Removal of Pharmaceuticals from Wastewater through Constructed Wetland-based systems in Rural Canada

by

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THESIS ABSTRACT

The main goal of the dissertation was to assess the occurrence and removal of pharmaceuticals in constructed wetlands (CWs) treating wastewaters under Arctic and temperate climates. To this end, we examined the relevance of observations and strength of methods found in the literature for the removal of 48 drugs through a weight-of-evidence-based rubric. Statistically greater relevance scores were obtained for hybrid CWs compared to single-step CWs for the removal of some pharmaceuticals. Also, CWs from all climates showed potential for pharmaceutical removal. Finally, studies from temperate climate (N=76) were stronger in methods than Arctic- (N=2) or tropical-based (N=9) efforts. Three case studies are also part of this dissertation. First, we examined a treatment system in Cambridge Bay, Nunavut, Canada, where common pharmaceuticals such as atenolol, carbamazepine, clarithromycin, metoprolol, sulfamethoxazole and trimethoprim were detected with greatest concentrations at the treatment lagoon, and limited to no offshore detection. Second, the dissipation of atenolol, carbamazepine, and sulfamethoxazole was observed in mesocosm modelled wetlands using glass or gravel as substrates. Atenolol dissipated the fastest from the system, followed by sulfamethoxazole, and carbamazepine, with no significant differences across treatments. Also, tertiary pilot-scale subsurface filters made of crushed recycled glass or sand were studied in a treatment facility in the village of Dunnottar, Manitoba, Canada. These materials showed no statistically significant differences for pharmaceutical removals. Finally, we assessed a full-scale lagoon-subsurface filter in Dunnottar to remove pharmaceuticals in 2015 and 2016. Out of six detected pharmaceuticals, atenolol, clarithromycin, metoprolol, propranolol were efficiently removed by lagoon treatment, while carbamazepine and sulfamethoxazole persisted to a certain extent after subsurface filtration.

Taken together, the thesis has shown that the removal of pharmaceuticals from wastewater is achievable in CWs from various scales, types and under various climates. We recommend that future research addresses the removal of pharmaceuticals under Arctic climate, as the currently available evidence is limited in strength of methods. The use of hybrid CWs of various configurations also constitutes an exciting opportunity to better understand and improve the quality of current wastewater treatment performance in remote locations around the globe.

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I dedicate this thesis to the memory of my Mom: Vera Barquero-Alvarado, who passed away unexpectedly two years into my graduate program. Without her profound love and care, I would not be the person that I am today.

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LIST OF ABBREVIATIONS

A – wetland area
A_{active} – area of wetland containing water in active flow
ANOVA – analysis of variance
ARG – antibiotic resistance genes
CHARS – Canadian High Arctic Research Station
CCME – Canadian Coucil of Ministers of the Environment
CRG – crushed recycled glass
D_{ow} – octanol-water distribution coefficient
EC_{50} – effective concentration causing 50% of the population to exhibit the observed end-point
ESI – electrospray ionization
f_w – fraction of chemical dissolved
f_{oc}^{sus} – fraction of organic carbon within suspended solids
h – wetland water depth
HLB – hydrophilic-lipophilic balance
HLR – hydraulic loading rate
HRT – hydraulic retention time
HQ – Hazard quotients
K_d – solid-water partition coefficient
k_a^t – total rate of light absorption
k_D – direct photolysis rate constant
k_{est} – overall dissipation rate
$k_{I,\cdot OH}$ – second-order hydroxyl radical rate constant
K_{oc} – organic carbon partition coefficient
K_{ow} – octanol-water partition coefficient
k_s - sedimentation rate constant*
LC – liquid chromatography
LC_{50} – lethal concentration required to kill 50% of the population
LC-MS/MS – liquid chromatography tandem mass spectrometry
LOD – limit of detection
LOQ – limit of quantification
M – molecular weight
PAR – photosynthetically active radiation
pKa – acid dissociation constant
POCIS – Polar Organic Chemical Integrative Samplers
Q – water flow rate
ROO – Relevance of Observation
R_s – sampling rate
r_{sw} – solid to solution phase ratio
SD – standard deviation
SE – standard error
SOM – strength of methods

t - time
TWA- time-weighted average
v_s *– particle settling velocity*
$W(\lambda)$ *– spectral photon fluence rate*
z_{mix} *– average depth of mesocosms*
$\alpha(\lambda)$ *– attenuation coefficient of the medium*
ε *– porosity*
$\varepsilon(\lambda)$ *– molar extinction coefficient*
Φ *– quantum yield*
$\Phi_{\lambda,direct}$ *– direct photolysis quantum yield*
$[\cdot OH]_{ss}$ – steady-state concentration of hydroxyl radicals

CONTRIBUTIONS OF AUTHORS

Chapter 1 is an introductory chapter written on its entirety by Luis Gerardo Chaves Barquero. Charles Wong and Mark Hanson reviewed the chapter and provided insights for its betterment.

Chapter 2 is currently unpublished work. Luis Gerardo Chaves Barquero wrote the manuscript, developed the customized rubric and performed the weight-of-evidence analysis. Charles Wong and Mark Hanson reviewed the manuscript and provided insights for its betterment.

Chapter 3 was previousy published, and it has been re-printed for this dissertation with permission from the copyright holder (Elsevier B.V):

> Chaves-Barquero, L.G.; Luong, K.H.; Mundy, C.J.; Knapp, C.W.; Hanson, M.L.; Wong, C.S. The release of wastewater contaminants in the Arctic: A case study from Cambridge Bay, Nunavut, Canada. *Environmental Pollution* **2016**; *218*: 542-550.
>
> DOI: 10.1016/j.envpol.2016.07.036

Luis Gerardo Chaves Barquero performed chemical analyses and wrote the manuscript. Kim H. Luong provided logistics support and performed chemical analyses. C.J. Mundy provided insights and experience for experimental design and sampling site selection; Charles W. Knapp conducted all genetic analyses at the University of Strathclyde in the United Kingdom; Charles Wong conducted the sampling, performed some chemical analyses and reviewed the manuscript. Mark Hanson reviewed the manuscript.

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case study of its potential to facilitate pharmaceutical removal. *Environmental Science and Pollution Research* **2021;** *28*: 62306-52318.

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Luis Gerardo Chaves Barquero wrote the manuscript, conducted sampling and all the chemical analyses. Braedon W. Humeniuk provided support for the experimental setup of the mesocosm-scale study, and conducted sampling. Kim Luong provided logistics support. Nazim Cicek provided insights on experimental design and sampling. Charles Wong and Mark Hanson provided advice on experimental design and reviewed the manuscript.

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Luis Gerardo Chaves Barquero wrote the manuscript, conducted sampling and chemical analyses. Kim Luong provided logistics support, conducted sampling and chemical analyses. Martina D. Rudy and Richard A. Frank conducted toxicity analyses. Charles Wong and Mark Hanson provided advice on experimental design and reviewed the manuscript.

Chapter 6 is a concluding chapter written on its entirety by Luis Gerardo Chaves Barquero. Charles Wong and Mark Hanson reviewed the chapter and provided insights for its betterment.

CHAPTER 1

1. PHARMACEUTICALS IN MUNICIPAL WASTEWATER AND THEIR REMOVAL BY CONSTRUCTED WETLANDS

1.1. Thesis Hypothesis and Objectives

The overall objective of this PhD dissertation was to assess the occurrence and removal of pharmaceuticals in constructed wetlands treating municipal wastewaters in rural and remote locations under various types of climate. Specific objectives and their proposed hypotheses are described as follows.

- Critically evaluate the available scientific evidence regarding pharmaceutical removal in engineered subsurface filters under Arctic, temperate and tropical climates. It was hypothesized that systems located in warmer climates are able to promote statistically better removal of pharmaceuticals in constructed wetland-based systems.
- Obtain concentration data for wastewater contaminants in Cambridge Bay, Nunavut to establish a baseline of the state of wastewater treatment practices in this Arctic community. It was hypothesized that the system in place is able to remove pharmaceutical analytes of interest down to levels where they do not pose a risk to the marine receiving environment.
- Assess the treatment efficiency of a full-scale engineered subsurface passive filter treating municipal wastewaters under prairie climatic conditions in Dunnottar, Manitoba, with a special interest in nutrients and pharmaceuticals. It was hypothesized that the full-scale system will perform more efficiently compared to a previously studied pilot-scale

system, and pharmaceutical analytes of interest will be attenuated down to exposure levels where they do not pose a risk for the receiving freshwater environment.

- Examine the potential of crushed recycled glass as a novel substrate material for removal of nutrients and pharmaceuticals from wastewater in a modelled mesocosm-scale system and a real-world pilot-scale subsurface filtration system. It was hypothesized that crushed recycled glass would remove pharmaceuticals with similar efficiency compared to traditional materials such as gravel and sand.

1.2. Introduction to this Dissertation

This dissertation focuses on the occurrence, fate, and removal of pharmaceuticals in municipal wastewaters from rural and remote communities in various types of climate, as well as the performance of low-cost materials as substrates for wastewater treatment applications in constructed wetland-based systems. This chapter presents context around the sources, history and environmental fate of pharmaceuticals, along with some theoretical background on the fundamentals of the design, components, and classification of constructed wetlands as wastewater treatment alternatives for pharmaceutical removal. In addition, some background is presented about the particular case studies addressed in this dissertation.

1.3. Pharmaceuticals in the Aquatic Environment

This section presents an overview on the history, sources, occurrence, environmental fate and ecotoxicology of most common pharmaceuticals, with an emphasis on the most important processes that govern pharmaceutical fate and degradation in the environment (i.e. photolysis, sorption, and biodegradation).

1.3.1. History

The modern pharmaceutical industry traces its origin to companies such as Merck, Eli Lilly, and Roche, that had previously been suppliers of products such as morphine, quinine and strychnine, and then moved into large-scale production of drugs in the middle of the 19th century. Newly established companies, such as Bayer, ICI, Pfizer, and Sandoz established research labs at the beginning of the 20th century and discovered medical applications for their products (Hester and Harrison, 2016). Growth of the industry was relatively limited, and at the start of the 1930s most medicines were still commercialized without a prescription. However, a number of important pharmacological advances were accomplished in the early- to mid- 20th century. For instance, a modified version of salicylic acid was demonstrated by Bayer scientists to show great efficacy in pain management, and the resultant product, aspirin, is still in widespread use today (Jeffreys, 2004). In the 1920s and 1930s, both penicillin and insulin were identified and manufactured at a modest scale. The Second World War brought a major boost to the developing industry, and increased demands from governments for research on treatments for a wide range of illnesses. Post-war, state-run healthcare systems were implemented in Europe, such as the UK's National Health Service (NHS), which created a more stable market both for the prescription of drugs and their reimbursement. From the 1950s to 1990s, major advances in drug development occurred, with the introduction of new antibiotics, new analgesics (e.g. acetaminophen, ibuprofen), and new classes of pharmaceuticals (e.g., oral contraceptives, beta-blockers, anti-cancer medicines). Prozac, the first selective serotonin re-uptake inhibitor was launched by Eli Lilly in 1987, and omeprazole, the first proton pump inhibitor, was introduced by Astra in 1989. Tagamet, an ulcer medication, earned its manufacturers, Glaxo Smith Kline, more than US$ 1 billion a year. Atorvastatin, marketed as Lipitor in 1996, became the world's best-selling drug of all time (Hester and Harrison, 2016). Increasing revenue from the

pharmaceutical industry, along setbacks such as the thalidomide scandal of 1961 (Sjöström and Nilsson, 1972), triggered demands for new regulations for efficacy, purity and safety of new drugs, leading to an important increase in the requirements and costs of research and development, particularly for clinical testing. More recently, developments in novel classes of medicines have built on a greater understanding of the human genome. In cancer, immunotherapies have been produced to strengthen the immune system against malignant tissues. This, along with other innovations, has led to drugs for rare diseases that were previously considered untreatable. There are drawbacks, though. The new generation of drugs are expensive and have pushed the limit of health care systems in terms of finance. Maintaining public trust and preventing deaths from antibiotic resistant bacterial strains are two major challenges to be overcome in the 21th century.

1.3.2. Sources and Occurrence

According to Health Canada, over 4,000 tablet or capsule pharmaceutical formulations are actively sold in Canada (Health Canada, 2018). Given the diversity of drugs available, and hence excreted daily, exposure measurements in field studies usually rely on suites of several analytes that can be tested across the wastewater treatment process (Hester and Harrison, 2016). Pharmaceutical analytes reported in the literature are commonly selected based on several criteria: their use patterns, ease of quantification and environmental relevance (Fatta-Kassinos et al., 2011).

Research on pharmaceuticals in the environment started in the 1970s, through an initial report funded by the United States Environmental Protection Agency, through which the concentration of several organic compounds in household effluents was determined (Garrison et al., 1976). Although the presence of pharmaceutical residues in surface waters had been

predicted by Richardson and Bowron in the mid-1980s (Richardson and Bowron, 1985), it took a decade until such residues began to be routinely measured, following the identification of clofibric acid in German rivers by Stan (Stan et al., 1994). Interest within the public and the international scientific community grew during mid to late 1990s. A landmark study was published in the early 2000s, detailing the results of a national reconnaissance study of U.S. surface waters (Kolpin et al., 2002). Eighty-two compounds were detected from a suite of ninety-five organic wastewater contaminants from a network of streams across thirty American states during 1999 and 2000. The compounds detected represented a wide range of residential, industrial, and agricultural origins.

Pharmaceuticals have been detected ubiquitously in surface and wastewaters, typically in the ng/L to µg/L range worldwide (Cizmas et al., 2015). Concentrations of pharmaceutical contaminants vary during their lifecycle, generally decreasing from wastewater treatment plant effluents to freshwater bodies. Global averages for commonly studied pharmaceuticals are in the ng/L range in typical surface waters, with maximum concentrations reaching µg/L levels in surface waters greatly impacted by wastewaters. Concentrations in wastewater treatment facilities are typically at least one order of magnitude greater than levels in receiving surface waters, though downstream concentrations depend on the extent of dilution occurring in receiving waters (Petrie et al., 2013). This occurrence is, for the most part, a consequence of their incomplete removal by wastewater treatment systems (Oulton et al., 2010; Uslu et al., 2013; Vidal-Dorsch et al., 2012). Concerns regarding pharmaceuticals as environmental contaminants are related to their bioactivity and continuous release into the aquatic environment (Brooks et al., 2009). Moreover, the diversity in chemical structure and function across many pharmaceutical

classes makes the study of these contaminants complex and multifaceted (Brooks et al., 2009; Daughton and Ternes, 1999).

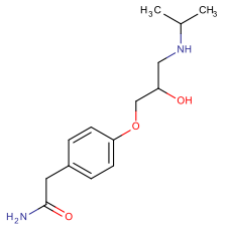
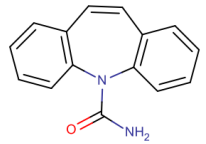
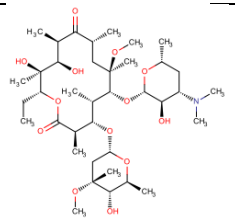
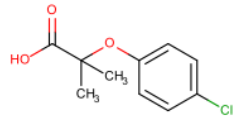
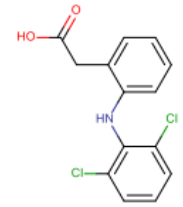
Considering the diversity of pharmaceutical analytes commonly found in the aquatic environment, this dissertation focused on the ones shown in Table 1.1. Several classes of pharmaceuticals are represented in this thesis: heart medicines (e.g., atenolol, metoprolol), anticonvulsant drugs (e.g., carbamazepine), antibiotics (e.g., clarithromycin, sulfamethoxazole), and others. Low concentrations (ng/L to µg/L) of pharmaceuticals in surface waters tend to pose little acute risk to aquatic organisms, but could exist the potential for chronic toxicity in non-target organisms and human receptors downstream of the effluent release, based on persistence and/or continuous release (Li et al., 2015; Zhu et al., 2013). For example, naproxen, carbamazepine and atenolol have been found to persist from a few days to several weeks into the aquatic environment (Brun et al., 2006; Carlson et al., 2013; Fent et al., 2006). Therefore, it is important to study the fate and effects of such contaminants to predict outcomes of chemical transport and partitioning in the environment, and to understand the potentially toxic effects on non-target organisms.

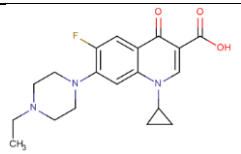
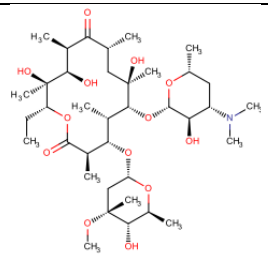
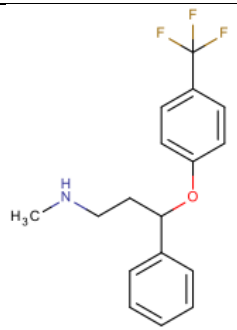
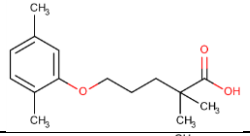
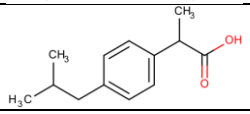
1.3.3. Environmental Fate

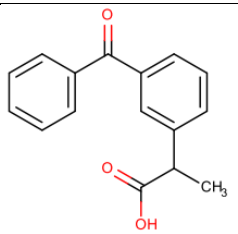
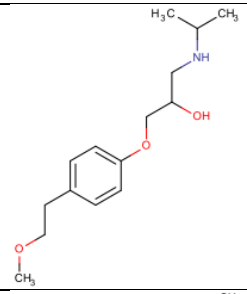
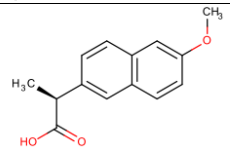
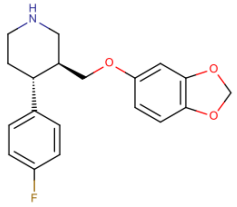
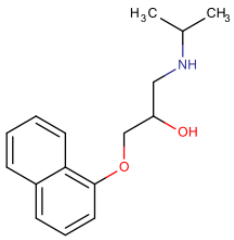
Various mathematical models and frameworks have been created to predict the environmental fate of organic contaminants, based on their physicochemical properties. The diversity of structures observed across pharmaceutical potentially classes dictate complex fate processes responsible for the behaviour of these contaminants in the environment. Particularly important are the properties dictating transfer of a chemical between phases (air-water and particle-water partitioning) and transformation of chemicals, both biotic (metabolism, conjugation, and microbial degradation) and abiotic (oxidation, reduction, hydrolysis and

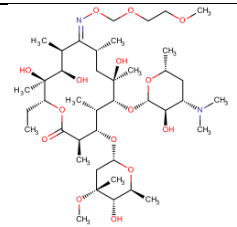
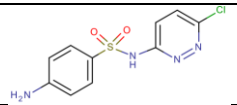
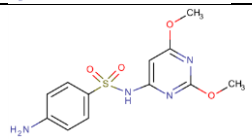
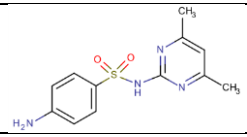
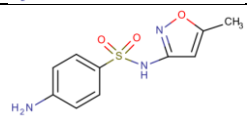
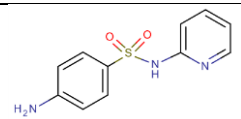
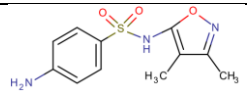
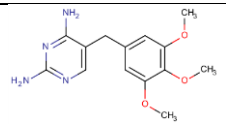
photolysis) (Fent et al., 2006; Heberer, 2002). Photolysis, sorption, and biotransformation represent the three most important mechanisms controlling the fate of pharmaceuticals in aquatic systems (Challis et al., 2014; Fent et al., 2006; Fono et al., 2006; Kummerer, 2009; Le-Minh et al., 2010; Löffler et al., 2005; Monteiro and Boxall, 2010; Petrie et al., 2015; Tixier et al., 2003; Tolls, 2001) Given the low rates of hydrolysis and vapour pressures for many pharmaceuticals, hydrolysis and volatilization typically play a minor role on the transport and transformation of pharmaceuticals in the environment (Tixier et al., 2003).

Table 1.1. Physical-chemical properties for the most important pharmaceuticals examined by this thesis.

Name	Formula	Molar mass (g/mol)	log K_{ow}	pK_a	Structure	Water solubility (mg/L)	Use
ATENOLOL	$C_{14}H_{22}N_2O_3$	266.3	0.16[b]	9.6[b]		13 330 at 25°C[i]	β-blocker
CARBAMAZEPINE	$C_{15}H_{12}N_2O$	236.3	2.45[a]	13.9[a]		17.7 at 25°C[i]	Anticonvulsant
CLARITHROMYCIN	$C_{38}H_{69}NO_{13}$	748.0	3.1[c]	9.0[c]		1.693 at 25°C[i]	Macrolide antibiotic
CLOFIBRIC ACID	$C_{10}H_{11}ClO_3$	214.6	2.6[h]	3.2[h]		583[j]	Lipid-regulator
DICLOFENAC	$C_{14}H_{11}Cl_2NO_2$	296.1	3.7[a]	4.2[a]		21.0[i]	Analgesic

Name	Formula	Molar mass (g/mol)	log K_{ow}	pK_a	Structure	Water solubility (mg/L)	Use
ENROFLOXACIN	$C_{19}H_{22}FN_3O_3$	359.4	0.28[g]	6.1[g]		53.9[i]	Antibiotic
ERYTHROMYCIN	$C_{37}H_{67}NO_{13}$	733.9	3.1[a]	8.8[a]		0.52 at 25°C[j]	Antibiotic
FLUOXETINE	$C_{17}H_{18}F_3NO$	309.3	3.8[a]	10.1[h]		17.0 at 25°C[i]	Anti-depressant
GEMFIBROZIL	$C_{15}H_{22}O_3$	250.3	4.8[a]	4.7[a]		11.0 at 25°C[i]	Lipid-regulator
IBUPROFEN	$C_{13}H_{18}O_2$	206.3	4.0[a]	4.9[a]		21.0 at 25°C[i]	Anti-inflammatory

Name	Formula	Molar mass (g/mol)	log K_{ow}	pK_a	Structure	Water solubility (mg/L)	Use
KETOPROFEN	$C_{16}H_{14}O_3$	254.3	3.1[b]	4.5[b]		51.0 at 22°C[i]	Anti-inflammatory
METOPROLOL	$C_{15}H_{25}NO_3$	267.4	1.7[b]	9.7[b]		16 900 at 25°C[i]	β-blocker
NAPROXEN	$C_{14}H_{14}O_3$	230.3	3.2[a]	4.2[a]		15.9[i]	Analgesic
PAROXETINE	$C_{19}H_{20}FNO_3$	329.4	4.0[g]	10.3[h]		1.1 at 25°C[i]	Anti-depressive
PROPRANOLOL	$C_{16}H_{21}NO_2$	259.3	3.5[g]	9.4[g]		61.7 at 25°C[i]	β-blocker

Name	Formula	Molar mass (g/mol)	log K_{ow}	pK_a	Structure	Water solubility (mg/L)	Use
ROXITHROMYCIN	$C_{41}H_{76}N_2O_{15}$	837.0	2.8[c]	9.2[c]		0.02 at 25°C[i]	Macrolide antibiotic
SULFACHLORPYRIDAZINE	$C_{10}H_9ClN_4O_2S$	284.7	0.89[h]	2.0, 5.9[f]		133 at 25°C[k]	Sulfonamide antibiotic
SULFADIMETHOXINE	$C_{12}H_{14}N_4O_4S$	310.3	1.5[c]	1.9, 5.9[c]		343 at 25°C[k]	Sulfonamide antibiotic
SULFAMETHAZINE	$C_{12}H_{14}N_4O_2S$	278.3	0.80[c]	2.3, 7.4		1500 at 29°C[k]	Sulfonamide antibiotic
SULFAMETHOXAZOLE	$C_{10}H_{11}N_3O_3S$	253.3	0.89[a]	2.1, 5.7[a]		610 at 37°C[i]	Sulfonamide antibiotic
SULFAPYRIDINE	$C_{11}H_{11}N_3O_2S$	249.3	0.35[h]	2.2, 8.6[d]		268 at 25°C[i]	Sulfonamide antibiotic
SULFISOXAZOLE	$C_{11}H_{13}N_3O_3S$	267.3	0.05[c]	1.5, 5.0[e]		300 at 37°C[i]	Sulfonamide antibiotic
TRIMETHOPRIM	$C_{14}H_{18}N_4O_3$	290.3	0.91[a]	4.0, 7.1[a]		400 at 25°C[i]	Antibiotic

References: [a] Westerhoff et al., 2005; [b] Vieno et al., 2007; [c] Le-Minh et al., 2010: [d] Challis et al., 2013; [e] Boreen et al., 2004; [f] Boreen et al., 2005; [g] Monteiro and Boxall, 2010; [h] Macleod et al., 2007; [i] PubChem database at URL: https://pubchem.ncbi.nlm.nih.gov/ (April 2019); [j] Estimations from EPI WEB version 4.1.; [k] Drugbank database at URL: https://www.drugbank.ca/drugs/DB01582 (April 2020).

1.3.3.1. Photolysis

Molecules that contain aromatic rings and conjugated-π systems, functional groups, and heteroatoms can experience appreciable absorbance in the UV-C wavelength range, often with tailing absorbance past 290 nm, and in some cases, into the UV-A range (>315 nm). Photodegradation is expected to play a key role on pharmaceutical degradation on sunlit waters (Boreen et al., 2003). In fact, some of these contaminants undergo direct photolysis when exposed to natural sunlight (Challis et al., 2014).

Many pharmaceuticals with minimal absorbance overlap with natural sunlight (>290 nm) may still undergo significant photodegradation via indirect photolysis. Through interaction with other light-absorbing species known as photosensitizers (e.g., organic matter, carbonate, nitrate, iron), transfer of energy from photo-excited species to the pharmaceutical can cause a chemical transformation (Leifer, 1988). These indirect photolysis mechanisms are inherently more complex as chemicals can interact through multiple pathways with photo-generated transient species, including triplet excited dissolved organic matter (^{3}DOM), singlet oxygen ($^1O_2$), hydroxyl radicals (·OH), and others (Boreen et al., 2003; Bodhipaksha et al., 2015; Challis et al., 2014; Lam and Mabury, 2005; Lam et al., 2003; Lam et al., 2004; Lam et al., 2005; McNeill and Canonica, 2016). Additionally, DOM can act through competing mechanisms, including scavenging of reactive oxygen species and light screening (Guerard et al., 2009; Lu et al., 2015; Miller and Chin, 2005), further complicating the characterization of photolytic mechanisms in natural waters.

The extent of direct photolysis for a given compound is generally reported as a pseudo first-order direct photolysis rate constant (k_D). Similarly, a direct photolysis quantum yield ($\Phi_{\lambda,direct}$) which describes the efficiency in which a chemical reacts or

transforms upon absorption of photons over a specific wavelength range (Leifer, 1988), can be determined using chemical actinometry (Dulin and Mill, 1982; Laszakovits et al., 2017). Photolysis rate constants are directly dependent upon the incident light intensity at the time of measurement, and thus have limited predictive utility under varying light conditions. The $\Phi_{\lambda,direct}$ is a powerful characteristic property of a chemical that allows k_D to be predicted, in principle, under any light conditions (Challis et al., 2014; Leifer, 1988).

The pharmaceuticals studied in this thesis (Table 1.1) undergo direct or indirect photolysis to some extent and for many of them, photolysis represents the major attenuation mechanism in natural surface waters. For example, sulfonamide antibiotics as a class have been studied extensively in the photochemistry literature (Challis et al., 2014). Despite the structural similarities of the sulfonamide class, k_D and $\Phi_{\lambda,direct}$ vary significantly from compound to compound, and kinetics can be highly dependent on solution pH (Boreen et al., 2004; Challis et al., 2013). Generally, sulfonamides can be characterized as photo-labile, undergoing rapid photo-degradation with half-lives of <1 day under natural sunlight (Boreen et al., 2004; Challis et al., 2013). In contrast, direct photo-degradation of carbamazepine is limited, with indirect photolysis mechanisms typically varying with type and concentration of water constituents (e.g. DOM) (Jasper and Sedlak, 2013). Reported half-lives in the literature are diverse, and range from 10 days in an outdoor mesocosm experiment (Cardinal et al., 2014; Lam et al., 2004) to 100 days in summer and 450 days in fall at 50°N latitude, calculated based on a measured $\Phi_{\lambda,direct}$. (Andreozzi et al., 2003).

As previously described, photolysis has been reported in the literature to be an important mechanism to explain fate and persistence of various pharmaceuticals in the environment. Nevertheless, thus far there is no reliable rule of thumb for predicting the photodegradation behaviour of pharmaceuticals in general, as its relative importance can

vary from study to study, depending on the design of the experiment, nature of the matrix, and the weather conditions, amongst other factors (Verlicchi and Zambello, 2014). That said, both direct and indirect photodegradation processes are key to analyze and discuss the results from the field studies presented in Chapters 3, 4, and 5 of this dissertation.

1.3.3.2. Sorption

Many drug classes are weak acids, bases or zwitterions. Traditional approaches describing organic contaminant partitioning to soils and sediments involve hydrophobic type interactions (Karickhoff et al., 1979; Schwarzenbach et al., 2017) and have often proven inadequate for pharmaceuticals (Brooks et al., 2009; Sassman and Lee, 2005; Tolls, 2001). As a result, hydrophobic-driven interactions can be less important for pharmaceuticals and their models tend to underestimate sorption (Ternes et al., 2004), when not also taking into account other binding processes, such as ion exchange, ion bridging, surface complexation and hydrogen-bonding (Sassman and Lee, 2005; Tolls, 2001).

Once in receiving waters, sorption of pharmaceuticals tends to become a less prevalent fate pathway since concentrations of suspended particles in most surface waters is far less than those in wastewater influent (Zhou and Broodbank, 2014). Additionally, large water-particle rations have the potential to shift equilibrium in chemical-particle interactions, thereby releasing pharmaceuticals back to the dissolved aqueous phase (Hajj-Mohamad et al., 2017). The extent of these sorption processes for individual pharmaceuticals will vary from system to system as specific sedimentation parameters like particle concentrations in the water column, particle composition (e.g., fraction organic carbon), and settling velocity can have significant impacts on the extent of sorption (Tixier et al., 2003).

Suspended particles from wastewater are retained in a wetland bed in which sorption of dissolved contaminants in organic matter and on biofilm coating the grains in the bed can be significant removal mechanisms (Matamoros et al., 2005). Adsorption of a compound onto a solid matrix, for instance the substrate of a CW or the sediment of a freshwater body, depends on its chemical nature and is correlated to K_{OW} and K_d, pH, ionic strength, the presence of cations, and the presence of other sorbable compounds (Verlicchi and Zambello, 2014). For instance, the overall sorption of ciprofloxacin could be influenced by a difference in soil pH (Vasudevan et al., 2009). The pH of soil influenced the sorption process of sulfamethazine due to its different ionization states under different pH conditions (Lertpaitoonpan et al., 2009). Similar trend was also found for sulfonamides to the anionic form of sulfonamides above pH 7.5 led to a lower sorption to soils (Kurwadkar et al., 2007). Competitive sorption phenomena can take place if multiple pharmaceutical compounds and other wastewater pollutants are present in the water at the same time (Conkle et al., 2010). The sorption capacity of one pharmaceutical may be decreased by another pharmaceutical and/or other contaminants due to competition to preferred binding sites on the solid matrices in CWs. Therefore, this competition effect may be important in CWs which are loaded with a wide range of pharmaceuticals.

Sorption is one of the most important mechanisms to explain pharmaceutical fate and removal in CWs. Although traditional substrates, such as sand or gravel, tend to predominate in CW design, the search for underexploited, novel materials could also be of interest for CW applications, especially if these materials can improve CW operation and maintenance. Important knowledge gaps persist, such as the study of competitive sorption, and warrant the need for future experiments in both laboratory and field settings.

1.3.3.3. Biodegradation

Bacteria and fungi are the two classes of organisms most relevant to biodegradation of pharmaceuticals, with the former typically playing the most significant role in aquatic systems (Caracciolo et al., 2015; Ghattas et al., 2017; Greskowiak et al., 2017; Kummerer, 2009). Biodegradation plays a very important role in the attenuation of pharmaceuticals in wastewater treatment plants (WWTPs) (Yamamoto et al., 2009; Yang et al., 2011) and while microbial activity is typically reduced significantly in natural surface waters due to lower bacterial density and diversity compared to undiluted wastewaters (Kummerer, 2009), it remains an important natural attenuation mechanism (Fono et al., 2006). Yamamoto et al. (2009) reported biodegradation half-lives in laboratory activated sludge experiments ranging from 5 days for propranolol to 125 days for carbamazepine, corresponding to relative removal rates of 60% and <0.1%, respectively. Conversely, Fono et al. (2006) studied the attenuation rates of pharmaceuticals in an effluent-dominated river and found that biotransformation was the major removal mechanism for most compounds, even surpassing photolysis, with half-lives ranging from 17 days for naproxen and ibuprofen to 53 days for metoprolol in microcosm incubations with river water (Fono et al., 2006).

Biofilms can also play relevant roles in the degradation and fate of pharmaceuticals in WWTPs (Casas et al., 2015) and receiving waters (Huerta et al., 2016; Writer et al., 2011). In fact, designed biofilm reactors have proven effective techniques for removing pharmaceuticals from wastewaters (Casas et al., 2015). Additionally, in-stream biofilms, generally forming layers on streambed sediments in wastewater influenced systems can be important compartments for accumulation of pharmaceuticals (Huerta et al., 2016) and even degradation of certain classes of compounds (e.g., endocrine-disrupting chemicals) (Writer et al., 2011).

While the available evidence suggests that microbial degradation is an important mechanism for pharmaceuticals (Caracciolo et al., 2015), biodegradation rates can be highly dependent upon the specific microbial and environmental conditions of an individual experiment or field site, varying as much as three orders of magnitude (Greskowiak et al., 2017), making compound-specific generalization, comparisons and applications across different systems challenging.

1.3.4. Ecotoxicology

The issues associated with the bioactivity of pharmaceuticals and their continuous release into the environment lead to a desire to better understand their occurrence and fate in surface waters around the world. More than 350 pharmaceutical molecules, excluding metabolites and transformation products, have been investigated in aquatic water bodies and terrestrial environments. These pharmaceuticals belong to several therapeutic groups but some of them attract more attention: 25% of the literature focuses on antibiotics, 21% on psychiatric drugs, 13% on analgesics/anti-inflammatories, 4% on beta-blockers and 3% on anti-hypertensives or lipid regulators (Hester and Harrison, 2016) . Sources and pathways of these compounds have been characterized: metabolic excretion from patients (wastewater and hospital effluents, septic tanks), industrial (industrial wastewater and waste) and livestock activities (waste lagoons, manure application to soil), as well as indirectly by improper disposal, untreated sewage, etc. (Kummerer, 2010). Effects of common pharmaceuticals on environmental receptors have also been investigated. For instance, effects of exposure of fish to contaminants with estrogenic activity (e.g. 17-α-ethynylestradiol) were examined in the Experimental Lakes Area (ELA) of northwestern Ontario, observing significant physiological effects (Palace et al., 2002).

Pharmaceuticals are biologically active compounds designed to elicit a biological response in the target organism (human or animal) at therapeutic doses. As such, concerns regarding interactions with non-target organisms has let to significant research to characterize the ecotoxicology of these contaminants (Santos et al., 2010). Most pharmaceuticals elicit effects via specific modes of action, and many physiological pathways are conserved across taxa, thus standard toxicity test methods are often used for measurement of certain endpoints (Boxall et al., 2012). Pharmaceutical concentrations in surface waters can be orders of magnitude below therapeutic doses and typically at least one order of magnitude below concentrations at which adverse effects have been reported in non-target organisms (Arnold et al., 2014; Corcoran et al., 2010; Santos et al., 2010). It is important to note that a common practice when estimating these environmental risks with added conservatism is using a safety factor of 1000. Though, there are some cases where low concentrations of contaminants can affect physiological aspects of aquatic species. For instance, a widely studied physiological response in exposed organisms is vitellogenin induction, an egg-yolk protein essential for reproduction in females. Feminization of male fish due to vitellogenin induction has been observed upon exposure to low and environmentally relevant concentrations (1-5 ng/L) of the potent birth control hormone 17α-ethinylestradiol (Caldwell et al., 2012; Kidd et al., 2007). The antidepressant fluoxetine and other lipophilic pharmaceuticals, including diphenhydramine, sertraline, and carbamazepine, have been measured in fish tissues from effluent-dominated streams (Brooks et al., 2005; Du et al., 2014), There is also evidence suggesting that these pharmaceuticals can bioconcentrate and bioaccumulate in aquatic food chains (Du et al., 2014) and lead to behavioural effects, for example modified aggression and reproductive behaviour in predatory fish (Brodin et al., 2014).

While effects for a wide range of organisms exposed to individual pharmaceuticals have been relatively well-studied (Arnold et al., 2014), a number of challenges and gaps regarding their ecotoxicology remain (Boxall et al., 2012). For example, because pharmaceuticals are always being released from WWTP as complex mixtures containing multiple bioactive compounds, the question of chronic mixture toxicity remains an area of significant interest (Backhaus, 2014; Boxall et al., 2012). Another area of pressing concern to researchers relates to the ubiquitous nature of antibiotics in the environment, which pose a very different, but potentially serious ecotoxicological challenge compared to other classes of pharmaceuticals. Namely, the selection and dissemination of anti microbial-resistant microorganisms (Boxall et al., 2012; Kummerer, 2009; Pepper et al., 2018). Although the presence of antibiotic resistance genes (ARGs) in most environments with wastewater influences is well-established (Anderson et al., 2013; Anderson et al., 2015; Huijbers et al., 2015; Pei et al., 2006), their relative importance as a vector and route of exposure to the human population remains to be seen (Huijbers et al., 2015). In response to this question, ARGs were characterized in different wastewater systems as part of the current thesis. Measurement of ARGs in a wastewater-influenced marine system is reported in Chapter 3, and in a rural temperate wastewater treatment facility in Chapter 5.

1.4. Constructed Wetlands for Wastewater Treatment

The present section will summarize the fundamental concepts on municipal wastewater treatment, constructed wetlands (CWs) as an alternative to conventional wastewater treatment, the types of CWs that exist, and the importance of CW components for contaminant removal efficiency. Finally, some advances in pharmaceutical removal through CW systems are discussed.

1.4.1. Municipal Wastewater and Its Treatment

Municipal wastewater is comprised of domestic sanitary sewage produced by households and businesses, and can also include storm runoff and infiltration water (Hopcroft, 2014). Municipal wastewater is discharged to publicly owned treatment works, and this differentiates it from other types of wastewater, such as the wastewater generated by agricultural or industrial activities, which is usually treated in a private treatment facility owned by the business.

Municipal wastewater typically consists of approximately 99.9% water and 0.1% solids, by weight (Di Bonito, 2008). The solid fraction contains both inorganic and organic compounds, which occur in suspended, colloidal, or dissolved physical states (Di Bonito, 2008). Organic substances typically constitute approximately 50% of the solids in domestic wastewaters; proteins, carbohydrates, and fats are the most abundant (Wentzel et al., 2003). Inorganic components comprise grit, silt, chlorides, and mineral and metallic salts. Nitrogen (N) and phosphorus (P) exist as both organic and inorganic compounds in municipal wastewater, with ammonia (NH_3-N) and phosphates being the most common forms (Wentzel et al., 2003). These wastewater effluents can also introduce pathogens, organic contaminants (e.g. pesticides, pharmaceuticals and personal care products) and metals into the environment, which could pose risks of acute or chronic effects in aquatic species (Fent et al., 2006; Kolpin et al., 2002).

Conventional municipal wastewater treatment is accomplished by physical, chemical, and biological processes. Many of these processes can function within a range of treatment schemes. First, pre-treatment with conventional processes is usually advisable before discharge into a wetland to prevent a solids or oxygen demand overload. Secondly, a wetland treatment technology may not be the most cost-effective or technically reliable for

a given location. Knowledge of conventional wastewater treatment methods as well as wetland-based technologies is essential to make a sound evaluation of the most appropriate technology or combination of technology. Primary treatment is considered the first line of defense in conventional wastewater treatment (Water Environmental Federation, 1998) as it sets the stage for the majority of biological treatment technologies that follow. Primary treatment consists of screening, grit removal and primary sedimentation (Kadlec et al., 2009). Secondary treatment generally consists of the removal of additional wastewater solids and dissolved organic matter through microbial uptake and growth. It is essentially a biological process in which bacteria and fungi are encouraged to grow. The principal secondary treatment technologies are facultative ponds, aerated lagoons, aeration basins with solids recycling (activated sludge), trickling filters and rotating biological contactors (Kadlec et al., 2009). Reductions of biochemical oxygen demand, total suspended solids, nitrogen, and phosphorus beyond those typically accomplished by secondary treatment are called tertiary or advanced treatment. Three forms of advanced wastewater treatment are nitrification, denitrification and phosphorus removal (Kadlec et al., 2009). Other methods include UV disinfection, and ozonation of the wastewaters. Disinfection for control of pathogenic microorganisms and viruses is the most common type of tertiary treatment in North America (U.S. National Research Council, 1996).

Conventional wastewater systems were designed to reduce regulatory parameters (e.g., nutrients, solids, microorganisms) below legal maximum levels. These systems were not built for the removal of organic contaminants, such as pesticides, personal care products, or pharmaceuticals. Hence, conventional wastewater treatment systems present variable performances for the removal of pharmaceuticals, ranging from no removal at all,

to complete removal depending on the nature of the analyte and the particular conditions of the treatment system.

A myriad of interactions can occur within treatment systems between organic contaminants and the various treatment components. For instance, pharmaceuticals with large solid-water partition coefficients (K_d) tend to sorb to solid suspended particles or activated sludge during primary or secondary wastewater treatment stages, ending up in the sewage sludge during settling (Le-Minh et al., 2010). For example, tetracycline and quinolone antibiotics and anti-depressants like fluoxetine have been found at elevated levels in suspended particulate matter and sludge of WWTP influents (Petrie et al., 2015), with sludge K_d values as large as 8000 L/kg (tetracycline) (Le-Minh et al., 2010). These compounds can have implications down the line where wastewater irrigation and/or biosolid amendments practices are used for agriculture (Prosser and Sibley, 2015), as these compounds have the potential to be re-mobilized through runoff from agricultural fields (Drillia et al., 2005; Sassman and Lee, 2005; Tolls, 2001). Additionally, veterinary pharmaceuticals are common as growth-promoters in livestock, and thus can enter agricultural soils more directly via excretion (Prosser and Sibley, 2015; Sarmah et al., 2006; Tolls, 2001).

1.4.2. Constructed Wetlands (CWs)

1.4.2.1. General aspects

Wetlands are land areas that are wet during part or all of the year because of their location in the landscape. Historically, wetlands were called swamps, marshes, bogs, fens or sloughs depending on existing plant and water conditions, and on geographic settings (Kadlec et al., 2009). Constructed wetlands (CWs) are engineered systems, designed and constructed to utilize the natural functions of wetland vegetation, soils and microbial

populations to treat wastewater pollutants. These wetlands consist of impermeable basins which use engineered structures to control flow direction, water retention time and water level (US EPA, 2000). Treatment wetlands can be constructed in a variety of hydrological modes, which means that wastewater can be pumped to flow either horizontally or vertically through the filter bed (Kadlec et al., 2009). CWs can be cost-effective treatment alternatives for the removal of contaminants from untreated and treated wastewater effluents (Conkle et al., 2008; Matamoros et al., 2007). There are various types of CWs that have been used in facilities around the globe. These systems differ amongst each other based on their configuration and wastewater flow patterns. A description in detail of these systems is available in section 1.4.2.2.

Effluents that have undergone primary or secondary treatment may be further treated in CWs. They have been used as secondary or tertiary treatment systems for polishing municipal wastewater around the world, making use of aerobic conditions, extended hydraulic residence times, and aquatic plants (Hijosa-Valsero et al., 2010a) to promote degradation of nutrients, pharmaceuticals and related contaminants. To date, most municipal wetlands have been restricted to small communities and pre-treatment systems. Common applications include: 1) secondary treatment for small communities (up to 2000 inhabitants); 2) add-ons to aging or overloaded conventional secondary plants, where the wetland serves as a buffer to complete the treatment; 3) add-ons to lagoons, on which the solids trapping properties of wetlands can provide further nutrient removal; and 4) tertiary and higher treatment of compliant secondary discharges (Kadlec et al., 2009).

Application and efficacy of these technologies in Manitoba (Anderson et al., 2015; Carlson et al., 2013), and elsewhere (Verlicchi and Zambello, 2014) have also been reported in the literature where either CWs or other engineered systems such as subsurface

filters have been used to efficiently treat municipal wastewaters with the aim of meeting regulatory requirements. In Manitoba, provincial guidelines have been established maximum limits for effluent parameters such as total phosphorus (1 mg/L) and ammonia (25 mg/L) (Government of Manitoba, 2011), while no guidelines are still available for emerging contaminants, such as pharmaceuticals and personal care products. As regulatory requirements are expected to continue changing, a more advanced level of treatment could be supported by the use of CWs, particularly in smaller communities (Kadlec et al., 2009).

1.4.2.2. Types of CWs

CWs can be classified, based on their design and flow patterns, in four types: surface free water CWs (SF-CWs), horizontal subsurface flow CWs (HSSF-CWs), vertical subsurface flow CWs (VSSF-CWs) and hybrid CWs (H-CWs) (Li et al., 2014).

The SF-CWs are composed of shallow channels or basins planted with vegetation, including rooted and floating plants, in which wastewater flows at relatively shallow depth over an impermeable bottom liner or a packed substrate layer. They have areas of open water and are similar in appearance to natural marshes (Kadlec et al., 2009). SF-CWs contain areas of open water, floating vegetation, and emergent plants, either by design or as an unavoidable consequence of design configuration. Depending upon local regulations and soil conditions, berms, dikes, and liners can be used to control flow and infiltration. As the wastewater flows through the wetland, it is treated by the processes of sedimentation, filtration, oxidation, reduction, adsorption and precipitation (Kadlec et al., 2009). SF-CWs closely mimic natural wetlands, attracting a wide variety of wildlife including insects, mollusks, fish, amphibians, reptiles, birds, and mammals (Kadlec and Knight, 1996). SF-CWs are suitable in most climates, including polar environments. However, ice formation can hydraulically rule out winter operation, and the rates of some removal processes are

slower for cold water temperatures. Treatment marshes are not inexpensive, but installation costs to be cost-competitive with alternative technologies, and the land operating costs are lower compared to alternative technologies (Kadlec et al., 2009).

In HSSF-CW systems, wastewater is pumped into the wetland at the inlet zone and flows horizontally through the substrate under the surface of the wetland bed, which can be planted with vegetation. After treatment, the effluent is collected at an outlet zone. Typically, these systems employ a gravel bed (Kadlec et al., 2009). HSSF-CWs consist of gravel or soil beds planted with wetland vegetation. They are typically designed to treat primary effluent prior to either soil dispersal or surface water discharge. The wastewater is intended to stay beneath the surface of the media and flows in and around the roots and rhizomes of the plants. Because the water is not exposed during the treatment process, the risk associated with human or wildlife exposure to pathogenic organisms is minimized. HSSF-CWs are generally more expensive that SF-CWs, and are commonly used for secondary treatment in small communities (Cooper et al., 1996). In general, HSSF have been utilized at lower flow rates than SF-CWs, because of cost and space considerations. HSSF-CWs are typically comprised of inlet piping, a clay or synthetic liner, filter media, emergent vegetation, berms, and outlet piping with water level control. These systems are capable of operating under colder conditions than SF-CWs because of the ability to insulate the top, though a key operational consideration is the propensity of clogging of the media.

In VSSF-CW systems, wastewater is applied onto the surface of the wetland bed and then flows vertically from the planted layer down through the substrate until it reaches the outlet zone. Water is treated as it percolates through the plant root zone (Kadlec et al., 2009).VSSF-CWs have been used in North America as vegetated recirculating gravel filters (Lemon et al., 1996). These systems have been used to minimize oxygen transfer (Kassenga

et al., 2004), while fill-and-drain systems have been implemented to treat water to oxidize ammonia (Austin and Lohan, 2005). The ability of VSSF-CWs to oxidize ammonia has resulted in their use in applications with higher ammonia than municipal or domestic wastewater, including food processing wastewaters (Burgoon et al., 1999).

Different types of CW may be combined in order to achieve higher removal efficiency. Hybrid CW systems are a combination of two or more types of CWs or the joint configuration of wetlands along with pond systems, such as facultative lagoons, to perform wastewater treatment in series or in parallel (Li et al., 2014). Most commonly used hybrid systems comprise a vertical flow stage followed by horizontal subsurface wetland cells. Hybrid systems are receiving attention in most European countries because of more stringent requirements for ammonia removal (Kadlec et al., 2009).

Overall, the selection of a particular type of CW depends on the nature of the contaminants to be removed, as well as land availability and the on-site technical capacities. As established previously, CWs were not designed for the removal of pharmaceuticals, though the study of every type of CW has advanced what is known in terms of the potential of each type to attenuate them.

1.4.2.3. Hydrology of CWs

The ability to control water depth is crucial for wetland operation. It is necessary to maintain the hydraulic regime within the needs of desired plant species and to avoid unintended operational consequences. Therefore, it is necessary to understand the basic hydraulic factors that relate depth and flow rate, including vegetation density and aspect ratio. Some other relevant issues have to do with the bed media size, hydraulic conductivity, and filter clogging (Kadlec et al., 2009).

Hydraulic loading rate (HLR) is defined as the rainfall equivalent of the considered flow. It does not imply uniform physical distribution of water over the wetland surface. In SF-CWs the wetted are is typically known with good accuracy because of confining features. The defining equation is:

$$HLR = \frac{Q}{A} \qquad \text{Equation 1.1}$$

where HLR = hydraulic loading rate, m/d; A = wetland area (wetted land area), m^2; Q = water flow rate, m^3/d.

The surface areas of CWs examined in the literature range from microcosm-scale (less than 0.5 m^2), mesocosm-scale (0.5 to 5 m^2), and pilot-scale (5 to 100 m^2) to full-scale systems (more than 100 m^2). Mesocosm-scale facilities have been studied the most, likely due to their flexibility for manipulations of the substrate, flow patterns, types of plants and convenience in controlling the concentrations of test substances (Li et al., 2014).

Hydraulic retention time (HRT), is defined as the wetland water volume involved in flow divided by the volumetric water flow:

$$HRT = \frac{\varepsilon h A_{active}}{Q} \qquad \text{Equation 1.2}$$

where Q = flow rate, m^3/d; A_{active} = area of wetland containing water in active flow, m^2; h = wetland water depth, m; ε = porosity (fraction of volume occupied by water); HRT = hydraulic retention time, d.

Although there are plenty of other hydraulic parameters that can be measured and studied in CWs, these are the most basic and also the most relevant for the scope of this dissertation work, especially for the field-based studies conducted in real-world wastewater treatment facilities (Chapters 3 to 5).

1.4.3. Removal of Pharmaceuticals in CWs

A large number of published research studies summarize the existing knowledge on the performance of different types of CWs for the removal of pharmaceuticals. However, only a few review studies with specific focus on pharmaceutical removal by CWs have been conducted in order to summarize current state of knowledge (Imfeld et al., 2009; Carvalho et al., 2014; Li et al., 2014; Verlicchi and Zambello, 2014; Zhang et al., 2014; Gorito et al., 2017; Ekperusi et al., 2019; Ilyas, 2020). For instance, Imfeld et al. (2009) provided a scientific description on removal processes in CWs, which was further advanced by Zhang et al. (2014) based on limited scientific evidence from available studies. Verlicchi and Zambello (2014) provided an overview of the removal of several pharmaceuticals by CW used for the primary, secondary and tertiary purposes. Li et al. (2014) summarized the role of design parameters (e.g. physical configuration, hydraulic mode, and vegetation species) in the removal of pharmaceuticals. Similarly, Gorito et al. (2017) discussed the removal processes and influence of design and operation parameters on the removal of four pharmaceuticals by CWs (azithromycin, clarithromycin, diclofenac and erythromycin), which are on the priority list of the European Union (EU). Carvalho et al. (2014) conducted a comprehensive review on the potential of CWs for phytoremediation. Consistent with that, Ekperusi et al. (2019) only reflected on the role of plants (duckweed- *Lemma minor*) in the removal of pharmaceuticals.

The size of investigated CWs involves microcosm-, mesocosm-, pilot-, and full-scale, of which the full-scale attracted the most attention. The wastewater introduced in the CWs includes secondary or tertiary effluent from urban or rural treatment facilities. Target pharmaceutical contaminants in wastewater covered analgesics, anti-inflammatories, antiallergics, antibiotics, anti-diabetics, anti-dysenterics, antifungals, anti-hypertensives, dementia drugs, barbiturates, beta-agonists, diurectics, hormone inhibitors, lipid regulators,

psychiatric drugs, receptor antagonists, stimulant/psychoactive drugs, and veterinary drugs. Among these pharmaceuticals, diclofenac, ibuprofen, ketoprofen, naproxen, sulfamethoxazole, triclosan, atenolol, clofibric acid, carbamazepine and caffeine were the most commonly investigated analytes.

The use of CWs applied as tertiary treatment systems to remove pharmaceutical contaminants from wastewater has been investigated in America and Europe. In comparison to the publications on the application of CWs as an alternative secondary wastewater treatment system for the removal of pharmaceuticals, the number of research studies on this application is relatively lower. Some of the most important aspects about the use of CWs for pharmaceutical removal are detailed in the following sections.

1.4.3.1. Design parameters of CWs

In the literature, the CWs selected for wastewater polishing treatment are SF-CWs, HSSF-CWs, VSSF-CWs, and hybrid CWs, for either controlled studies, or real-world scenarios. Most popular wetlands are SF-CWs and H-CW. The pollutant removal mechanisms which govern treatment process and resulting performance of CWs are different across CW types. The main mechanism for pharmaceutical removal is photodegradation, while microbial degradation and plant uptake also contribute to some extent in the removal. There are only a few pharmaceuticals, such as diclofenac, ketoprofen, naproxen and clarithromycin that are reported to be mainly removed by photodegradation (Ilyas et al., 2020). In HSSF-CWs, wastewater stays below the surface of the media and flows horizontally through the bed until it reaches the outlet. In this type of CW, anaerobic biodegradation is an important removal mechanism of pharmaceuticals, besides their removal by the filter media through sedimentation, adsorption and precipitation, and plant uptake. Anaerobic biodegradation was reported as a major removal

mechanism for naproxen, sulfamethoxazole, sulfapyridine, trimethoprim and atenolol (Ilyas et al., 2020). Considering that anaerobic biodegradation is slower than aerobic biodegradation, longer hydraulic retention time is needed to achieve same removal efficiency (Auvinen et al., 2017a). In VSSF-CWs, the beds are pulse-loaded with a large amount of water to temporarily flood the surface of the bed. The aerobic biodegradation is responsible for the removal of pharmaceuticals by VSSF-CWs among other dominant processes (e.g. sedimentation, adsorption, and plant uptake). Several pharmaceuticals biodegrade under aerobic conditions; hence, VSSF-CWs are suitable systems for the removal of, for instance, ibuprofen salicylic acid, acetaminophen, codeine, caffeine, metoprolol, and gemfibrozil. However, the removal of pharmaceuticals that are biodegraded under anaerobic conditions might be limited in these systems.

H-CWs are designed to achieve higher performance compared with only one setting of a CW. Research has been carried out to develop H-CWs by combining different CWs and hence a range of processes (e.g., reductive and oxidative processes) and different environments (e.g., aerobic and anaerobic) to achieve improved performance of CWs for the removal of pharmaceuticals (Ilyas et al., 2020).

1.4.3.2. The Role of Macrophytes in CWs

Macrophytes, or aquatic plants, play a role in various physical, chemical and biological processes in a wetland or CW-based system. They stabilize the substrate surface, insulate against freezing and frost through litter production, prevent clogging, shield algae from incoming solar radiation, adsorb and store nutrients and prevent channelled flow (Thomas et al., 2017). Many species of plants have been used in CWs: emergent plants such as common reed or cattails, submerged plants such as coontail and watermilfoil, as well as floating plants, rooted floating leaves and others. The most popular species of plants

used in CWs are *Phragmites australis*, *Typha* spp., *Typha angustifolia* and *Typha latifolia*, with plant densities ranging from 10-50 plants/m^2 (Li et al., 2014).

The presence of macrophytes in CWs has been reported to have a positive effect for attenuation of pharmaceuticals such as diclofenac, ibuprofen, ketoprofen, naproxen, salicylic acid, amoxicillin, ampicillin, erythromycin, sulfadiazine, sulfamethazine, sulfamethoxazole, atenolol, clofibric acid, carbamazepine and caffeine (Dordio et al., 2010, 2009a; Hijosa-Valsero et al., 2011a, 2011b, 2010b; Xian et al., 2010; Matamoros et al., 2012a, 2012; Yan et al., 2016; Zhang et al., 2012a; Zhang et al., 2012b, 2011, 2016, 2018).

Hijosa-Valsero et al. (2010, 2011) found that *P. australis* had better performance than *T. angustifolia* for the removal of several pharmaceuticals and personal care products (PPCPs). On the other hand, Dordio et al. (2009a) reported a superior performance of *Typha* spp. when compared to *Phragmites australis* for the removal of atenolol in light-expanded clay aggregates, and attributed this to the greater transpiration rate of *Typha* spp. Herrera-Cárdenas et al., (2016) reported differential performances between *T. latifolia*, *P. australis* and *Cyperus papyrus* for the attenuation of several PPCPs in a set of mesocosm-CWs, with removal rates between 70 and 99% for the studied contaminants.

Reyes-Contreras et al. (2012) indicated that recently established CWs are more efficient when they have plants (e.g. *Typha* spp., *Phragmites* spp.), but also reported that the contribution of these macrophytes to PPCP removal declines due to disturbing processes such as clogging or shading as the system ages, which ultimately minimizes their potential contributions. Dordio et al. (2010) reported that *Typha* spp. are able to contribute to the attenuation of ibuprofen, carbamazepine, and clofibric acid in mesocosm-scale CWs using a matrix of light expanded clay aggregates as a substrate. In contrast, Cardinal et al. (2014), on the other hand, reported no significant contribution from *Typha* spp. towards the

attenuation of carbamazepine, clofibric acid, naproxen, fluoxetine, sulfamethoxazole and sulfapyridine in mesocosm-scale CW systems treating spiked synthetic wastewater, as photolysis and sorption governed the fate of these pharmaceuticals over the course of their measurements.

1.4.3.3. The Role of Substrates in CWs

Substrates, also known as support matrices, are important components in CWs, especially in subsurface flow filters. Substrates provide support for the growth of plants and microorganisms and can interact directly with contaminants through sorption processes (Li et al., 2014). Sorption implies the transference and accumulation of sorptive molecules from the fluid phase to the interfacial layer or the bulk of the sorbent and can involve physical and/or chemical interactions. While physical adsorption originates from intermolecular forces, such as van der Waals, chemisorption implies chemical interactions with the transfer of electrons between adsorbent and adsorbate (de Andrade et al., 2018).

The flow of wastewater through the substrate bed depends on the hydraulic gradient and conductivity, particle size, and porosity of the materials used. Hydraulic conductivity should allow for an even distribution of the inlet flow and collection of the outlet flow (Kadlec et al., 2009). A low hydraulic conductivity will result in short-circuit flow of the wastewater between influent and effluent over the wetland surface or could lead to channelling, which both contribute to poor contact of the wastewater with the filter bed particles and hence to the lower effectiveness of the system (Netter, 1994). The bed porosity, defined as the ratio of pore water volume to the total volume of material, provides the space for wastewater treatment processes to occur. The typical porosity of sand and gravel media is in the range of 30-45%. Air-filled porosity may be involved in gas

exchange processes which are necessary to aerate the rhizosphere. This can influence both plant and microbial growth (Kadlec et al., 2009).

Gravel and sand have been reported as two of the most commonly used substrates in CW-based systems (both horizontal and vertical subsurface filter beds) for the removal of wastewater contaminants (Li et al., 2014). Crushed rock, gravel/sand mixtures, glass, anthracite, garnet, polonite, dolomite, and local soil have also been tested (Dordio and Carvalho, 2013). Selection of support matrix materials to be used in any given systems should be determined based on physical and chemical characteristics (e.g. composition, particle size distributions, porosity, effective particle size, hydraulic conductivity). This is crucial for the substrate to withstand the conditions of operation without significant degradation of its main properties. In addition, it should not release substances that could potentially be toxic for the biotic elements or that may contaminate the wastewater effluent (Dordio and Carvalho, 2013). Present and future research for novel substrates made of underexploited materials will conduct to study their potential removal interactions and performance with respect to pharmaceutical contaminants.

1.5. Environmental Data Quality and Relevance Assessments

This section addresses the fundamentals on environmental data quality assessment, and specifically, the weight-of-evidence philosophy. The concepts herein were applied through a critical review presented in Chapter 2 of this dissertation. An evaluation of the strength of methods and the relevance of observed effects was conducted for the whole body of literature on the performance of CWs for pharmaceutical removal from wastewater under various climates.

Improved understanding of pharmaceutical fate is needed to better characterize the risk these compounds pose to both ecosystems and human health via aquatic exposure. Specifically, defining their environmental fate processes and removal mechanism is crucial, as these influence the magnitude and duration of exposure to a particular pharmaceutical, and hence risk. Considering the importance of regulations for mitigating the ecological effects of pharmaceuticals, it is necessary that high-quality fate data can be generated to define and rank accurately the risks these compounds might pose so that they might be regulated appropriately. Characterizing strengths and weaknesses of available data on fate and removal processes for pharmaceuticals, specifically in CW systems, will allow researchers and regulators to direct their resources appropriately to address knowledge gaps.

To this end, the weight-of-evidence uses guidelines, known as the Bradford Hill criteria, to assign causality to particular observed effects (Hill, 1965). These guidelines evaluate a number of aspects of a study to adequately assign causality: strength, consistency, specificity, temporality, presence of a biological gradient, a plausible mechanism of action, coherence, experimental evidence, and analogy. For instance, causality is more likely to be demonstrated when there is a strong association between the independent and the dependent variables; when different studies show consistent results; when an effect is shown to have a specific cause; when cause precedes an observed effect; and when there is strong experimental evidence supporting the relationship. More recently, U.S. EPA has prepared recommendations for the application of weight of evidence principles in ecological assessment (USEPA, 2016).

The weight-of-evidence approach is increasingly being employed in ecotoxicology (e.g. Van der Kraak et al., 2014, Hanson et al., 2019) , and is often applied to toxicological

data to determine evidence for hazard in risk assessments, and to facilitate consistent and reliable data evaluation (Klimisch et al., 1997). The weight-of-evidence approach has been previously used to conduct a critical assessment of the photodegradation of pharmaceuticals in aquatic environments (Challis et al., 2014), which established an assessment of the quality of the data available up to that point in time, and pointed the knowledge gaps that needed to be addressed for future investigations.

Overall, there are a number of drivers that prompt the need for critical reviews into pharmaceutical removal in CWs. Many regulators are working towards the establishment of testing programs and strategies to assess human and wildlife health risks associated with pharmaceuticals in the environment (Hecker et al., 2011). As wastewater regulations become stricter, governments will need regulated and consistent testing protocols for their implementation. Therefore, a critical assessment of the data around fate and removal of pharmaceuticals in the environment is needed, with specific focus on testing criteria and guidelines that will facilitate consistent and reliable research in this area.

Most of the previous reviews about pharmaceutical removal in CWs, investigated a small number of analytes and selection of CWs, and their synthesis was often constrained by a limited number of available studies on a certain topic. Thus, comprehensive and critical reviews are needed to address knowledge gaps, especially on the treatment performance of different types of CWs for the removal of pharmaceuticals in order to present evidence-based conclusions. No previous study has applied the weight-of-evidence approach to evaluate the quality of data for pharmaceutical removal in CWs.

1.6. Case Studies relevant to the dissertation

Chapters 3, 4 and 5 of this dissertation were executed as case studies addressing pharmaceutical removal from wastewater under various types of climate, and through the use of diverse CW-based systems. This section will present some background on the communities and facilities on which the studies were conducted.

1.6.1. Dunnottar, Manitoba

Lake Winnipeg is the 10th largest freshwater lake in the world. Within the Lake Winnipeg watershed, there is intensive agriculture, and a relatively large population (approximately 7 million). Over the past few years, algal blooms have occurred on a regular basis throughout Lake Winnipeg during the summer months due to the discharge of excess nitrogen and phosphorus to surface waters (Environment Canada and Manitoba Water Stewardship, 2011). Furthermore, contaminants such as pharmaceuticals, pesticides, heavy metals, hospital waste and endocrine disruptors are also considered of concern in the Lake Winnipeg watershed (Lake Winnipeg Stewardship Board, 2006). There has been growing concern about the eutrophication and potential contamination of the lake, which has initiated a search for ways to reduce and minimize the adverse effects of anthropogenic activities on water quality and aquatic organisms.

The community of Dunnottar, Manitoba (population 763 as of 2016), near the shores of Winnipeg Beach, has installed a wastewater treatment facility, comprised of three wastewater lagoons performing primary and secondary treatment, followed by a pilot-scale subsurface filter installed in 2009. The purpose of the filter was to further polish the wastewater in order to achieve an effluent that would comply with provincial regulatory parameters. This system has been previously studied (Anderson et al., 2015), and it served as a basis for the construction of a full-scale vertical flow subsurface filter in 2014, which

has been operational ever since, and has achieved a similar wastewater treatment to its pilot-scale predecessor.

The addition of vertical subsurface filters after lagoon treatment was demonstrated to be a useful polishing step to reduce Dunnottar nutrient loadings into Lake Winnipeg and has provided a unique opportunity to conduct research on the occurrence, fate and removal of several contaminants along the wastewater path. Nevertheless, some challenges in this facility are still to be addressed. For example, as part of the full-scale system assessment and operation, it was discovered that clogging occurs in certain areas of the filter bed, which has led to occasional excavations for material replacement and temporary flow interruption. This presents an opportunity for the assessment of alternative, low-cost substrate materials in pilot-scale experiments aiming to achieve a similar wastewater treatment efficiency and also reduce the probability of clogging events. Development of such substrates could improve the management and efficiency of these municipal systems.

1.6.2. Cambridge Bay, Nunavut

In several Arctic regions, sewage is released to surface waters after minimal or no treatment. This can have impacts on the receiving environment, given the vulnerability of Arctic ecosystems to wastewater contaminants (Gunnarsdottir et al., 2013). Pharmaceutical residues are degraded more slowly in Arctic environments compared to locations in lower latitudes (Kallenborn et al., 2008). Removal of these contaminants through photodegradation is limited during the Arctic polar night and the intensity of sunlight at other times of the year is lesser than in more temperate regions. This can slow down the degradation rate of organic contaminants in the aquatic environment (Schwarzenbach et al., 2003).

Significant efforts to understand sources, fate and effects of contaminants in the Arctic have been conducted. However, most studies have focused on chemicals reaching the Arctic via long-range transport from more temperate regions where these chemicals are typically discharged into the environment. The potential impacts of chemical pollutants from regional human activities in northern communities has been significantly less studied, due to their sparse and relatively low populations.

Cambridge Bay is a community located in the territory of Nunavut in the Canadian Arctic. It has a population of approximately 1400. Mean monthly temperatures range from a daily maximum and minimum, respectively, of -28°C and -35°C in January to 13°C and 5°C in July (Government of Canada, 2014). The wastewater treatment system in place at Cambridge Bay is comprised of a wastewater lagoon, formerly a series of natural lakes, that performs primary treatment and is discharged once a year, during the summer, into a small hydrologically isolated natural tundra wetland, considered as a surface flow system for the purpose of the assessment. Wastewater is then released through an open channel into the marine environment. Municipal sewage from household sewage tanks is regularly transported to the lagoon by sewage trucks that perform dumping runs year-round. As of 2014, no study had addressed the performance of the wastewater system to remove pharmaceuticals and antibiotic resistance genes.

1.6.3. Mesocosms work – Prairie Wetland Research Facility, University of Manitoba

One of the projects in this dissertation made use of mesocosm systems to study the fate of pharmaceuticals in a modelled-wetland environment (Chapter 4). Mesocosms are model ecosystems that provide realistic scenarios to conduct toxicity studies and evaluate the fate of selected chemicals by measuring their dissipation over time including their degradation and bioavailability in aquatic systems. These are small, typically outdoor,

enclosures usually larger than 1000 litres that simulate a natural aquatic ecosystem where interactions occur at various trophic levels under environmentally relevant conditions (Sanderson, 2002). Each mesocosm aims to mimic a naturally-occurring shallow wetland, being comprised of natural sediments, a layer of water, and local flora and fauna. If a pharmaceutical is capable of partitioning to multiple compartments (e.g., soil, biota), a mass balance approach can often elucidate pharmaceutical transport provided the analytical methods have been developed for each compartment. For instance, the fate of the antibiotic sulfamethazine was investigated by attempting to determine its transport from manures to surface waters using pond water mesocosms (Henderson and Coats, 2009). The authors determined that sediments were potential sinks for this pharmaceutical and could have implications towards benthic organisms. Another similar experiment (Sanderson et al., 2007) showed a similar outcome for the pharmaceutical ivermectin partitioning to sediments where dissipation occurred from the water column with half-lives ranging from 3-5 days and concentrations stabilizing at 20-30 ng/kg in the sediment.

The fate of an analyte can vary widely depending on the system analyzed. For example, fluoxetine in distilled water undergoes photolysis when irradiated with simulated sunlight with a half-life of 55 hours (Lam et al., 2004). In synthetic field water (with added nitrate, carbonate and DOM), fluoxetine degrades more quickly with a half-life ranging from 5 to 22 hours (Lam et al., 2004). In water-sediment systems the half-life of fluoxetine (6 days) was 22 times shorter than in water alone (133 days) (Kwon and Armbrust, 2006). Thus, as a system increases in complexity, more factors come into play, and persistence of a particular compound varies. For example, a study by Knapp et al. (Knapp et al., 2003) investigated the fate of the pesticide alachlor in mesocosms over time. The activity of the bacteria biodegrading the analyte was attributed to increased rates of degradation of

alachlor. These types of experiments in wetland mesocosms represent reliable simulations of what actually occurs in the environment and could potentially be further compared to field-scale CW experiments (Hijosa-Valsero et al., 2011a; Hijosa-Valsero et al., 2010; Hijosa-Valsero et al., 2011b).

1.7. Dissertation Outline

The research projects contained within this dissertation are divided into four standalone manuscripts (Chapters 2 to 5) as below.

- ***Chapter 2: A critical review of the evidence for removal of pharmaceuticals in constructed wetlands treating municipal wastewaters.***
    - A standardized weight-of-evidence-based assessment of the literature on the performance of various types of constructed wetlands to remove pharmaceuticals from municipal wastewaters under various climates. Specifically, the relevance for pharmaceutical removal and the quality of the methods used were evaluated through a customized rubric, to compare between CW types and the various climates on which these studies have been carried out.
- ***Chapter 3: The release of wastewater contaminants in the Arctic: A case study from Cambridge Bay, Nunavut, Canada.***
    - A previously published field evaluation of the removal of nutrients, pharmaceuticals and ARGs in a lagoon-wetland system treating wastewater from the polar community of Cambridge Bay, Nunavut, Canada (Chaves-Barquero et al., 2016). This work represented a baseline status description of the performance of the wastewater treatment facility in the community, in anticipation of the installation of the Canadian High Arctic Research Station in the town.

- ***Chapter 4: Crushed recycled glass as a substrate for constructed wetland wastewater treatment: A case study of the potential to facilitate nutrients and pharmaceutical removals.***
    - This is a previously published study (Chaves-Barquero et al., 2021). Two sub-studies are presented. The first examined the removal of atenolol, carbamazepine and sulfamethoxazole in quiescent modelled mesocosm-scale tanks using recycled glass or limestone gravel as substrates. The second study looked at the removal of background pharmaceuticals from municipal wastewater on pilot-scale subsurface filter beds made of either recycled glass or sand.
- ***Chapter 5: Attenuation of pharmaceuticals, nutrients, and toxicity in a rural sewage lagoon system integrated with a subsurface filtration technology.***
    - A previously published two-year (2015-16) field study assessing the ability of a full-scale lagoon-wetland system to remove background nutrients, pharmaceuticals, and toxicity from municipal wastewater in Dunnottar, Manitoba, Canada through subsurface filtration using a mixture of sand and gravel as substrate (Chaves-Barquero, 2018).
- ***Chapter 6: Overall synthesis***
    - Summarizes the major findings and implications of the four research chapters and discusses research limitations and future recommendations.

1.8. References for Chapter 1

Anderson, J.C.; Carlson, J.C.; Low, JE, Challis, J.K.; Wong, C.S.; Knapp C.W.; Hanson, M.L. Performance of a constructed wetland in Grand Marais, Manitoba, Canada: Removal of nutrients, pharmaceuticals, and antibiotic resistance genes from municipal wastewater. *Chemistry Central Journal* **2013**; *7*: 54.

Anderson J.C.; Joudan S.; Shoichet, E.; Cuscito L.D.; Alipio, A.E.C.; Donaldson, C.S.; Khan, S.; Goltz, D.M.; Rudy, M.D.; Frank, R.A.; Knapp, C.W.; Hanson, M.L.; Wong, C.S. Reducing nutrients, organic micropollutants, antibiotic resistance, and toxicity in rural wastewater effluent with subsurface filtration treatment technology. *Ecological Engineering* **2015**; *84*: 375-385.

Arnold, K.E.; Brown, A.R.; Ankley, G.T.; Sumpter, J.P. Medicating the environment: assessing risks of pharmaceuticals to wildlife and ecosystems. *Philosophical Transactions of the Royal Society B-Biological Sciences* **2014**; *369*: 11.

Backhaus T. Medicines, shaken and stirred: a critical review on the ecotoxicology of pharmaceutical mixtures. *Philosophical Transactions of the Royal Society B-Biological Sciences* **2014**; *369*.

Bodhipaksha, L.C.; Sharpless, C.M.; Chin, Y.P.; Sander, M.; Langston, W.K.; MacKay, A.A. Triplet photochemistry of effluent and natural organic matter in whole water and isolates from effluent-receiving rivers. *Environmental Science & Technology* **2015**; *49*: 3453-63.

Boreen, A.L.; Arnold, W.A.; McNeill, K. Photodegradation of pharmaceuticals in the aquatic environment: A review. *Aquatic Sciences* **2003**; 65: 320-341.

Boreen, A.L.; Arnold, W.A.; McNeill, K. Photochemical fate of sulfa drugs in the aquatic environment: Sulfa drugs containing five-membered heterocyclic groups. *Environmental Science & Technology* **2004**; *38*: 3933-3940.

Boxall, A.B.A.; Rudd, M.A.; Brooks, B.W.; Caldwell, D.J.; Choi, K.; Hickmann, S.; Pharmaceuticals and Personal Care Products in the Environment: What Are the Big Questions? *Environmental Health Perspectives* **2012**; *120*: 1221-1229.

Brodin, T.; Piovano, S.; Fick, J.; Klaminder, J.; Heynen, M.; Jonsson, M. Ecological effects of pharmaceuticals in aquatic systems--impacts through behavioural alterations. *Philosophical Transactions of the Royal Society B-Biological Sciences* **2014**; *369*.

Brooks, B.W.; Chambliss, C.K.; Stanley, J.K.; Ramirez, A.; Banks, K.E.; Johnson, R.D.; Lewis, R.J. Determination of select antidepressants in fish from an effluent-dominated stream. *Environmental Toxicology and Chemistry* **2005**; 24: 464-9.

Brooks, B.W.; Huggett, D.B.; Boxall, A.B. Pharmaceuticals and personal care products: research needs for the next decade. *Environmental Toxicology and Chemistry* **2009**; *28*: 2469-72.

Brun, G.L.; Bernier, M.; Losier, R.; Doe, K.; Jackman, P.; Lee, H.B. Pharmaceutically active compounds in Atlantic Canadian sewage treatment plant effluents and receiving waters, and potential for environmental effects as measured by acute and chronic aquatic toxicity. *Environmental Toxicology and Chemistry* **2006**; *25*: 2163-2176.

Caldwell, D.J.; Mastrocco, F.; Anderson, P.D.; Länge, R.; Sumpter, J.P. Predicted-no-effect concentrations for the steroid estrogens estrone, 17β-estradiol, estriol, and 17α-ethinylestradiol. *Environmental Toxicology and Chemistry* **2012**; *31*: 1396-406.

Caracciolo, A.B.; Topp, E.; Grenni, P. Pharmaceuticals in the environment: Biodegradation and effects on natural microbial communities. A review. *Journal of Pharmaceutical and Biomedical Analysis* **2015**; *106*: 25-36.

Carlson, J.C.; Anderson, J.C.; Low, J.E.; Cardinal, P.; MacKenzie, S.D.; Beattie S.A.; Challis, J.K.; Bennett, R.J.; Meronek, S.S.; Wilks, R.P.A.; Buhay, W.M.; Wong, C.S.; Hanson, M.L. Presence and hazards of nutrients and emerging organic micropollutants from sewage lagoon discharges into Dead Horse Creek, Manitoba, Canada. *Science of the Total Environment* **2013**; *445*: 64-78.

Casas, M.E.; Chhetri, R.K.; Ooi, G.; Hansen, K.M.; Litty, K.; Christensson, M.; Kragelund, C.; Andersen, H.R.; Bester, K. Biodegradation of pharmaceuticals in hospital wastewater by staged Moving Bed Biofilm Reactors (MBBR). *Water Research* **2015**; *83*: 293-302.

Challis, J.K.; Carlson, J.C.; Friesen, K.J.; Hanson, M.L.; Wong, C.S. Aquatic photochemistry of the sulfonamide antibiotic sulfapyridine. *Journal of Photochemistry and Photobiology a-Chemistry* **2013**; *262*: 14-21.

Challis, J.K.; Hanson, M.L.; Friesen, K.J.; Wong, C.S. A critical assessment of the photodegradation of pharmaceuticals in aquatic environments: defining our current understanding and identifying knowledge gaps. *Environmental Science-Processes & Impacts* **2014**; *16*: 672-696.

Chaves-Barquero, L.G.; Humeniuk, B.W.; Luong, K.H.; Cicek, N.; Wong, C.S.; Hanson, M.L. Crushed recycled glass as a substrate for constructed wetland wastewater treatment: a case study of its potential to facilitate pharmaceutical removal. *Environmental Science and Pollution Research* **2021;** *28*: 62306-52318.

Chaves-Barquero, L.G.; Luong, K.H.; Mundy, C.J.; Knapp, C.W.; Hanson, M.L.; Wong, C.S. The release of wastewater contaminants in the Arctic: A case study from Cambridge Bay, Nunavut, Canada. *Environmental Pollution* **2016**; *218*: 542-550.

Chaves-Barquero, L.G.; Luong, K.H.; Rudy, M.D.; Frank, R.A.; Hanson, M.L.; Wong, C.S. Attenuation of pharmaceuticals, nutrients and toxicity in a rural sewage lagoon system integrated with a subsurface filtration technology. *Chemosphere* **2018**; *209*: 767-775.

Cizmas, L.; Sharma, V.K.; Gray, C.M.; McDonald, T.J. Pharmaceuticals and personal care products in waters: occurrence, toxicity, and risk. *Environmental Chemistry Letters* **2015**; *13*: 381-394.

Conkle, J.L.; Lattao, C.; White, J.R.; Cook, R.L. Competitive sorption and desorption behavior for three fluoroquinolone antibiotics in a wastewater treatment wetland soil. *Chemosphere* **2010**; *80*: 1353-1359.

Conkle, J.L.; White, J.R.; Metcalfe, C.D. Reduction of pharmaceutically active compounds by a lagoon wetland wastewater treatment system in Southeast Louisiana. *Chemosphere* **2008**; *73*: 1741-1748.

Corcoran, J.; Winter, M.J.; Tyler, C.R. Pharmaceuticals in the aquatic environment: a critical review of the evidence for health effects in fish. *Critical Reviews in Toxicology* **2010**; *40*: 287-304.

Daughton, C.G.; Ternes, T.A. Pharmaceuticals and personal care products in the environment: Agents of subtle change? *Environmental Health Perspectives* **1999**; *107*: 907-938.

de Andrade, J.R.; Oliveira, M.F.; da Silva, M.G.C.; Vieira, M.G.A. Adsorption of Pharmaceuticals from Water and Wastewater Using Nonconventional Low-Cost

Materials: A Review. *Industrial & Engineering Chemistry Research* **2018**; *57*: 3103-3127.

Di Bonito, M. Sewage sludge in europe and in the UK: environmental impact and improved standards for recycling and recovery to land. In: De Vivo, B.; Belkin, H.E.; Lima, A., editors. Environmental Geochemistry — Site Characterization, Data Analysis and Case Histories. Elsevier Inc., **2008**.

Dordio, A.V.; Carvalho, A.J.P. Organic xenobiotics removal in constructed wetlands, with emphasis on the importance of the support matrix. *Journal of Hazardous Materials* **2013**; *252*: 272-292.

Drillia, P.; Stamatelatou, K.; Lyberatos, G. Fate and mobility of pharmaceuticals in solid matrices. *Chemosphere* **2005**; *60*: 1034-44.

Du, B.; Haddad, S.P.; Luek, A.; Scott, W.C.; Saari, G.N.; Kristofco, L.A.; Connors, K.A.; Rash, C.; Rasmussen, J.B.; Chambliss, K.C.; Brooks, B.W. Bioaccumulation and trophic dilution of human pharmaceuticals across trophic positions of an effluent-dependent wadeable stream. *Philosophical Transactions of the Royal Society B-Biological Sciences* **2014**; *369*: 10.

Dulin, D.; Mill, T. Development and evaluation of sunlight actinometers. *Environmental Science & Technology* **1982**; *16*: 815-20.

Environment Canada, Manitoba Water Stewardship. State of Lake Winnipeg: 1999-2007, **2011.**

Fatta-Kassinos, D.; Meric, S.; Nikolaou, A. Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research. *Analytical and Bioanalytical Chemistry* **2011**; *399*: 251-275.

Fent, K.; Weston, A.A.; Caminada, D. Ecotoxicology of human pharmaceuticals. *Aquatic Toxicology* **2006**; *76*: 122-159.

Fono, L.J.; Kolodziej, E.P.; Sedlak, D.L. Attenuation of wastewater-derived contaminants in an effluent-dominated river. *Environmental Science & Technology* **2006**; *40*: 7257-62.

Garrison, A.W.; Pope, J.D.; Allen, F.R. GC/MS Analysis of organic compounds in domestic wastewaters. In: Keith, C.H.; Arbor, A., editors. Identification and Analysis of Organic Pollutants in Water. Ann Arbor Science Publishers, **1976,** pp. 516-556.

Ghattas, A.K.; Fischer, F.; Wick, A.; Ternes, T.A. Anaerobic biodegradation of (emerging) organic contaminants in the aquatic environment. *Water Research* **2017**; *116*: 268-295.

Government of Canada. Monthly climate report for Cambridge Bay. **2014**.

Greskowiak, J.; Hamann, E.; Burke, V.; Massmann, G. The uncertainty of biodegradation rate constants of emerging organic compounds in soil and groundwater - A compilation of literature values for 82 substances. *Water Research* **2017**; *126*: 122-133.

Guerard, J.J.; Miller, P.L.; Trouts, T.D.; Chin, Y.P. The role of fulvic acid composition in the photosensitized degradation of aquatic contaminants. *Aquatic Sciences* **2009**; *71*: 160-169.

Gunnarsdottir, R.; Jenssen, P.D.; Jensen, P.E.; Villumsen, A.; Kallenborn, R. A review of wastewater handling in the Arctic with special reference to pharmaceuticals and personal care products (PPCPs) and microbial pollution. *Ecological Engineering* **2013**; *50*: 76-85.

Hajj-Mohamad, M.; Darwano, H.; Duy, S.V.; Sauve, S.; Prevost, M.; Arp, H.P.H. Dorner, S. The distribution dynamics and desorption behaviour of mobile pharmaceuticals and caffeine to combined sewer sediments. *Water Research* **2017**; *108*: 57-67.

Health Canada. Drugs and Health Products - Drug Database Online Query, **2018.**

Heberer, T. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicology Letters* **2002**; *131*: 5-17.

Henderson, K.L.D.; Coats, J.R. Veterinary pharmaceuticals in the environment: an introduction. In: Veterinary Pharmaceuticals in the Environment **2009**; American Chemical Society Symposium Series; *1018*: 3-7.

Hester, R.E.; Harrison, R.M. Pharmaceuticals in the environment. Royal Society of Chemistry, **2016.**

Hijosa-Valsero, M.; Matamoros, V.; Sidrach-Cardona, R.; Martin-Villacorta, J.; Becares, E.; Bayona, J.M. Comprehensive assessment of the design configuration of constructed wetlands for the removal of pharmaceuticals and personal care products from urban wastewaters. *Water Research* **2010**; *44*: 3669-3678.

Hijosa-Valsero, M.; Matamoros, V.; Pedescoll, A.; Martin-Villacorta, J.; Becares, E.; Garcia, J.; Bayona, J.M. Evaluation of primary treatment and loading regimes in the removal of pharmaceuticals and personal care products from urban wastewaters by subsurface-flow constructed wetlands. *International Journal of Environmental Analytical Chemistry* **2011a**; *91*: 632-653.

Hijosa-Valsero, M.; Matamoros, V.; Sidrach-Cardona, R.; Pedescoll, A.; Martin-Villacorta, J.; Garcia, J.; Bayona, J.M.; Bécares, E. Influence of design, physico-chemical and environmental parameters on pharmaceuticals and fragrances removal by constructed wetlands. *Water Science and Technology* **2011b**; *63*: 2527-2534.

Hill, A.B. The environment and disease: association or causation? *Proceedings of the Royal Society of Medicine* **1965**; *58*: 295-300.

Hopcroft, F.J. Wastewater Treatment Concepts and Practices. New York, USA: Momentum Press, **2014.**

Huerta B.; Rodriguez-Mozaz, S.; Nannou, C.; Nakis, L.; Ruhí, A.; Acuña, V.; Sabater, S.; Barcelo, D. Determination of a broad spectrum of pharmaceuticals and endocrine disruptors in biofilm from a waste water treatment plant-impacted river. *Science of the Total Environment* **2016**; *540*: 241-9.

Huijbers, P.M.C.; Blaak, H.; de Jong, M.C.M.; Graat, E.A.M.; Vandenbroucke-Grauls, C.; Husman, A.M.D. Role of the Environment in the Transmission of Antimicrobial Resistance to Humans: A Review. *Environmental Science & Technology* **2015**; *49*: 11993-12004.

Ilyas, H.; Masih, I.; van Hullebusch, E.D. Pharmaceuticals' removal by constructed wetlands: a critical evaluation and meta-analysis on performance, risk reduction, and role of physicochemical properties on removal mechanisms. *Journal of Water and Health* **2020**; *18*: 253-291.

Jasper, J.T.; Sedlak, D.L. Phototransformation of wastewater-derived trace organic contaminants in open-water unit process treatment wetlands. *Environmental Science & Technology* **2013**; *47*: 10781-90.

Jeffreys, D. Aspirin: The Extraordinary Story of a Wonder Drug. London, UK: Bloomsbury, **2004**.

Kadlec, R.H.; Wallace, S. Treatment wetlands. CRC Press, Boca Raton, FL: ebrary Inc. **2009**.

Kallenborn, R.; Fick, J.; Lindberg, R.; Moe, M.; Nielsen, K.M.; Tysklind, M.; Vasskog, T. Pharmaceutical Residues in Northern European Environments: Consequences and Perspectives. In: Kummerer K., editor. Pharmaceuticals in the Environment: Sources, Fate, Effects and Risks, Third Edition. Springer-Verlag Berlin, Heidelberger Platz 3, D-14197 Berlin, Germany, **2008**.

Karickhoff, S.W.; Brown, D.S.; Scott, T.A. Sorption of hydrophobic pollutants on natural sediments. *Water Research* **1979**; *13*: 241-248.

Kidd, K.A.; Blanchfield, P.J.; Mills, K.H.; Palace, V.P.; Evans, R.E.; Lazorchak, J.M.; Flick, R.W. Collapse of a fish population after exposure to a synthetic estrogen. *Proceedings of the National Academy of Sciences of the United States of America* **2007**; *104*: 8897-901.

Klimisch, H.J.; Andreae, M.; Tillmann, U. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology* **1997**; *25*: 1-5.

Knapp, C.W.; Graham, D.W.; Berardesco, G.; deNoyelles, F.; Cutak, B.J.; Larive, C.K. Nutrient level, microbial activity, and alachlor transformation in aerobic aquatic systems. *Water Research* **2003**; *37*: 4761-9.

Kolpin, D.W.; Furlong, E.T.; Meyer, M.T.; Thurman, E.M.; Zaugg, S.D.; Barber, L.B.; Buxton, H.T. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A national reconnaissance. Environmental Science & Technology **2002**; *36*: 1202-1211.

Kummerer, K. Antibiotics in the aquatic environment - A review - Part II. *Chemosphere* **2009**; *75*: 435-441.

Kummerer, K. Pharmaceuticals in the Environment. In: Gadgil, A.; Liverman, D.M., editors. Annual Review of Environment and Resources, Vol 35. 35. Annual Reviews, Palo Alto, USA: **2010**.

Kurwadkar, S.T.; Adams, C.D.; Meyer, M.T.; Kolpin, D.W. Effects of sorbate speciation on sorption of selected sulfonamides in three loamy soils. *Journal of Agricultural and Food Chemistry* **2007**; *55*: 1370-1376.

Kwon, J.W.; Armbrust, K.L. Laboratory persistence and fate of fluoxetine in aquatic environments. *Environmental Toxicology and Chemistry* **2006**; *25*: 2561-8.

Lake Winnipeg Stewardship Board. Reducing Nutrient Loading to Lake Winnipeg and its Watershed: Our Collective Responsibility and Commitment to Action, **2006**.

Lam, M.W.; Mabury, S.A. Photodegradation of the pharmaceuticals atorvastatin, carbamazepine, levofloxacin, and sulfamethoxazole in natural waters. *Aquatic Sciences* **2005**; *67*: 177-188.

Lam, M.W.; Tantuco, K.; Mabury, S.A. PhotoFate: A new approach in accounting for the contribution of indirect photolysis of pesticides and pharmaceuticals in surface waters. *Environmental Science & Technology* **2003**; *37*: 899-907.

Lam, M.W.; Young, C.J.; Brain, R.A.; Johnson, D.J.; Hanson, M.A.; Wilson, C.J.; Richards, S.M.; Solomon, K.R.; Mabury, S.A.. Aquatic persistence of eight pharmaceuticals in a microcosm study. *Environmental Toxicology and Chemistry* **2004**; *23*: 1431-1440.

Lam, M.W.; Young, C.J.; Mabury, S.A. Aqueous photochemical reaction kinetics and transformations of fluoxetine. *Environmental Science & Technology* **2005**; *39*: 513-522.

Laszakovits, J.; Berg, S.; O'Brien, J.; Wammer, K.; Sharpless, C. p-Nitroanisole/pyridine and p-nitroacetophenone/pyridine actinometers revisited: Quantum yield corrections based on ferrioxalate. *Abstracts of Papers of the American Chemical Society* **2017**; *253*: 2.

Le-Minh, N.; Khan, S.J.; Drewes, J.E.; Stuetz, R.M. Fate of antibiotics during municipal water recycling treatment processes. *Water Research* **2010**; *44*: 4295-323.

Leifer, A. The Kinetics of Environmental Aquatic Photochemistry: Theory and Practice. USA: American Chemical Society, **1988**.

Lertpaitoonpan, W.; Ong, S.K.; Moorman, T.B. Effect of organic carbon and pH on soil sorption of sulfamethazine. *Chemosphere* **2009**; *76*: 558-564.

Li, Y.F.; Zhu, G.B.; Ng, W.J.; Tan, S.K. A review on removing pharmaceutical contaminants from wastewater by constructed wetlands: Design, performance and mechanism. *Science of the Total Environment* **2014**; *468*: 908-932.

Lu, Z.; Challis, J.K.; Wong, C.S. Quantum Yields for Direct Photolysis of Neonicotinoid Insecticides in Water: Implications for Exposure to Nontarget Aquatic Organisms. *Environmental Science & Technology Letters* **2015**; *2*: 188-192.

Löffler, D.; Römbke, J.; Meller, M.; Ternes, T.A. Environmental fate of pharmaceuticals in water/sediment systems. *Environmental Science & Technology* **2005**; *39*: 5209-18.

Matamoros, V.; Arias, C.; Brix, H.; Bayona, J.M. Removal of pharmaceuticals and personal care products (PPCPs) from urban wastewater in a pilot vertical flow constructed wetland and a sand filter. *Environmental Science & Technology* **2007**; *41*: 8171-8177.

Matamoros, V.; Garcia, J.; Bayona, J.M. Behavior of selected pharmaceuticals in subsurface flow constructed wetlands: A pilot-scale study. *Environmental Science & Technology* **2005**; *39*: 5449-5454.

McNeill, K.; Canonica, S. Triplet state dissolved organic matter in aquatic photochemistry: reaction mechanisms, substrate scope, and photophysical properties. *Environmental Science: Processes & Impacts* **2016**; *18*: 1381-1399.

Miller, P.L.; Chin, Y.P. Indirect photolysis promoted by natural and engineered wetland water constituents: processes leading to alachlor degradation. *Environmental Science & Technology* **2005**; *39*: 4454-62.

Monteiro, S.C.; Boxall, A.B.A. Occurrence and Fate of Human Pharmaceuticals in the Environment. *Reviews of Environmental Contamination and Toxicology* **2010**; 202: 53-154.

Netter, R. Flow characteristics of planted soil filters. *Water Science and Technology* **1994**; *29*: 37-44.

Oulton, R.L.; Kohn, T.; Cwiertny, D.M. Pharmaceuticals and personal care products in effluent matrices: A survey of transformation and removal during wastewater treatment and implications for wastewater management. *Journal of Environmental Monitoring* **2010**; *12*: 1956-1978.

Palace, V.P.; Evans, R.E.; Wautier, K.; Baron, C.; Vandenbyllardt, L.; Vandersteen, W.; Kidd, K. Induction of vitellogenin and histological effects in wild fathead minnows from a lake experimentally treated with the synthetic estrogen, ethynylestradiol. *Water Quality Research Journal of Canada* **2002**; *37*: 637-650.

Pei, R.; Kim, S.C.; Carlson, K.H.; Pruden A. Effect of River Landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG). *Water Research* **2006**; *40*: 2427-2435.

Pepper, I.L.; Brooks, J.P.; Gerba, C.P. Antibiotic Resistant Bacteria in Municipal Wastes: Is There Reason for Concern? *Environmental Science & Technology* **2018**; *52*: 3949-3959.

Petrie, B.; Barden, R.; Kasprzyk-Hordern, B. A review on emerging contaminants in wastewaters and the environment: current knowledge, understudied areas and recommendations for future monitoring. *Water Research* **2015**; *72*: 3-27.

Petrie, B.; McAdam, E.J.; Scrimshaw, M.D.; Lester, J.N.; Cartmell, E. Fate of drugs during wastewater treatment. *Trac-Trends in Analytical Chemistry* **2013**; *49*: 145-159.

Prosser, R.S.; Sibley, P.K. Human health risk assessment of pharmaceuticals and personal care products in plant tissue due to biosolids and manure amendments, and wastewater irrigation. *Environment International* **2015**; *75*: 223-33.

Richardson, M.L.; Bowron, J.M. The fate of pharmaceutical chemicals in the aquatic environment. *Journal of Pharmacy and Pharmacology* **1985**; *37*: 1-12.

Sanderson, H. Pesticide studies: replicability of micro/mesocosms. *Environmental Science and Pollution Research* **2002**; *9*: 429-435.

Sanderson, H.; Laird, B.; Pope, L.; Brain, R.; Wilson, C.; Johnson, D.; Bryning, G.; Peregrine, A.S.; Boxall, A.; Solomon, K.R. Assessment of the environmental fate and effects of ivermectin in aquatic mesocosms. *Aquatic Toxicology* **2007**; *85*: 229-40.

Santos, L.; Araujo, A.N.; Fachini, A.; Pena, A.; Delerue-Matos, C.; Montenegro, M. Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. *Journal of Hazardous Materials* **2010**; *175*: 45-95.

Sarmah, A.K.; Meyer, M.T.; Boxall, A.B. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere* **2006**; *65*: 725-59.

Sassman, S.A.; Lee, L.S. Sorption of three tetracyclines by several soils: assessing the role of pH and cation exchange. Environmental Science & Technology **2005**; *39*: 7452-9.

Schwarzenbach, R.; Gschwend, P.; Imboden, D. Environmental Organic Chemistry. 2nd ed, New Jersey, USA: John Wiley & Sons, Inc., **2003.**

Schwarzenbach, R.; Gschwend, P.; Imboden, D. Environmental Organic Chemistry. 3rd ed, New Jersey, USA: John Wiley & Sons, Inc., **2017.**

Sjöström, H.; Nilsson, R. Thalidomide and the Power of the Drug Companies. London: Penguin, **1972.**

Stan, H.J.; Heberer, T.; Linkerhägner, M. Vorkommen von Clofibrinsäure in aquatischen System: Führt die therapeutische Anwendung zu einer Belastung von Oberflächen-, Grund- und Trinkwasser? *Vom Wasser* **1994**; *83*: 57-68.

Ternes, T.A.; Joss, A.; Siegrist, H. Scrutinizing pharmaceuticals and personal care products in wastewater treatment. *Environmental Science & Technology* **2004**; *38*: 392A-399A.

Thomas, R.; Gough, R.; Freeman, C. Linear alkylbenzene sulfonate (LAS) removal in constructed wetlands: The role of plants in the treatment of a typical pharmaceutical and personal care product. *Ecological Engineering* **2017**; *106*: 415-422.

Tixier, C.; Singer, H.P.; Oellers, S.; Muller, S.R. Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, and naproxen in surface waters. *Environmental Science & Technology* **2003**; *37*: 1061-1068.

Tolls, J. Sorption of veterinary pharmaceuticals in soils: A review. *Environmental Science & Technology* **2001**; *35*: 3397-3406.

U.S. Environmental Protection Agency. Weight of evidence in ecological assessment. USEPA **2016**. Washington, DC. EPA/100/R16/001

U.S. National Research Council. Use of reclaimed water and sludge in food crop production. Washington, D.C.: National Academy Press, **1996**.

Uslu, M.O.; Jasim, S.; Arvai, A.; Bewtra, J.; Biswas, N. A Survey of Occurrence and Risk Assessment of Pharmaceutical Substances in the Great Lakes Basin. *Ozone-Science & Engineering* **2013**; *35*: 249-262.

Vasudevan, D.; Bruland, G.L.; Torrance, B.S.; Upchurch, V.G.; MacKay, A.A. pH-dependent ciprofloxacin sorption to soils: Interaction mechanisms and soil factors influencing sorption. *Geoderma* **2009**; *151*: 68-76.

Verlicchi, P.; Zambello, E. How efficient are constructed wetlands in removing pharmaceuticals from untreated and treated urban wastewaters? A review. *Science of the Total Environment* **2014**; *470*: 1281-1306.

Vidal-Dorsch, D.E.; Bay, S.M.; Maruya, K.; Snyder, S.A.; Trenholm, R.A.; Vanderford, B.J. Contaminants of emerging concern in municipal wastewater effluents and marine receiving water. *Environmental Toxicology and Chemistry* **2012**; *31*: 2674-2682.

Wentzel, M.C.; Ekama, G.A.; Loewenthal, R.E. Fundamentals of biological behaviour and wastewater strength tests. In: Mara, D.; Horan, N., editors. Handbook of Water and Wastewater Microbiology. Academic Press, London, UK, **2003**, pp. 145-173.

Writer, J.H.; Barber, L.B.; Ryan, J.N.; Bradley, P.M. Biodegradation and attenuation of steroidal hormones and alkylphenols by stream biofilms and sediments. Environmental Science & Technology **2011**; *45*: 4370-6.

Yamamoto, H.; Nakamura, Y.; Moriguchi, S.; Honda, Y.; Tamura, I.; Hirata Y.; Hayashi, A.; Sekizawa, J. Persistence and partitioning of eight selected pharmaceuticals in the aquatic environment: Laboratory photolysis, biodegradation, and sorption experiments. *Water Research* **2009**; *43*: 351-362.

Yang, X.; Flowers, R.C.; Weinberg, H.S.; Singer, P.C. Occurrence and removal of pharmaceuticals and personal care products (PPCPs) in an advanced wastewater reclamation plant. *Water Research* **2011**; *45*: 5218-5228.

Zhou, J.; Broodbank, N. Sediment-water interactions of pharmaceutical residues in the river environment. *Water Research* **2014**; *48*: 61-70.

1.9. Connecting text between Chapters 1 and 2

Chapter 1 laid the fundamental background for this dissertation. Next, in Chapter 2, a weight-of-evidence-based critical review evaluates the body of literature in terms of its relevance and strength of methods for the removal of pharmaceuticals from wastewater through the application of various types of constructed wetlands under diverse types of climate, with the intention of obtaining an integrative assessment of their performance and any relevant knowledge gaps.

CHAPTER 2

2. A CRITICAL REVIEW OF THE EVIDENCE FOR REMOVAL OF PHARMACEUTICALS IN CONSTRUCTED WETLANDS TREATING MUNICIPAL WASTEWATERS.

2.1. Abstract

The objective of this critical review was to evaluate evidence in the peer-reviewed literature for removal of pharmaceuticals in constructed wetlands (CWs) using a weight-of-evidence-based approach. We assessed by scoring rubrics the relevance of removal (ROO) and the strength of methods (SOM) of reported pharmaceutical attenuation by constructed wetland (CW) treatment in systems of various designs and climates. In total, 74 papers were scored for their ROO and SOM. The fates of 48 compounds from several drug classes were assessed. We found that hybrid CWs (which are sequential systems consisting of two or more single-step systems) showed evidence for meaningful removal of ibuprofen, diclofenac, sulfamethoxazole, acetaminophen, ciprofloxacin, clarithromycin, propranolol, and venlafaxine compared to single-step CWs. The ROO scores of contaminants across climate types (e.g, polar vs. temperate vs. tropical) suggest that CWs from all climates do have the potential to attain pharmaceutical removal ($p > 0.05$) to some extent. The SOM scores for studies from temperate climate were statistically significantly greater than polar- or tropical-based research ($p < 0.05$). We recommend stronger studies methodologically that aim to understand CW performance and the processes influencing the removal of specific pharmaceuticals, for tropical and polar climates. Overall, we conclude that CWs

can be tools for the removal of some pharmaceuticals in wastewater effluents regardless of climate conditions and seasonality. Nevertheless, our assessment found persistent knowledge gaps regarding the climate-based optimization of pharmaceutical removal through the manipulation of CW design and configurations.

2.2. Introduction

The release of pharmaceuticals into aquatic environments has been an ongoing regulatory and research concern for over two decades (Li et al., 2017). Despite the low concentrations at which these compounds are commonly found in the aquatic environment (ng L^{-1} to µg L^{-1}), pharmaceutical bioactivity and their potential chronic effects on aquatic species continue to be relevant ecological concerns (Carvalho et al., 2014; Gorito et al., 2017). Conventional wastewater treatment plants are not designed explicitly to eliminate pharmaceuticals and other organic contaminants, thus their removal by these systems is often incomplete (Hester and Harrison, 2016). Moreover, the large-scale application of conventional wastewater treatment is often cost-prohibitive (Li et al., 2014), which limits its application in poorer or remote regions. This means that pharmaceutical concentrations in effluents may be greater in such locales, and therefore their receiving environments may be at greatest ecological risk.

Constructed wetlands (CWs) have been increasingly seen as a financially viable alternative compared to conventional wastewater treatment practices for select communities and wastewater streams. CWs are low-cost and nature-based treatment technologies used to treat wastewater, especially in areas where land availability is not a limiting issue and budgets more restricted (Kadlec et al., 2009). CWs have been found to be efficient in removing conventional contaminants, such as nutrients, solids, and microorganisms from

diverse wastewater streams, including those of domestic, agricultural, and industrial origins (Kadlec et al., 2009; Vymazal, 2009). The feasibility of CWs for pharmaceutical removal from wastewater requires a comprehensive understanding of removal efficiencies, removal mechanisms, the influences of design and environmental factors, and toxicity of the compounds to the wetland itself (Li et al., 2014).

CWs are classified into surface free water CWs (SF-CWs), horizontal subsurface flow CWs (HSSF-CWs), vertical subsurface flow CWs (VSSF-CWs) and hybrid CWs (H-CWs) (Li et al., 2014), each of which comes with their own unique features, strengths and limitations, discussed further below.

SF-CWs are composed of shallow channels or basins planted with vegetation, including rooted and floating plants, in which wastewater flows at relatively shallow depth over an impermeable bottom liner or a packed substrate layer. They have areas of open water and are similar in appearance to natural marshes (Kadlec et al., 2009). SF-CWs contain areas of open water, floating vegetation, and emergent plants, either by design or as an unavoidable consequence of design configuration. SF-CWs closely mimic natural wetlands, attracting a wide variety of wildlife including insects, mollusks, fish, amphibians, reptiles, birds, and mammals (Kadlec and Knight, 1996). SF-CWs are suitable in most climates, including polar environments. However, ice formation can hydraulically rule out winter operation, and the rates of some removal processes are slower for cold water temperatures (Kadlec et al., 2009). For instance, when ice covers the open water, the transfer of oxygen from the atmosphere is reduced, decreasing oxygen-dependent treatment processes. It is generally more efficient to store water during winter and treat it during the warm part of the year (Kadlec et al., 2009).

In HSSF-CW systems, wastewater is pumped into the wetland at the inlet zone and flows horizontally through the substrate under the surface of the wetland bed, which can be planted with vegetation. After treatment, the effluent is collected at an outlet zone. Typically, these systems employ a gravel bed (Kadlec et al., 2009). HSSF-CWs consist of gravel or soil beds planted with wetland vegetation. They are typically designed to treat primary effluent prior to either soil dispersal or surface water discharge. The wastewater is intended to stay beneath the surface of the media and flows in and around the roots and rhizomes of the plants. Because the water is not exposed during the treatment process, the risk associated with human or wildlife exposure to pathogenic organisms is minimized (Kadlec et al., 2009). HSSF-CWs are generally more expensive that SF-CWs, and are commonly used for secondary treatment in small communities (Cooper et al., 1996). In general, HSSF have been utilized at lower flow rates than SF-CWs, because of cost and space considerations (Kadlec et al., 2009). HSSF-CWs are typically comprised of inlet piping, a clay or synthetic liner, filter media, emergent vegetation, berms, and outlet piping with water level control. These systems are capable of operating under colder conditions than SF-CWs because of the ability to insulate the top, though a key operational consideration is the propensity of clogging of the media (Kadlec et al., 2009).

In VSSF-CW systems, wastewater is applied onto the surface of the wetland bed and then flows vertically from the planted layer down through the substrate until it reaches the outlet zone. Water is treated as it percolates through the plant root zone VSSF-CWs have been used in North America as vegetated recirculating gravel filters. These systems have been used to minimize oxygen transfer, while fill-and-drain systems have been implemented to treat water to oxidize ammonia (Kadlec et al., 2009).

Various types of CW may be combined in order to achieve higher removal efficiency. Hybrid CW systems are a combination of two or more types of CWs or the joint configuration of wetlands along with pond systems, such as facultative lagoons, to perform wastewater treatment in series or in parallel (Li et al., 2014). Most commonly used hybrid systems comprise a vertical flow stage followed by horizontal subsurface wetland cells. Hybrid systems are receiving attention in most European countries because of more stringent requirements for ammonia removal (Kadlec et al., 2009).

Overall, the selection of a particular type of CW depends on the nature of the contaminants to be removed, as well as land availability and the on-site technical capacities. CWs were not designed for the removal of pharmaceuticals, though the study of every type of CW has advanced what is known in terms of the potential of each type to attenuate them. The study of contaminant removal efficiency through CWs is generally very complex, as many factors both biotic and abiotic can influence the reported performance (Li et al., 2014). For instance, temperature has been reported to play a role in the efficient functioning of macrophytes and microorganisms in CW systems (Kadlec et al., 2009). CW design aspects such as area, depth, hydraulic loading rate, hydraulic retention time, and physicochemical parameters such as temperature and pH have been highlighted to influence pharmaceutical degradation to a certain extent (Ilyas and van Hullenbusch, 2019). Seasonal variation is a primary aspect that affects the removal of some contaminants in CWs, as it has been previously reviewed for regulatory parameters (e.g., total nitrogen, biochemical oxygen demand, chemical oxygen demand) (Varma et al., 2021). Nevertheless, no comprehensive analysis has considered the suitability of CWs across varying seasons for pharmaceutical removal. Studies have observed simple wastewater treatment practices in the Arctic through the use of septic tanks, facultative ponds and small wetlands to achieve a

limited efficacy of treatment (for instance, Chaves-Barquero et al., 2016 and Stroski et al., 2020). More complex CW systems have been observed in temperate areas in Europe, America and Asia, either single step or hybrid CWs (e.g., Matamoros et al., 2009; Avila et al., 2015; Auvinen et al., 2017a). In the tropics, studies have used CW technologies at warm temperatures to make use of increased microbial activity to perform increased pharmaceutical degradation (e.g., Zhang et al., 2012; Moeder et al., 2017)

Previous review studies have summarized and critically reflected on the existing knowledge around the performance of different types of CWs for the removal of pharmaceuticals. Most reviews have been conducted in order to summarize the current state of knowledge (Carvalho et al., 2014; Ekperusi et al., 2019; Gorito et al., 2017; Ilyas and van Hullebusch, 2020; Imfeld et al., 2009; Li et al., 2014; Verlicchi and Zambello, 2014; Zhang et al., 2014), but did not quantitatively assess the evidence or the relevance of the observed effects of CW to remove pharmaceuticals. A summary of these reviews, their approaches, and main conclusions is available in Table 2.1. Other relevant efforts have been conducted to better understand CW performance towards meeting effluent quality goals for reuse, including monitoring of contaminants of emerging concern (WateReuse Research Foundation, 2011).

Overall, these reviews have reported persistent knowledge gaps associated with the effect of CW design parameters in pharmaceutical attenuation, mostly on pilot- and full-scale systems. Moreover, the diversity of pharmaceuticals in wastewater has rendered the study of individual compound removal a complex task. Some consensus has been established for the increased efficiency of hybrid CWs for overall pharmaceutical removal compared to surface and subsurface flow systems (Ilyas et al., 2020). An example is presented by Vymazal et al., (2013) through the study of the combination of two types of

CW to achieve increased removal efficiencies compared to single-step systems. Nevertheless, knowledge gaps persist regarding the optimization of CW design parameters to attain pharmaceutical removal from wastewater.

To our knowledge, no previous review has critically examined the available literature on pharmaceutical removal by CWs using any principles or tools based on the quantitative weight-of-evidence (QWoE) philosophy. QWoE offers an evidence-based framework to conduct a standardized evaluation of the relevance of reported observations (ROO) and strength of methods (SOM) used on each work to produce a comparative analysis and a summary of knowledge gaps on the matter. Weight of evidence (WoE) has previously been identified as a valuable process for evaluating the results of scientific research and combining these into an objective conclusion, as called by and editorial in *Nature*, which recommended WoE approaches to assess studies for the purposes of regulatory decision-making (Nature 2010). The term "weight of evidence" has been used in legal context as well as in the scientific literature. However, the term has been widely used and is most often used metaphorically, where WoE refers to a collection of studies or to an unspecified methodological approach, but the term and its associated process is seldom formalized (Weed, 2005). More recently, WoE has begun to be used in more formal and quantitative ways in the assessment of a variety of risks in ecotoxicology, for instance endocrine disruption (Borgert et al. 2012), and pesticides (van der Kraak et al. 2014, Hanson et al., 2019), and also in contaminant fate evaluation (Challis et al. 2014). The proposed application of the QWoE framework is valuable for CW study, as it can produce quantitative outcomes from the analysis of the evidence presented in various research items, hence opening the possibilities for comparisons (e.g., within types of CW, and climate) and knowledge gap elucidation for both ROO and SOM aspects. That having been

said, attention must be dedicated to minimize bias evaluation from systematic review processes in general. Recent concerns have been raised over the use of numeric based scoring to assess previously published papers in terms of their reported hazards to humans or ecosystems (National Academies of Sciences, Engineering and Medicine, 2021). Hence, it is important for researchers to continue re-evaluating the transparency and robustness of their systematic review processes moving forward.

The main objective of this critical review is to evaluate the available scientific peer-reviewed evidence regarding pharmaceutical removal in constructed wetlands (CWs) using a QWoE-based methodology. The specific objectives of this critical review, with regards to the body of literature, are: 1) assess the relevance of observed pharmaceutical attenuation through CW treatment; 2) evaluate the strength of methods used to determine pharmaceutical removal via CW treatment; 3) compare the removal efficiency of individual pharmaceuticals within and between various types of CW; and 4) test if there are seasonal or climate-based differences in pharmaceutical removal within and between various types of CW. Our hypotheses regarding the QWoE assessment are, respectively: 1) CW are relevant for the removal of some pharmaceuticals; 2) methods used for quantifying the removal of pharmaceuticals are generally strong; 3) overall, tandem-hybrid CWs perform more efficiently compared to single-stage CWs; and 4) an increased removal for pharmaceuticals is achieved at greater seasonal temperature.

Table 2.1. Summary of the approaches taken by previous reviews on pharmaceutical removal from constructed wetlands.

Reference	Description of included CW	Approach	Findings
Imfeld et al., 2009	Types: free-surface, and sub-surface. CW are described as shallow ecosystems designed to use intrinsic processes for water quality improvement.	A description of the removal processes is presented, with focus on physicochemical and biological mechanisms. Also, relevant characteristics of wetland systems are briefly discussed.	Points to the need for approaches and techniques from contaminant hydrology, environmental microbiology, and biotechnology, phytoremediation, statistics and environmental modelling to enhance future investigations.
Carvalho et al., 2014	Surface flow, subsurface flow; free-floating, with emergent and/or submerged macrophytes, and horizontal or vertical. Engineered wetlands that utilize natural processes involving vegetation, soil and their associated microbial assemblages to treat wastewater or other polluted water sources.	Summarized the available knowledge on pharmaceutical phytotoxicity, uptake and removal by plants.	Despite the reported occurrence of pharmaceutical plant uptake, it usually represents a low percentage of the mass depleted from the studied systems. Also, it is not expected that phytotoxic effects occur in phytoremediation designed systems.
Li et al., 2014	Low-cost systems used for the removal of contaminants from wastewater. Types: surface free water CWs, horizontal subsurface flow CWs, vertical subsurface flow CWs, hybrid CWs	Summarized the role of design parameters (e.g., physical configuration, hydraulic mode, and vegetation species) in the removal of pharmaceuticals.	CWs hold great potential of being used as an alternative secondary or tertiary wastewater polishing treatment system. Though, scarce data makes difficult to convey persuasive demonstrations for the performance and

Reference	Description of included CW	Approach	Findings
			effectiveness of CWs in these applications.
Verlicchi and Zambello, 2014	Surface flow, and subsurface flow CWs, hydroponic gravel beds and restoration wetlands. Studied systems were fed only by urban municipal wastewater, and acted as primary, secondary or tertiary steps. Also, hybrid systems were considered	Reviewed the removal of several pharmaceuticals by CWs used for primary, secondary, and tertiary treatment purposes	Further research is needed to better evaluate CW design parameters, to optimize removal of priority compounds (antibiotics and some analgesics and anti-inflammatories), and to better assess the risk posed in aquatic environment of pharmaceuticals
Zhang et al., 2014	The review refers to aquatic plant-based systems. Systems used as alternatives for secondary and tertiary wastewater treatment were included, both surface and subsurface wetlands.	Provided an overview of the state of research on the removal of pharmaceuticals and personal care products (PPCP) by secondary and tertiary CWs, focusing on an evaluation of the key mechanisms for the removal of these contaminants, and the effect of design parameters.	Detailed investigation of removal processes (e.g., sorption, plant uptake, biological degradation) are scarce and new research is needed. CW design parameters (e.g., flow, presence of vegetation, operational modes, hydraulic retention time) affect the fate of PPCP, but more field investigations are needed to better understand this influence, and to address a wider range of PPCP.
Gorito et al., 2017	CW of interest were either surface or subsurface flow systems treating wastewaters, with interest on specific contaminants.	Discussed the removal processes and influence of design and operation parameters on the removal of four pharmaceuticals by CWs (azithromycin,	This work suggests areas of research that have been poorly explored: seasonality of removal, varying operating conditions (e.g., configuration, light penetration,

Reference	Description of included CW	Approach	Findings
		clarithromycin, diclofenac and erythromycin), which are on the priority list of the European Union (EU).	residence time), CW experiments with inlet solutions spiked with pharmaceuticals for a multiple analyte removal analysis, and the study of potential transformation products appearing during treatment.
Ekperusi et al., 2019	Surface flow CW included horizontal floating macrophytes CW, emergent macrophytes CW, and submerged macrophytes CW. Subsurface flow CW included horizontal and vertical systems. All of the above contained *Lemna minor* macrophytes.	Reviewed the role of plants (duckweed-*Lemna minor*) in the removal of various pharmaceuticals.	*Lemna minor* is diagnosed as effective for the remediation of pharmaceuticals at laboratory scale. Further studies are required in greater scales to better understand the application of L. minor for wastewater treatment.
Ilyas et al., 2020	Four types of CW were studied: surface flow CW, horizontal flow CW, vertical flow CW and hybrid CW.	Conducted a review to evaluate and summarize the treatment performance of different types of CWs (surface flow, subsurface flow, hybrid) for the removal of 29 pharmaceuticals and 19 transformation products.	Hybrid CWs showed a better performance compared to surface and subsurface flow CWs, due to a co-existence of aerobic and anerobic conditions, and longer hydraulic retention times. The use of artificial aeration is reported to improve removal of pharmaceuticals.

2.3. Methods

2.3.1. Literature search

Papers for inclusion in the study were obtained via searches of the Web of Knowledge and SciFinder databases as of June 2021. The following search terms were used in various combinations: constructed, wetland, wastewater, pharmaceutical, removal. A total of six criteria were applied for paper inclusion/exclusion: 1) research papers must be peer-reviewed; 2) papers must be written in English; 3) papers must not be reviews or critical review papers; 4) papers must report on the fate of at least one pharmaceutical as analyte of interest by a CW in domestic or hospital wastewaters, 5) when the same research is published in more than one journal, only one of the references is included for evaluation; and 6) papers must study CW technologies: for instance, surface-flow SF-CW (e.g., Berglund et al., 2014; Dordio et al., 2010), HSSF-CW (e.g., Matamoros et al., 2006; Delgado et al., 2020), VSSF-CW (e.g., de Oliveira et al., 2019) or H-CW (e.g., Matamoros et al., 2016; Vystavna et al., 2017) at laboratory-, mesocosm-, pilot- or full-scale. For the purposes of this review, a CW is defined as a low-cost engineered ecosystem designed for the improvement of wastewater quality, as previously described elsewhere (Kadlec et al., 2009). Studies reporting on stabilization pond performance were also considered in our analysis to obtain a defined scope based on systems with fixed substrates. Thus, any other type of CW was excluded from this exercise (e.g., floating treatment wetlands).

2.3.2. Metadata collected from papers

A summary of the metadata collected from papers that screened in is shown in Table 2.2. General aspects were registered for research items, such as author information, year of publication, country where the research was conducted. Relevant aspects about CW design

were also collected, including scale of the CW, level of treatment achieved, type of wastewater, depth, surface area, hydraulic retention time, age of the system, type of substrate, seasonality and type of plants to provide context to interpret the findings (Ilyas and Hullenbusch, 2019).

Table 2.2. Metadata collected for evaluated peer-reviewed papers.

Aspect	Description
Author	Author(s) of the study.
Country	Country where research took place.
Year	Year of the study.
Scale	Scale of the studied CW (laboratory, mesocosm, pilot- or full-scale)
Level of treatment	Level of treatment intended to be achieved with the studied CW(s) (primary, secondary, or tertiary)
Wastewater type	A description of the origin of treated wastewater (e.g., synthetic, domestic).
Analyte	Pharmaceuticals that are quantified in the study.
Depth	Depth of the studied CW(s), in meters.
Area	Area of the studied CW(s), in square meters.
Temperature	Range of temperatures reported in the study, in Celsius degrees.
pH	Range of pH values reported in the study.
Hydraulic retention time (HRT)	Reported HRT of the studied CW(s), in days.
System age	Reported age of the studied CW(s), in months.
Experiment duration	Duration of the experiment(s), in months.
Type of substrate	Type of substrate used in the studied CW(s).
Type of plants	Type of plants used in the studied CW(s).
Influent and effluent concentration	Concentrations reported at the influent and effluent of the studied CW(s), in micrograms per liter.
Removal rate	Rate of removal of studied pharmaceuticals, in milligrams per square meter-day.
Removal percentage	Rate of removal of studied pharmaceuticals, expressed as percentage.

2.3.3. Scoring rubric description and rationale

The approach proposed in this critical review consists of a customized rubric (Table 2.3.) with separate sections to evaluate two main aspects of the research: relevance of the observation (ROO) and strength of methods (SOM). ROO aspects refer to how efficient can a CW be to remove a reported pharmaceutical, and include magnitude of removal, statistical significance of removal, consistency of removal, residence time needed for removal, and CW scale. SOM categories refer to quality of the study design and data reported in each study. A total of three sub-categories were evaluated for SOM: 1) analyte characterization and fate; 2) matrix and environmental monitoring; and 3) experimental

design and QA/QC. The rubric scale ranks from 0-4 as whole integers, with subsequent normalization of the scores to obtain final scores ranked from 0-1. The normalization consists on dividing the obtained score by the maximum score that the specific aspect can obtain (for instance, if the maximum score is 2, and the obtained score is 1, a normalized score of ½ = 0.5 is obtained). It is important to note that normalization of the scores is needed due to different maximum scores that are possible for the various evaluation aspects, providing equal weighting to all aspects. After normalization, the scores are summed, and final scores for ROE and SOM are obtained for each pharmaceutical on each paper. The decision to employ equal weighting is based on the current lack of evidence to establish some aspects as more critical than others regarding pharmaceutical-specific removal by CWs. The various evaluated aspects are described in detail in Table 2.4.

Table 2.3. Scoring rubric used to evaluate each study regarding pharmaceutical removal in constructed wetlands under various climates. Normalized scores are calculated in a scale of 0-1 on each question by dividing the score of each question by the maximum possible score the question can obtain. NS: normalized score.

Relevance of Observation (ROO)	**0 (No)**	**1 (Yes)**	**2**	**3**	**4**	**Normalized Score**
Magnitude of removal	No removal	Up to 25%	25-50%	50-75%	75-100%	
Statistical significance of removal	Not statistically significant	Inconsistent internally	Statistical significance			
Consistency of removal	Inconsistent	Consistent				
Residence time for removal	No removal / Not reported	> 24 h	< 24 h			
CW scale		Lab/Microcosm	Mesocosm	Pilot	Full	
SUM OF TOTAL NS SCORES FOR ROO CATEGORIES						
Strength of Methods (SOM)	**0 (No)**	**1 (Yes)**	**2**	**3**	**4**	**Normalized Score**
Group A – Analyte characterization and fate						
Description of the origin and purity of reagent and standards	Insufficient or non-existent	Sufficient details				
Environmental relevance of concentrations	Irrelevant	Close to environmental concentrations (i.e., µg/L)				
Hydraulic loading	Not characterized	Characterized				
Mass balance	Not presented	Presented				
Biotic/Abiotic CW processes	Not characterized	Sorption (1), Microbial degradation (1), Plant uptake (1), Photodegradation (1)				
Strength of Methods (SOM)	**0 (No)**	**1 (Yes)**	**2**	**3**	**4**	**Normalized Score**

Group B – Matrix and environmental conditions						
Environmental parameters characterization	Insufficient or non-existent	Sufficient description of pH (1), temperature (1), light-flux (1), weather conditions (1)				
Nature of the wastewater matrix	Pharms alone or in mixtures in water	Pharms mixtures with nutrients in water	Synthetic wastewaters with pharms	Natural wastewater streams with spiked pharms	Natural wastewater streams with background pharm levels	
Group C – Experimental design and QA/QC						
Sampling frequency	Not reported	Less than monthly	Monthly	Biweekly	Weekly or more	
Data transparency	Critical data not provided	Summary and aggregate provided	All raw data is available			
Instrumental analytical conditions, including LOQ and LOD	No details	Insufficient details	Sufficient details			
SUM OF TOTAL NS SCORES FOR SOM CATEGORIES						

Table 2.4. Scoring criteria for the weight-of-evidence-based analysis, its description, rationale, and examples. NS: normalized score; CW: constructed wetland(s); LOQ: Limits of Quantification; LOD: Limits of Detection.

Criteria	Description	Rationale	Examples
Relevance of Observation			
Magnitude of removal	Evaluates quantitatively the removal of pharmaceuticals. Greater percent removals suggest a greater relevance of observation.	Percent removals have been used as the main criteria to describe quantitatively how much of each analyte can be removed by CWs (e.g., Ilyas et al., 2020; Li et al., 2014).	A percent removal of 40% gets a score of 2, and a NS of 0.5.
Statistical Significance of removal	Evaluates the statistical significance of removal of pharmaceuticals. Significant removals suggest stronger relevance of the observation.	Statistical significance of pharmaceutical removal helps determine whether analyte removal is actually relevant, as seen in previous reviews (e.g., Li et al., 2014; Verlicchi and Zambello, 2014).	A statistically significant removal obtains a score of 2, and a NS of 1.
Consistency of removal	Assesses the observed consistency in pharmaceutical removal. Consistency is desirable to convey greater relevance.	When the performance of a CW is inconsistent, it becomes less relevant towards the observation. This would directly affect the assigned score for relevance of the observation.	If there are some inconsistencies in removal, a score of 1 is assigned, with a NS of 0.5.
Residence time for removal	Evaluates the residence time of wastewater in the CW to achieve the reported pharmaceutical removal.	Residence time is related with CW design and the efficiency of removal processes within the wetland. Hence, if removal is achieved in short residence time, the relevance of the observation increases.	If reported residence time is less than 24 hours, a score of 1 is assigned, with a NS of 0.5.
CW scale	Evaluates the scale of work of the reported CW(s) in the study.	Compared to smaller systems, full-scale studies are considered more representative of actual conditions in wastewater treatment facilities, as noted in previous reviews (e.g., Li et al., 2014).	Studies that report on a full-scale system obtain a score of 4, with a NS of 1.

Criteria	Description	Rationale	Examples
Strength of Methods Group A – Analyte characterization and fate			
Description of the origin and purity of reagents and standards	Evaluates the availability of a complete description of reagents and standards.	Robust studies are able to provide information on reagents and standards (e.g., their origin, purity and/or concentration) as these aspects are important for analyte quantification.	If sufficient details are provided, a score of 1 is assigned, with a NS of 1.
Environmental relevance of concentrations	Evaluates the pharmaceutical concentrations, as they relate to typical environmental levels.	It has been recommended that CW studies should mimic environmental concentrations to better represent actual conditions in real-world systems (e.g. Li et al., 2014; Imfeld et al., 2009).	If concentrations are environmentally relevant, a score of 1 is assigned, with a NS of 1.
Hydraulic loading	Evaluates the reporting of hydraulic loading in the study.	Hydraulic loading values are important to quantify the size and removal capacity of a determined CW (in volume per unit area per unit time). In order to better assess for removal of contaminants, hydraulic loading should be reported.	If hydraulic loading is reported, a score of 1 is assigned, with a NS of 1.
Mass balance	Evaluates the availability of mass balance data for the pharmaceuticals studied.	Mass balance calculations speak to considerations of mass conservation, and in turn provide a detailed analysis of pharmaceutical fate in CWs. A study that presents a mass balance is considered stronger than a study that does not present it.	If a mass balance is presented in the study, a score of 1 is assigned, with a NS of 1.
Biotic/abiotic processes	Evaluates whether biotic and abiotic processes were addressed specifically during the study.	The study of the potential influence of biotic/abiotic processes (e.g., sorption, microbial degradation, plant uptake, photodegradation) are important to understand their specific contributions to overall pharmaceutical removal. A study that can report on these aspects is considered of greater strength.	A study that only reports on sorption and photodegradation only, obtains a 2, with a NS of 0.5.

Criteria	Description	Rationale	Examples
Strength of Methods Group B – Matrix and environmental conditions			
Environmental parameters characterization	Addresses the level of clarity and availability of reported data for environmental parameters.	Monitoring of environmental parameters imply a good control of the parameters that could influence pharmaceutical removal in a CW. pH, temperature, light-flux and weather conditions are recommended to ensure data quality.	Whenever this information is clear and available in sufficient detail for the analyte being evaluated, a score of 2 will be obtained, with a NS of 1.
Nature of wastewater matrix	Evaluates the wastewater matrix type used in the study.	Systems reporting on natural wastewater streams are considered representative of matrices found in real-world conditions, as opposed to synthetic wastewater or stock solutions, as per previous reviews (e.g., Li et al., 2014).	A study that reports on natural streams containing background pharmaceutical levels obtains a score of 4, with a NS of 1.
Strength of Methods Group C – Experimental design and QA/QC			
Sampling frequency	Evaluates the sampling program used to collect data	Increased sampling frequency allows for better repeatability of the study observations, allowing for a more detailed evaluation of removal and performance consistency.	For biweekly sampling, a score of 2 is assigned, with a NS of 0.5.
Data transparency	Evaluates the availability of a complete description of reagents and standards. Information on reagents and standards (e.g., their origin, purity and/or concentration) must be clearly stated.	Stronger studies are able to report their raw data in a transparent way to make the information available for reprocessing by the scientific community if needed.	A study that reports all raw data obtains a score of 2, with a NS of 1.
Instrumental analytical conditions, including LOQ and LOD	Assesses a complete description of the analytical conditions used to conduct chemical analysis.	Stronger studies are careful of their good laboratory practices and QA/QC. A complete description of the analytical conditions, including LOD and LOQ is needed to ensure data quality.	Whenever this information is clear and available in sufficient detail for the analyte being evaluated, a score of 2 will be obtained, with a NS of 1.

2.3.4. Scoring interpretation and presentation

The maximum possible score for SOM is 10 and for ROO is 5. Studies were considered of "sufficient" quality and/or relevance if the obtained overall scores for SOM and ROO were greater than 6.0 and 3.0, respectively. While these rankings are somewhat arbitrary and specific to this rubric only, they are largely based on the average overall score for all of the included studies, which is approximately 60% of the maximum score. If the obtained scores for SOM or ROO for a specific paper were lower than these values, the evidence presented is considered of "insufficient" quality and/or relevance. An example score plot is shown in Figure 2.1., with mock SOM (y-axis) and ROO (x-axis) scores plotted for four pharmaceuticals, to orientate the reader as to how these score plots should be interpreted. The quality of data generated by a given study can be understood through the scores of given pharmaceuticals, based on where they fall on the plots. In general, compounds clustering closer to the upper right corner of these plots suggest "sufficient" relevance of the observation, and "sufficient" quality of the methods used, while the lower left corner implies low quality data. The purpose of the exercise is to represent graphically the quality of the totality of data for specific pharmaceuticals within a class, via the rubric scores. The scoring of studies in this case is a surrogate for the quality of data as defined by this exercise, so a low score implies low quality data, while a high score implies high quality data.

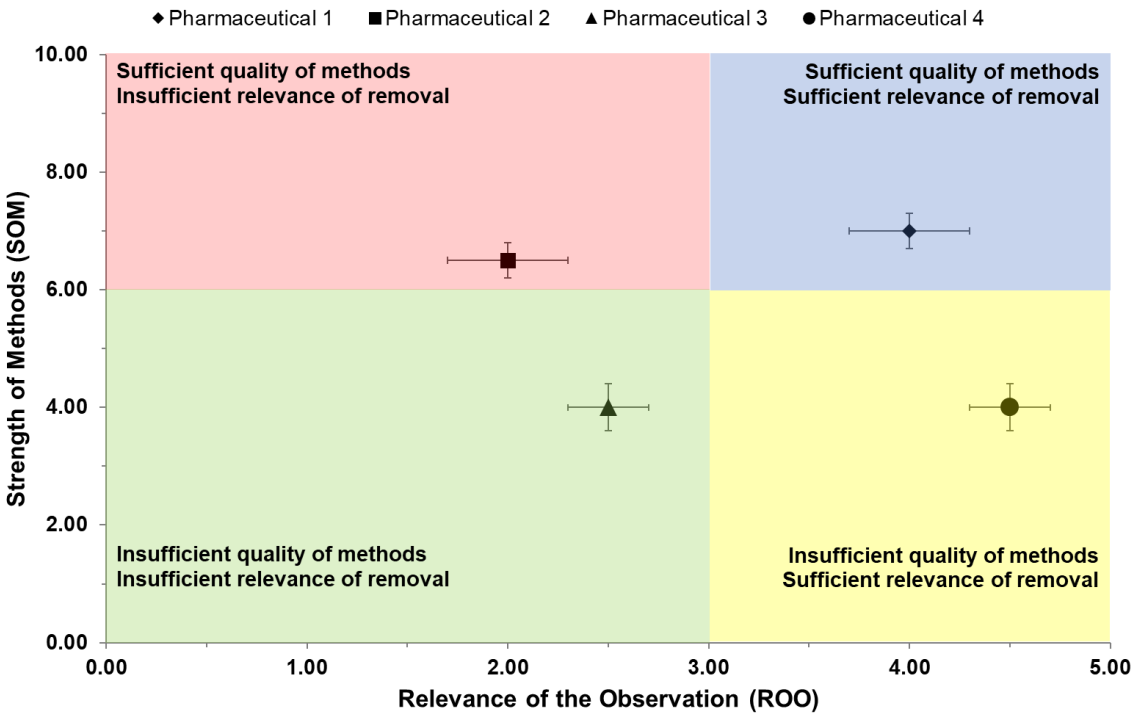


Figure 2.1. Model score plot of "mock" scores for strength of methods (SOM) and relevance of observation (ROO) for four hypothetical pharmaceuticals. The different colour areas represent different performance categories regarding ROO and SOM, defined through a normal distribution around the average scores for all research items included in the WoE analysis. Error bars represent the standard error of the mean score.

2.3.5. Statistical analyses

To test our hypotheses, the ROO and SOM scores were classified by analyte, by pharmaceutical class, by CW type (e.g., H-CWs vs. single-step CWs), by seasonality: warm season (ranging from late spring to late summer) vs. cold season (ranging from fall to early spring); and by climate: polar (i.e., cold regions that lack warm summers) vs. temperate (i.e., regions with a cold winter and a warm summer) vs. tropical (i.e., warm regions that lack cold winters) to evaluate whether our exercise can determine significant performance differences across the studies. Statistical comparison of independent data groups was performed by non-parametric Kruskal-Wallis tests, followed by post-hoc Nemenyi tests as needed. Differences were considered significant at $p < 0.05$.

2.4. Results and Discussion

2.4.1. Summary of included papers for WoE evaluation

A total of 93 journal articles were obtained using the defined search terms. After applying our inclusion/exclusion criteria, 74 papers were included for scoring and evaluation. An example of the application of our rubric is available in Table A1. A time-based cumulative description of the literature is shown in Figure 2.1. Of these papers, 81% were published in the last ten years (2011-2021) and 34% in the last four years (2018-2021), suggesting a sustained interest from the scientific community in the topic during the last decade (Figure 2.2). Of the 74 papers, 63 studies addressed pharmaceutical removal under temperate climate, nine under tropical conditions, and two were performed in polar regions.

A description of the geographical distribution of the research items can be found in Figure A1. Studies from North America, South America, Asia and Europe were included in our analysis, with Spain and Canada as the countries with the most included research items (18 and 10, respectively). The most investigated type of CW was HSSF-CW, with SF-CW as the second most studied (Figure 2.3). The most popular type of wastewater in the studies was domestic, with synthetic wastewater as the second most studied (Figure 2.4), the most used substrate was gravel (Figure 2.5), and the most popular plant included in the CW systems was the common reed (*Phragmites australis,* Figure 2.6).

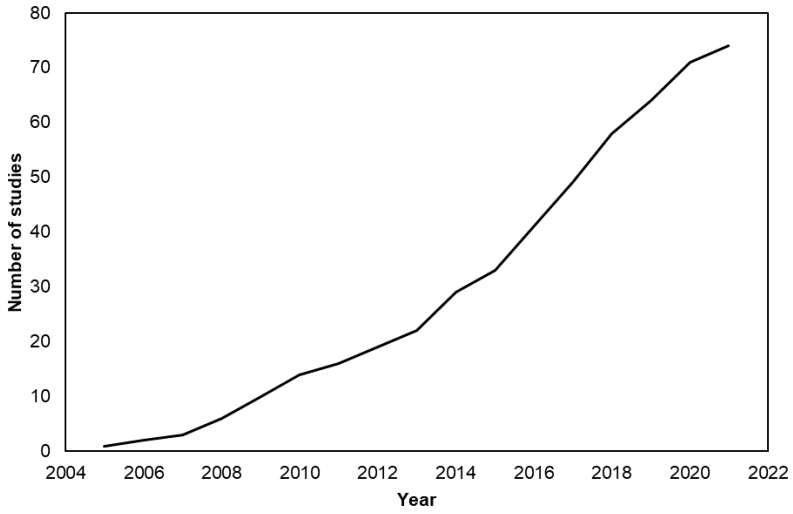


Figure 2.2. Cumulative total of publications over time for the various papers sampled and included in the present review.

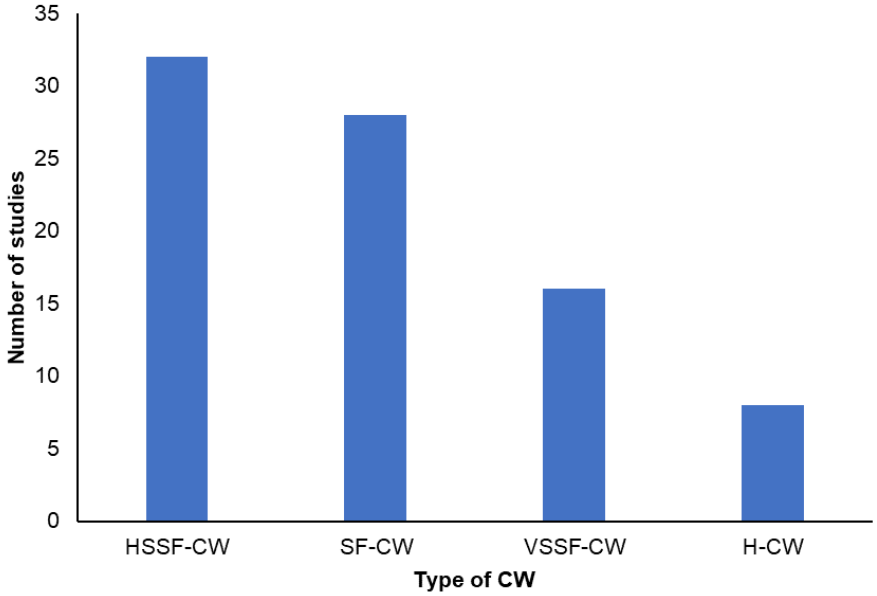


Figure 2.3. Description of the type of CW addressed in the selected papers for the present review (SF-CW: surface flow-CW, HSSF-CW: horizontal subsurface flow-CW, VSSF-CW: vertical subsurface flow-CW, H-CW: hybrid-CW). More than one type of CW was used in some of the papers, hence the sum is greater than the total number of papers.

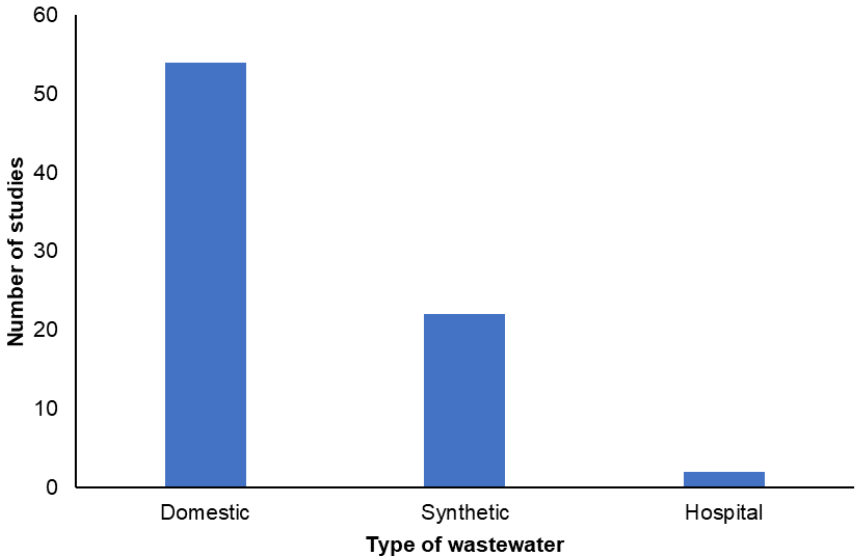


Figure 2.4. Description of the type of wastewater addressed in the selected papers for the present review. More than one type of wastewater was used in some of the papers, hence the sum is greater than the total number of papers.

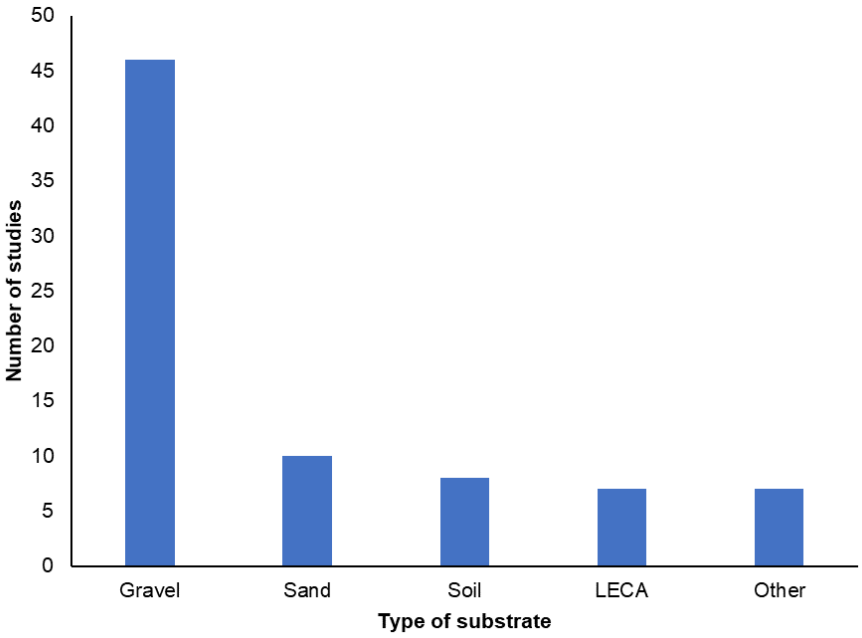


Figure 2.5. Description of the type of substrate used in the selected papers for the present critical review. More than one type of substrate was used in some of the papers, hence the sum is greater than the total number of papers. LECA: light expanded clay aggregate.

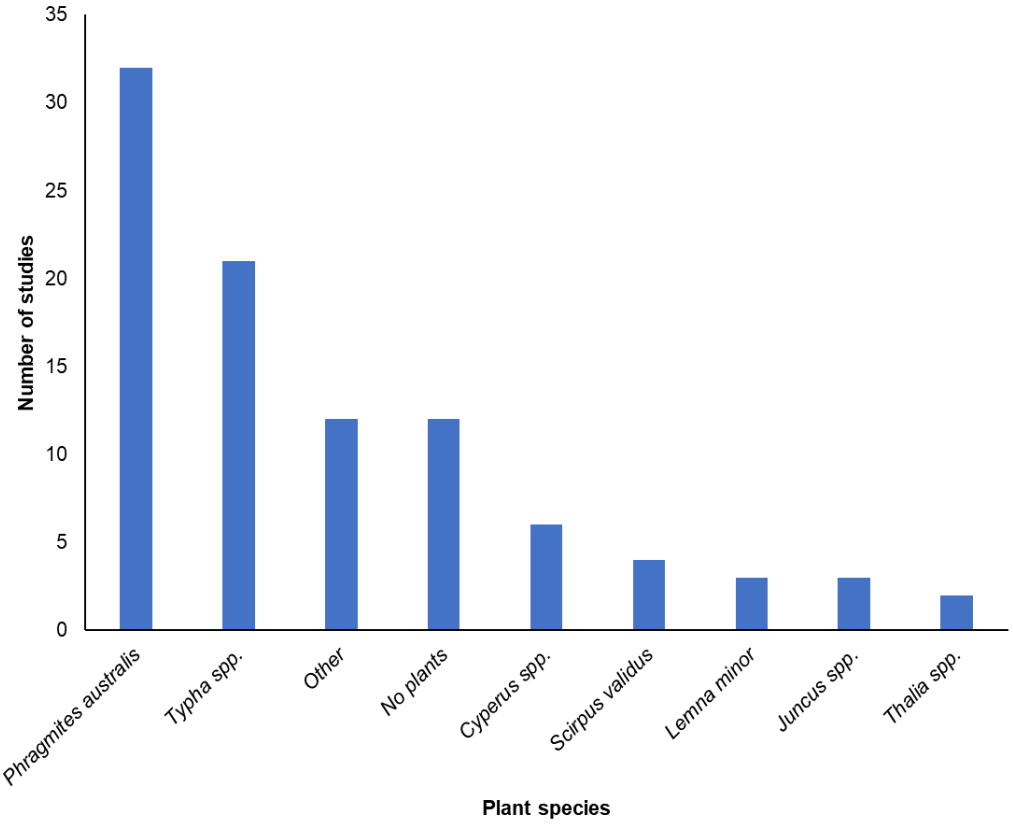


Figure 2.6. Description of the type of plants reported in the selected papers for the present review. More than one type of plant was used in some of the papers, hence the sum is greater than the total number of papers.

2.4.2. Occurrence and removal of pharmaceuticals in CWs

This review exercise allowed an evaluation of the removal of 48 compounds from CWs. The reported pharmaceuticals belong to several drug classes: steroidal anti-inflammatory drugs (NSAIDs; e.g., ibuprofen, diclofenac, naproxen, and ketoprofen); non-NSAID analgesics (e.g., salicylic acid, acetaminophen, tramadol, and codeine); β-blockers (e.g., metoprolol, atenolol, sotalol, propranolol, and bisoprolol); sulfonamide antibiotics (e.g., sulfamethoxazole, sulfapyridine, sulfadiazine); macrolide antibiotics (e.g., erythromycin, roxithromycin, clarithromycin, azithromycin and tylosin); tetracycline antibiotics (e.g., tetracycline, doxycycline); lipid-lowering fibrates (e.g., bezafibrate, gemfibrozil, clofibric acid); fluoroquinolones (e.g., ciprofloxacin, norfloxacin, ofloxacin,

and lomefloxacin); anticonvulsants (e.g., carbamazepine, gabapentin); diuretics (e.g., furosemide, hydrochlorothiazide); benzodiazepines (e.g., diazepam, alprazolam); lincosamide antibiotics (e.g., lincomycin, clindamycin); the alpha-blocker alfuzosine, the antihypertensive telmisartan, the antibiotic trimethoprim, the antidepressant venlafaxine, the antiviral acyclovir, the antifungal fluconazole, the H2 blocker ranitidine, the statin atorvastatin and the stimulant caffeine. Overall, 72 experiments were reported during warm seasons, while 14 were executed during cold seasons. Some research items included experiments conducted in more than one season. The top ten studied pharmaceuticals were ibuprofen, diclofenac, carbamazepine, naproxen, caffeine, ketoprofen, sulfamethoxazole, metoprolol, atenolol, and salicylic acid (Figure 2.7).

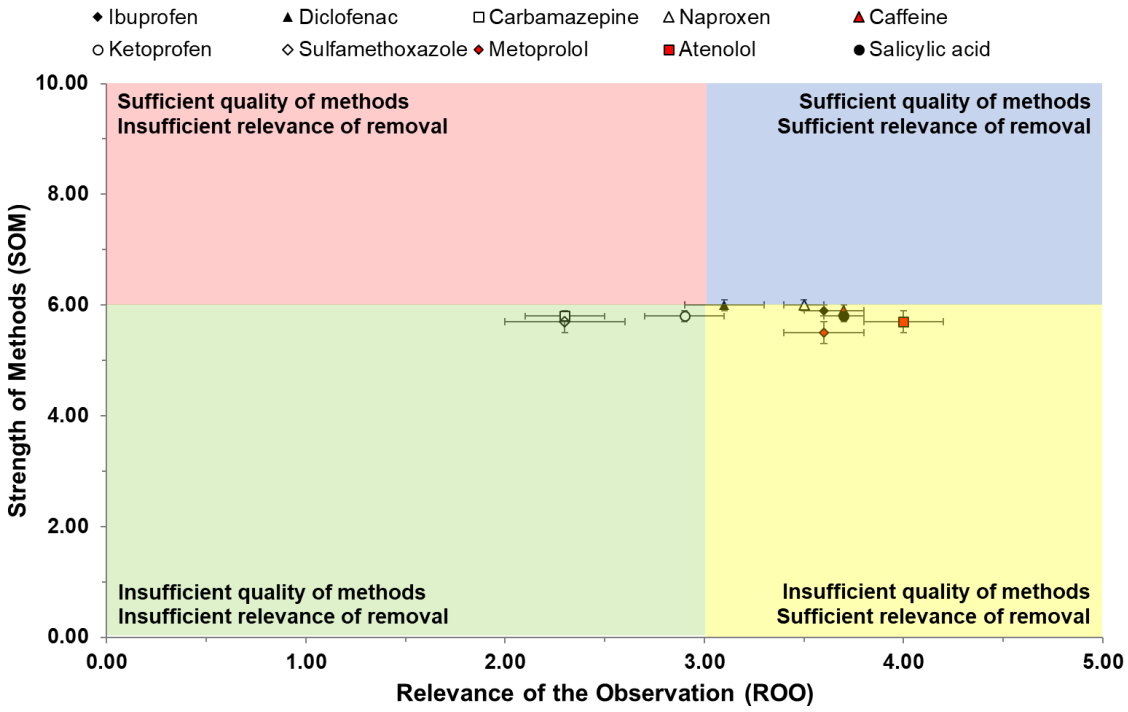


Figure 2.7. Mean score plot for the ten most studied pharmaceuticals in the present review. Error bars depict standard error of the mean.

Table 2.5. Summary of calculated ROO and SOM scores (maximum of 6 and 10, respectively) for most relevant pharmaceuticals in the present review, organized by frequency of scoring (n). The reference with the greatest SOM is highlighted in bold font. A full list of scores is available in Table A6.

Pharmaceutical	n	MEAN ROO	SE ROO	MEAN SOM	SE SOM	Drug class	References
Ibuprofen	101	3.6	0.1	5.9	0.1	NSAIDs	Matamoros et al. 2006; Chen et al., 2016; Ávila et al., 2013; Matamoros et al., 2008a; Matamoros et al., 2007; Hijosa-Valsero et al., 2010a; Matamoros et al., 2009; Ávila et al., 2015, **Zhu et al., 2014**; Hijosa-Valsero et al., 2010b; Ávila et al., 2014a; Moeder et al., 2017; Reyes-Contreras et al., 2011; Matamoros et al., 2016; Camacho-Muñoz et al., 2012; Vymazal et al., 2017; Carranza-Diaz et al., 2014; Nuel et al., 2018; Breitholtz et al., 2012; He et al., 2018; Verlicchi et al., 2013; Kahl et al., 2017; Ávila et al., 2014b; Zhang et al., 2011; Matamoros et al., 2005; Zhang et al., 2016; Dordio et al., 2010; Conkle et al., 2008; Park et al., 2018; de Oliveira et al., 2019; Vystavna et al., 2017; Matamoros et al., 2017; Dordio et al., 2009a; Lancheros et al., 2019; Zhang et al., 2012; Brenizova et al., 2018; Ozengin et al., 2016; Matamoros et al., 2008b
Diclofenac	99	3.1	0.2	6	0.1	NSAIDs	Matamoros et al., 2006; Chen et al., 2016; Ávila et al., 2013; Matamoros et al., 2008a; Matamoros et al., 2007; Hijosa-Valsero et al., 2010a; Ruhmland et al., 2015; Matamoros et al.; 2009; Ávila et al., 2015; **Zhu et al., 2014**; Hijosa-Valsero et al., 2010b; Avila et al., 2014b; Moeder et al., 2017; Matamoros et al., 2016; Vymazal et al., 2017; Carranza-Diaz et al., 2014; Nuel et al., 2018; Breitholtz et al., 2012; He et al., 2018; Verlicchi et al., 2013; Kahl et al., 2017; Ávila et al., 2010; Ávila et al., 2014; Zhang et al., 2011; Auvinen et al., 2017a; Mathon et al., 2019; Vystavna et al., 2017; Matamoros et al., 2017; Park et al., 2009; Ruppelt et al., 2020; Auvinen et al. 2017b; Francini et al., 2018; Zhang et al., 2012; Anderson et al., 2020; Ávila et al., 2021.
Carbamazepine	98	2.3	0.2	5.8	0.1	Anticonvulsant	Anderson et al., 2013; Cardinal et al., 2014; Matamoros et al., 2008a; Matamoros et al., 2007; Hijosa-Valsero et al., 2010a; Ruhmland et al., 2015; Matamoros et al., 2009; Zhu et al., 2014; Moeder et al., 2017; Reyes-Contreras et al., 2011; Matamoros et al., 2016; Camacho-Muñoz et al., 2012; Carranza-Diaz et al., 2014; Nuel et al., 2018; Breitholtz et al., 2012; He et al., 2018; Verlicchi et al., 2013; Kahl et al., 2017; Zhang et al., 2011; Matamoros et al., 2005; Dordio et al., 2010; Matamoros et al., 2008b; Conkle et al., 2008; Park et al., 2018; Auvinen et al., 2017a; Delgado et al., 2020; Mathon et al., 2019; Macci et al., 2015; **Sharif et al., 2014**; Vystavna et al., 2017; Matamoros et al., 2017; Park et al., 2009; Ruppelt et al.,

Pharmaceutical	n	MEAN ROO	SE ROO	MEAN SOM	SE SOM	Drug class	References
							2020; Dordio et al., 2009a; Auvinen et al., 2017b; Cardinal et al., 2016; Zhang et al., 2012; Anderson et al., 2020; Ozengin et al., 2016; Ávila et al., 2021; Stroski et al., 2020; Chaves-Barquero et al., 2016; Chaves-Barquero et al., 2018; Chaves-Barquero et al., 2021; Anderson et al., 2015.
Naproxen	77	3.5	0.1	6.0	0.1	NSAIDs	Matamoros et al., 2006; Chen et al., 2016; Cardinal et al., 2014; Matamoros et al., 2008a; Matamoros et al., 2007; Hijosa-Valsero et al., 2010a; Matamoros et al., 2009; **Zhu et al., 2014**; Hijosa-Valsero et al., 2010b; Moeder et al., 2017; Reyes-Contreras et al., 2011; Matamoros et al., 2016; Camacho-Muñoz et al., 2012; Carranza-Diaz et al; 2014; Nuel et al., 2018; Breitholtz et al., 2012; He et al; 2018; Verlicchi et al., 2013; Kahl et al., 2017; Ávila et al., 2010; Zhang et al., 2011; Conkle et al., 2008; Vystavna et al., 2017; Matamoros et al., 2017; Park et al., 2009; Lancheros et al., 2019; Cardinal et al., 2016; Zhang et al., 2012
Caffeine	67	3.7	0.1	5.9	0.1	Stimulant	Matamoros et al., 2006; Chen et al., 2016; Matamoros et al., 2007; Hijosa-Valsero et al., 2010a; Sgroi et al., 2018; Matamoros et al., 2009; **Zhu et al., 2014**; Li et al., 2017; Hijosa-Valsero et al., 2010b; Reyes-Contreras et al., 2011; Matamoros et al., 2016; Camacho-Muñoz et al., 2012; Vymazal et al., 2017; Carranza-Diaz et al., 2014; Nuel et al., 2018; He et al., 2018; Kahl et al., 2017; Conkle et al., 2008; de Oliveira et al., 2019; Vystavna et al., 2017; Herrera-Cárdenas et al., 2016; Zhang et al., 2012
Ketoprofen	50	2.9	0.2	5.8	0.1	NSAIDs	Matamoros et al., 2006; Chen et al., 2006; Matamoros et al., 2008a; Hijosa-Valsero et al., 2010a; Matamoros et al., 2009; Hijosa-Valsero et al., 2010b; Moeder et al., 2017; Reyes-Contreras et al., 2011; Matamoros et al., 2016; Vymazal et al., 2017; Carranza-Diaz et al., 2014; Nuel et al., 2018; Breitholtz et al., 2012; He et al., 2018; **Verlicchi et al., 2013**; Mathon et al., 2019; Vystavna et al., 2017; Matamoros et al., 2017; Francini et al., 2018; Zhang et al., 2012
Sulfamethoxazole	44	2.3	0.3	5.7	0.2	Sulfonamide antibiotic	Anderson et al., 2013; Cardinal et al., 2014; Berglund et al., 2014; Ruhmland et al., 2015; Sgroi et al., 2018; **Zhu et al., 2014**; Button et al., 2019; Camacho-Muñoz et al., 2012; Nuel et al., 2018; Breitholtz et al., 2012; He et al., 2018; Verlicchi et al., 2013; Ávila et al., 2014; Christofiloupoulos et al., 2020; Conkle et al., 2008; Auvinen et al., 2017a; Mathon et al., 2019; Sochaki et al., 2018; Park et al., 2009; Ruppelt et al., 2020; Cardinal et al., 2016; Dan et al., 2020b; Anderson et al., 2020; Stroski et al., 2020; Chaves-Barquero et al., 2016; Chaves-Barquero et al., 2018.; Chaves-Barquero et al., 2021; Sabri et al., 2021; Anderson et al., 2015.

Pharmaceutical	n	MEAN ROO	SE ROO	MEAN SOM	SE SOM	Drug class	References
Metoprolol	34	3.6	0.2	5.5	0.2	β-blockers	Chen et al., 2016; Ruhmland et al., 2015; Vymazal et al., 2017; Nuel et al., 2018; Breitholtz et al., 2012; He et al., 2018; **Verlicchi et al., 2013**; Conkle et al., 2008 Mathon et al., 2019; Ruppelt et al., 2020; Auvinen et al., 2017b; Anderson et al., 2020; Stroski et al., 2020; Chaves-Barquero et al., 2016; Chaves-Barquero et al., 2018; Chaves-Barquero et al., 2021
Atenolol	33	4.0	0.2	5.7	0.2	β-blockers	Chen et al., 2016; Ruhmland et al., 2015; Nuel et al., 2018; Breitholtz et al., 2012; **Verlicchi et al., 2013**; Conkle et al., 2008; Park et al., 2018; Auvinen et al., 2017a; Mathon et al., 2019; Park et al., 2009; Auvinen et al., 2017b; Francini et al., 2018; Anderson et al., 2020; Dordio et al., 2009b; Stroski et al., 2020; Chaves-Barquero et al., 2016; Chaves-Barquero et al., 2018; Chaves-Barquero et al., 2021
Salicylic acid	31	3.7	0.1	5.8	0.1	Non-NSAID analgesics	Matamoros et al., 2006; Hijosa-Valsero et al., 2010[a]; Matamoros et al., 2009; Hijosa-Valsero et al., 2010b; Reyes-Contreras et al., 2011; Camacho-Muñoz et al., 2012; **Verlicchi et al., 2013**; Zhang et al., 2012
Acetaminophen	27	3.9	0.2	5.7	0.2	Non-NSAID analgesics	Chen et al., 2016; Ávila et al., 2013; Ávila et al., 2015; Li et al., 2017; Matamoros et al., 2016; Vymazal et al., 2017; Nuel et al., 2018; **Verlicchi et al., 2013**; Ávila et al., 2014; Conkle et al., 2008; Vystavna et al., 2017; Vo et al., 2019; Vo et al., 2016; Ávila et al., 2021
Trimethoprim	27	3.4	0.3	5.8	0.2	Antibiotic	Chen et al., 2016; Berglund et al., 2014; Ruhmland et al., 2015; Sgroi et al., 2018; Zhu et al., 2014; Nuel et al., 2018; Breitholtz et al., 2012; **Verlicchi et al., 2013**; Mathon et al., 2019; Anderson et al., 2020; Stroski et al., 2020; Chaves-Barquero et al., 2016; Sabri et al., 2021
Ciprofloxacin	25	2.6	0.4	6.1	0.2	Fluoroquinolones	Berglund et al., 2014; Dan et al., 2020a; **Verlicchi et al., 2013**; Christofiloupoulos et al., 2019; Sabri et al., 2021
Norfloxacin	20	2.3	0.4	6.5	0.4	Fluoroquinolones	Berglund et al., 2014; Dan et al., 2020a; **Verlicchi et al., 2013**
Ofloxacin	20	3.7	0.2	6.6	0.1	Fluoroquinolones	Nuel et al., 2018; Dan et al., 2020a; **Verlicchi et al., 2013**
Tramadol	20	3.3	0.3	5.8	0.2	Non-NSAID analgesics	Chen et al., 2016; Ruhmland et al., 2015; Vymazal et al., 2017; **Nuel et al., 2018**; Breitholtz et al., 2012; Auvinen et al., 2017b
Erythromycin	19	3.3	0.3	6.3	0.1	Macrolide antibiotic	Berglund et al., 2014; Ruhmland et al., 2005; Dan et al., 2020a; He et al., 2018; **Verlicchi et al., 2013**; Ávila et al., 2014; Mathon et al., 2019
Tetracycline	19	3.3	0.2	6.4	0.1	Tetracycline antibiotic	Berglund et al., 2014; **Dan et al., 2020a**
Roxithromycin	19	2.6	0.3	6.6	0.1	Macrolide antibiotic	**Dan et al., 2020a**; Verlicchi et al., 2013
Clarithromycin	16	2.8	0.5	5.6	0.3	Macrolide antibiotic	Berglund et l., 2014; Ruhmland et al., 2015; Breitholtz et al., 2015; **Verlicchi et al., 2013**; Mathon et al., 2019; Anderson et al., 2020; Chaves-Barquero et al., 2016; Chaves-Barquero et al., 2018; Sabri et al., 2021; Anderson et al., 2015

Pharmaceutical	n	MEAN ROO	SE ROO	MEAN SOM	SE SOM	Drug class	References
Furosemide	15	3.5	0.3	5.2	0.2	Diuretic	Chen et al., 2016; Matamoros et al., 2009; Matamoros et al., 2016; Vymazal et al., 2017; **Verlicchi et al., 2013**
Clofibric acid	12	2.6	0.4	6.4	0.2	Lipid-lowering	Cardinal et al., 2014; Matamoros et al., 2008a; Nuel et al., 2018; He et al., 2018; **Verlicchi et al., 2013**; Matamoros et al., 2005; Dordio et al., 2010; Matamoros et al.,
Sulfapyridine	13	3.0	0.4	5.0	0.2	Sulfonamide antibiotic	Chen et al., 2016; Cardinal et al., 2014; Conkle et al., 2008; **Cardinal et al., 2016**; Anderson et al., 2020; Stroski et al., 2020; Sabri et al., 2021; Anderson et al., 2015
Sotalol	10	2.4	0.5	6.3	0.3	β-blockers	Nuel et al., 2018; Breitholtz et al., 2012; **Verlicchi et al., 2013**; Conkle et al., 2008; Mathon et al., 2019; Auvinen et al., 2017b
Sulfadiazine	10	1.7	0.6	5.7	0.4	Sulfonamide antibiotic	Nuel et al., 2018; **Verlicchi et al., 2013**; Dan et al., 2020b; Ozengin et al., 2016; Sabri et al., 2021
Bezafibrate	9	3.2	0.4	6.9	0.1	Lipid-lowering	Ruhmland et al., 2015; Breitholtz et al., 2012; **Verlicchi et al., 2013**; Ávila et al., 2021
Lincomycin	9	2.1	0.8	5.1	0.2	Lincosamide antibiotic	He et al., 2018; **Ávila et al., 2014**; Sabri et al., 2021
Gabapentin	9	2.3	0.7	5.4	0.1	Anticonvulsant	**Chen et al., 2016**; Auvinen et al., 2017a; Auvinen et al., 2017b
Venlafaxine	9	3.5	0.2	6.7	0.1	Antidepressant	Ruhmland et al., 2015; **Breitholtz et al., 2012**; Vystavna et al., 2017
Azithromycin	8	1.8	0.7	5.6	0.4	Macrolide antibiotic	Berglund et al., 2014; Breitholtz et al., 2012; **Verlicchi et al., 2013**; Sabri et al., 2021
Propranolol	8	2.9	1.7	5.7	0.9	β-blockers	Moeder et al., 2017; He et al., 2018; **Verlicchi et al., 2013**; Mathon et al., 2019; Vystavna et al., 2017; Chaves-Barquero et al., 2018
Codeine	8	3.5	0.3	7	0.1	Non-NSAID analgesics	Ruhmland et al., 2015; Breitholtz et al., 2012; **Verlicchi et al., 2013**
Bisoprolol	7	3.8	0.2	6.3	0.3	β-blockers	**Breitholtz et al., 2012**; Auvinen et al., 2017a; Auvinen et al., 2017b
Fluconazole	7	1.1	0.6	6.9	0.0	Azole antifungal	Ruhmland et al., 2015; **Breitholtz et al., 2012**
Gemfibrozil	7	2.9	0.7	6.0	0.4	Lipid-lowering fibrate	Anderson et al., 2013; Camacho-Muñoz et al., 2012; Nuel et al., 2018; **Verlicchi et al., 2013**; Conkle et al., 2008; Ávila et al., 2021; Anderson et al., 2015
Hydrochlorothiazide	7	4.2	0.1	5.8	0.3	Diuretic	Chen et al., 2016; **Verlicchi et al., 2013**
Clindamycin	6	1.3	0.7	6.6	0.3	Lincosamide antibiotic	Berglund et al., 2014; **Breitholtz et al., 2012**; Mathon et al., 2019
Oxazepam	5	1.5	0.5	6.9	0.1	Benzodiazepine	Ruhmland et al., 2015; **Breitholtz et al., 2012**
Telmisartan	5	3.5	0.5	6.7	0.3	Angiotensin II receptor antagonist	**Breitholtz et al., 2012**; Auvinen et al., 2017b
Ranitidine	5	3.1	0.7	7.1	0.1	H2 blocker	Breitholtz et al., 2012; **Verlicchi et al., 2013**
Tylosin	5	4.1	0.7	5.1	0.5	Macrolide antibiotic	Sabri et al., 2021; **Verlicchi et al., 2013**
Lomefloxacin	3	3.9	0.1	6.5	0.0	Fluoroquinolones	Dan et al., 2020a
Acyclovir	3	4	0.0	6.8	0.0	Antiviral	Ruhmland et al., 2015
Alfuzosine	3	3.9	0.1	7	0.0	Alpha-blocker	Breitholtz et al., 2012
Alprazolam	3	1.3	1.1	7	0.0	Benzodiazepine	Breitholtz et al., 2012
Atorvastatin	2	2.8	1.1	7.3	0.2	Statin	Breitholtz et al., 2012; **Verlicchi et al., 2013**

Pharmaceutical	n	MEAN ROO	SE ROO	MEAN SOM	SE SOM	Drug class	References
Diazepam	2	3.9	0.4	5.3	0.2	Benzodiazepine	Verlicchi et al., 2013; Mathon et al., 2019; Park et al., 2009; **Auvinen et al., 2017b**

Based on our rubric, Table 2.6 presents a classification of the calculated parameters for each pharmaceutical. Based on their obtained scores, pharmaceuticals are classified in one of four categories: sufficient SOM and ROO (13 compounds, 31% of total), sufficient SOM with insufficient ROO (8 compounds, 17% of total); insufficient SOM with sufficient ROO (18 compounds, 35% of total); and insufficient SOM and ROM (9 of compounds, 19% of total). The most studied pharmaceuticals in this study (Figure 2.7) obtained similar average scores for SOM (between 5 and 6), while a greater diversity of scores for ROO was observed, where either sufficient or insufficient evidence of removal was observed. More details on this are discussed below. Pharmaceuticals with sufficient ROO scores were ibuprofen, diclofenac, naproxen, caffeine, metoprolol, atenolol and salicylic acid. Drugs with insufficient ROO scores were: ketoprofen, sulfamethoxazole, and carbamazepine (Figure 2.7).

Table 2.7 presents the various proposed removal mechanisms on the included literature for the present assessment. These represent authors' own criteria based on removal mechanisms and limited evidence in the literature to explain their observed results. Aerobic biodegradation was reported as the dominant mechanism for several pharmaceuticals; for instance, ibuprofen, salicylic acid, acetaminophen, codeine, caffeine, metoprolol and gemfibrozil (Matamoros et al., 2007; Matamoros et al., 2008; Hijosa-Valsero et al., 2010a; Ávila et al., 2014; Zhu et al., 2014; Chen et al., 2016; Vymazal et al., 2017; Brezinova et al., 2018; Zhang et al., 2018; Reyes-Contreras et al., 2012; Zhang et al., 2012). In fact, aerobic biodegradation was the most commonly reported dominant removal

mechanism for pharmaceuticals from the top-ten that obtained sufficient ROO scores (Figure 2.7).

Pharmaceuticals that obtained insufficient ROO scores have been commonly reported as recalcitrant in the literature, for instance carbamazepine (e.g., Conkle et al., 2008), which often experiences incomplete removals in CW. Its most important interaction with the CW components is adsorption to organic surfaces and sorption (Kahl et al., 2017). An increased hydraulic retention time or the use of substrates with high sorptive capacities (e.g., LECA) may contribute to its removal in CWs (Auvinen et al., 2017). Limited removal was also observed for ketoprofen and sulfamethoxazole, which obtained insufficient ROO scores, which reflects their environmental persistence. There are only a few pharmaceuticals such as diclofenac, ketoprofen, naproxen, and clarithromycin that have been reported to be mainly removed by photodegradation (Matamoros et al., 2008; Ávila et al., 2014; Ávila et al., 2015; Ruhmland et al., 2015; Chen et al., 2016; Francini et al., 2018; Zhang et al., 2018; Reyes-Contreras et al., 2012; Hijosa-Valsero et al., 2011; Berglund et al., 2014).

Anaerobic biodegradation was reported as a dominant removal mechanism for sulfamethoxazole, sulfapyridine, and bezafibrate (Hijosa-Valsero et al., 2011; Conkle et al., 2008; Sgroi et al., 2018; Ruhmland et al., 2015). Since anaerobic biodegradation is usually slower than aerobic biodegradation, longer HRT are needed to achieve the same removal efficiency in the water column. That said, when the anaerobic biodegradation occurs within the sediment layer, HRT becomes mostly irrelevant, and the process is mass-transfer-controlled between the water column and sediment layer. It is important to note that there is currently a need for studies focused on specific removal processes using realistic matrices and environmentally relevant concentrations. Currently, the analysis of the aspects influencing pharmaceutical removal continues to be a complex task.

2.4.3. Main strengths and weaknesses of the studies

Scores for ROO and SOM varied greatly amongst the included studies. For ROO, scores ranging from 0 to 5 were obtained from our evaluation. These scores depended on the ability of the systems to show relevant evidence for the removal of each studied pharmaceutical, while for SOM, scores from 3.25 to 7.75 were obtained, depending on the quality of the data and experimental design.

The strongest studies (e.g., Sharif et al., 2014; Zhu et al., 2014; Verlicchi et al., 2013; Matamoros et al., 2008a; Dan et al., 2020a; Matamoros et al., 2007; Ávila et al., 2014a; Breitholtz et al., 2012; Kahl et al., 2017) were overall carefully designed to provide accurate information on the origin and purity of standards and reagents, as well as thoroughly describing the instrumental conditions for chemical analysis, including LODs and LOQs. The use of environmentally relevant concentrations of pharmaceuticals (i.e., µg/L or below) in real wastewater matrices helped these studies to be considered evidence of greater weight compared to others that used either stock solutions or synthetic wastewater loaded with pharmaceutical concentrations that were environmentally irrelevant. Strongest studies also involved a high sampling frequency (i.e., weekly or more), which is useful to characterize the dynamics in pharmaceutical concentrations over time in CWs, in comparison to biweekly or monthly sampling programs which could well miss events over time.

Table 2.6. Classification of evaluated pharmaceuticals based on their SOM and ROO scores. Scores are considered "sufficient" if SOM>6.0 or ROO>3.0, respectively, and "insufficient" if SOM<6.0 or ROO<3.0.

Pharmaceutical	Sufficient SOM Sufficient ROO	Sufficient SOM Insufficient ROO	Insufficient SOM Sufficient ROO	Insufficient SOM Insufficient ROO
Ibuprofen			X	
Diclofenac	X			
Carbamazepine				X
Naproxen	X			
Caffeine			X	
Ketoprofen				X
Sulfamethoxazole				X
Metoprolol			X	
Atenolol			X	
Salicylic acid			X	
Acetaminophen			X	
Trimethoprim			X	
Ciprofloxacin		X		
Norfloxacin		X		
Ofloxacin	X			
Tramadol			X	
Erythromycin	X			
Tetracycline	X			
Roxithromycin		X		
Clarithromycin				X
Furosemide			X	
Clofibric acid		X		
Sulfapyridine				X
Sotalol			X	
Sulfadiazine				X
Bezafibrate	X			
Lincomycin				X
Gabapentin			X	
Venlafaxine	X			
Azithromycin				X
Propranolol				X
Codeine	X			
Bisoprolol	X			
Fluconazole			X	
Gemfibrozil		X		
Hydrochlorothiazide	X			
Clindamycin		X		
Oxazepam			X	
Telmisartan	X			
Ranitidine	X			
Tylosin			X	
Lomefloxacin			X	
Acyclovir			X	
Alfuzosine	X			
Alprazolam		X		
Atorvastatin		X		
Diazepam			X	
Doxycycline			X	

There were also some weaknesses observed, even amongst these stronger studies. First, there was an overall lack of raw data availability for reprocessing or reanalysis if needed, and most studies presented an aggregate summary. More importantly, there was a general lack of research on specific biotic/abiotic processes effecting pharmaceutical removal: several studies focused on quantifying the overall pharmaceutical removal but did not focus on understanding the various processes and their contributions to total removal. Finally, it was noted an overall absence of mass balance estimations. This can be a very difficult task, as it needs accurate quantification of the distribution of the analytes in the water column, the sediments, the plant material and any other organic or inorganic surfaces where sorption and biodegradation could occur. Furthermore, it requires a strict monitoring of photodegradation to account for both direct and indirect photolysis processes. Summed up, these tasks represent great challenges from the standpoint of experimental and sampling design, and chemical analysis requirements and capabilities.

Studies that scored lower for their methodological reporting (e.g., Vo et al., 2019; Ruppelt et al., 2020; Park et al., 2009; Brezinova et al., 2018; Vymazal et al., 2017; Christofilopoulos e al., 2019; Moeder et al., 2017) tended to exhibit limitations in the detailing of instrumental conditions for chemical analysis, including LODs and LOQs, and the purity of reagents and standards. Also, these studies tended to focus on non-relevant pharmaceutical concentrations, either from stock solutions or synthetic wastewaters. Furthermore, the sampling programs observed on some of these studies involved a lesser frequency (e.g., biweekly, monthly or less) compared to the studies detailed above. Some weaknesses for these studies were shared with the strongest studies. For instance, limited raw data availability, and the lack of estimations of the contributions of specific

biotic/abiotic processes to overall removal, which hence rendered the calculations of mass balances unfeasible.

Based on this analysis, future studies should aim to provide raw data, detail instrumental conditions and QA/QC practices, design the experimental work to characterize CW performance by addressing biotic and abiotic processes that may influence the removal of specific pharmaceuticals. A mass-balance-based approach would be desirable to better understand the fate of these chemicals. Such efforts represent important challenges for better experimental design, moving forward into the advancement of the understanding of this topic.

2.4.4. Effect of CW type on pharmaceutical removal

The available evidence in the literature suggests that specific processes are involved in the removal of a specific type of pharmaceutical in CWs. These complex physical, chemical, and biological processes may occur simultaneously including photodegradation, volatilization, adsorption/sorption, plant uptake and accumulation, as well as biodegradation (aerobic and/or anaerobic), mainly depending on the design of the CW (Li et al., 2014; Gorito et al., 2017). Traditionally, CWs have been designed as surface flow, subsurface flow or hybrid systems. Due to variations in the dominant removal mechanisms of different types of pharmaceuticals, their removal efficiency for specific contaminants varies in different types of CWs (Kadlec et al., 2009). For instance, in SF-CWs, the main mechanism of pharmaceutical removal has been reported to be photodegradation. Microbial degradation and plant uptake also have been pointed to contribute to some extent in the removal. In HSSF-CW, wastewater stays below the surface of the media and flows horizontally through the bed until it reaches the outlet (Kadlec et al., 2009). Anaerobic biodegradation has been reported to be an important removal mechanism of

pharmaceuticals in this type of CW, along with sorption and, to some extent, plant uptake. In VSSF-CWs, the beds are intermittently loaded with a large amount of wastewater to temporarily flood the surface of the bed. Aerobic biodegradation is crucial for the removal of pharmaceuticals in these systems, among other important processes (e.g., adsorption, plant uptake).

H-CWs are designed to achieve greater performance compared to single-step CWs. For example, efforts have been reported for increasing nitrogen removal through the combination of a HSSF-CW and a VSSF-CW (Kadlec et al., 2009). Currently, one of the limitations of single-step CWs is their high footprint and their limited volumetric capacity. The combination in series of SF-CW with either HSSF-CW or VSSF-CW can enhance removal performance (e.g., Ávila et al., 2014), as the operation of a series of CW can work at greater hydraulic loading rates compared to single-step systems.

We evaluated the performance of H-CWs to remove pharmaceuticals versus single step CWs (e.g., SF-CWs, HSSF-CWs, and VSSF-CWs, see Tables A2 and A5), based on recent reports claiming a better performance to be expected from H-CWs (Ilyas and van Hullebusch, 2019). Results from our statistical analyses suggest that H-CWs had an increased removal of ibuprofen, diclofenac, sulfamethoxazole, acetaminophen, ciprofloxacin, clarithromycin, propranolol, and venlafaxine compared to single-step systems. In H-CWs it is possible to make use of better defined and explicit zones for aerobic and anaerobic treatment, as part of their design. This, in turn, provides a greater opportunity for the removal of some pharmaceuticals through the synergy of various mechanisms. The combination of various single-step CW can facilitate the design of hybrid systems that make use of increased hydraulic loading rates to attain greater treatment

capacity. Thus, the conditions offered by H-CWs are promising to obtain increased removal efficiencies compared to single-step CWs.

2.4.5. Effect of seasonality and climate on pharmaceutical removal

Temperature can play a key role on the various processes that occur within wastewater treatment systems (Garcia-Rodriguez et al., 2014; Hijosa-Valsero et al., 2010a; Li et al., 2014). The effect of microbial biomass on contaminant removal has been reported to be more important during the warm season, likely due to the greater biological activity as temperature increases, which promotes direct uptake by plants or the release of exudates that can directly or indirectly enhance microbial degradation (García-Rodríguez et al., 2014), as well increased removal from a greater biomass availability at higher temperatures. The effect of temperature on the removal of emerging organic contaminants (e.g., caffeine, ibuprofen, naproxen) by CWs has also been established on previous research efforts, as it influences plant productivity and consequently microbial and bacterial communities (Zhang et al., 2011). Moreover, biodegradation and photodegradation are processes of high seasonality previously studied in the literature (Garcia-Rodriguez et al., 2014). Organic contaminants susceptible to photodegradation processes in the aquatic environment are generally better removed in systems exposed to sunlight than in planted wetlands, due to the sunlight blocking effect of plants (Matamoros et al., 2009). During the summer, warmer temperatures tend to be related to greater light availability, both in intensity as well as in duration, which can both promote biodegradation. Also, an indirect effect could be observed by the generation of more photoinduced radicals that could take part in indirect photolysis processes.

Table 2.7. Removal mechanisms reported for most important analytes in the present review.

Pharmaceutical	Examples	Dominant removal mechanism*
Diclofenac	Hijosa-Valsero et al., 2010a, Kahl et al., 2017; Ávila et al., (2013, 2014)	Biodegradation (aerobic)
	Matamoros et al., 2008; Ávila et al., (2014, 2015); Ruhmland et al., 2015; Chen et al., 2016; Francini et al., 2018; Zhang et al., 2018.	Photodegradation
Ibuprofen	Matamoros et al., 2007; Matamoros et al., 2008; Hijosa-Valsero et al., 2010a; Ávila et al. 2014; Zhu et al., 2014; Chen et al., 2016; Vymazal et al., 2017; Brezinova et al., 2018; Zhang et al., 2018.	Biodegradation (aerobic)
Ketoprofen	Matamoros et al., 2008; Reyes-Contreras et al., 2012; Francini et al., 2018.	Photodegradation
Naproxen	Matamoros et al., 2007; Hijosa-Valsero et al., 2010a; Zhang et al., 2012a; Chen et al., 2016; He et al., 2018; Zhang et al., 2018.	Biodegradation (aerobic)
	Matamoros et al., 2008; Reyes-Contreras et al., 2012; Zhang et al., 2018	Photodegradation
Salicylic acid	Hijosa-Valsero et al., 2010a; Reyes-Contreras et al., 2012; Zhang et al., 2012.	Biodegradation (aerobic)
Acetaminophen	Ávila et al. (2013, 2015); Li et al., 2017; Vystavna et al., 2014.	Biodegradation (aerobic)
Codeine	Ruhmland et al., 2015	Biodegradation (aerobic)
Tramadol	Ruhmland et al., 2015; Chen et al., 2016.	Biological transformation
Clarithromycin	Hijosa-Valsero et al., 2011; Berglund et al., 2014	Photodegradation
	Hijosa-Valsero et al., 2011; Berglund et al., 2014	Sorption
Doxycycline	Hijosa-Valsero et al., 2011; Berglund et al., 2014.	Biodegradation (aerobic)
Sulfamethoxazole	Conkle et al., 2008; Sgroi et al., 2018; Button et al., 2019; Hijosa-Valsero et al., 2011; Ruhmland et al., 2015; Sgroi et al., 2018.	Biodegradation (aerobic; anaerobic)
Sulfapyridine	Conkle et al., 2008	Biodegradation (anaerobic)
Trimethoprim	Hijosa-Valsero et al., 2011; Ruhmland et al., 2015	Biodegradation (aerobic)
Caffeine	Matamoros et al., 2006; Hijosa-Valsero et al., 2010a; Zhang et al., 2014; Chen et al., 2016; Li et al., 2017; Vymazal et al., 2017;	Biodegradation (aerobic)

Pharmaceutical	Examples	Dominant removal mechanism*
	Vystavna et al., 2017; He et al., 2018; Zhu et al., 2014	
Carbamazepine	Matamoros et al., (2005, 2008); Hijosa-Valsero et al., 2011; Carranza-Diaz et al., 2014; Sharif et al., 2014; Vystavna et al., 2017; Park et al., 2018; Dordio et al., 2010.	Sorption
Atenolol	Park et al., 2018; Auvinen et al., 2017.	Sorption
Metoprolol	Conkle et al., 2008; Ruhmland et al., 2015; Chen et al., 2016; He et al., 2018.	Biodegradation (aerobic)
Bezafibrate	Ruhmland et al., 2015.	Biodegradation (anaerobic)
Clofibric acid	Dordio et al., 2010	Plant uptake
Gemfibrozil	Conkle et al., 2008; Zhang et al., 2018	Biodegradation (uptake)

*Author's own criteria based on removal mechanisms and limited evidence in the literature.

In this work, we evaluated the seasonal-based performance of CWs located under warm and cold seasons, and statistically compared their obtained scores. Climate and seasonality are expected to influence removal of all sorts of contaminants in CWs, based on the assumption of an increased biological activity as temperature rises. From our assessment (Tables A3 and A5), the compounds that showed statistically lower ($p < 0.05$) mean ROO scores under cold conditions compared to warm were caffeine, salicylic acid, trimethoprim, and tetracycline. These pharmaceuticals are reported to be removed from CWs by biodegradation as a predominant removal mechanism, based on authors' own criteria and limited previous evidence (see Table 2.7). An increased temperature favours microbial activity, which can then be a driver for wastewater polishing. Thus, it is expected to observe greater removal rates with increasing temperature. For the remaining studied pharmaceuticals, no significant differences could be found between the ROO scores. That having been said, our seasonal comparison considered all studied CWs that quantified each pharmaceutical on each type of season, without discriminating by CW type or design

parameters, mostly due to the scarcity of cold-season data to expand our analysis. This represented a source of uncertainty for our assessment.

With the aim of analyzing performance differences on varying climates, a statistical comparison was conducted for studies located in polar, tropical, and temperate climates, initially considering all of the analytes and all types of CWs to obtain a general picture of the evaluation (Figure 2.8). From this starting point, we observed sufficient ROO and SOM scores for studies conducted on temperate climate, and sufficient ROO with insufficient SOM scores for studies conducted in both polar and tropical climates. No significant differences were obtained amongst climate types for mean overall ROO scores ($p < 0.05$, Tables A4 and A5). This is a key finding of this work, as it suggests that CWs based on all types of climate have the potential to facilitate pharmaceutical removal. The polar-based studies were conducted during the relatively warmer summer seasons, after ice melt, at temperatures that are comparable to cold-season values for temperate climates. Tropical studies, on the other hand, had the greatest ROO scores overall, and hence evidence for pharmaceutical removal, which is expected due to their high temperatures and biological activity. This can help explain the lack of statistical differences when comparing ROO scores amongst climates.

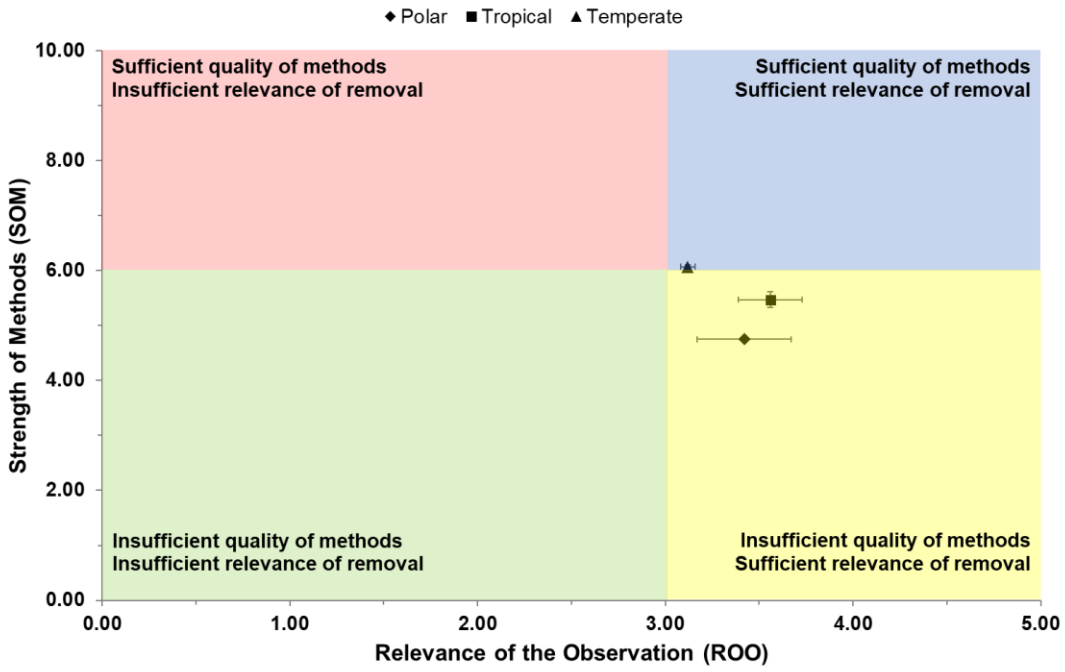


Figure 2.8. Mean score plot for all reviewed studies, grouped by type of climate: polar (n=21), tropical (n=36) or temperate (n=1178). Error bars depict standard error of the mean on each case.

Statistically significant differences were found for mean overall SOM scores ($p < 0.05$), which suggests a lower quality of data from studies based in polar or tropical locations, compared to temperate settings. Research carried out under tropical and polar climates tended to slightly be weaker on experimental design compared to temperate-based studies. Specifically, the studies tended to lack the use of environmentally relevant concentrations, even testing mg/L levels (e.g. Vo et al., 2019 in tropical climate); there was a general absence of process-driven research regarding the specific biotic and abiotic processes involved in the removal (e.g. Stroski et al., 2020 in polar climate; Zhang et al., 2012 in tropical climate), and finally, we observed a general lack of mass-balance approaches to the removal of pharmaceuticals from these systems, which is complex and difficult task across all types of climate. That having been said, the average score for SOM

indicates that there are generalized flaws in experimental design across all types of climate, as described under section 2.4.2, and based on the criteria designed for the present review.

To assess the differential removal for specific pharmaceuticals under various types of climate, compound-based comparisons were prepared (Figures 2.9 to 2.19). It is important to note that not all analytes were reported to occur in the three types of climate: the only pharmaceutical to comply with this was carbamazepine, commonly recognized for its ubiquitous nature and environmental persistence (Conkle et al., 2008). Ibuprofen, diclofenac, caffeine, ketoprofen and naproxen were reported in both tropical and temperate climates. Atenolol, metoprolol, sulfamethoxazole and trimethoprim were reported in both polar and temperate climates. Ibuprofen (Figure 2.9), diclofenac (Figure 2.10), naproxen (Figure 2.12), caffeine (Figure 2.13), metoprolol (Figure 2.16), atenolol (Figure 2.17), trimethoprim (Figure 2.18), and acetaminophen (Figure 2.19) showed, in average, sufficient relevance of removal and insufficient quality of methods across all studied climates. Carbamazepine (Figure 2.11), ketoprofen (Figure 2.14), and sulfamethoxazole (Figure 2.15) showed, in average, insufficient relevance of removal and insufficient quality of methods across the studied climates. Details on the statistical tests can be found in Table A4. Briefly, no significant differences ($p > 0.05$) were obtained from tests applied to the ROO and SOM scores across climate types, with the exception of acetaminophen, and carbamazepine (SOM only).

It is important to note that most CWs have the potential to score "sufficient" ROO scores for pharmaceutical removal under all studied types of climate, depending on specific design parameters that may be advantageous on each case. There is an abundance of studies conducted in temperate locations since 2005 and examining diverse design parameters. This has allowed researchers to test various hypotheses and evaluate a diversity of

pharmaceuticals and CWs in several locations. Tropical-based CW studies are scarcer, especially the ones studying pharmaceutical removal using these technologies. Although tropical and temperate systems rely on warmer temperatures to promote pharmaceutical removal through various mechanisms, as described above, polar systems experience extended hydraulic retention time through the use of lagoon-wetland systems to achieve pharmaceutical removal. The available polar studies for this assessment constituted pioneering efforts to evaluate pharmaceutical removal from real wastewater facilities in the Canadian Arctic. These studies were designed with the aim of obtaining a baseline assessment of wastewater polishing, without addressing or measuring specific mechanisms of removal, mostly due to logistical challenges. As seen in Stroski et al. (2020) and Chaves-Barquero et al. (2016), polar-based facilities have the potential to promote some pharmaceutical attenuation during their summer season. Future polar-based research should focus on evaluating the performance of other wastewater treatment facilities in polar areas both Arctic and Antarctic during the summer, to build and expand a comprehensive base of knowledge, in the context of global climate change. The strategy of using holding lagoons to contain the wastewater during winter months, with a subsequent discharge through natural or CWs during the summer seems to be a strategy that warrants further research.

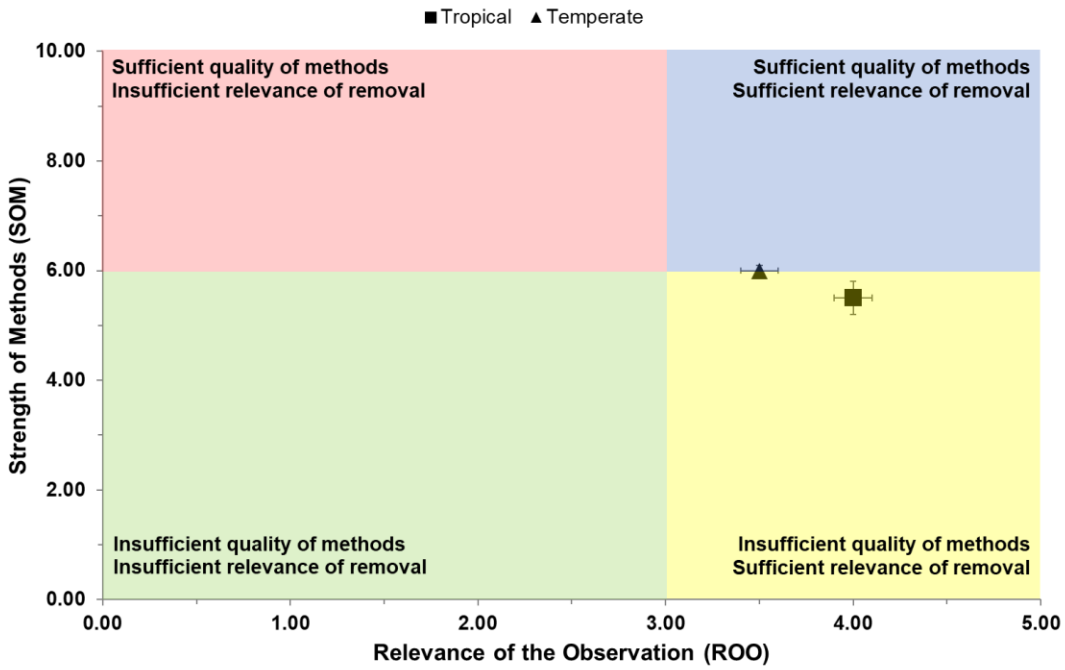


Figure 2.9. Score plot for ibuprofen, grouped by type of climate: tropical (n=9) and temperate (n=94). Error bars depict standard error of the mean. No data available for polar climate.

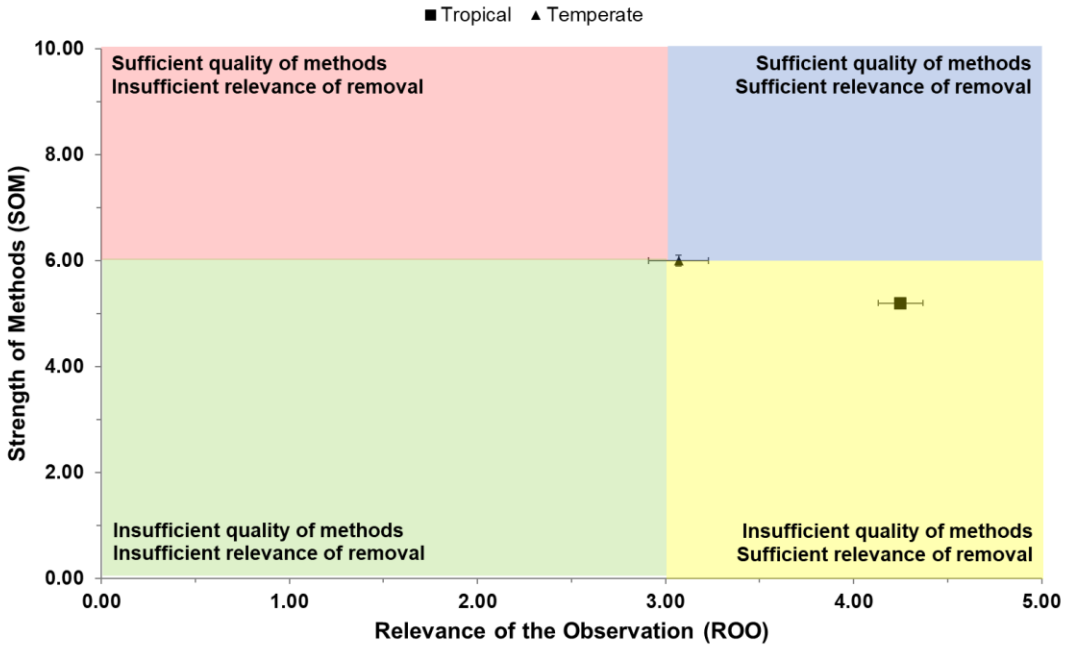


Figure 2.10. Score plot for diclofenac, grouped by type of climate: tropical (n=4) and temperate (n=95). Error bars depict standard error of the mean. No data available for polar climate.

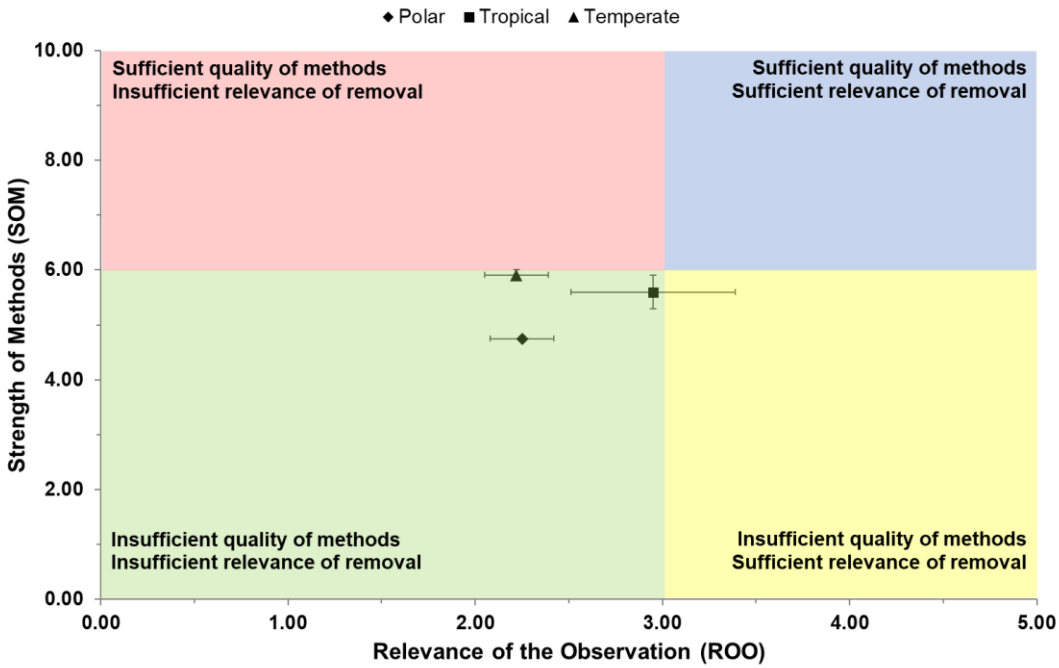


Figure 2.11. Score plot for carbamazepine, grouped by type of climate: polar (n=5), tropical (n=5), or temperate (n=89). Error bars depict standard error of the mean.

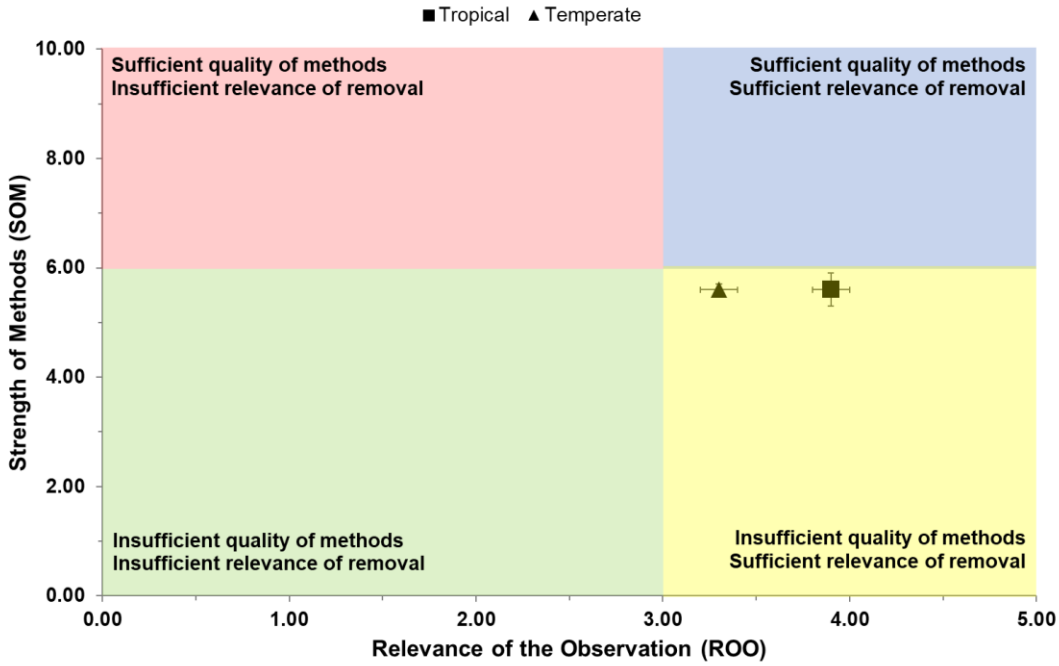


Figure 2.12. Score plot for naproxen, grouped by type of climate: polar (n=5) and temperate (n=70). Error bars depict standard error of the mean. No data available for polar climate.

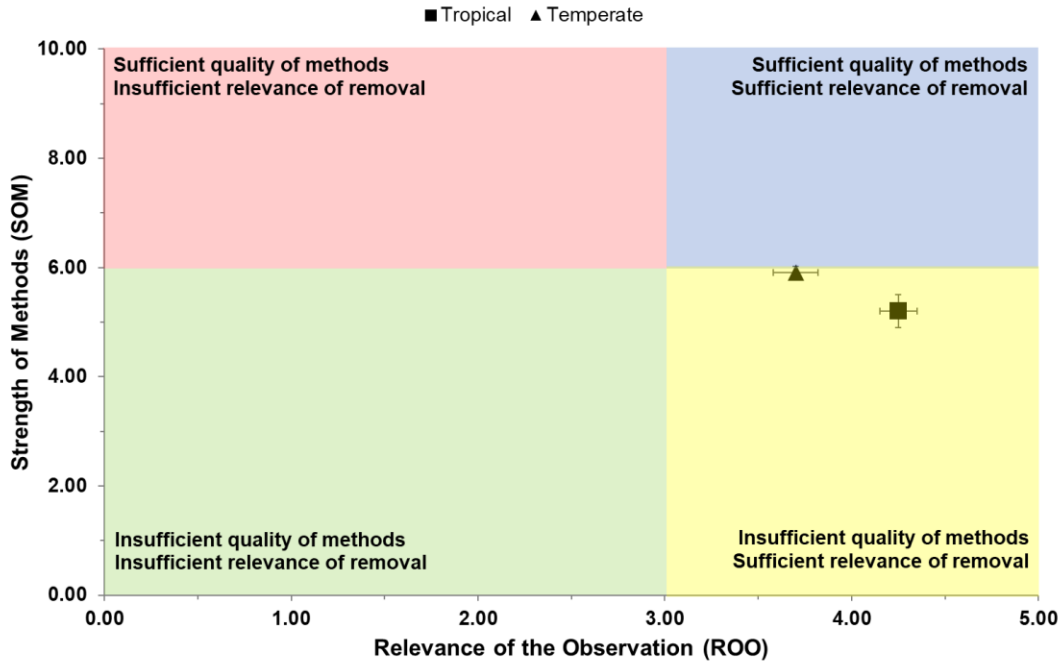


Figure 2.13. Score plot for caffeine, grouped by type of climate: polar (n=4) and temperate (n=63). Error bars depict standard error of the mean. No data available for polar climate.

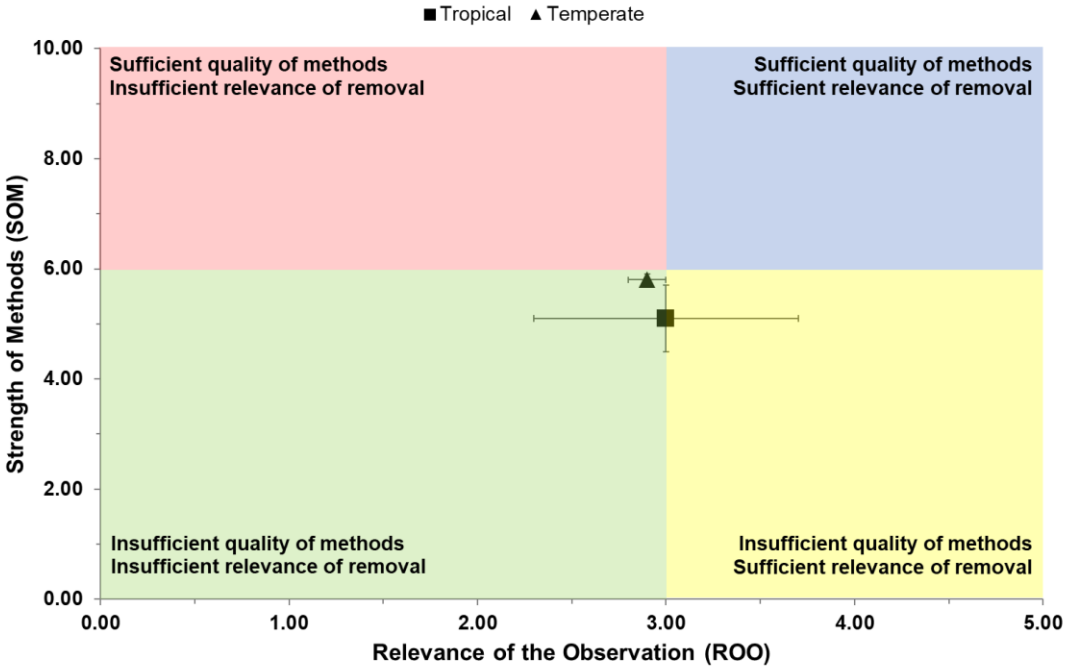


Figure 2.14. Score plot for ketoprofen, grouped by type of climate: polar (n=2) and temperate (n=48). Error bars depict standard error of the mean. No data available for polar climate.

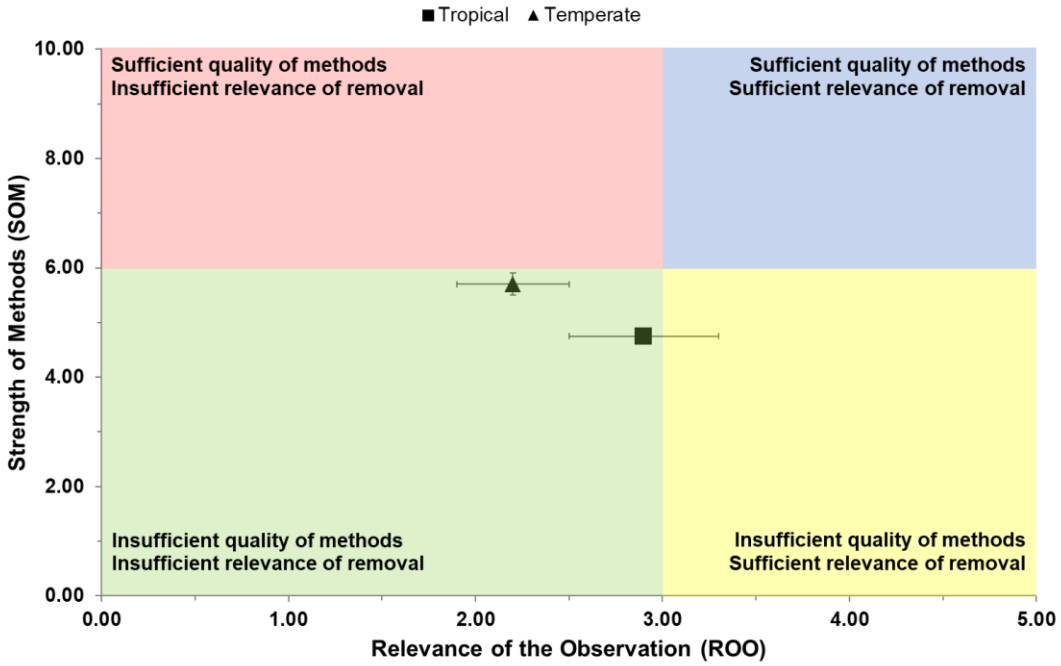


Figure 2.15. Score plot for sulfamethoxazole, grouped by type of climate: polar (n=2) and temperate (n=42). Error bars depict standard error of the mean. No data available for polar climate.

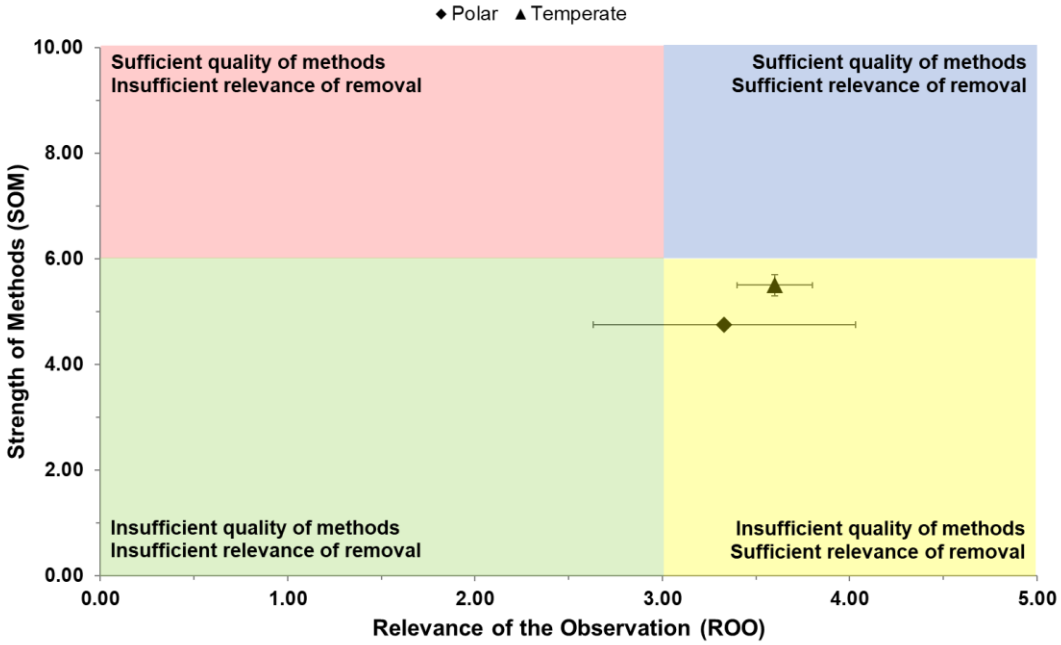


Figure 2.16. Score plot for metoprolol, grouped by type of climate: tropical (n=3) and temperate (n=30). Error bars depict standard error of the mean. No data available for tropical climate.

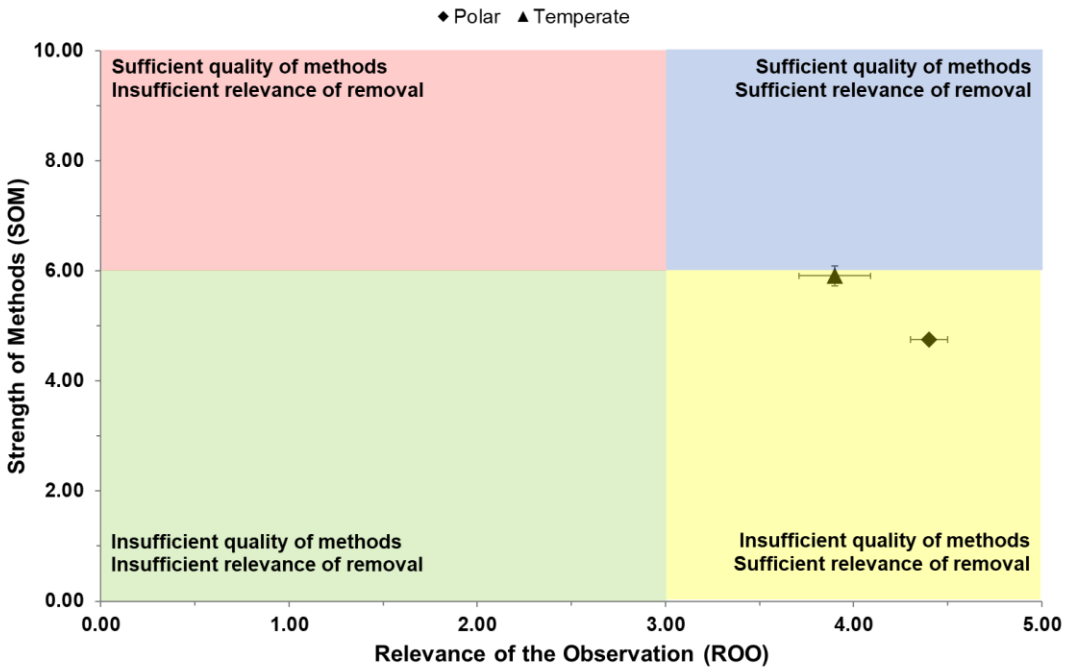


Figure 2.17. Score plot for atenolol, grouped by type of climate: polar (n=3) and temperate (n=30). Error bars depict standard error of the mean. No data available for tropical climate.

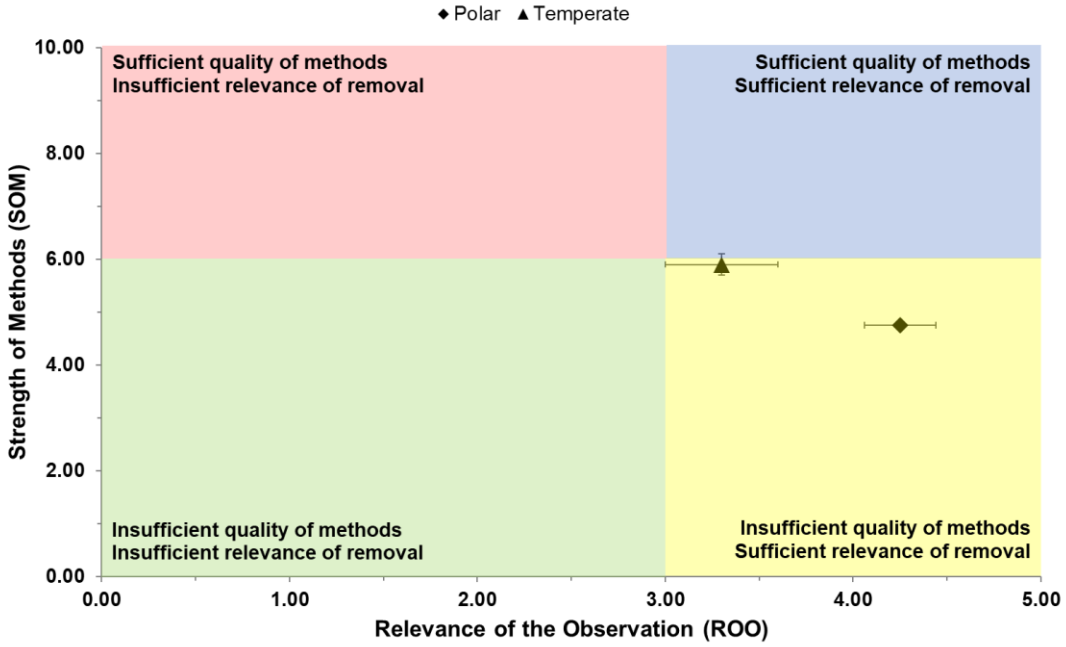


Figure 2.18. Score plot for trimethoprim, grouped by type of climate: polar (n=4) and temperate (n=23). Error bars depict standard error of the mean. No data available for tropical climate.

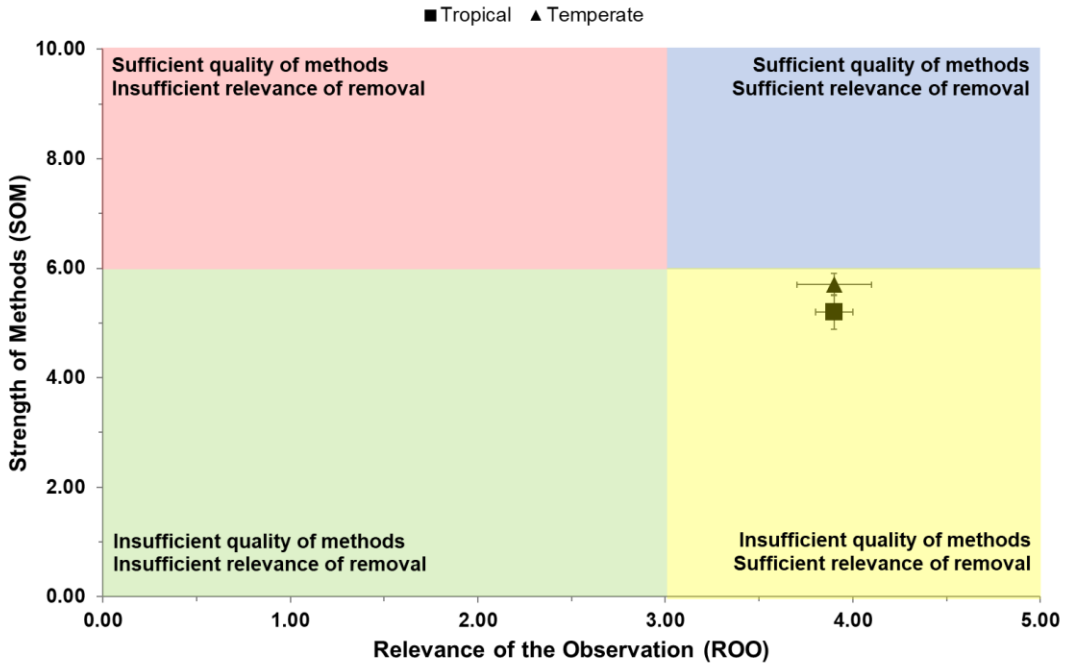


Figure 2.19. Score plot for acetaminophen, grouped by type of climate: tropical (n=2) and temperate (n=25). Error bars depict standard error of the mean. No data available for polar climate.

2.4.6. Caveats and limitations of this WoE assessment

The designed rubric allowed us to establish a baseline for evaluation of the relevance of pharmaceutical removal by CWs and the quality of methods used in the various research efforts. Still, this exercise was not exempt of some caveats and limitations due to the great complexity around the evaluation, based on the diversity of CW components and variables involved in the wastewater treatment of organic contaminants.

Some implicit weighting could be pointed out on the rubric, as the scoring for ROO had a lower maximum score (5) than the scoring for SOM (10). It could be suggested that this difference in scoring could imply different weighting, making emphasis on SOM over ROO. That having been said, the exercise aims to balance this situation by plotting ROO vs. SOM, and defining zones of "sufficient" and "insufficient" relevance and/or quality of

methods around the average of the scores obtained by all of the research papers. In this way, a performance threshold that considered both ROO and SOM scores was defined at 60% of the maximum score to conduct the assessment.

The ROO evaluation involved some implicit weighting for scoring the literature. This was particularly important when evaluating the characteristic time for removal, based on a somewhat arbitrary retention threshold of 24 hours. We acknowledge the fact that the threshold could have been established at different hydraulic retention times, since CWs can experience greater residence times, especially in full-scale systems. Nevertheless, the established threshold helped categorize various research items based on their readiness and efficiency.

The SOM evaluation involved the definition of three sub-groups that were not weighted equally (i.e., the maximum scores for SOM sub-groups A, B, and C are 5, 2, and 3 respectively). One of the greatest challenges of the overall assessment was defining the relative importance of the various aspects affecting the fate and removal of pharmaceuticals in complex systems such as CWs, given that important knowledge gaps around this aspect still exist. Based on this, normalization of the scores was proposed and executed to aim to balance the relative importance of each variable. Group A examined aspects around analyte characterization and fate, which can be often overlooked or omitted in the literature: stating the origin and purity of the reagents and standards, designing studies where the analytes concentration is realistic (i.e., close to environmental levels), measuring and reporting the hydraulic loading of the analytes into the system, providing a mass balance to account for the partition of the involved pharmaceuticals into the various CW compartments, and studying biotic and abiotic processes specifically to improve the knowledge around fate and kinetics of these contaminants. Of special difficulty was to evaluate the mass balance aspect

of the studies, as the rubric proposed a yes/no approach, without evaluating the quality of the mass balance more in depth. That said, given the myriad of possible experimental designs on this topic, such an assessment is very difficult and might need a more focused evaluation effort, which escapes the defined scope for the present work. Group B was geared towards the examination of matrix and environment-related aspects, while group C examined experimental design and quality control for data collection.

2.5. Conclusions and future research needs

A large number of research items on the removal of pharmaceuticals by CWs set the foundation of this assessment, which is based on critical review of literature and statistical analysis of available data from peer reviewed studies. We found all types of studied CWs have demonstrated some capacity for the removal of pharmaceuticals, across CW types and climates. Nevertheless, the various processes are involved in pharmaceutical removal by CWs, such as biodegradation, adsorption/sorption, plant uptake and photodegradation, render a comprehensive mechanistic description a very complex task, along with mass balance estimations.

This work showed overall significant differences between the quality of methods for pharmaceutical removal in polar, temperate and tropical climates, with temperate climate having the best quality of all the climates. Also, statistically significant differences were obtained for some of the pharmaceuticals when comparing their scores in warm vs cold seasons. These results suggest a need for the development of more research on pharmaceutical removal in CWs that operate at low temperatures, to increase the amount of available data and to continue evaluating the specific influence of CW design parameters at near-freezing temperatures.

From our assessment (Table A3), the compounds that showed statistically different ($p > 0.05$) mean ROO scores under cold conditions compared to warm were caffeine, salicylic acid, trimethoprim, and tetracycline. These pharmaceuticals are removed from CWs by biodegradation as a predominant removal mechanism (see Table 2.7). An increased temperature favours microbial activity and the formation of biofilms, which can then be drivers for removal of these contaminants. Thus, it is expected to observe greater removal rates with increasing temperature. For the remaining studied pharmaceuticals, no significant differences could be found between the ROO scores. That having been said, our seasonal comparison considered all studied CWs that quantified each pharmaceutical on each type of season, without discriminating by CW type or design parameters, mostly due to the scarcity of cold-season data to expand our analysis. This represented a major challenge for our assessment.

The comparatively better removal of various pharmaceuticals in H-CWs might be due to the co-existence of aerobic and anaerobic conditions and greater hydraulic loading rates in H-CWs, which can enhance the removal of some pharmaceuticals. The performance of H-CWs compared to single-step CWs (e.g., VSSF-CW, HSSF-CW and SF-CWs) suggests a potential the application of H-CWs for pharmaceutical removal when needed. However, H-CWs can be a combination of different types of conventional CWs such as VSSF-CW + HSSF-CW, HSSF-CW + VSSF-CW, VSSF-CW + VSSF-CW, HSSF-CW + HSSF-CW, other combinations with SF-CW, and also multi-stage of more than two types of CWs. Therefore, further research is needed regarding the evaluation of best possible integrated design of CWs to ensure various removal processes necessary to remove specific pharmaceuticals in a given context.

2.6. References for Chapter 2

Anderson, J.C.; Carlson, J.C.; Low, J.E.; Challis, J.K.; Wong, C.S.; Knapp, C.W.; Hanson, M.L. Performance of a constructed wetland in Grand Marais, Manitoba, Canada: Removal of nutrients, pharmaceuticals, and antibiotic resistance genes from municipal wastewater. *Chemistry Central Journal* **2013**; *7*: 54.

Anderson, J.C.; Joudan, S.; Shoichet, E.; Cuscito, L.D.; Alipio, A.E.C.; Donaldson, C.S, Khan S.; Goltz, D.M.; Rudy, M.D.; Frank, R.A.; Knapp, C.W.; Hanson, M.L.; Wong, C.S. Reducing nutrients, organic micropollutants, antibiotic resistance, and toxicity in rural wastewater effluent with subsurface filtration treatment technology. *Ecological Engineering* **2015**; *84*: 375-385.

Anderson, J.C.; Jabari, P.; Parajas, A.; Loeb, E.; Luong, K.H.; Vahedi, A.; Wong, C.S. Evaluation of cold-weather wastewater nitrification technology for removal of polar chemicals of emerging concern from rural Manitoba wastewaters. *Chemosphere* **2020**; *253*: 126711.

Auvinen, H.; Havran, I.; Hubau, L.; Vanseveren, L.; Gebhardt, W.; Linnemann, V, Van Oirschot, D.; Du Laing, G.; Rousseau, D.P.L. Removal of pharmaceuticals by a pilot aerated sub-surface flow constructed wetland treating municipal and hospital wastewater. *Ecological Engineering* **2017a**; *100*: 157-164.

Auvinen, H.; Gebhardt, W.; Linnemann, V.; Du Laing, G.; Rousseau, D.P.L. Laboratory- and full-scale studies on the removal of pharmaceuticals in an aerated constructed wetland: effects of aeration and hydraulic retention time on the removal efficiency and assessment of the aquatic risk. *Water Science and Technology* **2017b**; *76*: 1457-1465.

Avila, C.; Pedescoll, A.; Matamoros, V.; Bayona, J.M.; Garcia J. Capacity of a horizontal subsurface flow constructed wetland system for the removal of emerging pollutants: An injection experiment. *Chemosphere* **2010**; *81*: 1137-1142.

Avila, C.; Reyes, C.; Bayona, J.M.; Garcia, J. Emerging organic contaminant removal depending on primary treatment and operational strategy in horizontal subsurface flow constructed wetlands: Influence of redox. *Water Research* **2013**; *47*: 315-325.

Avila, C.; Nivala, J.; Olsson, L.; Kassa, K.; Headley, T.; Mueller, R.A.; Bayona, J.M.; García, J. Emerging organic contaminants in vertical subsurface flow constructed wetlands: Influence of media size, loading frequency and use of active aeration. *Science of the Total Environment* **2014a**; *494*: 211-217.

Avila, C.; Matamoros, V.; Reyes-Contreras, C.; Pina, B.; Casado, M.; Mita, L.; Rivetti, C.; Barata, C.; Garcia, J.; Bayona, J.M. Attenuation of emerging organic contaminants in a hybrid constructed wetland system under different hydraulic loading rates and their associated toxicological effects in wastewater. *Science of the Total Environment* **2014b**; *470*: 1272-1280.

Avila, C.; Bayona, J.M.; Martin, I.; Salas, J.J.; Garcia, J. Emerging organic contaminant removal in a full-scale hybrid constructed wetland system for wastewater treatment and reuse. *Ecological Engineering* **2015**; *80*: 108-116.

Avila, C.; Garcia-Galan, M.J.; Uggetti, E.; Montemurro, N.; Garcia-Vara, M.; Perez, S.; Garcia, J.; Postigo, C. Boosting pharmaceutical removal through aeration in constructed wetlands. *Journal of Hazardous Materials* **2021**; 125231.

Berglund, B.; Khan, G.A.; Weisner, S.E.B.; Ehde, P.M.; Fick, J.; Lindgren, P.E. Efficient removal of antibiotics in surface-flow constructed wetlands, with no observed impact on antibiotic resistance genes. *Science of the Total Environment* **2014**; *476*: 29-37.

Breitholtz, M.; Naslund, M.; Strae, D.; Borg, H.; Grabic, R.; Fick, J. An evaluation of free water surface wetlands as tertiary sewage water treatment of micro-pollutants. *Ecotoxicology and Environmental Safety* **2012**; *78*: 63-71.

Brezinova, T.D.; Vymazal, J.;, Kozeluh M, Kule L. Occurrence and removal of ibuprofen and its metabolites in full-scale constructed wetlands treating municipal wastewater. *Ecological Engineering* **2018**; *120*: 1-5.

Button, M.; Cosway, K.; Sui, J.; Weber, K. Impacts and fate of triclosan and sulfamethoxazole in intensified re-circulating vertical flow constructed wetlands. *Science of the Total Environment* **2019**; *649*: 1017-1028.

Camacho-Munoz, D.; Martin J.; Santos, J.L.; Aparicio, I.; Alonso, E. Effectiveness of Conventional and Low-Cost Wastewater Treatments in the Removal of Pharmaceutically Active Compounds. *Water Air and Soil Pollution* **2012**; *223*: 2611-2621.

Cardinal, P.; Anderson, J.C.; Carlson, J.C.; Low, J.E.; Challis, J.K.; Beattie, S.A.; Bartel, C.N; Elliot, A.D.; Montero, O.F.; Lokesh, S.; Favreau, A.; Kozlova, T.A.; Knapp, C.W.; Hanson, M.L.; Wong, C.S. Macrophytes may not contribute significantly to removal of nutrients, pharmaceuticals, and antibiotic resistance in model surface constructed wetlands. *Science of the Total Environment* **2014**; *482*: 294-304.

Cardinal, P.; Anderson, J.C.; Carlson, J.C.; Low, J.E.; Challis, J.K.; Wong, C.S.; Hanson, M.L. Late season pharmaceutical fate in wetland mesocosms with and without phosphorous addition. *Environmental Science and Pollution Research* **2016**; *23*: 22678-22690.

Carranza-Diaz, O.; Schultze-Nobre, L.; Moeder M.; Nivala J.; Kuschk, P.; Koeser, H. Removal of selected organic micropollutants in planted and unplanted pilot-scale

horizontal flow constructed wetlands under conditions of high organic load. *Ecological Engineering* **2014**; *71*: 234-245.

Carvalho, P.N.; Basto, M.C.P.; Almeida, C.M.R.; Brix, H. A review of plant-pharmaceutical interactions: from uptake and effects in crop plants to phytoremediation in constructed wetlands. *Environmental Science and Pollution Research* **2014**; *21*: 11729-11763.

Chaves-Barquero, L.G.; Humeniuk, B.W.; Luong, K.H.; Cicek, N.; Wong, C.S.; Hanson, M.L. Crushed recycled glass as a substrate for constructed wetland wastewater treatment: a case study of its potential to facilitate pharmaceutical removal. *Environmental Science and Pollution Research* **2021;** *28*: 62306-52318.

Chaves-Barquero, L.G.; Luong, K.H.; Mundy, C.J.; Knapp, C.W.; Hanson, M.L.; Wong, C.S. The release of wastewater contaminants in the Arctic: A case study from Cambridge Bay, Nunavut, Canada. *Environmental Pollution* **2016**; *218*: 542-550.

Chaves-Barquero, L.G.; Luong, K.H.; Rudy, M.D.; Frank, R.A.; Hanson, M.L.; Wong, C.S. Attenuation of pharmaceuticals, nutrients and toxicity in a rural sewage lagoon system integrated with a subsurface filtration technology. *Chemosphere* **2018**; *209*: 767-775.

Chen, Y.; Vymazal, J.; Brezinova, T.; Kozeluh, M.; Kule, L.; Huang, J.G.; Chen, Z. Occurrence, removal and environmental risk assessment of pharmaceuticals and personal care products in rural wastewater treatment wetlands. *Science of the Total Environment* **2016**; *566*: 1660-1669.

Christofilopoulos, S.; Kaliakatsos, A.; Triantafyllou, K.; Gounaki, I.; Venieri, D.; Kalogerakis, N. Evaluation of a constructed wetland for wastewater treatment:

Addressing emerging organic contaminants and antibiotic resistant bacteria. *New Biotechnology* **2019**; *52*: 94-103.

Conkle, J.L.; White, J.R.; Metcalfe, C.D. Reduction of pharmaceutically active compounds by a lagoon wetland wastewater treatment system in Southeast Louisiana. *Chemosphere* **2008**; *73*: 1741-1748.

Dan, A.; Zhang, X.M.; Dai, Y.V.; Chen, C.X.; Yang, Y. Occurrence and removal of quinolone, tetracycline, and macrolide antibiotics from urban wastewater in constructed wetlands. *Journal of Cleaner Production* **2020a**; *252*: 119677.

Dan, A.; Li, L.; Tai, Y.P.; Zhang, X.M.; Tao, R.; Yang, Y. Behavior assessment of sulfonamides and N-4-acetyl sulfonamides from wastewater effluent in subsurface constructed wetlands: Removal, distribution, and biotransformation. *Chemical Engineering Journal* **2020b**; *396*.

de Oliveira, M.; Atalla, A.A.; Frihling, B.E.F.; Cavalheri, P.S.; Migliolo, L.; Magalhaes, F.J.C. Ibuprofen and caffeine removal in vertical flow and free-floating macrophyte constructed wetlands with Heliconia rostrata and Eichornia crassipes. *Chemical Engineering Journal* **2019**; *373*: 458-467.

Delgado, N.; Bermeo, L.; Hoyos, D.A.; Penuela, G.A.; Capparelli, A.; Marino, D.; Navarro, A.; Casas-Zapata, J.C. Occurrence and removal of pharmaceutical and personal care products using subsurface horizontal flow constructed wetlands. *Water Research* **2020**; *187*: 116448.

Dordio, A.; Carvalho, A.J.P.; Teixeira DM, Dias CB, Pinto AP. Removal of pharmaceuticals in microcosm constructed wetlands using Typha spp. and LECA. *Bioresource Technology* **2010**; *101*: 886-892.

Dordio, A.V.; Candeias, A.J.E.; Pinto, A.P.; da Costa, C.T.; Carvalho, A.J.P. Preliminary media screening for application in the removal of clofibric acid, carbamazepine and ibuprofen by SSF-constructed wetlands. *Ecological Engineering* **2009a**; *35*: 290-302.

Dordio, A.; Pinto, J.; Dias, C.B.; Pinto, A.P.; Carvalho, A.J.P.; Teixeira, D.M. Atenolol removal in microcosm constructed wetlands. International Journal of Environmental *Analytical Chemistry* **2009b**; *89*: 835-848.

Ekperusi, A.O.; Sikoki, F.D.; Nwachukwu, E.O. Application of common duckweed (*Lemna minor*) in phytoremediation of chemicals in the environment: State and future perspective. *Chemosphere* **2019**; *223*: 285-309.

Francini, A.; Mariotti, L.; Di Gregorio, S.; Sebastiani, L.; Andreucci, A. Removal of micro-pollutants from urban wastewater by constructed wetlands with *Phragmites australis* and *Salix matsudana*. *Environmental Science and Pollution Research* **2018**; *25*: 36474-36484.

Garcia-Rodriguez, A.; Matamoros, V.; Fontas, C.; Salvado, V. The ability of biologically based wastewater treatment systems to remove emerging organic contaminants-a review. *Environmental Science and Pollution Research* **2014**; *21*: 11708-11728.

Gorito, A.M.; Ribeiro, A.R.; Almeida, C.M.R.; Silva, A.M.T. A review on the application of constructed wetlands for the removal of priority substances and contaminants of emerging concern listed in recently launched EU legislation. *Environmental Pollution* **2017**; *227*: 428-443.

Guedes-Alonso, R.; Montesdeoca-Esponda, S.; Herrera-Melian, J.A.; Rodriguez-Rodriguez, R.; Ojeda-Gonzalez, Z.; Landivar-Andrade, V.; Sosa-Ferrera, Z.; Santana-Rodríguez, J.J. Pharmaceutical and personal care product residues in a macrophyte pond-constructed wetland treating wastewater from a university campus: Presence,

removal and ecological risk assessment. *Science of the Total Environment* **2020**; *703*: 135596.

He, Y.J.; Nurul, S.; Schmitt, H.; Sutton, N.B.; Murk, T.A.J.; Blokland, M.H.; Rijnaarts, H.H.M.; Langenhoff, A.A.M. Evaluation of attenuation of pharmaceuticals, toxic potency, and antibiotic resistance genes in constructed wetlands treating wastewater effluents. *Science of the Total Environment* **2018**; *631-632*: 1572-1581.

Herrera-Cardenas, J.; Navarro, A.E.; Torres, E. Effects of porous media, macrophyte type and hydraulic retention time on the removal of organic load and micropollutants in constructed wetlands. *Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering* **2016**; *51*: 380-388.

Hester, R.E.; Harrison, R.M. Pharmaceuticals in the environment, **2016**. e-brary, Inc. London, England.

Hijosa-Valsero, M.; Matamoros, V.; Sidrach-Cardona, R.; Martin-Villacorta, J.; Becares, E.; Bayona, J.M. Comprehensive assessment of the design configuration of constructed wetlands for the removal of pharmaceuticals and personal care products from urban wastewaters. *Water Research* **2010a**; *44*: 3669-3678.

Hijosa-Valsero, M.; Matamoros, V.; Martin-Villacorta, J.; Becares, E.; Bayona, J.M. Assessment of full-scale natural systems for the removal of PPCPs from wastewater in small communities. *Water Research* **2010b**; *44*: 1429-1439.

Ilyas, H.; Masih, I.; van Hullebusch, E.D. Pharmaceuticals' removal by constructed wetlands: a critical evaluation and meta-analysis on performance, risk reduction, and role of physicochemical properties on removal mechanisms. *Journal of Water and Health* **2020**; *18*: 253-291.

Ilyas, H.; van Hullebusch, E.D. Role of Design and Operational Factors in the Removal of Pharmaceuticals by Constructed Wetlands. *Water* **2019**; *11*: 2356.

Ilyas, H.; van Hullebusch, E.D. Performance comparison of different types of constructed wetlands for the removal of pharmaceuticals and their transformation products: a review. *Environmental Science and Pollution Research* **2020**; *27*: 14342-14364.

Imfeld, G.; Braeckevelt, M.; Kuschk, P.; Richnow, H.H. Monitoring and assessing processes of organic chemicals removal in constructed wetlands. *Chemosphere* **2009**; *74*: 349-362.

Kadlec, R.H.; Wallace, S. Treatment wetlands. CRC Press, Boca Raton, FL: ebrary Inc. **2009**.

Kahl, S.; Nivala, J.; van Afferden, M.; Muller, R.A, Reemtsma, T. Effect of design and operational conditions on the performance of subsurface flow treatment wetlands: Emerging organic contaminants as indicators. *Water Research* **2017**; *125*: 490-500.

Lancheros, J.C.; Madera-Parra, C.A.; Caselles-Osorio, A.; Torres-Lopez, W.A.; Vargas-Ramirez, X.M. Ibuprofen and Naproxen removal from domestic wastewater using a Horizontal Subsurface Flow Constructed Wetland coupled to Ozonation. *Ecological Engineering* **2019**; *135*: 89-97.

Li, J.; Zhou, Q.; Campos, L.C. Removal of selected emerging PPCP compounds using greater duckweed (*Spirodela polyrhiza*) based lab-scale free water constructed wetland. *Water Research* **2017**; *126*: 252-261.

Li, Y.; Zhu, G.; Ng, W.J.; Tan, S.K. A review on removing pharmaceutical contaminants from wastewater by constructed wetlands: Design, performance and mechanism. *Science of the Total Environment* **2014**; *468*: 908-932.

Lima, M.X.; Carvalho, K.Q.; Passig, F.H.; Borges, A.C.; Filippe, T.C.; Azevedo, J.C.R.; Nagalli, A. Performance of different substrates in constructed wetlands planted with *E. crassipes* treating low-strength sewage under subtropical conditions. *Science of the Total Environment* **2018**; *630*: 1365-1373.

Macci, C.; Peruzzi, E.; Doni, S.; Iannelli, R.; Masciandaro, G. Ornamental plants for micropollutant removal in wetland systems. *Environmental Science and Pollution Research* **2015**; *22*: 2406-2415.

Matamoros, V.; Garcia, J.; Bayona, J.M. Behavior of selected pharmaceuticals in subsurface flow constructed wetlands: A pilot-scale study. *Environmental Science & Technology* **2005**; *39*: 5449-5454.

Matamoros, V.; Arias, C.; Brix, H.; Bayona, J.M. Removal of pharmaceuticals and personal care products (PPCPs) from urban wastewater in a pilot vertical flow constructed wetland and a sand filter. *Environmental Science & Technology* **2007**; *41*: 8171-8177.

Matamoros, V.; Bayona, J.M. Elimination of pharmaceuticals and personal care products in subsurface flow constructed wetlands. *Environmental Science & Technology* **2006**; *40*: 5811-5816.

Matamoros, V.; Garcia, J.; Bayona, J.M. Organic micropollutant removal in a full-scale surface flow constructed wetland fed with secondary effluent. *Water Research* **2008a**; *42*: 653-660.

Matamoros, V.; Caselles-Osorio, A.; Garcia, J.; Bayona, J.M. Behaviour of pharmaceutical products and biodegradation intermediates in horizontal subsurface flow constructed wetland. A microcosm experiment. *Science of the Total Environment* **2008b**; *394*: 171-176.

Matamoros, V.; Arias, C.; Brix, H.; Bayona, J.M.; Preliminary screening of small-scale domestic wastewater treatment systems for removal of pharmaceutical and personal care products. *Water Research* **2009**; *43*: 55-62.

Matamoros, V.; Rodriguez, Y.; Albaiges, J. A comparative assessment of intensive and extensive wastewater treatment technologies for removing emerging contaminants in small communities. *Water Research* **2016**; *88*: 777-785.

Matamoros, V.; Rodriguez, Y.; Bayona, J.M. Mitigation of emerging contaminants by full-scale horizontal flow constructed wetlands fed with secondary treated wastewater. *Ecological Engineering* **2017**; *99*: 222-227.

Mathon, B.; Coquery, M.; Miege, C.; Vandycke, A.; Choubert, J.M. Influence of water depth and season on the photodegradation of micropollutants in a free-water surface constructed wetland receiving treated wastewater. *Chemosphere* **2019**; *235*: 260-270.

Moeder, M.; Carranza-Diaz, O.; Lopez-Angulo, G.; Vega-Avina, R.; Chavez-Duran, F.A.; Jomaa, S.; Winkler, U.; Schrader, S.; Reemtsma, T.; Delgado-Vargas, F. Potential of vegetated ditches to manage organic pollutants derived from agricultural runoff and domestic sewage: A case study in Sinaloa (Mexico). *Science of the Total Environment* **2017**; *598*: 1106-1115.

National Academies of Science, Engineering, and Medicine. The Use of Systematic Review in EPA's Toxic Substances Control Act Risk Evaluations. The National Academies Press **2021**. DOI: 10.17226/25952

Nuel, M.; Laurent, J.; Bois, P.; Heintz, D.; Wanko, A. Seasonal and ageing effect on the behaviour of 86 drugs in a full-scale surface treatment wetland: Removal efficiencies and distribution in plants and sediments. *Science of the Total Environment* **2018**; *615*: 1099-1109.

Ozengin, N.; Elmaci, A. Removal of Pharmaceutical Products in a Constructed Wetland. *Iranian Journal of Biotechnology* **2016**; *14*: 221-229.

Park, J.; Cho, K.H.; Lee, E.; Lee, S.; Cho, J. Sorption of pharmaceuticals to soil organic matter in a constructed wetland by electrostatic interaction. *Science of the Total Environment* **2018**; *635*: 1345-1350.

Park, N.; Vanderford, B.J.; Snyder, S.A.; Sarp, S.; Kim, S.D.; Cho, J. Effective controls of micropollutants included in wastewater effluent using constructed wetlands under anoxic condition. *Ecological Engineering* **2009**; *35*: 418-423.

Phong, V.H.N.; Koottatep, T.; Chapagain, S.K.; Panuvatvanich, A.; Polprasert, C.; Ahn, K.H. Removal of acetaminophen from wastewater by constructed wetlands with Scirpus validus. *Environmental Engineering Research* **2016**; *21*: 164-170.

Reyes-Contreras, C.; Hijosa-Valsero, M.; Sidrach-Cardona, R.; Bayona, J.M.; Becares, E. Temporal evolution in PPCP removal from urban wastewater by constructed wetlands of different configuration: A medium-term study. *Chemosphere* **2012**; *88*: 161-167.

Ruhmland, S; Wick, A.; Ternes, T.A.; Barjenbruch, M. Fate of pharmaceuticals in a subsurface flow constructed wetland and two ponds. *Ecological Engineering* **2015**; *80*: 125-139.

Ruppelt, J.P.; Pinnekamp, J.; Tondera, K. Elimination of micropollutants in four test-scale constructed wetlands treating combined sewer overflow: Influence of filtration layer height and feeding regime. *Water Research* **2020**; *169*: 115214.

Sabri, N.A.; Schmitt, H.; van der Zaan, B.M.; Gerritsen, H.W.; Rijnaarts, H.H.M.; Langenhoff, A.A.M. Performance of full scale constructed wetlands in removing antibiotics and antibiotic resistance genes. *Science of the Total Environment* **2021**; *786*: 147368.

Sgroi, M.; Pelissari, C.; Roccaro, P.; Sezerino, P.H.; Garcia, J.; Vagliasindi, F.G.A.; Avila, C. Removal of organic carbon, nitrogen, emerging contaminants and fluorescing organic matter in different constructed wetland configurations. *Chemical Engineering Journal* **2018**; *332*: 619-627.

Sharif, F.; Westerhoff, P.; Herckes, P. Impact of hydraulic and carbon loading rates of constructed wetlands on contaminants of emerging concern (CECs) removal. *Environmental Pollution* **2014**; *185*: 107-115.

Sochacki, A.; Felis, E.; Bajkacz, S.; Nowrotek, M.; Miksch, K. Removal and transformations of diclofenac and sulfamethoxazole in a two-stage constructed wetland system. *Ecological Engineering* **2018**; *122*: 159-168.

Stroski, K.M.; Luong, K.H.; Challis, J.K.; Chaves-Barquero, L.G.; Hanson, M.L.; Wong, C.S. Wastewater sources of per- and polyfluorinated alkyl substances (PFAS) and pharmaceuticals in four Canadian Arctic communities. *Science of the Total Environment* **2020**; *708*: 134494.

U.S. Environmental Protection Agency. Weight of evidence in ecological assessment. USEPA **2016**. Washington, DC. EPA/100/R16/001

Varma, M.; Gupta, A.K.; Ghosal, P.S.; Majumder, A. A review on performance of constructed wetlands in tropical and cold climate: Insights of mechanism, role of influencing factors, and system modification in low temperature. *Science of the Total Environment* **2021**; *755*: 142540.

Verlicchi, P.; Galletti, A.; Petrovic, M.; Barcelo, D.; Al Aukidy, M.; Zambello, E. Removal of selected pharmaceuticals from domestic wastewater in an activated sludge system followed by a horizontal subsurface flow bed - Analysis of their respective contributions. *Science of the Total Environment* **2013**; *454*: 411-425.

Verlicchi, P.; Zambello, E. How efficient are constructed wetlands in removing pharmaceuticals from untreated and treated urban wastewaters? A review. *Science of the Total Environment* **2014**; *470*: 1281-1306.

Vo, H.N.P.; Koottatep, T.; Chapagain, S.K.; Panuvatvanich, A.; Polprasert, C.; Nguyen, T.M.H., Chaiwong, C.; Nguyen, N.L. Removal and monitoring acetaminophen-contaminated hospital wastewater by vertical flow constructed wetland and peroxidase enzymes. *Journal of Environmental Management* **2019**; *250*: 109526.

Vymazal, J. The use constructed wetlands with horizontal sub-surface flow for various types of wastewater. *Ecological Engineering* **2009**; *35*: 1-17.

Vymazal, J.; Brezinova, T.D.; Kozeluh, M.; Kule, L. Occurrence and removal of pharmaceuticals in four full-scale constructed wetlands in the Czech Republic - the first year of monitoring. *Ecological Engineering* **2017**; *98*: 354-364.

Vystavna, Y.; Frkova, Z.; Marchand, L.; Vergeles, Y.; Stolberg, F. Removal efficiency of pharmaceuticals in a full scale constructed wetland in East Ukraine. *Ecological Engineering* **2017**; *108*: 50-58.

WateReuse Research Foundation. Evaluate wetland systems for treated wastewater performance to meet competing effluent water quality goals. WateReuse Research Foundation **2011**.

Weed, D.L. Weight of evidence: A review of concept and methods. *Risk Analysis* **2005**; *25*: 1545-1557.

Zhang, D.; Gersberg, R.M.; Ng, W.J.; Tan, S.K. Removal of pharmaceuticals and personal care products in aquatic plant-based systems: A review. *Environmental Pollution* **2014**; *184*: 620-639.

Zhang, D.Q.; Gersberg, R.M.; Hua, T.; Zhu, J.F.; Nguyen, A.T.; Law, W.K.; Ng, W.J.; Tan, S.K. Effect of Feeding Strategies on Pharmaceutical Removal by Subsurface Flow Constructed Wetlands. *Journal of Environmental Quality* **2012**; *41*: 1674-1680.

Zhang, D.Q.; Luo, J.X.; Lee, Z.M.P.; Gersberg, R.M.; Liu, Y.; Tan, S.K.; Ng, J.W. Ibuprofen removal in horizontal subsurface flow constructed wetlands: treatment performance and fungal community dynamics. *Environmental Technology* **2016**; *37*: 1467-1479.

Zhang, D.Q.; Tan, S.K.; Gersberg, R.M.; Sadreddini, S.; Zhu, J.F.; Nguyen, A.T. Removal of pharmaceutical compounds in tropical constructed wetlands. *Ecological Engineering* **2011**; *37*: 460-464.

Zhu, S.C.; Chen, H. The fate and risk of selected pharmaceutical and personal care products in wastewater treatment plants and a pilot-scale multistage constructed wetland system. *Environmental Science and Pollution Research* **2014**; *21*: 1466-1479.

2.7. Connecting text between Chapters 2 and 3

Chapter 2 presented a weight-of-evidence assessment of the performance of CWs for pharmaceutical removal in various types of climate, and showed that these systems do have some potential to that purpose regardless of climate type. This dissertation continues on by providing three examples of these studies: one of them under Arctic climate, and two of them under temperate climate. First, we will examine the performance of a wastewater treatment facility located in the Canadian Arctic.

CHAPTER 3

3. THE RELEASE OF WASTEWATER CONTAMINANTS IN THE ARCTIC: A CASE STUDY FROM CAMBRIDGE BAY, NUNAVUT, CANADA

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The contribution from each author is detailed under the "Contributions of Authors" section in the preface of this dissertation.

3.1. Abstract

The treatment of municipal wastewater in the Arctic is challenging due to a variety of financial, operational, climatic and technical issues. To better understand the efficacy of current wastewater treatment in this region and the hazard posed to receiving waters, we assessed the occurrence of contaminants (i.e., pharmaceuticals, antibiotic resistance genes and nutrients) as they moved through a lagoon-based treatment system in Cambridge Bay in Nunavut, Canada. Wastewater treatment in this community is performed by the use of a lagoon-tundra wetland system that is discharged into the marine environment and is representative of current common practices throughout the region. In 2014, samples were collected before and during lagoon discharge from two locations in the main lagoon, one location downstream from the lagoon effluent and three locations offshore. Grab samples were collected to measure nutrients (e.g. total nitrogen and phosphorus) and the presence of antibiotic resistance gene-bearing microbes, and Polar Organic Chemical Integrative Samplers (POCIS) were deployed to collect passively organic contaminants in all locations. A total of six pharmaceuticals were detected from a screen of twenty-eight analytes during the study: atenolol, carbamazepine, clarithromycin, metoprolol, sulfamethoxazole and trimethoprim. The greatest concentrations of nutrients, antibiotic resistance genes (ARGs) and pharmaceuticals were found in sampling locations within the treatment lagoon. Offshore of the release point, we observed limited to no detection of pharmaceuticals and ARGs and no change in total nitrogen and phosphorus from pre-release. We conclude that the current concentrations of monitored pharmaceuticals do not pose a significant hazard at this time to aquatic organisms in Cambridge Bay.

3.2. Introduction

Organic contaminants in wastewater effluents, including pharmaceuticals, are released to aquatic ecosystems and have been found to pose a hazard under certain conditions to receiving waters; this poses a challenge for current wastewater treatment practices in many regions (Fent et al., 2006). Some pharmaceuticals such as anti-inflammatory drugs, antidepressants and antibiotics are not completely eliminated in the human body and can therefore enter the sewage system as parent compounds and their biologically active metabolites (Vasskog et al., 2009). Many current wastewater treatment systems are not specifically designed to eliminate organic contaminants and, as a consequence, many of these pollutants are able to persist through wastewater treatment processes (Gunnarsdottir et al., 2013). In addition, monitoring actions for most micropollutants have not been well established in most wastewater treatment facilities (Bolong et al., 2009). Another concern is the presence of organisms that carry antibiotic resistance genes (ARGs), which can threaten public health (Rowan, 2011). Antibiotic resistance genes have been detected in the environment as a result of the prevalent human and veterinary use of antibacterial and antimicrobial products (Kummerer, 2009). Nutrient enrichment has also been a potential hazard to the aquatic environment with increasing eutrophication in freshwater and enclosed marine systems downstream of areas of urbanization (Smith, 2003). Algal blooms can block light from getting into the aquatic environment. With enough overgrowth, they can prevent oxygen from getting into the water, thereby, endangering plants and animals. While the releases of effluents have been characterized for many countries and regions of the world (Luo et al., 2014), little to no work has been performed to quantify organic micropollutants and their risk in polar regions because of the difficulties associated with travel logistics and sample holding limitations. Therefore, the collection of sample replicates and comprehensive datasets suitable for

statistical analysis is highly constrained in these regions. As a consequence, there is a lack of understanding of environmental risks, system performance and treatment mechanisms associated with the treatment systems in polar regions (Chouinard et al., 2014).

Some studies have been performed in Arctic environments for the screening of pharmaceuticals and personal care products in wastewaters. For example, Weigel et al. (Weigel et al., 2004) studied the prevalence of selected pharmaceuticals in different sewage samples from Tromsø in Norway as well as in the seawater from Tromsø-sound, the recipient of wastewater. The selected pharmaceuticals were, among others, ibuprofen, and its metabolites, and the insect repellent N,N-diethyl-3-toluamide (DEET) as well as caffeine, which was included as a tracer for domestic sewage. Emnet et al. (Emnet et al., 2015) studied the occurrence of personal care products in two Antarctic research stations, detecting six analytes in treated wastewaters, including the UV filters 4-methyl-bezylidene camphor, 2-hydroxy-4-methoxybenzophenone and 2,4-dihydroxybenzophenone, the plastic monomer 2,2-bis(4-hydroxyphenyl)-propane, the steroid hormone estrone and the antimicrobial triclosan. These compounds were detected at concentrations comparable to those reported for international coastal waters adjacent to significantly greater human populations (Balmer et al., 2005).

In many regions of the Arctic, the release of sewage with minimum or no treatment can have consequences for the receiving environment due to high vulnerability of the Arctic ecosystem to environmental contaminants (Gunnarsdottir et al., 2013). (Kallenborn et al., 2008) reported that pharmaceutical residues are degraded slower in Arctic environments compared to release scenarios in lower latitudes. In their study a set of nine different antidepressants and their transformation products were analyzed in receiving seawater from two locations in Norway, one of them in a northern region. Increased

environmental stability of these compounds was detected in the Arctic environment compared to the temperate location. The removal of pharmaceutical residues by photodegradation is limited during the Arctic polar night and the intensity of sunlight (even continuously during periods of midnight sun) at other times of the year are less intense than that of more temperate regions. Both limited photodegradation during the winter and the cold Arctic climate can slow down the degradation rate of pharmaceutical residues in the environment (Schwarzenbach et al., 2003).

Arctic communities frequently experience several challenges in order to perform adequate treatment of their wastewaters. Characteristics such as geographical remoteness, adverse weather and lack of basic services are common in many communities and make wastewater treatment, whenever possible, a difficult task (Yates et al., 2012). The scarcity of accredited laboratories for compliance testing and the necessity for trained personnel to manage wastewater facilities are challenges that need to be overcome by these communities on a daily basis. The subsistence fishery is a significant industry in many Arctic coastal regions, for which pollutant contamination of marine species exploited for human consumption is a major concern. Exposure to micropollutants and their uptake in the food web can have hazardous effects on human health and the environment through bioaccumulation and biomagnification of chemicals (Gunnarsdottir et al., 2013).

The Canada Health Act ensures that the majority of health services are publicly funded for all Canadians, with administration occurring at the provincial and territorial level. Despite this universality of health care, some differences occur in the health status of aboriginal and non-aboriginal Canadians. First Nations populations experience greater rates of mental illness, suicide, diabetes, asthma, cardiovascular disease, tuberculosis, hepatitis, syphilis and HIV/AIDS than non-aboriginal populations (Romain, 2013). This can

influence the amount of pharmaceuticals that are needed to treat these diseases in Northern Canadian communities.

To begin to address the need for knowledge about wastewater contaminants exposure in the Arctic, it is needed to quantify the types and quantities of nutrients and micropollutants in lagoon discharge effluents and receiving waters. Such an effort would allow a partial understanding of the possible hazards associated with wastewater discharges into receiving environments. In this study, we examined the efficacy of wastewater treatment under arctic conditions, by assessing the occurrence of selected wastewater contaminants attenuation and release from a wastewater treatment facility in Cambridge Bay, Nunavut, Canada. Our objectives were: first, to obtain recent exposure data for the wastewater contaminants in Cambridge Bay, regarding to the concentrations of nutrients (total nitrogen and phosphorus), ARGs and pharmaceuticals; and second, to provide a baseline of the current state of wastewater treatment in Cambridge Bay, in anticipation of the eventual instalment, expected by 2017, of the Canadian High Arctic Research Station (CHARS), a scientific facility for Arctic research, as well for expanding populations in the Arctic in general. Of particular interest was the exposure data at the water intake point that CHARS will eventually use for research purposes. We were also interested on assessing the facility for evidence of any leaky sewage infrastructures, specifically at Finger Bay. We hypothesize that the wastewater contaminants in Cambridge Bay do not pose a significant risk at this time to the marine environment, and that the lagoon-wetland system in this community has the ability to perform partial attenuation on nutrients, pharmaceuticals and antibiotic resistance genes.

3.3. Materials and Methods

3.3.1. Study location

Cambridge Bay is located in the territory of Nunavut in the Canadian Arctic. It has a population of approximately 1,400. Mean monthly temperatures range from a maximum and minimum, respectively, of -28 °C and -35 °C in January to 13 °C and 5 °C in July (Government of Canada, 2014). In 2017 CHARS will become operational. This will likely have an impact in the community in terms of increases in population and use of water resources, including wastewater disposal and treatment. This study provides a baseline for current wastewater impacts prior to CHARS' opening.

The wastewater system monitored at Cambridge Bay is comprised of a wastewater lagoon, formerly a series of natural lakes, that performs primary treatment and is discharged once a year, during the summer, into a small hydrologically-isolated natural tundra wetland. Wastewater then is released through an open channel into the marine environment. Municipal sewage from household sewage tanks is regularly transported to the lagoon by sewage trucks that perform dumping runs year-round.

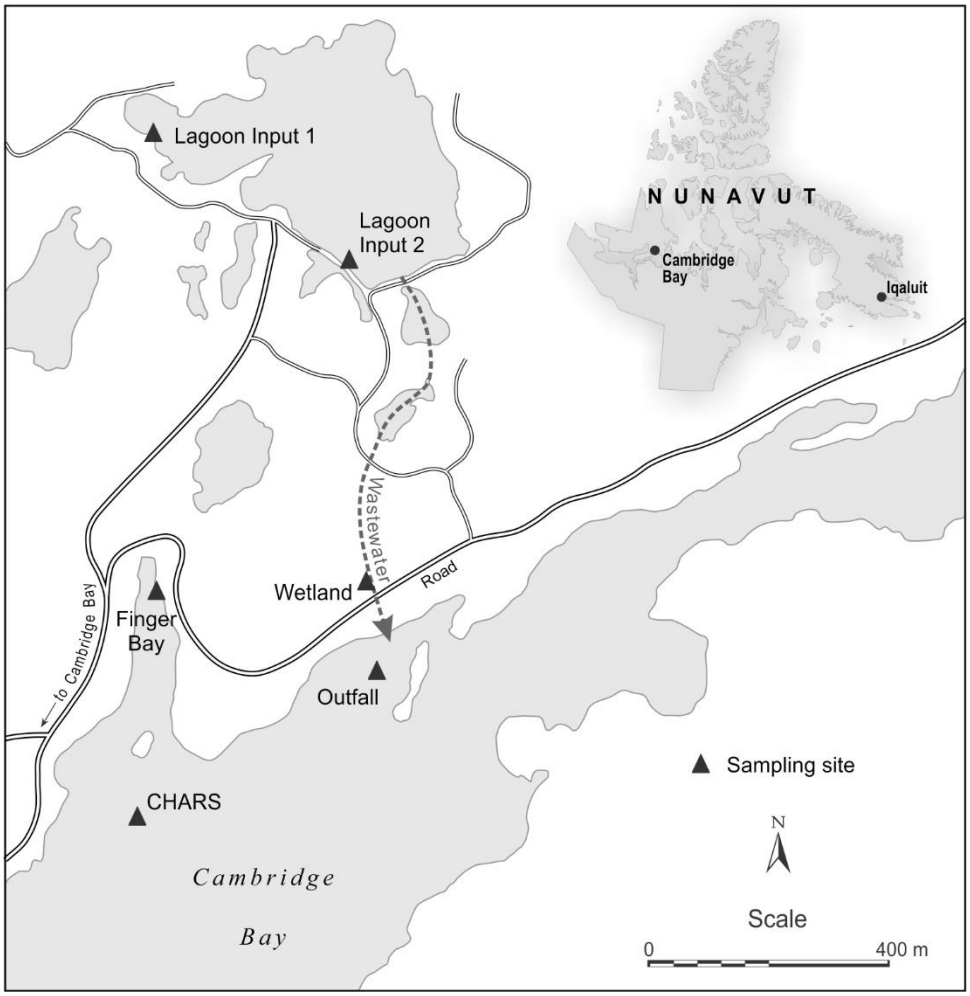

Figure 3.1. Sampling site locations at the Cambridge Bay wastewater treatment facility and receiving waters. Wastewater path from the lagoon to the bay is shown by means of a dotted line.

3.3.2. Sample collection

The selection of sampling sites was done in consultation with local municipal authorities with an overarching aim of characterizing the composition of wastewaters and receiving waters for the target analytes. We were also interested in assessing risk to CHARS intake by wastewater and the possibility of sewage leakiness into Finger Bay. Water was sampled from six selected locations around the study site (Figure 3.1). These were: approximately 20 meters away from a new wastewater drop off point (Lagoon Input 1); approximately 20 meters away from an older drop off point (Lagoon Input 2); at the

outflow of the natural tundra wetland (Wetland); approximately 100 meters offshore of the primary discharge point (approximately 100 meters from the Wetland site) to the bay (Outfall); at the seawater intake point for CHARS studies (CHARS); and at a previously used run-off discharge point culvert, currently closed, located around 300 meters west from the main discharge point (Finger Bay). No significant rain events were registered during the sampling. Pharmaceuticals were passively sampled using triplicate POCIS (Environmental Sampling Technologies, St Joseph, MO) in the "pharmaceutical" configuration as described by (MacLeod and Wong, 2010). Sampling was performed both before and during release of wastewater from the lagoon. Pre-release POCIS sampling was performed from July 25 to August 8, 2014 for inland locations, and from July 26 to August 9 for offshore locations. Wastewater release occurred from August 28 to September 5 with POCIS sampling performed from August 29 to September 8 at all locations. Grab-sampling for nutrients (composite sample) and antibiotic resistance genes (ARGs; triplicate sample) was conducted on July 25 in the pre-release stage, and on September 3 during release. Field blanks immersed in nanopure water (18 MΩ-cm, Millipore, Billerica, MA) in the appropriate containers were opened during sampling to determine the extent of contamination. Samples were kept on ice within 24 hours after the sampling for transport to the local laboratory, and shipped on ice back to Winnipeg for processing. Samples for nutrients were collected in 50 mL falcon tubes. Personnel wore gloves disinfected with 70% isopropanol while handling ARGs samples which were collected in autoclaved 500 mL polyethylene bottles pre-release (July 25) and during release (September 3) from all sampling locations. Bottles were rinsed three times with sample water before being filled to the top with no headspace. Field blanks filled with nanopure water (18 MΩ-cm, Millipore, Billerica, MA) in the appropriate containers were opened during sampling to determine the

extent of contamination. Samples were kept on ice within 24 hours after the sampling for transport to the laboratory, where ARGs samples were filtered in a sterile environment. These filters were kept at -20°C until shipment to the University of Strathclyde for antibiotic resistance genes analysis.

3.3.3. Determination of pharmaceuticals and nutrients

We followed the methods of Carlson et al., (2013), for the analysis of pharmaceuticals. Ultra high-performance liquid chromatography-tandem mass spectrometry (UHPLC/MS/MS) with isotope dilution was used to quantify chemicals of interest in water samples. These compounds included a suite of twenty-eight commonly-used micropollutants frequently found in wastewaters (Fatta-Kassinos et al., 2011), including β-blockers (e.g. metoprolol); antidepressants (e.g. fluoxetine); anticonvulsant drugs (e.g. carbamazepine) and macrolide (e.g. clarithromycin) and sulfonamide (e.g. sulfamethazine) antibiotics. Limits of quantification (LOQ) are found in Table B1, and other quality assurance/quality control parameters details in Carlson et al. (2013). Time-weighed average (TWA) concentrations were calculated by dividing the determined mass of chemical (ng) in the sampler by the sampling rate (L/d) times the deployment time (d) for all detected pharmaceuticals, making use of sampling rates (Table B2) found in the literature (MacLeod et al., 2007; Bartelt-Hunt et al., 2011). Concentrations of nutrients (e.g. total nitrogen and total phosphorus) were determined before and during discharge following standard methods (APHA, 2005).

3.3.4. Hazard assessment for detected pharmaceuticals

A hazard assessment was performed for detected compounds by calculating hazard quotients (HQs) using standard tests and endpoints from aquatic toxicological studies, particularly for primary producers, invertebrates and fish. Briefly, estimates for effective

concentrations (EC_{50}) or lethal concentrations (LC_{50}) were obtained through a literature search. For added conservatism, we employed an uncertainty factor of 1000 to the lowest EC_{50} or LC_{50} (Waiser et al., 2012). The maximum measured environmental concentration was then divided by the lowest reported effect concentration (typically freshwater, as marine organism tests were lacking) to obtain the hazard quotient. Hazard quotients greater than 1 were considered to be of concern while those compounds with HQ values less than 1 were considered less likely to pose a concern.

3.3.5. Determination of antibiotic resistance genes

Antibiotic resistance genes were quantified from cells harvested on filters following cell disruption (FastPrep, MP Biomedicals; 2 cycles at 30 s each at 6.0 setting) and DNA purification (MoBio PowerClean Soil DNA kit; Cambio, Cambridge, UK), similar to methods previously used (Anderson et al., 2015; Cardinal et al., 2014). A multiplex assay was used to target an array of tetracycline resistant genes (Ng et al., 2001), sulfonamide resistant genes (Pei et al., 2006) and 16S-rRNA was quantified as a measure of 'total bacteria'. Quantitative PCR was conducted using a BioRad iQ cycler (BioRad, Hercules, CA) using ssoFast EvaGreen reagents (BioRad) and 500 nM primer concentrations. All samples were diluted 1:100 with molecular grade water, as reactions were predetermined to be most efficient at those sample concentrations; standards and post-analytical melting curves were generated (Smith et al., 2004) to verify PCR reactions quality and quantify results.

3.3.6. Statistical analysis

Changes in concentrations of pharmaceuticals, as well as abundance of ARGs, from before and during release were assessed using a Student's paired *t*-test. Concentration data

are presented as mean ± standard deviation (St. Dev) unless otherwise indicated. Differences were considered significant at $p < 0.05$.

3.4. Results and Discussion

3.4.1. Nutrients

Nutrient samples had to be combined in order to measure concentrations at many sites, particularly those offshore given very low levels there (Table 3.1). Thus, only a single measurement was made per site per sampling time, so our discussion of changes in concentrations is qualitative in nature.

Total nitrogen showed values over 10 mg/L for the lagoon sites both before and during release measurements. Concentrations at Lagoon Input 1 and Lagoon Input 2 showed similar levels at both times, with the Wetland site having an increase in concentration after the wastewater discharge started. There was no apparent reduction in the concentration of nitrogen in the lagoon or the wetland in the two time periods. Locations offshore (e.g. Outfall, CHARS) showed much lower concentrations. Total phosphorus levels were approximately 2 mg/L for both lagoon sites prior to wastewater discharge. After the discharge commenced, phosphorus levels in the wetland were elevated to approximately 2 mg/L as well, with no apparent reduction from additional wetland treatment. Phosphorus levels appear to be greater and nitrogen values appear to be lesser than the maximum values recommended for Canadian provinces such as Manitoba, in which limits of 1 mg/L total phosphorus and 15 mg/L total nitrogen exist for wastewater effluents discharged to a water body (Manitoba Water Stewardship, 2011). However, policies for communities in the far north have not yet been defined and a joint governmental commission has been assigned to define them by 2019 (CCME, 2014).

Considerable dilution was observed for all locations offshore (e.g. Outfall, CHARS), which was consistent with the nitrogen measurements. Finger Bay showed reduced levels for both total nitrogen and phosphorus, which suggests that there is little possibility of runoff from the main lagoon to this location contrary to prior speculations that this was a route of contamination from the lagoon to the bay. The levels of phosphorus we measured pre-release are comparable to that in the water column at the center of Cambridge Bay and at Dease Strait, a waterway immediately west of Cambridge Bay (0.01-0.04 mg/L, C. J. Mundy, unpublished data). Concentrations of phosphorus at the Outfall site are roughly twice those levels, suggesting localized effects of phosphorus that are not evident at points farther away in the bay (Table 3.1). While nutrient levels during release are likely locally elevated relative to concentrations in the greater Canadian Arctic (Tremblay et al., 2015), more work is warranted to examine to what extent these added nutrients may influence the local ecosystem of Cambridge Bay and Dease Strait.

No apparent nutrient removal was observed during discharge as a result of lagoon-wetland treatment. As noted, statistical analysis of nutrient concentrations was not possible. Nor can we rule out the possibility that nutrient concentrations may have been affected by heterogeneous distributions within different locations of the lagoon. That having been said, the data obtained in this study differ from the results obtained in a previous work by (Yates et al., 2012), in which three larger lagoon-wetland systems in Nunavut (Arviat, Whale Cove and Coral Harbour) were studied, observing reductions up to 84-99% for NH_3-N and 80-99% for total phosphorus. It is known that the community of Arviat make use of berms and channels to direct wastewater flow away from the ocean and to keep a longer residence time in the wetland (Wooton et al., 2008), whereas in the Cambridge Bay wetland the residence time of wastewater is limited by the landscape topography and the scarce

available vegetation. It is yet unclear which mechanisms play the most important role in wastewater treatment in the Arctic. Wetland size and vegetation coverage as well as the potential for filtration and sedimentation of suspended solids and adsorption of nutrients within the soil and water column can play a significant role.

Table 3.1. Levels of total phosphorus and total nitrogen in the Cambridge Bay wastewater facility before and during wastewater discharge (n=1). Wastewater was grab-sampled on July 25 and September 3, 2014. The limit of detection (LOD) and limit of quantification (LOQ) values for total nitrogen were 8 µg/L and 27 µg/L, and those for total phosphorus were 0.58 µg/L and 1.85 µg/L, respectively.

	Before discharge		During discharge	
Sampling site	Total phosphorus (mg/L)	Total nitrogen (mg/L)	Total phosphorus (mg/L)	Total nitrogen (mg/L)
Lagoon Input 1	2.4	12.6	2.8	12.0
Lagoon Input 2	2.4	16.6	2.8	13.5
Wetland	0.7	0.4	2.5	13.3
Outfall	0.01	0.3	0.07	0.4
Finger Bay	0.01	0.3	0.03	0.3
CHARS	0.02	0.4	0.03	0.3

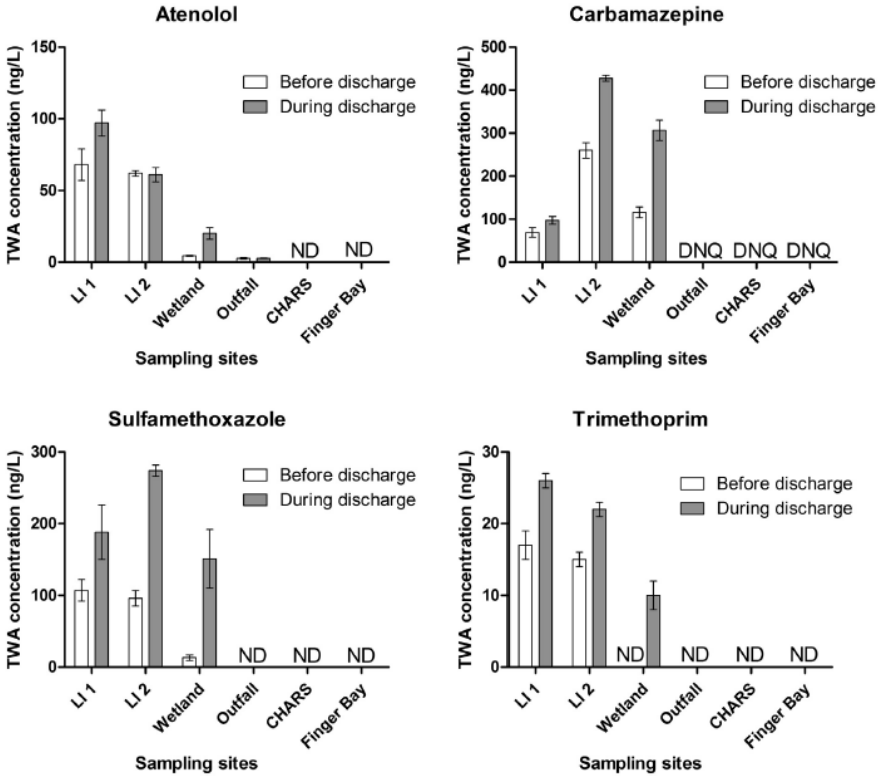

Figure 3.2. Mean time-weighted-average (TWA) levels of pharmaceuticals in Cambridge Bay wastewater facility, before and during the wastewater discharge process (n=3). Error bars depict the standard deviation on each case. Differences are significant for $p<0.05$ and were assessed using paired t-tests. Wastewater was collected using Polar Organic Chemical Integrative Samplers from July 25 to August 8, from July 26 to August 9, and from August 29 to September 8, 2014. ND: below limit of detection (LOD). DNQ: did not quantify.

3.4.2. Pharmaceuticals

Of the screened twenty-eight organic micropollutants, only six pharmaceuticals were detected above their LOQ at any of the locations. These were atenolol, clarithromycin, metoprolol, sulfamethoxazole, trimethoprim and carbamazepine, detected at least once in ng/L levels (Figure 3.2). We did not detect any significant interferences or matrix effects in our analyses. For the detected pharmaceuticals, the greatest concentrations were measured at the Lagoon Input 1 and 2 sites, although some differences in concentration could be seen between both of the dumping sites. Most locations offshore experienced considerable dilution with seawater, which was reflected in significantly lower concentrations for all of the passively sampled contaminants at Outfall, CHARS and Finger Bay. Although POCIS deployment times were different before and during discharge (14 days versus 9 days), steady state conditions for POCIS are typically reached within six days (Vermeirssen et al., 2012)

The greatest concentration of atenolol was 97 ng/L (Lagoon Input 1). Detected levels were significantly different ($p<0.05$) between Lagoon Input 1 and Lagoon Input 2 sites both before and during the discharge. There was a significant reduction of 45% observed between Lagoon Input 2 and Wetland ($p<0.05$) during the wastewater release, and heavy dilution at locations offshore, with most sites observed to be at non-detectable levels. These results suggest processes within the wetland (e.g. sorption to plants, microbial degradation) may reduce concentrations of atenolol under Arctic conditions. A more efficient removal of atenolol has been observed in more southern locations in Canada, with removal rates up to 98% for example within a sewage lagoon in Dunnottar, Manitoba under temperate conditions (Anderson et al., 2015).

Concentrations of carbamazepine were generally below 100 ng/L in both lagoon sites, with greater concentrations reported in the Wetland site both before and during discharge. No apparent removal was observed as a result of wastewater passage through the treatment wetland ($p<0.05$). Offshore locations showed levels below LOQ. Persistence of carbamazepine during the Arctic winter was observed, with a concentration of 116 ng/L in the hydrologically isolated wetland prior to discharge. While no measurements of pharmaceuticals occurred during the winter, we note that this shallow wetland system and the offshore locations are predominantly or completely frozen over the winter. This would presumably result in no removal of analytes by either microbial activity or photodegradation (i.e., light penetration would be prevented almost completely by ice cover) until summer melt.

The greatest concentration of sulfamethoxazole was 274 ng/L; this was detected at the Lagoon Input 2 site during wastewater discharge. Concentrations between lagoon sites were significantly different both before and during discharge ($p<0.05$), with some attenuation observed after wetland treatment, reaching 151 ng/L (45% removal, $p<0.05$). Levels offshore were non-detectable both before and during discharge. Unlike this study, (Conkle et al., 2008) noted over 90% removal of sulfonamides on a temperate wastewater facility, however, the differences may have been as a result of significantly greater temperatures and a 27-day retention period compared to a drastically colder weather and shorter retention time at Cambridge Bay facility of 1-2 days

Trimethoprim was detected in concentrations under 30 ng/L at the lagoon and wetland sites. During wastewater release, the wetland concentration was 9.8 ng/L after significant attenuation ($p<0.05$) occurred between the lagoon and the wetland. Finally, clarithromycin and metoprolol were detected at both of the lagoon sites and also in the

wetland at levels below LOQ. At the offshore sites, both compounds were non-detectable, which is consistent with what was observed for all contaminants studied.

The presence or absence of specific pharmaceuticals depends partially on the residence time within sewage holding tanks, prior to entry into sewage lagoons. While photodegradation is unable to occur in septic tanks, other degradative processes like anaerobic microbial-mediated biotransformation could occur. Consequently, the most labile compounds were likely partially degraded to an unknown extent. Sorption of pharmaceuticals to septic tank particulates may also occur. Photodegradation and biotransformation are typically the most important processes for the attenuation of organic micropollutants in effluent-receiving waters. Consequently, optimization of conditions for these processes (e.g. by using extended periods of treatment in sewage lagoons) can effectively minimize or prevent environmental exposure to biologically active levels of these contaminants (Ying et al., 2009).

To the best of our knowledge, there are no reported data for pharmaceuticals from wastewater systems from Northern Canada. Nevertheless, a larger amount of information (Table 3.3) is available for treated lagoon wastewaters using passive sampling of more southern regions in Canada, including various works done in the province of Manitoba (Anderson et al., 2013; Anderson et al., 2015; Carlson et al., 2013) and Alberta (MacLeod and Wong, 2010). At Cambridge Bay, all detectable compounds had greatest concentrations at either the lagoon or the wetland sites and were mostly non-detectable at locations offshore. Atenolol, carbamazepine, sulfamethoxazole and trimethoprim were detected in the Cambridge Bay facility at lower levels compared to the data obtained at Dunnottar, Manitoba (Anderson et al., 2015). On the other hand, atenolol, sulfamethoxazole and trimethoprim were detected in greater levels than in the Grand Marais wastewater treatment

facility. Levels of drugs were similar in wastewaters of Cambridge Bay and Lac la Biche, Alberta (MacLeod and Wong, 2010).

There are several factors that likely account for the differences in pharmaceutical levels among these locations. One factor is population, with greater populations implying greater loadings and impact on wastewater release. The populations served by the treatment facilities of the southern Canadian sites (Table 3.3) are all on the order of several thousand, with some seasonal variability. For example, the Dunnottar population, a popular regional summer resort (Anderson et al., 2015) is several times greater than that of Cambridge Bay during the summer. However, similar per capita use of drugs may result in similar concentrations in wastewaters (MacLeod and Wong, 2010), which appears to be the case based on our comparisons (Table 3.3). Another factor is temperature, given the fact that colder temperatures in Nunavut can cause treatment mechanisms such as sorption to be slower and less efficient when compared to temperate locations, as it has been previously observed in Norway (Kallenborn et al., 2008). Both factors are likely in play, confounding prediction of pharmaceutical levels in wastewaters.

3.4.3. Risk assessment for detected pharmaceuticals

Hazard quotients (HQs) were calculated for each organic contaminant based upon toxicity data reported in the literature for primary producers, invertebrates and fish (Table B3). Most compounds had an HQ less than 1, ranging from values between 10^{-6} and 10^{-1}. Only clarithromycin presented an HQ greater than 1 for the algae *Pseudokirchneriella subcapita*, which indicates that there is a potential for growth inhibition of algal species at concentrations such as those detected in lagoon and in the wetland. While the HQ for clarithromycin was greater than 1, the concentration level used for calculation may not be necessarily representative of what could be found in the entire lagoon, the use of 1000-fold

uncertainty factor adds a high degree of conservatism. As well, in eutrophic environments, such as these lagoons, excess nutrients can mitigate the effects of compounds that exhibit herbicidal activity (Baxter et al., 2013). For the rest of the detected pharmaceuticals, we can conclude that there is likely no significant hazard to aquatic life due to the low concentrations at which they were detected. Moermond et al. (2016) have estimated maximum acceptable concentrations for metoprolol and carbamazepine to protect the aquatic ecosystem against possible effects from short-term concentration peaks. The maximum concentrations found in our study are well below the values mentioned in said paper: 1.6 mg/L for carbamazepine and 0.76 mg/L for metoprolol We did lack Arctic and marine specific tests that would reduce the uncertainty and did not assess for the effect of mixtures of chemical stressors. We do recommend the development of standard toxicity tests with Arctic marine organisms to help address this uncertainty

3.4.4. Abundances and removal of ARGs

Total bacterial populations were determined by means of the abundances of 16S rRNA genes. Their presence was greatest in the lagoon sites in both sampling periods: before the wastewater discharge started ($10^{8.0}$ genes/mL in Lagoon Input 1 and $10^{7.8}$ copies/mL in Lagoon Input 2), and during wastewater discharge ($10^{7.4}$ genes/mL in Lagoon Input 1 and $10^{7.5}$ copies/mL in Lagoon Input 2) (Table 3.2). Overall, the abundances of 16S rRNA genes were similar (i.e., differences found were not greater than one order of magnitude) to levels reported at more southern locations in Canada (Anderson et al., 2015). Comparing concentrations before and during wastewater discharge, gene abundances did not change significantly (paired t-test, $t_5 = -1.46$, $p = 0.203$) and their distribution pattern remained similar ($r = 0.965$, $p = 0.002$) along the waste stream.

Clusters of tetracycline resistance and sulfonamide-resistance genes were analyzed and the results were summed to facilitate assessment of resistance patterns. The greatest abundances of tet^R (sum of tetracycline resistance genes) and sul^R (sum of sulfonamide resistance genes) were found in the primary lagoon, at the two wastewater drop-off locations, being Lagoon Input 2 the one with the greatest response, with some attenuation through the wetland (Table 3.2).

Specifically in terms of the tetracycline resistance genes, differences from lagoon levels to offshore levels were around one order of magnitude during discharge, with locations after the wetland having reductions most likely due to dilution. Outfall was the sampling spot with the lower amount of tet^R genes before and after discharge. Distribution of concentrations before and during discharge was similar ($r = 0.941$, $p = 0.005$), but became lower after discharge (paired t-test, $t_5 = 3.66$, $p = 0.015$) quite possibly due to dilution with water from the environment (e.g. existing surface water and/or groundwater seeps).

Table 3.2. Abundances of antibiotic resistance genes (log(genes/mL)) harvested from grab-samples taken at the Cambridge Bay wastewater treatment facility and receiving waters in 2014; standard deviation of sample replicates (n=3) are denoted in parentheses. Relative abundance of ARGs to 16S rRNA, calculated during discharge (A = tet^R/16S rRNA ratio, B = sul^R/16S rRNA ratio) is also shown.

	Before discharge			During discharge				
Sampling site	log total tet^R	log total sul^R	log 16S rRNA	log total tet^R	log total sul^R	log 16S rRNA	A (%)	B (%)
Lagoon Input 1	4.2 (0.3)	4.7 (0.5)	8.0 (0.5)	3.8 (0.2)	4.4 (0.3)	7.4 (0.4)	0.02	0.1
Lagoon Input 2	4.3 (0.3)	6.0 (0.7)	7.8 (0.9)	4.1 (0.3)	5.6 (0.3)	7.5 (0.8)	0.03	1.3
Wetland	3.9 (0.2)	4.4 (0.3)	6.8 (0.4)	3.8 (0.2)	5.6 (0.3)	7.3 (0.6)	0.03	2.0
Outfall	2.9 (0.2)	2.8 (0.2)	5.4 (0.4)	2.7 (0.2)	1.8 (0.1)	6.1 (0.4)	0.04	0.01
Finger Bay	3.0 (0.1)	2.8 (0.2)	5.3 (0.2)	2.7 (0.2)	2.0 (0.2)	6.3 (0.4)	0.02	0.01
CHARS	3.7 (0.2)	3.0 (0.2)	5.2 (0.4)	3.0 (0.3)	3.3 (0.3)	6.3 (0.4)	0.05	0.1

Sulfonamide resistance genes were more highly concentrated in the lagoon and wetland, and before discharge declined rapidly (2-3 orders of magnitude) following the wetland. During discharge, gene concentrations were variable at the two drop-off points in the lagoon, with minimum to no attenuation from the wetland. Gene distribution patterns along the waste stream were comparable (before-during discharge; $r = 0.887$, $p = 0.019$), but unlike 'total tet' there were significant pairwise changes (paired t-test, $t = 0.506$, $p = 0.634$) as most concentrations decline, except at the wetland and slightly in CHARS site.

Table 3.3. Comparison of concentrations of target pharmaceutical compounds in treated wastewaters of different Canadian lagoon wastewater systems (NA: not analyzed, ND: non-detectable). Lac la Biche data from MacLeod and Wong (2010), Dunnottar data from Anderson et al. (2015), Grand Marais data from Anderson et al. (2013), Cambridge Bay, this study. Populations are shown underneath the name of each location for comparison.

Location	Atenolol (ng/L)	Carbamazepine (ng/L)	Sulfamethoxazole (ng/L)	Trimethoprim (ng/L)
Lac la Biche, AB (8,402)	ND - 100	50 - 300	NA	10 -15
Dunnottar, MB (692)	ND - 856.5	20.1 - 426.1	ND - 1252.5	ND - 318.5
Grand Marais, MB (252)	ND	85-500	ND-21	ND
Cambridge Bay, NU (1,400)	ND - 97.4	1.2 - 306.7	ND - 274.2	ND - 25.7

To facilitate further analysis in prevalence of bacteria throughout the treatment process, abundances of resistance genes were divided by the abundance of 16S rRNA genes to represent relative gene abundances. Relative abundances of ARGs were low (e.g. less than 2% of the total as observed in the wetland) at all locations during discharge (see Table 3.2), which suggests a low potential for ARG-bearing bacteria to exist throughout the treatment system. Tetracycline resistance genes remain elevated in wastewater systems if there is a source of resistance microorganism and tetracycline usage (Peak et al., 2007), but can decline in sunlight-exposed systems over a relatively short period of time (Engemann et al., 2008; Zhang et al., 2009). This suggests that tetracycline may not have been extensively used in the Cambridge Bay population around the times of sampling. Gene concentrations were equivalent to wastewater lagoons with minimal tetracycline usage by source population e.g., (Peak et al., 2007), and could already represent near background levels (Engemann et al., 2006; Zhang et al., 2009). Whereas, detectable sulfonamide concentrations in this study may have been sufficient to maintain selective pressure for antibiotic-resistant bacteria, or their presence of elevated levels represent residual evidence

of previously higher levels of sulfonamide usage, as gene fate tends to differ from chemical fate (e.g. Engemann et al., 2006; Peak et al., 2007)

Wastewater systems have a variable ability to reduce antimicrobial resistance, given the fact that generally resistant bacteria numbers decline in wastewater treatment as bacteria are removed, but these patterns require further investigation, as they remain a function of bacterial community and operating conditions (Christgen et al., 2015). Further, no studies to date have examined the fate of antibiotic resistant bacteria in the wastewater stream at lower temperatures, such as in the Arctic. Well-studied coliform bacteria, which tend to carry ARGs, persist longer in colder temperatures (Solic and Krstulovic, 1992); however, gene persistence at lower temperatures could be exacerbated by slowed transformation rates of pharmaceutical compounds and prolonged selective pressures, reduced endonuclease activity, and lowered predation. Further investigations are required to fully elucidate gene fate under psychrophilic conditions.

3.5. Conclusions

Our assessment of the Cambridge Bay wastewater treatment facility allowed us to detect no apparent removal of nutrients as a result of lagoon-wetland treatment. Reduced nutrients concentrations at locations offshore occurred as a result of receiving environment dilution. Our data suggests that some attenuation mechanisms for pharmaceuticals exist in the treatment system, especially in the sewage lagoon and to some extent in the natural wetland. Distribution of the wastewater contaminants within the lagoon sites was not homogeneous, due to the presence of two different drop off points for sewage dumping and the topography of the lagoon. From all of the studied pharmaceuticals, only carbamazepine showed some persistence during the Arctic winter. Atenolol, sulfamethoxazole and

trimethoprim had dissipated prior to the first sampling campaign. Concentrations of detected pharmaceuticals and nutrients were minimal in the Finger Bay location, which suggests that there was minimal runoff of wastewater to this point. Hazard assessment for detected pharmaceuticals shows that current concentrations of monitored pharmaceuticals do not pose a significant hazard at this time to aquatic organisms in Cambridge Bay. Bacterial populations were detected in similar levels to more southern Canadian locations, with some ARGs attenuation observed in the lagoon-wetland system and considerable dilution at locations offshore. Finger Bay experienced non-detectable levels for all pharmaceuticals and very low levels of ARGs, which suggests that this location was not likely experiencing any sewage leaking at the time of this study. Overall, the CHARS scientific water supply location showed non-detectable levels for all pharmaceuticals and very low levels of ARGs, prior to the instalment of the facility at Cambridge Bay. This study constitutes one of first attempts ever made to understand the occurrence of pharmaceuticals, ARGs and nutrients on wastewater treatment facilities in the Canadian Arctic, as well as the removal performance of these systems under polar conditions.

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3.7. References for Chapter 3

Anderson, J.C.; Carlson, J.C.; Low, J.E.; Challis, J.K.; Wong, C.S.; Knapp, C.W.; Hanson, M.L. Performance of a constructed wetland in Grand Marais, Manitoba, Canada: Removal of nutrients, pharmaceuticals, and antibiotic resistance genes from municipal wastewater. *Chemistry Central Journal* **2013**; *7*: 54.

Anderson, J.C.; Joudan, S.; Shoichet, E.; Cuscito, L.D.; Alipio, A.E.C.; Donaldson, C.S.; Khan, S.; Goltz, D.M.; Rudy, M.D.; Frank, R.A.; Knapp, C.W.; Hanson M.L.; Wong, C.S. Reducing nutrients, organic micropollutants, antibiotic resistance, and toxicity in rural wastewater effluent with subsurface filtration treatment technology. *Ecological Engineering* **2015**; *84*: 375-385.

American Public Health Association (APHA). Standard Methods for the Examination of Water and Wastewater: American Public Health Association, **2005**.

Baxter, L.R.; Sibley, P.K.; Solomon, K.R.; Hanson, M.L. Interactions between atrazine and phosphorus in aquatic systems: Effects on phytoplankton and periphyton. *Chemosphere* **2013**; *90*: 1069-1076.

Bolong, N.; Ismail, A.F.; Salim, M.R.; Matsuura, T. A review of the effects of emerging contaminants in wastewater and options for their removal. *Desalination* **2009**; *239*: 229-246.

Cardinal, P.; Anderson, J.C.; Carlson, J.C.; Low, J.E.; Challis, J.K.; Beattie, S.A.; Bartel, C.N.; Elliot, A.D.; Montero, O.F.; Lokesh, S.; Favreau, A.; Kolzova, T.A.; Knapp, C.W.; Hanson, M.L.; Wong, C.S. Macrophytes may not contribute significantly to removal of nutrients, pharmaceuticals, and antibiotic resistance in model surface constructed wetlands. *Science of the Total Environment* **2014**; *482*: 294-304.

Carlson, J.C.; Anderson, J.C.; Low, J.E.; Cardinal, P.; MacKenzie, S.D.; Beattie S.A.; Challis, J.K.; Bennett, R.J.; Meronek, S.S.; Wilks, R.P.A.; Buhay, W.M.; Wong, C.S.; Hanson, M.L. Presence and hazards of nutrients and emerging organic micropollutants from sewage lagoon discharges into Dead Horse Creek, Manitoba, Canada. *Science of the Total Environment* **2013**; *445*: 64-78.

Canadian Council of Ministers of the Environment (CCME). Canada-wide strategy for the management of municipal wastewater effluent. 2014 progress report., **2014**.

Chouinard, A.; Yates, C.N.; Balch, G.C.; Jorgensen, S.E.; Wootton, B.C.; Anderson, B.C. Management of Tundra Wastewater Treatment Wetlands within a Lagoon/Wetland Hybridized Treatment System Using the SubWet 2.0 Wetland Model. *Water* **2014**; *6*: 439-454.

Christgen, B.; Yang, Y.; Ahammad, S.Z.; Li, B.; Rodriguez, D.C.; Zhang, T.; et al. Metagenomics Shows That Low-Energy Anaerobic-Aerobic Treatment Reactors Reduce Antibiotic Resistance Gene Levels from Domestic Wastewater. *Environmental Science & Technology* **2015**; *49*: 2577-2584.

Conkle, J.L.; White, J.R.; Metcalfe, C.D. Reduction of pharmaceutically active compounds by a lagoon wetland wastewater treatment system in Southeast Louisiana. *Chemosphere* **2008**; *73*: 1741-1748.

Emnet, P.; Gaw, S.; Northcott, G.; Storey, B.; Graham, L. Personal care products and steroid hormones in the Antarctic coastal environment associated with two Antarctic research stations, McMurdo Station and Scott Base. *Environmental Research* **2015**; *136*: 331-342.

Engemann, C.A.; Adams L, Knapp, C.W.; Graham, D.W. Disappearance of oxytetracycline resistance genes in aquatic systems. *FEMS Microbiology Letters* **2006**; *263*: 176-182.

Engemann, C.A.; Keen, P.L.; Knapp, C.W.; Hall, K.J.; Graham, D.W. Fate of tetracycline resistance genes in aquatic systems: Migration from the water column to peripheral biofilms. *Environmental Science & Technology* **2008**; *42*: 5131-5136.

Fent, K.; Weston, AA.; Caminada, D. Ecotoxicology of human pharmaceuticals. *Aquatic Toxicology* **2006**; *76*: 122-159.

Government of Canada. Monthly climate report for Cambridge Bay **2014**. URL: http://climate.weather.gc.ca/climateData/monthlydata_e.html?timeframe¼3&Prov¼NU%20%20&StationID¼1786&mlyRange¼1929-01-01j2014-12-01&Year¼2014&Month¼5&Day¼4 (accessed on January 2016)

Gunnarsdottir, R.; Jenssen, P.D.; Jensen, P.E.; Villumsen, A.; Kallenborn, R. A review of wastewater handling in the Arctic with special reference to pharmaceuticals and personal care products (PPCPs) and microbial pollution. *Ecological Engineering* **2013**; *50*: 76-85.

Kallenborn, R.; Fick, J.; Lindberg, R.; Moe, M.; Nielsen, K.M.; Tysklind, M.; Vasskog, T. Pharmaceutical Residues in Northern European Environments: Consequences and Perspectives. In: Kummerer, K., editor. Pharmaceuticals in the Environment: Sources, Fate, Effects and Risks, Third Edition. Springer-Verlag Berlin, Heidelberger Platz 3, D-14197 Berlin, Germany, **2008**, pp. 61-74.

Luo, Y.L.; Guo, W.S.; Ngo, H.H.; Nghiem, L.D.; Hai, F.I.; Zhang, J.; Liang, S.; Wang, X.C. A review on the occurrence of micropollutants in the aquatic environment and

their fate and removal during wastewater treatment. *Science of the Total Environment* **2014**; *473*: 619-641.

MacLeod, S.L.; Wong, C.S. Loadings, trends, comparisons, and fate of achiral and chiral pharmaceuticals in wastewaters from urban tertiary and rural aerated lagoon treatments. *Water Research* **2010**; *44*: 533-544.

Manitoba Water Stewardship. Manitoba Water Quality Standards, Objectives and Guidelines **2011**. URL: https://www.gov.mb.ca/waterstewardship/water_quality/quality/pdf/mb_water_quality_standard_final.pdf (Accessed on January 2016)

Ng, L.K.; Martin, I.; Alfa, M.; Mulvey, M. Multiplex PCR for the detection of tetracycline resistant genes. *Molecular and Cellular Probes* **2001**; *15*: 209-215.

Peak, N.; Knapp, C.W.; Yang, R.K.; Hanfelt, M.M.; Smith, M.S.; Aga, D.S.; Graham, D.W. Abundance of six tetracycline resistance genes in wastewater lagoons at cattle feedlots with different antibiotic use strategies. *Environmental Microbiology* **2007**; *9*: 143-151.

Pei, R.; Kim, S.C.; Carlson, K.H.; Pruden, A. Effect of River Landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG). *Water Research* **2006**; *40*: 2427-2435.

Romain, S.J. Pharmaceutical health care and Inuit language communications in Nunavut, Canada. *International Journal of Circumpolar Health* **2013**; *72*: 955-961.

Rowan, N.J. Defining established and emerging microbial risks in the aquatic environment: current knowledge, implications, and outlooks. *International Journal of Microbiology* **2011**; *2011*: 462832.

Schwarzenbach, R.; Gschwend, P.; Imboden, D. Environmental Organic Chemistry. New Jersey, USA: 2nd ed. John Wiley & Sons, Inc., **2003.**

Smith, M.S.; Yang, R.K.; Knapp, C.W.; Niu, Y.F.; Peak, N.; Hanfelt, M.M.; et al. Quantification of tetracycline resistance genes in feedlot lagoons by real-time PCR. *Applied and Environmental Microbiology* **2004**; *70*: 7372-7377.

Smith, V.H. Eutrophication of freshwater and coastal marine ecosystems - A global problem. *Environmental Science and Pollution Research* **2003**; *10*: 126-139.

Solic, M.; Krstulovic, N. Separate and combined effects of solar-radiation, temperature, salinity and pH on the survival of fecal-coliforms in seawater. *Marine Pollution Bulletin* **1992**; *24*: 411-416.

Tremblay, J.E.; Anderson, L.G.; Matrai, P.; Coupel, P.; Belanger, S.; Michel, C.; Reigstad, M. Global and regional drivers of nutrient supply, primary production and CO_2 drawdown in the changing Arctic Ocean. *Progress in Oceanography* **2015**; *139*: 171-196.

Vasskog, T.; Bergersen, O.; Anderssen, T.; Jensen, E.; Eggen, T. Depletion of selective serotonin reuptake inhibitors during sewage sludge composting. *Waste Management* **2009**; *29*: 2808-2815.

Weigel, S; Berger, U.; Jensen, E.; Kallenborn, R.; Thoresen, H.; Huhnerfuss, H. Determination of selected pharmaceuticals and caffeine in sewage and seawater from Tromso/Norway with emphasis on ibuprofen and its metabolites. *Chemosphere* **2004**; *56*: 583-592.

Wooton, B.; Durkalec, A.; Ashley, S. Canadian Council of Ministers of the Environment Canada-wide Strategy for the Management of Municipal Wastewater Effluent: Nunavut Regional Impact Analysis. Inuit Tapiriit, Katanami, Ottawa, ON., **2008**.

Yates, C.N.; Wootton, B.C.; Murphy, S.D. Performance assessment of arctic tundra municipal wastewater treatment wetlands through an arctic summer. *Ecological Engineering* **2012**; *44*: 160-173.

Ying, G.G.; Kookana, R.S.; Kolpin, D.W. Occurrence and removal of pharmaceutically active compounds in sewage treatment plants with different technologies. *Journal of Environmental Monitoring* **2009**; *11*: 1498-1505.

Zhang, W.; Sturm, B.S.M.; Knapp, C.W.; Graham, D.W.. Accumulation of Tetracycline Resistance Genes in Aquatic Biofilms Due to Periodic Waste Loadings from Swine Lagoons. *Environmental Science & Technology* **2009**; *43*: 7643-7650.

3.8. Connecting text between Chapters 3 and 4

Chapter 3 has presented a case study based in Arctic climate. Some pharmaceutical attenuation could be observed, though persistence of carbamazepine was reported even through the Arctic winter. Next, this dissertation continues to explore two temperate climate-based examples aiming to better understand the performance of CWs for pharmaceutical removal using low-cost substrates, starting with crushed glass on Chapter 4, and moving on to sand and gravel on Chapter 5.

CHAPTER 4

4. CRUSHED RECYCLED GLASS AS A SUBSTRATE FOR CONSTRUCTED WETLAND WASTEWATER TREATMENT: A CASE STUDY OF ITS POTENTIAL TO FACILITATE PHARMACEUTICAL REMOVAL

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The contribution from each author is detailed under the "Contributions of Authors" section in the preface of this dissertation.

4.1. Abstract

The use of recycled glass as a substrate for constructed wetlands was assessed through two studies. The first study examined the dissipation of atenolol, carbamazepine, and sulfamethoxazole in mesocosm modelled wetlands using glass or limestone gravel as substrates, with or without cattails (*Typha* spp.). Following pseudo-first order kinetics, atenolol dissipated the fastest from the water surface of the mesocosms ($t_{1/2}$ ~ 1 d), followed by sulfamethoxazole ($t_{1/2}$ ~ 14 d), and carbamazepine ($t_{1/2}$ ~ 48 d), with no significant differences across treatments. Increased half-lives were observed at greater depth, likely due to light screening. A Monte Carlo sensitivity analysis diagnosed sunlight absorption rates and second order hydroxyl-mediated indirect photolysis rates to be the main sources of uncertainty in our dissipation rate estimates, compared to our observed rates. The second study examined *in-situ* pharmaceutical removal in tertiary pilot-scale subsurface filters made of crushed recycled glass or sand in a wastewater treatment facility in Manitoba, Canada. Glass and sand showed no significant differences for pharmaceutical removals; atenolol and metoprolol were removed below limits of detection, while carbamazepine and sulfamethoxazole persisted over a retention time of 24 h. Overall, recycled glass performed similarly to traditional substrates for wetland-based wastewater treatment.

4.2. Introduction

The entry of pharmaceuticals into the aquatic environment occurs mostly through the discharge of wastewater effluents into surface waters. Pharmaceuticals and personal care products have been detected ubiquitously in surface and wastewaters worldwide as a consequence of their incomplete removal in wastewater treatment systems (Oulton et al., 2010; Uslu et al., 2013; Vidal-Dorsch et al., 2012) typically in the ng/L to µg/L range

(Cizmas et al., 2015),. Potential impacts of these contaminants, especially their chronic effects on the environment and human health are not fully understood (Li et al., 2015; Zhu et al., 2013). Currently, there are international efforts to develop wastewater guidelines for these ubiquitous emerging contaminants (Kolpin et al., 2002) since these are typically unregulated (Ávila and García, 2015).

Constructed wetlands (CWs) have provided a low-cost alternative to wastewater treatment in rural and remote regions, achieving adequate levels of treatment and reducing nutrients and other contaminant releases (Conkle et al., 2008; Li et al., 2014; Matamoros et al., 2007). CWs have been reported to remove pharmaceuticals on four categories of efficiency: readily removed (over 70% mean removal, e.g., sulfamethoxazole, atenolol, metoprolol); moderately removed (50%-70% mean removal, e.g., ibuprofen, naproxen, gemfibrozil); limitedly removed (20%-50% mean removal, e.g., diclofenac, clofibric acid, carbamazepine) and hardly removed (less than 20% mean removal, e.g., ampicillin, erythromycin) (Li et al., 2014). Current knowledge gaps exist around understanding the influence of design parameters (e.g., aeration conditions, hydraulic retention time, type of substrate used) on the performance of pilot- and full-scale treatment facilities for pharmaceutical removal. (Li et al., 2014; Verlicchi and Zambello, 2014)

Gravel and sand are the most common CW substrates reported in the literature for the removal of nutrients and other contaminants in horizontal, vertical, and hybrid subsurface filter beds (Li et al., 2014). Crushed rock, gravel and sand mixtures, glass, anthracite, dolomite, and soil have been tested as well (Wu et al., 2015). Retention behaviour is influenced by hydraulic properties, mechanical and chemical resistance, adequacy for supporting the development of plants and microorganisms, inertness, potential for release/leaching of additional contaminants, and ability for desorption/regeneration (Dordio

and Carvalho, 2013). Important factors to determine the applicability of candidate substrates in CWs are cost, local availability, saturation time, (i.e., the time needed to occupy all available sorption sites), and recyclability of saturated filter media (Dordio and Carvalho, 2013). The combination of hydraulic conductivity and adsorption capacity of the substrate, as determined by its surface area, are two factors that can drive the usefulness of a given substrate for CW applications. Moreover, particle size distributions, porosity, and effective particle size are all important factors that affect system performance (Kadlec et al., 2009; Vymazal, 2011).

Crushed recycled glass (CRG) is an abundant material in many jurisdictions. In Canada, it represents approximately 3% of national domestic solid waste (Statistics Canada, 2015). In Manitoba, Canada, residential and non-residential sources produced more than nine-thousand tonnes of waste glass in 2016, and over eight-thousand tonnes of glass were diverted from disposal sites for recycling purposes (Statistics Canada, 2018). The volume of waste transformed into new glass containers is minimal. Most collected glass are crushed and reused as sidewalk and road base aggregate (Morawski et al., 2016; Reindl, 2003), occasionally serving as fill-in for water and sewer trench systems. The limited use for CRG is not only an issue in Canada, but globally. In the United States, three million tonnes of container glass were recycled in 2017, which represents 27% of the total of glass waste generated for that year, while 60% was landfilled (U.S. EPA, 2018) . In Europe, 30 billion glass containers were recycled in 2017, for an average rate of 76% across 160 plants, with approximately a quarter of all glass being wasted during that year (European Container Glass Federation, 2018).

Considering the physical structure and properties of CRG, it has been proposed as a potentially suitable substrate for CWs. CRG is durable and inert, which can promote

aeration of the filter bed and potentially support biofilm growth while increasing backwash ability through increased percolation when compared to sand (Elliott, 2001; Gill et al., 2009; Gill et al., 2011). Increased aeration can enhance contaminant removal in CW-based application through promotion of biodegradation (Camacho-Munoz et al., 2012).

CRG has been tested as a wastewater filter medium within intermittent recirculating biofilters treating septic tank effluent (Elliott, 2001; Healy et al., 2010; Hu and Gagnon, 2006) and in polishing filters for domestic wastewater (Gill et al., 2011; Healy et al., 2010; Horan and Lowe, 2007). In recirculating biofilters, CRG has been reported to retain 79% to 98% of total suspended solids and remove over 94% and 96% of BOD and ammonium nitrogen, respectively (Elliott, 2001; Hu and Gagnon, 2006). As a tertiary filter media, Gill et al. (2009) reported removal of 73% of chemical oxygen demand and 28% of total nitrogen by CRG. Salzmann et al. (2020) studied CRG as a tertiary filter media for municipal lagoon water treatment, obtaining equal capacity for removal of suspended solids as achieved with sand, and similar performance for ammonia and chemical oxygen demand parameters.

To our knowledge, no previous studies have explored CRG as a substrate for the removal of pharmaceuticals from wastewater. This study sought to evaluate CRG as a substrate in CW-based applications and compare its performance to traditional materials such as gravel or sand. The studies were conducted at two scales of work (i.e., mesocosm and pilot-scale) to allow investigation of CRG removal of pharmaceutical analytes from both real and synthetic wastewaters. Mesocosm systems offer a controllable experimental setup to study and quantify processes and mechanisms (e.g., physical, chemical, biological, biogeochemical) for assessing removal and fate of wastewater contaminants. On the other hand, pilot-scale studies allow for observation of these processes using more realistic

matrices, flow conditions, and hydraulic retention times. We hypothesized that removal of pharmaceutical contaminants can be attained by CRG, and performance of CRG would not be significantly different from that of traditional substrates (i.e., gravel, sand), for both mesocosm- and pilot-scale systems.

4.3. Material and Methods

4.3.1. Study designs and treatment

4.3.1.1. Mesocosm-scale study

This study took place at the Prairie Wetland Research Facility (PWRF) at the University of Manitoba in Winnipeg, Canada (49°48'35.9''N, 97°07'33.0''W). Circular, flat-bottomed, low-density polyethylene tanks (2.7 m diameter × 0.72 m height; 3.49 m^3 total volume, Figure C1) from ACE Rotomolds (Hospers, Iowa) were installed in an array of twelve (Figure C2). The setup allowed for individual, isolated wetland mesocosms with quiescent waters having no inflows or outflows. Mesocosms were randomly assigned to one of three treatments in triplicate (Figure C2): limestone gravel (LG), glass (PCRG), or unplanted glass (CRG). Limestone gravel (3/4 inch) was sourced from a local materials vendor (Reimer Soils, Winnipeg), while CRG (3/4 inch) was sourced from a local recycling facility (Cascades Recovery+, Winnipeg). Glass was sieved through a tumbling screen to remove large waste impurities (e.g., plastic fragments, gravel particles, finer organic materials) originated from the recycling process. Some of these materials passed through the screen and were consequently combined with the crushed glass media. These materials accounted for approximately 12% of the glass media by weight (Salzmann et al., 2020). Physical characterization of the glass is reported in Table C1. The glass was given minimal treatment prior to use as filter media, in an attempt to employ post-consumer waste glass

that could be converted into wastewater filter media at low cost to facilitate system implementation. A layer of substrate (~27 cm) was placed at the bottom of the tanks. Winnipeg tap water was added on June 25th, 2018, to fill the tanks (average depth ~ 40 cm). Floating debris from CRG (e.g., plastic caps, labels, corks, etc.) were removed from the tanks upon filling and then periodically for three weeks. Mesocosms were then left unplanted or planted with approximately five cattails (*Typha* spp.) per square meter (25 plants per mesocosm). Cattails were collected from Oak Hammock Marsh (Stonewall, MB, 50° 11′ 15″ N, 97° 7′ 30″ W) on July 19th, 2018. Macrophytes were acclimated for 26 days prior to the start of the experiment.

Mesocosms were treated with synthetic wastewater and a stock solution of pharmaceuticals on August 14, 2018. The wastewater (2 L per mesocosm) contained (per liter): 32.0 g peptone, 19.0 g Lab Lemco powder meat extract, 3.0 g yeast extract, 3.0 g urea, 6.7 g $(NH_4)_2SO_4$, 2.9 g K_2HPO_4, 2.3 g KH_2PO_4, 0.27 g $CaCl_2 \cdot 2H_2O$, and 0.2 g $MgSO_4 \cdot 2H_2O$ (Knapp and Graham, 2007). Synthetic wastewater was prepared to control loadings of nutrient and BOD in the mesocosms, and to minimize human health risks associated with handling raw wastewater. The pharmaceutical additions consisted of 1 L of a stock solution of three common pharmaceuticals: atenolol, carbamazepine, and sulfamethoxazole. Pharmaceutical initial target concentrations were between 5 and 10 µg/L, selected both for ease of quantification and to reflect reported levels in wastewater influents in Manitoba municipal wastewater treatment facilities (Anderson et al., 2013; Anderson et al., 2015; Chaves-Barquero et al., 2018) and elsewhere (Verlicchi and Zambello, 2014). These pharmaceuticals were selected based on their frequency of detection in wetland-based treatment facilities, ease of quantification, low likelihood of acute effects to aquatic organisms, and comparison with previous field studies (Anderson et

al., 2015; Chaves-Barquero et al., 2018). All mesocosms also received a one-time 1-L amendment of secondary wastewater from a local wastewater treatment facility (Village of Dunnottar, Manitoba) to provide microorganism amendments. Based on mesocosm volumes, this represents approximately a 2000-fold dilution by volume.

4.3.1.2. Pilot-scale study

This study was conducted at the Village of Dunnottar, located on the southwestern shore of Lake Winnipeg, Manitoba. The village has approximately 763 permanent residents, with a several-fold increase during the summer due to the presence of vacationers (Anderson et al., 2015). Holding tanks have been installed in the community households, and sewage is hauled and discharged into the primary lagoon using septic trucks. The lagoon treatment system is comprised of a primary lagoon, a secondary lagoon, and a secondary overflow lagoon (Figure C3). After secondary treatment, water is polished by sending it through a pilot-scale tertiary subsurface filter (Figure 4.1) comprised of four cells (10 m long × 1.8 m wide × 1 m deep, bed volume of 18 m^3). In 2016, two of the cells were packed with three-quarter inch CRG (Cascades Recovery+, Winnipeg) and two with local river bottom sand as substrates. Cells were separated with plywood and individually lined with a polyethylene liner. Limestone cobble was placed on the bottom of the array, while a layer of wood chips was spread on top of the filter beds. Wastewater was pumped from the secondary lagoon through a transfer pipe, splitting water into the four filter beds with two parallel perforated PVC pipes delivering the water to the surface of each cell. Wastewater then percolated vertically through the bed, reaching a collector pipe at the bottom. A wastewater pump (Burcam 400500, Burke Water Systems Manufacturing, Inc.) was placed in the secondary lagoon to supply water to the filter system. It worked at a head of 1.5 m

and a flow rate of 17,000 L/h. A timer controlled the pump, leaving it on for 5 min and off for 99 min, resulting in a filter hydraulic residence time (HRT) of 24 hours.

4.3.2. Monitoring, sample collection, and analysis

4.3.2.1. Mesocosm-scale study

A suite of standard water quality parameters (dissolved oxygen, chlorophyll-a, pH, temperature, oxidation-reduction potential, and conductivity) were measured every weekday morning, and once a week in the afternoon, using a YSI 6600 V2 Sonde (Yellow Springs, OH). Measurements began on July 16th and continued until October 10th. Temperature was also monitored every thirty minutes using HOBO (Bourne, MA) water temperature data loggers. Depths were measured bi-weekly in five different locations for each tank, and then averaged to account for evaporation and to monitor the water volume. Photosynthetically active radiation (PAR) was measured at mid-day once a week.

Grab water samples for pharmaceutical analysis were taken pre-treatment on day -1 and post-treatment on days 0, 2, 7, 14, 28, and 56, with additional samples collected at 8 h, 24 h, and 30 h post-treatment. Samples were integrated from four to six random spots over the depth of the water column of each mesocosm tank and collected into pre-ashed amber glass bottles, using a PVC integrative water sampler (6 cm diameter × 1.6 m length; 4.5 L total capacity) retrofitted with PVC tubing (4 cm diameter × 30 cm length), based on previous models (Solomon et al., 1982). Piezometer wells were built using ½'' PVC piping and installed on each tank to monitor the concentration of pharmaceuticals at two depths: 2 cm and 22 cm below the depth of glass or gravel. Samples were collected from the wells on weeks 0, 1, 2, and 4 using an Masterflex E/S portable sampler (Cole-Parmer, IL). Cattail growth was monitored by measuring the height of the longest leaf at week 0 and week 4 after the pharmaceutical spike, and then calculating growth rate.

4.3.2.2. Pilot-scale study

Grab water samples for pharmaceutical analysis were taken in pre-ashed amber glass during the summer of 2018 on June 26th, July 24th, July 31st, August 7th, August 16th, August 21st, September 4th, and September 11th at the input of the filters and at each of the four output wells, with corresponding field blanks for each sampling date. Samples were preserved on ice and transported to the laboratory for analysis within 48 hours.

4.3.3. Sample processing and analysis

Water samples were stored in the dark at 4°C and processed within 48 hours. Samples were solid-phase extracted using 3 cc, 60 mg HLB cartridges from Waters Corporation (Milford, MA). Ultrahigh performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) was performed on an Agilent 1200 UHLPC coupled to an Agilent 6410 triple-quadrupole mass spectrometer, following the methods described on previous works (Carlson et al., 2013). Relevant QA/QC parameters (e.g., limits of detection and quantification, instrumental parameters for chemical analysis) can be found in Tables C2 and C3.

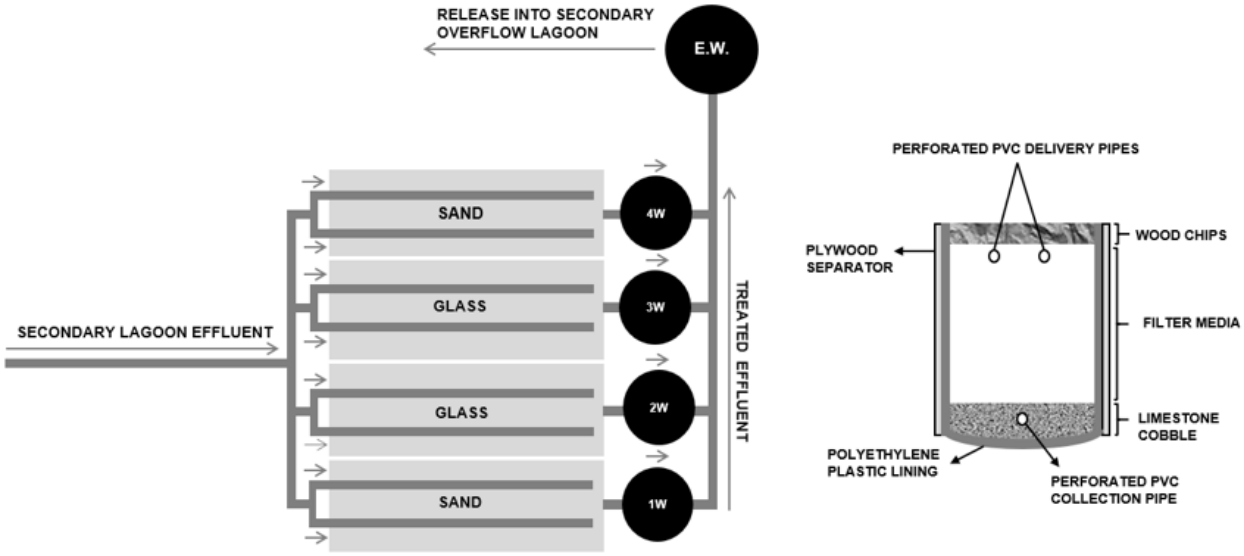

Figure 4.1 Top view of the pilot-scale subsurface filter system located in Dunnottar, Manitoba, Canada (left), and cross-sectional view of an individual filter bed (right).

4.3.4. Calculation of rate constants for fate processes

Estimates for sorption and direct/indirect photolysis were calculated by determining the compound-specific rates and half-lives (Schwarzenbach et al. 2017). The equations are described as follows:

4.3.4.1. Sedimentation

For sedimentation, the calculated rate constant (k_s^*) was:

$$k_s^* = (1 - f_w)k_s = \left(\frac{f_{oc}^{sus} r_{sw} K_{oc}}{1 + f_{oc}^{sus} r_{sw} K_{oc}}\right) k_s \qquad \text{(Equation 4.1)}$$

where f_w is the fraction of chemical dissolved, f_{oc}^{sus} is the fraction of organic carbon within the suspended particles, and r_{sw} is the solid-to-solution phase ratio, equivalent to the total suspended solids in the water column (Schwarzenbach et al. 2017). The sedimentation rate was estimated from:

$$k_s = \frac{v_s}{z_{mix}} \qquad \text{(Equation 4.2)}$$

where v_s is the particle settling velocity and z_{mix} is the average depth of the mesocosms (Schwarzenbach et al. 2017). For our purposes, z_{mix} is 0.4 m, v_s was estimated at 1.5 m d^{-1} for particulate organic carbon (Gustafsson et al. 2000) and f_{oc}^{sus} was estimated at 0.4 since most of the particulate matter was of plant origin (Schwarzenbach et al. 2017). No other sorption mechanisms were considered for our analysis. We estimated K_{oc} using a linear free energy relationship (Schwarzenbach et al. 2017) for alkylated and chlorinated benzenes, used previously by Carballa et al. (2008) as surrogate for sorption estimations on pharmaceuticals:

$$\log K_{oc} = 0.74 \log D_{ow} + 0.15 \qquad \text{(Equation 4.3)}$$

where $\log D_{ow}$ is the pH-dependent octanol-water distribution coefficient:

$$\log D_{ow} = \log K_{ow} - \log (1 + 10^{(pH-pKa)}) \qquad \text{(Equation 4.4)}$$

4.3.4.2. Direct Photolysis

Direct photolysis rates (k_D) were estimated by first determining the compound's wavelength-dependent specific rate of light absorption $k_a(\lambda)$ (Schwarzenbach et al. 2017):

$$k_a^t(\lambda) = \frac{W(\lambda)\varepsilon(\lambda)}{z_{mix}\alpha(\lambda)} \qquad \text{(Equation 4.5)}$$

where $W(\lambda)$ is the spectral photon fluence rate (Einstein cm^{-2} d^{-1}) for latitude 50°N during the fall (OECD, 1997), $\varepsilon(\lambda)$ is the molar extinction coefficient of the compound (M^{-1} cm^{-1}), and $\alpha(\lambda)$ is the attenuation coefficient of the medium. The total rate of light absorption (k_a^t) was estimated by summation of the rates over all wavelengths (Schwarzenbach et al., 2017):

$$k_a^t = \sum k_a^t(\lambda) = \frac{1}{z_{mix}} \sum \frac{W(\lambda)\varepsilon(\lambda)}{\alpha(\lambda)} \quad \text{(Equation 4.6)}$$

Finally, the direct photolysis rate constant (k_D) was calculated by:

$$k_D = k_a^t \Phi \quad \text{(Equation 4.7)}$$

where Φ is the quantum yield, which was retrieved from the literature (Table 4.3). Photolysis parameters (i.e. Φ) obtained from experiments under natural sunlight, or sunlight simulator with a cutoff filter below 290 nm, were favored for the calculation of our estimates. Direct photolysis is dependent on the incoming solar radiation and the ability of the medium to absorb some of this radiation.

4.3.4.3. Indirect photolysis

Indirect photolysis was estimated by assuming that pharmaceutical degradation occurred by reacting with hydroxyl radicals •OH ($k_{I\cdot OH}$):

$$k_{I,\cdot OH} = k'_{p,\cdot OH} [\bullet OH]_{SS} \quad \text{(Equation 4.8)}$$

where $k'_{p\bullet OH}$ is the second order hydroxyl radical rate constant and $[\bullet OH]_{ss}$ is the steady state concentration of •OH. Second order rate constants $k'_{p\bullet OH}$ were obtained from the literature and are displayed in Table 4.2 for each pharmaceutical. Steady-state concentrations of hydroxyl radicals have been reported in the literature ranging between 1×10^{-15} and 1×10^{-17} M (Schwarzenbach et al., 2017). The steady state of hydroxyl radical concentration was assumed to be 1×10^{-16} M in natural surface waters (Mabury and Crosby, 1994).

4.3.4.4. Overall dissipation rate

An overall dissipation rate can be estimated by adding up the rates calculated above:

$$k_{est} = k_s* + k_D + k_{I,\cdot OH} \quad \text{(Equation 4.9)}$$

4.3.5. Sensitivity analysis for the overall dissipation rate predictions

Oracle Crystal Ball software version 11.1.2.4.850 (Oracle Corporation, Redwood Shores, CA), was used to conduct sensitivity analyses of our fate predictions based on the estimated rate of dissipation defined in equation 4.9. We defined uncertainty distributions for every parameter based on our assumptions, and the available information in the literature. For k_S* we defined a conservative two order of magnitude triangular distribution. For k_D values, we considered horizontal distributions for the possible range of light absorption rates (k_a^t) considering from no medium absorption to full medium absorption, as well as the reported uncertainty from measured quantum yields in the literature (whenever reported). For $k_{I,\cdot OH}$ a conservative triangular distribution considering a variability of two orders of magnitude was used. Then, we performed a forecast simulation (100,000 iterations) for the estimated rate value (k_{est}) for each analyte and then obtained a sensitivity analysis information (i.e. how much each of the variables affects the final estimation) as described in Figure C11.

4.3.6. Statistical analysis

Results for pharmaceutical concentrations in both studies were assessed using GraphPad Prism 6 (Graph Pad Software, La Jolla, CA). Concentrations, half lives, and rate constants are reported as mean value ± standard deviation (SD) unless otherwise stated. Differences were deemed statistically significant at $p < 0.05$. Normality was assessed via Shapiro-Wilk test.

For the mesocosm study, Student's t-tests were applied to evaluate plant growth data, as well as general water quality parameters. One-way analysis of variance (ANOVA) followed by Tukey's test were applied to compare pharmaceutical concentrations and half-lives in the water column across treatments, as well as pharmaceutical concentrations and

half-lives in the water column, water-substrate interface, and within the substrate. For the pilot-scale study, one-way ANOVA tests followed by Tukey's post-hoc test were used to compare the concentrations of each pharmaceutical at the input and at each of the four outputs of the system.

4.4. Results

4.4.1. Mesocosm-scale project

4.4.1.1. Plant growth

After four weeks, no statistically significant differences were found for *Typha* spp. growth among substrates ($p > 0.05$; Figure C4). Plants were able to establish in both substrates and grow roots within the bulk of the materials with senescence of the macrophytes commencing during Week 4 (September 14th, 2018) for both substrates. During the course of the study, maintenance of the macrophytes was necessary during the course of the study, with some replanting labour conducted weekly due to tilting of the plants as a consequence of windy conditions.

4.4.1.2. Total phosphorus and water quality

Phosphorus was added to achieve an in-tank concentration of between 1.5 and 2 mg/L. After two weeks, it dissipated from the water column in all treatments (Figure C5). The greatest removal rates were observed for gravel treatments, followed by unplanted glass, and planted glass treatments. No significant differences ($p > 0.05$) in phosphorus removal rates were found between planted glass and unplanted glass treatments.

Temperatures for the mesocosms declined over time as the summer progressed into autumn (Figure C6). As expected, pH and dissolved oxygen values remained relatively consistent throughout the duration of the study, except for a quick decline and rebound in

all spiked treatments, due to the addition of synthetic wastewater and pharmaceuticals (Figure C7). A similar behaviour was observed for the evolution of dissolved oxygen in the mesocosms, with a quick decline and rebound upon spike (Figure C8). Treated mesocosms showed a brief increase in chlorophyll-a concentration following the addition of synthetic wastewater and pharmaceuticals, likely due to the increased availability of nutrients (Figure C9). As well, glass treatments experienced the greatest increase in chlorophyll-a following the pulse exposure of synthetic effluent.

4.4.1.3. Dissipation of pharmaceuticals

Atenolol dissipated rapidly from the water column in the tanks, while sulfamethoxazole and carbamazepine experienced greater half-lives compared to atenolol in the water column (Table 4.1). The half-life of atenolol averaged 0.8 days in gravel treatments, 1 day in planted glass treatments, and 1 day in unplanted glass treatments. Carbamazepine had average half-lives, respectively, of 46 days, 58 days, and 41 days. Sulfamethoxazole had average half-lives of 14 days, 14 days and 15 days. Among treatments, no significant differences ($p > 0.05$) were found for the dissipation rates of atenolol and carbamazepine, while sulfamethoxazole had a slightly shorter half-life in unplanted glass, compared to the other two treatments ($p < 0.05$).

Table 4.1. Observed mean half-lives ($t_{1/2}$) and dissipation rates (k_{obs}) of pharmaceuticals in the water column of modelled wetlands at the Prairie Wetland Research Facility using either gravel or crushed recycled glass as substrates. Values are presented as mean ± standard deviation

Compound	Gravel		Planted Glass		Unplanted Glass	
	Half-life (d)	k_{obs} (d^{-1})	Half-life (d)	k_{obs} (d^{-1})	Half-life (d)	k_{obs} (d^{-1})
Atenolol	0.8 ± 0.1	0.9 ± 0.1	1.0 ± 0.2	0.7 ± 0.1	0.97 ± 0.02	0.71 ± 0.01
Carbamazepine	46 ± 3	0.0150 ± 0.0006	58 ± 18	0.012 ± 0.004	41 ± 4	0.017 ± 0.002
Sulfamethoxazole	14 ± 1	0.050 ± 0.004	14 ± 1	0.050 ± 0.004	15 ± 1	0.046 ± 0.004

Dissipation of pharmaceuticals was also examined near the water-substrate interface (i.e., 2 cm depth) and within the substrate (i.e., 22 cm depth) in all tanks (Table 4.2). We observed significant differences ($p < 0.05$) between the half-lives of atenolol at different depths for all treatments. Briefly, a significantly lower half-life (~1 day) of atenolol was observed in the water surface, compared to the other depths near or within the substrate (2 to 3 days) pooled across treatments. No significant differences were found between atenolol half-lives at the interface and at the bulk of the substrates. In the case of carbamazepine and sulfamethoxazole, no statistically significant differences were found between the observed half-lives at different depths ($p > 0.05$).

Table 4.2. Observed mean-half lives (in days) of selected pharmaceuticals near the water-substrate interface (depth = 2 cm) and in the water around the bulk substrate (depth = 22 cm) at the Prairie Wetland Research Facility using either gravel or crushed recycled glass as substrates. Values are presented as mean ± standard deviation. G: gravel, I: interface, B: bulk, PG: planted glass, UG: unplanted glass.

Compound	G-I	G-B	PG-I	PG-B	UG-I	UG-B
Atenolol	2.8 ± 0.1	3.1 ± 0.3	3.5 ± 0.7	2.9 ± 0.2	2.7 ± 0.3	2.1 ± 0.2
Carbamazepine	65 ± 17	72 ± 24	40 ± 11	74 ± 12	47 ± 2	53 ± 27
Sulfamethoxazole	12 ± 2	14 ± 3	11 ± 1	11 ± 1	15 ± 1	17 ± 1

Table 4.3. Estimation of first-order direct photolysis rate constant (k_D) and pseudo-first order indirect photolysis rate constant due to reaction with hydroxyl radicals ($k_{I\cdot OH}$) for pharmaceuticals in mesocosm water from Eq. 8 (see SI). k_a^t = compound specific absorption rate, Φ = quantum yield, $k'_{p\cdot OH}$ = second order hydroxyl radical rate constant. N/A: the compound does not absorb radiation in the spectral range of 290 nm to 800 nm.

Compound	Direct photolysis			Indirect photolysis	
	k_a^t (Eins mol^{-1} d^{-1})	Φ (mol $Eins^{-1}$)	k_D (d^{-1})	$k'_{p\cdot OH}$ ($M^{-1}s^{-1}$)	$k_{I\cdot OH}$ [a] (d^{-1})
Atenolol	N/A	3.6×10^{-2} [b]	N/A	8.0×10^{9} [c]	6.9×10^{-2}
Carbamazepine	122	4.8×10^{-5}	0.006	8.8×10^{9}	7.6×10^{-2}
Sulfamethoxazole	9	0.09 [d]	0.81	5.5×10^{9}	4.8×10^{-2}

[a] Assuming $[\cdot OH]_{ss} = 1 \times 10^{-16}$ M (Mabury and Crosby, 1994); [b]Salgado et al. (2013); [c]Dong et al. (2015); [d]Boreen et al. (2004).

Table 4.4. Comparison of the estimated photolysis rate constants (k_{est}) with the observed dissipation rates (k_{obs}, averaged from values in Table 4.1) in the water column of mesocosm tanks at the Prairie Wetland Research Facility, and their associated half-lives for studied pharmaceuticals; k_{est} was calculated from $k_D + k_{I\cdot OH} + k_s^*$

Compound	k_D (d^{-1})	$k_{I\cdot OH}$ (d^{-1})	k_s^* (d^{-1})	k_{est} (d^{-1})	k_{obs} (d^{-1})	Estimated half-life (d)	Observed half-life (d)
Atenolol	N/A	6.9×10^{-2}	$\leq 1.2 \times 10^{-5}$	0.07	0.9	10	0.8
Carbamazepine	0.006	7.6×10^{-2}	1.3×10^{-4}	0.08	0.02	8.7	47.4
Sulfamethoxazole	0.81	4.8×10^{-2}	$\leq 1.2 \times 10^{-5}$	0.85	0.05	0.8	14

4.4.2. Pilot-scale study

From all pharmaceuticals of interest for this study (Table 4.5), only four were detected in the background at least once at the input of the filter beds or at any of the outputs of the pilot-scale system: atenolol, carbamazepine, metoprolol, and sulfamethoxazole, with maximum concentrations at the input of 13 ng/L, 247 ng/L, 72

ng/L, and 27 ng/L, respectively. Overall, carbamazepine was not effectively removed by any of the substrates during the season. Concentrations at the outputs of both types of subsurface filters were not significantly different from the levels detected at the input ($p > 0.05$). Sulfamethoxazole was neither removed by either sand or glass during this study ($p > 0.05$). Metoprolol was generally well removed, when detected, by both materials over the season. No significant differences were observed in the performance of the studied substrates with regards to pharmaceutical removal.

Table 4.5. Mean concentrations (ng L^{-1}, ± standard deviation, n=3) of consistently detected target compounds before and after the tertiary pilot-scale filter at the Dunnottar wastewater treatment facility during the summer of 2018. Subsurface filter cells were made of recycled glass or sand. LOD and LOQ (ng L^{-1}) are indicated below each analyte's name. ND: non-detectable.

Compound	Date	Sampling Site		
		Input	Glass	Sand
Atenolol LOD: 0.29 LOQ: 0.96	Jun 26	13±2	2.5±0.6	1.2±0.6
	Jul 24	4±2	3±1	4±2
	Jul 31	<LOQ	2.0±0.3	ND
	Aug 7	3±1	1.2±0.2	ND
	Aug 16	4±2	1.2±0.2	ND
	Aug 21	4±1	ND	ND
	Sep 4	4±1	1.4±0.3	ND
	Sep 11	6±2	1.2±0.2	2±1
Carbamazepine LOD: 1.7 LOQ: 5.7	Jun 26	120±5	120±3	90±10
	Jul 24	247±31	52±1	128±6
	Jul 31	115±12	125±8	99±13
	Aug 7	99±4	107±6	101±4
	Aug 16	88±9	66±2	78±13
	Aug 21	70±6	59±15	73±18
	Sep 4	ND	ND	ND
	Sep 11	ND	ND	ND
Metoprolol LOD: 3.4 LOQ: 11	Jun 26	24±2	22±1	26±1
	Jul 24	72±5	ND	ND
	Jul 31	19±2	11±2	ND
	Aug 7	30±1	<LOQ	ND
	Aug 16	<LOQ	ND	ND
	Aug 21	<LOQ	ND	ND
	Sep 4	ND	ND	ND
	Sep 11	21±4	<LOQ	ND
Sulfamethoxazole LOD: 0.25 LOQ: 0.83	Jun 26	24±5	22±1	26±1
	Jul 24	15±2	12±3	27±4
	Jul 31	ND	ND	ND
	Aug 7	ND	ND	ND
	Aug 16	13±3	9±4	14±3
	Aug 21	23±3	23±3	21±1
	Sep 4	9±1	12±2	9±1
	Sep 11	19±4	17±2	16±3

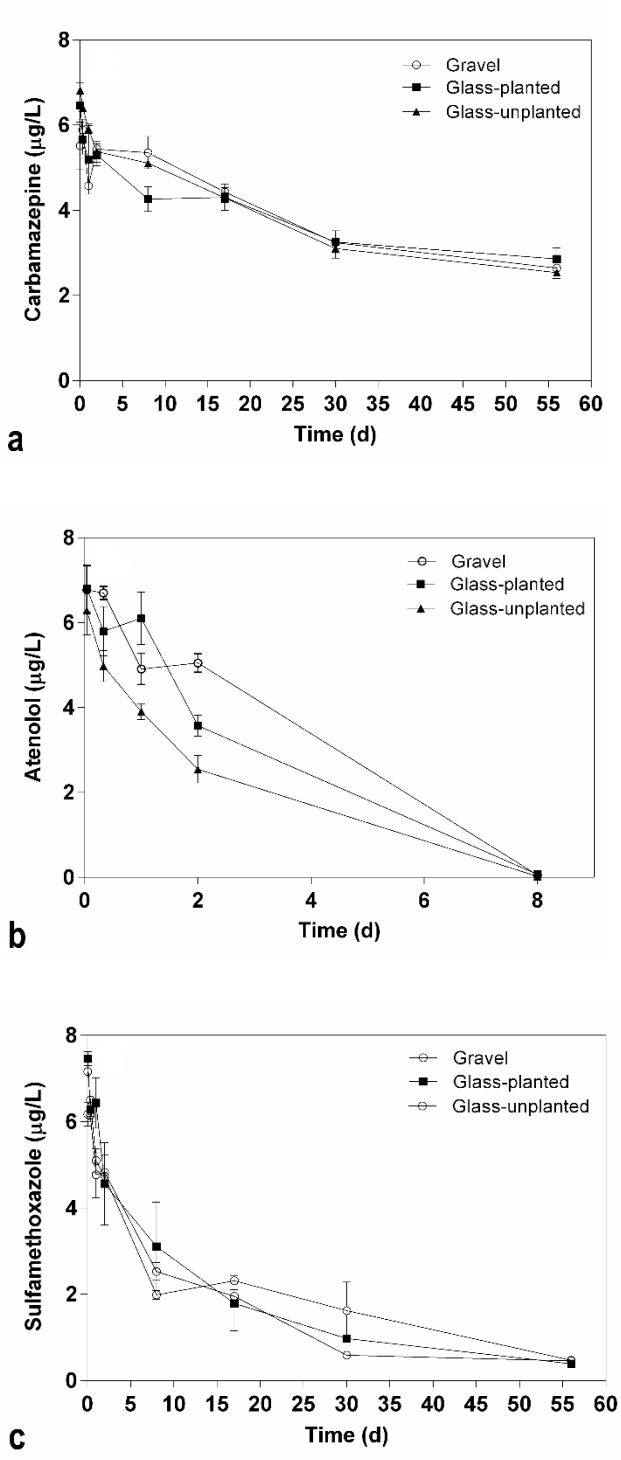

Figure 4.2. Dissipation over time of a) carbamazepine; b) atenolol, and c) sulfamethoxazole in the water column of modelled mesocosm-scale wetlands at the Prairie Wetland Research Facility using limestone gravel and either planted or unplanted crushed recycled glass as substrates.

4.5. Discussion

4.5.1. Mesocosm-scale project

4.5.1.1. Water quality and phosphorus

Temperature, pH, and conductivity values in the current study agreed with previous studies conducted at the same facility (Cardinal et al., 2014; Cardinal et al., 2016) and with other locations in Manitoba (Anderson et al., 2013; Anderson et al., 2015; Carlson et al., 2013; Chaves-Barquero et al., 2018). This demonstrates that the mesocosm system was representative of real systems (i.e. CWs) found in the province.

Our results suggest that a greater phosphorus removal was promoted in gravel (>90%) than glass due to co-precipitation and adsorption of calcium phosphates onto the calcite surface of the substrate, as observed elsewhere (Aulenbach and Nie, 1988; Xu et al., 2014). The crushed glass showed less affinity for phosphate removal during the course of the study (~70%), which agrees with a similar study where 50% removal was achieved for total phosphorus (Gill et al., 2011).

Table 4.6. Ranges of target concentrations (in ng L^{-1}) detected in grab-water samples collected in the current pilot-scale study and in previous studies of rural wastewater lagoon systems in Manitoba

Compound	Dunnottar[a]	Grand Marais[b]	Winkler/Morden[c]	Norway House[d]	Misipawistik[e]	Current study
Atenolol	4-856	< LOD	< 2-26	< LOD	44-2184	1-13
Carbamazepine	105-426	59-500	4-48	247-2770	6-150	6 – 247
Metoprolol	10-1122	< LOD	< LOD	1210-1230	30-600	11-73
Sulfamethoxazole	4-1253	10-21	6-48	186-7180	185-3701	0.8 - 27

[a] Anderson et al. (2015); [b] Anderson et al. (2013); [c] Carlson et al. (2013); [d] Challis et al. (2018); [e]Anderson et al. (2020)

4.5.1.2. Dissipation of pharmaceuticals

Atenolol, carbamazepine, and sulfamethoxazole presented pseudo-first-order dissipation kinetics over the course of the study, from the water column to the bottom of the system. No significant differences were found amongst treatments for observed half-lives, even in the case of planted and unplanted glass. Observed half-lives of carbamazepine at the water column were greater by approximately 6-fold compared to the half-lives found in previous studies in the same facility using topsoil as substrate during summer (Cardinal et al., 2014) and fall (Cardinal et al., 2016). Lam et al. (2004) observed a mean half-life of 82 days for carbamazepine in 12,000 L outdoor microcosms over the summer, which approximately doubles the observed half-lives in this study, likely due to increased light attenuation in their system compared to the mesocosm tanks used in our study. For sulfamethoxazole, we observed half-lives similar to the ones reported by Cardinal et al. (2016) during the fall season and 2-fold greater compared to the ones reported during the summer (Cardinal et al., 2014). In the case of atenolol, our results suggest that a subsurface concentration gradient occurred, with greater concentrations near the water-interface substrate and within the substrate than in those found in the water column during early

stages of the study. Incomplete mixing within the water column has been reported to affect the observed concentrations (Cardinal et al., 2014; Kunkel and Radke, 2011), particularly with the possibility of sorption to biofilms with subsequent release; however, it is not clear if this is the case for atenolol.

4.5.1.3. Pharmaceutical fate

In this study, the importance of fate processes responsible for the removal of pharmaceuticals were estimated. We used literature data from previous laboratory-based experiments to assess the processes that may affect removal efficiency of the three studied compounds. The processes considered for our predictions were sedimentation through sorption to particles, and direct and indirect photolysis. Our assumptions to conduct these fate predictions are described in the material and methods section. Briefly, sorption was estimated through particle sedimentation rates assuming most of the particulate matter being of plant origin; direct photolysis was estimated through the wavelength-dependent specific rate of light absorption for latitude 50°N, the total rate of light absorption was estimated by summation of the rates over all wavelengths; and indirect photolysis assumed pharmaceutical degradation due to hydroxyl radicals only.

Overall, the calculated and observed half-lives of all analytes in the water column were not in agreement, with observed values for all three compounds being significantly different than predicted ones (Table 4.4). Briefly, for atenolol we estimated a half-life of 10 days, versus an observed half-life of 0.8 days; for carbamazepine we estimated a half-life of 8.7 days, versus an observed half-life of 47.4 days; and for sulfamethoxazole we estimated a half-life of 0.8 days, versus an observed half-life of 14 days.

Sorption was quantified by the organic carbon normalized partition coefficient (log K_{oc}), which assumes neutral species binding mainly to the organic carbon content of the

particles. We used a single parameter linear free energy relationship (Schwarzenbach et al., 2017) to relate $\log D_{ow}$ to $\log K_{oc}$. The analytes in this study are polar, and can be charged at the pH values in the mesocosms (e.g., atenolol can be partially positively charged, and sulfamethoxazole negatively charged). Corresponding log K_{oc} values were calculated to be 0.16 for atenolol, 1.96 for carbamazepine and 0.004 for sulfamethoxazole within the pH range of our study. To determine the importance of sorption for dissipation of the analytes, we calculated compound-specific sedimentation rates (k_s*). We obtained a sedimentation rate of 1.3×10^{-4} day^{-1} for carbamazepine, and values lower than 1.2×10^{-5} day^{-1} for the other compounds, suggesting that sorption via sedimentation of particles could play a role for carbamazepine, as it was previously observed by Cardinal et al. (2014 and 2016). However, we expected photolysis to be dominant under the conditions of this study.

Direct photolysis of carbamazepine was estimated to contribute in 9% of its overall removal, and approximately 95% for sulfamethoxazole (Table 4.4). The extent of direct photolysis is dictated by both the quantum yield and the rate of light absorption, with compounds having a greater degree of conjugation being able to absorb more light in the UV-A range (Table 4.3). Atenolol is a compound that does not absorb radiation between 290 and 800 nm, so no direct photolysis in the natural environment was expected (Liu and Williams, 2007). Challis et al. (2014) highlighted the importance of identifying uncertainties in the estimated kinetic data, which can enhance research transparency in terms of precision of the experimental techniques and data variability. For instance, the incident light measurement can be a major source of uncertainty in studies addressing the fate of pharmaceuticals than can suffer photodegradation (Challis et al., 2014).

With the aim to understand and identify the greatest uncertainty sources in our estimations, we conducted a sensitivity analysis of our fate predictions through a Monte

Carlo (see Figure C4). For direct photolysis, we considered the potential variability in sunlight absorption rates for both carbamazepine and sulfamethoxazole, as well as the uncertainty in quantum yields obtained from the literature, whenever it was reported. Sunlight absorption rates variability proved to be important for sulfamethoxazole estimations (65.8%), and quantum yield uncertainty had a minor importance (2%). For carbamazepine, direct photolysis uncertainties in our estimations did not prove to be important for the overall estimated rates, according to the sensitivity analysis.

Hydroxyl radicals were expected to dominate indirect photolysis processes in this study. Their production was likely mediated by dissolved organic matter (Lam et al., 2005). As mentioned previously, carbamazepine and sulfamethoxazole dissipated considerably slower than predicted in this study (Table 4.4). This could be due to a number of processes including reactions with radical species such as singlet oxygen or carbonate radicals, increased light screening for direct photolysis in the mesocosm tanks, as previously observed at the same facility (Lu et al., 2015), or inaccurate estimations of the amount of light available during the course of the study. From the Monte Carlo analysis, uncertainties associated with second-order rate constants for reaction with hydroxyl radicals ($k'_{p\cdot OH}$) proved to be important for atenolol (100%), carbamazepine (100%), and sulfamethoxazole (33%).

Sorption mechanisms, including electrostatic interactions between analytes and sorbents, were probably in place to some extent to promote a delay in dissipation. Covalent and irreversible binding to sediments and suspended particulate matter has previously been observed for sulfonamide-type antibiotics (Carstens et al., 2013) and carbamazepine (Zhang et al., 2011), where sorption and photodegradation were important removal processes. The mineral surfaces used as substrates tend to be coated with negatively charged natural

organic matter (Schwarzenbach et al., 2017) and this was probably the case for our study. That said, we were unable to quantify electrostatic sorption of the compounds by the modelling approaches used.

4.5.2. Pilot-scale project

The number of consistently detected background pharmaceuticals during the course of the study was less than in previous efforts at the same facility (Anderson et al., 2015; Chaves-Barquero et al., 2018). Though, their detected levels (atenolol: 1-13 ng/L; carbamazepine: 5.7-247 ng/L; metoprolol: 11-73 ng/L; sulfamethoxazole 0.8-27 ng/L) were comparable to those measured in other municipal wastewater facilities in Manitoba and elsewhere in North America (Table 4.6). The ability of studied wastewater treatment system in removing wastewater contaminants continues to be relevant and potentially beneficial to the community.

Atenolol and metoprolol were efficiently removed (< 99%) from the pilot-scale subsurface beds, for both glass and sand, likely from biodegradation and sorption inside the filter beds as main drivers. Carbamazepine and sulfamethoxazole removals were non-existent across the season, with no significant differences between the performance of both studied substrates ($p < 0.05$) (Table 4.5). Inefficient removal of carbamazepine (i.e., very limited to no removal) by these types of systems has been reported in previous efforts at the same facility in Manitoba (Anderson et al., 2015; Chaves-Barquero et al., 2018), and elsewhere (Anderson et al., 2020; Carlson et al., 2013; Chaves-Barquero et al., 2016; Li et al., 2014). Carbamazepine is known for its recalcitrant behaviour and low biodegradation previously reported in municipal wastewater treatment systems (Metcalfe et al., 2003; Ngoc Han et al., 2018; Yang et al., 2017). Despite the susceptibility of sulfamethoxazole to undergo photodegradation in wetland systems (Cardinal et al., 2014), this was not the case

because of the light-limited conditions present in the subsurface system, which was covered by wooden chips. Variations in concentrations could be observed for all analytes depending on the sampling date, which is to be expected considering the use of grab-sampling on punctual dates to determine the concentrations, and the variability in population and in wastewater discharge from the community due to the of touristic activities during the summer as noted in previous studies (Anderson et al., 2015; Chaves-Barquero et al., 2018).

Finally, a comparison between the results obtained in the pilot-scale to the results reported in the mesocosm-scale study is presented. These systems were different in design: the mesocosms were configured as quiescent modelled wetlands with no inflows or outflows, treating spiked levels of contaminants, while the pilot-scale system was a vertical-flow subsurface filter treating secondary municipal wastewater with background levels of the contaminants of interest. In the mesocosm-scale project, we observed slow kinetics of removal, for typically recalcitrant pharmaceutical contaminants such as carbamazepine and sulfamethoxazole, while atenolol was dissipated efficiently. These dynamics agreed with the results obtained for removal of pharmaceuticals at the pilot-scale study, where efficient removals were observed for metoprolol, while carbamazepine and sulfamethoxazole were not removed by the substrates at the subsurface filter. Moreover, pharmaceutical half-lives obtained from the mesocosm-scale study were well above the hydraulic retention time that pharmaceuticals experienced in the pilot-scale system. Overall, CRG showed a good potential as substrate for CW applications, with similar performances compared to traditional materials such as sand or gravel. We recommend new studies consider the use of CRG either by itself or in mixtures with traditional materials to study the removal of nutrients and other wastewater contaminants though CW-based technologies in the field.

4.6. Conclusions

Our results suggest that CRG can be a suitable substrate for wetland-based wastewater treatment applications. Overall, CRG showed similar behaviour compared to gravel in terms of dissipation and obtained half-lives for studied pharmaceuticals in the mesocosm-scale systems, with photolysis being a predominant removal mechanism for sulfamethoxazole, based on our estimations. A similar behaviour in terms of interaction with pharmaceuticals was also observed for CRG and sand in the pilot-scale system. The observed performance of CRG suggests its potential to be used by itself or mixed with other traditional materials in substrates for CW application, especially in post-lagoon treatment systems such as the pilot-scale system studied on this manuscript. Lack of removal for carbamazepine and sulfamethoxazole in the pilot-scale system indicates a need for future studies that aim to optimize the operational conditions (e.g., substrate particle size, hydraulic retention time, flow rate) for achieving greater removal rates and better understanding removal mechanisms in the laboratory as well as in the field.

4.7. Acknowledgements

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4.8. References for Chapter 4

Anderson, J.C.; Carlson, J.C.; Low, J.E.; Challis, J.K.; Wong, C.S.; Knapp, C.W.; Hanson, M.L. Performance of a constructed wetland in Grand Marais, Manitoba, Canada: Removal of nutrients, pharmaceuticals, and antibiotic resistance genes from municipal wastewater. *Chemistry Central Journal* **2013**; *7*: 54.

Anderson, J.C.; Jabari, P.; Parajas, A.; Loeb, E.; Luong, K.H.; Vahedi, A.; Wong, C.S. Evaluation of cold-weather wastewater nitrification technology for removal of polar chemicals of emerging concern from rural Manitoba wastewaters. *Chemosphere* **2020**; *253*: 126711.

Anderson JC, Joudan S, Shoichet E, Cuscito LD, Alipio AEC, Donaldson CS, Khan S, Goltz DM, Rudy MD, Frank RA, Knapp CW, Hanson ML, Wong CS. Reducing nutrients, organic micropollutants, antibiotic resistance, and toxicity in rural wastewater effluent with subsurface filtration treatment technology. *Ecological Engineering* **2015**; *84*: 375-385.

Aulenbach, D.B.; Nie, M.S. Studies on the mechanism of phosphorus removal from trated wastewater by sand. Journal Water Pollution Control Federation **1988**; *60*: 2089-2094.

Camacho-Munoz, D.; Martin, J.; Santos, J.L.; Aparicio, I.; Alonso, E. Effectiveness of Conventional and Low-Cost Wastewater Treatments in the Removal of Pharmaceutically Active Compounds. *Water Air and Soil Pollution* **2012**; *223*: 2611-2621.

Cardinal, P.; Anderson, J.C.; Carlson, J.C.; Low, J.E.; Challis, J.K.; Beattie, S.A.; Bartel, C.N.; Elliot, A.D.; Montero, O.F.; Lokesh, S.; Favreau, A.; Kolzova, T.A.; Knapp,

C.W.; Hanson, M.L.; Wong, C.S. Macrophytes may not contribute significantly to removal of nutrients, pharmaceuticals, and antibiotic resistance in model surface constructed wetlands. *Science of the Total Environment* **2014**; *482*: 294-304.

Cardinal, P.; Anderson, J.C.; Carlson, J.C.; Low, J.E.; Challis, J.K.; Wong, C.S.; Hanson, M.L. Late season pharmaceutical fate in wetland mesocosms with and without phosphorous addition. *Environmental Science and Pollution Research* **2016**; *23*: 22678-22690.

Carlson, J.C.; Anderson, J.C.; Low, J.E.; Cardinal, P.; MacKenzie, S.D.; Beattie S.A.; Challis, J.K.; Bennett, R.J.; Meronek, S.S.; Wilks, R.P.A.; Buhay, W.M.; Wong, C.S.; Hanson, M.L. Presence and hazards of nutrients and emerging organic micropollutants from sewage lagoon discharges into Dead Horse Creek, Manitoba, Canada. *Science of the Total Environment* **2013**; *445*: 64-78.

Carstens, K.L.; Gross, A.D.; Moorman, T.B.; Coats, J.R. Sorption and Photodegradation Processes Govern Distribution and Fate of Sulfamethazine in Freshwater-Sediment Microcosms. *Environmental Science & Technology* **2013**; *47*: 10877-10883.

Challis, J.K.; Hanson, M.L; Friesen, K.J.; Wong, C.S. A critical assessment of the photodegradation of pharmaceuticals in aquatic environments: defining our current understanding and identifying knowledge gaps. *Environmental Science-Processes & Impacts* **2014**; *16*: 672-696.

Chaves-Barquero, L.G.; Luong, K.H.; Mundy, C.J.; Knapp, C.W.; Hanson, M.L.; Wong, C.S. The release of wastewater contaminants in the Arctic: A case study from Cambridge Bay, Nunavut, Canada. *Environmental Pollution* **2016**; *218*: 542-550.

Chaves-Barquero, L.G.; Luong, K.H.; Rudy, M.D.; Frank, R.A.; Hanson, M.L.; Wong, C.S. Attenuation of pharmaceuticals, nutrients and toxicity in a rural sewage lagoon

system integrated with a subsurface filtration technology. *Chemosphere* **2018**; *209*: 767-775.

Cizmas, L.; Sharma, V.K.; Gray, C.M, McDonald, T.J. Pharmaceuticals and personal care products in waters: occurrence, toxicity, and risk. *Environmental Chemistry Letters* **2015**; *13*: 381-394.

Conkle, J.L.; White, J.R.; Metcalfe, C.D. Reduction of pharmaceutically active compounds by a lagoon wetland wastewater treatment system in Southeast Louisiana. *Chemosphere* **2008**; *73*: 1741-1748.

Dordio, A.V.; Carvalho, A.J.P. Organic xenobiotics removal in constructed wetlands, with emphasis on the importance of the support matrix. *Journal of Hazardous Materials* **2013**; *252*: 272-292.

Elliott, R.W. Evaluation of the use of crushed recycled glass as a filter medium: Part 2. *Water-Engineering & Management* **2001**; *148*: 17-20.

European Container Glass Federation. Record collection of glass containers hits 76% in the EU **2018**. URL: https://feve.org/recordcollection-of-glass-containers-for-recycling-hits-76-in-the-eu/ (Accessed June 2020)

Gill, L.; Doran, C.; Misstear, D.; Sheahan, B. The use of recycled glass as a filter media for on-site wastewater treatment. *Desalination and Water Treatment* **2009**; *4*: 198-205.

Gill, L.W.; Veale, P.L.; Murray, M. Recycled glass compared to sand as a media in polishing filters for on-site wastewater treatment. *Water Practice and Technology* **2011**; *6*.

Healy, M.G.; Burke, P.; Rodgers, M. The use of laboratory sand, soil and crushed-glass filter columns for polishing domestic-strength synthetic wastewater that has

undergone secondary treatment. *Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering* **2010**; *45*: 1635-1641.

Horan, N.J.; Lowe, M. Full-scale trials of recycled glass as tertiary filter medium for wastewater treatment. *Water Research* **2007**; *41*: 253-259.

Hu, Z.F.; Gagnon, G.A. Impact of filter media on the performance of full-scale recirculating biofilters for treating multi-residential wastewater. *Water Research* **2006**; *40*: 1474-1480.

Kadlec RH, Wallace S. Treatment wetlands. CRC Press, Boca Raton, FL: ebrary Inc, **2009**.

Knapp, CW.; Graham, D.W. Nitrite-oxidizing bacteria guild ecology associated with nitrification failure in a continuous-flow reactor. *FEMS Microbiology Ecology* **2007**; *62*: 195-201.

Kolpin, D.W.; Furlong, E.T.; Meyer, M.T.; Thurman, E.M.; Zaugg, S.D.; Barber, L.B.; et al. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A national reconnaissance. *Environmental Science & Technology* **2002**; *36*: 1202-1211.

Kunkel, U.; Radke, M. Reactive Tracer Test To Evaluate the Fate of Pharmaceuticals in Rivers. *Environmental Science & Technology* **2011**; *45*: 6296-6302.

Lam, M.W.; Young, C.J.; Mabury, S.A. Aqueous photochemical reaction kinetics and transformations of fluoxetine. *Environmental Science & Technology* **2005**; 39: 513-522.

Li, J.A.; Cheng, W.X.; Xu, L.K.; Strong, P.J.; Chen H. Antibiotic-resistant genes and antibiotic-resistant bacteria in the effluent of urban residential areas, hospitals, and a municipal wastewater treatment plant system. *Environmental Science and Pollution Research* **2015**; *22*: 4587-4596.

Li, Y.; Zhu, G.; Ng, W.J.; Tan, S.K. A review on removing pharmaceutical contaminants from wastewater by constructed wetlands: Design, performance and mechanism. *Science of the Total Environment* **2014**; *468*: 908-932.

Liu, Q.T.; Williams, H.E. Kinetics and degradation products for direct photolysis of beta-blockers in water. *Environmental Science & Technology* **2007**; *41*: 803-810.

Lu, Z.; Challis, J.K.; Wong, C.S. Quantum Yields for Direct Photolysis of Neonicotinoid Insecticides in Water: Implications for Exposure to Nontarget Aquatic Organisms. *Environmental Science & Technology Letters* **2015**; *2*: 188-192.

Matamoros, V.; Arias, C.; Brix, H.; Bayona, J.M. Removal of pharmaceuticals and personal care products (PPCPs) from urban wastewater in a pilot vertical flow constructed wetland and a sand filter. *Environmental Science & Technology* **2007**; *41*: 8171-8177.

Metcalfe, C.D.; Koenig, B.G.; Bennie, D.T.; Servos, M.; Ternes, T.A.; Hirsch, R. Occurrence of neutral and acidic drugs in the effluents of Canadian sewage treatment plants. *Environmental Toxicology and Chemistry* **2003**; *22*: 2872-2880.

Morawski C, Wilcox J, Millette S. Who pays that — An analysis of beverage container collection and costs in Canada. CM Consulting, Barcelona, Spain, **2016**.

Ngoc Han, T.; Reinhard, M.; Gin, K.Y.H. Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions-a review. *Water Research* **2018**; *133*: 182-207.

Oulton, R.L.; Kohn, T.; Cwiertny, D.M. Pharmaceuticals and personal care products in effluent matrices: A survey of transformation and removal during wastewater treatment and implications for wastewater management. *Journal of Environmental Monitoring* **2010**; *12*: 1956-1978.

Reindl J. Reuse/recycling of glass cullet for non-container uses. Dane County Department of Public Works. Madison, WI **2003**. URL: https://archive.epa.gov/wastes/conserve/tools/greenscapes/web/pdf/glass.pdf (Accessed July 2020)

Salzmann, R.D.; Ackerman, J.N.; Cicek, N. Pilot-scale, on-site investigation of crushed recycled glass as tertiary filter media for municipal lagoon wastewater treatment. *Environmental Technology* **2020**: *9*.

Schwarzenbach, R.; Gschwend, P.; Imboden, D. Environmental Organic Chemistry. 3rd ed. New Jersey, USA: John Wiley & Sons, Inc., **2017**.

Solomon K.R.; Smith, K.; Stephenson, G.L. Depth integrating samplers for use in limnocorrals. *Hydrobiologia* **1982**; *94*: 71-75.

Statistics Canada. Human activity and the environment. Ottawa, ON **2015**. URL: https://www150.statcan.gc.ca/n1/en/pub/16-201-x/16-201-x2005000-eng.pdf?st=LvPhnqMU (Accessed July 2020)

Statistics Canada. Disposal of waste, by source (Table 38-10-0032-01). Ottawa, ON **2018**. Environmental Accounts and Statistics Division, System of National Accounts. URL: https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=3810003201 (Accessed July 2020)

U.S. EPA. Facts and figures about materials, waste and recycling. **2018**. URL: https://www.epa.gov/facts-and-figures-about-materialswaste-and-recycling/glass-material-specific-data (Accessed August 2020)

Uslu, M.O.; Jasim, S.; Arvai, A.; Bewtra, J.; Biswas, N. A Survey of Occurrence and Risk Assessment of Pharmaceutical Substances in the Great Lakes Basin. *Ozone-Science & Engineering* **2013**; *35*: 249-262.

Verlicchi, P.; Zambello, E. How efficient are constructed wetlands in removing pharmaceuticals from untreated and treated urban wastewaters? A review. *Science of the Total Environment* **2014**; 470: 1281-1306.

Vidal-Dorsch, D.E.; Bay, S.M.; Maruya, K.; Snyder, S.A.; Trenholm, R.A.; Vanderford BJ. Contaminants of emerging concern in municipal wastewater effluents and marine receiving water. *Environmental Toxicology and Chemistry* **2012**; *31*: 2674-2682.

Vymazal, J. Constructed wetlands for wastewater treatment: five decades of experience. *Environmental Science & Technology* **2011**; *45*: 61-9.

Wu, J.M.; Xu, D.; He, F.; He, J.; Wu, Z.B. Comprehensive evaluation of substrates in vertical-flow constructed wetlands for domestic wastewater treatment. *Water Practice and Technology* **2015**; *10*: 625-632.

Xu, N.; Chen, M.; Zhou, K.R.; Wang, Y.L.; Yin, H.W.; Chen, Z.G. Retention of phosphorus on calcite and dolomite: speciation and modeling. *RSC Advances* **2014**; *4*: 35205-35214.

Yang, Y.; Ok, Y.S.; Kim, K.H.; Kwon, E.E.; Tsang, Y.F. Occurrences and removal of pharmaceuticals and personal care products (PPCPs) in drinking water and water/sewage treatment plants: A review. *Science of the Total Environment* **2017**; *596*: 303-320.

Zhang, D.Q.; Tan, S.K.; Gersberg, R.M.; Sadreddini, S.; Zhu, J.F.; Nguyen, A.T. Removal of pharmaceutical compounds in tropical constructed wetlands. *Ecological Engineering* **2011**; *37*: 460-464.

Zhu, S.C.; Chen, H.; Li, J.N. Sources, distribution and potential risks of pharmaceuticals and personal care products in Qingshan Lake basin, Eastern China. *Ecotoxicology and Environmental Safety* **2013**; *96*: 154-159.

4.9. Connecting text between Chapters 4 and 5

Chapter 4 has presented novel research on the potential of crushed recycled glass to act as a low-cost substrate for mesocosm- and pilot-scale CW applications removing pharmaceuticals in temperate climate, finding no statistically significant differences between its performance and the one observed for more traditional materials in both mesocosm- and pilot-scale projects. Next, this dissertation focuses on the performance of a full-scale system based in temperate climate for pharmaceutical removal from real wastewater using other low-cost substrates such as sand and gravel. This constituted an exciting opportunity to better understand the processes therein and integrate them with the observations from previous chapters from this dissertation.

CHAPTER 5

5. ATTENUATION OF PHARMACEUTICALS, NUTRIENTS AND TOXICITY IN A RURAL SEWAGE LAGOON SYSTEM INTEGRATED WITH A SUBSURFACE FILTRATION TECHNOLOGY

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The contribution from each author is detailed under the "Contributions of Authors" section in the preface of this dissertation.

5.1. Abstract

Although many studies have addressed the ability of subsurface filtration systems to remove emerging contaminants from wastewater at micro- and mesocosm-scale, little is known about their performance on full-scale wastewater treatment facilities. To understand better how effective these systems can be for municipal wastewater polishing, we assessed the ability of a full-scale lagoon-subsurface filter system located in Dunnottar, Manitoba, Canada, to attenuate regulatory wastewater parameters, nutrients, pharmaceuticals, and toxicity over the course of the Summer discharge seasons of 2015 and 2016 (June-October). Pharmaceuticals included β-blockers, anticonvulsant drugs, and macrolide and sulfonamide antibiotics. Out of six consistently detected pharmaceuticals, four were efficiently removed through lagoon treatment (e.g. clarithromycin, metoprolol, propranolol), while two persisted to a certain extent (e.g. carbamazepine, sulfamethoxazole), even after subsurface filtration. Attenuation was observed for nutrients with averages of 40% and 60% for ammonia and total phosphorus respectively within the filter, consistent with previous pilot-scale studies at this facility. Compliance with regulations for conventional wastewater parameters at the effluent was observed, as well as reduced acute toxicity (as determined by Microtox®) from the primary lagoon to the effluent, and little likelihood of acute toxicity in receiving waters. Our results suggest that first, the full-scale system has an overall similar performance when compared to the previously studied pilot-scale system; second, there was no apparent effect of acclimation on the attenuation of studied contaminants or toxicity; and finally, the concentrations of contaminants do not appear to pose an acute risk for aquatic species in the receiving environment.

5.2. Introduction

The occurrence of contaminants in wastewater can represent a hazard to aquatic ecosystems and a challenge for wastewater treatment systems worldwide (Kolpin et al., 2002). More than 200 pharmaceutical active ingredients are found in aquatic compartments in concentrations ranging from a few nanograms per liter to thousands of micrograms per liter (Hughes et al., 2013). Excess nutrients (e.g. phosphorus and nitrogen) are also a concern in wastewater due to their promotion of eutrophication. To address the hazards that these contaminants pose to receiving environments, an improvement in current wastewater treatment practices is needed, along with a greater understanding of removal mechanisms. Wastewater lagoons are commonly used in rural North America for sewage treatment (Federation of Canadian Municipalities, 2004). They are bodies of water designed to hold and perform some treatment of wastewater for a defined amount of time and are typically far less costly than conventional activated sludge wastewater treatment. Thus, they are common in smaller communities with limited infrastructure and resources in North America (Chaves-Barquero et al., 2016; Conkle et al., 2008; Lishman et al., 2006). The implementation of lagoons occurred before a stricter regulatory environment emerged around wastewater discharges (Smith, 2003). Wastewater guidelines for contaminants have recently been published, for example the Canada-wide strategy for the Management of Municipal Wastewater Effluent, which aims to implement a strategy to develop a targeted research program that will encompass emerging substances, such as organochlorine pesticides, polychlorinated biphenyls, poly-aromatic hydrocarbons as well as pharmaceuticals and other personal care products (CCME, 2014). In Canada, regulations are becoming more stringent for BOD, P and total suspended solids (TSS) (Government of Canada, 2012). In Manitoba, where this research takes place, maximum levels have been

fixed to 25 mg L^{-1} for BOD and TSS (CCME, 2014), and 1 mg L^{-1} for total phosphorus (Manitoba Water Stewardship, 2011).

Constructed wetlands can provide a low-cost alternative for wastewater treatment in regions where availability of land is not a limiting issue. Nevertheless, no specific guidelines have been established for designing full-scale systems to remove pharmaceutical compounds from wastewater. Furthermore, attenuation of wastewater organic contaminants can be limited on these systems, depending on hydraulic loading (Zhang et al., 2012).. There are knowledge gaps related to the performance and design of these systems for the attenuation of pharmaceuticals and other organic contaminants in municipal wastewater, especially at pilot and full-scale facilities, where comprehensive evidence could be gathered in comparison to laboratory and mesocosm-scale experiments, which have been more frequently conducted in the literature (Li et al., 2014).

To address some of the treatment challenges described above, a pilot-scale subsurface filtration pilot-scale system was built in 2009 at the Village of Dunnottar, Manitoba, Canada, near the shore of Lake Winnipeg (the 10th largest freshwater lake in the world by surface area). The main purpose of this system was to reduce the release of traditional wastewater contaminants (e.g. phosphorus, coliforms) and its efficiency in this area has been previously reported (Anderson et al., 2015). The pilot-scale filter showed significant attenuation for nutrient loadings, such as phosphorus and nitrogen, but limited to no removal of most organic contaminants (e.g. pharmaceuticals), likely due to the short residence time of the wastewater in the filter bed (~ 6 h). This system served as a model for the construction of a full-scale filter at Dunnottar in 2014. It commenced operations in June 2015 with the purpose of achieving the same level of wastewater treatment. The full-scale system was not expressly designed to remove or attenuate organic contaminants (e.g.

pharmaceuticals, personal care products). Nevertheless, due to its design, it offered a valuable opportunity to study the concentrations of organic contaminants along the wastewater path with the aim of understanding the fate of these substances at all stages of the treatment. Such a system could be implemented as a model for similar rural communities as a low-cost, low-maintenance method to perform municipal wastewater treatment under current regulatory compliance. At the same time, it would serve as a platform for manipulation studies that help understand the effect of several design parameters on the potential attenuation and removal of emerging contaminants, should it be occurring.

In this study, we were interested in first, assessing the removal efficiency of the current full-scale wastewater treatment system at Dunnottar, Manitoba for pharmaceutical residues, phosphorus and toxicity; second, characterizing possible toxicological impacts on the surrounding aquatic environment from the release of wastewater; and third, comparing the full-scale system's performance over time (2 years) and with the pilot-scale system. We hypothesize that first, the full-scale treatment system will perform more efficiently than the pilot-scale system in attenuating wastewater contaminants of interest and toxicity due to larger scale and increased hydraulic retention time; second, detected contaminants will not presently pose a significant risk to the surrounding aquatic environment; and third, acclimation of the system will cause performance improvements during the second year of operations. This study will inform the design and management of subsurface treatment systems with similar characteristics in North America and around the globe.

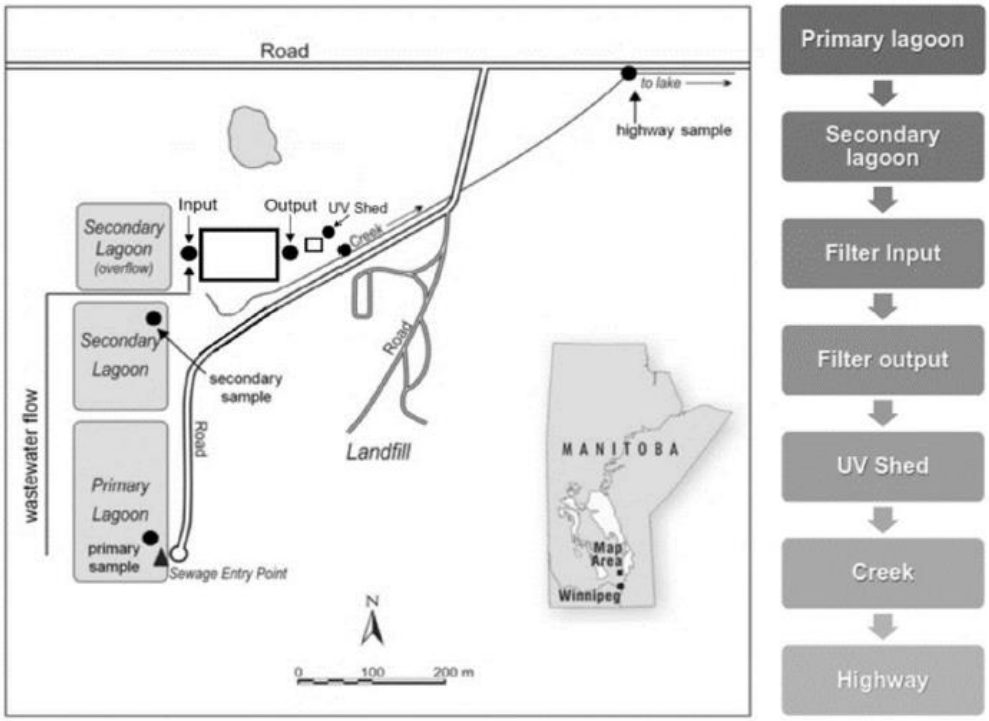


Figure 5.1. Dunnottar, Manitoba, Canada wastewater treatment system and sampling sites for the present study.

5.3. Materials and Methods

5.3.1. Study location

The municipal wastewater treatment system at Dunnottar consists of an array of three lagoons (Fig. 5.1) providing services for the Village of Dunnottar and the Provincial Park Camp Ground in Winnipeg Beach. The village has approximately 763 permanent residents, though summer season use from vacationers increases its population several-fold (Anderson et al., 2015). Residential holding tanks have been installed in the community, and sewage is hauled continuously and discharged in the primary lagoon using septic trucks.

In 2009, a four-cell pilot-scale subsurface passive filter was installed with the aim of further polishing the wastewater after lagoon treatment. Based on this pilot-scale design, a

two-cell full-scale subsurface filtration system was built (Fig. 5.1). Each of these cells is 50 m long × 25 m wide × 1.2 m deep for a total array volume of 3000 m^3 (Fig. 5.2), and can treat wastewater at a maximum rate of 500 m^3 d^{-1} (average ~265 m^3 d^{-1}). An intake well collects wastewater from the secondary lagoon and pumps it vertically into the filter bed through a distribution pipe. Wastewater is then redirected into an outflow well from where it is pumped through an in-line Trojan UV 3000 PTP ultraviolet treatment system, the output of which flows into Tegula creek for 1.5 km to Lake Winnipeg. Hydraulic retention time (HRT) can reach a maximum of 86 hours (average ~45 h). No chemical inputs (e.g. coagulants, flocculants, conditioners) were performed during the operation of this wastewater treatment facility in either 2015 or 2016.

5.3.2. Sample collection

In 2015 and 2016, water quality parameters (BOD, COD, total phosphorus, total suspended solids, total and fecal coliforms) in the final effluent were taken by the Village of Dunnottar in collaboration with Dillon Engineering Ltd. and measured by standard methods (APHA, 2005) at ALS Environmental. Sites chosen for pharmaceuticals and toxicity sample collection were: ~15 m away from the sewage discharge point in the primary lagoon ("primary lagoon"), at the former entry point into the pilot-scale filter from the secondary lagoon ("secondary lagoon"), at the input well to the full-scale filter ("input"), at the outflow well from the full-scale filter where treated water is collected ("output"), at the output of the in-line ultraviolet system ("UV shed"), at the output to Tegula creek ("creek") and ~200 m east from the main gate of the facility, downstream of Tegula creek ("highway") (Figure 5.1).

Sampling occurred during the licensed active treatment and discharge season in 2015 and in 2016 (Table 5.1). Grab sampling for toxicity assays (Microtox®) and determination

of organic compounds was performed in the form of single samples for every site and time, with a rotating triplicate (i.e. only one location was sampled by triplicate on each sampling day) for estimating measurement variability for individual sampling points. Pre-ashed glass amber bottles (1 L) were used to sample wastewater for organic contaminants measurement and 50 mL sterile Falcon tubes were used for Microtox® samples. Before sampling, bottles were rinsed three times with the sample and then filled up to the top with no headspace. In the case of Microtox® samples, some headspace was allowed for storage at -20°C in the laboratory. Field and laboratory blanks were prepared accordingly for each sampling day to guarantee an adequate quality of the determinations of both organic compounds and Microtox®. Triplicate Polar Organic Chemical Integrative Samplers (POCIS) (Environmental Sampling Technologies, St, Joseph, MO, USA) were used in the "pharmaceutical" configuration as described by MacLeod and Wong (2010) to perform passive sampling of the organic contaminants.

Table 5.1. Description of sampling dates and types taken at the Dunnottar wastewater treatment facility in 2015 and 2016.

Sample type	Sampling dates	Sampling locations
	2015	
Grab-samples for Pharmaceuticals	June 30, July 14, July 28, August 11, August 25, September 8, September 22, October 6.	Primary lagoon, secondary lagoon, intake, outflow, UV shed, highway.
POCIS samples for pharmaceuticals	June 15 – June 30, June 30 – July 14, July 14 – July 28, July 28 – August 11, August 11 – August 25, August 25 – September 8, September 8 – September 22, September 22 – October 6.	Intake, Outflow, UV shed
Grab-samples for Phosphorus and Ammonia	August 18, September 1, September 15, September 22, October 6.	Intake, Outflow
Grab-samples for regulatory parameters*	June 17, July 15, August 26, September 25, October 16.	Outflow
Grab-samples for Microtox®	June 30, July 14, July 28, August 11, August 25, September 8, September 22, October 6.	Primary lagoon, secondary lagoon, intake, outflow, UV shed, highway.
	2016	
Grab-sample, Pharmaceuticals	June 15, June 29, July 13, July 27, August 10, August 24, September 13, October 5.	Primary lagoon, secondary lagoon, intake, outflow, UV shed, highway.
POCIS, Pharmaceuticals	June 15 – June 29, June 29 – July 13, July 13 – July 27, July 27 – August 10, August 10 – August 24, August 24 – September 13, September 13 – October 6.	Intake, Outflow, UV shed
Grab-sample, regulatory parameters*	July 7, August 24, September 21, October 13	Outflow
Grab-sample, Microtox®	June 30, July 14, July 28, August 11, August 25, September 8, September 22, October 6	Primary lagoon, secondary lagoon, intake, outflow, UV shed, highway.

*sampled by the facility personnel at the effluent only.

5.3.3. Determination of pharmaceutical concentrations

Previous methods (Carlson et al., 2013) were followed for the analysis of pharmaceuticals. A suite of twenty commonly used pharmaceuticals frequently found in wastewater (Fatta-Kassinos et al., 2011) was analyzed using an Agilent 6410B ultra high-performance liquid chromatography tandem-mass spectrometer (UHPLC/MS/MS) with electrospray ionization and isotope dilution for quantification in all water samples. Examined analytes, their limits of quantification (LOQ), as well as their isotopic internal

standards, are found in the supplementary information (Table D1), and other quality assurance/quality control parameters details elsewhere (Carlson et al., 2013). For POCIS, time-weighted-average (TWA) concentrations were calculated by dividing the measured collected analyte mass (ng) by the sampling rate (L d^{-1}) using sampling rates (Table D2) found in the literature (Bartelt-Hunt et al., 2011; Challis et al., 2016; Macleod et al., 2007) multiplied by the deployment time (d) for all detected micropollutants, Specific attenuation ability from the subsurface filter was determined based on TWA POCIS concentrations pre- and post-filter.

5.3.4. Hazard assessment for detected pharmaceuticals.

For all detected organic compounds, risk assessment for acute effects was conducted by calculating hazard quotients (HQs) using standard endpoints derived from previous aquatic toxicological studies, mainly for primary producers, invertebrates and fish. In brief, effective concentration (EC50) or lethal concentration (LC50) estimates related to growth, reproduction and survivorship were collected from a literature review and the most sensitive response selected, without any further assessment of study quality. We defined an uncertainty factor of 1000 to the lowest EC50 or LC50 for added conservatism. The maximum measured environmental concentrations for each contaminant were then divided by the lowest reported effect concentration to obtain the HQ. Compounds for which HQs were greater than unity were considered a concern, while those with lower values of HQ were considered less likely to be of concern.

For organic contaminants consistently detected at the primary lagoon, we created exposure distributions to study the range of concentrations that were detected in 2013 (Anderson et al., 2015), in 2015 and 2016 at the same sampling location. Because the primary lagoon is the site where the greatest concentrations of contaminants are found, the

probability of finding hazardous conditions for aquatic species is greatest, and therefore we chose this site as a conservative location for exposures in the wastewater facility. Exposure distributions were created by organizing the non-detect values and detected concentrations from least to greatest using the Weibull ranking equation to establish a percent rank of these concentrations. Pharmaceuticals needed to be detected at least twelve times at levels greater than their LOQ over the course of the three seasons to be plotted. The ranked data were then plotted as cumulative frequency distributions using a probability scale on the y-axis as a function of the $\log_{10}$ concentration for the detected values only. Linear regression equations and 90^{th} centiles were obtained for every exposure distribution using Sigma Plot 11 (Jandel, San Rafael, CA, USA). A more recent example of exposure distribution application can be found elsewhere (Fedorova et al., 2022).

5.3.5. Toxicity assessment

Microtox® assays were performed to assess overall acute toxicity of collected samples. Measurement of relative bioluminescence of the marine bacterium *Vibrio fischeri* pre- and post-exposure was used for acute toxicity assessment following adapted standard procedures on a Microbics M500 Analyzer (Environment Canada, 1992). Briefly, individual frozen samples (-20°C) were thawed at 4°C and the change in *V. fischeri* bioluminescence was measured in triplicate at full sample strength. Our deviation from the standard protocol, which analyzes a serial dilution of the test mixture to generate an IC_{50} (Azur Environmental, 1995), was used to enable a more time- and cost-effective screening of the large sample set of the present study. Prior to analysis, samples were adjusted to optimal salinity for *V. fischeri,* with the response compared to control after 15 min of exposure as mean percent of control performance.

5.3.6. Statistical analysis

Microtox® bioluminescence and concentrations of pharmaceutical grab-samples between sequential sampling sites were assessed using analysis of variance (ANOVA) followed by Tukey's test. Comparisons were made between all sampling sites on a single sampling day. For pharmaceutical POCIS samples, filter input and output were compared using a Student's *t*-test, the same method being used for between year comparisons, based on removal efficiency percentages. Concentration data are reported as mean value ± standard deviation (SD), unless otherwise stated. Significant differences were considered at $p < 0.05$.

5.4. Results and Discussion

5.4.1. Wastewater treatment performance

5.4.1.1. Nutrients and other regulatory parameters

Select water quality parameters were measured nine times in 2015 and 2016 at the output of the full-scale treatment facility (Table D6). Reported BOD levels were less than 2.0 mg L^{-1} for all measurements, and COD oscillated between less than 2.0 mg L^{-1} and up to 53 mg L^{-1}. Total phosphorus showed values below 1 mg L^{-1} for all measurements, except for October 2016, when it was 1.9 mg L^{-1}. Total suspended solids and total and fecal coliforms were always below 6 mg L^{-1} and 5 counts L^{-1}, respectively. Overall, the system exhibited compliance with maximum permitted levels established for "very-small" wastewater treatment facilities (flow < 500 m^3 d^{-1}) by the Province of Manitoba (CCME, 2014) and the United States (EPA, 2002), with a few exceptions. Attenuation of total phosphorus and ammonia was measured five times during 2015 (Table 5.2). Removal efficiencies of 60% for total phosphorus and 40% for total ammonia were measured over the course of the 2015 season, which is consistent with previous results conducted in the

pilot scale system (Anderson et al., 2015) with observed removal efficiencies over the course of the season were 50% and 58%, respectively. Overall, the full-scale system performed wastewater treatment in compliance with regulatory levels for basic water quality parameters.

5.4.1.2. Pharmaceutical detection and attenuation

The concentrations of pharmaceuticals measured in grab-samples were similar to previously reported data from studies conducted in Manitoba (Table D7) (Anderson et al., 2013; Anderson et al., 2015; Carlson et al., 2013) and elsewhere (Conkle et al., 2008; MacLeod and Wong, 2010). Of the screened pharmaceuticals, only atenolol, carbamazepine, clarithromycin, metoprolol, propranolol and sulfamethoxazole were detected at least once at ng L^{-1} levels (Table D3). Figure 5.2 shows that atenolol and metoprolol were found in grab-samples predominantly at the primary lagoon in the order of hundreds of ng L^{-1}, with subsequent removal efficiencies of over 98% at the various treatment stages. Carbamazepine was detected at levels up to 327 ng L^{-1}, with some attenuation along the wastewater path. Concentrations in the order of tens of ng L^{-1} were detected consistently at the highway site. This observation suggests persistence of carbamazepine after treatment, consistent with steady use patterns for a relatively persistent compound in the aquatic environment (Conkle et al., 2008). Sulfamethoxazole was detected in grab-samples at levels up to 659 ng L^{-1} in the primary lagoon. Some persistence of this compound was observed along the wastewater path, as concentrations at the creek and highway sites were found at tens of ng L^{-1}. Concentrations of sulfamethoxazole varied over the course of summer seasons and then started declining towards the end, which may be due to photodegradation intensity and its variations over the season (Ryan et al., 2011). Clarithromycin and propranolol were rarely detected in grab-samples at the primary and/or

secondary lagoons (Table D3). Overall, attenuation processes for these pharmaceuticals occurred mostly in the primary and secondary lagoons, which is consistent with previous findings at the pilot-scale facility in 2013 (Anderson et al., 2015).

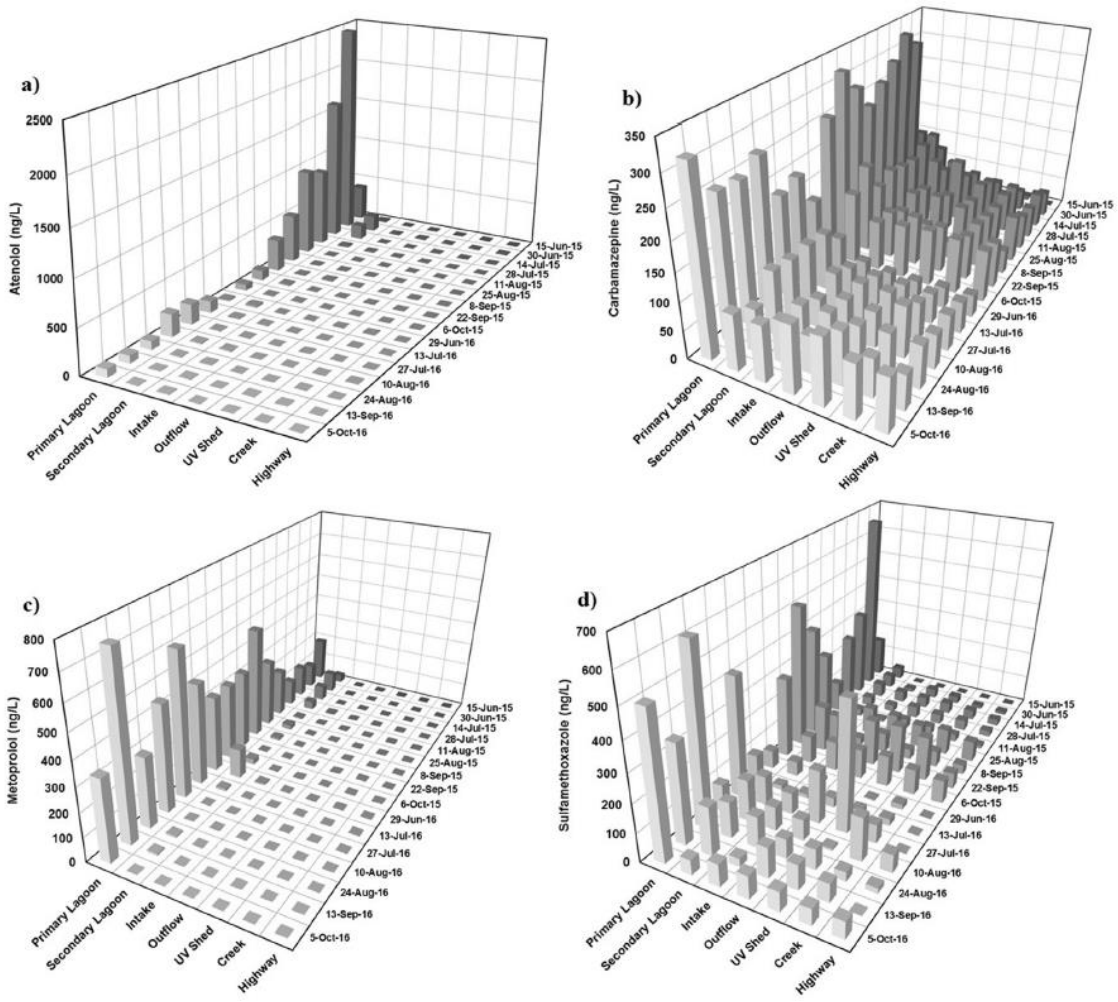


Figure 5.2. Grab sample concentrations of a) atenolol, b) carbamazepine, c) metoprolol, and d) sulfamethoxazole at sampling sites in the lagoons filter and discharge stream in the Dunnottar wastewater treatment system in 2015 and 2016.

Table 5.2. Levels of total phosphorus and ammonia (n=1) pre- and post-full-scale filtration at the Dunnottar wastewater treatment facility, measured in 2015.

Parameter ($mg\ L^{-1}$)	August 18		September 1		September 15		September 22		October 6	
	Input	Output	Input	Output	Input	Output	Input	Output	Input	Output
Total Phosphorus	1.1	0.32	0.69	0.29	1.0	0.43	0.88	0.39	1.1	0.39
Ammonia	0.041	0.032	0.056	0.03	0.036	0.029	0.038	0.028	0.045	0.026

Table 5.3. Mean percent removal of consistently detected pharmaceuticals in grab samples during 2013, 2015, and 2016 at the wastewater treatment facility in Dunnottar, Manitoba, between primary lagoon and creek sites. Only the pilot-scale subsurface filter was operational in 2013; this was decommissioned when the full-scale filter became operational in late 2014.

Compound	Mean removal (%)		
	2013*	2015	2016
Atenolol	> 98	> 98	> 98
Carbamazepine	49	71	70
Clarithromycin	NA	97	76
Metoprolol	> 99	> 99	> 99
Propranolol	77	NA	90
Sulfamethoxazole	76	77	64

* data collected from Anderson et al. (2015)

Table 5.3 shows mean removal percentages for consistently detected pharmaceuticals in the Dunnottar wastewater treatment facility in 2013 (Anderson et al., 2015), 2015, and 2016 based on the differences in grab-sample concentrations between the primary lagoon and the creek sites. These percentages consider the added effect of lagoon and filter treatments. For atenolol and metoprolol, removal efficiencies were, respectively, over 98% and 99% in all cases. Carbamazepine attenuation improved significantly from ~49% in 2013 to ~70% in 2015 ($p<0.05$). Sulfamethoxazole showed no significant differences in removal efficiency from pilot-scale to full-scale ($p>0.05$). No significant differences due to acclimation of the studied facility for pharmaceuticals attenuation between 2015 and 2016 seasons were found in grab-samples.

Although atenolol, carbamazepine and sulfamethoxazole were detected in POCIS samples (Fig. D2), no significant differences were found between concentrations pre- and

post-filtration. This observation is consistent with what was found at the pilot-scale filter in 2013 (Anderson et al., 2015), where a limited HRT was observed (~ 6 h) in comparison with the full-scale study (~ 45 h). The rest of the analytes showed non-detectable levels by the time they reached the filter input, and therefore, the potential for removal of these contaminants could not be determined. No significant differences due to acclimation were found between sampling sites pre- and post-filtration in 2015 and 2016. Fig. D3 shows a comparison between grab-sample and POCIS concentrations for atenolol, carbamazepine and sulfamethoxazole at the input and output of the filter. It must be noted that to examine the specific effect of the subsurface filter in pharmaceutical attenuation, we based our estimations on data from POCIS samples, as TWA concentrations even out the possibility of spuriously high or low concentrations that are usually seen in grab-samples, and therefore, provide a better estimation of the concentrations of these compounds over the sampling period. It should be acknowledged, however, that the presence of biofilms at the input of the filter, as well as fluctuations in flow rate, temperature and pH in wastewater from all three sites sampled at the Dunnottar wastewater treatment facility, can impact the sampling rates for the reported analytes and hence reported concentrations as well (Harman et al., 2012).

During 2015 and 2016, approximately 60,000 m^3 of wastewater were disposed annually into the Dunnottar primary lagoon. An exact correlation between population and the concentrations of pharmaceuticals is difficult to determine due to the great variability and transient nature of the cottager population, which fluctuates seasonally, as shown for other wastewater lagoons in Canada (MacLeod and Wong, 2010). Nevertheless, it is possible to compare qualitatively the detected concentrations in Dunnottar to other facilities in southern and Arctic Canada (Table 5.4). Increased population often results in increased

loadings of contaminants, which seems to be the case for Dunnottar (population 763), showing TWA concentrations for pharmaceuticals lower than the ones detected at Cambridge Bay in the Canadian Arctic (population 1,400) (Chaves-Barquero et al., 2016) and Lac la Biche in Alberta (population 8,402) (MacLeod and Wong, 2010). Seasonal trends indicated an increase in pharmaceuticals concentrations in the primary lagoon as the discharge season advances. This observation is consistent with the population increase expected during summer months due to tourism. It is worth mentioning that the facility experienced some maintenance labour during the 2016 season due to clogging of wastewater in certain areas of the subsurface filter bed, which lead to occasional excavations for material replacement and therefore temporary flow interruption. None of these events occurred during our sampling program.

5.4.1.3. Toxicity of wastewater.

Toxicity was represented by the assessment of *Vibrio fischeri* bioluminescence in test samples relative to controls (Fig. 5.4). At the Dunnottar wastewater treatment facility, an average of < 90% bioluminescence was detected after wastewater treatment in 2013 (Anderson et al., 2015). In 2015, the mean response at the primary lagoon was 71%, with similar response patterns along the wastewater path, except for the input site with 30%. Response of samples from the creek site averaged 73% (Table D8). In 2016, the mean response at the primary lagoon was 49% and the filter input site was 40% while the creek site averaged 100% (Table D9). These results suggest that, during both sampling seasons, the facility was able to attenuate toxicity and provide effective water treatment, which is consistent with the attenuation observed for other studied parameters. Inhibition has been previously reported in the Microtox® assay at levels between 15% and 100% in Greek raw wastewaters (Katsoyiannis and Samara, 2007). Therefore, the attenuation of toxicity

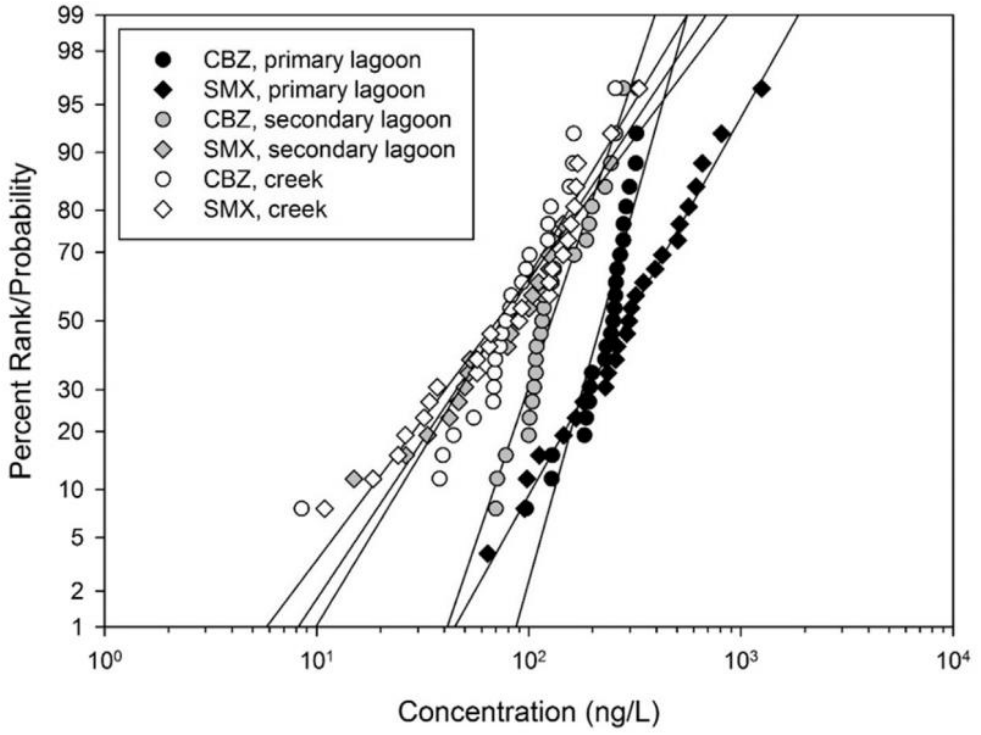
observed at the Dunnottar facility is reasonable and coherent with what could be expected for such a system.

5.4.1.4. Risk assessment for detected pharmaceuticals.

Exposure distributions at the primary lagoon, secondary lagoon and creek for carbamazepine and sulfamethoxazole are shown in Fig. 5.3. Plotted data represents grab-sample concentrations measured in 2013, 2015 and 2016. Cumulative data from these three seasons allowed observation of the greatest exposures for both analytes at the primary lagoon, with lower exposures at the secondary lagoon and creek sites. This was consistent for all studied seasons, regardless of the scale of the subsurface filter.

Table 5.4. Comparison of time-weighted average concentrations of pharmaceutical compounds in treated wastewaters of Canadian lagoon wastewater systems (NA: not analyzed, ND: non-detectable). Lac la Biche data from MacLeod and Wong (2010), Grand Marais data from Anderson et al. (2013), Cambridge Bay data from Chaves-Barquero et al. (2016), and Dunnottar data from this study. Populations are shown next to the name of each location for comparison.

Location	Atenolol (ng L^{-1})	Carbamazepine (ng L^{-1})	Sulfamethoxazole (ng L^{-1})
Lac la Biche, AB (8,402)	ND-100	50-300	NA
Grand Marais, MB (252)	ND	85-500	ND-21
Cambridge Bay, NU (1400)	ND-97	1-428	ND-274
Dunnottar, MB (763)	ND- 78	11-151	ND-316



PRIMARY LAGOON *CBZ: 5.76x-13.51, R^2=0.85, n=24, 90th centile = 370 ng/L; SMX: 2.87x-7.07, R^2=0.99, n=25, 90th centile = 813 ng/L*
SECONDARY LAGOON: *CBZ: 4.75x-10, R^2=0.95, n=24, 90th centile = 237 ng/L; SMX: 2.42x-4.54, R^2=0.95, n=24, 90th centile = 254 ng/L*
CREEK: *CBZ: 2.42x-4.53, R^2=0.95, n=24, 90th centile = 252 ng/L; SMX: 2.14x-3.96, R^2=0.95, n=24, 90th centile = 281 ng/L*

Figure 5.3. Exposure profiles for carbamazepine (CBZ) and sulfamethoxazole (SMX) at the primary lagoon, secondary lagoon and creek of the Dunnottar wastewater treatment system. Combined data were from grab-sample concentrations in 2013, 2015 and 2016. Regression equations, correlation coefficients, number of samples and 90th centiles are shown in the box below the figure.

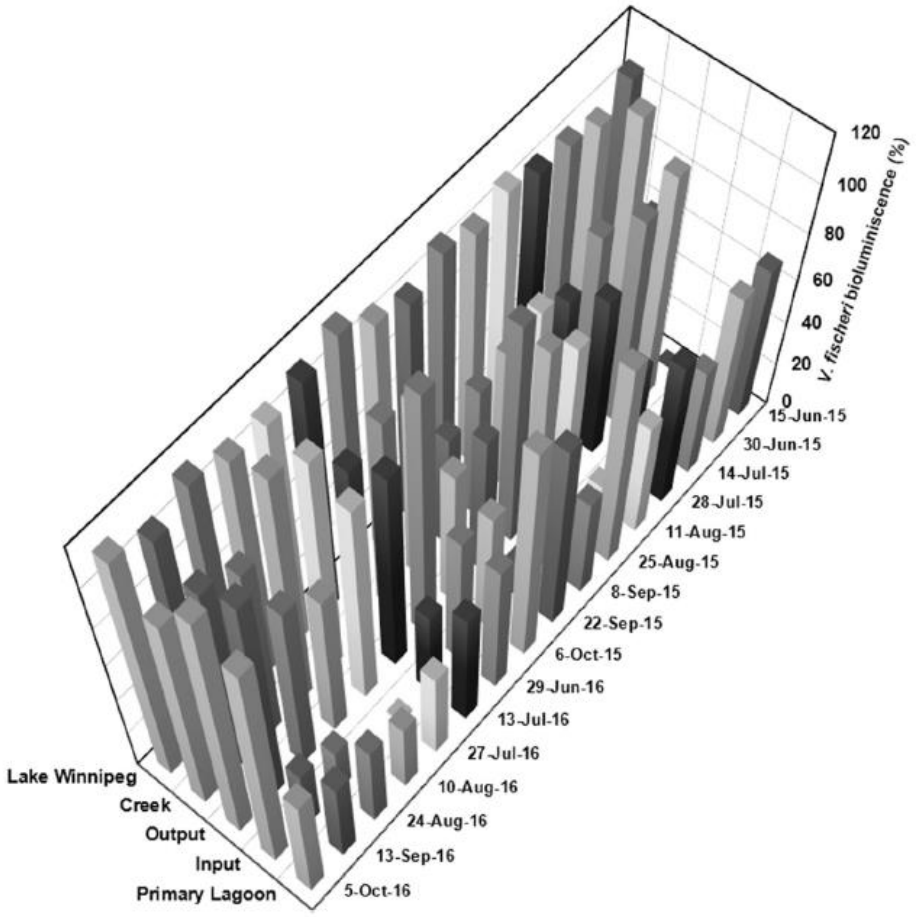


Figure 5.4. Vibrio fischeri (Microtox® assay) bioluminescence presented as mean percent of control after 15 minutes exposure to test water samples, including all triplicate samples collected at the Dunnottar wastewater treatment system in 2015 and 2016.

Previous studies in Manitoba (Anderson et al., 2013; Carlson et al., 2013) reported that hazard quotients (HQs) for all detected compounds in the outfall or downstream of the effluent discharge point would not pose a significant risk to fish, aquatic invertebrates or primary producers from acute exposures. This was also the case with HQs measured at the pilot-scale system in Dunnottar in 2013 (Anderson et al., 2015). Calculated HQs in this study were based on toxicity data reported in the literature for primary producers, invertebrates and fish (Table D5). Sampling sites evaluated were primary and secondary lagoons as well as the creek and highway sites. For most of the compounds that were

consistently detected on these locations, calculated HQs were less than 1 and ranged from 10^{-6} to 10^{-1}. For clarithromycin and sulfamethoxazole, worst-case scenario HQs at the primary lagoon, were of 9.0 (*Pseudokirchneriella subcapitata*) and 1.3 (*Selenastrum capricornutum*), while values of 0.15 and 0.2 were calculated for the highway site, respectively. Concentrations used for these calculations may not be representative of the average levels in time, especially for clarithromycin, where the value used comes from a unique peak event. Also, the use of a 1000-fold uncertainty factor introduces a high degree of conservatism on the calculations. These results suggest that concentrations of studied pharmaceuticals in this system's effluent would not be expected to pose a significant risk of acute toxicity to aquatic life in receiving waters.

5.5. Conclusions

The overall performance of the Dunnottar wastewater treatment facility allows for the discharge of effluent waters discharge into the receiving environment per current guidelines. Furthermore, wastewater overall toxicity attenuation is attained through lagoon and filter treatment. As previously seen in the pilot-scale system, most of the removal of pharmaceuticals took place between the primary and secondary lagoons, with negligible removals at the full-scale subsurface filter, despite the increased hydraulic retention time of this system (approx. 45 h) when compared to the previously studied pilot-scale project (approx. 6 h). From all detected pharmaceuticals, only carbamazepine and sulfamethoxazole persisted after lagoon and filter treatment, while atenolol, clarithromycin, metoprolol and propranolol were attenuated to non-detectable levels. No significant differences were found for pharmaceuticals attenuation between the first and second years of operation at the facility. No significant effect on atenolol, carbamazepine and sulfamethoxazole attenuation from the

subsurface filter could be thoroughly assessed for four detected pharmaceuticals due to an efficient pre-filter degradation process of four of them. A risk assessment for detected pharmaceuticals showed that detected concentrations do not pose a significant risk to aquatic organisms in the Dunnottar wastewater treatment facility nor at Teluga Creek.

While several attempts have been made in the past to characterize subsurface filtration technologies and their design parameters at laboratory and mesocosm scales, very few attempts have investigated real world facilities and their performance for wastewater polishing and this is still a knowledge gap to be addressed. This study allowed us to conduct an assessment on a full-scale functioning facility for municipal wastewater treatment and could serve as a reference for future studies in similar facilities in North America and around the globe. Further experimentation with design parameters of subsurface filters and constructed wetlands (e.g. substrate materials, flow direction, hydraulic retention time, recirculation) is worth conducting to evaluate the ability of these systems for nutrients and pharmaceuticals attenuation in pilot- and full-scale systems.

5.6. Acknowledgments

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5.7. References for Chapter 5

Anderson, J.C.; Carlson, J.C.; Low, J.E.; Challis, J.K.; Wong, C.S.; Knapp, C.W.; et al. Performance of a constructed wetland in Grand Marais, Manitoba, Canada: Removal of nutrients, pharmaceuticals, and antibiotic resistance genes from municipal wastewater. *Chemistry Central Journal* **2013**; *7*: 15.

Anderson, J.C.; Joudan, S.; Shoichet, E.; Cuscito, L.D.; Alipio, A.E.C. Donaldson, C.S.; Khan, S.; Goltz, D.M.; Rudy, M.D.; Frank, R.A.; Knapp, C.W.; Hanson, M.L.; Wong, C.S. Reducing nutrients, organic micropollutants, antibiotic resistance, and toxicity in rural wastewater effluent with subsurface filtration treatment technology. *Ecological Engineering* **2015**; *84*: 375-385.

American Public Health Association (APHA). Standard Methods for the Examination of Water and Wastewater: American Public Health Association, **2005**.

Bartelt-Hunt, S.L.; Snow, D.D.; Damon-Powell, T.; Brown, D.L.; Prasai, G.; Schwarz, M.; Kolok, A.S. Quantitative evaluation of laboratory uptake rates for pesticides, pharmaceuticals and steroid hormones using POCIS. *Environmental Toxicology and Chemistry* **2011**; *30*: 1412-1420.

Carlson, J.C.; Anderson, J.C.; Low, J.E.; Cardinal, P.; MacKenzie, S.D.; Beattie , S.A.; Challis, J.K.; Bennett, R.J.; Meronek, S.S.; Wilks, R.P.A.; Buhay, W.M.; Wong, C.S.; Hanson, M.L. Presence and hazards of nutrients and emerging organic micropollutants from sewage lagoon discharges into Dead Horse Creek, Manitoba, Canada. *Science of the Total Environment* **2013**; *445*: 64-78.

Canadian Council of Ministers of the Environment (CCME). Canada-wide strategy for the management of municipal wastewater effluent. 2014 progress report., **2014.**

Challis, J.K.; Hanson, M.L.; Wong, C.S. Development and Calibration of an Organic-Diffusive Gradients in Thin Films Aquatic Passive Sampler for a Diverse Suite of Polar Organic Contaminants. *Analytical Chemistry* **2016**; *88*: 10583-10591.

Chaves-Barquero, L.G.; Luong, K.H.; Mundy, C.J.; Knapp, C.W.; Hanson, M.L.; Wong, C.S. The release of wastewater contaminants in the Arctic: A case study from Cambridge Bay, Nunavut, Canada. *Environmental Pollution* **2016**; *218*: 542-550.

Conkle, J.L.; White, J.R.; Metcalfe, C.D. Reduction of pharmaceutically active compounds by a lagoon wetland wastewater treatment system in Southeast Louisiana. *Chemosphere* **2008**; *73*: 1741-1748.

Fatta-Kassinos, D.; Meric, S.; Nikolaou, A. Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research. *Analytical and Bioanalytical Chemistry* **2011**; *399*: 251-275.

Fedorova G.; Grabic, R.; Grabicova, K.; Turek, J.; Nguyen, T.V.; Randak, T.; Brooks, B.W. Water reuse for aquaculture: comparative removal efficacy and aquatic hazard reduction of pharmaceuticals by a pond treatment system during a one-year study. *Journal of Hazardous Materials* **2022;** *421*: 1266712.

Harman, C.; Allan, I.J.; Vermeirssen, E.L.M. Calibration and use of the polar organic chemical integrative sampler-a critical review. *Environmental Toxicology and Chemistry* **2012**; *31*: 2724-2738.

Hughes, S.R.; Kay, P.; Brown, L.E. Global Synthesis and Critical Evaluation of Pharmaceutical Data Sets Collected from River Systems. *Environmental Science & Technology* **2013**; *47*: 661-677.

Katsoyiannis A.; Samara C. Ecotoxicological evaluation of the wastewater treatment process of the sewage treatment plant of Thessaloniki, Greece. *Journal of Hazardous Materials* **2007**; *141*: 614-621.

Kolpin, D.W.; Furlong E.T.; Meyer, M.T.; Thurman, E.M.; Zaugg, S.D.; Barber, L.B.; Buxton, H.T. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A national reconnaissance. *Environmental Science & Technology* **2002**; *36*: 1202-1211.

Li, Y.F.; Zhu, G.B.; Ng, W.J.; Tan, S.K. A review on removing pharmaceutical contaminants from wastewater by constructed wetlands: Design, performance and mechanism. *Science of the Total Environment* **2014**; 468: 908-932.

Lishman, L.; Smyth, S.A.; Sarafin, K.; Kleywegt, S.; Toito, J.; Peart, T.; Lee, B.; Servos, M.; Beland, M.; Seto, P. Occurrence and reductions of pharmaceuticals and personal care products and estrogens by municipal wastewater treatment plants in Ontario, Canada. *Science of the Total Environment* **2006**; *367*: 544-558.

Macleod, S.L.; McClure, E.L.; Wong, C.S. Laboratory calibration and field deployment of the polar organic chemical integrative sampler for pharmaceuticals and personal care products in wastewater and surface water. *Environmental Toxicology and Chemistry* **2007**; *26*: 2517-2529.

MacLeod, S.L.; Wong, C.S. Loadings, trends, comparisons, and fate of achiral and chiral pharmaceuticals in wastewaters from urban tertiary and rural aerated lagoon treatments. *Water Research* **2010**; *44*: 533-544.

Manitoba Water Stewardship. Manitoba Water Quality Standards, Objectives and Guidelines **2011**. URL: https://www.gov.mb.ca/waterstewardship/

water_quality/quality/pdf/mb_water_quality_standard_final.pdf (Accessed on January 2016)

Ryan, C.C.; Tan, D.T.; Arnold, W.A. Direct and indirect photolysis of sulfamethoxazole and trimethoprim in wastewater treatment plant effluent. *Water Research* **2011**; 45: 1280-1286.

Smith, V.H. Eutrophication of freshwater and coastal marine ecosystems - A global problem. Environmental Science and Pollution Research **2003**; *10*: 126-139.

U.S. EPA. Wastewater Technology Fact Sheet: Facultative Lagoons, **2002**. URL: https://www3.epa.gov/npdes/pubs/faclagon.pdf (Accessed January 2018)

Zhang, D.Q.; Gersberg, R.M.; Hua, T.; Zhu, J.F.; Nguyen, A.T.; Law, W.K.; Ng, W.J.; Tan, S.K. Effect of Feeding Strategies on Pharmaceutical Removal by Subsurface Flow Constructed Wetlands. Journal of Environmental Quality **2012**; *41*: 1674-1680.

5.8. Connecting text between Chapters 5 and 6

This dissertation has presented a critical review and three case studies pertaining to the use of constructed wetlands for pharmaceutical removal in rural and remote locations under various types of climate. Next, Chapter 6 will present an integrative synthesis of the work, along with the main limitations of the dissertation and recommendations for future research.

CHAPTER 6

6. CONCLUSIONS AND SYNTHESIS

6.1. Summary and Novelty of Research Findings

The use of constructed wetlands (CWs) in rural and remote areas is crucial to providing low-cost effective wastewater treatment for these communities and protecting receiving waters. A diversity of constructed wetlands approaches can and have been applied for treatment of wastewater in order to comply with current treatment and release regulations in many jurisdictions. The selection of type and design will be informed by locale climate and ecological characteristics, as well as regulatory needs.

Recently, research efforts have been geared towards measuring and understanding how these systems can remove organic contaminants, such as pharmaceuticals and personal care products. For current constructed wetlands operating around the globe, an understanding of the effect of design parameters and wetland components on pharmaceutical removal efficiency is lacking, leading to significant challenges for environmental scientists and policy-making individuals. The research in this dissertation aims to contribute to the understanding of how constructed wetlands can be tools to remove these contaminants from wastewater. The focus was on systems in and for remote and rural communities, which often times are not able to finance the installation of conventional wastewater treatment systems, and must rely on cheaper, simpler designs instead. Special interest was placed into the study of wastewater removal practices under various climates

and CW designs, both through the critical evaluation of the available literature and the three experimental case studies.

One of the main contributions of this dissertation is the unique use of weight-of-evidence concepts to evaluate the relevance and strength of methods of the body of literature studying the efficiency of CWs to remove pharmaceuticals under various types of climates. The presented customized rubric could be taken as an example and be further modified to critically evaluate the removal of other contaminants in CWs and other alternative wastewater treatment systems. Overall, our assessment showed better removal efficiency for some pharmaceuticals treated on hybrid CWs compared to single-stage CWs. Significant differences were also found between pharmaceutical removal in polar, temperate and tropical climates. Additionally, statistically significant differences were obtained for some pharmaceuticals when comparing their scores in warm versus cold seasons. That having been said, it is important to stress the lack of Arctic-based data, which confers important uncertainties to our assessment. These results suggest a need for more research on pharmaceutical removal in CWs that operate at cold locations, to increase the amount of available data and to continue evaluating the specific influence of CW design parameters under these conditions.

This dissertation presents one of the first studies to ever report the occurrence of pharmaceuticals, antibiotic resistance genes, and nutrients in wastewater treatment facilities in the Canadian Arctic, as well as the removal performance of these contaminants under polar conditions. The data suggest that some attenuation mechanisms (e.g., microbial degradation, photolysis, sorption) for labile pharmaceuticals do exist in the treatment system, while more recalcitrant pharmaceuticals (e.g., carbamazepine) are not removed and do persist in the water phase over the winter and into the next summer season Overall, we

concluded that Arctic wetland-based wastewater treatment systems do have some potential to attenuate some organic contaminants and deserve continued study to help characterize and optimize their performance.

Another contribution from this thesis is a unique evaluation of crushed recycled glass (CRG) as a substrate for emerging organic contaminant removal in temperate CW-based applications. The ability to employ crushed recycled glass in this manner is a novel use for this material, currently underexploited in Canada and elsewhere. Overall, CRG showed similar behaviour compared to the standard gravel and sand in terms of dissipation and half-lives for studied pharmaceuticals in the mesocosm-scale systems, and overall removal in the pilot-scale system. The observed performance of CRG suggested its potential to be used by itself or mixed with other traditional materials in substrates for CW application in rural and remote areas, especially at post-lagoon treatment systems such as the subsurface pilot-scale system studied on this work. This is an interesting finding that could be further explored in future research.

This thesis also presents an assessment of a full-scale treatment facility for municipal wastewater operating in a temperate climate. While several attempts had been made previously to characterize vertical subsurface filtration technologies and their design parameters at laboratory and mesocosm scales, few attempts had investigated real world facilities and their performance for wastewater polishing at the time of the study. This work allowed us to confirm that the removal of pharmaceuticals took place mostly at the lagoons, likely due to microbial and photodegradation as main drivers, with negligible removals at the full-scale subsurface filter. Of all detected pharmaceuticals, only carbamazepine and sulfamethoxazole persisted after lagoon and filter treatment, while atenolol, clarithromycin,

metoprolol and propranolol were attenuated to non-detectable levels, which is in agreement with the other studies presented in this dissertation.

Taken together, the thesis has shown that the removal of some pharmaceuticals from wastewater is achievable through the use of CWs from various scales, types and under diverse climates. The presented case studies showed pharmaceutical removal for drug classes in both polar and temperate climates (β-blockers like atenolol and metoprolol), while environmental persistence was consistently found for other drugs in the various studied systems across scales and climates (e.g., sulfamethoxazole, carbamazepine). It was surprising to note the potential that CW have for pharmaceutical removal from wastewater even in cold climates, based on our weight-of-evidence assessment. Nevertheless, there are important knowledge gaps pertaining the importance of the various CW components for pharmaceutical removal across all types of climate. This continues to reaffirm the importance of compartmentalizing the study of specific interactions between the contaminants and the various components of the CW, aiming to attain removal through the many biotic and abiotic processes therein. We recommend that future research addresses the potential of CWs for removal of pharmaceuticals and other organic contaminants under arctic climates, as the currently available evidence is limited in strength of methods. The use of hybrid CWs of various configurations constitutes an exciting opportunity to better understand and improve the quality of current wastewater treatment practices in rural and remote communities around the globe.

6.2. Challenges and Future Directions

There were a number of challenges encountered during this dissertation. From these we can make reasonable recommendations for future work to further and improve our

understanding of the uncertainties inherent with the use of CWs for pharmaceutical removal from wastewater around the world. Many of the limitations of this work relate to a lack of extensive characterization regarding the specific performance aspects of studied CWs. Much of this could be attributed to sampling frequency and the difficulties to isolate and quantify the influence of each design parameter in the performance of the studied systems. Ultimately, many of the knowledge gaps relate to a lack of fundamental understanding of analyte interactions with the various components of a specific CW on a given time. The combined influence of photodegradation, microbial degradation, sorption/adsorption, and plant uptake are very difficult to characterize simultaneously in CW systems, especially when many analytes coexist. This aspect alone represents one of the most important difficulties to overcome, especially when studying real-world wastewater treatment facilities.

Lack of removal of recalcitrant pharmaceuticals (e.g., carbamazepine) across the various studies in this dissertation represents a different type of challenge, and indicates a need for research efforts that aim to optimize the operational conditions for greater removal rates and better understanding removal mechanisms for each analyte in the laboratory as well as in the field. For instance, the use of extended hydraulic retention times could be evaluated through recirculation studies; the influence of flow rate and type could be evaluated through the use of multiple combinations of single-step CWs in series to evaluate the effectiveness of the various configurations; the effect of intermittent and/or continuous aeration strategies could be studied by setting up aerators in CWs to promote aerobic biodegradation of contaminants. Ideally, these initiatives should focus on mesocosm-, pilot- or full-scale systems at environmentally relevant concentrations of pharmaceuticals. In the end, improved knowledge on how CWs can be applied and how can they be more effective

to achieve increased polishing of wastewater, especially in regions where industrial wastewater treatment is not possible, will benefit these communities and will reduce their impacts on the surrounding environments. Future studies should also conduct monitoring of pharmaceutical transformation products, as these compounds can be present in wastewater systems at non-negligible levels.

A recent study was conducted in our lab, for which I was involved in both the experimental design and sampling process (Stroski et al., 2020). This study built upon the work presented on Chapter 3 of this dissertation, by determining the pharmaceutical concentrations in wastewater treatment facilities in four arctic communities (Iqaluit, Baker Lake, Cambridge Bay, and Kugluktuk in Nunavut, Canada) before and after treatment. Results from the study suggest that some pharmaceutical attenuation was in place at the four treatment systems, which added to natural dilution during discharge led to negligible concentrations of pharmaceuticals post-discharge, and thus no ecological risk to the surrounding aquatic environments. Further studies are required, though, to conduct more in-depth testing of wastewater treatment efficiencies in these cold environments.Furthermore, our lab has also studied novel approaches to wastewater treatment in cold regions, through the use of submerged attached growth reactor (SAGR) technology (Anderson et al., 2020) for wastewater treatment, which is an insulated subsurface filtration system. Such efforts deserve continued exploring and evaluation in future endeavours.

The overall topic addressed by this dissertation relates to the occurrence and fate of organic contaminants in aquatic systems. Clearly, the presence of pharmaceuticals in the environment represents an ongoing problem that will be worsened by an increasing population in most corners of the world. Point-source pollution is a ubiquitous and ongoing problem around population centers. To continue investigating the performance of

wastewater treatment practices regarding emerging contaminants of concern, it is crucial to better understand which compounds are persisting post-treatment, hence contributing to the improvement of exposure and risk assessments, and water protection overall.

7. APPENDICES

- Appendix A – Additional information for Chapter 2
- Appendix B – Additional information for Chapter 3
- Appendix C – Additional information for Chapter 4
- Appendix D – Additional information for Chapter 5

7.1. APPENDIX A: ADDITIONAL INFORMATION FOR CHAPTER 2

A CRITICAL REVIEW OF THE EVIDENCE FOR REMOVAL OF PHARMACEUTICALS IN CONSTRUCTED WETLANDS TREATING MUNICIPAL WASTEWATERS

SUMMARY

The following includes geographical details on the included research for the critical review, an example of the rubric application is presented, as well as details on statistical tests. Finally, an extended list of scores obtained for the various research items, is presented, organized by pharmaceutical.

Figure A1. Geographical distribution of the selected studies reporting on pharmaceutical removal in CWs for the present weight-of-evidence assessment.

TABLE A1. Example of scoring for SOM and ROO of a pharmaceutical in a research item: acetaminophen on Verlicchi et al., 2013.

Authors:	Verlicchi et al.
Year:	2013
Title:	Removal of selected pharmaceuticals from domestic wastewater in an activated sludge system followed by a horizontal subsurface flow bed – Analysis of their respective contributions.
Bibliographic reference:	Science of the Total Environment 454-455 (2013) 411-425
DOI:	https://doi.org/10.1016/j.scitotenv.2013.03.044
Latitude:	44.6628
Longitude:	7.29615
Study objective:	**Provide useful information about the ability of wastewater treatment systems to mitigate pharmaceutical discharge into the environment.**

Relevance of Observation (ROO)	**Score**	**Normalized Score**	**Comments**
Magnitude of removal	3	**0.75**	Removal efficiency for acetaminophen was reported at 45%.
Statistical significance of removal	2	**1**	Removal of acetaminophen was statistically significant.
Consistency of removal	1	**1**	No inconsistencies were found in acetaminophen removal during the study.
Residence time for removal	1	**0.5**	HRT > 24 h
CW scale	3	**0.75**	Pilot-scale study
ROO SCORE (out of 5)		**4.0**	
Strength of Methods (SOM)	**Score**	**Normalized Score**	
Group A – Analyte characterization and fate			
Description of the origin and purity of reagent and standards	1	**1**	Information is presented under Section 2.3. on the manuscript.
Environmental relevance of concentrations	1	**1**	Background levels were monitored in natural wastewaters.
Hydraulic loading	1	**1**	Reported at 0.28 m/d.
Mass balance	0	**0**	The study did not present a mass balance.
Biotic/Abiotic CW processes	0	**0**	No specific study of biotic/abiotic processes was presented, just overall removal.
Group B – Matrix and environmental conditions			
Environmental parameters characterization	4	**1**	pH, temperature, light-flux, weather conditions were monitored.
Nature of the wastewater matrix	4	**1**	Natural with background levels of pharmaceuticals.
Group C – Experimental design and QA/QC			
Sampling frequency	4	**1**	Weekly sampling program.
Data transparency	1	**0.5**	Summary and aggregate of data was presented.
Instrumental analytical conditions, including LOQ and LOD	2	**1**	The information is presented under Section 2.3.
SOM SCORE (out of 10)		**7.5**	

TABLE A2. Kruskal-Wallis statistical tests for comparison of SOM and ROO scores between hybrid CWs and single-step CWs, organized by decreasing pharmaceutical detection. H: Kruskal-Wallis statistic term; χ2: chi-square statistic term. Null hypothesis of the test is rejected for H > χ2 (p < 0.05). NA: not-applicable.

Pharmaceutical-SCORE	H	χ2	p-value	Conclusion on null hypothesis
Ibuprofen-ROO	6.40	3.84	0.011	Reject
Ibuprofen-SOM	2.10	3.84	0.147	Fail to reject
Diclofenac-ROO	7.42	3.84	0.006	Reject
Diclofenac-SOM	0.007	3.84	0.933	Fail to reject
Carbamazepine-ROO	0.09	3.84	0.765	Fail to reject
Carbamazepine-SOM	0.62	3.84	0.432	Fail to reject
Naproxen-ROO	0.27	3.84	0.604	Fail to reject
Naproxen-SOM	0.17	3.84	0.676	Fail to reject
Caffeine-ROO	1.24	3.84	0.266	Fail to reject
Caffeine-SOM	2.39	3.84	0.122	Fail to reject
Ketoprofen-ROO	1.13	3.84	0.288	Fail to reject
Ketoprofen-SOM	1.93	3.84	0.164	Fail to reject
Sulfamethoxazole-ROO	5.18	3.84	0.022	Reject
Sulfamethoxazole-SOM	0.79	3.84	0.375	Fail to reject
Metoprolol-ROO	1.93	3.84	0.165	Fail to reject
Metoprolol-SOM	2.75	3.84	0.098	Fail to reject
Atenolol-ROO	0.66	3.84	0.416	Fail to reject
Atenolol-SOM	3.65	3.84	0.056	Fail to reject
Salicylic acid-ROO	0.70	3.84	0.404	Fail to reject
Salicylic acid-SOM	3.25	3.84	0.071	Reject
Erythromycin-ROO	NA	NA	NA	NA
Erythromycin-SOM	NA	NA	NA	NA
Acetaminophen-ROO	7.35	3.84	0.0067	Reject
Acetaminophen-SOM	0.94	3.84	0.333	Fail to reject
Trimethoprim-ROO	0.86	3.84	0.353	Fail to reject
Trimethoprim-SOM	4.97	3.84	0.026	Reject
Ciprofloxacin-ROO	9.69	3.84	0.0002	Reject
Ciprofloxacin-SOM	7.93	3.84	0.0049	Reject
Norfloxacin-ROO	NA	NA	NA	NA
Norfloxacin-SOM	NA	NA	NA	NA
Ofloxacin-ROO	NA	NA	NA	NA
Ofloxacin-SOM	NA	NA	NA	NA
Tetracycline-ROO	NA	NA	NA	NA
Tetracycline-SOM	NA	NA	NA	NA
Tramadol-ROO	NA	NA	NA	NA
Tramadol-SOM	NA	NA	NA	NA
Roxithromycin-ROO	NA	NA	NA	NA
Roxithromycin-SOM	NA	NA	NA	NA
Clarithromycin-ROO	4.75	3.84	0.029	Reject
Clarithromycin-SOM	10.12	3.84	0.0015	Reject
Lomefloxacin-ROO	NA	NA	NA	NA
Lomefloxacin-SOM	NA	NA	NA	NA
Clofibric acid-ROO	NA	NA	NA	NA
Clofibric acid-SOM	NA	NA	NA	NA
Furosemide-ROO	NA	NA	NA	NA
Furosemide-SOM	NA	NA	NA	NA
Sulfapyridine-ROO	0.05	3.841	0.830	Fail to reject
Sulfapyridine-SOM	9.00	3.841	0.003	Reject
Azithromycin-ROO	0.52	3.84	0.470	Fail to reject
Azithromycin-SOM	5.33	3.84	0.021	Reject
Lincomycin-ROO	1.09	3.84	0.297	Fail to reject
Lincomycin-SOM	1.80	3.84	0.180	Fail to reject
Sulfadiazine-ROO	0.00	3.84	1.000	Fail to reject
Sulfadiazine-SOM	1.64	3.84	0.201	Fail to reject
Propranolol-ROO	4.00	3.84	0.045	Reject
Propranolol-SOM	1.78	3.84	0.182	Fail to reject
Venlafaxine-ROO	4.20	3.84	0.040	Reject
Venlafaxine-SOM	4.20	3.84	0.040	Reject
Codeine-ROO	NA	NA	NA	NA
Codeine-SOM	NA	NA	NA	NA
Bisoprolol-ROO	NA	NA	NA	NA

Pharmaceutical-SCORE	H	$\chi 2$	p-value	Conclusion on null hypothesis
Bisoprolol-SOM	NA	NA	NA	NA
Fluconazole-ROO	NA	NA	NA	NA
Fluconazole-SOM	NA	NA	NA	NA
Gemfibrozil-ROO	NA	NA	NA	NA
Gemfibrozil-SOM	NA	NA	NA	NA
Hydrochlorothiazide-ROO	NA	NA	NA	NA
Hydrochlorothiazide-SOM	NA	NA	NA	NA
Oxazepam-ROO	NA	NA	NA	NA
Oxazepam-SOM	NA	NA	NA	NA
Clindamycin-ROO	NA	NA	NA	NA
Clindamycin-SOM	NA	NA	NA	NA
Telmisartan-ROO	NA	NA	NA	NA
Telmisartan-SOM	NA	NA	NA	NA
Atorvastatin-ROO	NA	NA	NA	NA
Atorvastatin-SOM	NA	NA	NA	NA
Diazepam-ROO	NA	NA	NA	NA
Diazepam-SOM	NA	NA	NA	NA
Doxycycline-ROO	NA	NA	NA	NA
Doxycycline-SOM	NA	NA	NA	NA
Ranitidine-ROO	NA	NA	NA	NA
Ranitidine-SOM	NA	NA	NA	NA
Tylosin-ROO	NA	NA	NA	NA
Tylosin-SOM	NA	NA	NA	NA
Acyclovir-ROO	NA	NA	NA	NA
Acyclovir-SOM	NA	NA	NA	NA
Alfuzosine-ROO	NA	NA	NA	NA
Alfuzosine-SOM	NA	NA	NA	NA
Alprazolam-ROO	NA	NA	NA	NA
Alprazolam-SOM	NA	NA	NA	NA

TABLE A3. Kruskal-Wallis statistical tests for comparison of SOM and ROO scores between warm and cold seasons, organized by decreasing pharmaceutical detection. H: Kruskal-Wallis statistic term; χ2: chi-square statistic term. Null hypothesis of the test is rejected for H > χ2 (p < 0.05). NA: not-applicable.

Pharmaceutical-SCORE	H	χ2	p-value	Conclusion on null hypothesis
Ibuprofen-ROO	0.74	3.841	0.389	Fail to reject
Ibuprofen-SOM	0.69	3.841	0.690	Fail to reject
Diclofenac-ROO	1.74	3.841	0.187	Fail to reject
Diclofenac-SOM	0.04	3.841	0.835	Fail to reject
Carbamazepine-ROO	0.27	3.841	0.606	Fail to reject
Carbamazepine-SOM	0.002	3.841	0.962	Fail to reject
Naproxen-ROO	0.10	3.841	0.754	Fail to reject
Naproxen-SOM	0.53	3.841	0.466	Fail to reject
Caffeine-ROO	4.59	3.841	0.032	Reject
Caffeine-SOM	0.05	3.841	0.821	Fail to reject
Ketoprofen-ROO	0.24	3.841	0.622	Fail to reject
Ketoprofen-SOM	2.46	3.841	0.117	Fail to reject
Sulfamethoxazole-ROO	2.77	3.841	0.096	Fail to reject
Sulfamethoxazole-SOM	1.77	3.841	0.183	Fail to reject
Metoprolol-ROO	1.33	3.841	0.249	Fail to reject
Metoprolol-SOM	0.10	3.841	0.752	Fail to reject
Atenolol-ROO	0.57	3.841	0.451	Fail to reject
Atenolol-SOM	1.28	3.841	0.2585	Fail to reject
Salicylic acid-ROO	10.63	3.841	0.001	Reject
Salicylic acid-SOM	3.31	3.841	0.069	Fail to reject
Erythromycin-ROO	1.31	3.841	0.253	Fail to reject
Erythromycin-SOM	1.71	3.841	0.191	Fail to reject
Acetaminophen-ROO	0.72	3.841	0.396	Fail to reject
Acetaminophen-SOM	0.66	3.841	0.417	Fail to reject
Trimethoprim-ROO	4.51	3.841	0.034	Reject
Trimethoprim-SOM	3.76	3.841	0.052	Fail to reject
Ciprofloxacin-ROO	2.43	3.841	0.119	Fail to reject
Ciprofloxacin-SOM	0.04	3.841	0.848	Fail to reject
Norfloxacin-ROO	2.09	3.841	0.149	Fail to reject
Norfloxacin-SOM	0.000	3.841	1.000	Fail to reject
Ofloxacin-ROO	0.05	3.841	0.820	Fail to reject
Ofloxacin-SOM	0.47	3.841	0.494	Fail to reject
Tetracycline-ROO	9.13	3.841	0.002	Reject
Tetracycline-SOM	0.14	3.841	0.713	Fail to reject
Tramadol-ROO	0.005	3.841	0.832	Fail to reject
Tramadol-SOM	0.003	3.841	0.958	Fail to reject
Roxithromycin-ROO	3.36	3.841	0.067	Fail to reject
Roxithromycin-SOM	0.14	3.841	0.713	Fail to reject
Clarithromycin-ROO	0.08	3.841	0.777	Fail to reject
Clarithromycin-SOM	0.43	3.841	0.514	Fail to reject
Lomefloxacin-ROO	NA	NA	NA	NA
Lomefloxacin-SOM	NA	NA	NA	NA
Clofibric acid-ROO	0.09	3.841	0.767	Fail to reject
Clofibric acid-SOM	0.79	3.841	0.374	Fail to reject
Furosemide-ROO	0.52	3.841	0.470	Fail to reject
Furosemide-SOM	1.17	3.841	0.279	Fail to reject
Sulfapyridine-ROO	0.02	3.841	0.877	Fail to reject
Sulfapyridine-SOM	0.000	3.841	1.000	Fail to reject
Azithromycin-ROO	2.14	3.841	0.143	Fail to reject
Azithromycin-SOM	1.78	3.841	0.182	Fail to reject
Lincomycin-ROO	3.37	3.841	0.069	Fail to reject
Lincomycin-SOM	1.78	3.841	0.182	Fail to reject
Sulfadiazine-ROO	1.09	3.841	0.296	Fail to reject
Sulfadiazine-SOM	2.45	3.841	0.117	Fail to reject
Propranolol-ROO	NA	NA	NA	NA
Propranolol-SOM	NA	NA	NA	NA
Venlafaxine-ROO	NA	NA	NA	NA
Venlafaxine-SOM	NA	NA	NA	NA
Codeine-ROO	NA	NA	NA	NA
Codeine-SOM	NA	NA	NA	NA
Bisoprolol-ROO	NA	NA	NA	NA

Pharmaceutical-SCORE	H	$\chi 2$	p-value	Conclusion on null hypothesis
Bisoprolol-SOM	NA	NA	NA	NA
Fluconazole-ROO	NA	NA	NA	NA
Fluconazole-SOM	NA	NA	NA	NA
Gemfibrozil-ROO	NA	NA	NA	NA
Gemfibrozil-SOM	NA	NA	NA	NA
Hydrochlorothiazide-ROO	NA	NA	NA	NA
Hydrochlorothiazide-SOM	NA	NA	NA	NA
Oxazepam-ROO	NA	NA	NA	NA
Oxazepam-SOM	NA	NA	NA	NA
Clindamycin-ROO	NA	NA	NA	NA
Clindamycin-SOM	NA	NA	NA	NA
Telmisartan-ROO	NA	NA	NA	NA
Telmisartan-SOM	NA	NA	NA	NA
Atorvastatin-ROO	NA	NA	NA	NA
Atorvastatin-SOM	NA	NA	NA	NA
Diazepam-ROO	NA	NA	NA	NA
Diazepam-SOM	NA	NA	NA	NA
Doxycycline-ROO	NA	NA	NA	NA
Doxycycline-SOM	NA	NA	NA	NA
Ranitidine-ROO	NA	NA	NA	NA
Ranitidine-SOM	NA	NA	NA	NA
Tylosin-ROO	NA	NA	NA	NA
Tylosin-SOM	NA	NA	NA	NA
Acyclovir-ROO	NA	NA	NA	NA
Acyclovir-SOM	NA	NA	NA	NA
Alfuzosine-ROO	NA	NA	NA	NA
Alfuzosine-SOM	NA	NA	NA	NA
Alprazolam-ROO	NA	NA	NA	NA
Alprazolam-SOM	NA	NA	NA	NA

TABLE A4. Kruskal-Wallis statistical tests for comparison of SOM and ROO scores between cold, tropical and temperate climates. H: Kruskal-Wallis statistic term; χ2: chi-square statistic term. Differences are considered significant for H > χ2 (p < 0.05). NA: not-applicable. The presented pharmaceuticals are the ones that were quantified at least in two types of climates.

Pharmaceutical-SCORE	H	χ2	p-value	Conclusion on null hypothesis
Mean-ROO*	3.34	6.00	0.19	Fail to reject
Mean-SOM*	44	6.00	<0.05	Reject
Carbamazepine-ROO*	1.87	6.00	0.39	Fail to reject
Carbamazepine-SOM*	6.59	6.00	0.04	Reject
Atenolol-ROO**	0.66	3.84	0.42	Fail to reject
Atenolol-SOM**	3.53	3.84	0.06	Fail to reject
Caffeine-ROO***	2.44	3.84	0.12	Fail to reject
Caffeine-SOM***	1.24	3.84	0.27	Fail to reject
Diclofenac-ROO***	0.10	3.84	0.76	Fail to reject
Diclofenac-SOM***	0.79	3.84	0.37	Fail to reject
Metoprolol-ROO**	0.19	3.84	0.66	Fail to reject
Metoprolol-SOM**	1.27	3.84	0.26	Fail to reject
Trimethoprim-ROO**	2.57	3.84	0.11	Fail to reject
Trimethoprim-SOM**	2.25	3.84	0.13	Fail to reject
Acetaminophen-ROO***	0.89	3.84	0.33	Fail to reject
Acetaminophen-SOM***	0.54	3.84	0.46	Fail to reject
Ibuprofen-ROO***	1.35	3.84	0.24	Fail to reject
Ibuprofen-SOM***	1.85	3.84	0.17	Fail to reject
Ketoprofen-ROO***	0.03	3.84	0.86	Fail to reject
Ketoprofen-SOM***	0.75	3.84	0.39	Fail to reject
Naproxen-ROO***	0.76	3.84	0.38	Fail to reject
Naproxen-SOM***	0.38	3.84	0.54	Fail to reject
Sulfamethoxazole-ROO***	0.05	3.84	0.82	Fail to reject
Sulfamethoxazole-SOM***	1.27	3.84	0.26	Fail to reject

*Compared between polar, temperate, and tropical climates.
**Compared between polar and temperate climates.
***Compared between tropical and temperate climates.

TABLE A5. Post-hoc Nemenyi tests applied to the data groups showing statistically significant differences per the Kruskal-Wallis tests detailed in tables A2, A3, and A4. Differences are considered significant for p < 0.05.

Pharmaceutical-Score	***p*-value**	**Conclusion on obtained score**
Hybrid (H) vs. single-step (SS) CWs		
Ibuprofen-ROO	0.01	H > SS
Diclofenac-ROO	0.007	H > SS
Sulfamethoxazole-ROO	0.02	H > SS
Salicylic acid-SOM	0.07	H > SS
Acetaminophen-ROO	0.007	H > SS
Trimethoprim-SOM	0.01	H < SS
Ciprofloxacin-ROO	0.002	H > SS
Ciprofloxacin-SOM	0.005	H < SS
Clarithromycin-ROO	0.03	H > SS
Clarithromycin-SOM	0.002	H < SS
Propranolol-ROO	0.04	H > SS
Venlafaxine-ROO	0.04	H > SS
Venlafaxine-SOM	0.04	H < SS
Seasonality comparisons: warm (W) vs. cold (C)		
Caffeine-ROO	0.03	C < W
Salicylic acid-ROO	0.001	C < W
Trimethoprim-ROO	0.03	C > W
Tetracycline-ROO	0.002	C < W
Climate comparisons: polar (P) vs. temperate (TEMP) vs. tropical (TRO)		
Mean-SOM* (P vs. TRO)	0.06	NA
Mean-SOM* (P vs. TEMP)	$7x10^{-8}$	P < TEMP
Mean-SOM* (TRO vs. TEMP)	0.001	TRO < TEMP
Carbamazepine-SOM* (P vs. TRO)	0.30	NA
Carbamazepine-SOM* (P vs. TEMP)	0.03	P < TEMP
Carbamazepine-SOM* (TRO vs. TEMP)	0.87	NA

*Compared between polar, temperate, and tropical climates.
***Compared between tropical and temperate climates.

TABLE A6. Complete list rubric-evaluated studies (74 total studies) and their respective ROO and SOM scores, organized by pharmaceutical, in alphabetical order. Means and standard errors of scores can be plotted for a given compound to produce scoring plots.

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Chen et al., 2016A (summer)	Acetaminophen	4.50	5.50	warm	HSSF-CW
Chen et al., 2016A (winter)	Acetaminophen	3.50	5.50	cold	HSSF-CW
Chen et al., 2016B (summer)	Acetaminophen	1.50	5.50	warm	HSSF-CW
Chen et al., 2016B (winter)	Acetaminophen	4.50	5.50	warm	HSSF-CW
Chen et al., 2016C (summer)	Acetaminophen	4.50	5.50	warm	HSSF-CW
Chen et al., 2016C (winter)	Acetaminophen	4.50	5.50	cold	HSSF-CW
Avila et al., 2013	Acetaminophen	4.50	6.50	warm	HSSF-CW
Avila et al., 2015	Acetaminophen	4.00	6.50	warm	H-CW
Li et al., 2017	Acetaminophen	3.25	6.50	warm	SF-CW
Matamoros et al., 2016 (summer)	Acetaminophen	3.75	5.50	warm	HSSF-CW
Matamoros et al., 2016 (winter)	Acetaminophen	3.75	5.50	cold	HSSF-CW
Vymazal et al., 2017A	Acetaminophen	4.00	4.25	warm	HSSF-CW
Vymazal et al., 2017B	Acetaminophen	4.00	4.25	warm	HSSF-CW
Vymazal et al., 2017C	Acetaminophen	4.00	4.25	warm	HSSF-CW
Vymazal et al., 2017D	Acetaminophen	4.00	4.25	warm	HSSF-CW
Nuel et al., 2018	Acetaminophen	0.00	7.00	warm	SF-CW
Verlicchi et al., 2013	Acetaminophen	4.00	7.50	warm	HSSF-CW
Ávila et al., 2014A	Acetaminophen	4.75	6.25	warm	H-CW
Ávila et al., 2014B	Acetaminophen	4.75	6.25	warm	H-CW
Ávila et al., 2014C	Acetaminophen	4.75	6.25	warm	H-CW
Conkle et al., 2008	Acetaminophen	4.50	4.50	warm	H-CW
Vystavna et al., 2017A	Acetaminophen	4.25	6.00	warm	H-CW
Vystavna et al., 2017B	Acetaminophen	4.50	6.00	warm	H-CW
Vo et al., 2019	Acetaminophen	4.00	3.25	warm	VSSF-CW
Phong et al., 2016	Acetaminophen	3.75	6.25	warm	VSSF-CW
Ávila et al., 2021A	Acetaminophen	4.25	6.50	warm	HSSF-CW
Ávila et al., 2021B	Acetaminophen	4.25	6.50	warm	HSSF-CW
MEAN(SE)		**3.9(0.2)**	**5.8(0.1)**		
Ruhmland et al., 2015A	Acyclovir	4.00	6.75	warm	HSSF-CW
Ruhmland et al., 2015B	Acyclovir	4.00	6.75	warm	HSSF-CW
Ruhmland et al., 2015C	Acyclovir	4.00	6.75	cold	HSSF-CW
MEAN(SE)		**4.0(0.0)**	**6.8(0.0)**		
Breitholtz et al., 2012A	Alfuzosine	3.75	7.00	warm	SF-CW
Breitholtz et al., 2012B	Alfuzosine	4.00	7.00	warm	SF-CW
Breitholtz et al., 2012C	Alfuzosine	4.00	7.00	warm	SF-CW
Breitholtz et al., 2012D	Alfuzosine	NA	NA	warm	SF-CW
MEAN(SE)		**3.9(0.1)**	**7.0(0.0)**		
Breitholtz et al., 2012A	Alprazolam	4.00	7.00	warm	SF-CW
Breitholtz et al., 2012B	Alprazolam	0.00	7.00	warm	SF-CW
Breitholtz et al., 2012C	Alprazolam	0.00	7.00	warm	SF-CW
Breitholtz et al., 2012D	Alprazolam	NA	NA	warm	SF-CW
MEAN(SE)		**1.3(1.1)**	**7.0(0.0)**		
Chen et al., 2016A (summer)	Atenolol	4.25	5.50	warm	HSSF-CW
Chen et al., 2016A (winter)	Atenolol	4.50	5.50	cold	HSSF-CW
Chen et al., 2016B (summer)	Atenolol	4.50	5.50	warm	HSSF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Chen et al., 2016B (winter)	Atenolol	4.50	5.50	warm	HSSF-CW
Ruhmland et al., 2015A	Atenolol	4.00	6.75	warm	HSSF-CW
Ruhmland et al., 2015B	Atenolol	4.00	6.75	warm	HSSF-CW
Ruhmland et al., 2015C	Atenolol	4.00	6.75	cold	HSSF-CW
Nuel et al., 2018	Atenolol	1.00	7.00	warm	SF-CW
Breitholtz et al., 2012A	Atenolol	3.00	7.00	warm	SF-CW
Breitholtz et al., 2012B	Atenolol	3.75	7.00	warm	SF-CW
Breitholtz et al., 2012C	Atenolol	3.75	7.00	warm	SF-CW
Breitholtz et al., 2012D	Atenolol	3.75	7.00	warm	SF-CW
Verlicchi et al., 2013	Atenolol	3.75	7.50	warm	HSSF-CW
Conkle et al., 2008	Atenolol	4.50	4.50	warm	H-CW
Park et al., 2018	Atenolol	4.50	4.75	warm	SF-CW
Auvinen et al., 2017a	Atenolol	3.75	5.00	warm	HSSF-CW
Mathon et al., 2019	Atenolol	3.25	5.00	warm	SF-CW
Park et al., 2009	Atenolol	5.00	3.75	warm	SF-CW
Auvinen et al., 2017b (full-scale)	Atenolol	4.50	5.50	warm	H-CW
Auvinen et al., 2017b (lab-scale)	Atenolol	4.50	5.50	warm	SF-CW
Francini et al., 2018A (summer)	Atenolol	4.50	6.25	warm	SF-CW
Francini et al., 2018B (summer)	Atenolol	4.50	6.25	warm	SF-CW
Anderson et al., 2020A	Atenolol	NA	NA	warm	H-CW
Anderson et al., 2020B	Atenolol	4.25	4.25	warm	H-CW
Dordio et al., 2009bA	Atenolol	4.75	4.75	warm	HSSF-CW
Dordio et al., 2009bB	Atenolol	4.75	4.75	warm	HSSF-CW
Dordio et al., 2009bC	Atenolol	4.75	4.75	warm	HSSF-CW
Stroski et al., 2020A	Atenolol	4.50	4.75	cold	SF-CW
Stroski et al., 2020B	Atenolol	4.50	4.75	cold	SF-CW
Chaves-Barquero et al., 2016	Atenolol	4.25	4.75	cold	SF-CW
Chaves-Barquero et al., 2018	Atenolol	NA	NA	warm	VSSF-CW
Chaves-Barquero et al., 2021A	Atenolol	3.50	5.75	warm	SF-CW
Chaves-Barquero et al., 2021B	Atenolol	4.25	5.75	warm	SF-CW
MEAN(SE)		**4.0(0.2)**	**5.7(0.2)**		
Breitholtz et al., 2012A	Atorvastatin	NA	NA	warm	SF-CW
Breitholtz et al., 2012B	Atorvastatin	1.25	7.00	warm	SF-CW
Breitholtz et al., 2012C	Atorvastatin	NA	NA	warm	SF-CW
Breitholtz et al., 2012D	Atorvastatin	NA	NA	warm	SF-CW
Verlicchi et al., 2013	Atorvastatin	4.25	7.50	warm	HSSF-CW
MEAN(SE)		**2.8(1.1)**	**7.3(0.2)**		
Berglund et al., 2014	Azithromycin	3.75	5.50	warm	SF-CW
Breitholtz et al., 2012A	Azithromycin	0.00	7.00	warm	SF-CW
Breitholtz et al., 2012B	Azithromycin	NA	NA	warm	SF-CW
Breitholtz et al., 2012C	Azithromycin	1.75	7.00	warm	SF-CW
Breitholtz et al., 2012D	Azithromycin	NA	NA	warm	SF-CW
Verlicchi et al., 2013	Azithromycin	4.25	7.50	warm	HSSF-CW
Sabri et al., 2021A (summer)	Azithromycin	4.75	4.50	warm	H-CW
Sabri et al., 2021A (winter)	Azithromycin	0.00	4.50	cold	H-CW
Sabri et al., 2021B (summer)	Azithromycin	0.00	4.50	warm	H-CW
Sabri et al., 2021B (winter)	Azithromycin	0.00	4.50	cold	H-CW
MEAN(SE)		**1.8(0.7)**	**5.6(0.4)**		
Ruhmland et al., 2015A	Bezafibrate	3.75	6.75	warm	HSSF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Ruhmland et al., 2015B	Bezafibrate	3.75	6.75	warm	HSSF-CW
Ruhmland et al., 2015C	Bezafibrate	3.00	6.75	cold	HSSF-CW
Breitholtz et al., 2012A	Bezafibrate	0.00	7.00	warm	SF-CW
Breitholtz et al., 2012B	Bezafibrate	2.75	7.00	warm	SF-CW
Breitholtz et al., 2012C	Bezafibrate	3.50	7.00	warm	SF-CW
Breitholtz et al., 2012D	Bezafibrate	NA	NA	warm	SF-CW
Verlicchi et al., 2013	Bezafibrate	3.75	7.50	warm	HSSF-CW
Ávila et al., 2021A	Bezafibrate	4.25	6.50	warm	HSSF-CW
Ávila et al., 2021B	Bezafibrate	4.25	6.50	warm	HSSF-CW
MEAN(SE)		**3.2(0.4)**	**6.9(0.1)**		
Breitholtz et al., 2012A	Bisoprolol	3.50	7.00	warm	SF-CW
Breitholtz et al., 2012B	Bisoprolol	3.25	7.00	warm	SF-CW
Breitholtz et al., 2012C	Bisoprolol	3.50	7.00	warm	SF-CW
Breitholtz et al., 2012D	Bisoprolol	3.50	7.00	warm	SF-CW
Auvinen et al., 2017a	Bisoprolol	3.75	5.00	warm	HSSF-CW
Auvinen et al., 2017b (full-scale)	Bisoprolol	4.50	5.50	warm	H-CW
Auvinen et al., 2017b (lab-scale)	Bisoprolol	4.50	5.50	warm	SF-CW
MEAN(SE)		**3.8(0.2)**	**6.3(0.3)**		
Matamoros et al., 2006A	Caffeine	3.75	5.75	warm	HSSF-CW
Matamoros et al., 2006B	Caffeine	3.75	5.75	warm	HSSF-CW
Matamoros et al., 2006C	Caffeine	3.75	5.75	warm	HSSF-CW
Matamoros et al., 2006D	Caffeine	3.75	5.75	warm	HSSF-CW
Chen et al., 2016A (summer)	Caffeine	4.50	5.50	warm	HSSF-CW
Chen et al., 2016A (winter)	Caffeine	3.50	5.50	cold	HSSF-CW
Chen et al., 2016B (summer)	Caffeine	4.50	5.50	warm	HSSF-CW
Chen et al., 2016B (winter)	Caffeine	4.50	5.50	warm	HSSF-CW
Chen et al., 2016C (summer)	Caffeine	4.50	5.50	warm	HSSF-CW
Chen et al., 2016C (winter)	Caffeine	4.50	5.50	cold	HSSF-CW
Matamoros et al., 2007aA	Caffeine	4.75	7.00	warm	SF-CW
Matamoros et al., 2007aB	Caffeine	4.75	7.00	warm	SF-CW
Matamoros et al., 2007aC	Caffeine	4.75	7.00	warm	VSSF-CW
Matamoros et al., 2007aD	Caffeine	4.75	7.00	warm	VSSF-CW
Hijosa-Valsero et al., 2010aA (summer)	Caffeine	3.50	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aA (winter)	Caffeine	3.00	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aB (summer)	Caffeine	3.50	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aB (winter)	Caffeine	3.00	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aC (summer)	Caffeine	3.50	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aC (winter)	Caffeine	3.00	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aD (summer)	Caffeine	3.50	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aD (winter)	Caffeine	3.00	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aE (summer)	Caffeine	3.50	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aE (winter)	Caffeine	3.00	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aF (summer)	Caffeine	3.50	5.50	warm	HSSF-CW
Hijosa-Valsero et al., 2010aF (winter)	Caffeine	3.00	5.50	cold	HSSF-CW
Hijosa-Valsero et al., 2010aG (summer)	Caffeine	3.50	5.50	warm	HSSF-CW
Hijosa-Valsero et al., 2010aG (winter)	Caffeine	3.25	5.50	cold	HSSF-CW
Sgroi et al., 2018A	Caffeine	3.50	6.50	warm	VSSF-CW
Sgroi et al., 2018B	Caffeine	3.50	6.50	warm	H-CW
Matamoros et al., 2009A	Caffeine	4.00	4.50	warm	HSSF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Matamoros et al., 2009B	Caffeine	3.75	4.50	warm	VSSF-CW
Zhu et al., 2014	Caffeine	4.00	7.50	warm	H-CW
Li et al., 2017	Caffeine	3.25	6.50	warm	SF-CW
Hijosa-Valsero et al., 2010bA	Caffeine	4.00	6.50	warm	H-CW
Hijosa-Valsero et al., 2010bB	Caffeine	4.00	6.50	warm	H-CW
Reyes-Contreras et al., 2011	Caffeine	3.50	6.50	warm	H-CW
Matamoros et al., 2016A	Caffeine	4.00	5.50	warm	HSSF-CW
Matamoros et al., 2016B	Caffeine	0.00	5.50	cold	HSSF-CW
Camacho-Muñoz et al., 2012	Caffeine	3.75	5.25	warm	VSSF-CW
Vymazal et al., 2017A	Caffeine	4.00	4.25	warm	HSSF-CW
Vymazal et al., 2017B	Caffeine	4.00	4.25	warm	HSSF-CW
Vymazal et al., 2017C	Caffeine	3.75	4.25	warm	HSSF-CW
Vymazal et al., 2017D	Caffeine	4.00	4.25	warm	HSSF-CW
Carranza-Diaz et al., 2014A	Caffeine	3.75	6.25	warm	HSSF-CW
Carranza-Diaz et al., 2014B	Caffeine	3.75	6.25	warm	HSSF-CW
Nuel et al., 2018	Caffeine	3.50	7.00	warm	SF-CW
He et al., 2018A	Caffeine	0.00	5.50	warm	VSSF-CW
He et al., 2018B	Caffeine	0.00	5.50	warm	SF-CW
He et al., 2018C	Caffeine	3.00	5.50	warm	SF-CW
Kahl et al., 2017A (warm)	Caffeine	4.25	7.00	warm	HSSF-CW
Kahl et al., 2017A (cold)	Caffeine	4.00	7.00	cold	HSSF-CW
Kahl et al., 2017B (warm)	Caffeine	4.25	7.00	warm	VSSF-CW
Kahl et al., 2017B (cold)	Caffeine	4.25	7.00	cold	VSSF-CW
Kahl et al., 2017C (warm)	Caffeine	4.25	7.00	warm	VSSF-CW
Kahl et al., 2017C (cold)	Caffeine	4.25	7.00	cold	VSSF-CW
Kahl et al., 2017D (warm)	Caffeine	4.25	7.00	warm	VSSF-CW
Kahl et al., 2017D (cold)	Caffeine	4.25	7.00	cold	VSSF-CW
Kahl et al., 2017E (warm)	Caffeine	4.25	7.00	warm	HSSF-CW
Kahl et al., 2017E (cold)	Caffeine	4.25	7.00	cold	HSSF-CW
Conkle et al., 2008	Caffeine	4.50	4.50	warm	H-CW
De Oliveira et al., 2019A	Caffeine	4.50	4.50	warm	VSSF-CW
De Oliveira et al., 2019B	Caffeine	4.50	4.50	warm	HSSF-CW
Vystavna et al., 2017A	Caffeine	4.50	6.00	warm	H-CW
Vystavna et al., 2017B	Caffeine	4.50	6.00	warm	H-CW
Herrera-Cardenas et al., 2016	Caffeine	4.00	5.75	warm	HSSF-CW
Zhang et al., 2012	Caffeine	4.00	6.00	warm	HSSF-CW
MEAN(SE)		**3.7(0.1)**	**5.9(0.1)**		
Anderson et al., 2013	Carbamazepine	5.00	5.00	warm	SF-CW
Cardinal et al., 2014	Carbamazepine	3.50	6.25	warm	SF-CW
Matamoros et al., 2008A	Carbamazepine	3.50	7.25	warm	SF-CW
Matamoros et al., 2008B	Carbamazepine	3.50	7.25	cold	SF-CW
Matamoros et al., 2007aA	Carbamazepine	3.00	7.00	warm	SF-CW
Matamoros et al., 2007aB	Carbamazepine	4.00	7.00	warm	SF-CW
Matamoros et al., 2007aC	Carbamazepine	4.00	7.00	warm	VSSF-CW
Matamoros et al., 2007aD	Carbamazepine	4.25	7.00	warm	VSSF-CW
Hijosa-Valsero et al., 2010aA (summer)	Carbamazepine	3.00	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aA (winter)	Carbamazepine	3.00	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aB (summer)	Carbamazepine	3.00	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aB (winter)	Carbamazepine	2.75	5.50	cold	SF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Hijosa-Valsero et al., 2010aC (summer)	Carbamazepine	3.00	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aC (winter)	Carbamazepine	3.00	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aD (summer)	Carbamazepine	3.00	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aD (winter)	Carbamazepine	0.00	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aE (summer)	Carbamazepine	3.00	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aE (winter)	Carbamazepine	0.00	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aF (summer)	Carbamazepine	3.00	5.50	warm	HSSF-CW
Hijosa-Valsero et al., 2010aF (winter)	Carbamazepine	3.00	5.50	cold	HSSF-CW
Hijosa-Valsero et al., 2010aG (summer)	Carbamazepine	3.00	5.50	warm	HSSF-CW
Hijosa-Valsero et al., 2010aG (winter)	Carbamazepine	2.75	5.50	cold	HSSF-CW
Ruhmland et al., 2015A	Carbamazepine	1.00	6.75	warm	HSSF-CW
Ruhmland et al., 2015B	Carbamazepine	0.00	6.75	warm	HSSF-CW
Ruhmland et al., 2015C	Carbamazepine	3.25	6.75	cold	HSSF-CW
Matamoros et al., 2009A	Carbamazepine	0.00	4.50	warm	HSSF-CW
Matamoros et al., 2009B	Carbamazepine	0.00	4.50	warm	VSSF-CW
Zhu et al., 2014	Carbamazepine	0.00	7.50	warm	H-CW
Moeder et al., 2017	Carbamazepine	3.75	4.25	warm	SF-CW
Reyes-Contreras et al., 2011	Carbamazepine	0.00	6.50	warm	H-CW
Matamoros et al., 2016A	Carbamazepine	3.50	5.50	warm	HSSF-CW
Matamoros et al., 2016B	Carbamazepine	0.00	5.50	cold	HSSF-CW
Camacho-Muñoz et al., 2012	Carbamazepine	3.50	5.25	warm	VSSF-CW
Carranza-Diaz et al., 2014A	Carbamazepine	2.25	6.25	warm	HSSF-CW
Carranza-Diaz et al., 2014B	Carbamazepine	2.25	6.25	warm	HSSF-CW
Nuel et al., 2018	Carbamazepine	0.00	7.00	warm	SF-CW
Breitholtz et al., 2012A	Carbamazepine	2.75	7.00	warm	SF-CW
Breitholtz et al., 2012B	Carbamazepine	1.75	7.00	warm	SF-CW
Breitholtz et al., 2012C	Carbamazepine	3.25	7.00	warm	SF-CW
Breitholtz et al., 2012D	Carbamazepine	0.00	7.00	warm	SF-CW
He et al., 2018A	Carbamazepine	0.00	5.50	warm	VSSF-CW
He et al., 2018B	Carbamazepine	0.00	5.50	warm	SF-CW
He et al., 2018C	Carbamazepine	0.00	5.50	warm	SF-CW
Verlicchi et al., 2013	Carbamazepine	0.00	7.50	warm	HSSF-CW
Kahl et al., 2017A (warm)	Carbamazepine	3.75	7.00	warm	HSSF-CW
Kahl et al., 2017A (cold)	Carbamazepine	1.50	7.00	cold	HSSF-CW
Kahl et al., 2017B (warm)	Carbamazepine	1.50	7.00	warm	VSSF-CW
Kahl et al., 2017B (cold)	Carbamazepine	0.00	7.00	cold	VSSF-CW
Kahl et al., 2017C (warm)	Carbamazepine	4.25	7.00	warm	VSSF-CW
Kahl et al., 2017C (cold)	Carbamazepine	0.00	7.00	cold	VSSF-CW
Kahl et al., 2017D (warm)	Carbamazepine	1.50	7.00	warm	VSSF-CW
Kahl et al., 2017D (cold)	Carbamazepine	0.00	7.00	cold	VSSF-CW
Kahl et al., 2017E (warm)	Carbamazepine	4.00	7.00	warm	HSSF-CW
Kahl et al., 2017E (cold)	Carbamazepine	0.00	7.00	cold	HSSF-CW
Zhang et al., 2011A	Carbamazepine	3.25	5.75	warm	HSSF-CW
Zhang et al., 2011B	Carbamazepine	3.25	5.75	warm	HSSF-CW
Matamoros et al., 2005A	Carbamazepine	2.75	5.75	warm	HSSF-CW
Matamoros et al., 2005B	Carbamazepine	2.75	5.75	warm	HSSF-CW
Dordio et al., 2010A	Carbamazepine	3.75	6.25	warm	VSSF-CW
Dordio et al., 2010B	Carbamazepine	3.75	6.25	cold	VSSF-CW
Matamoros et al., 2008	Carbamazepine	2.50	6.00	warm	HSSF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Conkle et al., 2008	Carbamazepine	4.25	4.50	warm	H-CW
Park et al., 2018	Carbamazepine	4.75	4.75	warm	SF-CW
Auvinen et al., 2017a	Carbamazepine	1.75	5.00	warm	HSSF-CW
Delgado et al., 2020	Carbamazepine	1.00	6.25	warm	HSSF-CW
Mathon et al., 2019	Carbamazepine	3.25	5.00	warm	SF-CW
Macci et al., 2015A	Carbamazepine	3.50	4.50	warm	VSSF-CW
Macci et al., 2015B	Carbamazepine	3.25	4.50	warm	VSSF-CW
Macci et al., 2015C	Carbamazepine	3.25	4.50	warm	VSSF-CW
Macci et al., 2015D	Carbamazepine	3.50	4.50	warm	VSSF-CW
Macci et al., 2015E	Carbamazepine	3.25	4.50	warm	VSSF-CW
Sharif et al., 2014	Carbamazepine	2.50	7.75	warm	SF-CW
Vystavna et al., 2017A	Carbamazepine	4.25	6.00	warm	H-CW
Vystavna et al., 2017B	Carbamazepine	4.50	6.00	warm	H-CW
Matamoros et al., 2007a	Carbamazepine	2.25	6.50	warm	HSSF-CW
Park et al., 2009	Carbamazepine	0.00	3.75	warm	SF-CW
Ruppelt et al., 2020A	Carbamazepine	0.00	3.75	warm	VSSF-CW
Ruppelt et al., 2020B	Carbamazepine	0.00	3.75	warm	VSSF-CW
Dordio et al., 2009a	Carbamazepine	3.75	5.25	warm	HSSF-CW
Auvinen et al., 2017A	Carbamazepine	4.50	5.50	warm	H-CW
Auvinen et al., 2017B	Carbamazepine	4.50	5.50	warm	SF-CW
Cardinal et al., 2016C	Carbamazepine	3.50	6.75	warm	SF-CW
Zhang et al., 2012	Carbamazepine	3.50	6.00	warm	HSSF-CW
Anderson et al., 2020A	Carbamazepine	0.00	4.25	warm	H-CW
Anderson et al., 2020B	Carbamazepine	0.00	4.25	warm	H-CW
Ozengin et al., 2016A	Carbamazepine	3.75	4.75	warm	HSSF-CW
Ozengin et al., 2016B	Carbamazepine	3.75	4.75	warm	HSSF-CW
Avila et al., 2021A	Carbamazepine	3.25	6.50	warm	HSSF-CW
Avila et al., 2021B	Carbamazepine	3.25	6.50	warm	HSSF-CW
Stroski et al., 2020A	Carbamazepine	4.50	4.75	cold	SF-CW
Stroski et al., 2020B	Carbamazepine	4.50	4.75	cold	SF-CW
Stroski et al., 2020C	Carbamazepine	4.50	4.75	cold	SF-CW
Stroski et al., 2020D	Carbamazepine	4.25	4.75	cold	SF-CW
Chaves-Barquero et al., 2016	Carbamazepine	1.50	4.75	cold	SF-CW
Chaves-Barquero et al., 2018	Carbamazepine	0.00	6.50	warm	VSSF-CW
Chaves-Barquero et al., 2021A	Carbamazepine	0.00	5.75	warm	SF-CW
Chaves-Barquero et al., 2021B	Carbamazepine	0.00	5.75	warm	VSSF-CW
Anderson et al., 2015	Carbamazepine	0.00	6.50	warm	VSSF-CW
MEAN(SE)		**2.3(0.2)**	**5.8(0.1)**		
Berglund et al., 2014	Ciprofloxacin	3.75	5.50	warm	SF-CW
Dan et al., 2020aA (summer)	Ciprofloxacin	0.00	6.50	warm	VSSF-CW
Dan et al., 2020aA (winter)	Ciprofloxacin	3.75	6.50	cold	VSSF-CW
Dan et al., 2020aB (summer)	Ciprofloxacin	0.00	6.50	warm	VSSF-CW
Dan et al., 2020aB (winter)	Ciprofloxacin	2.75	6.50	cold	VSSF-CW
Dan et al., 2020aC (summer)	Ciprofloxacin	2.75	6.50	warm	VSSF-CW
Dan et al., 2020aC (winter)	Ciprofloxacin	2.75	6.50	cold	VSSF-CW
Dan et al., 2020aD (summer)	Ciprofloxacin	1.75	6.50	warm	SF-CW
Dan et al., 2020aD (winter)	Ciprofloxacin	3.50	6.50	cold	SF-CW
Dan et al., 2020aE (summer)	Ciprofloxacin	0.00	6.50	warm	SF-CW
Dan et al., 2020aE (winter)	Ciprofloxacin	3.50	6.50	cold	SF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Dan et al., 2020aF (summer)	Ciprofloxacin	0.00	6.50	warm	SF-CW
Dan et al., 2020aF (winter)	Ciprofloxacin	3.50	6.50	cold	SF-CW
Dan et al., 2020aG (summer)	Ciprofloxacin	0.00	6.50	warm	HSSF-CW
Dan et al., 2020aG (winter)	Ciprofloxacin	3.50	6.50	cold	HSSF-CW
Dan et al., 2020aH (summer)	Ciprofloxacin	0.00	6.50	warm	HSSF-CW
Dan et al., 2020aH (winter)	Ciprofloxacin	3.00	6.50	cold	HSSF-CW
Dan et al., 2020aI (summer)	Ciprofloxacin	0.00	6.50	warm	HSSF-CW
Dan et al., 2020aI (winter)	Ciprofloxacin	3.50	6.50	cold	HSSF-CW
Verlicchi et al., 2013	Ciprofloxacin	4.00	7.50	warm	HSSF-CW
Christofiloupoulos et al., 2019	Ciprofloxacin	4.00	4.25	warm	HSSF-CW
Sabri et al., 2021A (summer)	Ciprofloxacin	5.00	4.50	warm	H-CW
Sabri et al., 2021A (winter)	Ciprofloxacin	5.00	4.50	cold	H-CW
Sabri et al., 2021B (summer)	Ciprofloxacin	5.00	4.50	warm	H-CW
Sabri et al., 2021B (winter)	Ciprofloxacin	4.50	4.50	cold	H-CW
MEAN(SE)		**2.6(0.4)**	**6.1(0.2)**		
Berglund et al., 2014	Clarithromycin	3.50	5.50	warm	SF-CW
Ruhmland et al., 2015A	Clarithromycin	0.00	6.75	warm	HSSF-CW
Ruhmland et al., 2015B	Clarithromycin	1.25	6.75	warm	HSSF-CW
Ruhmland et al., 2015C	Clarithromycin	4.00	6.75	cold	HSSF-CW
Breitholtz et al., 2012A	Clarithromycin	0.00	7.00	warm	SF-CW
Breitholtz et al., 2012B	Clarithromycin	3.75	7.00	warm	SF-CW
Breitholtz et al., 2012C	Clarithromycin	NA	NA	warm	SF-CW
Breitholtz et al., 2012D	Clarithromycin	NA	NA	warm	SF-CW
Verlicchi et al., 2013	Clarithromycin	2.50	7.50	warm	HSSF-CW
Mathon et al., 2019	Clarithromycin	3.25	5.00	warm	SF-CW
Anderson et al., 2020A	Clarithromycin	4.00	4.25	warm	H-CW
Anderson et al., 2020B	Clarithromycin	4.25	4.25	warm	H-CW
Chaves-Barquero et al., 2016	Clarithromycin	1.00	4.75	cold	SF-CW
Chaves-Barquero et al., 2018	Clarithromycin	NA	NA	warm	VSSF-CW
Sabri et al., 2021A (summer)	Clarithromycin	4.75	4.50	warm	H-CW
Sabri et al., 2021A (winter)	Clarithromycin	5.00	4.50	cold	H-CW
Sabri et al., 2021B (summer)	Clarithromycin	4.75	4.50	warm	H-CW
Sabri et al., 2021B (winter)	Clarithromycin	0.00	4.50	cold	H-CW
Anderson et al., 2015	Clarithromycin	NA	6.50	warm	VSSF-CW
MEAN(SE)		**2.8(0.5)**	**5.6(0.3)**		
Berglund et al., 2014	Clindamycin	3.50	5.50	warm	SF-CW
Breitholtz et al., 2012A	Clindamycin	2.75	7.00	warm	SF-CW
Breitholtz et al., 2012B	Clindamycin	0.00	7.00	warm	SF-CW
Breitholtz et al., 2012C	Clindamycin	0.00	7.00	warm	SF-CW
Breitholtz et al., 2012D	Clindamycin	0.00	7.00	warm	SF-CW
Mathon et al., 2019	Clindamycin	3.75	5.00	warm	SF-CW
MEAN(SE)		**1.3(0.7)**	**6.6(0.3)**		
Cardinal et al., 2014	Clofibric acid	3.50	6.25	warm	SF-CW
Matamoros et al., 2008A	Clofibric acid	3.50	7.25	warm	SF-CW
Matamoros et al., 2008B	Clofibric acid	3.50	7.25	cold	SF-CW
Nuel et al., 2018	Clofibric acid	3.25	7.00	warm	SF-CW
He et al., 2018A	Clofibric acid	NA	NA	warm	VSSF-CW
He et al., 2018B	Clofibric acid	NA	NA	warm	SF-CW
He et al., 2018C	Clofibric acid	NA	NA	warm	SF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Verlicchi et al., 2013	Clofibric acid	4.25	7.50	warm	HSSF-CW
Matamoros et al., 2005A	Clofibric acid	0.00	5.75	warm	HSSF-CW
Matamoros et al., 2005B	Clofibric acid	0.00	5.75	warm	HSSF-CW
Dordio et al., 2010A	Clofibric acid	3.75	6.25	warm	VSSF-CW
Dordio et al., 2010B	Clofibric acid	3.25	6.25	cold	VSSF-CW
Matamoros et al., 2008a	Clofibric acid	1.00	6.00	warm	HSSF-CW
Dordio et al., 2009a	Clofibric acid	3.25	5.25	warm	HSSF-CW
Cardinal et al., 2016	Clofibric acid	3.50	6.75	warm	SF-CW
Zhang et al., 2012	Clofibric acid	1.00	6.00	warm	HSSF-CW
MEAN(SE)		**2.6(0.4)**	**6.4(0.2)**		
Ruhmland et al., 2015A	Codeine	4.00	6.75	warm	HSSF-CW
Ruhmland et al., 2015B	Codeine	1.00	6.75	warm	HSSF-CW
Ruhmland et al., 2015C	Codeine	4.00	6.75	cold	HSSF-CW
Breitholtz et al., 2012A	Codeine	3.50	7.00	warm	SF-CW
Breitholtz et al., 2012B	Codeine	4.00	7.00	warm	SF-CW
Breitholtz et al., 2012C	Codeine	4.00	7.00	warm	SF-CW
Breitholtz et al., 2012D	Codeine	3.50	7.00	warm	SF-CW
Verlicchi et al., 2013	Codeine	4.00	7.50	warm	HSSF-CW
MEAN(SE)		**3.5(0.3)**	**7.0(0.1)**		
Verlicchi et al., 2013	Diazepam	NA	NA	warm	HSSF-CW
Mathon et al., 2019	Diazepam	3.25	5.00	warm	SF-CW
Park et al., 2009	Diazepam	NA	NA	warm	SF-CW
Auvinen et al., 2017b	Diazepam	4.50	5.50	warm	H-CW
Auvinen et al., 2017b	Diazepam	NA	NA	warm	SF-CW
MEAN(SE)		**3.9(0.4)**	**5.3(0.2)**		
Matamoros et al., 2006A	Diclofenac	3.00	5.75	warm	HSSF-CW
Matamoros et al., 2006B	Diclofenac	0.00	5.75	warm	HSSF-CW
Matamoros et al., 2006C	Diclofenac	0.00	5.75	warm	HSSF-CW
Matamoros et al., 2006D	Diclofenac	2.25	5.75	warm	HSSF-CW
Matamoros et al., 2006E	Diclofenac	0.00	5.75	warm	HSSF-CW
Chen et al., 2016A (summer)	Diclofenac	3.75	5.50	warm	HSSF-CW
Chen et al., 2016A (winter)	Diclofenac	4.00	5.50	cold	HSSF-CW
Chen et al., 2016B (summer)	Diclofenac	4.00	5.50	warm	HSSF-CW
Chen et al., 2016B (winter)	Diclofenac	4.50	5.50	warm	HSSF-CW
Chen et al., 2016C (summer)	Diclofenac	4.50	5.50	warm	HSSF-CW
Chen et al., 2016C (winter)	Diclofenac	4.50	5.50	cold	HSSF-CW
Avila et al., 2013	Diclofenac	4.25	6.50	warm	HSSF-CW
Matamoros et al., 2008A	Diclofenac	4.00	7.25	warm	SF-CW
Matamoros et al., 2008B	Diclofenac	3.75	7.25	cold	SF-CW
Matamoros et al., 2007A	Diclofenac	4.25	7.00	warm	SF-CW
Matamoros et al., 2007B	Diclofenac	4.75	7.00	warm	SF-CW
Matamoros et al., 2007C	Diclofenac	4.50	7.00	warm	VSSF-CW
Matamoros et al., 2007D	Diclofenac	4.50	7.00	warm	VSSF-CW
Hijosa-Valsero et al., 2010aA (summer)	Diclofenac	0.00	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aA (winter)	Diclofenac	3.00	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aB (summer)	Diclofenac	0.00	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aB (winter)	Diclofenac	3.00	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aC (summer)	Diclofenac	0.00	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aC (winter)	Diclofenac	0.00	5.50	cold	SF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Hijosa-Valsero et al., 2010aD (summer)	Diclofenac	0.00	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aD (winter)	Diclofenac	3.00	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aE (summer)	Diclofenac	0.00	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aE (winter)	Diclofenac	3.00	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aF (summer)	Diclofenac	0.00	5.50	warm	HSSF-CW
Hijosa-Valsero et al., 2010aF (winter)	Diclofenac	3.00	5.50	cold	HSSF-CW
Hijosa-Valsero et al., 2010aG (summer)	Diclofenac	3.00	5.50	warm	HSSF-CW
Hijosa-Valsero et al., 2010aG (winter)	Diclofenac	2.75	5.50	cold	HSSF-CW
Ruhmland et al., 2015A	Diclofenac	2.25	6.75	warm	HSSF-CW
Ruhmland et al., 2015B	Diclofenac	1.75	6.75	warm	HSSF-CW
Ruhmland et al., 2015C	Diclofenac	1.75	6.75	cold	HSSF-CW
Matamoros et al., 2009A	Diclofenac	1.25	4.50	warm	HSSF-CW
Matamoros et al., 2009B	Diclofenac	0.00	4.50	warm	VSSF-CW
Avila et al., 2015	Diclofenac	4.00	6.50	warm	H-CW
Zhu et al., 2014	Diclofenac	1.25	7.50	warm	H-CW
Hijosa-Valsero et al., 2010bA	Diclofenac	4.00	6.50	warm	H-CW
Hijosa-Valsero et al., 2010bB	Diclofenac	4.00	6.50	warm	H-CW
Avila et al., 2014aA	Diclofenac	4.00	7.00	warm	VSSF-CW
Avila et al., 2014aB	Diclofenac	4.00	7.00	warm	VSSF-CW
Avila et al., 2014aC	Diclofenac	4.00	7.00	warm	VSSF-CW
Avila et al., 2014aD	Diclofenac	4.25	7.00	warm	VSSF-CW
Moeder et al., 2017	Diclofenac	4.00	4.25	warm	SF-CW
Matamoros et al., 2016A	Diclofenac	3.75	5.50	warm	HSSF-CW
Matamoros et al., 2016A	Diclofenac	1.25	5.50	cold	HSSF-CW
Vymazal et al., 2017A	Diclofenac	1.25	4.25	warm	HSSF-CW
Vymazal et al., 2017B	Diclofenac	3.75	4.25	warm	HSSF-CW
Vymazal et al., 2017C	Diclofenac	3.50	4.25	warm	HSSF-CW
Vymazal et al., 2017D	Diclofenac	3.75	4.25	warm	HSSF-CW
Carranza-Diaz et al., 2014A	Diclofenac	0.00	6.25	warm	HSSF-CW
Carranza-Diaz et al., 2014B	Diclofenac	0.00	6.25	warm	HSSF-CW
Nuel et al., 2018	Diclofenac	0.00	7.00	warm	SF-CW
Breitholtz et al., 2012A	Diclofenac	3.50	7.00	warm	SF-CW
Breitholtz et al., 2012B	Diclofenac	3.25	7.00	warm	SF-CW
Breitholtz et al., 2012C	Diclofenac	3.50	7.00	warm	SF-CW
Breitholtz et al., 2012D	Diclofenac	3.50	7.00	warm	SF-CW
He et al., 2018A	Diclofenac	3.25	5.50	warm	VSSF-CW
He et al., 2018B	Diclofenac	1.75	5.50	warm	SF-CW
He et al., 2018C	Diclofenac	3.00	5.50	warm	SF-CW
Verlicchi et al., 2013	Diclofenac	1.50	7.50	warm	HSSF-CW
Kahl et al., 2017A (warm)	Diclofenac	4.00	7.00	warm	HSSF-CW
Kahl et al., 2017A (cold)	Diclofenac	1.50	7.00	cold	HSSF-CW
Kahl et al., 2017B (warm)	Diclofenac	4.00	7.00	warm	VSSF-CW
Kahl et al., 2017B (cold)	Diclofenac	3.75	7.00	cold	VSSF-CW
Kahl et al., 2017C (warm)	Diclofenac	4.25	7.00	warm	VSSF-CW
Kahl et al., 2017C (cold)	Diclofenac	4.00	7.00	cold	VSSF-CW
Kahl et al., 2017D (warm)	Diclofenac	4.25	7.00	warm	VSSF-CW
Kahl et al., 2017D (cold)	Diclofenac	4.00	7.00	cold	VSSF-CW
Kahl et al., 2017E (warm)	Diclofenac	4.25	7.00	warm	HSSF-CW
Kahl et al., 2017E (cold)	Diclofenac	4.25	7.00	cold	HSSF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Avila et al., 2010	Diclofenac	4.25	6.25	warm	HSSF-CW
Avila et al., 2014bA	Diclofenac	4.75	6.25	warm	H-CW
Avila et al., 2014bB	Diclofenac	4.75	6.25	warm	H-CW
Avila et al., 2014bC	Diclofenac	4.75	6.25	warm	H-CW
Zhang et al., 2011A	Diclofenac	3.25	5.75	warm	HSSF-CW
Zhang et al., 2011B	Diclofenac	3.50	5.75	warm	HSSF-CW
Auvinen et al., 2017a	Diclofenac	3.25	5.00	warm	HSSF-CW
Mathon et al., 2019	Diclofenac	3.75	5.00	warm	SF-CW
Vystavna et al., 2017A	Diclofenac	4.25	6.00	warm	H-CW
Vystavna et al., 2017A	Diclofenac	4.50	6.00	warm	H-CW
Sochaki et al., 2018	Diclofenac	4.00	5.50	warm	H-CW
Matamoros et al., 2007	Diclofenac	2.25	6.50	warm	HSSF-CW
Park et al., 2009	Diclofenac	4.50	3.75	warm	SF-CW
Ruppelt et al., 2020A	Diclofenac	4.25	3.75	warm	VSSF-CW
Ruppelt et al., 2020B	Diclofenac	4.50	3.75	warm	VSSF-CW
Auvinen et al., 2017bA	Diclofenac	4.50	5.50	warm	H-CW
Auvinen et al., 2017bB	Diclofenac	4.25	5.50	warm	SF-CW
Francini et al., 2018A	Diclofenac	4.50	6.25	warm	SF-CW
Francini et al., 2018B	Diclofenac	4.50	6.25	warm	SF-CW
Zhang et al., 2012	Diclofenac	3.25	6.00	warm	HSSF-CW
Anderson et al., 2020A	Diclofenac	3.75	4.25	warm	H-CW
Anderson et al., 2020B	Diclofenac	3.50	4.25	warm	H-CW
Avila et al., 2021A	Diclofenac	4.00	6.50	warm	HSSF-CW
Avila et al., 2021B	Diclofenac	4.25	6.50	warm	HSSF-CW
MEAN(SE)		**3.1(0.2)**	**6.0(0.1)**		
Berglund et al., 2014	Erythromycin	3.75	5.50	warm	SF-CW
Ruhmland et al., 2015A	Erythromycin	4.00	6.75	warm	HSSF-CW
Ruhmland et al., 2015B	Erythromycin	4.00	6.75	warm	HSSF-CW
Ruhmland et al., 2015C	Erythromycin	4.00	6.75	cold	HSSF-CW
Dan et al., 2020aA (summer)	Erythromycin	NA	NA	warm	VSSF-CW
Dan et al., 2020aA (winter)	Erythromycin	4.00	6.50	cold	VSSF-CW
Dan et al., 2020aB (summer)	Erythromycin	NA	NA	warm	VSSF-CW
Dan et al., 2020aB (winter)	Erythromycin	3.75	6.50	cold	VSSF-CW
Dan et al., 2020aC (summer)	Erythromycin	NA	NA	warm	VSSF-CW
Dan et al., 2020aC (winter)	Erythromycin	3.75	6.50	cold	VSSF-CW
Dan et al., 2020aD (summer)	Erythromycin	NA	NA	warm	SF-CW
Dan et al., 2020aD (winter)	Erythromycin	3.50	6.50	cold	SF-CW
Dan et al., 2020aE (summer)	Erythromycin	NA	NA	warm	SF-CW
Dan et al., 2020aE (winter)	Erythromycin	3.50	6.50	cold	SF-CW
Dan et al., 2020aF (summer)	Erythromycin	NA	NA	warm	SF-CW
Dan et al., 2020aF (winter)	Erythromycin	4.00	6.50	cold	SF-CW
Dan et al., 2020aG (summer)	Erythromycin	NA	NA	warm	HSSF-CW
Dan et al., 2020aG (winter)	Erythromycin	4.00	6.50	cold	HSSF-CW
Dan et al., 2020aH (summer)	Erythromycin	NA	NA	warm	HSSF-CW
Dan et al., 2020aH (winter)	Erythromycin	3.50	6.50	cold	HSSF-CW
Dan et al., 2020aI (summer)	Erythromycin	NA	NA	warm	HSSF-CW
Dan et al., 2020aI (winter)	Erythromycin	3.50	6.50	cold	HSSF-CW
He et al., 2018A	Erythromycin	3.50	5.50	warm	VSSF-CW
He et al., 2018B	Erythromycin	1.75	5.50	warm	SF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
He et al., 2018C	Erythromycin	0.00	5.50	warm	SF-CW
Verlicchi et al., 2013	Erythromycin	0.00	7.50	warm	HSSF-CW
Avila et al., 2014bA	Erythromycin	NA	NA	warm	H-CW
Avila et al., 2014bB	Erythromycin	NA	NA	warm	H-CW
Avila et al., 2014bC	Erythromycin	4.00	6.25	warm	H-CW
Mathon et al., 2019	Erythromycin	3.25	5.00	warm	SF-CW
MEAN(SE)		**3.3(0.3)**	**6.3(0.1)**		
Ruhmland et al., 2015A	Fluconazole	1.00	6.75	warm	HSSF-CW
Ruhmland et al., 2015B	Fluconazole	0.00	6.75	warm	HSSF-CW
Ruhmland et al., 2015C	Fluconazole	0.00	6.75	cold	HSSF-CW
Breitholtz et al., 2012A	Fluconazole	0.00	7.00	warm	SF-CW
Breitholtz et al., 2012B	Fluconazole	3.50	7.00	warm	SF-CW
Breitholtz et al., 2012C	Fluconazole	3.50	7.00	warm	SF-CW
Breitholtz et al., 2012D	Fluconazole	0.00	7.00	warm	SF-CW
MEAN(SE)		**1.1(0.6)**	**6.9(0.0)**		
Chen et al., 2016A (summer)	Furosemide	4.50	5.50	warm	HSSF-CW
Chen et al., 2016A (winter)	Furosemide	3.50	5.50	cold	HSSF-CW
Chen et al., 2016B (summer)	Furosemide	4.50	5.50	warm	HSSF-CW
Chen et al., 2016B (winter)	Furosemide	4.50	5.50	warm	HSSF-CW
Chen et al., 2016C (summer)	Furosemide	4.50	5.50	warm	HSSF-CW
Chen et al., 2016C (winter)	Furosemide	4.50	5.50	cold	HSSF-CW
Matamoros et al., 2009A	Furosemide	1.50	4.50	warm	HSSF-CW
Matamoros et al., 2009B	Furosemide	0.00	4.50	warm	VSSF-CW
Matamoros et al., 2016A	Furosemide	3.75	5.50	warm	HSSF-CW
Matamoros et al., 2016B	Furosemide	1.25	5.50	cold	HSSF-CW
Vymazal et al., 2017A	Furosemide	4.00	4.25	warm	HSSF-CW
Vymazal et al., 2017B	Furosemide	4.00	4.25	warm	HSSF-CW
Vymazal et al., 2017C	Furosemide	3.75	4.25	warm	HSSF-CW
Vymazal et al., 2017D	Furosemide	4.00	4.25	warm	HSSF-CW
Verlicchi et al., 2013	Furosemide	3.75	7.50	warm	HSSF-CW
MEAN(SE)		**3.5(0.3)**	**5.2(0.2)**		
Chen et al., 2016A (summer)	Gabapentin	4.25	5.50	warm	HSSF-CW
Chen et al., 2016A (winter)	Gabapentin	0.00	5.50	cold	HSSF-CW
Chen et al., 2016B (summer)	Gabapentin	4.25	5.50	warm	HSSF-CW
Chen et al., 2016B (winter)	Gabapentin	0.00	5.50	warm	HSSF-CW
Chen et al., 2016C (summer)	Gabapentin	0.00	5.50	warm	HSSF-CW
Chen et al., 2016C (winter)	Gabapentin	0.00	5.50	cold	HSSF-CW
Auvinen et al., 2017a	Gabapentin	3.25	5.00	warm	HSSF-CW
Auvinen et al., 2017bA	Gabapentin	4.50	5.50	warm	H-CW
Auvinen et al., 2017bB	Gabapentin	4.50	5.50	warm	SF-CW
MEAN(SE)		**2.3(0.7)**	**5.4(0.1)**		
Anderson et al., 2013	Gemfibrozil	0.00	5.00	warm	SF-CW
Camacho-Muñoz et al., 2012	Gemfibrozil	3.25	5.25	warm	VSSF-CW
Nuel et al., 2018	Gemfibrozil	0.00	7.00	warm	SF-CW
Verlicchi et al., 2013	Gemfibrozil	3.75	7.50	warm	HSSF-CW
Conkle et al., 2008	Gemfibrozil	4.50	4.50	warm	H-CW
Avila et al., 2021A	Gemfibrozil	4.25	6.50	warm	HSSF-CW
Avila et al., 2021B	Gemfibrozil	4.25	6.50	warm	HSSF-CW
Anderson et al., 2015	Gemfibrozil	NA	6.50	warm	VSSF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
MEAN(SE)		**2.9(0.7)**	**6.0(0.4)**		
Chen et al., 2016A (summer)	Hydrochlorothiazide	4.00	5.50	warm	HSSF-CW
Chen et al., 2016A (winter)	Hydrochlorothiazide	3.50	5.50	cold	HSSF-CW
Chen et al., 2016B (summer)	Hydrochlorothiazide	4.25	5.50	warm	HSSF-CW
Chen et al., 2016B (winter)	Hydrochlorothiazide	4.50	5.50	warm	HSSF-CW
Chen et al., 2016C (summer)	Hydrochlorothiazide	4.50	5.50	warm	HSSF-CW
Chen et al., 2016C (winter)	Hydrochlorothiazide	4.50	5.50	cold	HSSF-CW
Verlicchi et al., 2013	Hydrochlorothiazide	4.00	7.50	warm	HSSF-CW
MEAN(SE)		**4.2(0.1)**	**5.8(0.3)**		
Matamoros et al., 2006A	Ibuprofen	3.50	5.75	warm	HSSF-CW
Matamoros et al., 2006B	Ibuprofen	3.00	5.75	warm	HSSF-CW
Matamoros et al., 2006C	Ibuprofen	3.50	5.75	warm	HSSF-CW
Matamoros et al., 2006D	Ibuprofen	3.75	5.75	warm	HSSF-CW
Matamoros et al., 2006E	Ibuprofen	3.50	5.75	warm	HSSF-CW
Chen et al., 2016A (summer)	Ibuprofen	4.50	5.50	warm	HSSF-CW
Chen et al., 2016A (winter)	Ibuprofen	4.50	5.50	cold	HSSF-CW
Chen et al., 2016B (summer)	Ibuprofen	4.50	5.50	warm	HSSF-CW
Chen et al., 2016B (winter)	Ibuprofen	4.50	5.50	warm	HSSF-CW
Chen et al., 2016C (summer)	Ibuprofen	4.50	5.50	warm	HSSF-CW
Chen et al., 2016C (winter)	Ibuprofen	4.50	5.50	cold	HSSF-CW
Avila et al., 2013	Ibuprofen	4.50	6.50	warm	HSSF-CW
Matamoros et al., 2008aA	Ibuprofen	3.75	7.25	warm	SF-CW
Matamoros et al., 2008aB	Ibuprofen	4.00	7.25	cold	SF-CW
Matamoros et al., 2007A	Ibuprofen	4.25	7.00	warm	SF-CW
Matamoros et al., 2007B	Ibuprofen	4.75	7.00	warm	SF-CW
Matamoros et al., 2007C	Ibuprofen	4.50	7.00	warm	VSSF-CW
Matamoros et al., 2007D	Ibuprofen	4.75	7.00	warm	VSSF-CW
Hijosa-Valsero et al., 2010aA (summer)	Ibuprofen	3.50	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aA (winter)	Ibuprofen	3.25	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aB (summer)	Ibuprofen	3.50	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aB (winter)	Ibuprofen	3.25	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aC (summer)	Ibuprofen	3.25	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aC (winter)	Ibuprofen	3.25	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aD (summer)	Ibuprofen	3.25	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aD (winter)	Ibuprofen	3.25	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aE (summer)	Ibuprofen	3.25	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aE (winter)	Ibuprofen	3.25	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aF (summer)	Ibuprofen	3.25	5.50	warm	HSSF-CW
Hijosa-Valsero et al., 2010aF (winter)	Ibuprofen	3.25	5.50	cold	HSSF-CW
Hijosa-Valsero et al., 2010aG (summer)	Ibuprofen	3.25	5.50	warm	HSSF-CW
Hijosa-Valsero et al., 2010aG (winter)	Ibuprofen	3.00	5.50	cold	HSSF-CW
Matamoros et al., 2009A	Ibuprofen	3.75	4.50	warm	HSSF-CW
Matamoros et al., 2009B	Ibuprofen	3.75	4.50	warm	VSSF-CW
Avila et al., 2015	Ibuprofen	4.00	6.50	warm	H-CW
Zhu et al., 2014	Ibuprofen	4.00	7.50	warm	H-CW
Hijosa-Valsero et al., 2010b	Ibuprofen	3.50	6.50	warm	H-CW
Hijosa-Valsero et al., 2010b	Ibuprofen	4.00	6.50	warm	H-CW
Avila et al., 2014aA	Ibuprofen	4.25	7.00	warm	VSSF-CW
Avila et al., 2014aB	Ibuprofen	4.25	7.00	warm	VSSF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Avila et al., 2014aC	Ibuprofen	4.25	7.00	warm	VSSF-CW
Avila et al., 2014aD	Ibuprofen	4.50	7.00	warm	VSSF-CW
Moeder et al., 2017	Ibuprofen	4.00	4.25	warm	SF-CW
Reyes-Contreras et al., 2011	Ibuprofen	3.25	6.50	warm	H-CW
Matamoros et al., 2016A	Ibuprofen	3.25	5.50	warm	HSSF-CW
Matamoros et al., 2016B	Ibuprofen	1.75	5.50	cold	HSSF-CW
Camacho-Muñoz et al., 2012	Ibuprofen	3.75	5.25	warm	VSSF-CW
Vymazal et al., 2017A	Ibuprofen	2.00	4.25	warm	HSSF-CW
Vymazal et al., 2017B	Ibuprofen	3.75	4.25	warm	HSSF-CW
Vymazal et al., 2017C	Ibuprofen	3.50	4.25	warm	HSSF-CW
Vymazal et al., 2017D	Ibuprofen	3.50	4.25	warm	HSSF-CW
Carranza-Diaz et al., 2014A	Ibuprofen	0.00	6.25	warm	HSSF-CW
Carranza-Diaz et al., 2014B	Ibuprofen	0.00	6.25	warm	HSSF-CW
Nuel et al., 2018	Ibuprofen	3.50	7.00	warm	SF-CW
Breitholtz et al., 2012A	Ibuprofen	3.50	7.00	warm	SF-CW
Breitholtz et al., 2012B	Ibuprofen	4.00	7.00	warm	SF-CW
Breitholtz et al., 2012C	Ibuprofen	4.00	7.00	warm	SF-CW
Breitholtz et al., 2012D	Ibuprofen	1.25	7.00	warm	SF-CW
He et al., 2018A	Ibuprofen	2.25	5.50	warm	VSSF-CW
He et al., 2018B	Ibuprofen	0.00	5.50	warm	SF-CW
He et al., 2018C	Ibuprofen	3.00	5.50	warm	SF-CW
Verlicchi et al., 2013	Ibuprofen	3.75	7.50	warm	HSSF-CW
Kahl et al., 2017A (warm)	Ibuprofen	4.00	7.00	warm	HSSF-CW
Kahl et al., 2017A (cold)	Ibuprofen	1.50	7.00	cold	HSSF-CW
Kahl et al., 2017B (warm)	Ibuprofen	4.25	7.00	warm	VSSF-CW
Kahl et al., 2017B (cold)	Ibuprofen	4.25	7.00	cold	VSSF-CW
Kahl et al., 2017C (warm)	Ibuprofen	4.25	7.00	warm	VSSF-CW
Kahl et al., 2017C (cold)	Ibuprofen	4.25	7.00	cold	VSSF-CW
Kahl et al., 2017D (warm)	Ibuprofen	4.25	7.00	warm	VSSF-CW
Kahl et al., 2017D (cold)	Ibuprofen	4.25	7.00	cold	VSSF-CW
Kahl et al., 2017E (warm)	Ibuprofen	4.25	7.00	warm	HSSF-CW
Kahl et al., 2017E (cold)	Ibuprofen	4.25	7.00	cold	HSSF-CW
Avila et al., 2010	Ibuprofen	4.25	6.25	warm	HSSF-CW
Avila et al., 2014bA	Ibuprofen	4.75	6.25	warm	H-CW
Avila et al., 2014bB	Ibuprofen	4.75	6.25	warm	H-CW
Avila et al., 2014bC	Ibuprofen	4.75	6.25	warm	H-CW
Zhang et al., 2011A	Ibuprofen	3.50	5.75	warm	HSSF-CW
Zhang et al., 2011B	Ibuprofen	3.75	5.75	warm	HSSF-CW
Matamoros et al., 2005A	Ibuprofen	3.25	5.75	warm	HSSF-CW
Matamoros et al., 2005B	Ibuprofen	3.25	5.75	warm	HSSF-CW
Zhang et al., 2016A	Ibuprofen	4.00	6.50	warm	HSSF-CW
Zhang et al., 2016B	Ibuprofen	3.75	6.50	warm	HSSF-CW
Dordio et al., 2010A	Ibuprofen	3.75	6.25	warm	VSSF-CW
Dordio et al., 2010C	Ibuprofen	3.75	6.25	cold	VSSF-CW
Matamoros et al., 2008	Ibuprofen	1.00	6.00	warm	HSSF-CW
Conkle et al., 2008	Ibuprofen	4.50	4.50	warm	H-CW
Park et al., 2018	Ibuprofen	0.00	4.75	warm	SF-CW
De Oliveira et al., 2019A	Ibuprofen	4.50	4.50	warm	VSSF-CW
De Oliveira et al., 2019B	Ibuprofen	5.00	4.50	warm	HSSF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Vystavna et al., 2017A	Ibuprofen	4.25	6.00	warm	H-CW
Vystavna et al., 2017B	Ibuprofen	4.25	6.00	warm	H-CW
Matamoros et al., 2007	Ibuprofen	2.50	6.50	warm	HSSF-CW
Dordio et al., 2009a	Ibuprofen	3.75	5.25	warm	HSSF-CW
Lancheros et al., 2019	Ibuprofen	3.75	6.00	warm	VSSF-CW
Zhang et al., 2012	Ibuprofen	3.75	6.00	warm	HSSF-CW
Brezinova et al., 2018A	Ibuprofen	2.00	4.25	warm	HSSF-CW
Brezinova et al., 2018B	Ibuprofen	3.75	4.25	warm	HSSF-CW
Brezinova et al., 2018C	Ibuprofen	3.50	4.25	warm	HSSF-CW
Brezinova et al., 2018D	Ibuprofen	3.50	4.25	warm	HSSF-CW
Ozengin et al., 2016A	Ibuprofen	3.75	4.75	warm	HSSF-CW
Ozengin et al., 2016B	Ibuprofen	3.75	4.75	warm	HSSF-CW
MEAN(SE)		**3.6(0.1)**	**5.9(0.1)**		
Matamoros et al., 2006A	Ketoprofen	0.00	5.75	warm	HSSF-CW
Matamoros et al., 2006B	Ketoprofen	0.00	5.75	warm	HSSF-CW
Matamoros et al., 2006C	Ketoprofen	3.25	5.75	warm	HSSF-CW
Matamoros et al., 2006D	Ketoprofen	2.50	5.75	warm	HSSF-CW
Matamoros et al., 2006E	Ketoprofen	0.00	5.75	warm	HSSF-CW
Chen et al., 2016A (summer)	Ketoprofen	4.25	5.50	warm	HSSF-CW
Chen et al., 2016A (winter)	Ketoprofen	4.00	5.50	cold	HSSF-CW
Chen et al., 2016B (summer)	Ketoprofen	4.50	5.50	warm	HSSF-CW
Chen et al., 2016B (winter)	Ketoprofen	4.50	5.50	warm	HSSF-CW
Chen et al., 2016C (summer)	Ketoprofen	4.50	5.50	warm	HSSF-CW
Chen et al., 2016C (winter)	Ketoprofen	4.50	5.50	cold	HSSF-CW
Matamoros et al., 2008bA	Ketoprofen	4.00	7.25	warm	SF-CW
Matamoros et al., 2008bB	Ketoprofen	4.00	7.25	cold	SF-CW
Hijosa-Valsero et al., 2010aA (summer)	Ketoprofen	NA	NA	warm	SF-CW
Hijosa-Valsero et al., 2010aA (winter)	Ketoprofen	3.00	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aB (summer)	Ketoprofen	NA	NA	warm	SF-CW
Hijosa-Valsero et al., 2010aB (winter)	Ketoprofen	3.00	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aC (summer)	Ketoprofen	NA	NA	warm	SF-CW
Hijosa-Valsero et al., 2010aC (winter)	Ketoprofen	3.25	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aD (summer)	Ketoprofen	NA	NA	warm	SF-CW
Hijosa-Valsero et al., 2010aD (winter)	Ketoprofen	3.00	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aE (summer)	Ketoprofen	NA	NA	warm	SF-CW
Hijosa-Valsero et al., 2010aE (winter)	Ketoprofen	3.00	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aF (summer)	Ketoprofen	NA	NA	warm	HSSF-CW
Hijosa-Valsero et al., 2010aF (winter)	Ketoprofen	3.00	5.50	cold	HSSF-CW
Hijosa-Valsero et al., 2010aG (summer)	Ketoprofen	NA	NA	warm	HSSF-CW
Hijosa-Valsero et al., 2010aG (winter)	Ketoprofen	2.75	5.50	cold	HSSF-CW
Matamoros et al., 2009A	Ketoprofen	2.00	4.50	warm	HSSF-CW
Matamoros et al., 2009B	Ketoprofen	0.00	4.50	warm	VSSF-CW
Hijosa-Valsero et al., 2010bA	Ketoprofen	NA	NA	warm	H-CW
Hijosa-Valsero et al., 2010bB	Ketoprofen	4.00	6.50	warm	H-CW
Moeder et al., 2017	Ketoprofen	2.00	4.25	warm	SF-CW
Reyes-Contreras et al., 2011	Ketoprofen	3.25	6.50	warm	H-CW
Matamoros et al., 2016A	Ketoprofen	3.25	5.50	warm	HSSF-CW
Matamoros et al., 2016B	Ketoprofen	0.00	5.50	cold	HSSF-CW
Vymazal et al., 2017A	Ketoprofen	4.00	4.25	warm	HSSF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Vymazal et al., 2017B	Ketoprofen	3.50	4.25	warm	HSSF-CW
Vymazal et al., 2017C	Ketoprofen	0.00	4.25	warm	HSSF-CW
Vymazal et al., 2017D	Ketoprofen	3.25	4.25	warm	HSSF-CW
Carranza-Diaz et al., 2014A	Ketoprofen	0.00	6.25	warm	HSSF-CW
Carranza-Diaz et al., 2014B	Ketoprofen	0.00	6.25	warm	HSSF-CW
Nuel et al., 2018	Ketoprofen	3.50	7.00	warm	SF-CW
Breitholtz et al., 2012A	Ketoprofen	3.75	7.00	warm	SF-CW
Breitholtz et al., 2012B	Ketoprofen	1.25	7.00	warm	SF-CW
Breitholtz et al., 2012C	Ketoprofen	3.50	7.00	warm	SF-CW
Breitholtz et al., 2012D	Ketoprofen	2.75	7.00	warm	SF-CW
He et al., 2018A	Ketoprofen	NA	NA	warm	VSSF-CW
He et al., 2018B	Ketoprofen	0.00	5.50	warm	SF-CW
He et al., 2018C	Ketoprofen	NA	NA	warm	SF-CW
Verlicchi et al., 2013	Ketoprofen	3.50	7.50	warm	HSSF-CW
Mathon et al., 2019	Ketoprofen	3.75	5.00	warm	SF-CW
Vystavna et al., 2017A	Ketoprofen	4.25	6.00	warm	H-CW
Vystavna et al., 2017B	Ketoprofen	NA	NA	warm	H-CW
Matamoros et al., 2007	Ketoprofen	2.25	6.50	warm	HSSF-CW
Francini et al., 2018A	Ketoprofen	4.50	6.25	warm	SF-CW
Francini et al., 2018B	Ketoprofen	4.25	6.25	warm	SF-CW
Francini et al., 2018C	Ketoprofen	4.25	6.25	warm	SF-CW
Francini et al., 2018D	Ketoprofen	4.25	6.25	warm	SF-CW
Zhang et al., 2012	Ketoprofen	4.00	6.00	warm	HSSF-CW
Avila et al., 2021A	Ketoprofen	4.00	6.50	warm	HSSF-CW
Avila et al., 2021B	Ketoprofen	4.25	6.50	warm	HSSF-CW
MEAN(SE)		**2.9(0.2)**	**5.8(0.1)**		
He et al., 2018A	Lincomycin	3.00	5.50	warm	VSSF-CW
He et al., 2018B	Lincomycin	0.00	5.50	warm	SF-CW
He et al., 2018C	Lincomycin	0.00	5.50	warm	SF-CW
Avila et al., 2014bA	Lincomycin	NA	NA	warm	H-CW
Avila et al., 2014bB	Lincomycin	NA	NA	warm	H-CW
Avila et al., 2014bC	Lincomycin	4.00	6.25	warm	H-CW
Sabri et al., 2021A (summer)	Lincomycin	0.00	4.50	warm	H-CW
Sabri et al., 2021A (winter)	Lincomycin	5.00	4.50	cold	H-CW
Sabri et al., 2021B (summer)	Lincomycin	0.00	4.50	warm	H-CW
Sabri et al., 2021B (winter)	Lincomycin	4.75	4.50	cold	H-CW
MEAN(SE)		**2.1(0.2)**	**5.1(0.2)**		
Dan et al., 2020aA (summer)	Lomefloxacin	NA	NA	warm	VSSF-CW
Dan et al., 2020aA (winter)	Lomefloxacin	4.00	6.50	cold	VSSF-CW
Dan et al., 2020aB (summer)	Lomefloxacin	NA	NA	warm	VSSF-CW
Dan et al., 2020aB (winter)	Lomefloxacin	NA	NA	cold	VSSF-CW
Dan et al., 2020aC (summer)	Lomefloxacin	NA	NA	warm	VSSF-CW
Dan et al., 2020aC (winter)	Lomefloxacin	NA	NA	cold	VSSF-CW
Dan et al., 2020aD (summer)	Lomefloxacin	NA	NA	warm	SF-CW
Dan et al., 2020aD (winter)	Lomefloxacin	3.75	6.50	cold	SF-CW
Dan et al., 2020aE (summer)	Lomefloxacin	NA	NA	warm	SF-CW
Dan et al., 2020aE (winter)	Lomefloxacin	NA	NA	cold	SF-CW
Dan et al., 2020aF (summer)	Lomefloxacin	NA	NA	warm	SF-CW
Dan et al., 2020aF (winter)	Lomefloxacin	NA	NA	cold	SF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Dan et al., 2020aG (summer)	Lomefloxacin	NA	NA	warm	HSSF-CW
Dan et al., 2020aG (winter)	Lomefloxacin	3,75	6.50	cold	HSSF-CW
Dan et al., 2020aH (summer)	Lomefloxacin	NA	NA	warm	HSSF-CW
Dan et al., 2020aH (winter)	Lomefloxacin	NA	NA	cold	HSSF-CW
Dan et al., 2020aI (summer)	Lomefloxacin	NA	NA	warm	HSSF-CW
Dan et al., 2020aI (winter)	Lomefloxacin	NA	NA	cold	HSSF-CW
MEAN(SE)		**3.9(0.1)**	**6.5(0.0)**		
Chen et al., 2016A (summer)	Metoprolol	4.25	5.50	warm	HSSF-CW
Chen et al., 2016A (winter)	Metoprolol	4.25	5.50	cold	HSSF-CW
Chen et al., 2016B (summer)	Metoprolol	4.25	5.50	warm	HSSF-CW
Chen et al., 2016B (winter)	Metoprolol	4.50	5.50	warm	HSSF-CW
Chen et al., 2016C (summer)	Metoprolol	4.50	5.50	warm	HSSF-CW
Chen et al., 2016C (winter)	Metoprolol	4.50	5.50	cold	HSSF-CW
Ruhmland et al., 2015A	Metoprolol	3.75	6.75	warm	HSSF-CW
Ruhmland et al., 2015B	Metoprolol	3.75	6.75	warm	HSSF-CW
Ruhmland et al., 2015C	Metoprolol	4.00	6.75	cold	HSSF-CW
Vymazal et al., 2017A	Metoprolol	3.75	4.25	warm	HSSF-CW
Vymazal et al., 2017B	Metoprolol	3.75	4.25	warm	HSSF-CW
Vymazal et al., 2017C	Metoprolol	3.50	4.25	warm	HSSF-CW
Vymazal et al., 2017D	Metoprolol	3.50	4.25	warm	HSSF-CW
Nuel et al., 2018	Metoprolol	1.25	7.00	warm	SF-CW
Breitholtz et al., 2012A	Metoprolol	0.00	7.00	warm	SF-CW
Breitholtz et al., 2012B	Metoprolol	3.50	7.00	warm	SF-CW
Breitholtz et al., 2012C	Metoprolol	3.25	7.00	warm	SF-CW
Breitholtz et al., 2012D	Metoprolol	3.50	7.00	warm	SF-CW
He et al., 2018A	Metoprolol	3.50	5.50	warm	VSSF-CW
He et al., 2018B	Metoprolol	1.75	5.50	warm	SF-CW
He et al., 2018C	Metoprolol	3.25	5.50	warm	SF-CW
Verlicchi et al., 2013	Metoprolol	2.50	7.50	warm	HSSF-CW
Conkle et al., 2008	Metoprolol	4.50	4.50	warm	H-CW
Mathon et al., 2019	Metoprolol	3.25	5.00	warm	SF-CW
Ruppelt et al., 2020A	Metoprolol	4.50	3.75	warm	VSSF-CW
Ruppelt et al., 2020B	Metoprolol	4.75	3.75	warm	VSSF-CW
Auvinen et al., 2017bA	Metoprolol	4.50	5.50	warm	H-CW
Auvinen et al., 2017bB	Metoprolol	4.50	5.50	warm	SF-CW
Anderson et al., 2020A	Metoprolol	4.00	4.25	warm	H-CW
Anderson et al., 2020B	Metoprolol	4.00	4.25	warm	H-CW
Stroski et al., 2020A	Metoprolol	4.50	4.75	cold	SF-CW
Stroski et al., 2020B	Metoprolol	4.50	4.75	cold	SF-CW
Chaves-Barquero et al., 2016	Metoprolol	1.00	4.75	cold	SF-CW
Chaves-Barquero et al., 2018	Metoprolol	NA	NA	warm	VSSF-CW
Chaves-Barquero et al., 2021	Metoprolol	4.25	5.75	warm	VSSF-CW
MEAN(SE)		**3.6(0.2)**	**5.5(0.2)**		
Matamoros et al., 2006A	Naproxen	0.00	5.75	warm	HSSF-CW
Matamoros et al., 2006B	Naproxen	3.25	5.75	warm	HSSF-CW
Matamoros et al., 2006C	Naproxen	3.75	5.75	warm	HSSF-CW
Matamoros et al., 2006D	Naproxen	2.75	5.75	warm	HSSF-CW
Matamoros et al., 2006E	Naproxen	2.75	5.75	warm	HSSF-CW
Chen et al., 2016A (summer)	Naproxen	4.25	5.50	warm	HSSF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Chen et al., 2016A (winter)	Naproxen	3.50	5.50	cold	HSSF-CW
Chen et al., 2016B (summer)	Naproxen	3.50	5.50	warm	HSSF-CW
Chen et al., 2016B (winter)	Naproxen	3.25	5.50	warm	HSSF-CW
Chen et al., 2016C (summer)	Naproxen	4.50	5.50	warm	HSSF-CW
Chen et al., 2016C (winter)	Naproxen	4.50	5.50	cold	HSSF-CW
Cardinal et al., 2014	Naproxen	3.50	6.25	warm	SF-CW
Matamoros et al., 2008bA	Naproxen	4.00	7.25	warm	SF-CW
Matamoros et al., 2008bB	Naproxen	3.75	7.25	cold	SF-CW
Matamoros et al., 2007A	Naproxen	4.50	7.00	warm	SF-CW
Matamoros et al., 2007B	Naproxen	4.75	7.00	warm	SF-CW
Matamoros et al., 2007C	Naproxen	4.50	7.00	warm	VSSF-CW
Matamoros et al., 2007D	Naproxen	4.75	7.00	warm	VSSF-CW
Hijosa-Valsero et al., 2010aA (summer)	Naproxen	3.25	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aA (winter)	Naproxen	3.00	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aB (summer)	Naproxen	3.25	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aB (winter)	Naproxen	3.00	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aC (summer)	Naproxen	3.25	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aC (winter)	Naproxen	3.25	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aD (summer)	Naproxen	3.25	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aD (winter)	Naproxen	3.25	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aE (summer)	Naproxen	3.25	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aE (winter)	Naproxen	3.25	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aF (summer)	Naproxen	3.25	5.50	warm	HSSF-CW
Hijosa-Valsero et al., 2010aF (winter)	Naproxen	3.00	5.50	cold	HSSF-CW
Hijosa-Valsero et al., 2010aG (summer)	Naproxen	3.50	5.50	warm	HSSF-CW
Hijosa-Valsero et al., 2010aG (winter)	Naproxen	3.00	5.50	cold	HSSF-CW
Matamoros et al., 2009A	Naproxen	1.50	4.50	warm	HSSF-CW
Matamoros et al., 2009B	Naproxen	3.75	4.50	warm	VSSF-CW
Zhu et al., 2014	Naproxen	0.00	7.50	warm	H-CW
Hijosa-Valsero et al., 2010bA	Naproxen	4.00	6.50	warm	H-CW
Hijosa-Valsero et al., 2010bB	Naproxen	3.75	6.50	warm	H-CW
Moeder et al., 2017	Naproxen	4.00	4.25	warm	SF-CW
Reyes-Contreras et al., 2011	Naproxen	3.25	6.50	warm	H-CW
Matamoros et al., 2016A	Naproxen	3.50	5.50	warm	HSSF-CW
Matamoros et al., 2016B	Naproxen	3.25	5.50	cold	HSSF-CW
Camacho-Muñoz et al., 2012	Naproxen	4.00	5.25	warm	VSSF-CW
Carranza-Diaz et al., 2014A	Naproxen	0.00	6.25	warm	HSSF-CW
Carranza-Diaz et al., 2014B	Naproxen	0.00	6.25	warm	HSSF-CW
Nuel et al., 2018	Naproxen	1.25	7.00	warm	SF-CW
Breitholtz et al., 2012A	Naproxen	3.50	7.00	warm	SF-CW
Breitholtz et al., 2012B	Naproxen	3.50	7.00	warm	SF-CW
Breitholtz et al., 2012C	Naproxen	4.00	7.00	warm	SF-CW
Breitholtz et al., 2012D	Naproxen	3.75	7.00	warm	SF-CW
He et al., 2018A	Naproxen	3.25	5.50	warm	VSSF-CW
He et al., 2018B	Naproxen	1.75	5.50	warm	SF-CW
He et al., 2018C	Naproxen	2.25	5.50	warm	SF-CW
Verlicchi et al., 2013	Naproxen	3.75	7.50	warm	HSSF-CW
Kahl et al., 2017A (warm)	Naproxen	4.25	7.00	warm	HSSF-CW
Kahl et al., 2017A (cold)	Naproxen	1.50	7.00	cold	HSSF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Kahl et al., 2017B (warm)	Naproxen	4.25	7.00	warm	VSSF-CW
Kahl et al., 2017B (cold)	Naproxen	4.25	7.00	cold	VSSF-CW
Kahl et al., 2017C (warm)	Naproxen	4.25	7.00	warm	VSSF-CW
Kahl et al., 2017C (cold)	Naproxen	4.25	7.00	cold	VSSF-CW
Kahl et al., 2017D (warm)	Naproxen	4.25	7.00	warm	VSSF-CW
Kahl et al., 2017D (cold)	Naproxen	4.25	7.00	cold	VSSF-CW
Kahl et al., 2017E (warm)	Naproxen	4.25	7.00	warm	HSSF-CW
Kahl et al., 2017E (cold)	Naproxen	4.25	7.00	cold	HSSF-CW
Avila et al., 2010	Naproxen	4.25	6.25	warm	HSSF-CW
Zhang et al., 2011A	Naproxen	3.75	5.75	warm	HSSF-CW
Zhang et al., 2011B	Naproxen	3.75	5.75	warm	HSSF-CW
Conkle et al., 2008	Naproxen	4.50	4.50	warm	H-CW
Vystavna et al., 2017A	Naproxen	NA	NA	warm	H-CW
Vystavna et al., 2017B	Naproxen	4.50	6.00	warm	H-CW
Matamoros et al., 2007	Naproxen	4.75	6.50	warm	HSSF-CW
Park et al., 2009	Naproxen	5.00	3.75	warm	SF-CW
Lancheros et al., 2019	Naproxen	3.75	6.00	warm	VSSF-CW
Cardinal et al., 2016	Naproxen	3.50	6.75	warm	SF-CW
Zhang et al., 2012	Naproxen	4.00	6.00	warm	HSSF-CW
Anderson et al., 2020A	Naproxen	4.00	4.25	warm	H-CW
Anderson et al., 2020B	Naproxen	3.50	4.25	warm	H-CW
Stroski et al., 2020A	Naproxen	NA	NA	cold	SF-CW
Stroski et al., 2020B	Naproxen	4.50	4.75	cold	SF-CW
Stroski et al., 2020C	Naproxen	4.50	4.75	cold	SF-CW
MEAN(SE)		**3.5(0.1)**	**6.0(0.1)**		
Berglund et al., 2014	Norfloxacin	3.75	5.50	warm	SF-CW
Dan et al., 2020aA (summer)	Norfloxacin	0.00	6.50	warm	VSSF-CW
Dan et al., 2020aA (winter)	Norfloxacin	0.00	6.50	cold	VSSF-CW
Dan et al., 2020aB (summer)	Norfloxacin	0.00	6.50	warm	VSSF-CW
Dan et al., 2020aB (winter)	Norfloxacin	0.00	6.50	cold	VSSF-CW
Dan et al., 2020aC (summer)	Norfloxacin	0.00	6.50	warm	VSSF-CW
Dan et al., 2020aC (winter)	Norfloxacin	0.00	6.50	cold	VSSF-CW
Dan et al., 2020aD (summer)	Norfloxacin	3.75	6.50	warm	SF-CW
Dan et al., 2020aD (winter)	Norfloxacin	1.50	6.50	cold	SF-CW
Dan et al., 2020aE (summer)	Norfloxacin	3.75	6.50	warm	SF-CW
Dan et al., 2020aE (winter)	Norfloxacin	3.50	6.50	cold	SF-CW
Dan et al., 2020aF (summer)	Norfloxacin	4.00	6.50	warm	SF-CW
Dan et al., 2020aF (winter)	Norfloxacin	4.00	6.50	cold	SF-CW
Dan et al., 2020aG (summer)	Norfloxacin	3.50	6.50	warm	HSSF-CW
Dan et al., 2020aG (winter)	Norfloxacin	0.00	6.50	cold	HSSF-CW
Dan et al., 2020aH (summer)	Norfloxacin	3.50	6.50	warm	HSSF-CW
Dan et al., 2020aH (winter)	Norfloxacin	3.50	6.50	cold	HSSF-CW
Dan et al., 2020aI (summer)	Norfloxacin	3.50	6.50	warm	HSSF-CW
Dan et al., 2020aI (winter)	Norfloxacin	3.25	6.50	cold	HSSF-CW
Verlicchi et al., 2013	Norfloxacin	4.25	7.50	warm	HSSF-CW
MEAN(SE)		**2.3(0.4)**	**6.5(0.4)**		
Nuel et al., 2018	Ofloxacin	0.00	7.00	warm	SF-CW
Dan et al., 2020aA (summer)	Ofloxacin	4.25	6.50	warm	VSSF-CW
Dan et al., 2020aA (winter)	Ofloxacin	4.00	6.50	cold	VSSF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Dan et al., 2020aB (summer)	Ofloxacin	3.25	6.50	warm	VSSF-CW
Dan et al., 2020aB (winter)	Ofloxacin	3.75	6.50	cold	VSSF-CW
Dan et al., 2020aC (summer)	Ofloxacin	3.75	6.50	warm	VSSF-CW
Dan et al., 2020aC (winter)	Ofloxacin	3.75	6.50	cold	VSSF-CW
Dan et al., 2020aD (summer)	Ofloxacin	3.75	6.50	warm	SF-CW
Dan et al., 2020aD (winter)	Ofloxacin	3.75	6.50	cold	SF-CW
Dan et al., 2020aE (summer)	Ofloxacin	3.75	6.50	warm	SF-CW
Dan et al., 2020aE (winter)	Ofloxacin	3.75	6.50	cold	SF-CW
Dan et al., 2020aF (summer)	Ofloxacin	4.25	6.50	warm	SF-CW
Dan et al., 2020aF (winter)	Ofloxacin	4.25	6.50	cold	SF-CW
Dan et al., 2020aG (summer)	Ofloxacin	3.75	6.50	warm	HSSF-CW
Dan et al., 2020aG (winter)	Ofloxacin	3.75	6.50	cold	HSSF-CW
Dan et al., 2020aH (summer)	Ofloxacin	3.75	6.50	warm	HSSF-CW
Dan et al., 2020aH (winter)	Ofloxacin	3.75	6.50	cold	HSSF-CW
Dan et al., 2020aI (summer)	Ofloxacin	3.75	6.50	warm	HSSF-CW
Dan et al., 2020aI (winter)	Ofloxacin	3.75	6.50	cold	HSSF-CW
Verlicchi et al., 2013	Ofloxacin	4.25	7.50	warm	HSSF-CW
MEAN(SE)		**3.7(0.2)**	**6.6(0.1)**		
Ruhmland et al., 2015A	Oxazepam	1.25	6.75	warm	HSSF-CW
Ruhmland et al., 2015B	Oxazepam	2.00	6.75	warm	HSSF-CW
Ruhmland et al., 2015C	Oxazepam	1.75	6.75	cold	HSSF-CW
Breitholtz et al., 2012A	Oxazepam	NA	NA	warm	SF-CW
Breitholtz et al., 2012B	Oxazepam	0.00	7.00	warm	SF-CW
Breitholtz et al., 2012C	Oxazepam	NA	NA	warm	SF-CW
Breitholtz et al., 2012D	Oxazepam	2.75	7.00	warm	SF-CW
MEAN(SE)		**1.5(0.5)**	**6.9(0.1)**		
Moeder et al., 2017	Propranolol	0.00	4.25	warm	SF-CW
He et al., 2018A	Propranolol	3.50	5.50	warm	VSSF-CW
He et al., 2018B	Propranolol	0.00	5.50	warm	SF-CW
He et al., 2018C	Propranolol	3.25	5.50	warm	SF-CW
Verlicchi et al., 2013	Propranolol	3.75	7.50	warm	HSSF-CW
Mathon et al., 2019	Propranolol	3.75	5.00	warm	SF-CW
Vystavna et al., 2017A	Propranolol	4.50	6.00	warm	H-CW
Vystavna2017B	Propranolol	4.50	6.00	warm	H-CW
Chaves-Barquero et al., 2018	Propranolol	NA	NA	warm	VSSF-CW
MEAN(SE)		**2.9(1.7)**	**5.7(0.9)**		
Breitholtz et al., 2012A	Ranitidine	0.00	7.00	warm	SF-CW
Breitholtz et al., 2012B	Ranitidine	4.00	7.00	warm	SF-CW
Breitholtz et al., 2012C	Ranitidine	4.00	7.00	warm	SF-CW
Breitholtz et al., 2012D	Ranitidine	3.75	7.00	warm	SF-CW
Verlicchi et al., 2013	Ranitidine	3.75	7.50	warm	HSSF-CW
MEAN(SE)		**3.1(0.7)**	**7.1(0.1)**		
Dan et al., 2020aA (summer)	Roxithromycin	4.00	6.50	warm	VSSF-CW
Dan et al., 2020aA (winter)	Roxithromycin	0.00	6.50	cold	VSSF-CW
Dan et al., 2020aB (summer)	Roxithromycin	3.50	6.50	warm	VSSF-CW
Dan et al., 2020aB (winter)	Roxithromycin	2.50	6.50	cold	VSSF-CW
Dan et al., 2020aC (summer)	Roxithromycin	3.75	6.50	warm	VSSF-CW
Dan et al., 2020aC (winter)	Roxithromycin	2.50	6.50	cold	VSSF-CW
Dan et al., 2020aD (summer)	Roxithromycin	3.75	6.50	warm	SF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Dan et al., 2020aD (winter)	Roxithromycin	3.25	6.50	cold	SF-CW
Dan et al., 2020aE (summer)	Roxithromycin	3.25	6.50	warm	SF-CW
Dan et al., 2020aE (winter)	Roxithromycin	0.00	6.50	cold	SF-CW
Dan et al., 2020aF (summer)	Roxithromycin	2.50	6.50	warm	SF-CW
Dan et al., 2020aF (winter)	Roxithromycin	3.00	6.50	cold	SF-CW
Dan et al., 2020aG (summer)	Roxithromycin	3.50	6.50	warm	HSSF-CW
Dan et al., 2020aG (winter)	Roxithromycin	3.50	6.50	cold	HSSF-CW
Dan et al., 2020aH (summer)	Roxithromycin	3.50	6.50	warm	HSSF-CW
Dan et al., 2020aH (winter)	Roxithromycin	3.25	6.50	cold	HSSF-CW
Dan et al., 2020aI (summer)	Roxithromycin	3.50	6.50	warm	HSSF-CW
Dan et al., 2020aI (winter)	Roxithromycin	0.00	6.50	cold	HSSF-CW
Verlicchi et al., 2013	Roxithromycin	0.00	7.50	warm	HSSF-CW
MEAN(SE)		**2.6(0.3)**	**6.6(0.1)**		
Matamoros et al., 2006A	Salicylic acid	3.75	5.75	warm	HSSF-CW
Matamoros et al., 2006B	Salicylic acid	3.75	5.75	warm	HSSF-CW
Matamoros et al., 2006C	Salicylic acid	3.75	5.75	warm	HSSF-CW
Matamoros et al., 2006D	Salicylic acid	3.75	5.75	warm	HSSF-CW
Matamoros et al., 2006E	Salicylic acid	3.75	5.75	warm	HSSF-CW
Matamoros et al., 2007A	Salicylic acid	4.75	7.00	warm	SF-CW
Matamoros et al., 2007B	Salicylic acid	4.75	7.00	warm	SF-CW
Matamoros et al., 2007C	Salicylic acid	4.75	7.00	warm	VSSF-CW
Matamoros et al., 2007D	Salicylic acid	4.75	7.00	warm	VSSF-CW
Hijosa-Valsero et al., 2010aA (summer)	Salicylic acid	3.50	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aA (winter)	Salicylic acid	3.50	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aB (summer)	Salicylic acid	3.50	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aB (winter)	Salicylic acid	3.25	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aC (summer)	Salicylic acid	3.50	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aC (winter)	Salicylic acid	3.50	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aD (summer)	Salicylic acid	3.50	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aD (winter)	Salicylic acid	3.25	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aE (summer)	Salicylic acid	3.50	5.50	warm	SF-CW
Hijosa-Valsero et al., 2010aE (winter)	Salicylic acid	3.25	5.50	cold	SF-CW
Hijosa-Valsero et al., 2010aF (summer)	Salicylic acid	3.50	5.50	warm	HSSF-CW
Hijosa-Valsero et al., 2010aF (winter)	Salicylic acid	3.25	5.50	cold	HSSF-CW
Hijosa-Valsero et al., 2010aG (summer)	Salicylic acid	3.50	5.50	warm	HSSF-CW
Hijosa-Valsero et al., 2010aG (winter)	Salicylic acid	3.25	5.50	cold	HSSF-CW
Matamoros et al., 2009A	Salicylic acid	4.50	4.50	warm	HSSF-CW
Matamoros et al., 2009B	Salicylic acid	3.75	4.50	warm	VSSF-CW
Hijosa-Valsero et al., 2010bA	Salicylic acid	4.00	6.50	warm	H-CW
Hijosa-Valsero et al., 2010bB	Salicylic acid	4.00	6.50	warm	H-CW
Reyes-Contreras et al., 2011	Salicylic acid	3.50	6.50	warm	H-CW
Camacho-Muñoz et al., 2012	Salicylic acid	4.00	5.25	warm	VSSF-CW
Verlicchi et al., 2013	Salicylic acid	2.50	7.50	warm	HSSF-CW
Zhang et al., 2012	Salycylic acid	4.00	6.00	warm	HSSF-CW
MEAN(SE)		**3.7(0.1)**	**5.8(0.1)**		
Nuel et al., 2018	Sotalol	1.25	7.00	warm	SF-CW
Breitholtz et al., 2012A	Sotalol	2.75	7.00	warm	SF-CW
Breitholtz et al., 2012B	Sotalol	0.00	7.00	warm	SF-CW
Breitholtz et al., 2012C	Sotalol	1.75	7.00	warm	SF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Breitholtz et al., 2012D	Sotalol	0.00	7.00	warm	SF-CW
Verlicchi et al., 2013	Sotalol	1.50	7.50	warm	HSSF-CW
Conkle et al., 2008	Sotalol	4.50	4.50	warm	H-CW
Mathon et al., 2019	Sotalol	3.50	5.00	warm	SF-CW
Auvinen et al., 2017bA	Sotalol	4.50	5.50	warm	H-CW
Auvinen et al., 2017bB	Sotalol	4.50	5.50	warm	SF-CW
MEAN(SE)		**2.4(0.5)**	**6.3(0.3)**		
Nuel et al., 2018	Sulfadiazine	0.00	7.00	warm	SF-CW
Verlicchi et al., 2013	Sulfadiazine	0.00	7.50	warm	HSSF-CW
Dan et al., 2020bA	Sulfadiazine	4.75	7.25	warm	H-CW
Dan et al., 2020bB	Sulfadiazine	4.50	7.25	warm	H-CW
Ozengin et al., 2016A	Sulfadiazine	3.50	4.75	warm	HSSF-CW
Ozengin et al., 2016B	Sulfadiazine	3.75	4.75	warm	HSSF-CW
Sabri et al., 2021A (summer)	Sulfadiazine	0.00	4.50	warm	H-CW
Sabri et al., 2021A (winter)	Sulfadiazine	0.00	4.50	cold	H-CW
Sabri et al., 2021B (summer)	Sulfadiazine	0.00	4.50	warm	H-CW
Sabri et al., 2021B (winter)	Sulfadiazine	0.00	4.50	cold	H-CW
MEAN(SE)		**1.7(0.6)**	**6.3(0.3)**		
Anderson et al., 2013	Sulfamethoxazole	0.00	5.00	warm	SF-CW
Cardinal et al., 2014	Sulfamethoxazole	3.50	6.25	warm	SF-CW
Berglund et al., 2014	Sulfamethoxazole	3.75	5.50	warm	SF-CW
Ruhmland et al., 2015A	Sulfamethoxazole	3.75	6.75	warm	HSSF-CW
Ruhmland et al., 2015B	Sulfamethoxazole	3.75	6.75	warm	HSSF-CW
Ruhmland et al., 2015C	Sulfamethoxazole	0.00	6.75	cold	HSSF-CW
Sgroi et al., 2018A	Sulfamethoxazole	3.25	6.50	warm	VSSF-CW
Sgroi et al., 2018B	Sulfamethoxazole	2.50	6.50	warm	H-CW
Zhu et al., 2014	Sulfamethoxazole	1.25	7.50	warm	H-CW
Button et al., 2019	Sulfamethoxazole	4.00	5.50	warm	VSSF-CW
Camacho-Muñoz et al., 2012	Sulfamethoxazole	0.00	5.25	warm	VSSF-CW
Nuel et al., 2018	Sulfamethoxazole	0.00	7.00	warm	SF-CW
Breitholtz et al., 2012A	Sulfamethoxazole	0.00	7.00	warm	SF-CW
Breitholtz et al., 2012B	Sulfamethoxazole	0.00	7.00	warm	SF-CW
Breitholtz et al., 2012C	Sulfamethoxazole	2.75	7.00	warm	SF-CW
Breitholtz et al., 2012D	Sulfamethoxazole	0.00	7.00	warm	SF-CW
He et al., 2018A	Sulfamethoxazole	3.50	5.50	warm	VSSF-CW
He et al., 2018B	Sulfamethoxazole	1.75	5.50	warm	SF-CW
He et al., 2018C	Sulfamethoxazole	3.00	5.50	warm	SF-CW
Verlicchi et al., 2013	Sulfamethoxazole	3.00	7.50	warm	HSSF-CW
Avila et al., 2014bA	Sulfamethoxazole	NA	NA	warm	H-CW
Avila et al., 2014bB	Sulfamethoxazole	NA	NA	warm	H-CW
Avila et al., 2014bC	Sulfamethoxazole	4.25	6.25	warm	H-CW
Christofiloupoulos et al., 2020	Sulfamethoxazole	0.00	4.25	warm	HSSF-CW
Conkle et al., 2008	Sulfamethoxazole	4.50	4.50	warm	H-CW
Auvinen et al., 2017a	Sulfamethoxazole	3.25	5.00	warm	HSSF-CW
Mathon et al., 2019	Sulfamethoxazole	3.25	5.00	warm	SF-CW
Sochaki et al., 2018	Sulfamethoxazole	4.00	5.50	warm	H-CW
Park et al., 2009	Sulfamethoxazole	5.00	3.75	warm	SF-CW
Ruppelt et al., 2020A	Sulfamethoxazole	1.50	3.75	warm	VSSF-CW
Ruppelt et al., 2020B	Sulfamethoxazole	0.00	3.75	warm	VSSF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Cardinal et al., 2016	Sulfamethoxazole	3.50	6.75	warm	SF-CW
Dan et al., 2020bA	Sulfamethoxazole	4.25	7.25	warm	H-CW
Dan et al., 2020bB	Sulfamethoxazole	4.50	7.25	warm	H-CW
Anderson et al., 2020A	Sulfamethoxazole	0.00	4.25	warm	H-CW
Anderson et al., 2020B	Sulfamethoxazole	3.75	4.25	warm	H-CW
Stroski et al., 2020C	Sulfamethoxazole	4.25	4.75	cold	SF-CW
Chaves-Barquero et al., 2016	Sulfamethoxazole	3.50	4.75	cold	SF-CW
Chaves-Barquero et al., 2018	Sulfamethoxazole	0.00	6.50	warm	VSSF-CW
Sabri et al., 2021A (summer)	Sulfamethoxazole	2.25	4.50	warm	H-CW
Sabri et al., 2021A (winter)	Sulfamethoxazole	4.50	4.50	cold	H-CW
Sabri et al., 2021B (summer)	Sulfamethoxazole	0.00	4.50	warm	H-CW
Sabri et al., 2021B (winter)	Sulfamethoxazole	4.50	4.50	cold	H-CW
Chaves-Barquero et al., 2021	Sulfamethoxazole	0.00	5.75	warm	SF-CW
Chaves-Barquero et al., 2021	Sulfamethoxazole	0.00	5.75	warm	VSSF-CW
Anderson et al., 2015	Sulfamethoxazole	0.00	6.50	warm	VSSF-CW
MEAN(SE)		**2.3(0.3)**	**5.7(0.2)**		
Chen et al., 2016A (summer)	Sulfapyridine	3.75	5.50	warm	HSSF-CW
Chen et al., 2016A (winter)	Sulfapyridine	0.00	5.50	cold	HSSF-CW
Chen et al., 2016B (summer)	Sulfapyridine	3.50	5.50	warm	HSSF-CW
Cardinal et al., 2014	Sulfapyridine	3.50	6.25	warm	SF-CW
Conkle et al., 2008	Sulfapyridine	4.50	4.50	warm	H-CW
Cardinal et al., 2016	Sulfapyridine	3.50	6.75	warm	SF-CW
Anderson et al., 2020A	Sulfapyridine	1.75	4.25	warm	H-CW
Anderson et al., 2020B	Sulfapyridine	3.75	4.25	warm	H-CW
Stroski et al., 2020A	Sulfapyridine	4.50	4.75	cold	SF-CW
Sabri et al., 2021A (summer)	Sulfapyridine	2.25	4.50	warm	H-CW
Sabri et al., 2021A (winter)	Sulfapyridine	4.50	4.50	cold	H-CW
Sabri et al., 2021B (summer)	Sulfapyridine	2.25	4.50	warm	H-CW
Sabri et al., 2021B (winter)	Sulfapyridine	1.25	4.50	cold	H-CW
Anderson et al., 2015	Sulfapyridine	NA	6.50	warm	VSSF-CW
MEAN(SE)		**3.0(0.4)**	**5.0(0.2)**		
Breitholtz et al., 2012A	Telmisartan	3.75	7.00	warm	SF-CW
Breitholtz et al., 2012B	Telmisartan	3.75	7.00	warm	SF-CW
Breitholtz et al., 2012C	Telmisartan	1.25	7.00	warm	SF-CW
Breitholtz et al., 2012D	Telmisartan	4.00	7.00	warm	SF-CW
Auvinen et al., 2017bA	Telmisartan	NA	NA	warm	H-CW
Auvinen et al., 2017bB	Telmisartan	4.50	5.50	warm	SF-CW
MEAN(SE)		**3.5(0.5)**	**6.7(0.3)**		
Berglund et al., 2014	Tetracycline	3.75	5.50	warm	SF-CW
Dan et al., 2020aA (summer)	Tetracycline	4.00	6.50	warm	VSSF-CW
Dan et al., 2020aA (winter)	Tetracycline	3.75	6.50	cold	VSSF-CW
Dan et al., 2020aB (summer)	Tetracycline	3.75	6.50	warm	VSSF-CW
Dan et al., 2020aB (winter)	Tetracycline	3.25	6.50	cold	VSSF-CW
Dan et al., 2020aC (summer)	Tetracycline	3.75	6.50	warm	VSSF-CW
Dan et al., 2020aC (winter)	Tetracycline	3.25	6.50	cold	VSSF-CW
Dan et al., 2020aD (summer)	Tetracycline	3.75	6.50	warm	SF-CW
Dan et al., 2020aD (winter)	Tetracycline	3.25	6.50	cold	SF-CW
Dan et al., 2020aE (summer)	Tetracycline	3.75	6.50	warm	SF-CW
Dan et al., 2020aE (winter)	Tetracycline	0.00	6.50	cold	SF-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Dan et al., 2020aF (summer)	Tetracycline	4.25	6.50	warm	SF-CW
Dan et al., 2020aF (winter)	Tetracycline	3.75	6.50	cold	SF-CW
Dan et al., 2020aG (summer)	Tetracycline	3.75	6.50	warm	HSSF-CW
Dan et al., 2020aG (winter)	Tetracycline	2.00	6.50	cold	HSSF-CW
Dan et al., 2020aH (summer)	Tetracycline	3.75	6.50	warm	HSSF-CW
Dan et al., 2020aH (winter)	Tetracycline	3.25	6.50	cold	HSSF-CW
Dan et al., 2020aI (summer)	Tetracycline	3.75	6.50	warm	HSSF-CW
Dan et al., 2020aI (winter)	Tetracycline	2.00	6.50	cold	HSSF-CW
Verlicchi et al., 2013	Tetracycline	NA	NA	warm	HSSF-CW
MEAN(SE)		**3.3(0.2)**	**6.4(0.1)**		
Chen et al., 2016A (summer)	Tramadol	4.50	5.50	warm	HSSF-CW
Chen et al., 2016A (winter)	Tramadol	3.50	5.50	cold	HSSF-CW
Chen et al., 2016B (summer)	Tramadol	4.25	5.50	warm	HSSF-CW
Chen et al., 2016B (winter)	Tramadol	4.25	5.50	warm	HSSF-CW
Chen et al., 2016C (summer)	Tramadol	4.50	5.50	warm	HSSF-CW
Chen et al., 2016C (winter)	Tramadol	4.50	5.50	cold	HSSF-CW
Ruhmland et al., 2015A	Tramadol	3.75	6.75	warm	HSSF-CW
Ruhmland et al., 2015B	Tramadol	4.00	6.75	warm	HSSF-CW
Ruhmland et al., 2015C	Tramadol	1.75	6.75	cold	HSSF-CW
Vymazal et al., 2017A	Tramadol	3.75	4.25	warm	HSSF-CW
Vymazal et al., 2017B	Tramadol	3.75	4.25	warm	HSSF-CW
Vymazal et al., 2017C	Tramadol	3.50	4.25	warm	HSSF-CW
Vymazal et al., 2017D	Tramadol	3.75	4.25	warm	HSSF-CW
Nuel et al., 2018	Tramadol	1.25	7.00	warm	SF-CW
Breitholtz et al., 2012A	Tramadol	1.25	7.00	warm	SF-CW
Breitholtz et al., 2012B	Tramadol	1.75	7.00	warm	SF-CW
Breitholtz et al., 2012C	Tramadol	3.50	7.00	warm	SF-CW
Breitholtz et al., 2012D	Tramadol	0.00	7.00	warm	SF-CW
Auvinen et al., 2017bA	Tramadol	4.50	5.50	warm	H-CW
Auvinen et al., 2017bB	Tramadol	4.50	5.50	warm	SF-CW
MEAN(SE)		**3.3(0.3)**	**5.8(0.2)**		
Chen et al., 2016A (summer)	Trimethoprim	2.50	5.50	warm	HSSF-CW
Chen et al., 2016A (winter)	Trimethoprim	2.50	5.50	cold	HSSF-CW
Chen et al., 2016B (summer)	Trimethoprim	3.50	5.50	warm	HSSF-CW
Berglund et al., 2014	Trimethoprim	3.50	5.50	warm	SF-CW
Ruhmland et al., 2015A	Trimethoprim	4.00	6.75	warm	HSSF-CW
Ruhmland et al., 2015B	Trimethoprim	4.00	6.75	warm	HSSF-CW
Ruhmland et al., 2015C	Trimethoprim	4.00	6.75	cold	HSSF-CW
Sgroi et al., 2018A	Trimethoprim	3.50	6.50	warm	VSSF-CW
Sgroi et al., 2018B	Trimethoprim	3.50	6.50	warm	H-CW
Zhu et al., 2014	Trimethoprim	0.00	7.50	warm	H-CW
Nuel et al., 2018	Trimethoprim	0.00	7.00	warm	SF-CW
Breitholtz et al., 2012A	Trimethoprim	3.75	7.00	warm	SF-CW
Breitholtz et al., 2012B	Trimethoprim	3.75	7.00	warm	SF-CW
Breitholtz et al., 2012C	Trimethoprim	3.75	7.00	warm	SF-CW
Breitholtz et al., 2012D	Trimethoprim	4.00	7.00	warm	SF-CW
Verlicchi et al., 2013	Trimethoprim	3.75	7.50	warm	HSSF-CW
Mathon et al., 2019	Trimethoprim	3.25	5.00	warm	SF-CW
Anderson et al., 2020A	Trimethoprim	4.25	4.25	warm	H-CW

Reference	PHARMACEUTICAL	ROO	SOM	SEASON	CW TYPE
Anderson et al., 2020B	Trimethoprim	4.00	4.25	warm	H-CW
Stroski et al., 2020A	Trimethoprim	4.50	4.75	cold	SF-CW
Stroski et al., 2020B	Trimethoprim	4.50	4.75	cold	SF-CW
Stroski et al., 2020C	Trimethoprim	4.50	4.75	cold	SF-CW
Chaves-Barquero2016	Trimethoprim	3.50	4.75	cold	SF-CW
Sabri et al., 2021A (summer)	Trimethoprim	4.75	4.50	warm	H-CW
Sabri et al., 2021A (winter)	Trimethoprim	4.75	4.50	cold	H-CW
Sabri et al., 2021B (summer)	Trimethoprim	0.00	4.50	warm	H-CW
Sabri et al., 2021B (winter)	Trimethoprim	4.50	4.50	cold	H-CW
MEAN(SE)		**3.4(0.3)**	**5.8(0.2)**		
Sabri et al., 2021A (summer)	Tylosin	5.00	4.50	warm	H-CW
Sabri et al., 2021A (winter)	Tylosin	4.75	4.50	cold	H-CW
Sabri et al., 2021B (summer)	Tylosin	5.00	4.50	warm	H-CW
Sabri et al., 2021B (winter)	Tylosin	4.50	4.50	cold	H-CW
Verlicchi et al., 2013	Tylosin	1.00	7.50	warm	HSSF-CW
MEAN(SE)		**4.1(0.7)**	**5.1(0.5)**		
Ruhmland et al., 2015A	Venlafaxine	3.75	6.75	warm	HSSF-CW
Ruhmland et al., 2015B	Venlafaxine	3.25	6.75	warm	HSSF-CW
Ruhmland et al., 2015C	Venlafaxine	2.00	6.75	cold	HSSF-CW
Breitholtz et al., 2012A	Venlafaxine	3.50	7.00	warm	SF-CW
Breitholtz et al., 2012B	Venlafaxine	3.25	7.00	warm	SF-CW
Breitholtz et al., 2012C	Venlafaxine	2.75	7.00	warm	SF-CW
Breitholtz et al., 2012D	Venlafaxine	3.75	7.00	warm	SF-CW
Vystavna et al., 2017A	Venlafaxine	4.50	6.00	warm	H-CW
Vystavna et al., 2017B	Venlafaxine	4.50	6.00	warm	H-CW
MEAN(SE)		**3.5(0.2)**	**6.7(0.1)**		

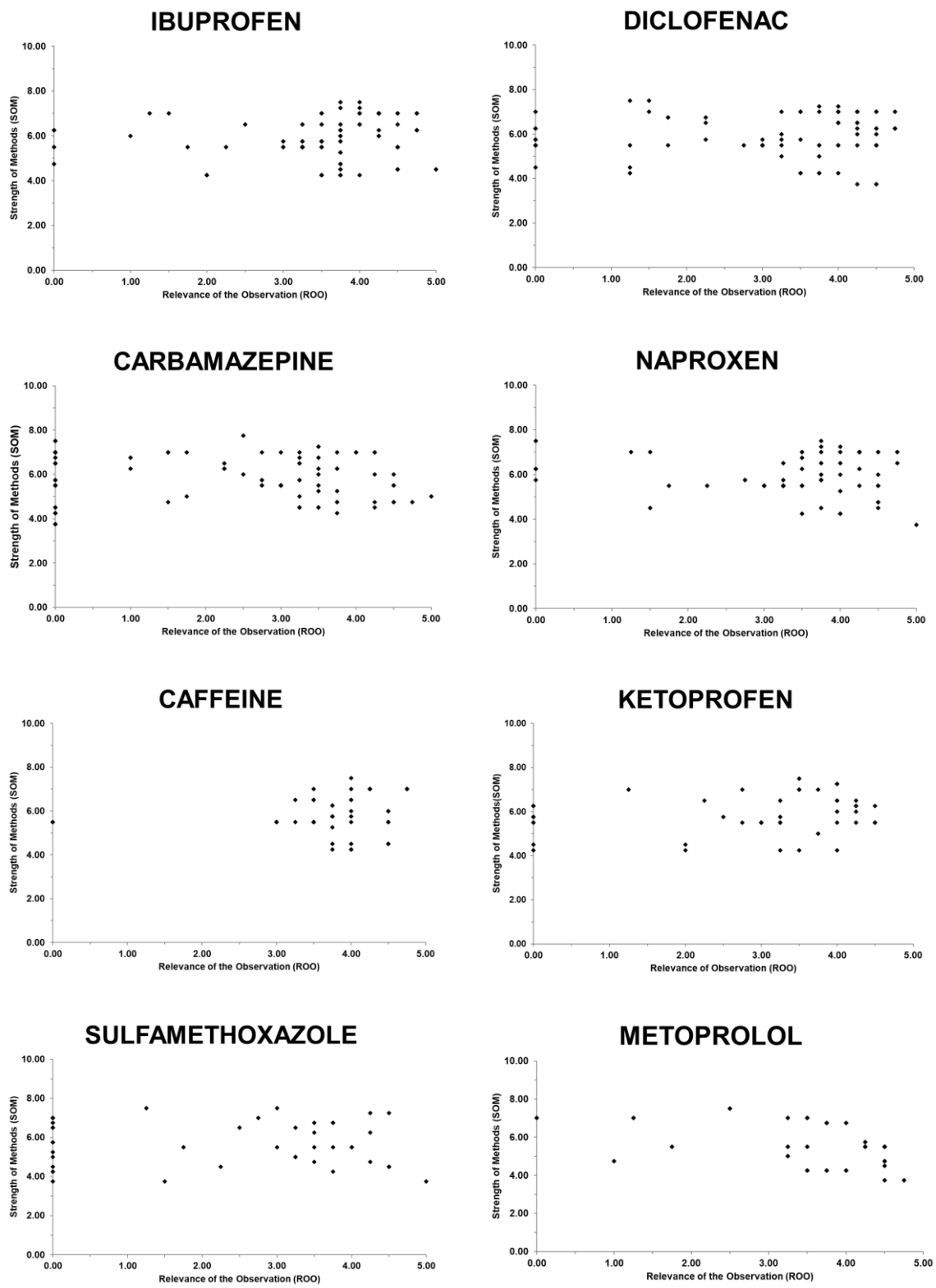


Figure A2. WoE scoring plots for ibuprofen, diclofenac, carbamazepine, naproxen, caffeine, ketoprofen, sulfamethoxazole and metoprolol. Some of the data points could be covering others with the same scores.

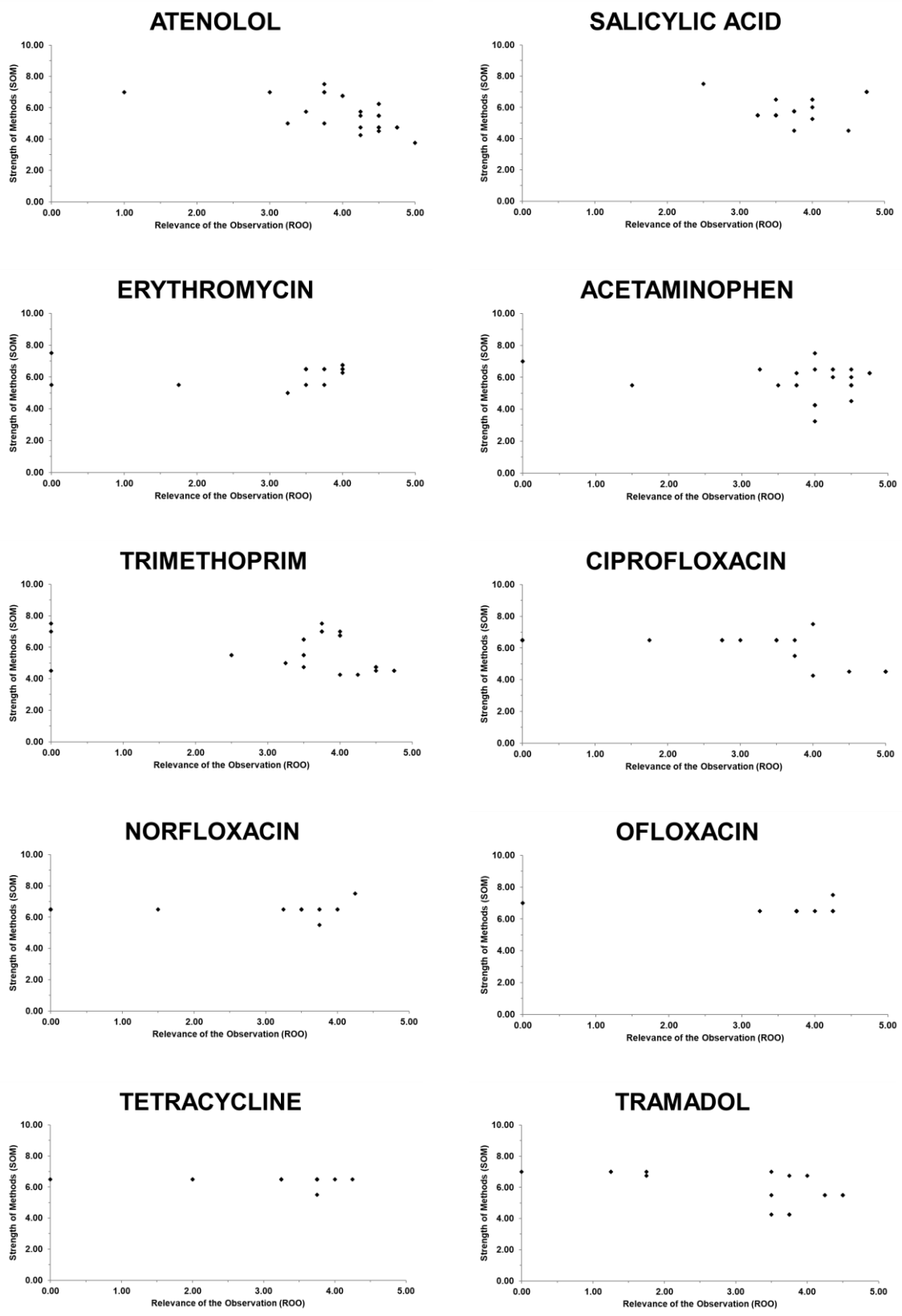


Figure A3. WoE scoring plots for atenolol, salicylic acid, erythromycin, acetaminophen, trimethoprim, ciprofloxacin, norfloxacin, ofloxacin, tetracycline and tramadol. Some of the data points could be covering others with the same scores.

7.2. APPENDIX B: ADDITIONAL INFORMATION FOR CHAPTER 3

THE RELEASE OF WASTEWATER CONTAMINANTS IN THE ARCTIC: A CASE STUDY FROM CAMBRIDGE BAY, NUNAVUT, CANADA

SUMMARY

The following includes further details on the pharmaceutical concentrations found in the studied facility both before and during wastewater discharge, as well as additional details on the sampling rates chosen for the calculation of time-weighted-average concentrations of pharmaceuticals collected by POCIS samplers. Finally, details on the numbers used for our hazard quotient calculations are shown.

Table B1. Mean concentrations (ng/L) of detected organic micropollutants in the Cambridge Bay wastewater treatment facility and receiving waters in 2014. All other compounds were below their limits of detection. LOQs and LODs (ng/L) are indicated below each compound's name. Standard deviations are denoted in parentheses.

Compound	Site						
	Time frame (Discharge)	LI1	LI2	Wetland	Outfall	CHARS	Finger Bay
Atenolol	Before	68 (11)	62 (2)	4.3 (0.4)	2.6 (0.4)	ND	ND
(LOQ: 2.4, LOD: 0.7)	During	97 (9)	61 (5)	20 (4)	2.7 (0.2)	ND	ND
Carbamazepine	Before	68 (11)	260 (18)	116 (12)	< 1.2	< 1.2	< 1.2
(LOQ: 1.2, LOD: 0.4)	During	97 (9)	428 (10)	307 (24)	< 1.2	< 1.2	<1.2
Clarithromycin	Before	< 9.0	< 9.0	ND	ND	ND	ND
(LOQ: 9.0, LOD: 3.0)	During	< 9.0	< 9.0	< 9.0	ND	ND	ND
Metoprolol	Before	< 2.1	< 2.1	< 2.1	ND	ND	ND
(LOQ: 2.1, LOD: 0.6)	During	< 2.1	< 2.1	< 2.1	ND	ND	ND
Sulfamethoxazole	Before	107 (15)	96 (11)	13 (4)	ND	ND	ND
(LOQ: 2.1, LOD: 0.6)	During	188 (38)	274 (8)	151 (41)	ND	ND	ND
Trimethoprim	Before	17 (2)	15 (1)	< 2.7	ND	ND	ND
(LOQ: 2.7, LOD: 0.8)	During	26 (1)	22 (1)	10 (2)	ND	ND	ND

Analysis of micropollutants were for the following analytes: atenolol, atrazine, carbamazepine, chlorpyrifos, ciprofloxacin, clarithromycin, diazinon, enrofloxacin, erythromycin, fluoxetine, imidacloprid, malathion, metoprolol, paroxetine, propranolol, roxithromycin, spiramycin, sulfamethazine, sulfamethoxazole, sulfapyridine, sulfisoxazole, sulphachloropyridazine, sulphadimetoxine, trimethoprim, tylosin, clothianidin and thiamethoxam.

Table B2. Sampling rates for the detected organic micropollutants in the Cambridge Bay wastewater treatment facility and receiving waters in 2014. Values were taken from MacLeod et al. (2007), except for sulfamethoxazole, which was taken from Bartelt-Hunt et al., (2011).

Compound	**Sampling rate (L/day)**
Atenolol	0.040
Carbamazepine	0.348
Clarithromycin	0.668
Metoprolol	0.599
Sulfamethoxazole	0.066
Trimethoprim	0.36

Table B3. Calculated hazard quotients for organic micropollutants detected by POCIS samplers in the Cambridge Bay wastewater treatment facility and receiving waters from July 25 to August 8 and from August 29 to September 8, 2014. Full data set of measured concentrations available in Table B1.

Compound	Site type	Species	Toxicity endpoint	Toxicity value (mg/L)	MEC (mg/L)	HQ	Reference
Atenolol	Lagoon Input 1	*Lemna spp.* (Duckweed)	EC50 - 7 day growth inhibition	> 320	9.74×10^{-5}	3.04×10^{-4}	(Cleuvers, 2005)
		Daphnia magna	EC50 - 48 h immobilization	313	9.74×10^{-5}	3.11×10^{-4}	(Cleuvers, 2005)
		Pimephales promelas (Fathead minnow)	NOEC - 32 day growth inhibition	3.2	9.74×10^{-5}	3.04×10^{-2}	(Kuester et al., 2010)
	Lagoon Input 2	*Lemna spp.*	EC50 - 7 day growth inhibition	> 320	6.19×10^{-5}	1.93×10^{-4}	(Cleuvers, 2005)
		Daphnia magna	EC50 - 48 h immobilization	313	6.19×10^{-5}	1.98×10^{-4}	(Cleuvers, 2005)
		Pimephales promelas	NOEC - 32 day growth inhibition	3.2	6.19×10^{-5}	1.93×10^{-2}	(Kuester et al., 2010)
	Wetland	*Lemna spp.*	EC50 - 7 day growth inhibition	> 320	1.98×10^{-5}	6.19×10^{-5}	(Cleuvers, 2005)
		Daphnia magna	EC50 - 48 h immobilization	313	1.98×10^{-5}	6.33×10^{-5}	(Cleuvers, 2005)
		Pimephales promelas	NOEC - 32 day growth inhibition	3.2	1.98×10^{-5}	6.19×10^{-5}	(Kuester et al., 2010)
Carbamazepine	Lagoon Input 1	*Lemna minor*	EC50 - 7 day growth inhibition	22.5	9.74×10^{-5}	4.33×10^{-3}	(Cleuvers, 2003)
		Daphnia magna	EC50 - 48 h immobilization	> 100	9.74×10^{-5}	9.74×10^{-4}	(Cleuvers, 2003)
		Oryzias latipes (Japanese medaka)	LC50 - 28 day exposure	35.4	9.74×10^{-5}	2.75×10^{-3}	(Kim et al., 2007)
	Lagoon Input 2	*Lemna minor*	EC50 - 7 day growth inhibition	22.5	4.28×10^{-4}	1.90×10^{-2}	(Cleuvers, 2003)
		Daphnia magna	EC50 - 48 h immobilization	> 100	4.28×10^{-4}	4.28×10^{-3}	(Cleuvers, 2003)
		Oryzias latipes	LC50 - 28 day exposure	35.4	4.28×10^{-4}	1.21×10^{-2}	(Kim et al., 2007)
	Wetland	*Lemna minor*	EC50 - 7 day growth inhibition	22.5	3.07×10^{-4}	1.36×10^{-2}	(Cleuvers, 2003)
		Daphnia magna	EC50 - 48 h immobilization	> 100	3.07×10^{-4}	3.07×10^{-3}	(Cleuvers, 2003)
		Oryzias latipes	LC50 - 28 day exposure	35.4	3.07×10^{-4}	8.67×10^{-3}	(Kim et al., 2007)
Clarithromycin	Lagoon Input 1	*Pseudokirchneriella subcapita*	EC50 - 72 h growth inhibition	0.005	9.00×10^{-6}	1.8	(Yang et al., 2008)
		Daphnia magna	EC50 - 24 h immobilization	25.7	9.00×10^{-6}	3.50×10^{-4}	(Isidori et al., 2005)
		Danio rerio (zebrafish)	LC50 - 96 h exposure	> 1000	9.00×10^{-6}	9.00×10^{-6}	(Isidori et al., 2005)
	Lagoon Input 2	*Pseudokirchneriella subcapita*	EC50 - 72 h growth inhibition	0.005	9.00×10^{-6}	1.8	(Yang et al., 2008)
		Daphnia magna	EC50 - 24 h immobilization	25.7	9.00×10^{-6}	3.50×10^{-4}	(Isidori et al., 2005)
		Danio rerio	LC50 - 96 h exposure	> 1000	9.00×10^{-6}	9.00×10^{-6}	(Isidori et al., 2005)
	Wetland	*Pseudokirchneriella subcapita*	EC50 - 72 h growth inhibition	0.005	9.00×10^{-6}	1.8	(Yang et al., 2008)
		Daphnia magna	EC50 - 24 h immobilization	25.7	9.00×10^{-6}	3.50×10^{-4}	(Isidori et al., 2005)
		Danio rerio	LC50 - 96 h exposure	> 1000	9.00×10^{-6}	9.00×10^{-6}	(Isidori et al., 2005)
Metoprolol	Lagoon Input 1	*Lemna spp.*	EC50 - 7 day growth inhibition	> 320	2.10×10^{-6}	6.56×10^{-6}	(Cleuvers, 2005)

Compound	Site type	Species	Toxicity endpoint	Toxicity value (mg/L)	MEC (mg/L)	HQ	Reference
		Daphnia magna	EC50 - 48 h immobilization	438	2.10×10^{-6}	4.79×10^{-6}	(Cleuvers, 2005)
		Danio rerio	EC50 – 72 h embryo exposure	12.6	2.10×10^{-6}	1.66×10^{-4}	(van den Brandhof and Montforts, 2010)
	Lagoon Input 2	*Lemna spp.*	EC50 - 7 day growth inhibition	> 320	2.10×10^{-6}	6.56×10^{-6}	(Cleuvers, 2005)
		Daphnia magna	EC50 - 48 h immobilization	438	2.10×10^{-6}	4.79×10^{-6}	(Cleuvers, 2005)
		Danio rerio	EC50 - 72 h embryo exposure	12.6	2.10×10^{-6}	1.66×10^{-4}	(van den Brandhof and Montforts, 2010)
	Wetland	*Lemna spp.*	EC50 - 7 day growth inhibition	> 320	2.10×10^{-6}	6.56×10^{-6}	(Cleuvers, 2005)
		Daphnia magna	EC50 - 48 h immobilization	438	2.10×10^{-6}	4.79×10^{-6}	(Cleuvers, 2005)
		Danio rerio	EC50 - 72 h growth inhibition	12.6	2.10×10^{-6}	1.66×10^{-4}	(van den Brandhof and Montforts, 2010)
Sulfamethoxazole	Lagoon Input 1	*Pseudokirchneriella subcapitata*	EC50 - 72 h growth inhibition	0.52	1.88×10^{-4}	3.62×10^{-1}	(Isidori et al., 2005)
		Daphnia magna	EC50 - 48 h immobilization	25.2	1.88×10^{-4}	7.46×10^{-3}	(Isidori et al., 2005)
		Oryzias latipes	LC50 - 48 h exposure	562.5	1.88×10^{-4}	3.34×10^{-4}	(Kim et al., 2007)
	Lagoon Input 2	*Pseudokirchneriella subcapitata*	EC50 - 72 h growth inhibition	0.52	2.74×10^{-4}	5.27×10^{-1}	(Isidori et al., 2005)
		Daphnia magna	EC50 - 48 h immobilization	25.2	2.74×10^{-4}	1.09×10^{-2}	(Isidori et al., 2005)
		Oryzias latipes	LC50 - 48 h exposure	562.5	2.74×10^{-4}	4.87×10^{-4}	(Kim et al., 2007)
	Wetland	*Pseudokirchneriella subcapitata*	EC50 - 72 h growth inhibition	0.52	1.51×10^{-4}	2.90×10^{-1}	(Isidori et al., 2005)
		Daphnia magna	EC50 - 48 h immobilization	25.2	1.51×10^{-4}	5.99×10^{-3}	(Isidori et al., 2005)
		Oryzias latipes	LC50 - 48 h exposure	562.5	1.51×10^{-4}	2.68×10^{-4}	(Kim et al., 2007)
Trimethoprim	Lagoon Input 1	*Selenastrum capricornutum*	EC50 - 72 h growth inhibition	80.3	2.57×10^{-5}	3.20×10^{-4}	(Eguchi et al., 2004)
		Daphnia magna	EC50 - 48 h immobilization	120.7	2.57×10^{-5}	2.13×10^{-4}	(Kim et al., 2007)
		Oryzias latipes	LC50 - 48 h exposure	> 100	2.57×10^{-5}	2.57×10^{-4}	(Kim et al., 2007)
	Lagoon Input 2	*Selenastrum capricornutum*	EC50 - 72 h growth inhibition	80.3	2.22×10^{-5}	2.76×10^{-4}	(Eguchi et al., 2004)
		Daphnia magna	EC50 - 48 h immobilization	120.7	2.22×10^{-5}	1.83×10^{-4}	(Kim et al., 2007)
		Oryzias latipes	LC50 - 48 h exposure	> 100	2.22×10^{-5}	2.22×10^{-4}	(Kim et al., 2007)
	Wetland	*Selenastrum capricornutum*	EC50 - 72 h growth inhibition	80.3	9.80×10^{-6}	1.22×10^{-4}	(Eguchi et al., 2004)
		Daphnia magna	EC50 - 48 h immobilization	120.7	9.80×10^{-6}	8.12×10^{-5}	(Kim et al., 2007)
		Oryzias latipes	LC50 - 48 h exposure	> 100	9.80×10^{-6}	9.80×10^{-5}	(Kim et al., 2007)

References for Appendix B

Bartelt-Hunt, S.L.; Snow, D.D.; Damon-Powell, T.; Brown, D.L.; Prasai, G.; Schwarz, M.; Kolok, A.S. Quantitative evaluation of laboratory uptake rates for pesticides, pharmaceuticals and steroid hormones using POCIS. *Environmental Toxicology and Chemistry* **2011**; *30*: 1412-1420.

Cleuvers, M. Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects. *Toxicology Letters* **2003**; *142*: 185-194.

Cleuvers, M. Initial risk assessment for three beta-blockers found in the aquatic environment. *Chemosphere* **2005**; *59*: 199-205.

Eguchi, K.; Nagase, H.; Ozawa, M.; Endoh, Y.S.; Goto, K.; Hirata, K.; Miyamoto, K.; Yoshimura, H. Evaluation of antimicrobial agents for veterinary use in the ecotoxicity test using microalgae. Chemosphere **2004**; *57*: 1733-1738.

Isidori, M.; Lavorgna, M.; Nardelli, A.; Pascarella, L.; Parrella, A. Toxic and genotoxic evaluation of six antibiotics on non-target organisms. *Science of the Total Environment* **2005**; *346*: 87-98.

Kim, Y.; Choi, K.; Jung, J.Y.; Park, S.; Kim, P.G.; Park, J. Aquatic toxicity of acetaminophen, carbamazepine, cimetidine, diltiazem and six major sulfonamides, and their potential ecological risks in Korea. *Environment International* **2007**; *33*: 370-375.

Küster, A.; Alder, A.C.; Escher, B.I.; Duis, K.; Fenner, K.; Garric, J.; Hutchinson, T.H.; Lapen, D.R.; Péry, A.; Römbke, J.; Snape, J.; Ternes, T.; Topp, E.; Wehrhan, A.; Knacker, T. Environmental Risk Assessment of Human Pharmaceuticals in the European Union: A Case Study with the beta-Blocker Atenolol. *Integrated Environmental Assessment and Management* **2010**; *6*: 514-523.

van den Brandhof, E.J.; Montforts, M. Fish embryo toxicity of carbamazepine, diclofenac and metoprolol. *Ecotoxicology and Environmental Safety* **2010**; 73: 1862-1866.

Yang, L.H.; Ying, G.G.; Su, H.C.; Stauber, J.L.; Adams, M.S.; Binet, M.T. Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga *Pseudokirchneriella subcapitata*. *Environmental Toxicology and Chemistry* **2008**; *27*: 1201-1208.

7.3. APPENDIX C: ADDITIONAL INFORMATION FOR CHAPTER 4

CRUSHED RECYCLED GLASS AS A SUBSTRATE FOR CONSTRUCTED WETLAND WASTEWATER TREATMENT: A CASE STUDY OF ITS POTENTIAL TO FACILITATE PHARMACEUTICAL REMOVAL

SUMMARY

The following includes further details on the experimental setup of mesocosm- and pilot-scale studies, including the monitoring of environmental parameters, as well as data on plant growth and phosphorus removal for the mesocosm-scale study. Next, details on instrumental parameters for pharmaceutical quantification for both studies are presented, including the totality of measured concentrations over the course of the mesocosm-study. Finally, additional data for our pharmaceutical fate estimations are presented, including the Monte Carlo sensitivity analysis.

Figure C1. Mesocosm-scale modelled wetland installed at the Prairie Wetland Research Facility, University of Manitoba, Winnipeg, Canada as pictured on August 17th, 2018.

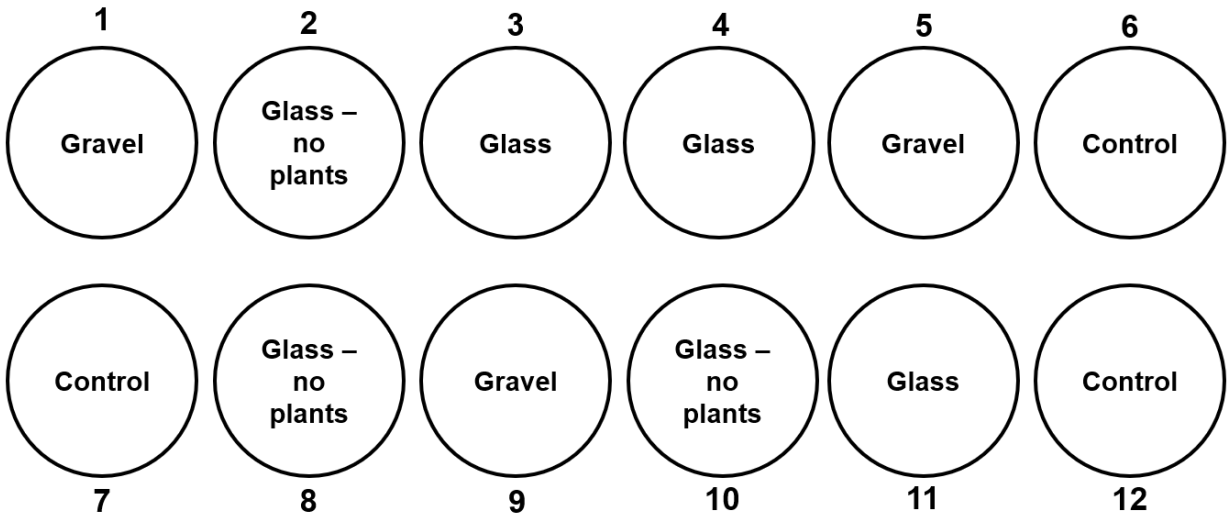


Figure C2. Layout of randomly assigned treatments in twelve mesocosms. The treatments consisted of: control (crushed glass as substrate, unplanted, not treated), gravel (gravel as substrate, planted with Typha spp., addition of pharmaceuticals and synthetic wastewater), glass (PCRG, crushed glass as substrate, planted with Typha spp., addition of pharmaceuticals and synthetic wastewater), and glass – no plants (crushed glass as substrate, unplanted, addition of pharmaceuticals and synthetic wastewater).

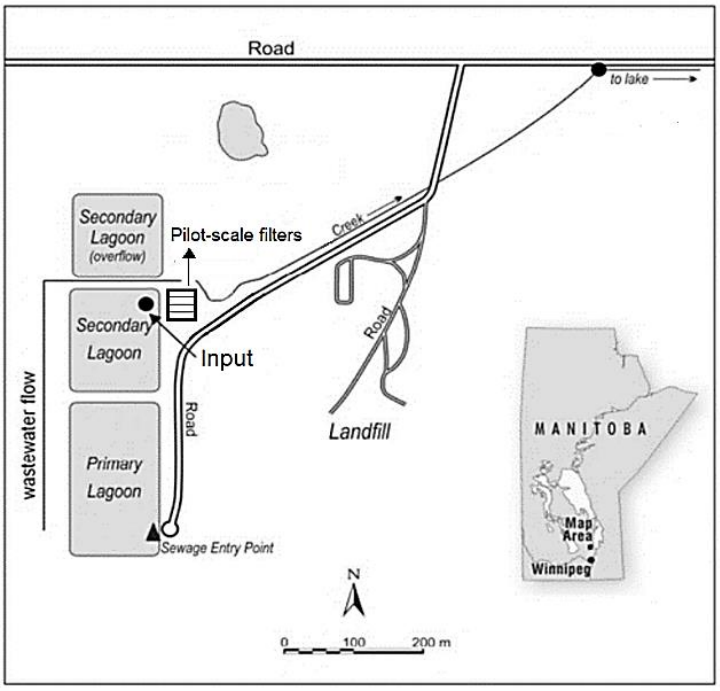


Figure C3. Dunnottar wastewater treatment facility as of 2018. Pilot-scale filters received effluent from the secondary lagoon. Figure adapted from Anderson et al., 2015.

Plant growth and phosphorus removal in mesocosm-study

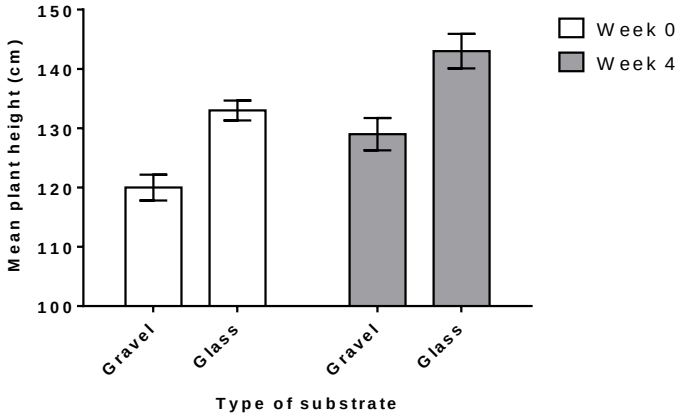


Figure C4. Typha spp. height (n=48) over a 4-week period in modelled-CW tanks at the PWRF using limestone gravel (LG) or crushed recycled grass (CRG) as substrates. Average growth was 5.5 cm for gravel and 10.5 cm for glass, and the differences between treatments were not statistically significant (p > 0.05, two-tailed Student's t-test).

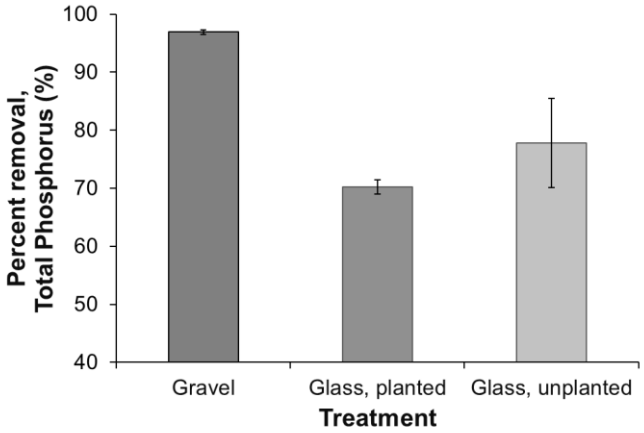


Figure C5. Phosphorus removal over a 4-week period in modelled-CW tanks at the PWRF using LG or CRG as substrates. Statistically significant differences were found for Gravel vs. Glass treatments (p < 0.05), while no statistically significant differences were found between Planted Glass and Unplanted Glass treatments, based on a two-tailed Student's t-test.

Table C1. Physical characteristics of sand and crushed recycled glass media used as substrates in the pilot-scale system (adapted from Salzmann et al., 2020). Values are presented as mean ± standard deviation. All characteristics were measured in triplicates, except for bulk density, where no replication was performed.

	D_{10} (mm)	UC	Bulk density (g/cm^3)	Particle density (g/cm^3)	Permeability (cm/s)	Porosity (%)	Void ratio
Sand	0.170±0.004	3.13±0.04	1.82	2.53±0.05	0.020±0.002	28±1	0.39±0.03
Glass	3.6±1.7	4.2±1.7	1.77	2.43±0.08	0.12±0.05	27±2	0.37±0.04

Table C2. Pharmaceuticals analyzed for in grab samples collected in the presented studies. Limits of detection (LOD) and quantification (LOQ) were previously published by Challis et al. (2016), and are presented in ng/L.

Compound Name (CAS#)	Compound class	Use	LOD	LOQ
Atenolol (29122-68-7)	Beta-Adrenergic Receptor Antagonists	β-blocker	0.29	0.96
Carbamazepine (298-46-4)	Tricylics	Anticonvulsant	1.7	5.7
Clarithromycin (81103-11-9)	Macrolides	Antibiotic	0.6	2
Enrofloxacin (93106-60-6)	Fluoroquinolones	Antibiotic	15	48
Erythromycin (114-07-8)	Macrolides	Antibiotic	0.18	0.61
Erythromycin-H2O (114-07-8)	Macrolides	Antibiotic	0.18	0.61
Fluoxetine (54910-89-3)	Selective Serotonin-Reuptake Inhibitors (SSRIs)	Antidepressant	1.7	5.7
Metoprolol (37350-58-6)	Beta-Adrenergic Receptor Antagonists	β-blocker	3.4	11
Paroxetine (61869-08-7)	SSRIs	Antidepressant	1.3	4.2
Propranolol (525-66-6)	Beta-Adrenergic Receptor Antagonists	β-blocker	1.2	4
Roxithromycin (80214-83-1)	Macrolides	Antibiotic	0.44	1.5
Sulfadimethoxine (122-11-2)	Sulfonamides	Antibiotic	13	43
Sulfamethazine (57-68-1)	Sulfonamides	Antibiotic	1.6	5.2
Sulfamethoxazole (723-46-6)	Sulfonamides	Antibiotic	0.25	0.83
Sulfapyridine (144-83-2)	Sulfonamides	Antibiotic	20	66
Sulfisoxazole (127-69-5)	Sulfonamides	Antibiotic	0.57	1.9
Sulfachloropyridazine (80-32-0)	Sulfonamides	Antimicrobial	0.24	0.78
Trimethoprim (738-70-5)	Pyrimidines	Antibiotic	3.6	12

LC/MS Methods and Parameters:

- LC: Agilent 1200 Series (Agilent Technologies, Mississauga, ON) binary pump, degasser, and column heater
- Column: Phenomenex (Torrance, CA) Kinetex XB-C18 column (50 mm × 2.1 mm × 1.7 μm particle size)
- MS: Agilent 6410B MS with electrospray ionization source positive
- Source parameters:
    - Gas temp (°C): 300
    - Nebulizer (psi): 50,
    - Gas Flow (L/min): 10.5
    - Capillary (V): 4000
- Calibration Standards: 14 (0.01 – 750 ug/L)
- Flow rate: 0.45 ml/min
- Column temp: 42°C
- Injection Volume: 20 uL

Positive Mode (all studied pharmaceuticals)

- Run Time: 11.5 min
- Solvent A: 95% H_2O:5% MeOH w/ 0.05% Formic acid
- Solvent B: MeOH w/ 0.05% Formic acid

Time	**B%**
0.00	6.0
1.00	6.0
2.80	50.0
3.50	50.0
6.50	95.0
7.50	95.0
7.51	6.0
11.50	6.0

Instrumental analysis

Details on internal standards, including sources, are as follows: Atenolol[a], ATE (ATE-d_7)[f]; Carbamazepine[b], CBZ (CBZ-d_{10})[f]; Clarithromycin[a], CLA (Josamycin)[b]; Enrofloxacin[a], ENR (ENR-d_5)[f]; Erythromycin[a], ERY (Josamycin)[b]; Fluoxetine[a], FLU (FLU-d_6)[g]; Metoprolol[a], MET (MET-d_7)[f]; Paroxetine[e], PAR (FLU-d_6)[g]; Propranolol[a], PRO (PRO-d_7)[f]; Roxithromycin[a], ROX (Josamycin)[b]; Sulfadimethoxine[a], SDM (SDM-d_6)[d]; Sulfamethazine[a], SMZ (SMZ-$^{13}C_6$)[g]; Sulfamethoxazole[a], SMX (SMX-d_4)[d]; Sulfapyridine[e], SPY (SPY-d_4)[e]; Sulfisoxazole[a], SXZ (SMX-d_4)[d]; Sulfachloropyridazine[a], SCP (SMZ-$^{13}C_6$)[g]; Trimethoprim[a], TRI (TRI-d_3)[f]. All target chemicals were of >98% purity except for ERY, which was 95% pure. Stable isotope standards were all of >99% isotopic purity. Target analytes were obtained from (a) Sigma-Aldrich (Oakville, ON); (b) MP Biomedicals (Montreal, QC); (c) EQ Laboratories Inc. (Atlanta, GA); (d)

ICN Biomedicals (Irvine, CA); (e) Toronto Research Chemicals (Toronto, ON); (f) C/D/N Isotopes Inc. (Pointe-Claire, QC); (g) Cambridge Isotopes (Andover, MA)

Batch analyses of sample sets were conducted by running 13 calibration standards (ranging of 0.01 – 500 µg/L) along with the samples. Blanks were run between triplicate sets of samples and single calibration standards (10, 25, or 50 µg/L) were run every 15 samples as a QA/QC protocol (concentration to be within 20% of target). Linearity (R^2) of calibration standards was ≥0.98 over all analyses and all analytes.

Table C3: m/z transition, fragmentor voltage (Frag), collision energy (CE), and retention time details for the MS/MS positive mode dynamic MRM method.

Compound Name	**Precursor Ion**	**Product Ion**	**Frag (V)**	**CE (V)**	**RT (min)**
Atenolol (Q)	267.2	190.2	135	16	0.7
Atenolol (q)	267.2	145.2	135	16	0.7
Atenolol-d_7 (IS)	274.2	145.1	135	24	0.7
Carbamazepine (Q)	237.1	194.2	145	18	4.1
Carbamazepine (q)	237.1	179.2	145	36	4.1
Carbamazepine-d_{10} (IS)	247.1	204.2	145	36	4.1
Clarithromycin (Q)	748.5	158.1	165	11	4.9
Enrofloxacin (Q)	360.1	342.1	140	18	2.7
Enrofloxacin (q)	360.1	316.2	140	14	2.7
Enrofloxacin-d_5 (IS)	365.1	347.1	140	19	2.7
Erythromycin (Q)	743.5	158.0	155	33	4.2
Erythromycin-H20	716.5	558.4	155	11	4.5
Fluoxetine (Q)	310.3	148.1	92	5	4.4
Fluoxetine-d_6 (IS)	316.2	154.2	90	4	4.4
Josamycin (IS)	828.0	174.3	80	35	5.0
Metoprolol (Q)	268.2	191.1	133	15	2.8
Metoprolol (q)	268.2	133.1	133	17	2.8
Metoprolol-d_7 (IS)	275.1	123.1	125	19	2.8
Paroxetine (Q)	330.2	192.2	145	16	3.9
Propranolol (Q)	260.1	183.1	130	14	3.4
Propranolol (q)	260.1	155.1	130	23	3.4
Propranolol-d_7 (IS)	267.2	189.1	130	16	3.4
Roxithromycin (Q)	837.5	158	180	30	5.1
Sulfadimethoxine (Q)	311.1	156.0	125	17	3.2
Sulfadimethoxine (q)	311.1	245.0	125	15	3.2
Sulfadimethoxine-d_6 (IS)	317.1	162.1	125	19	3.2
Sulfamethazine (Q)	279.1	186.1	120	13	2.5
Sulfamethazine (q)	279.1	156.1	120	14	2.5
Sulfamethazine-$^{13}C_6$ (IS)	285.1	186.1	120	14	2.5
Sulfamethoxazole (Q)	254.0	156.1	110	11	2.7
Sulfamethoxazole (q)	254.0	108.1	110	22	2.7

Compound Name	Precursor Ion	Product Ion	Frag (V)	CE (V)	RT (min)
Sulfamethoxazole-d_4 (IS)	258.0	160.0	110	13	2.7
Sulfapyridine (Q)	250.1	156.1	110	12	1.5
Sulfapyridine (q)	250.1	184.1	110	13	1.5
Sulfapyridine-d_4 (IS)	254.1	160.1	110	12	1.5
Sulfisoxazole (Q)	268.1	156.1	105	8	2.9
Sulfisoxazole (q)	268.1	113.1	105	12	2.9
Sulfachloropyridazine (Q)	285.1	156.1	105	10	2.7
Sulfachloropyridazine (q)	285.1	108.2	105	20	2.7
Trimethoprim (Q)	291.1	230.1	150	21	2.0
Trimethoprim (q)	291.1	261.1	150	20	2.0
Trimethoprim-d_3 (IS)	294.1	230.1	150	22	2.0

Q = quantifier ion; q = qualifier ion; IS = internal standard

Table C4. Concentrations (µg L^{-1}, n=1) of target compounds in the water column of mesocosm-scale tanks at the Prairie Wetland Research Facility of the University of Manitoba between August and October of 2018. LOD and LOQ (ng L^{-1}) are indicated below each analyte's name. NA: results above LOD but below LOQ. "-": not measured. ND: non-detectable.

		Tank Number											
Compound	**Date**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Atenolol LOD: 0.29 LOQ: 0.96	Aug 14 (1 h)	7.27	6.84	5.85	6.79	7.43	ND	ND	5.72	5.63	4.62	7.77	ND
	Aug 14 (8 h)	3.69	5.45	4.64	6.47	6.87	ND	ND	5.22	6.83	3.66	6.28	ND
	Aug 15	4.65	4.27	3.30	4.95	4.44	ND	ND	3.78	5.64	-	6.07	ND
	Aug 16	4.63	3.17	3.54	3.16	5.16	ND	ND	2.09	5.36	2.39	4.01	ND
	Aug 21	0.014	0.03	0.019	0.01	0.005	ND	ND	0.02	0.02	0.02	0.17	ND
	Aug 31	0.011	0.002	0.002	0.003	0.004	ND	ND	0.009	0.002	0.005	0.01	ND
	Sept 13	0.002	0.001	0.001	0.004	0.001	ND	ND	0.002	0.002	0.004	0.004	ND
	Oct 08	0.001	0.001	0.001	0.001	0.001	ND	ND	0.001	0.001	0.001	0.001	ND
Carbamazepine LOD: 1.7 LOQ: 5.7	Aug 14 (1 h)	6.08	6.58	5.66	6.22	6.06	ND	ND	6.78	4.39	6.36	7.49	ND
	Aug 14 (8 h)	5.80	6.57	5.00	6.10	5.50	ND	ND	6.29	6.35	6.12	5.89	ND
	Aug 15	4.50	5.73	4.71	4.83	4.71	ND	ND	5.89	5.33	-	5.20	ND
	Aug 16	5.27	5.46	4.35	5.71	5.50	ND	ND	4.90	5.53	5.76	4.84	ND
	Aug 21	6.09	4.84	3.94	4.84	5.15	ND	ND	5.26	4.82	5.22	4.00	ND
	Aug 31	4.30	4.50	4.19	4.88	4.64	ND	ND	4.12	4.35	4.25	3.84	ND
	Sept 13	3.16	3.19	3.60	3.52	3.37	ND	ND	3.43	3.18	2.68	2.99	ND
	Oct 08	2.79	2.32	3.23	2.99	2.66	ND	ND	2.79	2.48	2.49	2.35	ND
Sulfamethoxazole LOD: 0.25 LOQ: 0.83	Aug 14 (1 h)	6.45	7.07	7.63	7.13	6.43	ND	ND	7.25	5.62	6.41	7.63	ND
	Aug 14 (8 h)	6.20	6.69	6.61	6.08	6.37	ND	ND	6.40	5.88	4.56	6.59	ND
	Aug 15	4.48	5.33	6.87	5.28	4.04	ND	ND	5.40	5.80	-	6.16	ND
	Aug 16	4.16	4.56	6.33	4.28	5.53	ND	ND	4.88	4.78	4.77	3.78	ND
	Aug 21	2.93	2.13	1.85	2.32	2.32	ND	ND	1.79	2.32	2.04	3.15	ND
	Aug 31	1.87	2.23	2.15	3.06	2.26	ND	ND	2.25	1.73	2.45	1.16	ND
	Sept 13	0.55	0.94	0.45	0.98	0.76	ND	ND	0.99	0.45	0.94	0.97	ND
	Oct 08	0.38	0.38	0.29	0.44	0.57	ND	ND	0.54	0.41	0.49	0.44	ND

Table C5. Concentrations of target compounds at two depths (2 cm: interface, 22 cm: bulk) in the substrate of mesocosm-scale tanks at the Prairie Wetland Research Facility of the University of Manitoba between August and September of 2018. LOD and LOQ (ng L^{-1}) are indicated below each analyte's name. "-": not measured. ND: non-detectable.

		Tank Number											
Compound	**Date**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Atenolol (interface)	Aug 14	4.8	4.0	4.7	6.5	3.9	ND	ND	2.8	4.4	3.0	4.0	ND
	Aug 21	0.006	0.002	0.02	0.002	0.006	ND	ND	0.002	0.02	0.009	0.001	ND
	Aug 31	0.005	0.001	0.001	0.02	0.009	ND	ND	0.002	0.002	0.002	0.001	ND
	Sep 13	0.001	0.001	0.001	0.005	0.001	ND	ND	ND	0.001	0.001	0.002	ND
Atenolol (bulk)	Aug 14	4.8	4.8	2.7	3.2	3.9	ND	ND	3.9	4.4	4.4	4.4	ND
	Aug 21	0.006	0.014	0.02	0.02	0.006	ND	ND	0.01	0.02	0.002	0.02	ND
	Aug 31	0.005	0.001	0.006	0.01	0.009	ND	ND	0.001	0.002	0.001	0.004	ND
	Sep 13	0.003	0.001	0.002	0.002	0.002	ND	ND	0.001	0.001	0.001	0.001	ND
Carbamazepine (interface)	Aug 14	4.8	5.5	5.5	7.7	4.3	ND	ND	5.1	5.1	4.9	5.4	ND
	Aug 21	4.7	4.4	5.5	5.5	5.0	ND	ND	4.6	4.8	4.2	3.9	ND
	Aug 31	4.9	4.6	4.3	4.3	4.5	ND	ND	4.2	4.2	4.6	3.9	ND
	Sep 13	3.6	3.4	3.1	3.3	3.4	ND	ND	3.2	3.1	3.0	3.5	ND
Carbamazepine (bulk)	Aug 14	4.8	4.8	4.1	4.6	4.3	ND	ND	-	5.1	5.1	4.2	ND
	Aug 21	4.7	3.8	5.0	4.4	5.0	ND	ND	4.7	4.8	4.5	3.9	ND
	Aug 31	4.9	4.3	4.2	3.8	4.5	ND	ND	4.5	4.2	4.1	4.3	ND
	Sep 13	3.9	2.1	3.9	3.1	3.4	ND	ND	3.8	3.1	3.0	2.1	ND
Sulfamethoxazole (interface)	Aug 14	5.1	6.9	5.9	7.8	5.4	ND	ND	6.0	5.3	5.8	5.8	ND
	Aug 21	1.7	3.0	2.1	3.0	2.8	ND	ND	3.9	1.4	3.9	2.2	ND
	Aug 31	2.4	2.4	2.2	2.6	2.1	ND	ND	3.8	1.9	3.8	2.1	ND
	Sep 13	0.9	1.6	0.5	1.0	0.9	ND	ND	1.4	0.5	1.4	0.7	ND
Sulfamethoxazole (bulk)	Aug 14	5.1	5.1	4.9	7.6	5.4	ND	ND	5.4	5.3	5.3	4.9	ND
	Aug 21	1.7	3.0	2.4	3.2	2.8	ND	ND	3.5	1.4	2.0	1.4	ND
	Aug 31	2.4	2.7	1.4	1.2	2.1	ND	ND	1.8	1.9	1.3	1.4	ND
	Sep 13	1.2	1.3	0.7	0.9	1.2	ND	ND	1.6	0.7	1.5	0.7	ND

Table C6. Numbers used for estimations of K_{oc} and prediction of sedimentation rates for the present study.

Compound	pK_a	log K_{ow}	log D_{ow}	log K_{oc}	K_{oc}	k_s*
Atenolol	9.6	0.16	0.014	0.16	1.4	2.1×10^{-6}
Carbamazepine	13.9	2.45	2.45	1.96	92	1.3×10^{-4}
Sulfamethoxazole	5.7	0.89	-3.41	-2.37	0.004	6.1×10^{-9}

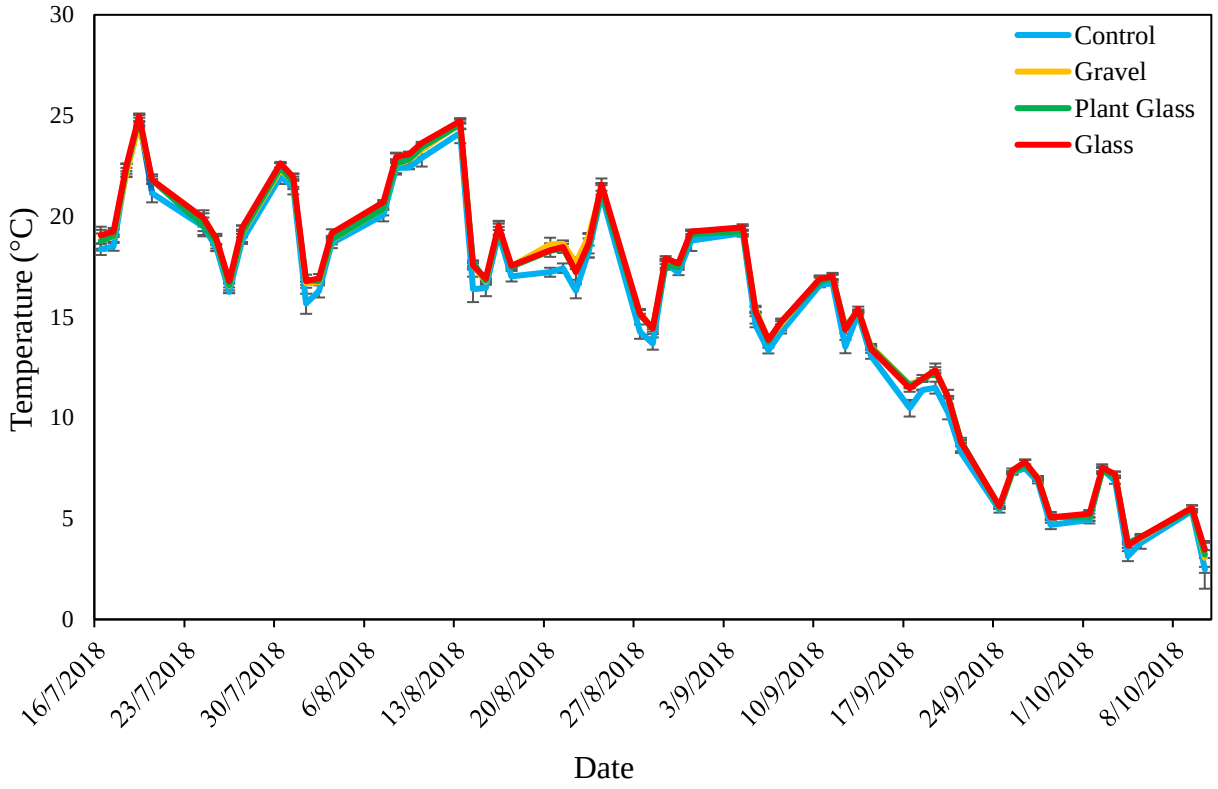


Figure C6. Mean temperature (°C) of treated mesocosms at the Prairie Wetland Research Facility (PWRF). Temperature values were averaged across replicates (n=3) for each of the four treatments (control, LG, planted CRG, and unplanted CRG). Measurements were made using a YSI 6600 V2 Sonde every weekday morning from July 16, 2018 to October 10, 2018. Treatment took place on August 14, 2018. Error bars represent standard deviation.

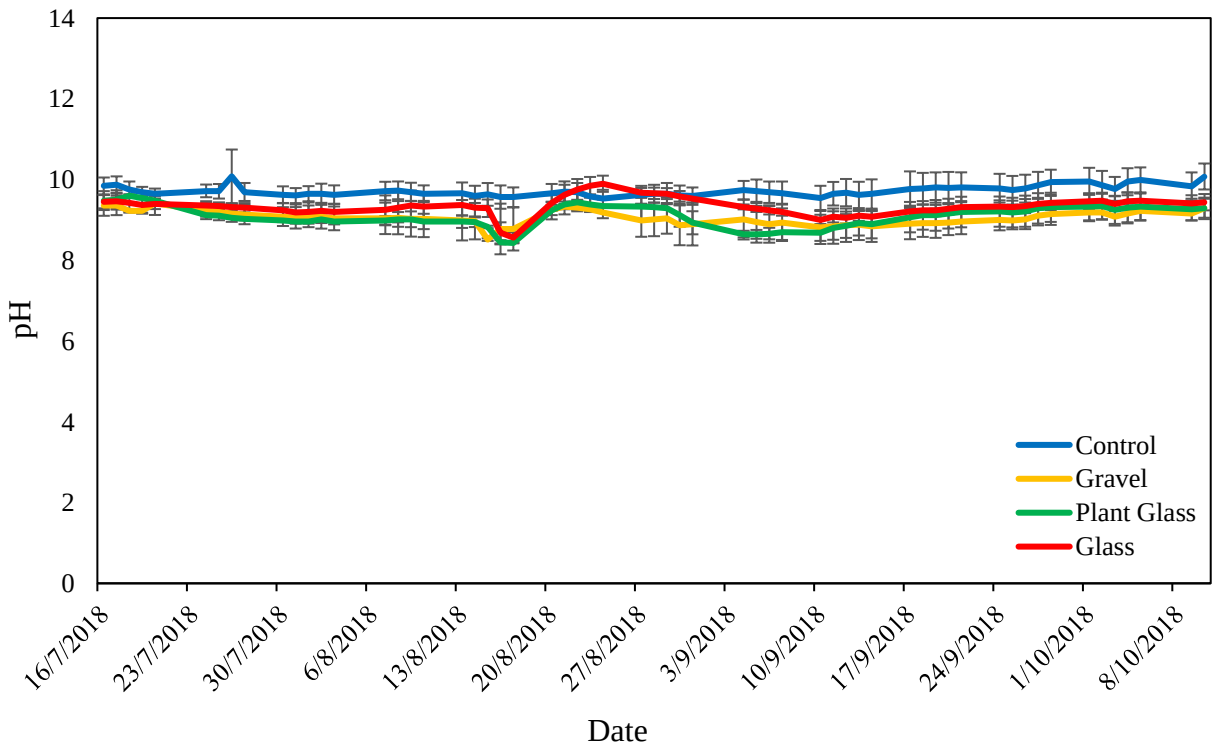


Figure C7. Mean pH of treated mesocosms at the Prairie Wetland Research Facility (PWRF). pH values were averaged across replicates (n=3) for each of the four treatments (control, LG, planted CRG, and unplanted CRG). Measurements were made using a YSI 6600 V2 Sonde every weekday morning from July 16, 2018 to October 10, 2018. Treatment took place on August 14, 2018. Error bars represent standard deviation.

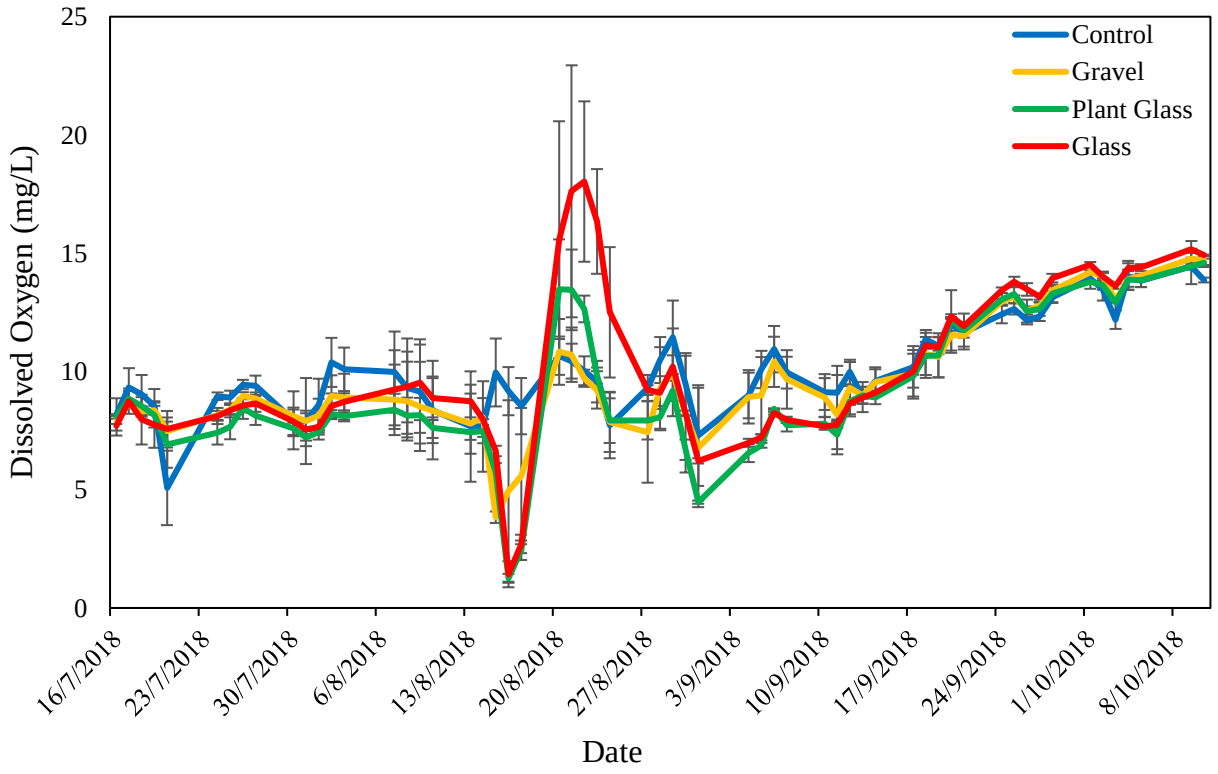


Figure C8. Mean dissolved oxygen concentration (mg/L) of treated mesocosms at the Prairie Wetland Research Facility (PWRF). Dissolved oxygen values were averaged across replicates (n=3) for each of the four treatments (control, LG, planted CRG, and unplanted CRG). Measurements were made using a YSI 6600 V2 Sonde every weekday morning from July 16, 2018 to October 10, 2018. Treatment took place on August 14, 2018. Error bars represent standard deviation.

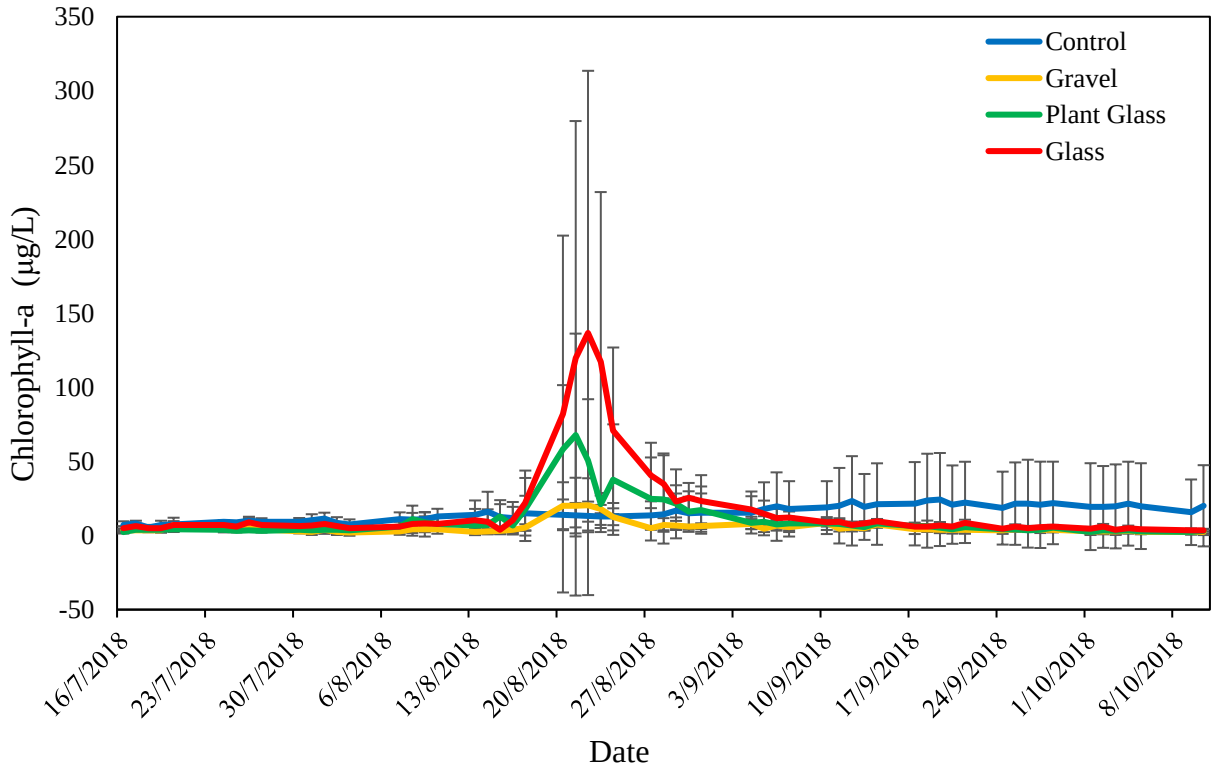
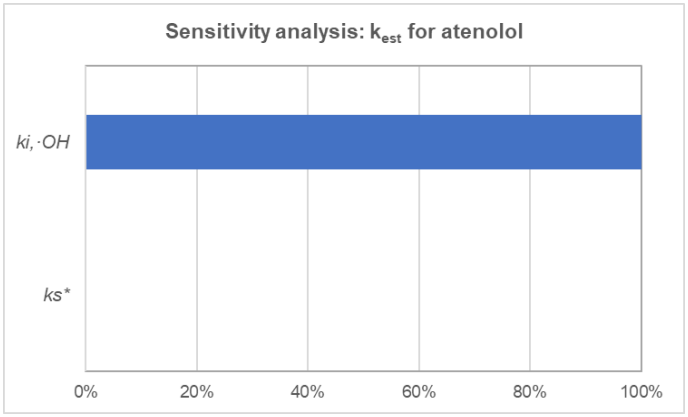
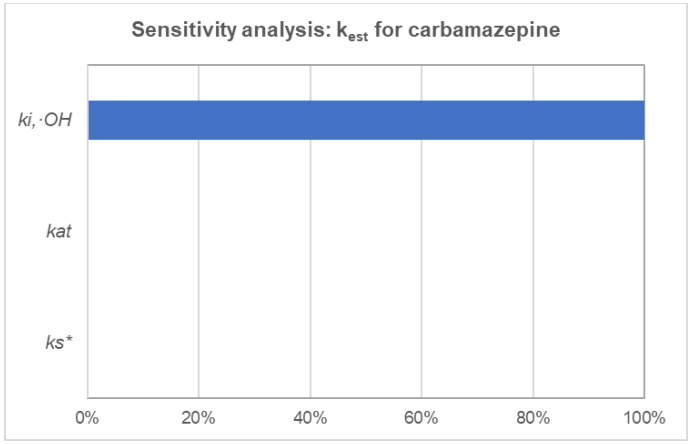


Figure C9. Mean chlorophyll-a concentration (µg/L) of treated mesocosms at the Prairie Wetland Research Facility (PWRF). Chlorophyll values were averaged across replicates (n=3) for each of the four treatments (control, LG, planted CRG, and unplanted CRG). Measurements were made using a YSI 6600 V2 Sonde every weekday morning from July 16, 2018 to October 10, 2018. Error bars represent standard deviation.

a)



b)



c)

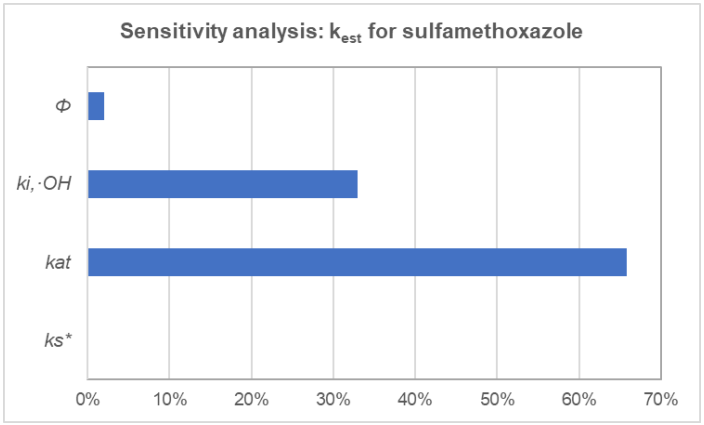


Figure C10. Sensitivity analysis (Monte Carlo simulations in Oracle Crystal Ball, 100,000 iterations) for the estimated overall dissipation rates (k_{est}) of a) atenolol, b) carbamazepine, and c) sulfamethoxazole in mesocosm-scale tanks at the Prairie Wetland Research Facility during the summer of 2018.

References for Appendix C

Challis, J.K., Hanson, M.L., Wong, C.S.: Development and calibration of an organic-diffusive gradients in thin films aquatic passive sampler for a diverse suite of polar organic contaminants. *Analytical Chemistry* **2016**; *88*: 10583-10591.

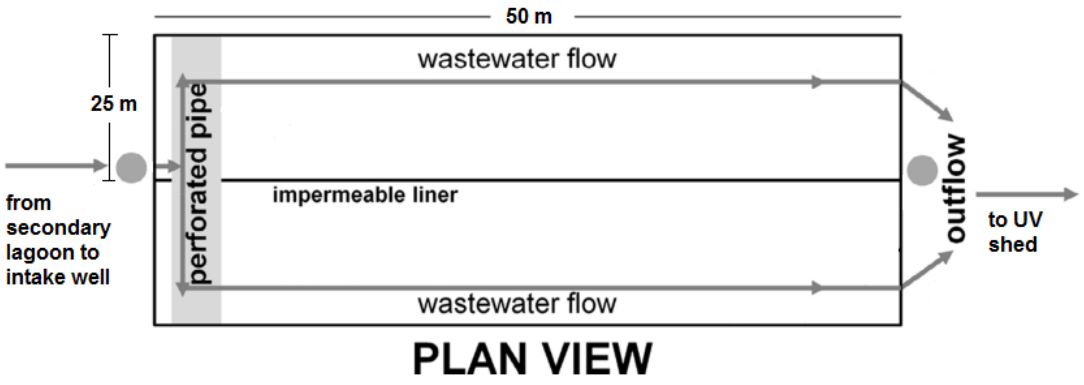
Salzmann, R.D., Ackerman, J.N., Cicek, N. Pilot-scale, on-site investigation of crushed recycled glass as tertiary filter media for municipal lagoon wastewater treatment. *Environmental Technology* **2020**; *9*.

7.4. APPENDIX D: ADDITIONAL INFORMATION FOR CHAPTER 5

ATTENUATION OF PHARMACEUTICALS, NUTRIENTS AND TOXICITY IN A RURAL SEWAGE LAGOON SYSTEM INTEGRATED WITH A SUBSURFACE FILTRATION TECHNOLOGY

SUMMARY

The following includes the schematics for the full-scale subsurface passive filter located in Dunnottar. Also, the full range of measured pharmaceutical concentrations are presented, ialong with details on internal standards, limits of detection and quantification, sampling rates. Finally, the information on the calculation of hazard quotients is available, along with data for the toxicity tests.



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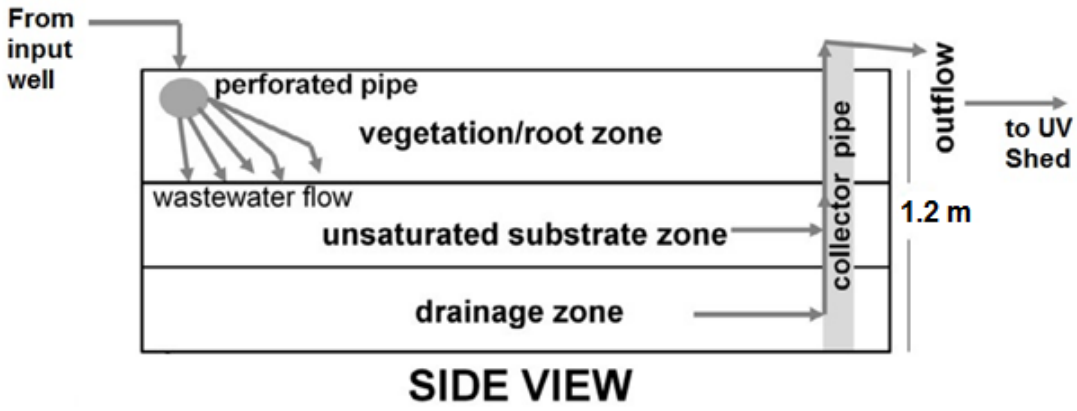


c)

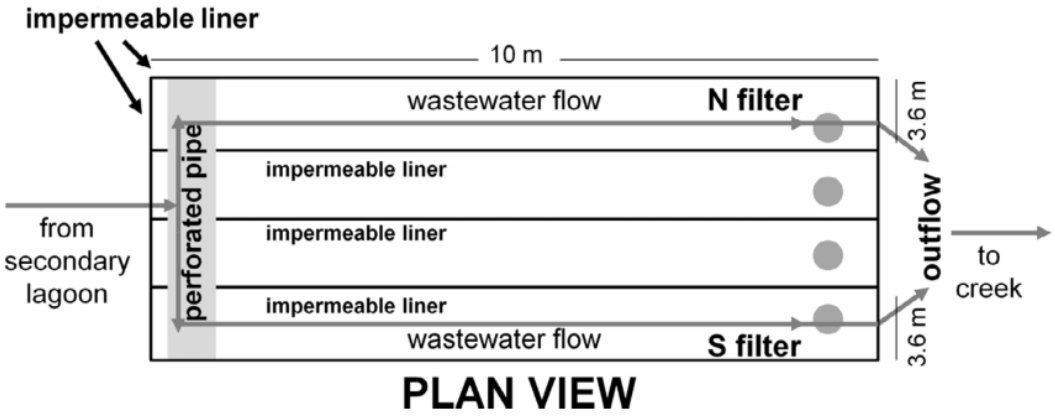
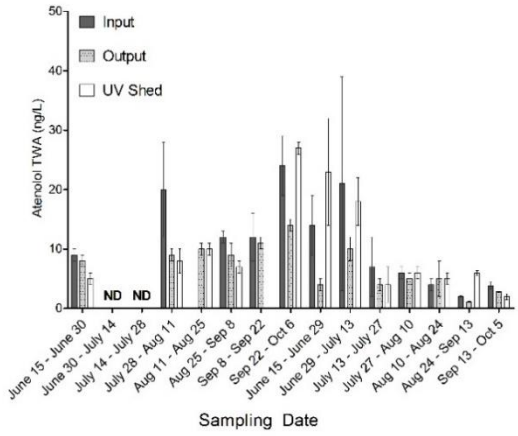
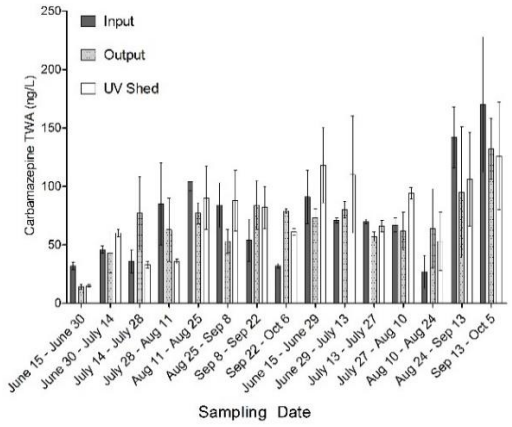


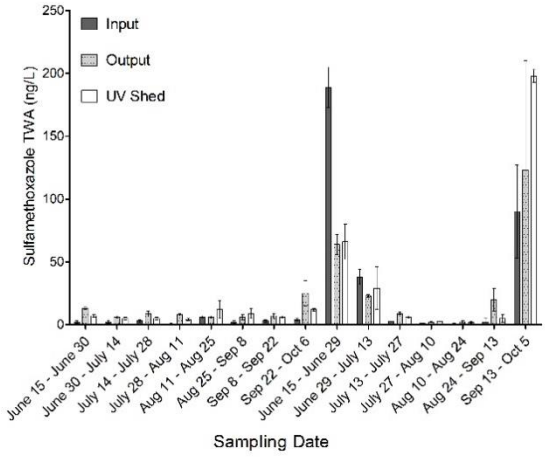
Figure D1. Schematic of full-scale subsurface passive filter (not to scale). Wastewater flow path indicated by gray arrows. 2a) plan view full-scale; 2b) side view full-scale, and 2c) plan view pilot-scale.



a)



b)



c)

Figure D2. Time-weighted average concentrations of a) atenolol b) carbamazepine, and c) sulfamethoxazole in the Dunnottar wastewater treatment system in 2015 and 2016 sampled with passive organic chemical integrative samplers (POCIS). Error bars depict standard deviation.

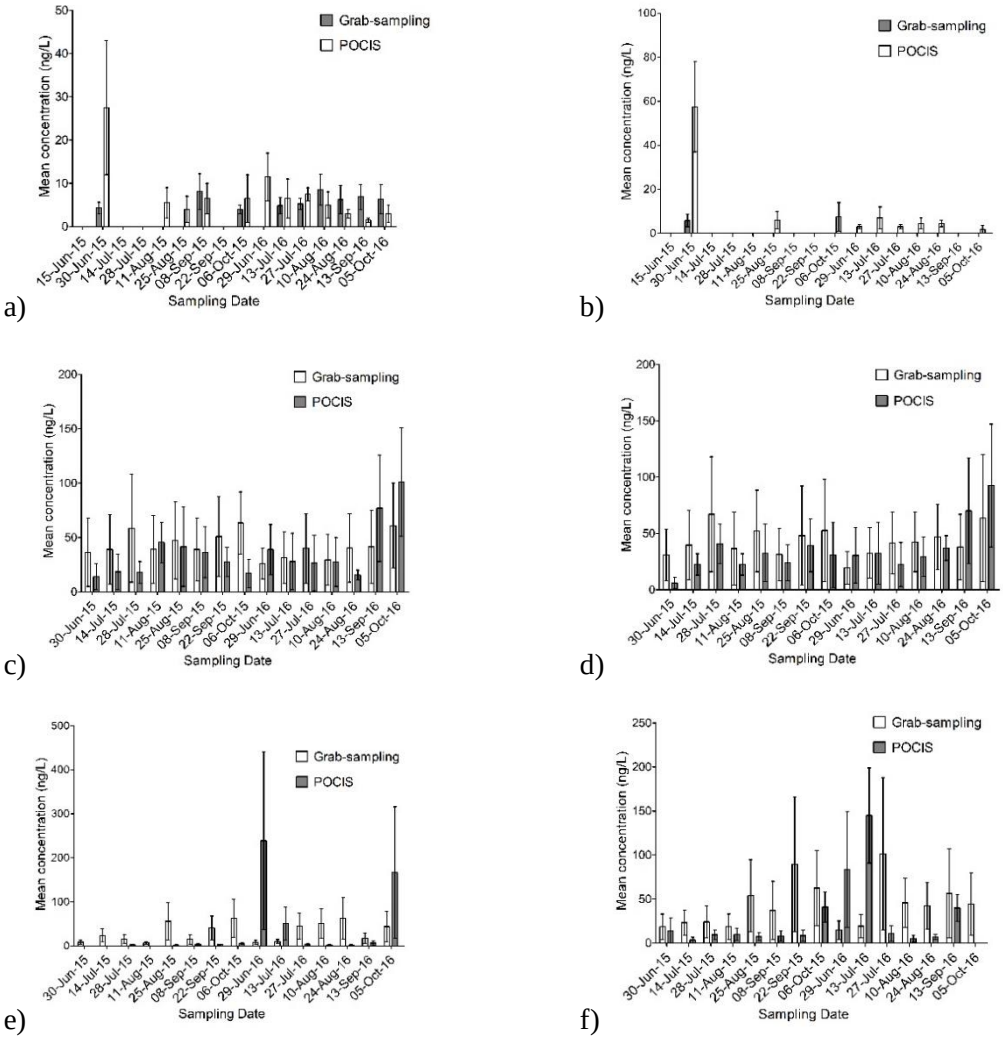


Figure D3. Mean concentrations of pharmaceuticals in grab and passive organic chemical integrative samples at the input and output of the sub-surface passive filter in the Dunnottar wastewater treatment facility: a) atenolol-input, b) atenolol-output, c) carbamazepine-input, d) carbamazepine-output, e) sulfamethoxazole-input, and f) sulfamethoxazole-output.

Table D1. List of analytes, their reference internal standards and limits of quantification for grab- and POCIS samples in wastewater. All compounds were measured in positive ionization mode.

Analyte	**Reference internal standard**	**Limit of quantification in grab-samples (ng L^{-1})**	**Limit of quantification in POCIS (ng L^{-1})**
Atenolol	Atenolol-d_7	2.4	2
Carbamazepine	Carbamazepine-d_{10}	1	1.2
Ciprofloxacin	Ciprofloxacin-d_8	12	9
Clarithromycin	Josamycin	1	9
Enrofloxacin	Enrofloxacin-d_5	9	12
Erythromycin	Josamycin	9	3
Fluoxetine	Fluoxetine-d_6	15	18
Metoprolol	Metoprolol-d_7	5	2.1
Paroxetine	Fluoxetine-d_6	12	9
Propranolol	Propranolol-d_7	12	9
Roxithromycin	Josamycin	12	9
Spiramycin	Josamycin	12	9
Sulfamethazine	Sulfamethazine-$^{13}C_6$	1	1.8
Sulfamethoxazole	Sulfamethoxazole-d_4	3	2.1
Sulfisoxazole	Sulfamethoxazole-d_4	12	9
Sulphachloropyridazine	Sulfamethazine-$^{13}C_6$	12	9
Sulphadimethoxine	Sulphadimethoxine-d_6	5	9
Sulfapyridine	Sulfapyridine-d_4	3	9
Trimethoprim	Trimethoprim-d_3	2	2.7
Tylosin	No IS	30	30

Table D2. Sampling rates for passively sampled pharmaceuticals in the Dunnottar wastewater treatment facility in 2015 and 2016. Sampling rates shown are for detected compounds only.

Compound	**Sampling rate ($L\ d^{-1}$)**	**Reference**
Atenolol	0.05	MacLeod et al. (2007)
Carbamazepine	0.28	Challis et al. (2016)
Clarithromycin	0.38	Challis et al. (2016)
Erythromycin	0.183	MacLeod et al. (2007)
Metoprolol	0.599	MacLeod et al. (2007)
Propranolol	0.147	MacLeod et al. (2007)
Sulfamethoxazole	0.22	Challis et al. (2016)
Sulfapyridine	0.15	Challis et al. (2016)
Trimethoprim	0.26	Challis et al. (2016)

Table D3: Mean concentrations (ng L^{-1}) of organic micropollutants in grab-samples from the Dunnottar wastewater treatment system in 2015 and 2016. All other compounds had non-detectable concentrations. LOD and LOQ (ng L^{-1}) are indicated below each analyte's name. NA: results above LOD, but below LOQ. "-": not measured. ND: non-detectable.

Compound	Sampling Date	Sampling Site						
		Primary Lagoon	Secondary Lagoon	Intake	Outflow	UV Shed	Creek	Highway
Atenolol LOD 0.6 LOQ 2.4	**June 15, 2015**	403	16	-	-	-	ND	-
	June 30, 2015	2470	177	6	9	6	6	9
	July 14, 2015	1630	164	ND	ND	ND	ND	ND
	July 28, 2015	888	14	ND	ND	ND	ND	ND
	Aug. 11, 2015	983	4	NA	NA	NF	NA	NA
	Aug. 25, 2015	546	3	ND	ND	ND	ND	ND
	Sept. 8, 2015	362	9	12	ND	ND	ND	ND
	Sept. 22, 2015	112	15	ND	ND	ND	ND	ND
	Oct. 6, 2015	67	6	5	ND	ND	ND	ND
	June 29, 2016	15.3	21	ND	ND	ND	6	ND
	July 13, 2016	129	11	7	NA	3	NA	2
	July 27, 2016	226	8	6	NA	NA	NA	ND
	Aug. 10, 2016	248	12	12	NA	3	5	5
	Aug. 24, 2016	95	10	10	ND	NA	NA	NA
	Sept. 13, 2016	85	9	10	ND	6	5	ND
	Oct. 5, 2016	91	9	10	NA	NA	NA	NA
Carbamazepine	**June 15, 2015**	97	70	NA	NA	NA	8	NA

LOD 0.3	**June 30, 2015**	299	118	68	54	46	39	47
LOQ 1	**July 14, 2015**	327	114	71	70	69	68	65
	July 28, 2015	288	101	108	118	83	73	58
	Aug. 11, 2015	261	108	70	69	70	70	65
	Aug. 25, 2015	232	118	83	88	78	75	85
	Sept. 8, 2015	280	106	68	55	31	82	44
	Sept. 22, 2015	322	164	88	92	86	83	83
	Oct. 6, 2015	258	132	92	98	103	101	94
	June 29, 2016	129	71	40	34	34	38	40
	July 13, 2016	194	104	55	55	55	55	56
	July 27, 2016	183	109	72	69	70	68	64
	Aug. 10, 2016	270	108	53	69	65	93	62
	Aug. 24, 2016	250	116	72	76	72	78	77
	Sept. 13, 2016	254	78	75	67	96	69	63
	Oct. 5, 2016	320	100	100	120	120	97	96
	June 15, 2015	10	ND	-	-	-	ND	-
	June 30, 2015	ND	ND	ND	ND	ND	ND	ND
Clarithromycin	**July 14, 2015**	ND	ND	ND	ND	ND	ND	ND
LOD 0.3	**July 28, 2015**	ND	ND	ND	ND	ND	ND	ND
LOQ 1	**Aug. 11, 2015**	8	ND	ND	ND	ND	ND	ND
	Aug. 25, 2015	ND	ND	ND	ND	ND	ND	ND
	Sept. 8, 2015	18	ND	ND	ND	ND	ND	ND

	Sept. 22, 2015	ND	ND	ND	ND	ND	ND	ND
	Oct. 6, 2015	ND	ND	ND	ND	ND	ND	ND
	June 29, 2016	1	1	ND	ND	ND	ND	ND
	July 13, 2016	1	ND	ND	ND	ND	ND	ND
	July 27, 2016	1	NA	ND	ND	ND	ND	ND
	Aug. 10, 2016	NA	ND	ND	ND	ND	ND	ND
	Aug. 24, 2016	1	ND	ND	NA	ND	ND	ND
	Sept. 13, 2016	12	NA	ND	ND	ND	ND	ND
	Oct. 5, 2016	1	ND	ND	ND	ND	ND	ND
	June 15, 2015	ND	ND	-	-	-	ND	-
	June 30, 2015	ND	ND	ND	ND	ND	ND	ND
	July 14, 2015	ND	ND	ND	ND	ND	ND	ND
	July 28, 2015	ND	ND	ND	ND	ND	ND	ND
	Aug. 11, 2015	ND	ND	ND	ND	ND	ND	ND
Erythromycin	**Aug. 25, 2015**	ND	ND	ND	ND	ND	ND	ND
LOD 2.7	**Sept. 8, 2015**	ND	ND	ND	ND	ND	ND	ND
LOQ 9	**Sept. 22, 2015**	ND	ND	ND	ND	ND	ND	ND
	Oct. 6, 2015	ND	ND	ND	ND	ND	ND	ND
	June 29, 2016	ND	ND	ND	ND	ND	ND	ND
	July 13, 2016	ND	ND	ND	ND	ND	ND	ND
	July 27, 2016	ND	ND	ND	ND	ND	ND	ND
	Aug. 10, 2016	ND	ND	ND	ND	ND	ND	ND

	Aug. 24, 2016	ND	ND	ND	ND	ND	ND	ND
	Sept. 13, 2016	NA	ND	ND	ND	ND	ND	ND
	Oct. 5, 2016	ND	ND	ND	ND	ND	ND	ND
Metoprolol LOD 1.7 LOD 5	**June 15, 2015**	190	34	-	-	-	NA	-
	June 30, 2015	103	84	ND	ND	ND	ND	ND
	July 14, 2015	135	64	ND	ND	ND	ND	ND
	July 28, 2015	111	35	5.0	ND	ND	ND	ND
	Aug. 11, 2015	202	6	ND	ND	ND	ND	ND
	Aug. 25, 2015	291	20	ND	ND	ND	ND	ND
	Sept. 8, 2015	480	11	ND	NA	NA	NA	ND
	Sept. 22, 2015	331	NA	ND	NA	NA	ND	ND
	Oct. 6, 2015	324	22	ND	ND	NA	ND	ND
	June 29, 2016	321	124	7	ND	ND	ND	ND
	July 13, 2016	427	10	ND	ND	ND	ND	ND
	July 27, 2016	610	NA	ND	ND	ND	ND	ND
	Aug. 10, 2016	448	NA	ND	ND	ND	ND	ND
	Aug. 24, 2016	295	8	ND	ND	ND	ND	ND
	Sept. 13, 2016	745	8	ND	ND	ND	ND	ND
	Oct. 5, 2016	340	NA	ND	ND	ND	ND	ND
Propranolol LOD 3.6 LOQ 12	**June 15, 2015**	NA	ND	-	-	-	ND	-
	June 30, 2015	NA	ND	ND	ND	ND	ND	ND
	July 14, 2015	NA	ND	ND	ND	ND	ND	ND

	July 28, 2015	NA	ND	ND	ND	ND	ND	ND
	Aug. 11, 2015	12	ND	ND	ND	ND	ND	ND
	Aug. 25, 2015	NA	ND	ND	ND	ND	ND	ND
	Sept. 8, 2015	NA	ND	ND	ND	ND	ND	ND
	Sept. 22, 2015	NA	ND	ND	ND	ND	ND	ND
	Oct. 6, 2015	NA	ND	ND	ND	ND	ND	ND
	June 29, 2016	ND	ND	ND	ND	ND	ND	ND
	July 13, 2016	ND	ND	ND	ND	ND	ND	ND
	July 27, 2016	16	ND	ND	ND	ND	ND	ND
	Aug. 10, 2016	74	ND	ND	ND	ND	ND	ND
	Aug. 24, 2016	351	ND	NA	ND	ND	ND	ND
	Sept. 13, 2016	28	ND	ND	ND	ND	ND	ND
	Oct. 5, 2016	36	ND	ND	ND	ND	ND	ND
	June 15, 2015	146	42	-	-	-	NA	-
	June 30, 2015	659	47	13	33	25	11	10
	July 14, 2015	320	50	39	38	40	34	22
Sulfamethoxazole	**July 28, 2015**	257	33	26	42	36	37	21
LOD 0.9	**Aug. 11, 2015**	112	26	10	33	32	26	11
LOQ 3	**Aug. 25, 2015**	261	125	99	95	83	93	73
	Sept. 8, 2015	394	88.8	26	70	36	57	34
	Sept. 22, 2015	514	167	68	166	173	158	55
	Oct. 6, 2015	290	100	106	105	110	90	81

	June 29, 2016	64	52	13	25	4	18	NA
	July 13, 2016	98	15	15	33	22	24	NA
	July 27, 2016	426	104	75	188	452	65	NA
	Aug. 10, 2016	96	145	84	74	8	153	64
	Aug. 24, 2016	616	125	110	69	72	32	19
	Sept. 13, 2016	348	170	29	107	88	66	NA
	Oct. 5, 2016	504	53	79	80	70	57	61
	June 15, 2015	NA	NA	-	-	-	NA	-
	June 30, 2015	NA	NA	NA	NA	NA	NA	NA
	July 14, 2015	NA	NA	NA	NA	NA	NA	NA
	July 28, 2015	NA	NA	NA	NA	NA	NA	NA
	Aug. 11, 2015	NA	NA	NA	NA	NA	NA	NA
	Aug. 25, 2015	NA	NA	NA	NA	NA	NA	NA
Sulfapyridine	**Sept. 8, 2015**	NA	NA	NA	NA	NA	NA	NA
LOD 0.9	**Sept. 22, 2015**	NA	NA	NA	NA	NA	NA	NA
LOQ 3	**Oct. 6, 2015**	NA	NA	NA	NA	NA	NA	NA
	June 29, 2016	NA	NA	NA	ND	ND	ND	ND
	July 13, 2016	NA	NA	NA	ND	ND	ND	ND
	July 27, 2016	NA	NA	NA	NA	ND	ND	ND
	Aug. 10, 2016	NA	NA	NA	NA	ND	ND	ND
	Aug. 24, 2016	NA	NA	NA	NA	ND	ND	ND
	Sept. 13, 2016	NA	NA	NA	NA	6	3	ND

	Oct. 5, 2016	NA	NA	NA	5	ND	NA	ND
Trimethoprim LOD 0.6 LOQ 2	**June 15, 2015**	NA	NA	-	-	-	NA	-
	June 30, 2015	NA	NA	NA	NA	NA	NA	NA
	July 14, 2015	NA	NA	NA	NA	NA	NA	NA
	July 28, 2015	NA	NA	NA	NA	NA	NA	NA
	Aug. 11, 2015	NA	NA	NA	NA	NA	NA	NA
	Aug. 25, 2015	NA	NA	NA	NA	NA	NA	NA
	Sept. 8, 2015	NA	NA	NA	NA	NA	NA	NA
	Sept. 22, 2015	NA	NA	NA	NA	NA	NA	NA
	Oct. 6, 2015	NA	NA	NA	NA	NA	NA	NA
	June 29, 2016	NA	NA	NA	ND	ND	ND	ND
	July 13, 2016	NA	NA	NA	NA	ND	2	2
	July 27, 2016	NA	NA	NA	NA	ND	NA	2
	Aug. 10, 2016	NA	NA	NA	NA	NA	NA	NA
	Aug. 24, 2016	NA	NA	NA	NA	NA	NA	NA
	Sept. 13, 2016	NA	NA	NA	NA	NA	NA	NA
	Oct. 5, 2016	NA	NA	NA	NA	3	NA	ND

Table D4: Time-weighted average concentrations (ng L^{-1}) of passively collected organic micropollutants in the Dunnottar wastewater treatment system full-scale passive filter in 2015 and 2016. Standard deviations are depicted in parentheses. All other compounds had non-detectable concentrations. LOD and LOQ (ng L^{-1}) are indicated below each compound's name. NA: results above LOD, but below LOQ. ND: non-detectable.

Compound	Sampling period	Sampling Sites		
		Input	**Output**	**UV Shed**
Atenolol LOD 0.6 LOQ 2	**June 15 – June 30, 2015**	43 (12)	78 (37)	13 (1)
	June 30 – July 14, 2015	ND	ND	ND
	July 14 – July 28, 2015	ND	ND	ND
	July 28 – Aug. 11, 2015	9 (2)	NA	50 (10)
	Aug. 11 – Aug. 25, 2015	7 (1)	10 (2)	9 (2)
	Aug. 25 – Sept. 8, 2015	10 (3)	NA	7 (1)
	Sept. 8 – Sept. 22, 2015	ND	ND	ND
	Sept. 22 – Oct. 6, 2015	12 (1)	14 (1)	10 (1)
	June 15 – June 29, 2016	17 (6)	4 (2)	30 (10)
	June 29 – July 13, 2016	11 (2)	12 (2)	22 (5)
	July 13 – July 27, 2016	9 (6)	4 (2)	10 (1)
	July 27 – Aug. 10, 2016	8 (2)	7 (2)	7 (1)
	Aug. 10 – Aug. 24, 2016	4 (2)	6 (3)	6 (1)
	Aug. 24 – Sept. 13, 2016	2 (1)	NA	5 (1)
	Sept. 13 – Oct. 5, 2016	5 (1)	3.5 (0.1)	3 (1)
Carbamazepine	**June 15 – June 30, 2015**	26 (2)	11 (1)	11 (1)

LOD 0.4 LOQ 1.2	**June 30 – July 14, 2015**	35 (2)	32 (13)	45 (2)
	July 14 – July 28, 2015	28 (8)	58 (23)	25 (2)
	July 28 – Aug. 11, 2015	64 (27)	32 (13)	27 (4)
	Aug. 11 – Aug. 25, 2015	78 (5)	58 (7)	75 (16)
	Aug. 25 – Sept. 8, 2015	60 (13)	40 (8)	68 (21)
	Sept. 8 – Sept. 22, 2015	41 (14)	63 (16)	62 (14)
	Sept. 22 – Oct. 6, 2015	30 (4)	60 (2)	44 (1)
	June 15 – June 29, 2016	62 (16)	55 (6)	73 (14)
	June 29 – July 13, 2016	54 (2)	60 (5)	56 (7)
	July 13 – July 27, 2016	52 (1)	42 (3)	50 (4)
	July 27 – Aug. 10, 2016	50 (5)	47 (12)	71 (3)
	Aug. 10 – Aug. 24, 2016	20 (11)	48 (26)	40 (19)
	Aug. 24 – Sept. 13, 2016	126 (28)	117 (23)	33 (4)
	Sept. 13 – Oct. 5, 2016	151 (51)	147 (38)	141 (24)
Clarithromycin LOD 2.7 LOQ 9	**June 15 – June 30, 2015**	ND	ND	ND
	June 30 – July 14, 2015	ND	ND	ND
	July 14 – July 28, 2015	ND	ND	ND
	July 28 – Aug. 11, 2015	ND	ND	ND
	Aug. 11 – Aug. 25, 2015	ND	ND	ND
	Aug. 25 – Sept. 8, 2015	ND	ND	ND
	Sept. 8 – Sept. 22, 2015	ND	ND	ND
	Sept. 22 – Oct. 6, 2015	ND	ND	ND

	June 15 – June 29, 2016	ND	ND	ND
	June 29 – July 13, 2016	ND	ND	ND
	July 13 – July 27, 2016	ND	ND	ND
	July 27 – Aug. 10, 2016	ND	ND	ND
	Aug. 10 – Aug. 24, 2016	NA	ND	ND
	Aug. 24 – Sept. 13, 2016	ND	ND	ND
	Sept. 13 – Oct. 5, 2016	NA	ND	ND
	June 15 – June 30, 2015	ND	ND	ND
	June 30 – July 14, 2015	ND	ND	ND
	July 14 – July 28, 2015	ND	ND	ND
	July 28 – Aug. 11, 2015	ND	ND	ND
	Aug. 11 – Aug. 25, 2015	NA	ND	ND
	Aug. 25 – Sept. 8, 2015	NA	ND	ND
Metoprolol	**Sept. 8 – Sept. 22, 2015**	NA	NA	NA
LOD 0.6	**Sept. 22 – Oct. 6, 2015**	ND	ND	ND
LOQ 2.1	**June 15 – June 29, 2016**	3.6 (1.7)	1.7 (0.5)	2.6 (1.3)
	June 29 – July 13, 2016	0.77 (0.03)	1.3 (0.3)	1.1 (0.6)
	July 13 – July 27, 2016	ND	NA	ND
	July 27 – Aug. 10, 2016	ND	ND	ND
	Aug. 10 – Aug. 24, 2016	ND	NA	ND
	Aug. 24 – Sept. 13, 2016	ND	ND	ND
	Sept. 13 – Oct. 5, 2016	ND	ND	ND

	June 15 – June 30, 2015	ND	ND	ND
	June 30 – July 14, 2015	ND	ND	ND
	July 14 – July 28, 2015	ND	ND	ND
	July 28 – Aug. 11, 2015	ND	ND	ND
	Aug. 11 – Aug. 25, 2015	ND	ND	ND
	Aug. 25 – Sept. 8, 2015	NA	NA	ND
Propranolol	**Sept. 8 – Sept. 22, 2015**	ND	ND	ND
LOD 2.7	**Sept. 22 – Oct. 6, 2015**	ND	ND	ND
LOQ 9	**June 15 – June 29, 2016**	ND	ND	ND
	June 29 – July 13, 2016	ND	ND	ND
	July 13 – July 27, 2016	ND	ND	ND
	July 27 – Aug. 10, 2016	ND	ND	ND
	Aug. 10 – Aug. 24, 2016	ND	ND	ND
	Aug. 24 – Sept. 13, 2016	ND	ND	ND
	Sept. 13 – Oct. 5, 2016	ND	ND	ND
	June 15 – June 30, 2015	ND	28.4 (0.1)	15 (2)
	June 30 – July 14, 2015	NA	6.7 (0.4)	7 (1)
Sulfamethoxazole	**July 14 – July 28, 2015**	4 (1)	15 (4)	6.8 (1.4)
LOD 0.6	**July 28 – Aug. 11, 2015**	ND	17 (3)	6 (2)
LOQ 2.1	**Aug. 11 – Aug. 25, 2015**	3 (1)	12 (3)	44 (5)
	Aug. 25 – Sept. 8, 2015	5 (2)	14 (2)	21 (10)
	Sept. 8 – Sept. 22, 2015	4 (2)	15 (2)	14 (1)

	Sept. 22 – Oct. 6, 2015	8 (3)	58 (24)	27 (2)
	June 15 – June 29, 2016	441 (37)	149 (18)	155 (32)
	June 29 – July 13, 2016	88 (14)	199 (91)	66 (39)
	July 13 – July 27, 2016	5 (1)	20 (2)	14 (1)
	July 27 – Aug. 10, 2016	4 (1)	9 (1)	10 (1)
	Aug. 10 – Aug. 24, 2016	3 (1)	10 (4)	8 (2)
	Aug. 24 – Sept. 13, 2016	12 (1)	55 (25)	38 (27)
	Sept. 13 – Oct. 5, 2016	316 (17)	NA	NA
Sulfapyridine LOD 2.7 LOQ 9	**June 15 – June 30, 2015**	ND	ND	ND
	June 30 – July 14, 2015	ND	ND	ND
	July 14 – July 28, 2015	ND	NA	ND
	July 28 – Aug. 11, 2015	NA	NA	NA
	Aug. 11 – Aug. 25, 2015	NA	NA	NA
	Aug. 25 – Sept. 8, 2015	NA	NA	NA
	Sept. 8 – Sept. 22, 2015	NA	NA	NA
	Sept. 22 – Oct. 6, 2015	NA	NA	NA
	June 15 – June 29, 2016	NA	NA	NA
	June 29 – July 13, 2016	NA	NA	NA
	July 13 – July 27, 2016	NA	NA	NA
	July 27 – Aug. 10, 2016	NA	10 (1)	9 (2)
	Aug. 10 – Aug. 24, 2016	ND	NA	NA
	Aug. 24 – Sept. 13, 2016	NA	NA	NA

	Sept. 13 – Oct. 5, 2016	NA	NA	NA
	June 15 – June 30, 2015	NA	NA	NA
	June 30 – July 14, 2015	NA	NA	NA
	July 14 – July 28, 2015	NA	NA	NA
	July 28 – Aug. 11, 2015	NA	NA	NA
	Aug. 11 – Aug. 25, 2015	NA	NA	NA
	Aug. 25 – Sept. 8, 2015	NA	NA	NA
Trimethoprim	**Sept. 8 – Sept. 22, 2015**	NA	NA	NA
LOD 0.8	**Sept. 22 – Oct. 6, 2015**	NA	NA	NA
LOQ 2.7	**June 15 – June 29, 2016**	10 (1)	4 (1)	6 (2)
	June 29 – July 13, 2016	7 (1)	9 (5)	9 (5)
	July 13 – July 27, 2016	10 (1)	3 (1)	3 (1)
	July 27 – Aug. 10, 2016	4 (1)	2 (1)	9 (1)
	Aug. 10 – Aug. 24, 2016	NA	3 (1)	NA
	Aug. 24 – Sept. 13, 2016	ND	ND	ND
	Sept. 13 – Oct. 5, 2016	ND	ND	ND

Table D5. Calculated hazard quotients for organic micropollutants detected by grab-sampling in the Dunnottar wastewater treatment facility in 2015 and 2016. Full data set of measured concentrations available in Table D2.

Compound	Site type	Species	Toxicity endpoint	Toxicity value (mg L^{-1})	MEC (mg L^{-1})	HQ	Reference
Atenolol	Primary Lagoon	*Lemna spp.* (Duckweed)	EC50 - 7 day growth inhibition	> 320	2.5×10^{-3}	7.8×10^{-3}	(Cleuvers, 2005)
		Daphnia magna	EC50 - 48 h immobilization	313	2.5×10^{-3}	8.0×10^{-3}	(Cleuvers, 2005)
		Pimephales promelas (Fathead minnow)	NOEC - 32 day growth inhibition	3.2	2.5×10^{-3}	0.8	(Kuester et al., 2010)
	Secondary Lagoon	*Lemna spp.*	EC50 - 7 day growth inhibition	> 320	1.8×10^{-4}	5.6×10^{-4}	(Cleuvers, 2005)
		Daphnia magna	EC50 - 48 h immobilization	313	1.8×10^{-4}	5.8×10^{-4}	(Cleuvers, 2005)
		Pimephales promelas	NOEC - 32 day growth inhibition	3.2	1.8×10^{-4}	5.6×10^{-2}	(Kuester et al., 2010)
	Creek	*Lemna spp.*	EC50 - 7 day growth inhibition	> 320	5.9×10^{-6}	1.8×10^{-5}	(Cleuvers, 2005)
		Daphnia magna	EC50 - 48 h immobilization	313	5.9×10^{-6}	1.9×10^{-5}	(Cleuvers, 2005)
		Pimephales promelas	NOEC - 32 day growth inhibition	3.2	5.9×10^{-6}	1.8×10^{-3}	(Kuester et al., 2010)
	Highway	*Lemna spp.*	EC50 - 7 day growth inhibition	> 320	8.8×10^{-6}	2.7×10^{-5}	(Cleuvers, 2005)
		Daphnia magna	EC50 - 48 h immobilization	313	8.8×10^{-6}	2.8×10^{-5}	(Cleuvers, 2005)
		Pimephales promelas	NOEC - 32 day growth inhibition	3.2	8.8×10^{-6}	2.8×10^{-3}	(Kuester et al., 2010)
Carbamazepine	Primary Lagoon	*Lemna minor*	EC50 - 7 day growth inhibition	22.5	3.3×10^{-4}	4.3×10^{-3}	(Cleuvers, 2003)
		Daphnia magna	EC50 - 48 h immobilization	> 100	3.3×10^{-4}	9.7×10^{-4}	(Cleuvers, 2003)
		Oryzias latipes (Japanese medaka)	LC50 - 28 day exposure	35.4	3.3×10^{-4}	2.8×10^{-3}	(Kim et al., 2007)
	Secondary Lagoon	*Lemna minor*	EC50 - 7 day growth inhibition	22.5	1.6×10^{-4}	1.9×10^{-3}	(Cleuvers, 2003)
		Daphnia magna	EC50 - 48 h immobilization	> 100	1.6×10^{-4}	4.3×10^{-4}	(Cleuvers, 2003)
		Oryzias latipes	LC50 - 28 day exposure	35.4	1.6×10^{-4}	1.2×10^{-3}	(Kim et al., 2007)
	Creek	*Lemna spp.*	EC50 - 7 day growth inhibition	22.5	1.0×10^{-4}	4.4×10^{-3}	(Cleuvers, 2003)

Compound	Site type	Species	Toxicity endpoint	Toxicity value (mg L^{-1})	MEC (mg L^{-1})	HQ	Reference
		Daphnia magna	EC50 - 48 h immobilization	> 100	1.0×10^{-4}	1.0×10^{-3}	(Cleuvers, 2003)
		Pimephales promelas	NOEC - 32 day growth inhibition	35.4	1.0×10^{-4}	2.8×10^{-3}	(Kim et al., 2007)
	Highway	*Lemna spp.*	EC50 - 7 day growth inhibition	22.5	9.4×10^{-5}	4.2×10^{-3}	(Cleuvers, 2003)
		Daphnia magna	EC50 - 48 h immobilization	> 100	9.4×10^{-5}	9.4×10^{-4}	(Cleuvers, 2003)
		Pimephales promelas	NOEC - 32 day growth inhibition	35.4	9.4×10^{-5}	2.7×10^{-3}	(Kim et al., 2007)
Clarithromycin	Primary Lagoon	*Pseudokirchneriella subcapita*	EC50 - 72 h growth inhibition	0.002	1.8×10^{-5}	9.0	(Isidori et al., 2005)
		Daphnia magna	EC50 - 24 h immobilization	25.7	1.8×10^{-5}	7.0×10^{-4}	(Isidori et al., 2005)
		Danio rerio (zebrafish)	LC50 - 96 h exposure	> 1000	1.8×10^{-5}	1.8×10^{-5}	(Isidori et al., 2005)
	Secondary Lagoon	*Pseudokirchneriella subcapita*	EC50 - 72 h growth inhibition	0.002	1.1×10^{-6}	0.55	(Isidori et al., 2005)
		Daphnia magna	EC50 - 24 h immobilization	25.7	1.1×10^{-6}	4.3×10^{-5}	(Isidori et al., 2005)
		Danio rerio	LC50 - 96 h exposure	> 1000	1.1×10^{-6}	1.0×10^{-6}	(Isidori et al., 2005)
	Creek	*Lemna spp.*	EC50 - 7 day growth inhibition	0.002	$< 3 \times 10^{-7}$	0.15	(Isidori et al., 2005)
		Daphnia magna	EC50 - 48 h immobilization	25.7	$< 3 \times 10^{-7}$	1.17×10^{-5}	(Isidori et al., 2005)
		Pimephales promelas	NOEC - 32 day growth inhibition	> 1000	$< 3 \times 10^{-7}$	3.0×10^{-7}	(Isidori et al., 2005)
	Highway	*Lemna spp.*	EC50 - 7 day growth inhibition	0.002	$< 3 \times 10^{-7}$	0.15	(Isidori et al., 2005)
		Daphnia magna	EC50 - 48 h immobilization	25.7	$< 3 \times 10^{-7}$	1.17×10^{-5}	(Isidori et al., 2005)
		Pimephales promelas	NOEC - 32 day growth inhibition	> 1000	$< 3 \times 10^{-7}$	3.0×10^{-7}	(Isidori et al., 2005)
Metoprolol	Primary Lagoon	*Pseudokirchneriella subcapitata*	EC50 - 72 h growth inhibition	> 320	7.5×10^{-4}	2.3×10^{-3}	(Cleuvers, 2005)
		Daphnia magna	EC50 - 48 h immobilization	438	7.5×10^{-4}	1.7×10^{-3}	(Cleuvers, 2005)
		Oryzias latipes	LC50 - 48 h exposure	31.0	7.5×10^{-4}	2.4×10^{-2}	(van den Brandhof and Montforts, 2010)
	Secondary Lagoon	*Pseudokirchneriella subcapitata*	EC50 - 72 h growth inhibition	> 320	1.2×10^{-4}	3.8×10^{-4}	(Cleuvers, 2005)

Compound	Site type	Species	Toxicity endpoint	Toxicity value (mg L^{-1})	MEC (mg L^{-1})	HQ	Reference
		Daphnia magna	EC50 - 48 h immobilization	438	1.2×10^{-4}	2.7×10^{-4}	(Cleuvers, 2005)
		Oryzias latipes	LC50 - 48 h exposure	31.0	1.2×10^{-4}	3.9×10^{-3}	(van den Brandhof and Montforts, 2010)
	Creek	*Pseudokirchneriella subcapitata*	EC50 - 72 h growth inhibition	> 320	$< 1.7 \times 10^{-6}$	5.3×10^{-6}	(Cleuvers, 2005)
		Daphnia magna	EC50 - 48 h immobilization	438	$< 1.7 \times 10^{-6}$	3.9×10^{-6}	(Cleuvers, 2005)
		Oryzias latipes	LC50 - 48 h exposure	31.0	$< 1.7 \times 10^{-6}$	5.5×10^{-5}	(van den Brandhof and Montforts, 2010)
	Highway	*Pseudokirchneriella subcapitata*	EC50 - 72 h growth inhibition	> 320	$< 1.7 \times 10^{-6}$	5.3×10^{-6}	(Cleuvers, 2005)
		Daphnia magna	EC50 - 48 h immobilization	438	$< 1.7 \times 10^{-6}$	3.9×10^{-6}	(Cleuvers, 2005)
		Oryzias latipes	LC50 - 48 h exposure	31.0	$< 1.7 \times 10^{-6}$	5.5×10^{-5}	(van den Brandhof and Montforts, 2010)
Sulfamethoxazole	Primary Lagoon	*Selenastrum capricornutum*	EC50 - 72 h growth inhibition	0.52	6.6×10^{-4}	1.3	(Isidori et al., 2005)
		Daphnia magna	EC50 - 48 h immobilization	25.2	6.6×10^{-4}	2.6×10^{-2}	(Isidori et al., 2005)
		Oryzias latipes	LC50 - 48 h exposure	562.5	6.6×10^{-4}	1.2×10^{-3}	(Kim et al., 2007)
	Secondary Lagoon	*Selenastrum capricornutum*	EC50 - 72 h growth inhibition	0.52	1.7×10^{-4}	0.3	(Isidori et al., 2005)
		Daphnia magna	EC50 - 48 h immobilization	25.2	1.7×10^{-4}	6.8×10^{-3}	(Isidori et al., 2005)
		Oryzias latipes	LC50 - 48 h exposure	562.5	1.7×10^{-4}	3.0×10^{-4}	(Kim et al., 2007)
	Creek	*Selenastrum capricornutum*	EC50 - 72 h growth inhibition	0.52	1.6×10^{-4}	0.3	(Isidori et al., 2005)
		Daphnia magna	EC50 - 48 h immobilization	25.2	1.6×10^{-4}	6.3×10^{-3}	(Isidori et al., 2005)
		Oryzias latipes	LC50 - 48 h exposure	562.5	1.6×10^{-4}	2.8×10^{-4}	(Kim et al., 2007)
	Highway	*Selenastrum capricornutum*	EC50 - 72 h growth inhibition	0.52	8.1×10^{-5}	0.2	(Isidori et al., 2005)
		Daphnia magna	EC50 - 48 h immobilization	25.2	8.1×10^{-5}	3.2×10^{-3}	(Isidori et al., 2005)
		Oryzias latipes	LC50 - 48 h exposure	562.5	8.1×10^{-5}	1.4×10^{-4}	(Kim et al., 2007)

Table D6. Levels of standard wastewater parameters measured in the effluent of the passive filter at Dunnottar, Manitoba, Canada in 2015 and 2016. The regulatory maximum levels are: BOD 25 mg L^{-1}; TSS 25 mg L^{-1}; TP 1 mg L^{-1}

	2015					**2016**			
Parameter	June 17	July 15	August 26	September 25	October 16	July 7	August 24	September 21	October 13
BOD (mg L^{-1})	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0
COD (mg L^{-1})	28	46	49	48	46	43	53	< 2.0	< 2.0
Total phosphorus (mg L^{-1})	0.019	0.041	0.280	0.303	0.378	0.319	0.167	0.685	1.89
TSS (mg L^{-1})	6.0	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0
Total coliforms (CFU L^{-1})	< 3	< 3	< 3	< 3	< 3	< 3	< 3	4	< 3
Fecal coliforms (CFU L^{-1})	< 3	< 3	< 3	< 3	< 3	< 3	< 3	4	< 3

Table D7. Comparison of concentrations of target pharmaceutical compounds (ng L^{-1}) in water grab-samples from receiving waters of different Manitoban wastewater systems. "ND": not detected.

Compound	Dunnottar Full-Scale	Dunnottar Pilot-Scale[a]	Grand Marais[b]	Winkler/Morden[c]
Atenolol	ND-2476	ND-856	ND	ND-270
Carbamazepine	34-327	44-256	85-500	18-85
Metoprolol	ND-745	ND-26.7	ND	ND-19
Sulfamethoxazole	ND-659	ND-403	ND-21	ND-70

[a]Anderson et al., (2015); [b] Anderson et al., (2013); [c]Carlson et al., (2013)

Table D8. <u>Vibrio fischeri</u> (Microtox ® assay) bioluminescence presented as percent of control (±SD) after 15 minutes exposure to test water samples, including all triplicate samples collected as part of rotating triplicate sample schedule at the Dunnottar system in 2015.

Sample Locations	June 10	June 30	July 14	July 28	August 11	August 25	September 8	September 22	October 6	Average
Primary lagoon	69 (±2)	68 (±1) 77 (±1) 65 (±1)	50 (±1)	67 (±4)	53 (±2)	95 (±8)	47 (±1)	89 (±1)	104 (±2)	71
Input	-	0 (±0)	4 (±1)	49 (±1) 62 (±3) 48 (±3)	10 (±1)	7 (±1)	43 (±1)	17 (±1)	59 (±1)	30
Output	-	100 (±3)	94 (±1)	73 (±1)	62 (±1) 70 (±3) 67 (±2)	79 (±2)	104 (±1)	67 (±2)	66 (±3)	78
Creek	60 (±1)	113 (±2)	77 (±6)	62 (±1)	71 (±1)	65 (±2)	61 (±1) 64 (±2) 63 (±1)	55 (±1)	99 (±1) 76 (±4) 85 (±2)	73
Highway	-	91 (±3)	107 (±3)	91 (±6)	98 (±2)	80 (±2)	103 (±2)	102 (±2) 99 (±1) 68 (±2)	100 (±5)	94
Field Blank (milli-q)	74 (±3)	44 (±2)	106 (±4)	90 (±2)	68 (±2)	84 (±2)	108 (±2)	102 (±1)	92 (±1)	85
Lake Blank	107 (±3)	100 (±2)	104 (±2)	105 (±1)	110 (±2)	106 (±3)	111 (±2)	104 (±3)	109 (±1)	106

Table D9. Vibrio fischeri (Microtox ® assay) bioluminescence presented as percent of control (±SD) after 15 minutes exposure to test water samples, including all triplicate samples collected as part of rotating triplicate sample schedule at the Dunnottar system in 2016.

Sample Locations	June 29	July 13	July 27	August 10	August 24	September 13	October 5	Average
Primary lagoon	64 (±2)	57 (±2)	44 (±1)	35 (±2)	43 (±1)	43 (±1)	56 (±1)	49
Input	64 (±1)	43 (±2)	1.3 (±0.9)	1.21 (±0.03)	24 (±10)	32 (±4)	111 (±4)	40
Output	119 (±7)	98 (±2)	100 (±2)	73 (±3)	86 (±4)	108 (±5)	120 (±6)	101
Creek	94 (±8)	88 (±3)	108 (±7)	117 (±4)	90 (±11)	99 (±15)	102 (±5)	100
Field Blank (milli-q)	112 (±5)	108 (±6)	104 (±4)	104 (±4)	114 (±3)	106 (±1)	109 (±1)	108
Lake Blank	119 (±2)	114 (±6)	111 (±4)	111 (±3)	117 (±4)	109 (±3)	117 (±3)	114

References for Appendix D

Anderson, J.C.; Carlson, J.C.; Low, JE, Challis, J.K.; Wong, C.S.; Knapp C.W.; Hanson, M.L. Performance of a constructed wetland in Grand Marais, Manitoba, Canada: Removal of nutrients, pharmaceuticals, and antibiotic resistance genes from municipal wastewater. *Chemistry Central Journal* **2013**; *7*: 54.

Anderson J.C.; Joudan S.; Shoichet, E.; Cuscito L.D.; Alipio, A.E.C.; Donaldson, C.S.; Khan, S.; Goltz, D.M.; Rudy, M.D.; Frank, R.A.; Knapp, C.W.; Hanson, M.L.; Wong, C.S. Reducing nutrients, organic micropollutants, antibiotic resistance, and toxicity in rural wastewater effluent with subsurface filtration treatment technology. *Ecological Engineering* **2015**; *84*: 375-385.

Carlson, J.C.; Anderson, J.C.; Low, J.E.; Cardinal, P.; MacKenzie, S.D.; Beattie S.A.; Challis, J.K.; Bennett, R.J.; Meronek, S.S.; Wilks, R.P.A.; Buhay, W.M.; Wong, C.S.; Hanson, M.L. Presence and hazards of nutrients and emerging organic micropollutants from sewage lagoon discharges into Dead Horse Creek, Manitoba, Canada. *Science of the Total Environment* **2013**; *445*: 64-78.

Cleuvers M. Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects. *Toxicology Letters* **2003**; *142*: 185-194.

Cleuvers M. Initial risk assessment for three beta-blockers found in the aquatic environment. *Chemosphere* **2005**; *59*: 199-205.

Isidori, M.; Lavorgna, M.; Nardelli, A.; Pascarella, L.; Parrella, A. Toxic and genotoxic evaluation of six antibiotics on non-target organisms. *Science of the Total Environment* **2005**; *346*: 87-98.

Kim, Y.; Choi, K.; Jung, J.Y.; Park, S.; Kim, P.G.; Park, J. Aquatic toxicity of acetaminophen, carbamazepine, cimetidine, diltiazem and six major sulfonamides, and their potential ecological risks in Korea. *Environment International* **2007**; *33*: 370-375.

Küster, A.; Alder, A.C.; Escher, B.I.; Duis, K.; Fenner, K.; Garric, J.; Hutchinson, T.H.; Lapen, D.R.; Péry, A.; Römbke, J.; Snape, J.; Ternes, T.; Topp, E.; Wehrhan, A.; Knacker, T. Environmental Risk Assessment of Human Pharmaceuticals in the European Union: A Case Study with the beta-Blocker Atenolol. *Integrated Environmental Assessment and Management* **2010**; *6*: 514-523.

van den Brandhof, E.J.; Montforts M. Fish embryo toxicity of carbamazepine, diclofenac and metoprolol. *Ecotoxicology and Environmental Safety* **2010**; *73*: 1862-1866.