412.1	56.5	N.D.–BQL	N/A	
Ibuprofen	Greece	BQL-612.2	494.6	N.D.–BQL	N/A	(Kosma *et al.*, 2014)
		N.D.	N/A	N.D.	N/A	
		N.D.	N/A	N.D.	N/A	
		LOD-36375	8414	LOD-10771	2440	(Papageorgiou *et al.*, 2019)
		<LOD-4623.0	1264	<LOD-1557.5	159.9	
		<LOD-7752.00	2861.33	<LOD-6016	1027	
		<LOD-5752.3	3711.6	<LOD-4727.8	505.2	
		17100-113200	N/A	1900-13900	N/A	
		2800-25400	12500	500-2600	1500	(Kosma *et al.*, 2010)
		7000-8900	7800	500-900	600	

Pharmaceuticals	Locations	Influent Range (ng L^{-1})	Mean (ng L^{-1})	Effluent Range (ng L^{-1})	Mean (ng L^{-1})	References
Ibuprofen	UK	968-2986	1681	131-424	263	(Kasprzyk-Hordern *et al.*, 2009)
		984-6328	2294	65-491	143	
	Spain	1978	N/A	10	N/A	Carmona et al. (2017)
	Portugal	127-7681	689	80-358	196	(Paíga *et al.*, 2019)
	Poland	4198.4-10,864	6586.1	24.1-644.2	141.7	(Kot-Wasik *et al.*, 2016)
	India	<LOQ-2799	1200	270-1940	980	(Subedi *et al.*, 2017)
		970-1900	1400	290-710	630	
	US	5749-16328	N/A	9-749	N/A	(Mohapatra *et al.*, 2016)
		6072-16883	N/A	N.D.-48	N/A	
	Mexico	1840-2835	1983	N.D.	N/A	(Rivera-Jaimes *et al.*, 2018)
		370-1310	810	N.D.	N/A	
	US	8600-56500	22300	13-92	55.8	(Yu *et al.*, 2013)
	China	75.6-262	219	7.66-35.8	20.1	(Ashfaq *et al.*, 2017a)
	Tunisia	N/A	N/A	N.D.	N/A	(Afsa *et al.*, 2020)
	Egypt	N/A	N/A	1497	N/A	(Abdallah *et al.*, 2019)
		N/A	N/A	1661	N/A	
		N/A	N/A	6702	N/A	
		N/A	N/A	812	N/A	
		N/A	N/A	1092	N/A	

Pharmaceuticals	Locations	Influent Range (ng L^{-1})	Mean (ng L^{-1})	Effluent Range (ng L^{-1})	Mean (ng L^{-1})	References
Ibuprofen	Algeria	N/A	607.8	N/A	341.4	(Kermia *et al.*, 2016)
		N/A	8612.9	N/A	431.3	
Metformin	Greece	97036-176417	135,149	885-4806	2,448	(Dasenaki and Thomaidis, 2015)
		137.82-1589.9	308.1	<LOD-238	18.7	
		<LOD-478.9	185.9	<LOD-30.3	5	
	Spain		5927		1252	(Carmona *et al.*, 2017)
	Poland	3818.7-16,790.7	8101.4	7.5-62.9	24.3	(Kot-Wasik *et al.*, 2016)
	Egypt	N/A	N/A	219	N/A	(Abdallah *et al.*, 2019)
		N/A	N/A	589	N/A	
		N/A	N/A	5613	N/A	
		N/A	N/A	1109	N/A	
		N/A	N/A	168	N/A	
Ofloxacin	Greece	116-180	133	62-194	142	(Dasenaki and Thomaidis, 2015)
	Spain		7		5	(Carmona *et al.*, 2017)
	Portugal	N.D.-39		N.D.-233	174	(Paíga *et al.*, 2019)
	Poland	39.4-363.9	125.7	9.5-203.4	35.2	(Kot-Wasik *et al.*, 2016)
	Hong Kong	750-1170	1020	740-1220	980	(Leung *et al.*, 2012)

Pharmaceuticals	Locations	Influent Range (ng L^{-1})	Mean (ng L^{-1})	Effluent Range (ng L^{-1})	Mean (ng L^{-1})	References
Ofloxacin	Hong Kong	672-742	707	397-914	708	(Leung *et al.*, 2012)
		142-281	220	95.8-352	202	
		3830-5670	4750	3530-7870	5700	
		7480-7900	7690	5900-7780	6840	
		5280-7680	6480	5810-6830	6320	
		3660-4120	3890	4150-4650	4400	
	China	320-616	495	103-162	129	(Ashfaq *et al.*, 2017a)
	Tunisia	N/A	392.02	N/A	239.1	(Harrabi *et al.*, 2018)
		N/A	442	N/A	509	(Moslah *et al.*, 2018)
		N/A	599	N/A	629	
		N/A	419	N/A	648	
		N/A	284	N/A	367	
		N/A	284	N/A	392	
		N/A	868	N/A	260	
		N/A	138	N/A	190	
	UAE	N/A	846	N/A	511	(Semreen *et al.*, 2019)
		680-1012	845.9	345-676	510.8	(Semerjian *et al.*, 2018)
Oxypurinol	Germany	N/A	2800	N/A	5200	(Funke *et al.*, 2015)
		N/A	3100	N/A	5000	

Pharmaceuticals	Locations	Influent Range (ng L^{-1})	Mean (ng L^{-1})	Effluent Range (ng L^{-1})	Mean (ng L^{-1})	References
Oxypurinol	Germany	N/A	5900	N/A	6800	(Funke *et al.*, 2015)
		N/A	8000	N/A	8200	(Funke *et al.*, 2015)
		N/A	16800	N/A	18300	
		N/A	25300	N/A	9100	
		N/A	26600	N/A	21700	
		N/A	15400	N/A	14200	
		N/A	11400	N/A	9000	
		N/A	9700	N/A	6700	
		N/A	13100	N/A	3400	
		N/A	9100	N/A	7900	
		N/A	22000	N/A	3400	(Hermes *et al.*, 2018)
		N/A	5600	N/A	1000	
		N/A	21000	N/A	20500	
Trimethoprim	Greece	133-309	194	119-154	134	(Dasenaki and Thomaidis, 2015)
		36.7-180.3	132.1	BQL-111.2	59.8	(Kosma *et al.*, 2014)
		31.7-1866.2	621.8	BQL-533.2	186.7	
		BQL-54.5	23.1	BQL-27.4	11.6	
		BQL-42.1	16.2	BQL-23.1	8	
		BQL-39.1	16.7	N.D.-20.0	8.3	
		N.D.-19.6	7.9	N.D.-BQL		
		BQL-72.9	33.7	BQL-7.0	BQL	

Pharmaceuticals	Locations	Influent Range (ng L^{-1})	Mean (ng L^{-1})	Effluent Range (ng L^{-1})	Mean (ng L^{-1})	References
Trimethoprim	Greece	BQL-39.4	22.9	N.D.-18.6	10	(Kosma *et al.*, 2014)
		2.00-47.84	11.7	LOQ-69	7.82	(Papageorgiou *et al.*, 2019)
		10.8-54.0	19.4	<LOD-23.6	13.1	
		< LOQ-200	138	N.D.—95.8	46.7	(Papageorgiou *et al.*, 2016)
	UK	464-6796	2192	625-3052	1152	(Kasprzyk-Hordern *et al.*, 2009)
		1514-4673	2925	385-1218	876	
	Portugal	N.D.-<MDL	N/A	N.D.-108	62	(Paíga *et al.*, 2019)
	Hong Kong	72-118	95	59-113	91	(Leung *et al.*, 2012)
		110-120	114	63-71	68	
		116-138	124	60-73	68	
		250-363	307	216-445	331	
		220-235	228	209-451	330	
		410-700	555	347-425	386	
		397-458	428	424-465	445	
	India	68-400	180	N.D.-<LOQ	<LOQ	(Subedi *et al.*, 2017)
		<LOQ-51	29	N.D.-25.0/29	25	
	US	409-1620	N/A	8-894	N/A	Mahapatra et al. (2016)
		401-1417	N/A	9-410	N/A	
	Mexico	125-165	145	135–300	143	(Rivera-Jaimes *et al.*, 2018)
		440-790	680	290-395	338	
	China		11.2		18	(Zhang *et al.*, 2018)

Pharmaceuticals	Locations	Influent Range (ng L^{-1})	Mean (ng L^{-1})	Effluent Range (ng L^{-1})	Mean (ng L^{-1})	References
Trimethoprim	China	N/A	192.9	N/A	4.3	(Zhang *et al.*, 2018)
		N/A	423.2	N/A	427.8	
		N/A	252.8	N/A	10.8	
		N/A	155.5	N/A	9.6	
		N/A	252.6	N/A	41.4	
		N/A	97.2	N/A	14.5	
		N/A	285.6	N/A	37.3	
	Tunisia	N/A	160.69	N/A	25.85	(Harrabi *et al.*, 2018)
		N/A	<LOD	N/A	<LOQ	(Moslah *et al.*, 2018)
	Tunisia	N/A	<LOD	N/A	<LOQ	(Moslah *et al.*, 2018)
		N/A	<LOD	N/A	<LOQ	
		N/A	<LOD	N/A	<LOQ	
		N/A	<LOD	N/A	<LOD	
		N/A	<LOD	N/A	<LOD	
		N/A	<LOD	N/A	<LOD	
	Egypt	N/A	N/A	1060	N/A	(Abdallah *et al.*, 2019)
		N/A	N/A	271	N/A	
		N/A	N/A	2738	N/A	
		N/A	N/A	459	N/A	
		N/A	N/A	650	N/A	

Pharmaceuticals	Locations	Influent Range (ng L^{-1})	Mean (ng L^{-1})	Effluent Range (ng L^{-1})	Mean (ng L^{-1})	References
Trimethoprim	Jordan	N/A	171	N/A	<0.005	(Al-Mashaqbeh *et al.*, 2019)
	Iran	7.65	N/A	3.05	N/A	(Aydin *et al.*, 2019)
		27.4	N/A	15.4	N/A	

MDL: Method detection limit, BQL: Below quantification limit, LOD: Limit of detection, N.D.: Not detected, LOQ: Limit of quantification, BLD: Below limit of detection, DL: Detection limit.

Table A-3-4: Removal efficiency (%) of wastewater quality sample parameters in the studied WWTPs.

WWTPs	Years	Seasons	Months	COD	BOD5	TSS
H-WWTP R (%)	2018	Spring	March	96.18	96.19	97.50
			April	93.20	92.31	98.01
			May	95.10	95.71	96.46
		Summer	June	98.69	98.53	96.06
			July	94.15	93.91	96.74
		Autumn	September	95.85	97.33	98.66
			October	94.28	94.29	95.45
			November	94.01	94.10	90.34
	2019	Spring	April	93.76	93.78	95.43
			May	96.18	96.35	96.57
		Summer	June	96.32	95.20	96.79
			July	95.36	95.83	97.50
	Maximum			98.69	98.53	98.66
	Minimum			93.20	92.31	90.34
	Mean			95.26	95.30	96.29
	Standard deviation			1.51	1.72	2.11
M-WWTP R (%)	2018	Spring	March	96.71	96.70	98.78
			April	95.41	95.63	96.92
			May	96.40	96.92	96.08
		Summer	June	97.82	97.49	97.47
			July	97.18	97.41	96.46
		Autumn	September	93.49	96.51	98.17
			October	96.40	96.92	96.08
			November	96.81	97.31	97.98
	2019	Spring	April	97.44	97.21	97.29
			May	97.22	98.06	97.79
		Summer	June	96.99	98.08	98.08
			July	90.32	97.21	94.12
	Maximum			97.82	98.08	98.78
	Minimum			90.32	95.63	94.12
	Mean			96.02	97.12	97.10
	Standard deviation			2.12	0.67	1.27
KSU-WWTP R (%)	2018	Spring	March	96.67	97.38	91.67
			April	96.80	97.20	92.00
			May	96.67	96.90	92.19
		Summer	June	96.88	97.13	93.60
			July	96.89	97.14	91.67
		Autumn	September	96.20	97.04	92.19
			October	96.48	97.68	92.31
			November	96.72	97.25	95.83
	2019	Spring	April	96.52	97.39	92.13
			May	96.77	97.00	92.31

WWTPs	Years	Seasons	Months	COD	BOD5	TSS
KSU-WWTP R (%)	2019	Summer	June	96.83	97.43	92.19
			July	96.53	97.50	91.67
	Maximum			96.89	97.68	95.83
	Minimum			96.20	96.90	91.67
	Mean			96.66	97.25	92.48
	Standard deviation			0.20	0.23	1.17

Table A-3-5: Removal efficiency (%) in the selected WWTPs for the detected pharmaceutical compounds.

H-WWTP												
Years	**Seasons**	**Months**	**ACE**	**ATE**	**BAC**	**CAF**	**CEP**	**CIP**	**DIC**	**MET**	**OFL**	**TRI**
2018	Spring	Mar.	99.93	85.54	N/A	99.99	N/A	81.55	95.76	99.69	68.44	N/A
		Apr.	99.99	99.42	N/A	99.99	N/A	83.98	99.93	99.99	71.85	99.24
		May.	99.98	99.26	N/A	99.99	N/A	82.53	99.95	99.97	72.61	98.92
	Summer	Jun.	99.99	99.25	N/A	99.99	N/A	91.53	99.76	99.82	87.11	98.57
		Jul.	100.00	99.30	N/A	99.97	N/A	97.89	99.91	96.63	98.47	98.87
	Autumn	Sep.	100.00	96.65	N/A	99.98	N/A	76.96	97.65	93.80	81.13	97.55
		Oct.	100.00	98.00	N/A	99.98	N/A	94.72	99.34	97.52	94.21	97.86
		Nov.	100.00	97.73	N/A	97.65	94.57	97.18	99.82	98.95	96.29	97.70
2019	Spring	Apr.	99.97	98.74	N/A	99.99	N/A	80.70	99.91	99.98	73.81	98.83
		May.	99.99	95.72	N/A	99.22	N/A	12.44	95.38	97.26	N/A	N/A
	Summer	Jun.	99.99	82.23	N/A	99.96	N/A	N/A	95.28	99.63	58.76	N/A
		Jul.	100.00	97.30	N/A	99.98	N/A	83.51	99.28	99.02	80.18	95.92
Maximum			100.00	99.42	N/A	99.99	94.57	97.89	99.95	99.99	98.47	99.24
Minimum			99.93	82.23	N/A	97.65	94.57	12.44	95.28	93.80	58.76	95.92
Average			99.99	95.76	N/A	99.72	94.57	80.27	98.50	98.52	80.26	98.16
M-WWTP												
2018	Spring	Mar.	100.00	91.09	N/A	99.98	N/A	99.55	97.94	99.38	91.45	87.63
		Apr.	100.00	99.87	N/A	99.99	95.20	100.00	99.92	99.98	99.81	99.70
		May.	99.99	99.24	N/A	99.99	98.29	93.76	99.63	99.95	89.16	99.32
	Summer	Jun.	100.00	99.81	N/A	99.98	99.70	99.99	99.95	99.83	99.87	99.61
		Jul.	100.00	97.49	N/A	99.98	N/A	99.97	99.48	98.46	98.52	96.47

M-WWTP												
Years	**Seasons**	**Months**	**ACE**	**ATE**	**BAC**	**CAF**	**CEP**	**CIP**	**DIC**	**MET**	**OFL**	**TRI**
2018	Autumn	Sep.	100.00	94.05	N/A	97.00	96.80	97.12	99.25	99.67	98.66	98.18
		Oct.	100.00	97.85	N/A	99.06	N/A	99.95	99.74	98.72	99.18	97.78
		Nov.	100.00	95.82	N/A	99.93	N/A	99.98	98.64	99.96	99.07	95.86
2019	Spring	Apr.	100.00	99.77	N/A	99.98	N/A	99.99	99.89	99.98	99.75	99.55
		May.	99.97	85.72	N/A	99.98	N/A	99.60	95.94	99.21	94.51	N/A
	Summer	Jun.	100.00	N/A	N/A	99.97	N/A	99.74	94.27	99.08	87.29	N/A
		Jul.	100.00	97.39	N/A	99.98	N/A	99.90	99.34	99.98	95.10	97.25
Maximum			100.00	99.87	N/A	99.99	99.70	100.00	99.95	99.98	99.87	99.70
Minimum			99.97	85.72	N/A	97.00	95.20	93.76	94.27	98.46	87.29	87.63
Average			100.00	96.19	N/A	99.65	97.50	99.13	98.66	99.52	96.03	97.13
KSU-WWTP												
2018	Spring	Mar.	99.95	86.67	N/A	99.95	95.68	99.67	92.37	98.24	86.12	96.13
		Apr.	100.00	99.13	81.33	99.97	N/A	99.10	99.55	99.96	99.12	99.70
		May.	99.97	99.40	N/A	99.97	86.44	93.63	97.09	99.97	91.45	99.65
	Summer	Jun.	100.00	98.42	N/A	99.76	89.82	-48.04	98.95	99.60	N/A	98.81
		Jul.	100.00	93.47	96.73	99.78	N/A	78.30	96.14	90.57	N/A	99.21
	Autumn	Sep.	99.97	92.79	97.82	99.64	N/A	75.73	93.70	89.70	69.13	97.29
		Oct.	99.69	94.25	N/A	99.90	N/A	35.66	98.92	16.90	-63.48	94.04
		Nov.	99.21	83.51	N/A	99.76	N/A	64.60	97.80	99.78	42.52	92.89
2019	Spring	Apr.	100.00	98.63	N/A	99.96	N/A	99.52	99.50	99.96	98.81	99.60
		May.	99.99	89.02	N/A	99.95	N/A	99.71	88.10	97.66	N/A	88.06
	Summer	Jun.	99.99	93.86	N/A	99.96	N/A	99.19	93.03	93.11	N/A	89.15
		Jul.	100.00	96.79	N/A	99.96	N/A	98.49	97.14	99.98	98.61	99.37

KSU-WWTP										
	ACE	**ATE**	**BAC**	**CAF**	**CEP**	**CIP**	**DIC**	**MET**	**OFL**	**TRI**
Maximum	100.00	99.40	97.82	99.97	95.68	99.71	99.55	99.98	99.12	99.70
Minimum	99.21	83.51	81.33	99.64	86.44	-48.04	88.10	16.90	-63.48	88.06
Average	99.90	93.83	91.96	99.88	90.65	74.63	96.02	90.45	65.29	96.16

ACE: Acetaminophen, ATE: Atenolol, BAC: Baclofen, CAF: Caffeine, CEP: Cephalexin, CIP: Ciprofloxacin, DIC: Diclofenac, MET: Metformin, OFL: Ofloxacin, TRI: Trimethoprim, N/A: Not available.

Table A-3-6: Average seasonal concentrations (μg L^{-1}) of detected compounds and their seasonal variations (%) in the studied WWTPs influent and effluent.

H-WWTP Effluent											
Season average concentrations (μg L^{-1})											
		ACE	ATE	BAC	CAF	CEP	CIP	DIC	MET	OFL	TRI
2018	Spring	0.09	N/A	N/A	N/A	N/A	16.06	N/A	N/A	5.74	N/A
	Summer	0.04	N/A	N/A	N/A	N/A	4.93	N/A	8.77	1.33	N/A
	Fall	N/A	N/A	N/A	16.56	N/A	1.85	N/A	3.49	<LOD	N/A
2019	Spring	0.19	N/A	N/A	4.92	N/A	15.76	N/A	<LOD	5.37	N/A
	Summer	N/A	N/A	N/A	N/A	N/A	9.70	N/A	1.38	1.34	N/A
Seasonal variations (%)											
2018	Spring	-6.60	N/A	N/A	N/A	N/A	68.49	N/A	N/A	47.02	N/A
	Summer	-63.04	N/A	N/A	N/A	N/A	-48.23	N/A	112.59	-65.80	N/A
	Fall	N/A	N/A	N/A	54.16	N/A	-80.55	N/A	-15.36	N/A	N/A
2019	Spring	82.84	N/A	N/A	-54.16	N/A	65.42	N/A	N/A	37.49	N/A
	Summer	N/A	N/A	N/A	N/A	N/A	1.79	N/A	-66.51	-65.73	N/A
H-WWTP Influent											
Season average concentrations (μg L^{-1})											
2018	Spring	599.20	4.18	N/A	2053.10	N/A	97.47	27.65	175.14	14.09	1.59
	Summer	383.82	5.55	N/A	978.17	N/A	107.42	18.05	154.81	16.30	1.13
	Fall	335.98	1.65	N/A	871.34	0.37	31.46	5.90	138.89	5.61	0.62
2019	Spring	338.94	2.05	N/A	1192.06	N/A	75.03	13.10	85.46	10.56	1.22
	Summer	99.67	0.85	N/A	933.21	N/A	32.43	1.92	74.37	3.79	0.35
Seasonal variations (%)											
2018	Spring	61.57	45.83	N/A	64.47	N/A	43.24	98.94	33.75	40.45	61.83
	Summer	3.49	93.61	N/A	-21.64	N/A	57.86	29.90	18.22	62.42	14.44
	Fall	-9.41	-42.43	N/A	-30.20	0.00	-53.76	-57.57	6.07	-44.04	-37.07

H-WWTP Influent											
Seasonal variations (%)											
		ACE	ATE	BAC	CAF	CEP	CIP	DIC	MET	OFL	TRI
2019	Spring	-8.61	-28.46	N/A	-4.51	N/A	10.26	-5.77	-34.73	5.23	23.31
	Summer	-73.13	-70.25	N/A	-25.24	N/A	-52.34	-86.19	-43.21	-62.26	-64.64
M-WWTP Effluent											
Season average concentrations (μg L^{-1})											
		ACE	ATE	BAC	CAF	CEP	CIP	DIC	MET	OFL	TRI
2018	Spring	0.05	N/A	N/A	N/A	N/A	19.92	N/A	N/A	3.96	N/A
	Summer	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1.55	N/A	N/A
	Fall	N/A	N/A	N/A	7.31	N/A	1.49	N/A	1.73	N/A	N/A
2019	Spring	0.01	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Summer	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Seasonal variations (%)											
2018	Spring	57.33	N/A	N/A	N/A	N/A	86.09	N/A	N/A	0.00	N/A
	Summer	N/A	N/A	N/A	N/A	N/A	N/A	N/A	-7.45	N/A	N/A
	Fall	N/A	N/A	N/A	0.00	N/A	-86.09	N/A	3.73	N/A	N/A
2019	Spring	-57.33	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Summer	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
M-WWTP Influent											
Season average concentrations (μg L^{-1})											
2018	Spring	608.31	11.92	N/A	1182.19	0.79	283.43	16.57	120.13	32.62	2.33
	Summer	394.15	11.36	N/A	906.75	6.75	391.76	24.94	74.78	47.92	2.03
	Fall	501.03	1.16	N/A	607.77	0.62	80.49	4.73	309.39	11.40	0.59
2019	Spring	343.28	8.67	N/A	859.87	N/A	158.53	11.03	69.64	23.72	1.61
	Summer	254.58	1.53	N/A	757.73	N/A	31.24	2.05	177.52	3.89	0.28

M-WWTP Influent											
Seasonal variations (%)											
		ACE	ATE	BAC	CAF	CEP	CIP	DIC	MET	OFL	TRI
2018	Spring	37.42	62.18	N/A	36.16	-64.61	50.84	42.07	-25.40	38.26	68.31
	Summer	-10.96	54.58	N/A	4.44	201.33	108.49	113.86	-53.56	103.11	46.88
	Fall	13.18	-84.17	N/A	-30.00	-72.11	-57.16	-59.44	92.13	-51.69	-57.55
2019	Spring	-22.45	17.97	N/A	-0.96	N/A	-15.63	-5.38	-56.76	0.55	16.41
	Summer	-42.49	-79.14	N/A	-12.72	N/A	-83.38	-82.42	10.23	-83.52	-79.43
KSU-WWTP Effluent											
Season average concentrations (μg L^{-1})											
		ACE	ATE	BAC	CAF	CEP	CIP	DIC	MET	OFL	TRI
2018	Spring	0.17	N/A	N/A	N/A	N/A	4.41	N/A	N/A	N/A	N/A
	Summer	N/A	N/A	N/A	N/A	N/A	9.79	N/A	3.58	1.15	N/A
	Fall	0.12	N/A	N/A	N/A	N/A	12.28	N/A	4.22	2.48	N/A
2019	Spring	N/A	N/A	N/A	N/A	N/A	1.68	N/A	N/A	N/A	N/A
	Summer	N/A	N/A	N/A	N/A	N/A	1.64	N/A	N/A	N/A	N/A
Seasonal variations (%)											
2018	Spring	22.80	N/A	N/A	N/A	N/A	-42.12	N/A	N/A	N/A	N/A
	Summer	N/A	N/A	N/A	N/A	N/A	28.53	N/A	-10.63	-36.63	N/A
	Fall	-15.20	N/A	N/A	N/A	N/A	61.20	N/A	5.31	36.63	N/A
2019	Spring	N/A	N/A	N/A	N/A	N/A	-78.01	N/A	N/A	N/A	N/A
	Summer	N/A	N/A	N/A	N/A	N/A	-78.42	N/A	N/A	N/A	N/A
KSU-WWTP Influent											
Season average concentrations (μg L^{-1})											
2018	Spring	737.46	3.71	0.33	1371.08	0.24	184.39	2.72	180.25	14.42	3.05
	Summer	300.78	1.51	1.88	222.94	0.16	19.72	2.09	29.77	0.52	1.50
	Fall	58.51	0.48	2.82	289.95	N/A	29.54	1.97	23.23	2.25	0.32

KSU-WWTP Influent											
Season average concentrations (μg L^{-1})											
		ACE	ATE	BAC	CAF	CEP	CIP	DIC	MET	OFL	TRI
2019	Spring	395.24	1.58	N/A	767.68	N/A	179.30	2.52	98.22	14.38	1.84
	Summer	231.92	0.91	N/A	456.51	N/A	55.70	0.59	190.35	4.18	1.19
Seasonal variations (%)											
2018	Spring	108.53	116.35	-80.35	108.86	13.46	92.20	33.28	73.44	96.20	90.92
	Summer	-14.95	-11.86	12.21	-66.04	-26.92	-79.45	2.43	-71.35	-92.88	-6.30
	Fall	-83.46	-72.05	68.14	-55.83	N/A	-69.20	-3.32	-77.64	-69.32	-79.95
2019	Spring	11.76	-7.91	N/A	16.94	N/A	86.89	23.46	-5.50	95.62	15.43
	Summer	-34.42	-46.69	N/A	-30.46	N/A	-41.94	-70.84	83.16	-43.06	-25.61

ACE: Acetaminophen, ATE: Atenolol, BAC: Baclofen, CAF: Caffeine, CEP: Cephalexin, CIP: Ciprofloxacin, DIC: Diclofenac, MET: Metformin, OFL: Ofloxacin, TRI: Trimethoprim, N/A: Not available.

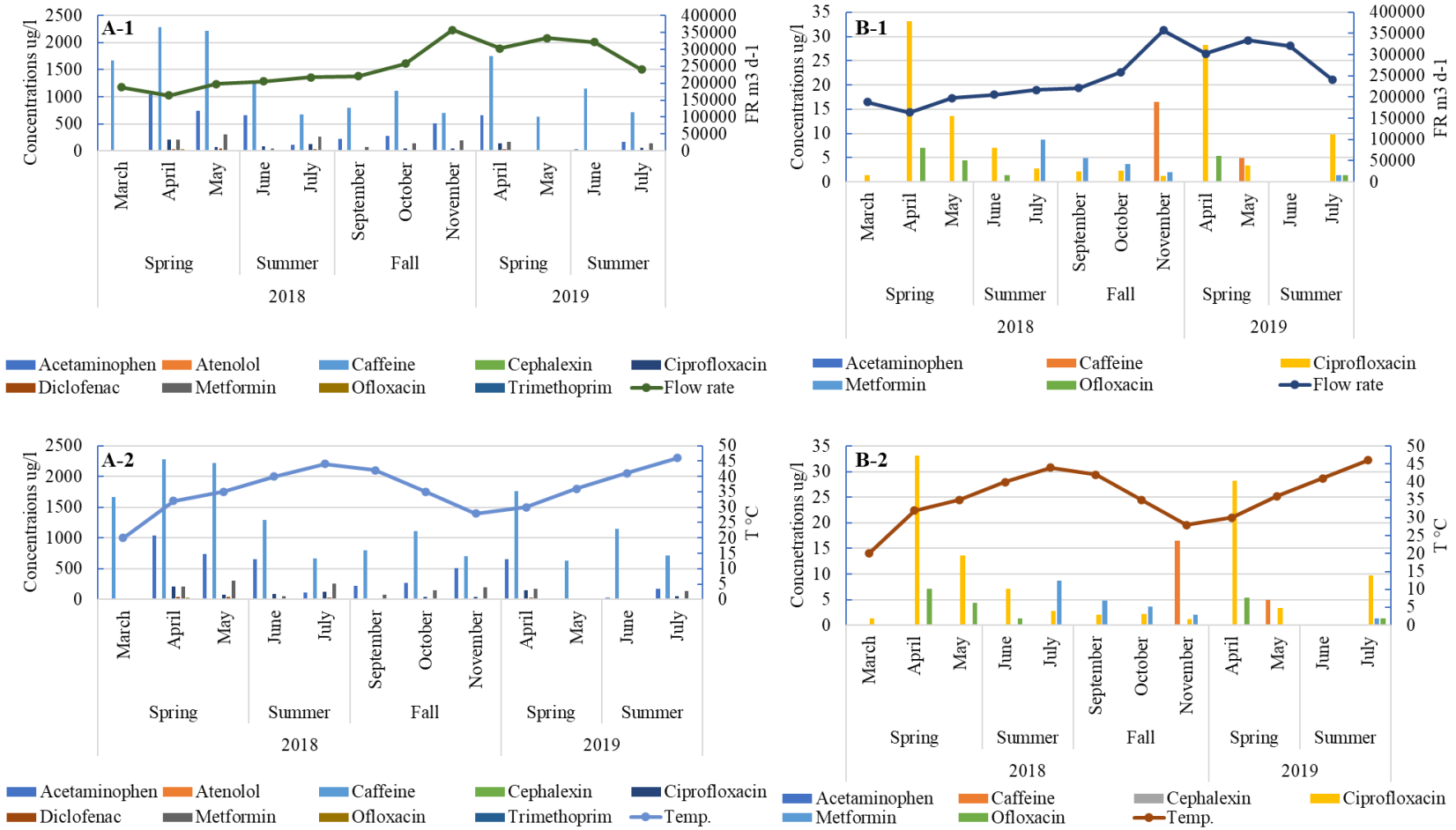

Figure A-3-1: Monthly variations in pharmaceutical concentrations (µg L^{-1}) in H-WWTP influent, together with flow rate (m^3 d^{-1}) (A-1), temperature (°C) (A-2) and H-WWTP effluent with flow rate (B-1), temperature (B-2).

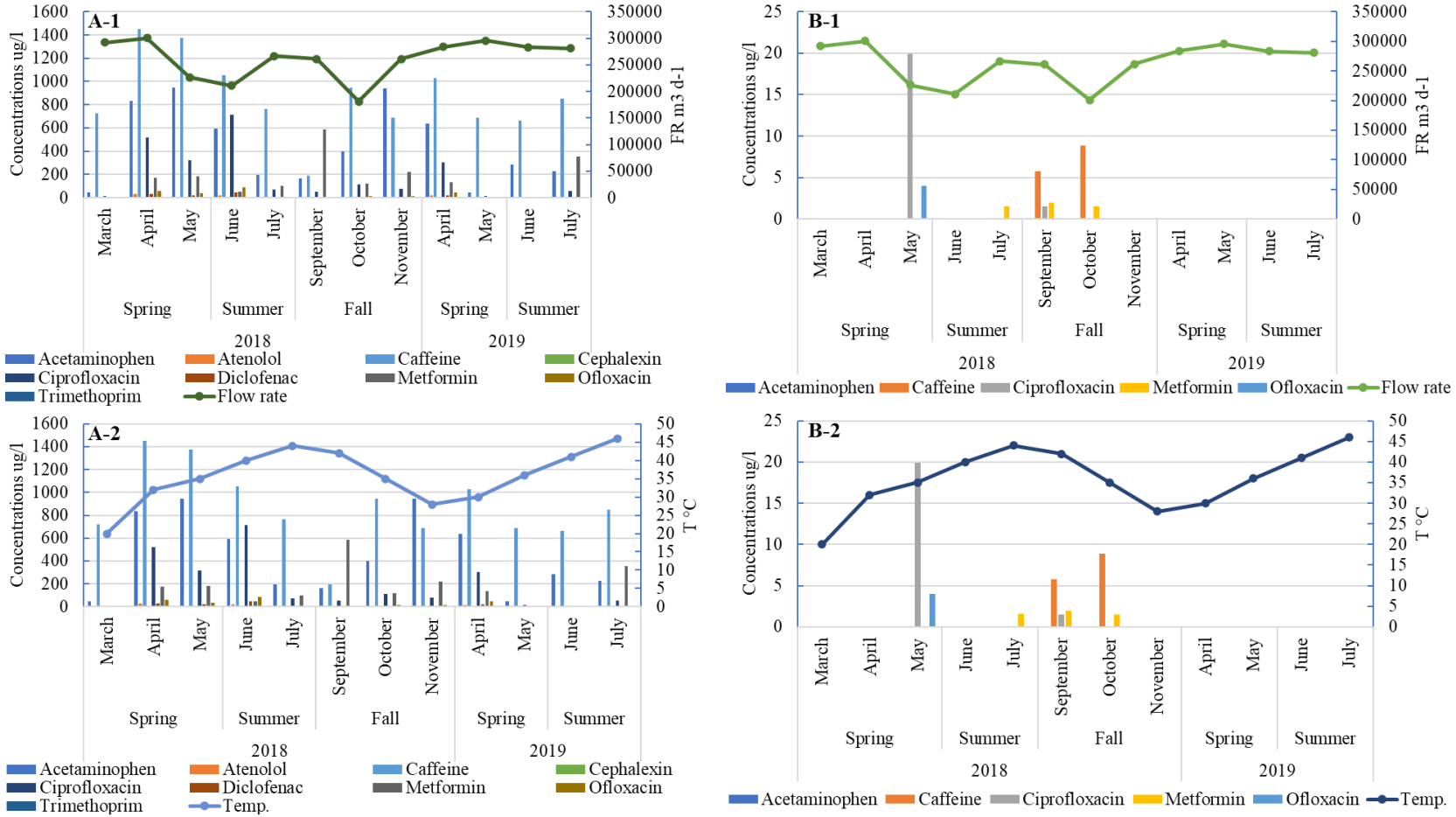

Figure A-3-2: Monthly variations in pharmaceutical concentrations (µg L^{-1}) in M-WWTP influent together with flow rate (m^3 d^{-1}) (A-1), temperature (°C) (A-2) and M-WWTP effluent with flow rate (B-1), temperature (B-2).

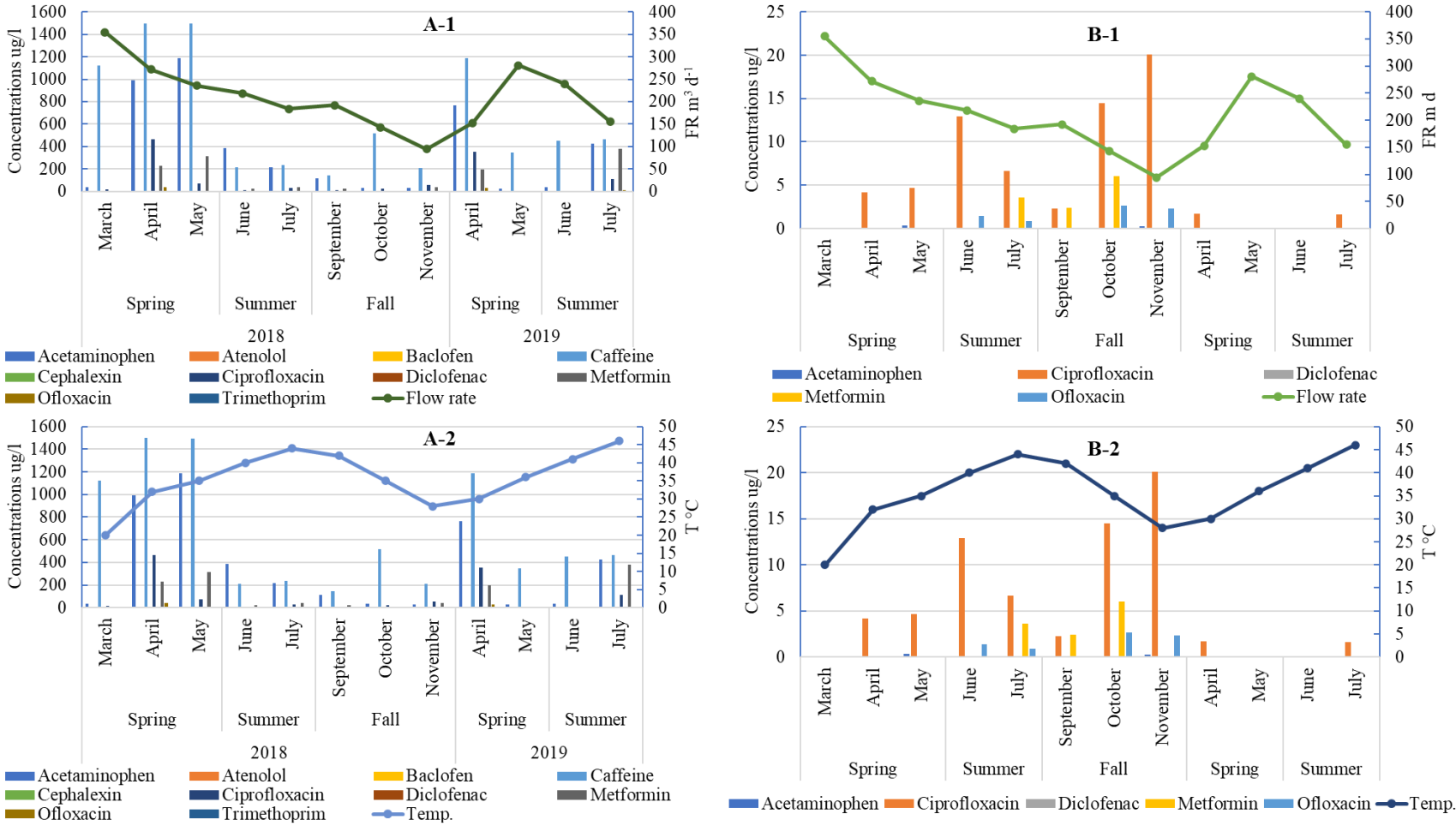

Figure A-3-3: Monthly variations in pharmaceutical concentrations (µg L^{-1}) in KSU-WWTP influent together with flow rate (m^3 d^{-1}) (A-1), temperature (°C) (A-2) and KSU-WWTP effluent with flow rate (B-1), temperature (B-2).

Table A-4-1: Ecotoxicological data for the studied pharmaceutical compounds and their different corresponding assayed species. Lowest toxic values are in bold.

Compounds	L(E)C50 (µg/l)								
	Algae	Species	Ref.	Daphnia	Species	Ref.	Fish	Species	Ref.
Acetaminophen	134000		(Mendoza *et al.*, 2015)	2040		(Mendoza *et al.*, 2015)	378000		(Grung *et al.*, 2008)
	134000		(Grung *et al.*, 2008)	9200		(Grung *et al.*, 2008)	378000		(Henschel *et al.*, 1997)
	134000	S. subcapitata	(Henschel *et al.*, 1997)	9200	D.magna	(Kühn *et al.*, 1989)	**40000**	B. rerio	(Sanderson *et al.*, 2003)
	2549000	ECOSAR	(Sanderson *et al.*, 2003)	50000	D.magna	(Henschel *et al.*, 1997)	900000		(Verlicchi *et al.*, 2012a)
	105000		(Verlicchi *et al.*, 2012a)	2400	Daphnid	(Dave and Herger, 2012)	47000	QSARINS	(Bu *et al.*, 2020)
	583		(Escher *et al.*, 2011)	136000	D.magna	(Stuer-Lauridsen *et al.*, 2000)			
	17000	QSARINS	(Bu *et al.*, 2020)	9200	D.magna	(Stuer-Lauridsen *et al.*, 2000)			
				42000	ECOSAR	(Sanderson *et al.*, 2003)			
				300000		(Verlicchi *et al.*, 2012a)			

Compounds	L(E)C50 (µg/l)								
	Algae	Species	Ref.	Daphnia	Species	Ref.	Fish	Species	Ref.
Acetaminophen				12900	D. magna	(Bouissou-Schurtz *et al.*, 2014)			
				1000	Daphnia	(Verlicchi *et al.*, 2012a)			
				28650	QSARINS	(Bu *et al.*, 2020)			
Diclofenac	14500		(Grung *et al.*, 2008)	22000		(Grung *et al.*, 2008)	532000		(Grung *et al.*, 2008)
	14500		(Ginebreda *et al.*, 2010)	22000		(Ginebreda *et al.*, 2010)	5300		(van den Brandhof and Montforts, 2010)
	71900		(Vestel *et al.*, 2016)	80100		(Vestel *et al.*, 2016)	166600		(Vestel *et al.*, 2016)
	3800	V. fisheri	(Adame and Rocha, 2014)	22000	D. magna	(Ginebreda *et al.*, 2010)	7800	D. reri	(van den Brandhof and Montforts, 2010)
	21300	V. fischeri	(Wieczerzak *et al.*, 2016)	**520**	D. magna	(Du *et al.*, 2016)	532000	D. rerio	(Ginebreda *et al.*, 2010)
	14500		(Ginebreda *et al.*, 2010)	5057000	Daphnid	(Sanderson *et al.*, 2003)	90000	D. rerio	(Haap *et al.*, 2008)
	72000	D. subspicatus	(Grung *et al.*, 2008)	68000	D. magna	(Grung *et al.*, 2008)	7800	D. rerio	(Papageorgiou *et al.*, 2019)

Compounds	L(E)C50 (µg/l)								
	Algae	Species	Ref.	Daphnia	Species	Ref.	Fish	Species	Ref.
Diclofenac	16000	P. subcapitata	(Grung *et al.*, 2008)	41000	T. Platyurus	(Grung *et al.*, 2008)	90000	D. rerio	(Nassef *et al.*, 2010)
	19000	C. meneghiniana	(Grung *et al.*, 2008)	80000	D. magna	(Grung *et al.*, 2008)	53200	ECOSAR	(Sanderson *et al.*, 2003)
	72000	D. suspicatus	(Santos *et al.*, 2013)	23000	C. dubia	(Grung *et al.*, 2008)	7800	D. rerio	(Whitacre, 2012)
	16300		(Ferrari *et al.*, 2004)	224300	D. magna	(Ferrari *et al.*, 2003)	5000	ECOTOX	(Kuzmanović *et al.*, 2015)
	16000	P. subcapitata	(Kosma *et al.*, 2014)	22700	C. dubia	(Ferrari *et al.*, 2003)	173000	L. macrochirus	(Whitacre, 2012)
	7500		(Cleuvers, 2004)	22700	C. dubia	(Papageorgiou *et al.*, 2019)	**200**		(Lucas *et al.*, 2016)
	4000	ECOTOX	(Kuzmanović *et al.*, 2015)	15800	T.battagliai	(Papageorgiou *et al.*, 2019)	532000	ECOSAR	(Sanderson *et al.*, 2003)
	710	S.costatum (96h EC50)	(Whitacre, 2012)	15800	T. battagili	(Schmidt *et al.*, 2011)	700	QSARINS	(Bu *et al.*, 2020)
	315000	D. subspicatus (72h EC50)	(Whitacre, 2012)	22700	C. Dubia	(Whitacre, 2012)			
	2300		(Harada *et al.*, 2008)	15800	T.battagliai	(Whitacre, 2012)			
	72000		(Cleuvers, 2004)	35000	ECOTOX	(Kuzmanović *et al.*, 2015)			

Compounds	L(E)C50 (µg/l)								
	Algae	Species	Ref.	Daphnia	Species	Ref.	Fish	Species	Ref.
Diclofenac	14500		(Ferrari *et al.*, 2004)	9060	D. magna	(Whitacre, 2012)			
	2911000	ECOSAR	(Sanderson *et al.*, 2003)	108000	D. magna	(Whitacre, 2012)			
	16300		(Ferrari *et al.*, 2004)	5700		(Dave and Herger, 2012)			
	1940	QSARINS	(Bu *et al.*, 2020)	22700	C. dubia	(Ferrari *et al.*, 2003)			
				5057000	ECOSAR	(Sanderson *et al.*, 2003)			
				22400	Daphnia	(Ferrari *et al.*, 2004)			
				22430	Daphnia	(Ferrari *et al.*, 2003)			
				68000	Daphnia	(Cleuvers, 2004)			
				90000	Invertebrates	(Verlicchi *et al.*, 2012a)			
				11320	QSARINS	(Bu *et al.*, 2020)			
Ibuprofen	4000	ECOTOX	(Kuzmanović *et al.*, 2015)	34000	ECOTOX	(Kuzmanović *et al.*, 2015)	5000	ECOTOX	(Kuzmanović *et al.*, 2015)
	4000		(Ginebreda *et al.*, 2010)	9020		(Ginebreda *et al.*, 2010)	42036	ECOSAR	(Thomaidi *et al.*, 2015)

Compounds	L(E)C50 (µg/l)								
	Algae	Species	Ref.	Daphnia	Species	Ref.	Fish	Species	Ref.
Ibuprofen	7100	S.costatum	(Whitacre, 2012)	9060	D. magna	(Whitacre, 2012)	173000	L. macrochirus	(Whitacre, 2012)
	315000	D. subspicatus	(Whitacre, 2012)	108000	D. magna	(Whitacre, 2012)	**200**		(Lucas *et al.*, 2016)
	2300		(Harada *et al.*, 2008)	5700		(Dave and Herger, 2012)	5000	ECOSAR	(Sanderson *et al.*, 2003)
	342200		(Cleuvers, 2004)	38000	Daphnia	(Sanderson *et al.*, 2003)	110000		(Verlicchi *et al.*, 2012a)
	500000		(Verlicchi *et al.*, 2012a)	9060		(Halling-Sørensen *et al.*, 1998)	1650		(Verlicchi *et al.*, 2012a)
	26000	ECOSAR	(Sanderson *et al.*, 2003)	**1650**		(Quinn *et al.*, 2008)	3200	QSARINS	(Bu *et al.*, 2020)
	6600		(Escher *et al.*, 2011)	100000		(Verlicchi *et al.*, 2012a)			
	2600	QSARINS	(Bu *et al.*, 2020)	101200	Daphnia	(Cleuvers, 2004)			
				25430	QSARINS	(Bu *et al.*, 2020)			
Atenolol	190000	ECOTOX	(Kuzmanović *et al.*, 2015)	205000	ECOTOX	(Kuzmanović *et al.*, 2015)	**1096000**	ECOTOX	(Kuzmanović *et al.*, 2015)
	110000		(Yamamoto *et al.*, 2007)	33400		(Fraysse and Garric, 2005)	1800000		(Yamamoto *et al.*, 2007)

Compounds	L(E)C50 (µg/l)								
	Algae	Species	Ref.	Daphnia	Species	Ref.	Fish	Species	Ref.
Atenolol	620000		(Santos *et al.*, 2013)	313000		(Vestel *et al.*, 2016)	>100		(Santos *et al.*, 2013)
				205000	ECOTOX	(Kuzmanović *et al.*, 2015)			
				33400					
				30000		(Verlicchi *et al.*, 2012a)			
Azithromycin	1874	ECOSAR	(Kuzmanović *et al.*, 2015)	3070	ECOSAR	(Kuzmanović *et al.*, 2015)	**1970**	ECOSAR	(Kuzmanović *et al.*, 2015)
	19000		(Harada *et al.*, 2008)	120000		(Montforts, 2005)	18882	ECOSAR	(Zhang *et al.*, 2017)
	840		(Vestel *et al.*, 2016)	120000		(Vestel *et al.*, 2016)	90000		(Tousova *et al.*, 2017)
	1874	ECOSAR	(Zhang *et al.*, 2017)	**3070**	ECOSAR	(Kuzmanović *et al.*, 2015)			
	1874	ECOSAR	(Kuzmanović *et al.*, 2015)	>120000		(Cunningham *et al.*, 2006)			
	19000		(Harada *et al.*, 2008)	120000		(Cunningham *et al.*, 2006)			
	19000		(Harada *et al.*, 2008)	255610	QSARINS	(Bu *et al.*, 2020)			

Compounds	L(E)C50 (µg/l)								
	Algae	Species	Ref.	Daphnia	Species	Ref.	Fish	Species	Ref.
Azithromycin	**100**	QSARINS	(Bu *et al.*, 2020)						
Cephalexin	**45650**	QSARINS	(Bu *et al.*, 2020)	**228730**	QSARINS	(Bu *et al.*, 2020)	**19000**	QSARINS	(Bu *et al.*, 2020)
Ciprofloxacin	**2970**		(Kuzmanović *et al.*, 2015)	60000		(Kuzmanović *et al.*, 2015)	100000		(Kuzmanović *et al.*, 2015)
	6700		Yang et al., 2008	**12800**		(Santos *et al.*, 2013)	13131424	ECOSAR	(Thomaidi *et al.*, 2015)
	5000	M. aeruginosa	(Halling-Sørensen *et al.*, 2000)	60000	D. magna	(Halling-Sørensen *et al.*, 2000)	100000	B. rerio	(Halling-Sørensen *et al.*, 2000)
	938000	ECOSAR	(Sanderson *et al.*, 2003)	991000	ECOSAR	(Sanderson *et al.*, 2003)	**10000**	P. Promelas	(Whitacre, 2012)
	20680	QSARINS	(Bu *et al.*, 2020)	77020	QSARINS	(Bu *et al.*, 2020)	246000000	ECOSAR	(Sanderson *et al.*, 2003)
							13000	QSARINS	(Bu *et al.*, 2020)
Ofloxacin	2444544	ECOSAR	(Kuzmanović *et al.*, 2015)	31750	ECOSAR	(Kuzmanović *et al.*, 2015)	19352000	ECOSAR	(Kuzmanović *et al.*, 2015)
	16000		(Ferrari *et al.*, 2004)	31750		(Isidori *et al.*, 2005)	10000		(Verlicchi *et al.*, 2012a)

Compounds	L(E)C50 (µg/l)								
	Algae	Species	Ref.	Daphnia	Species	Ref.	Fish	Species	Ref.
Ofloxacin	**600**	P. subcapitata	(van der Grinten *et al.*, 2010)	3130	C. dubia	(Isidori *et al.*, 2005)	1000000	B. rerio	(Isidori *et al.*, 2005)
	16000		(Ferrari *et al.*, 2004)	30000		(Verlicchi *et al.*, 2012a)	10000		(Verlicchi *et al.*, 2012a)
	1500		(Verlicchi *et al.*, 2012a)	**1440**	Daphnia	(Iatrou *et al.*, 2014)	**530**		(Iatrou *et al.*, 2014)
	20000	Green algae	(Iatrou *et al.*, 2014)	3415000	Daphnia	(Iatrou *et al.*, 2014)	7285000		(Iatrou *et al.*, 2014)
	10000	Green algae	(Iatrou *et al.*, 2014)	57100	QSARINS	(Bu *et al.*, 2020)	7100	QSARINS	(Bu *et al.*, 2020)
	6980	QSARINS	(Bu *et al.*, 2020)						
Trimethoprim	16000	ECOTOX	(Kuzmanović *et al.*, 2015)	121000	ECOTOX	(Kuzmanović *et al.*, 2015)	795000	ECOTOX	(Kuzmanović *et al.*, 2015)
	16000	R. salina	(Lützhøft *et al.*, 1999)	92000	D.Magna	(Park and Choi, 2008)	3179100	ECOSAR	(Thomaidi *et al.*, 2015)
	110000	P. subcapitata	(Sanderson and Thomsen, 2009)	>100000 (48h)	Daphnid	(Sanderson and Thomsen, 2009)	100000	B. rerio	(Halling-Sørensen *et al.*, 2000)
	16000		(Ginebreda *et al.*, 2010)	120700	D. magna	(Ginebreda *et al.*, 2010)	>100000		(Sanderson and Thomsen, 2009)

Compounds	L(E)C50 (µg/l)								
	Algae	Species	Ref.	Daphnia	Species	Ref.	Fish	Species	Ref.
Trimethoprim	83800	P. subcapitata	(De Liguoro *et al.*, 2012)	123000	D. magna	(Grung *et al.*, 2008)	795000		(Ginebreda *et al.*, 2010)
	16000		(Stuer-Lauridsen *et al.*, 2000)	8210	D. magna	(De Liguoro *et al.*, 2012)	92660	P. reticulata	(De Liguoro *et al.*, 2012)
	90000		(Verlicchi *et al.*, 2012a)	121000	ECOTOX	(Kuzmanović *et al.*, 2015)	92660		(De Liguoro *et al.*, 2012)
	2600	ECOSAR	(Sanderson *et al.*, 2003)	**4800**	ECOSAR	(Sanderson *et al.*, 2003)	>100000		(Kim *et al.*, 2007)
	6470	QSARINS	(Bu *et al.*, 2020)	121000		(Kim *et al.*, 2007)	100000		(Verlicchi *et al.*, 2012a)
				>100000		(Quinn *et al.*, 2008)	795000	ECOSAR	(Sanderson *et al.*, 2003)
				110000		(Verlicchi *et al.*, 2012a)	**310**	QSARINS	(Bu *et al.*, 2020)
				17480	QSARINS	(Bu *et al.*, 2020)			
Tetracycline	6000		(Grung *et al.*, 2008)	**6000**		(Grung *et al.*, 2008)	220000		(Grung *et al.*, 2008)
	2200	P. subcapitata	(Halling-Sørensen *et al.*, 2000)	31850	D. magna	(Kim *et al.*, 2014)	356000	ECOSAR	(Zhang *et al.*, 2017)

Compounds	L(E)C50 (µg/l)								
	Algae	Species	Ref.	Daphnia	Species	Ref.	Fish	Species	Ref.
Tetracycline	**1330**	QSARINS	(Bu *et al.*, 2020)	460380	QSARINS	(Bu *et al.*, 2020)	**22000**	QSARINS	(Bu *et al.*, 2020)
Triclosan	530		(Brausch and Rand, 2011)	390		(Brausch and Rand, 2011)	270		(Brausch and Rand, 2011)
	228	V. fischeri	(Carbajo *et al.*, 2015)	1000	T. thermophila	(Carbajo *et al.*, 2015)	**260**	P. promelas	(Orvos *et al.*, 2002)
	450	S. capricornutum	(Orvos *et al.*, 2002)	391	D. magna	(Orvos *et al.*, 2002)	370	L. macrochirus	(Orvos *et al.*, 2002)
	70	S. subspicatus	(Orvos *et al.*, 2002)	200	C. dubia	(Bedoux *et al.*, 2012)	350	O. mykiss	(Bedoux *et al.*, 2012)
	140	Scenedesmus	(Orvos *et al.*, 2002)	390	D. magn	(Orvos *et al.*, 2002)	399		(Brausch and Rand, 2011)
	450	LC50-48h	(Sanderson and Thomsen, 2009)	**130**	Daphnid	(Sanderson and Thomsen, 2009)			
Metformin	110000		(Santos *et al.*, 2013)	64000	ECOSAR	(Zhou *et al.*, 2019)	27737.17	ECOSAR	(Zhou *et al.*, 2019)
	4624.59	ECOSAR	(Mansour *et al.*, 2016)	64000	D. magna	(Cleuvers, 2004)	>100000		(Sanderson and Thomsen, 2009)
	511	EC50 ECOSAR	(Sanderson *et al.*, 2003)	211.67	QSARINS	(Bu *et al.*, 2020)	**1900**	QSARINS	(Bu *et al.*, 2020)

Compounds	L(E)C50 (µg/l)								
	Algae	Species	Ref.	Daphnia	Species	Ref.	Fish	Species	Ref.
Metformin	58900	Lemna minor	(Godoy *et al.*, 2018)	1927.74	ECOSAR	(Mansour *et al.*, 2016)	27737.17	ECOSAR	(Mansour *et al.*, 2016)
	53700	Lemna minor	(Godoy *et al.*, 2018)	**64**	EC50	(Cleuvers, 2003)	33200	ECOSAR	(Sanderson *et al.*, 2003)
	58700	Lemna minor	(Godoy *et al.*, 2018)	130	EC50	(Montforts, 2005)	1315500	Danio rerio	(Godoy *et al.*, 2018)
				1345	ECOSAR	(Sanderson *et al.*, 2003)			
				14300		(Godoy *et al.*, 2018)			
				3918000	H. attenuata	(Godoy *et al.*, 2018)			
				2709000	H. attenuata	(Godoy *et al.*, 2018)			
Baclofen	**8985.51**	EC50 (96h, ECOSAR)	This study	**5070.55**	ECOSAR	This study	**62600.38**	ECOSAR	This study
Caffeine	**760**	ECOSAR	(Kuzmanović *et al.*, 2015)	46000	ECOSAR	(Kuzmanović *et al.*, 2015)	**4600**	ECOSAR	(Kuzmanović *et al.*, 2015)
	46000		(Sanderson *et al.*, 2003)	182000		(Fernández *et al.*, 2010)	87500		(Fernández *et al.*, 2010)
	86000	EC 50 (24h)	(Kosma *et al.*, 2014)	**46000**	Daphnid	(Sanderson *et al.*, 2003)	15000	ECOSAR	(Thomaidi *et al.*, 2015)

Compounds	L(E)C50 (µg/l)								
	Algae	Species	Ref.	Daphnia	Species	Ref.	Fish	Species	Ref.
Caffeine	9040	QSARINS	(Bu *et al.*, 2020)	182000	Daphnid	(Sanderson and Thomsen, 2009)	805000		(Sanderson *et al.*, 2003)
							87000		(Sanderson and Thomsen, 2009)
				395000	D. magna	(Di Lorenzo *et al.*, 2019)	53000		(Ginebreda *et al.*, 2010)
				5280000		(Di Lorenzo *et al.*, 2019)	120000	QSARINS	(Bu *et al.*, 2020)
				64760	QSARINS	(Bu *et al.*, 2020)			

Table A-5-1: Percentage retained from spiked concentrations in the soil column sections and in the leachates.

Selected soils	Column depths	Analytes[1]											
		ACE	ATE	AZI	BAC	CAF	CEP	CIP	DIC	OFL	TRI	IBU	TET
Silt loam	0-5cm	<LOD	88.30	<LOD	72.64	78.21	<LOD	12.26	95.98	29.22	85.79	38.86	84.30
	5-10cm	<LOD	12.45	<LOD	16.28	6.38	<LOD	<LOD	4.19	3.76	0.95	<LOD	<LOD
	10-20cm	<LOD	<LOD	<LOD	1.67	3.32	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	20-30cm	<LOD	<LOD	<LOD	<LOD	2.95	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Leachate	<LOD	<LOD	<LOD	<LOD	0.09	0.05	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Clay	0-5cm	<LOD	66.69	30.50	93.95	77.19	24.92	52.17	59.38	35.87	71.63	64.65	<LOD
	5-10cm	<LOD	30.83	<LOD	<LOD	4.06	<LOD	<LOD	<LOD	<LOD	1.54	<LOD	<LOD
	10-20cm	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	20-30cm	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Leachate	<LOD	<LOD	<LOD	<LOD	0.06	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Silty clay loam	0-5cm	<LOD	94.50	45.82	72.31	75.85	<LOD	34.72	44.44	58.12	71.12	27.14	96.31
	5-10cm	<LOD	3.35	<LOD	12.01	12.38	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	10-20cm	<LOD	<LOD	<LOD	<LOD	11.29	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	20-30cm	<LOD	<LOD	<LOD	<LOD	6.63	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Leachate	<LOD	<LOD	<LOD	<LOD	0.08	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Sandy loam	0-5cm	<LOD	84.90	27.90	81.21	83.94	8.48	36.27	34.15	69.64	94.68	76.90	92.14
	5-10cm	<LOD	9.49	<LOD	17.28	4.73	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	10-20cm	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	20-30cm	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Leachate	<LOD	<LOD	<LOD	<LOD	0.14	0.42	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

ACE: Acetaminophen, ATE: Atenolol, AZI: Azithromycin, BAC: Baclofen, CAF: Caffeine, CEP: Cephalexin, CIP: Ciprofloxacin DIC: Diclofenac, OFL: Ofloxacin, TRI: Trimethoprim, IBU: Ibuprofen, TET: Tetracycline, [1]: Metformin, Oxypurinol, Triclosan and Allopurinol compounds were not analysis due to their low recoveries from selected soils.