IMPACTS OF WASTEWATER EFFLUENTS AND SEASONAL TRENDS ON LEVELS OF EMERGING CONTAMINANTS IN TWO COLD-REGION RIVERS

A Thesis Submitted to the
College of Graduate and Postdoctoral Studies
In Partial Fulfillment of the Requirements
For the Degree of Master of Science
In the School of Environmental and Sustainability of
the University of Saskatchewan
Saskatoon, Saskatchewan, Canada

By

ANA SHARELYS CARDENAS PEREZ

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis/dissertation in any manner, in whole or in part, for scholarly purposes may be granted by the professor or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

DISCLAIMER

This thesis was exclusively created to meet the thesis and/or exhibition requirements for the degree of Masters of Environment and Sustainability at the University of Saskatchewan. Any reference in this thesis to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not constitute or imply its endorsement, recommendation, or favoring by the University of Saskatchewan. The views ad opinions of the author expressed herein do not state or reflect those of the University of Saskatchewan and shall not be used for advertising or product endorsement purposes. Requests for permission to copy or to make other uses of materials in this thesis/dissertation in whole or part should be addressed to:

Graduate Chair of the School of Environment and Sustainability
117 Science Place
University of Saskatchewan
Saskatoon, Saskatchewan, S7N 5B3, Canada

Dean
College of Graduate and Postdoctoral Studies
University of Saskatchewan
116 Thorvaldson Building, 110 Science Place
Saskatoon, Saskatchewan S7N 5C9 Canada

ABSTRACT

Emerging contaminants such as pharmaceutical drugs have been detected in waters across the globe and are of concern for human and aquatic ecosystems health. Most pharmaceuticals are found at trace concentrations, but the continuous use and potential accumulation of some of these compounds can potentially lead to effects in aquatic organisms. The principal aim of this research was to enhance our understanding of the environmental risks associated with pharmaceuticals as one group of emerging contaminants. Many pharmaceuticals are ionizable organic chemicals (IOCs), which makes their environmental and toxicological behavior particularly challenging to predict due to their partitioning mechanism which is useful to estimate the distribution of the chemical. Therefore, the objective of this thesis was to evaluate the hypothesis that uptake and effects of IOCs in aquatic organisms are influenced by the interaction between environmental, physicochemical, and biological factors. To this end, first, field studies were conducted during spring, summer, and fall of 2021 on water (diffusive gradient in thin film and conventional grab) and sediments at four locations including upstream and downstream of the wastewater treatment plants (WWTPs) of the cities of Saskatoon and Regina in the South Saskatchewan River and Wascana Creek, Saskatchewan, Canada, respectively. Second, seven representative antipsychotic pharmaceuticals were measured in water, sediment, and fish samples up- and downstream of the City of Regina WWTP. Data collected from this research effort indicate contamination with antipsychotic pharmaceuticals, with the potential to adversely impact exposed organisms. Third,

non-target chemical analysis was conducted in water, sediments, and fish samples, at the two locations in Wascana Creek and throughout the three seasons. Data collected from non-target analysis indicated that pharmaceuticals, rubber components and personal care products were the priority pollutants in all the matrices and their transcriptomics changes were also supported by the qPCR analysis. Finally, transcripts of several genes of interest were determined in brain and liver samples from in fathead minnow (*Pimephales promelas*) exposed to the wastewater effluents in Wascana Creek during summer and fall in 2021, using a qPCR gene expression array (the EcoToxChips). The integrative approach used in this study, strongly supports the need to combine chemical analysis with transcriptomics-based approaches as useful tools for assessing of complex mixtures of contaminants in wastewater discharges and their effects in aquatic organisms.

This research provides a better understanding of the risks that pharmaceuticals may pose to aquatic organisms under varying environmental conditions and thereby aid in better protecting aquatic ecosystems in the future.

Keywords: Pharmaceuticals, fathead minnow, *Pimephales promelas*, monitoring, wastewater discharge

ACKNOWLEDGMENTS

I would like to thank my graduate supervisor Dr. Markus Brinkmann for his continued guidance, support, and mentorship throughout my Masters. I would like to extend my thanks to the members of my graduate committee, Dr. Markus Hecker, and Dr. Paul Jones for their comments, support, and advice. I would also like to thank my external examiner, Dr. Robert Laprairie.

I would like to thank Dr. Jonathan Challis, Xiaowen Ji, Jenna Cantin, Dr. Alper Alcaraz, Hannah Mahoney, and Saurabh Prajapati, for their help with lab and field work. Also thank you to Alexis Valery for his assistance with statical analysis.

I would like to acknowledge my funding sources for this project including the NSERC Discovery Grants to Drs. Brinkmann and Giesy, as well as a grant from Western Economic Diversification Canada (6578, 6807, and 000012711). Also, the Canada Foundation for Innovation for the support through an instrumentation grant.

DEDICATION

I dedicate my dissertation work to God, my family, and friends. I dedicate this chapter of my life to God, my strength and light even in the darkest moments. My deepest gratitude to my Mom and Dad. Thanks for being always there for me, thanks for believing in me, and for always being such a great inspiration.

I also dedicate this dissertation to many of my friends, including Jordi and Alexis for all your help, patience, collaboration, great conversations, and for being there during this journey. Thanks Bibi and Gigi, for all your hospitalities, cordially, and support from the very beginning. I really appreciate to have people like you in my life.

I dedicate this work and give special thanks to Cale and Nairn family, for your love, patience, help, constant support and understanding. Your support made this achievement joyful and an excellent experience for me. Thanks for everything.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

^{o}C	degrees Celsius
µg/kg	micrograms per kilogram
µg/L	micrograms per liter
g/day	gram per day
ACN	acetonitrile
AGC	automatic gain control
ANOVA	analysis of variance
AMI	amitriptyline
AREB	the University of Saskatchewan Animal Research Ethics Board
BAF	Bioconcentration
BSAF	Biota-sediment accumulation
BUP	bupropion
CAS	Chemical Abstract Service
CBZ	carbamazepine
CD	Compound Discoverer 3.2
cDNA	is DNA synthesized
CECs	chemicals of emerging concern
CLO	clozapine
cm	centimeter
Ct	the number of cycles of amplification
COVID-19	Coronavirus disease
ddMS2	data dependent MS2
DGT	diffusive gradients in thin films
DO	dissolved oxygen
DOC	dissolved organic carbon
ECHA	European Chemicals Agency
EC_{50}	half maximal effective concentration
ECs	emerging contaminants
FDA	US Food and Drug Administration
FLX	fluoxetine
HESI	Heating electrospray source
HKG	housekeeping genes

HQs	Hazard quotients
IOCs	organic chemicals
Kd	The sediment-water partitioning coefficient
$KgKg^{-1}$	Kilograms per kilograms
LAM	lamotrigine
L/kg	Liters per kilogram
LC	Liquid Chromatography
LC-HRMS	liquid chromatography coupled with high-resolution mass spectrometry
m^2	square meter
M1 and M2	Mixed models
MS/MS	mass spectrometry
mg/L	miligram per liter
mL/min	mililiters per minute
mm	milimeters
n	number of samples
NAM	new approach method
NCE	new chemical entity
ng/L	nanogram per liter
ng/g	nanogram per gram
NTC	non-target chemical
p	p-value
PCPs	personal care products
PES	polyethersulfone
pKa	acidity constant
POCIS	polar organic chemical integrative samplers
PTFE	polytetrafluoroethylene
qPCR	quantitative real-time polymerase chain reaction
REACH	Registration, Evaluation, Authorization, and Restriction of Chemicals
RNA	Ribonucleic acid
RQs	risk quotients
SC	specific conductivity
SDF	supporting data file
SPE	solid-phase extraction
SSR	The South Saskatchewan River
STU	Sum of Toxic Units
TDS	dissolved solids

TP	total phosphorous
TWA	time-weighted averages
UACC	the University of Saskatchewan Animal Care Committee
UHPLC	Ultra high-performance liquid Chromatography
VEN	venlafaxine
v/v	Volume per volume
WWTPs	wastewater treatment plants
WC	Wascana Creek

PREFACE

This thesis is organized and formatted to follow the University of Saskatchewan College of Graduate Studies and Postdoctoral Research guidelines for a manuscript-style thesis. Therefore, there is some repetition between the content presented in each chapter. Chapter 1 is a general introduction and literature review, including project goals and objectives. Chapter 2 is organized as a manuscript for publication in a peer-reviewed scientific journal and a description of author contributions is provided in the preface of this chapter. Chapter 3 is organized as a manuscript for publication in a peer-reviewed scientific journal and a description of author contributions is provided in the preface of this chapter. Supplementary materials related to Chapter 2 that was published and Chapter 3 that will be published have been included in the Appendices section at the end of this thesis. Chapter 4 is a general discussion, overall conclusion, and recommendations for future research. References cited were included at the end of each chapter.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Water security threats

Water is one of the essential natural resources on our planet. It is the basis of all life on Earth and necessary for human well-being (Anderson et al., 2019). At the same time, human activities are negatively affecting water bodies and the aquatic ecosystems that rely on them (Boelee et al., 2019; Falkenmark et al., 2020). To prevent overexploitation and permanent damage to our planetary life-support systems, we need to strike a balance between the use of water and ecosystem conservation (Ogunkunle et al., 2019). A comprehensive evaluation of threats to our global freshwater resources will ultimately allow us to improve water management to achieve water security for humans and ecosystems (Mishra et al., 2021; Cooke et al., 2022).

Water resources are vulnerable to the impacts of urbanization, industry, land use variations, and changes in flow patterns (Gorelick et al., 2020**;** Lin et al., 2021). The persistence and long-term effects of these impacts can differ. Poorly planned infrastructure projects have affected many natural reservoirs and their biodiversity (Hanson 2021). In addition, global climate change is now a central driver of alterations in hydrology, thermal regimes, acidification, and nutrient loading (Foley et al., 2019; Khangaonkar et al., 2019; Chaturvedi et al., 2021). Industrialization, urbanization, and agriculture have resulted in increasing discharges of complex mixtures of chemicals into water bodies (Akhtar et al., 2021**;** Morin-Crini et al., 2022).

As a result, water systems that sustain ecosystems and human populations are under stress (Hoekstra et al., 2018; Moyle et al., 2022). Rivers, aquifers, and lakes are among the water bodies most affected by pollution and high consumption rates that reduce water reserves (Du Plessis, 2019; Cantonati et al., 2020). An adequate balance between economic growth, protection of ecosystems, and biodiversity are essential for preventing water scarcity (Tzanakakis et al., 2020; Albert et al., 2021). The increasing complexity of human development motivates public authorities and researchers to innovate by developing new concepts, equipment, and methods in environmental chemistry and toxicology to improve the management of chemicals in the water cycle (Fairbrother et al., 2019; Leung et al., 2020).

Many pollutants are released into aquatic systems due to human activities, but only a small proportion of chemicals used by society are currently regulated (Fu et al., 2019; Du et al., 2022). Consequently, human, and environmental health concerns not only relate to "classic" and well-studied chemical pollutants, such as metals, dioxins, polychlorinated biphenyls, and pesticides; there is also an increasing focus on emerging contaminants (ECs) that have only recently been shown to enter the environment through domestic pathways and can be present at low, yet biologically active, concentrations (Geissen et al., 2015; Kumar et al., 2022).

1.2 Water quality

A wide range of parameters determine the quality of surface water bodies, such as rivers, which can change from one region to another (Zeinalzadeh and Rezaei, 2017; Uddin et al., 2021).

Physicochemical characteristics such as pH, dissolved oxygen (DO), temperature, and specific conductivity (SC) can influence chemical characteristics (e.g., chemical structure, toxicity, persistence in the environment, and bioaccumulation) of some metals, pharmaceuticals, ammonia, and other chemicals (Saidulu et al., 2021; Pérez et al., 2022a). Additionally, aquatic species can be more or less sensitive under specific physical, chemical, and biological conditions (Brezonik et al., 2020).

Variations in water quality can be manageable for some ecosystems (Murdoch et al., 2000). However, many ecosystems are susceptible to even slight variations in physicochemical properties, leading to losses in biodiversity and associated ecosystem services (e.g., water supply) (Mori et al., 2013). Environmental monitoring of physical, chemical, and biological elements of water bodies can help identify and prevent conditions that reduce ecosystem resilience, i.e., its capacity to revert to a "normal" state following a perturbation (Behera and Prasad, 2020; Vannevel and Goethals, 2020).

Water quality guidelines or standards are used in many jurisdictions internationally to establish water quality by comparing measured physical, chemical, or biological properties of water samples with defined threshold values (Slavik et al., 2020; Torres et al., 2022). These standards can differ from one country to another and are usually categorized according to water uses (e.g., human consumption, agriculture, industry, and recreation) (Marttunen et al., 2019). Regarding the concentrations of chemical contaminants, establishing water quality guidelines typically involves a methodical assessment of toxicant levels tolerable for humans or aquatic organisms (Altenburger

et al., 2019; Posthuma et al., 2019). However, due to the high number of chemicals used to date, not all contaminants are included in these guidelines. Additionally, regulators are facing challenges of establishing new guidelines for an ever-increasing number of new chemicals (Dulio et al., 2018) which is practically impossible based on current risk assessment schemes.

The rapid production of new chemicals and the increasing complexity of chemical mixtures found in the aquatic environment require improvements to how we monitor and prioritize hazards and risks of chemicals beyond focusing exclusively on lists of priority contaminants (Munthe et al., 2017; Been et al., 2021; Berthiaume et al., 2022). Consequently, the focus has shifted away from "classic" contaminants to ECs such as pharmaceuticals, personal care products (PCPs), and perfluorinated alkyl substances (Richardson and Kimura, 2019). The term "emerging" in this context is not only used to indicate the recent discovery of some of these chemicals but can also be used for chemicals with recent scientific interest due to their potential hazards to people or the environment (Stefanakis & Becker, 2016**).**

1.3 Emerging contaminants

ECs are a widely defined and heterogeneous group of chemical compounds used in industrial, agricultural, and domestic applications (Yap et al., 2019; Rout et al., 2021; Kumar et al., 2022). Not only are new chemicals part of the ECs, but those for which presence and relevance are only recently being accepted (Dulio et al., 2018; Tavengwa and Dalu, 2022**)**. People use chemical products in their households daily, many of which are disposed of through municipal wastewater.

Most municipal wastewater treatment plants (WWTPs) are not designed to eliminate these chemicals efficiently (Ngo et al., 2019; Ikonen et al., 2021; Tadsuwan and Babel, 2022). In this way, these chemicals are released into the environment, and scientists and regulators are concerned about their impact on humans and aquatic ecosystems (Bashir et al., 2020; Mehinto et al., 2022).

Identification and quantification of ECs are critical to determining their occurrence and fate in various environmental compartments (Tong et al., 2022). Some ECs are hydrophobic chemicals, which leads to their accumulation in sediments and aquatic organisms and can lead to trophic magnification through the food chain (Hiranmai and Kamaraj, 2021). Most ECs are found in the environment at trace levels, making it challenging to detect and eliminate them from the environment (Gomes et al., 2020; Priya et al., 2022). Many laboratories do not have standardized methods and availability of reference standards to detect ECs, resulting in "blind spots" in water quality monitoring (Umemneku et al., 2019).

ECs can have sustained toxicological impacts on aquatic ecosystems, some at very low concentrations (Boxall et al., 2012; Mahesh et al., 2022). Pharmaceuticals, for example, are intended to have biological effects on humans, yet, little is known regarding the risks they pose to aquatic life (Nair et al., 2018). A growing number of studies on the environmental risks of pharmaceuticals point toward potential environmental risks of ECs (Richmond et al., 2018; Schwartz et al., 2021). However, more studies are urgently needed to close gaps in our knowledge and support their regulation (Boxall et al., 2012; Foulkes et al., 2020; Narayanan et al., 2022).

The extensive presence of ECs in municipal wastewater and the harmful ecological and health effects of this group of chemicals are increasing the concern among science, engineering, and the public (Gogoi et al., 2018; Ahammad et al., 2022). This group of chemicals basically consists of contaminants such as pharmaceuticals, PCPs, biocides, surfactants, reagents, solvents, and food additives frequently used in our daily life (Rodriguez-Narvaez et al., 2017; Martín-Girela et al., 2020).

Although this study, in part, is focused on the targeted analysis of pharmaceuticals, a non-target analysis of compounds suspected to be present in the wastewater effluents studied here was conducted, identifying a broad range of chemicals and their biological impacts, which are summarized in the following sections.

1.3.1 Pharmaceuticals

Pharmaceuticals are intended to improve human and animal health but might end up causing impacts on natural ecosystems and consequently negatively affecting human health (Li et al., 2014; Borja et al., 2020). Residues of pharmaceuticals reach aquatic ecosystems through municipal, hospital, industrial, and agricultural wastewater effluents (Khan et al., 2021). Municipal wastewater is a major point source that contributes to the occurrence of pharmaceuticals in aquatic systems caused by existing treatment plants that are unsuited for the removal of this type of contaminant (Olasupo and Suah, 2021). Pharmaceuticals are very bioactive, and their polarity makes them quite mobile within aquatic ecosystems; even at low concentrations, they can cause severe environmental impacts (Branchet et al., 2021).

Antipsychotics, also known as neuroleptics, are one group of pharmaceuticals that have raised great concern in the last several years (Correll et al., 2018; Calsolaro et al., 2021). These chemicals are continuously released into the environment via effluent discharges from WWTPs with insufficient removal efficiencies (Yuan et al., 2013; Paíga et al., 2019). Low-level exposure of aquatic organisms to antipsychotics can result in increased risks of chronic ecotoxicological effects (Beghin et al., 2021). Many of the human receptors specifically targeted by antipsychotic drugs are conserved across vertebrates; consequently, long-term low-level exposure to these chemicals might result in similar effects in fish (Chan et al., 2021). Huggett et al. (2003) evaluated the potential for chronic receptor-mediated responses in fish. They found that lower effective plasma concentrations in humans were correlated with greater potential for a pharmacological response in fish.

Fish exposure to antipsychotics from wastewater effluents has been recently studied due to the importance of identifying the health effects of pharmaceuticals in water (David et al., 2018; Cerveny et al., 2021; Sumpter and Margiotta-Casaluci, 2022). Katare et al. (2015) observed elevated levels of antipsychotic pharmaceuticals in the brain and plasma of round goby (*Neogobius melanostomus*) after exposure to wastewater effluent, which was associated with altered behaviors. David et al. (2018) evaluated a complex mixture of neuroactive pharmaceuticals accumulated in the brain and plasma of effluent-exposed fish and found a disruption in neurotransmitter concentrations in brain regions of roach (*Rutilus rutilus*). Cerveny et al. (2021) found a potential of these exposures to alter natural fish behavior and moderate or high risk related to neuroactive compounds (e.g., flupentixol, haloperidol, and risperidone). Also, surprisingly, plasma

concentrations of risperidone and flupentixol were higher in fish than concentrations usually reached in the blood of human patients taking these medications (Cerveny et al., 2021).

Previous studies have indicated the lack of information related to many antipsychotics often used by humans for medical treatments. Therefore, more ecotoxicity data with relevance to their mode of action in non-target organisms is essential to understand the toxic nature of these chemicals. This study will help gain a better understanding of the potential effects of selected antipsychotics based on a combination of exposure and effect data following environmental exposure to these chemicals.

1.3.2 Personal care products

For decades, PCPs have been widely used, and their abundance in the ecosystems is increasing due to the limitations in conventional wastewater treatment plants (Ahamad et al., 2020). PCPs are made with bisphenol A and other esters found in cosmetics, skin care products, dental products, washing lotions, and shampoos (Galindo-Miranda et al., 2019). Industries, hospitals, and domestic wastewater effluents discharged into surface water constitutes the main direct pathway of PCPs entering aquatic environments (Kuroda and Kobayashi, 2021).

Sediment and soil within the fresh water can absorb these chemicals and act like sink for PCPs (Ohoro et al., 2019). Several research have indicated that concentrations of PCPs varying between ng/L and μg/L in water (Pai et al., 2020) and μg/kg in sediment (Avellan et al., 2022) and soil (Dai et al., 2020). Water quality and ecosystem health can be negatively affected by PCPs, their constant release made then act as pseudo persistent organic contaminants with a similar mode of action than

persistent contaminants (Sungur, 2022). Some investigations have been conducted to provide a theoretical baseline for determining the environmental risk of PCPs (Sharma et al., 2019; Khan et al., 2022a), but more research is needed to understand the full scope of the problem.

1.3.3 Biocides

Biocides are also known as antimicrobial pesticides or microbicides including fungicides, herbicides, bactericides, and insecticides according to the target organism to eradicate. These products are widely used for disinfection by households, industries, and healthcare (Paul et al., 2019). Biocides products contain a wide variety of components and chemicals such as oxidants (ozone, chlorinated substances, hydrogen peroxide) and non-oxidants (copper salts, sulfur compounds, isothiazoiones, etc.) (Du et al., 2020). Biocides usually end up in aquatic ecosystems through wastewater effluents or direct disposal in water bodies (Paun et al., 2022).

Biocides have been detected in WWTPs discharges all over the world (Paun et al., 2022). Juksu et al. in 2019 found biocides in wastewater discharges in Europe, Australia, and South Africa ranged from 13 μg/L to 50 ng/L. Also, biocides have been detected in sediment samples with concentrations up to the order of magnitude of μg/g and fish samples with mean concentration between 0.2 and 2.2 ng/g wet weight (Vorkamp et al., 2014).

1.3.4 Surfactants

Some surfactants are contaminants with ionic behaviour in solution with a hydrophilic (polar) and hydrophobic (lipophilic) portions that are present in household cleaning products (detergents, surface cleaners) and care products (shampoos, soaps) (Dutta et al., 2022). After use, surfactants

are commonly detected in municipal wastewater and receiving aquatic environments (Zhu et al., 2018; Schinkel et al., 2022). Their complex chemical activity in the environment and higher concentrations in the environment have made them one of the most challenging ECs to assess (Gomes et al., 2020).

Many studies have shown the occurrence of different classes of surfactants in water all over the world, concentrations of non-ionic surfactants were detected in commercial laundries in Canada and industrial wastewaters in Italy with values up to 108,937 μg/L and 3,370 μg/L, respectively (Nunes et al., 2022). Also, cationic surfactants were detected in hospital wastewaters in Europe and municipal wastewater in Austria with values up to 6.03 mg/L and 6.7 mg/L, respectively (Nunes et al., 2022). Fish samples collected in Netherland have showed concentrations of surfactants with 80.12 ng/g wet weight (Chu et al., 2016).

1.3.5 Solvents

Solvents are heavily used in commercial, industrial, and household environments. They can be classified as aliphatic, aromatic, and paraffinic based on their chemical structure. They are commonly use in dissolving paint, oil, and grease, to mixing or thinning pigments, pesticides, glues, epoxy resins and paints, to cleaning automotive parts, tools, and electronics (Montemayor, 2010). Municipal and industrial wastewater effluents are the main sources of these contaminants in aquatic ecosystems (Crini and Lichtfouse, 2018).

Several studies have found solvents such as methylene chloride and trichloroethylene in drinking water and groundwater (DeWeerd et al., 1998; Pope et al., 2018; Emsbo-Mattingly et al.,

2022). These contaminants can be toxic and negatively affect human health. For instance, chlorinated solvents tend to damage the kidneys and trichloroethylene can affect cardiovascular health (Schwenk & Burr, 2020). Different biological studies in bacteria, human cells, and plants have shown negative effects of ionic liquids previously called “green solvents”. Also, toxic effects were identified by Costa et al. 2015 according to bioassays conducted where *Daphnia magna* and *Raphidocelis subcapitata* showed higher sensitivity to solvents than *Haworthiopsis attenuata* species.

1.3.6 Food additives

Food additives are chemicals added to maintain or improve the safety, taste, texture, or quality during food production (Carocho et al., 2014; Awuchi et al., 2020). These chemicals have different uses, i.e., triethyl citrate foam stabiliser in egg white, butylated hydroxytoluene to avoid fat in products, and some may include oxidants and other endocrine disruptors (Carocho et al., 2014; Baig et al., 2018).

Different publications have indicated a high persistence and occurrence of food additives’ components in different aquatic ecosystems (Birch et al., 2015; Pressman, 2017; Bellani et al., 2020; Zheng et al., 2021). Studies conducted in water bodies across Europe and North America have found sweeteners up to μg/L levels (Tollefsen et al., 2012). The degradation of these chemicals is very slow, suggesting a high persistence in the environment and their potential accumulation, including by-product from natural degradation (Gillois et al., 2018; Khan et al., 2022b). Toxicity tests have also been conducted to evaluate the correlation between temperature and UV sensitivity

of sucralose indicating that molecular activity relies on the reaction conditions (i.e., reactive species and presence of water) (Sang et al., 2014). Also, toxicity in aquatic organisms at concentrations above 1123 mg/L have been suggested by ecotoxicological studies conducted with sucralose (Tollefsen et al., 2012).

1.3.7 Other emerging contaminants

Reagents, lubricant additive, and cigarettes are part of a group of less common studied chemicals which are highly used by industrial, commercial, household, and research activities (Brusseau and Artiola, 2019). These products are made with components with high potential to affect the environment but are not usually monitored which led to high challenges to develop alternative to understand more about them and provide approaches to promote a safety used on a local and large scale (Petrie and Kasprzyk-Hordern, 2015; Cheng et al., 2021). Reagents are mostly organic molecules or inorganic compounds to facilitate a chemical reaction in a wide range of industrial processes including Peroxyacetic acid in food products and acetic acid a common reagent used in laboratory analysis.

Lubricant additive industries are a small group with the petroleum industry but have significant effects on environmental ecosystems and human health. These products are used as a fluid for improving the movement of certain elements within equipment and the main components include organic, metal-organic, and/or synthetic chemicals produced during crude oil processing. Although there are regulations to manage this type of waste, still it is challenging to reduce spills and disposal of lubricants in the environment (Nowak et al., 2019). Robert et al. conducted a study in 2000 to

study the fate and effects of cumylphenol (commonly used in lubricants), indicating concentrations up to 6300 μg/L in wastewater, 70,000 μg/kg in sediments, and behaviour effects in shrimp exposed to concentrations below 10 μg/L in Virginia, USA (Hale et al., 2000).

Cigarettes and e-cigarettes refills are single use products with different forms of nicotine, polycyclic aromatic hydrocarbons, metals usually disposed into soil and water. Cigarette butts also contain fibrous plastic contaminants made with a wide range of contaminants to the environment. Nicotine is in the top of pharmaceuticals with toxic effects in the environment and recent studies have found that only one cigarette but can contaminate 1000 L of water (Green et al., 2014). The complex mixture of chemicals in cigarettes can also negatively affect microorganisms such a *Vibrio fischeri,* according to a bioassay conducted using different cigarette brands (Beutel et al., 2021).

1.4 Ionizable organic chemicals

Most of ECs are ionizable organic chemicals (IOCs). This simple fact has received increasing interest from risk assessors, as it has a number of important implications for the assessment of their environmental chemodynamics, as well as uptake and toxicity (Armitage et al., 2017; Sigmund et al., 2020). IOCs can dissociate to a varying extent depending on environmental factors (e.g., pH, alkalinity, hardness). It is generally accepted that neutral, undissociated forms of organic chemicals are less water soluble but there are exceptions including organic compounds that contain ionizable groups where hydrogen bonding occurs making them change their bioavailability (Vitale et al., 2019). If the bioavailable fraction is highly variable due to variations in environmental factors,

however, this can potentially affect our ability to extrapolate from experimental observations in the laboratory to situations in the field (Boxall et al., 2012; Vitale et al., 2019).

Furthermore, many models used in ecotoxicological risk assessment of organic chemicals were originally developed for neutral organic chemicals and can lead to unreliable predictions of uptake and effects of chemicals when applied to IOCs (Armitage et al., 2017; Su et al., 2019; Escher et al., 2020). In addition to potential differences in bioaccumulation and toxicity of IOCs between laboratory and field exposures, gradual changes in physicochemical water quality characteristics may occur under natural exposure conditions, e.g., as a result of snowmelt or precipitation events, leading to variable levels and effects in exposed organisms that might be erroneously interpreted as analytical artifacts (Armitage et al., 2017).

IOCs are monovalent, multivalent, or zwitterionic (i.e., contain an equal number of positively- and negatively charged functional groups) molecules that can be present as either neutral or organic species charged, or both of them simultaneously, depending on environmental conditions. Thus, toxicity testing needs to consider environmentally relevant ranges of environmental factors. Environmental factors (pH, alkalinity, DO, hardness, temperature) might modify the bioavailability and toxicity of IOCs in water, controlling chemical speciation and complexation (Xin et al., 2021). Different studies have demonstrated the impact of pH variations on bioaccumulation in aquatic organisms under field and laboratory conditions (Armitage et al., 2017; Brinkmann et al., 2020), but little is known about the effects of pH on the availability of pharmaceuticals in fish species.

Uptake of IOCs by different organisms has received much lesser scientific attention compared to non-ionizable and neutral chemicals (Boxall et al., 2012; Armitage et al., 2017). Thus, establishing suitable animal models and exposure protocols is a necessary first step to achieving better assessments of the uptake and effects of IOCs in aquatic organisms (Huchthausen et al., 2020).

1.5 Effects of emerging contaminants

An increasing number of studies on ECs remark that they are a threat to natural environments, human health, and wildlife (Gavrilescu et al., 2015; Khan et al., 2020). Effects of ECs can be observed even though the mechanisms concerning their effects on humans and animals are not completely comprehended (Peña-Guzmán et al., 2019). For instance, the occurrence of some classes of ECs at low concentrations in water bodies has been linked to endocrine-disruptive effects, genotoxicity, metabolism, behavior, reproduction, growth, and body development, and even decreasing natural populations, among others (Kumar et al., 2022).

Some ECs are endocrine-disruptive compounds (EDCs) and can interfere with the controlled hormonal systems at very low concentrations (Kasonga et al., 2021). EDCs interfere with or mimic the body's hormones responsible for the development or function of diverse organs (Viera et al., 2021). The endocrine system is present in all vertebrates to control body functions and processes to keep the organism in balance. Similarly, signaling between nerves to tissues and organs that are part of the nervous system can be affected by ECs (Kasonga et al., 2021; Kumar et al., 2022). Genetic expression plays a fundamental role during the transcription and translation of information

stored in genes which means that gene encode proteins and proteins dictate cell function and alteration in these processes could lead to a different result in gene expression. (Almeida et al., 2019; Baines et al., 2021; Varsha et al., 2022)

The absence of regulations to control ECs due to a lack of data on the impacts, fate, and concentration of ECs makes it challenging for authorities to control their use and management (Ouda et al., 2021). There are no limits on EC concentrations in WWTP effluents or the environment (Gaston et al., 2019). This study is focused on chemical analysis (target and non-target) and biological analysis to provide relevant data that can help to support the need to mitigate ECs from wastewater and develop guidelines and regulations to improve wastewater treatment technologies.

1.6 Previous studies of WWTP effluents in Southern Saskatchewan

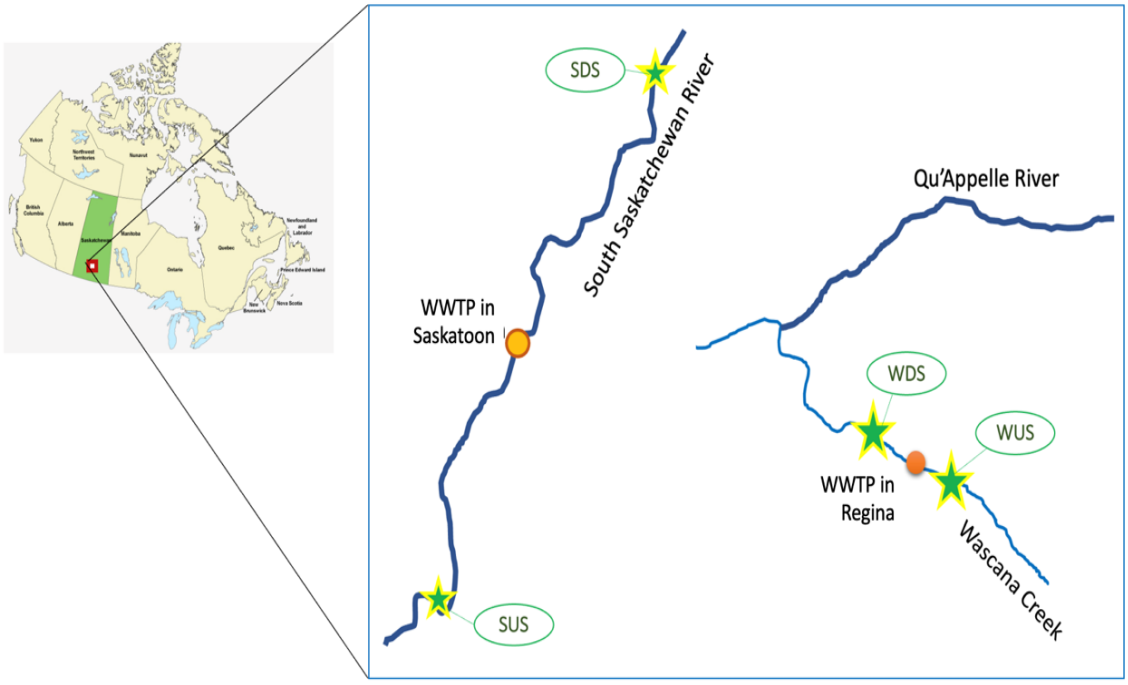
Saskatchewan is one of the prairie provinces of Canada, with an estimated population of 1.12 million. Saskatoon and Regina are the two major cities in the province. Even though both cities have advanced wastewater treatment processes in place, more research is needed to understand the potential impacts of WWTP effluents on rivers in southern Saskatchewan (Fung, 1999). Southern Saskatchewan is a semi-arid region with unique features to consider for assessing contaminants in water bodies (Fung, 1999). For example, in the summer season, dry conditions can result in reduced water flows of streams and rivers. As a result, low flow in the river leads to low dilution of wastewater effluents and, consequently, high concentrations of pollutants. In the case of Wascana

Creek (WC) in Regina, almost 100% of the streamflow can result from effluents between late October and March (Hanson et al., 2021).

The South Saskatchewan River (SSR) and Qu'Appelle River are important rivers in Saskatchewan with similarities regarding the contaminant sources. The Qu'Appelle River receives Regina's effluent discharges from Regina's WWTP through WC. Since dilution factors significantly differ between both systems, both SSR and WC were selected for conducting fieldwork. Due to it being highly influenced by WWTP discharges, the effluent-dominated WC is exceptionally well-suited for this proof-of-concept study.

Characterizations of endocrine disrupting potentials of WWTPs discharges have been conducted in previous studies focusing on WC treatment plants. One of the first studies in WC was conducted in 2011 by Waiser, who identified a mixture of PCPs (including pharmaceuticals) in the proximity of the WWTP and great distances downstream from the WWTP in WC. Then in 2012, Tetreault et al. (2012) found kidney and gill alterations in fathead minnows exposed to effluents during winter months in WC. Later in 2021, Hanson et al. (2021) observed effects in growth, development, and reproduction of wild fathead minnow populations downstream of the City of Regina's WWTP. Both studies were able to demonstrate that a variety of human pharmaceuticals are found in WWTP effluents in Saskatchewan, which can have a marked impact on fish in the receiving aquatic environments. This research helps to deepen previous studies further and adds urgently needed information on neuroactive pharmaceuticals, specifically antipsychotics (Figure 1.1).

Figure 1.1 Sampling locations on the South Saskatchewan River and Wascana Creek. SUS (South Saskatchewan Upstream Site: 51°98'37.60"N, 106°73'49.73"W); SDS (South Saskatchewan Downstream Site: 52°31'79.98"N, 106°46'10.99"W); WUS (Wascana Creek Upstream Site: 50°47'65.42"N, 104°73'26.30"W); WDS (Wascana Creek Downstream Site: 50°48'42.01"N, 104°77'80.66"W).



1.7 Chemical analysis and non-target screening

ECs encompass a diverse group of compounds, including pharmaceuticals, PCPs, hormones, surfactants, drugs of abuse, endocrine disrupting compounds, industrial additives and agents, gasoline additives, food additives, perfluorinated compounds, biocides. After being released into the environment, biotic and abiotic processes can lead to transformation products with different behavior and ecotoxicology (Li, 2021; Anagnostopoulou, 2022). These transformations can occur even after leaving WWTPs (Hena, 2021; Bonnot, 2022). Therefore, the qualitative and quantitative occurrence of ECs and their transformation products in the environment need to be evaluated (Mhuka, 2020; Ibáñez, 2021; El-Deen, 2022).

Highly sensitive analytical methods are usually required to conduct chemical analysis of ECs (Tran, 2019). Ultra high-performance liquid Chromatography (UHPLC) coupled with mass spectrometry (MS/MS) is a common alternative for environmental analysis (López-Ruiz, 2019; Perez de Souza, 2021). High complexity samples like WWTPs discharges can be evaluated using targeted and non-target approaches, where target analysis is most commonly used (Kiefer et al., 2019). Suspect screening is based on predicted compounds that could be present in environmental samples according to previous studies (Kiefer et al., 2019). This technique uses a database with the accurate mass and isotope information of the suspected compounds (Sobus, 2018; Aalizadeh, 2019). However, many compounds can remain unnoticed after target and suspect screening approaches due to their co-extraction in the analysis process and cost-time limitations to purchasing

hundreds or thousands of reference standards (Schwarzbauer, 2010; Aalizadeh; 2019). Instead, non-target screening methods provide a full picture of the product ions, chemical compounds, or their metabolites after transformation processes in complex matrices (Bletsou, 2015; Wang, 2020). This high-resolution mass spectrometry screening method can detect and provide comprehensive data to identify novel ECs in aquatic systems, particularly polar organic pollutants (Hajeb, 2022).

This study applied and assessed targeted and non-target screening approaches for the identification of micropollutants in WWTPs effluents. Initially, the quantitative target screening approach was used to obtain an overview of the occurrence of antipsychotic pharmaceuticals, including amitriptyline (AMI), bupropion (BUP), carbamazepine (CBZ), clozapine (CLO), fluoxetine (FLX), and lamotrigine (LAM), venlafaxine (VEN). Then, a non-target screening approach considered a systematic selection of peaks for identification and exclusion of structure for each peak. Both approaches used automated software-based procedures to support and optimize manual data processing.

1.8 Biological Endpoints in Fathead Minnows

Biotesting conducted on surface water samples has has demonstrated biological impacts in aquatic ecosystems, and this has been supported by the detection of EDCs (Yusuf et al., 2021; Robitaille et al., 2022). Currently, biological endpoints can be assessed through *in vitro*, *in silico*, and *in vivo* assays (Forest et al., 2019; Audouze et al., 2020). However, there is a limited understanding of the effects observed in bioassays with respect to the chemicals detected in environmental samples (Neale, et al., 2020; Schuijt et al., 2021). Using systems biology-based

approaches looking at larger sets of genes or whole transcriptomes, it is possible to obtain a more holistic profile of the chemical effects in water samples (Bylemans et al., 2018; Yates et al., 2021).

Quantitative PCR is a cost-effective molecular technique to facilitate rapid and sensitive analysis of effects caused by wastewater contaminants (Zulkifli et al., 2018; Paruch, 2022). Previous toxicological investigations have indicated that contaminants can be distributed throughout the body after ingestion; depending on the type of chemical compound evaluated, it is suggested to evaluate specific tissues (Abdel-Shafy et al., 2016; Yusuf et al., 2021; Li et al., 2021). For instance, there are studies suggesting severe toxic effects of pharmaceuticals on the fish liver and brain (Li e al., 2011; Tanoue et al., 2015; Nowakowska et al., 2020).

The EcoToxChip is a next generation toxicogenomic tool for rapid characterization, prioritization, and management of environmental chemicals and complex mixtures (Basu et al., 2019). It is composed of a 384-well qPCR array (EcoToxChips) and an intuitive web-based bioinformatics tool and analytical platform (EcoToxXplorer.ca; Soufan et al., 2022). Specifically, the EcoToxChip array consists of 8 quality control wells, 6 housekeeping genes, and 370 manually curated, evidence-based, omics- and expert-informed target genes, representing EcoToxModules - ecotoxicology-relevant gene sets and pathways (Ewald et al., 2020). The genes assessed using the EcoToxChip represent key molecular pathways that have been shown to inform specific toxic responses or regulatory relevance (e.g., genotoxicity, reproductive toxicity, endocrine toxicity, neurotoxicity, immunotoxicity). Results of the EcoToxChips may help identify biological processes that may be affected by compound or a mixture, or whether a compound in question may

be of potential significant concern. Overall, the EcoToxChips represent a new approach method (NAM) that utilize a suite of target genes (and molecular pathways) as non-traditional endpoint in predicting apical outcomes of regulatory relevance that do not rely on conventional testing approaches and end-points such as survival, growth, and development, among others, that tend to be expensive, tedious, and ethically concerning due to the use of large numbers of live animals.

Fathead minnow is an ideal bioindicator species to assess the toxicity of contaminants and the immune responses of fish (Tanoue et al., 2015; Cozzola et al., 2022). EcoToxChips are qPCR arrays designed for six ecologically relevant species, including fathead minnows as a representative native specie of Canadian ecosystems and was monitored in this study.

1.9 Risk assessment and prioritization of detected chemicals

Originally, risk assessments for environmental systems are based on measuring priority pollutants in the environment. The effects of complex mixtures of contaminants in the environment, however, can differ markedly from those of individual chemicals through antagonistic or synergistic effects, thereby posing challenges to assess the risk and prioritization of contaminants (Escher et al., 2020; Goutam et al., 2022). Due to the drawbacks introduced above, this is not always efficient, and researchers and regulators are looking toward using effect-based tools.

Chemical/biological interactions of contaminant mixtures in the environment can be explained with combined approaches. Bioassays are complementary to chemical analyses in aquatic systems and have been increasingly applied for water quality monitoring to measure the combined effects of low-level mixtures of chemicals (Barceló and Ginebreda., et al., 2020). Different biological

systems including isolated receptors, cell models, tissues or small organisms are useful tools for measuring negative effects of chemicals on generic and specific biological processes (Nivala et al., 2018). Also, many of these methods are used as bioanalytical methods for water quality (Neale and Escher, 2019; Barceló and Ginebreda., et al., 2020). Therefore, identification of chemicals of concern requires to implement a suitable combination of monitoring and assessment between exposure and effect as well as prediction of exposures at molecular levels.

Previous studies have provided some practical approaches to address the abovementioned drawbacks and data gaps. One alternative is improving the standards under the Water Framework Directive for mixtures of chemicals instead of chemical-by-chemical risk assessment, which can provide a more holistic approach (Kortenkamp et al., 2019). Another alternative is to improve monitoring strategies by using passive and biota sampling for bioaccumulative and polar substances, which can help to close the gaps between exposure and risk to aquatic ecosystems including temporal variations in concentration of contaminants (Brack et al., 2017).

In consequence, this study integrates non-target chemical and biological analysis to provide a powerful approach for merging both streams of evidence collected in the field to improve the risk assessment and prioritisation of ECs.

1.10 Objectives and hypothesis

Despite the heightened concerns about the accumulation of ECs in the environment, additional data are needed to understand their mobility and ecotoxicological effects on wildlife and human health. Therefore, the main purpose of the proposed research was to reduce the knowledge gaps of the

toxicological risks associated with IOCs in general, and pharmaceuticals specifically, to aquatic environments by assessing (1) the impact of physicochemical parameters (i.e., pH, temperature, DO, SC) on the uptake and toxicity of these compounds, and (2) how this interaction affects toxicity under field conditions. The ultimate goal of this research was to contribute to our current knowledge that can be used to improve water quality guidelines and assist chemical regulators to better understand the chemodynamics and toxicological impacts of ECs under changing environmental conditions and thereby protect aquatic ecosystems from their potentially harmful impacts. The specific objectives of this research were to:

1) Characterize the presence of a suite of IOCs (with an emphasis on antipsychotic pharmaceuticals) originating from Saskatoon and Regina WWTPs, as well as physicochemical parameters, upstream and downstream of the WWTPs in Wascana Creek and the South Saskatchewan River and determine the levels and resulting effects of these chemicals in fish inhabiting the downstream environment.

   Ho-1: There is no statistically significant correlation between the presence of antipsychotic pharmaceuticals upstream and downstream of the WWTPs on Wascana Creek and the South Saskatchewan River and the levels and resulting effects of these chemicals on fish inhabiting the downstream environment.

2) Develop an approach for integrating non-target chemical analysis and molecular biology using fish species (*Pimephales promelas*) exposed to complex chemical mixtures in

WWTP effluents to assess the bioavailability and ecotoxicological effects of these chemicals.

Ho-2: There is no statistically significant correlation between non-target chemical analysis and molecular biology of chemical mixtures in WWTP effluents.

References

Aalizadeh, R., Nika, M. C., & Thomaidis, N. S. (2019). Development and application of retention time prediction models in the suspect and non-target screening of emerging contaminants. *Journal of Hazardous materials*, 363, 277-285.

Abdel-Shafy, H. I., & Mansour, M. S. (2016). A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. *Egyptian journal of petroleum, 25*(1), 107-123.

Ahamad, A., Madhav, S., Singh, A. K., Kumar, A., & Singh, P. (2020). Types of water pollutants: Conventional and emerging. In Sensors in water pollutants monitoring: *Role of material* (pp. 21-41). Springer, Singapore.

Ahammad, N. A., Ahmad, M. A., Hameed, B. H., & Mohd Din, A. T. (2022). A mini review of recent progress in the removal of emerging contaminants from pharmaceutical waste using various adsorbents. *Environmental Science and Pollution Research*, 1-15.

Akhtar, N., Syakir Ishak, M. I., Bhawani, S. A., & Umar, K. (2021). Various natural and anthropogenic factors responsible for water quality degradation: *A review. Water, 13*(19), 2660.

Albert, J. S., Destouni, G., Duke-Sylvester, S. M., Magurran, A. E., Oberdorff, T., Reis, R. E., ... & Ripple, W. J. (2021). *Scientists' warning to humanity on the freshwater biodiversity crisis. Ambio, 50*(1), 85-94.

Almeida, D. L., Pavanello, A., Saavedra, L. P., Pereira, T. S., de Castro-Prado, M. A. A., & de Freitas Mathias, P. C. (2019). *Environmental monitoring and the developmental origins of health and disease. Journal of developmental origins of health and disease, 10*(6), 608-615.

Altenburger, R., Brack, W., Burgess, R. M., Busch, W., Escher, B. I., Focks, A., ... & Krauss, M. (2019). Future water quality monitoring: improving the balance between exposure and toxicity assessments of real-world pollutant mixtures. *Environmental Sciences Europe, 31*(1), 1-17.

Anagnostopoulou, K., Nannou, C., Evgenidou, E., & Lambropoulou, D. (2022). Overarching issues on relevant pesticide transformation products in the aquatic environment: A review. *Science of The Total Environment*, 152863.

Anderson, E. P., Jackson, S., Tharme, R. E., Douglas, M., Flotemersch, J. E., Zwarteveen, M., ... & Arthington, A. H. (2019). Understanding rivers and their social relations: A critical step to advance environmental water management. *Wiley Interdisciplinary Reviews: Water, 6*(6), e1381.

Armitage, J. M., Erickson, R. J., Luckenbach, T., Ng, C. A., Prosser, R. S., Arnot, J. A., Schirmer, K., Nichols, J. W. (2017). Assessing the bioaccumulation potential of ionizable organic compounds: Current knowledge and research priorities. *Environmental Toxicology and Chemistry*, *36*(4), 882-897. doi:10.1002/etc.3680

Audouze, K., Sarigiannis, D., Alonso-Magdalena, P., Brochot, C., Casas, M., Vrijheid, M., ... & Barouki, R. (2020). Integrative strategy of testing systems for identification of endocrine

disruptors inducing metabolic disorders—An introduction to the oberon project. *International Journal of Molecular Sciences, 21*(8), 2988.

Avellan, A., Duarte, A., & Rocha-Santos, T. (2022). Organic contaminants in marine sediments and seawater: A review for drawing environmental diagnostics and searching for informative predictors. *Science of The Total Environment*, 808, 152012.

Awuchi, C. G., Twinomuhwezi, H., Igwe, V. S., & Amagwula, I. O. (2020). Food Additives and Food Preservatives for Domestic and Industrial Food Applications. Journal of Animal Health, 2(1), 1-16.

Baig, S. K. M. S., & Kasim, S. S. (2018). Study of Harmful Effects of Consuming Food Additives and Public Awareness. *IJSRST, 2*(4), 1071-1074.

Baines, C., Lerebours, A., Thomas, F., Fort, J., Kreitsberg, R., Gentes, S., ... & Sepp, T. (2021). Linking pollution and cancer in aquatic environments: A review. *Environment International,* 149, 106391.

Barceló, D., Žonja, B., & Ginebreda, A. (2020). Toxicity tests in wastewater and drinking water treatment processes: a complementary assessment tool to be on your radar. *Journal of Environmental Chemical Engineering, 8*(5), 104262.

Bashir, I., Lone, F. A., Bhat, R. A., Mir, S. A., Dar, Z. A., & Dar, S. A. (2020). Concerns and threats of contamination on aquatic ecosystems. *In Bioremediation and Biotechnology* (pp. 1-26). Springer, Cham.

Basu, N., Crump, D., Head, J., Hickey, G., Hogan, N., Maguire, S., ... & Hecker, M. (2019). EcoToxChip: A next-generation toxicogenomics tool for chemical prioritization and environmental management. *Environmental Toxicology and Chemistry, 38*(2), 279-288.

Been, F., Kruve, A., Vughs, D., Meekel, N., Reus, A., Zwartsen, A., ... & Brunner, A. M. (2021). Risk-based prioritization of suspects detected in riverine water using complementary chromatographic techniques. *Water Research*, 204, 117612.

Beghin, M., Schmitz, M., Betoulle, S., Palluel, O., Baekelandt, S., Mandiki, S. N., ... & Kestemont, P. (2021). Integrated multi-biomarker responses of juvenile rainbow trout (Oncorhynchus mykiss) to an environmentally relevant pharmaceutical mixture. *Ecotoxicology and Environmental Safety*, 221, 112454.

Behera, B. K., & Prasad, R. (2020). Environmental technology and sustainability: Physical, chemical and biological technologies for clean environmental management. *Elsevier.*

Bellani, L., Muccifora, S., Barbieri, F., Tassi, E., Castiglione, M. R., & Giorgetti, L. (2020). Genotoxicity of the food additive E171, titanium dioxide, in the plants Lens culinaris L. and Allium cepa L. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 849, 503142.

Berthiaume, A., Arnot, J. A., & Toose, L. (2022). Risk-based prioritization of organic substances in the Canadian National Pollutant Release Inventory using an evaluative regional-scale multimedia mass balance model. *Integrated Environmental Assessment and Management.*

Beutel, M. W., Harmon, T. C., Novotny, T. E., Mock, J., Gilmore, M. E., Hart, S. C., ... & Holden, P. A. (2021). *A Review of Environmental Pollution from the Use and Disposal of Cigarettes and Electronic Cigarettes: Contaminants, Sources, and Impacts. Sustainability, 13*(23), 12994.

Birch, G. F., Drage, D. S., Thompson, K., Eaglesham, G., & Mueller, J. F. (2015). Emerging contaminants (pharmaceuticals, personal care products, a food additive and pesticides) in waters of Sydney estuary, Australia. *Marine pollution bulletin, 97*(1-2), 56-66.

Bletsou, Anna A., Junho Jeon, Juliane Hollender, Eleni Archontaki, and Nikolaos S. Thomaidis. "Targeted and non-targeted liquid chromatography-mass spectrometric workflows for identification of transformation products of emerging pollutants in the aquatic environment." *TrAC Trends in Analytical Chemistry 66* (2015): 32-44.

Boelee, E., Geerling, G., van der Zaan, B., Blauw, A., & Vethaak, A. D. (2019). Water and health: From environmental pressures to integrated responses. *Acta tropica*, 193, 217-226.

Bonnot, K., Benoit, P., Mamy, L., & Patureau, D. (2022). Transformation of PPCPs in the environment: Review of knowledge and classification of pathways according to parent molecule structures. *Critical Reviews in Environmental Science and Technology*, 1-23.

Borja, A., White, M. P., Berdalet, E., Bock, N., Eatock, C., Kristensen, P., ... & Fleming, L. E. (2020). Moving toward an agenda on ocean health and human health in Europe. *Frontiers in Marine Science*, 7, 37.

Boxall, A. B. A., Rudd, M. A., Brooks, B. W., Caldwell, D. J., Choi, K., Hickmann, S., Van Der Kraak, G. (2012). Pharmaceuticals and Personal Care Products in the environment: What Are the Big Questions? *Environmental Health Perspectives, 120*(9), 1221-1229. doi:10.1289/ehp.1104477

Branchet, P., Arpin-Pont, L., Piram, A., Boissery, P., Wong-Wah-Chung, P., & Doumenq, P. (2021). Pharmaceuticals in the marine environment: What are the present challenges in their monitoring?. *Science of the Total Environment*, 766, 142644.

Brack, W., Dulio, V., Ågerstrand, M., Allan, I., Altenburger, R., Brinkmann, M., ... & Vrana, B. (2017). Towards the review of the European Union Water Framework Directive: recommendations for more efficient assessment and management of chemical contamination in European surface water resources. Science of the Total Environment, 576, 720-737.

Brezonik, P. L., King, S. O., & Mach, C. E. (2020). The influence of water chemistry on trace metal bioavailability and toxicity to aquatic organisms. *In Metal ecotoxicology* (pp. 1-31). CRC Press.

Brinkmann, M., Alharbi, H., Fuchylo, U., Wiseman, S., Morandi, G., Peng, H., & Hecker, M. (2020). Mechanisms of pH-dependent uptake of ionizable organic chemicals by fish from oil sands process-affected water (OSPW). *Environmental Science & Technology, 54*(15), 9547-9555. https://doi.org/10.1021/acs.est.0c02522

Brusseau, M. L., & Artiola, J. F. (2019). Chemical contaminants. *In Environmental and pollution science* (pp. 175-190). Academic Press.

Bylemans, J., Furlan, E. M., Gleeson, D. M., Hardy, C. M., & Duncan, R. P. (2018). Does size matter? An experimental evaluation of the relative abundance and decay rates of aquatic environmental DNA. *Environmental Science & Technology, 52*(11), 6408-6416.

Calsolaro, V., Femminella, G. D., Rogani, S., Esposito, S., Franchi, R., Okoye, C., ... & Monzani, F. (2021). Behavioral and psychological symptoms in dementia (BPSD) and the use of antipsychotics. *Pharmaceuticals, 14*(3), 246.

Cantonati, M., Poikane, S., Pringle, C. M., Stevens, L. E., Turak, E., Heino, J., ... & Znachor, P. (2020). Characteristics, main impacts, and stewardship of natural and artificial freshwater environments: consequences for biodiversity conservation. *Water, 12*(1), 260.

Carocho, M., Barreiro, M. F., Morales, P., & Ferreira, I. C. (2014). Adding molecules to food, pros and cons: A review on synthetic and natural food additives. *Comprehensive reviews in food science and food safety, 13*(4), 377-399.

Cerveny, D., Grabic, R., Grabicová, K., Randák, T., Larsson, D. J., Johnson, A. C., ... & Fick, J. (2021). Neuroactive drugs and other pharmaceuticals found in blood plasma of wild European fish. *Environment International*, 146, 106188.

Chan, S. J., Nutting, V. I., Natterson, T. A., & Horowitz, B. N. (2021). Impacts of Psychopharmaceuticals on the Neurodevelopment of Aquatic Wildlife: A Call for Increased Knowledge Exchange across Disciplines to Highlight Implications for Human Health. *International journal of environmental research and public health, 18*(10), 5094.

Chaturvedi, A., Pandey, B., Yadav, A. K., & Saroj, S. (2021). An overview of the potential impacts of global climate change on water resources. *Water Conservation in the Era of Global Climate Change*, 99-120.

Cheng, N., Wang, B., Wu, P., Lee, X., Xing, Y., Chen, M., & Gao, B. (2021). Adsorption of emerging contaminants from water and wastewater by modified biochar: A review. *Environmental Pollution*, 273, 116448.

Chu, S., Letcher, R. J., McGoldrick, D. J., & Backus, S. M. (2016). A new fluorinated surfactant contaminant in biota: perfluorobutane sulfonamide in several fish species. *Environmental Science & Technology, 50*(2), 669-675.

Cooke, S. J., Frempong-Manso, A., Piczak, M. L., Karathanou, E., Clavijo, C., Ajagbe, S. O., ... & Piccolo, J. (2022). A freshwater perspective on the United Nations decade for ecosystem restoration. *Conservation Science and Practice*, e12787.

Correll, C. U., Rubio, J. M., & Kane, J. M. (2018). What is the risk-benefit ratio of long-term antipsychotic treatment in people with schizophrenia?. *World Psychiatry, 17*(2), 149-160.

Costa, S. P., Pinto, P. C., Saraiva, M. L. M., Rocha, F. R., Santos, J. R., & Monteiro, R. T. (2015). The aquatic impact of ionic liquids on freshwater organisms. *Chemosphere,* 139, 288-294.

Cozzola, A. J., Dehnert, G. K., White, A. M., & Karasov, W. H. (2022). Effects of subchronic exposure to environmentally relevant concentrations of a commercial fluridone formulation on fathead minnows (*Pimephales promelas*). *Aquatic Toxicology*, 244, 106098.

Crini, G., & Lichtfouse, E. (2018). Wastewater treatment: an overview. *Green adsorbents for pollutant removal*, 1-21.

Dai, Y., Zhuang, J., & Chen, X. (2020). Synergistic effects of unsaturated flow and soil organic matter on retention and transport of PPCPs in soils. *Environmental Research*, 191, 110135.

David, A., Lange, A., Tyler, C. R., & Hill, E. M. (2018). Concentrating mixtures of neuroactive pharmaceuticals and altered neurotransmitter levels in the brain of fish exposed to a wastewater effluent. *Science of the Total Environment*, 621, 782-790.

DeWeerd, K. A., Flanagan, W. P., Brennan, M. J., Principe, J. M., & Spivack, J. L. (1998). Biodegradation of trichloroethylene and dichloromethane in contaminated soil and groundwater. *Bioremediation Journal, 2*(1), 29-42.

Du Plessis, A. (2019). Current and future water scarcity and stress. In Water as an Inescapable Risk (pp. 13-25). *Springer,* Cham.

Du, Y., Wang, W. L., Zhang, D. Y., Zhou, T. H., Lee, M. Y., Wu, Q. Y., ... & Huang, T. Y. (2020). Degradation of non-oxidizing biocide benzalkonium chloride and bulk dissolved organic matter in reverse osmosis concentrate by UV/chlorine oxidation. *Journal of hazardous materials*, 396, 122669.

Du, Y., Xu, X., Liu, Q., Bai, L., Hang, K., & Wang, D. (2022). Identification of organic pollutants with potential ecological and health risks in aquatic environments: Progress and challenges. *Science of the total environment*, 806, 150691.

Dulio, V., van Bavel, B., Brorström-Lundén, E., Harmsen, J., Hollender, J., Schlabach, M., ... & Koschorreck, J. (2018). Emerging pollutants in the EU: 10 years of NORMAN in support of environmental policies and regulations. *Environmental Sciences Europe, 30*(1), 1-13.

Dutta, D., Gaur, N., Barman, P., Ghosh, D., Dubey, R., & Dwivedi, S. K. (2022). A Review on the Degradation of Ionic and Non-Ionic Surfactants in Water.

El-Deen, A. K., & Shimizu, K. (2022). Suspect and non-target screening workflow for studying the occurrence, fate, and environmental risk of contaminants in wastewater using data-independent acquisition. *Journal of Chromatography A*, 1667, 462905.

Emsbo-Mattingly, S. D., Flanders, K. L., & Litman, E. R. (2022). Integrated differentiation of multiple trichloroethylene and tetrachloroethylene groundwater impacts using spatial concentration, biodegradation indices, chemical fingerprinting and carbon/chlorine isotope patterns. *Environmental Forensics*, 1-22.

Escher, B. I., Stapleton, H. M., & Schymanski, E. L. (2020). Tracking complex mixtures of chemicals in our changing environment. *Science, 367*(6476), 388–392. doi:10.1126/science.aay6636

Ewald, J. D., Soufan, O., Crump, D., Hecker, M., Xia, J., & Basu, N. (2020). EcoToxModules: custom gene sets to organize and analyze toxicogenomics data from ecological species. *Environmental Science & Technology, 54*(7), 4376-4387.

Fairbrother, A., Muir, D., Solomon, K. R., Ankley, G. T., Rudd, M. A., Boxall, A. B., ... & Brooks, B. W. (2019). Toward sustainable environmental quality: priority research questions for North America. *Environmental toxicology and chemistry, 38*(8), 1606-1624.

Falkenmark, M. (2020). Water resilience and human life support-global outlook for the next half century. *International Journal of Water Resources Development, 36*(2-3), 377-396.

Foley, M. M., & Carbines, M. (2019). Climate Change Risk Assessment for Auckland's Marine and Freshwater Ecosystems. Auckland Council, Te Kaunihera o Tāmaki Makaurau.

Forest, V., Hochepied, J. F., & Pourchez, J. (2019). Importance of choosing relevant biological end points to predict nanoparticle toxicity with computational approaches for human health risk assessment. *Chemical research in toxicology, 32*(7), 1320-1326.

Foulkes, R., Man, E., Thind, J., Yeung, S., Joy, A., & Hoskins, C. (2020). The regulation of nanomaterials and nanomedicines for clinical application: Current and future perspectives. *Biomaterials science, 8*(17), 4653-4664.

Fu, Z., & Wang, J. (2019). Current practices and future perspectives of microplastic pollution in freshwater ecosystems in China. *Science of the Total Environment*, 691, 697-712.

Fung, K. I., 1999. Atlas of Saskatchewan. Millennium Edition, University of Saskatchewan, Saskatoon.

Galindo-Miranda, J. M., Guízar-González, C., Becerril-Bravo, E. J., Moeller-Chávez, G., León-Becerril, E., & Vallejo-Rodríguez, R. (2019). Occurrence of emerging contaminants in

environmental surface waters and their analytical methodology–a review. *Water Supply, 19*(7), 1871-1884.

Gaston, L., Lapworth, D. J., Stuart, M., & Arnscheidt, J. (2019). Prioritization approaches for substances of emerging concern in groundwater: a critical review. *Environmental science & technology, 53*(11), 6107-6122.

Gavrilescu, M., Demnerová, K., Aamand, J., Agathos, S., & Fava, F. (2015). Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation. *New biotechnology, 32*(1), 147-156.

Geissen, V., Mol, H., Klumpp, E., Umlauf, G., Nadal, M., Van Der Ploeg, M., Van De Zee, S. E. A. T. M., & Ritsema, C. J. (2015). Emerging pollutants in the environment: A challenge for water resource management. *International Soil and Water Conservation Research, 3*(1), 57–65. https://doi.org/10.1016/j.iswcr.2015.03.002

Gillois, K., Lévêque, M., Théodorou, V., Robert, H., & Mercier-Bonin, M. (2018). Mucus: an underestimated gut target for environmental pollutants and food additives. Microorganisms, 6(2), 53.

Gogoi, A., Mazumder, P., Tyagi, V. K., Chaminda, G. T., An, A. K., & Kumar, M. (2018). Occurrence and fate of emerging contaminants in water environment: a review. *Groundwater for Sustainable Development,* 6, 169-180.

Gomes, I. B., Maillard, J. Y., Simões, L. C., & Simões, M. (2020). Emerging contaminants affect the microbiome of water systems—strategies for their mitigation. *NPJ Clean Water, 3*(1), 1-11.

Gorelick, D. E., Lin, L., Zeff, H. B., Kim, Y., Vose, J. M., Coulston, J. W., ... & Characklis, G. W. (2020). Accounting for adaptive water supply management when quantifying climate and land cover change vulnerability. *Water Resources Research, 56*(1), e2019WR025614.

Goutam Mukherjee, A., Ramesh Wanjari, U., Eladl, M. A., El-Sherbiny, M., Elsherbini, D. M. A., Sukumar, A., ... & Valsala Gopalakrishnan, A. (2022). *Mixed Contaminants: Occurrence, Interactions, Toxicity, Detection, and Remediation. Molecules, 27*(8), 2577.

Green, A. L. R., Putschew, A., & Nehls, T. (2014). Littered cigarette butts as a source of nicotine in urban waters. *Journal of hydrology*, 519, 3466-3474.

Hajeb, P., Zhu, L., Bossi, R., & Vorkamp, K. (2022). Sample preparation techniques for suspect and non-target screening of emerging contaminants. *Chemosphere*, 287, 132306.

Hale, R. C., Smith, C. L., De Fur, P. O., Harvey, E., Bush, E. O., La Guardia, M. J., & Vadas, G. G.. (2000). Nonylphenols in sediments and effluents associated with diverse wastewater outfalls. *Environmental Toxicology and Chemistry, 19*(4), 946–952. https://doi.org/10.1002/etc.5620190423

Hanson, S., Steeves, K., Bagatim, T., Hogan, N., Wiseman, S., Hontela, A., Giesy, J., Paul, J., & Hecker, M. (2021). Health status of fathead minnow (*Pimephales promelas*) populations in a

municipal wastewater effluent-dominated stream in the Canadian prairies, Wascana Creek, Saskatchewan. *Aquatic Toxicology*, 238, 105933.

Hena, S., Gutierrez, L., & Croué, J. P. (2021). Removal of pharmaceutical and personal care products (PPCPs) from wastewater using microalgae: A review. Journal of hazardous materials, 403, 124041.

Hiranmai, R. Y., & Kamaraj, M. (2021). Occurrence, fate, and toxicity of emerging contaminants in a diverse ecosystem. *Physical Sciences Reviews*.

Hoekstra, A. Y., Buurman, J., & Van Ginkel, K. C. (2018). Urban water security: A review. *Environmental research letters, 13*(5), 053002.

Huchthausen, J., Mühlenbrink, M., König, M., Escher, B. I., & Henneberger, L. (2020). Experimental exposure assessment of ionizable organic chemicals in in vitro cell-based bioassays. *Chemical Research in Toxicology, 33*(7), 1845-1854.

Huggett, D. B., Cook, J. C., Ericson, J. F., & Williams, R. T. (2003). A theoretical model for utilizing mammalian pharmacology and safety data to prioritize potential impacts of human pharmaceuticals to fish. *Human and Ecological Risk Assessment, 9*(7), 1789-1799.

Ibáñez, M., Bijlsma, L., Pitarch, E., López, F. J., & Hernandez, F. (2021). Occurrence of pharmaceutical metabolites and transformation products in the aquatic environment of the Mediterranean area. *Trends in Environmental Analytical Chemistry*, 29, e00118.

Ikonen, J., Nuutinen, I., Niittynen, M., Hokajärvi, A. M., Pitkänen, T., Antikainen, E., & Miettinen, I. T. (2021). Presence and Reduction of Anthropogenic Substances with UV Light

and Oxidizing Disinfectants in Wastewater—A Case Study at Kuopio, Finland. *Water, 13*(3), 360.

Juksu, K., Zhao, J. L., Liu, Y. S., Yao, L., Sarin, C., Sreesai, S., ... & Ying, G. G. (2019). Occurrence, fate and risk assessment of biocides in wastewater treatment plants and aquatic environments in Thailand. *Science of the Total Environment*, 690, 1110-1119.

Kasonga, T. K., Coetzee, M. A., Kamika, I., Ngole-Jeme, V. M., & Momba, M. N. B. (2021). Endocrine-disruptive chemicals as contaminants of emerging concern in wastewater and surface water: A review. *Journal of Environmental Management*, 277, 111485.

Katare, Y. K., Daya, R. P., Sookram Gray, C., Luckham, R. E., Bhandari, J., Chauhan, A. S., & Mishra, R. K. (2015). Brain targeting of a water insoluble antipsychotic drug haloperidol via the intranasal route using PAMAM dendrimer. *Molecular pharmaceutics, 12*(9), 3380-3388.

Khan, N. A., Khan, S. U., Ahmed, S., Farooqi, I. H., Yousefi, M., Mohammadi, A. A., & Changani, F. (2020). Recent trends in disposal and treatment technologies of emerging-pollutants-A critical review. *TrAC Trends in Analytical Chemistry*, 122, 115744.

Khan, A. H., Aziz, H. A., Khan, N. A., Hasan, M. A., Ahmed, S., Farooqi, I. H., ... & Mahtab, M. S. (2021). Impact, disease outbreak and the eco-hazards associated with pharmaceutical residues: A Critical review. *International Journal of Environmental Science and Technology*, 1-12.

Khan, H. K., Rehman, M. Y. A., Junaid, M., Lv, M., Yue, L., Haq, I. U., ... & Malik, R. N. (2022a). Occurrence, source apportionment and potential risks of selected PPCPs in

groundwater used as a source of drinking water from key urban-rural settings of Pakistan. *Science of The Total Environment*, 807, 151010.

Khan, N. H., ur Rahman, A., Zuljalal, F., Saeed, T., Aziz, S., & Ilyas, M. (2022b). Food additives as environmental micropollutants. In Environmental Micropollutants (pp. 63-79). *Elsevier.*

Khangaonkar, T., Nugraha, A., Xu, W., & Balaguru, K. (2019). Salish Sea response to global climate change, sea level rise, and future nutrient loads. *Journal of Geophysical Research: Oceans, 124*(6), 3876-3904.

Kiefer, K., Müller, A., Singer, H., & Hollender, J. (2019). New relevant pesticide transformation products in groundwater detected using target and suspect screening for agricultural and urban micropollutants with LC-HRMS. *Water Research*, 165, 114972.

Kortenkamp, A., Faust, M., Backhaus, T., Altenburger, R., Scholze, M., Müller, C., Ermler, S., Posthuma, L., & Brack, W.. (2019). Mixture risks threaten water quality: the European Collaborative Project SOLUTIONS recommends changes to the WFD and better coordination across all pieces of European chemicals legislation to improve protection from exposure of the aquatic environment to . *Environmental Sciences Europe, 31*(1). https://doi.org/10.1186/s12302-019-0245-6

Kumar, N., Singh, A., Sharma, D. K., & Kishore, K. (2019). Toxicity of food additives. *In Food safety and human health* (pp. 67-98). Academic Press.

Kumar, R., Qureshi, M., Vishwakarma, D. K., Al-Ansari, N., Kuriqi, A., Elbeltagi, A., & Saraswat, A. (2022). A review on emerging water contaminants and the application of

sustainable removal technologies. *Case Studies in Chemical and Environmental Engineering*, 6, 100219.

Kuroda, K., & Kobayashi, J. (2021). Pharmaceuticals, personal care products, and artificial sweeteners in Asian groundwater: a review. *Contaminants in Drinking and Wastewater Sources*, 3-36.

Leung, K. M., Yeung, K. W., You, J., Choi, K., Zhang, X., Smith, R., ... & Brooks, B. W. (2020). Toward sustainable environmental quality: priority research questions for Asia. *Environmental toxicology and chemistry, 39*(8), 1485-1505.

Li, Z. H., Zlabek, V., Velisek, J., Grabic, R., Machova, J., Kolarova, J., ... & Randak, T. (2011). Acute toxicity of carbamazepine to juvenile rainbow trout (Oncorhynchus mykiss): effects on antioxidant responses, hematological parameters and hepatic EROD. *Ecotoxicology and environmental safety, 74*(3), 319-327.

Li, W. C. (2014). Occurrence, sources, and fate of pharmaceuticals in aquatic environment and soil. *Environmental pollution*, 187, 193-201.

Li, N., Zhang, T., Chen, G., Xu, J., Ouyang, G., & Zhu, F. (2021). Recent advances in sample preparation techniques for quantitative detection of pharmaceuticals in biological samples. *TrAC Trends in Analytical Chemistry*, 142, 116318.

Lin, J., Huang, J., Prell, C., & Bryan, B. A. (2021). Changes in supply and demand mediate the effects of land-use change on freshwater ecosystem services flows. *Science of the Total Environment*, 763, 143012.

López-Ruiz, R., Romero-González, R., & Frenich, A. G. (2019). Ultrahigh-pressure liquid chromatography-mass spectrometry: an overview of the last decade. *TrAC Trends in Analytical Chemistry*, 118, 170-181.

Martín-Girela, I., Albero, B., Tiwari, B. K., Miguel, E., & Aznar, R. (2020). Screening of contaminants of emerging concern in microalgae food supplements. *Separations, 7*(2), 28.

Marttunen, M., Mustajoki, J., Sojamo, S., Ahopelto, L., & Keskinen, M. (2019). A framework for assessing water security and the water–energy–food nexus—the case of Finland. *Sustainability, 11*(10), 2900.

Mahesh, N., Balakumar, S., Danya, U., Shyamalagowri, S., Babu, P. S., Aravind, J., ... & Govarthanan, M. (2022). A review on mitigation of emerging contaminants in an aqueous environment using microbial bio-machines as sustainable tools: Progress and limitations. *Journal of Water Process Engineering,* 47, 102712.

Mehinto, A. C., Coffin, S., Koelmans, A. A., Brander, S. M., Wagner, M., Thornton Hampton, L. M., ... & Rochman, C. M. (2022). Risk-based management framework for microplastics in aquatic ecosystems. *Microplastics and Nanoplastics, 2*(1), 1-10.

Mhuka, V., Dube, S., & Nindi, M. M. (2020). Occurrence of pharmaceutical and personal care products (PPCPs) in wastewater and receiving waters in South Africa using LC-Orbitrap™ MS. *Emerging Contaminants*, 6, 250-258.

Mishra, B. K., Kumar, P., Saraswat, C., Chakraborty, S., & Gautam, A. (2021). Water security in a changing environment: Concept, challenges and solutions. *Water, 13*(4), 490.

Montemayor, R. G. (2010). Petroleum Solvents. West Conshohocken, PA, USA: ASTM International.

Mori, A. S., Furukawa, T., & Sasaki, T. (2013). *Response diversity determines the resilience of ecosystems to environmental change. Biological reviews, 88*(2), 349-364.

Morin-Crini, N., Lichtfouse, E., Liu, G., Balaram, V., Ribeiro, A. R. L., Lu, Z., ... & Crini, G. (2022). Worldwide cases of water pollution by emerging contaminants: a review. *Environmental Chemistry Letters*, 1-28.

Moyle, B. D., Weaver, D. B., Gössling, S., McLennan, C. L., & Hadinejad, A. (2022). Are water-centric themes in sustainable tourism research congruent with the UN Sustainable Development Goals?. *Journal of Sustainable Tourism, 30*(8), 1821-1836.

Munthe, J., Brorström-Lundén, E., Rahmberg, M., Posthuma, L., Altenburger, R., Brack, W., ... & van Wezel, A. (2017). An expanded conceptual framework for solution-focused management of chemical pollution in European waters. *Environmental Sciences Europe, 29*(1), 1-16.

Murdoch, P. S., Baron, J. S., & Miller, T. L. (2000). Potential effects of climate change on surface-water quality in North America 1. *JAWRA Journal of the American Water Resources Association, 36*(2), 347-366.

Nair, A., Morsy, M. A., & Jacob, S. (2018). Dose translation between laboratory animals and human in preclinical and clinical phases of drug development. *Drug development research, 79*(8), 373-382.

Narayanan, M., El-Sheekh, M., Ma, Y., Pugazhendhi, A., Natarajan, D., Kandasamy, G., ... & Kandasamy, S. (2022). Current status of microbes involved in the degradation of pharmaceutical and personal care products (PPCPs) pollutants in the aquatic ecosystem. *Environmental Pollution*, 118922.

Neale, P. A., & Escher, B. I. (2019). In vitro bioassays to assess drinking water quality. *Current Opinion in Environmental Science & Health*, 7, 1-7.

Neale, P. A., O'Brien, J. W., Glauch, L., König, M., Krauss, M., Mueller, J. F., ... & Escher, B. I. (2020). Wastewater treatment efficacy evaluated with in vitro bioassays. *Water research X*, 9, 100072.

Ngo, P. L., Pramanik, B. K., Shah, K., & Roychand, R. (2019). Pathway, classification and removal efficiency of microplastics in wastewater treatment plants. *Environmental Pollution,* 255, 113326.

Nivala, J., Neale, P. A., Haasis, T., Kahl, S., König, M., Müller, R. A., ... & Escher, B. I. (2018). Application of cell-based bioassays to evaluate treatment efficacy of conventional and intensified treatment wetlands. *Environmental Science: Water Research & Technology, 4*(2), 206-217.

Nowak, P., Kucharska, K., & Kamiński, M.. (2019). Ecological and Health Effects of Lubricant Oils Emitted into the Environment. *International Journal of Environmental Research and Public Health, 16(*16), 3002. https://doi.org/10.3390/ijerph16163002

Nowakowska, K., Giebułtowicz, J., Kamaszewski, M., Adamski, A., Szudrowicz, H., Ostaszewska, T., ... & Drobniewska, A. (2020). Acute exposure of zebrafish (Danio rerio) larvae to environmental concentrations of selected antidepressants: Bioaccumulation, physiological and histological changes. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 229, 108670.

Nunes, R. F., & Teixeira, A. C. S. C. (2022). An overview on surfactants as pollutants of concern: Occurrence, impacts and persulfate-based remediation technologies. *Chemosphere,* 134507.

Ogunkunle, T. J., Adewumi, A., & Adepoju, A. O. (2019). Biodiversity: overexploited but underutilized natural resource for human existence and economic development. *Environment & Ecosystem Science, 3*(1), 26-34.

Ohoro, C. R., Adeniji, A. O., Okoh, A. I., & Okoh, O. O. (2019). Distribution and chemical analysis of pharmaceuticals and personal care products (PPCPs) in the environmental systems: A review. *International Journal of Environmental Research and Public Health, 16*(17), 3026.

Olasupo, A., & Suah, F. B. M. (2021). Recent advances in the removal of pharmaceuticals and endocrine-disrupting compounds in the aquatic system: A case of polymer inclusion membranes. *Journal of hazardous materials*, 406, 124317.

Ouda, M., Kadadou, D., Swaidan, B., Al-Othman, A., Al-Asheh, S., Banat, F., & Hasan, S. W. (2021). Emerging contaminants in the water bodies of the Middle East and North Africa (MENA): A critical review. *Science of the Total Environment*, 754, 142177.

Pai, C. W., Leong, D., Chen, C. Y., & Wang, G. S. (2020). Occurrences of pharmaceuticals and personal care products in the drinking water of Taiwan and their removal in conventional water treatment processes. *Chemosphere,* 256, 127002.

Paruch, L. (2022). Molecular Diagnostic Tools Applied for Assessing Microbial Water Quality. *International Journal of Environmental Research and Public Health, 19*(9), 5128.

Paíga, P., Correia, M., Fernandes, M. J., Silva, A., Carvalho, M., Vieira, J., Jorge, S., Silva, J. G., Freire, C., & Delerue-Matos, C.. (2019). Assessment of 83 pharmaceuticals in WWTP influent and effluent samples by UHPLC-MS/MS: Hourly variation. *Science of the Total Environment,* 648, 582–600. https://doi.org/10.1016/j.scitotenv.2018.08.129

Paul, D., Chakraborty, R., & Mandal, S. M. (2019). Biocides and health-care agents are more than just antibiotics: Inducing cross to co-resistance in microbes. *Ecotoxicology and environmental safety*, 174, 601-610.

Paun, I., Pirvu, F., Iancu, V. I., & Chiriac, F. L. (2022). Occurrence and Transport of Isothiazolinone-Type Biocides from Commercial Products to Aquatic Environment and Environmental Risk Assessment. *International journal of environmental research and public health, 19*(13), 7777.

Peña-Guzmán, C., Ulloa-Sánchez, S., Mora, K., Helena-Bustos, R., Lopez-Barrera, E., Alvarez, J., & Rodriguez-Pinzón, M. (2019). Emerging pollutants in the urban water cycle in Latin America: a review of the current literature. *Journal of environmental management,* 237, 408-423.

Perez de Souza, L., Alseekh, S., Scossa, F., & Fernie, A. R. (2021). Ultra-high-performance liquid chromatography high-resolution mass spectrometry variants for metabolomics research. *Nature Methods, 18*(7), 733-746.

Pérez, D. J., Doucette, W. J., & Moore, M. T. (2022a). Contaminants of emerging concern (CECs) in Zea mays: Uptake, translocation and distribution tissue patterns over the time and its relation with physicochemical properties and plant transpiration rate. *Chemosphere*, 288, 132480.

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

Pressman, P., Clemens, R., Hayes, W., & Reddy, C. (2017). Food additive safety: A review of toxicologic and regulatory issues. *Toxicology Research and application*, 1, 2397847317723572.

Priya, A. K., Gnanasekaran, L., Rajendran, S., Qin, J., & Vasseghian, Y. (2022). Occurrences and removal of pharmaceutical and personal care products from aquatic systems using advanced treatment-A review. *Environmental Research*, 204, 112298.

Pope, J. L., & Osantowski, R. A. (2018, May). Case History–Use of a Mobile Advanced Water Treatment System to Treat Groundwater Contaminated with Volatile Organic Compounds. In Proceedings of the 41st Industrial Waste Conference May 13, 14, 15, 1986 (pp. 408-414). CRC Press.

Posthuma, L., Altenburger, R., Backhaus, T., Kortenkamp, A., Müller, C., Focks, A., ... & Brack, W. (2019). Improved component-based methods for mixture risk assessment are key to characterize complex chemical pollution in surface waters. *Environmental Sciences Europe, 31*(1), 1-11.

Richardson, S. D., & Kimura, S. Y. (2019). Water analysis: emerging contaminants and current issues. *Analytical Chemistry, 92*(1), 473-505.

Richmond, E. K., Rosi, E. J., Walters, D. M., Fick, J., Hamilton, S. K., Brodin, T., ... & Grace, M. R. (2018). A diverse suite of pharmaceuticals contaminates stream and riparian food webs. *Nature Communications, 9*(1), 1-9.

Robitaille, J., Denslow, N. D., Escher, B. I., Kurita-Oyamada, H. G., Marlatt, V., Martyniuk, C. J., ... & Langlois, V. S. (2022). Towards regulation of Endocrine Disrupting chemicals (EDCs) in water resources using bioassays–A guide to developing a testing strategy. Environmental research, 205, 112483.

Rodriguez-Narvaez, O. M., Peralta-Hernandez, J. M., Goonetilleke, A., & Bandala, E. R. (2017). Treatment technologies for emerging contaminants in water: A review. *Chemical Engineering Journal,* 323, 361-380.

Rout, P. R., Zhang, T. C., Bhunia, P., & Surampalli, R. Y. (2021). Treatment technologies for emerging contaminants in wastewater treatment plants: A review. *Science of the Total Environment*, 753, 141990.

Saidulu, D., Gupta, B., Gupta, A. K., & Ghosal, P. S. (2021). A review on occurrences, eco-toxic effects, and remediation of emerging contaminants from wastewater: special emphasis on biological treatment based hybrid systems. *Journal of Environmental Chemical Engineering, 9*(4), 105282.

Sang, Z., Jiang, Y., Tsoi, Y. K., & Leung, K. S. Y. (2014). Evaluating the environmental impact of artificial sweeteners: a study of their distributions, photodegradation and toxicities. *Water Research*, 52, 260-274.

Schinkel, L., Lara-Martín, P. A., Giger, W., Hollender, J., & Berg, M. (2022). Synthetic surfactants in Swiss sewage sludges: Analytical challenges, concentrations and per capita loads. *Science of the Total Environment*, 808, 151361.

Schuijt, L. M., Peng, F. J., van den Berg, S. J., Dingemans, M. M., & Van den Brink, P. J. (2021). (Eco) toxicological tests for assessing impacts of chemical stress to aquatic ecosystems: Facts, challenges, and future. *Science of the total environment*, 795, 148776.

Schwartz, H., Marushka, L., Chan, H. M., Batal, M., Sadik, T., Fediuk, K., & Tikhonov, C. (2021). Pharmaceuticals in source waters of 95 First Nations in Canada. *Canadian Journal of Public Health, 112*(1), 133-153.

Schwarzbauer, J., & Ricking, M. (2010). Non-target screening analysis of river water as compound-related base for monitoring measures. Environmental science and pollution research, 17(4), 934-947.

Schwenk, M., & Burr, R. (2020). Intrinsic Toxicity of Substances: Aspects for Risk Assessment. *Regulatory Toxicology*, 1-27.

Sharma, B. M., Bečanová, J., Scheringer, M., Sharma, A., Bharat, G. K., Whitehead, P. G., ... & Nizzetto, L. (2019). Health and ecological risk assessment of emerging contaminants (pharmaceuticals, personal care products, and artificial sweeteners) in surface and groundwater (drinking water) in the Ganges River Basin, India. *Science of the Total Environment*, 646, 1459-1467.

Sigmund, G., Gharasoo, M., Hüffer, T., & Hofmann, T. (2020). Deep learning neural network approach for predicting the sorption of ionizable and polar organic pollutants to a wide range of carbonaceous materials. *Environmental science & technology, 54*(7), 4583-4591.

Slavik, I., Oliveira, K. R., Cheung, P. B., & Uhl, W. (2020). Water quality aspects related to domestic drinking water storage tanks and consideration in current standards and guidelines throughout the world–a review. *Journal of water and health, 18*(4), 439-463.

Sobus, J. R., Wambaugh, J. F., Isaacs, K. K., Williams, A. J., McEachran, A. D., Richard, A. M., ... & Newton, S. R. (2018). Integrating tools for non-targeted analysis research and chemical safety evaluations at the US EPA. *Journal of exposure science & environmental epidemiology, 28*(5), 411-426.

Soufan, O., Ewald, J., Zhou, G., Hacariz, O., Boulanger, E., Alcaraz, A. J., ... & Xia, J. (2022). EcoToxXplorer: Leveraging Design Thinking to Develop a Standardized Web-Based

Transcriptomics Analytics Platform for Diverse Users. *Environmental toxicology and chemistry, 41*(1), 21-29.

Stefanakis, A. I., & Becker, J. A. (2016). A review of emerging contaminants in water: classification, sources, and potential risks. *Impact of water pollution on human health and environmental sustainability,* 55-80.

Su, C., Zhang, H., Cridge, C., & Liang, R. (2019). A review of multimedia transport and fate models for chemicals: Principles, features and applicability. *Science of the total environment,* 668, 881-892.

Sumpter, J. P., & Margiotta-Casaluci, L. (2022). Environmental occurrence and predicted pharmacological risk to freshwater fish of over 200 neuroactive pharmaceuticals in widespread use. *Toxics, 10*(5), 233.

Sungur, Ş. (2022). Pharmaceutical and personal care products in the environment: occurrence and impact on the functioning of the ecosystem. In Emerging Contaminants in the Environment (pp. 137-157). *Elsevier.*

Tadsuwan, K., & Babel, S. (2022). Microplastic abundance and removal via an ultrafiltration system coupled to a conventional municipal wastewater treatment plant in Thailand. *Journal of Environmental Chemical Engineering, 10*(2), 107142.

Tanoue, R., Nomiyama, K., Nakamura, H., Kim, J. W., Isobe, T., Shinohara, R., ... & Tanabe, S. (2015). Uptake and tissue distribution of pharmaceuticals and personal care products in wild

fish from treated-wastewater-impacted streams. *Environmental science & technology, 49*(19), 11649-11658.

Tavengwa, N. T., & Dalu, T. (2022). Introduction to emerging freshwater pollutants. In Emerging Freshwater Pollutants (pp. 1-6). *Elsevier.*

Tetreault, G. R., Bennett, C. J., Cheng, C., Servos, M. R., & McMaster, M. E. (2012). Reproductive and histopathological effects in wild fish inhabiting an effluent-dominated stream, Wascana Creek, SK, Canada. *Aquatic Toxicology*, 110, 149-161.

Tollefsen, K. E., Nizzetto, L., & Huggett, D. B. (2012). Presence, fate and effects of the intense sweetener sucralose in the aquatic environment. *Science of the Total Environment*, 438, 510-516.

Tong, X., Mohapatra, S., Zhang, J., Tran, N. H., You, L., He, Y., & Gin, K. Y. H. (2022). Source, fate, transport and modelling of selected emerging contaminants in the aquatic environment: Current status and future perspectives. *Water Research*, 118418.

Torres, C., Gitau, M. W., Paredes-Cuervo, D., & Engel, B. (2022). Evaluation of sampling frequency impact on the accuracy of water quality status as determined considering different water quality monitoring objectives. *Environmental Monitoring and Assessment, 194*(7), 1-23.

Tzanakakis, V. A., Paranychianakis, N. V., & Angelakis, A. N. (2020). Water supply and water scarcity. *Water, 12*(9), 2347.

Uddin, M. G., Nash, S., & Olbert, A. I. (2021). A review of water quality index models and their use for assessing surface water quality. *Ecological Indicators*, 122, 107218.

Umemneku Chikere, C. M., Wilson, K., Graziadio, S., Vale, L., & Allen, A. J. (2019). Diagnostic test evaluation methodology: a systematic review of methods employed to evaluate diagnostic tests in the absence of gold standard–an update. *PLoS One, 14*(10), e0223832.

Vannevel, R., & Goethals, P. L. (2020). Identifying ecosystem key factors to support sustainable water management. *Sustainability, 12*(3), 1148.

Varsha, M., Kumar, P. S., & Rathi, B. S. (2022). A review on recent trends in the removal of emerging contaminants from aquatic environment using low-cost adsorbents. *Chemosphere,* 287, 132270.

Vieira, W. T., de Farias, M. B., Spaolonzi, M. P., da Silva, M. G. C., & Vieira, M. G. A. (2021). Endocrine-disrupting compounds: Occurrence, detection methods, effects and promising treatment pathways—A critical review. *Journal of Environmental Chemical Engineering, 9*(1), 104558.

Vitale, C. M., & Di Guardo, A. (2019). A review of the predictive models estimating association of neutral and ionizable organic chemicals with dissolved organic carbon. *Science of the Total Environment,* 666, 1022-1032.

Vorkamp, K., Bossi, R., Bester, K., Bollmann, U. E., & Boutrup, S. (2014). New priority substances of the European Water Framework Directive: biocides, pesticides and brominated

flame retardants in the aquatic environment of Denmark. *Science of the Total Environment*, 470, 459-468.

Waiser, M. J., Tumber, V., & Holm, J.. (2011a). Effluent-dominated streams. Part 1: Presence and effects of excess nitrogen and phosphorus in Wascana Creek, Saskatchewan, Canada. *Environmental Toxicology and Chemistry, 30*(2), 496–507. https://doi.org/10.1002/etc.399

Wang, X., Yu, N., Yang, J., Jin, L., Guo, H., Shi, W., ... & Wei, S. (2020). Suspect and non-target screening of pesticides and pharmaceuticals transformation products in wastewater using QTOF-MS. *Environment international*, 137, 105599.

Xin, X., Huang, G., & Zhang, B. (2021). Review of aquatic toxicity of pharmaceuticals and personal care products to algae. *Journal of Hazardous Materials*, 410, 124619.

Yap, H. C., Pang, Y. L., Lim, S., Abdullah, A. Z., Ong, H. C., & Wu, C. H. (2019). A comprehensive review on state-of-the-art photo-, sono-, and sonophotocatalytic treatments to degrade emerging contaminants. *International Journal of Environmental Science and Technology, 16*(1), 601-628.

Yates, M. C., Derry, A. M., & Cristescu, M. E. (2021). Environmental RNA: a revolution in ecological resolution?. Trends in Ecology & Evolution, 36(7), 601-609.

Yuan, S., Jiang, X., Xia, X., Zhang, H., & Zheng, S. (2013). Detection, occurrence, and fate of 22 psychiatric pharmaceuticals in psychiatric hospital and municipal wastewater treatment plants in Beijing, China. *Chemosphere, 90*(10), 2520–2525. https://doi.org/10.1016/j.chemosphere.2012.10.089

Yusuf, A., O'Flynn, D., White, B., Holland, L., Parle-McDermott, A., Lawler, J., ... & Regan, F. (2021). Monitoring of emerging contaminants of concern in the aquatic environment: a review of studies showing the application of effect-based measures. *Analytical Methods*.

Zeinalzadeh, K., & Rezaei, E. (2017). Determining spatial and temporal changes of surface water quality using principal component analysis. *Journal of Hydrology: Regional Studies*, 13, 1-10.

Zheng, Y., & Nowack, B. (2021). Size-specific, dynamic, probabilistic material flow analysis of titanium dioxide releases into the environment. Environmental Science & Technology, 55(4), 2392-2402.

Zhu, F. J., Ma, W. L., Xu, T. F., Ding, Y., Zhao, X., Li, W. L., ... & Zhang, Z. F. (2018). Removal characteristic of surfactants in typical industrial and domestic wastewater treatment plants in Northeast China. *Ecotoxicology and environmental safety*, 153, 84-90.

Zulkifli, S. N., Rahim, H. A., & Lau, W. J. (2018). Detection of contaminants in water supply: A review on state-of-the-art monitoring technologies and their applications. *Sensors and Actuators B: Chemical*, 255, 2657-2689

CHAPTER 2: IMPACTS OF WASTEWATER EFFLUENTS AND SEASONAL TRENDS ON LEVELS OF ANTIPSYCHOTIC PHARMACEUTICALS IN WATER AND SEDIMENTS FROM TWO COLD-REGION RIVERS

Overview

A version of this chapter has been published in Journal Science of The Total Environment with the following details: Perez, A. S. C., Challis, J. K., Ji, X., Giesy, J. P., & Brinkmann, M. (2022). Impacts of wastewater effluents and seasonal trends on levels of antipsychotic pharmaceuticals in water and sediments from two cold-region rivers. Science of The Total Environment, 851, 158247.

Contributions

Ana Cardenas: Research conceptualization, study design and implementation, methodology, software, validation, formal analysis, investigation, data curation, writing - original draft, reviewing, and editing, visualization.

Jonathan Challis: Data curation, methodology, writing, review, and editing.

Xiaowen Ji: Methodology, writing, review, and editing.

Jon Giesy: Resources, writing, review, and editing.

Markus Brinkmann: Research conceptualization, data curation, formal analysis, funding acquisition, Investigation, methodology, project administration, resources, supervision, writing – review & editing.

2.1 Abstract

Most pharmaceuticals are found at trace concentrations in aquatic systems, but their continuous release and potential accumulation can lead to adverse health effects in exposed organisms. Concentrations can vary temporally, driven by variations in discharges of receiving waters, sorption to sediments, and other biotic and abiotic exchange processes. The principal aim of this research was to better understand the occurrence, trends, and dynamics of pharmaceuticals in a cold-climate, riverine environment. To this end, a suite of seven representative antipsychotic pharmaceuticals was measured upstream and downstream of two wastewater treatment plants in Saskatchewan, Canada, located in the South Saskatchewan River and Wascana Creek, respectively, across three seasons. Concentrations of analytes were in the ng/L range and generally greater downstream of both wastewater treatment plants compared to upstream. Some compounds, including the tricyclic antidepressant amitriptyline, which was the most abundant analyte in water and sediment from both sites and across seasons, reached low μg/L concentrations. Data collected from this research effort indicate contamination with antipsychotic pharmaceuticals, with the potential to adversely impact exposed organisms.

Keywords: Pharmaceutical, diffusive gradients in thin films, DGT, wastewater treatment plants, drugs, sewage.

2.2 Introduction

Rivers provide a number of critical ecosystem services, by providing a source of fresh water and a habitat for aquatic organisms. Due in part to the widespread installation of wastewater treatment

plants (WWTPs), qualities of water in rivers has improved significantly in developed countries (Eggen et al., 2014; Kosek et al., 2020). However, WWTPs were not primarily designed to remove organic chemicals like pharmaceuticals (Reichert et al., 2019; Lopez-Herguedas et al., 2022), and their effluents can still contain complex mixtures of chemicals. Effluents of WWTPs contain emerging pollutants, such as pharmaceuticals, personal care products, and plasticizers that can negatively affect aquatic organisms (Laville et al, 2004; Yuan et al., 2013; Geissen et al., 2015; Reichert et al., 2019; Lopez-Herguedas et al., 2022). In receiving waters, these emerging pollutants are found at trace concentrations in the ng/L to μg/L range (Brady et al., 2017; Tran et al., 2018; Yang et al., 2019; Valdez-Carrillo et al., 2020). However, due to their continuous release, potential for biological transfer, and possible bioaccumulation by aquatic organisms, they might be an important driver of ecotoxicological risks of effluents (Hanson, 2019; Paiga et al., 2019). There has been an increasing number of studies on the environmental risks of pharmaceuticals, but further studies are still needed to close the remaining knowledge gaps and inform the necessary regulations (Mejias et al., 2021).

One group that has recently received increasing scientific and public attention are antipsychotic drugs, which have been observed in the ng/L to μg/L concentration range in WWTP effluents from across Canada (Metcalfe et al., 2010). Additionally, several of these compounds and their environmental transformation products have been detected in surface and drinking waters (Caldas et al., 2016; Nannou et al., 2015; Silveira et al., 2013), sediments (Nunes et al., 2019), and fish

(Kalichak et al., 2017). Furthermore, these antipsychotics can adversely affect aquatic ecosystems. For example, expressions of genes, related to neuroendocrine functions in zebrafish were altered after exposure to fluoxetine (Wu et al., 2017). The tricyclic antidepressant amitriptyline triggered alterations in concentrations of hormones related to the hypothalamic-pituitary-adrenal axis, biochemical parameters related to the antioxidant defense system, and amounts of immune system modulators in zebrafish (Yang et al., 2014). Consequently, there is an urgent need for more information on occurrences of these chemicals in support of environmental risk assessments.

Concentrations of pharmaceuticals in environmental media can be affected by several factors, including temporal variations, discharges of receiving water bodies, sorption capacity of sediments, and other abiotic dynamic processes that operate in aquatic ecosystems. Currently, most experimental approaches have ignored these dynamics when trying to evaluate chemical activities, bioavailability, and toxic potencies of these compounds (López-Herguedas et al., 2022). Since some pharmaceuticals are ionisable, organic chemicals (IOCs), they can be dissociated to variable degrees, depending on environmental factors, including pH, alkalinity and hardness, in turn modulating bioavailable fractions, as well as sorption to sediment constituents (Boxall et al., 2012). All of these factors lead to variability in concentrations of pharmaceuticals in the environment, which is a challenge for reliable environmental risk assessments.

Achieving accurate monitoring of pharmaceuticals in the environment necessitates the use of advanced tools that not only provide discrete measurements over time, but also allow calculating

time-weighted averages (TWA) of fluctuating concentrations of target analytes and allow evaluation of cumulative risks of pollutants (Bunting et al., 2021). One such tool is passive sampling devices, which can be used to determine concentrations of various compounds in aquatic environments over time (Wang et al., 2020; Caban et al., 2021). For example, diffusive gradients in thin films (DGT) passive samplers have been successfully applied to monitor TWA concentrations of various pharmaceuticals (Gong et al., 2018; Ji et al., 2022; Niti et al., 2022).

Human health risk evaluation of pharmaceuticals through both water intake and fish consumption in Saskatchewan, Canada is very limited, especially at river-basin scale. Over the past decade, some studies conducted in WC have been published focusing on monitoring seasonal trends of mixture of personal care products (Waiser et al., 2011a, 2011b) and evaluating the effects of municipal WWTPs effluents on wild fish (Tetreault et al., 2012), and investigating the health and molecular disruptions in resident fishes (Hanson et al., 2021). However, the toxicity data studies in recent years have not been showed the environmental media effects on concentrations of pharmaceuticals, and risk assessment for different classes of organisms (including algae, invertebrate, and fish). Due to the variability in concentrations of emerging pollutants, the main objective of this research was to investigate their temporal dynamics and their relationships with physicochemical water parameters upstream and downstream of two WWTPs located in the South Saskatchewan River (SSR) and Wascana Creek (WC), Saskatchewan, Canada. Specifically, a suite of antipsychotic pharmaceuticals was selected for this research, including amitriptyline (AMI),

bupropion (BUP), carbamazepine (CBZ), clozapine (CLO), fluoxetine (FLX), and lamotrigine (LAM), venlafaxine (VEN), which were monitored in sediments and water by conventional grab and passive sampler (to assess different sampling methods) during three seasons (Spring, Summer, and Fall of 2021).

2.3 Materials and methods

2.3.1 Sampling locations

The SSR is one of the largest and most important rivers in Saskatchewan, which begins in the Rocky Mountains of Alberta, flows across Saskatchewan including Saskatoon (with estimated population of 282,900 in 2021) and then flows into Manitoba. Wascana Creek, an effluent-dominated stream near the City of Regina (with estimated population of 249,217 in 2021), Saskatchewan, constantly receives effluents from the Regina WWTP, which can make up to over 90% of the creek's flow under low flow conditions and thus, might affect water quality downstream. The Qu´Appelle River Basin is located in the southern part of Saskatchewan. The basin has an extension of 74,589 square kilometers from the border of SSR basin to just inside the province of Manitoba, where the Qu´Appelle River joins the Assiniboine River (Akomeah and University of Saskatchewan, 2021).

Some threats to water quality in the SSR and WC include contaminants entering from major cities, industrial areas, and agricultural activities. Municipal sewage is a significant contributor to

nutrient loadings and is most detrimental during warm periods, causing significant deterioration of water quality. Water quality downstream in the SSR and WC can be expected to be affected by the constant discharge of effluents from Saskatoon and Regina WWTPs (Waiser et al., 2011a, 2011b). In the present study, samples were collected from four sites that are located both upstream and downstream of the WWTPs in Saskatoon and Regina, respectively: SUS (South Saskatchewan Upstream Site: 51°98’37.60 “N, 106°73’49.73 “W); SDS (South Saskatchewan Downstream Site: 52°31’79.98 "N, 106°46'10.99"W); WUS (Wascana Creek Upstream Site: 50°47'65.42"N, 104°73'26.30"W); WDS (Wascana Creek Downstream Site: 50°48'42.01"N, 104°77'80.66"W) (Figure 1.1).

2.3.2 Water and sediment sampling

Duplicate samples of water and sediments, as well as six replicate DGT samples, were collected for each of the four sampling sites during the spring, summer, and fall of 2021. Measurements of *in situ* physicochemical parameters and environmental factors, including temperature, pH, SC, DO, and TDS, were obtained using a Data Sonde 4a (Hydrolab, Campbell Scientific, Edmonton, Canada). Water samples were collected manually at each location below the water surface, considering the points with maximum water mixing, approximately 1.5 m deep, and DGT were deployed during 14 days (per season). Pre-cleaned (washed with liquinox and mili-Q water and then rinsed with methanol) 2-L and 1-L glass jars were used independently to collect 1 L and 0.5 L of water and sediment, respectively, for the analysis of contaminants. The sediments were

collected using a stainless-steel spoon, ensuring the representativeness of the sample by sampling the top 0 to 10 cm across a representative area (5 spots within 10 m^2). Supernatant water was decanted from the sample, and the sediment was homogenized in the container using a stainless-steel spoon prior to sealing the container. Samples were preserved at 4ºC immediately after sampling and frozen at -20ºC once brought to the laboratory.

2.3.3 Water and sediment sample processing and preparation for chemical analysis

Sediments were extracted using a method developed by Ji et al. (2022). Sediments were transferred to a freezer (-20°C) before lyophilization (Dura-Dry MP FD2085 microprocessor-controlled freeze-dryer, Stone Ridge, NY). Dried sediments were ground and passed through a 2-mm sieve to remove large fragments and plant materials. Aliquots of 10-g of sediment were placed in a 50-mL conical centrifuge tube and 50 µL of internal standard (mixture of Amitriptyline-D6, Bupropion-D9, Carbamazepine-D10, Clozapine-D4, Fluoxetine-D5, Lamotrigine-[13C;15N4], and Venlafaxine-D6 at 1 mg/L) in methanol were added. Then, 10 mL of a methanol: dichloromethane mixture (50:50, v/v) for ultrasound-assisted extraction. The mixture was sonicated for 5 min and centrifuged at 3,000 ×*g* for 15 min. Supernatants were collected, and the sequence was repeated three times. Afterward, combined extracts were evaporated and re-dissolved in 500 mL ultrapure water in methanol in wide-mouth amber glass bottles before solid-phase extraction (SPE). Water samples were prepared using a method previously published by Challis et al. (2016). Samples were filtered, and approximately 500 mL of filtrate was fortified with 50 µg/L of internal

standard (1 mg/L in methanol) and transferred to wide-mouth amber glass bottles for SPE. Ultrapure water was treated the same way as water samples or sediment extracts to obtain procedural blank samples.

SPE for both water and sediment samples was conducted using 3-mL Oasis HLB SPE cartridges with 60 mg sorbent per cartridge (Waters Corp, MA). The cartridges were preconditioned with 5 mL methanol followed by 10 mL ultrapure water. Diluted sediment extracts or samples of water were then passed through the cartridge at a stable flow (approximately 4 mL/min). Afterward, SPE cartridges were rinsed in10 mL of 10% methanol and drained completely for 30 minutes using vacuum suction. The drained sample bottles were rinsed thoroughly with 20 mL of a mixture of dichloromethane and methanol (50:50, v/v) that was then passed through the corresponding SPE cartridges for elution. Another volume of 5 mL of methanol was used for final elution. The combined eluates were concentrated close to dryness under a gentle stream of nitrogen gas, reconstituted in 1 mL of 50:50 MeOH: water (v/v), and filtered through a 0.45-µm PTFE syringe filter (Pall Life Sciences, Mississauga, ON) into amber LC vials.

2.3.4 Fundamental principle of DGT

Diffusive gradients in thin films (DGT) passive samplers are used for monitoring of chemicals in aquatic ecosystems (Chen et al., 2018). DGT devices are composed of a sorbent binding phase and an outer agarose diffusive gel (Chen et al., 2018). The DGT technique is based on Fick's first law of diffusion (Davison and Zhang, 2012; Zhang and Davison, 1995). Considering the time of

deployment of the DGT, it is possible to obtain an *in situ* measurement of TWA concentrations, C_{DGT}. This principle can be expressed through Equations (1) and (2) after correlating C_{DGT} to the mass of analyte on the sampler (M_{DGT}), the thickness of the diffusive layer (Δg), exposed area (A), deployment time (t), and analyte diffusion coefficient through the diffusive gel (D) (Equation 1).

$$C_{DGT} = \frac{M_{DGT}\Delta g}{DAt} \qquad (1)$$

Application of this relationship is valid considering natural flow conditions (≥≈2 cm/s) and Δg ≥ 0.8 mm with a relative error under 10%. However, the influence of the diffusive boundary layer thickness (δ) on DGT uptake of the analytes of interest needs to be corrected (Equation 2). Its thickness was calculated considering static conditions and boundary layer measurements for the analytes in flowing water (see Challis, 2018).

$$C_{DGT} = \frac{M_{DGT}(\Delta g + \delta)}{DAt} \qquad (2)$$

The standard o-DGT configuration used in this research was a 0.75-mm binding gel containing 25 mg Sepra™-ZT (30 µm, 85A, Phenomenex) and a 1-mm agarose diffusive gel without polyethersulfone (PES) membrane, considering the optimization of the method developed by Challis in 2018. All gels were prepared using 1.5% agarose (molecular biology grade, Sigma-Aldrich, Oakville, ON).

2.3.5 DGT sample processing and preparation for chemical analyses

DGT samples were prepared using a previously described method (Challis et al., 2016). The binding gels were transferred to 50-mL glass tubes with an addition of 50 µg/L internal standard (1

mg/L in methanol). A first extraction was conducted using approximately 3 mL of methanol, followed by a 2-min sonication. Using glass pipettes, all the liquid was removed, keeping the gel to a side, to a second tube. The gel was placed again on the bottom of the glass tube for conducting a second and third extraction following the same steps as indicated for the first. Combined extracts were concentrated to near dryness under a gentle stream of nitrogen gas, reconstituted in 1 mL of 50:50 MeOH: water (v/v), and filtered through a 0.45-µm polytetrafluoroethylene (PTFE) syringe filter (Pall Life Sciences) into amber LC vials. MeOH was treated in the same way as samplers to obtain procedural blank samples.

2.3.6 Instrumental analysis

Extracts were analyzed by use of a Vanquish UHPLC Liquid Chromatography system coupled to a QExactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer (Thermo Fisher Scientific). Liquid chromatography separation was achieved with a Kinetex 1.7 µm XB-C18 LC column (100 × 2.1 mm) (Phenomenex, Torrance, CA) by gradient elution with 95% water + 5% methanol (A) and 100% methanol (B), both containing 0.1% formic acid (Optima MS grade) at a flow rate of 0.2 mL min^{-1} and a column temperature of 40ºC. The gradient method started at 10% B, ramping linearly to 100% B over 7 min, where it was held for 1.5 min and returned to the starting conditions for column re-equilibration between 8.5 – 11 min.

Positive mode heated electrospray ionization was used to ionize the samples. The Q-Exactive Orbitrap method implemented the following the parameters indicated by Ji et al. (2022).

Calibration standards were placed before each batch of samples, followed by a blank run every ten samples and a 50-µg/L single calibration standard after running the whole batch of samples as a QA/QC protocol. For quantification purposes, a 7-point calibration curve from 0.01-500 µg/L and spiked with 50 µg/L internal standards were used for quantification by isotope dilution. All data acquisition and processing were conducted using Xcalibur v. 4.2 (Qual and Quan browser).

2.3.7 Calculation of sediment-water partitioning coefficient (Kd) and risk quotient (RQ)

The sediment-water partitioning coefficient (Kd) is an important parameter in the study of the sorption or mobility of contaminants between sediment and water (Bavumiragira & Yin, 2022). Kd for each pharmaceutical was determined by dividing concentrations of pharmaceuticals in sediments by that estimated for water, based on DGT results.

Risks associated with concentrations of pharmaceuticals in water were evaluated using the Sum of Toxic Units (STU) approach, combining effect of multi-component mixtures on overall toxicity rather than each chemical individually (Backhaus & Karlsson 2014). This approach incorporates pre-defined regulatory guidelines for risk assessment based on the European REACH (Registration, Evaluation, Authorization, and Restriction of Chemicals) regulation, as well as European Chemicals Agency (ECHA) and the US Food and Drug Administration (FDA) guidelines for the release of chemicals into surface waters. Since pharmaceuticals do not occur as individual substances in the effluents, this approach was selected as the most appropriate. STU were calculated

for each class of organism (algae, invertebrates, and fish) based on toxicity data available for each organism (Table 2.1, Equation 3).

$$STU = \sum \frac{MEC}{LC/EC50} \qquad (3)$$

Where MEC is the measured environmental concentration of each analyte, and LC/EC50 is the half-maximal effect concentration of the respective analyte in a given class of organisms. Minimum and maximum risk quotients (RQs) were then calculated by multiplying the minimum and maximum STUs for a given site and season with a safety factor of 1,000 to account for the potential effects of chronic toxicity, multiple stressors and mixture effects, and indirect ecological effects. RQs greater than 1.0 suggest adverse effects to exposed organisms due to exposure concentrations that exceed those expected to cause toxicity.

Table 2.1. Toxicity estimates (half-maximal lethal or effective concentrations, LC_{50}/EC_{50} selected for the calculation of toxic units in WWTP effluent for each class of organisms.

Compound	**Organism**	**LC/EC_{50} (mg/L)**	**Reference**
Amitriptyline	Algae	0.72	Minguez et al., 2016
	Invertebrates	4.82	Minguez et al., 2016
	Fish	320.00	Díaz-Garduño et al., 2017
Bupropion	Algae	4.50	Kosma et al., 2019
	Invertebrates	3.80	Kosma et al., 2019
	Fish	33.00	Kosma et al., 2019
Carbamazepine	Algae	31.60	Backhaus et al., 2014
	Invertebrates	3.76	Backhaus et al., 2014
	Fish	35.40	Backhaus et al., 2014
Clozapine	Algae	3.13	Minguez et al., 2016
	Invertebrates	8.63	Minguez et al., 2016
	Fish	26.00	Kosma et al., 2019
Fluoxetine	Algae	0.02	Kosma et al., 2019
	Invertebrates	0.17	Kosma et al., 2019
	Fish	0.20	Kosma et al., 2019
Lamotrigine	Algae	37.90	GlaxoSmithKline 2004
	Invertebrates	56.00	GlaxoSmithKline 2004
	Fish	85.00	GlaxoSmithKline 2004
Venlafaxine	Algae	47.80	Minguez et al., 2016
	Invertebrates	141.28	Minguez et al., 2016
	Fish	0.30	Thompson et al., 2020

2.3.8 Statistical analysis

Various statistical methods were used to process results from field and lab measurements of pharmaceuticals and to facilitate data interpretation. Physicochemical parameters were evaluated descriptively. Concentrations obtained from conventional samples of water and sediments were first analyzed descriptively and later through a multi-level model, which seeks to simulate processes that vary on more than one level; That is, data are assumed to have a hierarchical or nested structure, which allow residual components at each level. The full multi-level regression model assumes that there is a hierarchical data set, with a single dependent variable and explanatory variables that exist at various levels. Conceptually, the model can be viewed as a hierarchical system of regression equations (Raudenbush and Bryk, 2002).

The results obtained using DGT devices were analyzed in the same way through a multi-level model that incorporated the responses of water, sediments, and physicochemical parameters in the developed model. Comparisons were conducted considering each pharmaceutical (individually), sites (WC and SSR), time (spring, summer, fall), and location (upstream and downstream of WWTPs) through pairwise comparisons using t-tests with pooled standard deviation with Bonferroni correction (Laird & Ware, 1982; Verbeke et al., 2010). The analyses were performed using package *lme4* in the language R (Bates et al., 2022; CRAN - Package lme4 (r-project.org)).

2.4 Results and discussion

2.4.1 Physicochemical parameters

Changes in environmental parameters and physicochemical variations in aquatic environments are considered factors that can influence the presence, as well as temporal and spatial dynamics of emerging contaminants, such as pharmaceuticals. The results obtained in this study showed distinct differences in temperatures of water between upstream and downstream sites across both WWTPs (Figures B.1 and B.2). On the SSR, an increase in temperature between up- and downstream sites was observed in spring and fall, and a decrease was observed during summer. It should be noted that the cooling water discharge of the Queen Elisabeth Natural Gas Power Station with a capacity of 623 megawatts is located along the same stretch of the river, potentially affecting temperatures of water. Conversely, temperatures in WC decreased between upstream and downstream sites during both spring and summer and increased slightly in the fall. Overall, pH was greater in the SSR compared to WC (Figure B.1 and B.2), and upstream pHs on the SSR were consistently less than downstream. In contrast, pH values are greater upstream than downstream in WC, except for samples obtained during summer. These variations might be caused by discharges from the treatment plants and their interactions with seasonal water quality characteristics, which may modify the behavior of pharmaceutical compounds and their effect on the aquatic environments (Bijlsma et al., 2021; Comber et al., 2020; He et al., 2018; Lei et al., 2019; Wells, 2006).

Concentrations of DO in the SSR were constant within the three seasons. In the SSR, results were always less upstream than downstream, while in WC, the values varied among seasons (Figures B.1 and B.2). In the fall, concentrations of dissolved oxygen were greater compared to spring and summer for the upstream site, while for downstream, the greatest concentration of DO was observed in spring. The results obtained for the SSR might be related to a weir in downtown Saskatoon, which causes turbulence and thus reoxygenates water. In WC, DO in the summer decreased to concerning hypoxic conditions less than 50% oxygen saturation that might become a risk to local fish and invertebrate species (Maccallum et al., 2019). Due to higher concentrations of organic matter, it is common for concentrations of DO below WWTP outfalls to be less (Barrenha et al., 2018; Maccallum et al., 2019).

SC and total dissolved solids (TDS) were consistently greater in WC compared to the SSR, and values increased for both rivers between upstream and downstream sites on average by 119% (Figure B.1 and B.2). These findings indicate an increase in salt concentrations, including nutrients and other chemical compounds. Similar studies have evaluated variations in water quality, reporting an increase downstream compared to upstream, with increases in total suspended solids, TDS, nitrate and nitrite, and SC ranging from 18.7% to 60.3% downstream of the WWTPs in Bayou Lacassine in Louisiana, the United States (Poudel et al., 2020).

Monitoring physicochemical parameters is useful for evaluating overall water quality. Previous studies have assessed how variations in physicochemical parameters can lead to changes in the

behavior of pollutants (Sierra, 2017; Smith and Jeong, 2021). These changes include variations in the sediment-water partition coefficients (Chen and Lin, 2016), as well as in the ability of pollutants to spread and be detectable in the environment (Lis et al., 2019) or in the degree to which they can cause toxicity in exposed organisms (Sierra et al., 2017). Therefore, it is important to consider the concentrations of contaminants relative to these physicochemical parameters.

2.4.2 Chemical concentrations in water samples (grab samples and DGT extracts)

Six out of seven target analytes were detected by both conventional grab samples of water and extracts of DGT samplers. CBZ was detected in the majority of the samples, however in SSR for fall season, it was only detected by DGT (Figures 2.1 and 2.2). Overall, the following order of abundances was found from the greatest to least concentrations in water samples by conventional grab from SSR was LAM > VEN > CBZ > AMI > FLX > BUP > CLO, ranged from 1.79 ng/L to 92.48 ng/L, meanwhile in WC was AMI > VEN > LAM > CBZ > BUP > CLO > FLX, ranged from 2.07 ng/L to 1,118.64 ng/L. DGT analysis of pharmaceuticals showed the following order of abundance for SSR, LAM > AMI > VEN > CBZ > CLO > FLX > BUP, ranged between 3.40 ng/L and 75.59 ng/L, for WC the order was the same as the obtained for water samples by conventional grab, ranged between 4.09 ng/L and 2,137.51 ng/L. Overall, concentrations of pharmaceuticals were detected in water samples by conventional grab and passive samplers in both sites and across the three seasons.

The sampling location and time of the fieldwork for monitoring are determining factors in the presence, absence, and/or variations of pharmaceuticals in samples. In the applied multilevel model concentrations varied significantly (p <0.05) among compounds, therefore individual multilevel models were developed for each compound. Concentrations of CBZ and LAM in water varied considerably, which could be attributed to the site (SSR and WC), season (spring, summer, and fall), and location from the WWTP (upstream and downstream). Instead for AMI and BUP, the multilevel model based on the sites (WC and SSR), time (spring, summer, and fall), and location (upstream and downstream of the WWTPs) and the physicochemical parameters are showing that only the time and the site were significant. In the same way, the multilevel analysis conducted for CLO with the same variables showed that the time and site were significant to explain the changes in the concentration of pharmaceuticals. Meanwhile, the site and the location were significant for VEN. None of the factors were significant determinants of concentrations for FLX.

Correlations between environmental factors and concentrations of pharmaceuticals were evaluated to understand their contributions to the variability of results. Nevertheless, it was not found any correlation between the physicochemical parameter (temperature, pH, SC, DO, TDS) evaluated individually with each one of the pharmaceuticals. Even though the physicochemical parameters evaluated individually through the multilevel model did not show any correlation with the pharmaceuticals, the parameters were evaluated in groups including the site (WC and SSR), the time (spring, summer, fall), and locations (upstream and downstream of WWTPs) showing

variations in the concentration of pharmaceuticals. The use of parameters as a group instead of evaluating the parameters individually improved the approach of the multilevel model used, this is supported by a better coefficient of Akaike in comparison with models where these parameters are not included. For example, considering the results for DGT, temperature was positively correlated with concentrations of pharmaceuticals but the concentration of DO was negatively correlated with temperature. Results of the simulation model, together with other variables like the site (SSR or WC), the time (spring, summer, or fall), and the location from the WWTP (upstream or downstream), explained between 71.8% and 93.1% of the variability of the concentrations depending on the pharmaceutical. Similar patterns were reported previously (Burns *et al.,* 2018) where it was found that temporal and spatial differences explained variations in concentrations of pharmaceuticals in wastewater and river water in the United Kingdom. Previous studies have indicated the correlation between pH and temperature as two environmental factors of water and concentrations of pharmaceuticals (Conley et al., 2008; Lis et al., 2019). Similarly for this study, pH and temperature may explain the results obtained for CBZ in SSR, which was not detectable in fall.

Seasonal variations of pharmaceuticals were also influenced by seasonal variations, even though the providence of Saskatchewan had an abnormally dry year in 2021. In the SSR, lesser concentrations of pharmaceuticals (except FLX) were found in the samples collected during summer 2021, in comparison to spring 2021. Conversely, there were greater concentrations of

pharmaceuticals, except CLO and FLX, during fall 2021, compared to summer 2021. All of the above suggests that not only temperature variations will affect concentrations of pharmaceuticals but also environmental factors. In WC, lesser concentrations of AMI, BUP, and CBZ were observed during summer, 2021 compared to spring, 2021. Meanwhile, greater concentrations of pharmaceuticals, except CLO and FLX, were observed during fall, 2021 compared to spring, 2021. According to the results obtained, dilution is another aspect influencing the changes in concentrations of pharmaceuticals during the three seasons evaluated in 2021. Similar trends have been reported previously, indicating that the increase of dilution effect in chemical concentrations caused by water levels, irrigation, rain, and snow melting (Huber et al., 2016; Afonso-Olivares et al., 2017; Bertucci et al., 2018; Charuaud et al., 2019; Wang et al., 2019; Nyaga et al., 2020).

The dilution factor was also considered for evaluating the results for the SSR and WC. Greater concentrations of pharmaceuticals were observed in WC compared to SSR; the average discharge of the SSR was 925 m^3/s (Prajapati et al., 2021), which was 1000-fold greater than that in WC, which had a mean, annual discharge of only 0.9 m^3/s (Hanson et al., 2021). Consequently, variations in discharge can be related to the greater concentrations of pharmaceuticals in WC compared to SSR. For example, the mean concentration of CBZ in SSR was 14.7 ng/L, while in WC it was 128 ng/L. Water flow is related to the concentrations of pharmaceuticals, considering the volume of discharge at each site, for SSR, the compound would be passing at a concentration of 1,177.63 g/day, while for WC would be 9,953.3 g/day.

2.4.3 Concentrations in sediments

Sediment is another important component of aquatic ecosystems that was considered for chemical analysis. Sediments can act as a sink for retention and storage of emerging contaminants, reinforcing the concerns and necessity for determining the toxicological risks in aquatic ecosystems (Petrie et al., 2015; Muz et al., 2020). Similar to collections of water samples, AMI was the most abundant compound in sediment samples at the four sampling sites and during the three seasons. The following order of abundance was found from the greatest to least concentrations in sediments: AMI > CLO > LAM > CBZ > BUP > FLX > VEN. Overall, concentrations of pharmaceuticals in sediments makes them a critical environmental compartment with concentrations of most of the compounds above critical levels i.e., the predicted water concentrations that would elevate the plasma concentration in exposed fish to a level equal to the human therapeutic plasma concentration (Fick et al., 2010).

Evaluations of pharmaceuticals in sediments indicated significant differences in the mixed models for the variability among concentrations, related to location, temperature, and DO. Also, "site" was significant for most pharmaceuticals, except for CLO. Only time was significant for AMI and BUP. After comparing the results of the sediments from both sites, SSR and WC, it was observed that under all conditions and sampling times, concentrations were greater in WC (Figure 2.1 and 2.2).

Concentrations of CBZ in sediments were positively correlated with temperature and SC, but negatively correlated with pH, dissolved oxygen, and total dissolved solids. Previous investigations have indicated that adsorption is affected by the diffusion processes of the compounds, which is related to the temperature of the medium (Ten Hulscher and Cornelissen, 1996; Česen et al., 2018; Pietrzak et al., 2020). Similarly, physicochemical parameters also influenced this variability for CLO, as well as the concentrations determined by conventional grab samples in water and sediments (a positive relationship with temperature and total dissolved solids, and the rest being negative). When performing the means tests, carried out by the presence of a relationship with multiple parameters in the evaluated models, a difference was observed (p <0.05) between upstream and downstream for most of the cases (Figures 2.1 and 2.2).

Figure 2.1 Concentrations of pharmaceuticals in water (conventional grab), DGT, and sediments, as well as sediment-water distribution coefficients (Kd) based on sediment and DGT concentrations, collected in the South Saskatchewan River near Saskatoon, Saskatchewan, Canada. Note logarithmic scaling of the y-axes of the graphs (Upstream = data in red, Downstream = data in blue)

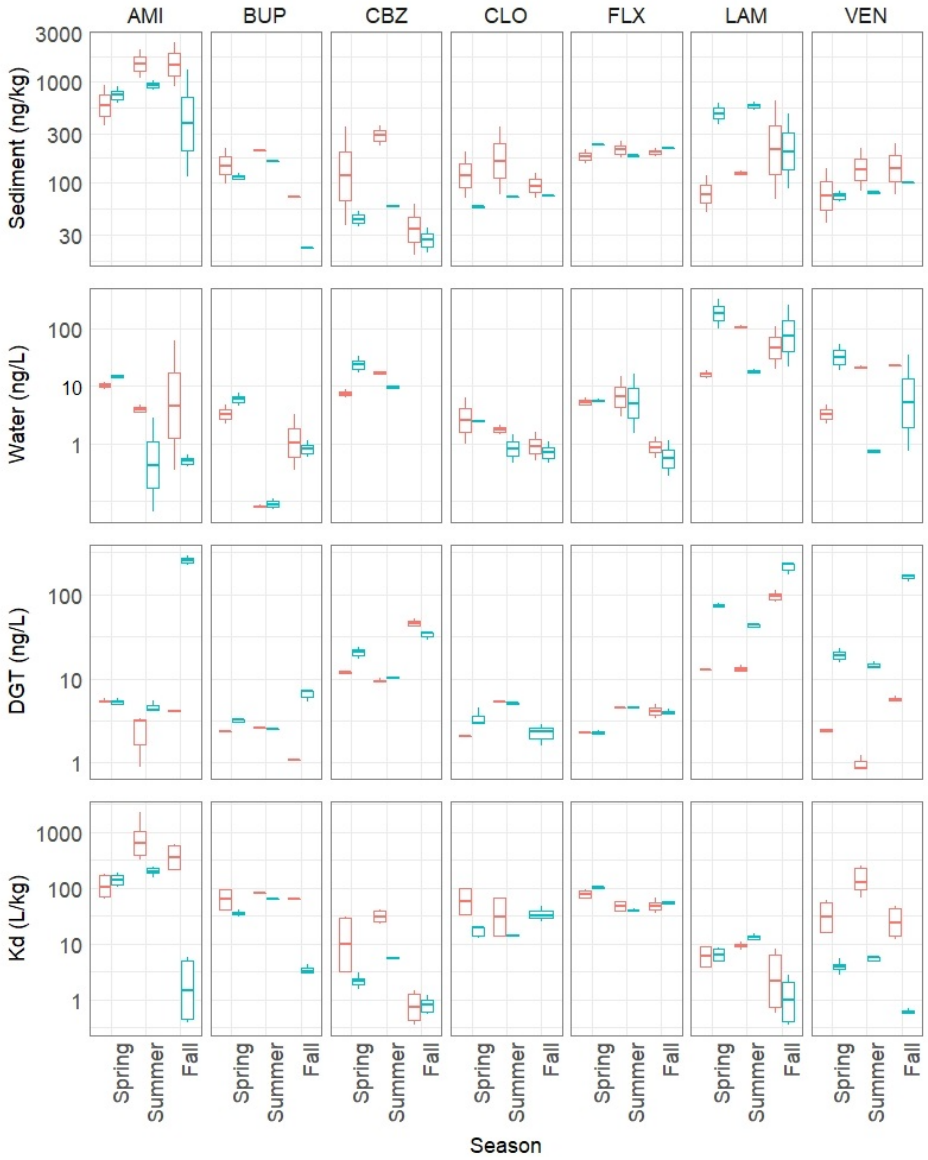
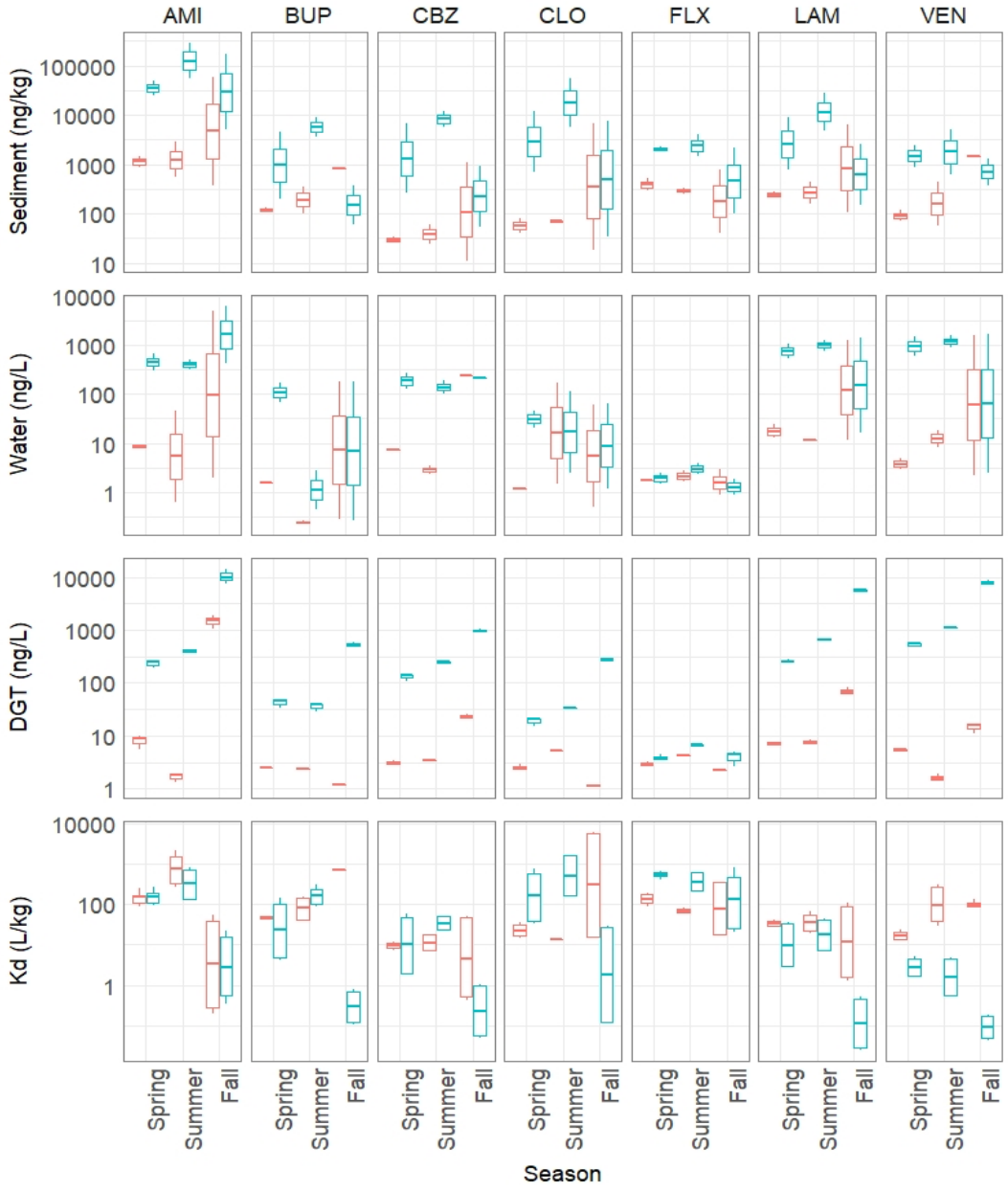


Figure 2.2 Concentrations of pharmaceuticals in water (conventional grab), DGT, and sediments, as well as sediment-water distribution coefficients (Kd) based on sediment and DGT concentrations, collected in Wascana Creek near Regina, Saskatchewan, Canada. Note logarithmic scaling of the y-axes of the graphs (Upstream = data in red, Downstream = data in blue).



2.4.4. Sediment-water partitioning

The sediment-water distribution coefficient (K_d) is a parameter that describes the partitioning of chemicals between water and sediment matrix in an aquatic system. The apparent K_d observed under field conditions is driven by properties of the sediment, as well as properties of the chemical, such as its lipophilicity (Hörsing et al., 2011). It also allows for estimation of the hysteresis of sediments, once a contaminant source has been removed from the overlying water. For example, lesser values of K_d (*log* K_d<2) are indicative of more mobile chemicals, that would be expected to be found mainly in the aqueous phase, while those with greater values of K_d (*log* K_d>2) are more strongly adsorbed to sediments and only have potential to become remobilized when sediments are disturbed (Müller et al., 2021). In the SSR, all analytes exhibited *log* K_d values of less than 2.0 in spring, while those of AMI and BUP were greater than 2.0 during summer, as well as AMI and FLX during fall 2021 (Figures 2.1 and 2.2). In WC, *log* K_d exceeded 2 for AMI during spring and summer, BUP in summer, and FLX in all seasons of 2021. Information related to K_d for pharmaceuticals in the literature is generally limited; however, K_d values obtained in the present study for CBZ (0.98-64.2 L/kg) were similar to or slightly less than those reported in the literature (10.0-50.7 L/kg), while those reported for LAM and VEN were greater here (0.70-38.1 L/kg and 0.59-151 L/kg, respectively) compared to those reported in the literature (6.91-20.0 L/kg and 437-1,260 L/kg, respectively) (Golovko et al., 2020; Koba et al., 2018a, 2018b). Since these contaminants evidently originate from WWTP effluents, these findings indicate that they have

potential to remain abundant in the receiving water bodies for extended periods of time even if their emission should eventually cease due to the installation of advanced treatment facilities (Golovko et al., 2020).

2.4.5. Comparison of pharmaceutical concentrations with findings from previous studies

Data collected during previous studies were compared with the results obtained in this study. Concentrations of pharmaceuticals in water, excluding FLX and CLO, were greater than those reported by other authors (Table B.1). Also, AMI and BUP presented values above critical levels reported by Fick et al. (2010).

Previous publications include different types of passive samplers for analysis of pharmaceuticals. The results obtained in this study were compared with various types of passive samplers, including polar organic chemical integrative samplers (POCIS), DGT, and a combination of different configurations of samplers. Overall, most concentrations of pharmaceuticals were greater than those reported for other locations by at least one of the references reviewed.

As has been demonstrated for antiallergic drugs, consumption rates of these pharmaceuticals tend to change depending on the season of the year (Philip et al., 2002; Häder et al., 2020; Muz et al., 2020). It is reasonable to expect that at least a subset of antipsychotic drugs might also show seasonal trends that mirror prescription numbers (Kurian et al., 2007; Kamble et al., 2015).

2.4.6. Risk assessment

The SSR and WC are aquatic ecosystems with a diversity of species, including fish, invertebrates, and algae. Organisms downstream are exposed to chemicals present in WWTP discharge. Consequently, a screening-level hazard assessment (US EPA, 1997) was conducted to establish potential risks to aquatic species related to the combined concentrations of the measured analytes (Table 2.2). Here, we applied a simple STU approach, which follows a concentration addition mixture model, which is generally adequate for chemicals that exert their biological effects through similar modes of action (Backhaus & Karsson, 2014).

Hazard quotients (HQs) calculated for downstream of the Saskatoon WWTP did not exceed 1 for any organisms group and sampling season, indicating that the levels of the measured analytes were well below known toxicity thresholds in aquatic organisms. Downstream of the Regina WWTP, however, HQs exceeded the threshold level of 1 for all classes of organisms and all sampling seasons, indicating that the levels of pharmaceuticals were sufficiently high to be considered a toxicological risk to the receiving aquatic ecosystem. The most sensitive group was fish, with a maximum HQ of 26.8. AMI was the pharmaceutical with the greatest toxic units in algae and invertebrates and VEN in fish.

Table 2.2. Mixture toxicity assessment based on measured pharmaceutical concentrations (MEC) at the downstream site and half-maximal lethal or effective concentration (LC50/EC50) data for freshwater organisms (algae, invertebrates, fish). Toxic units (TUs) were calculated for each group of organisms and pharmaceutical and then summed to obtain one sum of toxic units (STU) per organism group per sampling season and location. Minimum and maximum hazard quotients (HQs) for each group were determined by selecting the minimum and maximum STU out of the three sampling seasons and applying a safety factor of 1,000.

Compound	**Season**	**Algae**	**Invertebrates**	**Fish**
Saskatoon				
STU	Spring	1.07E-04	2.28E-05	7.67E-05
	Summer	1.97E-04	3.24E-05	7.29E-05
	Fall	5.34E-04	9.13E-05	5.61E-04
RQ STU min	–	0.11	0.02	0.07
RQ STU max	–	0.53	0.09	0.56
Regina				
STU	Spring	5.27E-04	1.29E-04	1.80E-03
	Summer	8.99E-04	2.21E-04	3.85E-03
	Fall	1.55E-02	2.80E-03	2.68E-02
RQ STU min	–	0.53	0.13	1.80
RQ STU max	–	15.5	2.8	26.8

2.5 Conclusions

Increase of human and animal population are leading to increase of pharmaceuticals for prevention or treatment of diseases (Waiser et al., 2011a, 2011b; Patel et al., 2019; Bavumiragira et al., 2022). Due to COVID-19 pandemic and the subsequent lockdowns, there is an increase in mental health issues supported by the increase in treatments using antipsychotic medications (Rossi et al., 2020; Townsend et al., 2022). Pharmaceuticals are under not yet regulated Canada's Wastewater Systems Effluent Regulations and class of emerging contaminants that treatment plants across Canada have started to evaluate due to the growing public concern. The City of Saskatoon's and Regina's WWP's are part of the treatment and monitoring of traditional contaminants including organic matter, bacteria, nutrients, etc. However, there was a gap in understanding of pharmaceuticals in wastewater and their impacts. To our best knowledge, this study was the first to comprehensively investigate the occurrence and fate of pharmaceuticals upstream and downstream of WWTPs in Saskatchewan. The data obtained on the distribution of pharmaceuticals in the SSR and WC indicates exposure of aquatic organisms to mixtures of pharmaceuticals at concentrations in the ng/L to μg/L range. Differences were observed in the concentration of the evaluated compounds between WC and the SSR, with higher values in WC for most of the pharmaceuticals. Amitriptyline was the compound with the greatest concentration out of all seven monitored pharmaceuticals in water and sediment from the four sampling sites and across the three seasons.

Maximum concentrations of pharmaceuticals detected in our study were several orders of magnitude greater than those measured in comparable studies at other locations.

Currently, studies considering the mobility of pharmaceuticals in the environment are quite limited (Miller et al., 2018; Obeiro et al., 2019). Our investigation provides insights into the distribution coefficient (Kd) values trends obtained on the sorption of pharmaceuticals as a key parameter not only for environmental fate of contaminants but also transport processes such as bioavailability and degradation rates. In this study, Kd results indicated the potential that the pharmaceuticals detected have to persist in the water bodies for long periods of time. This complex approach that included water and sediment interactions with environmental factors can help to better understand the fate of pharmaceuticals and their effects on the aquatic systems (Koba et al., 2018a, 2018b; Zhou et al., 2019)

Giving the results of the chemical analysis, we decided to conduct a screening-level risk assessment to evaluate the potential toxicological risk to aquatic life in the SSR and WC. Pharmaceutical concentrations detected downstream WC, for all classes of organisms and all sampling seasons indicated immediate toxicological risks. The data supported the significance of conducting an integrative risk assessment including species from different trophic levels of the environmental systems (USEPA, 2002) and showed that water resource management should give priority to high-risk pharmaceuticals.

The compounds evaluated are not the only pharmaceutical products present, nor are pharmaceutical products the only ecotoxicologically relevant compounds present (Backhaus and Karlsson, 2014), this shows the importance of any study regarding the review of their behavior in terms of seasonal trends and the effect of treatment plants on them, which allow revealing strategies for managing them in all tributaries worldwide, especially due to the increasing needs of drinking water supplies and improving of drinking water quality (Escudero et al., 2021).

The data obtained will be the baseline for further studies on the possible effects that they can generate on aquatic organisms, and the present food chain, which ultimately could reach food consumed by people (Hanson et al.., 2021; Tetreault et al., 2012; Escudero et al., 2021)

References

Afonso-Olivares, C., Sosa-Ferrera, Z., & Santana-Rodríguez, J. J. (2017). Occurrence and environmental impact of pharmaceutical residues from conventional and natural wastewater treatment plants in Gran Canaria (Spain). *Science of the total environment*, 599, 934-943. http://dx.doi.org/10.1016/j.scitotenv.2017.05.058

Akomeah, Eric, and University of Saskatchewan, College of Graduate Studies Research. Water quality modelling of The Qu'appelle River Basin for long-term management (2021). Website: https://harvest.usask.ca/handle/10388/13260.

Alygizakis, N. A., Urik, J., Beretsou, V. G., Kampouris, I., Galani, A., Oswaldova, M., Berendok, T., Oswald, P., Thomaidis, N., Slobodnik, J., Vrana, B., & Fatta-Kassinos, D. (2020). Evaluation of chemical and biological contaminants of emerging concern in treated wastewater intended for agricultural reuse. *Environment International, 138*, 105597. https://doi.org/10.1016/j.envint.2020.105597

Backhaus, T., & Karlsson, M. (2014). Screening level mixture risk assessment of pharmaceuticals in STP effluents. Water research, 49, 157-165. https://doi.org/10.1016/j.watres.2013.11.005

Barrenha, P. I. I., Tanaka, M. O., Hanai, F. Y., Pantano, G., Moraes, G. H., Xavier, C., Awan, A. T., Grosseli, G. M., Fadini, P. S., & Mozeto, A. A.. (2018). Multivariate analyses of the effect of an urban wastewater treatment plant on spatial and temporal variation of water quality and

nutrient distribution of a tropical mid-order river. Environmental Monitoring and Assessment, 190(1). https://doi.org/10.1007/s10661-017-6386-4

Bates, D., Maechler, M., Bolker, B., Walker, S., Bojesen Christensen R.H., Singmann, H., Dai, B., Scheipl, F., Grothendieck, G., Green, P., Fox, J., Bauer, A., Krivitsky, P.N. (2022). Package 'lme4' Linear Mixed-Effects Models using 'Eigen' and S4. R package version 1.1-29. CRAN - Package lme4 (r-project.org).

Bavumiragira, J. P., & Yin, H. (2022). Fate and transport of pharmaceuticals in water systems: A processes review. *Science of The Total Environment*, 153635.

Bertucci, A., Pierron, F., Gourves, P. Y., Klopp, C., Lagarde, G., Pereto, C., ... & Baudrimont, M. (2018). Whole-transcriptome response to wastewater treatment plant and stormwater effluents in the Asian clam, Corbicula fluminea. *Ecotoxicology and Environmental Safety*, 165, 96-106. doi: 10.1016/j.ecoenv.2018.08.090

Bijlsma, L., Pitarch, E., Fonseca, E., Ibanez, M., Botero, A. M., Claros, J., ... & Hernandez, F. (2021). Investigation of pharmaceuticals in a conventional wastewater treatment plant: Removal efficiency, seasonal variation and impact of a nearby hospital. *Journal of Environmental Chemical Engineering, 9*(4), 105548. https://doi.org/10.1016/j.jece.2021.105548

Boxall, A. B. A., Rudd, M. A., Brooks, B. W., Caldwell, D. J., Choi, K., Hickmann, S., Van Der Kraak, G. (2012). Pharmaceuticals and Personal Care Products in the environment: What Are

the Big Questions? Environmental Health Perspectives, 120(9), 1221-1229. doi:10.1289/ehp.1104477

Brady, S. P., Richardson, J. L., & Kunz, B. K. (2017). Incorporating evolutionary insights to improve ecotoxicology for freshwater species. *Evolutionary Applications, 10*(8), 829–838. doi:10.1111/eva.12507

Bunting, S. Y., Lapworth, D. J., Crane, E. J., Grima-Olmedo, J., Koroša, A., Kuczyńska, A., & Lopez, B. (2021). Emerging organic compounds in Europeangroundwater. *Environmental Pollution*, 269, 115945.https://doi.org/10.1016/j.envpol.2020.115945

Burns, E. E., Carter, L. J., Kolpin, D. W., Thomas-Oates, J., & Boxall, A. B. A.. (2018).Temporal and spatial variation in pharmaceutical concentrations in an urban river system. *Water Research,* 137, 72–85. https://doi.org/10.1016/j.watres.2018.02.066

Caban, M., Lis, H., & Stepnowski, P. (2021). Limitations of Integrative Passive Samplers as a Tool for the Quantification of Pharmaceuticals in the Environment–A Critical Review with the Latest Innovations. Critical Reviews in Analytical Chemistry, 1-40.https://doi.org/10.1080/10408347.2021.1881755

Caldas, S.S., Rombaldi, C., de Oliveira, A.J.L., Cardoso,M.L., Primel, E.G., 2016. Multi-residue method for determination of 58 pesticides, pharmaceuticals and personal care products in water using solvent demulsification dispersive liquid–liquid microextraction combined with

liquid chromatography-tandem mass spectrometry. *Talanta* 146, 676–688. https://doi.org/10.1016/j.talanta.2015.06.047.

Cardoso-Vera, J. D., Elizalde-Velázquez, G. A., Islas-Flores, H., Mejía-García, A., Ortega-Olvera, J. M., & Gómez-Oliván, L. M. (2021). A review of antiepileptic drugs: Part 1 occurrence, fate in aquatic environments and removal during different treatment technologies. *Science of The Total Environment*, 768, 145487. doi:10.1016/j.scitotenv.2021.145487.

Česen, M., Heath, D., Krivec, M., Košmrlj, J., Kosjek, T., & Heath, E. (2018). Seasonal and spatial variations in the occurrence, mass loadings and removal of compounds of emerging concern in the Slovene aqueous environment and environmental risk assessment. Environmental pollution, 242, 143-154.

Challis, J. K., Hanson, M. L., & Wong, C. S. (2016). Development and calibration of an organic-diffusive gradients in thin films aquatic passive sampler for a diverse suite of polar organic contaminants. *Analytical chemistry, 88*(21), 10583-10591.

Challis, J. K. (2018). Development and evaluation of passive sampling devices to characterize the sources, occurrence, and fate of polar organic contaminants in aquatic systems. (Doctor in Philosophy). University of Manitoba, Department of Chemistry.

Challis, J. K., Almirall, X. O., Helm, P. A., & Wong, C. S. (2020). Performance of the organic-diffusive gradients in thin-films passive sampler for measurement of target and suspect

wastewater contaminants. *Environmental Pollution, 261*, 114092. https://doi.org/10.1016/j.envpol.2020.114092

Charuaud, L., Jardé, E., Jaffrézic, A., Liotaud, M., Goyat, Q., Mercier, F., & Le Bot, B. (2019). Veterinary pharmaceutical residues in water resources and tap water in an intensive husbandry area in France. *Science of the Total Environment*, 664, 605-615. https://doi.org/10.1016/j.scitotenv.2019.01.303

Chen, C. S., & Lin, S. T. (2016). Prediction of pH effect on the octanol–water partition coefficient of ionizable pharmaceuticals. *Industrial & Engineering Chemistry Research, 55*(34), 9284-9294.https://doi.org/10.1021/acs.iecr.6b02040

Chen, W., Pan, S., Cheng, H., Sweetman, A. J., Zhang, H., & Jones, K. C.. (2018). Diffusive gradients in thin-films (DGT) for in situ sampling of selected endocrine disrupting chemicals (EDCs) in waters. Water Research, 137, 211–219. https://doi.org/10.1016/j.watres.2018.03.029

Comber, S. D. W., Gardner, M. J., & Ellor, B.. (2020). Seasonal variation of contaminant concentrations in wastewater treatment works effluents and river waters. *Environmental Technology, 41*(21), 2716–2730. https://doi.org/10.1080/09593330.2019.1579872

Conley, J. M., Symes, S. J., Schorr, M. S., & Richards, S. M. (2008). Spatial and temporal analysis of pharmaceutical concentrations in the upper Tennessee River basin. *Chemosphere, 73*(8), 1178-1187.https://doi.org/10.1016/j.chemosphere.2008.07.062

Costa, A., Schaider, L., Phillips, P., Kolpin, D., Furlong, E., Alvarez, D., Lohmann, R., Becanova, J., Gardiner, Ch., Robuck, A. & Tokranov, A. (2017). *Evaluating land use impacts on contaminants of emerging concern in Cape Cod Bay estuaries.* Commonwealth of Massachusetts. https://www.mass.gov/service-details/evaluating-land-use-impacts-on-contaminants-of-emerging-concern-in-cape-cod-bay-estuaries.

Davison, W., & Zhang, H. (2012). Progress in understanding the use of diffusive gradients in thin films (DGT)–back to basics. Environmental Chemistry, 9(1), 1-13.

Díaz-Garduño, B., Pintado-Herrera, M. G., Biel-Maeso, M., Rueda-Márquez, J. J., Lara-Martín, P. A., Perales, J. A., ... & Martín-Díaz, M. L. (2017). Environmental risk assessment of effluents as a whole emerging contaminant: Efficiency of alternative tertiary treatments for wastewater depuration. *Water research*, 119, 136-149.

Eggen, R., Hollender, J., Joss, A., 2014. Reducing the discharge of micropollutants in the aquatic environment: the benefits of upgrading wastewater treatment plants. Environ. Sci. Technol. 48 (14), 7683–7689. https://doi.org/10.1021/es500907n.

Escher, B. I., Stapleton, H. M., & Schymanski, E. L. (2020). Tracking complex mixtures of chemicals in our changing environment. *Science, 367*(6476), 388–392. doi:10.1126/science.aay6636

Escudero, J., Muñoz, J. L., Morera-Herreras, T., Hernandez, R., Medrano, J., Domingo-Echaburu, S., ... & Lertxundi, U. (2021). Antipsychotics as environmental pollutants: An underrated threat?. *Science of The Total Environment*, 769, 144634.

Fernandes, M. J., Paíga, P., Silva, A., Llaguno, C. P., Carvalho, M., Vázquez, F. M., & Delerue-Matos, C. (2019). Antibiotics and antidepressants occurrence in surface waters and sediments collected in the north of Portugal. *Chemosphere,* 124729. doi:10.1016/j.chemosphere.2019.12

Fick, J., Lindberg, R. H., Tysklind, M., & Larsson, D. J. (2010). Predicted critical environmental concentrations for 500 pharmaceuticals. Regulatory Toxicology and Pharmacology, 58(3), 516-523. https://doi.org/10.1016/j.yrtph.2010.08.025

Geissen, V., Mol, H., Klumpp, E., Umlauf, G., Nadal, M., Van Der Ploeg, M., Van De Zee, S. E. A. T. M., & Ritsema, C. J. (2015). Emerging pollutants in the environment: A challenge for water resource management. *International Soil and Water Conservation Research, 3*(1), 57–65. https://doi.org/10.1016/j.iswcr.2015.03.002

Golovko, O., Rehrl, A.-L., Köhler, S., & Ahrens, L. (2020). Organic micropollutants in water and sediment from Lake Mälaren, Sweden. *Chemosphere,* 258, 127293. doi:10.1016/j.chemosphere.2020.127293

Gonzalez-Rey, M., Tapie, N., Le Menach, K., Devier, M. H., Budzinski, H., & Bebianno, M. J. (2015). Occurrence of pharmaceutical compounds and pesticides in aquatic systems. *Marine Pollution Bulletin, 96*(1-2), 384-400. https://doi.org/10.1016/j.marpolbul.2015.04.029

Gong, X., Li, K., Wu, C., Wang, L., & Sun, H. (2018). Passive sampling for monitoring polar organic pollutants in water by three typical samplers. Trends in Environmental Analytical Chemistry, 17, 23-33.

Gould, S. L., Winter, M. J., Norton, W. H. J., & Tyler, C. R.. (2021). The potential for adverse effects in fish exposed to antidepressants in the aquatic environment. *Environmental Science & Technology, 55*(24), 16299–16312. https://doi.org/10.1021/acs.est.1c04724

GSK - GlaxoSmithKline plc, 03-0200/C, 2004. Lamotrigine: Toxicity to the Green Alga Selenastrum capricornutum. https://www.fass.se/LIF/product;jsessionid=4UuuPSHtxwL47TjAg15JCdH1M9H1RB6lOkHZYqXPVG639QD9Znz!1388213712?-1.ILinkListener-documentTabPanel-tabs-panel-article~tools~bottom-articletoolsprintlink&userType=0&nplId=19940128000088&docType=78&docTypeDynTab=78&autoScroll=true&scrollPosition=528. (Accessed 23 August 2022).

Hanson, S. (2019). *Reproductive health assessment of fathead minnow (Pimephales promelas) populations inhabiting an effluent dominated stream, Wascana Creek, SK*. (Master in Environment and Sustainability). University of Saskatchewan, College of Graduate Studies and Research.

Hanson, S., Steeves, K., Bagatim, T., Hogan, N., Wiseman, S., Hontela, A., Giesy, J., Paul, J., & Hecker, M. (2021). Health status of fathead minnow (Pimephales promelas) populations in a

municipal wastewater effluent-dominated stream in the Canadian prairies, Wascana Creek, Saskatchewan. Aquatic Toxicology, 238, 105933.

Häder, D. P., Banaszak, A. T., Villafañe, V. E., Narvarte, M. A., González, R. A., & Helbling, E. W. (2020). Anthropogenic pollution of aquatic ecosystems: Emerging problems with global implications. *Science of the Total environment*, 713, 136586. https://doi.org/10.1016/j.scitotenv.2020.136586

He, S., Dong, D., Zhang, X., Sun, C., Wang, C., Hua, X., Zhang, L., & Guo, Z.. (2018). Occurrence and ecological risk assessment of 22 emerging contaminants in the Jilin Songhua River (Northeast China). *Environmental Science and Pollution Research*, *25*(24), 24003–24012. https://doi.org/10.1007/s11356-018-2459-3

Hörsing, M., Ledin, A., Grabic, R., Fick, J., Tysklind, M., la Cour Jansen, J., & Andersen, H. R. (2011). Determination of sorption of seventy-five pharmaceuticals in sewage sludge. *Water research,* 45(15), 4470-4482. https://doi.org/10.1016/j.watres.2011.05.033.

Huber, M., Welker, A., & Helmreich, B. (2016). Critical review of heavy metal pollution of traffic area runoff: Occurrence, influencing factors, and partitioning. Science of the Total *Environment*, 541, 895-919. doi: 10.1016/j.scitotenv.2015.09.033

Ji, X., Challis, J. K., Cantin, J., Perez, A. S. C., Gong, Y., Giesy, J. P., & Brinkmann, M. (2022). Desorption kinetics of antipsychotic drugs from sandy sediments by diffusive gradients in

thin-films technique. Science of The Total Environment, 155104. https://doi.org/10.1016/j.scitotenv.2022.155104

Kamble, P., Chen, H., Johnson, M. L., Bhatara, V., & Aparasu, R. R. (2015). Concurrent Use of Stimulants and Second-Generation Antipsychotics Among Children With ADHD Enrolled in Medicaid. Psychiatric Services, 66(4), 404–410. doi:10.1176/appi.ps.201300391

Koba, O., Grabicova, K., Cerveny, D., Turek, J., Kolarova, J., Randak, T., Zlabek, V., & Grabic, R. (2018). Transport of pharmaceuticals and their metabolites between water and sediments as a further potential exposure for aquatic organisms. *Journal of hazardous materials,* 342, 401-407. https://doi.org/10.1016/j.jhazmat.2017.08.039.

Kosek, K., Luczkiewicz, A., Fudala-Ksiazek, S., Jankowska, K., 2020. Implementation of advanced micropollutants removal technologies in wastewater treatment plants (WWTPs): examples and challenges based on selected EU countries. *Environ. Sci. Pol*. 112, 213–226. https://doi.org/10.1016/j.envsci.2020.06.011.

Kosma, C. I., Nannou, C. I., Boti, V. I., & Albanis, T. A. (2019). Psychiatrics and selected metabolites in hospital and urban wastewaters: occurrence, removal, mass loading, seasonal influence and risk assessment. Science of the Total Environment, 659, 1473-1483.

Kurian, B. T., Ray, W. A., Arbogast, P. G., Fuchs, D. C., Dudley, J. A., & Cooper, W. O. (2007). Effect of Regulatory Warnings on Antidepressant Prescribing for Children and Adolescents. Archives of Pediatrics & Adolescent Medicine, 161(7), 690. doi:10.1001/archpedi.161.7.690

Laird, N. M., & Ware, J. H. (1982). Random-effects models for longitudinal data. *Biometrics*, 963-974.

Laville, N., AïT-AïSsa, S., Gomez, E., Casellas, C., & Porcher, J. M. (2004). Effects of human pharmaceuticals on cytotoxicity, EROD activity and ROS production in fish hepatocytes. *Toxicology*, 196(1-2), 41–55. https://doi.org/10.1016/j.tox.2003.11.002

Lei, H. J., Yang, B., Ye, P., Yang, Y. Y., Zhao, J. L., Liu, Y. S., & Ying, G. G. (2021). Occurrence, fate and mass loading of benzodiazepines and their transformation products in eleven wastewater treatment plants in Guangdong province, China. *Science of The Total Environment*, 755, 142648.https://doi.org/10.1016/j.scitotenv.2020.142648

Lei, K., Zhu, Y., Chen, W., Pan, H. Y., Cao, Y. X., Zhang, X., ... & Liu, X. T. (2019). Spatial and seasonal variations of antibiotics in river waters in the Haihe River Catchment in China and ecotoxicological risk assessment. *Environment international*, 130, 104919. https://doi.org/10.1016/j.envint.2019.104919

Lis, H., Stepnowski, P., & Caban, M. (2019). Salinity and pH as factors affecting the passive sampling and extraction of pharmaceuticals from water. *Journal of separation science, 42*(18), 2949-2956.https://doi.org/10.1002/jssc.201900346

Long, E. R., Dutch, M., Weakland, S., Chandramouli, B., & Benskin, J. P. (2013). Quantification of pharmaceuticals, personal care products, and perfluoroalkyl substances in the marine

sediments of Puget Sound, Washington, USA. *Environmental toxicology and chemistry, 32*(8), 1701-1710. doi:10.1002/etc.2281

Lopez-Herguedas, N., González-Gaya, B., Castelblanco-Boyacá, N., Rico, A., Etxebarria, N., Olivares, M., & Zuloaga, O. (2022). Characterization of the contamination fingerprint of wastewater treatment plant effluents in the Henares River Basin (central Spain) based on target and suspect screening analysis. *Science of the Total Environment,* 806, 151262.

Ma, L., Li, J., Li, J., Liu, M., Yan, D., Shi, W., & Xu, G. (2018). Occurrence and source analysis of selected antidepressants and their metabolites in municipal wastewater and receiving surface water. Environmental Science: Processes & Impacts, 20(7), 1020–1029. https://doi.org/10.1039/c8em00077h.

Matongo, S., Birungi, G., Moodley, B., & Ndungu, P. (2015). Pharmaceutical residues in water and sediment of Msunduzi River, kwazulu-natal, South Africa. *Chemosphere*, 134, 133-140. https://doi.org/10.1016/j.chemosphere.2015.03.093

Mccallum, E. S., Nikel, K. E., Mehdi, H., Du, S. N. N., Bowman, J. E., Midwood, J. D., Kidd, K. A., Scott, G. R., & Balshine, S.. (2019). Municipal wastewater effluent affects fish communities: A multi-year study involving two wastewater treatment plants. Environmental Pollution, 252, 1730–1741. https://doi.org/10.1016/j.envpol.2019.06.075.

Mejias, C., Martín, J., Santos, J. L., Aparicio, I., & Alonso, E. (2021). Occurrence of pharmaceuticals and their metabolites in sewage sludge and soil: A review on their

distribution and environmental risk assessment. Trends in Environmental Analytical Chemistry, 30, e00125.

Metcalfe, C., Tindale, K., Li, H., Rodayan, A., Yargeau, V. (2010). Illicit drugs in Canadian municipal wastewater and estimates of community drug use. *Environ. Pollut.* 158 (10), 3179–3185. https://doi.org/10.1016/j.envpol.2010.07.002.

Miller, T. H., Bury, N. R., Owen, S. F., MacRae, J. I., & Barron, L. P. (2018). A review of the pharmaceutical exposome in aquatic fauna. Environmental pollution, 239, 129-146.

Minguez, L., Pedelucq, J., Farcy, E., Ballandonne, C., Budzinski, H., & Halm-Lemeille, M. P. (2016). Toxicities of 48 pharmaceuticals and their freshwater and marine environmental assessment in northwestern France. Environmental Science and Pollution Research, 23(6), 4992-5001.

Moschet, C., Vermeirssen, E. L., Singer, H., Stamm, C., & Hollender, J. (2015). Evaluation of in-situ calibration of Chemcatcher passive samplers for 322 micropollutants in agricultural and urban affected rivers. *Water Research, 71,* 306-317. https://doi.org/10.1016/j.watres.2014. 12.043

Muz, M., Escher, B. I., & Jahnke, A. (2020). Bioavailable Environmental Pollutant Patterns in Sediments from Passive Equilibrium Sampling. *Environmental Science & Technology, 54*(24), 15861-15871. https://doi.org/10.1021/acs.est.0c05537

Nannou, C., Kosma, C., Albanis, T., 2015. Occurrence of pharmaceuticals in surface waters:

analytical method development and environmental risk assessment. *Int. J. Environ. Anal. Chem. 95* (13), 1242–1262. https://doi.org/10.1080/03067319.2015.1085520.

Nitti, F., Kapitan, O. B., Ola, P. D., & Siswanta, D. (2022). Passive Sampling Techniques for Monitoring of Pharmaceuticals and Personal Care Products in Water Matrix: Trends from 2016 to 2020. Impact of COVID-19 on Emerging Contaminants, 17-44.

Nunes, C., dos Anjos, V., Quináiaa, S., 2019. Are there pharmaceutical compounds in sediments or in water? Determination of the distribution coefficient of benzodiazepine drugs in aquatic environment. *Environ. Pollut. 251*, 522–529. https://doi.org/10.1016/j.envpol.2019.05.015.

Nyaga, M. N., Nyagah, D. M., & Njagi, A. (2020). Pharmaceutical waste: Overview, management, and impact of improper disposal. *Preprints*. 202010024.

Oberoi, A. S., Jia, Y., Zhang, H., Khanal, S. K., & Lu, H. (2019). Insights into the fate and removal of antibiotics in engineered biological treatment systems: a critical review. Environmental Science & Technology, 53(13), 7234-7264.

Patel, M., Kumar, R., Kishor, K., Mlsna, T., Pittman Jr, C. U., & Mohan, D. (2019). Pharmaceuticals of emerging concern in aquatic systems: chemistry, occurrence, effects, and removal methods. Chemical reviews, 119(6), 3510-3673.

Paíga, P., Correia, M., Fernandes, M. J., Silva, A., Carvalho, M., Vieira, J., Jorge, S., Silva, J. G., Freire, C., & Delerue-Matos, C.. (2019). Assessment of 83 pharmaceuticals in WWTP

influent and effluent samples by UHPLC-MS/MS: Hourly variation. *Science of the Total Environment,* 648, 582–600. https://doi.org/10.1016/j.scitotenv.2018.08.129

Petrie, B., Barden, R., & Kasprzyk-Hordern, B.. (2015). A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring. *Water Research*, 72, 3–27. https://doi.org/10.1016/j.watres.2014.08.053

Philip, G., Malmstrom, K., Hampel, F. C., Weinstein, S. F., Laforce, C. F., Ratner, P. H., Malice, M.-P., & Reiss, T. F.. (2002). Montelukast for treating seasonal allergic rhinitis: a randomized, double-blind, placebo-controlled trial performed in the spring. *Clinical & Experimental Allergy*, 32(7), 1020–1028. https://doi.org/10.1046/j.1365-2222.2002.01422.x

Pietrzak, D., Kania, J., Kmiecik, E., Malina, G., & Wątor, K. (2020). Fate of selected neonicotinoid insecticides in soil–water systems: Current state of the art and knowledge gaps. Chemosphere, 255, 126981.

Poudel, D. D., Cazan, A. M., Oguma, A. Y., & Klerks, P. L.. (2020). Monitoring fish, benthic invertebrates, and physicochemical properties of surface water for evaluating nonpoint source pollution control in coastal agricultural watersheds. *Journal of Soil and Water Conservation, 75*(2), 177–190. https://doi.org/10.2489/jswc.75.2.177

Prajapati, S., Beal, M., Maley, J., & Brinkmann, M. (2021). Qualitative and quantitative analysis of microplastics and microfiber contamination in effluents of the City of Saskatoon

wastewater treatment plant. *Environmental Science and Pollution Research, 28*(25), 32545-32553. https://doi.org/10.1007/s11356-021-12898-7

Quesada, H. B., Baptista, A. T. A., Cusioli, L. F., Seibert, D., de Oliveira Bezerra, C., & Bergamasco, R. (2019). Surface water pollution by pharmaceuticals and an alternative of removal by low-cost adsorbents: A review. *Chemosphere,* 222, 766-780. https://doi.org/10.1016/j.chemosphere.2019.02.009Get

Raudenbush, S. W., & Bryk, A. S. (2002). Hierarchical linear models: Applications and data analysis methods (Vol. 1). sage.

Reichert, J. F., Souza, D. M., & Martins, A. F. (2019). Antipsychotic drugs in hospital wastewater and a preliminary risk assessment. *Ecotoxicology and Environmental Safety*, 170, 559–567. https://doi.org/10.1016/j.ecoenv.2018.12.021

Rossi, R., Socci, V., Talevi, D., Mensi, S., Niolu, C., Pacitti, F., ... & Di Lorenzo, G. (2020). COVID-19 pandemic and lockdown measures impact on mental health among the general population in Italy. Frontiers in psychiatry, 790.

Schultz, M. M., Furlong, E. T., Kolpin, D. W., Werner, S. L., Schoenfuss, H. L., Barber, L. B., & Vajda, A. M. (2010). Antidepressant pharmaceuticals in two US effluent-impacted streams: occurrence and fate in water and sediment, and selective uptake in fish neural tissue. *Environmental science & technology, 44*(6), 1918-1925.

Sierra, J., Roig, N., Papiol, G. G., Pérez-Gallego, E., & Schuhmacher, M. (2017). Prediction of the bioavailability of potentially toxic elements in freshwaters. Comparison between speciation models and passive samplers. *Science of the Total Environment, 605*, 211-218. https://doi.org/10.1016/j.scitotenv.2017.06.136

Smith, K. E. C., & Jeong, Y.. (2021). Passive Sampling and Dosing of Aquatic Organic Contaminant Mixtures for Ecotoxicological Analyses. *Environmental Science & Technology*, *55*(14), 9538–9547. https://doi.org/10.1021/acs.est.0c08067

Ten Hulscher, T. E. M., & Cornelissen, G. (1996). Effect of temperature on sorption equilibrium and sorption kinetics of organic micropollutants - a review. Chemosphere, 32(4), 609–626. doi:10.1016/0045-6535(95)00345-2.

Tetreault, G. R., Bennett, C. J., Cheng, C., Servos, M. R., & McMaster, M. E. (2012). Reproductive and histopathological effects in wild fish inhabiting an effluent-dominated stream, Wascana Creek, SK, Canada. Aquatic Toxicology, 110, 149-161.

Tran, N. H., Reinhard, M., & Gin, K. Y.-H.. (2018). Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions-a review. *Water Research*, 133, 182–207. https://doi.org/10.1016/j.watres.2017.12.029

Thompson, W. Andrew, and Mathilakath M. Vijayan. "Environmental levels of venlafaxine impact larval behavioural performance in fathead minnows." Chemosphere 259 (2020): 127437.

Townsend, M., Pareja, K., Buchanan-Hughes, A., Worthington, E., Pritchett, D., Brubaker, M., ... & Waters, H. (2022). Antipsychotic-Related Stigma and the Impact on Treatment Choices: A Systematic Review and Framework Synthesis. Patient preference and adherence, 16, 373.

US EPA, 1997. Guidance on Cumulative Risk Assessment – Part 1. Planning and Scoping. https://www.epa.gov/expobox/exposure-assessment-tools-tiers-and-types-screeninglevel-and-refined. (Accessed 24 August 2022).

USEPA, E. (2002). Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. United States Environmental Protection Agency, Office of Water, Washington.

Valdez-Carrillo, M., Abrell, L., Ramírez-Hernández, J., Reyes-López, J. A., & Carreón-Diazconti, C.. (2020). Pharmaceuticals as emerging contaminants in the aquatic environment of Latin America: a review. *Environmental Science and Pollution Research, 27*(36), 44863–44891. https://doi.org/10.1007/s11356-020-10842-9

Verbeke, G., Molenberghs, G., & Rizopoulos, D. (2010). Random effects models for longitudinal data. *In Longitudinal research with latent variables* (pp. 37-96). Springer, Berlin, Heidelberg.

Vystavna, Y., Huneau, F., Grynenko, V., Vergeles, Y., Celle-Jeanton, H., Tapie, N., & Le Coustumer, P. (2012). Pharmaceuticals in rivers of two regions with contrasted socio-economic conditions: occurrence, accumulation, and comparison for Ukraine and France. *Water, Air, & Soil Pollution, 223*(5), 2111-2124. https://doi.org/10.1007/s11270-011-1008-1

Waiser, M. J., Tumber, V., & Holm, J.. (2011a). Effluent-dominated streams. Part 1: Presence and effects of excess nitrogen and phosphorus in Wascana Creek, Saskatchewan, Canada. *Environmental Toxicology and Chemistry, 30*(2), 496–507. https://doi.org/10.1002/etc.399

Waiser, M. J., Humphries, D., Tumber, V., & Holm, J. (2011b). Effluent-dominated streams. Part 2: Presence and possible effects of pharmaceuticals and personal care products in Wascana Creek, Saskatchewan, Canada. *Environmental Toxicology and Chemistry, 30*(2), 508-519. https://doi.org/10.1002/etc.398

Wang, Y., Liu, Y., Lu, S., Liu, X., Meng, Y., Zhang, G., ... & Guo, X. (2019). Occurrence and ecological risk of pharmaceutical and personal care products in surface water of the Dongting Lake, China-during rainstorm period. *Environmental Science and Pollution Research*, 26(28), 28796-28807. doi: 10.1007/s11356-019-06047-4

Wang, L., Liu, R., Liu, X., & Gao, H. (2020). Sampling rate of polar organic chemical integrative sampler (POCIS): influence factors and calibration methods. *Applied Sciences, 10*(16), 5548. https://doi.org/10.3390/app10165548

Wells, M. J. (2006). Log DOW: key to understanding and regulating wastewater-derived contaminants. Environmental Chemistry, 3(6), 439-449.

Wu, M., Liu, S., Hu, L., Qu, H., Pan, C., Lei, P., She, Y., & Yang, M. (2017). Global transcriptomic analysis of zebrafish in response to embryonic exposure to three

antidepressants, amitriptyline, fluoxetine and mianserin. Aquatic Toxicology, 192, 274-283. https://doi.org/10.1016/j.aquatox.2017.09.027

Yang, M., Qiu, W., Chen, J., Zhan, J., Pan, C., Lei, X., & Wu, M. (2014). Growth inhibition and coordinated physiological regulation of zebrafish (Danio rerio) embryos upon sublethal exposure to antidepressant amitriptyline. Aquatic Toxicology, 151, 68-76. https://doi.org/10.1016/j.aquatox.2013.12.029

Yang, J., Zhao, Y., Li, M., Du, M., Li, X., & Li, Y. (2019). A Review of a Class of Emerging Contaminants: The Classification, Distribution, Intensity of Consumption, Synthesis Routes, Environmental Effects and Expectation of Pollution Abatement to Organophosphate Flame Retardants (OPFRs). *International Journal of Molecular Sciences, 20*(12), 2874. https://doi.org/10.3390/ijms20122874

Yuan, S., Jiang, X., Xia, X., Zhang, H., & Zheng, S. (2013). Detection, occurrence, and fate of 22 psychiatric pharmaceuticals in psychiatric hospital and municipal wastewater treatment plants in Beijing, China. *Chemosphere, 90*(10), 2520–2525. https://doi.org/10.1016/j.chemosphere.2012.10.089

Zhang, H., & Davison, W. (1995). Performance characteristics of diffusion gradients in thin films for the in situ measurement of trace metals in aqueous solution. *Analytical chemistry, 67*(19), 3391-3400.

Zhou, J., & Broodbank, N. (2014). Sediment-water interactions of pharmaceutical residues in the river environment. *Water research*, 48, 61-70. https://doi.org/10.1016/j.watres.2013.09.026.

Zhou, S., Di Paolo, C., Wu, X., Shao, Y., Seiler, T. B., & Hollert, H. (2019). Optimization of screening-level risk assessment and priority selection of emerging pollutants–the case of pharmaceuticals in European surface waters. Environment international, 128, 1-10.

CHAPTER 3: DEVELOPING AN APPROACH FOR INTEGRATING CHEMICAL ANALYSIS AND TRANSCRIPTIONAL CHANGES TO ASSESS CONTAMINANTS IN WATER, SEDIMENT, AND FISH.

Overview

A version of this chapter will be published with the following details: Perez, A. S. C., Challis, J. K., Alcaraz, J.A., Valery Ramirez, A. V., Ji, X., Giesy, J. P., Hecker, M. & Brinkmann, M. (2022). Impacts of wastewater effluents and seasonal trends on levels of antipsychotic pharmaceuticals in water and sediments from two cold-region rivers. Science of The Total Environment.

Contributions

Ana Cardenas: Research conceptualization, study design and implementation, methodology, software, validation, formal analysis, investigation, data curation, writing - original draft, reviewing, and editing, visualization.

Jonathan Challis: Data curation, methodology, writing, review, and editing.

James Alcaraz: Resources, writing, review, and editing.

Xiaowen Ji: Methodology, writing, review, and editing.

Alexis Valery: Statistical analysis, writing, review, and editing.

Jon Giesy: Resources, writing, review, and editing.

Markus Hecker: Resources, writing, review, and editing.

Markus Brinkmann: Research conceptualization, data curation, formal analysis, funding acquisition, Investigation, methodology, project administration, resources, supervision, writing – review & editing.

3.1 Abstract

Pharmaceuticals have been found in water sources across the world and can adversely impact exposed aquatic organisms due to their continuous release and potential accumulation, therefore requiring adequate methodologies for their monitoring. Targeted analyses of chemicals alone are insufficient to comprehensively assess water quality and potential resulting effects in biota. Instead, integrative strategies combining non-target and targeted chemical analyses and effect-based tools, such as molecular biomarker approaches, can provide a more robust characterization of the risks of complex chemical mixtures occurring in the environment. Therefore, this study aimed to develop a strategy for integrating chemical analysis and transcriptome changes in exposed fish (fathead minnows [*Pimephales promelas*]) to prioritize contaminants and their toxic effects and test this strategy in Wascana Creek, an effluent-dominated stream in Saskatchewan, Canada. Concentrations of pharmaceuticals were in the ng/L to µg/L range, and generally greater downstream of the WWTP compared to upstream. Clozapine was the pharmaceutical with the greatest concentration in fathead minnow and the most bioavailable from both water and sediment. Pharmaceuticals, personal care products, and rubber components were the prioritized chemicals according to the non-target chemical analysis and supported by the perturbed pathways (signalling and cellular processes) identified through transcriptomic analysis in exposed fish. The strategy

developed here can serve as a blueprint for other watersheds and has the potential to streamline risk assessment lead to identify environmental hazards and reduce or eliminate the risks they pose.

Keywords: Pharmaceutical, wastewater treatment plants, bioaccumulation, transcriptomic.

3.2 Introduction

The release of human-made chemicals has been one of the most critical environmental issues affecting aquatic ecosystem health of our time. Although most of these chemicals occur at trace concentrations, the bioactivity of complex mixtures, transformation products, and unknown chemicals is still raising significant ecotoxicological concerns (Danforth et al., 2020; Schuijt et al., 2021). Traditionally, chemicals in the environment have been monitored using conventional chemical analytical techniques; however, these measurements are neither indicative of a chemical's bioavailability nor its toxicological effects in exposed organisms (Chmiel et al., 2019). Therefore, chemical analytical monitoring strategies need to be combined with effect-based tools to assess the potential risks of chemical mixtures to aquatic ecosystems (Brack et al., 2017).

Comparison of target compound concentrations with environmental quality standards is often insufficient for achieving a comprehensive risk assessment of contaminants due to the complexity of chemical mixtures, including interactions of chemicals within mixtures that may alter their bioactivity (i.e., antagonism or synergism) (Xiao et al., 2016; Altenburger, et al., 2019; Pourchet et al., 2020). More comprehensive approaches are therefore needed to assess cause-effect

relationships and risks of chemical mixtures present in aquatic ecosystems. Integrating methods such as targeted and non-target chemical analysis and ecotoxicity tests may offer a rational approach to identify known and unknown toxicants in the environment, linking chemical contamination to measurable ecotoxicological effects (Kim et al., 2018; Sussman et al., 2022).

Targeted chemical analysis is most commonly used by regulators to detect and monitor chemical contaminants. However, the entirety of compounds of potential environmental relevance cannot possibly be analyzed using targeted analysis due to the time and costs related to purchasing and evaluating hundreds or even thousands of reference standards (Park et al., 2018; Purschke et al., 2020). Furthermore, targeted analysis can omit critical information about some complex samples, such as those containing products of transformation through biotic and abiotic processes (Miller et al., 2018). Detection of the presence or absence of a wide range of substances in wastewater is now possible due to the evolution of analytical technologies like liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS). Qualitative or semi-quantitative analysis can be conducted through non-target chemical (NTC) screening approaches by comparing mass spectra to those obtained from a variety of databases (Dürig et al., 2020; Goessens et al., 2020), which often contain a combination of accurate mass and isotope information, as well as fragment ions of suspected chemicals for tentative identification (Guo et al., 2020). This approach can support the detection and analysis of a wide range of contaminants providing information to understand the

occurrence, fate, bioaccumulation, and transformation of chemicals of emerging concern (CECs) in aquatic ecosystems (Brunelle, 2022).

Over the past few years, the monitoring of unknown chemicals in wastewater discharges based on non-target approaches has received increasing interest. Recent studies have highlighted increases in the numbers of emerging and non-regulated contaminants such as pharmaceuticals, personal care products, pesticides, and their transformation products (Ccanccapa-Cartagena et al., 2019; Llamas-Dios et al., 2021; Sumpter et al., 2022**).** However, little is known about their effects on aquatic organisms (Miller et al., 2018; Park et al., 2018). Testing each of these chemicals in each potentially impacted species using traditional toxicity assays requiring large number of live animals, however, is not feasible. Thus, a comprehensive assessment of potential risks will require the use of alternative methods. New technologies have not only advanced chemical analysis but also high-throughput next-generation characterization of effects in exposed organisms (Ghosh et al., 2018; Martínez et al., 2020). A wide range of chemical effects can be determined through untargeted and unbiased methods, such as transcriptomics, in diverse species, including fish (Martínez et al., 2020).

Mechanism-based assessments such as bioanalytical approaches can go beyond analytical detection linking exposure and effects to assess how organisms respond stress caused by molecular and cellular responses related to chemical classes and indicator of toxic action mode (Groh et al., 2015). Gene expression data alone is often not sufficient for predicting apical outcomes (Vinken,

2019; Jagiello et al., 2021). As the current regulatory landscape requires assessment of biologically relevant apical outcomes (i.e., survival, growth/development, reproduction) there is a need to establish clear links between molecular response patterns and physiological changes that are indicative of these outcomes. There are various frameworks, such as the AOP (Adverse Outcome Pathways) framework that have been designed to establish those links across biological levels of organization to facilitate the prediction of toxicant responses in whole organisms and to facilitate the interpretation of mechanistic information needed to extrapolate effects across species (Brockmeier et al., 2017).

The aims of this study were to 1) apply a high-resolution LC-MS/MS method to conduct non-target screening of a complex, effluent-dominated exposure scenario (Wascana Creek), and 2) to characterize molecular disturbance patterns in fish collected from the same sites to link exposure patterns with biological perturbations.

3.3 Materials and methods

3.3.1 Sampling locations

Southern Saskatchewan is a semi-arid region with seasonal drying that can cause low water flows and consequently low dilution of effluents. Wascana Creek (WC) is located downstream of the City of Regina, Saskatchewan, selected for sampling as a representative system for small semi-arid prairie and cold regions. Furthermore, WC is an effluent-dominated stream that receives constant

discharges of effluents from the Regina WWTP, which can make up to over 90% of the creek's flow under low flow conditions, and thus, may affect downstream water quality. WC discharges in the Qu´Appelle River approx. 60 km downstream of the WWTP (Hanson et al., 2021**)**. Previous studies have shown downstream water quality in WC was affected by effluents from Regina WWTPs (Waiser et al., 2011; Hanson et al., 2021; Cardenas Perez et al., 2022). It is therefore urgent to better understand the diversity, levels, and spatiotemporal distributions of pollutants in WC.

Few studies have examined the impacts of WWTP effluent on fish in WC using targeted screening analysis (Tetreault et al., 2012; Hanson et al., 2021); predominantly during warm periods, WWTP effluent was most harmful and caused negative impacts on water quality and aquatic organisms. This is the first study combining target and non-target compounds to conduct chemical and biological analysis on samples that were collected from two sites that are located both upstream and downstream of the WWTPs in Regina, respectively. Sampling sites will be referred to as WUS (WC Upstream Site: 50°47'65.42"N 104°73'26.30"W) and WDS (WC Downstream Site: 50°48'42.01"N 104°77'80.66"W) (Cardenas Perez et al., 2022).

3.3.2 Sample collection

All samples at both sampling sites were collected during the spring (May and June), summer (July and August) and fall (September and October) of 2021 (Supporting data file (SDF) 1). Water samples were obtained using conventional grab sampling and using diffusive gradients in thin films

(DGT) passive samplers, and sediment samples were obtained as described in the Chapter 2. Fathead minnows were sampled using seine nets. At each site and for each sampling event, a total of 20 fathead minnows were collected, mostly juveniles and some adults. Where possible, females and males were visually identified. After capturing the fish, individuals were sorted and inspected to characterize their health conditions and to determine mass (ranging from 0.196 to 3.36 g) and total length (ranging from 2.6 to 7.2 cm). All non-target individuals were released immediately if individuals were of good health status. Sampled fish were humanely euthanized using buffered tricaine methanesulfonate (MS-222). A subset of 5 males and 5 females were frozen for subsequent chemical analysis. The remaining fish were also separated by sex including a subset of 5 males and 5 females which were dissected to excise brain and liver tissues that were snap-frozen and transported in liquid nitrogen and subsequently stored at -80 °C until the determination of various biological endpoints (Section 3.6). All fieldwork data per sample are provided in Table B.2. Fish sampling considered animal care and field safety guidelines, environmental conditions, and effective techniques to capture fish including the guidelines established by the University of Saskatchewan Animal Care Committee (UACC). The research has reviewed and been approved by the University of Saskatchewan Animal Research Ethics Board (AREB), animal use protocol No. 20200044.

3.3.3 Sample processing and preparation for chemical analysis

Fish samples were extracted for chemical analysis using a method developed by Fedorova et al. (2014) (Table B.3). Whole-body fish samples were weighed in centrifuge tubes and lyophilized (Dura-Dry MP FD2085 microprocessor-controlled freeze-dryer, Stone Ridge, NY). The dried samples were weighted and homogenized using an immersion blender (SCILOGEX D160 Homogenizer). Samples were fortified by addition of 50 µL internal standard mixture (Amitriptyline-D6, Bupropion-D9, Carbamazepine-D10, Clozapine-D4, Fluoxetine-D5, Lamotrigine-[13C;15N4], and Venlafaxine-D6 at 1 mg/L each). After adding methanol (2:1, methanol/sample), the mixture was sonicated for 10 min, and centrifuged at 4,000 × g and 4°C for 30 min. The supernatants were collected, ice-cold acetonitrile (ACN), acidified with 0.1 vol. % formic acid) was added to the methanol extract (1:1, sample/ACN), and stored at -20 °C. After 12 h, the sample was centrifugated at 4,000 × g and 4°C for 30 min for a 2nd step extraction and the supernatant was removed to a new tube, leaving behind any precipitate. The combined extracts were concentrated to dryness under a gentle stream of nitrogen gas, reconstituted in 1 mL of 50:50 MeOH: water (v/v), and filtered through a 0.45 µm PTFE syringe filter (Pall Life Sciences, Mississauga, ON) into amber LC vials.

Water (conventional grab and DGT) and sediments samples were obtained and treated as described in Chapter 2.

3.4 Instrumental chemical analysis

3.4.1 Target screening

The resulting sample extracts from water (conventional grab and DGT), sediments, and fish were analyzed using a Vanquish UHPLC Liquid Chromatography system coupled to a QExactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer (Thermo Fisher Scientific). These analyses were conducted using the same method described in Chapter 2.

Positive mode heated electrospray ionization was used to ionize the samples. The QExactive Orbitrap method implemented the following parameters indicated by Ji et al. (2022).

Calibration standards were placed before each batch of samples, followed by a blank run every 10 samples and a 50 µg/L single calibration standard after running the whole batch of samples as a QA/QC protocol. The same instrument was used for targeted and non-targeted screening.

3.4.2 Non-target (NTC) screening

The analyzed sample extracts, instrumentation, analytical column, and Liquid Chromatography (LC) solvents were the same as for the targeted analysis method. The LC gradient elution is listed in Table B.2. The suspect screening method used the following positive mode HESI (heating electrospray source) source parameters: sheath gas flow = 35; aux gas flow = 10; sweep gas flow = 1; aux gas heater = 400 ^{o}C; spray voltage = 3.8 kV; S-lens RF = 60; capillary temperature = 350 ^{o}C. A Full MS- Top 10 ddMS2 (data-dependent MS2) method was used with the following Full MS/ddMS2 scan settings: 60,000/15,000 resolution, AGC (automatic gain control) target =

$5x10^5/1x10^5$, max injection time = 100 ms/100 ms, full scan range of 70-1000 m/z, MS2 isolation window = 2.0 m/z, loop count = 5, and a stepped NCE (new chemical entity) = 15, 30, 45.

Instrument blanks (clean solvent) were run in between each triplicate set of samples from a single sampling site. Site specific field blanks were run immediately before the samples from that specific site. The native standards from the targeted method were used as quality control samples to assess the performance of the suspect screening method. A subset of the calibration standards was analyzed with each batch of samples (2, 5, 50, 100 ng/mL).

Processing of the suspect screening data was conducted in Compound Discoverer 3.2 (CD) (ThermoFisher Scientific) using the CD processing workflow and relied on mzCloud spectra for suspect identification (all workflow details are provided in Figures B.8 and B.9). A tiered approach for data reduction started with filtering using CD filter options. From the identified features per matrix, chromatographic peaks were inspected manually and either confirmed or removed as a tentative identification based on the expert opinion of the user. Chromatographic peaks were removed based on high background noise surrounding the peak, poor peak shape, resolution, sharpness, and double/split peaks. Major feature filtering criteria included: remove the background compounds (the compounds found in the blank samples), area (max.) greater than or equal to 1,000,000, must include mzCloud results, and mass accuracy between -5 and +5 ppm (Figure B.10). This reduced the number of identified features from 14,683 to 186 for DGT, from 34,928 to 278 for water samples by conventional grab, 7,842 to 134 for sediments, from 104,506

to 175 for fish collected in spring 2021, from 23,984 to 60 for fish collected in summer 2021, and from 41,844 to 76 for fish samples collected in fall 2021. From these totals the Chemical Abstract Service (CAS) could not be found for 13.3%, 13.9%, 17,2%, 26.3%, 35%, and 19.7%, respectively (SDF-2 NTC data).

3.5 Relative abundance of analytes from non-target screening in water (conventional grab and DGT), sediment, and fish samples

Using the final numbers of identified features from the non-target analysis for all the matrices and both sampling locations (up- and down-stream), the CAS numbers were identified for each record using the CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard/) (SDF-2 NTC data). Later, a table was created with those that matched to existing CAS numbers (SDF-2 NTC data), including the relative abundance (area) of each compound for up and down stream locations, as well as the replicates per season. This abundance was averaged for each substance to express them as parts of the summed total abundance averages (100%).

3.5.1 Determination of chemical uses linked to non-target compounds (NTC)

The CompTox Chemicals Dashboard also allowed to link each CAS number with chemical uses and biological effects stored in the database. This information was associated with the relative abundance determined for each compound. For the use, the relative abundance was associated per compound (up- and down-stream, separately) to each reference of chemical use of the compound,

then the relative abundances of each substance use were added, creating a summary table of the use and the total percentage obtained for each use (SDF-2 NTC data). The table obtained allowed the uses to be grouped into 8 categories: pharmaceuticals, personal care products, food, biocides, reagents, solvents, surfactants, and other (additives in cigarettes, oil field additive oil, etc.) (Table S2e in SDF-2 NTC data). The results were normalized using the total sum of the percentages (100%) and determining the final percentage of each category of use, by matrix, for up- and downstream.

3.5.2 Determination of biological effects linked to non-target compounds (NTC)

The same methodology indicated above for determining the chemical uses per substance was used for determining the biological effects linked to each substance per matrix and per sampling location. In this case, the endpoint assay list generated by the CompTox Chemicals Dashboard was associated with 18 subcategories (biological endpoints) in a general way (SDF-2 NTC data), which allowed for each compound to add its presence in each of these subcategories (SDF-2 NTC data). For each of the determined compounds, the results were associated with the percent abundance of the compounds based on their CAS number (SDF-2 NTC data). The sum of the total percentages for each site (100%) was used to normalize the percentages of each effect according to the site. The percentages obtained from the normalization for the different subcategories were added, creating a table with 5 categories: signaling, cellular processes, metabolism, endocrine, and immune (Table S2e in SDF-2).

3.6 Transcriptomic analysis using EcoToxChips

Total RNA was extracted from individual brain tissues in summer season 2021 (n = 6 upstream samples, n = 5 downstream samples) and fall season 2021 (n = 8 upstream samples, n = 8 downstream samples); from liver tissues in summer 2021 (n = 2 upstream samples, n = 2 downstream samples) and from fall season (n = 8 upstream samples, n = 8 downstream samples). RNA was extracted using the RNeasy Plus Universal Mini kit (Qiagen), following the manufacturer's protocol. RNA concentration and quality were assessed by spectral profiling using a QIAxpert instrument (Qiagen). Then, 700 ng of total RNA in liver and 1000 ng in brain samples were reverse transcribed to synthesize cDNA using the RT2 First Strand kit (Qiagen) and then mixed with RT2 SYBR® Green (Qiagen) to prepare the master mix for EcoToxChip analysis. Individual samples were then run in the FHM v1 EcoToxChip custom RT2 profiler PCR array (Qiagen CAPU14176E) using a QuantStudio 6 Flex instrument, following the manufacturer's protocol.

Reduced transcriptome analysis was conducted in EcoToxXplorer (ecotoxxplorer.ca) using the EcoToxChip *P. promelas* v.1 384-well plate module. Liver samples collected were very heterogeneous and did not pass the quality check (qPCR data summary in SDF-3). All brain samples passed quality check (6 housekeeping genes (HKG); reverse transcription control – positive PCR control < 5). Ct values > 35 were filtered to remove values with higher uncertainties and variance and were considered as non-detects. All non-detects were imputed by randomly

drawing values from the normal distribution that has a mean Ct = 35 (Ct cut-off) and a standard deviation of the data surrounding this cut-off. The EcoToxXplorer generates an analysis of differential gene expression where, due to the behavior of the data (not normal), a non-parametric test (Kruskal Wallis/H-test) was used, using the results of upstream samples as a control group, which allowed generating the comparison of the variation of the expression of the 384 genes between upstream and downstream samples. This analysis yielded as a result a Sankey Plot showing enriched modules including multiple genes associated with a certain biological function (e.g., endocrine system, reproduction, neurotoxicity, signal transduction).

3.7 Results and discussion

Several investigations (Santos et al., 2010; Matus et al., 2018; Nilsen et al., 2019; Wang et al., 2021) have indicated the relevance of negative effects of pharmaceuticals on aquatic organisms, including various fish species. The results of the chemical analysis conducted by Cardenas Perez et al. (2022) indicated a high toxicological risk to the receiving aquatic ecosystem at Wascana Creek, Saskatchewan, Canada, with fish as the most sensitive group. Therefore, this investigation focussed further on evaluating the concentrations of pharmaceuticals in fish and the assessment of their behaviour in terms of fate and uptake in fish, as well as the biotic and abiotic factors that might influence them. In this study, the fathead minnow was selected as the model species considering

their significant role in toxicological research over the last decades (Ankley and Villeneuve, 2006; Chibwe et al., 2022).

3.7.1. Target screening analysis in fish samples

Not all the target pharmaceuticals were always detected in fathead minnows at the two sites (up- and down-stream of the WWTP) and across the three seasons (spring, summer, and fall 2021). The results indicated that only during spring the seven pharmaceuticals were consistently detected at both sites (Figure 3.1), with concentrations ranging from 0.2 to 4,290 ng/g. Conversely, target analysis for water (by DGT) and sediments showed that all the pharmaceuticals were identified at both sites (Cardenas Perez et al., 2022). CLO and FLX were found at both sites and across the three seasons and CLO was the pharmaceutical with the overall highest concentration up- and downstream during spring and summer (from 9.2 to 4,290 ng/g). In general, the speciation of ionizable organic chemicals can be predicted by pH and the acidity constant (pKa) (Chang et al., 2021). For CLO the pKa (25 ^{0}C) was 7.6 the closest to the pH measured in WC (between 7.3 and 8.1 upstream and downstream) (Table B.4). The fraction of the uncharged form has been suggested to be correlated with higher bioaccumulation and toxicity in the fish at the upstream sampling location. The results obtained for CLO are consistent with a potential impact of pH on the charge and bioavailability of this molecule. The pH in Wascana Creek upstream of the WWTP was 7.3, while that downstream of it was 8.0 (Cardenas Perez et al., 2022). CLO shows a marked difference

in the relative abundance of the neutrally charged (blue line in Figure B.7) in this pH range, suggesting greater bioavailability at greater pH.

Fish analysis of pharmaceuticals showed the following order of abundance in WC, CLO>AMI>VEN>FLX>BUP>LAM>CBZ, with concentrations ranging from 0.32 to 773 µg/g (Table 3.1). Data collected in this targeted analysis suggests that concentrations of AMI, CLO, and VEN were by 1-3 orders of magnitude greater than those measured in previous investigations (Table 3.1). In contrast, BUP, CBZ, FLX, and LAM showed similar concentrations in comparison with previous studies, including results from both laboratory and field. Differences between the data obtained in this study and previous research may be due to the nature of the experiments: the data collected for this study were based on field samples, while the results available from the literature were from laboratory experiments performed under controlled physicochemical and biological conditions.

Differences in length and weight of sampled fish were also considered in the assessment of the results (Table B.3). The fish samples collected in WC during three seasons showed that CLO had a positive correlation ($r>0.93$) upstream with respect to the morphological characteristics of the fathead minnows, while downstream showed a positive correlation ($r>0.75$) with AMI, FLX, and VEN, and negative ($r<-0.97$) correlation with BUP and CBZ. The variability observed in the findings may be related to the nutrients available (TP, NH_4), the availability of food may affect the metabolism of contaminants in fish, and this is supported by Breitmeyer et al. (2022) who found

changes in the morphology of bluegills and redbreast sunfish after being exposed to WWTP discharges.

Missing data due to field conditions did not allow the application of a classic analysis of variance, which required the use of mixed models. These models considered the different study variables (physicochemical factors, water concentrations of pharmaceuticals determined using DGTs, and concentrations in sediments), and allowed us to observe that depending on the compound, they could explain between 43 and 97% of the variability of their concentration found in fish (Table B.5).

Two statistical mixed models (M1 and M2) were analysed (without and with including physicochemical factors, respectively) (Appendix A). For AMI, BUP, CBZ, CLO, and VEN, model M2 could explain the variability of pharmaceutical concentrations in fish accurately (R^2 between 0.43 and 0.97, Table B.5). Instead, variations in fish concentrations for FLX and LAM were explained better by model M1 (R^2 between 0.82 and 0.97, Table B.5).

With respect to the significant variables in the models for each pharmaceutical, in most cases, M2 did not suggest significant correlation between concentrations in sediment, DGT, and physicochemical parameters, but their incorporation in the model allowed to obtain a better fit of the data. In the case of AMI and BUP in fish, the variability of the concentrations was only significant in terms of time of year, showing changes related to the season of fish collection. Also, M2 showed a significant correlation with the concentrations of BUP in sediments, as well as with

the physicochemical parameters of temperature, pH, and SC. Similarly, for CBZ and VEN, the location (up- and downstream of the WWTP) and the time of year were significant. Conversely, M2 for CLO did not show significance in the variables used to describe its variability. On the other hand, M1 showed significant correlation between the sampling location and seasons for FLX and LAM.

Using Pearson's correlation coefficient, the results obtained showed differences for most of the pharmaceuticals in fish between the seasons and sampling locations. Although there were no differences with respect to physicochemical parameters, correlations were identified between the temperature, DOC (dissolved organic carbon), NH_4, TP (total phosphorous) evaluated in water samples and the behaviour of each pharmaceutical in fish samples up- and downstream of the WWTP (Figure B.3 to B.6). The Pearson Product-Moment Correlation for upstream samples showed a negative correlation (-0.75) between CLO and DOC, while FLX and DOC had a positive correlation (0.99). Also, variations of NH_4 were associated with CLO (0.97), while variations TP and temperature were related to FLX for the samples collected upstream (-0.99 and -0.98, respectively). In the case of downstream samples, variations were found for TP with respect to the concentrations of AMI (0.77), BUP (-0.98), CBZ (-0.99), FLX (0.99), and VEN (0.91). Meanwhile, DOC and NH_4 were positively (>0.96) and temperature was negatively correlated (-0.90 and 0.93, respectively) with CLO and LAM concentrations (Table B.6).

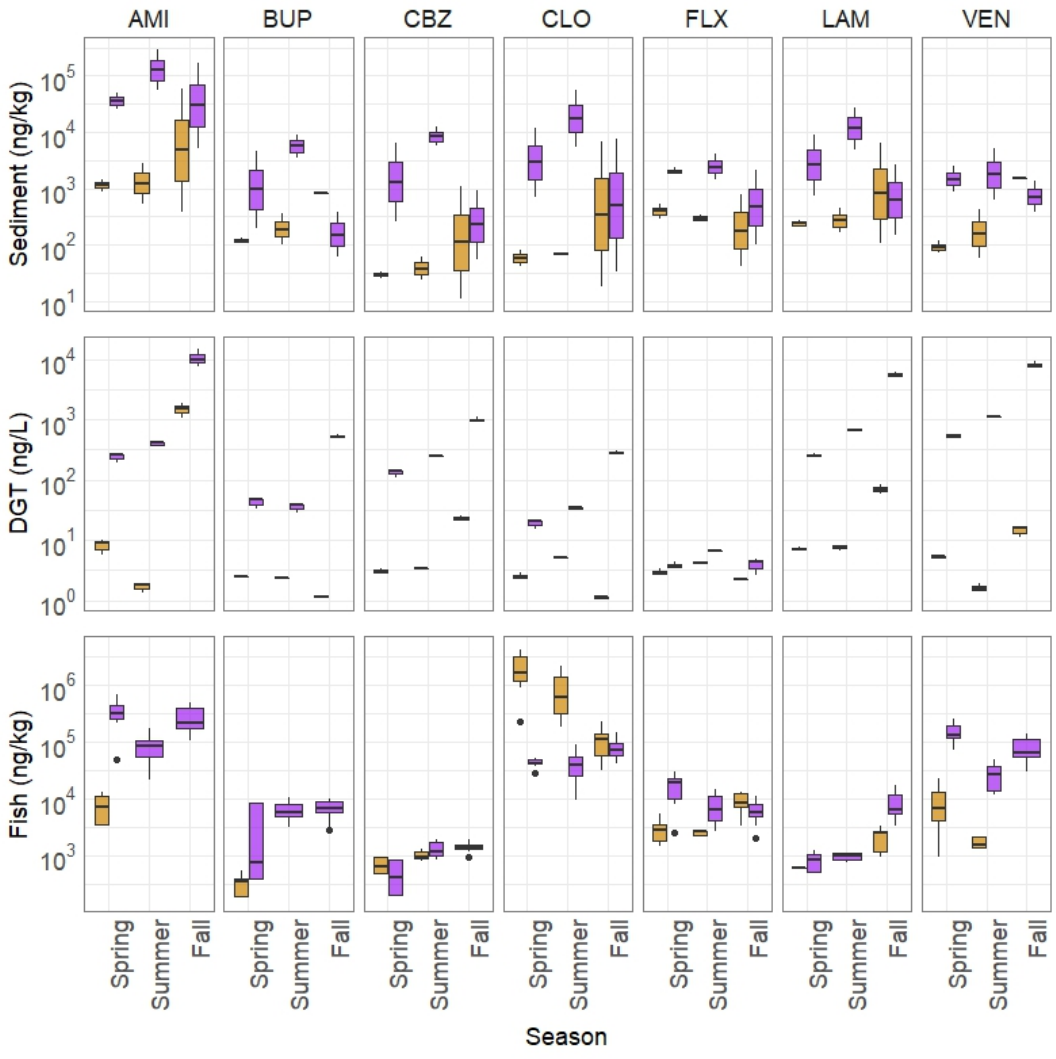
Multilevel models incorporate all the possible sources of variation, in this case a complex system of changes in the concentration of the compounds due to the effects of all the variables involved and can help identify which are the most significant. In general, this research found that season and sampling location were the variables with the most significant influence, and the physicochemical parameters included in the model allowed a better description of model predictions to describe changes in concentrations of pharmaceuticals.

Table 3.1. Comparison of concentrations of pharmaceuticals in fish samples.

Pharmaceutical	**Fish (ng/g)**			**Type of exposure**	**Fish species**
	This study	**Previous studies**	**Location**		
AMI	3.3-691	7.1-58 (1)	Spain	Laboratory	*Sparus aurata*
		6-8 (2)	UK	Laboratory	*Rutilus rutilus*
BUP	0.2-10.5	0.14-22.0 (3)	Canada	Laboratory	*Neogobius melanostomus*
CBZ	0.2-1.9	1.26-1.36 (3)	Canada	Laboratory	*Neogobius melanostomus*
		0.83-1.44 (4)	Czech Republic	Field	*Salmo trutta fario*
CLO	9.2-4,290	2 (2)	UK	Laboratory	*Rutilus rutilus*
FLX	1.4-29.3	19-70 (5)	USA	Field	Various
		5 (2)	UK	Laboratory	*Rutilus rutilus*
LAM	0.5-16.7	0.17-0.40 (7)	Czech Republic	Field	Various
VEN	0.9-259	0.9-10.8 (3)	Canada	Laboratory	*Neogobius melanostomus*
		0.5-20 (6)	Czech Republic	Field samples	*Cyprinus carpio L.*

(1) Mijangos et al., 2019; (2) David et al., 2018; (3) McCallum et al 2017; (4) Grabicova et al., 2017; (5) Ramírez et al., 2009; (6) Grabicova et al., 2018, (7) Grabicova et al., 2022

Figure 3.1. Comparison of concentrations of pharmaceuticals in fish, water (by DGT), and sediments collected in Wascana Creek near Regina Saskatchewan, Canada. DGT and sediment data were taken from Cardenas Perez et al., 2022 and are shown for reference. The boxplot shows the distribution of numerical data and skewness through displaying the data quartiles (sediment n=2; DGT n=3; fish n-3 to 20 depending on the season and site) and interquartile ranges. Note logarithmic scaling of the y-axes of the graphs. (Upstream = data in orange, Downstream = data in purple)



3.7.2. Bioconcentration (BAF) and biota-sediment accumulation (BSAF)

Considering the importance of BAF and BSAF in risk assessment, these coefficients were calculated based on field data collected during spring, summer, and fall seasons in 2021. Bioaccumulation was classified as not bioaccumulative BAF for a value <5,000 L Kg^{-1} while chemicals with >5,000 L Kg^{-1} were considered very bioaccumulative, suggesting their ability to accumulate in aquatic organisms. This classification was selected using the criteria established by guidelines **(**U.S. EPA, 2010). BSAF values > 1 indicate that the accumulation in the organism is greater than that of the medium and were compared to BAF.

3.7.2.1 BAF and target analysis

BAFs obtained between the fish concentrations across the three sampling seasons and the DGT-derived water concentrations presented by Cardenas Perez et al. (2022) showed that only CLO bioaccumulated in fish (Figure 3.2). Additionally, depending on the pharmaceutical, the season and the location, some BAF values were close to 5,000 L Kg^{-1} as in the case of FLX (upstream in summer and downstream in fall) or getting lower as was the case upstream during the three seasons for CBZ and LAM. Overall, the following order was observed for field-derived BAFs from the greatest to lowest: CLO>FLX>VEN>AMI>CBZ>BUP>LAM.

Data collected during previous studies were compared with the results obtained in this study (Table 3.2). BAF of pharmaceuticals, excluding FLX, were greater than those reported by other authors. BAF variations may be related to the different exposure conditions (environmental factors,

locations, pharmaceutical consumption in the area of interest, etc.). For example, data reported by Duarte et al. (2022) suggested that BAFs ranged from 0.0135 to 7,590 L Kg^{-1} for 22 neuroactive pharmaceuticals and could be additionally be impacted by temperature and pH. BAFs ranged from 11.35 to 142,000 L Kg^{-1} for the 6 pharmaceuticals that were evaluated in this study from the literature and the present study. The discrepancies between these data show the complexity of antipsychotic pharmaceuticals and the varying effects of environmental factors on the uptake of these chemicals.

Correlations analysis between BAF values, nutrients, and physicochemical parameters were carried out in the same way as was done for the concentrations of pharmaceuticals in fish, through the use of generalized mixed models. M1 had a better fit to describe the variability of BAF for all the pharmaceuticals ((R^2 between 0.18 and 0.93, Table B.7). These correlations with time, place, and nutrients were significant depending on the pharmaceutical.

Table 3.2. Comparison of concentrations of pharmaceuticals assessed in this study and data collected from previous publications for the same group of pharmaceuticals.

Pharmaceutical	BAF (L Kg^{-1})		Data source
	This study	Previous studies	
AMI	7.1-3,640	30-110 (1)	Field
		4.95 (7)	Review
		1,500 (8)	Field
		231.9-274.1 (2)	Laboratory
BUP	4.7-363	3.85 (7)	Review
CBZ	0.9-374	2.5-3.8 (3)	Laboratory
		3.4-265 (4)	Laboratory
		2.25 (7)	Review
CLO	134-1,920,000	0.7-46.5 (5)	Laboratory
		2.84 (7)	Review
		105,000 (8)	Field
FLX	382.6-8,538.7	80-927 (1)	Field
		4.65 (7)	Review
		68,000 (8)	Field
LAM	0.5-87.9	0.17-20 (9)	Field
VEN	3.3-4,410	13-90 (1)	Field
		3.3-22 (6)	Field
		3.28 (7)	Review
		700 (8)	Field

(1) Muir et al., 2017; (2) Gilroy et al., 2017; (3) Garcia et al., 2012; (4) Xie et al., 2015; (5) Nallani et al., 2016; (6) Grabicova et al., 2017; (7) Duarte et al., 2022; (8) David et al., 2018; (9) Grabicova et al., 2022

3.7.2.2 BSAF and target analysis

The BSAF was calculated to evaluate whether partitioning of pharmaceutical contamination from sediment to fish species could be relevant. The values were compared to the commonly used 1,000 Kg Kg^{-1} threshold (Froehner et al., 2018; Yan et al., 2020; Gu et al, 2021). The BSAFs of pharmaceuticals ranged from 13.3 to 13,900 Kg Kg^{-1}. Only the BSAF of CLO is above the threshold (1,000 Kg Kg^{-1}) and it can be considered bioaccumulative, indicating that partitioning from sediment could serve as a route for bioaccumulation of this pharmaceutical. BSAFs was lower than BAF for all pharmaceuticals, except LAM. Overall, the following order of BSAF values was observed: CLO>VEN>FLX>CBZ>BUP>LAM>AMI. (Figure 3.2). Data on BSAFs of pharmaceuticals was generally scarce: one study conducted in a river system in China showed BSAF ranged between 0.1 and 1 Kg Kg^{-1} for CLO in plankton and snails (Yang et al., 2020**).** Although scarce studies support the influences BSAF of antipsychotic drugs such as CLO, our results showed how sampling design influences bioaccumulation of these contaminants and subsequently toxicity assessment.

Both coefficients, BAF and BSAF, were above the 5,000 L Kg^{-1} or 1,000 Kg Kg^{-1} for CLO and were consistently greater downstream compared to upstream. It is common to see fluctuations between these two factors (Adeogun et al., 2015; Van Den Brink et al., 2019) but for this compound we did not find such systematic differences, which could indicate that something else changes downstream of the treatment plant in terms of physical chemical properties of the water that drives

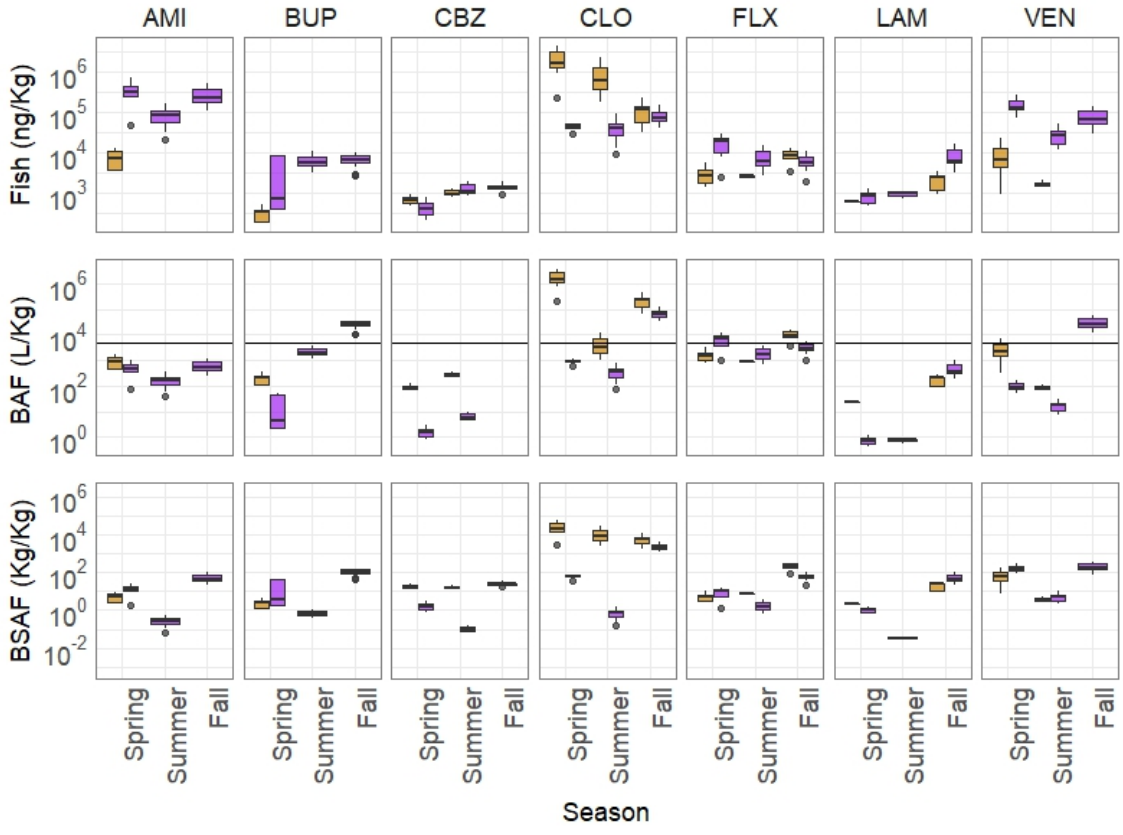
a higher accumulation. The results obtained for CLO are consistent with a potential impact of pH on the charge and bioavailability of this molecule and this is supported by the field data collected (see section 3.7.1), even though greater relative bioavailability of chemicals downstream of WWTPs is also often observed when discharges result in decreases in dissolved oxygen, leading to greater ventilation rates in fish and greater potential chemical flux between water and fish (Pan et al., 2019).

Correlations between BSAF values, nutrients, and physicochemical parameters were evaluated and showed that M2 was the best for evaluating variability for most pharmaceuticals, except CBZ and FLX. These findings were similar to the results obtained for BAF (Table B.8). The comparison of the results of the two coefficients (BAF and BSAF) with the physical chemical parameters (temperature, TP, NH_4 and DOC) (Table B.9 and B.10), as in fish, it is indicating that there is only a correlation for CLO and FLX. For samples collected upstream of the WWTP, CLO and NH_4 had a correlation of 0.99, while for FLX the correlation with DOC, TP and temperature was 0.99, -0.98 and -0.97, respectively. The data obtained for CLO is supporting that variations not only for BAF but also for BSAF can be related to dynamics and interactions of nutrients and chemicals in sediments and microbial functions which serves as an indicator of changes in ecological processes influencing the behaviour of the contaminants (Bolan et al., 2011**).**

Currently, there are many studies detecting pharmaceuticals in the environment, but the data related to their mobility is in general very scarce and/or absence for the majority of the

pharmaceuticals (Miller et al., 2018; Oberoi et al., 2019; Khan et al., 2020; Duarte et al., 2022). To our best knowledge this is the first research determining the BAF and BSAFs correlations between pharmaceuticals from field samples. Despite the increasing number of publications about toxicity of pharmaceuticals in fish, few data are available to evaluate the link between BAFs and BSAFs. Our results indicated a high correlation between BAFs and BSAFs in single chemical, CLO, after finding that aquatic organisms were simultaneously affected by water and sediment. Therefore, it can be concluded that CLO was the most bioavailable pharmaceutical in water and sediment to organisms, and that this increased bioavailability might be linked to the different physical-chemical features and slow metabolism of CLO in wild fish (Diao et al., 2017). These coefficients BAF and BSAF differed from those previously reported (del Carmen Gomez-Regalado et al., 2022; Duarte et al., 2022), which has significant implications for the ecological risk assessment of CLO that was shown in this study to accumulate in fish under specific environmental conditions (e.g., pH values between 7.3 and 8.1 and DOC between 8 and 270 mg/L measured in WC).

Figure 3.2. Comparison between concentration of pharmaceuticals in fish, bioconcentration factor (BAF), and biota-sediment accumulation factor (BSAF). Bioaccumulation was classified as bioaccumulative (BAF) for values >5,000 L Kg^{-1} which is the limit included in the plot). Note logarithmic scaling of the y-axes of the graphs (U.S. EPA, 2010). The boxplot shows the distribution of numerical data and skewness through displaying the data quartiles (n=3 to 20 depending on the season and site) and interquartile ranges. Note logarithmic scaling of the y-axes of the graphs. (Upstream = data in orange, Downstream = data in purple)



3.7.3. Non-target chemical analysis

Non-target chemical analysis tentatively identified 278 compounds in water collected by conventional grab, 186 in water sampled by DGTs, 134 in sediments, and 175, 60, and 76 in fish collected in spring, summer, and fall, respectively. From these total numbers of chemicals, CAS numbers could not be found for 13.3, 13.9, 17,2, 26.3, 35.0, and 19.7%, respectively (Chapter 3, Section 3.3.2).

3.7.3.1 NTC categorization in terms of chemical use and biological effects

Relative abundances determined by non-target analysis were calculated using the average abundance (peak area) of each compound found divided by the summed peak areas of all compounds for each matrix separately to categorize the substances identified (SDF-2 NTC data). The abundances obtained per matrix were evaluated with respect to the chemical use and biological effects according to the CompTox Chemicals Dashboard (Table S2e in SDF-2 NTC data).

The most abundant groups of chemicals identified in the non-target analysis (Figure 3) included pharmaceuticals, personal care products, biocides, food additives, reagents, solvents, surfactants, and others (e.g., additives in cigarettes and oil field additives). For all matrices and locations, pharmaceuticals were the chemical use class with the greatest relative abundance (between 48 and 79%), followed by personal care products that ranged between 10 and 25%. Biocides had a lower relative abundance in water and sediments, but it was the second most abundant chemical group in fish samples, and this can be related to the bioaccumulation processes.

The CompTox Chemicals Dashboard also includes high-throughput screening (HTS) data collected in ToxCast and Tox21 programs providing information in the assessment of bioassays. All the assays available were selected for each NTC and the resulting predictions of the biological endpoints were exported into an Excel file for additional review and analysis. The results obtained from the predictions were correlated to the relative abundance of the NTC, the main biological effects were related to alterations in the endocrine system for all the matrices and all the seasons with a relative abundance between 34 and 79%, followed by cellular processes with a relative abundance between 10 and 35%. However, for fish samples, signalling and cellular processes showed very similar abundance across the three seasons (Figure 3.4).

3.7.3.2 NTC categorization in terms of chemical relationships

Venn diagrams were used to determine logical relationships between time and sampling matrices including water (conventional grab and DGT), sediments, and fish samples. Two main groups were obtained to evaluate the correlations in terms of the presence and absence of the pharmaceuticals. The first group comparing results from water collected by conventional grab, sediments, and fish (Figure 3.4) showed 10 compounds across all three matrices during spring, only 2 in summer, and 6 in fall. The second group comparing water collected using DGTs, sediments, and fish (Figure B.11) showed similar results compared to the first group, except one substance (propranolol) that was only found in the first group (Table 3.3).

A correlation analysis was performed for the relative abundances of the common substances found in all three matrices (water by conventional grab, sediment, and fish obtained from the non-target analysis). The results showed a correlation (>0.85) between water and sediments for both sampling locations (up- and downstream). However, a correlation (0.98) for fish was only evident with respect to the sampling locations and not with the other two matrices. These same trends were also found for the second group (DGT, sediment, and fish) (Figure 3.4).

The similarities between the substances identified in the table 3.3 were determine through principal component analysis (PCA) (Figure 3.4). Four groups of substances were obtained after correlating their individual relative abundance in the different matrices. Two groups were identified from clusters in the PCA (Figure 3.4), represented by a group of pharmaceuticals (C2, C8, C6, C5, and C10), and another group represented by different types of substances including pharmaceuticals, rubber additives, and personal care products (C9, C3, and C7; respectively). The group of pharmaceuticals showed the greatest relative abundance in water with 94%, followed of 68% for sediments, and 17% for fish samples. Two substances (C1 and C4) did not show any correlations with other compounds, even though C1 had the greatest relative abundance in water (upstream of the WWTP), DTG, and sediments (both downstream the WWTP), and C4 was more abundant in fish (downstream), previous studies showed low cytotoxicity (EC_{50} > 10 mM for cell lines) (Radošević et al., 2015).

Non-target analysis found that not only pharmaceuticals were present in all studied water (by conventional grab and DGT), sediments, and fish but also rubber tire components and personal care products, indicating that these emerging contaminants may be classified as ubiquitous in Wascana Creek. Pharmaceuticals were the group with relevant priority between the top 10 of compounds found. This group showed the highest relative abundance in water samples.

Table 3.3. Chemical compounds prioritized from relationships between time and sampling matrices including water (conventional grab and DGT), sediments, and fish samples using Venn diagrams.

Name	**CAS No.**	**Chemical use**	**Water-Sediment-Fish**	**DGT-Sediment-Fish[1]**
2-Propyl-1H-benzimidazole	5465-29-2	Pharmaceutical (various)	C1	C1d
3,5-Dimethyl-1-phenylpyrazole	1131-16-4	Pharmaceutical (anticancer)	C2	C2d
5,6-Dimethylbenzimidazole	582-60-5	Pharmaceutical (vitamin B12)	C3	C3d
Choline chloride	62-49-7	Pharmaceutical (B-complex)	C4	C4d
Clozapine	5786-21-0	Pharmaceutical (antipsychotic)	C5	C5d
Desmethylcitalopram	62498-67-3	Pharmaceutical (antidepressant)	C6	C6d
Galaxolidone	507442-49-1	PCPs (various)	C7	C7d
Lidocaine	137-58-6	Pharmaceutical (anesthetic)	C8	C8d
N,N'-Diphenylguanidine	20277-92-3	Rubber industry (accelerator)	C9	C9d
Propranolol	525-66-6	Pharmaceutical (hypertension)	C10	--

[1] The ‘d’ added to the conventions of DGT-Sediment-Fish group was only to show that those compounds belong to that group, but the compounds are the same (C# = C#d).

PCPs = Personal Care Products

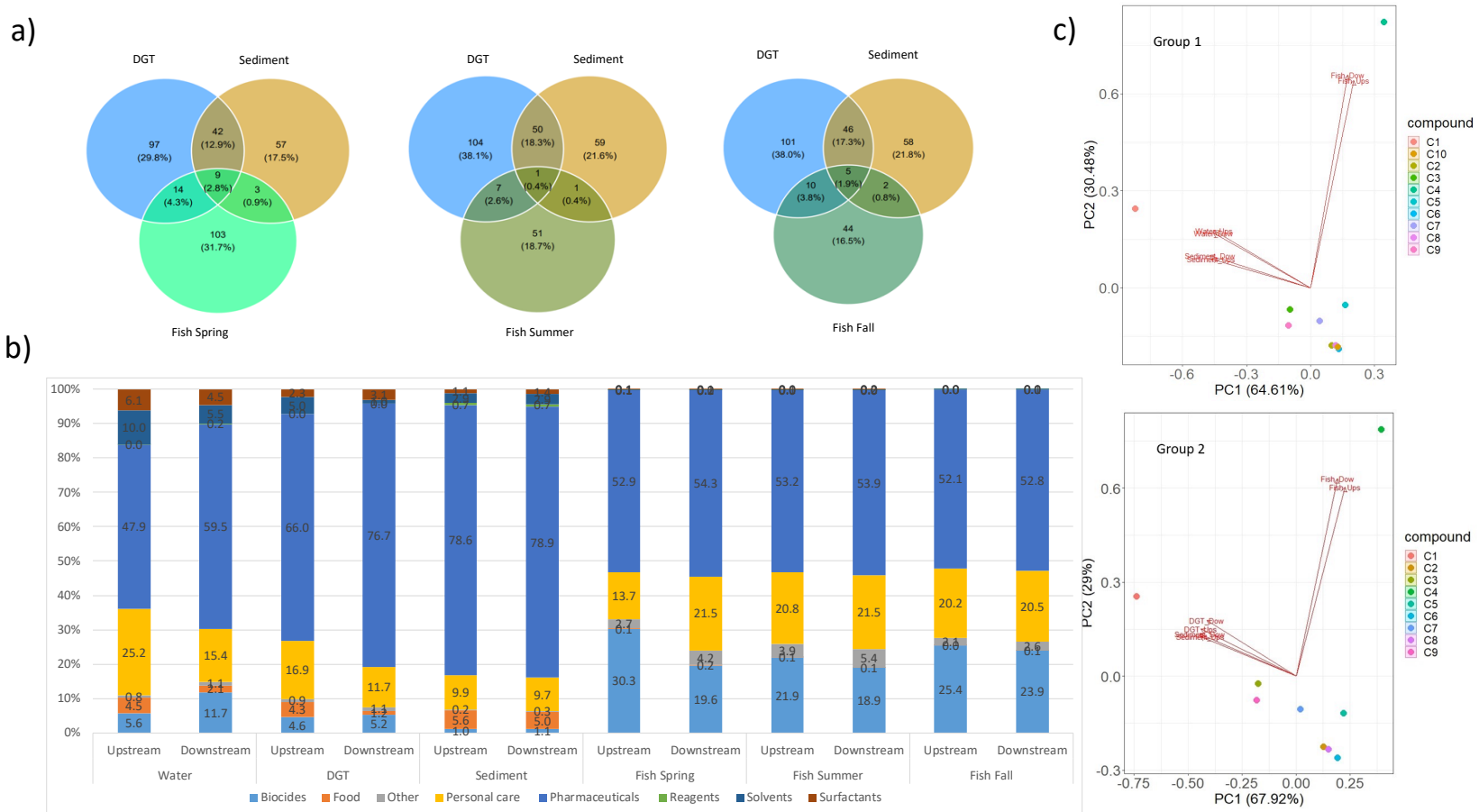


Figure 3.3. Chemical analysis of water, sediment, and fish samples collected in spring, summer and fall 2021 in Wascana Creek a) Venn diagrams considering the presence/absence of non-target compounds in water (by DGT), sediments, and fish samples, b) Bar charts with the chemical use categories identified through semi-quantitative assessment of the non-target compounds in water, sediment, and fish samples, c) Principal component analysis (PCA) with the result of a clustering calculations indicating the similarities between non-target compounds in both groups, Group 1 of water (by conventional sampling)-sediment-fish and Group 2 of water (by DGT)-sediment.

3.7.4. Transcriptomic effects in fathead minnow

The data obtained showed an acceptable coefficient of variation (<20%, low variability) only for the brain samples across sampling locations (up- and downstream) and seasons (Table B.11). The results of the qPCR analysis showed that 36 genes presented significant differences in abundance between upstream and downstream in summer, while in the fall, there were 18 dysregulated genes (Figure 4.4; Tables B.12 and B.13 in the Supporting Information).

The main biological processes perturbed following exposure to WWTP effluents from the City of Regina during summer included: cellular communication (signalling), cellular growth and death (signalling), metabolism (carbohydrates and other compounds), endocrine effects (corticosteroid), and immune system. Instead, during fall carbohydrates metabolism and Peroxisome proliferator-activated receptors (endocrine) were the predominant processes altered. Endocrine alterations (specifically reproduction processes) identified during summer and fall 2021 in our study were also supported by previous observations of decreases in the abundance of transcripts of 13 genes involved in normal reproductive functioning and maturation (Hanson et al., 2021), and alterations in gill and kidney that can affect their reproduction and ability to survive (Tetreault et al., 2012).

The relevance of the priority pollutants obtained from non-target analysis was confirmed through the results of the qPCR analysis. The main effect in qPCR analysis was signalling which correspond to the main effect of pharmaceuticals, the main chemical category and top pollutant prioritized correspond to a pharmaceutical. Also, signalling is the for non-target analysis fish

samples the second higher effect were signalling and cellular processes which is consistent with the results obtained in the qPCR analysis. The results obtained in our study are supporting the relevance of antipsychotic pharmaceuticals that affect chemical signalling related with brain functions (Duarte et al., 2022). Previous studies have showed correlations between the relative abundance of antipsychotic pharmaceuticals and biological effects including nervous system compromising physical, behavioural and reproduction processes (Munawar et al. 2021; Peng et al., 2022; Chang et al., 2023).

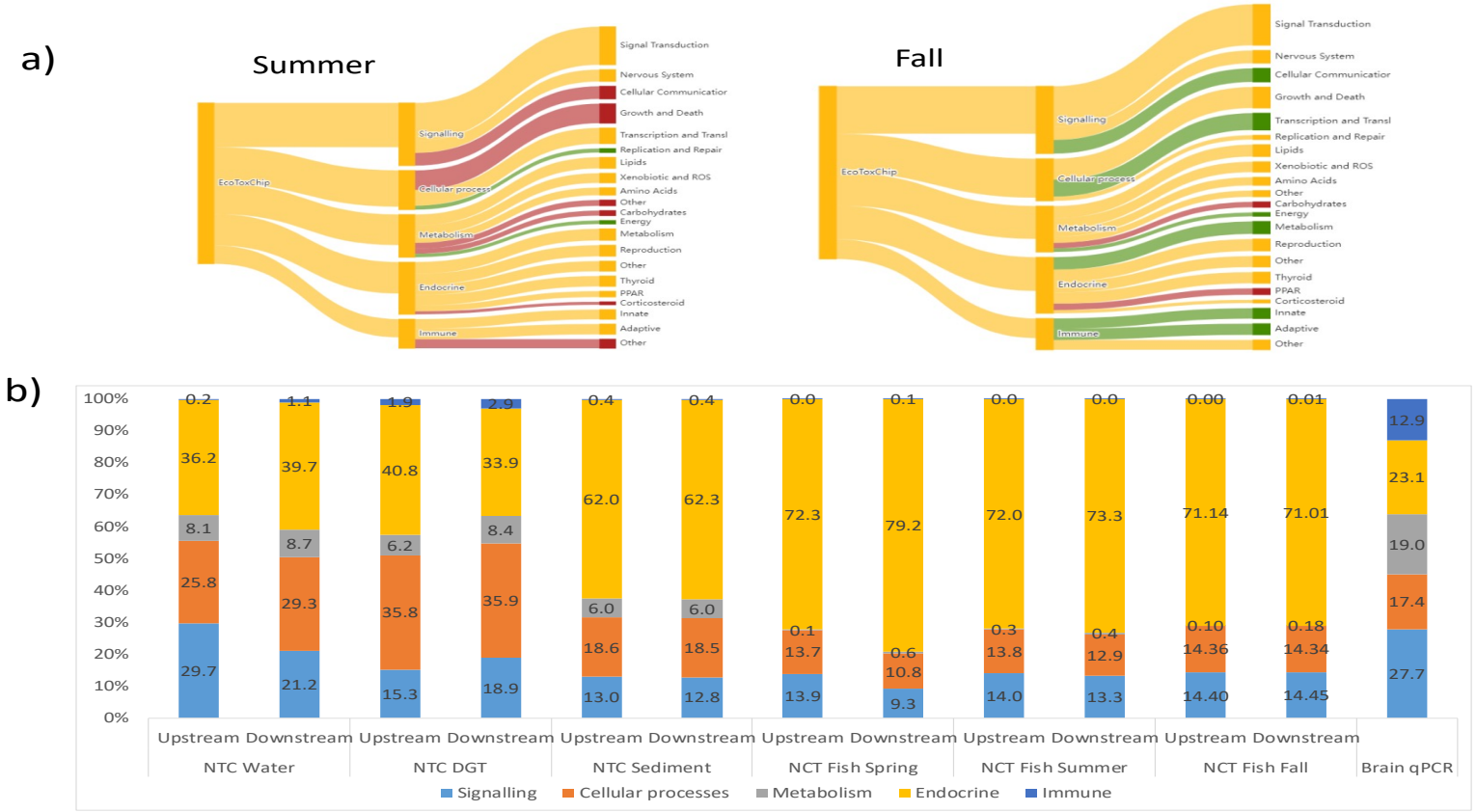


Figure 3.4. Biological analysis of P. promelas collected in summer and fall 2021 in Wascana Creek a) Sankey plot obtained from qPCR analysis of brain samples collected in summer and fall season in 2021, indicating gene expression organized with the EcoToxModule hierarchy (yellow threshold: 1.5; Red threshold: 2.0), b) Bar charts showing the comparison of the biological effects identified through the assessment of non-target analysis and biological effects determined through the qPCR analysis

3.7.5. Integrated approach biological endpoints and non-target analysis

Based on the results obtained through the combination of statistical models it was possible to prioritize several contaminants identified using non-target analysis in the environmental samples collected in wastewater of the City of Regina. Pharmaceuticals were the chemical class with the greatest relative abundance across all matrices, all seasons, and both sampling locations. This top group comprises 8 of the 10 contaminants prioritized. According to the literature review conducted to interpretate our results in context with previous publications it was found that the majority of the pharmaceuticals identified carry a significant human risk (Cardoso-Vera et al., 2021; Duarte et al., 2022**)**. C1 and C3 pose a medium to high ecotoxicological risk (Zhou ET AL., 2020; Chen et al., 2021), C2 has endocrine disrupting effects in rat and mouse models (Lakhey et al., 2021**)**, C4 is an essential vitamin (Das et al., 2022) and one of the most common components used for green designer solvents with low cytoxocity in fish and human cells (Radošević et al., 2015), and C5 (CLO) under laboratory conditions shows bioconcentration in brain and liver with critical concentrations exceeding minimum response concentrations (MRC) by up to 17-fold (Nallani et al., 2016). C6 can also bioconcentrate in fish tissues causing disturbances in behaviour, growth, condition and/or reproduction (Writer et al., 2013). C8 is susceptible to cause neuronal damage (Cano-Europa et al., 2008), and C10 may affect the mobilization of liver glycogen, which can negatively affect the energy balance and mobility of fish (Matus et al., 2018; Pereira et al., 2018). The other groups of chemical categories within the list of prioritized pollutants include a personal

care product (C7) and a rubber tire-derived contaminant (C9). Studies conducted in oyster tissues indicated that C7 had a low health risk (Gadelha et al., 2019). Conversely, C9 is an environmental contaminant present in tires with a potential persistence, high mobility, and toxicity according to the German Environment Agency (Challis et al., 2021).

After comparing the relevant responses from toxicity data using predictive models from the CompTox database for NTC versus those identified by analysis of qPCR results (by means of the EcoToxXplorer), it was found that the highest percentage corresponds to alterations in the endocrine system for all the matrices and both locations, while for qPCR this system would be the second in importance of effect (the greatest percentage was identified for signaling). However, the qPCR gene alterations showed very similar relative trends in measured effects compared to those predicted from the CompTox database, with the following order (highest to lowest): signaling>endocrine>metabolism>cellular processes>immune. The variations between the effects identified through non-target analysis and qPCR analysis may be related to the nature of both approaches, semiquantitative and quantitative, which can lead to variations in the estimation of contaminants; specially when semiquantitative assessments are conducted for categorization of contaminants (Hollender et al., 2019). The CompTox database includes results of experimental chemical assays and models, including those from ToxCast and Tox21, which are screening programs designed to identify assays and responses to level toxicity to develop predictive models for chemicals with little or no available data, and these data were correlated to the NTC (Albergamo

et al. 2020; Lai et al., 2021). qPCR analysis for RNA quantification using EcoToxChips based on evidence from 370 gene targets for the characterization, prioritization, and management of complex chemical mixtures in the environment, the core and design of this tool are driven by regulatory principles of Canada government (Basu et al. 2019; Soufan et al., 2022). Both approaches, CompTox and qPCR differ in the nature and design of the data but the main purpose of using them was to provide additional tools to obtain relevant information linking the mode of action of contaminants and their biological pathways of regulatory relevance.

Overall, data collected and assessed provided a list of chemicals to prioritize and this approach is intended to be fit to support screening level activities and solid evaluations of chemical safety that can improve understanding of chemicals.

3.6 Conclusions

This study provided the first comprehensive spatiotemporal dataset on the contamination of Wascana Creek, an important water body in the Qu'Appelle River basin, with emerging contaminants, including pharmaceuticals, personal care products, biocides, reagents, solvents, surfactants, food additives, and others.

In conclusion, it can be stated that chemical analysis and transcriptomics-based approaches are useful and complementary tools for assessing risks of complex mixtures of contaminants in wastewater discharges and their effects in aquatic organisms. This initial tiered analytical approach

using NTC analyses and transcriptomics in fathead minnows as a bioindicator of chemical stress was demonstrated to be capable of identifying and prioritizing toxicologically relevant substances in complex environmental samples. With this information, more targeted analyses of individual chemicals can be performed, which will ultimately aid in establishing water quality guidelines. Additionally, the analytical approaches conducted can help to understand interactions between complex mixtures of contaminants (especially at trace levels) and aquatic ecosystems (Brinkmann et al., 2018).

The approach applied in this study can serve as a blueprint for similar studies in other systems around the globe, which will ultimately assist governments and regulators in the effective management of risks associated with emerging contaminants.

References

Adeogun, A. O., Ibor, O. R., Omogbemi, E. D., Chukwuka, A. V., Adegbola, R. A., Adewuyi, G. A., & Arukwe, A. (2015). Environmental occurrence and biota concentration of phthalate esters in Epe and Lagos Lagoons, Nigeria. Marine environmental research, 108, 24-32.

Albergamo, V., Escher, B. I., Schymanski, E. L., Helmus, R., Dingemans, M. M., Cornelissen, E. R., ... & De Voogt, P. (2020). Evaluation of reverse osmosis drinking water treatment of riverbank filtrate using bioanalytical tools and non-target screening. Environmental Science: Water Research & Technology, 6(1), 103-116.

Altenburger, R., Brack, W., Burgess, R. M., Busch, W., Escher, B. I., Focks, A., ... & Krauss, M. (2019). Future water quality monitoring: improving the balance between exposure and toxicity assessments of real-world pollutant mixtures. Environmental Sciences Europe, 31(1), 1-17.

Ankley, G. T., & Villeneuve, D. L.. (2006). The fathead minnow in aquatic toxicology: Past, present and future. Aquatic Toxicology, 78(1), 91–102. https://doi.org/10.1016/j.aquatox.2006.01.018

Brack, W., Dulio, V., Ågerstrand, M., Allan, I., Altenburger, R., Brinkmann, M., ... & Vrana, B. (2017). Towards the review of the European Union Water Framework Directive: recommendations for more efficient assessment and management of chemical contamination in European surface water resources. Science of the Total Environment, 576, 720-737.

Basu, N., Crump, D., Head, J., Hickey, G., Hogan, N., Maguire, S., Xia, J., & Hecker, M.. (2019). EcoToxChip: A next-generation toxicogenomics tool for chemical prioritization and environmental management. Environmental Toxicology and Chemistry, 38(2), 279–288. https://doi.org/10.1002/etc.4309

Brinkmann, M., Hecker, M., Giesy, J. P., Jones, P. D., Ratte, H. T., Hollert, H., & Preuss, T. G. (2018). Generalized concentration addition accurately predicts estrogenic potentials of mixtures and environmental samples containing partial agonists. Toxicology in Vitro, 46, 294-303.

Bolan, N. S., Adriano, D. C., Kunhikrishnan, A., James, T., McDowell, R., & Senesi, N. (2011). Dissolved organic matter: biogeochemistry, dynamics, and environmental significance in soils. Advances in agronomy, 110, 1-75.

Bombaywala, S., Dafale, N. A., Jha, V., Bajaj, A., & Purohit, H. J.. (2021). Study of indiscriminate distribution of restrained antimicrobial resistome of different environmental niches. Environmental Science and Pollution Research, 28(9), 10780–10790. https://doi.org/10.1007/s11356-020-11318-6

Breitmeyer, S. E., Walsh, H. L., Blazer, V. S., Bunnell, J. F., Burritt, P. M., Dragon, J., ... & Smalling, K. L. (2022). Potential health effects of contaminant mixtures from point and nonpoint sources on fish and frogs in the New Jersey Pinelands. Science of the Total Environment, 851, 158205.

Brockmeier, E. K., Hodges, G., Hutchinson, T. H., Butler, E., Hecker, M., Tollefsen, K. E., ... & Falciani, F. (2017). The role of omics in the application of adverse outcome pathways for chemical risk assessment. Toxicological Sciences, 158(2), 252-262.

Brunelle, L. D., Huang, I. J., Angeles, L. F., Running, L. S., Sirotkin, H. I., McElroy, A. E., & Aga, D. S. (2022). Comprehensive assessment of chemical residues in surface and wastewater using passive sampling, chemical, biological, and fish behavioral assays. Science of The Total Environment, 828, 154176.

Cano-Europa, E., López-Galindo, G. E., Hernández-García, A., Blas-Valdivia, V., Gallardo-Casas, C. A., Vargas-Lascari, M., & Ortiz-Butrón, R. (2008). Lidocaine affects the redox environment and the antioxidant enzymatic system causing oxidative stress in the hippocampus and amygdala of adult rats. Life sciences, 83(19-20), 681-685.

Cardenas Perez, A. S., Challis, J. K., Ji, X., Giesy, J. P., & Brinkmann, M. (2022). Impacts of wastewater effluents and seasonal trends on levels of antipsychotic pharmaceuticals in water and sediments from two cold-region rivers. Science of The Total Environment, 158247.

Cardoso-Vera, J. D., Elizalde-Velázquez, G. A., Islas-Flores, H., Mejía-García, A., Ortega-Olvera, J. M., & Gómez-Oliván, L. M. (2021). A review of antiepileptic drugs: Part 1 occurrence, fate in aquatic environments and removal during different treatment technologies. Science of The Total Environment, 768, 145487.

Ccanccapa-Cartagena, A., Pico, Y., Ortiz, X., & Reiner, E. J. (2019). Suspect, non-target and target screening of emerging pollutants using data independent acquisition: Assessment of a Mediterranean River basin. Science of the total environment, 687, 355-368.

Challis, J. K., Popick, H., Prajapati, S., Harder, P., Giesy, J. P., Mcphedran, K., & Brinkmann, M.. (2021). Occurrences of Tire Rubber-Derived Contaminants in Cold-Climate Urban Runoff. Environmental Science & Technology Letters, 8(11), 961–967. https://doi.org/10.1021/acs.estlett.1c00682

Chang, X., Shen, Y., Yun, L., Wang, X., Feng, J., Yang, G., ... & Su, X. (2023). The antipsychotic drug olanzapine altered lipid metabolism in the common carp (Cyprinus carpio L.): Insight from the gut microbiota-scfas-liver axis. Science of The Total Environment, 856, 159054.

Chang, E. D., Town, R. M., Owen, S. F., Hogstrand, C., & Bury, N. R. (2021). Effect of Water PH on the Uptake of Acidic (Ibuprofen) and Basic (Propranolol) Drugs in a Fish Gill Cell Culture Model. Environmental Science & Technology, 55(10), 6848-6856.

Chen, S., Gan, Z., Li, Z., Li, Y., Ma, X., Chen, M., ... & Su, S. (2021). Occurrence and risk assessment of anthelmintics in Tuojiang River in Sichuan, China. Ecotoxicology and Environmental Safety, 220, 112360.

Chmiel, T., Mieszkowska, A., Kempińska-Kupczyk, D., Kot-Wasik, A., Namieśnik, J., & Mazerska, Z. (2019). The impact of lipophilicity on environmental processes, drug delivery

and bioavailability of food components. Microchemical Journal, 146, 393-406. https://doi.org/10.1016/j.microc.2019.01.030

Chibwe, L., Parrott, J. L., Shires, K., Khan, H., Clarence, S., Lavalle, C., ... & Rochman, C. M. (2022). A deep dive into the complex chemical mixture and toxicity of tire wear particle leachate in fathead minnow. Environmental toxicology and chemistry, 41(5), 1144-1153.

Danforth, C., Chiu, W. A., Rusyn, I., Schultz, K., Bolden, A., Kwiatkowski, C., & Craft, E. (2020). An integrative method for identification and prioritization of constituents of concern in produced water from onshore oil and gas extraction. Environment international, 134, 105280. https://doi.org/10.1016/j.envint.2019.105280

Das, S., Patra, A., Mandal, A., Mondal, N. S., Dey, S., Mondal, A. K., ... & Ghosh, A. R. (2022). Choline Chloride Induces Growth Performance of Indian Major Carps and Air-Breathing Fish Species with an Outcome of Quality Food-Fish under a Semi-Intensive Culture System: A Biochemical Investigation. ACS omega, 7(17), 14579-14590.

David, A., Lange, A., Tyler, C. R., & Hill, E. M. (2018). Concentrating mixtures of neuroactive pharmaceuticals and altered neurotransmitter levels in the brain of fish exposed to a wastewater effluent. Science of the Total Environment, 621, 782-790. https://doi.org/10.1016/j.scitotenv.2017.11.265

del Carmen Gómez-Regalado, M., Martín, J., Santos, J. L., Aparicio, I., Alonso, E., & Zafra-Gómez, A. (2022). Bioaccumulation/bioconcentration of pharmaceutical active compounds in

aquatic organisms: Assessment and factors database. Science of The Total Environment, 160638.

Diao, P., Chen, Q., Wang, R., Sun, D., Cai, Z., Wu, H., & Duan, S. (2017). Phenolic endocrine-disrupting compounds in the Pearl River Estuary: Occurrence, bioaccumulation and risk assessment. Science of the Total Environment, 584, 1100-1107.

Duarte, I. A., Fick, J., Cabral, H. N., & Fonseca, V. F. (2022). Bioconcentration of neuroactive pharmaceuticals in fish: Relation to lipophilicity, experimental design and toxicity in the aquatic environment. Science of The Total Environment, 812, 152543.

Dürig, W., Kintzi, A., Golovko, O., Wiberg, K., & Ahrens, L. (2020). New extraction method prior to screening of organic micropollutants in various biota matrices using liquid chromatography coupled to high-resolution time-of-flight mass spectrometry. Talanta, 219, 121294.

U.S. EPA. (2010). TSCA New Chemicals Program (NCP) Chemical Categories. Washington, DC.

Fedorova, G., Nebesky, V., Randak, T., & Grabic, R. (2014). Simultaneous determination of 32 antibiotics in aquaculture products using LC-MS/MS. Chemical Papers, 68(1), 29-36. https://doi.org/10.2478/s11696-013-0428-3

Fitzgerald, J. A., Könemann, S., Krümpelmann, L., Županič, A., & Vom Berg, C. (2021). Approaches to test the neurotoxicity of environmental contaminants in the zebrafish model: From behavior to molecular mechanisms. Environmental Toxicology and Chemistry, 40(4), 989-1006.

Froehner, S., Rizzi, J., Vieira, L. M., & Sanez, J. (2018). PAHs in water, sediment and biota in an area with port activities. Archives of environmental contamination and toxicology, 75(2), 236-246.

Gadelha, J. R., Rocha, A. C., Camacho, C., Eljarrat, E., Peris, A., Aminot, Y., ... & Almeida, C. M. R. (2019). Persistent and emerging pollutants assessment on aquaculture oysters (Crassostrea gigas) from NW Portuguese coast (Ria De Aveiro). Science of the Total Environment, 666, 731-742.

Garcia, S. N., Foster, M., Constantine, L. A., & Huggett, D. B. (2012). Field and laboratory fish tissue accumulation of the anti-convulsant drug carbamazepine. Ecotoxicology and environmental safety, 84, 207-211. https://doi.org/10.1016/j.ecoenv.2012.07.013

Grabicova, K., Grabic, R., Fedorova, G., Fick, J., Cerveny, D., Kolarova, J., ... & Randak, T. (2017). Bioaccumulation of psychoactive pharmaceuticals in fish in an effluent dominated stream. Water research, 124, 654-662. https://doi.org/10.1016/j.watres.2017.08.018

Grabicova, K., Staňová, A. V., Ucun, O. K., Borik, A., Randak, T., & Grabic, R. (2018). Development of a robust extraction procedure for the HPLC-ESI-HRPS determination of multi-residual pharmaceuticals in biota samples. Analytica chimica acta, 1022, 53-60. https://doi.org/10.1016/j.aca.2018.04.011

Grabicová, K., Staňová, A. V., Švecová, H., Nováková, P., Kodeš, V., Leontovyčová, D., ... & Grabic, R. (2022). Invertebrates differentially bioaccumulate pharmaceuticals: Implications for

routine biomonitoring. Environmental Pollution, 309, 119715. https://doi.org/10.1016/j.envpol.2022.119715

Ghosh, M., Sharma, N., Singh, A. K., Gera, M., Pulicherla, K. K., & Jeong, D. K. (2018). Transformation of animal genomics by next-generation sequencing technologies: a decade of challenges and their impact on genetic architecture. Critical Reviews in Biotechnology, 38(8), 1157-1175.

Gilroy ÈAM, Gillis PL, King LE, Bendo NA, Salerno J, Gioacomin M, de Solla SR (2017) The effects of pharmaceuticals on a unionid mussel (Lampsilis siliquoidea): an examination of acute and chronic endpoints of toxicity across life stages. Environ Toxicol Chem 36:1572–1593. https://doi.org/10.1002/etc.3683

Goessens, T., Huysman, S., De Troyer, N., Deknock, A., Goethals, P., Lens, L., ... & Croubels, S. (2020). Multi-class analysis of 46 antimicrobial drug residues in pond water using UHPLC-Orbitrap-HRMS and application to freshwater ponds in Flanders, Belgium. Talanta, 220, 121326.

Groh, K. J., Carvalho, R. N., Chipman, J. K., Denslow, N. D., Halder, M., Murphy, C. A., ... & Watanabe, K. H. (2015). Development and application of the adverse outcome pathway framework for understanding and predicting chronic toxicity: I. Challenges and research needs in ecotoxicology. Chemosphere, 120, 764-777.

Gu, X., Xu, L., Wang, Z., Ming, X., Dang, P., Ouyang, W., ... & Wang, B. (2021). Assessment of cadmium pollution and subsequent ecological and health risks in Jiaozhou Bay of the Yellow Sea. Science of the Total Environment, 774, 145016.

Guo, C., Gong, L., Wang, W., Leng, J., Zhou, L., Xing, S., Zhao, Y., Xian, R., Zhang, X. & Shi, F. (2020). Rapid screening and identification of targeted or non-targeted antitussive adulterants in herbal medicines by Q-Orbitrap HRMS and screening database. International Journal of Mass Spectrometry, 447, 116250.

Habibi, N., Uddin, S., Lyons, B., Al-Sarawi, H. A., Behbehani, M., Shajan, A., Razzack, N. A., Zakir, F., & Alam, F.. (2022). Antibiotic Resistance Genes Associated with Marine Surface Sediments: A Baseline from the Shores of Kuwait. Sustainability, 14(13), 8029. https://doi.org/10.3390/su14138029

Hanson, S., Steeves, K., Bagatim, T., Hogan, N., Wiseman, S., Hontela, A., Giesy, J., Paul, J., & Hecker, M. (2021). Health status of fathead minnow (Pimephales promelas) populations in a municipal wastewater effluent-dominated stream in the Canadian prairies, Wascana Creek, Saskatchewan. Aquatic Toxicology, 238, 105933.

Hollender, J., Van Bavel, B., Dulio, V., Farmen, E., Furtmann, K., Koschorreck, J., ... & Tornero, V. (2019). High resolution mass spectrometry-based non-target screening can support regulatory environmental monitoring and chemicals management. Environmental Sciences Europe, 31(1), 1-11.

Jagiello, K., Halappanavar, S., Rybińska-Fryca, A., Willliams, A., Vogel, U., & Puzyn, T. (2021). Transcriptomics-Based and AOP-Informed Structure–Activity Relationships to Predict Pulmonary Pathology Induced by Multiwalled Carbon Nanotubes. Small, 17(15), 2003465.

Ji, X., Challis, J. K., Cantin, J., Perez, A. S. C., Gong, Y., Giesy, J. P., & Brinkmann, M. (2022). Desorption kinetics of antipsychotic drugs from sandy sediments by diffusive gradients in thin-films technique. Science of The Total Environment, 155104. https://doi.org/10.1016/j.scitotenv.2022.155104

Khan, H. K., Rehman, M. Y. A., & Malik, R. N. (2020). Fate and toxicity of pharmaceuticals in water environment: An insight on their occurrence in South Asia. Journal of Environmental Management, 271, 111030.

Kim, J.-H., Kong, T. Y., Moon, J.-Y., Choi, K. H., Cho, Y.-Y., Kang, H. C., Lee, J. Y., & Lee, H. S.. (2018). Targeted and non-targeted metabolite identification of MAM-2201 in human, mouse, and rat hepatocytes. Drug Testing and Analysis, 10(8), 1328–1335. https://doi.org/10.1002/dta.2389

Lai, A., Singh, R. R., Kovalova, L., Jaeggi, O., Kondić, T., & Schymanski, E. L. (2021). Retrospective non-target analysis to support regulatory water monitoring: from masses of interest to recommendations via in silico workflows. Environmental Sciences Europe, 33(1), 1-21.

Lakhey, N., Sierra-Alvarez, R., Couger, M. B., Krzmarzick, M. J., & Field, J. A. (2021). Anammox enrichment culture has unexpected capabilities to biotransform azole contaminants of emerging concern. Chemosphere, 264, 128550.

Llamas-Dios, M. I., Vadillo, I., Jiménez-Gavilán, P., Candela, L., & Corada-Fernández, C. (2021). Assessment of a wide array of contaminants of emerging concern in a Mediterranean water basin (Guadalhorce river, Spain): Motivations for an improvement of water management and pollutants surveillance. Science of the Total Environment, 788, 147822.

Martínez, R., Codina, A. E., Barata, C., Tauler, R., Piña, B., & Navarro-Martín, L. (2020). Transcriptomic effects of tributyltin (TBT) in zebrafish eleutheroembryos. A functional benchmark dose analysis. Journal of hazardous materials, 398, 122881.

Matus, G. N., Pereira, B. V., Silva-Zacarin, E., Costa, M. J., Cordeiro Alves dos Santos, A., & Nunes, B. (2018). Behavior and histopathology as biomarkers for evaluation of the effects of paracetamol and propranolol in the neotropical fish species Phalloceros harpagos. Environmental Science and Pollution Research, 25(28), 28601-28618.

McCallum, E. S., Krutzelmann, E., Brodin, T., Fick, J., Sundelin, A., & Balshine, S. (2017). Exposure to wastewater effluent affects fish behaviour and tissue-specific uptake of pharmaceuticals. Science of the Total Environment, 605, 578-588. https://doi.org/10.1016/j.scitotenv.2017.06.073

Mijangos, L., Ziarrusta, H., Zabaleta, I., Usobiaga, A., Olivares, M., Zuloaga, O., Etxebarria, N. & Prieto, A. (2019). Multiresidue analytical method for the determination of 41 multiclass organic pollutants in mussel and fish tissues and biofluids by liquid chromatography coupled to tandem mass spectrometry. Analytical and bioanalytical chemistry, 411(2), 493-506. https://doi.org/10.1007/s00216-018-1474-z

Miller, T. H., Bury, N. R., Owen, S. F., MacRae, J. I., & Barron, L. P. (2018). A review of the pharmaceutical exposome in aquatic fauna. Environmental Pollution, 239, 129-146.

Muir, D., Simmons, D., Wang, X., Peart, T., Villella, M., Miller, J., & Sherry, J. (2017). Bioaccumulation of pharmaceuticals and personal care product chemicals in fish exposed to wastewater effluent in an urban wetland. Scientific reports, 7(1), 1-11. https://doi.org/10.1038/s41598-017-15462-x

Munawar, N., Ahsan, K., Muhammad, K., Ahmad, A., Anwar, M. A., Shah, I., ... & Al Mughairbi, F. (2021). Hidden role of gut microbiome dysbiosis in schizophrenia: Antipsychotics or psychobiotics as therapeutics?. International Journal of Molecular Sciences, 22(14), 7671.

Nallani, G. C., Edziyie, R. E., Paulos, P. M., Venables, B. J., Constantine, L. A., & Huggett, D. B. (2016). Bioconcentration of two basic pharmaceuticals, verapamil and clozapine, in fish. Environmental Toxicology and Chemistry, 35(3), 593-603.

Nilsen, E., Smalling, K. L., Ahrens, L., Gros, M., Miglioranza, K. S., Picó, Y., & Schoenfuss, H. L. (2019). Critical review: grand challenges in assessing the adverse effects of contaminants of

emerging concern on aquatic food webs. Environmental Toxicology and Chemistry, 38(1), 46-60.

Nikel, K. E., McCallum, E. S., Mehdi, H., Du, S. N., Bowman, J. E., Midwood, J. D., ... & Balshine, S. (2021). Fish living near two wastewater treatment plants have unaltered thermal tolerance but show changes in organ and tissue traits. Journal of Great Lakes Research, 47(2), 522-533.

Oberoi, A. S., Jia, Y., Zhang, H., Khanal, S. K., & Lu, H. (2019). Insights into the fate and removal of antibiotics in engineered biological treatment systems: a critical review. Environmental Science & Technology, 53(13), 7234-7264.

Pan, Y. K., Mandic, M., Zimmer, A. M., & Perry, S. F. (2019). Evaluating the physiological significance of hypoxic hyperventilation in larval zebrafish (Danio rerio). Journal of Experimental Biology, 222(13), jeb204800.

Park, N., Choi, Y., Kim, D., Kim, K., & Jeon, J. (2018). Prioritization of highly exposable pharmaceuticals via a suspect/non-target screening approach: a case study for Yeongsan River, Korea. Science of the total environment, 639, 570-579.

Peng, X., Xu, Q., Guo, Y., & Zhang, B. (2022). Effects of Environmentally Relevant Concentrations of Antipsychotic Drugs (Sulpiride and Clozapine) on Serotonergic and Dopaminergic Neurotransmitter Systems in Octopus Brain Tissue. Water, 14(17), 2608.

Pereira, B. V. R., Matus, G. N., Costa, M. J., Santos, A. C. A. D., Silva-Zacarin, E. C. M., Do Carmo, J. B., & Nunes, B.. (2018). Assessment of biochemical alterations in the neotropical

fish species Phalloceros harpagos after acute and chronic exposure to the drugs paracetamol and propranolol. Environmental Science and Pollution Research, 25(15), 14899–14910. https://doi.org/10.1007/s11356-018-1699-6

Pourchet, M., Debrauwer, L., Klanova, J., Price, E. J., Covaci, A., Caballero-Casero, N., ... & Antignac, J. P. (2020). Suspect and non-targeted screening of chemicals of emerging concern for human biomonitoring, environmental health studies and support to risk assessment: from promises to challenges and harmonisation issues. Environment international, 139, 105545.

Purschke, K., Zoell, C., Leonhardt, J., Weber, M., & Schmidt, T. C. (2020). Identification of unknowns in industrial wastewater using offline 2D chromatography and non-target screening. Science of The Total Environment, 706, 135835.

Radošević, K., Bubalo, M. C., Srček, V. G., Grgas, D., Dragičević, T. L., & Redovniković, I. R. (2015). Evaluation of toxicity and biodegradability of choline chloride based deep eutectic solvents. Ecotoxicology and environmental safety, 112, 46-53.

Ramirez, A. J., Brain, R. A., Usenko, S., Mottaleb, M. A., O'Donnell, J. G., Stahl, L. L., ... & Chambliss, C. K. (2009). Occurrence of pharmaceuticals and personal care products in fish: results of a national pilot study in the United States. Environmental Toxicology and Chemistry, 28(12), 2587-2597.

Santos, L. H., Araújo, A. N., Fachini, A., Pena, A., Delerue-Matos, C., & Montenegro, M. C. B. S. M. (2010). Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. Journal of hazardous materials, 175(1-3), 45-95.

Schuijt, L. M., Peng, F. J., van den Berg, S. J., Dingemans, M. M., & Van den Brink, P. J. (2021). (Eco) toxicological tests for assessing impacts of chemical stress to aquatic ecosystems: Facts, challenges, and future. Science of the Total Environment, 795, 148776. https://doi.org/10.1016/j.scitotenv.2021.148776

Soufan, O., Ewald, J., Zhou, G., Hacariz, O., Boulanger, E., Alcaraz, A. J., ... & Xia, J. (2022). EcoToxXplorer: Leveraging Design Thinking to Develop a Standardized Web-Based Transcriptomics Analytics Platform for Diverse Users. Environmental toxicology and chemistry, 41(1), 21-29.

Sumpter, J. P., & Margiotta-Casaluci, L. (2022). Environmental occurrence and predicted pharmacological risk to freshwater fish of over 200 neuroactive pharmaceuticals in widespread use. Toxics, 10(5), 233.

Sussman, E. M., Oktem, B., Isayeva, I. S., Liu, J., Wickramasekara, S., Chandrasekar, V., Nahan, K., Shin, H. Y., & Zheng, J.. (2022). Chemical Characterization and Non-targeted Analysis of Medical Device Extracts: A Review of Current Approaches, Gaps, and Emerging Practices. ACS Biomaterials Science & Engineering, 8(3), 939–963. https://doi.org/10.1021/acsbiomaterials.1c01119

Tetreault, G. R., Bennett, C. J., Cheng, C., Servos, M. R., & McMaster, M. E. (2012). Reproductive and histopathological effects in wild fish inhabiting an effluent-dominated stream, Wascana Creek, SK, Canada. Aquatic Toxicology, 110, 149-161.

Van Den Brink, N. W., Kokalj, A. J., Silva, P. V., Lahive, E., Norrfors, K., Baccaro, M., ... & Van Gestel, C. A. (2019). Tools and rules for modelling uptake and bioaccumulation of nanomaterials in invertebrate organisms. Environmental Science: Nano, 6(7), 1985-2001.

Vinken, M. (2019). Omics-based input and output in the development and use of adverse outcome pathways. Current Opinion in Toxicology, 18, 8-12.

Waiser, M. J., Tumber, V., & Holm, J.. (2011). Effluent-dominated streams. Part 1: Presence and effects of excess nitrogen and phosphorus in Wascana Creek, Saskatchewan, Canada. Environmental Toxicology and Chemistry, 30(2), 496–507. https://doi.org/10.1002/etc.399

Wang, H., Xi, H., Xu, L., Jin, M., Zhao, W., & Liu, H. (2021). Ecotoxicological effects, environmental fate and risks of pharmaceutical and personal care products in the water environment: a review. Science of The Total Environment, 788, 147819.

Writer, J. H., Ferrer, I., Barber, L. B., & Thurman, E. M.. (2013). Widespread occurrence of neuro-active pharmaceuticals and metabolites in 24 Minnesota rivers and wastewaters. Science of the Total Environment, 461-462, 519–527. https://doi.org/10.1016/j.scitotenv.2013.04.099

Xiao, H., Krauss, M., Floehr, T., Yan, Y., Bahlmann, A., Eichbaum, K., ... & Hollert, H. (2016). Effect-directed analysis of aryl hydrocarbon receptor agonists in sediments from the Three Gorges Reservoir, China. Environmental Science & Technology, 50(20), 11319-11328.

Xie, Z., Lu, G., Liu, J., Yan, Z., Ma, B., Zhang, Z., & Chen, W. (2015). Occurrence, bioaccumulation, and trophic magnification of pharmaceutically active compounds in Taihu Lake, China. Chemosphere, 138, 140-147.

Xie, Z., Lu, G., Yan, Z., Liu, J., Wang, P., & Wang, Y. (2017). Bioaccumulation and trophic transfer of pharmaceuticals in food webs from a large freshwater lake. Environmental Pollution, 222, 356-366. https://doi.org/10.1016/j.envpol.2016.12.026

Yang, H., Lu, G., Yan, Z., Liu, J., Dong, H., Bao, X., ... & Sun, Y. (2020). Residues, bioaccumulation, and trophic transfer of pharmaceuticals and personal care products in highly urbanized rivers affected by water diversion. Journal of hazardous materials, 391, 122245.

Zhou, Y., Wu, J., Wang, B., Duan, L., Zhang, Y., Zhao, W., ... & Yu, G. (2020). Occurrence, source and ecotoxicological risk assessment of pesticides in surface water of Wujin District (northwest of Taihu Lake), China. Environmental Pollution, 265, 114953.

CHAPTER 4: SYNTHESIS AND RECOMMENDATIONS FOR FUTURE WORK

4.1 Summary of findings

The increasing production and consumption of new chemicals released into aquatic ecosystems are raising concerns of unknown toxic effects. This is especially common for wastewater discharges that reach surface water bodies. This study used a holistic approach to evaluate potential impacts of environmental factors (physicochemical parameters, nutrients, seasonal variations) on the levels, bioavailability, and potential effects of emerging contaminants such as pharmaceuticals. As discussed in previous chapters, concentrations of pharmaceuticals were evaluated from chemical and biological perspectives.

Based on field investigations, the occurrence, trends, and seasonal dynamics of pharmaceuticals and their interactions with physicochemical parameters upstream and downstream of two WWTPs located in the SSR and WC, allowed to determine their levels and resulting effects in fish. Antipsychotic pharmaceuticals were the selected group for this research, including AMI, BUP, CBZ, CLO, FLX, LAM, VEN that were monitored in sediments, water by conventional grab and passive sampler (to assess different sampling methods) during three seasons (Spring, Summer, and Fall of 2021). The distribution of pharmaceuticals in the SSR and WC according to the data collected ranged from 0.1 ng/L to 10.7 µg/L in water and 0.02 ng/kg to 173 ng/kg in sediment. Most pharmaceuticals showed higher levels in WC than the SSR. AMI was the pharmaceutical with

maximum concentration in water and sediment, up and downstream of both sites, and during the three seasons. Concentrations detected here were among the highest ever detected globally, thereby indicating the pressing need for environmental regulation of these chemicals.

Kd values provide insights into the partitioning of pharmaceuticals between water and sediment (Koba et al., 2018; Zhou et al., 2019), to determine the mobility and persistence of compounds in different study matrices. In this case, even though the concentrations of pharmaceuticals varied considerably both in time and space, Kd values confirmed that the detected pharmaceuticals had the potential to persist in the water bodies for long periods of time even if their emission should eventually cease due to the installation of advanced treatment facilities (Golovko et al., 2020).

Given the high levels identified in the SSR and WC, a screening-level risk assessment was conducted to evaluate potential toxicological risks to aquatic life. An immediate toxicological risk was detected for pharmaceutical concentrations downstream WC, for all organisms and all sampling seasons. These results reinforced the importance of integrative risk assessment considering species from relevant trophic levels of the environmental systems (USEPA, 2002) and showed a challenge for water resource management that should give priority to pharmaceuticals.

The initial findings showed a high toxicological risk to the receiving aquatic ecosystem at WC, with fish as the most sensitive group. Therefore, this study focusses all the efforts on developing

an integrated approach for chemical analysis and transcriptome changes to prioritize contaminants that are contributing to the pollution of WC and their resulting toxic effects.

In general, target chemical analysis showed that time and location were variables with significant influence on pharmaceutical concentrations, and the inclusion of physicochemical parameters in the statistical analysis allowed for a better accuracy of model predictions to define changes and trends. The data obtained showed a wide distribution of pharmaceuticals in water, sediment, and fish samples from WC. CLO was the target pharmaceutical with higher concentrations in fathead minnows across the three seasons and both sampling locations (up and downstream in WC). Among water, sediment, and fish samples, maximum concentrations of pharmaceuticals detected were several orders of magnitude greater than those measured in comparable studies at other locations. As has been demonstrated for antiallergic drugs, consumption rates of these pharmaceuticals tend to change depending on the season of the year (Philip et al., 2002; Häder et al., 2020; Muz et al., 2020). It is reasonable to expect that at least a subset of antipsychotic drugs might also show seasonal trends that mirror prescription numbers (Kurian et al., 2007; Kamble et al., 2015).

BAF and BSAF coefficients are useful criteria to determine the availability of contaminants for uptake from environmental compartments into biota. Studies related to the bioaccumulation of pharmaceuticals are scarce and almost absent under field conditions for most of these chemicals (Miller et al., 2018; Obeiro et al., 2019, Duarte et al., 2022). To our best knowledge, this is the first

study determining BAFs and BSAFs of pharmaceuticals under field conditions in WC. BAF and BSAF showed that CLO was accumulating in fish under specific environmental conditions (like pH and DOC). These differences in bioavailability might be linked to the different ionization behaviour of the chemical at varying pHs (Diao et al., 2017).

Non-target analysis found that pharmaceuticals, rubber components, and PCPs, were present in all studied water (by conventional grab and DGT), sediment, and fish samples, indicating that these emerging contaminants may be classified as ubiquitous in WC. Pharmaceuticals were the group with relevant priority between the top 10 of compounds found. This group showed the highest relative abundance in water and sediment samples. Data obtained through qPCR analysis were consistent with the most affected toxicity pathways predicted from non-target analysis. Effects on signalling, which was the top most affected pathway in the present study, are mostly driven by exposure to pharmaceuticals, which was the main chemical category and top pollutant class prioritized in the present study.

Overall, the integrative approach used in this study strongly supports the need to combine chemical analysis with effect-based transcriptomic approaches as a useful tool for assessing the risks of complex mixtures of contaminants in wastewater discharges.

4.2 Recommendations for future works

This research constitutes a comprehensive study of the impacts of wastewater effluents and seasonal trends on levels of ECs, specifically pharmaceuticals. However, additional efforts could be done to further evaluate some of the findings.

Comparisons between concentrations of pharmaceuticals in the SSR and WC (Chapter 2) showed that results may influenced by the flow conditions at each sampling locations and season. Future investigations could evaluate additional sampling locations further downstream of the wastewater discharge to evaluate the severity and extent of the impact of contaminants from wastewater effluents.

Water sampling was conducted using two methodologies (Chapter 2 and 3), conventional grab sampling and DGT. The latter showed the lowest variability between the replicates which provides support for the broader application of this technique for future research. This will ultimately help improve the quality of the collected samples and the correlation with changes caused by interactions between chemicals and environmental factors.

Conducting both target and non-target chemical analyses (Chapter 2 and 3) showed the importance of including environmental factors in the mixed models to obtain a better description of the variability of pharmaceuticals concentrations. Even though the environmental factors were not significant with respect to the concentrations of pharmaceuticals, the results supported the relevance of physicochemical parameters to understand chemical variations in aquatic ecosystems.

This study provides an initial analytical approach using non-target chemical analysis and transcriptomics in fathead minnows as a bioindicator of chemical stress to help prioritize contaminants that are typically found in increasingly complex mixtures in environmental samples and potentially causing negative effects. Therefore, this study provides a blueprint for similar efforts in different aquatic ecosystems impacted by wastewater effluents.

References

Diao, P., Chen, Q., Wang, R., Sun, D., Cai, Z., Wu, H., & Duan, S. (2017). Phenolic endocrine-disrupting compounds in the Pearl River Estuary: Occurrence, bioaccumulation and risk assessment. Science of the Total Environment, 584, 1100-1107.

Duarte, I. A., Fick, J., Cabral, H. N., & Fonseca, V. F. (2022). Bioconcentration of neuroactive pharmaceuticals in fish: Relation to lipophilicity, experimental design and toxicity in the aquatic environment. Science of The Total Environment, 812, 152543.

Golovko, O., Rehrl, A.-L., Köhler, S., & Ahrens, L. (2020). Organic micropollutants in water and sediment from Lake Mälaren, Sweden. *Chemosphere,* 258, 127293. doi:10.1016/j.chemosphere.2020.127293

Häder, D. P., Banaszak, A. T., Villafañe, V. E., Narvarte, M. A., González, R. A., & Helbling, E. W. (2020). Anthropogenic pollution of aquatic ecosystems: Emerging problems with global implications. *Science of the Total environment*, 713, 136586. https://doi.org/10.1016/j.scitotenv.2020.136586

Kamble, P., Chen, H., Johnson, M. L., Bhatara, V., & Aparasu, R. R. (2015). Concurrent Use of Stimulants and Second-Generation Antipsychotics Among Children With ADHD Enrolled in Medicaid. Psychiatric Services, 66(4), 404–410. doi:10.1176/appi.ps.201300391

Koba, O., Grabicova, K., Cerveny, D., Turek, J., Kolarova, J., Randak, T., Zlabek, V., & Grabic, R. (2018). Transport of pharmaceuticals and their metabolites between water and sediments

as a further potential exposure for aquatic organisms. *Journal of hazardous materials,* 342, 401-407. https://doi.org/10.1016/j.jhazmat.2017.08.039.

Kurian, B. T., Ray, W. A., Arbogast, P. G., Fuchs, D. C., Dudley, J. A., & Cooper, W. O. (2007). Effect of Regulatory Warnings on Antidepressant Prescribing for Children and Adolescents. Archives of Pediatrics & Adolescent Medicine, 161(7), 690. doi:10.1001/archpedi.161.7.690

Miller, T. H., Bury, N. R., Owen, S. F., MacRae, J. I., & Barron, L. P. (2018). A review of the pharmaceutical exposome in aquatic fauna. Environmental pollution, 239, 129-146.

Muz, M., Escher, B. I., & Jahnke, A. (2020). Bioavailable Environmental Pollutant Patterns in Sediments from Passive Equilibrium Sampling. *Environmental Science & Technology, 54*(24), 15861-15871. https://doi.org/10.1021/acs.est.0c05537

Oberoi, A. S., Jia, Y., Zhang, H., Khanal, S. K., & Lu, H. (2019). Insights into the fate and removal of antibiotics in engineered biological treatment systems: a critical review. Environmental Science & Technology, 53(13), 7234-7264.

Philip, G., Malmstrom, K., Hampel, F. C., Weinstein, S. F., Laforce, C. F., Ratner, P. H., Malice, M.-P., & Reiss, T. F.. (2002). Montelukast for treating seasonal allergic rhinitis: a randomized, double-blind, placebo-controlled trial performed in the spring. *Clinical & Experimental Allergy*, 32(7), 1020–1028. https://doi.org/10.1046/j.1365-2222.2002.01422.x

USEPA, E. (2002). Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. United States Environmental Protection Agency, Office of Water, Washington.

Zhou, S., Di Paolo, C., Wu, X., Shao, Y., Seiler, T. B., & Hollert, H. (2019). Optimization of screening-level risk assessment and priority selection of emerging pollutants–the case of pharmaceuticals in European surface waters. Environment international, 128, 1-10.

APPENDICES

Appendix A: Statistical analysis

Statistical analysis of target screening results was conducted using analysis of variance (ANOVA) test, correlations, and mixed models were conducted using package lme4 in the language R (Bates et al., 2022; CRAN-Package lme4 (r-project.org)). In the case, of non-target screening results excel was used for calculations, as well as package ggvenn in the language R (Bates et al., 2022; CRAN-Package ggvenn (r-project.org)).

The qPCR results were analyzed in the EcoToxXprorer (EcoToxXplorer) using the EcoToxChip Analysis (EcoToxXplorer) where the Differential expression analysis option was selected for *P. promelas* (fathead minnow) version 1.0 in Specify chip size of 384, applying a test non-parametric (KW/H-test), using the results of upstream as a control group.

The results of the characteristics of the fish used for the analysis of target compounds were compared by means of an analysis of variance. the results of the target pharmaceutical compounds, as well as the BAF and BSAF coefficients, as they did not present conditions to perform a classic analysis of variance, were evaluated through the use of multilevel mixed models (Pleil et al., 2018), where two options were proposed, a first, called M1, which does not incorporate the physical chemical parameters of the water samples (temperature, pH, SC, DO and TDS) and a second, called M2, which does incorporate them. For the analysis of each compound in the targets in fish, both models will incorporate the variables of the place of sample collection (Ups-Down-streams), the

collection season (spring, summer and fall), as well as the reported values of these compounds in water, DGT and sediment. While the models that seek to describe the variability of the coefficients only had the site and the time of year, since they are determined based on the concentrations of pharmaceuticals in DGT and sediments. Similarly, the Pearson correlation coefficient was obtained between the results of the targets and the physical-chemical parameters of TP, NH_4, DOC, and temperature, as well as for each of the bioaccumulation coefficients (BAF and BASF).

Appendix B: Tables and Figures

Table B.1. Comparison of concentrations of pharmaceuticals in water and sediments.

Pharmaceutical	This study	Literature			This study	Literature			This study	Literature	
	Water (grab sample) (ng/L)			*CEC (ng/L)	Water (passive sampler) (ng/L)			Technique	Sediments (ng/g)		
AMI	0.50-3,350	2.00 - 10.3 (1)	UK	48.0	1.70 - 10,700	15.0 - 500 (9)	Ukraine France	POCIS	0.60 - 173	0.23 - 2.45 (15)	USA
		196 (2)	Brazil			1.00 - 2.20 (10)	Portugal			0.10 – 1.00 (8)	Sweden
BUP	0.10-122	0.74 - 3.70(3)	Shanghai	116	1.09 - 529	3.60 – 18.0 (12)	USA	POCIS	0.02 - 6.30	1.08 - 2.12 (16)	USA
CBZ	3.00-244	2,300 (4)	Europe	346,000	3.10 - 990	7,000 - 7,750 (9)	Ukraine France	POCIS	0.03 - 9.00	0.95 - 6.16 (17)	South Africa
		0.94 - 350 (5)	USA			280 - 450 (14)	Canada	DGT		46.5 (18)	UK
CLO	0.80-88.1	45.5 (6)	Vietnam	321,000	1.10 - 281	68.0 - 113 (11)	Cyprus	Hydrogel	0.06 - 30.3	17.9 - 18.5 (17)	South Africa
FLX	0.70-9.00	2.01 - 25.4 (5)	Portugal	489	2.30 - 6.7	35.0 – 50.0 (9)	Ukraine France	POCIS	0.20 - 2.80	0.40 - 19.4 (16)	USA
		410 (2)	Brazil			0.50 - 2.50 (10)	Portugal			1.58 - 2.53 (19)	Portugal
LAM	11.6-1,010	1.20 - 2,780 (7)	USA Europe	1.40 10^6	7.20 - 5,580	6.10 - 220 (13)	Swiss	**PES	0.08 - 16.4	0.70 - 1.20 (8)	Sweden
		50.0 (8)	Sweden			90.0 - 590 (14)	Canada	DGT			
VEN	0.70-1,220	2.20 - 20.6 (1)	UK	6,110	1.00 - 7,980	2.40 – 94.0 (13)	Swiss	**PES	0.07 - 2.90	1.60 - 26.1 (16)	USA
		1,310 (2)	Brazil			70.0 - 730 (14)	Canada	DGT		0.25 - 5.56 (19)	Portugal

(*) CEC = critical environmental concentrations (Fick et al., 2010); (**) Empore™ disks/ PES membranes; (1) Burns et al., 2018; (2) Gould et al., 2021; (3) Bunting et al., 2021; (4) Ma et al., 2018; (5) Quesada et al., 2019; (6) Lei et al., 2021; (7) Cardoso-Vera et al., 2021; (8) Golovko et al., 2020; (9) Vystavna*et al*., 2012; (10) Gonzalez-rey *et al.,*2015; (11) Alygizakis *et al.,* 2020; (12) Costa *et al.,*2017; (13) Moschet *et al.,* 2015; (14) Challis *et al.,* 2020; (15) Long et al., 2013; (16) Schultz et al., 2010; (17) Matongo et al. 2015; (18) Zhou and Broodbank, 2014; (19) Fernandes et al., 2019.

Table B.2. Positive mode suspect screening gradient elution method. Flow rate = 0.2 mL/min, column temperature = 40 oC, solvent A = 95% H2O: 5% MeOH + 0.1% formic acid and B = 100% MeOH + 0.1% formic acid.

Time (min)	%B
0.00	5
7.50	40
15.00	100
20.00	100
20.10	5
25.00	5

Table B.3. Morphometric measurements and sex of fish samples collected in up- and downstream the City of Regina's WWTP.

Season	Location	Sex	Standard length (cm)	Total length (cm)	Weight (dry) (g)
Spring	Upstream	Fem/Mal	4.25 ± 0.92 [a]	5.20 ± 1.11 [a]	0.34 ± 0.19 [a]
	Downstream	Fem/Mal	4.47 ± 0.49 [a]	5.33 ± 0.53 [a]	0.47 ± 0.12 [a]
Summer	Upstream	Juvenile	2.88 ± 0.18 [a]	3.51 ± 0.21 [a]	0.07 ± 0.02 [a]
	Downstream	Juvenile	2.30 ± 0.12 [a]	2.76 ± 0.11 [b]	0.05 ± 0.01 [a]
	Downstream	Fem/Mal	2.94 ± 0.25 [a]	3.55 ± 0.25 [a]	0.10 ± 0.02 [a]
Fall	Upstream	Fem/Mal	2.83 ± 0.15 [a]	3.42 ± 0.23 [a]	0.08 ± 0.02 [a]
	Downstream	Fem/Mal	2.84 ± 0.16 [a]	3.41 ± 0.19 [a]	0.08 ± 0.03 [a]

Note: same letter between measurements indicates significant differences for a p-value < 0.05

Table B.4. Physicochemical properties of targeted pharmaceuticals.

Compound	Abbreviation	CAS	Molecular weight (g/mol)	log K_{ow}	pKa
Carbamazepine	CBZ	298-46-4	236.27	2.45	15.96
Fluoxetine	FLX	56296-78-7	345.79	4.65	9.80

Compound	Abbreviation	CAS	Molecular weight (g/mol)	log K_{ow}	pKa
Venlafaxine	VLF	99300-78-4	227.604	3.28	8.91/14.42
Bupropion	BPP	31677-93-7	276.2	3.85	8.22
Amitriptyline	AMT	549-18-8	313.86	4.95	9.76
Clozapine	CLZ	5786-21-0	326.82	3.35	7.35/ 15.9
Lamotrigine	LAM	84057-84-1	256.09	2.57	5.7

pKa was from Chemicalize.org, http://www.chemicalize.org; *log* K_{ow} was estimated values from database of ChemSpider (EPISuite), http://www.chemspider.com; *log* D_{ow} was calculated referred to Scherrer and Howard (1977).

Table B.5. Statistics of mixed models (M1 and M2) related to concentration of pharmaceuticals in target screening analysis of fathead minnow samples.

Pharmaceutical	Model	AIC	R^2
AMI	M1	-6032.1	0.97
	M2	-5492.9	0.97
BUP	M1	17814.4	0.43
	M2	17802.3	0.43
CBZ	M1	-10474.5	0.96
	M2	-5940.1	0.96
CLO	M1	-10474.5	0.96
	M2	-1812.0	0.93
FLX	M1	-3051.7	0.82
	M2	-14668.1	0.77
LAM	M1	-3485.7	0.97
	M2	-10033.1	0.97
VEN	M1	-8299.4	0.97
	M2	1154.7	0.97

Table B.6. Pearson correlation between environmental factors and concentrations of pharmaceuticals in fathead minnow samples.

Sampling location[1]	Pharmaceutical/ environmental factors/morphology	AMI	BUP	CBZ	CLO	FXL	LAM	VEN
Upstream	DOC				-0.745	0.999		
	NH_4				0.979	-0.570		
	TP				0.649	-0.994		
	Temperature				0.572	-0.979		
	Standard length				0.947	-0.467		
	Toral length				0.952	-0.479		
	Wet weight				0.944	-0.460		
	Dry weight				0.933	-0.431		
Downstream	DOC	0.285	0.541	0.468	0.999	-0.535	0.993	0.024
	NH_4	0.430	0.403	0.325	0.980	-0.398	0.964	0.179
	TP	0.769	-0.985	-0.996	-0.429	0.986	-0.492	0.910
	Temperature	-0.581	-0.238	-0.156	-0.931	0.232	-0.903	-0.347
	Standard length	0.822	-0.968	-0.985	-0.350	0.969	-0.416	0.942
	Toral length	0.821	-0.968	-0.986	-0.352	0.970	-0.418	0.942
	Wet weight	0.753	-0.990	-0.998	-0.452	0.991	-0.514	0.899
	Dry weight	0.763	-0.987	-0.997	-0.438	0.988	-0.501	0.906

[1]With respect to the City of Regina WWTP

Table B.7. Statistics of mixed models (M1 and M2) related to bioconcentration (BAF) of pharmaceuticals in target screening analysis for fathead minnow samples.

Pharmaceutical	Model	AIC	R^2
AMI	M1	4117	0.64
	M2	4125	0.64
BUP	M1	5454.5	0.55
	M2	5457.9	0.55
CBZ	M1	1181.4	0.93
	M2	1189.4	0.93
CLO	M1	11938	0.45
	M2	11945.7	0.45
FLX	M1	5078.5	0.18
	M2	5085.2	0.18

Pharmaceutical	Model	AIC	R^2
LAM	M1	2203.1	0.73
	M2	2210.7	0.73
VEN	M1	7363.1	0.57
	M2	7371.1	0.57

Table B.8. Statistics of mixed models (M1 and M2) related to biota-sediment accumulation (BSAF) of pharmaceuticals in target screening analysis of fathead minnow samples.

Pharmaceutical	Model	AIC	R^2
AMI	M1	-5280.9	0.90
	M2	-5373.5	0.93
BUP	M1	5294.3	0.47
	M2	5302.3	0.47
CBZ	M1	-3331.1	0.98
	M2	-3310.4	0.98
CLO	M1	863.1	0.82
	M2	974.0	0.82
FLX	M1	-5228.2	0.91
	M2	-2769.4	0.90
LAM	M1	3895.3	0.22
	M2	3901.5	0.23
VEN	M1	7096.6	0.30
	M2	7104.6	0.30

Table B.9. Pearson correlation between environmental factor and BAF of target analysis in fathead minnow

Sampling location[1]	Pharmaceutical/ Environmental factors	AMI	BUP	CBZ	CLO	FXL	LAM	VEN
Upstream	DOC				-0.534	0.996		
	NH_4				0.997	-0.525		
	TP				0.415	-0.998		
	Temperature				0.325	-0.988		
Dowstream	DOC	-0.510	-0.854	-0.937	-0.774	-0.271	-0.383	-0.451

Sampling location[1]	Pharmaceutical/ Environmental factors	AMI	BUP	CBZ	CLO	FXL	LAM	VEN
	NH_4	-0.370	-0.925	-0.980	-0.666	-0.118	-0.235	-0.307
	TP	0.991	-0.141	0.048	0.885	0.991	0.999	0.997
	Temperature	0.203	0.977	0.999	0.526	-0.055	0.062	0.137

[1]With respect to the City of Regina WWTP

Table B.10. Pearson correlation between environmental factor and BSAF of target analysis in fathead minnow

Sampling location[1]	Chemical / EF	AMI	BUP	CBZ	CLO	FXL	LAM	VEN
Upstream	DOC				-0.659	0.999		
	NH_4				0.996	-0.630		
	TP				0.551	-0.983		
	Temperature				0.467	-0.961		
Dowstream	DOC	0.958	0.999	0.998	0.997	0.998	0.996	0.704
	NH_4	0.991	0.989	0.979	0.972	0.994	0.971	0.805
	TP	-0.114	-0.384	-0.433	-0.461	-0.342	-0.467	0.3774
	Temperature	-0.999	-0.948	-0.929	-0.917	-0.961	-0.915	-0.896

[1]With respect to the City of Regina WWTP

EF = Environmental Factor

Table B.11. Coefficient of variation (%) for qPCR analysis across replicates.

Season	Location	Brain	Liver
Summer	Ups	0.38-13.15	0.025-14.93
	Dow	0.56-35.84	0.002-18.23
Fall	Ups	0.5-25.16	0.69-28.22
	Dow	0.7-18.05	1.68-15.64

Table B.12. List of significantly dysregulated genes obtained from the qPCR analysis conducted in brain samples during summer 2021.

Gene	Description of gene's functions	Upstream	Downstream
abcc6b.2	Multidrug resistance-associated protein 1-like	31.55±0.92	30.68±1.72
apoba	Apolipoprotein Ba	30.41±0.85	29.98±1.17
gngt2b	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-T2-like	30.28±1.38	30.39±1.7
sdha	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	24.59±0.61	30.1±4.04
tcf7l2	Transcription factor 7 like 2	25.49±1.25	29.14±2.35
ccl20a.3	Chemokine (C-C motif) ligand 20a, duplicate 3	32.87±1.15	32.19±1.52
gapdh	Glyceraldehyde-3-phosphate dehydrogenase	29.4±1.27	29.29±1.73
hsp90aa1.1	Heat shock protein HSP 90-alpha	30.65±1.42	30.38±1.56
lct	Lactase	32.63±0.96	31.36±1.76
adcy1	Adenylate cyclase type gene 1	27.38±0.27	28.48±1.37
ccnd2b	Cyclin D2, b	33.24±0.77	31.88±1.66
ahrrb	Aryl-hydrocarbon receptor repressor b	36.86±0.14	32.14±0
cdk4	Cyclin-dependent kinase 4	31.44±1.77	31.15±1.07
cyp11c1	Cytochrome P450, family 11, subfamily C, polypeptide 1	33.05±0.96	31.4±2.18
cyp17a1	Cytochrome P450, family 17, subfamily A, polypeptide 1	31.77±0.54	30.79±1.27
cyp21a2	Cytochrome P450, family 21, subfamily A, polypeptide 2	28.79±0.9	29.06±0.43
epha2b	Ephrin type-A receptor 2-like	33.08±3.61	32.35±1.17
fam114a1	Family with sequence similarity 114 member A1	35.13±1.67	35.49±2.46
g6pd	Glucose-6-phosphate dehydrogenase	34.55±1.13	31.76±1.01
hnf4g	Hepatocyte nuclear factor 4, gamma	32.56±1.21	32.42±2.27
plk4	Serine/threonine-protein kinase PLK4 isoform X1	36.04±0.56	34.09±2.08
rock1	Rho-associated, coiled-coil containing protein kinase 1	33.05±1.19	31.55±2.8
rpl13	60S ribosomal protein L13	33.06±1.18	32.13±1.29
tg	Thyroglobulin	32.08±1.41	31.9±1.59
casp3a	Caspase 3, apoptosis-related cysteine peptidase a	29.78±1.08	30.45±0.99
dio1	Iodothyronine deiodinase 1	35.59±1.41	35.8±2.21
fosab	V-fos FBJ murine osteosarcoma viral oncogene homolog Ab	32.61±1.14	32.12±1.56

Gene	Description of gene's functions	Upstream	Downstream
gsto1	Glutathione S-transferase omega gene 1	31.11±1.33	30.27±1.04
irf1a	Interferon regulatory factor 1a	25.56±1.14	28.4±2.37
nedd4l	E3 ubiquitin-protein ligase NEDD4-like isoform X14	31.8±0.43	32.14±1.92
nr1h4	Nuclear receptor subfamily 1, group H, member 4	25.27±1.57	28.33±2.2
nr2f2	Nuclear receptor subfamily 2, group F, member 2	34.06±2.89	31.64±1.78
pparda	Peroxisome proliferator-activated receptor delta a	26.83±1.05	29.49±1.67
tfap2a	Transcription factor AP-2 alpha	31.17±1.7	31.34±1.22
ttr	Transthyretin	29.83±1.74	29.05±1.66
vtg1	Vitellogenin 1	32.25±0.48	31.97±1.49

Table B.13. List of significantly dysregulated genes obtained from the qPCR analysis conducted in brain samples during fall 2021.

Gene	Description of gene's functions	Upstream	Downstream
cyp1a	Cytochrome P450, family 1, subfamily A	26.31±1.22	21.15±1.13
fabp3	Fatty acid binding protein 3, muscle and heart	25.97±1.34	25.69±1.6
agt	Angiotensinogen	27.02±1.83	27.49±2.41
hsp70.3	Heat shock cognate 70-kd protein, tandem-like gene 3	26.07±1.22	27.29±1.91
il6	Interleukin 6 (interferon, beta 2)	27.45±1.36	28.63±1.96
ccnt1	Cyclin T1	28.37±1.15	29.74±2.72
cyb561	Cytochrome b561	26.08±1.32	27.3±2.04
spata2	Spermatogenesis-associated protein gene 6	26.05±1.26	26.98±1.9
sec61al1	Protein transport protein Sec61 subunit alpha-like 1	25.64±1.43	25.56±1.82
apoba	Apolipoprotein Ba	30.12±1.73	31.47±1.34
g6pd	Glucose-6-phosphate dehydrogenase	31.22±2.51	32.87±1.4
mgmt	O-6-methylguanine-DNA methyltransferase	31.43±1.68	32.48±2.13
tapbp	Tpsn protein	26.18±1.46	27.75±2.55
tspo	Translocator protein-like	26.89±1.24	28.17±2.09
atr	ATR serine/threonine kinase	27.22±1.26	28.3±2.62
hmgcra	3-hydroxy-3-methylglutaryl-CoA reductase a	25.73±1.15	25.17±1.63
nrxn3a	Neurexin-3a-like isoform X2	25.6±1.4	27.42±3.47
rorb	RAR-related orphan receptor B	28.18±1.96	28.63±2.46

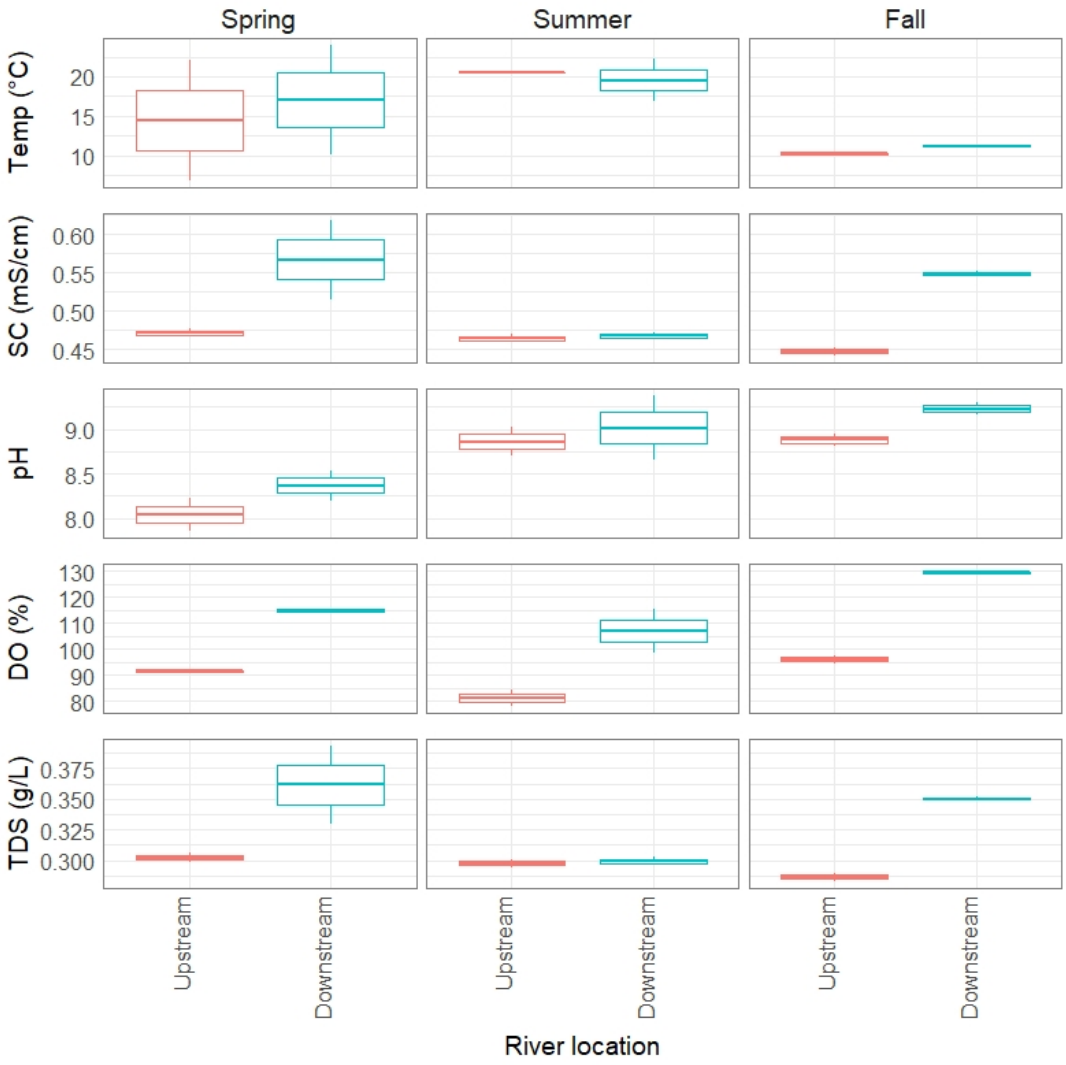


Figure B.1. Physicochemical parameters of the water of the collected samples in South Saskatchewan River near Saskatoon, Saskatchewan, Canada.

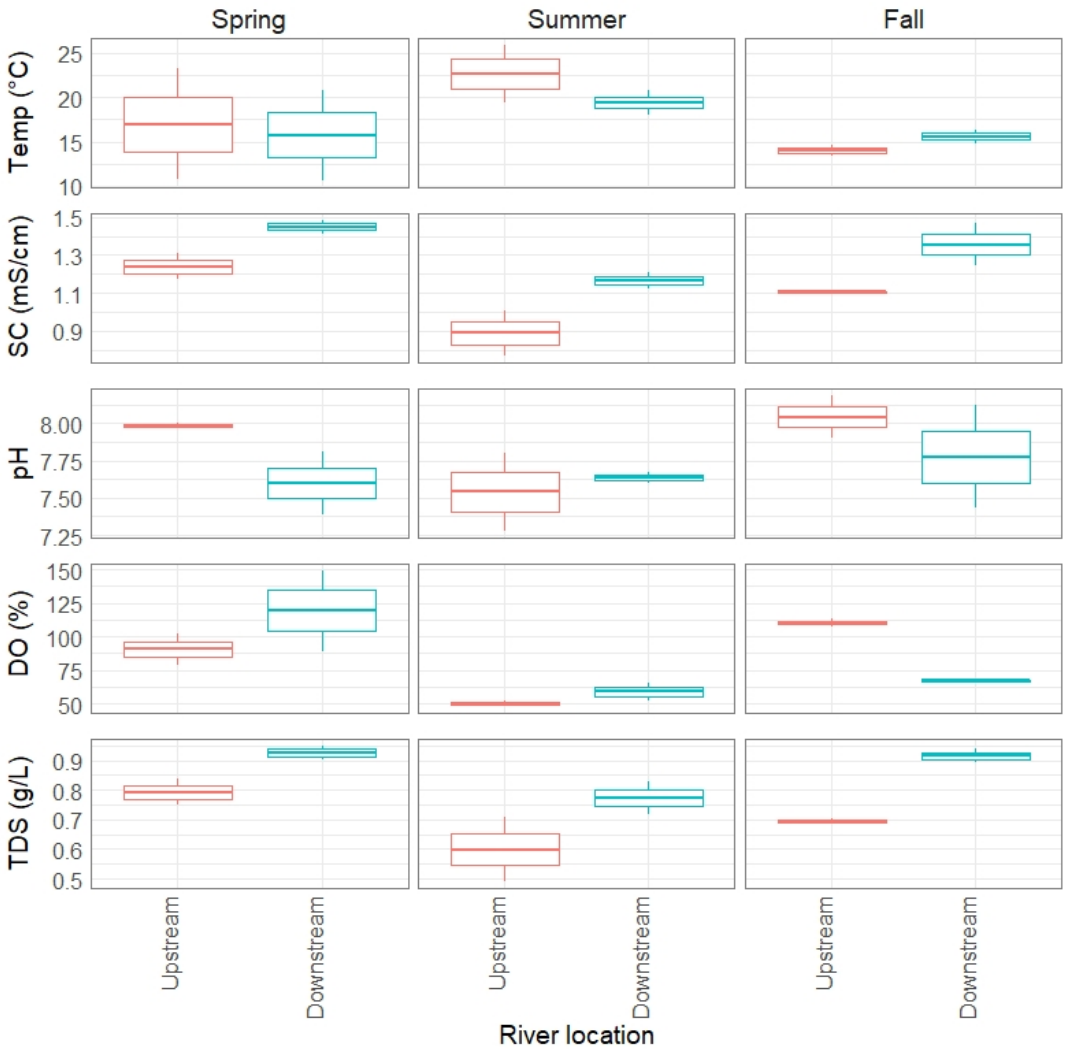


Figure B.2. Physicochemical parameters of the water of the collected samples in Wascana Creek near Regina, Saskatchewan, Canada.

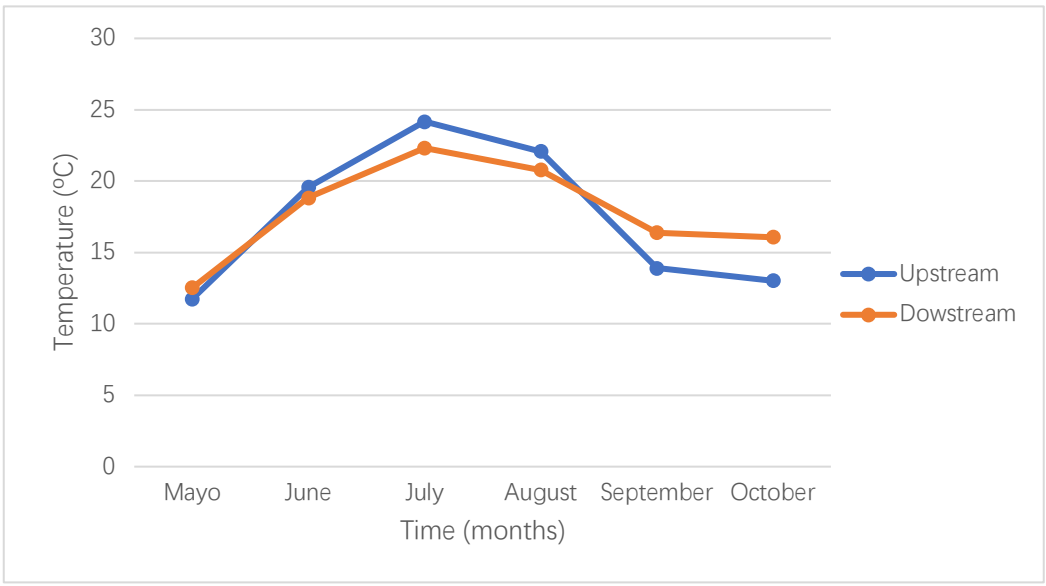


Figure B.1. Temperature variation during spring, summer, and fall 2021.

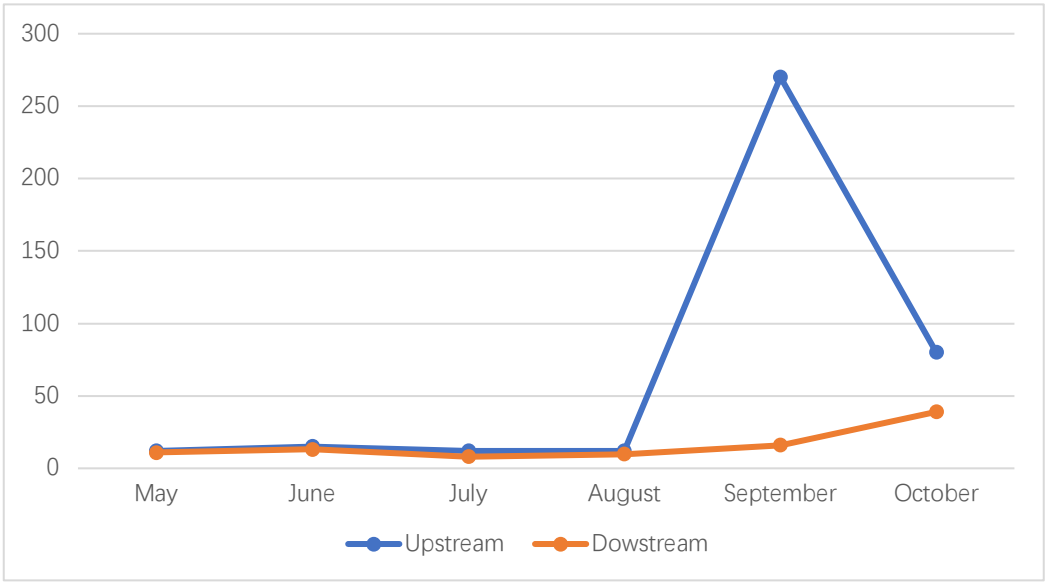


Figure B.2. Dissolved Organic Carbon (DOC) variation during spring, summer, and fall 2021.

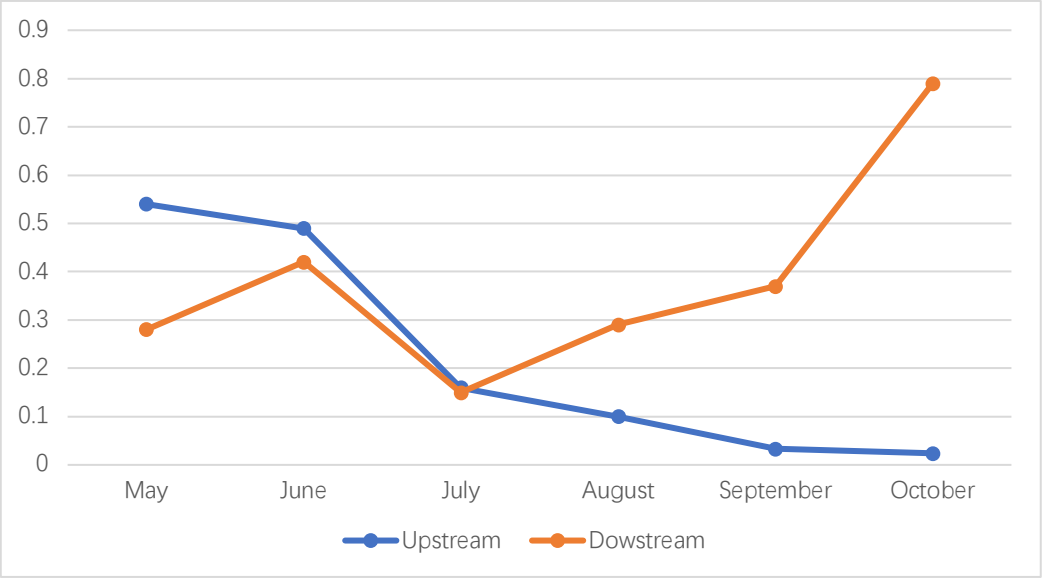


Figure B.3. Total ammonia variation during spring, summer, and fall 2021.

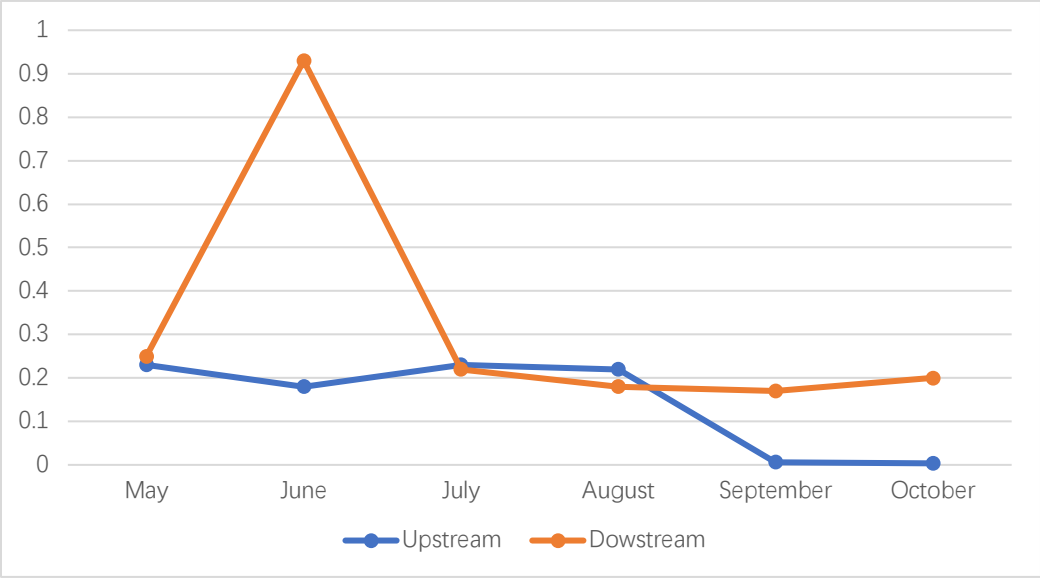


Figure B.4. Total Phosphorous variation during spring, summer, and fall 2021.

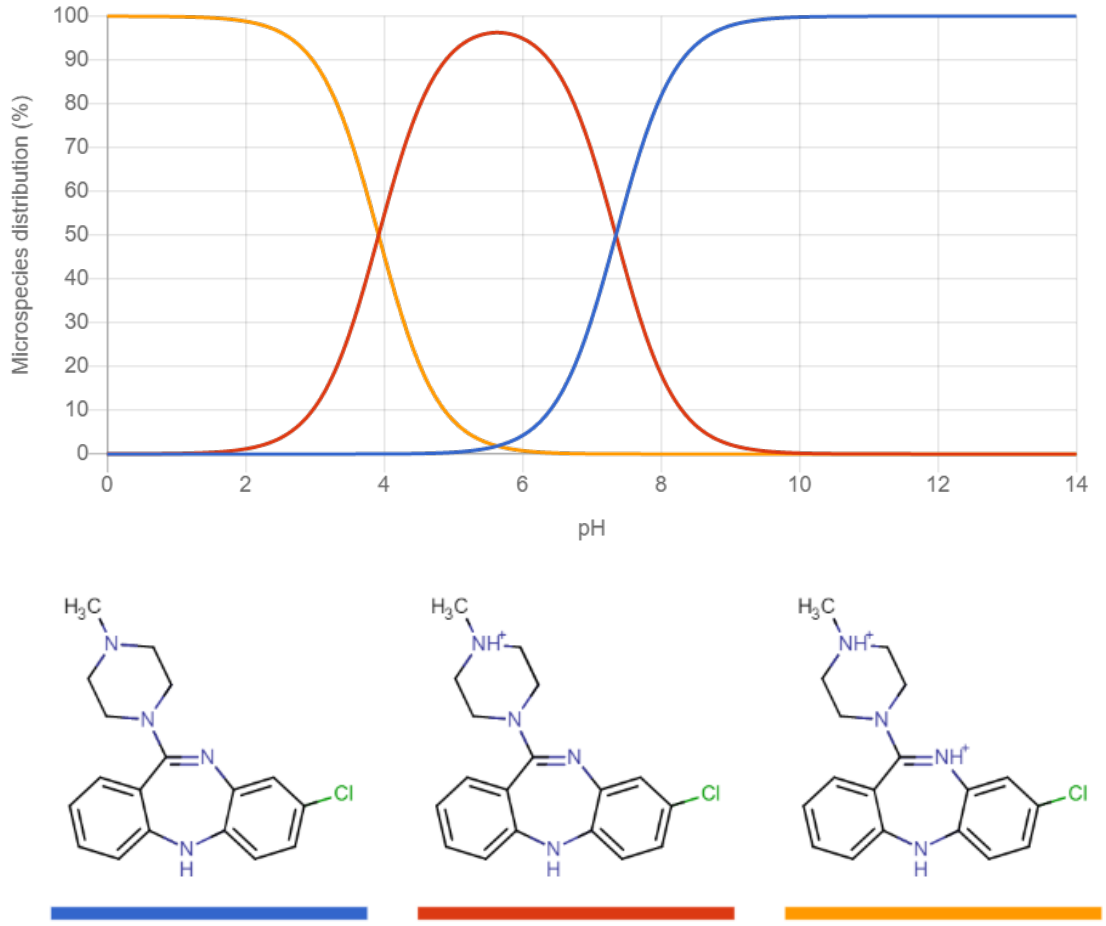


Figure B.5. The microspecies distribution of clozapine with varying pHs predicted using Chemicalize software (accessed in November, 2022; https://chemicalize.com developed by ChemAxon, http://www.chemaxon.com).

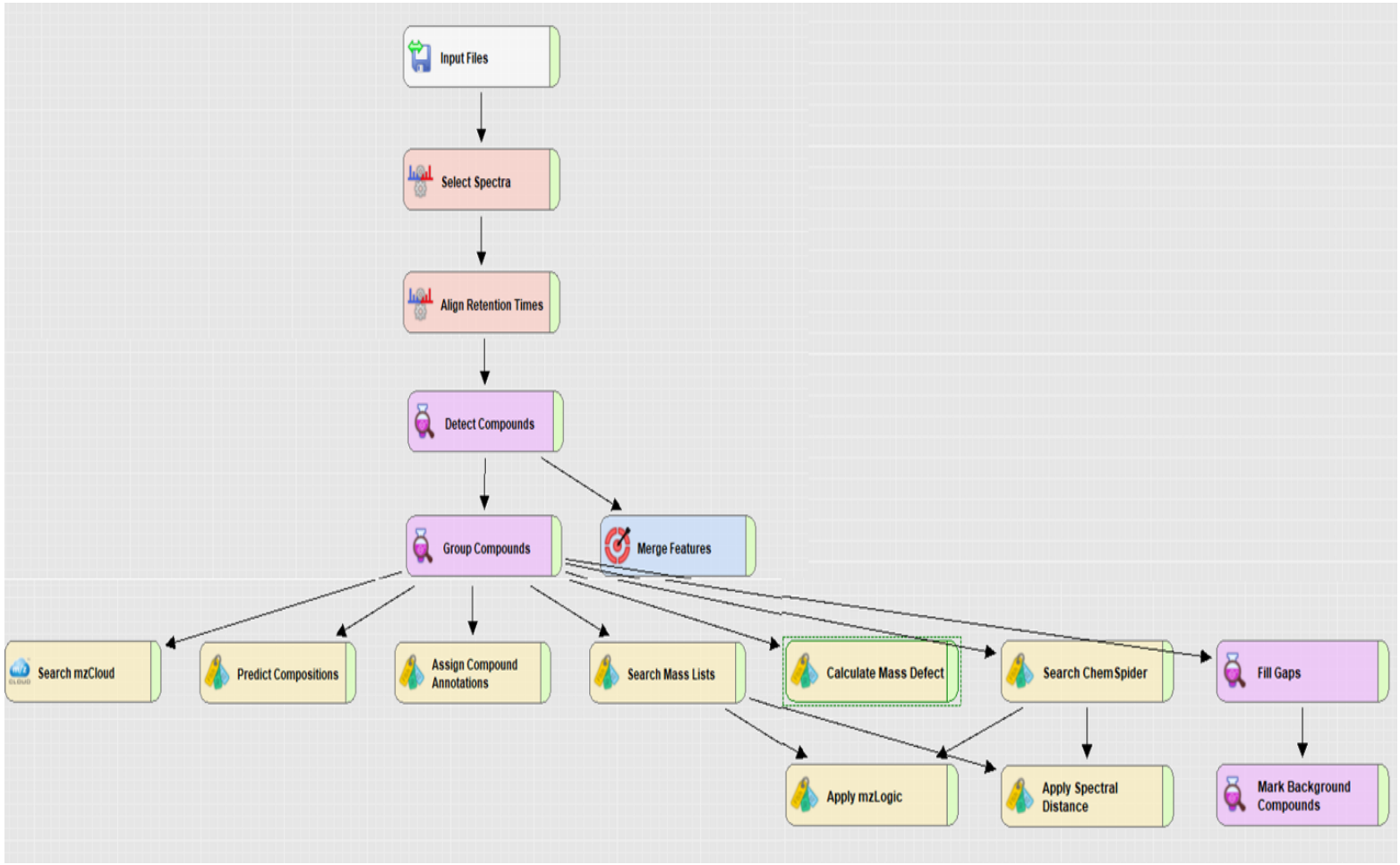
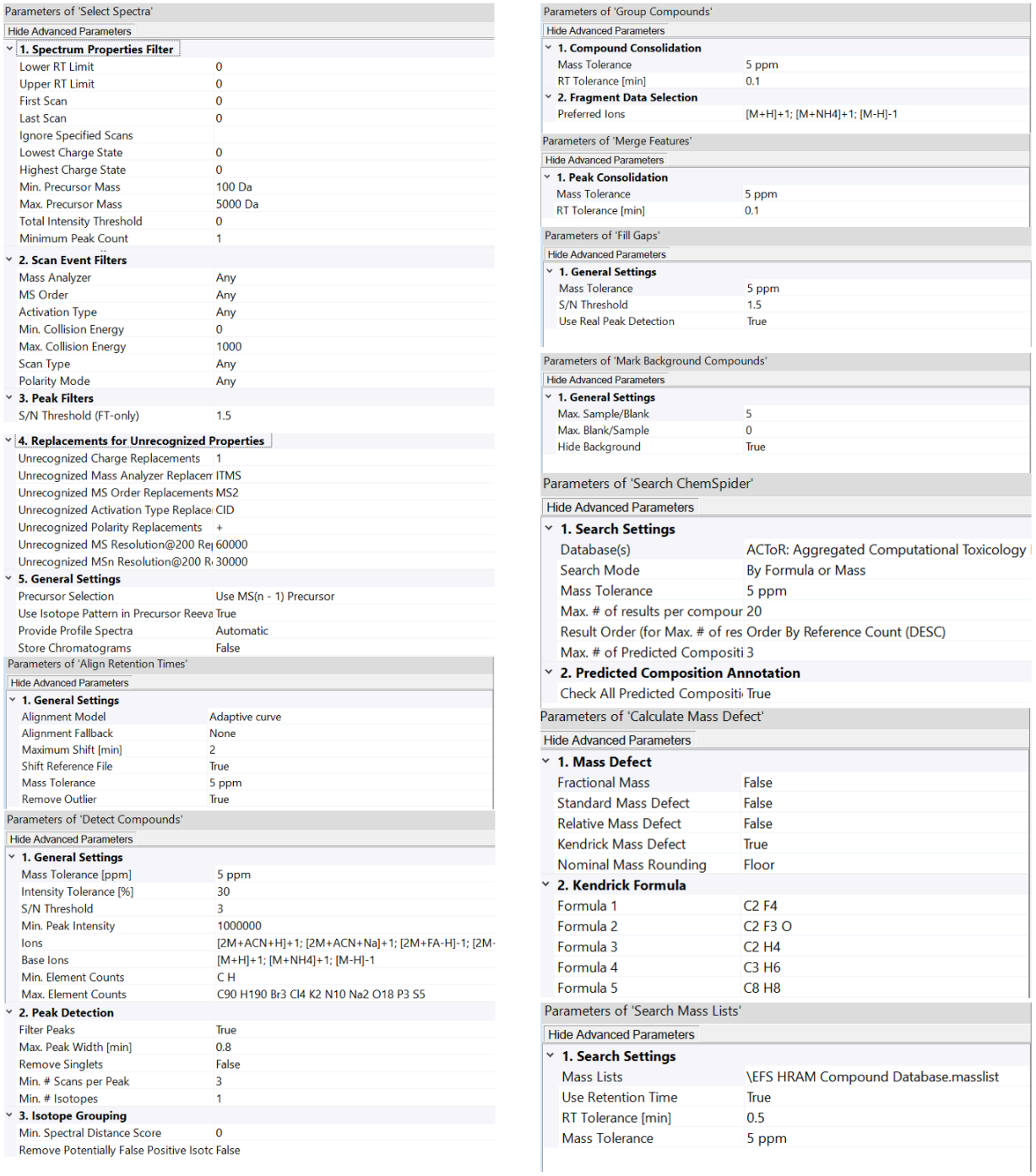


Figure B.6. Compound Discoverer v 3.2 data workflow



Parameters of 'Select Spectra'

Hide Advanced Parameters

1. Spectrum Properties Filter	
Lower RT Limit	0
Upper RT Limit	0
First Scan	0
Last Scan	0
Ignore Specified Scans	
Lowest Charge State	0
Highest Charge State	0
Min. Precursor Mass	100 Da
Max. Precursor Mass	5000 Da
Total Intensity Threshold	0
Minimum Peak Count	1
2. Scan Event Filters	
Mass Analyzer	Any
MS Order	Any
Activation Type	Any
Min. Collision Energy	0
Max. Collision Energy	1000
Scan Type	Any
Polarity Mode	Any
3. Peak Filters	
S/N Threshold (FT-only)	1.5
4. Replacements for Unrecognized Properties	
Unrecognized Charge Replacements	1
Unrecognized Mass Analyzer Replacem	ITMS
Unrecognized MS Order Replacements	MS2
Unrecognized Activation Type Replace	CID
Unrecognized Polarity Replacements	+
Unrecognized MS Resolution@200 Re	60000
Unrecognized MSn Resolution@200 R	30000
5. General Settings	
Precursor Selection	Use MS(n - 1) Precursor
Use Isotope Pattern in Precursor Reeva	True
Provide Profile Spectra	Automatic
Store Chromatograms	False

Parameters of 'Align Retention Times'

Hide Advanced Parameters

1. General Settings	
Alignment Model	Adaptive curve
Alignment Fallback	None
Maximum Shift [min]	2
Shift Reference File	True
Mass Tolerance	5 ppm
Remove Outlier	True

Parameters of 'Detect Compounds'

Hide Advanced Parameters

1. General Settings	
Mass Tolerance [ppm]	5 ppm
Intensity Tolerance [%]	30
S/N Threshold	3
Min. Peak Intensity	1000000
Ions	[2M+ACN+H]+1; [2M+ACN+Na]+1; [2M+FA-H]-1; [2M-
Base Ions	[M+H]+1; [M+NH4]+1; [M-H]-1
Min. Element Counts	C H
Max. Element Counts	C90 H190 Br3 Cl4 K2 N10 Na2 O18 P3 S5
2. Peak Detection	
Filter Peaks	True
Max. Peak Width [min]	0.8
Remove Singlets	False
Min. # Scans per Peak	3
Min. # Isotopes	1
3. Isotope Grouping	
Min. Spectral Distance Score	0
Remove Potentially False Positive Isotc	False

Parameters of 'Group Compounds'

Hide Advanced Parameters

1. Compound Consolidation	
Mass Tolerance	5 ppm
RT Tolerance [min]	0.1
2. Fragment Data Selection	
Preferred Ions	[M+H]+1; [M+NH4]+1; [M-H]-1

Parameters of 'Merge Features'

Hide Advanced Parameters

1. Peak Consolidation	
Mass Tolerance	5 ppm
RT Tolerance [min]	0.1

Parameters of 'Fill Gaps'

Hide Advanced Parameters

1. General Settings	
Mass Tolerance	5 ppm
S/N Threshold	1.5
Use Real Peak Detection	True

Parameters of 'Mark Background Compounds'

Hide Advanced Parameters

1. General Settings	
Max. Sample/Blank	5
Max. Blank/Sample	0
Hide Background	True

Parameters of 'Search ChemSpider'

Hide Advanced Parameters

1. Search Settings	
Database(s)	ACToR: Aggregated Computational Toxicology
Search Mode	By Formula or Mass
Mass Tolerance	5 ppm
Max. # of results per compour	20
Result Order (for Max. # of res	Order By Reference Count (DESC)
Max. # of Predicted Compositi	3
2. Predicted Composition Annotation	
Check All Predicted Compositi	True

Parameters of 'Calculate Mass Defect'

Hide Advanced Parameters

1. Mass Defect	
Fractional Mass	False
Standard Mass Defect	False
Relative Mass Defect	False
Kendrick Mass Defect	True
Nominal Mass Rounding	Floor
2. Kendrick Formula	
Formula 1	C2 F4
Formula 2	C2 F3 O
Formula 3	C2 H4
Formula 4	C3 H6
Formula 5	C8 H8

Parameters of 'Search Mass Lists'

Hide Advanced Parameters

1. Search Settings	
Mass Lists	\EFS HRAM Compound Database.masslist
Use Retention Time	True
RT Tolerance [min]	0.5
Mass Tolerance	5 ppm

Figure B.7a. Compound Discoverer v 3.2 data workflow settings.

Parameters of 'Assign Compound Annotations'

Hide Advanced Parameters

1. General Settings	
Mass Tolerance	5 ppm
2. Data Sources	
Data Source #1	mzCloud Search
Data Source #2	mzVault Search
Data Source #3	MassList Search
Data Source #4	Predicted Compositions
Data Source #5	ChemSpider Search
Data Source #6	
Data Source #7	
3. Scoring Rules	
Use mzLogic	True
Use Spectral Distance	True
SFit Threshold	20
SFit Range	20

Parameters of 'Predict Compositions'

Hide Advanced Parameters

1. Prediction Settings	
Mass Tolerance	5 ppm
Min. Element Counts	C H
Max. Element Counts	C90 H190 Br3 Cl8 F18 N10 O18 P3 S5
Min. RDBE	0
Max. RDBE	40
Min. H/C	0.1
Max. H/C	3.5
Max. # Candidates	10
Max. # Internal Candidates	500
2. Pattern Matching	
Intensity Tolerance [%]	30
Intensity Threshold [%]	0.1
S/N Threshold	3
Min. Spectral Fit [%]	30
Min. Pattern Cov. [%]	80
Use Dynamic Recalibration	True
3. Fragments Matching	
Use Fragments Matching	True
Mass Tolerance	5 ppm
S/N Threshold	3

Parameters of 'Search mzCloud'

Hide Advanced Parameters

1. General Settings	
Compound Classes	All
Precursor Mass Tolerance	10 ppm
FT Fragment Mass Tolerance	10 ppm
IT Fragment Mass Tolerance	0.4 Da
Library	Autoprocessed; Reference
Post Processing	Recalibrated
Max. # Results	10
Annotate Matching Fragments	True

Parameters of 'Search mzCloud'

Hide Advanced Parameters

2. DDA Search	
Identity Search	Cosine
Match Activation Type	True
Match Activation Energy	Match with Tolerance
Activation Energy Tolerance	20
Apply Intensity Threshold	True
Similarity Search	None
Match Factor Threshold	30
3. DIA Search	
Use DIA Scans for Search	True
Max. Isolation Width [Da]	500
Match Activation Type	False
Match Activation Energy	Any
Activation Energy Tolerance	100
Apply Intensity Threshold	True
Match Factor Threshold	20

Parameters of 'Apply Spectral Distance'

Hide Advanced Parameters

1. Pattern Matching	
Mass Tolerance	5 ppm
Intensity Tolerance [%]	30
Intensity Threshold [%]	0.1
S/N Threshold	3
Use Dynamic Recalibration	True

Parameters of 'Apply mzLogic'

Hide Advanced Parameters

1. Search Settings	
FT Fragment Mass Tolerance	10 ppm
IT Fragment Mass Tolerance	0.4 Da
Max. # Compounds	0
Max. # mzCloud Similarity Resu	10
Match Factor Threshold	30

Figure B.7b. Compound Discoverer v 3.2 data workflow settings.

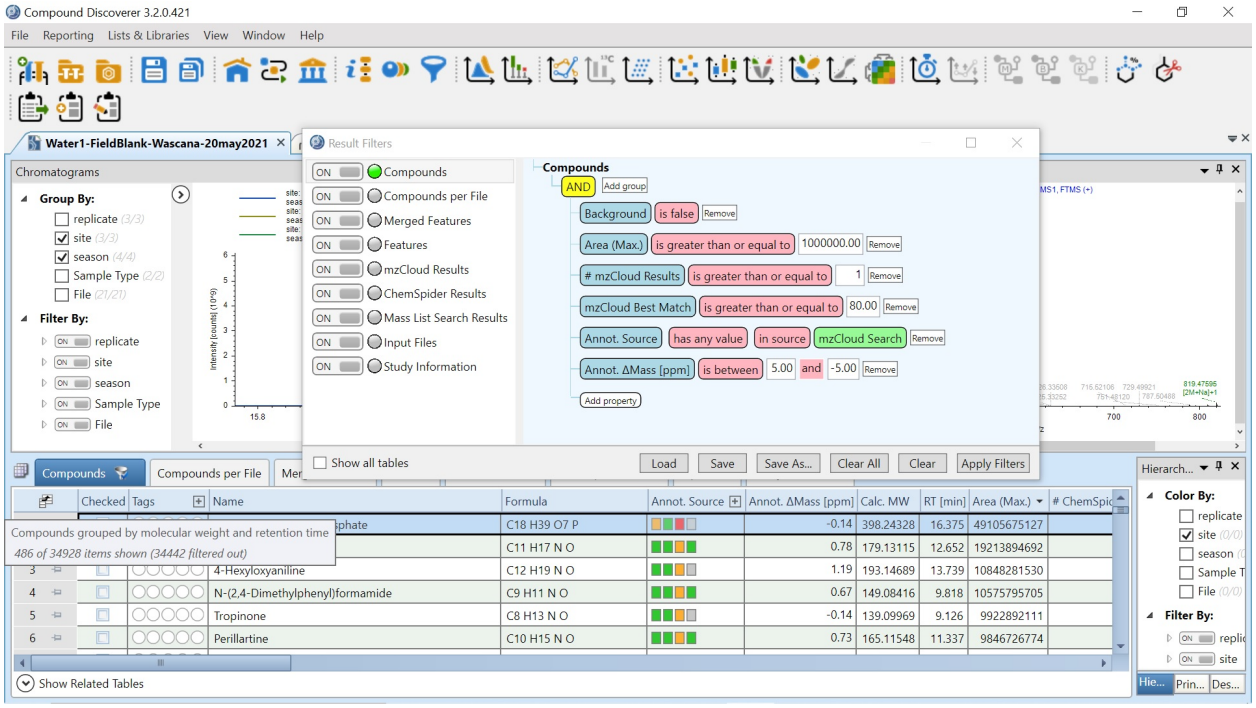


Figure B.8. Compound Discoverer v3.2 data filters.

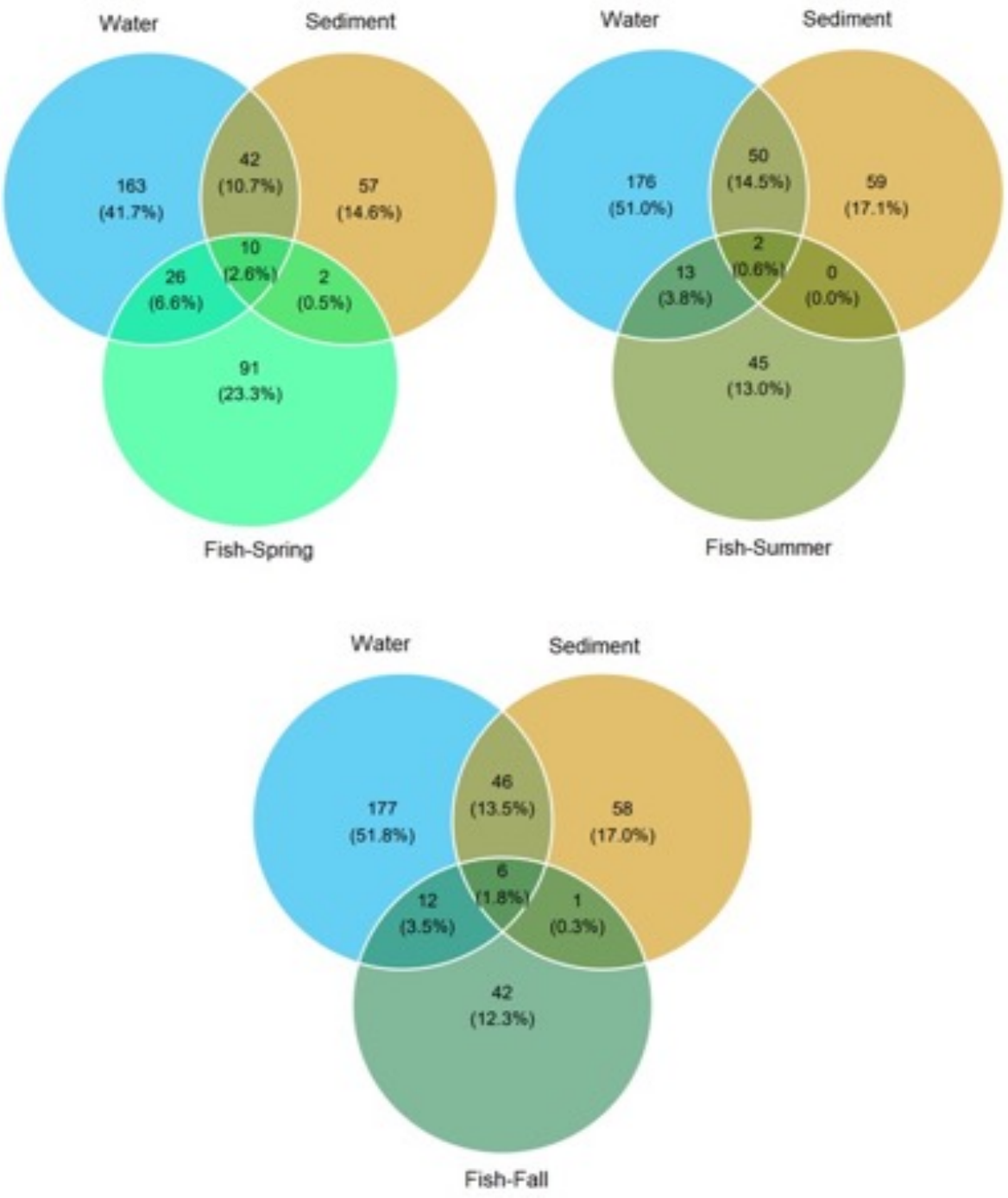


Figure B.9. Venn diagrams indicating the overlap between chemicals detected in water (by conventional grab), sediment, and fish (spring, summer, and fall seasons) samples collected in up- and down-stream the WWTP in the City of Regina.

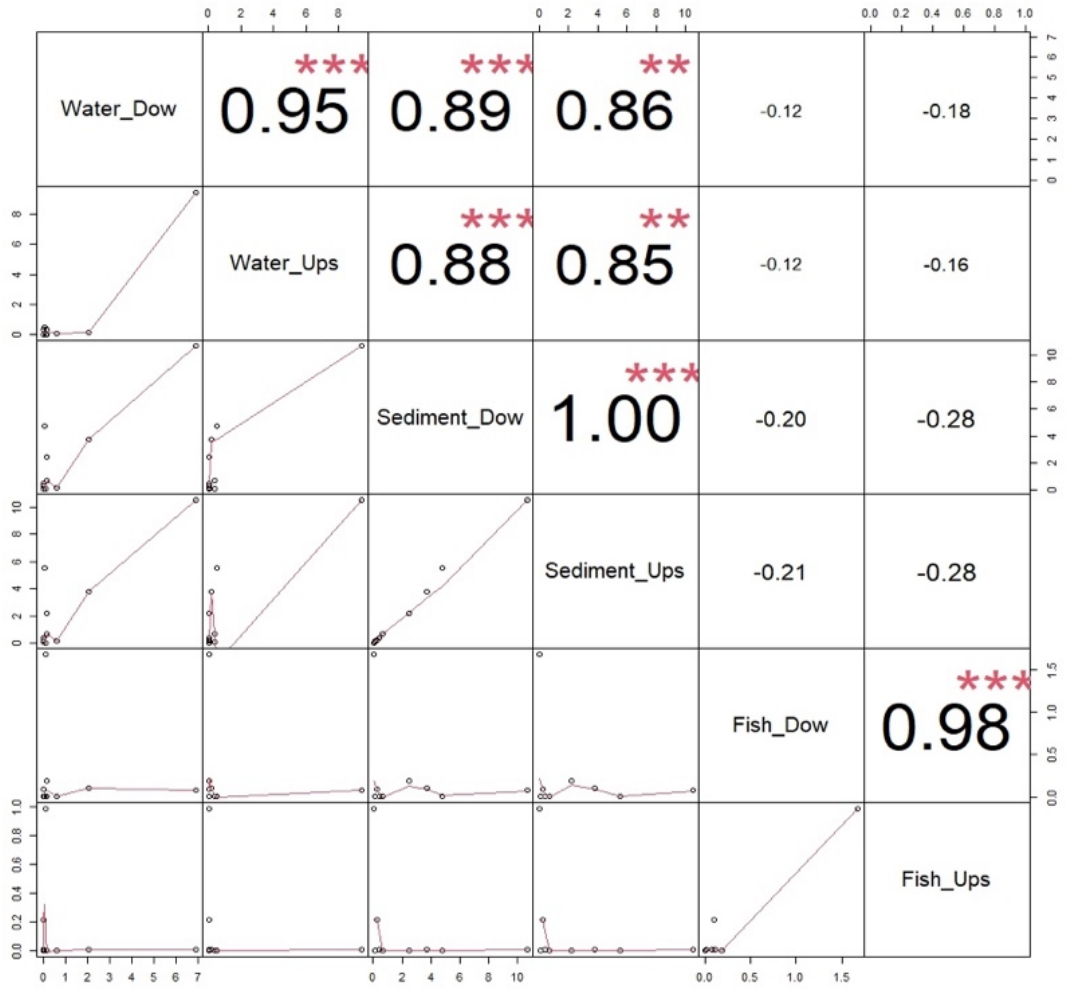


Figure B.10. Correlations between compounds found by non-target screening in all matrices and seasons.

References

Basu, N., Crump, D., Head, J., Hickey, G., Hogan, N., Maguire, S., Xia, J., Hecker, M., 2019. EcoToxChip: A next-generation toxicogenomics tool for chemical prioritization and environmental management. Environ. Toxicol. Chem. 38, 279–288. https://doi.org/10.1002/etc.4309

Bates, D., Maechler, M., Bolker, B., Walker, S., Bojesen Christensen R.H., Singmann, H., Dai, B., Scheipl, F., Grothendieck, G., Green, P., Fox, J., Bauer, A., Krivitsky, P.N. (2022). Package 'lme4' Linear Mixed-Effects Models using 'Eigen' and S4. R package version 1.1-29. CRAN - Package lme4 (r-project.org).

Horton, N. J., & Kleinman, K. (2015). *Using R and RStudio for data management, statistical analysis, and graphics*. CRC Press. 229 page.

Jia, A., Xu, L., & Wang, Y. (2021). Venn diagrams in bioinformatics. *Briefings in Bioinformatics*, *22*(5), bbab108. https://doi.org/10.1093/bib/bbab108

Spyroglou, I., Skalák, J., Balakhonova, V., Benedikty, Z., Rigas, A. G., & Hejátko, J. (2021). Mixed models as a tool for comparing groups of time series in plant sciences. *Plants*, *10*(2), 362. https://doi.org/10.3390/plants10020362