Investigating micropollutant partitioning in five environmental and biological matrices collected in replicate artificial streams

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Environmental Engineering

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Abstract

The presence of micropollutants (substances occurring in sub-ng/L concentrations) is a growing concern due to their potential risks to both the ecosystem and human health. Although they can be introduced into the environment *via* point (wastewater treatment plants) and non-point sources (urban and agriculture runoff), a lack of comprehensive regulation has overlooked their potential persistence, mobility, and adverse impacts on aquatic ecosystems. The main objective of this thesis was to investigate the partitioning of a diverse group of micropollutants, including pharmaceuticals and personal care products in the following environmental compartments: (1) water, (2) sediments, (3) invertebrates, (4) biofilm, and (5) fish. Then, the impact of more advanced levels of wastewater treatment (i.e., ultrafiltration, ozonation, reverse osmosis) on the occurrence and partitioning of these compounds was evaluated. Sampling campaigns were carried out at the Advancing Canadian Water Assets (ACWA) in Calgary, Canada as this facility is equipped with 12 naturalized artificial streams (320 m long) that receive 95% Bow River water and 5% effluent v/v from an operational municipal (Pine Creek) wastewater treatment plant (WWTP) and two pilot WWTPs (reverse osmosis and ozonation). This thesis first focused on improving sample preparation methods in these complex environmental matrices so defensible analytical data (*via* liquid chromatography, triple quadrupole mass spectrometry) on trace concentrations can be obtained. After evaluating different sampling preparation techniques for the solid matrices, the QuEChERS method (Quick, Easy, Cheap, Rugged, and Safe) for sediment, biofilm, invertebrate (*Gammaridae spp*), and fish (longnose dace [*Rhinichthys cataractae*] and spoonhead sculpin [*Cottus ricei*]) tissues were found to be an appropriate sample extraction method with analytical recoveries from 70% to 120% for most of the compounds analyzed. Overall, the compounds that were frequently detected at high concentrations in all the matrices include analgesics (diclofenac),

antibiotics (sulfamethoxazole), antiepileptics (carbamazepine), and antidepressants (venlafaxine). Furthermore, 18 of the 22 compounds were detected in the water matrix, and <10 compounds were detected in the solid matrices (sediment, biofilm, fish, gammarids). High concentrations were observed in the water matrix for diclofenac, venlafaxine, O-desmethylvenlafaxine (venlafaxine metabolite), and carbamazepine at 162 ± 3 ng/L, 381 ± 28 ng/L, 149 ± 3 ng/L, and 45 ± 1 ng/L, respectively. Concentrations in the streams as well as the seasonal trends observed were linked to the Bow River conditions given that it represents a large portion of the stream volume. The Bow River near the ACWA facility has already accumulated micropollutants as a result of WWTP discharges from two Calgary WWTPs that service ~75% of the population. It was also clear that the streams receiving effluent from the Pine Creek WWTP had higher levels of micropollutants compared to effluents that underwent ultrafiltration, reverse osmosis, and ozonation. Finally, an increase in effluent contribution (5% to 15%) was also reflected in the streams and was more detectable in solid matrices. The concentrations ranged from below the limits of quantification (<LOQ) –57 ± 14 ng/g_{dw} in sediments, <LOQ –198 ± 55 ng/g_{dw} in biofilm, <LOQ –18 ± 3 ng/g_{dw} in gammarids and <LOQ –3 ± 1 ng/g_{dw} in fish, suggesting that these substances can be transported into the sediment and/or be taken up by exposed aquatic organisms. The calculated bioconcentration factors (BCF) further indicate that the antidepressant fluoxetine is potentially bioaccumulative (BCF>2000 L/kg in fish, gammarids, and biofilm). For sediment, the solid-water distribution coefficient (K_d) for fluoxetine and triclosan have the highest values (>4000 L/kg), indicating that these compounds tend to sorb more into the solids as they were non-detected or present at low concentrations in the streams. Overall, the results suggest that certain micropollutants partition in the solid matrices more and monitoring of the water alone can underestimate the overall pollution levels and potential risks.

Preface

This thesis is an original work by Daniela Pulgarin Zapata under the supervision of Dr. Maricor Arlos. Aspects of this work has been presented to conferences including Society of Environmental Toxicology and Chemistry (SETAC) Pittsburgh in November 2022, Western Canada Water Conference & Exhibition in September 2023 and the Canadian Ecotoxicity Workshop (CEW) Ottawa in October 2023. No part of this thesis has been previously published.

Acknowledgments

I would like to express my sincere gratitude and appreciation to everyone who has contributed to the completion of this thesis. First, I am deeply thankful to my supervisor Dr. Maricor Arlos, for her guidance, support, patience, and kindness. Thank you for your dedication and knowledge – you have made this experience invaluable.

I also want to thank all the people that made possible this project. To Dr. Mark Servos and Leslie Bragg from the University of Waterloo for sharing all your knowledge on analytical instrumentation, and Dr. Kelly Munkittrick and Dr. Frederick Wrona for allowing a collaborative experience with your research lab. Thank you Dr. Patricija Marjan (Munkittrick Lab) for providing us with the fish samples and Breanna Sayles (Wrona lab) for assisting with the rock basket deployment. I am also grateful to Christine O'Grady from the Advance Canadian Water Assets (ACWA) who helped us with access to the artificial streams. I am grateful for Jela Burkus and Garreth Lambkin during my sediment extraction and sample preparation. Finally, I also would like to show my deep gratitude to all my lab mates from the Arlos Research Group who supported me, especially J. Seth Bumagat, Fei Cheng, and Demi Meier for all your assistance with my experiments and sampling campaigns. I am grateful for the financial support of the Colfuturo Scholarship (Foundation for the Future of Colombia) and Western Canada Water for the conference travel scholarship.

I would like to express my heartfelt gratitude to my family. My parents Ana and Reison and my sister Sara for your support, guidance, and love. Especially, my mother, Ana who despite of going through one of the hardest battles of her life, kept filling mine with joy, strength, and courage to finish my Master's.

Finally, thanks to all my friends near and far for all your motivation and love.

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Chapter 1 – Introduction

1.1 Brief Background

Prior to the 1990s, research on organic pollutants was mostly focused on traditional compounds (e.g., petroleum derivates, PCBs, dioxins, and furans) because of their well-known risks to exposed aquatic organisms. However, the development of increasingly sophisticated analytical techniques has uncovered the presence of previously unknown chemical classes at ubiquitous quantities, further revealing the scale and scope of their impact (Beretta et al. 2014). These substances are commonly referred to as **micropollutants.** These compounds stem from diverse sources, including natural or anthropogenic origins, and are typically categorized based on their chemical properties and potential environmental effects (Bhatt et al. 2022; Luo et al. 2014). Examples of such categories include pharmaceuticals, endocrine-disrupting compounds, personal care products, industrial chemicals such as flame retardants and plasticizers, pesticides, and disinfection by-products.

The occurrence of micropollutants in the environment is an emerging concern due to their potential risk to the environment and human health (Luo et al. 2014). These substances enter the environment mostly via waterways and are found at low concentrations ranging from ng/L to µg/L (Nannou et al. 2015; Wang et al. 2016). Hence, when released into the environment, micropollutants could reach different environmental compartments including the sediments and biota such as biofilm, invertebrates, and fish (Jeon et al. 2013; Kalogeropoulou et al. 2021; Phong Vo et al. 2019). Furthermore, due to the lack of regulation and knowledge gaps about their environmental impact, their emissions into the environment is increasing, ignoring their potential mobility, persistence, and the overall damage on aquatic environment (Beretta et al. 2014).

Micropollutants receive special attention because they are designed to modify biochemical pathways in humans (or animals as in the case of veterinary applications) at low concentrations. Some of these physiological receptors such as hormone receptors are conserved across different animal species, including fish (Álvarez-Muñoz et al. 2015). Hence, when they are released into the waterways, they can bind to similar receptors in exposed organisms, leading to a range of effects on their physiology and behavior (Nannou et al. 2015). Furthermore, their occurrence in the environment has been associated with short- and long-term toxicities, including microbial resistance to antibiotics and endocrine disruption that causes disturbances in the hormonal systems of the organisms including reproduction (e.g., fish intersex) (Oluwole et al. 2020; Phong Vo et al. 2019). Although the consequences are not yet clear in all scenarios, the risks are evident for other cases. For instance, there is evidence that diclofenac (anti-inflammatory) was the major cause of vulture population decline in South Asia (after scavenging through carcasses of animals treated with diclofenac) and an exposure to 5 ng/L 17α-ethinylestradiol in the experimental lakes area in Ontario showed a fish population collapse (Fent et al. 2006; Kidd et al. 2007).

Many studies have examined the presence of micropollutants in the water column only, and the vast majority of these investigations have taken place in Europe and North America. However, regions that are less frequently studied may be at greater risk for micropollutant contamination due poor wastewater treatment. In 2022, Wilkinson et al. gathered monitoring data of 61 substances in rivers from 104 countries, considering different characteristics such as size, weather/climate, and political and economic situation. In this study, low-middle-income regions had the most contaminated sites. Pakistan and Bolivia had the highest mean cumulative concentrations of the 61 active pharmaceuticals ingredients studied, with 70.8 µg/L and 68.9 µg/L, respectively. The largest global concentration range was observed for analgesic, antibiotic, and

anticonvulsant classes, and substances such as carbamazepine, metformin, and caffeine which had a detection frequency of >50%. Most of the concentrations were lower than the proposed levels for causing an ecological effect. However, environmental concentrations exceeded the predicted no-effect concentrations proposed by European guidelines in 25.7% of the study sites, which might represent a risk (Wilkinson et al. 2022).

Few countries and jurisdictions have strict guidelines or regulations for specific micropollutants. The European Union (EU) issued one of the first proposed regulations via its Directive 2000/60/EC that defined and prioritized high-risk substances (Directive 2000; Luo et al. 2014). In 2008, Directive 2008/105/EC highlighted 33 priority substances and later, Directive 2013/ 39/EU suggested the monitoring and treatment options for 45 priority substances (Commission 2013; Khan et al. 2021; Parliament 2008). In Canada, the Federal environmental quality guidelines are established for bisphenol A (plasticizer) and triclosan (antimicrobial) of 3.5 and 0.47 μg/L, respectively. Furthermore, the province of Alberta through the Environmental Quality Guidelines for Surface Water presented the regulations adopted by the province, which include guidelines for carbamazepine (10 μg/L), 17α- Ethinylestradiol (0.5 ng/L), Di(2-ethylhexyl) phthalate (16 μg/L) and Di-n-butyl phthalate (19 μg/L).

Numerous studies have focused on understanding the potential adverse effects of micropollutants in the environment, as well as their fate, behaviour, and transport, but studies that also include other environmental compartments such as sediments and biota are rare. Additionally, different extraction methods and advanced analytical techniques have been developed to allow the detection of these substances in water, but sample preparation for other environmental compartments continue to be a challenge.

1.2 Thesis Objectives

The main purpose of this thesis is to investigate the partitioning and bioaccumulation (i.e., accumulation of substances in an organism) of a diverse group of micropollutants, including pharmaceuticals and personal care products in replicate artificial streams and five environmental compartments: (i) water column, (ii) sediments, (iii) invertebrates, (iv) biofilm, and (v) fish tissue. To achieve this, the following objectives were developed:

i) improve sample preparation methods to obtain trace concentration of micropollutants in environmental compartments; and

ii) evaluate the impact of different types of wastewater treatment (e.g., ultrafiltration, ozonation, reverse osmosis) on the occurrence and partitioning of these compounds in the environment.

During the development of this study, it was observed that most of the data concerning micropollutants in the Bow River watershed (main study site) was available for the aqueous phase only (i.e., dissolved in the water column) and information is very limited to assess bioaccumulation and partitioning for sediment and biota. Hence, improving the extraction methods and detection in other compartments is important to determine the general behaviour of these substances in the environment.

1.3 Thesis Scope

This thesis first focuses on the method development related to sample clean-up prior to detection of 22 pharmaceuticals and personal care products via liquid-chromatography, tandem mass-spectrometry (LC-MS/MS). Chapter 2 provides a brief literature review on the fate and occurrence of target micropollutants in environmental compartments. Chapter 3 discusses the sampling campaign details and the sample extraction methods evaluated and developed for this

work. Finally, Chapter 4 evaluates the concentrations detected when the effluent contribution in an artificial stream facility associated with an operational wastewater treatment plant (WWTP) was increased to 15%.

1.4 Site Description

This research was carried out in Calgary (southern Alberta) with a population of ~1.4 million people (2022) (Government of Alberta). Calgary has three WWTPs currently in operation: Bonnybrook, Fish Creek, and Pine Creek WWTPs, which discharge into the Bow River (Figure 1.1) (Bash el al. 2014). This river originates from the Canadian Rockies and is a major source of water in southern Alberta. Its path involves three main geographic regions: the mountains, the foothills, and the prairies (Veiga et al. 2015). The Pine Creek WWTP is Calgary's newest plant which incorporates biological nutrient removal (BNR) whereas Bonnybrook and Fish Creek WWPTs only operate as partial BNR and full activated sludge system, respectively.

Most of the sampling campaigns for this project were completed at the Advancing Canadian Water Assets (ACWA) facility located at Pine Creek WWTP (Figure 1.2). ACWA is equipped with 12 naturalized artificial streams (320 m long with 10 pools and 10 riffles) integrated into the fully functional WWTP (Pine Creek) and 2 pilot treatment plants (ozonation, reverse osmosis). These streams have hydraulic and ecological parameters that mimic natural local systems (Jackson 2020).

The facility is divided into 4 different treatment processes in triplicates. The first group receives 100% water from the Bow River (control) and the three extra groups receive 5% treatment process effluent (i.e., ozonation or reverse osmosis pilot treatment (with ultrafiltration) and Pine Creek WWTP effluent) and 95% Bow River water. ACWA is a world class facility and is the only

one in Canada that has these replicate systems. Further information about the sampling campaigns will be provided in the methodology of this thesis.

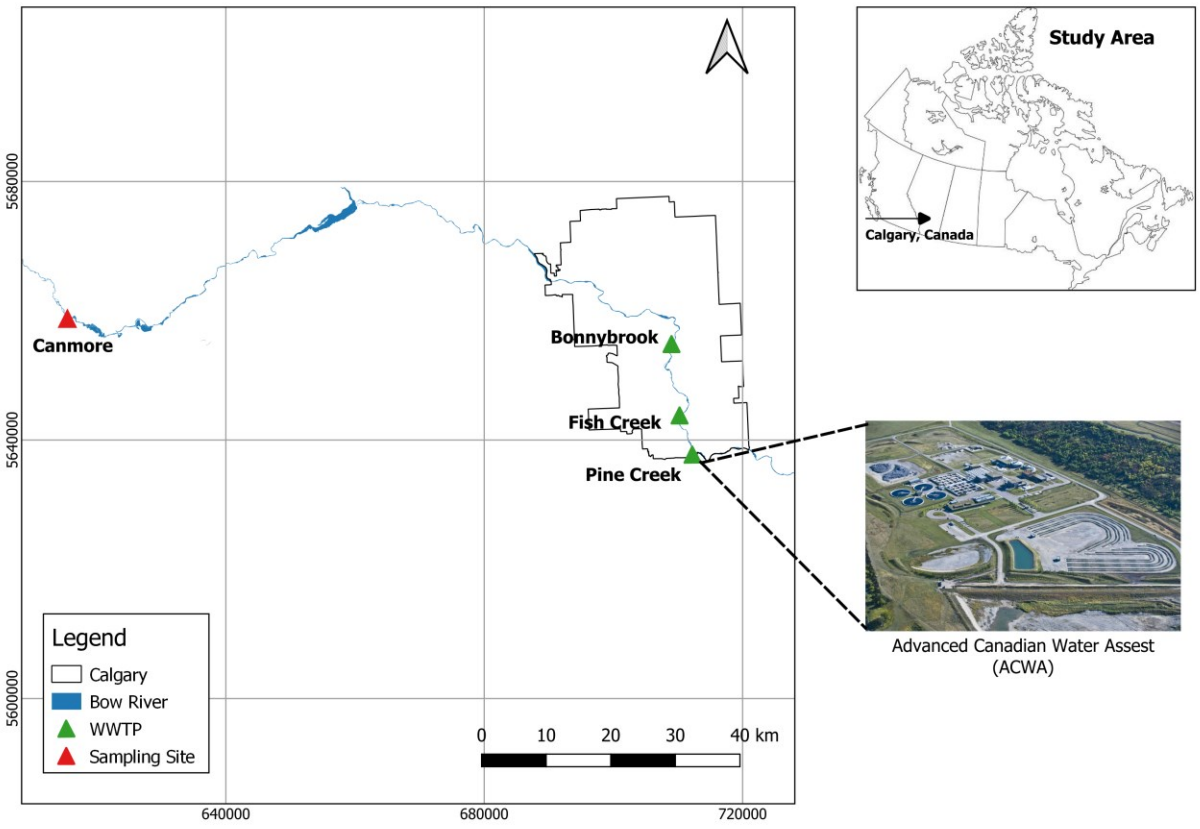

Figure 1.1. *Map of the study area in southern Alberta. Wastewater treatment plants (WWTP) are located inside of the Calgary city limits and the site in red is the upstream reference site. Samples of this project were collected at Advancing Canadian Water Assets (ACWA) and Canmore.*

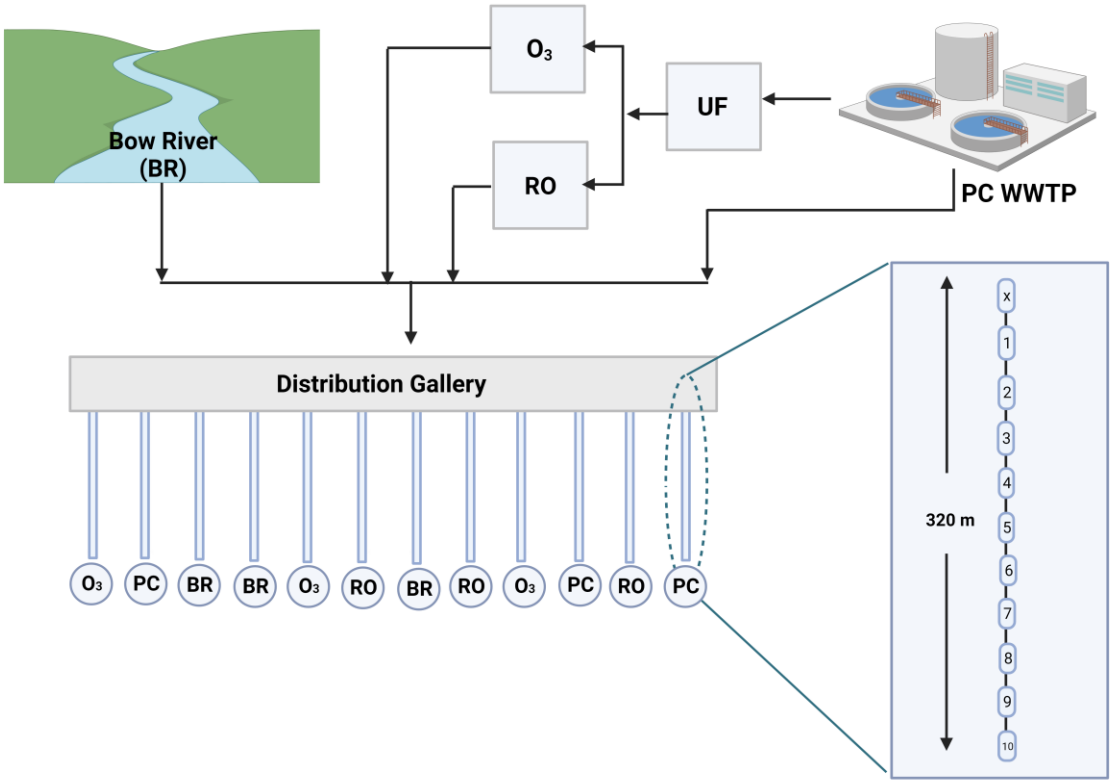

Figure 1.2 *Layout of the replicate streams at the Advancing Canadian Water Assets (ACWA). Bow River (BR) streams are piped directly from the river. The Pine Creek (PC) streams contain 5% treated wastewater (biological nutrient removal) from the operational Pine Creek WWTP. The Reverse Osmosis (RO) and Ozonation (O_3) streams receive 5% of effluent that had undergone ultrafiltration of the secondary effluent from PC WWTP, and then piped to either RO or O_3. Figure created via Biorender.*

Chapter 2 – Literature Review

2.1 Brief overview of micropollutant fate

Conventional WWTPs have the potential to remove micropollutants via sorption to solids/sludge and biodegradation (Fent et al. 2006) as the physico-chemical properties can render them susceptible to the removal/degradation processes that naturally occur in WWTPs (i.e., biodegradation, sorption, hydrolysis). However, these conventional processes are mainly designed to remove solid waste, suspended solids, biodegradable dissolved organic matter, and nutrients. Since treatment plants are not specifically developed to remove trace organic compounds, WWTPs are recognized as one of the main sources of micropollutants in the aquatic environment (Margot et al. 2015; Su et al. 2021).

Sorption is defined as the process in which compounds are associated with solid phases. In this case, micropollutant sorption into the solids has a potential to remove them in the wastewater before entering the aquatic environment, thus accumulating in the sludge. This could also represent a risks for the soil matrix, such as micropollutants can be transported to terrestrial via the application of biosolids as fertilizers (Bolesta et al. 2022). There are two main mechanisms of sorption of a chemical into the activated sludge. Adsorption is an electrostatic interaction between positively charged compounds and negatively charged surface of the microorganisms, while absorption is related to hydrophobic interactions of chemicals and lipophilic cell membrane of the microorganisms and the lipid fractions of the sludge (Besha et al. 2017).

Biodegradation of micropollutants typically requires carbon and energy sources for their transformation. This process does not occur easily for many micropollutants and in some cases, it can take up to several months (Li 2014). Nonetheless, micropollutant biodegradation can occur in aerobic zones in activated sludge treatment or anaerobically in sewage sludge digestion (Fent et

al. 2006) where micropollutants can be further utilised by microorganisms as a growth substrate or are transformed by side reactions catalyzed by enzymes or cofactors. Note, however, that micropollutant removal during wastewater treatment is still poorly understood despite decades of research, making micropollutant treatment efficiency difficult to predict. A recent study by Zumstein et al. (2022) suggested that micropollutant functional groups could be used as predictors of biotransformation, and could act either as biotransformation promoters or inhibitors. More specifically, certain functional groups may be recalcitrant and biotransformable depending on redox conditions (e.g., aerobic vs. anaerobic) (Alvarino et al. 2018), which led to many arguing that diverse microbial communities in wastewater treatment systems can enhance micropollutant degradation and the presence of unique and specialist microbial communities are crucial for micropollutant biotransformation (Alvarino et al. 2018; Rich et al. 2022; Wolff et al. 2018).

Correlating micropollutant concentrations with typical wastewater parameters (e.g., ammonia, suspended solids) is sparse with an exception of a study by J. Wang and Wang (2016) that linked micropollutant physicochemical properties and conventional activated sludge (CAS) process parameters with the biotransformation potential. They found that in conventional activated sludge (CAS) systems, solids retention time, influent concentration, and the presence nitrification/denitrification are predictors of micropollutant removals in WWTPs.

The fate of organic chemicals, including micropollutants, are typically assessed against their physicochemical properties (Li 2014). A list of the pharmaceuticals and personal care products studied in this thesis is shown in Table 2.1. Compounds with higher molecular weight and high octanol-water partition coefficient (Log K_{ow} >4) are predicted to easily sorb to suspended solids and therefore, easily removed by conventional wastewater treatment (Li 2014).

Table 2.1 *Pharmaceuticals and personal care products analyzed. It indicates the molecular weight, dissociation constant (pK_a), and octanol-water partition coefficient (Log K_{OW}) at pH 7.4 and solubility (at 6.5 pH). Data was adapted from Chemicalize - Instant Cheminformatics Solutions[a], retrieved June 2023 from https://chemicalize.com/app/calculation ; PubChem n.d.[b], retrieved June 2023 from https://pubchem.ncbi.nlm.nih.gov/. *Metabolites.*

Compound	Class	Molar mass (g/mol)[a]	LogKow[a]	pK_a[b]	Solubility (mg/mL)[a]
O-desmethylvenlafaxine*	Antidepressant	263.38	0.98	9.45	263.38
Venlafaxine		277.41	1.12	9.50	236.18
Fluoxetine		309.33	2.19	10.10	20.92
Norfluoxetine*		295.30	2.19	9.05	0.009
Diclofenac	Analgesic	296.15	1.10	4.20	4.66
Ibuprofen		206.29	1.34	5.30	2.69
Naproxen		230.26	-0.02	4.15	15.07
Carbamazepine	Anticonvulsant	236.27	2.77	13.90	0.04
10, 11 Epoxide Carbamazepine*		252.27	2.77	N/A	N/A
Atorvastatin	Cardiovascular	558.65	2.43	4.54	0.01
p-hydroxy atorvastatin*		556.60	2.43	N/A	N/A
o-hydroxy atorvastatin*		556.68	2.43	N/A	N/A
Gemfibrozil		250.34	1.51	4.50	14.97
Sulfamethoxazole	Antibiotic	253.28	-0.07	1.60	8.52
Trimethoprim		290.32	1.10	7.12	2.54
Sulfamethazine		278.33	0.21	7.59	0.56
Caffeine	Stimulant	194.19	-0.55	14.00	70.94
Triclocarban	Antibacterial	315.58	4.93	12.70	0
Triclosan		289.54	4.80	7.90	0.01

2.2 Potential treatment of micropollutants

Since both adsorption to solids and biodegradation are not capable of removing micropollutants, other advanced treatment processes have been studied to assess their treatment efficacy. For instance, ultrafiltration, advanced oxidation processes (ozonation), adsorption via activated carbon, and membrane bioreactors, which have been shown to efficiently remove these compounds (Bhatt et al. 2022; Paucar et al. 2019; Schaar et al. 2010). This chapter focuses on

micropollutant removal via ultrafiltration, ozonation, and reverse osmosis as these are the treatment technologies that were investigated at the ACWA facility.

Ozonation is one of the most suitable advanced oxidation treatments for micropollutant removal and abatement. As an oxidant, ozone reacts either directly with the compound or indirectly after the formation of hydroxyl radicals from the ozone decomposition (Altmann et al. 2014; Paucar et al. 2019). The first pathway consist in the direct attack of the ozone to the acidic sites and involves electrophilic aromatic substitution, whereas in the second pathway, ozone decomposition produces radical species, often hydroxyl radicals that can act as secondary oxidants, which interact with organic molecules more quickly (Almomani et al. 2016). Some studies have found a relationship between the ozonation order kinetics and micropollutant removal. Almomani et al. (2016) found that ozonation is an efficient treatment technology due to its second-order kinetics, thus, its higher reaction rate facilitated the removal of antibiotics, analgesics, and stimulants. A similar approach was presented by Huber et al. (2005).

Lester et al. (2013) studied the removal efficiency of ozonation for wastewater at a pharmaceutical formulation facility and observed removals above 98% for carbamazepine and venlafaxine. Furthermore, Huber et al. (2005) found that the removal of antibiotics and analgesics including diclofenac, naproxen, and sulfamethoxazole, were removed by 90% by using ozonation for wastewater. In addition, Kim and Tanaka (2010) reported removal of >90% for pharmaceuticals that belong to different classes (analgesics, cardiovascular, antibiotics, and anticonvulsants). Some of the compounds studied included diclofenac, acetaminophen, naproxen, atenolol, trimethoprim, sulfamethoxazole, and carbamazepine. Size exclusion or separation processes such as reverse osmosis have also been shown to efficiently remove pharmaceuticals from water sources as shown in Table 2.2.

Table 2.2 *Removal efficiency of micropollutants from water matrix for different treatments. O_3 = ozonation, RO = reverse osmosis, UF = ultrafiltration, DOC=dissolved organic carbon, DEET=N,N-diethyl-meta-toluamide; EE2 = 17 α-ethinylestradiol.*

Tertiary Treatment	Micropollutant	Class	Water Matrix	Treatment parameters	Treatment efficiency (%)	Reference
O_3	Carbamazepine	Anticonvulsant	Municipal Wastewater	Full-scale 5 mg/L	> 90	(Sui et al. 2010)
	Diclofenac	Analgesics			> 90	
	Metropolol	Cardiovascular			80-90	
	Benzafibrate				0-50	
	Trimethoprim	Antibiotics			> 90	
	DEET	Personal care			50-80	
O_3	Venlafaxine	Antidepressant	Wastewater from formulation facility	Bench-scale 0.87 O_3/DOC	~98	(Lester et al. 2013)
	Carbamazepine	Anticonvulsant		0.55 O_3/DOC	> 90	
O_3	Sulfamethoxazole	Antibiotics	Municipal wastewater	Pilot-scale 2 mg/L	≥90-99	(Huber et al. 2005)
	Diclofenac	Analgesics				
	Naproxen					
	EE2	Estrogen				
O_3	Diclofenac	Analgesics	Municipal wastewater	Bench-scale 6 mg/L	> 90	(I. Kim and Tanaka 2010)
	Acetaminophen					
	Naproxen					
	Caffeine	Stimulant drug			78	
	Atenolol	Cardiovascular			99	
	Trimethoprim	Antibiotics			> 96	
	Sulfamethoxazole					
	Carbamazepine	Anticonvulsant			> 90	
O_3	Sulfamethoxazole	Antibiotics	Municipal wastewater	Bench-scale 0.42 (±0.15) mg O_3/mg DOC	> 90	(Almomani et al. 2016)
	Diclofenac	Analgesics		0.35 (±0.15) mg O_3/mg DOC		
	Caffeine	Stimulant drug		0.43 (±0.15) mg O_3/mg DOC		
O_3	Triclosan	Antibacterial	Surface water	Bench-scale 5 mg/L	> 99	(Orhon et al. 2017)
RO	Gemfibrozil	Cardiovascular	Municipal wastewater	Full-scale	> 97	(Al-Rifai, Khabbaz, and Schäfer 2011)
	Diclofenac	Analgesics				
	Ibuprofen					
	Naproxen					
	Acetaminophen					
	Ketoprofen					
	Carbamazepine	Anticonvulsant				

Table 2.2 Continued

Tertiary Treatment	Micropollutant	Class	Water Matrix	Treatment parameters	Treatment efficiency (%)	Reference
RO	Dypirone	Analgesics	Non-specified	Bench-scale	> 98	(Licona et al. 2018)
	Ibuprofen					
	Diclofenac					
	Acetaminophen				85-95	
	Caffeine	Stimulant drug			85-98	
RO	Carbamazepine	Anticonvulsant	Groundwater	Full-scale	> 85	(Radjenović et al. 2008)
	Diclofenac	Analgesics			> 95	
	Ketoprofen				> 95	
	Sulfamethozaxole	Antibiotics			> 95	
	Gemfibrozil	Cardiovascular			50–70	
UF+RO	Acetaminophen	Analgesics	Drinking water	Full-scale	~99	(Boleda, Galceran, and Ventura 2011)
	Diclofenac				~99	
	Ibuprofen				>99	
	Naproxen					
	Gemfibrozil	Cardiovascular				
	Sulfamethoxazole	Antibiotics				
	Trimethoprim					
UF	Carbamazepine	Anticonvulsant	Drinking water	Bench-scale	<30	(Yoon et al. 2007)
	Diclofenac	Analgesics				
	Acetaminophen					
	Sulfamethoxazole	Antibiotics				
	Caffeine	Stimulant drug				
	Oxybenzone	Personal care			>60	
	Triclosan	Antibacterial			>80	
UF	Acetaminophen	Analgesics	Municipal wastewater	Bench-scale	<30	(Sheng et al. 2016)
	Diclofenac					
	Caffeine	Stimulant drug				
	Sulfamethoxazole	Antibiotics				
	Trimethoprim					
	Naproxen	Analgesics				
	Ibuprofen					
	Gemfibrozil	Cardiovascular			>40	
	Carbamazepine	Anticonvulsant			>60	
	Triclosan	Antibacterial			>95	
UF	Trimethoprim	Antibiotics	Municipal wastewater		<50	(Sui et al. 2010)
	Gemfibrozil	Cardiovascular				
	Diclofenac	Analgesics				
	Carbamazepine	Anticonvulsant				
	Caffeine	Stimulant drug				

This process with a pore size of typically 0.2-1 nm removes micropollutants by a size exclusion mechanism (Dolar and Košutić 2013). The removal efficiency of this treatment process is related to molecular weight, molecular size, and the charge of the compounds, as well as, hydrophobicity, which is given by the octanol-water partition coefficient (Log K_{OW}) (Agenson et al. 2003; Licona et al. 2018; Radjenović et al. 2008). Licona et al. (2018) studied the removal of a selected group of micropollutants, including acetaminophen, ibuprofen, diclofenac, and caffeine. It found a removal of greater than 97% for most of the compounds, except for acetaminophen and caffeine. This was attributed to their low hydrophobicity and their molecular size.

Other studies reported the removal of micropollutants using reverse osmosis and compared the process with their physicochemical properties. For example, Al-Rifai et al. (2011) studied the removal of pharmaceuticals in a recycling facility for industrial users via RO and found that gemfibrozil, diclofenac, naproxen, acetaminophen, ibuprofen, and carbamazepine were removed above 97% after the advanced treatment (Al-Rifai et al. 2011). Moreover, Radjenović et al. (2008) tested the removal of pharmaceuticals for a drinking water facility that uses groundwater. It found a removal >95% for a group of analgesics and antibiotics.

Another separation process evaluated to remove micropollutants is ultrafiltration. The pore size of these filters usually ranges from 0.05 μm to 2 nm (Dolar and Košutić 2013). This process is usually designed for particles with larger molecular weight than micropollutant particles. The molecular weight cutoff of UF membranes (10–100 kDa), while the molecular weight of most micropollutants is <1 kDa, which causes low retention capacity for micropollutants (Dolar and Košutić 2013; Sheng et al. 2016). When applied alone, Yoon et al. (2007) found that the retention of 27 micropollutants was <30% for most of the compounds including carbamazepine, diclofenac, acetaminophen, sulfamethoxazole, and caffeine, and >60% and >80% for oxybenzone and

triclosan, respectively. Similar results were obtained by Sheng et al. (2016), where removal efficiency was between 30% to 60%, except for triclosan at over 80% and carbamazepine at 70%. Therefore, the removal mechanism of ultrafiltration is usually accompanied by other treatments (Dolar and Košutić 2013). In the case of ACWA, reverse osmosis and ozonation streams are preceded by ultrafiltration process as shown in Figure 1.2.

2.3 Micropollutants in the water column

The presence of micropollutants in the environment can be attributed to various factors including increase in urbanization and industrial activities driven by humans needs for well-being, health, and agriculture (Bhatt et al. 2022; Luo et al. 2014). One of the main pathways of these substances to the water environment is through the administration of health and/or veterinary care pharmaceuticals, which are subsequently excreted, remain untreated in WWTPs and eventually are released in the environment (Bhatt et al. 2022). Furthermore, other common pathways of micropollutants can be point sources, including WWTP effluent from industrial or domestic activities, spills and leaching from landfills or non point sources such as run off that comes from agricultural (e.g. pesticides) and urban activities (e.g. biocides, stormwater) (Warner, Licha, and Nödler 2019) as shown in Figure 2.1. Thus, micropollutants make their way to the environment and are easily detected in surface water, urban wastewater effluents, drinking water, and groundwater (Bhatt et al. 2022; Caliman et al. 2009).

The first studies reporting the concentration of micropollutants in the water environment are registered in the mid-70s. The occurrence of clorific acid was reported in 1976 in the USA in treated wastewater at a range of 0.8-2 μg/L (Fent et al. 2006). Later, other pharmaceuticals were detected in the UK, including antibiotics, antidepressants, and analgesics, by monitoring the river, sewage effluent, and drinking water samples (Richardson and Bowron 1985). Subsequently, in

1981 caffeine, ibuprofen, and naproxen were detected in municipal wastewater in British Columbia, Canada (Rogers et al. 1986). In the following years, the number of studies that have reported micropollutants in the water environment has increased, as well as, more compounds are being detected due to the improvement of techniques that allow determining these substances at trace concentrations (Díaz-Cruz et al. 2005; Nikolaou et al. 2007).

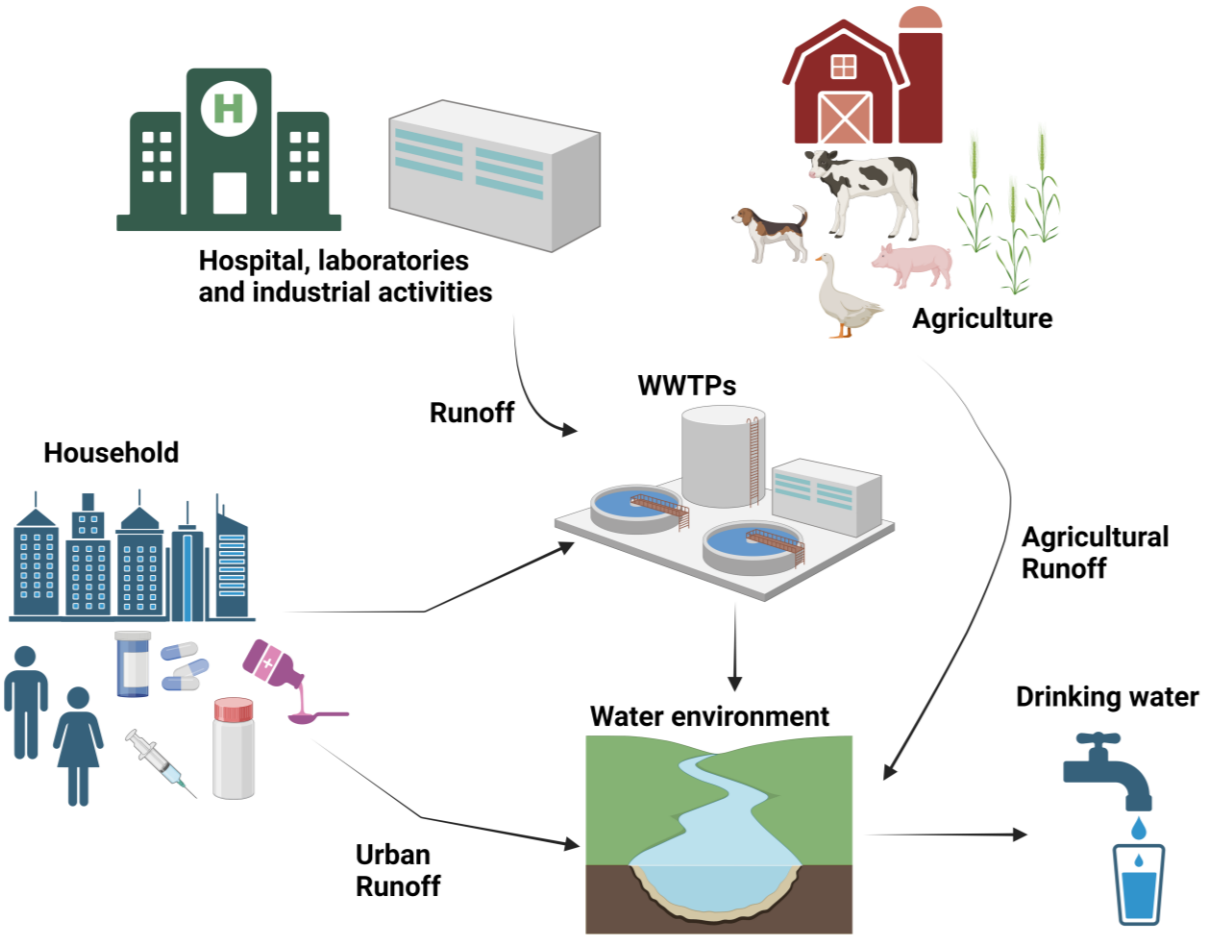

Figure 2.1 *Fate of micropollutants in the aquatic environment. Created via Biorender.*

Other studies reported micropollutants in the water environment in different locations around the globe (Caliman et al. 2009; Jiang et al. 2013). Sacher et al. (2001) established a database on the occurrence of pharmaceuticals in groundwater in Baden-Württemberg, Germany. There were 60 compounds monitored including diclofenac, carbamazepine, ibuprofen, naproxen, gemfibrozil, sulfamethoxazole, and trimethoprim. Concentrations ranged from 10 ng/L to 100 ng/L for most of the compounds but went up to several hundred for some of them (Sacher et al. 2001). Another study by Zhou et al. (2009) in the river Ouse, England determined the concentration of 5 micropollutants in the influent and effluent of three WWTPs and the receiving river. This study found that WWTPs cause a substantial increase in the micropollutant concentration downstream of the effluent, which suggests WWTP as an important source of micropollutants in this riverine environment. In addition, carbamazepine and diclofenac were frequently detected due to their persistence and extensive use. Carbamazepine showed the highest concentrations ranging from 167 to 334 ng/L (Zhou et al. 2009), as shown in Figure 2.2.

Micropollutants also occur at varying ranges in water bodies worldwide (Caliman and Gavrilescu 2009). Acetaminophen, sulfamethoxazole, trimethoprim, venlafaxine, carbamazepine, diclofenac, and naproxen were found in a Mediterranean river in Spain at concentrations ranging from 0.2 to 0.94 μg/L (Fonseca et al. 2020). Monitoring in the Lagos States, Nigeria that include samples from rivers, canals, lagoons, groundwater, and drinking water detected acetaminophen, caffeine, gemfibrozil, and ibuprofen concentrations were reported from 1-12,430, <4-1,080, <4-552, and <4-2,740 ng/L, respectively (Ebele et al. 2020). Pivetta et al. (2020) studied the occurrence of psychotropic drugs in surface water (amitriptyline, bupropion, carbamazepine, escitalopram, fluoxetine, and trazodone) in Campinas, Brazil. Samples collected from the Atibaia River ranged from 25 to 3,530 ng/L (Pivetta et al. 2020). In river Torsa, India, personal care

products (synthetic antimicrobials), triclosan, and triclocarban were monitored with concentrations ranging from 0.055–0.184 µg/L and 0.041–0.077 µg/L respectively (Das Sarkar et al. 2020).

2.4 Micropollutants in suspended solids and/or sediments.

Sediments are natural sinks of many chemical substances that are discharged into water bodies. During sedimentation, micropollutants can have contact with suspended material, which in higher concentrations can facilitate the pollutant sorption to solids. Afterward, this is integrated into the sedimentary deposits (Beretta et al. 2014; Salomons and Stigliani 1995). This generally occurs in low-flow velocity areas (Salomons and Stigliani 1995). These characteristics can facilitate pollutants to bind into sediments and bioaccumulate in benthic organisms (e.g., macroinvertebrates such as gammarids) (Pan and Xing 2011).

Micropollutants with high hydrophobicity (Log K_{OW}) attach to sediments and suspended solids more easily (Zoppini et al. 2014). This facilitates their occurrence in the sediment environment (Kim and Zoh 2016). Some examples for this study are listed in Table 2.1, including triclosan and triclocarban (more information regarding their accumulation in the sediment environment will be given in chapter 4). The partition coefficient (K_d), defined as the ratio between water concentration and sediment concentration, is considered to determine the capacity of absorption and predict the concentration of different micropollutants in water and sediment. Substances with low K_d are expected to occur principally in the water phase and those with higher values tend to have a stronger sorption into sediments (Golovko et al. 2020; Koba et al. 2018). In a study by Golovko et al. (2020), antidepressants and antihistamine were found to have the highest accumulation in solids with the highest K_d values.

Many studies only report the occurrence of pharmaceutical and personal care products in the water environment but rarely focus the occurrence of these substances in the sediment

compartment (Pan and Xing 2011). However, sediments are crucial to determine the fate of chemicals in the environment as they provide an important environmental layer understand partitioning processes (Bagnis et al. 2018).

Golovko et al. (2020) reported the occurrence of 24 micropollutants in sediments collected from three different locations at Lake Malaren, Sweden. Carbamazepine, venlafaxine, desvenlafaxine, and caffeine were some of the compounds found during the study. Venlafaxine had the highest concentration with 7.8 ng/$g_{dry\ weight\ (dw)}$ and its metabolite desvenlafaxine was found in all locations with a highest of 3.7 ng/g_{dw} (Golovko et al. 2020). Another monitoring of pharmaceuticals in the north coast of Salvador, Brazil have observed concentrations of 23.4 ng/g_{dw}, 14.3 ng/g_{dw}, 9.84 ng/g_{dw}, 4.81 ng/g_{dw}, 1.06 ng/g_{dw} for caffeine, ibuprofen, atenolol, carbamazepine, diclofenac, respectively (Beretta et al. 2014). Furthermore, the Cezarka pond in the Czech Republic was designed to retain and treat effluent from the Vodnany WWTP. A study carried out in this experimental ecosystem found a concentration of analgesics, antidepressants, neuroinhibitors, metabolites, and cardiovascular substances in the sediment environment. It was reported a maximum concentration for venlafaxine and carbamazepine of 140 ng/g_{dw} and 16 ng/g_{dw}, their metabolites, 10,11 epoxide carbamazepine was below the detection limit and O-desmethylvenlafaxine at a maximum of 290 ng/g_{dw} (Koba et al. 2018). Even though, research on micropollutants in sediment is not as extensive as studies focused on the water matrix, numerous investigations indicate that sediments can serve as a potential secondary reservoir of micropollutants in the environment. Therefore, these compounds may also find their way into biota matrices, resulting in possible accumulation.

2.5 Micropollutants in Biofilm.

Biofilm (a consortia of bacteria, fungi, algae, and other microorganisms) can serve as an indicator to evaluate the impact of WWTPs discharge and its effects on aquatic ecosystems as they can sequester micropollutants away from the water column (Aubertheau et al. 2017). In the case of micropollutants, sorption by biofilm is not necessarily attributed to chemical properties such as octanol-water partition coefficient (Log K_{OW}) as in the case of other environmental matrices (Aubertheau et al. 2017; Huerta et al. 2016). Instead, partitioning of micropollutants in biofilm is related to sorption mechanisms through the ionization of the different functional groups of micropollutants. Thus, given the negatively charged surface of biomass, sorption is likely influenced by the charge of chemicals, giving higher sorption potential to positively charged compounds (Torresi et al. 2017; Yamamoto et al. 2009). Other than playing a role in the biogeochemical cycles, ecosystem respiration, and working as primary producers in the food web (Battin et al. 2016), biofilms have been considered as important contributors to the bioremediation of aquatic environments by sorption, biotransformation, or bioaccumulation of substances (Desiante et al. 2021). However, the occurrence of micropollutant substances might alter microbial communities by promoting antibiotic-resistance genes and eliminating algal growth (Ricart et al. 2010; Aubertheau et al. 2017).

Aubertheau et al. (2017) reported the occurrence of 11 micropollutants from biofilm samples collected downstream of 12 WWTPs in the Vienne River watershed in central France. Anticonvulsants, antibiotics, cardiovascular, analgesics, and beta-blockers were found in the samples. Carbamazepine and diclofenac had the highest concentrations, ranging from 2.1-583.5 ng/g_{dw} (Figure 2.2) and 4-190.3 ng/g_{dw}, respectively. The widespread presence of these chemicals in biofilms could be attributed to their large usage and poor degradation in receiving environments (Aubertheau et al. 2017). Another study by Huerta et al. (2016) focused on the bioaccumulation of

micropollutants in river Segre in Spain in different groups of pharmaceuticals and endocrine disruptors. Some of the compounds detected include diclofenac, venlafaxine, carbamazepine, gemfibrozil, and triclosan. Diclofenac once again was reported to have the highest concentration with 100 ng/g_{dw}. Although, very little information is available in micropollutant occurrence in biofilm, it is clear that these are important contributors to the bioremediation of aquatic environments but could also contribute to the distribution of micropollutants in the environment.

2.6 Micropollutants in Gammarids (benthic macroinvertebrates).

The presence and abundance of certain aquatic macroinvertebrates such as gammarids are usually good bioindicators of the aquatic environment as they are sensitive to environmental degradation (Chaumot et al. 2015; Garcia-Galan et al. 2017). They are also less mobile than fish and can therefore be more representative of exposure to substances stemming from wastewater pollution (Munz et al. 2018; Vrana et al. 2005). Gammarus species, (i.e., gammarids) belong to the family Gammaridae, a group of amphipod crustaceans. These organisms play an important role in food webs as they are preyed on by fish, turtles, or birds and are abundant in freshwater systems (Munz et al. 2018).

Many of the bioaccumulation studies of aquatic organisms are focused on superior taxa such as fish. However, Lagesson et al. (2016) studied the bioaccumulation of pharmaceuticals, including analgesics, antibiotics, and antidepressants for different trophic levels. Finding a higher concentration for benthic species than species at superior taxa (i.e., fish). Therefore, it shows that lower trophic species are the primary recipients of pharmaceuticals. Moreover, some other studies have studied the bioaccumulation of micropollutants in different benthic species. In Kitchener, Canada, 27 out of 43 compounds studied in fresh mussels were detected at the downstream collection sites (receiving effluent from 30 WWTPs), including analgesics, anti-bacterial agents,

antibiotics, antidepressants, and antihistamines (de Solla et al. 2016). In a different study by Huerta et al. (2015), analgesics, ibuprofen, and diclofenac reached concentrations of 183 and 12.4 ng/g_{dw} in different species of macroinvertebrates from the Segre River in Spain.

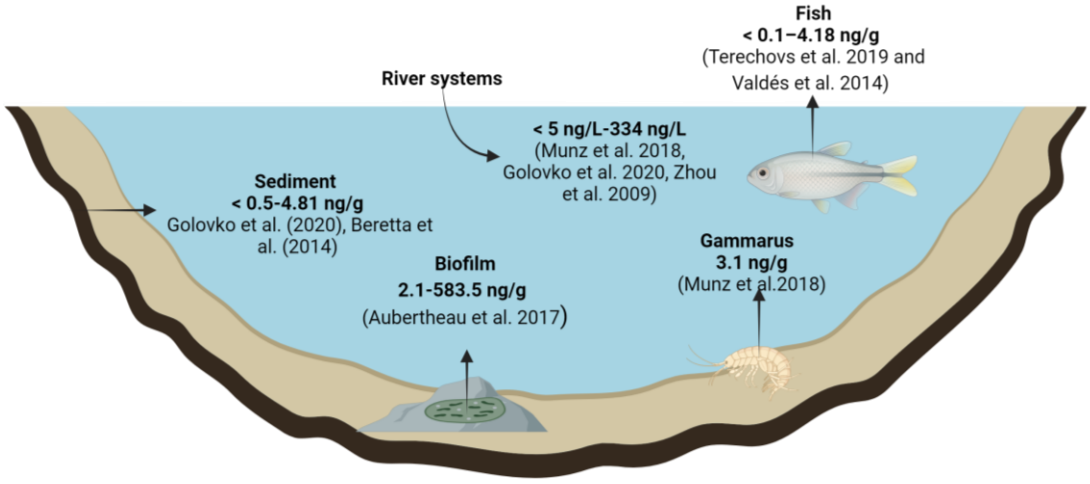

Figure 2.2 *Representation of micropollutant partitioning in different environmental compartments and several biota classes. Information is shown for carbamazepine in biofilm, fish, water, gammarids, and sediments. Created via BioRender.*

Garcia-Galan et al. (2017) reported the bioaccumulation of pharmaceuticals in gammarids, finding an average concentration of 106 ng/$g_{wet\ weight\ (ww)}$ for oxazepam and 44.4 ng/g_{ww} for sulfamethoxazole after two weeks of exposure. In a similar study, 63 compounds were found in field gammarids, including antidepressants, analgesics, and cardiovascular. Some of the maximum concentrations found were 1 ng/g_{dw}, 5.4 ng/g_{dw}, and 3.1 ng/g_{dw} for carbamazepine, diclofenac, and venlafaxine respectively (Munz et al. 2018).

2.7 Micropollutants in Fish

Concerns related to micropollutant exposure and bioaccumulation in fish are related to reproductive health impacts that may impact populations (Overturf et al. 2015). Many studies have reported the occurrence of pharmaceutical and personal care products in fish tissue (Kalogeropoulou et al. 2021; Ramirez et al. 2009; Terechovs et al. 2019; Valdés et al. 2014). Ramirez et al. (2009) studied the occurrence of micropollutants in 5 rivers that receive a direct discharge from WWTPs in different locations in the United States (US). They detected antidepressants, anticonvulsants, and antihypertension products in fish tissue, and the concentrations were typically lower in river locations that receive tertiary-treated/advanced treatment. Another study completed in Suquia River Basin in Argentina focused on the bioaccumulation of carbamazepine and atenolol on the fish *Gambusia affini*s in exposure experiments, showing that the highest concentrations found were in average 95 ng/g_{ww} at 100 ug/L exposure levels and 53 ng/g_{ww} at 1000 ug/L exposure levels (Valdés et al. 2014). This study considered the n-octanol/water partition coefficients (Log K_{OW}). Therefore, carbamazepine has a higher Log K_{OW} (2.77) and is expected to bioaccumulate more than atenolol (0.16) (Valdés et al. 2014). In addition, it was concluded that due to the high frequency of occurrence of these compounds even at low concentrations, it might still facilitate bioaccumulation in aquatic species. Terechovs et al. (2019) examined the occurrence of 49 micropollutants in reclaimed water reservoirs and fish in the Shoalhaven region, Australia. This study reported 20 compounds in total, including antibacterial, caffeine, and neuroinhibitory substances with concentrations between < 0.1–2.72, < 0.1–2.42, and <0.1–4.18 ng/g_{ww}, respectively. Another study reported antibacterial, triclosan, and triclocarban in the Niche of River Torsa, India, ranging from 91.1–589 ng/g_{ww} and 29.1–285.5 ng/g_{ww} respectively were found (Das Sarkar et al. 2020). Hence, it is observed that in

developing countries where wastewater treatment is scarce (Wilkinson et al. 2022), higher concentrations have been found in fish tissue as in the case of India.

2.8 Sample Preparation Methods

Due to low concentrations of micropollutants in the environment coupled with the complexity of the environmental matrices, micropollutants are often difficult to detect and quantify (Sánchez-Avila et al. 2011). Thus, advanced multi-residue analytical methods with high standards of sensitivity and reproducibility, such as gas chromatography (GC) or liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) are required (Barbieri et al. 2019; Godfrey et al. 2022; Picó 2016). Furthermore, efficient extraction and clean-up of the samples are essential to optimize analytical methods, avoid ionization and background noise and determine trace concentrations (Sánchez-Avila et al. 2011; Serrano et al. 2003). These methods have been applied successfully to different environmental matrices, including invertebrates, sediment fish, and biota, which will be discussed in detail in the methodology of this thesis. Some sample preparation methods used for pharmaceutical and personal care products detection are:

i) **QuEChERs (Quick, Easy, Cheap, Effective, Rugged, and Safe):** it is a low resource extraction technique that offers a high throughput (Godfrey et al. 2022). In addition, other advantages that this method includes are high analyte recoveries, accurate results, little use of sources, and requires simple lab equipment (Barbieri et al. 2019).

   This method usually requires the samples to be vortexed, homogenized (manually or automatically), and centrifuged (Desiante et al. 2021). In addition, it uses water and a solvent, as well as, salts, sorbents, or buffers, depending on the analyte of interest, this is usually followed by a clean-up step to clean samples from lipids, color, humic acids, and other interferences (Anastassiades et al. 2003; Kalogeropoulou et al. 2021). This procedure

aims to obtain a clean supernatant that is reconstituted into a certain volume and subsequently analyzed (Anastassiades et al. 2003; Desiante et al. 2021).

ii) **SPE (Solid Phase Extraction):** it is a technique that allows the separation and isolation of different analytes from a liquid matrix (Lehotay and Schenck 2000). Thus, this method allows to enrichment and purify micropollutants from water samples for many compounds (Tran et al. 2013). SPE columns are packed with different chromatographic sorbents, including silica, florisil, or alumina (Lehotay and Schenck 2000). The selection typically depends on the physicochemical properties of the analytes and the sorbent characteristics (Tran et al. 2013). The experimental procedure typically consists of 4 steps, conditioning, sample introduction, washing, and eluting either via a manual vacuum manifold systems that use disposable columns (Verette 2000) or automated SPE instrumentation.

iii) **ASE (Accelerated Solvent Extraction):** is a sample preparation technique that is applied to detect different analytes of interest, including pharmaceutical and personal care products in solid or semi-solid matrices (Xia et al. 2005). It uses high organic solvents at high temperatures to increase the capacity to solubilize analytes and the diffusion rates and pressures to keep the solvent in liquid state (Sun et al. 2012). It is an optimum method to remove unwanted matrix components from the samples, providing a cleaner sample prior to chromatography. Automated ASE provides some advantages compared to conventional methods (e.g., Soxhlet), requires 15-30 minutes and 10-30 mL of solvent per solvent, depending on the application (Sparr Eskilsson and Björklund 2000).

Table 2.3. *Sample preparation and analysis methods for different environmental matrices. PAHs= Polycyclic aromatic hydrocarbons, PCBs= Polychlorinated biphenyls, PFAs=Per- and polyfluoroalkyl substances, ASE=Accelerated solvent extraction, SPE=Solid phase extraction, N/A=not applicable. SW= surface water, GW = groundwater, DW = drinking water, WWTP = wastewater treatment plant. MeOH = Methanol, ACNN = acetonitrile; GC = gas chromatography, LC = liquid chromatography, MS = mass spectrometry, TOF=time of flight. HPLC/UPLC = high or ultra performance LC.*

Matrix	Location	Type of sample	Analyte	Extraction method	Extraction Solvent	Analytical method	Analyte Recovery (%)	Reference
Water	Seine river Estuary, France	SW, WWTP effluent	Analgesics, stimulants, anticonvulsants, cardiovascular	SPE	MeOH	GC-MS	53 to 99	(Togola and Budzinski 2007)
Water	River Ouse, England	WWTP effluent	Analgesics	SPE	MeOH	LC-MS/MS	71–95 for most of the compounds	(J. L. Zhou et al. 2009)
Water	Lagos State, Nigeria	SW, GW, DW	Analgesics, antibiotics, anticonvulsants, antidepressants, stimulants, cardiovascular.	SPE	MeOH	UPLC-QExactive Orbitrap MS	>70	(Ebele et al. 2020)
Sediment	Bourbre River, France	N/A	Pharmaceuticals (antibacterial, antifungal, analgesic, anticonvulsants, antiestrogen), pesticides, UV filter, hormones.	QuEChERs	ACN	LC-MS/MS	>50	(Berlioz-Barbier et al. 2014)
Sediment	Adour estuary and Capbreton submarine canyon, France	N/A	51 priority and emerging pollutants including, pharmaceuticals (carbamazepine), PAHs, PCBs, pesticides, musks and sunscreen.	QuEChERs	Ethyl acetate-toluene	GC-MS	62 –131 for all the compounds	(Miossec, Lanceleur, and Monperrus 2018)

Table 2.3 continued

Matrix	Location	Type of sample	Analyte	Extraction method	Extraction Solvent	Analytical method	Analyte Recovery (%)	Reference
Fish	Aquaculture located in the Mediterranean Sea, Greece	N/A	Antibiotics, stimulants, analgesics, anticonvulsants, antidepressants	QuEChERs	Acetonitrile	UHPLC-Orbitrap MS	62-107	(Kalogeropoulou et al. 2021)
Fish	Hainan Province, China	N/A	151 organic compounds including, pharmaceuticals (stimulants, anticonvulsats, antibiotics, antibacterial), PFAs, pesticides.	QuEChERs	Acetonitrile	LC-QTOF-MS/MS	89.3, 76.6, and 67.9 at three different concentration levels (10, 50, and 100 ng g−1)	(Zhao et al. 2022)
Fish	Dongjiang River, south China	N/A	Personal care products	QuEChERs	Acetonitrile	UPLC-MS/MS and GC-MS	45-150 for most of the compounds	(Yao et al. 2016)
Gammarids	Bourbre River, France	N/A	Pharmaceuticals (anxiolytic and antibiotic) and surfactants	microQuEChERs	Acetonitrile and Hexane	nanoLC-MS/MS	N/A	(Garcia-Galan et al. 2017)
Gammarids	Switzerland	N/A	63 compounds, including antidepressants, analgesics and cardiovascular.	QuEChERs	Acetonitrile	UPLC-QExactive Orbitrap MS	43-182	(Munz et al. 2018)
Biofilm	Switzerland	N/A	63 compounds, including antidepressants, analgesics and cardiovascular	QuEChERs	Acetonitrile	LC- Orbitrap MS	80 and 120 for 50 of the compounds	(Desiante et al. 2021)

Table 2.3 continued

Matrix	Location	Type of sample	Analyte	Extraction method	Extraction Solvent	Analytical method	Analyte Recovery (%)	Reference
Biofilm	Switzerland	N/A	75 compounds including antibiotics, pharmaceuticals, anti-corrosion agents, artificial sweeteners, fungicides, herbicides, insecticides.	QuEChERs	ACN	LC- Orbitrap MS	Not specified.	Desiante et al. 2022
Sediment	China	N/A	186 compounds were detected, including antihistamines, anti-infective, analgesics, cardiovascular, hormones, urinary system, respiratory, central nervous system.	ASE+SPE	MeOH/water (50/50, v/v)	LC-MS/MS	62.4 to 107.1	Chen et al. 2013
Sediment	Augusta Bay, Italy	N/A	46 pharmaceuticals including antibiotics, cardiovascular, analgesics, antidepressants, lipid regulators, gastrointestinal drugs.	ASE+SPE	MeOH/water (50/50, v/v)	HPLC – MS	>75	Feo et al. 2020

Chapter 3 – Methodology

3.1 Reagents and materials

Ibuprofen, carbamazepine, venlafaxine, trimethoprim, sulfamethoxazole, norfluoxetine, fluoxetine, 10, 11 epoxide carbamazepine, atenolol, gemfibrozil, naproxen, lorazepam, chloramphenicol and triclocarban were purchased from Sigma-Aldrich (Oakville, ON). Atorvastatin, atrazine, caffeine, and triclosan were from Syn-Finechem, Chem Service (West Chester, PA), Thermo Fisher (Waltham, MA), and Alfa Aesar (Wardhill, MA) respectively. The isotopically labeled standards (d-atorvastatin, d-carbamazepine, d-ibuprofen, d-triclosan, d-venlafaxine, d-diclofenac, d-gemfibrozil, d-naproxen, d-triclocarban, d-caffeine, d-epoxide carbamazepine, d-fluoxetine, d-norfluoxetine, d-trimethoprim) were purchased from CDN Isotopes Inc (Pointe-Claire, QC) and d-Atorvastatin, d-p-Hydroxy Atorvastatin, and d-o-Hydroxy Atorvastatin from Toronto Research Chemicals (Toronto, ON). The stock solutions for all compounds were prepared in HPLC grade methanol from Fisher Scientific (Toronto, ON). Acetonitrile (HPLC grade), heptane (HPLC grade), and hydrochloric acid (10 M) were purchased from Fisher Scientific (Toronto, On). Ammonium acetate was obtained from Sigma-Aldrich (Oakville, ON). Ultrapure water for sample preparation and mobile phase preparation was obtained from a Milli-Q ultrapure water system (IQ 7000) with a specific resistance of 18 MΩ·cm and total organic carbon of <50 ppb. QuEChERS salts, dispersive SPE extraction kits (sample cleanup method development), and Bond Elut Plexa SPE cartridges were purchased from Agilent Technologies (Mississauga, ON).

3.2 Sample collection

A total of twelve sampling campaigns were carried out (Table 3.1) to (1) characterize the occurrence of micropollutants in 12 replicate streams (September 2020); (2) develop and validate

sample cleanup methods for biofilms, fish, gammarids, and sediments (Fall 2021); (3) survey the changes in micropollutant concentrations in the streams over time; and (4) assess the impact of increasing effluent contribution (5% to 15%) in five environmental matrices (Fall 2022). The schematic of sampling procedures is found in Figure 3.1. Field water quality parameters (temperature, pH, and conductivity) were measured using a calibrated portable multiprobe meter (Thermo Scientific, Orion 8107UWMMD ROSS Ultra pH/ATC triode and YSI 21F105206). Furthermore, additional water samples were collected in February, April, May and July 2022 to determine the monthly variation of the concentrations and fish (*via* collaborators from the University of Calgary) were collected at Bowness Park as a control site.

Table 3.1 *Sampling campaign details. ACWA = Advancing Canadian Water Assets, O_3 = ozonation, RO = reverse osmosis, UF = ultrafiltration. For stream number and types see Figure 1.2.W = Water, S = Sediments, BF = Biofilm, G = Gammarids, F = Fish. For ACWA stream information, see Section 1.4.*

Date	Matrix	Purpose	Location
September 2020	W	Characterization and assessment of replication	All ACWA streams
June 2021	W	Characterization and Seasonal Survey	All streams and O_3, RO, and UF effluent.
	S, BF, G	Method development	Streams 1, 2, 3, and 6
October 1, 2021 October 29, 2021	W	Seasonal Survey	Streams 2, 3, 4, 7, 10, and 12 and UF effluent.
November 26, 2021	S, BF, G, F	Method development and validation	Streams 2, 3, 4, 7, 10, and 12.
February 2022 April 2022 May 2022 July 2022	W	To determine monthly variation of the concentrations	Streams 2, 3, 4, 7, 10, 12

August 30, 2022 September 21, 2022 October 13, 2022	W, S, BF	Compare upstream Bow River site to ACWA streams	Canmore
August 31, 2022 September 20, 2022 October 12, 2022	W S, BF, G, F	Seasonal Survey Assess bioaccumulation	Streams 2, 3, 4, 7, 10 (now at 15% v/v effluent), 12

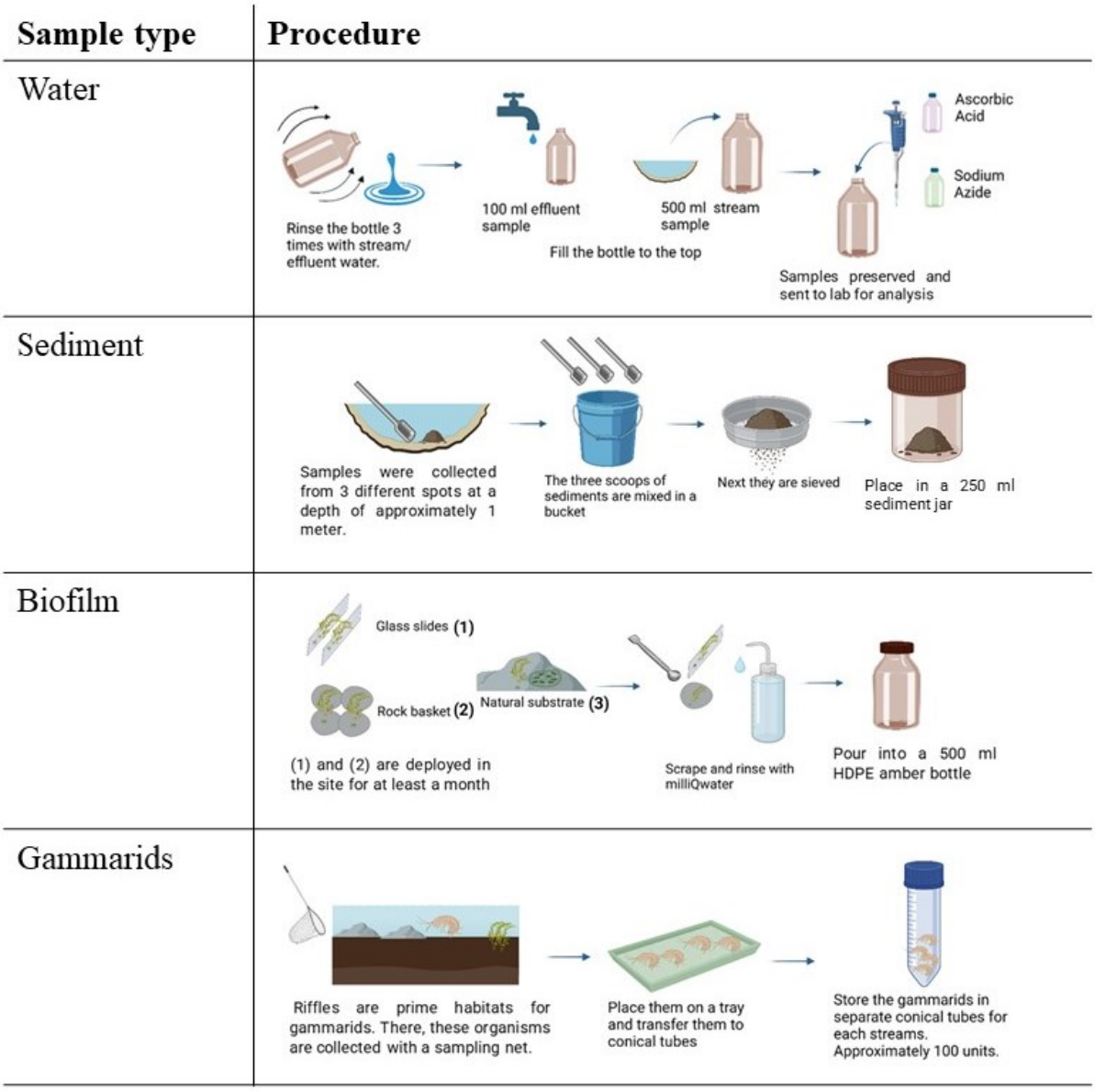

Figure 3.1 *Summarized schematic and sampling collection protocols for each environmental matrix considered in this study.*

In Fall 2022, Stream 10 (Pine Creek) had an experimental increase in the effluent concentration from 5% to 15% while the other 2 replicate streams maintained the effluent contributions (i.e., 5%). At this sampling campaign, a control site was included in Canmore to compare the differences between the upstream and downstream Bow River (after inputs from two major municipal wastewater treatment plants). Water samples (n=3), sediment samples, and biofilms were collected at the Canmore site in August, September and October 2022. Locations of the Canmore samples are found in Table 3.2. For biofilm samples, only 1 replicate was collected due to the low water level one month after rock basket deployment.

Table 3.2 *Latitudinal and longitudinal coordinates for Canmore water samples.*

Sampling date	Sample 1	Sample 2	Sample 3
August 31, 2022	51.07432°N, -115.35424°W	51.07443°N, -115.35374°W	51.07450°N, -115.35352°W
September 20, 2022	51.07437°N, -115.35374°W	51.07449°N, -115.35345°W	51.07454°N, -115.35314°W
October 12, 2022	51.07425°N, -115.35437°W	51.07436°N, -115.35389°W	51.07448°N, -115.35339°W

The ACWA stream samples were collected from the third pond of each stream in a 500 mL glass amber bottle. For stream characterization (September 2020), triplicates were first collected and after a preliminary assessment of the replication, collecting one sample from each stream is considered sufficient (Table S.3). The bottle was first rinsed three times with stream water before filling it up to the top with no headspace. Effluent samples (Reverse Osmosis, Ozonation, Pine Creek WWTP) were collected in a 100 mL glass amber bottle directly from the sample port prior to mixing with the stream water. All water samples were preserved with 1g/L mg/L 5% v/v sodium azide solution and 1.25 mL 2.5% v/v of 50 mg/L ascorbic acid and stored in 4°C until extraction.

Sediment samples were also collected from the same pond as the water samples, but three different locations were subsampled (using a shovel) and then mixed for a homogenous, composite sample. The samples were then sieved (2.36mm), scooped into 250 mL amber glass jar, transported in ice, stored in -20°C, and freeze-dried prior to extraction.

Initially, three different methods were used to assess the suitability of the biofilm collection technique. Glass substrates (using Wildco periphyton sampler) and rock baskets made in-house using store-bought ceramic briquettes enclosed in a stainless mesh grill were deployed in the third riffle of the streams for one month to allow biofilm growth. The third method relied on the collection of biofilms from the substrates naturally present in the streams (rocks). In the later sampling campaigns (Fall 2022), only rock basket samples were deployed as it was found that there were no substantial differences in the accumulation of micropollutants between glass and rock basket substrates (See chapter 4, Figure 4.4). Regardless of the method, biofilm was scraped from the substrate and rinsed with ultrapure water into a 500 ml High Density Polyethylene (HDPE) amber bottle. The samples were then centrifuged in 50 mL conical tubes for 10 min at 4000 rpm to separate the biofilms. The biofilms were freeze-dried prior to extraction.

Gammarids were collected in the riffles via kick-net sampling. Approximately 100 adult gammarids (1-1.5 cm long) were collected in each stream, placed in conical tubes, transported in dry ice, and then freeze-dried prior to extraction. Fish (spoonhead sculpin and longnose dace) were collected at a control site and then caged in the streams (Stream 3, 4, 7 and 10) for up to 28 days. Whole fish tissue samples were transported in dry ice and freeze-dried prior to extraction.

3.3 Sample extraction – water samples

All water samples were extracted via solid phase extraction (SPE), a common extraction method for trace organics from liquids by passing them through a sorbent (Neale, Leusch, and

Escher 2021). The sample preparation and extraction methods are similar to previously published methods (Arlos et al. 2016) and are summarized in Figure 3.2. Briefly, water samples were filtered with 1µm Pall glass fibre filters and acidified to a pH of ~2 using drops of 1 or 10N HCL.

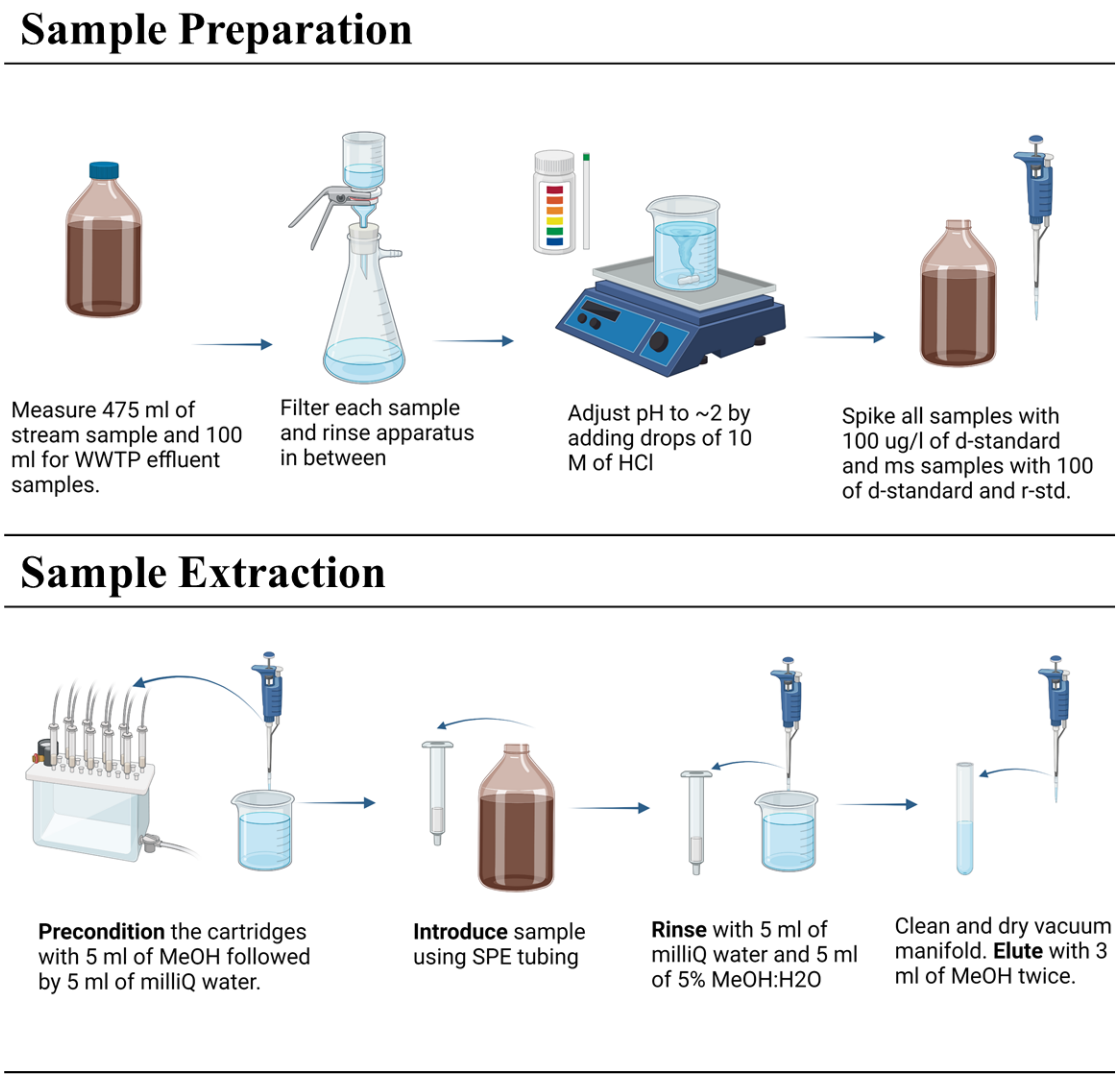

Figure 3.2 *Sample preparation and extraction procedures for water samples. MS= Matrix Spiked, MeOH=Methanol, d=deuterated standards, r=regular standards, pharmamix=pharmaceutical mixture. HCl = hydrochloric acid.*

A manual SPE vacuum manifold (12-position Visiprep, Supelco) was set up with Bond Elut Plexa (6 cc, 500 mg) cartridges which were then preconditioned with 5 mL of methanol and 5 mL ultrapure water. Next, water samples were introduced into the vacuum manifold via large volume samplers (Visiprep, Supelco). The cartridges were then rinsed with 5 mL of ultrapure water and 5% v/v methanol:ultrapure water, and dried under vacuum for ~1h. Sample elution was done with 2 × 3 mL methanol. Finally, samples were evaporated to dryness under a gentle stream of nitrogen and reconstituted to 500 µL of reconstitution solution containing 10 mg/L lorazepam-chloramphenicol prepared in methanol. A 100 µL aliquot was transferred to a 2 mL HPLC vial with glass inserts and stored at -20°C until analysis. For SPE quality control (QA/QC), 500 mL of ultrapure water were acidified and spiked with 100 µl of 100 µg/L of deuterated standard (d-std) and 100 µl of 100 µg/L of regular standards (r-std). SPE blanks were also prepared (spiked with d-std) and analysed for any process background.

3.4 Sample extraction – sediment samples

Two extraction methods were assessed for sediment extraction: (1) accelerated solvent extraction (ASE) via Dionex ASE 350 and (2) QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe). ASE extracts organic compounds from solid to semi-solid samples by using elevated temperature and pressure at a relatively short time and as a result, only requires low volume of solvent in comparison to liquid-liquid extraction (Gan et al. 1999). The ASE was set up by placing one ASE filter (Thermo Scientific, glass fiber for 1, 5, 10, 22 ml ASE 350/150 Cell) at the bottom of the 10 mL ASE cell and then packed with 1 g of Florisil (Supelco, 60-100 Mesh, Activated magnesium silicate), a second ASE filter, and finally, 6 g_{dw} of sediment samples or 6 g

of sand (Fisher Chemical, 20-30 Mesh, for cement testing) for control samples mixed with 0.5 g hydromatrix (Agilent Technologies, diatomaceous earth) (Figure 3.3). The cell was capped and then installed into the instrument for extraction. The automated extraction cycle begins by bringing the temperature to 80°C and filling the cell with extraction solvent (70:30 ethyl acetate:acetone (EtOAc:Ace)) at 1500 psi. Two static extraction cycles were then completed for 5 min each and the cells were rinsed with fresh extraction solvent (60% of the cell volume) and purged with nitrogen (150 psi). A different extraction solvent (1:1 MeOH:EtOAc) and temperature (70°C) was also assessed to see any differences in extraction methods. More details related to the results obtained with this method is explained in Chapter 4.

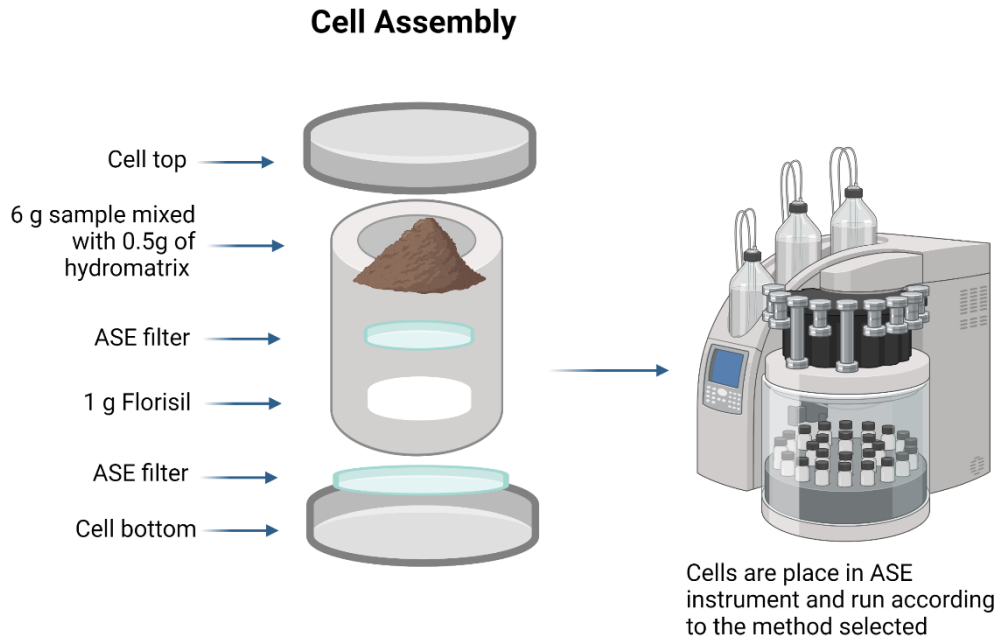

Figure 3.3 *Visual of the Accelerated Solvent Extraction (ASE) sample preparation, assembly, and extraction.*

The QuEChERS method for the sediments was based on Berlioz-Barbier et al. (2014) with acetonitrile (ACN) as the extraction solvent. ACN is an adequate solvent to extract a broad range

of analytes and it is efficient to remove matrix interferences (Kalogeropoulou et al. 2021). Briefly, 2 g_{dw} of sediment were pre-weighed, placed in a 50 mL conical tube, and spiked with 100 µl of 100 µg/L of d-std and QA/QC with 100 µl of 100 µg/L of r- and d-std. The tube was shaken manually and vortexed for 30 s, and 10 mL of ultrapure water was then added to hydrate the samples. A similar procedure (shake-vortex) was followed after adding 10 mL of ACN and QuEChERS acetate buffer (6:1.5 Magnesium sulfate: Sodium Acetate ($MgSO_4$:NaOAc)). The tube was then centrifuged for 5 min at 5000 rpm and 8 mL of the supernatant was carefully transferred to a 15 mL conical tube containing 150 mg Primary Secondary Amine (PSA), 45 mg Graphitized Carbon Black (GCB), 855 mg $MgSO_4$ for another clean up step to remove pigments and fats. The tube was then shaken manually for 30 s, vortexed for 30 s and centrifuged for 5 min at 5000 rpm (Figure 3.4). Finally, 5 ml of the top layer was transferred to a test tube, evaporated at 40°C under gentle nitrogen stream and reconstituted to 500 µL in reconstitution solution (10 mg/L Lorazepam-Chloramphenicol prepared in MeOH).

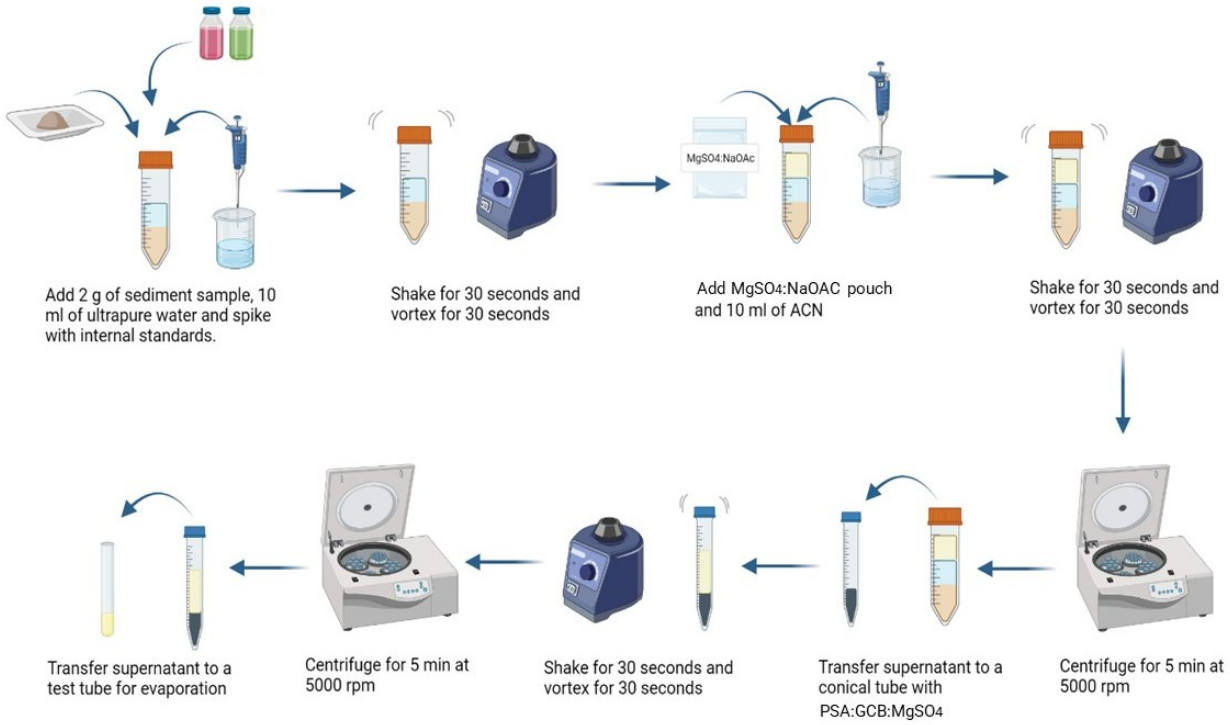

Figure 3.4 *QuEChERS sample extraction and preparation method used for sediment samples.*

3.5 Sample Extraction – fish, gammarid, and biofilm samples

Method development was initially carried out for the three matrices using the QuEChERS procedure described by Munz et al. (2018). For the purposes of this thesis, this method is labeled as Biota Extraction Method (BEM) 1 (initial method) and has the following steps: 125 mg_{dw} of gammarids, 100 mg_{dw} of biofilm or fish were pre-weighed in a 2 mL vial (MP Biomedicals, FastPrep Tubes). Next, 0.5 g of silica beads, 500 µl of ACN and 500 µl of ultrapure water were added. The sample was homogenized for 20 s at 6.5 m/s twice using a 24-position Fast Prep bead beater (MP Biomedicals) and cooled in ice for 5 min in between and centrifuged for 6 min at 10,000 rpm. Later, the supernatant was transferred to a second vial. A second extraction was completed on the same sample but only adding 500 µl of ACN this time. In the second vial, 0.3 g of 4:1 $MgSO_4$:NaCl QuEChERS salts were added and vortexed for 1 min and centrifuged for 6 min. The supernatant was transferred to a third vial, where 500 µl of heptane were added as a clean-up step for lipophilic substances (fats, oils), then the vial was vortexed and centrifuged for 6 min (these steps were repeated twice). The final extract was then transferred to a test tube and evaporated to dryness under a gentle stream of nitrogen. Finally, the sample was reconstituted to 500 µl of reconstitution solution and 100 µl were transferred to a 2 ml vial plus insert.

Low concentrations of analytes were expected as explained in Chapter 2. Therefore, different sample mass and reconstitution volumes were explored to obtain a more concentrated extract for a better detection as shown in Table 3.3. Additional extractions were completed using the initial method but with the mass and volume modifications (BEM 1.2 to 1.5). However, for BEM 1.3 the clean-up step was changed by using Z sep+ bulk salts (Sigma Aldrich) instead of heptane. The gammarids and fish samples were run for all the BEM 1 experiments while biofilms were only via BEM 1.3 and 1.4 due to limited amounts.

Table 3.3 *QuEChERS experiments with different reconstitution volume, mass, regular standard concentration and clean up step. [a]Expected concentration in the tissue extract assuming they are from a clean site). dw = dry weight.*

Method	Amount of Regular standard spiked (µL)	Regular Standard concentration (µg/L)	Sample Weight (mg_{dw})	Reconstitution volume (µL)	Clean-up Step	Expected concentration in the extract (µg/L)[a]
BEM 1.0	100	100	~100/~125	500	Heptane	20
BEM 1.2	32	10	~300	80	Heptane	4
BEM 1.3	35	10	~300	50	Z sept +	7
BEM 1.4	15	10	~125	80	Heptane	3
BEM 1.5	16	10	~125	50	Heptane	2

The results from the experiments on Table 3.3 suggested a further sample clean up and are subsequently discussed in chapter 4. Therefore, an additional method development included the 4 QuEChERS experiments shown in Table 3.4, which were adapted from Godfrey et al (2022) (BEM 2), Kalogeropoulou et al. (2022) (BEM 3), Barbieri et al. (2019) (BEM 4) and Munz et al (2018) (BEM 5) respectively. All samples were spiked with 16 µl of 100 µg/L r-pharmamix standard and 100 µL of 100 µg/L d-pharmamix. Additionally, these experiments were tested with biofilm, fish and gammarids to determine which one works best for each matrix. Moreover, to enhance the sample clean up, all the extracts were filtered with centrifugal filter (Amicon, 10 kDa molecular weight cut off (MWCO)). Finally, the extractions utilised for the Fall 2022 sampling campaign samples is shown in Figure 3.5.

Table 3.4 *Method development for further clean up of fish, gammarids and biofilm. NaCitrate=Sodium Citrate, C18=Trifunctionally-bonded C18 silica, NaCl=Sodium chloride, DCS=Disodium citrate sesquihydrate.*

Method	Weight (mg)	Reconstitution volume (ul)	Extraction Salts	dispersed-SPE (dSPE) /Clean up
Experiment 1	~500	80	6:1.5 $MgSO_4$:NaOAc	1:6 PSA:$MgSO_4$ and Z-sep+
Experiment 2	~500	80	4:1 $MgSO_4$:NaCl	6:1:1 $MgSO_4$:PSA:C18 + Z sep+
Experiment 3	~500	80	4:1:1:0.5 $MgSO_4$:NaCl: NaCitrate:DCS	6:1:1 $MgSO_4$:PSA:C18
Experiment 4	~400	80	4:1 $MgSO_4$:NaCl	Z sep+

Initial QuEChERS method was used to extract biofilm samples with some modifications. Instead of using heptane for the cleanup procedure, Z sep+ salts were utilised. In addition, 0.2 g_{dw} of biofilm were extracted, and the reconstitution volume was 80 µl as shown in Figure 3.6.

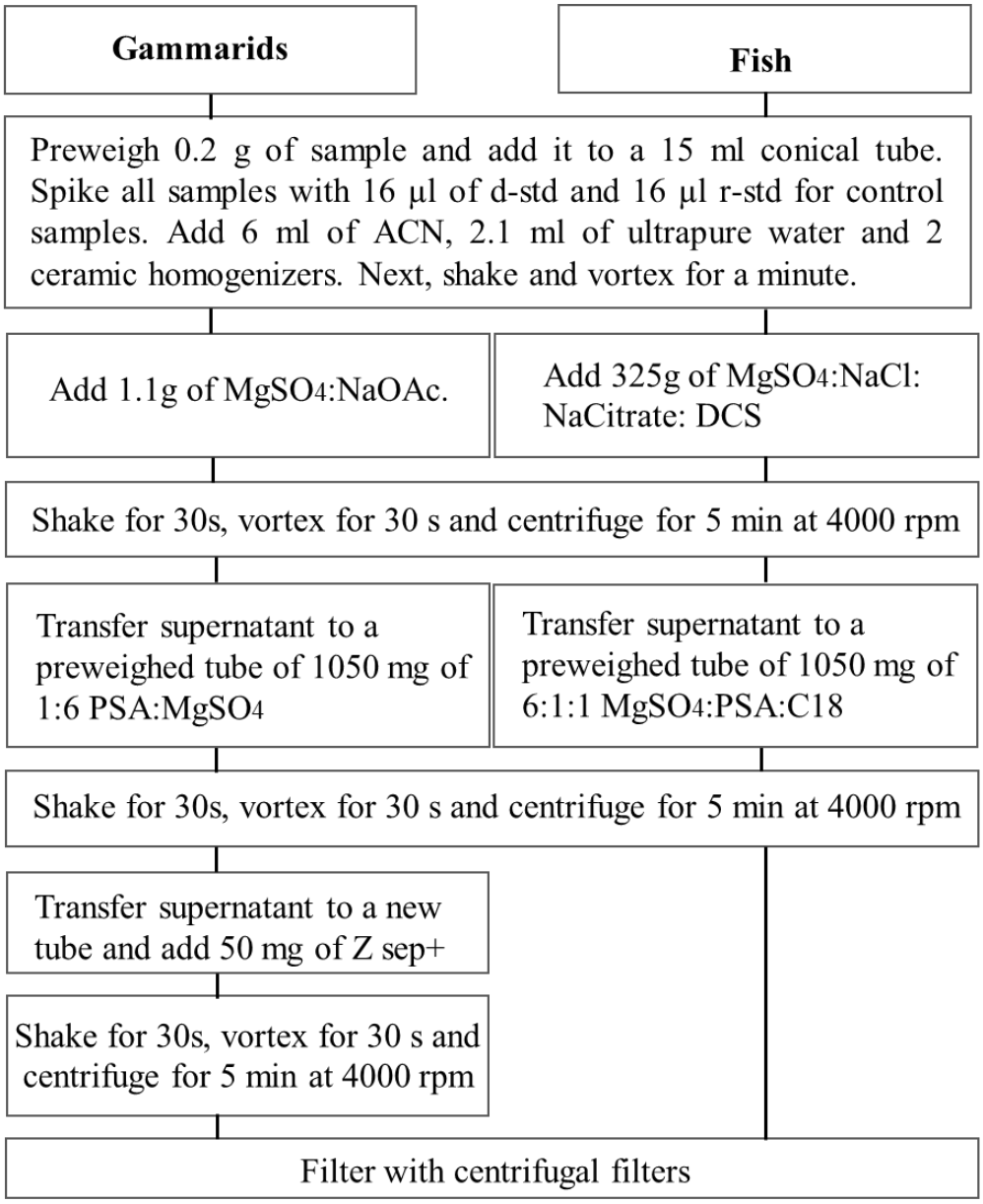

Figure 3.5 *Final extraction methods used for gammarids and fish. Samples were spiked with 16 µL of 100 µg/L regular-pharmamix standard and 16 µL of 100 µg/L deuterated-pharmamix. ACN=acetonitrile, NaCitrate=Sodium Citrate, DCS=Disodium citrate sesquihydrate.*

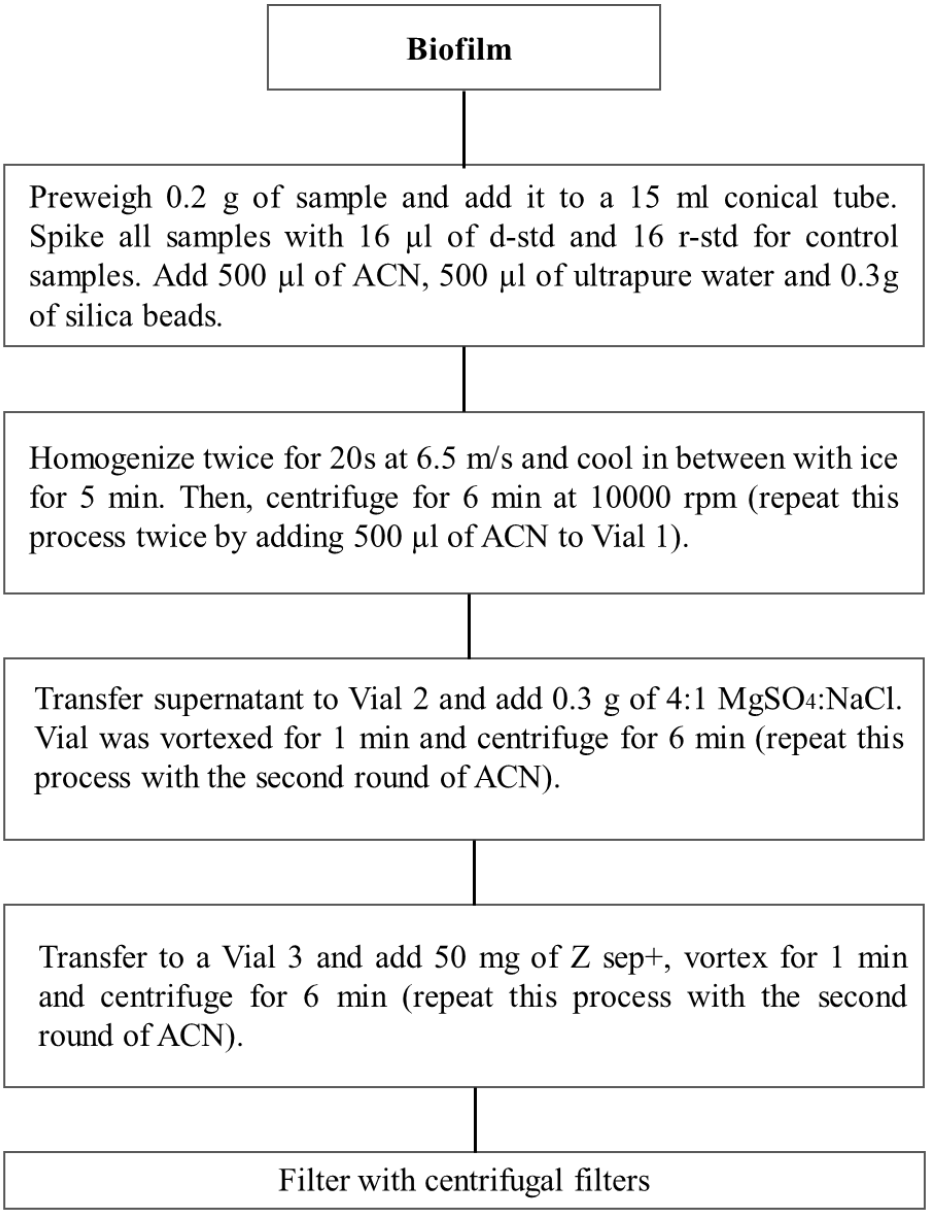

Figure 3.6 *Final QuEChERS experiment for biofilm extraction.*

3.6 Sample Analysis

Sample analyses for pharmaceuticals was completed via liquid chromatography and tandem mass spectrometry (LC-MS/MS) using an Agilent 1260 liquid chromatograph (LC) with a 6460 Triple Quad mass spectrometer (MS) system equipped with an electrospray interface (ESI). The column used for this method is a 2.1 mm × 50 mm × 1.8µm ZORBAX Eclipse Plus C18 (Agilent Technologies). The analytical method has been previously developed and the LC and MS/MS parameters are discussed in detail elsewhere (Arlos et al. 2015; Mehdi et al. 2021).

3.7 Bioaccumulation Assessment

Bioaccumulation is defined as the partitioning of different compounds via aqueous, sediment, or dietary exposure. Bioconcentration factor is defined as the ratio of the concentration in the organism to that in the water (Xie et al. 2019). The following formula is used to estimate the Bioconcentration Factor, BCF:

$$BCF = \frac{C_{Internal}}{C_{Exposure}} \qquad (2)$$

where the unit of BCF is given in L kg-1, C internal (ng/kg) is the concentration of compounds in the organisms and C exposure (ng/L) is the concentration of the compounds in water (Munz et al. 2018). According to USEPA (1999), a compound is bioaccumulative if the BCF is greater than 5000 L/kg and as potentially bioaccumulative if it is between 2000 and 5000 L/kg (USEPA, 1999).

3.8 Sediment/water partition coefficient (K_d)

The sediment/water partition coefficient is calculated to determine the affinity of substances to sediments (Baker et al. 1986). This coefficient is calculated with the following formula:

$$\mathrm{K_d} = \frac{\mathrm{C_s}}{\mathrm{C_w}} \qquad (3)$$

Where, K_d is given in L/kg, C_s is the concentration in sediments and C_w is the concentration in water.

3.9 Quality Controls

3.9.1 Relative Recovery

The relative recovery was calculated as the concentration recovered from the spiked samples and the theoretical spiked concentration (equation 3).

$$\%\ Relative\ recovery = \frac{Spiked\ matrix\ sample - Unspiked\ matrix\ sample}{Theoretically\ spiked} \times 100 \quad (4)$$

where spiked matrix sample is the concentration found in the spiked samples, unspiked matrix samples is the concentration found in the samples that were not spiked, and theoretically spiked is the expected concentration.

The samples from this thesis were collected from sites with high background (e.g., Pine Creek, Bow River) (See Chapter 2, Figure 2.1). Therefore, spiked samples and Canmore (reference) samples were also considered to estimate the recoveries. In addition, due to impact of different matrices on analysis, recovery calculations are considered only if the response area of the unspiked sample was lower than the response area of the spiked sample multiplied by a factor of 1.7 (Lauper et al. 2022). The recovery from these samples is compared to a theoretical concentration of 20 µg/L.

Table 3.5 *Standard and reconstitution values used to obtain expected concentration from final experiments.*

Sample	Reconstitution Volume (µl)	Standard concentration (µg/L)	Standard spiked (µl)
Water	500	100	100
Sediment	500	100	100
Fish	80	100	16
Gammarids	80	100	16
Biofilm	80	100	16

3.9.2 Limit of quantification

The limit of quantitation (LOQ) is the lowest measured concentration of an analyte that can be reported given an analytical procedure with a determined degree of certainty (Lister 2005). An absolute recovery was first calculated by averaging the internal standard responses in the sample and the average of internal standard responses in the calibration curve (equation 5).

$$Absolute\ recovery\ (\%) = \frac{Average\ ISTD\ response\ in\ sample}{Average\ ISTD\ response\ in\ calibration\ curve} * 100 \qquad (5)$$

Subsequently, the LOQs for each analyte at the time the samples were injected was calculated as the ratio of the lowest point in the calibration curve and the absolute recovery (equation 6).

$$LOQ = \frac{Lowest\ std\ found\ in\ calibration\ curve}{Absolute\ recovery} \qquad (6)$$

3.9.3 Matrix Effect Factor

LC-MS techniques are very sensitive and often suffer from matrix effects, which can cause ion suppression or ion enhancement. Thus, the following approach by (Zhou et al. 2017) was used to determine the matrix effect factor for the samples analysed in this study:

$$Matrix\ effects\ factor\ (MEF) = \frac{A - B}{A} * 100 \qquad (7)$$

where A is the average of the response area of the calibration curve and B is the average of the response area of the sample. If ME ~0%, there is no matrix effect. If ME> 0%, an ion-suppression occurs and, if ME <0%, ion-enhancement occurs. In addition, the result of a sample is considered acceptable if it is ≤85%.

Chapter 4 – Results and Discussions

This chapter first discusses the outcome of the sample preparation method development for sediments, gammarids, biofilm, and fish, followed by a thorough evaluation of the partitioning and bioaccumulation conditions of micropollutants in environmental matrices including biota (biofilm, gammarids, and fish). No method development on the preparation of river and wastewater effluent samples was completed as it has already been evaluated in a prior study (i.e., Arlos et al. 2015). The quality of the analytical data derived from different sample preparation methods (e.g., ASE vs. QuEChERS for sediments) are described in detail, including the justification of which approach was finally employed.

4.1 Evaluation of the sample preparation methods

4.1.1 Sediment

As described in Chapter 3, two extraction methods were evaluated for sediment sample preparation prior to LC-MS/MS analysis. Based on the quality of the chromatograms (Figure 4.1a), the QuEChERS method was deemed to be more acceptable as they have symmetrical peak shapes (Gaussian) and flatter and more stable baselines, indicating minimal interferences or noise. Background interferences are more pronounced in sediment when employing the ASE (initial) method. More specifically, multiple peaks showed up before and after the compound retention time, suggesting poor resolution between different peaks. In addition, the ISTD signal for the unspiked sample for venlafaxine is two orders of magnitude lower compared to the average ISTD area in the calibration curve, and the compound response area for the spiked samples (MS) is also very low (Table 4.1). Thus, the method is not able to recover the spiked amount, suggesting that the cleanup method is not amenable for chemical analysis.

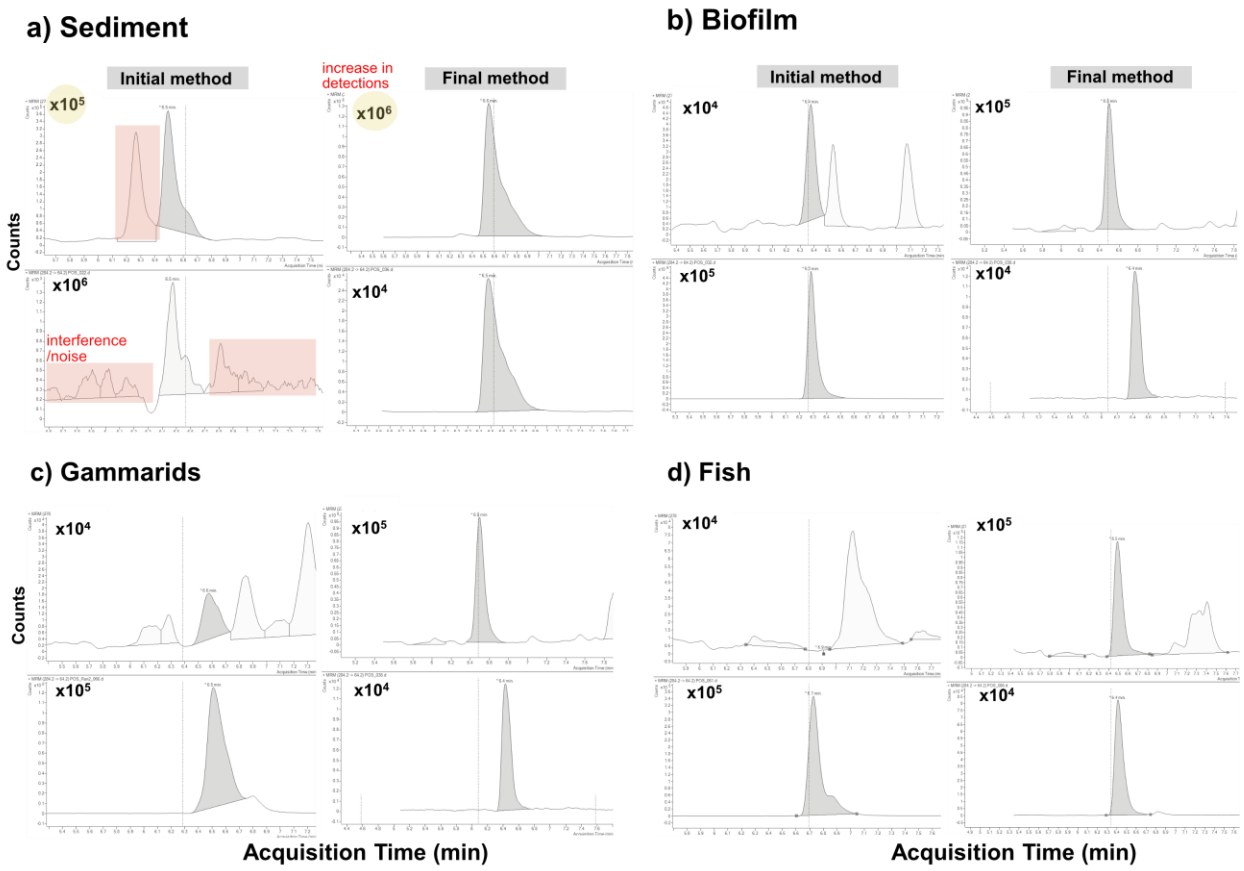

Figure 4.1 *Sample chromatogram comparison (venlafaxine) between initial (BEM 1.0 [gammarids, biofilm, fish] and ASE [sediments]) and final sample preparation method (QuEChERS) for (a) sediment, (b) biofilm, (c) gammarids and (d) fish (see chapter 3, Section 3.4 and 3.5). Top and bottom chromatograms are regular/native standard and ISTD response areas, respectively. The response area for comparisons between the two methods for venlafaxine are found in Table 4.1. Good LC-MS/MS chromatogram is considered to have a good Gaussian (bell) curve with very little to no tailing. However, chromatograms can have different shapes and pass the integration as long as the tailing is consistent (Zhang et al. 2009; Mičová et al. 2012). S=Stream.*

Table 4.1. *Comparison of the LC-MS/MS responses in the internal standard and concentrations detected for venlafaxine. For fish, SED=Sediment, BF=Biofilm, GAM=Gammarids, MS=Matrix spiked, ISTD=Internal Standard, BEM=Biota extraction method, "- "= not applicable. Response area for ISTD is lower for final methods due to different reconstitution volumes (see Chapter 2, section 2.4.). Experiments 1,4, and 3 were all variations of QuEChERS method (see Chapter 2) with an addition of colour or fat layer removal (in the case of fish). Cal curve = calibration curve. *Average ISTD Area Cal Curve considered the same for both spiked and unspiked samples except for fish.*

Matrix	**SED**		**BF**		**GAM**		**FISH**	
Type of Sample	**MS Spiked**	**Stream 2 Unspiked**	**Stream 3 Spiked**	**Stream 2 Unspiked**	**Stream 2 Spiked**	**Stream 3 Unspiked**	**Stream 10 Spiked**	**Stream 2/ 3 Unspiked**
Initial Method	**ASE**		**BEM 1.0**		**BEM 1.0**		**BEM 1.0**	
Expected concentration (µg/L)	20	-	20	-	20	N/A	20	-
Measured Concentration (µg/L)	0.7	1110.5	27.6	9.53	0	1.44	0	0
Compound response area	8086	200141	852271	314015	0	30790	0	0
ISTD Area	6029618	17976	2147845	1648983	2912230	1477330	6033866	2045516
Average ISTD Calibration Curve	8344998	8344998	8344998	5790911	8344998	6663612	8344998	5703331
Final Method	**QuECheRS**		**Experiment 4**		**Experiment 1**		**Experiment 3**	
Expected concentration (µg/L)	20	N/A	20	-	20	-	20	-
Measured Concentration (µg/L)	22	498	65.2	37.3	44.2	10.3	18.7	2.3
Compound response area	1318618	9600639	537761	377422	1866331	415118	1125762	112270
ISTD Area	981297	242007	120927	149426	599915	582787	759272	691118
Average ISTD Area Cal Curve*	2619159	2619159	20962990	20962990	2221018	20962990	31125576	20962990

ASE has been successfully utilised in the past for micropollutant extraction in environmental samples, including sediments (Hossain et al. 2011; Okuda et al. 2009). However, this method is typically followed up with an additional cleanup step such as solid phase extraction (SPE), solid phase microextraction (SPME), and dialysis to pre-concentrate the analytes of interest and most importantly, to minimize interferences during LC-MS/MS analysis (Jelić et al. 2009; Llompart et al. 2019; Wenzel et al. 2004). SPE is a more common approach and can be done offline or online. However, the automated approach (i.e., online SPE) is considered more superior due to its efficiency (e.g., reagent consumption, speed) and improved sensitivity and precision (Pan et al. 2014).

Although it is now common to utilise online SPE, this approach was not available for this thesis. Hence, the QuEChERS method was explored due to its practicality and reported applicability with the LC-MS/MS method (Ferreira et al. 2016; Park et al. 2021; Stubbings et al. 2009). The first stage of this method utilises addition of an organic solvent and salts to regulate pH and control polarity, thereby facilitating better separation and the recovery of the analytes (Gómez-Regalado et al. 2022). The QuEChERS method initially selected for this study is from the Association of Official Agricultural Chemists International (AOAC) 2007.01 (6:1.5 $MgSO_4$: NaOAc modified with sample rehydration using 10 mL of ultrapure water) due to the more favourable compound recoveries for sediment samples reported in the literature (Nannou et al. 2019; Vulliet et al. 2014). Furthermore, ACN was selected as the extraction solvent based on its capacity to extract a wide variety of analytes and its ability to reduce matrix interferences. Finally, this method was followed by an additional clean up step via dispersed SPE (dSPE) with PSA, GCB, and $MgSO_4$ (see Chapter 3, section 3.4). PSA removes fatty acids, sugars and polar interferences, $MgSO_4$ removes excess water and GCB is used for pigment and non-polar

interferences removal (Nannou et al. 2019). This dSPE sorbent has shown to have an acceptable recovery for a larger number of micropollutants and has reduced the matrix effects (loss or increase in response in the analysis), compared to other sorbents such as single PSA or PSA/C18 sorbents (Berlioz-Barbier et al. 2014). Fernandes et al. (2020) obtained lower average recovery (72.3%) for pharmaceutical detections using original QuEChERS buffer ($MgSO_4$:NaCl) and dSPE C18:$MgSO_4$, compared to the values observed in this study, suggesting the suitability of the approach for sediment sample preparation.

The final sediment extraction method provided LOQs ranging from 0.04–1.01 ng/g_{dw}, analyte recoveries of 75±14%–134±15% and a visually cleaner extract (Table 4.2 and Figure 4.2). These values are comparable with the results obtained by Berlioz-Barbier et al. (2014), where a similar method was used for the detection of pharmaceuticals and obtained recoveries between 40–98% and LOQs between 0.06-0.64 ng/g for sediment samples. Ben Salem et al. (2016) found similar values with by using $MgSO_4$:NaCl:NaCitrate:DCS and dSPE with $MgSO_4$:PSA, the recoveries were between 81–137% and LOQs 0.01–1.27 ng/g_{dw} for pesticides detection.

Liquid chromatography with a combination of mass spectrometry is a good analytical tool for the detection of trace-level analytes. However, the high selectivity and sensitivity of this tool does not guarantee the elimination of interferences or background in the matrices. This can cause ion enhancement or suppression (known as the Matrix Effect [ME]) and can impact the accuracy of quantitative data (Trufelli et al. 2011). Although the method performance is considered acceptable for this study, the ME and therefore the process efficiency cannot be calculated due to the lack of "pristine" sediment samples that could be utilised for this analysis. This is also the case for other matrices incorporated in this study (gammarids, biofilms, and fish). More specifically, the Bow River stream (Stream 3, 4, 7), which was considered the "control" stream, but the inputs

of treated WWTP effluents upstream (Bonnybrook and Fish Creek WWTPs, Figure 1.1) suggested that these streams are not true "reference" sites as the sediments have already accumulated micropollutants which impede the effective ME analysis.

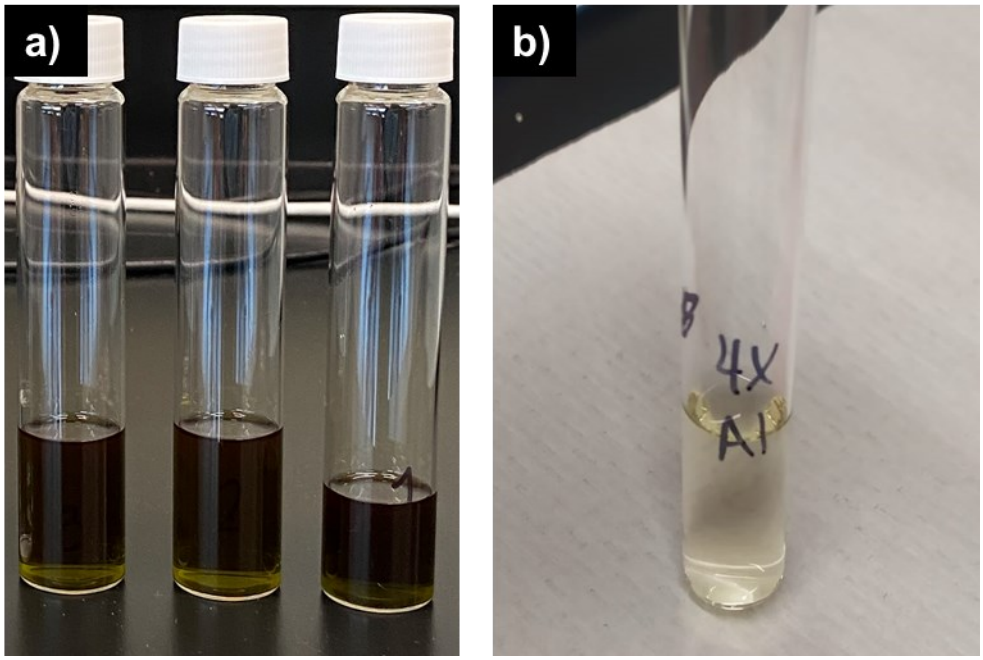

Figure 4.2 *Comparison between a) ASE extract and b) QuEChERS extracts for sediment samples. The clarity in sediment extracts not only impact the quality of LC-MS/MS analysis but also introduces high background in the instruments.*

However, the analytical workflow employed labeled internal standards prior to sample extraction, which allows the calculation of matrix effect factor (MEF). MEF is the approach utilised by Zhou et al. (2017) that incorporates the combined impact of the sample matrix during sample preparation and ionization in the MS ion source. If MEF is ~0%, then there is a negligible ion source suppression and a value of ≤85% is considered acceptable. Once the sample is outside of this threshold, the sample might require further sample preparation to improve analyte detection.

Although some MEFs values are slightly higher than the acceptable threshold as observed here (Table 4.2), these values are still considered acceptable for the purposes of this study (i.e., matrix spike recoveries and use of internal standards). In multi-residue analysis *via* LC-MS/MS, there needs to be a balance between further improvements in sample preparation and the value that the analytical method can provide for assessing partitioning of micropollutants in several environmental matrices. The relevance of the concentrations detected in the sediments along with other environmental matrices are explained in Section 4.2 of this chapter.

Table 4.2 *Average recovery, standard deviation (n=7) and LOQ for sediment samples. LOQ=Limit of quantitation, MEF=Matrix effect factor. Acceptable compound recoveries range from 70-120%. High recoveries (>120%) in samples are likely from the background contamination from the sediment samples (e.g., venlafaxine, gemfibrozil).*

Compound	**Recovery (%)**	**LOQ (ng/g)**	**MEF %**
Sulfamethazine	99 ± 6	0.13	88
Trimethoprim	115 ± 5	0.13	88
O-desmethylvenlafaxine	94 ± 7	0.18	91
Venlafaxine	103± 26	0.12	86
Carbamazepine	103±6	0.08	80
Norfluoxetine	76 ± 14	0.24	93
Fluoxetine	99 ±7	0.11	86
Gemfibrozil	134 ± 15	0.16	90
Triclocarban	101 ± 9	1.01	98
Triclosan	105 ± 15	0.04	59

4.1.2 Biofilm, Gammarids, and Fish Sample Clean up

BEM 1.0 (QuEChERS approach, Chapter 3, Table 3.3) was initially used for biofilm, gammarids and fish, but poor regular and ISTD recoveries were obtained based on the quality of the chromatograms especially for gammarids and fish (Figure 4.1, b-d). Using venlafaxine as an example, the BEM 1.0 obtained poor recoveries for fish and gammarids, with spiked samples showing no peaks and very low ISTD response areas (Table 4.1). Similar outcomes were obtained for other analytes of interest in gammarids and fish (Table S.2).

Although there were some detections in the biofilm *via* the BEM 1.0 method, they were also considered not the most optimal ("dirty" extract [Figure S.1]). Nevertheless, these initial positive results provided a starting point for QuEChERs method improvement for all matrices, and most importantly, allowed the comparison of different biofilm deployment and collection methods that were trialed based on the literature (Head et al. 2004; Martínez-Campos et al. 2023; Tan et al. 2015). These rock baskets, glass slides, and natural rock substrates (Chapter 3, Section 3.2) were tested for deployment/collection in June 2021 (Figure 4.3). The BEM 1.0 results showed that there were no substantial differences among the methods, as the concentration spiked in the samples were recovered (except for venlafaxine) suggesting that either one of them would be appropriate (Figure 4.4). The rock basket deployment approach was chosen due to the ease of preparation and the larger amounts of biofilm (dry weight) obtained compared to glass slides (Figure 4.3).

Note that BEM 1.0 has been successfully used in the past via original QuEChERS salts and heptane clean up, but it is typically coupled with an online SPE to minimize interferences (Munz et al. 2018) which is not available for the current LC-MS/MS setup in this thesis. Given that BEM 1.0 had the potential for use in biofilms, the subsequent cleanup step after the addition of ACN (solvent) and salts was explored, primarily via the use of Z sep+ and centrifugal filters instead of

the heptane extraction step. Improvements in the chromatogram were observed using this approach (Figure 4.1b), which is further characterized by higher recoveries and detectable concentrations in both spiked and unspiked samples. Z sep or Z sep+ is a sorbent based on silica gel modified with zirconium dioxide used to remove lipids and pigments in the extraction for different types of complex matrices (Kaczyński et al. 2017; Rejczak et al. 2017), including fish (Kalogeropoulou et al. 2021), edible oils (Moreno-González et al. 2014), breast milk (Tuzimski et al. 2019). Kalogeropoulou et al. (2021) used Z sep+ at different amounts (25 mg, 50 mg, and 100 mg) and obtained the highest recoveries with 50 mg (63%-103%) and LOQs between 0.5 to 19 ng/g for fish tissue. In this study, the mean recoveries (51±20 – 94 ±15%) and LOQs (0.14–1.20 ng/g_{dw}) were obtained using 50 mg of Z sep+ (Table 4.3). Although they were lower compared to other studies such as that of Desiante et al. (2021) who observed recoveries between 80–120% using heptane as a clean up step, experiment 4 method for biofilms is an acceptable approach due to acceptable MEF values (Table 4.3).

Figure 4.3 *Comparison between a) glass slides biofilm and b) rock baskets during method development sampling campaign in June 2021. Larger amounts of biofilms can be obtained through the rock basket method.*

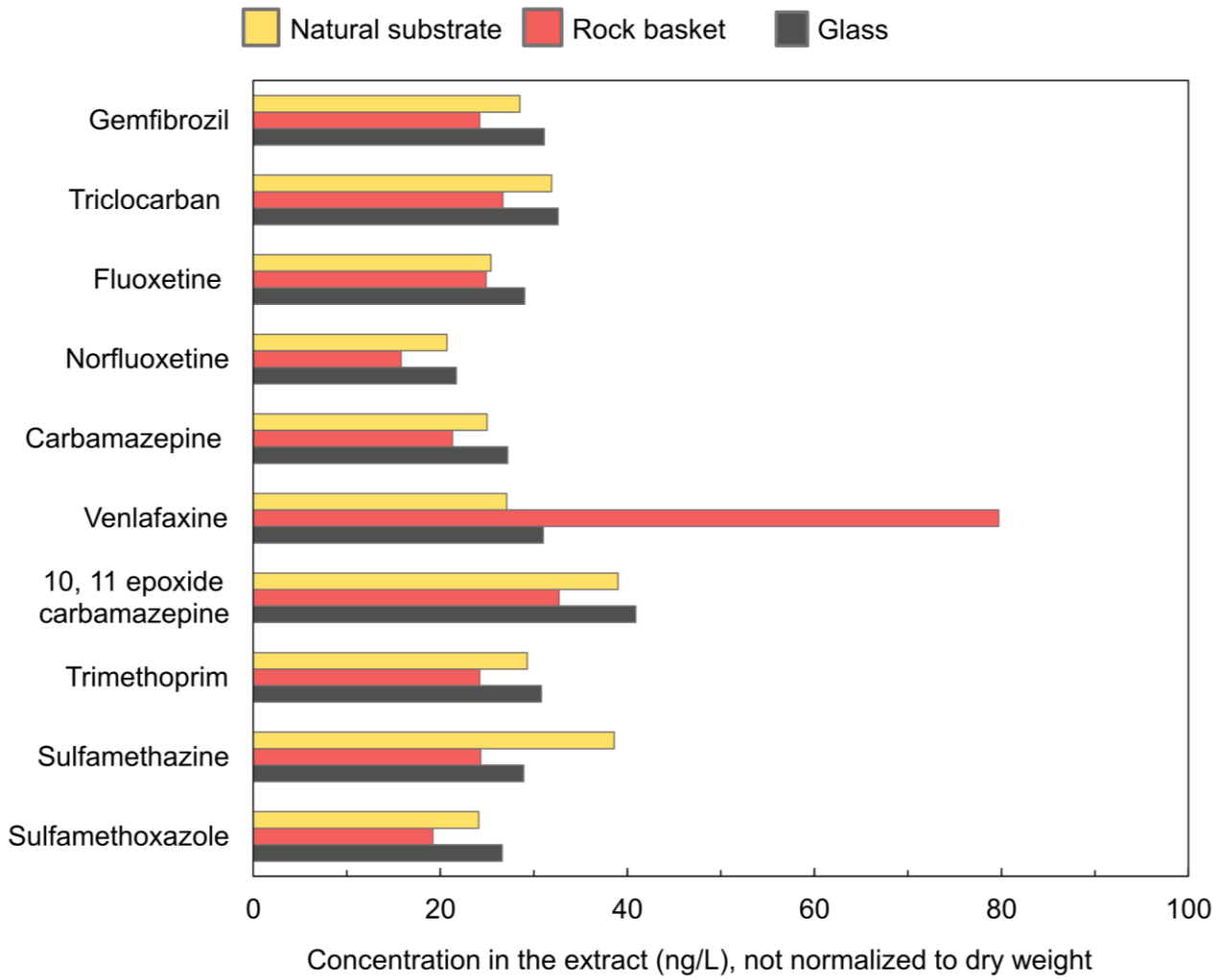

Figure 4.4 *Compound detection for rock basket, glass and natural substrates extracted for method development. Samples were spiked at an expected extract concentration of 20 ng/L. Although the initial method (BEM 1.0) did not provide the best recoveries, it was sufficient in aiding the choice of using rock baskets for future sampling campaign.*

Table 4.3 *Analytical performance of the clean up method for biofilm, gammarids, and fish. LOQs = limit of quantification, ND=no detected and MEF = matrix effect factor. ODMV= O-desmethylvenlafaxine.*

	Biofilm (Experiment 4)			Gammarids (Experiment 1)			Fish (Experiment 3)		
Compound	**Recovery (%)**	**LOQ (ng/g)**	**MEF (%)**	**Recovery (%)**	**LOQ (ng/g)**	**MEF (%)**	**Recovery (%)**	**LOQ (ng/g)**	**MEF (%)**
Trimethoprim	80±24	0.14	71	90±13	0.14	88	ND	ND	ND
Sulfamethazine	ND	ND	ND	83±32	0.64	97	ND	ND	ND
Sulfamethoxazole	79±16	0.52	92	ND	ND	ND	ND	ND	ND
ODMV	62±50	1.20	97	112±34	0.13	87	77±23	0.10	84
10, 11 epoxide-carbamazepine	94±15	0.17	77	117±17	0.14	89	86±16	0.19	91
Venlafaxine	82±45	0.79	95	96±31	0.07	77	89±16	0.10	83
Carbamazepine	88±17	0.21	81	97±23	0.15	89	116±17	0.24	93
Norfluoxetine	51±20	0.35	88	58±11	0.10	83	68±24	0.6	97
Fluoxetine	71±34	0.16	76	98±42	0.07	78	75±33	0.22	92
Triclocarban	65±18	0.34	88	ND	ND	ND	123±23	0.09	82

For gammarids samples, Experiment 1 was selected as the optimal sample preparation method (See chapter 3, Table 3.4) as it did not only provide a cleaner extract visually (Figure 4.5), it also showed lower background (Figure 4.1). Here, the $MgSO_4$:NaOAc QuEChERs salts were used with 2.1 mL of ultrapure water for rehydration, followed by 1:6 PSA:$MgSO_4$ d-SPE and Z-sep+. Improvement was evident based on the chromatogram (Figure 4.1c) and recoveries ranging from 90±13%–117±17%, except for trimethoprim with a recovery of 58±10%. LOQs were observed from 0.07–0.17 ng/g_{dw}. Godfrey et al. (2022) used a similar method (but without a Z sep+ clean up) for the detection of pharmaceuticals and biocides in soil, biota, and clay, and found recoveries >62% for soil and biota and between 88-131.1% for clay. Townsend et al. (2020) found recoveries between 39–100% for soil samples using a similar method.

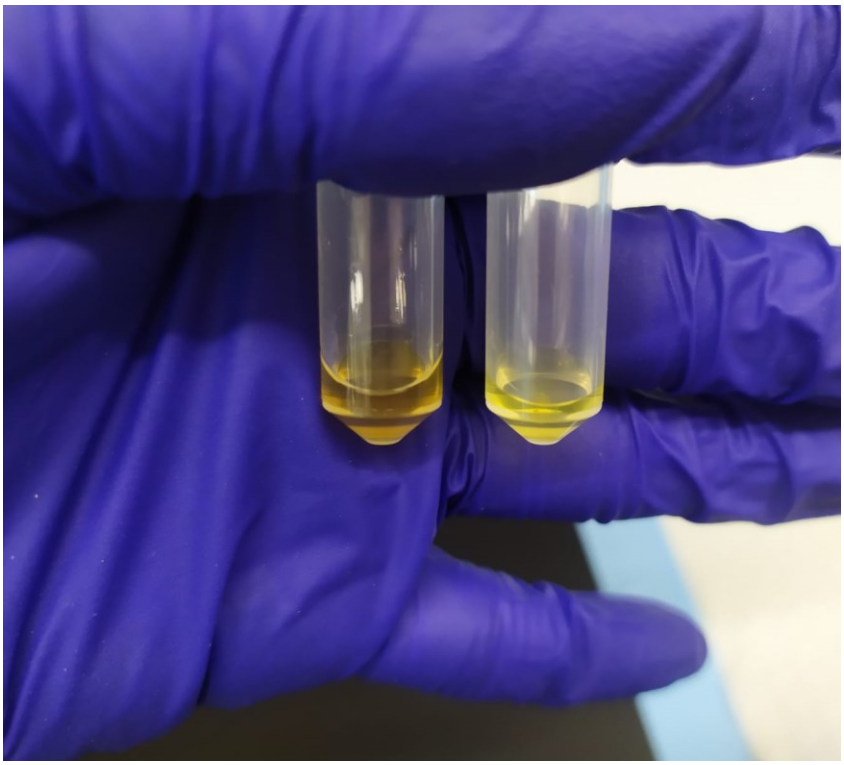

Figure 4.5 *Visual comparison between heptane clean up (left) and d-SPE/ Z sep+ (right) for gammarids extraction.*

Fish samples were extracted using $MgSO_4$:NaCl:NaCitrate:DCS as extraction salts (see Chapter 3, Table 3.4) and was based on Barbieri et al. (2019) where they reported compound recoveries of 71-120% in fish tissue. $MgSO_4$:PSA:C18 salts were employed for additional dSPE clean up step. The addition of C18 in this mixture was mainly to remove fats and non-polar components (Kaczyński et al. 2017).

The recoveries for fish samples were within an acceptable range between 75±33%–116±17% except for trimethoprim where <70% recovery was observed (62±28%). Furthermore, LOQs were also observed and were found to be between 0.07–0.83 ng/g_{dw} for the compounds detected. A similar method has been used by López-García et al. (2019) for the detection of psychoactive substances in mussels, with a reported recovery of 77–118% and the limits of detection were <2 ng/g_{ww} for all compounds analysed. Baesu et al. (2021) used the same clean up step ($MgSO_4$:PSA:C18) but with different extraction salts, $MgSO_4$:AcONa (magnesium sulfate heptahydrate) for the detection of pharmaceuticals in different fish species, and reported recoveries between 67–148%. The MEF approach was done for the biofilm, gammarids and fish with values close to the acceptable threshold (Table 4.3). Although these are slightly higher than 85%, they are still sufficient for the purposes of this thesis, as already discussed for the sediment samples.

Given the complexity of the matrices considered in this study (sediment, gammarids, biofilm, and fish), the sample preparation methods evaluated were considered (overall) acceptable for assessing the partitioning of substances in various environmental compartments as well as their bioaccumulation in exposed aquatic organism. Note that 22 compounds were spiked and only 10, can be detected in the sediment with confidence, 9 in biofilm, 8 in gammarids and 7 in fish (as will be discussed in Sections 4.3, 4.4 and 4.5). For instance, caffeine showed poor ionization and high background. Triclosan detections in solid matrices showed a low ISTD response area and

compounds like gemfibrozil showed signals on the spiked samples, but no concentration was detected for the unspiked samples. Indeed, additional work can be employed to improve detection, but the current methods are sufficient to address the research objective outlined initially. To standardize the comparison across all compartments, the compounds detected in each matrix will be evaluated for partitioning and bioaccumulation objectives (Section 4.3, 4.4 and 4.5). The next section illustrates this during sampling campaign when the ACWA Stream 10 effluent contribution was increased to 15% (from 5%).

4.2 Occurrence of micropollutants in environmental matrices

This section discusses the micropollutant detection for each environmental matrix. Of the 22 target compounds, 18 were detected in the water matrix, 10 in sediments, 9 in biofilms, 8 in gammarids and 7 in fish. Overall, venlafaxine (antidepressant) and carbamazepine (antiepileptic) were detected in all the matrices, while diclofenac was detected at high concentrations in water samples only. The following sections show the specific trends observed for each environmental compartment followed by an assessment of partitioning and bioaccumulation.

4.2.1 ACWA streams characterization

All the 12 streams associated with four experimental conditions (100% Bow River, 5% Pine Creek WWTP, 5% RO, and 5% O_3 effluent) were first characterized for micropollutant concentrations in September 2020 and June 2021. The September 2020 water sampling campaign was the first time the streams were assessed for micropollutant occurrence since the ACWA facility was established in 2015. Of the 22 compounds analysed, 18 were detected in all streams in 2020, with diclofenac, venlafaxine, and its metabolite O-desmethylvenlafaxine dominating the total concentrations contributing to 31.36%, 14.05% and 13.64%, respectively. It was also observed

that triplicate sampling in each stream was not necessary due to the low variability observed in concentrations during the September sampling campaign (Table S.3). Hence, only 1 replicate was collected at each stream for subsequent sampling campaigns.

For both stream characterization campaigns (September 2020 and June 2021), BR streams ("control" streams) consistently showed high concentrations suggesting that the micropollutants found in PC, RO, and O_3 streams were contributed by the Bow River since it represents 95% v/v in each stream. Furthermore, the 5% PC streams were found to have the highest concentrations compared to other experimental streams for most micropollutants detected (e.g., venlafaxine, diclofenac, sulfamethoxazole, Figure 4.6).

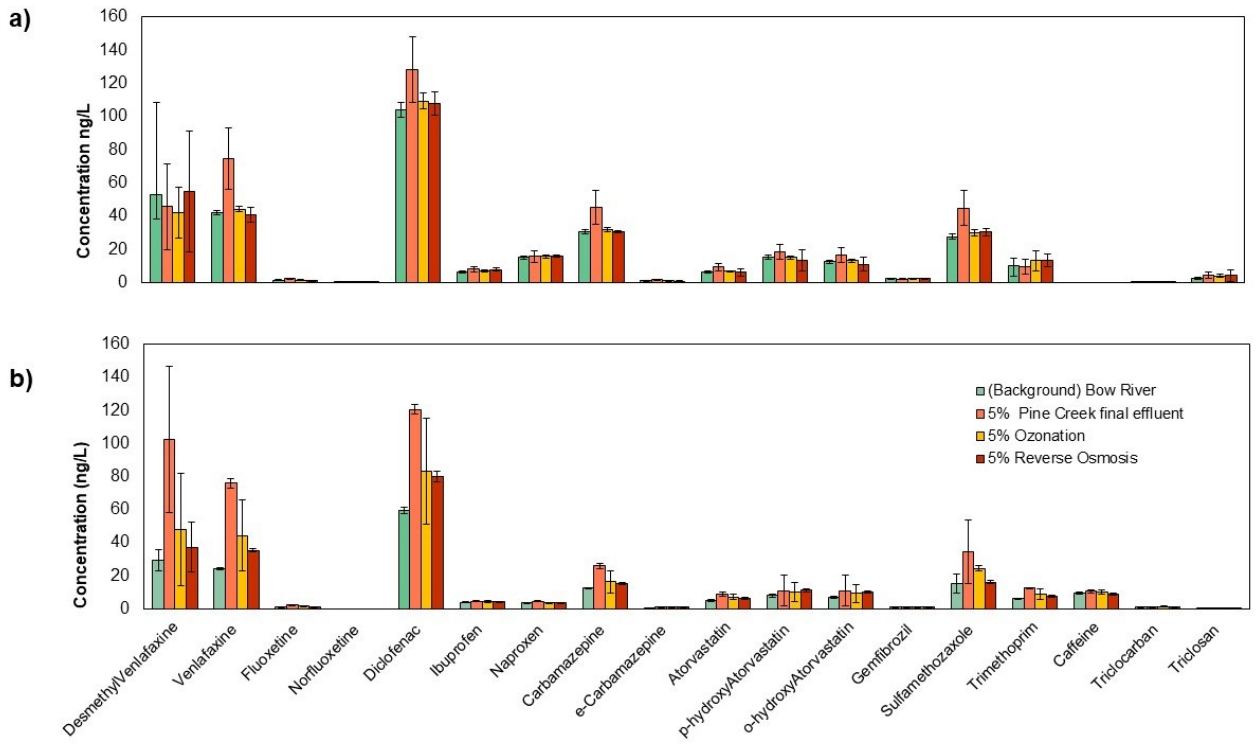

Figure 4.6 *(a) September 2020 characterization, n=9 and (b) June 2021 n=3. All 12 replicate streams were analysed for micropollutant concentrations. e-Carbamazepine=10, 11 epoxide*

carbamazepine. Treatment streams (Pine Creek, Ozonation, and Reverse Osmosis) contain 5% effluent mixed with 95% Bow River water v/v.

There was no statistical significant difference among the BR, RO, and O_3 stream concentrations (One-Way ANOVA, $p>0.05$, $\alpha=0.05$) suggesting that they perform similarly. Given that the BR contributes 95% of the total concentration, it is likely that the concentrations in the RO and O_3 streams are driven by the Bow River background concentrations. However, there was a substantial difference in diclofenac, venlafaxine, and O-desmethylvenlafaxine concentrations in PC streams compared to BR, RO, O_3 and were on average two times higher in June 2021 and up to 45% higher in September 2020. These results suggest that biological nutrient removal (BNR) currently operated at the PC WWTP followed by ultrafiltration (UF) and RO or O_3 at the ACWA pilot treatment were effective at removing these compounds. No observable differences were seen for other substances such as ibuprofen and naproxen.

The PC WWTP is the newest plant in Calgary and operates using BNR. It has been recently shown that BNR is effective at removing micropollutants in comparison to more conventional wastewater treatment such as activated sludge (Dubey et al. 2020; T. Okuda et al. 2008). The recent work by Arlos et al. (2023) that assessed contribution of micropollutants in the Bow and Elbow River watersheds further supports this, with the conventional activated sludge system (Fish Creek WWTP) operating worse than the PC WWTP (although the population serviced are similar). Although the City of Calgary is not currently planning on upgrading the PC WWTP further, the results show that advanced treatment options such as what are currently set up at ACWA can be implemented at a modular scale when potential regulations are in place for micropollutants, and compliance is mandatory for their approval to operate.

A review paper by Dolar and Košutić (2013) suggested that ultrafiltration is usually accompanied by an additional treatment (e.g., RO, O_3) for better removal efficiency. More specifically, Yoon et al. 2007 evaluated the retention of 27 micropollutants and found that UF treatment had only <30% removal for most of their target compounds including carbamazepine, diclofenac, acetaminophen, sulfamethoxazole, and caffeine. These results are consistent with Chon et al. (2013), where UF and RO were tested for the removal of micropollutants. They observed that the removal efficiency with UF was 33% for diclofenac and 28% for sulfamethoxazole; for all other compounds, removals were <17% for UF only but increased to >65% when UF and RO were both implemented.

Among the anti-inflammatories, diclofenac showed the highest concentrations at 128±20 ng/L in 2020 and 121±3 ng/L in 2021 in all the streams (Figure 4.6, Table S.3 and Table S.4). Diclofenac is commonly found in aquatic environments, which can be attributed to its limited biodegradability and thus, its effective removal through conventional wastewater treatment methods can be challenging (Stepanova et al. 2013; Vieno and Sillanpää 2014). For example, inconsistent removals have been reported between 3–60% (Verlicchi et al. 2012) or no removal at all (Zorita et al. 2009). In addition to high usage in human and veterinary care (Bonnefille et al. 2018; He et al. 2017), other explanations for diclofenac persistence and mobility are related to hydrophilicity (Log K_{ow}=1.1 at pH 7.4) (Ziylan and Ince 2011), parent molecule reformation, pharmaceutical desorption from suspended material, or metabolite deconjugation (of the orally administrated dose 65–70% is eliminated through urine and 20–30% in feces) (Bonnefille et al. 2018; Davies and Anderson 1997).

For antidepressants, venlafaxine and its metabolite O-desmethylvenlafaxine were observed to have the highest concentrations in water compared to the other antidepressants, fluoxetine and

norfluoxetine (metabolite). In 2020, venlafaxine was detected at 74±18 ng/L and O-desmethylvenlafaxine at 55±37 ng/L and in 2021, 76±3 ng/L for venlafaxine and for its metabolite 102±44 ng/L. Venlafaxine is a frequently prescribed antidepressant in the world (Schlüsener et al. 2015), and with an applied dose of venlafaxine, 29% is excreted in the urine as its metabolite O-desmethylvenlafaxine and 5% is unchanged parent compound (Martínez Bueno et al. 2014). Additionally, its low hydrophobicity (Log K_{OW}=1.12, pH 7.4) and high solubility indicate a tendency to remain in the water phase (Rúa-Gómez and Püttmann 2012).

Carbamazepine (antiepileptic) was detected at 45±10 ng/L and 26±2 ng/L in 2020 and 2021, respectively. Despite being entirely metabolized by humans (<2% is eliminated), it has high global consumption (Tolou-Ghamari et al. 2013; Almeida et al. 2021). In addition, carbamazepine is a very persistent compound, and the removal efficiency has been reported to be <10% in different studies (Zhang, Geißen, and Gal 2008) due to its resistance to biodegradation at low concentrations (Keen et al. 2012).

For compounds from the antibacterial group, triclocarban and triclosan were found at concentrations <1.7 ng/L for both characterization studies. These compounds have high Log Kow values (>4.8 at pH 7.4), thus they are more hydrophobic and will likely bind more to solid matrices such as suspended solids (Chen et al. 2018). Activated sludge/biological treatment could be present a major removal mechanism (in addition to biodegradation) due to the sorption to the particles (Lehutso et al. 2017) and elimination of up to 99% through this treatment has been reported (Kumar et al. 2010). Although not measured in this study, it is possible that these compounds might partition into the biosolids during wastewater treatment depending on the compound affinity to solid matrices. As will be discussed in sections 4.3, the concentrations of anti-inflammatories,

antidepressants, carbamazepine and anti-bacterials in the sediments vary indicating the differences in compound fate/behaviour in different environmental compartments.

The Canmore site was added in the Fall 2022 sampling campaign to show contrast between the more upstream (approximate distance of 134 km between Canmore site and PC WWTP) (Figure S2, Table 4.4) and the Bow River streams at the ACWA facility that have already received effluent discharges from the two Calgary WWTPs (Bonnybrook [BB]and Fish Creek [FC]). For O-desmethylvenlafaxine, venlafaxine, diclofenac, carbamazepine, concentrations at Canmore were <LOQ (0.1)–1.2 ng/L whereas in the Bow River streams at ACWA, they were detected from

Table 4.4 *Average concentrations in ng/L for sampling campaign in June 2021, Fall 2021 and Fall 2022. Samples collected in Canmore, 5% PC Effluent (Stream 2, 12), and BR (Stream 3, 4, 7). ND=no detected. Triclocarban LOQ for June 2021 is 0.2 ng/L and for Fall 2022 is 0.1 ng/L. For June 2021, n=3 and Fall 2021 and 2022, n=9; except for PC 2022 with n=2. *Metabolites.*

Compound	Class	June 2021		Fall 2021		Fall 2022		
		PC	BR	PC	BR	Canmore	PC	BR
O-desmethylvenlafaxine*	Antidepressant	102±44	29±7	109±60	63±43	1±0.4	245±147	190±70
Venlafaxine		76±3	24±1	126±24	76±18	1±1	74±11	47±9
Fluoxetine		2±0.1	1±0.05	3±1	2±0.5	0.1±0.04	2±0.5	1±0.2
Norfluoxetine		ND	ND	ND	ND	ND	ND	ND
Diclofenac	Analgesic	121±3	59±2	133±34	123±24	1.2±0.7	110±14	107±57
Ibuprofen		4±0.2	4±0.25	7±1	6±1	0.4±0.2	3±2	3±2
Naproxen		4±0.4	4±0.1	8±3	7±1	0.4±0.1	10±3	10±4
Carbamazepine	Antiepileptic	26±2	12±0.3	39±4	26±3	0.20±0.1	35±4	23±4
11,12 epoxide Carbamazepine*		1±0.1	1±0.03	2±0.3	1±0.2	ND	ND	ND
Atorvastatin	Cardiovascular	9±1	5±0.8	7±2	5±2	0.5±0.1	3±1	3±1
p-hydroxyatorvastatin*		16±1	8±0.8	15±3	13±4	0.7±0.1	12±3	13±3
o-hydroxyatorvastatin*		16±1	7±0.7	13±4	11±4	0.6±0.1	8±2	8±2
Gemfibrozil		1±0.1	1±0.04	2±2	1±0.3	0.2±0.1	1±0.4	1±0.4
Sulfamethoxazole	Antibiotic	34±19	15±6	45±7	32±5	0.6±0.1	46±15	33±10
Trimethoprim		12±0.1	6±0.9	22±2	14±2	0.2±0.1	19±5	14±3
Sulfamethazine		ND	ND	ND	ND	0.7±0.2	1±1	2±0.4
Caffeine	Stimulant	10±0.9	9±0.4	33±24	24±9	ND	ND	ND
Triclocarban	Antibacterial	>LOQ	>LOQ	0.3±0.01	0.3±0.01	>LOQ	>LOQ	>LOQ
Triclosan		0.2±0.1	0.2±0.06	1.4±0.6	1±0.3	0.7±0.5	1±0.2	1±0.1

1–190 ng/L (Table 4.4, mean concentration). This result suggests that BB and FC WWTPs upstream of ACWA contributed to the Bow River micropollutant concentrations near the facility and increased the concentrations in the Bow River at least by two orders of magnitude. In the PC 5% ACWA streams, these compounds were detected at 245 ng/L, 74 ng/L, 110 ng/L and 35 ng/L, respectively (Table 4.4) and were already high due to the background (BR streams, Figure 4.6). This result is not surprising given that Bartelt-Hunt et al. (2009) found WWTP effluents represented a significant load of micropollutants concentrations in river that includes antibiotics, analgesics, anticonvulsants, and stimulants, with concentrations of micropollutants went from 1.9–30.3 ng/L upstream to 2.3 ng/L–1600 ng/L downstream.

The concentrations in the Bow River streams are similar to other studies in Canada and around the globe, including those of Arlos et al. (2015) in the Grand River, Ontario, Silva et al. (2011) in the Ebro river basin in Spain, and Rúa-Gómez and Püttmann (2012) in Hesse, Germany. Micropollutants have a global presence and among the environmental compartments, surface water is the only compartment that have recommended and/or proposed guidelines. In Europe, diclofenac has a proposed guideline of 0.040 µg/L (40 ng/L) (SCHEER 2022). In Canada, triclosan has a guideline of 0.47 µg/L (470 ng/L) and in Alberta, carbamazepine has a guideline of 10 µg/L (10^3 ng/L). Triclosan and carbamazepine concentrations were all below the Canadian and Alberta guidelines, but diclofenac exceeded in over 80% of the samples the European Union (EU) Water Framework Directive proposed guideline in all ACWA streams characterized, except at the Canmore site (Fall 2022 only). Note that the development of diclofenac guidelines is still contentious in the EU, with some parties suggesting much higher standard of 126 ng/L(Maack et al. 2022). Hence, it is currently difficult to assess the true impact of diclofenac in the streams given that the development of water quality standards related to micropollutants are still emerging.

4.2.2 Temporal variations in water concentrations

Additional water sampling campaigns (12 monthly time points) were completed for the 5% PC and BR streams between 2021 and 2022 (Figure 4.7). The dominant groups are anti-inflammatories and antidepressants, which is consistent with the results presented in Figure 4.6. For both streams (PC and BR), the antibiotics were detected at higher concentrations in 2020 and 2021 campaigns but were observed to be on average two times lower in the 2022 sampling campaign. The summer months (June and July) had the lowest total concentrations with 438 ng/L and 185 ng/L in 2021, and 225 ng/L and 94 ng/L in 2022 for PC and BR, respectively. High concentrations were detected in 5% PC streams in February and October 2022 (i.e., 906 ng/L and 794 ng/L respectively), and similarly for BR streams (518 ng/L and 664 ng/L, respectively) (Figure 4.7). This overall fluctuations in the concentrations of micropollutants can be explained by natural attenuation, including sorption to solids, bio- and photodegradation, and dilution (Luo et al. 2014). However, it appears that the high concentrations in the streams corresponded to low river flows and vice-versa when flows were high. This observation suggests that flows have an implication on micropollutant distribution in the streams and in the Bow River in general. A similar observation was observed by Arlos et al. (2023) at a site 20 km downstream of the ACWA facility, further supporting the impacts of river flows (via dilution) in pollutant attenuation in large river streams such as the Bow River.

Wang et al. (2011) evaluated the seasonal occurrence of 16 pharmaceuticals, including antibiotics, hormones, analgesics, stimulants and antiepileptics, and further observed that concentrations were lower during the summer months because of water dilution and/or improved

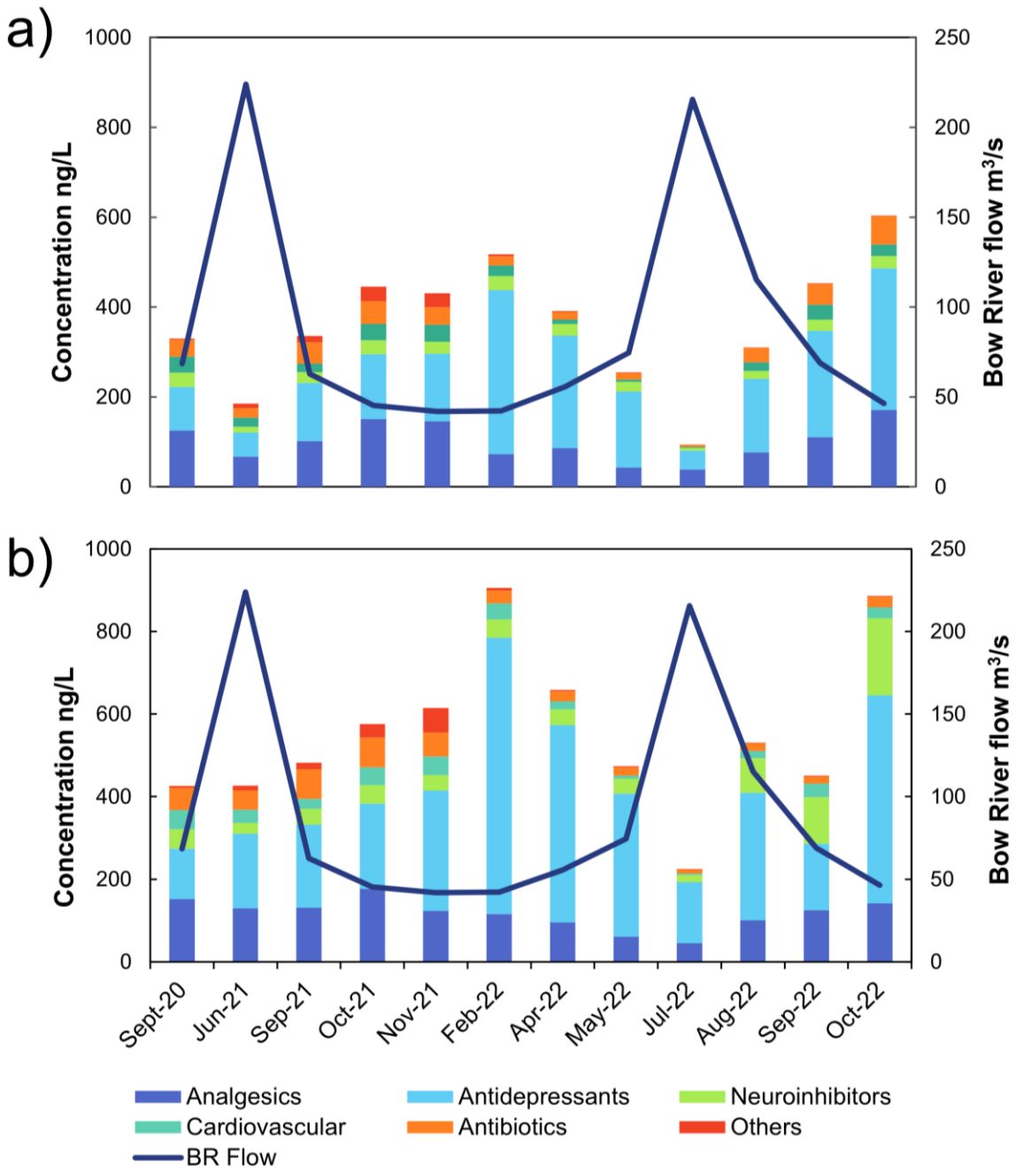

Figure 4.7 *Comparison of Bow River flow (m^3/s) and sum concentrations of target micropollutants grouped by class in (a) Bow River and (b) Pine Creek Streams at various water sampling campaigns between 2020 and 2022.*

Table 4.5 *Mean sediment concentration (ng/g_{dw}) per site and month from sampling campaign in Fall 2022. n=number of samples. Limits of quantification (LOQs) are listed on Table 4.2. dw= dry weight. * metabolites and put full names. ODMV = O-desmethylvenlafaxine.*

Month	**August (ng/g_{dw})**				**September (ng/g_{dw})**				**October (ng/g_{dw})**			
Site	**Canmore (upstream)**	**Pine Creek 5%**	**Pine Creek 15%**	**Bow River**	**Canmore (upstream)**	**Pine Creek 5%**	**Pine Creek 15%**	**Bow River**	**Canmore (upstream)**	**Pine Creek 5%**	**Pine Creek 15%**	**Bow River**
n	**3**	**6**	**3**	**6**	**3**	**6**	**3**	**6**	**3**	**6**	**3**	**6**
Sulfamethazine	<LOQ	0.4±0.01	0.3±0.04	0.3±0.04	<LOQ	0.3±0.04	0.3±0.1	0.2±0.04	<LOQ	0.2±0.1	0.2±0.1	0.2±002
Trimethoprim	<LOQ	0.9±0.1	1±0.2	1±0.1	<LOQ	1±0.1	1±0.1	1±0.1	<LOQ	0.9±0.2	1.7±0.3	0.6±0.04
ODMV	0.3±0.01	58±2	77±3	24±7	0.2±0.01	54±3	50±2	25±2	0.2±0.02	52±9	70±4	26±4
Venlafaxine	0.2±0.04	106±4	121±5	63±11	0.1	88±8	84±2	52±4	<LOQ	147±5	195±3	106±18
Carbamazepine	<LOQ	1.2±0.1	1±0.1	1±0.1	<LOQ	1±0.1	2±0.1	1±0.1	<LOQ	1±0.2	2±0.03	0.7±0.1
Norfluoxetine	<LOQ	7±1	8±0.5	4±0.1	<LOQ	6±0.7	6±0.4	5±0.4	<LOQ	5±1	9±1	4±1
Fluoxetine	<LOQ	46±1	50±2	21±3	<LOQ	47±4	46±2	29±1	<LOQ	54±4	76±3	34±3
Gemfibrozil	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Triclocarban	<LOQ	14±1	16±1	13±3	<LOQ	20±1	17±1	12±1	<LOQ	23±6	14±1	15±2
Triclosan	<LOQ	17±2	16±2	12±3	0.3±0.2	24±1	21±1	13±1	0.4±0.04	27±6	19±1	15±2

biodegradation of the compounds during warmer temperature. Azzouz et al. (2013) found that there are higher levels of these pharmaceuticals in the colder periods (12– 314 ng/L in the fall and winter) than warmer months (18–127 ng/L in spring and summer). Different river systems will obviously have unique trends and patterns but given that the highest concentrations in the PC and BR streams were observed during the colder periods, the conditions are likely exacerbated by lower river flows during this time in addition to lower rates of bio- and photo-degradation (Figure 4.7).

4.2.3 Impact of the 15% increase in PC effluent streams

In the Fall 2022 sampling campaign, the effluent contribution in one of the PC streams (Stream 10) was increased from 5% to 15% (Figure 4.8) given that there are relatively lower differences between RO and O_3 streams behaviour. Most of the compounds were, as expected, three times higher on average in PC 15% streams than PC 5% streams, except for the metabolites (10,11 epoxide carbamazepine, p-hydroxy Atorvastatin, o-hydroxy Atorvastatin) and the analgesics (diclofenac, naproxen, and ibuprofen), which are ~1 order of magnitude higher in PC 15% on average.

Sulfamethoxazole, triclocarban, and gemfibrozil did not fall within this ratio, possibly due to their low detections, which were close to the LOQs of 0.4, 0.3, and 0.1 ng/L, respectively (therefore, there is larger analytical measurement variability). The highest concentrations found in PC 15% were for O-desmethylvenlafaxine, venlafaxine and diclofenac at 811 ± 449 ng/L, 221 ± 4 ng/L and 178 ± 31 ng/L and for PC 5% were 245 ± 164 ng/L, 74 ± 12 ng/L and 110 ± 16 ng/L, respectively. Additional samples were also collected in January 2023 from 100% PC WWTP to confirm its contribution (Figure 4.8). Effluent values ranged from 0.3 to 3518 ng/L, with the

highest values for O-desmethylvenlafaxine, venlafaxine, diclofenac, and carbamazepine at 3014 ± 436, 1007 ± 26, 655 ±7, 410 ±11 ng/L respectively (Table S.5).

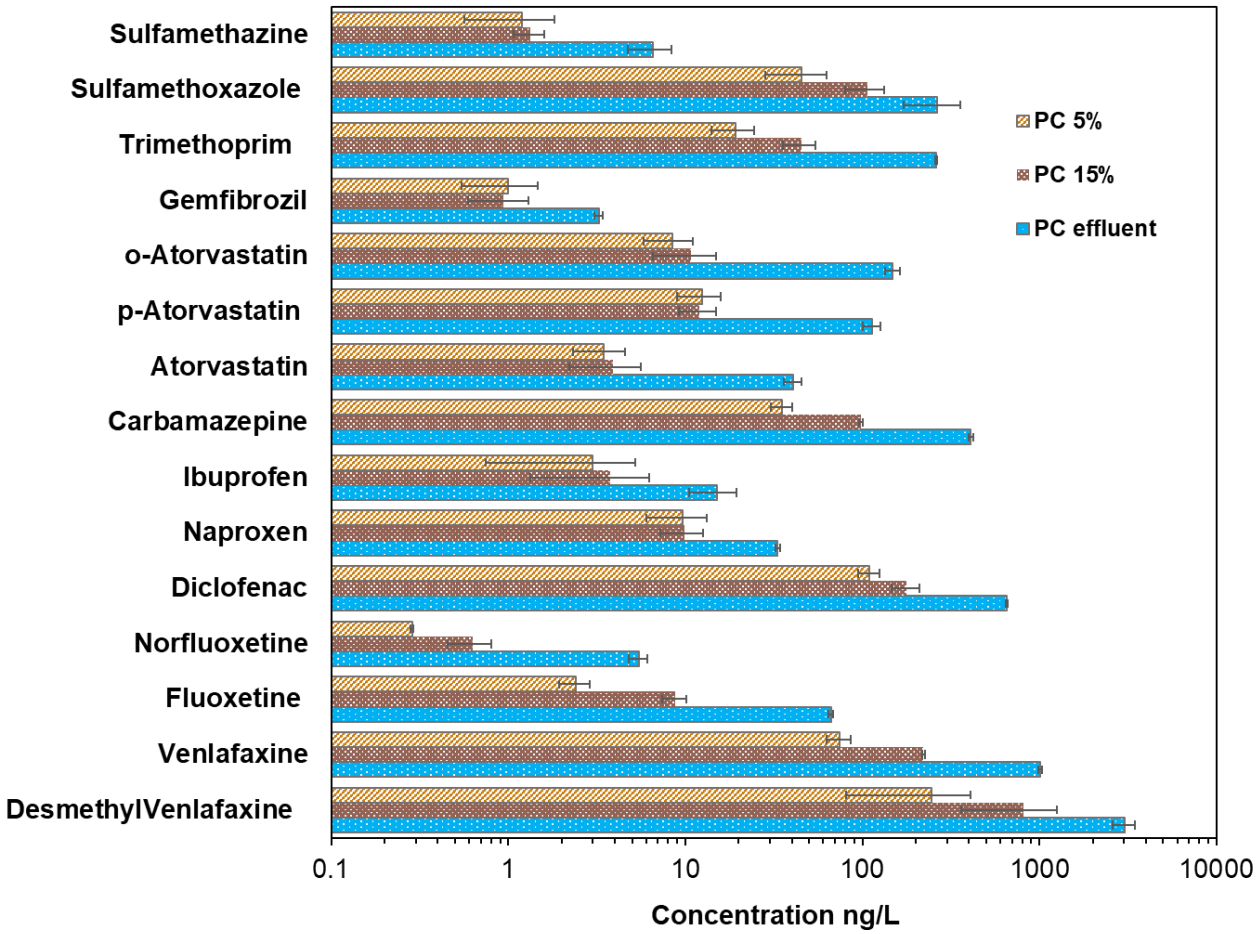

Figure 4.8 *Micropollutant concentrations at different PC effluent contributions. Average concentrations from August to October 2022. For PC 5%, n=6, PC 15%=3 and PC effluent=3. Effluent samples were collected January 24th, 2023. Note that only a selected list of compounds is shown here for visualization purposes. Please see Table S.5 and Table S.9 for the raw data.*

Many studies have reported a consistent correlation in the dilution between the concentrations of effluent and surface water. For instance, Kim et al. (2007) found a significant difference in the ratio of carbamazepine and diclofenac concentrations, with these compounds

being on average ~9 and 13 orders of magnitude more concentrated in WWTP effluent compared to surface water. Rúa-Gómez and Püttmann (2012) confirmed the influence of WWTP effluent in the concentration in a group of pharmaceuticals, including antidepressants and analgesics and its metabolites, where the concentration of the compounds, such as venlafaxine went from <LOQ (upstream) to 28 ng/L (downstream) in February 2010. Gros et al. (2007) studied the occurrence of 28 micropollutants in WWTP effluent and receiving waters of the Ebro River basin in Spain, and found that dilution of micropollutants was generally found at concentrations at least one order of magnitude lower than the effluent. The most frequently detected compounds include analgesics and anti-inflammatories (ibuprofen, diclofenac, and naproxen), the lipid regulators (bezafibrate and gemfibrozil), the antibiotics (sulfamethoxazole and trimethoprim) and carbamazepine at relatively lower concentration. The increase in concentration in the 15% PC effluent is more pronounced than in 5% and it is hypothesized then that the concentrations in the sediments and the biota (biofilm, gammarids, and fish) will also increase, as discussed subsequently below.

4.3 Occurrence in the sediment environment

In total, 10 out of the 18 compounds that were consistently detected in the water matrix were found in the sediment samples, with higher and frequent detections in 5% and 15% PC streams than the BR streams (Table 4.5). The antidepressants (O-desmethylvenlafaxine, venlafaxine, fluoxetine) and antibacterials (triclosan and triclocarban) were found up to an order magnitude higher in comparison to other analytes (Figure 4.9). More specifically, the average concentrations of the Fall 2022 sampling campaign for O-desmethylvenlafaxine were 55±6 ng/g, venlafaxine at 114±26 ng/g_{dw} and fluoxetine at 49±5 ng/g_{dw} in PC 15% (Table S.9), whereas triclosan and triclocarban were detected at 22±6 and 19±5 ng/g_{dw} respectively. Fewer (3/9) and

lower sediment concentrations (<LOQ–0.35±0.1 ng/g_{dw}) were observed at the Canmore site as it located upstream, and the impact of WWTP effluent discharges was expected to be minimal.

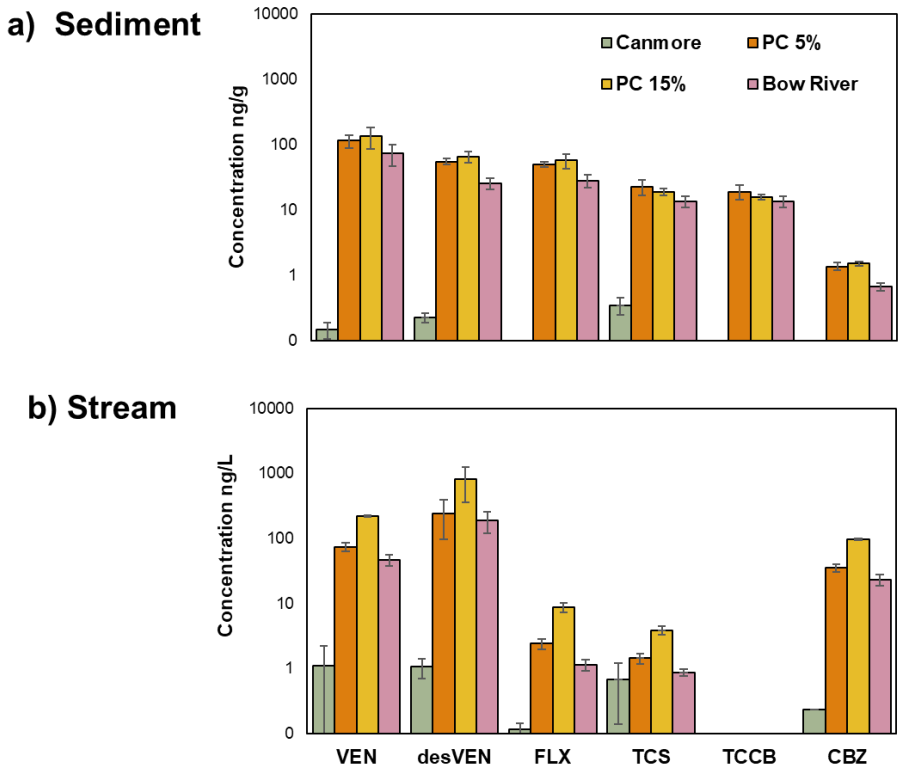

Figure 4.9 *Sediment (a) concentrations of select micropollutants in sediment in comparison to stream concentrations (b). Canmore is the upstream site, prior to Calgary WWTPs into the Bow River. VEN = Venlafaxine, desVEN = O-desmethylvenlafaxine, FLX=Fluoxetine, TCS=Triclosan, TCCB=Triclocarban, CBZ=Carbamazepine.*

When the PC WWTP effluent contribution was increased to 15% v/v, the concentrations also increased for 4/9 compounds in PC 15% with a difference of 10–30% compared to PC 5%. However, sulfamethazine, trimethoprim, and the antibacterials did not change substantially among the streams (both 5 and 15% PC, BR). For sulfamethazine, carbamazepine, and trimethoprim, the

detections were close to the LOQs, and it is difficult to assess the differences among the streams as the concentrations are likely showing analytical variability (expected to occur within ±20% of the analytical detections). For triclosan and triclocarban, an increase in concentration for 15% stream was not observed (Table 4.5). It is likely that for hydrophobic compounds at lower concentrations, the sorption (and therefore sedimentation) may have already reached equilibrium conditions. In addition, these compounds are present in water at low concentrations, suggesting an efficient removal, hence there is not a substantial difference observed between PC 5% and 15%. Discussions related to hydrophobicity will be explained further at the end of this section.

Micropollutants have been detected in the sediments, although studies related to their partitioning into the sediments/suspended solids are not as frequent as in surface waters. Schultz et al. (2010) found venlafaxine, fluoxetine and norfluoxetine at 25.29 ng/g_{dw}, 19.37 ng/g_{dw}, and 3.17 ng/g_{dw}, respectively. Their concentrations in the sediments have also been associated with the WWTP effluent discharges (Golovko et al. 2020), with the highest concentrations typically detected at sites that are in close proximity to WWTP outfalls. For instance, Fernandes et al. (2020) reported antidepressant concentrations to be below detection limits (0.002–0.036 ng/g_{dw}) upstream of the WWTP in Leça river, Portugal and increased at a site immediately downstream (i.e., 5.56 ng/g_{dw} for venlafaxine and 2.53 ng/g_{dw} for fluoxetine). Moreover, Venkatesan et al. (2012) found concentrations for triclosan ranging from 0.4–85 ng/g_{dw} and for triclocarban from 5–822 ng/g_{dw}, again showing the highest concentrations near WWTP effluent discharge points.

The occurrence of pharmaceuticals in the sediment may be attributed to different properties, such as hydrophobic partitioning and acid-base dissociation (pKa) (Kwon and Armbrust 2008). Silva et al. (2011) correlated the sorption of chemicals pKa, and found that compounds with pKa>7 tend to sorb onto the suspended solids more (which subsequently settles

onto the stream bed). Suspended solids are also typically negatively charged, and cationic and basic compounds at environmental pH (ACWA streams pH ~8.2) will likely sorb onto solids due to electrostatic interactions. Antidepressants such as venlafaxine and fluoxetine particularly behave as a base with high pKa values. Hence, they will remain positively charged at pH conditions below 8.2, further improving their sorption to solids.

Octanol-water partition coefficients (Log K_{ow}) play an important role in sorption processes, as substances with high Log Kow's have a higher likelihood of sorption onto suspended solids (Venkatesan et al. 2012; Zind et al. 2021). Here, triclocarban and triclosan have low solubility in water (insoluble–0.01 g/L) and high log K_{OW} (4.8–4.93). As a result, these substances tend to accumulate more in sediments and their concentration was more detectable in this matrix than in water, similar to what were reported in Venkatesan et al. (2012) and Amigun Taiwo et al. (2022). By contrast, diclofenac has a low Log K_{ow} (1.1 at pH 7.4), is negatively charged at environmental pH (pKa = 4.01), and more soluble than triclosan or triclocarban (146.8 g/L at pH 8.0). Hence, these properties could explain why diclofenac was not detected in any of the sediment samples but is found in high concentrations in the water.

Furthermore, the water/sediment partition coefficient K_d was calculated and the highest values were obtained for triclosan (4850 L/kg) and fluoxetine (6506 L/kg), which indicates that these compounds tend to sorb more into sediments (Table S.6b). For other compounds that are more hydrophilic (Log K_{OW} <4) and neutral such as carbamazepine, their presence in the sediment is proportional to their concentrations in the water where sites that have concentrations ~100 ug/L in the stream will also have detections in the sediments (Figure 4.9). A stark difference between the antibacterials and carbamazepine is also observed here such that carbamazepine occurs at ~2 orders of magnitude higher than the triclocarban in the stream/river (Figure 4.9b) but the opposite

was observed in the sediment (Figure 4.9a). Overall, the results show the clear trend related to the compounds physico-chemical properties are observed in the sediments and can be used further to hypothesize the partitioning of chemicals between water and sediment compartments.

4.4 Occurrence in biofilm and gammarids

This section focuses on the occurrence of micropollutants in the biota samples. For biofilm and gammarids, the frequently detected compounds were venlafaxine and its metabolite, carbamazepine, fluoxetine, and trimethoprim. The concentrations for the compounds detected in gammarids ranged from <LOQ (0.1)–20±0.01 ng/g_{dw} and biofilm from <LOQ (0.1)–152±20 ng/g_{dw} (Table 4.6). For fish, desmethylvenlafaxine, venlafaxine and carbamazepine were the most frequently detected compounds and the concentrations were between <LOQ (0.1)–3±1 ng/g_{dw}. Fish results will be addressed separately, as they were collected and exposed to the water at different times (see Chapter 3, Section 3.2).

In contrast to sediments, chemical properties do not determine whether compounds are going to bind to biofilm or be taken up by gammarids. Based in Log Kow, O-desmethylvenlafaxine and trimethoprim have low sorption potential, Log Kow<2. However, O-desmethylvenlafaxine was found at the highest concentrations in these matrices and trimethoprim was found in over 70% of the samples. Carbamazepine has been also detected despite of the low sorption potential (Log Kow=2.77). These inconsistencies were similar to the results in Munz et al. (2018) where they analyzed >50 micropollutants with a broad range of compounds with different Log K_{ow} in gammarids. Likewise, Aubertheau et al. (2017) obtained comparable results in the detection of micropollutants in biofilms. Although the mechanisms of partitioning of micropollutants are complex processes (and are outside the scope of this study), this thesis attempts to explain the observations in the subsequent sections.

Table 4.6 *Monthly average concentration (ng/g_{dw}) for micropollutants in biofilm and gammarids during the Fall 2022 sampling campaign. dw=dry weight. LOQ = limit of quantification, ND= no detected, NC= no collected.*

Compound	Month	August			September			October		
	Site	BR	PC 5%	PC 15%	BR	PC 5%	PC 15%	BR	PC 5%	PC 15%
Trimethoprim	Biofilm	NC	NC	NC	1	2	2	1	2	10
	Gammarids	0.3	0.2	2	0.2	0.3	1	0.2	0.2	1
O-desmethylvenlafaxine	Biofilm	NC	NC	NC	12	33	43	13	28	70
	Gammarids	2	4	16	4	6	13	4	5	7
10,11 epoxide Carbamazepine	Biofilm	NC	NC	NC	<LOQ	<LOQ	<LOQ	0.2	<LOQ	0.2
	Gammarids	<LOQ	<LOQ	2	<LOQ	0.2	0.3	<LOQ	<LOQ	0.2
Venlafaxine	Biofilm	NC	NC	NC	13	28	45	16	25	48
	Gammarids	2	5	16	4	5	11	3	5	8
Carbamazepine	Biofilm	NC	NC	NC	0.3	0.5	0.5	0.2	0.4	1
	Gammarids	0.2	0.4	3	0.4	0.5	1	0.3	0.5	1
Fluoxetine	Biofilm	NC	NC	NC	28	74	152	31	65	244
	Gammarids	2	6	20	3	6	16	3	8	16
Triclocarban	Biofilm	NC	NC	NC	3	5	4	4	5	8
	Gammarids	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfamethoxazole	Biofilm	NC	NC	NC	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1
	Gammarids	ND	ND	ND	ND	ND	ND	ND	ND	ND
Norfluoxetine	Biofilm	NC	NC	NC	4	8	10	4	6	19
	Gammarids	1	2	5	2	2	3	1	2	4
Sulfamethazine	Biofilm	NC	NC	NC	ND	ND	ND	ND	ND	ND
	Gammarids	3	7	6	7	<LOQ	4	1	1	<LOQ

4.4.1 Biofilms

Nine pharmaceuticals out of the 18 detected in the water matrix were found in biofilm. The chemical classes that were detected at the highest concentration were the antidepressants during the October 2022 sampling campaign in (PC 15%) (Table 4.6). The concentrations for O-desmethylvenlafaxine, venlafaxine, norfluoxetine and fluoxetine were 69 ±5 ng/g_{dw}, 48 ±3 ng/g_{dw}, 19 ±3 ng/g_{dw} and 244 ±16 ng/g_{dw}, respectively. Carbamazepine, 10, 11 epoxide-carbamazepine and trimethoprim were detected at lower concentrations ranging from <LOQ to 3.08 ±1.54 ng/g_{dw} (Table 4.6).

Studies have evaluated the occurrence of micropollutants in biofilm, again suggesting that influence of WWTPs effluent discharges. Huerta et al. (2016) assessed the occurrence of 44 micropollutants in river biofilm and reported venlafaxine and triclosan in biofilm at 43.7 ng/g_{dw} and 76.5 ng/g_{dw} respectively, which is similar to what was observed at the ACWA streams. Carbamazepine and trimethoprim were found to be at higher concentrations in a study (583.5 ng/g_{dw} and 10.4 ng/g_{dw} respectively) by Aubertheau et al. (2017), but the difference is likely due to the type of activated sludge biological treatment which is known to have poorer performance in micropollutant removals in comparison to BNR treatment at the PC WWTP (Dubey et al. 2020; Okuda et al. 2008). Mastrángelo et al. (2022) further detected 11 of 39 studied compounds in biofilm including, analgesics, antibiotics, antidepressants and antiepileptics (ranged from 1-179 ng/g_{dw}). In this study, carbamazepine, fluoxetine, and venlafaxine were detected at 2 ng/g_{dw}, 54 ng/g_{dw} and 44 ng/g_{dw} respectively, in the ACWA streams, while the concentrations detected at Canmore (upstream) were either not detected or <LOQs (Figure 4.10).

Regardless of the chemical, it appears that the there is a proportional increase in biofilm concentrations with increasing micropollutant concentrations in the river (correlation coefficient,

r>0.97) (Figure 4.10), suggesting that micropollutants can be adsorbed by biofilms (passively) via the cell surfaces. A key observation here is the presence of fluoxetine in biofilms at ~1–2 orders of magnitude higher than any other chemical (Figure 4.10), although the concentrations in the streams are among the lowest in comparison to venlafaxine, carbamazepine, and trimethoprim as examples (Figure 4.7).

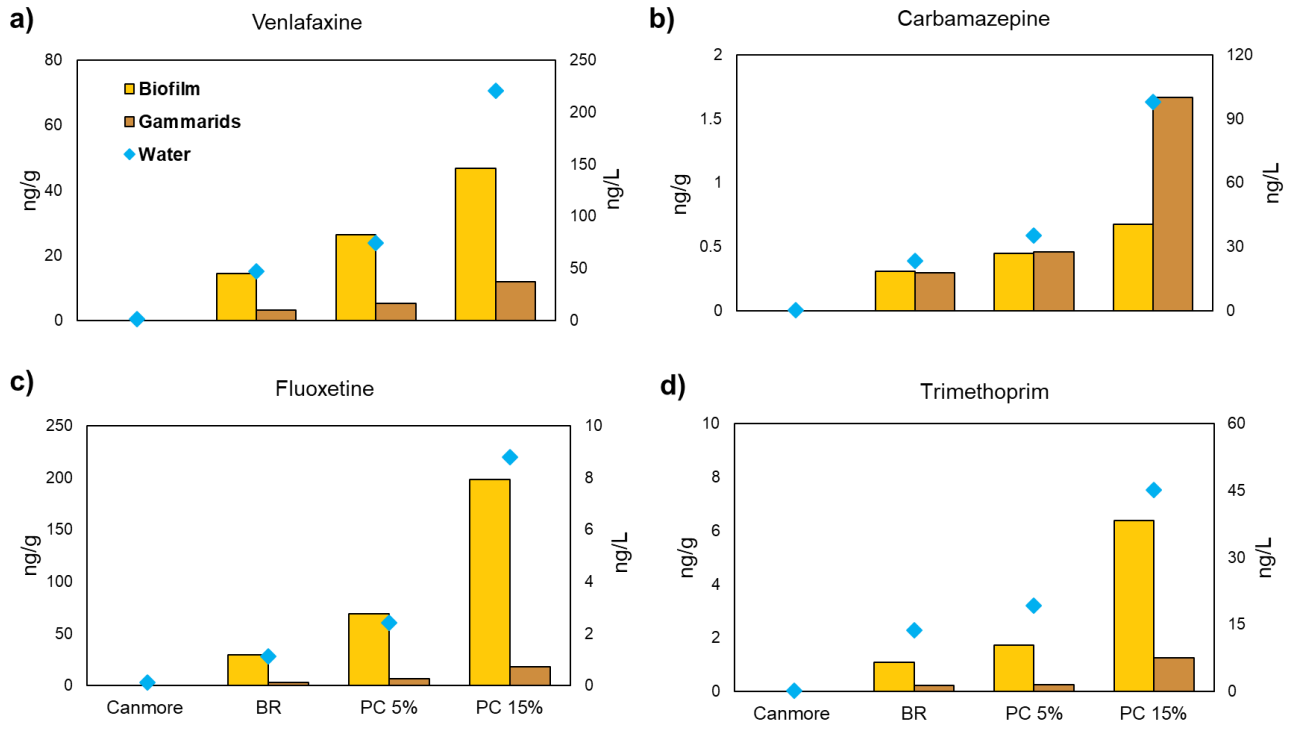

Figure 4.10 *Comparison of mean concentrations for Fall 2022 in biofilm and gammarids for a) venlafaxine, b) carbamazepine, c) fluoxetine, and d) trimethoprim in different sites.*

The fate mechanisms of fluoxetine in biofilm are still unclear. However, these results are consistent with Mastrángelo et al. (2022) who detected fluoxetine in biofilm (54 ng/g_{dw}) but no concentration in the water matrix. Another study by A. Silva et al. (2019) established that fluoxetine is more adsorptive than venlafaxine, which could explain the low concentrations of this

compound in the water matrix and high presence in the biofilm. In addition, the bioconcentration factor (BCF) was calculated for this matrix and fluoxetine and its metabolite were found to be bioaccumulative (BCF>5000 L/kg), which is different from the other compounds detected (low bioaccumulative potential, BCF<2000 L/kg)), suggesting that the concentration detected for these compounds is attributed to exposure (Table S.6).

Studies have also indicated that micropollutants can further be biotransformed and/or bioaccumulated by the diverse consortia of algae, bacteria, fungi and other microorganisms that comprise the biofilms (Desiante et al. 2021). The exact mechanisms of chemical fate in the biofilms are outside the scope of this thesis, but it is clear that they are an important sink of micropollutants in natural environments. It is also unsurprising that biofilm-based water/wastewater treatment technologies (e.g., biofilm bioreactors) have been assessed for their ability to bio-adsorb, bioaccumulate and bioremediate pollutants (Bhatt et al. 2022). In fact, it has been suggested that stream biofilms can be used for micropollutant remediation due to their large potential to sequester micropollutants from the aqueous phase (Peng et al. 2020). Given the benefit of being a micropollutant sink in aquatic ecosystems, adsorption/absorption of these compounds to biofilms could cause changes in the microbial communities present in the aqueous phase (Aubertheau et al. 2017) and therefore, the bioaccumulation of these compounds throughout the trophic web may become more important (briefly explained in the following sections in gammarids and fish).

4.4.2 Occurrence in Gammarids

Eight of the 22 compounds were detected in gammarids, including antidepressants, antibiotics, cardiovascular and antibacterial. The concentrations found in this matrix are generally lower than the ones detected in biofilms and ranged between <LOQ and 20±0.01 ng/g_{dw} (Figure

4.7). The highest concentrations were reported for PC 15% in August for fluoxetine and O-desmethylvenlafaxine at 20±0.01 ng/g_{dw} and 16±0.4 ng/g_{dw}, respectively (Table 4.6). Furthermore, the concentrations of O-desmethylvenlafaxine, venlafaxine, carbamazepine, and fluoxetine were three times higher in PC 15% stream than PC 5%. Also, micropollutants were generally detected at lower concentration in BR streams (<LOQ–3±3 ng/g_{dw}) in comparison to the PC 5% streams (<LOQ–6±4 ng/g_{dw}) and PC 15% (1±1–18±2 ng/g_{dw}). In addition, the BCFs were also calculated for 7 compounds (Table S.6) and were found to be higher for PC 15% than PC 5% and BR streams. More specifically, fluoxetine, norfluoxetine and sulfamethazine showed a higher BCF at these experimental streams and showed the highest values for PC 15% at 2018 L/kg, 6929 L/kg and 3794 L/kg respectively. Therefore, the results suggest that fluoxetine, norfluoxetine, and sulfamethazine are potentially bioaccumulative (USEPA, 1999). For the rest of the compounds, BCFs in biofilms were <2000 L/kg. The exposure of gammarids to high concentrations of O-desmethylvenlafaxine, venlafaxine, carbamazepine and trimethoprim correspond to their high concentrations in the streams but not the case for fluoxetine.

Other studies have focused on the detection of organic contaminants in benthic organisms. For example, De Solla et al. (2016) detected 43 micropollutants in wild mussels collected from a river that receives WWTP effluent, including fluoxetine (5.81–8.98 ng/g_{ww}), norfluoxetine (1.18–2 ng/g_{ww}), triclocarban (3.32–5.37 ng/g) and venlafaxine (14.3–24.9 ng/g). Additionally, Munz et al. (2018) studied the bioaccumulation of micropollutants in gammarids, where 63 compounds were detected, including, O-desmethylvenlafaxine, carbamazepine and venlafaxine at 0.3 ng/g_{dw}, 1 ng/g_{dw} and 0.9 ng/g_{dw}, respectively. Miller et al. (2015) found concentrations up to 36 ng/g_{dw} for pharmaceuticals, including antibiotics, antidepressants, antiepileptics and analgesics in gammarids collected in eight tributaries of the River Thames, UK; trimethoprim was detected at 5 ng/g_{dw} and

carbamazepine at 6 ng/g_{dw}. In general, the literature points to a consistent pattern where organisms collected near effluent discharge points were found to have higher micropollutant concentrations that the ones collected further downstream.

4.5 Occurrence in fish

For the fish matrix, 7 micropollutants were detected in fish tissue, from which 1 of them (10, 11 epoxide carbamazepine) was below the LOQ (0.1 ng/g). The concentrations ranged from <LOQ (0.1) to 3±1 ng/g (Figure 4.8). Two species were considered in this study, spoonhead sculpin (SS) and longnose dace (LND) and three sites were compared: BR, PC 15%, and Bowness Park (original source of SS and LND). Note that in comparison to other matrices, the fish collected here had different exposure times. Due to low amount of fish tissue needed for this study, the samples were pooled from the exposure period (7 d, 14 d, and 28 d). The most prevalent compounds were O-desmethylvenlafaxine, norfluoxetine and carbamazepine with highest concentrations of 2±1 ng/g_{dw} (LND), 3±1 ng/g_{dw} (LND), and 1±0.5 ng/g_{dw} (SS), respectively (Table S.7). The highest concentration was found in PC 15% for norfluoxetine in LND. In addition, these compounds were detected in both species and 10,11 epoxide carbamazepine was only found at >LOQ only in LND. Fluoxetine, triclocarban and venlafaxine were detected only in SS at 1±0.5 ng/g_{dw}, 0.1±0 ng/g_{dw} and 0.3±0.1 ng/g_{dw}. Hence, the detection frequency was higher for SS as 6 compounds detected and only 4 in LND. A correlation between the compounds and the detection frequency of the two fish species of this study cannot be derived the same way it was for the biofilms and gammarids.

In addition, the BCFs were calculated in fish tissue for 5 compounds (Table S.6). For this matrix, norfluoxetine is potentially bioaccumulative with the highest BCF at 4912 L/kg in Bow River for spoonhead sculpin and 4895 L/kg in longnose dace in PC 15%. The rest of the

compounds also had BCFs>2000 L/kg, which means a higher influence of the exposure to the water concentration. However, note that the water and fish samples were not collected at the same time, which could change these results. Hence a simultaneous sample collection is suggested.

Many studies have reported the occurrence of micropollutants in fish in similar values. Huerta et al. (2018) studied the occurrence of pharmaceuticals in 8 different species of wild fish in 25 rivers in the USA. Eleven compounds were detected with concentrations <10 ng/g_{dw}, including analgesics, antidepressants, and β-blockers. Venlafaxine and carbamazepine were detected at 4.6 ng/g_{dw} and 3 ng/g_{dw}. Schultz et al. (2010) found venlafaxine (0.1 ng/g), fluoxetine (0.6 ng/g_{dw}) and norfluoxetine (0.9 ng/g_{dw}) in brain tissue from fish collected in WWTP effluent affected river. Moreno-González et al. (2016) detected 18 pharmaceuticals in fish collected in coastal lagoon, the frequently detected groups were b-blockers and psychiatric drugs. Several samples were collected in different seasons and the highest concentration was detected in spring with an average concentration for carbamazepine of 1.2 ng/g_{dw}, while venlafaxine was not detected in the muscle tissue. Micropollutants were also found in samples collected in Canada. Chu and Metcalfe (2007) studied the occurrence of antidepressants in fish from the Hamilton Harbour in Ontario with concentrations up to 1 ng/g_{ww}. Fluoxetine was reported at 1.02 ng/g_{ww} and norfluoxetine 1.02 ng/g_{ww}.

Another important point to consider, it is that most of the studies including this thesis have focused on fish muscle. However, various studies have reported higher concentrations of compounds such as antidepressants and their metabolites in tissues such as liver and brain (Huerta et al. 2013). For example, in a study by Valdés et al. (2016), carbamazepine was found to accumulate more in brain and liver than muscle. Furthermore, in an exposure experiment of water

effluent by McCallum et al. (2017), the concentrations of pharmaceuticals were compared in different fish tissue, and found venlafaxine to have higher concentrations in brain than muscle.

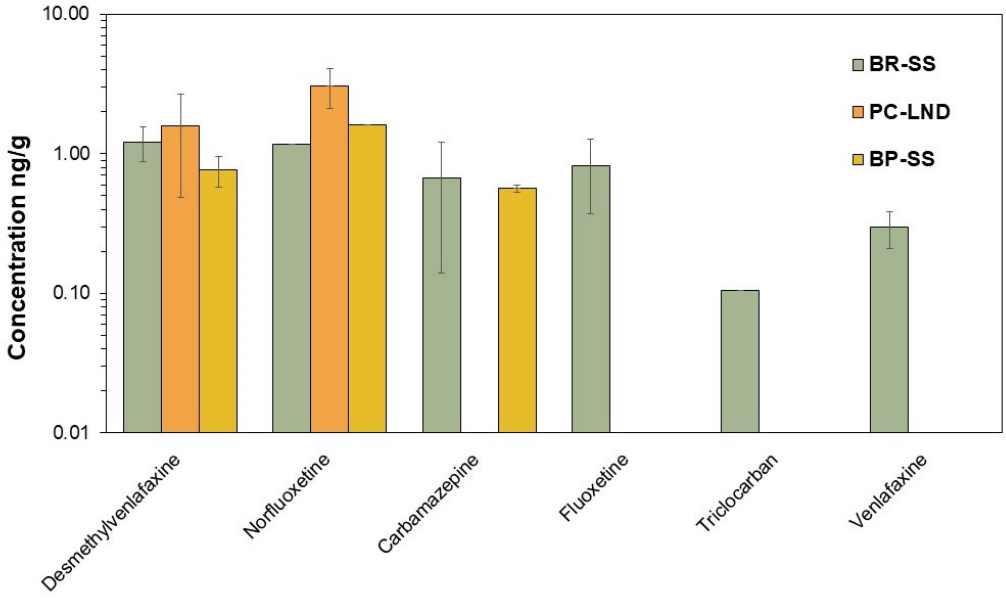

Figure 4.11 *Fish concentration for frequently detected compounds compounds. Frequency of detection (5) went from 2% up to 97%. Bowness Park is the control site for the fish collection.*

4.6 Why do internal concentrations matter?

Water concentrations provide a starting point for understanding potential risks of micropollutant accumulation in different environmental matrices. However, many of them partition strongly in solid matrices (sediments, biofilms, gammarids), thus the water concentrations are not always sufficient to accurately assess their impact on fish and invertebrates. In this study, for example fluoxetine and norfluoxetine were not relevant in the water matrix but were dominant in the solid matrices and showed a higher bioaccumulation potential. On the other hand, diclofenac was detected at high concentrations in water and was not detected in any of the solid matrices.

Therefore, the whole-body burden (internal concentrations) cannot simply be assessed using the water concentrations and monitoring of invertebrate or fish tissue samples might be of relevance, especially for antidepressants that have been considered to impact exposed aquatic organisms (e.g., behaviour-related impacts) (Wiles et al. 2020; Hedgespeth et al. 2014; Brodin et al. 2017).

Additionally, concentrations in the organisms of a lower trophic position (benthic organisms and biofilm) were found to be higher than the concentration found in fish (Table 4.6), suggesting that the bioaccumulation potential in fish is lower (Table S.7). These results are consistent with previous studies (de Solla et al. 2016; Lagesson et al. 2016) and further suggests that invertebrates may be a good class of organisms to monitor. Studies have also pointed towards the bioaccumulation of micropollutants via dietary uptake (Adriaens et al. 2007). Given that gammarids are detritus feeders, they may be exposed to leaf matter that has accumulated biofilms (which have now also accumulated micropollutants).

Overall, measuring internal concentrations in fish, gammarids and biofilm is a starting point to understand what compounds should be regulated as they are all connected in the food web. Most of the regulations available for micropollutants are focused on water. However, this thesis has shown that animal tissue is also an important factor to consider. Although, some guidelines are already established, an expanded regulatory framework should be contemplated to the compounds that tend to bioaccumulate more in tissue.

Chapter 5 – Conclusions and Recommendations

This study focused on the partitioning of a diverse group of micropollutants in 5 environmental matrices (water, sediment, biofilm, gammarids and fish) in an artificial stream facility (ACWA) with four types of streams, PC, BR, RO and O_3. To achieve this goal, sample preparation methods based on different QuEChERS and SPE (only for water samples), and the detection via LC-QQQ were used for the detection of 22 micropollutants. The recoveries, LOQs and MEFs were within acceptable ranges for most of the compounds, indicating that sample preparation approach developed for the solid matrices (sediment, biofilm, gammarids, and fish) is suitable for the objectives of this study.

In the water matrix, 18 out of 22 compounds were detected in the sampling campaigns of 2020, 2021 and 2022. Diclofenac, venlafaxine O-desmethylvenlafaxine and carbamazepine were the most frequently detected and were at the highest concentrations in PC experimental streams. Although the BR streams are currently reflected as a "control stream", they showed consistently high concentrations suggesting that the micropollutants found in PC, RO, and O_3 streams were contributed by the Bow River that already received discharges from upstream WWTPs (Fish Creek and Bonnybrook). Furthermore, these results suggested that BNR treatment currently operated at the PC WWTP followed by ultrafiltration (UF) and RO or O_3 were effective at removing micropollutants as concentrations in RO and O_3 streams were similar and lower than PC streams (e.g., venlafaxine, diclofenac, sulfamethoxazole). Nonetheless, WWTP effluents are an important source of micropollutant concentration in the streams as observed by the difference in concentration between Canmore (upstream reference site), BR and PC streams. Moreover, when the PC effluent was increased to 15%, most of the compounds were detected three times higher on average in PC 15% streams. A similar trend was observed in the sediment and biota matrix.

The concentrations detected for the sampling campaigns from 2020 to 2022 followed a seasonal trend, where the lowest concentrations were detected for both BR and PC streams in the summer months when the dilutions were high while high concentrations corresponded to low river flows during the colder periods. Although, these concentrations fluctuated in different times of the year, the compounds regulated in Canada, included in this study (triclosan and carbamazepine) met with the guideline. However, diclofenac was detected in concentrations higher than 40 ng/L, which exceeds the European proposed regulations.

For the sediment matrix, 10 compounds were detected with most of them following the same pattern observed in the streams, i.e., PC 15% > PC 5% > BR. In this case, O-desmethylvenlafaxine, venlafaxine, carbamazepine, fluoxetine, triclocarban and triclosan were the dominant compounds. The latter three were observed to have low concentrations in the water matrix which indicates that some micropollutants partition well onto the suspended solid/sediments.

For biofilm and gammarids, 9 and 8 compounds were detected respectively. Here, there were inconsistencies in the trends as they relate to the water sample detections. Moreover, it was observed that regardless of the chemical, there is a proportional increase in biofilm and gammarids concentrations with increasing micropollutant concentrations in the river (PC 15%). Additionally, fluoxetine showed the highest concentrations in both matrices, although the concentrations in the streams are among the lowest in comparison to venlafaxine, carbamazepine, and trimethoprim. For gammarids, it is suggested that the take up of micropollutants is via respiration or dietary (via biofilm). On the other hand, the fate mechanisms of fluoxetine in biofilm are still unclear. However, fluoxetine is more adsorptive than venlafaxine, which could explain the low concentrations of this compound in the water matrix and high presence in the biofilm.

For fish, 7 compounds were detected, which had lower concentrations than biofilm and gammarid tissues extracted. In addition, spoonhead sculpin had a higher frequency than longnose dace, but the highest concentrations were found for longnose dace.

For the bioaccumulation assessment, fluoxetine and norfluoxetine were found to have a bioaccumulative potential for the biota matrices (BCF>2000 L/kg), while in the sediments triclosan and fluoxetine were found to have the highest sorption potential to these particles. In addition, diclofenac showed constantly the highest concentrations in the water samples, but it was not detected in the solid matrices.

Finally, the following recommendations have been summarized to improve on future research related to micropollutant partitioning:

- Although, acceptable recoveries were obtained for the preparation methods, further cleaning is recommended for the extraction methods as the MEFs is close to the threshold or slightly higher for some compounds.
- Matrix effects experiments are recommended for these types of extractions since the approach used for this study was theoretical, which will allow to obtain a broader range of compounds that fit in the quality measures.
- Analysis of different fish tissue such as liver and brain may be required to properly assess their bioaccumulation. Muscle tissues may not have the properties to show accumulation of micropollutants but other studies have shown them to accumulate at higher concentrations in organs (Huerta et al. 2013; Valdés et al. 2016; McCallum et al. 2017).
- Simultaneous sampling campaigns for fish and water samples are recommended, as it will facilitate a comparison between the findings in this matrix with the rest of them.

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Appendix: Supplementary Information

Table S.1 *Method development using the ASE method for sediment samples. Samples were collected from stream 1. Four replicates were extracted.*

Compound	Name	Matrix Spiked 1	Matrix Spiked 2	Stream 1-1	Stream 1-2	Stream 1-3	Stream 1-4	Response Area Cal curve
	Measured concentration (ng/L)	23.6		32.3	41.1	16.9	30.8	
Sulfamethazine	**Compound Response Area**	118744	0	42470	44506	17308	18349	792711
	ISTD response Area	190593	316013	50121	41460	38375	22675	
	Measured concentration (ng/L)	26.4		31.1	28.6	0	0	
Trimethoprim	**Compound Response Area**	442988	0	9727	7456	0	0	568184
	ISTD response Area	249543	402879	4653	3870	4516	4494	
	Measured concentration (ng/L)	31	0	0	0	0	0	
O-desmethylvenlafaxine	**Compound Response Area**	2687023	0	0	0	0	0	6791928
	ISTD response Area	1844045	2784105	5942	1131	1372	805	
	Measured concentration (ng/L)	25.4	0	0	0	0	0	
Venlafaxine	**Compound Response Area**	884801	0	0	0	0	0	8905422
	ISTD response Area	2428057	3697309	11184	5630	6195	5800	
	Measured concentration (ng/L)	21.6		25.3	26.2	2.8	3.2	
Carbamazepine	**Compound Response Area**	2382662	0	1042304	1431212	99962	92186	2139671
	ISTD response Area	1387746	2405443	517819	687479	487779	397099	
	Measured concentration (ng/L)	23.1		0	0	0	0	
Norfluoxetine	**Compound Response Area**	103421	0	0	0	0	0	450562
	ISTD response Area	82325	95588	115	1092	1534	54	
	Measured concentration (ng/L)	24.9	0	0	0	0	0	
Fluoxetine	**Compound Response Area**	210075	0	0	0	0	0	2884191
	ISTD response Area	199871	443683	1946	779	1682	1273	
	Measured concentration (ng/L)	19.8						
Naproxen	**Compound Response Area**	2412	0	0	0	0	0	90537
	ISTD response Area	2191	2759	0	816	957	1073	

Table S.1. *Continued.*

Compound	Name	Matrix Spiked 1	Matrix Spiked 2	Stream 1-1	Stream 1-2	Stream 1-3	Stream 1-4	Response Area Cal curve
	Measured concentration (ng/L)		52.4	0	0	0	0	
Diclofenac	**Compound Response Area**	18355	9326	0	0	0	0	449114
	ISTD response Area	4836	6373	20931	31051	6681	21927	
	Measured concentration (ng/L)	12.3	0	0	0	0	0	
Ibuprofen	**Compound Response Area**	2378	0	0	0	0	0	78476
	ISTD response Area	3660	5012	3313	2364	2862	1454	
	Measured concentration (ng/L)	25.6	1.4	26.5	29.2	9.8	10	
Gemfibrozil	**Compound Response Area**	35295	3057	9524	4781	1465	1390	463110
	ISTD response Area	31791	63940	8293	3771	3539	3272	
	Measured concentration (ng/L)	26	0	36.5	39.7	13.7	14	
Triclocarban	**Compound Response Area**	1516731	0	1276261	1474642	454164	462341	3112036
	ISTD response Area	1971032	3278460	1185327	1258032	1125462	1119827	
	Measured concentration (ng/L)	25.9	0	53.2	56.2	29	29	
Triclosan	**Compound Response Area**	29857	0	44412	48647	26528	26676	63913
	ISTD response Area	39857	71711	29985	31261	31760	31905	

Table S.2. *Comparison of the signal in the internal standard and concentrations detected after the analysis for a) carbamazepine and b) fluoxetine. For fish in carbamazepine Stream 2 was analyzed for BEM1.0 and Stream 3 for Experiment 3 for the unspiked sample. and for fluoxetine stream 10 for BEM 1.0 and Stream 3 for experiment 3 for the spiked sample. SED=Sediment, BF=Biofilm, GAM=Gammarids, MS=Matrix spiked, ISTD=Internal Standard, BEM=Biota extraction method, "- "= not applicable.*

a) Carbamazepine

Matrix	**SED**		**BF**		**GAM**		**FISH**	
Type of Sample	**MS**	**Stream 4**	**Stream 3**	**Stream 2**	**Stream 2**	**Stream 3**	**Stream 10**	**Stream 2/ Stream 3**
	Spiked	**Unspiked**	**Spiked**	**Unspiked**	**Spiked**	**Unspiked**	**Spiked**	**Unspiked**
Method	**ASE**		**BEM 1.0**		**BEM 1.0**		**BEM 1.0**	
Expected concentration (ug/L)	20	N/A	20	N/A	20	N/A	20	N/A
Measured Concentration (ug/L)	0	6	21	0.30	0	0.8	0	0
Compound response area	0	267851	1544604	14937	0	5381	0	2097240
ISTD Area	4283471	616558	919650	1640169	866726	145394	1129757	0
Average ISTD Calibration Curve	4317922		1998832	3730326	1998832	3469150	1998832	6441106
Method	**QuECheRS**		**Experiment 4**		**Experiment 1**		**Experiment 3**	
Expected concentration (ug/l)	20	N/A	20	N/A	20	N/A	20	N/A
Measured Concentration (ug/l)	20.8	2.8	21.2	1.2	31.5	1.2	18.4	4.1
Compound response area	1556107	81340	457525	16341	315826	18918	398185	73828
ISTD Area	1029676	434103	340991	268789	162340	333948	404706	356944
Average ISTD Area Cal Curve	2406662		2310596		1743569		4568699	

b) Fluoxetine

Matrix	**SED**		**BF**		**GAM**		**FISH**	
Type of Sample	**MS**	**Stream 4**	**Stream 3**	**Stream 2**	**Stream 2**	**Stream 3**	**Stream 10/Stream 3**	**Stream 3**
	Spiked	**Unspiked**	**Spiked**	**Unspiked**	**Spiked**	**Unspiked**	**Spiked**	**Unspiked**
Method	**ASE**		**BEM 1.0**		**BEM 1.0**		**BEM 1.0**	
Expected concentration (ug/L)	20	N/A	20	N/A	20	N/A	20	N/A
Measured Concentration (ug/L)	0	0	32	8.00	1.3	0	0	0
Compound response area	0	0	1214098	512823	50541	0	0	0
ISTD Area	1399621	3866	904726	1793295	1250699	1086050	1365519	1326051
Average ISTD Calibration Curve	5165731		2646949	4367741	2646949	4450456	2646949	5718499
Method	**QuECheRS**		**Experiment 4**		**Experiment 1**		**Experiment 3**	
Expected concentration (ug/l)	20	N/A	20	N/A	20	N/A	20	N/A
Measured Concentration (ug/l)	20.2	96.5	77.9	114.9	52	8	23.9	5.3
Compound response area	767393	1144081	2037648	3591590	1340330	170287	428788	49857
ISTD Area	777212	227089	476950	555531	496421	446531	304950	167534
Average ISTD Area Cal Curve	2201388		2103094		1889049		3565502	

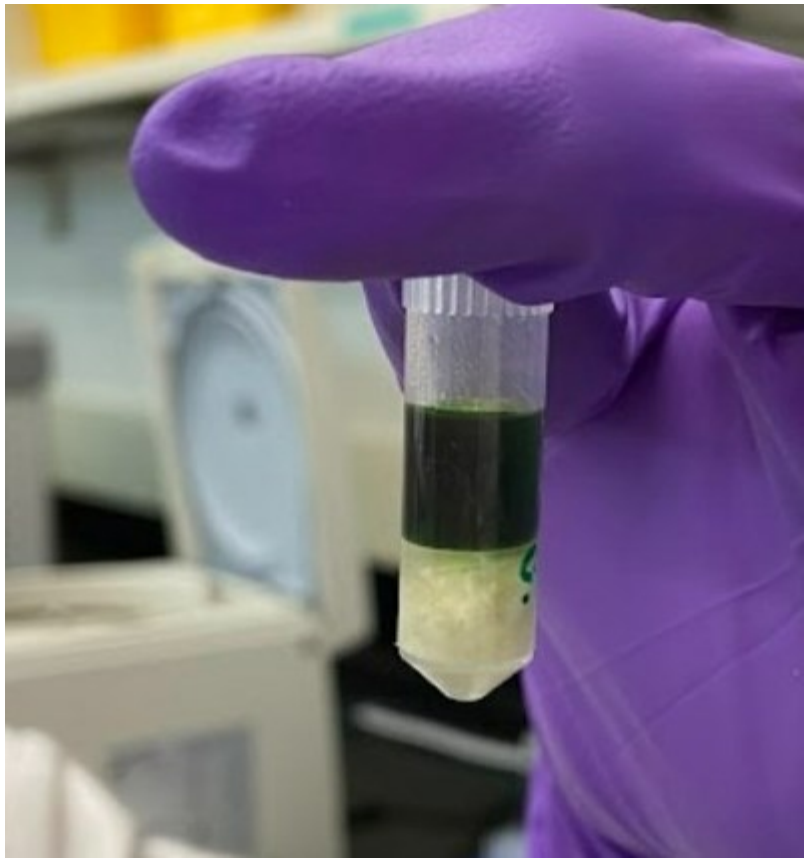

Figure S.1. *Visual of biofilm extract with BEM 1.0 method.*

Table S.3 R*aw data* from *Characterization 2020 (ng/L)*, n=*3. S=stream, desVEN=O-desmethylvenlafaxine, VEN=venlafaxine, FLX=Fluoxetine, NFLX=Norfluoxetine, DCF=Diclofenac, IBU=Ibuprofen, NPX=Naproxen, CBZ=Carbamazepine, e-CBZ=10,11 epoxide carbamazepine, ATOR=Atorvastatin, p-ATOR=p-hydroxy atorvastatin, o-ATOR=p-hydroxy atorvastatin GFZ=Gemfibrozil, SULF=Sulfamethoxazole, TRIM=Trimethoprim, TCCB=Triclocarban, TCS=Triclosan.*

Treatment	Stream	desVEN	VEN	FLX	NFLX	DCF	IBU	NPX	CBZ	e-CBZ	ATOR	p-ATOR	o-ATOR	GFZ	SULF	TRIM	TCCB	TCS
PC	**S2**	36±31	59±25	2±1	0.5±0.1	116±34	8±3	13±5	38±16	1±1	8±3	15±7	13±6	2±1	36±16	9±2	0.2±0	5±4
	S10	27±36	25±35	22±34	23±34	25±35	16±16	17±17	17±18	13±14	15±15	15±16	14±17	13±19	16±20	6±6	2±2	4±1
	S12	44±8	88±8	2±0.1	0.4±0.1	136±10	8±1	17±2	50±4	2±0.2	10±1	20±2	17±2	2±0.4	50±4	9±2	0.2±0	4±1
O_3	**S6**	71±34	37±4	1±0.1	1±0	100±4	8±1	16±0.3	30±1	1±0.1	7±1	17±7	12±2	2±0.2	31±1	15±6	0.2±0	3±1
	S8	56±54	39±2	1±0.1	0.4±0.1	108±4	7±1	16±1	30±1	1±0	6±0.4	14±0.4	12±1	2±0.1	27±1	11±2	0.2±0	6±6
	S11	37±20	45±2	1±0.1	0.5±0.1	115±5	8±1	16±0.2	30±1	1±0	8±0.1	15±0.2	13±0.1	2±0.2	33±1	14±3	0.2±0	3±1
BR	**S3**	31±1	41±0.4	1±0.1	0.5±0.1	108±3	6±1	16±1	32±1	1±0	7±1	16±0.4	13±1	2±0.1	28±1	12±1	0.2±0	2±1
	S4	47±19	41±1	1±0.3	0.4±0.1	102±4	6±1	15±1	30±1	1±0.1	6±0.4	14±1	12±0.4	2±0.1	26±1	13±1	0.2±0	2±0.4
	S7	81±100	43±1	1±0	0.4±0.1	102±4	7±1	14±0.1	30±0.1	1±0.1	6±1	15±1	12±1	2±0.1	28±2	10±0.3	0.2±0	3±1
RO	**S1**	49±17	45±1	2±0.2	0.4±0	112±6	7±0.4	16±1	33±2	1±0.1	7±0.4	16±1.4	14±1	2±0.1	30±1	17±11	0.2±0	4±0.4
	S5	31±8	44±1	1±0.1	0.4±0.1	109±5	6±0.3	15±1	31±1	1±0.1	7±0.2	15±1	12±1	2±0.1	31±2	11±2	0.2±0	3±1
	S9	46±17	43±1	1±0.1	0.4±0.2	107±2	8±1	16±0.2	31±0.6	1±0.1	7±0.2	14±0.2	13±0.4	2±0	28±1	11±3	0.2±0	4±1

Table S. 4 *Replicate streams concentration (ng/L) per site in 2021*, n=*3*.

Stream	PC	BR	O_3	RO
desVEN	102±7	29±44	48±34	37±15
VEN	76±1	24±3	44±22	35±1
FLX	2±0.1	1±0.1	1±0.2	1±0.1
NFLX	<LOQ	<LOQ	<LOQ	<LOQ
DCF	121±2	59±3	83±32	80±3
IBU	4±0.2	4±0.2	4±0.4	4±0.1
NPX	4±0.1	4±0.4	3±1	4±0.1
CBZ	26±0.3	12±2	16±7	15±1
e-CBZ	1±0	1±0.1	1±0.2	1±0.1
ATOR	9±1	5±1	7±2	6±1
p-ATOR	16±1	8±0.3	10±6	11±1
o-ATOR	16±1	7±1	9±6	10±1
GFZ	1±0	1±0.1	1±0.1	1±0
SULF	34±6	15±20	24±2	16±1
TRIM	12±0.2	6±0.1	9±3	7±1
CAFF	10±0.4	9±1	10±1	9±0.4
TCCB	<LOQ	<LOQ	0.4±0	<LOQ
TCS	1±0.1	1±0.1	1±0.4	1±0.1

Table S.5 *PC effluent concentration (ng/L) collected in January 2023. STDV= Standard Deviation.*

Compound	ng/L	STDV (±)
SULF	264	93
TRIM	261	4
desVEN	3014	436
VEN	1007	26
CBZ	410	11
p-ATOR	114	13
o-ATOR	149	14
ATOR	41	5
FLX	66	2
NPX	33	1
DCF	655	7
IBU	15	5
GFZ	3	0
TCCB	0	0
TCS	27	5
NFLX	5	1
SMZ	7	2

Table S.6 *Bioaccumulation factor BCF for a) biofilm, gammarids, fish and* solid-water distribution coefficient K_d *for b) sediment.*

a)

Sample	Site	TRIM	desVEN	VEN	CBZ	NFLX	FLX	TCS	SMZ	SULF
Biofilm	PC 5%	90	124	353	13	23666	28639	N/A	N/A	6
	PC 15%	141	69	211	7	22910	22576	N/A	N/A	5
	BR	80	68	304	13	15831	25764	N/A	N/A	1
Gammarids	PC 5%	13	20	70	13	7218	2707	617	4783	N/A
	PC 15%	28	15	53	17	6929	2018	531	3794	N/A
	BR	16	16	64	13	5499	2371	984	2206	N/A
Fish	BR-SS	N/A	6	6	29	4912	727	N/A	N/A	N/A
	PC-LND	N/A	2	N/A	N/A	4896	N/A	N/A	N/A	N/A

b)

Sediment	TRIM	desVEN	VEN	CBZ	NFLX	FLX	TCS	SMZ
PC 5%	44	224	1527	38	21414	20374	15634.7	248
PC 15%	27	81	605	15	12439	6507	4850	194
Bow River	49	134	1560	28	16861	24743	15311	155

Table S.7. *Fish concentration for SS and LND in Bow River, Pine Creek and Bownness park (Control site). ND=not detected.*

Site+ Fish specie	desVEN	e-CBZ	VEN	CBZ	NFLX	TCCB	FLX
BR spoonhead sculpin	1.2±0.3	ND	0.3±0.1	0.7±0.5	1.2	0.1	0.8±0.4
PC longnose dace	1.6±1	<LOQ	ND	ND	3.1±1	ND	ND
Bowness Park spoonhead sculpin	0.8±0.2	ND	ND	0.6±0.03	1.6	ND	ND

Table S.8. *LOQs for sampling campaigns in 2020, 2021 and 2022. For 2022, a) samples from February, April, May and July and b) for Fall 2022 (August, September, October).*

Sampling campaign	2021	2020	2022 (a)	2022 (b)
desVEN	0.4	0.5	1.2	0.5
VEN	0.2	0.2	0.1	0.1
SULF	0.4	0.8	ND	0.4
CAFF	0.3	ND	ND	0.2
TRIM	0.3	0.3	0.2	0.2
e-CBZ	0.2	0.3	0.3	ND
CBZ	0.2	0.2	0.1	0.2
p-ATOR	0.6	0.4	0.4	0.4
NFLX	0.3	0.3	0.2	0.2
FLX	0.2	0.2	0.1	0.1
o-ATOR	0.7	0.4	0.4	0.4
ATOR	0.6	0.3	0.3	0.4
NPX	0.2	0.2	0.2	0.2
DCF	0.1	0.1	0.4	0.2
IBU	0.2	0.2	0.2	0.1
GFZ	0.1	0.1	0.1	0.1
TCCB	0.2	0.2	0.3	0.3
TCS	0.1	0.1	0.2	0.1
SMZ	ND	ND	ND	0.6

Table S.9. *Sampling campaign concentrations for sampling campaigns in 2022, a) is for sampling campaigns from winter to summer and b) is for fall.* n=*3, except for BR samples in February (n=2) and Pine Creek samples in Fall (n=2).*

a)

Site	Month	TRIM	desVEN	VEN	CBZ	NFLX	o-ATOR	p-ATOR	ATOR	FLX	NPX	DCF	IBU	GFZ	TCS
Bow River	February	20±1	280±5	80±1	31±1	1±0.1	7±0.02	12±0.3	2±0.1	4±0.1	18±0.1	38±0	16±0.3	3±0.2	5±0.1
	April	17±1	193±12	54±3	26±1	1±0.2	2±0.2	4±0.3	1±0.04	2±0.2	22±1	48±42	17±1	3±0.2	2±0.3
	May	15±0.2	128±5	39±2	22±1	<LOQ	1±0.2	3±0.4	<LOQ	2±0.2	6±0.4	28±7	8±0.1	1±0.1	2±1
	July	5±0.2	31±1	11±1	6±0.2	<LOQ	1±0.2	1±1	<LOQ	1±0.1	2±0.1	36±39	<LOQ	0.5±0	1±0.2
Pine Creek	February	31±1	532±24	129±3	45±1	1±0.3	12±1	20±1	4±0.2	7±0.4	21±1	76±23	19±1	4±0.1	6±0.4
	April	25±1	381±28	91±3	38±2	1±0.1	5±1	9±2	2±0.5	4±0.3	26±1	50±22	20±2	3±0.2	3±0.3
	May	21±0.4	267±14	75±1	36±1	1±0.3	2±0.03	4±1	<LOQ	3±0.1	7±0.4	43±2	10±1	1±1	2±0.2
	July	10±0.5	108±6	37±3	17±1	1±0	1±0.2	2±0.3	<LOQ	2±0.3	3±0.1	37±4	5±1	1±0	2±0.3

b)

Site		**PC**			**BR**	
Month	**August**	**September**	**October**	**August**	**September**	**October**
desVEN	247±8	80±13	409±9	127±7	183±79	260±11
VEN	61±1	78±4	84±0.3	36±1	52±2	54±2
SULF	31±3	41±2	65±1	22±2	31±2	45±4
TRIM	15±0.4	17±1	25±1	10±0.4	14±1	17±1
CBZ	30±0.2	36±3	40±1	18±0.5	25±1	27±1
p-ATOR	9±0.3	16±1	12±0.3	10±0.4	17±1	12±1
o-ATOR	6±0	11±1	8±0.4	6±0.4	11±1	8±1
ATOR	2±0.1	5±0.4	4±0.1	2±0.2	4±0	4±0.2
FLX	2±0.2	3±0.2	3±0.2	1±0.1	1±0.1	1±0.2
NPX	5±0.1	11±0.4	12±1	5±0.1	12±0.5	13±2
DCF	95±5	109±6	126±2	70±5	94±7	154±77
IBU	1±0.1	5±0.2	3±1	2±0.3	4±1	4±1
GFZ	1±0.1	1±0.1	1±0.1	1±0.1	1±0.1	1±0.1
TCCB	>LOQ	>LOQ0	>LOQ	>LOQ	>LOQ	>LOQ
TCS	1±0	2±0.2	1±0.1	1±0.1	1±0	1±0.2
NFLX	0.3±0	0.3±0	>LOQ	0.2±0.1	0.3±0	>LOQ
SMZ	1±0	2±1	1±0	>LOQ	2±0.4	1±0

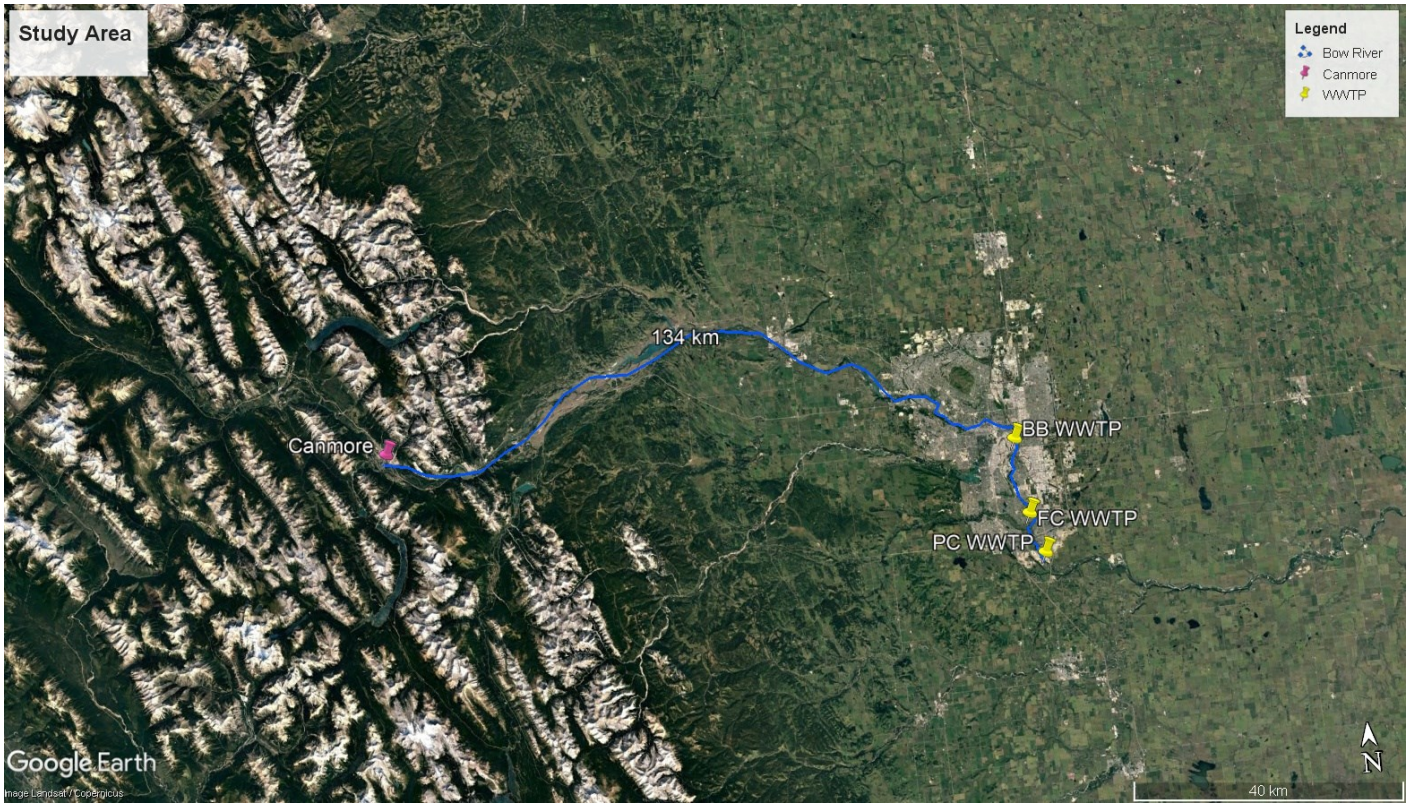

Figure S.2. *Study Area.* Approximate d*istance between Canmore site and PC WWTP.*

Table S. 10 *Monthly average concentration (ng/g_{dw}) with standard deviation for micropollutants in biofilm and gammarids during the Fall 2022 sampling campaign. dw=dry weight. LOQ = limit of quantification, ND= no detected, NC= no collected.*

Compound	Month	August			September			October		
	Site	BR	PC 5%	PC 15%	BR	PC 5%	PC 15%	BR	PC 5%	PC 15%
Trimethoprim	Biofilm	NC	NC	NC	1±0.1	2±1	2±0.3	1±0.3	2±0.3	10±0.5
	Gammarids	0.3±0.04	0.2±0.03	2±2	0.2±0.02	0.3±0.1	1±0.1	0.2±0.1	0.2±0.1	1±0.1
O-desmethylvenlafaxine	Biofilm	NC	NC	NC	12±5	33±5	43±2	13±2	28±14	70±5
	Gammarids	2±1	4±0.2	16±0.4	4±0.3	6±1	13±0.3	4±1	5±1	7±0.2
10,11 epoxide Carbamazepine	Biofilm	NC	NC	NC	<LOQ	<LOQ	<LOQ	0.2±0	<LOQ	0.2±0
	Gammarids	<LOQ	<LOQ	2±2	<LOQ	0.2±0	0.3±0	<LOQ	<LOQ	0.2±0
Venlafaxine	Biofilm	NC	NC	NC	13±8	28±5	45±5	16±2	25±11	48±3
	Gammarids	2±1	5±0.3	16±0.03	4±1	5±1	11±1	3±1	5±1	8±1
Carbamazepine	Biofilm	NC	NC	NC	0.3±0.1	0.5±0.1	0.5±0.1	0.2±0.02	0.4±0.1	1±0.03
	Gammarids	0.2±0.1	0.4±0.01	3±2	0.4±0.1	0.5±0.1	1±0.35	0.3±0.1	0.5±0.2	1±0.2
Fluoxetine	Biofilm	NC	NC	NC	28±8	74±3	152±21	31±10	65±29	244±16
	Gammarids	2±1	6±0.3	20±0.01	3±0.3	6±2	16±2	3±1	8±0.5	16±3
Triclocarban	Biofilm	NC	NC	NC	3±1	5±1	4±0.5	4±1	5±2	8±1
	Gammarids	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfamethoxazole	Biofilm	NC	NC	NC	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1±0.03
	Gammarids	ND	ND	ND	ND	ND	ND	ND	ND	ND
Norfluoxetine	Biofilm	NC	NC	NC	4±1	8±2	10±2	4±0.5	6±3	19±3
	Gammarids	1±0.5	2±0.3	5±2	2±0.2	2±0.5	3±1	1±0.3	2±0.2	4±0.1
Sulfamethazine	Biofilm	NC	NC	NC	ND	ND	ND	ND	ND	ND
	Gammarids	3±1	7±4	6±3	7±4	<LOQ	4±0	1±1	1±0	<LOQ