Sources, Transport, and Sediment-Water Distributions of Contaminants of Emerging Concern in a Mixed-Use Watershed

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David Joel Fairbairn

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Dr. Deborah L. Swackhamer, Advisor and Dr. Pamela J. Rice, Co-advisor

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Dedication

I dedicate this work to all of my family and all of my friends. You know who you are, and you each blend the best elements of both. As in life, there is no way this would have been possible without you. There has been much crying on your shoulders. Thankfully, there has also been a lot of laughing. Thank you so much for being who you are and for your support. I love you and hope that I return the favor every day.

This is especially dedicated to my sons, Owen and Leif. I hope that what I do will contribute in some small way to a continuation of the astounding natural beauty and health with which we've been blessed, that it shows you an example of a possible path for one interested in science, and that it has made me better able to care for and be an all-around good father to you. Thanks for putting up with it! You are amazing kids and kind young men. I love you both very much.

In biology, the concept of symbiosis really stuck with me. In philosophy, it was holism and causality. Thank you for including me as one of the links in your chain of being. I hope that I have been and will continue to be able to return the consideration.

Abstract

Understanding the sources and transport of contaminants of emerging concern (CECs) is crucial for risk assessment and mitigation. The goal of this research was to augment this knowledge by characterizing the spatiotemporal variability of a diverse set of CECs in a mixed land-use watershed. The study area (South Zumbro Watershed, Minnesota) presented a gradient of land uses that facilitated the investigation of agricultural and urban/residential sources and transport of CECs. Concentrations and loadings of CECs in water samples were analyzed in light of spatial, temporal, hydrologic, and physicochemical variables. Contaminant mass balances were analyzed to characterize the proportional sources and instream transport of CECs. Sediment-water distributions of CECs were assessed in terms of their magnitude, variability, and predictability.

Three distinct CEC groups emerged with respect to sources, transport, and seasonal/temporal patterns. The first group (i.e., atrazine, acetochlor, metolachlor, and daidzein) was characterized by agricultural/upstream-dominated sources and transport. These CECs were input primarily via upstream routes with loadings and concentrations that were greatest during high flows. For the second group (i.e., acetaminophen, trimethoprim, DEET, caffeine, cotinine, and mecoprop), a mix of wastewater and runoff transport was demonstrated by frequent detections in effluent and upstream samples, with peak loadings/concentrations associated with high flows and areas of greater population density/development. For the third group (i.e., sulfamethoxazole, carbamazepine, erythromycin, tylosin, carbaryl, and 4-nonylphenol), the detections, concentrations, and loadings were explained by effluent-dominated sources and transport. These CECs showed expected trends of stable loading across events, with the greatest concentrations and detection frequencies associated with low flows and the wastewater treatment plant.

Average measured sediment-water distributions exceeded equilibrium hydrophobic-based predictions for five of seven detected CECs by at least an order of magnitude. The consistency and predictability of the measured distributions improved with increasing CEC hydrophobicity and persistence.

Thus, spatiotemporal analysis can be used to characterize and track CEC sources and transport, even for ubiquitous CECs. These results augment existing

knowledge of CEC sources, fate, and transport by describing dominant sources, transport, and temporal patterns for different types of CECs. This will enhance monitoring, exposure/risk assessments, and management of CECs in surface water ecosystems.

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CHAPTER 1. Introduction

This research project focused on contaminants of emerging concern (CEC) in surface waters of the South Fork of the Zumbro River watershed in southeastern Minnesota. CECs are synthetic or natural, chemical or microbial contaminants that are not commonly monitored in the environment nor commonly subject to health-based water quality standards, but that have the potential to enter the environment and cause known or suspected adverse human or ecological effects [1-2]. Thus, CECs may be candidates for future regulation, depending on their environmental occurrence, ecotoxicity, potential health effects, and public perception [3].

Chemicals that have been identified as CECs include natural/synthetic hormones, pharmaceuticals and personal care products (PPCP), veterinary medicines, industrial/household products, pesticides, and many other chemical and physical agents. This equates to tens of thousands of individual chemicals [4]. Major inputs of CECs to surface waters stem from the use and disposal of compounds that benefit agriculture, industry, health, hygiene, commerce, and residences [5-8]. However, little work has characterized the contributions of individual sources to cumulative CEC loads and concentrations in surface waters. Thus, the major goal of this project was to augment this understanding by characterizing the sources and variability of CECs in surface waters in a mixed-use watershed. The objectives were to identify patterns of CEC occurrence, concentration, and loading, and to characterize the CEC profiles due to different sources and transport mechanisms over time in the study area. To that end, land use and seasonal influences were investigated through the spatiotemporal variability in concentrations and loadings of CECs in water samples through the two-year study. Also, the instream sources and transport of CECs were characterized with contaminant mass balances through different seasonal and hydrological conditions. The importance and predictability of the sediment phase was investigated for the CECs of interest. The results of these investigations will provide valuable information for researchers, regulators, and stakeholders engaged in understanding the sources, fate/transport, impacts, and environmental management of CECs.

Chapter 2 comprises a review of the literature and knowledge gaps related to the project goals and objectives. Chapter 3 provides detail on the project materials and methods. Each of Chapters 4-6 presents a research article that has been published or submitted for publication by a peer-reviewed journal as a part of this project.

Chapter 4, titled "Contaminants of Emerging Concern in a Mixed Land Use Watershed: A Two Year Study of Fluvial Occurrence and Spatiotemporal Variation", describes the occurrence and spatiotemporal variation of twenty-six CECs in water samples. The focus is on the effects of land use, season/hydrology, and CEC type on the spatiotemporal patterns of CEC concentrations and loadings.

Chapter 5, titled "Sediment-Water Distribution of Contaminants of Emerging Concern in a Mixed Use Watershed", describes the occurrence and distribution of fifteen CECs in water and sediment samples. The focus is on the predictability of the sediment-water distributions, and the effects of site, season, and physicochemical parameters on the concentrations and sediment-water distributions of the detected CECs.

Chapter 6, titled "Contaminants of Emerging Concern: Mass Balance and Comparison of Wastewater Effluent and Upstream Sources in a Mixed-Use Watershed", describes the application of contaminant mass balances to compare the proportional source loading and transport of CECs to an instream site. Results include characterizations of the CEC loadings from the Rochester Water Reclamation Plant and the upstream areas, the total loading in the watershed in various seasonal and hydrological conditions, and the common sources and patterns among CEC types.

Chapter 7 provides a summary and synthesis of the project. The References section presents a complete bibliography. The Appendix includes supporting data, tables, and figures.

CHAPTER 2. Literature Review

2.1 Introduction

Numerous reviews have been published on the sources, occurrences, fate, transport, and effects of CECs [6-7, 9-29]. Rather than provide an exhaustive review, the purpose of the current chapter is to illustrate the knowledge gaps related to the current work. First, an overview of relevant types, occurrences, sources, and fate processes of the CECs of interest is provided. Then, current knowledge and gaps related to spatiotemporal occurrence patterns, source characterizations, and *in situ* sediment-water distribution behavior of these CECs in mixed-use freshwater systems are discussed.

2.1.1 Overview of types, sources, occurrence, and transport of CECs

Commonly cited categories of CEC include [2]:

- Commercial/industrial chemicals (e.g., plasticizers, surfactants, and flame retardants)
- Human/veterinary pharmaceuticals (e.g., antibiotics, synthetic hormones, antidepressants, pain relievers, and other prescription/non-prescription drugs)
- Personal care products (e.g., antimicrobials, sunscreens, fragrances, and insect repellants)
- Food components (e.g., caffeine, artificial sweeteners)
- Pesticides (e.g., herbicides, insecticides, and fungicides)
- Naturally-occurring plant and animal hormones
- Legacy contaminants with newly recognized effects (e.g., polycyclic aromatic hydrocarbons and chlorinated paraffins)
- Other endocrine disrupting compounds (EDCs).

Numerous studies have reported CECs in surface waters in the United States [30-38] and globally [39-47]. One of the first large-scale U.S. studies targeted 139 wastewater-impacted streams in 30 states [30]. Organic contaminants were detected at 80% of these sites. In an early study in Minnesota, CECs were detected in more than

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90% of samples from 65 sites. Detections included 74 of the 91 targeted compounds, 13 of which were known EDCs [32]. Also, sediment can be a CEC repository. A Minnesota study of almost 400 miles of the Mississippi River detected 24 CECs in at least one water sample and 40 CECs in at least one sediment sample. Individual water and sediment samples contained up to 8 and 31 CECs, respectively [37].

The solubilities of many CECs allow aqueous transport. Depending on compound-specific physicochemical parameters, CECs may also associate with sediments [7, 48] or biota [49-50], or volatilize and undergo atmospheric transport [51-53]. Contaminants of emerging concern are transported to surface waters from agricultural areas [32]; municipal wastewater treatment plant (WWTP) discharges; urban/residential runoff and storm sewers [54-56]; industrial wastewater discharges [39, 57-61]; groundwater polluted with septic system, landfill, or feedlot waste [62-65]; and atmospheric deposition and drift [66]. Transport from WWTPs has been by far the most-studied, with other sources and transport pathways recently receiving greater attention. These diverse sources and pathways often result in complex mixtures of tens to hundreds of CECs being detected in surface waters in a wide range of settings [17, 25, 30, 32].

Despite numerous studies, significant gaps remain in our knowledge of the fate and transport [57, 60-62, 67-68], effects [57, 69-70], and mitigation potential of CECs in complex environmental systems [2, 10, 21, 47, 57-58, 60-62, 67-69, 71-74]. The sheer number of CECs that have been identified and the complexity of the associated natural systems pose serious challenges to addressing these gaps [24, 70, 75].

2.1.2 Overview of CEC effects

Several authors have reviewed CEC effects [6-7, 10-12, 18-19, 21, 76-78], which include endocrine disruption and associated impacts in aquatic systems [6-7, 79-81] and human populations [82], induction of antibiotic resistance [71, 83-87], and direct aquatic toxicity [6, 88]. Many CECs are known or suspected EDCs, so named because they have the capacity to mimic hormones, block receptors, affect neurotransmitters involved with hormone release, or otherwise disrupt endocrine signaling [7, 13, 24, 76]. Exposures to ng/L concentrations of EDCs have been linked to abnormal sexual development and reproduction in fish, amphibians, reptiles, birds, and mammals [2, 89-93]. Effects of endocrine disruption in aquatic species include immunosuppression [94],

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vitellogenin induction and feminization of male fish [92], altered gene expression [95], decreased testosterone [96], behavioral changes [97], and population crashes [98]. EDCs may cause adverse individual and population effects in humans (e.g., reduced sperm counts and increased prevalence of certain cancers) [80, 99-102]. Environmental occurrences of antibiotics have been associated with antibiotic resistance in soils and manure [84, 103-105], plant growth inhibition [106], and bioaccumulation and sub-acute effects in aquatic organisms [107].

Some CECs are known pollutants with newly recognized effects. Pesticides have demonstrated endocrine activity and other emerging effects in aquatic organisms and humans [100, 108-112]. Many CECs are lipophilic/hydrophobic, which can lead to their biomagnification. CECs often undergo significant attenuation in surface waters. Nevertheless, numerous continuous sources of CECs result in "pseudo-persistence" (persistent low-level concentrations that result from equivalent input and attenuation rates) [89, 113]. Thus, chronic low-dose exposures of aquatic organisms and humans to mixtures of CECs may be expected [89, 114-115].

2.2 Environmental fate processes

This section presents an overview of relevant fate processes for the CECs of interest to this study. The relevance of a particular process depends on the environmental context and CEC-specific properties.

2.2.1 Photolysis

Direct photolysis refers to the photoexcitation and subsequent molecular transformation of a molecule by sunlight. Indirect photolysis refers to the molecular transformation of a chemical by photoexcited reactive intermediates of co-occurring environmental constituents (e.g., dissolved organic matter, nitrate, and nitrite) [9]. Environmental factors that affect photolysis rates include latitude, season, time of day, cloud cover, shade, water depth, and turbidity [116]. Variable environmental conditions result in situation-dependent photolysis rates even for readily photodegradable CECs [117-118].

2.2.2 Biodegradation

Microbes can extract energy from organic chemicals via electron-transfer (redox) reactions or via metabolism and carbon assimilation. Biodegradability and biodegradation rates depend on compound-specific parameters and concentrations, microorganism communities and populations, and environmental factors such as temperature, pH, and oxygen and nutrient availability. Biodegradation is unimportant for many antibiotics, but can be significant for other CECs in surface waters [113, 119]. In general, biodegradation half-lives are longer in fluvial systems than in WWTPs because of smaller chemical concentrations, smaller bacterial population densities, and a less nutrient-rich growth medium [21, 120]. Thus, CEC loads that persist through a WWTP and are discharged in effluent will likely persist downstream for a period of time greater than the hydraulic retention time of the WWTP [121].

2.2.3 Sorption

Sorption to soils and sediments is one of the most important factors affecting CEC fate, transport, and bioavailability in natural systems [7, 122-123]. Generally, sediment concentrations of CECs are less likely than aqueous concentrations to show short-term variability due to changing temperatures, inputs, dilution, and fate processes including photodegradation and biodegradation [7, 9, 28, 124]. Thus, sediment contamination may indicate chronic pollution across broad time scales [125]. Sorption of hydrophobic organic contaminants (HOC) has been well-characterized, but relatively little information is available on sorption of hydrophilic and moderately hydrophobic contaminants [126].

2.2.4 Hydrolysis and dilution

Hydrolysis is the cleavage of a molecule through reaction with water. Generally, if an organic chemical is less polar than water, hydrolysis will be insignificant except when an acid or base catalyst is present. However, for highly soluble and polar molecules, hydrolysis can cause significant attenuation, especially as pH and temperature increase [127].

Aqueous dilution does not involve molecular or phase transformation, but can directly affect environmental concentrations. Dilution may also indirectly affect

concentrations by affecting concentration-dependent transformation rates (e.g., biodegradation) [78].

2.3 CECs of interest

This section provides an overview of the types of CECs that are of interest to the current study.

2.3.1 Pharmaceuticals and personal care products (PPCPs)

In general, many PPCPs exhibit at least moderate persistence (>12hr) in aquatic systems [117, 119, 124, 128-132], which suggests the need for environmental monitoring [124]. Photodegradation and sorption of PPCPs is typically more important that biodegradation or hydrolysis in aqueous systems [78, 113, 119, 129, 133-135].

2.3.1.1 Prescription human/veterinary pharmaceuticals

Over the last decade, several reviews have discussed the occurrence, fate, and transport of antibiotics and other human/veterinary pharmaceuticals in agricultural settings [28, 136-137], WWTPs [6, 16, 27, 138] and surface waters [7, 21, 73, 139]. Most pharmaceuticals administered for therapeutic, subtherapeutic, and other purposes to humans and livestock are only partially absorbed in the body. Consequently, large fractions of the administered dose are excreted unchanged in the urine and feces [21, 140] from where they may be transported to surface waters via WWTPs, septic systems, or runoff of land-applied manure and other biosolids [14, 84, 141-142]. The livestock industry accounts for the majority of total antibiotic use in many countries [15]. The most important inputs of veterinary pharmaceuticals to surface waters may occur by mobilization from land-applied manure by precipitation and from aquaculture operations [10]. Primary sources of human pharmaceuticals to surface waters are thought to be WWTPs, septic systems, and land-applied septage [57-58].

Many antibiotics are used both in medical and veterinary applications, while others are restricted to certain species. Pertaining to this study, sulfamethoxazole, trimethoprim, and erythromycin are approved for veterinary and human uses in the United States. Tylosin and monensin are only approved for veterinary use. Trenbolone is a potent synthetic androgen (and EDC) that is used for bovine growth promotion but is also used illegally as an anabolic steroid by humans. Trenbolone has been detected in

the environment at toxicologically-relevant concentrations [143]. Carbamazepine is a human anticonvulsant that is recalcitrant in the environment and is frequently detected in wastewater-influenced surface waters [144]. Often, veterinary antibiotics (e.g., tylosin) are detected in WWTP effluents [145-147] and human pharmaceuticals are detected in runoff from fields where wastewater has been applied [148]. These are a few examples of the mixed sources and transport pathways that exist in the environment for many CECs [145, 149].

In addition to endocrine disruption, potential effects of concern regarding antibiotics and other pharmaceuticals include antibiotic resistance, alteration to microbial communities, and bioaccumulation and sub-acute effects on aquatic organisms [10]. Indeed, elevated microbial resistance to macrolide, sulfonamide, and tetracycline antibiotics has been reported in concentrated animal feeding operation (CAFO) lagoons [84, 103-105]. These effects may pose health threats to aquatic and terrestrial communities and possibly human populations [10, 21, 104, 106, 150].

2.3.1.2 Personal care products

The umbrella term "personal care products" has been applied to many non-prescription CECs or their byproducts that are ingested (e.g., artificial sweeteners) or topically applied (e.g., fragrances, sunscreens) for heath, cosmetic, hygienic, or dietary purposes. Acetaminophen, caffeine, N,N-diethyl-meta-toluamide (DEET), and cotinine (a nicotine metabolite) occur at concentrations of nanograms to hundreds of nanograms per liter in a wide range of settings [59-60, 62, 151-153]. Caffeine has been reported as a potential stressor to algal and mussel populations [154-155] and as a marker of domestic wastewater contamination in surface waters [129]. Likewise, microbial or invertebrate impacts may be relevant for acetaminophen at environmentally relevant concentrations [156]. These PPCPs, like many other CECs, may contribute to deleterious effects in aquatic systems in the context of the mixtures in which they typically occur, although the concentrations of the individual CECs are not likely to be toxic [157-158].

2.3.2 Phytoestrogens and mycoestrogens

Phytoestrogens are naturally-occurring plant-derived compounds that are structurally or functionally similar to mammalian estrogens and their estrogenic

metabolites and have been shown to interfere with endocrine functions [23]. The dominant sources of phytoestrogens to surface waters are WWTPs, pulp mills, food processing plants, biodiesel plants, and land-applied manure [159-161]. Daidzein and genistein are produced by soybeans and other legumes such as clover [45]. Formononetin is produced primarily by red clover, but additional sources have also been suggested [162]. Equol is a byproduct of mammalian digestion of daidzein [45]. Zeranol is a potent mycoestrogen produced by crop fungi and is also used as a bovine growth promoter [162]. The low or undetectable concentrations of phyto/mycoestrogens reported in many studies suggests that they may not contribute significantly to environmental estrogenicity. Nonetheless, *in vitro* assays have shown that they may contribute substantially to estrogenic activity in sites that are more heavily contaminated with municipal wastewater or agricultural runoff [163]

2.3.3 Pesticides

Pesticides (e.g., herbicides, insecticides, and fungicides) are used in a variety of settings and are widely detected in the environment [24, 54, 110-111, 164-166]. Pesticides of interest to the current study may be subject to surface runoff/overland flow, volatilization, atmospheric transport and deposition, and groundwater transport [52, 66, 167-168], with half-lives in surface water and sediment from weeks to months [165, 169-174]. Although ecotoxicity varies by the type of compound, many pesticides have been associated with endocrine disruption or direct aquatic toxicity [110-111, 175]. Agricultural herbicides such as atrazine, acetochlor, and metolachlor are commonly detected in surface waters in many regions, including Minnesota [33, 54, 165-166, 176-177]. Runoff from residential, commercial, recreational, municipal, and other non-agricultural areas may contain significant loads of insecticides (e.g., carbaryl), fungicides (e.g., iprodione), and herbicides (e.g., mecoprop) that are used for turf/plant management and pest control [178-179]. Insecticides often pose greater threats than herbicides to aquatic life in urban areas [110-111]. Aquatic toxicity criteria for pesticides are exceeded in a majority of U.S. urban and agricultural streams [110-111].

2.4 Knowledge gaps and research needs

Despite their intensive use and ubiquitous environmental occurrences, knowledge of the sources, temporal variability, fate, transport, effects, and risks of many

CECs in the environment remains limited [9-10, 12, 21, 47, 60, 74, 78, 113, 180-181]. Environmental systems are complex, with numerous interrelated variables that often result in unpredictable behavior and confound the identification of existing patterns and drivers. Many field studies have included a limited number of samples and may thus provide little information on spatial and temporal variability of CECs and associated risks [182-183]. Limited numbers of sampling periods or sample sizes inhibit the identification of significant trends [184-185]. Many authors have cited the need for further investigation into the sources, occurrence, fate, and transport of CECs [73, 113, 186-188] to better understand and predict impacts in aquatic systems and human populations [189].

2.4.1 Environmental and public health effects of CECs

There are several lines of evidence that connect to suggest that environmental estrogenicity may be affecting human populations [190]:

- Laboratory studies have identified many EDCs, the exposures to which are associated with biological effects that are relevant to humans and wildlife
- Endocrine disruption due to EDCs has been observed in aquatic and terrestrial species and systems
- There is evidence of increased prevalence of endocrine-related disorders in humans.

Thus, it is inferred that environmental estrogens may be a cause of increased incidence of endocrine-related disorders in humans. Nonetheless, significant epidemiological gaps exist in the associations of exposure to CECs with human disease [2, 190]. Assessments are complicated by several factors, including [2, 190]:

- Only a small fraction of suspected EDCs have been investigated for effects at the organismal level
- Currently validated and widely used assessments capture only a limited range of known endocrine-related effects
- Humans and other organisms are typically exposed to mixtures of CECs that may exert synergistic or additive effects that may not be evident for the isolated CECs at similar concentrations
- Chronic effects of CECs may occur at far lower concentrations than threshold concentrations for acute toxicity

- Sensitivity to CEC exposure may vary by species or life stage (e.g., humans are more sensitive to EDCs during prenatal and puberty stages)
- Temporal gaps between exposures and effects of concern can span years or generations.

In general, exposure regimes of CECs in surface waters are poorly understood [189]. The existence of many diffuse and/or continual sources suggests that aquatic organisms and humans are chronically exposed to low-level mixtures of CECs [89, 114-115]. Traditional toxicity assessments are often insufficient to adequately characterize the effects of some CECs [191]. Nonetheless, the collective laboratory and epidemiological evidence suggests that fetal or pubescent exposure to EDCs may be associated with several recently observed changes to human populations, including early onset of puberty, reduced fertility rates, the increased prevalence of certain cancers (e.g., breast, childhood leukemia, prostate), and other physical or developmental problems such as ADHD, asthma, obesity, diabetes, or susceptibility to infections [190] .

2.4.2 Source characterization and spatial variation

Most source characterizations of CECs have focused on WWTPs. A multitude of CECs are present in WWTP influents as a result of residential, commercial, industrial, and medical uses [32]. Many of these CECs are not completely removed by WWTPs [27, 81, 181, 192]. Key factors determining WWTP removal efficiencies include WWTP processes, *in situ* conditions, and CEC size, shape, charge, and polarity [27, 81, 138, 193-196]. Thus, many studies have detected wastewater-associated CECs at greater frequencies and concentrations downstream than upstream of WWTPs [151, 177, 181, 197-198]. Proximity to a WWTP has been identified as the most influential factor on the detection frequencies and concentrations of some CECs [30, 60, 166]. Indeed, sewage effluents have been observed to be the prime contributor of many EDCs and other CECs to surface waters [199-200].

The release of CECs in agricultural areas results from activities including pesticide applications, livestock rearing, and land spreading of manure, septage, and other biosolids [57]. Subsequent CEC transport to surface waters may occur via precipitation runoff, tile drainage, volatilization, and other mechanisms [52, 66, 167-168].

The greatest concentrations, loads, and detection frequencies of herbicides are typically measured in agricultural areas [54, 175]. However, agricultural herbicides are also among the most frequently detected pesticides in urban areas [33, 54, 110-111, 176-177, 201-202].

Feedlots are increasingly being recognized as on a par with WWTPs in terms of their inputs of estrogens, antibiotics, and other CECs [203]. Indeed, surface water sites near CAFOs and aquaculture operations have exhibited greater CEC concentrations than nearby reference sites [204-207]. Veterinary use has been identified as the most important contributor of some mixed-use antibiotics to surface waters [77]. Nonetheless, agricultural sources of CECs (other than pesticides) have been less studied than WWTP sources [57, 60-61].

2.4.2.1 Source markers

Certain CECs have potential utility as markers for tracking contaminant sources [208] without the disadvantages common to some microbial markers (e.g., lack of specificity or sensitivity, short holding times, or expensive analysis) [209-211]. Ideal markers of contaminant sources are source-specific, rapidly/sensitively measurable, quantitatively reflective of contamination by the source, and released in detectable quantities to the environment [211-212].

Qualitative source markers can indicate the presence and possibly the magnitude of contamination from a given source. Quantitative markers allow source-specific contaminant or discharge loadings to be calculated based on the marker concentration or loading [129, 209, 213].

Hydrophilic markers are useful in tracking water-soluble contaminants in surface and groundwater [214]. Hydrophobic markers are useful in detecting particulate contamination of surface waters but may be retained in soil, which limits their utility in detecting groundwater contamination [211].

Conservative markers persist through different processes and compartments and can indicate the presence of a given source. However, conservative markers may persist through otherwise effective treatment processes. Thus, they may not distinguish between untreated and treated wastewater nor reflect pollution with labile contaminants. In contrast, labile markers can distinguish between untreated and treated wastewater sources. But, because labile markers may be naturally attenuated even in contaminated

areas, other more recalcitrant contaminants from the same source remain influential [213]. Thus, the simultaneous assessment of conservative and labile tracers provides complementary information on the influence of a given source, and the magnitude and extent of the associated contamination [208, 212, 215].

Carbamazepine, sucralose, and acesulfame have shown utility as conservative tracers of residential wastewater [27, 43, 208, 212, 216-219]. Likewise, caffeine and ibuprofen have been proposed as labile residential tracers [208, 220]. However, the ubiquity of caffeine poses specificity issues [152]. Other CECs that have shown promise as quantitative markers in surface waters include synthetic fragrances and antibiotics (WWTP effluent markers), total PAHs (urban/suburban runoff markers), ionophore antibiotics (CAFO markers), and animal steroids (markers of livestock, wildlife, and human waste) [43, 152, 208, 212, 215, 220-222]. Nonetheless, many of these proposed markers have shown disadvantages such as seasonal variations in biodegradability, photodegradability, varying or limited usage, or a tendency associate with solids [208, 212]. Additional research on appropriate CAFO markers is especially necessary [104, 220, 223].

2.4.2.2 Source comparisons

Quantitative characterization of CEC sources and transport is important for environmental monitoring, risk assessment, and mitigation [61-62, 69, 221, 223]. While we understand that myriad sources contribute CECs to surface waters, relatively little work has quantified point- and non-point sources of CECs in mixed-use areas [12, 61-62, 72]. Knowledge of these sources and their relative contributions is necessary for developing effective and efficient pollution prevention and mitigation strategies [209].

Efforts to characterize sources and transport can be complicated by the ubiquity, overlapping sources, and myriad transport routes of many CECs [166, 189]. For example, biosolids generated by WWTPs are often spread on agricultural fields from where CECs may migrate to surface or groundwater. Transport of CECs from urban/residential areas to surface waters via septic systems [64, 224-226] and stormwater conveyances [227-228] has been reported. Varying local WWTP processes, chemical usage, and other parameters linked to fate and transport can increase uncertainty.

Indeed, several studies have reported poor source differentiation or significant but undefined non-WWTP sources [60-62, 69, 151, 177, 198]. For example, acetaminophen, caffeine, DEET, and cotinine occur in a wide range of settings and are not exclusively associated with WWTPs [59-60, 62, 151-153, 185]. Acetaminophen was detected in CAFO effluents [229]. Lack of spatial trends in a mixed-use area was explained by diffuse PPCP sources such as septic systems and biosolids runoff [61]. Insignificant correlations of PPCP concentrations with flow in an urban watershed were explained by mixed sources such as WWTPs, septic systems, and landfills. The WWTP accounted for ~2% of the load of many CECs to the watershed [69].

Some studies have successfully characterized contaminant sources to surface waters via correlation of land use data with instream water chemistry [230-231], by the identification of source-specific marker compounds [209, 220], and by statistical relationships of land use data with CEC detections, concentrations, or loads in streams. For example, a non-parametric analysis showed that concentrations of sulfamethoxazole were greater at WWTP-influenced sites while concentrations of tylosin were greater at agricultural sites [232]. In another study, agricultural extent was significantly correlated with instream concentrations of phytoestrogens, mycotoxins, and pesticides, based on land use data developed from satellite imagery [162]. However, most comparisons of CEC sources have relied on instream concentrations or detection frequencies. Although useful, the information provided by source characterizations based on CEC concentrations without corollary loading data is limited [183]. A few studies have compared mass fluxes of CECs from different sources [61-62].

2.4.3 Temporal variation

Temporal variability of CEC concentrations and loads in surface waters results from a number of environmental (e.g., precipitation, discharge, temperature, or turbidity), source (e.g., usage patterns, distance from source), and physicochemical factors (e.g., potential for sorption or degradation) [41, 60, 181, 189, 233]. Indeed, several studies have reported significant temporal variation in CEC concentrations due to these factors at a given site [47, 60, 62, 184, 233]. However, compound- and condition-specific attenuation rates are sometimes not readily modeled or transferrable [39, 57, 62, 67-68, 197, 234]. Longitudinal studies have reported instream transport of a wide range of persistent and degradable CECs over kilometer-scale distances [187, 197, 235-236].

Continual loading is considered likely even in large streams when multiple sources exist [36, 43, 166, 187, 236-237].

Increased water concentrations and detection frequencies of agricultural herbicides and veterinary pharmaceuticals in spring and summer months were explained by increased seasonal usage and transport in precipitation-runoff from agricultural fields and CAFOs [61, 181, 233, 238]. Instream concentrations of pesticides are typically greatest after early growing season precipitation, and smaller during low flows and other times of year [166, 184, 233, 238-239].

Concentrations of wastewater-associated CECs have often been reported to increase in cold and low-flow conditions [62, 151]. This has been ascribed to smaller instream degradation rates and WWTP removal efficiencies during colder periods, reduced instream dilution of effluents in low flows, and greater pharmaceutical use in winter [47, 60-62, 151, 181, 189, 204, 218, 232].

However, several studies have reported negative or insignificant correlations of concentrations of PPCPs with flow, temperature, or precipitation [60, 62, 69, 151-152]. Likewise, inconsistent or insignificant seasonality of instream PPCP profiles has been reported [61, 233]. Measured instream half-lives of triclosan varied by a factor of 4-5 across studies, which was explained by variable photolysis rates due to varying turbidity, flow, and depth [240-241]. Increased instream concentrations of caffeine and other PPCPs in high-flow spring conditions were explained by reduced WWTP removal efficiencies due to reduced temperature and hydraulic retention times [35, 189]. Mobilization from land-applied biosolids was suggested as an explanation for greater concentrations and loads of some PPCPs in warm, high flow conditions compared to cold, low flow conditions [60].

2.4.4 Sorption to sediments in rivers

Sorption is a dynamic process that occurs primarily through hydrophobic interactions or attractive force and is related to the physicochemical properties of the sorbent and the sorbate [242]. Understanding the sediment-water distributions of CECs is important as these are directly related to the fate and bioavailability of CECs, the sediment's function as a CEC source or sink [243], the exposures of benthic organisms to CECs [78, 244-245], and the development of antimicrobial resistance in sediments [137, 236].

The octanol-water partition coefficient (K_{ow}) is a common parameter used to describe the hydrophobicity of a chemical, and can be measured in the laboratory or estimated from chemical structure. Hydrophobicity is largely related to the energy cost of cavity formation in water and to van der Waals interactions between sorbent and sorbate. Hydrophobic interactions are especially important for non-polar sorbates and sorbents with high organic and low mineral content [246]. For neutral HOCs, observed empirical relationships between the soil organic carbon-water partition coefficient (K_{oc}) and K_{ow} are often able to predict the overall solid-water distribution coefficient (K_d) within an order of magnitude [247]. This approach has also been attempted with a wide variety of chemical and soil types due to convenience and a lack of common, feasible alternatives [247]. However, several factors related to the chemistry of the sediment and the sorbate may affect the accuracy of these predictions [242]. Thus, this simple model is often inconsistent with the wide range of observed sorption phenomena [248]. Its output often represents a conservative or minimum estimate for the sorption of even hydrophobic organics [247].

Non-hydrophobic interactions between CECs and sediment can significantly affect sorption, especially for polar/ionizable compounds (log K_{ow}<3) when soil organic content is <2% [242]. Potential interactions include cation exchange, cation bridging, hydrogen-bonding, and mineral complexation with aluminosilicates, metal hydroxides, or ionizable components of soil organic matter [89, 249-251]. Clay content can be predictive of significant mineral contributions to sorption for molecules with polar moieties [252]. These "multiple sorptive components" typically show additive effects on total sorption [246, 248]. These unaccounted non-hydrophobic interactions often result in under-prediction of CEC sorption by K_{ow}-based models [28, 253-257].

Many laboratory and the few existing field studies have reported spatiotemporally variable sediment-water distributions of polar PPCPs that often deviated from predictions by an order of magnitude or more [89, 124, 126, 128, 186, 188, 221, 232, 236, 253, 255-256, 258-261]. This variation has been ascribed to non-hydrophobic interactions, hydrologic factors, chemical usage variations, and phase-dependent susceptibility to fate processes [28, 124, 126, 221, 232, 236]. In contrast, distributions of (less polar and more hydrophobic) atrazine, carbamazepine, and synthetic hormones were well-predicted by the HOC sorption model [124, 256, 259, 262-264].

Few studies have analyzed CECs in water and sediment samples from the same time and place [48, 188, 221, 232, 236]. Although non-hydrophobic mechanisms can be determinative of sorption for many CECs [246, 249, 265], a consistent and broadly applicable predictive approach has not been defined [122]. But, models are being developed [246, 257, 266]. Further laboratory and field studies are necessary to characterize the relative influences of hydrophobic and non-hydrophobic interactions on sorption for a range of CECs and instream conditions [246].

CHAPTER 3. Study Area and Methods

3.1 Study location – South Fork of the Zumbro River (SFZR) The South Zumbro Watershed encompasses 1202 km^2 in southern Olmsted and eastern Dodge Counties in southeastern Minnesota [267]. The study area (786 km^2, Figure 3-1) is drained by the South Fork of the Zumbro River (SFZR) and is delineated upstream by the boundaries of the South Zumbro Watershed. The downstream boundaries of the study area are defined by the subwatershed boundaries that drain to a point approximately 200 m downstream from the Rochester Water Reclamation Plant on the SFZR. This discrete drainage area is dominated by agriculture in the uplands and the City of Rochester in the lowlands. Seven subwatersheds with a gradient of land use allocations come to a confluence in the City of Rochester. This subwatershed configuration allowed us to compare instream water chemistry based on the particular agricultural and urban/residential land uses associated with different sampling sites and the wastewater treatment plant (WWTP).
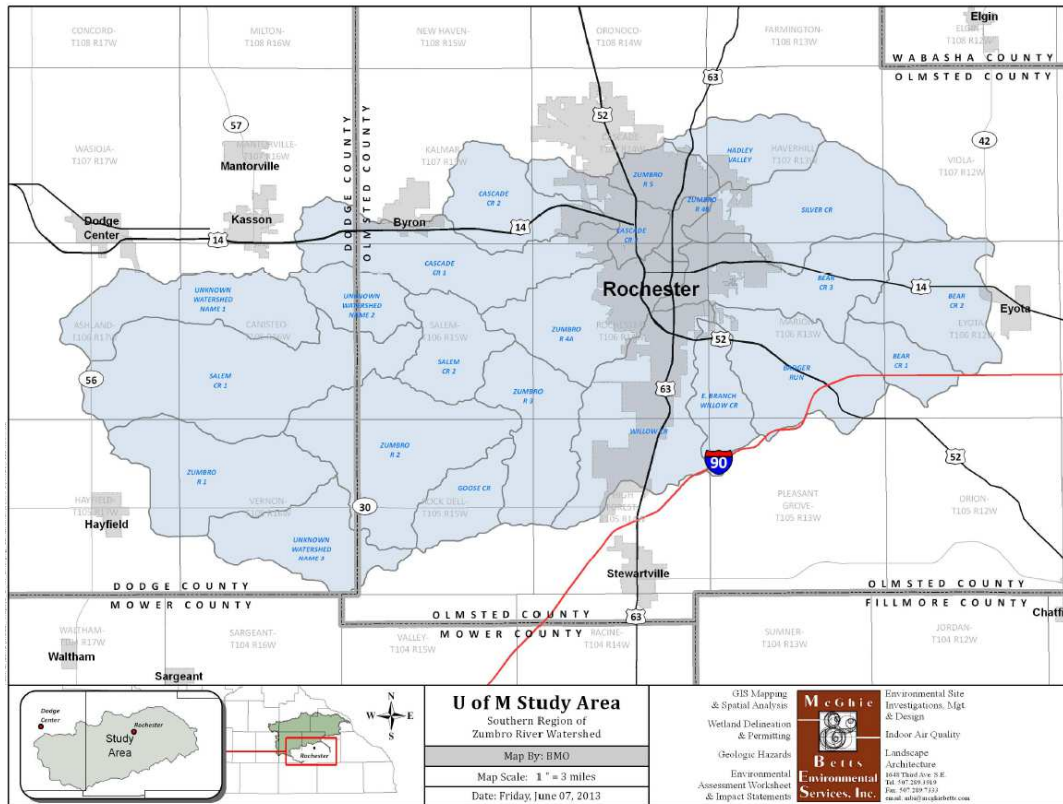
Figure 3-1. Map of the South Fork of the Zumbro River (SFZR) study area.

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3.1.1 Geography, geology, hydrology, and climate

This gently rolling area is part of the Rochester Plateau, which is characterized by loamy/silty loess in upland areas that overlays coarser glacial till. The Rochester area is characterized as a mature landscape dissected by a dendritic drainage network flowing to the SFZR. The SFZR is intersected by four first-order and one second-order stream in the study area. The surface elevation (above mean sea level) in the study area falls from approximately 415 meters in the headlands to 297 meters at the downstream boundary. Toward the east, drainage is characterized by sedimentary bedrock and karst features. Baseflow is contributed by aquifer-surficial interactions and agricultural drain tile. The hydrology of the area is highly modified and reflects rapid urbanization, suburban development, and agriculture. There are no natural lakes and over 80% of the pre-settlement wetlands have been drained [268]. The area receives approximately 84 cm of precipitation annually, with a mean temperature of 20.8°C in summer and -7.5°C in winter. The study area is in the interior climate region of North America characterized by cold winters and mild summers with greatly variable daily and weekly temperature extremes. The United States Geological Survey estimates that 25% of the annual precipitation infiltrates and recharges the groundwater aquifers [4].

3.1.2 Land uses

The SFZR study area is composed of approximately 64% agricultural area, 9.4% developed area, 19% grassland, and less than 10% woodland and surface water (Figure 3-2). Corn and soybeans are the dominant crops. There are 269 registered feedlots in the SFZR area that house approximately 212,000 total livestock. The number and types of animals in CAFOs varies by subwatershed and includes hogs, poultry, beef/dairy cattle, goats/sheep, and mink. In Minnesota, a large proportion of manure is land-applied in winter on frozen ground with less than 6% slopes [269]. In the spring (pre-planting) and fall (post-harvest), manure is injected or surface-applied. Some manure is spread in the summer following the harvest of vegetable crops.

The City of Rochester is an Urban Service Area measuring 142 km^2 with a total population 110,742 (43,226 households) [270] located in the valley at the confluence of the subwatersheds of this study. Rochester is home to the Mayo Clinic, a medical center that boasts more than 1500 staff, 2100 hospital beds, 300,000 annual patient visits, and 500,000 annual visitors. Near the downstream boundary of the study area, the

Rochester Water Reclamation Plant treats the wastewater of sewered Rochester customers with an activated sludge and chlorine disinfection system. The plant discharges an average of 49.2 million liters/day of treated effluent from a single outfall into the SFZR. Approximately 15,000 residents (5,400 households) use septic systems in the unsewered upland agricultural and suburban areas. The entire region relies on groundwater for the potable water supply, which is susceptible to contamination due to the karst geology.
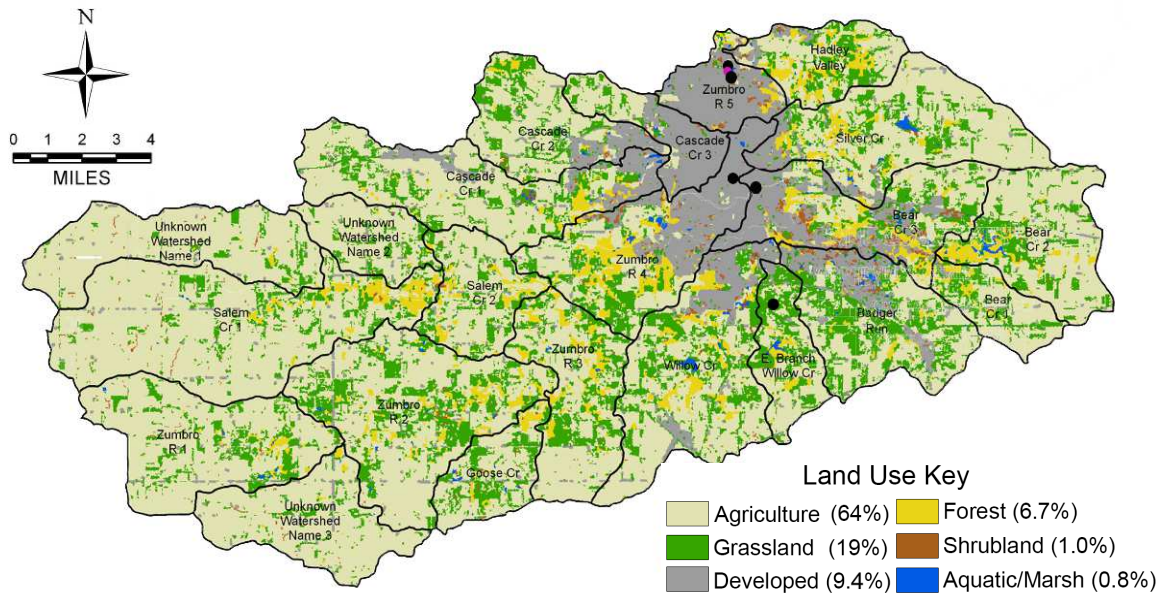
Figure 3-2. Map of land use allocation in the SFZR study area. Percentages in the key indicate the percentage of the total SFZR that is dedicated to that land use.

3.1.3 Sampling locations

To identify potential sampling sites, McGhie Betts, Inc. (Rochester) conducted a preliminary watershed analysis to estimate the land allocations and stream characteristics of the subwatersheds in the study area. Ten candidate sites were initially identified. From these, four primary sites (Figure 4-1) were selected to represent a range of land uses based on the percentages allocated to agricultural, urban/sewered, and suburban/septic system areas (Table A-1). Other factors considered in the decision-making included perennial stream flows and the presence of USGS or Minnesota DNR/MPCA stream monitoring network stations near the sites. Also, samples were occasionally collected from a fifth instream location 250 m upstream from the WWTP and from an effluent sampling point with the WWTP.

3.2 CECs of interest

CECs of interest to this study were selected based on reported occurrences, fate and transport, environmental effects, and associations with specific land uses. The primary goal was to select CECs that may serve to indicate the influences of cropland, different types of livestock, WWTP effluent, septic systems, and urban/residential runoff. Based on these priorities, twenty-six CECs were selected and analyzed in samples in this study (Table A-2).

3.3 Field methods

3.3.1 Sample types, schedule, and collection

Grab and automated surface water samples, passive water samples, and sediment samples were collected from the four primary sites in the SFZR watershed and analyzed for selected CECs. Multiple sample types were analyzed (a) to increase our ability to detect CECs that may not have been present in a single sample type, (b) to compare the utility of the sampling methods, and (c) to assess *in situ* sediment distributions of these CECs.

Samples were collected during five seasonal periods in both 2011 and 2012 and reflected a variety of seasons, flows, and terrestrial activities. Spring (March) sampling corresponded with snowmelt runoff. Early summer (May-June) sampling corresponded with the first predicted rainfall (>3cm) after >90% of cropland was planted [271]. Late summer (August-September), fall (October-November), and winter (January-February) sampling was conducted during low flows without predicted precipitation or snowmelt. Also, fall sampling was completed within a week of when 90% of corn had been harvested and land-spreading of manure was expected [271]. During low flows, it was expected that greater influences of WWTP and septic systems would be evident. During high flows, it was expected that the influences of urban or agricultural runoff and land-applied manure or biosolids would be evident. During most of the 2012 sampling events, grab water samples were collected from the fifth upstream site and from the effluent sampling location.

A sampling period consisted of either a single grab sampling event or a two-week deployment in which automated, passive, and grab samples were collected at each site. In winter and spring sampling periods, a single grab sample was collected from each site

due to equipment and safety constraints. In all other early summer, late summer, and fall periods, the two-week deployment strategy was employed.

3.3.1.1 Grab water samples

Grab water samples for CEC analyses were collected by submerging a cleaned container directly into the stream. A stainless steel keg or a four-liter amber glass bottle was used for collecting grab water samples at instream sites. Effluent samples were collected from within the WWTP in one-liter amber glass bottles. Grab water samples were collected on days 8 and 14 of the two-week deployment periods. Grab samples were also collected on one day in winter and spring in each year. Samples were sealed with Teflon-lined or stainless steel lids at the site, the security and accuracy of their labels was confirmed, and they were placed on ice in coolers for transport to the sample storage facility. High-density polyethylene containers were used to collect water samples for analysis of total suspended solids, nutrients, and other major ions and elements.

3.3.1.2 Grab sediment samples

Grab sediment samples were collected using a stainless steel scoop. Whenever possible, sediment was collected from multiple depositional areas across the width of the stream. In some cases, the depth of the stream or the presence of bedrock or riprap on the streambed limited the area from which sediments were collected. In these instances, sediment was collected from multiple areas, but these were grouped closer to the stream bank or were spread out along the length of the stream. Sediment was collected from the top 5cm of the streambed and deposited into a clean one-quart mason jar. Two of these sediment composite samples were collected at along with each grab water sample. All samples were sealed, their labels were secured and verified, and they were placed on ice in coolers for transport to the storage facility.

3.3.1.3 Automated samplers

Automated samplers (Teledyne-ISCO, Lincoln, NE) are capable of drawing a specific volume of water that can be paced by parameters such as time or streamflow when used with peripheral equipment. Automated water samples were collected through a Teflon-lined suction head and sampling hoses. Suction heads were secured four to six inches above the streambed with stainless steel posts or concrete blocks and wire. Ice was maintained in the samplers for the duration of the sampler deployments. Full sample

bottles were collected every two to three days from the samplers during sampling events. Following collection, samples were sealed with Teflon-lined lids, their labels were secured, and they were placed on ice in coolers for transport to the storage facility.

For this project, a time-based sampling protocol was established. Time-composited samples represent instream conditions as integrated over time. In this case, the automated sampling protocol specified that 170 mL of water was collected every 7 hours for the duration of each two-week deployment. The sample bottles had a 350 mL capacity. Thus, two 170 mL samples were collected in each bottle over 14 hours. The two-week deployment periods were each represented by 24 bottles. An equal portion from each of the first 12 bottles was composited to represent a "week 1" composite. Likewise, a "week 2" composite was generated from the last 12 bottles. Additional composite samples representing fractions of weeks (3.5 or 2.3 days) were generated when sample volumes allowed.

Some automated sampling errors occurred to instances of improper pump calibration, freezing of sample lines, power loss, damaged sample bottles, chewed sample lines (by wildlife), and other sampler malfunction. In these cases, the remaining bottles from the time period were used to generate the composite, if possible. For example, if one bottle was lost, then a weekly composite was generated from the 11 remaining bottles. In some cases, several sample bottles were missing and thus a given composite was not generated or included in the dataset. Each missing sample and adjustment to the compositing procedure was recorded in the sample processing log book.

3.3.1.4 Passive water samples

For this study, Polar Organic Contaminant Integrative Samplers (POCIS) samplers were used both to broaden the dataset and to assess their utility for routine CEC monitoring programs. These samplers have the potential for long-term deployment and low detection limits for many CECs [272]. In this project, a cage containing six POCIS samplers was deployed at each site during each two-week deployment. Three POCIS samplers were retrieved from each site after the first week. The remaining three POCIS samplers were retrieved at the end of the second week. The purpose of this strategy was to (a) distinguish week one from week two, and (b) to provide a safeguard in the event that one deployment duration was optimal in terms of contaminant uptake

rates or sampler durability. Following collection, samplers were wrapped in methanol-rinsed and dried aluminum foil, and placed in plastic bags on ice in coolers for transport to the storage facility.

Each POCIS sampler consisted of two semi-permeable membranes surrounding 0.200 g of Oasis HLB sorbent (Waters, Milford, MA). The membranes were sealed at the edges by two stainless steel rings fastened with stainless steel nuts and bolts. The POCIS sampler allowed water and dissolved contaminants to pass through the membranes and interact with the interior Oasis HLB sorbent. Following sampler collection, the POCIS were transported back to the laboratory and the sorbent was removed. The CECs that associated with the Oasis HLB sorbent during sampling were then eluted with solvents with procedures similar to those of the laboratory solid-phase extraction (SPE) methods used in this study. Time-integrated concentrations of CECs of interest were calculated based on the length of sampler deployment, the measured concentration of each CEC per mass of Oasis HLB sorbent, and published uptake rates for each CEC in POCIS samplers [272].

3.3.2 *In situ* data collection

In situ monitoring probes (YSI, Yellow Springs, OH, and Hach Hydrolab, Loveland, CO) were deployed at all stream sites to measure pH, temperature, dissolved oxygen, and conductivity. At Willow Creek, a Doppler-based velocity probe connected to a data logger (Teledyne-ISCO, Lincoln, NE) measured the flow velocity, which was multiplied by the cross-sectional area of the stream to yield instream discharge. These measurements were collected every 15 minutes during deployment. The instruments were cleaned, calibrated, and tested prior to each deployment according to the manufacturers' specifications. At the other stream sites, flow data were acquired from nearby USGS and Minnesota DNR/MPCA stream monitoring stations from agency web portals.

3.3.3 Additional field equipment and incidentals

Methanol (HPLC-grade), ultrapure water, Kim-wipes, nitrile gloves, coolers, and ice were transported to each field site. Deep-cycle marine batteries were used to power each automated sampler and probe setup. Samplers were chained and padlocked to

fences or other features. An all-terrain cart was used to transport equipment, coolers, and stainless steel kegs between the field vehicle and the riparian sampling locations.

3.3.4 Sampling equipment cleaning

A pre-deployment cleaning protocol was applied to all sampling containers and equipment. These items were sequentially washed with phosphorus-free soap and tap water, rinsed with tap water, rinsed with distilled water, triple rinsed with ultrapure water, allowed to dry, rinsed with methanol, allowed to dry, and heated to 400 °C for 3 hours. Items that were cleaned in this manner included all field and laboratory glassware, stainless steel POCIS rings, and other metallic items. For materials not amenable to being heated (e.g., Teflon-lined lids), the cleaning procedure was completed through the methanol rinse and drying steps. High-density polyethylene containers that were used to collect samples for TSS, nutrient, ion, and elemental analyses were washed with phosphorus-free soap and tap water, soaked for 2h in a 0.1 N HCl an acid bath, then rinsed once with distilled water and three times with ultrapure water. In addition to the pre-deployment cleaning procedures, all sampling equipment that was transported between sites was sequentially rinsed at each site with methanol, ultrapure water, and instream water from the site of interest prior to sample collection.

3.3.5 Sample handling, transport, and storage

Sterile gloves were worn by project personnel during all equipment deployment and sample collection of handling activities. This included cleaning and setup of sampling equipment, handling of sample containers prior to, during, following sample collection, and during all phases of sample processing and extraction. Staff followed procedures designed to maintain sample integrity, such as omitting the use and presence of tobacco, pharmaceuticals, personal care products, and caffeine before and during laboratory and field activities.

Following collection, samples were placed in coolers with ice at each site and transported to the storage location. Water samples for CEC analysis were stored in refrigerators at 4 °C. Grab water samples were processed within 72 hours of collection. Automated water samples were processed within 72 hours of collection of the last sample needed to generate the relevant composite sample. Sediment and POCIS samples were stored in sealed containers in freezers until processing. Water samples for

TSS analysis were kept at room temperature or frozen until analysis. Water samples for nutrient/ionic/elemental analysis were frozen until analysis.

3.3.6 Field quality assurance/quality control data collection

Field blanks were created, processed, and analyzed alongside actual samples in order to assess sample contamination that might occur during collection and transport. Field blanks consisted of ultrapure water that was transported from the laboratory in a glass container that had been cleaned and stored with the typical equipment cleaning procedures of this study. The container was opened at a sampling site, and the water was poured from one container to another cleaned container. Automated sampler blanks were collected on three occasions by rinsing and purging the automated sampler and its tubing with ultrapure water and then drawing ultrapure water into the sampler through the suction head to collect the blank sample. The containers containing blank samples were handled, stored, processed, and analyzed with procedures identical to grab water samples. Duplicate grab samples of water and sediment were collected, processed, and analyzed at a rate of one duplicate for every ten samples. All POCIS samples were collected, processed, and analyzed in triplicate for all sites and events.

3.4 Laboratory and analytical methods

3.4.1 Laboratory chemical standards and materials.

HPLC-grade solvents, formic acid, metazachlor (used as a surrogate standard), and CEC standards were purchased from Fisher Scientific (Waltham, MA) and Sigma Aldrich (St. Louis, MO). Additional internal standards (deuterated atrazine and deuterated mecoprop) and surrogate standards (deuterated ibuprofen and deuterated cotinine) were purchased from CDN Isotopes (Pointe-Claire, QC, Canada). Individual stock solutions (5-50 mg/L) were prepared in acetonitrile (ACN). These were mixed to create a stock solution of all CECs that was diluted to create a 6-point working standard curve. Ultrapure water was produced by a Milli-Q Advantage A10 system (EMD Millipore, Inc., Billerica, MA).

3.4.2 Water sample processing

Water samples (2 L grab, 1 L composite) were homogenized by inversion and shaking, filtered through 0.7 µm glass-fiber filters, spiked with a surrogate standard (metazachlor), and processed via SPE using an Autotrace 280 (Dionex, Sunnyvale, CA) equipped with Oasis HLB cartridges (6 cm^3, with 500 mg HLB sorbent) (Waters, Milford, MA).

For SPE, the Oasis HLB cartridges were sequentially conditioned with 3.0 mL of ACN/0.1% formic acid, 3.0 mL of ACN, and 3.0 mL of ultrapure water at a flow rate of 5.0 mL/minute. The filtered, weighed sample was then loaded onto an SPE cartridge at 5.0 mL/minute. The cartridge was then dried with nitrogen gas for 15 minutes. The cartridge was sequentially eluted with 6.0 mL each of ACN and ACN/0.1% formic acid at a flow rate of 5.0 mL/minute. The eluate was collected in a glass culture tube to which 400µL of ultrapure water was added. This extract was then evaporated under nitrogen to 400 +/- 10 µL. Following evaporation, the sample was reconstituted to 1.5mL with ACN and ultrapure water (1:1), transferred to an amber liquid chromatography vial, and stored at -18°C until analysis. All sample masses were recorded to 0.00x g prior to filtration, post-filtration, post-SPE, post-evaporation, post-reconstitution, and again during storage.

3.4.3 POCIS sample processing

In the laboratory, POCIS samplers were gently rinsed with ultrapure water to remove debris and dismantled. Then, the Oasis HLB sorbent was rinsed from the semi-permeable membranes with 6mL of ACN directly into an SPE cartridge equipped with a Teflon frit and a small amount of clean, ACN-rinsed and dried glass wool. The SPE cartridges containing the Oasis HLB sampling medium were placed on a vacuum SPE manifold with the syringes closed to soak the Oasis HLB sorbent in ACN for five minutes. The syringes were then opened and the solvent was eluted under vacuum into glass culture tubes. The syringes were then closed and 3 mL of acidified ACN (pH 3 with formic acid) was added to the SPE cartridge. After soaking again for five minutes, the syringes were opened and the acidified solvent was eluted under vacuum into the same glass culture tube as the initial eluate for that sample. The combined eluates were then spiked with metazachlor and evaporated under a gentle stream of nitrogen. All subsequent steps for processing, storage, and analysis of POCIS samples were identical to those of the water samples as described above. Following elution, the Oasis

HLB sorbent from each POCIS sampler was dried in the SPE cartridges under vacuum, and its dried mass was recorded.

3.4.4 Sediment sample processing

Sediment samples were thawed and homogenized by mixing the sample with a stainless steel scoop on methanol-rinsed and dried aluminum foil. Sediment samples were passed through a 2mm sieve to remove larger grains and debris. A subsample was then transferred to a 60 mL glass jar. The jars were placed with loosely sealed lids into a freeze-dryer until they were sufficiently dry, as indicated by a lack of condensate accumulation in the collection area of the freeze-dryer. The freeze-dried sediments were then homogenized, and a 10 g (dry weight) subsample was transferred to an accelerated solvent extraction (ASE) cylinder. The remaining freeze-dried samples were sealed and stored in a freezer as duplicates. Surrogate standards of metazachlor, deuterated ibuprofen, and deuterated cotinine were added to the subsample in the ASE cylinders and allowed to equilibrate overnight. Then, extraction of the samples was completed with a Dionex ASE 350 (Dionex, Sunnyvale, CA).

An optimization assessment was conducted to determine the ASE solvents, ASE settings, and clean-up steps for sediment processing. Based on ranked and absolute recoveries for all CECs, it was determined that the optimal ASE solvent was a 3:1 mixture of ACN and ultrapure water. The ASE parameters were as follows: 100 °C, 1600 psi, 3 static cycles of 5 minutes each, 100% purge volume, and 120s purge time. The ASE extracts (45-60 mL) were evaporated under nitrogen until the remaining solvent was <1% ACN (i.e., >99% ultrapure water) and were then diluted with 140 mL ultrapure water. The extracts were then cleaned and concentrated using SPE with Oasis HLB cartridges via the same procedures as applied to the water samples. The subsequent elution, processing, storage, and analysis procedures were performed according to the methods applied for the water samples.

3.4.5 Liquid chromatography tandem mass spectrometry (LC-MS/MS)

All liquid chromatography tandem mass spectrometry (LC-MS/MS) was conducted by Brian Barber (University of Minnesota, Department of Soil, Water, and Climate). Concentrations of the CECs were quantified on a Shimadzu (Kyoto, Japan) high performance liquid chromatograph (HPLC), with an Agilent (Santa Clara, CA) C8

2.1 x 150mm x 5µm film thickness Zorbax column and an Agilent Eclipse Plus C8 2.1 x 12.5mm x 5µm film thickness narrow bore guard column used for analyte separation. The HPLC was coupled to an Applied Biosystems (Carlsbad, CA) API 3200 triple quadrupole mass spectrometer using turbo spray in scheduled multiple reaction monitoring mode either positive or negative mode for compound identification. The column was maintained at 40° C. The mobile phase was a gradient of 80% water (0.1% formic acid) and 20% ACN (0.1% formic acid) to 80% ACN at 15 min, held at 80% ACN to 20 min, to 90% ACN at 24 min, with column re-equilibration to 20% ACN from 24 to 30 min. A flow rate of 0.2 mL/min was used for all runs. The sample injection volume was 50µL. Samples were maintained at 15 °C in the auto sampler to minimize decomposition. Tuning parameters were optimized for each analyte by direct infusion and are presented Table A-3.

3.4.6 Nutrient, major ion, and elemental analysis

During each grab sampling event, a separate grab sample was collected and submitted to the University of Minnesota Soil Testing Research Analytical Laboratory (St. Paul, MN) for analysis of total organic carbon, metals, nutrients, and other major ions.

3.5 Data quality assurance/quality control (QA/QC)

Laboratory, procedural, and matrix spikes, blanks, and duplicates were used to calculate analytical recoveries and precision, to assess losses due to sample processing, assess matrix effects, and to ensure that laboratory methods did not result in a loss of sample integrity. Laboratory spikes and blanks were included in all analytical runs. Procedural blanks were generated from ultrapure water and were analyzed at a rate of 1 for every 10 environmental samples. Procedural water blanks were typically subjected to each processing step starting with glass-fiber filtration through LC-MS/MS analysis. Procedural spikes consisting of ultrapure water spiked with CECs of interest were subjected to processing and analysis to quantify the effects of each processing step on the recoveries of individual CECs. Procedural sediment blanks and spikes were generated by heating Ottawa sand to 400 °C for 3 hours, allowing the sand to cool, and then spiking it with surrogate standards and (for procedural spikes) standards of CECs of interest. Sediment blanks and spikes were then processed with ASE and subsequent

procedures as described for environmental sediment samples. Matrix spikes of sediment and water were generated by duplicating extracted environmental samples, spiking one of the duplicates, analyzing both duplicates, and then subtracting the difference of the CEC masses measured in the spiked and unspiked duplicates to determine the spiked mass recovery. Duplicate analysis of water and sediment samples was conducted for 10% of all samples to assess analytical precision.

Concentrations of CECs in water and sediment samples were quantified using external calibration. The external calibration method accounts for CEC-specific recovery because the calibration curves are based on the analytical response of each CEC in laboratory spikes. Thus, additional correction against recovery of a given CEC in laboratory spikes was not necessary. Measurement precision of replicate analyses of environmental samples and spiked environmental samples was acceptable (RSDs and RPDs <25% for detected CECs). Linear regression coefficients of determination (r^2) were greater than 0.98 for all detected analyte calibration curves. Any anomalous surrogate or internal standard responses were investigated for possible sampling or measurement error.

The QA/QC policy was to flag and review data if a CEC was quantified above the MRL in a laboratory or procedural blank at a concentration greater than 20% of that for an environmental sample from the same dataset. If a CEC was quantified in a laboratory or procedural blank at a concentration greater than 50% of that for an environmental sample from the same dataset, the environmental sample datum would be reported as non-detected. No CEC detected in environmental water samples was quantified in equivalent procedural blanks above the MRL. Caffeine and trimethoprim were each detected in one field water blank above the MRL (4.1 ng/L and 2.8 ng/L, respectively). These concentrations were less than those quantified in environmental samples from the same event, and environmental data were not censored against these field blank detections. The calculated value of caffeine was censored (reported as non-detect) in one sediment sample due to the quantification of caffeine in a procedural sediment blank at a value within 50% of the environmental sample.

CHAPTER 4. Contaminants of Emerging Concern in a Mixed Land Use Watershed: A Two Year Study of Fluvial Occurrence and Spatiotemporal Variation

Abstract

The occurrence and spatiotemporal variation of twenty-six contaminants of emerging concern (CECs) were evaluated in water samples collected over two years in the Zumbro River watershed, Minnesota, U.S.A. Selected CECs included pharmaceuticals and personal care products (PPCPs), pesticides, veterinary pharmaceuticals, phytoestrogens, and commercial/industrial compounds. Detection frequencies of atrazine, metolachlor, acetaminophen, caffeine, DEET, and trimethoprim were greater than 70%. Concentrations and loadings of agricultural herbicides were greatest during early summer periods of increased use and precipitation, with concentrations typically greatest at sites upstream from the wastewater treatment plant (WWTP). Spatiotemporal patterns of concentration and loading of five PPCPs (acetaminophen, trimethoprim, DEET, caffeine, and cotinine) and an urban/residential herbicide (mecoprop) suggested a mix of wastewater and runoff transport that varied among CECs and season. The remaining PPCPs showed expected trends of stable year-round loading with the greatest concentrations during low-flow seasons and downstream of the WWTP. Spatiotemporal patterns for carbamazepine and 4-nonylphenol suggested smaller upstream sources along with the dominant WWTP source. Surface water studies of CECs often focus on areas near WWTPs. In this study, however, several CECs often characterized as effluent-associated occurred frequently upstream from the WWTP. Our results indicate that CEC monitoring studies should incorporate spatiotemporality of concentrations and loadings, especially when investigating CECs that may have mixed sources or transport. This knowledge can augment CEC monitoring programs to result in more accurate source and occurrence characterizations, and will ultimately enhance environmental decision-making.

4.1 Introduction

Over the past two decades, enhanced awareness of ecotoxicological issues and the refinement of analytical techniques have resulted in the identification and assessment of numerous contaminants of emerging concern (CEC) in freshwater

ecosystems. These CECs include, but are not limited to, natural and synthetic hormones, veterinary pharmaceuticals, pesticides, human pharmaceuticals and personal care products (PPCP), and other industrial/commercial compounds [6, 8]. Numerous agricultural, residential, commercial, and industrial sources contribute CECs to surface waters [8, 10, 166]. CECs are detected throughout the environment and biota worldwide, even in remote, "pristine" areas and in treated drinking water [144]. Several authors have reviewed potential CEC effects [6, 10, 19], which include endocrine disruption and associated biological fitness issues in aquatic systems [6] and human populations [82], induction of antibiotic resistance [10], and direct aquatic toxicity [6].

Despite recent advances, knowledge regarding the sources, fate, transport, and effects of many CECs in the environment is still limited [10, 47, 60, 70, 185, 197]. Concentrations differences among sites have been linked to varying land uses and the influences of wastewater treatment plants (WWTP) [8, 60, 69, 101, 166, 185, 198]. Longitudinal studies have reported instream transport of a wide range of persistent and labile CECs over kilometer-scale distances [197, 236]. Similarly, several studies have reported significant temporal trends in CEC concentrations at individual sites [47, 60, 62, 184, 189, 233]. Reports have explained increased concentrations of PPCPs in cold or low-flow conditions by reduced degradation, dilution, and/or increased use of certain compounds (e.g., antibiotics) at these times [61-62, 151]. Other researchers have explained elevated concentrations of some PPCPs in high-flow spring conditions by lower temperatures and hydraulic retention times that reduced removal efficiencies and increased inputs from WWTPs [189]. Finally, increased concentrations and detection frequencies of agricultural herbicides and veterinary pharmaceuticals in spring and summer have been explained by increased usage and runoff transport [61, 233, 238]. Overall, variation of CECs is dependent on physicochemical, societal, and/or environmental variables such as temperature, sunlight, precipitation, chemical use, source proximity, flow, dissolved oxygen, specific conductance, pH, solubility, sorption, photodegradability, biodegradability, and wastewater treatment processes [60, 185, 189, 233]. Environmental systems are complex, and these processes are not easily modeled or extrapolated from one site to another [39, 57, 62, 67-68, 197, 234]. Limited sampling periods or sample sizes also inhibit the identification of significant trends [183-185]. Thus, seasonality and associations of CEC concentrations with flow or temperature are

often unclear, insignificant, or inconsistent within or between study areas [60-62, 69, 151, 233].

A greater understanding of spatiotemporal patterns in CEC concentration and loading is therefore clearly important for characterizing CEC sources, fate/transport, and ultimately, risk [61, 185]. The objectives of this inland, freshwater study were to assess these patterns and their drivers for a diverse set of CECs in rivers of a mixed-use watershed. Samples were collected during all seasons for two years to facilitate trend detection. It was hypothesized that a range of spatiotemporal patterns of CEC occurrence, concentration, and loading would be evident due to environmental factors, compound characteristics, and land use. We expected that some CECs would be transported mainly from upstream areas during high flows (e.g., agricultural herbicides) or from the WWTP (e.g., some prescription pharmaceuticals), while we expected that others (e.g., mixed-use or ubiquitous PPCPs) would show mixed patterns that have heretofore been vaguely defined. Understanding these spatiotemporal patterns can aid the development of appropriate sampling regimes and knowledge of occurrences, sources, and risks of CECs.

4.2 Methods

4.2.1 Study area and CECs of interest

The study area (approximately 786 km^2) is part of the Zumbro River watershed (southeastern Minnesota, U.S.A.), and contains a gradient of land uses across its subwatersheds that provides a unique opportunity to study CEC sources. The City of Rochester lies in the downstream portion of the watershed. Rochester's WWTP serves approximately 110,000 residents and other commercial/industrial customers, including a medical complex that provides more than 2,100 beds and 225,000 annual outpatient visits. In the adjacent suburban and agricultural areas, approximately 15,000 people use onsite septic systems. Approximately 64% of the study area is agricultural and is comprised mainly of corn/soybean crops and an estimated 212,000 livestock.

Land use and hydrologic information was provided by McGhie Betts, Inc. (Rochester, MN) (detailed in Karpuzcu et al. [273]). Four sampling sites (Figure 4-1) were selected and categorized by land use allocations (Table A-1) for data analysis. Sites with >50% agricultural area were considered agricultural, sites with >5% developed area were considered to have significant urban/suburban/residential influences, and

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sites meeting both of these criteria were considered mixed-use [101]. By this strategy, Willow Creek and SFZR-US2 sites were agricultural, while Bear Creek and SFZR-DS were mixed-use sites. Human population densities were 4-5 times greater at Bear Creek and SFZR-DS than at Willow Creek and SFZR-US2. Bear Creek had the greatest density of septic systems among the study sites. SFZR-DS was the only WWTP-influenced site, and was approximately 250 m downstream of the WWTP outfall. In 2012, a fifth site (SFZR-US1), approximately 300 m upstream from the WWTP, was sampled to provide additional resolution between the WWTP and upstream areas for comparing CEC sources/transport at the downstream site (SFZR-DS).

Twenty-six urban/residential, agricultural, and mixed use CECs (Table A-2) were selected based on previous freshwater occurrences [8, 165], chemical usage patterns [165], potential effects [6, 10, 19], and associated land uses [8].

4.2.2 Sample collection, processing, and analysis

Grab water samples were collected during five seasonal periods in both 2011 and 2012 and reflected a variety of seasons, flows, and terrestrial activities. Spring (March) sampling corresponded with snowmelt runoff. Early summer (May-June) sampling corresponded with the first predicted rainfall (>3cm) after >90% of cropland was planted [271]. Late summer (August-September), fall (October-November), and winter (January-February) sampling occurred during low flows without predicted precipitation or snowmelt. Also, fall sampling occurred within a week of when 90% of corn was harvested and land-spreading of manure was expected [271].

Samples (2-L) were collected and refrigerated at 4°C until processing, which was initiated within 72 hours. Equipment cleaning and sample collection, handling, processing, and analysis were as previously described [273-274]. Briefly, water samples were filtered through 0.7 μm glass-fiber filters, spiked with a surrogate standard (metazachlor), and processed via solid phase extraction using Oasis HLB cartridges (Waters, Milford, MA, USA). Eluates were evaporated under nitrogen, reconstituted to 1:1 (v/v) acetonitrile/ultrapure water, and analyzed with high performance liquid chromatography tandem-mass spectrometry. Additional information regarding chemical standards, equipment, sampling, processing, extraction, and analysis is presented in the Tables A-2 and A-3.

4.2.2.1 Data quality assurance/quality control

Field and laboratory quality assurance/quality control (QA/QC) and external calibration procedures are described in [274]. QA/QC results are presented in Table A-3. The range of mean analytical recoveries for individual CEC was 53%-161% in laboratory spikes, 48%-149% in matrix water samples spiked before injection, and 35%-131% in matrix water samples spiked before SPE. For individual detected CECs, mean relative percent differences of (RPD) of duplicate analyses of environmental water samples ranged from 2.8%-25% (n=12) and for spiked water samples, relative standard deviations (RSD) ranged from 0.9-%-19% (n=4). Method reporting limits (MRL) were determined using U.S. EPA methods [275]. These MRLs were compared with results of a USGS method [276] and the minimum CEC mass that produced a signal-to-noise ratio of at least nine to ensure consistency across numerous analytical runs.

Laboratory blanks were included in all analytical runs. Procedural and field blanks were each generated and analyzed at a rate of 1 for every 10 environmental samples. The QA/QC policy was to flag and review data if a CEC was quantified above the MRL in a laboratory or procedural blank at a concentration greater than 20% of that for an environmental sample from the same dataset. If a CEC was quantified in a laboratory or procedural blank at a concentration greater than 50% of that for an environmental sample from the same dataset, the environmental sample datum would be reported as non-detected. No CEC detected in environmental samples was quantified in procedural blanks above the MRL. Caffeine and trimethoprim were each detected in one field blank above the MRL (4.1 ng/L and 2.8 ng/L, respectively). These concentrations were less than those quantified in environmental samples from the same event, and environmental data were not censored against these field blank detections.

4.2.2.2 Analytical method considerations

Concentrations of CECs in water samples were quantified using external calibration. The external calibration method accounted for CEC-specific recovery because the calibration curves are based on the analytical response of each CEC in laboratory spikes. Thus, additional correction against recovery of a given CEC in laboratory spikes was not necessary. Measurement precision of replicate analyses of environmental samples and spiked environmental samples was acceptable (RSDs and

RPDs <25% for detected CECs). Linear regression coefficients of determination (r^2) were greater than 0.98 for all detected analyte calibration curves.

Correction for surrogate recoveries can account for sample-specific issues that may arise during sample processing and analysis. While it is preferable to use the internal standard calibration approach and correct for surrogate standard recoveries in most instances, it was not feasible to include the number of standards that would be required to represent each of these CECs for these procedures. However, any anomalous surrogate or internal standard responses were investigated for possible sampling or measurement error.

The difference in recoveries between ultrapure water and matrix water spikes indicated that recoveries of most studied CECs were within an acceptable and expected range for a multi-residue method for diverse CECs (Table A-2). For most CECs in this study, corrected concentrations would be equal to or greater than the uncorrected concentrations, because recoveries were somewhat lower in matrix water than ultrapure water. Generally, the potential differences between calculated and actual concentration were equivalent to what is normally anticipated for measurement of organic compounds at part-per-trillion concentrations. For a given CEC, correction for matrix effects would apply the same correction factor to each calculated concentration. Although the range of detected concentrations could be affected, the differences in proportion and rank between among data points for a given CEC would not. The statistical methods used in this study compared the ranks of concentrations or loadings across seasons and sites for a given CEC. Thus, matrix correction would not affect the statistical outcomes. Given these alternatives, it was decided that a conservative approach would be to report the uncorrected concentrations. Because matrix correction was not conducted, the calculated CEC concentrations should be considered in light of the differences between recovery in ultrapure water and matrix water.

4.2.3 Statistical analysis

Statistical analysis was conducted using SPSS (IBM, Armonk, NY, USA) and Stata (StataCorp LP, College Station, TX, USA). Concentration and mass loading data were rounded to two significant digits prior to ranking and statistical analysis to reflect the calculated analytical precision. Categorical statistical analysis was utilized due to the number of sites (n=4). Multi-way analysis of variance (ANOVA) on ranks is a

nonparametric statistical method suitable for assessing the variation of a numerical dependent variable due to categorical independent variables when data are censored or do not meet the assumptions of parametric methods [277]. The main effects and interaction of season and site on the concentration and loading of each CEC were assessed with two-way ANOVA on the censored/ranked data (with α=0.05). When main effects were significant, the Protected Least Significant Difference procedure was used assess significant differences between individual seasons and sites. Significant interactions were included in the final ANOVA models and investigated with interaction plots. The influence of numerical independent variables was assessed using Spearman's rank-order correlation.

4.3 Result and discussion

4.3.1 CEC detection frequencies and concentrations

In total, CEC data for 68 grab water samples were analyzed for spatiotemporal patterns in this study. Concentrations of CECs measured in each grab sample and associated flow data are presented in Table A-4. Summary concentration data and detection frequencies of CECs are presented in Figure 4-2.

Generally, agricultural herbicides and non-prescription PPCPs were more commonly detected than prescription/veterinary pharmaceuticals, phytoestrogens, and mixed-use pesticides. The six most commonly detected CECs (detected in over 70% of grab samples) were the agricultural herbicides atrazine and metolachlor, the non-prescription PPCPs caffeine, acetaminophen, and DEET, and the prescription/veterinary pharmaceutical trimethoprim. Four CECs were detected in 35%-50% of samples (acetochlor, mecoprop, carbamazepine, and daidzein), four were detected in 20%-30% of samples (4-nonylphenol, cotinine, sulfamethoxazole, and erythromycin), and two were detected in 10%-20% of samples (tylosin and carbaryl). The remaining ten CECs (chlorpyrifos, formononetin, genistein, ibuprofen, iprodione, monensin, oxytetracycline, trenbolone, virginiamycin, and zeranol) were not detected in any sample. Some of the non-detected CECs showed greater analytical variability or weaker instrumental responses than the other CECs, which resulted in greater MRLs that may have affected their detection (*e.g.*, chlorpyrifos, monensin). For others, occurrence was not expected to be as widespread (*e.g.*, formononetin, virginiamycin). These issues were also evident for

some of the detected CECs. For example, 4-nonylphenol and erythromycin had higher MRLs (range of 10^2 ng/L) than most other detected CECs (range of 10^{-1} ng/L). Thus, the reduced detection frequencies of 4-nonylphenol and erythromycin compared to, e.g., caffeine, in this study could be due to their greater MRLs and do not necessarily indicate reduced environmental occurrence. The different MRLs should be considered when comparing the detection frequencies of different CECs.

4-Nonylphenol (commercial/industrial compound), erythromycin and sulfamethoxazole (prescription/veterinary pharmaceuticals) were not among the most frequently detected CECs, but were measured at maximum concentrations (10^3-10^4 ng/L) that were generally 1-2 orders of magnitude greater than other detected CECs. Atrazine, metolachlor, trimethoprim, caffeine, and DEET were frequently detected with median concentrations of 5-50 ng/L. Their maximum concentrations, and those of acetochlor and carbamazepine, were 110-440 ng/L. The remaining six detected CECs had median and maximum concentrations of <MRL-5 ng/L and 3-30 ng/L, respectively. Typically, the concentration range of a given CEC spanned about two orders of magnitude throughout the study.

4.3.2 Spatial and temporal patterns

Box plots, detection frequencies, and results of statistical comparisons are presented for site and seasonal concentrations (Figures 4-3 and 4-4) and loadings (Figures 4-5 and 4-6) of CECs. The five most-detected CECs (atrazine, metolachlor, acetaminophen, caffeine, and DEET) showed spatial and temporal ubiquity (>70% detection frequency for each site and seasonal period). When grouping all studied CECs (including CECs not detected in any sample), SFZR-DS had the greatest mean detection frequency (49%), followed by Bear Creek (29%), SFZR-US2 (24%), and Willow Creek (22%).

There was significant seasonality in concentration and/or loading of trimethoprim, acetaminophen, DEET, daidzein, and 4-nonylphenol, in addition to that expected for the herbicides. Also, concentrations of all but two CECs (atrazine and daidzein) showed significant spatial variation. Interactions of site and season on concentration were evident for trimethoprim, acetochlor, and carbaryl (Figure A-1).

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4.3.2.1 Wastewater-dominated CECs

4-Nonylphenol, carbaryl, and most of the prescription pharmaceuticals showed spatial and seasonal patterns that together demonstrate the dominance of wastewater inputs on their instream profiles, discussed in the following sections.

4.3.2.1.1 Spatial patterns – wastewater-dominated CECs

The greatest concentrations and loadings of all PPCPs and 4-nonylphenol occurred at SFZR-DS ($p < 0.05$, Figures 4-3 and 4-5). Several of these CECs were detected almost exclusively at SFZR-DS (4-nonylphenol, erythromycin, sulfamethoxazole, tylosin, and carbaryl). This may be due to the WWTP influence but could also reflect additional upstream sources, given the location of SFZR-DS at the mouth of the study area (Figure 4-1). Water samples collected at SFZR-US1 (350 m upstream from the WWTP) allowed us to distinguish these influences. Sulfamethoxazole, erythromycin, and tylosin were each detected in more than 70% of SFZR-DS samples, no SFZR-US1 samples, and only a single sample from any other upstream site (Table A-4). Thus, WWTP effluent is the dominant source of these antibiotics to SFZR-DS. Likewise, most of the 4-nonylphenol and carbaryl detections were at SFZR-DS (respective detection frequencies were 88% and 29% at SFZR-DS). Meanwhile, they were infrequently detected upstream (8% and 4%, respectively, of 51 total samples from upstream sites). Carbamazepine was frequently detected at SFZR-DS and Bear Creek, although the concentrations were much greater at SFZR-DS. This suggests that additional smaller upstream sources such as septic systems or land-applied biosolids were more influential for carbamazepine than for the other effluent-dominated CECs discussed in this section.

4.3.2.1.2 Temporal patterns – wastewater-dominated CECs

Concentrations of wastewater-derived CECs (e.g., prescription pharmaceuticals and 4-nonylphenol) were expected to increase in low-flow seasons due to increased pharmaceutical use, reduced instream flows, and/or reduced degradation rates. No prescription pharmaceuticals, however, showed seasonality in concentration ($p > 0.20$ for each, Figure 4-4). Trends of smaller concentrations of carbamazepine during spring and early summer are consistent with predicted dilution.

Tylosin (a veterinary antibiotic) was expected to associate with agricultural runoff and perhaps pet waste. Nonetheless, 93% of tylosin detections were at SFZR-DS and the greatest seasonal detection frequency was in fall. Indeed, tylosin has been detected in WWTP influents and effluents in the United States [145] and elsewhere [146-147], which suggests the influences of veterinary clinics, pet owners, or runoff and infiltration from CAFOs on the WWTP [145].

4-nonylphenol had significantly increased concentrations and loading in spring and late summer, with concentrations also increased in fall (Figures 4-4 and 4-6). Although typically wastewater-associated, 4-nonylphenol is also used in pesticide formulations and may be found in agricultural areas [278]. This may explain its occasional detections and high concentrations at agricultural sites and during spring sampling. The seasonality in 4-nonylphenol concentration and loading could have resulted from occasional runoff transport related to agricultural use or from biotransformation of nonylphenol ethoxylates to nonylphenol in WWTPs, which should occur more rapidly in warmer seasons [26].

Carbaryl is an insecticide approved for some indoor and outdoor mixed-use settings in the U.S. Its infrequent detection and low concentrations in this study agreed with expectations based on its smaller annual usage and more limited geographic application compared to the studied agricultural herbicides [279]. Carbaryl appeared to show trends of greater concentration and loading in early summer and at SFZR-DS (Figures 4-3 to 4-6), as demonstrated by the significant interaction (<0.001) between site and season. The interaction plot (Figure A-1) shows that the greatest carbaryl concentrations occurred at SFZR-DS in early summer and winter followed by Bear Creek in spring and fall. This suggests the influence of urban/residential sources that may stem from effluent and other seasonally variable transport routes.

4.3.2.2 Mixed-transport CECs

In contrast to the CECs discussed in Section 3.2.1, spatiotemporal concentration and loading profiles of acetaminophen, DEET, caffeine, cotinine, trimethoprim (PPCPs) and mecoprop (urban/residential herbicide) suggest the influence of runoff/stormwater or septic system inputs in addition to wastewater effluent inputs. These CECs were frequently detected at upstream sites and (except caffeine) showed temporal patterns of greater concentration and loading in high-flow periods (Figures 4-4 and 4-6). Also, their

concentrations and loading were greatest at SFZR-DS, but the differences between SFZR-DS and the other sites were not as extreme as were those of the effluent-dominated CECs discussed in section 3.2.1 (Figures 4-3 and 4-5).

4.3.2.2.1 Spatial patterns - mixed-transport CECs

Acetaminophen, caffeine, and DEET were each detected in more than 80% of samples from each site. Trimethoprim was detected in more than 50% of samples from each site. The median concentrations of each of these CECs varied by less than a factor of five across sites (Figure 4-3), and median loadings at SFZR-US2 and Bear Creek were within an order of magnitude of those at SFZR-DS (Figure 4-5). Likewise, cotinine concentrations were greatest at SFZR-DS, but cotinine was also detected in 18% of samples at Bear Creek as opposed to 6% of samples at Willow Creek and SFZR-US2. These diffuse spatial patterns suggest non-WWTP sources and pathways such as septic systems or runoff of land-applied biosolids or manure in addition to a wastewater source.

Similarly, mecoprop concentrations were highest at SFZR-DS and second-highest at Bear Creek compared to the agricultural sites (Figure 4-3). The U.S. EPA estimated that >97% of annual mecoprop use in the U.S. is on residential lawns [280]. This agrees with the expected transport of this herbicide in runoff or stormwater from urban/residential areas.

4.3.2.2.2 Temporal patterns – mixed-transport CECs

The ubiquity and pseudo-persistence of acetaminophen, caffeine, and DEET was demonstrated by high detection frequencies (>65%) in all seasons in this study. However, like the agricultural herbicides, acetaminophen and DEET also appear influenced by runoff in this study (*i.e.*, greater concentrations and loadings in spring and/or early summer, Figures 4-4 and 4-6). Acetaminophen concentrations and loadings were greatest in spring and early summer (p<0.001), and acetaminophen concentrations were correlated with flow ($\rho=0.380$, p=0.001). Although DEET showed insignificant concentration seasonality, upward trends were evident in early and late summer when usage was expected to be greatest. DEET loadings showed a corresponding early summer peak (p=0.002). Mecoprop showed its greatest concentrations and loadings in early summer (p<0.01, Figures 4-4 and 4-6), consistent with its outdoor applications.

Trimethoprim concentrations were greatest in late summer and fall compared to all other seasons, but the greatest individual concentrations occurred in early summer (Figure 4-4). Interactions of site and season (Figure A-1) indicated that concentrations at upstream sites were greatest in late summer and fall, while at SFZR-DS they were greatest in early summer and winter. The greater concentrations at upstream sites in late summer and fall could be due to terrestrial sources such as manure or land-applied biosolids, while the concentration patterns at SFZR-DS could be due to variable WWTP or upstream inputs. Likewise, loadings of trimethoprim were greater in early summer, late summer, and fall compared to winter and spring ($p=0.001$, Figure 4-6).

4.3.2.3 Runoff-dominated CECs

Agricultural herbicides, veterinary pharmaceuticals, and phytoestrogens were expected to show greater occurrences, concentrations, and loadings from agricultural sites and during periods of increased application and precipitation. Patterns of the agricultural herbicides generally corresponded to expectations, as discussed in the following sections.

4.3.2.3.1 Spatial patterns – runoff-dominated CECs

Runoff influences were expected to be evident in greater concentrations and loadings of agricultural CECs at agricultural sites compared to mixed-use sites. Spatial patterns of the herbicides supported this expectation, although the associations were not as obvious as those of urban/residential or mixed-use CECs and the mixed-use sites. Concentrations of all agricultural herbicides varied spatially ($p<0.05$, Figure 4-3). Atrazine concentrations were greater at (agricultural) SFZR-US2 than SFZR-DS ($p=0.007$), but metolachlor concentrations were greatest at (mixed-use) Bear Creek ($p<0.001$). Concentrations of acetochlor were elevated at Bear Creek and SFZR-DS compared to Willow Creek, but not compared to SFZR-US2 (Figure 4-3). Also, detection frequencies and median loadings of the agricultural herbicides showed smaller ranges across sites than did many of the urban/residential or mixed-use CECs (Figures 4-3 and 4-5). These patterns accord with the >50% agricultural area at each site and demonstrate the influence of upstream sources of these CECs in the watershed. Daidzein was also regular in its spatial distribution (35%-42% detection among sites and insignificant concentration and loading variation). This agrees with associations of

phytoestrogens with agricultural and industrial-commercial activities (*e.g.*, food processing) [281].

4.3.2.3.2 Temporal patterns – runoff-dominated CECs

Concentrations and loadings of acetochlor, atrazine, and metolachlor varied seasonally ($p < 0.05$, Figures 4-4 and 4-6), peaking in early summer. These patterns agree with the expectation that these agricultural herbicides would be transported in precipitation runoff following pre-emergent crop applications. Daidzein showed a seasonal pattern somewhat similar to the agricultural herbicides, with its greatest concentrations in spring, early summer, and winter (Figures 4-4 and 4-6).

In early summer, acetochlor concentrations, loadings, and detection frequencies were much greater than in late summer and fall when it was infrequently detected (Figures 4-4 and 4-6). In contrast, atrazine and metolachlor were frequently detected in all seasons at median concentrations and loadings within an order of magnitude of the early summer peaks. This could be due to the lower persistence of acetochlor compared to atrazine and metolachlor [169, 171]. Acetochlor is also approved for some post-harvest applications in Minnesota. Concentrations and loadings of acetochlor were greater in winter and spring compared to late summer, which may be due to transport in snowmelt runoff associated with post-harvest applications or atmospheric deposition [282].

4.3.3 Research synopsis

Atrazine, acetochlor, and metolachlor are commonly detected in many areas at concentrations similar to this study [165-166]. The greatest agricultural herbicide concentrations in streams are typically reported when precipitation follows their application during the growing season [166, 184, 233, 238-239]. Similar to this study, concentrations of agricultural herbicides are often an order of magnitude greater than many PPCPs [233]. Certain PPCPs (*e.g.*, some antibiotics, carbamazepine) and 4-nonylphenol, however, frequently exceed herbicide concentrations, occurring at up to several µg/L near WWTPs [47], as observed in this study. Although erythromycin and sulfamethoxazole are approved for human and veterinary use, our results agree with reports that WWTP effluents are a greater source of these antibiotics to surface waters [144, 283]. The greater instream persistence of trimethoprim compared to (typically co-

prescribed) sulfamethoxazole may result in longer-range trimethoprim transport [41]. In this study, the greater persistence of carbamazepine [284] and trimethoprim [41] is evident in their more frequent detection across sites and seasons compared to sulfamethoxazole and erythromycin.

In the current study, all PPCPs were detected most frequently and with the greatest concentrations and loadings downstream from the WWTP. Some PPCPs (e.g., acetaminophen, trimethoprim, caffeine, DEET) were also influenced by upstream sources. Caffeine, acetaminophen, and DEET have been detected ubiquitously in surface water, independently of WWTP influences [60, 62, 151-152]. Lack of spatial trends in one U.S. study was explained by diffuse PPCP sources such as septic systems and land-applied biosolids [61]. Insignificant correlations of PPCP concentrations with flow were explained by mixed sources, with WWTPs accounting for only ~2% of the annual load of many CECs in an urban watershed [69]. Other studies, however, have identified WWTP proximity as the primary influence on detection frequencies and concentrations of prescription pharmaceuticals [8, 60, 166, 185].

Seasonality and associations of PPCP concentrations with flow or temperature are often unclear, insignificant, or inconsistent within or between study areas [60-62, 69, 151, 233]. For example, variations of acetaminophen, carbamazepine, DEET, and caffeine concentrations due to precipitation were statistically insignificant [60, 152]. In a year-round study, however, caffeine and sulfamethoxazole concentrations increased in spring [189]. DEET concentrations have been variously reported to increase in the summer when usage is greater [61] and to show a lack of seasonality similar to wastewater-derived PPCPs such as caffeine and cotinine [235]. In contrast, many reports confirm predictions that PPCP concentrations would be enhanced during dry or cold seasons due to reduced dilution and/or degradation [47, 60, 62, 151]. Carbamazepine has shown stable instream and effluent concentrations and loads with little year-round variation due to seasonality or fate processes [185, 189, 219].

The CEC groups that were identified by spatiotemporal analysis in this study agreed with a principal component analysis completed in this area in which occurrences of some CECs were well-explained by effluent-sources (e.g., sulfamethoxazole, carbamazepine) or agricultural sources (e.g., atrazine, acetochlor), while others showed mixed patterns (e.g., acetaminophen, caffeine) [273].

4.3.4 Implications for CEC monitoring

It is necessary to characterize the spatiotemporal variability of different CECs to understand their sources and impacts and to inform monitoring and mitigation efforts. Otherwise, incorrect patterns may be identified. The importance of using appropriate sampling regimes to assess diverse CECs is highlighted by the different spatiotemporal patterns of CEC loadings and concentrations observed in this study. Several CECs identified as having mixed sources or transport routes did not correspond to typically understood patterns of effluent-dominated or runoff-dominated CECs.

The limited seasonality of many of the effluent-dominated CECs in this study may reflect consistent seasonal use and inputs to surface waters. On the other hand, it may also suggest the need for alternative sampling regimes to detect trends. Detection frequencies of most of the prescription/veterinary pharmaceuticals were among the lowest of studied CECs, which may have affected trend analysis. Also, most of their detections were at SFZR-DS. If WWTP effluent was indeed their primary source, then the proximity of SFZR-DS (approximately 200 m downstream) to the WWTP outfall would have allowed a short time for instream attenuation before sample collection. This may have reduced the influence of seasonally variable fate processes on their concentrations and loading compared to what might have been observed further downstream.

4-Nonylphenol did show seasonality in loading and concentration. Likewise, the greatest detection frequencies and median concentrations of the other effluent-dominated CECs typically occurred during low-flow seasons. This may reflect the influence of concentration-dilution processes, seasonal variation in WWTP removal efficiencies, or, less likely, instream fate processes.

Our results indicate that seasonal concentration and loading assessments of CECs should be incorporated into monitoring, especially for runoff-associated pesticides and mixed-transport PPCPs such as acetaminophen, DEET, and trimethoprim. Likewise, although seasonality was not as evident for other prescription and non-prescription PPCPs, we observed evidence of concentration-dilution effects and (for specific CECs such as carbamazepine and 4-nonylphenol) additional upstream sources. Loadings and concentrations of many of the mixed-transport CECs were within an order of magnitude at upstream sites compared to the WWTP site. This suggests that pollution prevention

efforts that target these sources in addition to WWTPs may be needed if mitigation of these CECs is desired.

Periods of increased loads or concentrations may result in increased environmental or ecological health risks. For CEC programs assessing peak concentrations or loading, runoff-associated CECs should be monitored during high-flow periods when the greatest concentrations and loadings will occur. However, concentrations of effluent-associated CECs are likely to be reduced at these times. They should be measured during low flows if instream exposures are of concern. Monitoring at watershed collection points is suitable to survey the loading and concentrations of effluent- and mixed-transport CECs. Upstream monitoring is advisable if concentrations of runoff-associated CECs are of interest, or if downstream surveys suggest that upstream sources of mixed or urban/residential CECs may be influential in addition to WWTPs.

The flow monitoring data and grab sampling methods utilized in this study are commonly available to water monitoring programs. The source and transport comparisons were strengthened by robust CEC, drainage area, land use, and streamflow data. These types of data should be employed in concert with concentration data to characterize CEC sources and transport and to measure the effectiveness of mitigation efforts. Loading and temporal analysis can assist in identifying the influential sources, pathways, and periods on instream CEC profiles.

This study focused on hydrophilic or moderately hydrophobic CECs (generally with log K_{ow}<4). Several of these CECs were frequently detected in sediments in this area [274]. Therefore, sediment monitoring may be considered alongside water monitoring if mass balances, sediment as a source/sink, or risk to benthic organisms are of interest. Similarly, for CECs with higher log K_{ow} values, alternate or additional matrices (e.g., sediments, organisms, terrestrial matrices) should be considered for occurrence, fate, and risk assessments.

Many surface water studies of CECs have focused on areas near WWTPs. However, many urban/residential and mixed-use CECs occurred frequently at sites not influenced by a WWTP at concentrations that varied with land use. Incorporating this understanding of CEC occurrence variability into monitoring programs can result in more accurate source and transport characterization to inform environmental decision-making.

46
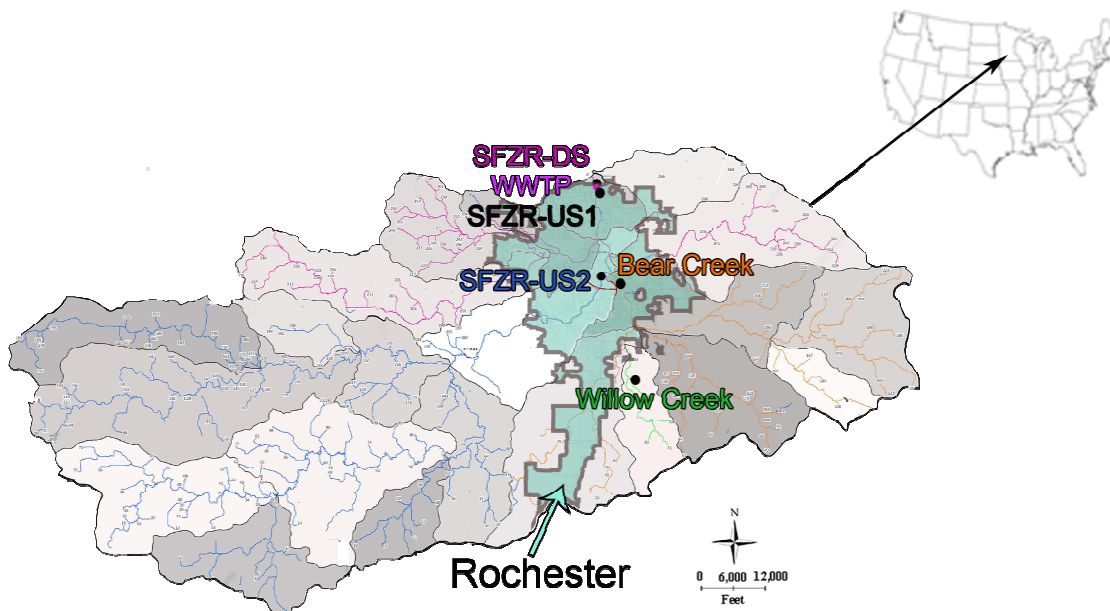
Figure 4-1. Map of the SFZR study area. Labels indicate sampling site locations. Streams that influence each sampling site are color coded – Willow Creek = Green, Bear Creek = Orange + Green, SFZR-US2 = Blue, and SFZR-DS = All colored streams. The city of Rochester is indicated by the green shaded area. The SFZR flow is generally northward from the southwest. Willow Creek flows into Bear Creek, which flows into the SFZR just downstream of SFZR-US2, which then flows toward the SFZR-DS site.

47
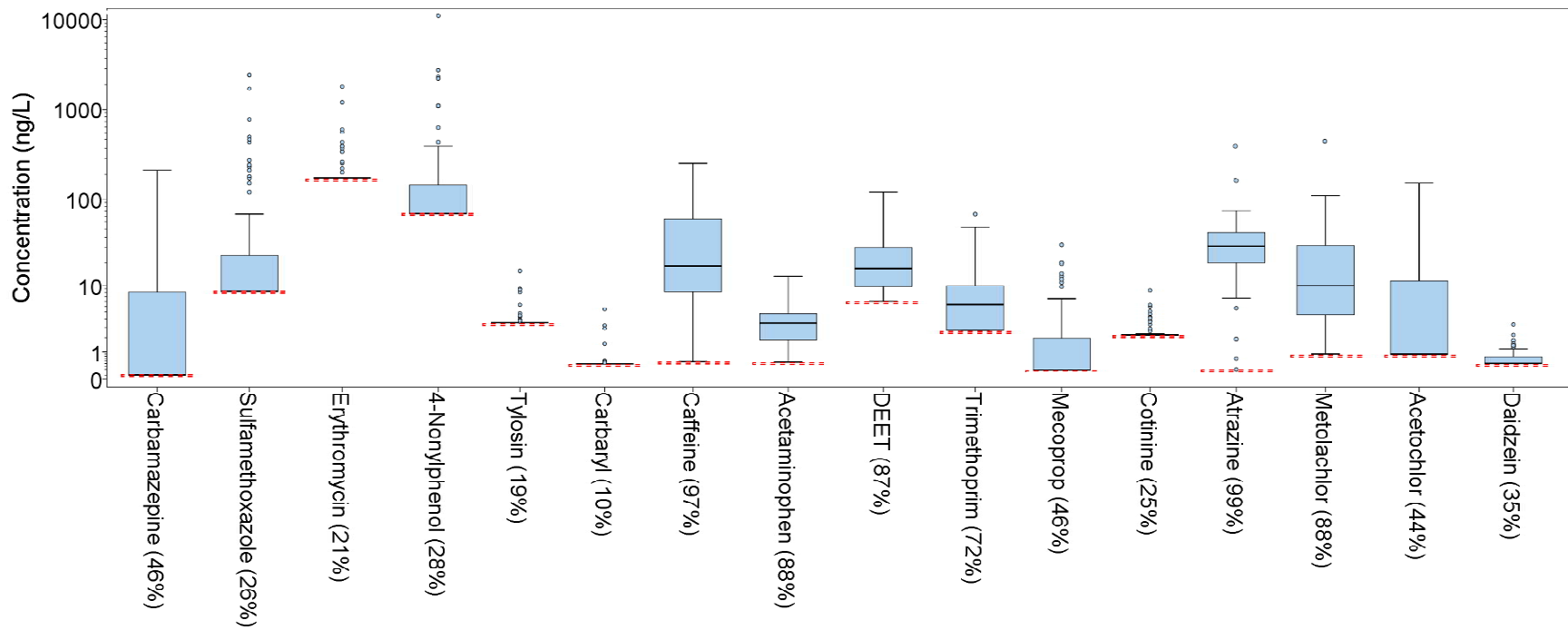
Figure 4-2. Box plots of CEC concentrations in 68 water samples collected from four sites in 2011-2012. Detection frequencies are shown in parentheses in x-axis labels. Boxes represent the interquartile ranges (IQR) of concentration data for each CEC. Median concentrations are represented by the black lines inside each box. Whiskers extend to the minimum and maximum values, up to 1.5 times the IRQ from each box. Circles indicate values beyond that range. Values below the method reporting limit (MRL, indicated by dotted red lines) were coded with a value equal to the MRL for the graphical representation.
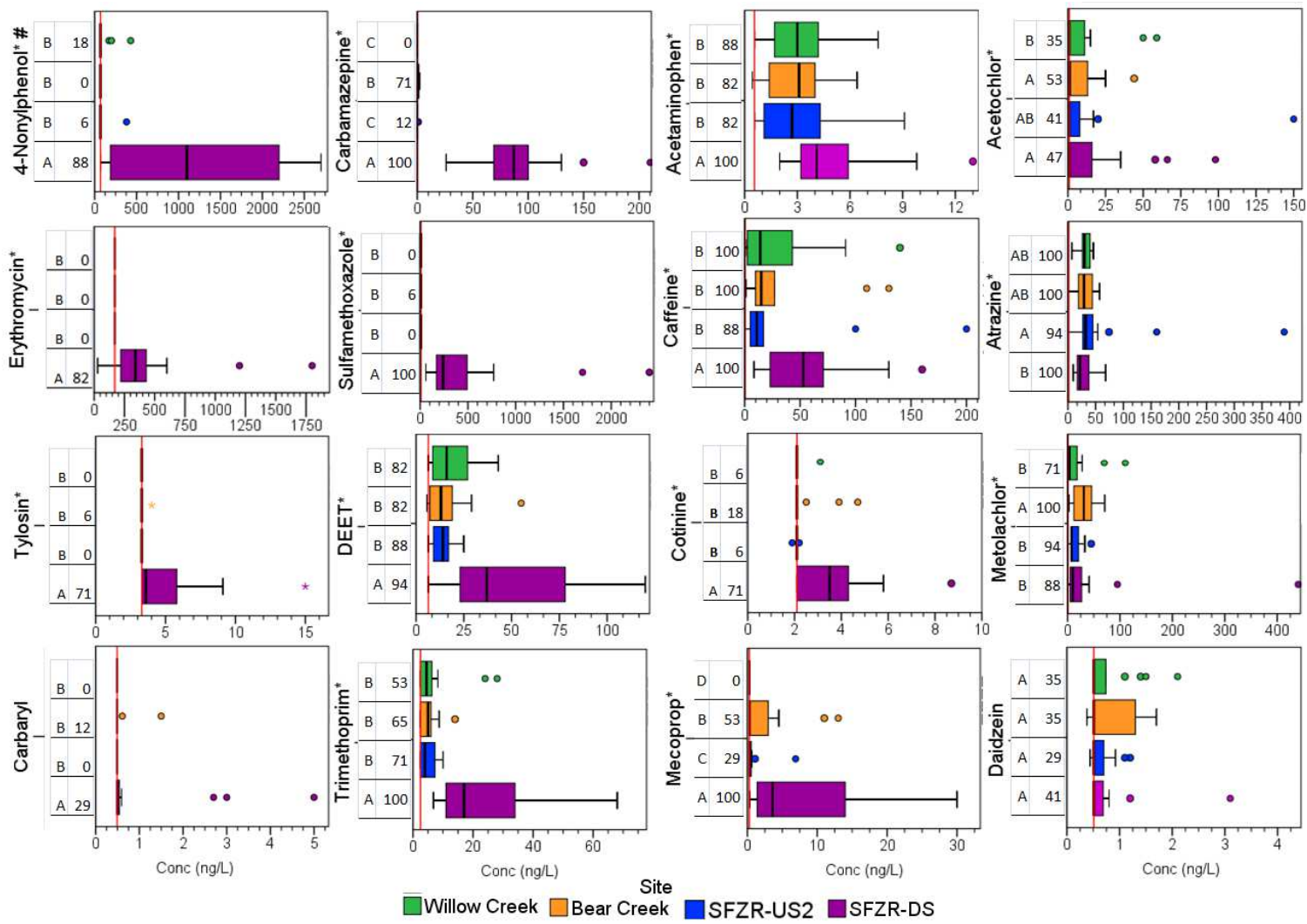
Figure 4-3. Box plots of CEC concentrations (ng/L) by site in 68 water samples. The site legend is at the bottom of the chart. Boxes represent the interquartile ranges (IQR) of concentration data for each CEC by site. Median concentrations are represented by the black lines inside each box. Whiskers extend to the minimum and maximum values, up to 1.5 times the IRQ from each box. Circles indicate values beyond that range. The method reporting limit (MRL) for each CEC is indicated by the red line in each plot. Detection frequencies for each site are indicated by the numbers along the y-axis of each plot. Data below the MRL were given a value equal to the MRL for graphical representation and ranked ANOVA. CECs with significant spatial differences in concentrations ($\alpha=0.05$) are indicated with an asterisk (*) next to the CEC name. For a given CEC, sampling sites that share a common letter on the y-axis did not have significantly different concentrations. Sites that do not share a common letter on the y-axis had significantly different concentration for that CEC. Letters earlier in the alphabetical sequence indicate greater concentrations. Using atrazine as an example, SFZR-US2 is coded with an 'A' on the y-axis, indicating a significantly greater atrazine concentration at SFZR-US2 than at SFZR-DS (coded with a 'B'). Willow Creek and Bear Creek are coded with 'AB', indicating that they are not different from any of the sites (because all sites are coded with either an 'A' or 'B' for atrazine). An octothorp symbol (#) next to the chemical name indicates that there was one large detected concentration that was omitted for display purposes. These data are presented in the Table A-4.

50

Season

■ Winter (Jan/Feb) ■ Spring (March) ■ Early Summer (May/June) ■ Late Summer (Aug/Sept) ■ Fall (Oct/Nov)

51
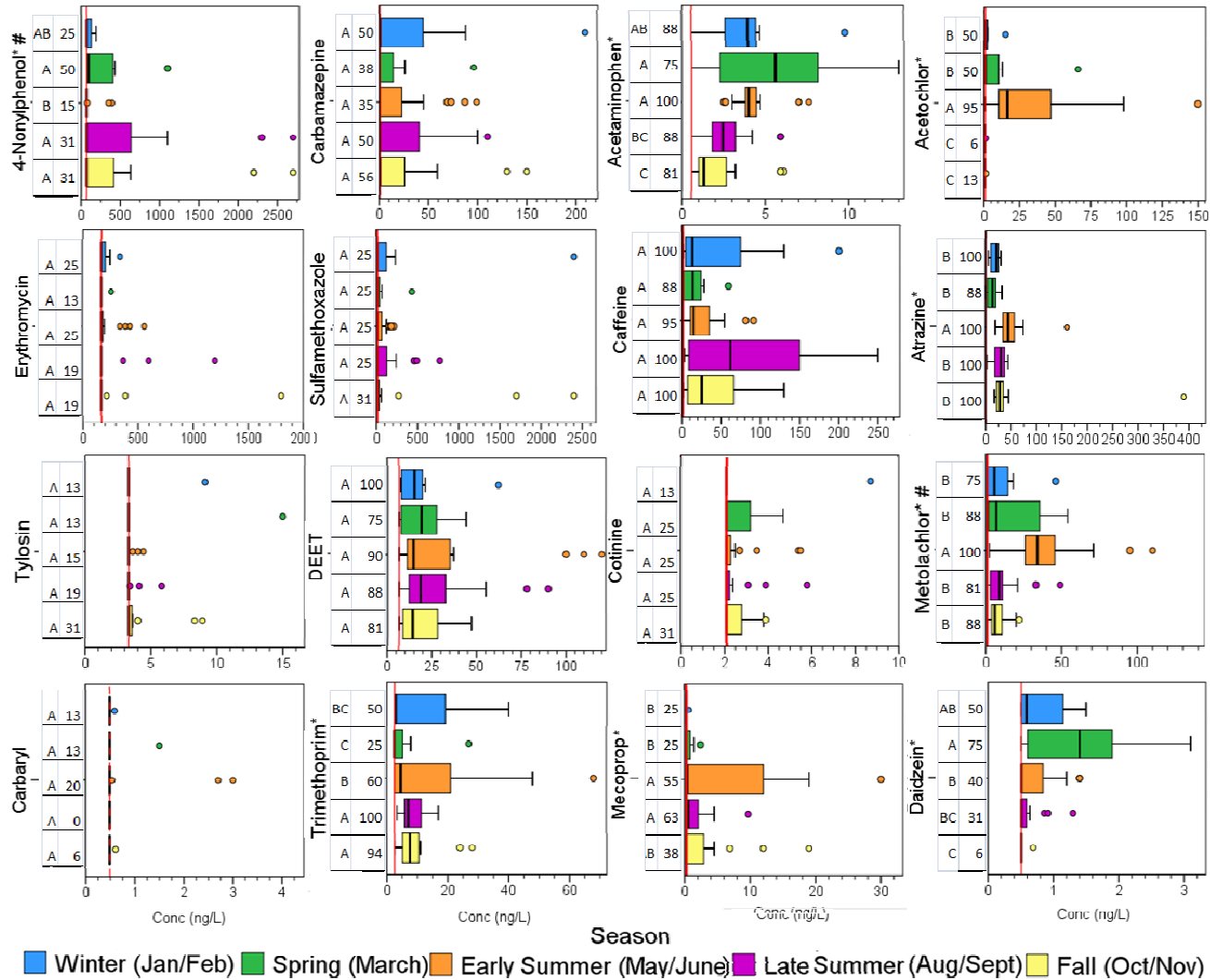
Figure 4-4. Box plots of CEC concentrations (ng/L) by season in 68 water samples. The seasonal legend is at the bottom of the chart. Boxes represent the interquartile ranges (IQR) of concentration data for each CEC by season. Median concentrations are represented by the black lines inside each box. Whiskers extend to the minimum and maximum values, up to 1.5 times the IRQ from each box. Circles indicate values beyond that range. The method reporting limit (MRL) for each CEC is indicated by the red line in each plot. Detection frequencies for each season are indicated by the numbers along the y-axis of each plot. Data below the MRL were given a value equal to the MRL for graphical representation and ranked ANOVA. CECs with significant seasonal differences in concentrations ($\alpha=0.05$) are indicated with an asterisk (*) next to the CEC name. For a given CEC, seasons that share a common letter on the y-axis did not have significantly different concentrations. Seasons that do not share a common letter on the y-axis had significantly different concentration for that CEC. Letters earlier in the alphabetical sequence indicate greater concentrations. Using atrazine as an example, the greatest concentrations occurred in early summer, which is indicated by an 'A' in the left-most column. All other seasons are coded with a 'B'', which indicates that they had smaller concentrations than early summer (i.e., they do not share a letter with early summer) but did not differ from each other (i.e., they share a common letter ('B')). An octothorp symbol (#) next to the chemical name indicates that there was one large detected concentration that was omitted for display purposes. These data are presented in the Table A-4.

Site: ■ Willow Creek ■ Bear Creek ■ SFZR-US2 ■ SFZR-DS

53
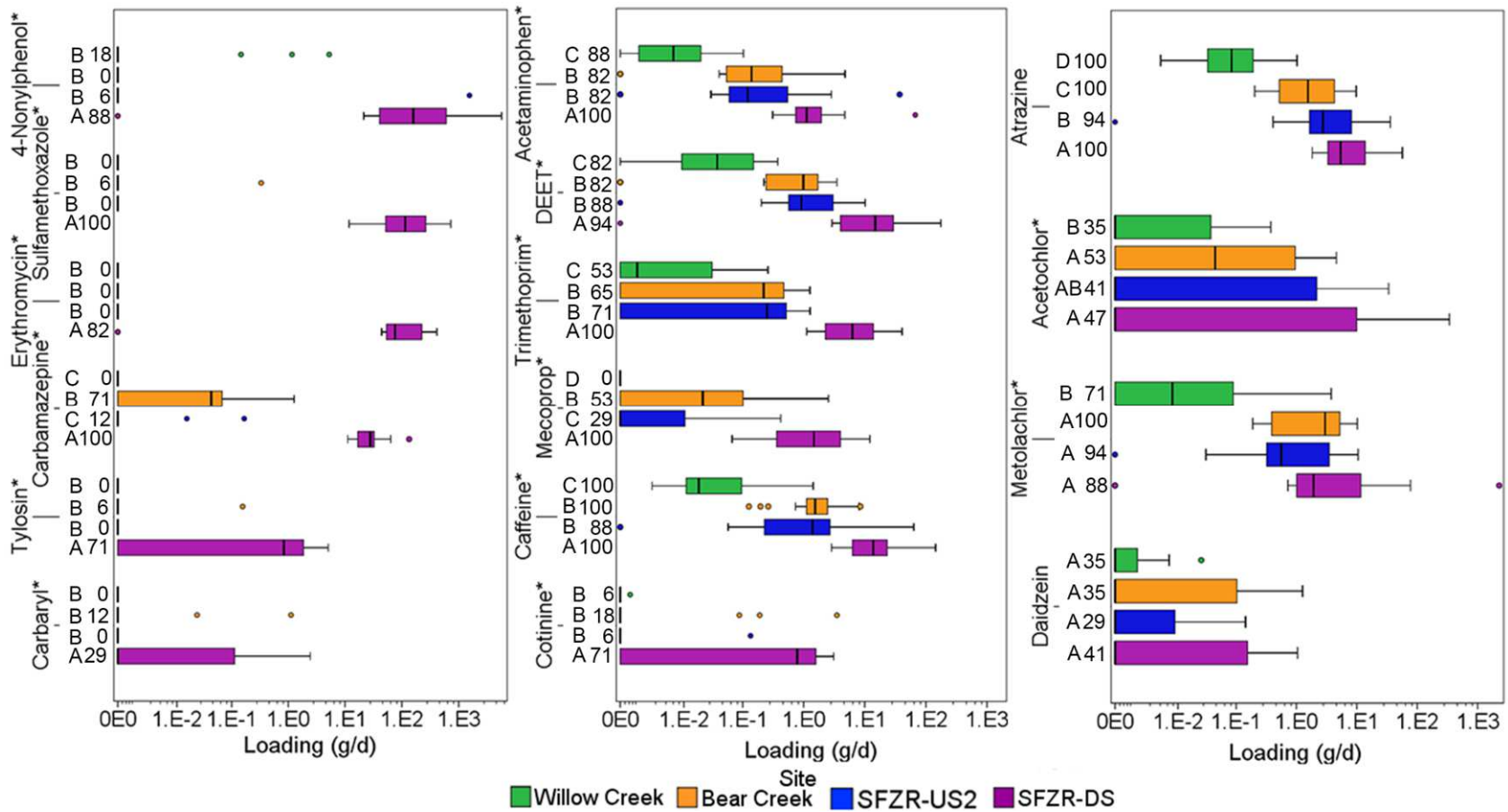
Figure 4-5. Box plots of CEC loadings (g/d) by site in 68 water samples. CECs are grouped in each panel according to the groupings identified in the study. An asterisk (*) beside the CEC label on the x-axis denotes a significant effect of season on the loading variation for that CEC ($\alpha=0.05$). Data below the method reporting limit were given a value of zero for the graphical representation and ranked ANOVA. Boxes represent the interquartile ranges (IQR) of loading data for each CEC by site. Median loadings are represented by the black lines inside each box. Whiskers extend to the minimum and maximum values, up to 1.5 times the IRQ from each box. Circles indicate values beyond that range. For a given CEC, sites that share a common letter on the y-axis did not have significantly different loadings. Sites that do not share a common letter on the y-axis had significantly different loadings for that CEC. Letters earlier in the alphabetical sequence indicate greater loadings.

Season
Winter (Jan/Feb)   Spring (March)   Early Summer (May/June)   Late Summer (Aug/Sept)   Fall (Oct/Nov)

55
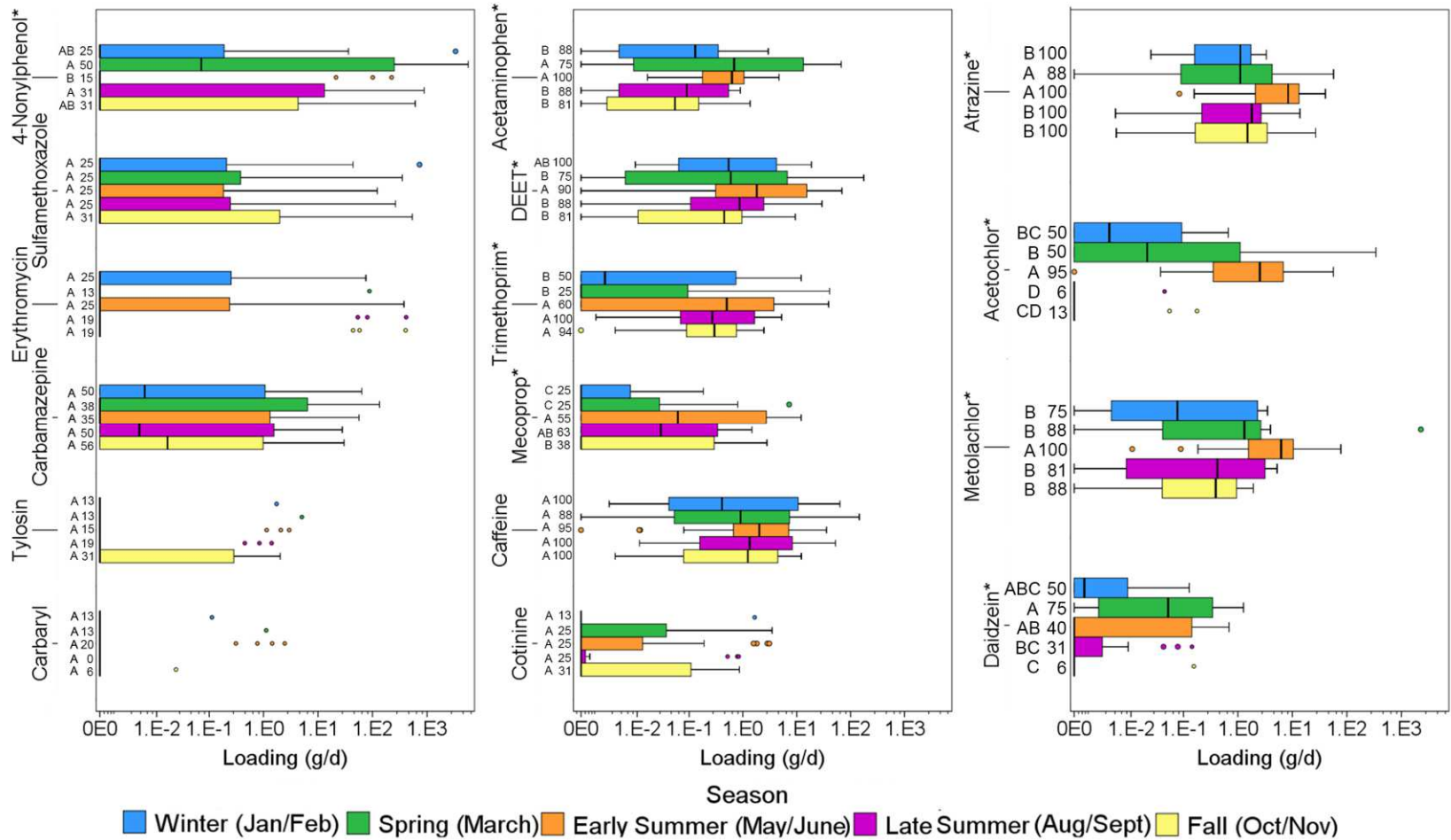
Figure 4-6. Box plots of CEC loadings (g/d) by season in 68 water samples. CECs are grouped in each panel according to the groupings identified in the study. An asterisk (*) beside the CEC label on the x-axis denotes a significant effect of season on the loading variation for that CEC ($\alpha=0.05$). Data below the method reporting limit were given a value of zero for the graphical representation and ranked ANOVA. Boxes represent the interquartile ranges (IQR) of loading data for each CEC by season. Median loadings are represented by the black lines inside each box. Whiskers extend to the minimum and maximum values, up to 1.5 times the IRQ from each box. Circles indicate values beyond that range. For a given CEC, seasons that share a common letter on the y-axis did not have significantly different loadings. Seasons that do not share a common letter on the y-axis had significantly different loadings for that CEC. Letters earlier in the alphabetical sequence indicate greater loadings.

CHAPTER 5. Sediment-Water Distribution of Contaminants of Emerging Concern in a Mixed Use Watershed

Abstract

This study evaluated the occurrence and distribution of 15 contaminants of emerging concern (CEC) in stream water and sediments in the Zumbro River watershed in Minnesota and compared these with sub-watershed land uses. Sixty pairs of sediment and water samples were collected across all seasons from four stream sites over two years and analyzed for selected personal care products, pesticides, human and veterinary medications, and phytoestrogens. Spatial and temporal analyses indicate that pharmaceuticals and personal care products (urban/residential CECs) are significantly elevated in water and/or sediment at sites with greater population density (>100 people/km^2) and percentage of developed land use (>8% of subwatershed area) than those with less population density and land area under development. Significant spatial variations of agricultural pesticides in water and sediment were detectable, even though all sites had a high percentage of agricultural land use. Seasonality in CEC concentration was observed in water but not in sediment, although sediment concentrations of three CECs did vary between years. Average measured non-equilibrium distribution coefficients exceeded equilibrium hydrophobic partitioning-based predictions for 5 of the 7 detected CECs by at least an order of magnitude. Agreement of measured and predicted distribution coefficients improved with increasing hydrophobicity and instream persistence. The more polar and degradable CECs showed greater variability in measured distributions across different sampling events. Our results confirm that CECs are present in urban and agricultural stream sediments, including those CECs that would typically be thought of as non-sorptive based on their log K_{ow} values. These results and the observed patterns of sediment and water distribution augment existing information to improve prediction of CEC fate and transport, leading to more accurate assessments of exposure and risk to surface water ecosystems.

5.1 Introduction

Contaminants of emerging concern (CECs) are widely found in the environment and include natural/synthetic hormones, pharmaceuticals and personal care products

(PPCP), veterinary medicines, industrial/household products, pesticides, and other chemical and physical agents [7-8]. CECs are not commonly subject to environmental monitoring or regulations, but they have potential adverse effects on human and ecosystem health [3], which include endocrine disruption in aquatic systems [7] and human populations [82], induction of antibiotic resistance [78], and direct aquatic toxicity [88]. Numerous sources of CECs to surface water ecosystems include concentrated animal feeding operations (CAFOs) [28], row crops [110], lawns and golf courses [110], industry [8], roads [285], landfills [286], land-applied sludge or biosolids [254], and wastewater treatment system effluent [250, 286]. National and international reviews of CEC occurrence in surface waters are available [7, 286] with far fewer reports of CECs in river sediments [37, 232, 259, 287].

Sorption is a dynamic process that occurs primarily through hydrophobic interactions or attractive force and is related to the physicochemical properties of the sediment and the sorbate [242]. Observed empirical relationships between the soil organic carbon-water partition coefficient (K_{oc}) and the octanol-water partition coefficient (K_{ow}) for neutral hydrophobic organic contaminants (HOCs) are often able to predict the overall solid-water distribution coefficient (K_d) within an order of magnitude [247]. Although HOC sorption has been studied extensively, relatively little information is available on sorption of hydrophilic and moderately hydrophobic contaminants, including many CECs [126]. Sorption of polar/ionizable compounds is influenced strongly by non-hydrophobic interactions when relevant mineral or soil organic matter components are present [28, 126, 246, 257, 261, 288]. For these compounds, K_d is often poorly predicted by K_{ow} and K_{oc} relationships, with non-hydrophobic interactions driving sorption for compounds with relevant functionalities (e.g., amine, carboxylic acid, or hydroxyl groups). Models are being developed for sorption of such compounds [246, 257, 266].

Few studies have analyzed CECs in water and sediment samples collected at the same time and place [48, 188, 221, 232, 236]. Measured instream K_d values for low-K_{ow} antibiotics and natural hormones exceeded K_{ow}-based predictions by at least an order of magnitude [232, 236, 259]. In contrast, hydrophobic synthetic hormones (log K_{ow}>4) agreed more closely with predictions [259]. Similarly, the distributions of neutral CECs with log K_{ow}>2 (e.g., carbamazepine [124, 255] and atrazine [264]) have been well-predicted by K_{ow}-K_{oc} relationships. Wide "within-compound" spatial and temporal

variations in K_d, often up to three orders of magnitude, have been observed for individual CECs across field sampling events [186, 221]. It has been suggested that this variation is due to non-hydrophobic interactions, hydrologic factors, chemical usage variations, and variable attenuation rates between aqueous and sediment-bound fractions [28, 124, 126, 221, 232, 236]. Laboratory studies have corroborated both the within-compound variations and the deviation from K_{ow}-based predictions (up to 4 orders of magnitude) for many low-K_{ow} CECs [124, 256, 258]. Nevertheless, most field studies of sediment-water distribution of CECs have analyzed only a few pairs of samples or sampling periods. Thus, there is little available information on the spatial-temporal variation of sediment concentrations and sediment-water distributions in the environment.

The objectives of this study were to assess the occurrence, distribution, and spatial-temporal variation of CECs measured in stream water and sediments of a mixed-use watershed and to evaluate the use of equilibrium partition coefficients to predict non-equilibrium sediment-water distribution of CECs in streams. Understanding CEC sediment-water distributions and variation is important because these processes influence the fate and bioavailability of CECs, thus affecting the sediment's function as a CEC source or sink [287]. In addition, CEC presence in the sediment will influence benthic organisms' exposures to CECs as well as the development of antimicrobial resistance [78]. The results of this study will ultimately enhance future CEC research, predictive methods, and effective targeting of monitoring, management, and mitigation solutions.

5.2 Methods

5.2.1 Study area and CECs of interest

The South Fork of the Zumbro River (SFZR) watershed (Figure 5-1) in southeastern Minnesota contains a gradient of land uses across its subwatersheds. This provides a unique opportunity to study CECs from both agricultural and urban sources. Four sampling sites in four subwatersheds were chosen to reflect different types and extents of land uses, different human and animal populations, and use of septic systems versus a city sanitary sewer system (Table A-1).

A detailed land use and hydrologic characterization was conducted by McGhie Betts, Inc. (Rochester, MN) (discussed in Karpuzcu et al. [273]). Pertinent information is presented in Table A-1, and was used to categorize sites as being primarily agricultural or urban/residential for categorical data analysis and interpretation.

Fifteen CECs were selected based on surface water occurrences reported in the literature [8], chemical usage patterns [32, 37, 78, 165, 289], potential effects [78, 141], and land-use associations [8, 37]. The suite includes three agricultural herbicides, one veterinary antibiotic, five urban/residential PPCPs, and six mixed use compounds (pesticides, phytoestrogens, and antibiotics) (Table A-2).

5.2.2 Sample collection, processing, and analysis

Sampling periods (four in 2011 and five in 2012) were chosen to reflect a variety of seasonal, flow, and land use conditions. In the late summer (August-September), fall (October-November), and winter (January-February) periods, low flows were expected to result in enhanced CEC signals associated with sanitary sewers and with reduced contributions from runoff. During spring melt (March), CECs associated with land-applied manure and other terrestrial components were expected to be transported with snowmelt runoff. During early summer (May-June), runoff of row crop herbicides was expected to be associated with precipitation events.

Two sampling days occurred one week apart for all sites in early summer, late summer, and fall periods. Prior to use, all field, laboratory, and storage equipment was cleaned with CEC-free soap and distilled water, triple rinsed with ultrapure water, methanol-rinsed, and (for glassware and metals) heated to 400 °C for 3 hours. Sediment samples were collected from the top 5cm of multiple areas across the width of the stream using a stainless steel scoop. Samples were deposited into cleaned 1-L glass jars. Jars were sealed with Teflon-lined lids and transported on ice to the storage facility, where they were frozen (-18°C) until processing. Water samples were collected a few minutes prior to sediment samples by submerging a clean amber glass or stainless steel container into the stream to a depth of 10-20cm. Water samples were sealed with Teflon-lined or stainless steel lids and transported on ice to the laboratory. Water samples were refrigerated at 4°C until processing, which was initiated with 72 hours of collection. Corollary physical and chemical data were acquired using *in situ* sondes (YSI

Inc, Yellow Springs, OH, USA; Hach Hydromet, Loveland, CO, USA), YSI flowmeters, and existing USGS monitoring stations.

Water samples (2 L) were filtered through 0.7μm glass-fiber filters, spiked with a surrogate standard (metazachlor, which is not approved for use in the U.S.A.), and processed via solid phase extraction (SPE) using an Autotrace 280 (Dionex, Sunnyvale, CA, USA) equipped with Oasis HLB cartridges (6cc, with 500mg HLB sorbent) (Waters, Milford, MA, USA). Extraction solvents were acetonitrile and acetonitrile/0.1% formic acid. Complete SPE parameters are presented in Chapter 3, Section 4. The eluate was collected in glass culture tubes and evaporated under nitrogen to near-dryness. Following evaporation, the sample was reconstituted to 1.5mL with acetonitrile and ultrapure water (1:1), transferred to amber liquid chromatography vials, and stored at -18°C until analysis.

Sediment samples (10g dry weight) were processed by: (a) sieving the wet sediment through a 2mm sieve, (b) homogenizing, (c) freeze-drying, (d) adding surrogate standards, (e) allowing overnight equilibration, (f) extracting using a Dionex ASE 350 (Dionex, Sunnyvale, CA, USA) with 33-mL ASE cells, and (g) evaporation, cleanup, and subsequent processing as described in Chapter 3.

Concentrations of the CECs were quantified on a Shimadzu (Kyoto, Japan) high performance liquid chromatograph coupled to an Applied Biosystems (Carlsbad, CA, USA) API 3200 triple quadrupole mass spectrometer using electrospray ionization in scheduled multiple reaction monitoring mode in either positive or negative mode. Tuning parameters were optimized for each analyte by direct infusion and are detailed along with other parameters in Table A-3.

Two sediment samples from each site were analyzed for total organic carbon (TOC), composition, and major elements and ions at the University of Minnesota Soil Testing Research Analytical Laboratory (St. Paul, MN, USA).

5.2.3 Modeling and statistical analysis

The KocWIN application in EPI Suite [290] was used to generate predicted log K_{oc} values (using the K_{ow} and Molecular Connectivity Index approaches). Sediment-water distributions from previous field studies and laboratory partitioning data for the CECs were collected from available publications (citations listed in Table A-11).

Statistical analysis was conducted using Stata (StataCorp LP, College Station, TX, USA), and SPSS (IBM, Armonk, NY, USA). Box-Cox assessments and transformations were used to satisfy assumptions of normality and constant variance. Categorical analysis was the primary focus of statistical analysis due to the small number of sites (n=4). Two-way Analysis of Variance (ANOVA) and the Protected Least Significant Difference multiple comparison procedure was used to determine the significance and magnitude of between-group variation for the investigated factors using both censored/ranked and uncensored/transformed datasets (with $\alpha=0.05$). Spearman's rank correlation analysis was used to assess the influence of numerical independent variables and corroborate the categorical analysis and interpretation.

5.2.4 Data quality assurance/quality control and presentation

Method reporting limits (MRL) were established using published U.S. EPA methods [275]. These were compared with USGS methods [276] and the minimum CEC mass that consistently produced a signal-to-noise ratio of at least nine in order to ensure consistency across numerous analytical runs. Calibration curves were generated using six standard levels across three orders of magnitude of analyte concentration. R-squared values were greater than 0.98 for all detected analyte calibration curves.

Each analytical run included laboratory spikes to assess analytical accuracy and precision and laboratory blanks to assess contamination and instrument carryover. Laboratory spikes were ultrapure water/HPLC-grade acetonitrile (1:1) samples that were spiked with compounds of interest directly into the liquid chromatography vials before injection into the liquid chromatograph. Laboratory blanks were identical to laboratory spikes except that they were not spiked with compounds of interest. Procedural spikes and blanks were created and subjected to the various extraction steps in order to assess contamination or loss of recovery resulting from sample processing. Field blanks were included to assess potential contamination resulting from sample collection, handling, and storage. Spiked environmental samples were used to assess matrix interference. If a laboratory blank response was more than 20% of that in an associated environmental sample, data were flagged and reviewed. If a laboratory blank response was above 50% of that in an associated environmental sample, the data was reported as "non-detect".

This occurred once for caffeine in a single sediment sample. No other blank responses were above 20% of the MRL of any detected CEC.

Analytical recoveries for all detected CECs were between 64-170% in laboratory spikes, 48-150% in matrix water samples spiked before injection, 60-110% in sediment samples spiked prior to SPE cleanup, and 21-103% in sediment samples spiked prior to ASE. For individual detected CECs, relative standard deviations (RSDs) ranged from 4.5-17% for repeated analyses of environmental sediment samples (n=3) and from 5.2-9.8% for replicate spiked sediment samples (n=3). Quality assurance/quality control results are presented in Table A-2.

5.2.5 Analytical method considerations

Samples were quantified using the external calibration approach (quantification of unknown CEC concentrations in environmental samples by comparison with calibration curves that are generated from analysis of laboratory standards of known concentration). It was not possible to use the internal standard calibration approach due to the diversity of compounds included in this study. It was not feasible to include the number of standards that would be required to confidently represent each of these CECs for internal standard calibration and correction against surrogate standard recoveries.

Comparison of recoveries from ultrapure water, matrix water, and matrix sediment samples that were spiked with CECs of interest indicated that recoveries of most CECs were acceptable and within a range that might be expected for a multi-residue method for diverse CECs (Table A-2). Measurement precision as indicated by relative standard deviations (RSD) of replicate analyses of the same unspiked environmental samples and spiked environmental samples was also considered acceptable (RSD<20% in most instances). Coefficients of determination (r^2) of linear regressions of external calibration curves were consistently greater than 0.992 for all CECs.

We did not correct data for matrix effects or surrogate recovery. Some losses of recovery are expected to occur due to matrix effects and sample processing, and will vary for different CECs. Correction for these losses would tend to increase the reported concentrations of CECs in environmental samples. Thus, not correcting for matrix effects is a conservative approach. In reality, the actual environmental concentrations may be

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greater than those reported herein. Also, correction for matrix effects will not affect the spatiotemporal comparison of water or sediment samples when comparing the same CEC; each CEC would receive the same correction in a given matrix such that different samples would maintain approximately the same proportionality. In developing the $K_{d\text{-}obs}$, reduced recovery due to matrix effects would be likely to affect sediment values more than water values, which would result in actual $K_{d\text{-}obs}$ values that may be greater than those reported herein.

Correction for surrogate recoveries can account for sample-specific issues that may arise during sample processing and analysis. While it is preferable to correct for surrogate losses in most instances, the number of surrogate samples that would be required in this study in order to properly conduct this procedure precluded the use of surrogate corrections. However, any anomalous surrogate and internal standard responses were examined for possible sampling or measurement error.

5.3 Results and discussion

5.3.1 Detection and concentrations of CECs in sediment and water

In total, 60 paired sets of water and sediment samples were analyzed for CECs (15 pairs from each of 4 sites). Eight of the fifteen investigated CECs were detected in at least one sediment sample, and thirteen CECs were detected in at least one water sample (Table A-8). All of the CECs found in sediment were also found in water, but not necessarily in the same sample pair. Individual sediment samples contained between two and six CECs, with an average of four CECs per sample (Table A-8). Metolachlor was only detected in one sediment sample and is not discussed further. All sample data are presented in Table A-8, including basic sediment and water characteristics (Table A-9).

5.3.2 Seasonal and spatial differences in CECs present in sediment versus water

Figures 5-2 (A,B) and 5-3 (A,B) express mean CEC concentrations by season and site, respectively, in sediment and water. The ANOVA and pairwise comparison results are provided in Table A-5.

No CECs exhibited seasonality in sediment (Figure 5-2A), but several CECs exhibited seasonality in water (denoted with an asterisk (*) in Figure 5-2B). Elevated water concentrations occurred in summer periods for four CECs ($p < 0.05$), including caffeine, acetaminophen, and the agricultural herbicides acetochlor and atrazine. Daidzein exhibited its highest water concentrations in spring. The seasonality in water as compared to sediment is likely explained by water concentrations responding more rapidly to changes in aqueous inputs and instream flows, as well as to seasonal fate-related parameters such as sunlight and temperature variation, than do sediment concentrations [125]. In fact, dissolved and sorbed CEC fractions are affected differently by common transformation/transport processes including photodegradation [7], biodegradation [141], concentration/dilution, and temporal input variations. Concentrations of some CECs in sediment did occasionally exhibit variations of an order of magnitude or more in between weekly sampling events. These tended to follow precipitation events in early and late summer when instream flows exhibited large variations. This correlation of sediment concentrations with hydrologic flow may be due to deposition of fresh sediments having differing concentrations of CECs than existing sediments, or due to resuspension and mixing of sediments with different concentrations.

None of the CECs exhibited significant annual variation in water concentrations. Sediment CEC concentrations did exhibit changes on this longer, annual time scale (Table A-7), as has been previously observed [125]. Acetaminophen, caffeine, and acetochlor varied annually in sediment, and all exhibited higher concentrations in 2012 than 2011. These yearly trends and high detection frequencies may be reflective of increasing inputs, ongoing accumulation of these CECs in sediment, or other factors. For instance, total flow at the study sites was smaller in 2012 than 2011. This would increase the effective exposure time of sediments to aqueous CEC concentrations, resulting in higher sediment concentrations. Additionally, annual sales of acetochlor in Minnesota increased from 2009-2011, and were (in millions of pounds): 2.63 (2009), 3.35 (2010), and 3.98 (2011); 2012 sales data were not available at the time of writing [279]. Increased pesticide application may therefore also explain the increased concentrations of acetochlor and other CECs in sediments between years. Longer-term studies would be required to confirm annual patterns.

Location (site) affected similar numbers of CECs in water and sediment, but with divergent patterns between phases. Sites with elevated CEC concentrations in sediment generally did not exhibit elevated concentrations in water, and vice versa; nevertheless, patterns of CEC detection with respect to location could be discerned. Land use associations of PPCPs were stronger compared to agricultural pesticides (Table A-7). For example, the site downstream from the WWTP (SFZR-WWTP) exhibited significantly elevated concentrations of all PPCPs (acetaminophen, caffeine, carbamazepine, DEET) in water (Figure 5-3A), and of carbamazepine in sediment (Figure 5-3B). Bear Creek was associated with the highest human population density amongst sites, and exhibited elevated sediment concentrations of acetaminophen and caffeine. Indeed, even with only four sites for analysis, sediment concentrations of caffeine were positively correlated with population density (Spearman's rank correlation coefficient (ρ) = 0.297, p=0.021) and percentage of residential land use (ρ =0.319, p=0.013). Other location-based influences on CEC detection appeared to be hydrological or morphological in nature. For example, Bear Creek exhibited a uniquely shallow (~30cm) and wide (~15m) morphology, with a primarily bedrock streambed and frequent low-level turbulence. These characteristics affect sediment deposition and CEC concentrations. Sediment deposition appeared to occur only along the stream bank and in isolated ridges and clefts of the streambed. Complete light penetration of the water column at Bear Creek, particularly in the summer, helps explain the lack of correspondingly elevated water concentrations of caffeine and acetaminophen, both of which are photodegradable [119, 131].

Although less clear and consistent, spatial variations of agricultural CECs were evident in this study. These more ambiguous occurrence patterns of agricultural versus urban/residential CECs reflect a more consistent allocation of agricultural land use among sites, in contrast to the greater spread in urban/residential land use allocation among sites (Table A-1). Acetochlor concentrations were elevated in water and sediments at Bear Creek, a site with 52.3% agricultural land use (along with its aforementioned high human population density). Atrazine concentrations were elevated in sediments at Willow Creek (a site with 53.2% agricultural land use and the lowest human population density among study sites). Atrazine showed marginally significant spatial variation in water concentrations (p=0.083), which speaks to diffuse sources such

as atmospheric deposition in addition to agricultural runoff. Acetochlor and atrazine are used on the same crops, with acetochlor use increasing and atrazine use decreasing in Minnesota. The lack of covariance of these CECs across sites could be due to varying usage among farmers in the subwatersheds or to differences in their relative persistence and transport. The elevation of atrazine concentrations in Willow Creek sediments but not in water compared to other sites may reflect a historical input and record in sediment in this subwatershed that is less evident in water concentrations with atrazine's recent sales decline. Karpuzcu et al. [273] used a multivariate technique (principal component analysis (PCA)) with an expanded set of water samples collected from this study area to discern patterns among groups of CECs that are not as apparent when investigating individual CECs. Atrazine, acetochlor, and metolachlor were strongly associated with a principal component that was attributed to agricultural land uses. The results of Karpuzcu et al. [273] thus reinforce our observation that occurrence patterns of agricultural CECs in this mixed use area are evidence of a gradient of seasonal agricultural land use influences, and associated inputs to streams. This also highlights the importance of robust statistical and sampling methodologies to discern such patterns, especially in watersheds such as the SFZR where all studied sites are associated with more than 50% agricultural land use.

5.3.3 Sediment-water distributions ($K_{d\text{-}obs}$)

An observed sediment-water distribution coefficient, $K_{d\text{-}obs}$, was calculated each time that a CEC was detected in sediment and water samples from the same sampling event (Figure 5-4, Tables A-6 and A-7). In Figure 5-4, two trends are apparent: (1) most of the CEC $K_{d\text{-}obs}$ values are above the K_{ow}-based line, and (2) $K_{d\text{-}obs}$ values generally span orders of magnitude for a given CEC.

The $K_{d\text{-}obs}$ values are not assumed to be true equilibrium partitioning coefficients, for flowing systems cannot be assumed to be at equilibrium. However, understanding sediment-water distribution behavior is desirable for chemical fate, ecotoxicological, and risk assessment considerations. Models relating K_{ow} to K_{oc} are well-established, use readily available information, and perform reasonably well for HOCs [247]. This approach has been attempted with a wide variety of chemical and soil types due to convenience and a lack of common, feasible alternatives [247]. Other than temporal and

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spatial influences, as discussed above, several factors related to sediment chemistry or to the chemistry of the sorbing compounds themselves affect the accuracy of these predictions [242]. Indeed, numerous field and laboratory CEC studies have reported $K_{d\text{-}obs}$ values that deviate widely from equilibrium predictions and each other, often by an order of magnitude or more [124, 126, 188, 232, 236, 255-256, 258-259, 261]. Therefore, it is useful to assess for which compounds sorption is governed primarily by hydrophobic partitioning, by polar-ionic interactions with mineral components, or by a mix of influences, and under what conditions [246].

Figure 5-4 and Table A-6 indicate that $K_{d\text{-}obs}$ approaches K_{ow}-based predictions with increasing K_{ow}. The observed deviations of $K_{d\text{-}obs}$ may be due to seasonally and/or spatially influenced variations as discussed above, the fact that the system is not truly at equilibrium, or non-hydrophobic interactions between CECs and sediment. In this research, in all instances when the $K_{d\text{-}obs}$ values of a given CEC exhibited significant seasonal or spatial variation, so too did its water concentration (Table A-7). Moreover, in only two cases did water concentration exhibit significant variation without corresponding $K_{d\text{-}obs}$ variation. Sediment concentration variations did not exhibit similar association with $K_{d\text{-}obs}$ variation. Thus, variation of $K_{d\text{-}obs}$ is partially driven by variations in instream water concentration for these CECs, which supports the assumption that the system is not at equilibrium. This could explain some of the discrepancies between laboratory and field results for these and other organic compounds.

Non-hydrophobic interactions between CECs and sediment exert significant effects on sorption behavior. It is known that sorbate polarity, ionizability, and pK_a strongly influence sorption. Potential interactions include cation exchange, cation bridging, hydrogen-bonding, and mineral complexation [89, 250]. Amines have been noted to consistently exceed estimated K_d's by an order of magnitude due to cation exchange [242]. Hydroxyl, carboxylic acid, and other N- and O- containing functionalities exert similar effects through cation bridging, anion exchange, and mineral complexation. Most CECs in the current study exhibit at least one such functionality and the majority of PPCPs are polar and hydrophilic, with low K_{ow} [7]. Thus, predictions based solely on K_{ow} are unlikely to accurately predict CEC sorption due to unaccounted non-hydrophobic interactions [28, 254-257]. In addition, a high clay content (e.g., clay to organic carbon ratio > 30) indicates that significant mineral contributions to sorption are likely, especially

for molecules with polar functional groups [252]. For pesticides, a clay to organic matter ratio as low as 15 was found to result in significant mineral contributions to sorption [291], an order of magnitude above organic carbon-based predictions [292]. The average clay to organic carbon ratio in the current study was 39, again suggesting the importance of non-hydrophobic interactions.

Considering a few specific compounds in light of their physicochemical properties and spatiotemporal variability provides further insight into when the use of equilibrium partition coefficients might be useful to estimate non-equilibrium sediment-water distribution in streams, as well as potential sources of K_{d-obs} variability. For example, acetaminophen and caffeine log K_{d-obs} values deviated farthest from equilibrium-based log K_d predictions (by 3-6 orders of magnitude). This parallels previous literature reports that suggested the deviation is explained by non-hydrophobic mechanisms [89, 124, 256]. These CECs are polar and hydrophilic as demonstrated by their high solubilities, log K_{ow} less than one, and amine/amide functional groups. Photolytic and overall instream half-lives for these hydrophilic CECs are on the order of a few days [119, 124, 129-130]. These CECs may not desorb or degrade as quickly in sediment compared to water (due to reduced exposure to sunlight or bioavailablity for microbial degradation, for example), resulting in increased K_{d-obs}. Additionally, fluctuations in CEC inputs would result in water concentrations that change more rapidly than sorption-desorption processes. This is seen in the seasonality in water concentration for several CECs, whereas no such seasonality was evident in sediment concentrations.

For daidzein, DEET, and acetochlor (log K_{ow}>2), K_{d-obs} values diverge from predictions by 1-3 orders of magnitude, in agreement with some previous literature reports (Figure 5-4). These CECs are moderately hydrophobic and weakly polar as exhibited by log K_{ow} range of 2-3 and solubilities of 200-600 mg/L. The instream half-lives of these moderately hydrophobic CECs, including photolytic half-lives, are reported to be on the order of hours-to-weeks [45, 131-132, 173, 281]. Thus, in comparison with acetaminophen and caffeine, the more moderate aqueous attenuation rates and increased hydrophobicity of these CECs may explain the somewhat better agreement of their K_{d-obs} with equilibrium predictions.

For the more hydrophobic CECs atrazine and carbamazepine, sediment-water distributions were generally well-predicted by K_{ow} and had the lowest magnitude and

variability of K_{d-obs} among the CECs in this study. These results agree with previous literature reports (Figure 5-4). These CECs are neutral at the pHs of this study [293-294], are the least soluble among detected CECs (Table A-2) [290], and have log $K_{ow}>2$. Carbamazepine's sorption is dominated by organic carbon-partitioning [293] and well-predicted by K_{ow} [255]. Although atrazine sorbs to both organic carbon and clay fractions [294], organic carbon partitioning dominates total sorption at the clay to organic carbon ratios observed in this study [295]. Photolytic and overall aqueous environmental half-lives are on the order of months or greater for these hydrophobic CECs [119, 124, 169]. The increased hydrophobicity and much greater aqueous persistence of atrazine and carbamazepine likely explains why their K_{d-obs} values exhibited reduced variability and better agreement with equilibrium predictions than did the other CECs in this study.

Based on these observations, it is suggested that polarity and degradability in the water column influence the consistency of K_{d-obs} and its predictability by K_{ow}. Atrazine and carbamazepine had the best–predicted and least variable K_{d-obs} values. Compared to the other CECs, they are also more persistent and less soluble, with sorption dominated by hydrophobic mechanisms. The CECs that have been observed to participate in non-hydrophobic interactions and generally have higher rates of degradation (acetaminophen, acetochlor, caffeine, daidzein and DEET) are those that exhibited greater deviations of log K_{d-obs} from K_{ow}-based predictions, often by orders of magnitude. Indeed, as the degradability and spatial-temporal variability in both phases increased, a greater spread in the K_{d-obs} values was observed. Likewise, when the polarity or hydrophilicity of a compound increased, a lower correlation of K_{d-obs} to K_{ow} was observed.

5.3.4 Implications for CEC monitoring

This study addresses the importance of the sediment phase for the reported CECs. These results provide information that aids in the consideration and development of sediment monitoring activities. This study indicates that CECs are present in sediments, even those traditionally thought of as non-sorptive. Generally, K_{ow} underestimated sorption of these CECs to sediments. The absence of seasonality in CEC sediment concentrations indicates that selection of sampling time may not be crucial when planning sediment monitoring, although increases of CEC concentration in

the sediment from 2011 to 2012 for several CECs suggests that external source functions must also be considered. It also suggests that sediment might provide a more stable marker of CEC sources and land use influences with fewer samples than would be required to ascertain similar differences amongst water samples. However, it may be desirable to consider finer-scale runoff events when planning monitoring activities, as these may affect sediment CEC concentrations by temporarily affecting sediment deposition and resuspension. Sediment CEC concentrations were linked with land use, with PPCPs showing strong associations with more urban/residential sites. Occurrence profiles of agricultural CECs are consistent with the prevalence of upstream agricultural land use at the study sites and exhibited temporal increases in water concentrations during seasons of increased usage and runoff.

Traditional predictive parameters like K_{ow} often fail to accurately estimate CEC distributions in sediment in the field or laboratory. Non-hydrophobic interactions are commonly identified as factors contributing to underestimation of sediment distributions. Our results indicate that spatial and temporal variability in the water column are significant drivers of variability in K_{d-obs}. A "one-size-fits-all" approach to CEC monitoring may be unlikely to adequately capture the variability of different classes of CECs, especially if only a few grab samples are collected, which may lead to incorrect interpretations. Aqueous persistence and other temporal factors of individual CECs should be considered when designing field studies to properly characterize CEC sources, fate, transport, and risk.
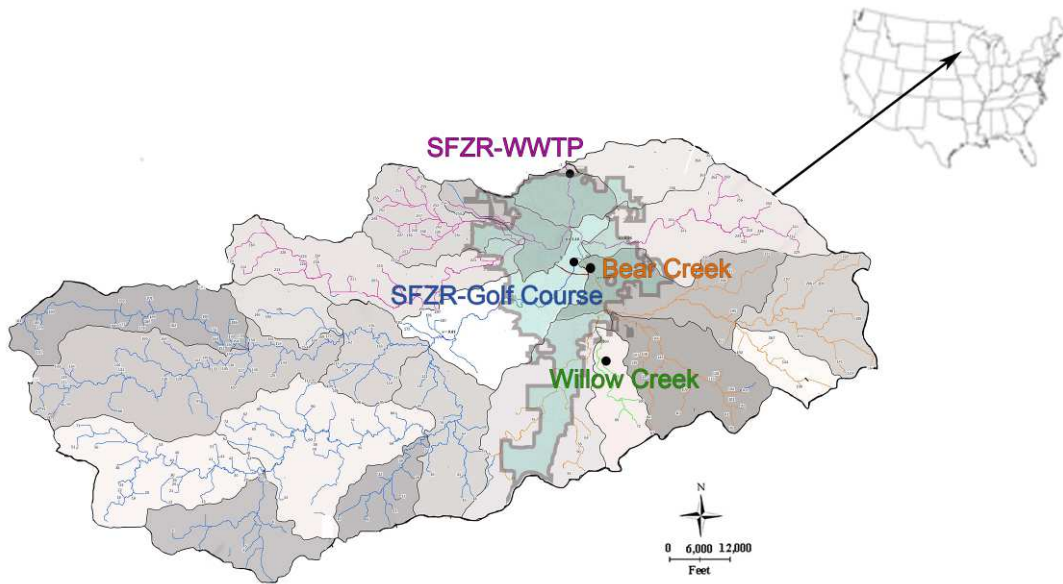
Figure 5-1. Map of SFZR study area. Labels indicate sampling site locations. Streams that influence each sampling site are color coded – Willow Creek = Green, Bear Creek = Orange + Green, SFZR-Golf Course = Blue, and SFZR-WWTP = All colored streams. The City of Rochester is indicated by the green shaded area. The SFZR flow is generally northward, such that Willow Creek flows into Bear Creek, which flows into the SFZR just downstream of SFZR-Golf Course, which then flows toward the SFZR-WWTP site.
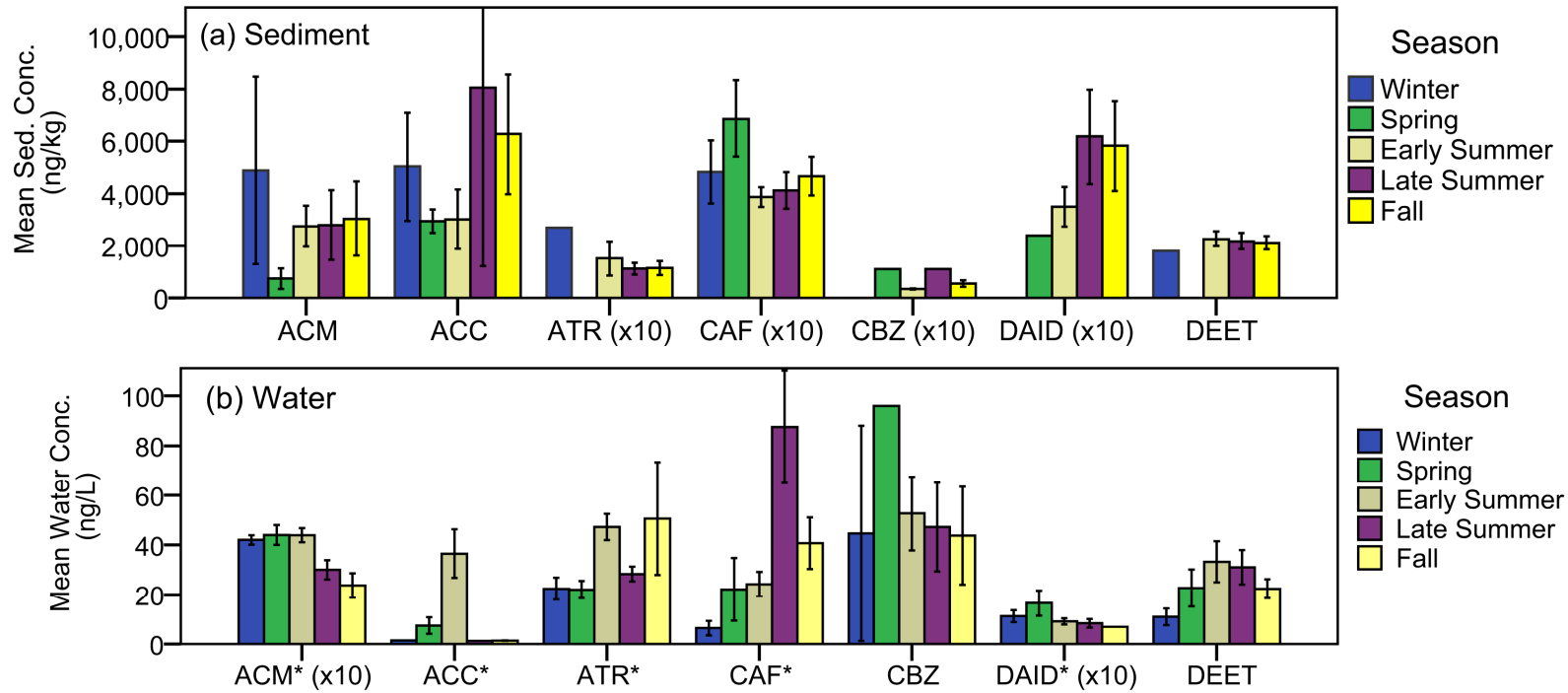
Figure 5-2 (A,B). Seasonal mean CEC concentrations in (a) sediment (ng/kg dry weight) and (b) water (ng/L). A (x10) notation indicates that the displayed concentration is ten times greater than actual concentration. Asterisks (*) indicate significant seasonality for that CEC. Error bars represent standard error of the mean. If no error bar is present, this is due to lack of sufficient number of detections during that season to calculate standard error. Seasonal detection frequencies are presented in Table A-10. Acetaminophen (ACM), acetochlor (ACC), atrazine (ATR), caffeine (CAF), carbamazepine (CBZ), daidzein (DAID).

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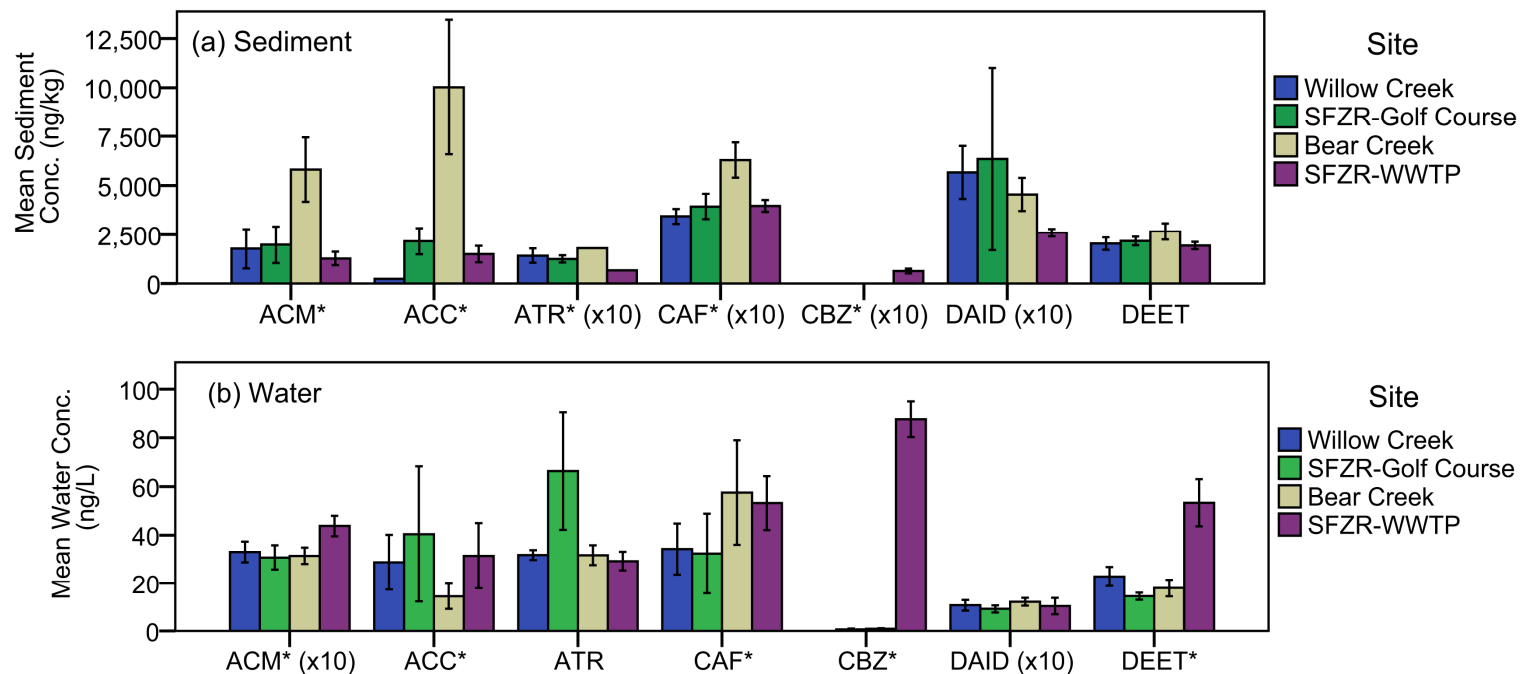
Figure 5-3 (A,B). Mean concentrations of CECs by site in (a) sediment (ng/kg dry weight) and (b) water (ng/L). A (x10) notation indicates that the displayed concentration is ten times greater than actual concentration. Asterisks (*) indicate significant variation across sites for that CEC. Error bars represent standard error of the mean. If no error bar is present, this is due to lack of sufficient number of detections at that site to calculate standard error. Detection frequencies by site are presented in Table A-10. Acetaminophen (ACM), acetochlor (ACC), atrazine (ATR), caffeine (CAF), carbamazepine (CBZ), Daidzein (DAID), South Fork of the Zumbro River (SFZR).

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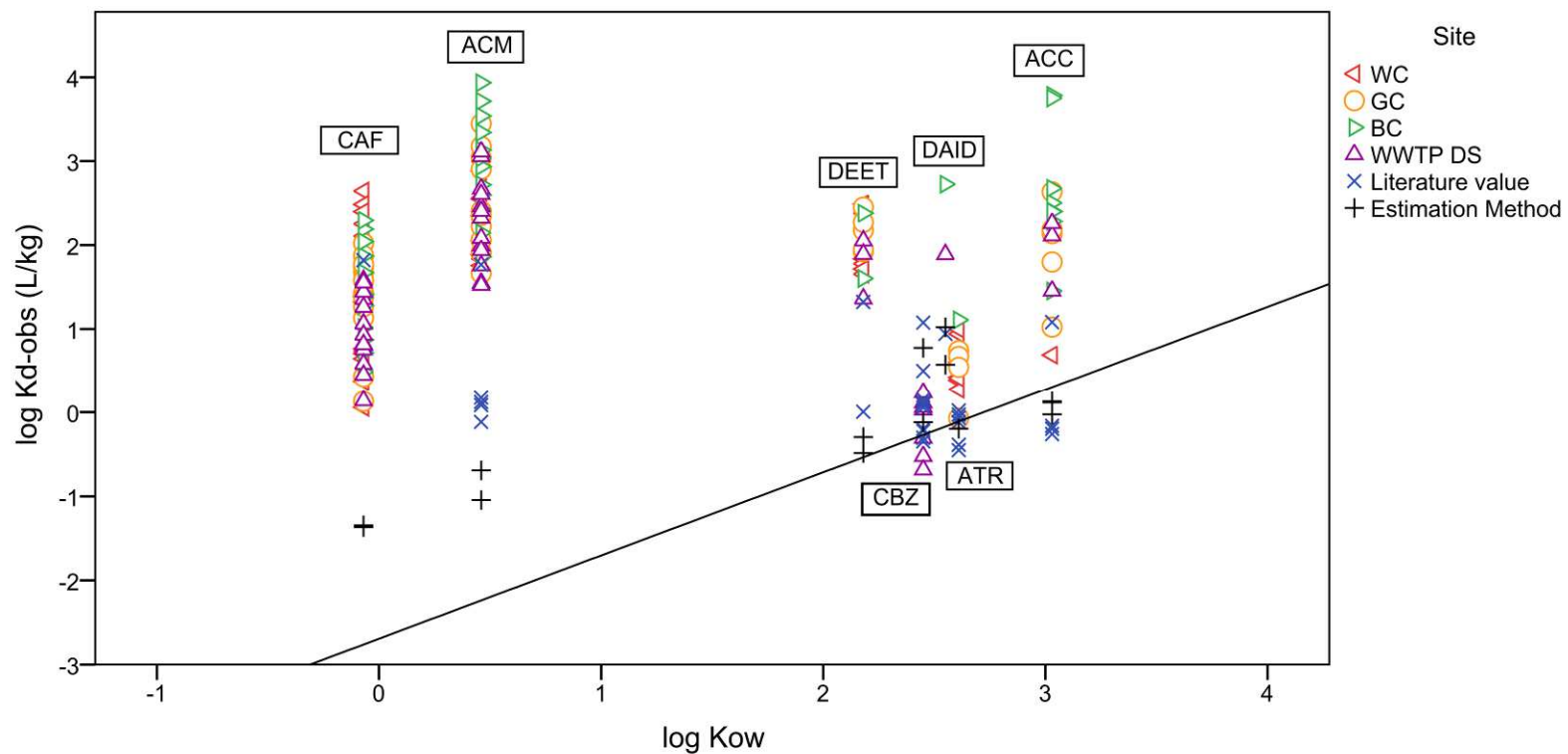
Figure 5-4. Plot of calculated log K_{d-obs} vs. literature log K_{ow} for CECs detected in water and sediment across the study sites. Results from the current study are provided, along with batch equilibrium and field studies results reported in the literature, predicted values (based on EpiSuite's MCI and K_{ow} methods), and a K_{ow}-K_{oc} relationship observed by Karickhoff [288] (converted to K_d, and represented by the black line) for comparison. All numerical data and associated citations are presented in Tables A-8 and A-11. ACM = acetaminophen, CAF = caffeine, CBZ = carbamazepine, ACC = acetochlor, ATR = atrazine, DAID = Daidzein. K_{ow} was used rather than K_{oc} because the range of sediment TOC was low and similar across sites.

CHAPTER 6. Contaminants of Emerging Concern: Mass Balance and Comparison of Wastewater Effluent and Upstream Sources in a Mixed-Use Watershed

Abstract

      Understanding the sources, transport, and spatiotemporal variability of contaminants of emerging concern (CECs) is important for understanding risks and developing monitoring and mitigation strategies. This study compared CEC loading and transport from a wastewater treatment plant and upstream areas in a mixed-use watershed in Minnesota, USA, under different seasonal and hydrological conditions. Mass balances were used to characterize the sources, loadings, and variability of sixteen CECs. Three distinct CEC groups emerged with respect to their total loading and source proportionality. Agricultural herbicides and daidzein inputs were primarily via upstream routes and their loadings and concentrations were greatest during high flows. Trimethoprim, mecoprop, non-prescription pharmaceuticals, and personal care products entered the system via balanced/mixed pathways with peak loadings and concentrations in high flows. Carbaryl, 4-nonylphenol, and the remaining prescription pharmaceuticals entered the system via wastewater treatment plant effluent. Loadings of the effluent-dominated pharmaceuticals were relatively stable across sampling events. Mass balance analysis based on multiple sampling events and sites facilitated CEC source comparison and may therefore prove to be a powerful tool for exploring mitigation strategies.

6.1 Introduction

      Chemicals classified as contaminants of emerging concern (CECs) have been found in most environmental compartments, including polar ice caps, groundwater, treated drinking water, soil, the atmosphere, precipitation, animal tissues, breast milk, and the blood and urine of infants [24, 58, 101, 197]. This is problematic because CECs have been linked with numerous effects in biological systems [58], including endocrine [24], reproductive [58, 93], neurologic [24], and carcinogenic [24, 58] effects. Despite numerous studies on CECs, significant gaps remain in our knowledge of their fate and transport [57, 60-62, 67-68], effects [57, 69], and mitigation potential in complex environmental systems [58]. The sheer number of chemicals that have been identified as

CECs or as potential endocrine-active compounds, and the fact that many of them exert effects through similar mechanisms, poses serious challenges to addressing these gaps [24, 75].

Major sources of CECs to surface waters include municipal wastewater treatment plants (WWTPs), industrial and commercial facilities, croplands, concentrated animal feeding operations (CAFOs), urban exterior landscapes, landfills, and septic systems [39, 57-61]. Transport to surface waters occurs via point and nonpoint mechanisms including pipe discharges, surface runoff, atmospheric deposition, and groundwater baseflow [62]. Instream transport and attenuation of CECs has been studied with longitudinal techniques such as Lagrangian sampling and conservative tracer methods [67, 234]. Results often indicate the potential for long-range transport. Nevertheless, fate and transport depend on the CEC and local environmental characteristics. Thus, these processes are not easily modeled or extrapolated from one site to another [39, 57, 62, 67-68, 197, 234].

WWTP discharges often account for significant portions of discharge in rivers downstream of urban and mixed-use areas [67, 197] and act as point sources of CEC fractions that are not removed in the treatment plant [47]. Thus, wastewater-associated CECs are often reported at greater frequencies and concentrations downstream than upstream of WWTPs and in low-flow versus high-flow conditions [151, 177, 197]. Other studies, however, report poor source differentiation, variable associations with flow rates, and/or significant non-WWTP sources of CECs [60-62, 69, 151, 177]. Although sometimes unexpected, these patterns may be explained by ubiquitous mixed sources that create a myriad of transport routes for CECs.

Indeed, transport of CECs to surface waters can occur via a number of additional routes. In urban or mixed-use surface waters, occurrences of CECs have been linked to stormwater-runoff conveyances, leaking sewer pipes, managed aquifer recharge, and septic systems [101, 197, 296-297]. Agricultural activities such as pesticide applications, livestock rearing, and land spreading of manure, sewage, and other biosolids may contribute CECs to agricultural landscapes [57, 298]. Transport to surface waters then occurs via runoff, tile drainage, volatilization, baseflow, and other routes [14, 298]. Livestock operations account for the majority of antibiotic use in many countries [15]. The majorities of manure and other wastewater-derived solids are land-applied, and high

concentrations of antibiotics have been reported in these matrices [15, 137]. Thus, runoff of CECs associated with land-applied manure and biosolids has been proposed to explain some unexpectedly greater concentrations and loads of PPCPs in high-flow versus low-flow conditions in agricultural areas [60]. Inputs from croplands and CAFOs remain less-studied than WWTPs [57, 60-61].

Given the large number of compounds identified as CECs and their varied transport routes into surface waters, it is important to provide quantitative characterization and differentiation of these sources. Risk assessment and mitigation depend on understanding both the adverse effects of CECs and their spatiotemporal occurrence patterns. Spatiotemporal occurrence patterns depend on sources, fate, and transport. Retrofitting WWTPs and other pollution prevention strategies (e.g., reduced veterinary pharmaceutical use, changes to pesticide practice, consumer product changes) currently being considered to reduce CEC exposure risk may entail considerable expense [61-62, 69]. Thus, an understanding of proportional source contributions to CEC loads is critical to ensure that these efforts are applied wisely and effectively.

Despite the need to characterize CEC sources, transport, and seasonal/hydrologic variability, relatively few studies have compared mass loadings from different sources under a range of conditions [61-62]. The objectives of the current field-based study were to use a mass balance approach to compare CECs loads, sources, and transport in a mixed-use watershed for different types of CECs under different seasonal and hydrologic conditions. We assessed the loadings of twenty-six CECs at downstream and upstream sites and in WWTP effluent across seven sampling events in the South Fork of the Zumbro River in Rochester, MN, U.S.A. CECs were expected to occur ubiquitously, and we anticipated that groupings would be evident based on CEC behavior, typical use, land uses, and seasonal-hydrologic variations in the study area. This comparative characterization of CEC sources, loads, and transport increases our ability to conduct risk assessments, predict areas of impact, identify aquatic stressors, and mitigate CEC exposure.

6.2 Materials and methods

6.2.1 Study area and CECs of interest

The study area is part of the Zumbro River Watershed (Figure 6-1), in southeastern Minnesota, and encompasses an area of approximately 786 km^2 that includes the City of Rochester. The South Fork of the Zumbro River (SFZR) is a second-order stream that is intersected by only a few other streams in this well-defined, mid-sized, mixed-use, and gently rolling drainage area. Baseflow is contributed by aquifer-surficial interactions and agricultural drain tile. This area is part of the Rochester Plateau, which is characterized by glacial till and loess in the western portion that thins toward the east where some bedrock-controlled and karst features occur. The area receives approximately 84 cm of precipitation annually, with a mean temperature of 20.8°C in summer and -7.5 °C in winter. Near the mouth of this drainage area, the Rochester Water Reclamation Plant uses an activated sludge and chlorine disinfection system to treat the wastewater of approximately 110,000 residents and other commercial/industrial entities, including a world-renowned medical complex with more than 2,000 beds and 225,000 annual outpatient visits. An estimated 15,000 residents use septic systems in unsewered areas. Agriculture accounts for approximately 64% of the study area. Corn and soybeans are the dominant crops. Approximately 212,000 livestock exist on 269 feedlots in the study area

Water samples were collected from four stream sites and from a treated effluent sampling location inside the WWTP. A detailed land use analysis conducted by McGhie Betts, Inc. (Rochester, MN, USA) was previously described [273]. Sites were categorized based on land use characteristics (Table A-1). Sites with >50% associated agricultural area were categorized as having agricultural influences, sites with >5% associated residential/urban area were categorized as having residential/urban influences, and sites meeting both of these criteria were categorized as having mixed uses [101]. Thus, SFZR-US2 was agricultural and Bear Creek, SFZR-US1, and SFZR-DS were mixed-use sites. SFZR-US1 and SFZR-DS have similar drainage areas but were just upstream and downstream of the WWTP discharge, respectively.

Twenty-six CECs were selected for analysis as previously described [273, 299]. These include CECs used primarily in agriculture (herbicides and veterinary

pharmaceuticals), urban/residential applications (PPCPs and industrial/commercial ingredients), and mixed settings (pesticides, phytoestrogens, and pharmaceuticals). CEC characteristics are described in Table A-2.

6.2.2 Sample collection, processing, and analysis

Grab water samples (2-L) were collected from all sites on seven days from March to October 2012 to represent different seasonal and hydrologic conditions. Effluent, upstream, and downstream samples were collected as part of a broader sampling campaign previously described [273-274, 299]. Quantitative precipitation forecasts [300], USGS streamflow data, and USDA crop reports [271] were used to target a snowmelt event (March), the first predicted precipitation event at which at least 90% of corn cropland had been planted (May), late summer baseflow conditions (September), and post-harvest fall conditions (October) for sampling. Equipment, equipment cleaning, chemical standards, and sample collection, handling, processing, and analytical methods were performed as previously described [273-274]. Flow data were obtained from USGS monitoring stations and WWTP records.

6.2.3 Data quality assurance/quality control, mass balance, and statistical analysis

Method reporting limits (MRL, Table A-2) were determined using U.S. EPA methods [275]. Quantification of CECs concentrations and other data quality assurance/quality control procedures were completed as previously described [274, 299] and are summarized in the Table A-2.

Mass balances were used to determine if the CEC loadings measured at SFZR-DS were sufficiently accounted for by those measured at the WWTP effluent and SFZR-US1 sites. The loading through a stream cross-section was modeled as a plug-flow reactor in which the loading into the cross-section equaled the loading out of the cross-section plus or minus mass transformation processes. Uncertainty in chemical mass balances of this type may be introduced by heterogeneous instream conditions and fine-scale temporal variation of CEC concentrations or flow measurements [67]. To complete the mass balance for each CEC and sampling event, the observed instantaneous loading (g/d) at SFZR-DS was calculated and compared to the predicted loading at

SFZR-DS as calculated by: $Loading_{(SFZR\text{-}DS,\ Pred)} = Loading_{(SFZR\text{-}US1)} + Loading_{(Effluent)}$, where $Loading_{(i)} = Concentration_{(i)} * Discharge_{(i)}$.

  To validate the mass balances, the relationship between predicted and observed loading at SFZR-DS was analyzed by a linear regression that included all CECs and events. Wilcoxon Signed Rank tests were used to compare the agreement of all pairs of observed and predicted loadings at SFZR-DS for each CEC (with $\alpha = 0.05$). Loadings to SFZR-DS from the individual upstream sites and WWTP effluent were compared with one-way analysis of variance (ANOVA) on ranks and the Protected Least Significant Difference multiple comparison procedure ($\alpha=0.05$). Data below the MRL were given the lowest rank in the dataset for the respective CEC. Statistical analysis was conducted using SPSS (IBM) and Stata (StataCorp).

6.3 Results and discussion

  Sixteen of the twenty-six studied CECs were detected in water samples. Summary and individual CEC concentration data are presented in the Figure 6-6 and Table A-12. The most frequently detected CECs in this study (>50% detection frequency) were the herbicides atrazine, acetochlor, metolachlor, and mecoprop and the PPCPs caffeine, DEET, acetaminophen, trimethoprim, and carbamazepine. 4-Nonylphenol and the prescription drugs erythromycin, sulfamethoxazole, and carbamazepine were detected at the greatest concentrations (median >100 ng/L). The MRLs varied by CEC, which should be considered when comparing the detection frequencies among CECs in this study

6.3.1 Mass balances

  The agreement of observed and predicted loading was evident in the strong and nearly 1:1 relationship ($r^2 = 0.881$, $p<0.01$) of a scatterplot (Figure 6-2A) of these data for all CECs and sampling events. Comparing instream flows (Figure 6-5) with the regression in Figure 6-2A, it was apparent that the greatest discrepancies between observed and predicted loadings occurred at times of greater flow. When high-flow (March and May) and low-flow (September and October) events were separated, even better agreement of observed and predicted loadings was evident for low flow periods ($r^2=0.988$, $p<0.01$, Figure 6-2B).

Despite the good agreement between all predicted and observed CEC loadings, loading predictions were not significantly accurate in the case of 5 individual CECs, according to the Wilcoxon Signed-Rank test. These included the three agricultural herbicides and two of the prescription/veterinary antibiotics (sulfamethoxazole and tylosin). During high flows, uncertainty in the loading calculations could have been introduced by flow variations, concentration variations (e.g., due to rapid concentration/dilution), or influences of runoff, storm sewers, or ephemeral streams. These variations would have affected the differences between predicted and observed loadings of these 5 CECs more than others because they had greater spatial differences in concentrations that would magnify any flow-based variability. The agricultural herbicides were detected more frequently and at much greater concentrations in upstream samples than in effluent samples. Conversely, sulfamethoxazole and tylosin were detected more frequently and at much greater concentrations in effluent and at SFZR-DS than upstream (data and statistical results in Tables A-12 and A-13

Considering this analysis, the observed and predicted CEC loadings were in good agreement. Comparison of the calculated loadings among different sites was therefore a reasonable first-order estimate of the actual proportional loading from different sources in this area.

6.3.2 CEC groups with similar sources and spatiotemporal loading patterns

The effects of dilution were obvious where the WWTP joins the SFZR. Some CECs occurred at greater concentrations at SFZR-US1 and were diluted by effluent (e.g., atrazine, acetochlor, and caffeine), whereas others occurred at greater concentrations in the effluent and were diluted by upstream flows (e.g., 4-nonylphenol, erythromycin, and carbamazepine). For almost all detected CECs, the median concentrations at SFZR-DS were between those in effluent and SFZR-US1 (Figure 6-6).

Three distinct CEC groupings (upstream-dominated, mixed, or WWTP-dominated) were evident in the concentrations (Figure 6-6) and loadings (Figures 6-3 and 6-4) of CECs by site. For this loading analysis, Bear Creek and SFZR-US2 data were utilized because they represent distinct upstream subwatersheds that together comprise 78% of the study area. Figure 6-4 compares the loading at the downstream site (SFZR-DS) to the aggregated loadings from effluent and upstream sites for each

event and detected CEC. Clearly, flow or other temporal dynamics affected not only the total loadings but also the dominant sources/transport of certain CECs in the watershed. At SFZR-DS, the greatest loadings of all but one CEC (4-nonylphenol) occurred in high-flow periods (May or March) compared to low-flow periods (September-October). Also, most CECs showed notable differences between SFZR-DS loading and the aggregated loading from other sites for one or more high flow (May/March) events. Subtler effects of flow variation appear to depend on which dominant transport group the CEC fell into.

6.3.2.1 Upstream-dominated sources and transport

As expected, upstream sources dominated for the agricultural herbicides and daidzein (a phytoestrogen). Atrazine, acetochlor, and metolachlor were among the most frequently detected CECs at all stream sites, with much greater concentrations and loadings upstream than in effluent (Figures 6-3 and 6-6). Atrazine was detected in all effluent samples but always at smaller concentrations than at every instream site. Acetochlor was detected in two effluent samples, while metolachlor was not detected in effluents. Daidzein was detected less frequently than the herbicides at all sites and was not detected in effluents. More than 95% of the total loading of each of these CECs was contributed by the aggregated (SFZR-US2 + Bear Creek) upstream areas (p<0.05, Table A-13).

Spatiotemporally, the loadings of these CECs were greatest from upstream areas and during high flows (Figure 6-4). Increased loadings of all agricultural herbicides at all instream sites on May 10th likely resulted from the temporal proximity of their application to 4cm precipitation on May 4th-6th (Figure 6-6). Increased loadings were evident to a lesser degree on May 3rd and 17th, which also showed higher flows and corresponded with seasonal application periods of these herbicides in Minnesota [301]. In low-flow periods, total loadings of these runoff-associated CECs were greatly reduced.

At SFZR-US2 on October 29th, a hydrograph peak associated with post-harvest precipitation (Figure 6-5) affected the concentration and loading of atrazine but not the other herbicides (Figure 6-4). Atrazine did not show this pattern at other sites on October 29th or at any site for the other September/October sampling events. Fall applications of atrazine to croplands are not approved in Minnesota [301]. Nonetheless, atrazine has been detected year-round in Minnesota precipitation [168] and is

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considered to be more persistent and mobile than acetochlor or metolachlor [169, 171]. October precipitation must have mobilized terrestrial fractions persisting from earlier in the growing season or resulted in wet deposition, explaining this peak.

6.3.2.2 Balanced/mixed sources and transport

Balanced/mixed sources and transport were evident for mecoprop and several of the PPCPs (caffeine, acetaminophen, cotinine, DEET, and trimethoprim). Indeed, their loadings were more balanced between upstream sites and effluent than were those of the other studied CECs (Figure 6-3). Except for caffeine, their mean concentrations in effluent were equal to or greater than at SFZR-US1, but within an order of magnitude (Figure 6-6, Table A-13).

Spatiotemporal loading patterns also indicated mixed sources and transport routes (i.e., runoff and effluent) of these CECs (Figure 6-4). Runoff influences were demonstrated by greater total loading and greater contributions from upstream areas during high flows. Effluent influences were demonstrated by a greater proportion of effluent loading to total loading during low flows, and smaller changes to total loading between high and low flows, compared to the upstream-dominated CECs. Effluent loadings of most of these CECs increased during high flows and remained influential even as their upstream loadings increased. The peak loadings of these CECs in May and March thus appear to result from a combination of greater effluent and greater upstream loadings at these times.

Among these CECs, caffeine showed the greatest proportional upstream influences. Mean concentrations of caffeine were greater at upstream sites than in effluent (Figure 6-6), and 75% of its mean total loading was due to transport from aggregated upstream areas (Figure 6-3, p=0.001). When comparing individual sites, the loadings of caffeine from Bear Creek, SFZR-US2, and effluent were not significantly different (p=0.481). The total loading of caffeine was dominated by upstream sources during low flow periods, with upstream and effluent sources showing balance during high flows (Figure 6-4). Caffeine showed pronounced differences between SFZR-DS loading and aggregated loading from the effluent and upstream sites for several high flow events (Figure 6-4). This patterns was evident to a lesser degree for some other CECs (e.g.,

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cotinine and carbamazepine) and may suggest additional unmeasured instream sources at these times.

Effluent loadings of acetaminophen, trimethoprim, and mecoprop were not significantly different than aggregated loadings from upstream sites (p= 0.687, p=0.247, and p=0.059, respectively) when considering all events (Figure 6-3). When comparing effluent to the individual sites, loadings of acetaminophen and mecoprop in effluent were greater than at either SFZR-US2 (p=0.020 and p= 0.002, respectively) or Bear Creek (p=0.027 and p=0.028, respectively). Loading of trimethoprim in effluent was greater than at Bear Creek (p=0.004) but not SFZR-US2 (p=0.108). Effluent loadings of cotinine and DEET were significantly greater (p=0.015 and p=0.004, respectively) than loadings from aggregated and individual upstream sites.

In contrast caffeine, the total loading of acetaminophen and other mixed-source CECs was effluent-dominated in low flow periods (Figure 6-4). During high flows, however, total loadings of acetaminophen were much greater and upstream sources dominated. Mecoprop, trimethoprim, and DEET also showed these patterns of increased upstream and total loading in high flows, but their upstream loadings did not typically exceed effluent loadings.

Mecoprop concentrations and loadings showed a pulse at all instream sites in October events that were associated with precipitation (Figure 6-6). Also, mecoprop was the only CEC that showed significantly greater loading (p=0.023) at SFZR-US1 compared to the aggregated loading from the further upstream sites (Bear Creek and SFZR-US2). Together, these patterns suggest urban inputs via storm sewers or direct runoff of this primarily residential herbicide in Rochester [280].

Interestingly, the greatest effluent concentrations of all of these "mixed-source" CECs occurred during (high-flow) May events (Table A-12). Effluent concentrations of caffeine in high flow were almost double those in the low flow periods. This pattern also held true for atrazine but for none of the WWTP-dominated CECs. WWTP influent flow rates in early May were the highest that had yet occurred in 2012. The greater effluent concentrations of the mixed-source CECs and atrazine could have resulted from reduced WWTP removal efficiencies (due to reduced hydraulic retention time or temperature) or increased loadings to the WWTP from additional terrestrial sources such as leaky sewers during high flow periods. The latter would explain why the effluent

concentrations of the mixed-source CECs and atrazine increased during high flows while those of effluent-dominated CECs did not.

6.3.2.3 WWTP-dominated sources and transport

Indoor urban use and subsequent effluent transport was evidently the primary source of the remaining prescription pharmaceuticals (sulfamethoxazole, erythromycin, carbamazepine, and tylosin), surfactant metabolite (4-nonylphenol), and mixed-use insecticide (carbaryl) to the watershed. Loadings (Figure 6-3) and concentrations (Figure 6-6) of these CECs were at least an order of magnitude greater in effluent than from upstream sites ($p < 0.05$, Table A-13). At the downstream site (SFZR-DS), the loadings of 4-nonylphenol, sulfamethoxazole, erythromycin, and carbamazepine were among the greatest of the detected CECs (Figure 6-4). These CECs were detected in all effluent and SFZR-DS samples, while upstream detections were infrequent and at much smaller concentrations (Figure 6-6, Table A-12). Likewise, carbaryl was only detected in three instream samples (all at SFZR-DS), which coincided with effluent detections on two occasions. Although tylosin only has veterinary applications, it has been detected in WWTP influents and effluents in the United States [145] and elsewhere [146-147]. Thus, tylosin detections in WWTP effluent in this study suggest wastewater inputs from veterinary clinics or pet owners, or infiltration of CAFO runoff or wastewater to the municipal sewer system [145].

Compared to the upstream-dominated and mixed-source groups, the proportional loading and pathways of the WWTP-dominated CECs to the watershed were not as affected by high-flow events. Like the other groups, the total loadings of WWTP-dominated CECs were generally greatest in higher-flow (May or March) events. Nonetheless, their total instream loadings were overwhelmingly dominated by effluent in all flow conditions. Effluent loadings of erythromycin and tylosin were relatively consistent across sampling events, with ranges that varied by less than a factor of two. Effluent loadings of 4-nonylphenol and carbamazepine were greatest in September/October when those of most other CECs were smallest. For 4-nonylphenol, greater hydraulic retention times and temperatures in the WWTP may have served to enhance microbial transformation of nonylphenol ethoxylates to 4-nonylphenol at these times [62].

6.3.3 Research implications

Two related SFZR studies analyzed larger sample sets, focused on CEC concentrations, and did not include WWTP effluents or mass balance assessments [273, 299]. In those studies, the concentrations of agricultural herbicides were explained by agricultural runoff sources. Clustering of some prescription and non-prescription PPCP concentrations (e.g., carbamazepine and cotinine) was explained by urban wastewater sources. A final cluster, of other non-prescription PPCP and mixed-use CEC concentrations (e.g., acetaminophen and trimethoprim) was observed and attributed to a mixture of urban/wastewater and runoff sources. In the current study, the analysis of effluent samples and instream mass balances confirms similar groupings of CECs by source/transport. The current study also provides a better understanding of the proportional loading from the WWTP and upstream drainage areas to the total downstream load of CECs.

Agricultural herbicides have been commonly observed to have diffuse sources and greater concentrations or loads in agricultural areas during growing seasons and other periods of application and runoff [166, 168]. As anticipated, the current study also showed that transport of agricultural herbicides was clearly dominated by upstream sources. The much smaller loadings observed in September/October in this study accord with the expected low flows and concentrations at that time.

The WWTP exerted a year-round influence on the CECs at SFZR-DS, but the magnitude and relative influence of the effluent loading on downstream loadings varied temporally and by CEC type. Non-prescription PPCPs frequently occur in a wide range of settings influenced by runoff or other diffuse sources as well as WWTPs [32, 59-62, 151, 153, 197, 273-274, 299, 302]. In this study, the mixed sources and transport of these CECs were illustrated by additional non-WWTP loading from upstream sources in high-flow periods when effluent comprised a smaller portion of downstream flow. This has been observed elsewhere [60]. Similarly, two studies of WWTP effluents and upstream/downstream sites in multiple streams (including the SFZR) reported influential upstream sources of many CECs (e.g., acetaminophen and caffeine) [32, 59]. The loading of these widely used CECs in upstream areas may have resulted from their greater usage and mobility compared to prescription pharmaceuticals. Acetaminophen, caffeine, and cotinine have smaller K_{ow} and much greater solubilities than most of the

studied prescription pharmaceuticals (Table A-2). This may therefore lead to enhanced transport from diffuse sources such as septic systems and land-applied manure, sludge, or biosolids.

Prescription PPCPs often exhibit enhanced concentrations at WWTP-influenced sites [32, 153, 197] and in low-flow periods. The latter is often explained by concentration-dilution effects [47, 151, 166]. Human-use pharmaceuticals were anticipated to enter this watershed primarily via the WWTP and secondarily via septic systems, runoff of land-applied biosolids, or other sources. Mixed-use and veterinary pharmaceuticals were expected to occur due to transport from agricultural fields, the WWTP, or other secondary sources. Trimethoprim and tylosin showed some temporal patterns that suggest veterinary sources (e.g., greater concentrations and loadings during high flows). Except for trimethoprim, however, the detection frequencies and/or concentrations of all prescription pharmaceuticals were far greater downstream than upstream of the WWTP. Loading analysis confirmed that effluent transport dominated their loading to the watershed. Thus, while non-WWTP sources of antibiotics may exist in the watershed, except for trimethoprim, they did not significantly influence the study sites. Trimethoprim is more persistent than the commonly co-prescribed sulfamethoxazole, which may explain its greater instream detection frequencies and transport [41]. Further study with additional sites could determine whether antibiotic ratios vary with downstream distance from the WWTP in this watershed.

6.3.4 Recommendations for monitoring and risk assessment

Many CEC monitoring studies have been conducted in surface waters [14-15, 57-58], but quantitative temporal and statistical comparisons of CEC loadings and transport from different sources are relatively rare [60, 62, 68-69]. The methods of sampling and analysis used in this study allowed the differentiation of myriad sources of CECs in a complex system and therefore should be transferrable to other monitoring efforts for proportional source assessments. For risk assessments and programs targeting peak CEC concentrations or loadings, upstream-dominated CECs should be monitored during high-flow events and seasons because the increased concentrations that occur at these times will also represent the greatest loadings. Also, monitoring in upstream areas is advisable for risk assessments of agriculturally-associated CECs, as

the potential for dilution by WWTP effluents exists. WWTP-dominated CECs should be measured in low-flow periods if instream exposures are of concern; relatively consistent loading can be expected year-round. Monitoring at watershed collection points below WWTPs is suitable for screening assessments of WWTP-dominated and mixed-transport CECs. If mass balance assessments, temporal sampling, or other monitoring suggests upstream sources of mixed-transport CECs, then upstream and seasonal monitoring in light of land use data may help track their sources.

This study demonstrated that the majority of loadings and concentrations of several CECs that are typically identified as effluent-associated were indeed transported via the WWTP (e.g., 4-nonylphenol, carbamazepine, and sulfamethoxazole). These included the CECs that had the greatest downstream concentrations and loadings of studied CECs. Thus, source controls or additional WWTP-based treatment may be recommended. Further study would be necessary to ascertain whether these CECs remain proportionally elevated compared to other CECs further downstream. Likewise, concentrations and loadings of agricultural herbicides were predominantly a result of higher-flow periods when usage is greater. Significant upstream and flow-based influences were also evident for some PPCPs (e.g., trimethoprim, DEET, and caffeine). For these CECs, mixed sources and transport suggest that WWTP retrofits alone may not sufficiently reduce their loads. There is evidence that some agricultural and stormwater best management practices may prevent mobilization and promote attenuation of certain CECs through filtration, sorption, and biodegradation [303]. These practices may be useful in concert with increasingly proposed CEC source reductions and WWTP-based mitigation strategies, especially in mixed-use areas where additional sources, high-flow events, and runoff components are likely to be important.
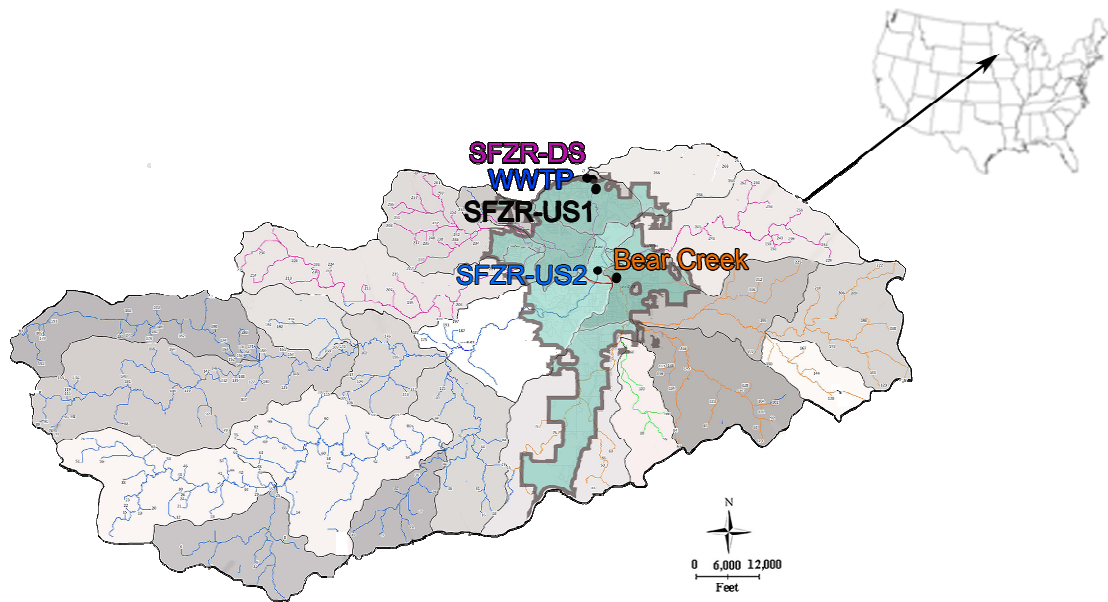
Figure 6-1. Map of the SFZR study area. Labels indicate sampling site locations. Streams that influence each sampling site are color coded – Bear Creek = Orange + Green, SFZR-US2 = Blue, and SFZR-DS and SFZR-US1 = All colored streams. The SFZR generally flows northeast, joins with Bear Creek just downstream from SFZR-US2, and then flows toward SFZR-US1 and SFZR-DS. The city of Rochester is indicated by the green shaded area. WWTP = wastewater treatment plant
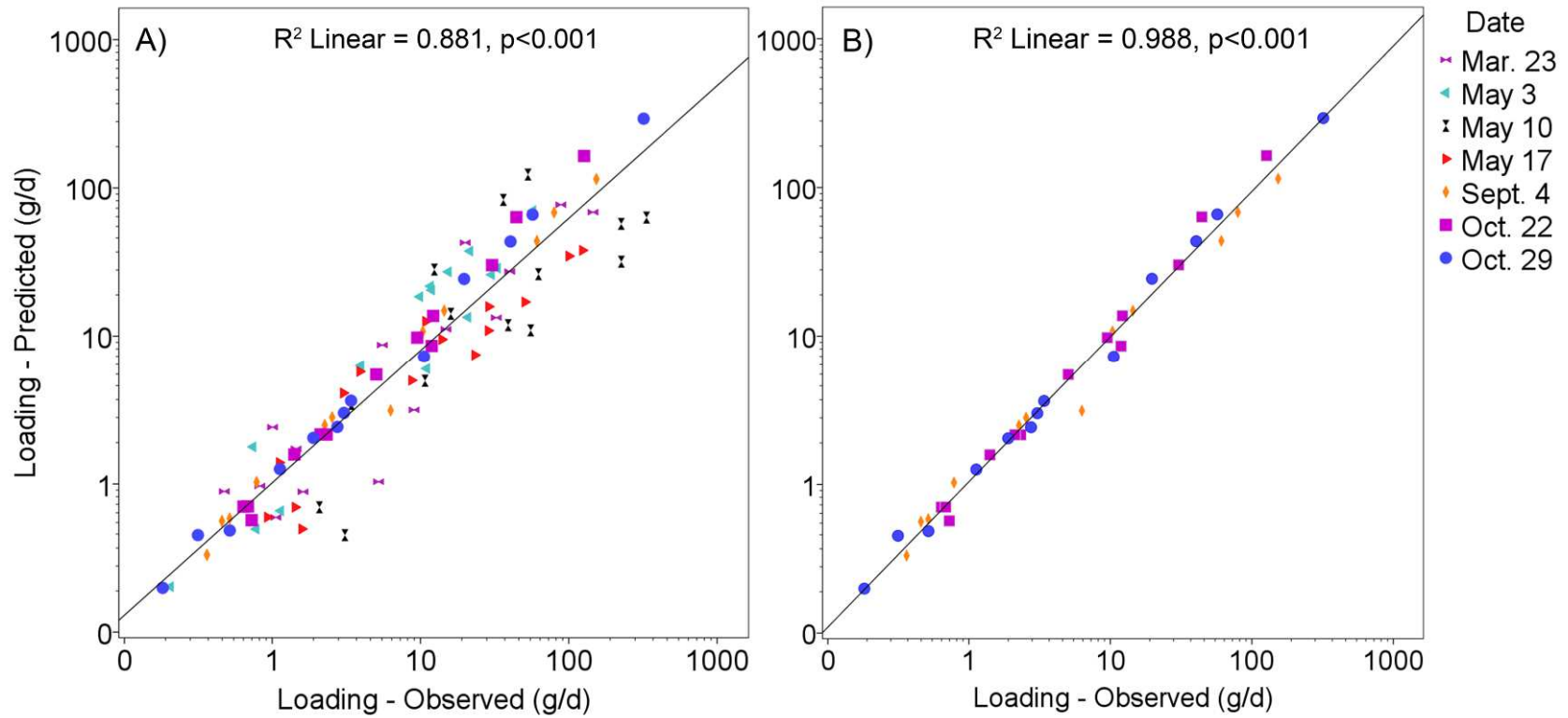
Figure 6-2 (A,B). Scatterplots of predicted versus observed loading of individual CECs at SFZR-DS for (A) all events and (B) low flow events only.

Predicted loading was calculated as the sum of loadings from the wastewater treatment plant and upstream (SFZR-US1) samples.
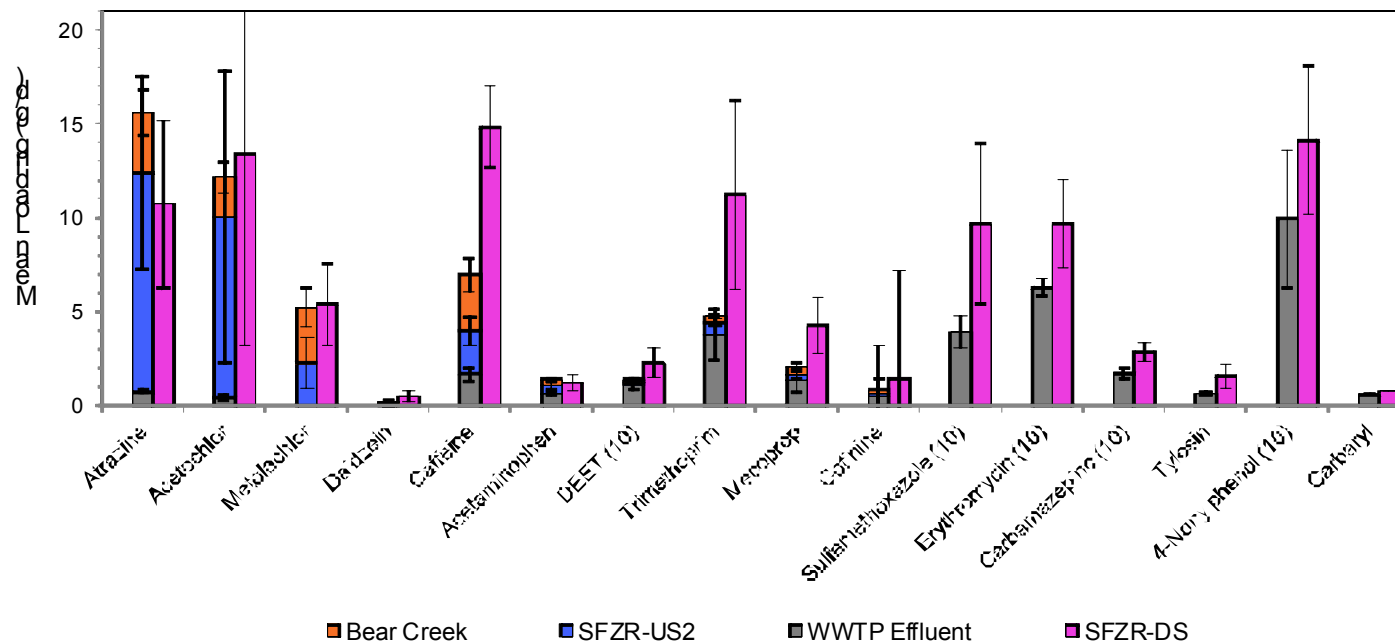
Figure 6-3. Comparison of mean CEC loadings from upstream sites and the downstream site. The graph displays the mean loadings (g/d) of CECs measured at the downstream site (SFZR-DS) versus additive loadings (stacked bars) measured in the effluent and upstream sites across all events. A (10) in the x-axis label of a given CEC indicates that its actual concentrations were 10 times greater than the displayed concentrations for that CEC (rescaled for display purposes). Error bars indicate standard error of the mean. SFZR = South Fork of the Zumbro River.
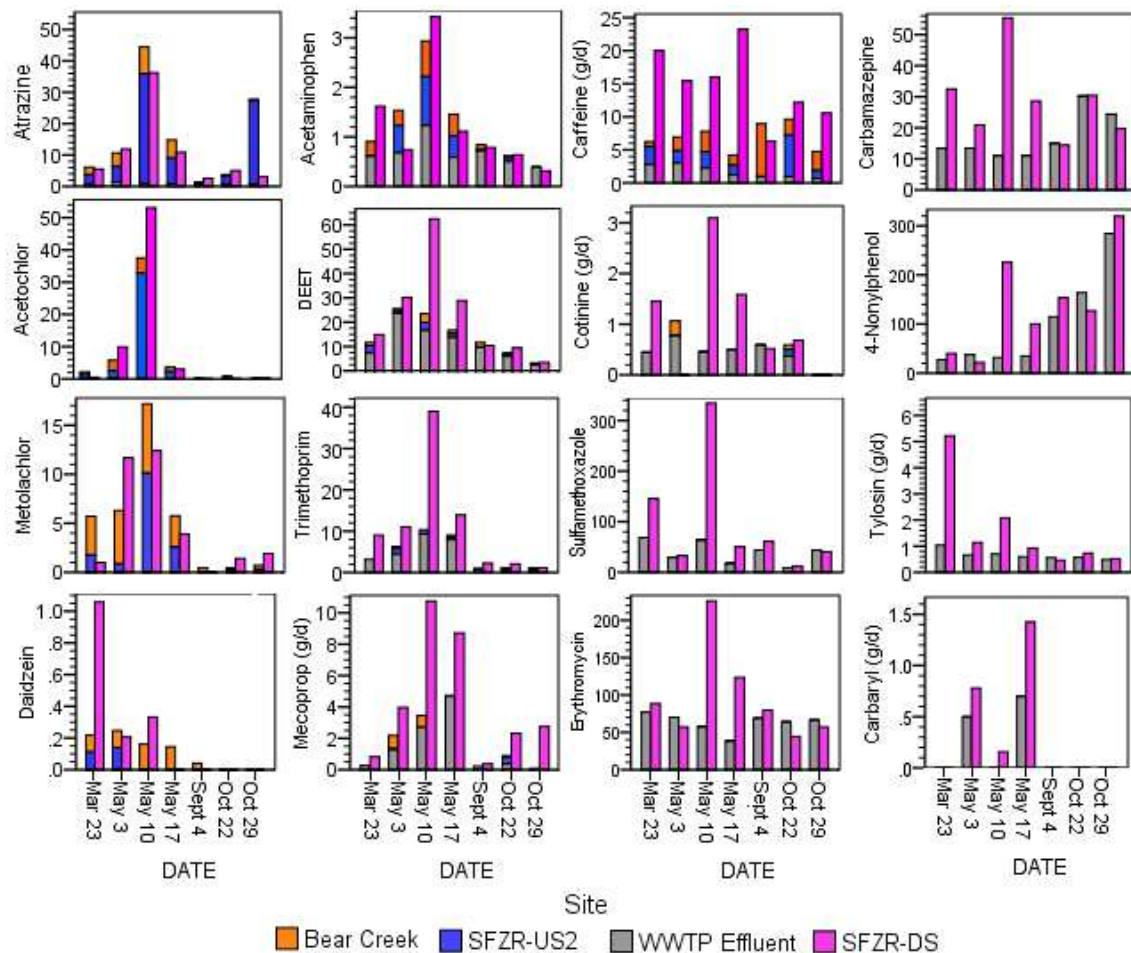
Figure 6-4. Comparison of CEC loadings from upstream sites and the downstream site over time.
The graphs displays the loadings (g/d) of contaminants of emerging concern (CEC) at the downstream site (SFZR-DS) versus additive loadings (stacked bars) from effluent and upstream sites (Bear Creek and SFZR-US2) by site for each event. The stacked bar in each cluster shows the loadings from wastewater treatment plant effluent and upstream sites. The second (purple) bar in each cluster represents the loading at SFZR-DS. SFZR = South Fork of the Zumbro River.
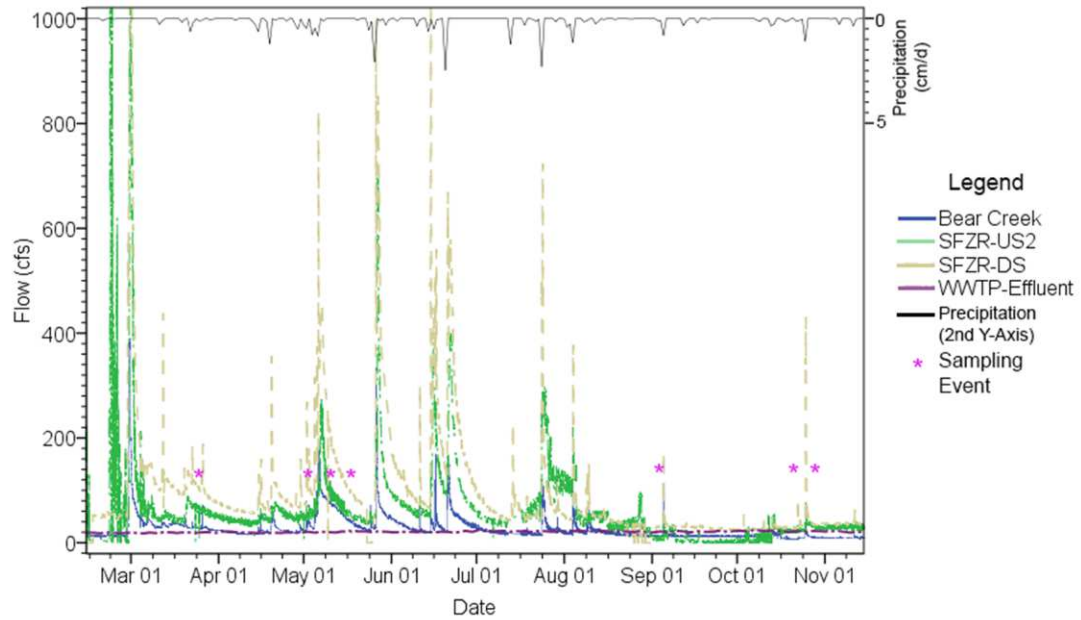
Figure 6-5. Instream flow and precipitation, March-November 2012. The graph displays the flow rates (primary y-axis) at stream sites and from the Rochester Water Reclamation Plant and precipitation (secondary, inverted y-axis) in Rochester during the 2012 sampling events. Sampling events are indicated with pink asterisks (*) in the figure. SFZR = South Fork of the Zumbro River. Flow and precipitation data were obtained from the USGS National Streamflow Information Program, the Minnesota Department of Natural Resources/Minnesota Pollution Control Agency Cooperative Stream Gaging Program, and the Rochester Water Reclamation Plant staff, and the Minnesota Climatology Working Group.
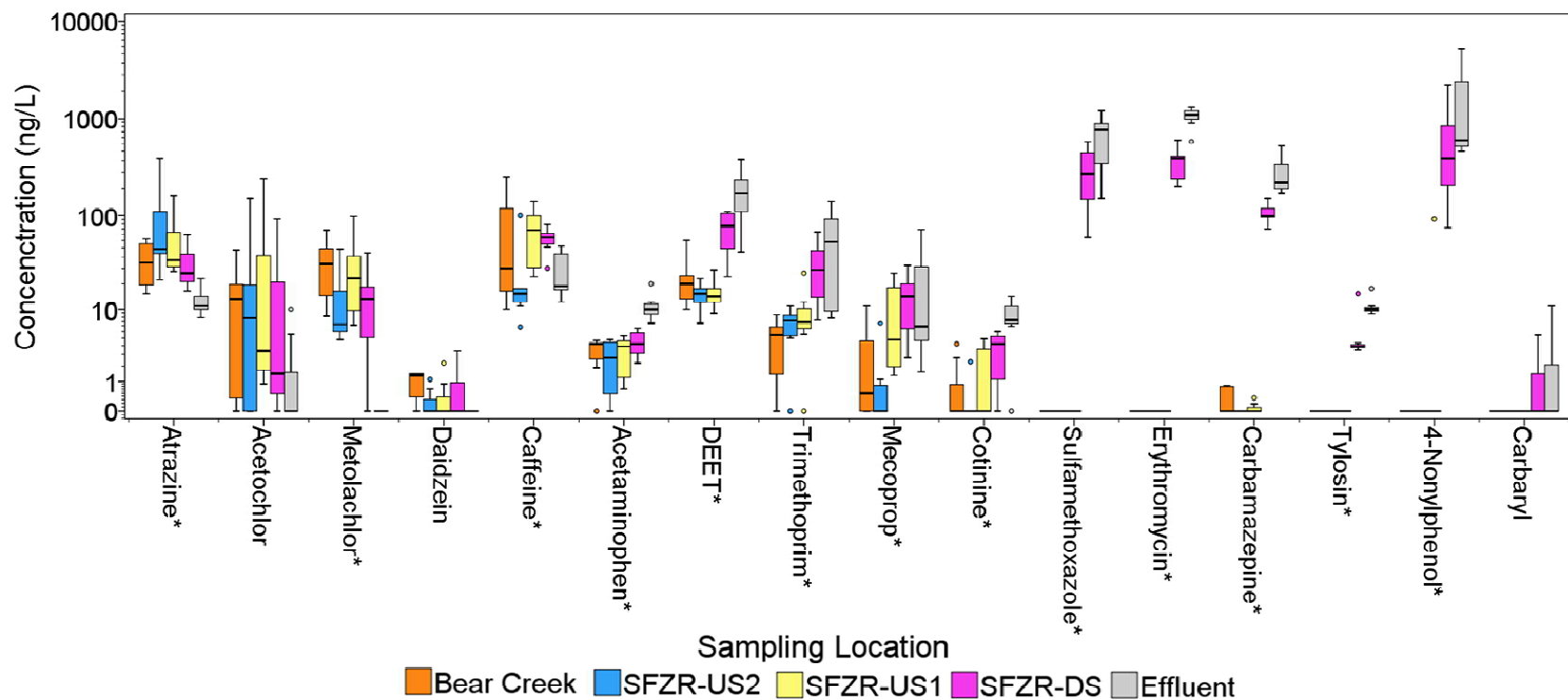
Figure 6-6. Box plots of concentrations of contaminants of emerging concern (CEC) measured in 35 water samples collected from four instream sites and wastewater treatment plant effluent in the SFZR study area. Boxes represent the interquartile ranges (IQR) of concentration data for each CEC by site. Medians concentrations are represented by the black lines inside each box. Whiskers extend to the minimum and maximum values, up to 1.5 times the IRQ from each box. Circles indicate values beyond that range. Data below the method reporting limit were ranked lowest in the statistical analysis and given a value of zero for the graphical representation. Compounds with an asterisk (*) in their x-axis label showed significant differences in concentration across sites by analysis of variance on ranks (data and p-values in Table A-12 and A-13).

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CHAPTER 7. Project Synthesis

7.1 Introduction

      Understanding the spatiotemporal occurrence patterns of contaminants of emerging concern (CECs) is crucial for assessing the sources, context, and impacts of CECs [47, 61, 197]. Tens of thousands of organic chemicals have been identified as CECs, which is considered a conservative estimate [70]. This multitude of compounds is associated with numerous and sometimes overlapping sources and transport routes, and a wide range of physicochemical properties and associated fates. Despite numerous studies, significant gaps remain in our knowledge of CEC sources, occurrence, fate, and transport in complex environmental systems [24, 57, 60-62, 67-68, 70, 75, 185].

      This study characterized the sources, transport, seasonality, and sediment-water distributions of a diverse set of CECs in a mixed-use watershed. Concentrations and loadings of CECs in water and sediment samples were analyzed in light of spatial/land use, seasonal, hydrologic, and physicochemical variables. Data analysis was completed with a range of statistical and graphical methods. Distinct groups of CECs that exhibited similar instream profiles and sources were identified based on statistical linkages, proportional source loading, spatiotemporal patterns in water concentrations and loading, and sediment-water distributions.

7.2 Findings

      Three distinct CEC groups emerged with respect to their patterns of detection, concentration, and loading via the different sample sets, research questions, and methods of analysis of this study. These overarching patterns underscored the results of the individual chapters. The CECs within each group demonstrated similar sources, seasonality, transport, and variability thereof. Effluent-dominated sources and transport explained the detections, concentrations, and loadings of one group (i.e., sulfamethoxazole, carbamazepine, erythromycin, tylosin, carbaryl, and 4-nonylphenol). Agricultural/upstream-dominated sources and transport explained those of a second group (i.e., atrazine, acetochlor, metolachlor, and daidzein). The third group showed mixed sources and transport that varied somewhat by the typical use and properties of the CEC (i.e., acetaminophen, caffeine, trimethoprim, DEET, cotinine, and mecoprop).

The effects of site, season, and other hydrologic parameters (i.e., flow and precipitation) on occurrence, concentration, and loading were evident for many CECs in water and provided resolution among the groups of CECs.

Agricultural CECs showed similar within-group behavior, and greater concentrations and loadings from agricultural sites and during periods of herbicide application and high flows. More than 90% of the downstream loading of the each of the CECs in this group was due to upstream sites compared to effluent.

The effluent-dominated CECs showed the almost singular influence of the WWTP on their instream profiles. Spatially, the concentrations and loadings of each of these CECs in WWTP effluent were at least an order of magnitude greater than from any of the upstream sites (including the Bear Creek site, which had similar population density and percentage of developed land but was upstream from the WWTP discharge). Likewise, temporal patterns of the effluent-dominated group were demonstrated by similar/greater concentrations in low flows compared to high flows, and consistent loadings across seasons and flows in comparison with other CECs.

The mixed-sources/transport group showed concentrations and loadings that were significantly greater at the mixed-use sites (SFZR-DS and Bear Creek) compared with the agricultural sites, but were also more spatially balanced than the other groups. The proportional loadings of this group did not differ by more than an order of magnitude between effluent and combined upstream sites, in contrast with the agricultural and effluent-dominated groups. Also, the temporal patterns of this group indicated mixed sources and transport. For example, effluent inputs were continually evident, but concentrations and loadings from upstream sites increased during high flows, which demonstrated the influence of runoff or other upstream transport. Thus, the results of the spatiotemporal analysis for this group suggest mixed sources and pathways such as septic systems, stormwater, or runoff of land-applied biosolids in addition to the WWTP.

This study indicates that CECs are present in urban and agricultural sediments, even those CECs traditionally thought of as non-sorptive. As in water, the effects of land use on sediment concentrations were evident. Acetaminophen, caffeine, carbamazepine, and DEET concentrations in sediment concentrations were greatest at mixed-use sites (Bear Creek and/or SFZR-DS). For acetochlor, the greatest sediment concentrations, water concentrations, and aqueous loadings were evident at Bear

Creek, which has 52% agricultural area. Sediment concentrations of atrazine were greatest at an agricultural site (Willow Creek). Seasonal patterns of CEC concentrations were not evident in sediment, but some CECs showed changes in sediment concentrations between years. This agrees with the characterization of the sediment phase as an integrative marker of contamination. The more polar and labile CECs (e.g., caffeine, acetaminophen, DEET, and acetochlor) showed significant variability in their observed sediment-water distribution coefficients across sites and seasons, which was mainly due to variability in water concentrations. These CECs were also distributed to sediment in much higher proportions than would be predicted based on their hydrophobic activities alone. In contrast, the sediment-water distributions of the less polar and more persistent CECs (e.g., atrazine and carbamazepine) were less spatiotemporally variable and were well-predicted by octanol-water partition coefficients derived in laboratory studies.

7.3 Implications for research

The capacities to characterize and track contaminant sources are crucial to our abilities to identify and mitigate pollution [220]. Recent studies have identified numerous patterns and determinants of CEC occurrences in urban, agricultural, and mixed-use areas. Nonetheless, the sources and transport of CECs are not fully characterized or consistently predictable in freshwater systems. This is understandable considering the multitude and diversity of CECs that have been identified and the complexity of natural systems. Source characterizations and comparisons were recently identified by MPCA staff as priority research topics regarding CEC occurrences that remain to be addressed [304].

Further CEC source and transport characterization studies are thus warranted. The design elements that were included in this study (e.g., multiple sites, discrete drainage areas, discharge measurements, land use data, and multiple events and sample types) should be applied in studies in other watersheds to verify and refine the observed spatiotemporal and source characterizations. Linkages between sources and instream occurrences should be investigated by directly sampling sources, other off-stream matrices (e.g., land-applied biosolids, manure lagoon slurries, urban runoff, agricultural tile drainage, and septic systems), and receiving matrices (e.g., river water,

river sediment, and fish tissues). Agricultural and urban stormwater BMPs should be assessed for their performance at preventing CEC transport to receiving waters. Mass balances that include additional flow pathways and matrices would serve to refine CEC source characterizations. For example, the inclusion of WWTP influent samples could be used to assess per-capita outputs and WWTP removal efficiencies for various CECs, and thus provide additional context for the influence of the WWTP on instream loading. Proportional source assessments that include population data could determine whether the per-capita loading of CECs to receiving waters is affected by the transport route (e.g., WWTP discharge or other urban/residential transport). Analysis of additional sites would indicate if proportional source loadings of CECs remain similar at locations further downstream from the WWTP, along with the relative influences of fate processes for different CECs. Correlation of CEC concentrations and loadings with effect screening data (e.g., genomic or metabolomic parameters) would be valuable for linking specific CECs and sources to effects.

7.4 Implications for monitoring and risk assessment

The importance of appropriate study designs to assess diverse CECs was highlighted by the variety of spatiotemporal patterns of CEC loadings and concentrations evident in this research. This study demonstrated the value of appropriate data reduction and statistical analysis for identifying patterns in a robust field dataset. The results provided new understanding of how certain types of CECs behave in this mixed land-use system.

The mass balance approach utilized in this study showed that proportional source loading of CECs can be quantified when discharge and land use data sufficiently represent the watershed of interest. Mass balances and spatiotemporal analysis confirmed the typically reported dominance of the WWTP on the instream profiles of several CECs (e.g., 4-nonylphenol and carbamazepine). Moreover, the downstream concentrations and loadings of these CECs were among the greatest of all studied CECs. Also, some veterinary and mixed-use antibiotics (e.g., sulfamethoxazole, erythromycin, and tylosin) that have been previously detected in agricultural or other non-WWTP-associated areas were WWTP-dominated in this study. In contrast, several PPCPs (e.g., caffeine, acetaminophen, DEET, and trimethoprim) that have been

reported elsewhere as wastewater-dominated showed additional influential sources in this study.

Aqueous persistence influenced CEC profiles in water and sediment and should be considered when designing field studies to properly characterize CEC sources, fate, transport, and risk. Some more persistent CECs showed greater detection frequencies across seasons or sites than more labile CECs of the same category. For example, atrazine is more persistent than acetochlor and was more frequently detected in all seasons and sites. The same was true for trimethoprim in relation to sulfamethoxazole. In contrast, caffeine is readily degradable but was nonetheless ubiquitous in this study. Moreover, caffeine and acetaminophen were detected in more than 80% of water and 90% of sediment samples. This suggests continual, long-term, and diffuse inputs that lead to pseudo-persistence of these CECs in both phases. If long-term sources and occurrences of CECs are of interest, monitoring of the sediment matrix will be beneficial. Sediment may provide a more stable marker of CEC sources and land use influences with fewer samples than would be required to ascertain similar differences with water samples. However, lack of detection in the sediment phase is not necessarily predictive of lack of detection in the water phase.

A "one-size-fits-all" approach to CEC monitoring may be unlikely to adequately capture the variability of different classes of CECs, especially if only a few grab samples are collected. Our results indicate that CEC monitoring studies should measure concentrations and loading and incorporate seasonal/hydrologic variability, especially when assessing CECs that have runoff-associated or mixed sources and transport. The inclusion of a range of seasonal conditions was important in understanding the influences of these sources over time. For studies assessing peak concentrations or loading, runoff-associated CECs should be monitored during high-flow periods at upstream areas when the greatest concentrations and loadings will occur. Effluent-associated CECs should be monitored in low-flows to show the greatest detectability and potential concentrations of concern. Mixed-source/transport CECs should be monitored in a range of temporal conditions. If mixed-use CECs are detected at watershed collection points, then source characterization should assess WWTPs and upstream areas.

Our results demonstrated that certain CECs may indeed serve as markers of related CECs or of specific sources related to CEC contamination or water quality impairments (e.g., metal, nutrient, biotic). The utility of carbamazepine as a recalcitrant marker of urban/residential land uses was confirmed by its high concentrations and loadings at SFZR-DS and its frequent low-level detections at Bear Creek. Sulfamethoxazole may be a useful labile wastewater marker based on its exclusive detections at SFZR-DS and in effluent. Metolachlor or acetochlor should be considered over atrazine as markers of agricultural influences. Although they are not ideal WWTP markers, acetaminophen and trimethoprim were associated with mixed-use over agricultural areas, and may thus represent diffuse urban/residential sources such as septic systems, stormwater discharges, or land-applied sludge.

Effective and efficient risk assessment and pollution mitigation strategies require information on CEC sources and transport [209], yet significant gaps remain in these areas [73, 113, 186-189]. While we understand that myriad sources contribute CECs to surface waters, relatively little work has quantified CEC sources in mixed-use [12, 61-62, 72] and agricultural areas [57, 60-61]. The source characterizations and comparisons that were developed in this study provided insight on locations and times that may show increased concentrations and loadings of different types of CECs. This information can be used to target locations for contaminant or effect-based monitoring, to identify particular CECs of concern in a given setting, and to track CEC sources in impacted areas. This exposure and risk assessment knowledge can in turn be applied to focus mitigation efforts (e.g., pollutant prevention, fish consumption advisories) on locations, CECs, sources, and transport pathways that may pose the greatest risks and opportunities for risk reduction. If certain CECs are deemed candidates for regulation, these tools and data will assist in stressor identifications, source assessments, load allocations, and implementation and monitoring activities.

Together, the outcomes of this study have characterized the dominant sources, temporal patterns, and clustering behavior in the aqueous occurrences, concentrations, and loadings of a diverse set of CECs in this mixed-use watershed. We have confirmed some previously observed patterns in the instream profiles of agricultural and wastewater-dominated CECs. For the wastewater-dominated CECs, source controls or additional WWTP-based treatment may be recommended. For the agricultural and

mixed-source/transport CECs, WWTP retrofits alone may not sufficiently reduce their loads. This suggests that pollution prevention efforts that target upstream sources and transport may be needed if mitigation of these CECs is desired. Agricultural and stormwater best management practices may be useful in concert with source reductions and WWTP-based mitigation strategies in agricultural or mixed-use areas where additional sources and transport mechanisms will likely be important [303].

While some CECs have previously shown ubiquitous and mixed sources that were difficult to characterize [61, 69], we have shown that spatiotemporal patterns in concentration and loading can be used to trace their sources and transport. Therefore, prediction of influential source, pathways, and events is possible even for ubiquitous CECs. These methods and characterizations can be applied in a wide range of settings to inform CEC monitoring, models, screening/risk assessments, and pollution prevention/mitigation strategies. This will increase our ability to predict impacts and allow us to focus future efforts on relevant CECs and their likely sources and transport to surface waters.

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APPENDIX

Table A-1. Characteristics of South Fork of the Zumbro River (SFZR) sampling sites and associated drainage areas. WWTP = wastewater treatment plant.

Sampling Sites	Characteristics of Associated Drainage Area							
Site	Median Flow [a] (m^3/s)	Area (km^2)	Land Use Category	Extent Developed (%)	Agricultural Extent (%)	Est. Human Pop. (% Sewer/%Septic) [b]	Human Pop. Density (ppl/km^2)	Livestock Types (Pop.)
Willow Creek	0.08	18.7	Agricultural	2.6	53.2	432 (15/85)	23	Poultry (35k)
Bear Creek	1.5	212	Mixed	12.0	52.3	28.2k (75/25)	133	Poultry (50k)/Swine (20k)/Cattle (7k)
SFZR Upstream 2 (SFZR-US2)	3.7	404	Agricultural	3.0	73.3	13.2k (70/30)	33	Poultry (8k)/Swine (47k)/ Cattle (12k)
SFZR Upstream 1 (SFZR-US1)	5.1	785	Mixed	8.1	64.9	97.2k (85/15)		Poultry (101k)/Swine (68k)/Cattle (22k)
WWTP Effluent	0.62	142	Urban/Residential	64.1	23.6	106.8k[c](100/0)	124	N/A[d]
SFZR Downstream (SFZR-DS)	5.7	786	Mixed	9.4	63.9	97.2k (85/15)	122	Poultry (101k)/Swine (73k)/Cattle (22k)/Mink (13k)

[a] Average flows are for 2011-2012.

[b] Percent sewer or septic indicates the portion of the given population using municipal sewer or private onsite sanitary (septic) systems, respectively.

[c] the population served by the WWTP is greater that of the SFZR drainage area because a portion of the WWTP service area lies outside of the SFZR drainage area

[d] N/A: not applicable

Table A-2. Quality assurance/quality control data and selected characteristics of studied compounds.

Compound	Primary Use - Class	CAS#	MRL (ng/L), grab and composite water samples	Laboratory Spike Recovery (%)	RSD, laboratory spikes (n=7)	Matrix Recovery, mean (%) (n=4)	RPD, mean, duplicate water sample analyses (n=3 pairs)	Solubility (mg/L)	Log K_{ow}	pK_a
Acetochlor	Agricultural - Herbicide	34256-82-1	0.89/1.8	97.8	2.6	72.6	10.6	223[a]	3.03[a]	N/A
Atrazine	Agricultural - Herbicide	1912-24-9	0.30/0.62	91.2	2.0	85.1	11.3	30[a]	2.61[a]	1.68
Metolachlor	Agricultural - Herbicide	51218-45-2	0.90/1.9	104.8	1.9	77.2	24.5	480, 530[a]	3.13[e]	N/A
Monensin	Veterinary – Antibiotic	17090-79-8	12/24	89.6	35	65.1	N/A	0.003[a]	5.43[b]	4.3[e]
Tylosin	Veterinary – Antibiotic	1401-69-0	3.3/6.8	53.3	3.9	35.3	6.0	5[a]	1.63[a]	7.7[e]
Virginiamycin M1	Veterinary – Antibiotic	21411-53-0	1.8/3.6	101.6	2.0	86.6	N/A	1.6[k]	0.06[k]	N/A
Zeranol	Veterinary – Synthetic hormone	26538-44-3	0.59/1.2	91.3	12.5	85.3	N/A	2.9[d]	5.37[b]	8.4, 11.4[c]
Carbaryl	Mixed[g] – Insecticide	63-25-2	0.49/1.0	161.2	0.7	130.9	25.3	110[a]	2.36[a]	10.4[e]
Chlorpyrifos	Mixed[g] – Insecticide	2921-88-2	700/1400	116.6	22	97.5	N/A	1.12[a]	4.94[a]	F/A
Iprodione	Mixed[g] – Fungicide	36734-19-7	14/30	100.6	17.1	71.4	N/A	13.9[a]	3.00[a]	F/A
Mecoprop	Urban/Residential – Herbicide	93-65-2	0.28/0.57	92.3	9.6	100.7	11.0	620-860[a]	3.13[a]	3.1[e]
Daidzein	Mixed[h] – Phytoestrogen	486-66-8	0.51/1.0	103.8	2.1	85.7	14.5	570, 620[a]	2.51, 2.55[b]	7.4, 9.9[f]
Formononetin	Mixed[h] – Phytoestrogen	485-72-3	0.61/1.2	90.6	16.1	96.6	N/A	52.2[d]	3.11[b]	7.5
Genistein	Mixed[h] – Phytoestrogen	446-72-0	15/31	98.3	4.3	78.9	N/A	258, 412	2.84[b]	6.7, 9.6, 13.0[f]
Erythromycin	Mixed[i] - PPCP prescription antibiotic	114-07-8	170/340	94.5	8.3	82.5	2.8	25[a]	1.6, 3.06[a]	8.8[e]
Oxytetracycline	Mixed[i] – PPCP prescription antibiotic	79-57-2	3.8/7.9	130.8	4.3	114.0	N/A	313[a]	-1.22[e]	3.27, 7.32, 9.11[e]
Sulfamethoxazole	Mixed[i] - PPCP prescription antibiotic	723-46-6	8.5/17	99.8	5.4	87.1	7.2	610[a]	0.89[a]	5.6[e]
17-β-Trenbolone	Mixed[i] – Synthetic hormone	10161-33-8	3.4/6.9	87.6	2.4	81.7	N/A	40-42[c]	2.7 [c]	N/A
Trimethoprim	Mixed[i] – PPCP prescription antibiotic	738-70-5	2.5/5.1	102.6	2.8	95.8	3.8	400[a]	0.91[a]	7.1[e]

4-Nonylphenol	Urban/Residential – Industrial/commercial surfactant	104-40-5	69/140	112.9	14.0	101.6	6.7	6.35[a]	5.76[a]	10.7[e]
Acetaminophen	Urban/Residential – PPCP nonprescription analgesic/antipyretic	103-90-2	0.56/1.2	98.6	22.1	63.5	17.0	14000[a]	0.46[a]	9.4[e]
Caffeine	Urban/Residential - PPCP nonprescription stimulant	58-08-2	0.58/1.2	103.8	7.4	88.0	8.7	21600[a]	-0.07[a]	3.6[e]
Carbamazepine	Urban/Residential - PPCP prescription anticonvulsant	298-46-4	0.11/.0.23	106.4	2.1	84.8	11.7	112[e]	2.45[a]	13.9[a]
Cotinine	Urban/Residential - PPCP metabolite	486-56-6	2.1/4.2	102.5	7.7	58.8	15.6	~50000[a]	0.07[a]	4.5
Ibuprofen	Urban/residential – PPCP nonprescription non-steroidal anti-inflammatory drug	15687-27-1	0.97/2.0	104.7	10.9	92.8	N/A	21[a]	3.97[a]	4.91[a]
N,N-Diethyl-meta-toluamide (DEET)	Urban/Residential – PPCP insect repellant	134-62-3	6.4/13	96.6	4.1	75.8	12.1	912[e]	2.18[a]	N/A
Metazachlor (surrogate)	Herbicide – not approved for use in the U.S.	67129-08-2	17/35	93.9	18.7	73.4	3.2	420[a]	2.13[a]	N/A

[a] US EPA (EPI Suite experimental database) [290], [b] EPI Suite KOWWIN v1.68 [290], [c] U.S. Food and Drug Administration [305], [d] EPI Suite WSKOW [290], [e] Environmental Science SRC PhysProp Database [306], [f] Kelly and Arnold [281], [g] agricultural and urban/residential pesticide, [h] may result from agriculture and industrial food processing (urban), [i] approved for human and veterinary use. PPCP = pharmaceuticals and personal care products; N/A = Not Applicable.

Table A-3. Liquid chromatography-tandem mass spectrometry parameters.[a]

Positive mode scan conditions								
Compound	Q1 mass (Da)	Q3 mass (Da)	scan time (min)	Parameter				
				DP	EP	CEP	CE	CXP
Cotinine	177.194	146.100	2.170	51.00	4.00	14.56	23.00	4.00
Acetaminophen	152.171	110.200	2.250	46.00	8.00	10.00	23.00	4.00
Caffeine	195.135	138.100	2.410	46.00	6.50	15.15	23.00	4.00
Sulfamethoxazole	254.157	156.100	9.500	70.00	10.00	17.10	23.00	4.00
Daidzein	255.136	199.300	9.600	61.00	9.50	17.13	23.00	4.00
Erythromycin	734.388	158.300	10.000	46.00	9.00	32.94	23.00	4.00
Genistein	271.137	153.100	10.000	61.00	11.00	17.66	23.00	4.00

Compound	Q1 mass (Da)	Q3 mass (Da)	scan time (min)	DP	EP	CEP	CE	CXP
Carbamazepine	237.223	194.300	10.740	46.00	4.50	16.54	23.00	4.00
Carbaryl	202.250	145.200	12.090	26.00	3.50	15.38	23.00	4.00
Atrazine	216.216	174.100	12.240	36.00	15.38	23.00	23.00	4.00
Deet	192.235	119.100	12.550	41.00	8.00	15.05	23.00	4.00
Metolachlor	284.295	252.200	15.750	46.00	2.00	18.09	23.00	4.00
Acetochlor	270.253	224.200	15.870	26.00	6.50	13.00	23.00	6.00
Iprodione	331.277	246.100	16.700	36.00	1.50	19.64	23.00	6.00
Monensin	693.443	479.400	25.720	86.00	12.00	31.59	23.00	4.00
Trimethoprim	291.160	230.200	2.300	61.00	11.00	14.0	31.00	4.00
Oxytetracycline	461.000	426.300	3.400	36.00	5.00	18.00	25.00	6.00
Tylosin	916.500	174.207	10.300	66.00	4.00	30.00	51.00	4.00
Metazachlor	278.260	134.200	13.400	26.00	5.00	12.00	27.00	4.00
Trenbolone	271.300	115.161	13.500	56.00	6.5	14.00	89.00	4.00
Virginamcin M1	824.171	205.200	14.500	70.00	10.00	29.338	50.00	4.00
Chlorpyrifos	350.054	198.100	18.900	70.00	10.00	16.536	50.00	4.00

Negative mode scan conditions								
Compound	Q1 mass (Da)	Q3 mass (Da)	scan time (min)	Parameter				
				DE	EP	CEP	CE	CXP
Genistein	268.792	132.800	10.620	-65.00	-8.00	-20.00	-42.00	-2.00
Formononetin	266.864	251.900	11.830	-60.00	-8.00	-22.00	-30.00	-4.00
Zeranol	320.938	276.781	12.620	-85.00	-10.00	-14.00	-32.00	-4.00
MCPP-d3	215.832	143.747	13.150	-25.00	-4.00	-12.00	-22.00	-2.00
MCPP	212.773	140.691	13.150	-30.00	-4.00	-16.33	-20.00	-2.00
Ibuprofen	204.937	161.000	14.900	-20.00	-10.00	-16.06	-10.00	-4.00
4-Nonlyphenol	219.000	105.900	18.900	-75.00	-7.50	-16.00	-28.00	0.00

[a] Curtain gas = 25 psi, GS1 gas = 30 psi, GS2 gas 30 psi, positive mode ionization voltage 5500.00, negative mode ionization voltage -4500.00, temperature 300°C. Collision energy (CE), CXP = collision cell exit potential, declustering potential (DP), entrance potential (EP) collision cell entrance potential (CEP).

Table A-4. CEC data for grab water samples (n=68) (Chapter 4). Concentration values (ng/L) are listed with two significant figures.

Sample Date	Site	Season	Flow (cfs)	Acetaminophen	Acetochlor	Atrazine	Caffeine	Carbamazepine	Carbaryl	Cotinine	Daidzein	DEET	Erythromycin	Metolachlor	Sulfamethoxazole	4-Nonylphenol	Mecoprop	Trimethoprim	Tylosin
02.10.2011	BC	W	27.6	2.5	2.7	23	19	1.0	<MRL	<MRL	<MRL	18	<MRL	46	<MRL	<MRL	<MRL	<MRL	<MRL
01.30.2012	BC	W	13.6	4.2	1.4	23	3.8	1.3	<MRL	<MRL	<MRL	7.3	<MRL	5.7	<MRL	<MRL	<MRL	<MRL	<MRL
02.10.2011	US2	W	130	<MRL	2.1	5.2	200	<MRL	<MRL	<MRL	<MRL	14	<MRL	11	<MRL	<MRL	<MRL	<MRL	<MRL
01.30.2012	US2	W	10.5	4.7	<MRL	31	5.1	<MRL	<MRL	<MRL	1.2	7.9	<MRL	1.2	<MRL	<MRL	<MRL	3.4	<MRL
02.10.2011	WC	W	0.5	2.7	15	20	11	<MRL	<MRL	<MRL	1.1	16	<MRL	18	<MRL	<MRL	<MRL	<MRL	<MRL
01.30.2012	WC	W	0.5	4.1	<MRL	27	1.9	<MRL	<MRL	<MRL	1.5	7.5	<MRL	<MRL	<MRL	<MRL	<MRL	5.5	<MRL
02.10.2011	DS	W	124	9.8	<MRL	11	130	210	<MRL	<MRL	<MRL	62	250	5.6	2400	11000	0.61	40	<MRL
01.30.2012	DS	W	77.9	3.8	<MRL	9.6	15	88	0.59	8.7	0.67	21	340	<MRL	230	190	0.35	33	9.1
03.18.2011	BC	Sp	305	6.4	<MRL	0.7	1.5	1.7	1.5	4.7	1.7	<MRL	<MRL	2.3	<MRL	<MRL	<MRL	<MRL	<MRL
03.23.2012	BC	Sp	30	4.0	13	32	10	<MRL	<MRL	<MRL	1.4	19	<MRL	54	<MRL	<MRL	<MRL	<MRL	<MRL
03.18.2011	US2	Sp	1670	9.1	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	380	<MRL	<MRL	<MRL
03.23.2012	US2	Sp	65.3	<MRL	8.0	21	17	<MRL	<MRL	<MRL	0.7	19	<MRL	11	<MRL	<MRL	<MRL	<MRL	<MRL
03.18.2011	WC	Sp	5.0	7.2	<MRL	7.0	20	<MRL	<MRL	<MRL	2.1	21	<MRL	17	<MRL	430	<MRL	<MRL	<MRL
03.23.2012	WC	Sp	2.0	<MRL	<MRL	19	2.2	<MRL	<MRL	<MRL	1.4	8.7	<MRL	1.5	<MRL	<MRL	<MRL	<MRL	<MRL
03.18.2011	DS	Sp	2110	13	66	11	28	26	<MRL	<MRL	<MRL	34	<MRL	440	68	1100	1.4	7.9	<MRL
03.23.2012	DS	Sp	138	4.8	1.4	16	59	96	<MRL	4.3	3.1	44	260	3.0	430	120	2.4	27	15
05.03.2012	BC	ES	31.2	4.0	44	56	28	<MRL	<MRL	2.5	1.4	13	<MRL	72	<MRL	<MRL	11	7.0	<MRL
05.03.2012	US2	ES	52.2	4.3	17	39	15	<MRL	<MRL	<MRL	1.1	7.2	<MRL	6.7	<MRL	<MRL	0.58	10	<MRL
05.03.2012	WC	ES	1.8	4.5	8.5	19	2.5	<MRL	<MRL	<MRL	0.74	<MRL	<MRL	2.4	<MRL	<MRL	<MRL	<MRL	<MRL
05.03.2012	DS	ES	117	2.6	35	42	54	73	2.7	<MRL	0.72	110	200	41	120	75	14	39	4.0
06.03.2011	BC	ES	91	3.7	13	30	10	0.37	<MRL	<MRL	<MRL	9.6	<MRL	46	<MRL	<MRL	0.33	<MRL	<MRL
05.10.2012	BC	ES	75.4	3.9	25	46	17	<MRL	<MRL	<MRL	0.88	19	<MRL	38	<MRL	<MRL	4.0	<MRL	<MRL
06.03.2011	US2	ES	166	7	7.3	36	11	<MRL	<MRL	<MRL	<MRL	25	<MRL	26	<MRL	<MRL	<MRL	<MRL	<MRL
05.10.2012	US2	ES	91.7	4.4	150	160	11	<MRL	<MRL	<MRL	<MRL	15	<MRL	45	<MRL	<MRL	<MRL	4.6	<MRL
06.3.2011	WC	ES	14	3	11	30	42	<MRL	<MRL	<MRL	<MRL	11	<MRL	110	<MRL	<MRL	<MRL	7.6	<MRL
05.10.2012	WC	ES	2.7	7.6	50	42	12	<MRL	<MRL	<MRL	<MRL	35	<MRL	28	<MRL	<MRL	<MRL	<MRL	<MRL
06.03.2011	DS	ES	334	4.1	58	27	14	69	3.0	3.5	<MRL	37	340	95	150	<MRL	3.6	32	<MRL
05.10.2012	DS	ES	235	3.4	98	68	24	87	0.54	5.4	1.2	120	390	27	210	390	19	68	3.6
06.10.2011	BC	ES	80	2.5	<MRL	50	9.8	0.28	<MRL	<MRL	<MRL	5.7	<MRL	36	<MRL	<MRL	13	3.2	<MRL
05.17.2012	BC	ES	42.1	4.3	14	57	15	<MRL	<MRL	<MRL	1.4	13	<MRL	31	<MRL	<MRL	0.5	4.6	<MRL
06.10.2011	US2	ES	90	4	10	54	<MRL	<MRL	<MRL	<MRL	0.44	14	<MRL	33	<MRL	<MRL	<MRL	<MRL	<MRL
05.17.2012	US2	ES	45.2	3.9	20	74	13	<MRL	<MRL	<MRL	<MRL	14	<MRL	23	<MRL	<MRL	<MRL	5.2	<MRL
06.10.2011	WC	ES	5.6	3.9	12	32	0.84	<MRL	<MRL	<MRL	<MRL	19	<MRL	70	<MRL	<MRL	<MRL	<MRL	<MRL
05.17.2012	WC	ES	1.4	4.7	59	46	91	<MRL	<MRL	<MRL	<MRL	11	<MRL	26	<MRL	<MRL	<MRL	<MRL	<MRL

06.10.2011	DS	ES	275	7	16	60	53	45	<MRL	2.7	0.8	35	560	35	170	<MRL	18	34	4.4
05.17.2012	DS	ES	118	3.9	11	38	81	99	5.0	5.5	<MRL	100	430	14	180	350	30	48	<MRL
08.23.2011	BC	LS	43.7	<MRL	<MRL	1.9	12	0.52	<MRL	<MRL	<MRL	16	<MRL	49	<MRL	<MRL	3.0	5.7	<MRL
08.27.2012	BC	LS	15.1	3.3	1.2	44	230	<MRL	<MRL	<MRL	<MRL	13	<MRL	9.1	<MRL	<MRL	0.58	6.0	<MRL
08.23.2011	US2	LS	27.3	1.9	<MRL	32	230	<MRL	<MRL	<MRL	<MRL	17	<MRL	6.8	<MRL	<MRL	0.65	3.9	<MRL
08.27.2012	US2	LS	68.2	2.7	<MRL	17	8.4	1.0	<MRL	<MRL	0.86	<MRL	<MRL	21	<MRL	<MRL	0.55	7.3	<MRL
08.23.2011	WC	LS	2.8	2.3	<MRL	34	140	<MRL	<MRL	<MRL	<MRL	22	<MRL	<MRL	<MRL	170	<MRL	8.3	<MRL
08.27.2012	WC	LS	0.06	2.2	<MRL	32	76	<MRL	<MRL	3.1	0.64	<MRL	<MRL	1.2	<MRL	<MRL	<MRL	6.1	<MRL
08.23.2011	DS	LS	134	2	<MRL	29	160	85	<MRL	2.4	<MRL	90	110	10	240	2700	4.5	16	<MRL
08.27.2012	DS	LS	59.2	5.9	<MRL	17	120	100	<MRL	5.8	0.54	23	370	5.0	490	1100	9.7	15	5.8
08.30.2011	BC	LS	37	0.44	<MRL	31	2.9	0.53	<MRL	<MRL	0.38	20	<MRL	33	<MRL	<MRL	0.44	14	<MRL
09.04.2012	BC	LS	13.3	3.1	<MRL	15	250	0.83	<MRL	<MRL	1.3	55	<MRL	12	<MRL	<MRL	<MRL	8.7	<MRL
08.30.2011	US2	LS	30.2	1.1	<MRL	27	7.2	<MRL	<MRL	<MRL	<MRL	9.1	<MRL	7.6	<MRL	<MRL	<MRL	3.4	<MRL
09.04.2012	US2	LS	3.9	3.1	<MRL	43	6.0	<MRL	<MRL	<MRL	0.92	23	<MRL	8.8	<MRL	<MRL	1.1	8.7	<MRL
08.30.2011	WC	LS	2.8	1.7	<MRL	28	14	<MRL	<MRL	<MRL	<MRL	11	<MRL	10	<MRL	<MRL	<MRL	4.5	<MRL
09.04.2012	WC	LS	0.1	4.2	<MRL	44	89	<MRL	<MRL	<MRL	<MRL	42	<MRL	<MRL	<MRL	<MRL	<MRL	6.3	<MRL
08.30.2011	DS	LS	141	2.6	<MRL	40	8.4	80	<MRL	<MRL	<MRL	15	1200	10	770	2300	1.4	6.8	4.1
09.04.2012	DS	LS	54.6	5.9	<MRL	19	47	110	<MRL	3.9	<MRL	78	600	<MRL	460	1100	2.7	17	3.4
11.23.2011	BC	F	20	1.1	0.85	29	27	1.7	<MRL	<MRL	<MRL	7.3	<MRL	20	<MRL	<MRL	<MRL	5.3	<MRL
10.22.2012	BC	F	9.2	1.8	<MRL	18	110	0.81	<MRL	3.9	<MRL	29	<MRL	8.4	<MRL	<MRL	4.5	5.5	<MRL
11.23.2011	US2	F	24.3	1.0	<MRL	29	4.9	<MRL	<MRL	<MRL	<MRL	17	<MRL	7.5	<MRL	<MRL	<MRL	4	<MRL
10.22.2012	US2	F	25	1.2	<MRL	45	100	<MRL	<MRL	2.2	<MRL	15	<MRL	4.4	<MRL	<MRL	6.9	8.5	<MRL
11.23.2011	WC	F	0.8	3.2	<MRL	28	9.1	<MRL	<MRL	<MRL	<MRL	43	<MRL	3.8	<MRL	<MRL	<MRL	24	<MRL
10.22.2012	WC	F	0.3	0.99	<MRL	45	43	<MRL	<MRL	<MRL	<MRL	40	<MRL	<MRL	<MRL	200	<MRL	4.5	<MRL
11.23.2011	DS	F	91.8	6.1	<MRL	22	23	59	<MRL	3.5	0.69	13	1800	5	2400	2700	1.3	11	8.3
10.22.2012	DS	F	81.9	3.2	<MRL	25	61	150	<MRL	3.4	<MRL	47	220	7	59	630	12	11	3.6
11.30.2011	BC	F	16	1.4	1.4	28	5.0	2.2	0.61	<MRL	<MRL	<MRL	<MRL	22	8.5	<MRL	<MRL	8.7	4.0
10.29.2012	BC	F	9.3	<MRL	0.84	19	130	0.77	<MRL	<MRL	<MRL	9.8	<MRL	17	<MRL	<MRL	<MRL	5	<MRL
11.30.2011	US2	F	23.3	1.1	<MRL	28	4.0	0.27	<MRL	1.9	0.44	10	<MRL	7	<MRL	<MRL	<MRL	6.8	<MRL
10.29.2012	US2	F	28	<MRL	<MRL	390	17	<MRL	<MRL	<MRL	<MRL	9.9	<MRL	4.7	<MRL	<MRL	<MRL	7.5	<MRL
11.30.2011	WC	F	1.04	1.7	<MRL	26	1.9	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	3.1	<MRL	<MRL	<MRL	28	<MRL
10.29.2012	WC	F	0.05	<MRL	<MRL	40	27	<MRL	<MRL	<MRL	<MRL	27	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
11.30.2011	DS	F	93	5.9	<MRL	17	17	49	<MRL	3.8	<MRL	<MRL	28	4.0	1700	2200	1.3	10	8.9
10.29.2012	DS	F	60.4	2.1	1.2	21	71	130	<MRL	<MRL	<MRL	23	390	13	270	2200	19	7.6	3.5
03.23.2012	US1	Sp	116	0.97	3.1	29	140	<MRL	<MRL	4.5	2.1	13	<MRL	<MRL	<MRL	<MRL	2.4	<MRL	<MRL
05.03.2012	US1	ES	90	4.9	82	86	110	<MRL	<MRL	4.4	0.91	11	<MRL	<MRL	<MRL	<MRL	23	10	<MRL
05.10.2012	US1	ES	209	4.6	220	160	19	<MRL	<MRL	<MRL	<MRL	18	<MRL	<MRL	<MRL	<MRL	4.4	6.7	<MRL

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05.17.2012	US1	ES	91	3.6	18	53	28	<MRL	<MRL	<MRL	<MRL	9	<MRL	<MRL	<MRL	<MRL	1.4	9.9	<MRL
08.27.2012	US1	LS	36	4.5	1.2	31	61	<MRL	<MRL	4.8	1.49	18	<MRL	<MRL	<MRL	<MRL	17	15	<MRL
09.04.2012	US1	LS	31	4.1	1.2	29	29	<MRL	<MRL	<MRL	<MRL	15	<MRL	<MRL	<MRL	<MRL	1.3	34	<MRL
10.22.2012	US1	F	58	1.4	0.88	34	90	0.35	<MRL	2.4	<MRL	27	<MRL	64	<MRL	<MRL	13	16	<MRL
10.29.2012	US1	F	38	0.7	2.1	26	71	0.18	<MRL	<MRL	<MRL	14	<MRL	40	<MRL	91	25	13	<MRL

Table A-5. Results of statistical comparisons (non-parametric multi-factor ANOVA) of grab water sample data (Chapter 4).[a]

Parameter	Factor	Acetaminophen	Acetochlor	Atrazine	Caffeine	Carbamazepine	Carbaryl	Cotinine	Daidzein	DEET	Erythromycin	Metolachlor	Sulfamethoxazole	4-Nonylphenol	MCPP	Trimethoprim	Tylosin
Concentration	Site	0..013*	0.05*	0.153	0.03*	<0.001*	0.008*	<0.001*	0.867	<0.001*	<0.001*	0.005*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Concentration	Season	0.001*	<0.001*	0.002*	0.23	0.243	0.115	0.931	0.002*	0.796	0.623	<0.001*	0.180	0.026*	0.003*	0.001*	0.397
Concentration	Site*Season		<0.001*				<0.001*									0.041*	
Flux	Site	<0.001*	0.008*	<0.001*	<0.001*	<0.001*	0.007*	<0.001*	0.833	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Flux	Season	<0.001*	<0.001*	<0.001*	0.579	0.771	0.100	0.799	0.040*	0.002*	0.155	0.001*	0.680	0.020*	0.013*	0.002*	0.466
Flux	Site*Season		<0.001*				<0.001*									<0.001*	0.039*

[a] An asterisk (*) indicates that the results is statistically significant at the α=0.05 level. Figures 4-3 to 4-6 in the manuscript display the significant differences among groups by sites and seasons. The interaction term is indicated by the factor "site*season". P-values for interactions are only presented if they were significant. If interactions were not significant, they were removed from the ANOVA model, and the p-values presented for the main effects of site and season for that CEC are the p-values for the model that did not include the interaction term.

Figure A-1. Plots of significant interactions of season and site on CEC concentrations (Chapter 4)**.**

Trimethoprim - Interaction Plot

Acetochlor - Interaction Plot

Carbaryl - Interaction Plot
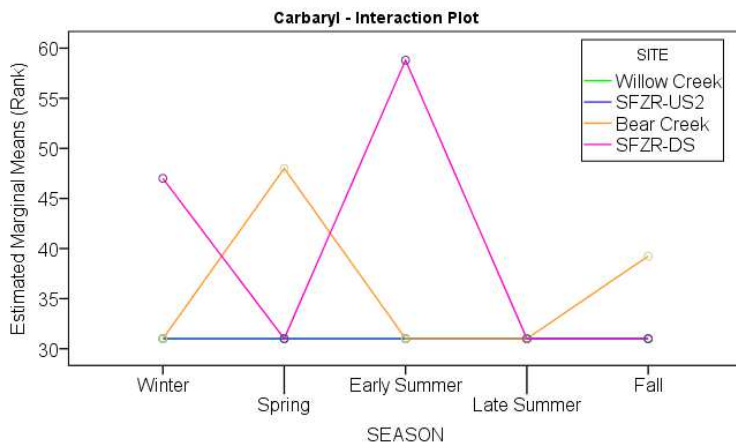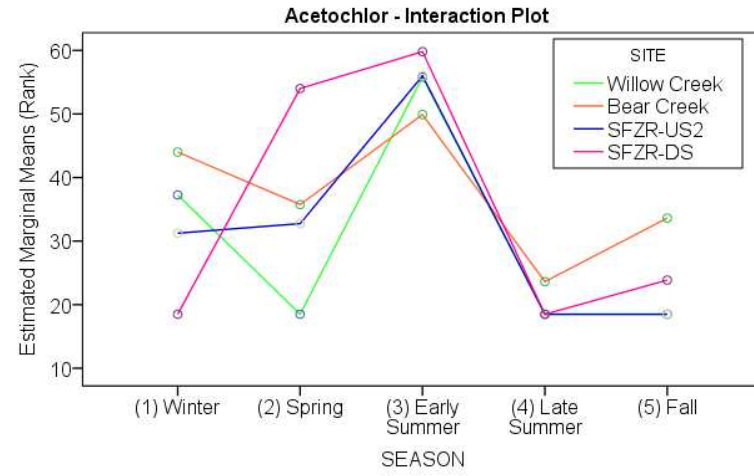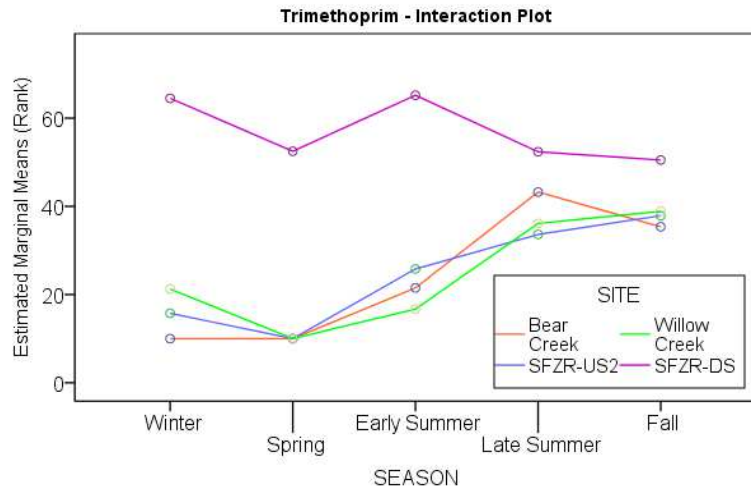
Table A-6. Occurrence data for in sediment (n=60) and water (n=60) samples for the 7 CECs detected in sediment (Chapter 5).[a]

CEC	MRL in Sediment (ng/g)/ Water (ng/L)	Detection Frequency Sediment/ Water, %	Concentration Range (Mean) in Sediment (ng/g)	Concentration Range (Mean) in Water (ng/L)	Avg. log K_{d-obs}	Difference between ave. measured log K_{d-obs} and predicted log K_d
Caffeine	0.12/0.58	100/98.3	0.16-1.3 (0.44)	0.84-250 (44)	1.3	4.1
Acetaminophen	0.11/0.56	90.0/88.3	0.16-21 (2.8)	0.99-7.0 (3.5)	2.5	4.8
DEET	1.3 /6.4	30.0/88.3	1.3-3.5 (2.2)	7.2-110 (27)	2.0	2.6
Carbamazepine	0.02/0.11	13.3/45.0	0.03-0.11 (0.06)	0.27-150 (49)	-0.2	0.1
Daidzein	0.10/0.51	21.7/31.7	0.17-1.1 (0.50)	0.54-3.1 (1.1)	2.3	2.5
Atrazine	0.06/0.30	21.7/100	0.06-0.28 (0.13)	1.8-390 (40)	0.6	0.7
Acetochlor	0.18/0.89	56.7/43.3	0.21-49 (5.2)	1.2-180 (28)	2.2	1.9

[a]All sediment concentrations are in units of ng/g dry weight. Method reporting limits (MRL), detection frequencies, summary concentration data, and mean K_{d-obs} (over all sampling events in which the CEC was detected in both water and sediment) for the CECs in sediment and water are included. For each CEC, the difference between its mean log K_{d-obs} and a K_{ow}-based prediction [288] is given. Rows are arranged in order of increasing K_{ow}.

Table A-7. P-values of ANOVA results and pairwise comparisons for the significance of site, year, and seasonal factors on CEC variability (Chapter 5).[a]

Factor	Parameter	Acetaminophen	Acetochlor	Atrazine	Caffeine	Carbamazepine	Daidzein	DEET
Season	$[C]_{sed}$	0.072	0.192	0.829	0.125	0.577	0.334	0.342
	$[C]_{water}$	0.004*	0.001*	0.001*	0.005*	0.170	0.010*	0.208
	K_{d-obs}	0.013*	0.0001* (F/W/LS)	0.109	<0.0001*	0.084	NC	0.443
Site	$[C]_{sed}$	0.0003*	<.0001*	0.021*	0.001*	0.031*	0.449	0.462
	$[C]_{water}$	<0.0001*	0.017*	0.083	0.003*	<0.0001*	0.641	0.0004*
	K_{d-obs}	0.015* (BC)	0.023* (BC)	0.071	0.082	0.0084* (WWTP)	NC	0.765
Year	$[C]_{sed}$	0.003* (2012)	0.016* (2012)	0.292	0.032* (2012)	0.939	0.404	0.656
	$[C]_{water}$	0.236	0.133	0.063	0.094	0.169	0.956	0.134
	K_{d-obs}	0.317	0.854	0.227	0.347	0.024* (2011)	NC	0.153

[a] Significant p-values are indicated with an asterisk (*). For significant effects on K_{d-obs}, the factor level listed in parenthesis indicates the group(s) that was significantly higher than other groups. For significant effects of site or season on water or sediment concentrations, see Figures 5-2 and 5-3 for point estimates of the mean; for effect of year on water or sediment concentrations, the higher of the two years is listed in parentheses. NC = not calculated due to lack of sufficient number of data points; winter (W), late summer (LS), fall (F), Bear Creek (BC), SFZR-WWTP (WWTP).

Table A-8. Sediment data, water data, and observed sediment-water distribution coefficients (K$_{d-obs}$) for detected CECs (Chapter 5). A K$_{d-obs}$ was only calculated if the CEC was quantified above the MRL in both water and sediment at the same site and time (an empty cell indicates no K$_{d-obs}$ was calculated for that paired sampling event). Note: additional water samples were collected, but only sampling events that have a paired water and sediment sample are reported herein. Metolachlor was detected in a single sediment sample at a concentration of 0.21 ng/g at site BC on 9.4.2012 (not displayed in this table). K$_{d-obs}$ values were calculated by: [CEC]$_{sed}$ (ng/g) / [CEC]$_{water}$ (ng/L) * (1000g/kg). BC = Bear Creek, GC = South Fork Zumbro River – Golf Course, WC = Willow Creek, WWTP = South Fork Zumbro River – Wastewater Treatment Plant, Acetaminophen (ACM), acetochlor (ACC), atrazine (ATR), caffeine (CAF), carbamazepine (CBZ), daidzein (DAID). Early Summer (ES), Late Summer (LS), Winter (W), Spring (Sp)

Sampling Time and Location			Sediment CEC Concentrations (ng/g dry weight)							Water CEC Concentrations (ng/L) of CECs detected in sediment							Log K$_{d-obs}$						
Date	Site	Season	ACM	ACC	ATR	CAF	CBZ	DAID	DEET	ACM	ACC	ATR	CAF	CBZ	DAID	DEET	ACM	ACC	ATR	CAF	CBZ	DAID	DEET
6.3.11	BC	ES	3.2	0.37	<MRL	0.25	<MRL	<MRL	2.3	3.7	13	30	10	0.37	<MRL	9.6	2.9	1.4		1.4			2.4
6.10.11	BC	ES	0.66	0.77	<MRL	0.46	<MRL	<MRL	3.5	2.5	<MRL	50	9.8	0.28	<MRL	<MRL	2.4			1.7			
8.23.11	BC	LS	7.6	2.1	<MRL	0.23	<MRL	<MRL	<MRL	<MRL	<MRL	1.8	12	0.52	<MRL	16				1.3			
8.30.11	BC	LS	0.27	<MRL	<MRL	0.45	<MRL	0.38	<MRL	<MRL	<MRL	31	2.9	0.53	<MRL	20				2.2			
11.23.11	BC	Fall	1.2	10	<MRL	0.28	<MRL	<MRL	<MRL	1.1	<MRL	29	27	1.6	<MRL	7.3	3.0			1.0			
11.30.11	BC	Fall	12	8.5	<MRL	0.37	<MRL	<MRL	<MRL	1.4	1.4	28	5.0	2.2	<MRL	<MRL	3.9	3.8		1.9			
1.30.12	BC	W	2.2	7.9	<MRL	0.75	<MRL	<MRL	<MRL	4.2	1.4	23	3.8	1.2	<MRL	7.3	2.7	3.7		2.3			
3.23.12	BC	Sp	0.56	2.5	<MRL	1.1	<MRL	<MRL	<MRL	4.0	13	32	10	<MRL	1.4	19	2.2	2.3		2.1			
5.3.12	BC	ES	8.8	14	<MRL	0.65	<MRL	<MRL	<MRL	4.0	44	56	27	<MRL	1.4	13	3.3	2.5		1.4			
5.10.12	BC	ES	5.8	7.1	<MRL	0.48	<MRL	<MRL	<MRL	4.3	28	43	20	<MRL	0.88	22	3.1	2.4		1.4			
5.17.12	BC	ES	0.31	3.5	<MRL	0.39	<MRL	<MRL	<MRL	4.3	14	57	15	<MRL	1.4	13	1.9	2.4		1.4			
8.27.12	BC	LS	1.3	0.57	<MRL	0.71	<MRL	0.30	<MRL	3.3	1.2	44	230	<MRL	<MRL	13	2.6	2.7		0.49			
9.4.12	BC	LS	16	49	0.18	1.3	<MRL	0.69	2.2	3.1	<MRL	14	250	0.83	1.3	55	3.7		1.1	0.71		2.7	1.6
10.2.12	BC	Fall	6.2	23	<MRL	0.82	<MRL	<MRL	<MRL	1.8	<MRL	18	110	0.81	<MRL	29	3.5			0.88			
0.29.12	BC	Fall	21	11	<MRL	1.2	<MRL	0.45	<MRL	<MRL	<MRL	19	130	0.77	<MRL	9.8				0.96			
6.3.11	GC	ES	0.57	1.0	<MRL	0.28	<MRL	<MRL	2.1	7.0	7.3	36	11	<MRL	<MRL	25	1.9	2.1		1.4			1.9
6.10.11	GC	ES	0.46	0.63	<MRL	0.93	<MRL	<MRL	2.1	4.0	10	54	<MRL	<MRL	<MRL	14	2.1	1.8					2.2
8.23.11	GC	LS	0.42	<MRL	<MRL	0.31	<MRL	<MRL	<MRL	1.9	<MRL	32	230	<MRL	<MRL	17	2.4			0.14			
8.30.11	GC	LS	<MRL	<MRL	<MRL	0.34	<MRL	<MRL	1.7	1.1	<MRL	27	7.2	<MRL	<MRL	9.1				1.7			2.3
11.23.11	GC	Fall	1.1	6.2	0.16	0.27	<MRL	<MRL	1.5	1.0	<MRL	29	4.9	<MRL	<MRL	17	3.0		0.73	1.7			2.0
11.30.11	GC	Fall	0.28	<MRL	<MRL	0.42	<MRL	<MRL	<MRL	1.1	<MRL	28	4.0	0.27	<MRL	10	2.4			2.0			
1.30.12	GC	W	<MRL	6.2	<MRL	0.19	<MRL	<MRL	<MRL	4.7	<MRL	30	5.0	<MRL	1.2	7.9				1.6			
3.23.12	GC	Sp	1.9	3.4	<MRL	0.42	<MRL	<MRL	<MRL	<MRL	7.9	21	17	<MRL	0.70	19		2.6		1.4			
5.3.12	GC	ES	12	2.6	<MRL	0.40	<MRL	<MRL	<MRL	4.3	17	39	15	<MRL	<MRL	7.2	3.4	2.2		1.4			
5.10.12	GC	ES	6.0	1.9	0.11	0.19	<MRL	<MRL	<MRL	4.0	180	130	14	<MRL	<MRL	15	3.2	1.0	-0.073	1.1			
5.17.12	GC	ES	0.18	<MRL	<MRL	0.24	<MRL	0.17	<MRL	3.9	20	74	13	<MRL	<MRL	14	1.7			1.3			

Date	Site	Season																					
8.27.12	GC	LS	0.45	0.49	0.08	0.20	<MRL	<MRL	<MRL	2.7	<MRL	17	8.4	1.0	0.86	<MRL	2.2			0.67	1.4		
9.4.12	GC	LS	0.70	0.63	0.15	0.45	<MRL	1.1	<MRL	3.1	<MRL	43	6.0	<MRL	<MRL	23	2.4			0.54	1.9		
10.22.12	GC	Fall	0.96	0.21	<MRL	0.27	<MRL	<MRL	2.8	1.2	<MRL	45	100	<MRL	<MRL	15	2.9			0.42			2.3
10.29.12	GC	Fall	0.66	0.49	<MRL	1.0	<MRL	<MRL	2.8	<MRL	<MRL	390	17	<MRL	<MRL	9.9					1.8		2.4
6.3.11	WC	ES	<MRL	<MRL	<MRL	0.23	<MRL	<MRL	<MRL	3.0	11	30	42	<MRL	<MRL	11				0.74			
6.10.11	WC	ES	0.30	<MRL	0.28	0.37	<MRL	<MRL	1.3	3.9	12	32	0.84	<MRL	<MRL	19	1.9			0.95	2.6		1.8
8.23.11	WC	LS	<MRL	<MRL	<MRL	0.16	<MRL	<MRL	<MRL	2.3	<MRL	34	140	<MRL	<MRL	22				0.05			
8.30.11	WC	LS	0.19	<MRL	<MRL	0.34	<MRL	<MRL	3.4	1.7	<MRL	28	14	<MRL	<MRL	11	2.1				1.4		2.5
11.23.11	WC	Fall	0.24	<MRL	0.068	0.24	<MRL	<MRL	<MRL	3.2	<MRL	28	9.1	<MRL	<MRL	43	1.9			0.38	1.4		
11.30.11	WC	Fall	0.38	<MRL	0.069	0.34	<MRL	0.69	<MRL	1.7	<MRL	26	1.9	<MRL	<MRL	<MRL	2.3			0.43	2.3		
1.30.12	WC	W	12	<MRL	0.27	0.58	<MRL	<MRL	1.8	4.1	<MRL	27	1.9	<MRL	1.5	7.5	3.5			1.0	2.5		2.4
3.23.12	WC	Sp	0.33	<MRL	<MRL	0.55	<MRL	<MRL	<MRL	<MRL	<MRL	19	2.2	<MRL	1.4	8.7					2.4		
5.3.12	WC	ES	0.31	<MRL	<MRL	0.32	<MRL	<MRL	<MRL	4.5	8.5	19	2.5	<MRL	0.74	<MRL	1.8				2.1		
5.10.12	WC	ES	5.1	0.24	0.064	0.31	<MRL	0.49	<MRL	6.7	49	34	13	<MRL	<MRL	33	2.9	0.70		0.28	1.4		
5.17.12	WC	ES	0.25	<MRL	<MRL	0.26	<MRL	0.46	<MRL	4.4	63	38	51	<MRL	<MRL	9.7	1.8			0.71			
8.27.12	WC	LS	0.78	<MRL	0.083	0.18	<MRL	<MRL	<MRL	2.2	<MRL	32	76	<MRL	0.64	<MRL	2.6			0.41	0.37		
9.4.12	WC	LS	<MRL	<MRL	<MRL	0.52	<MRL	<MRL	1.9	4.2	<MRL	44	89	<MRL	<MRL	42				0.77			1.7
10.22.12	WC	Fall	0.36	<MRL	<MRL	0.19	<MRL	0.19	2.4	0.99	<MRL	45	43	<MRL	<MRL	40	2.6			0.63	1.4		1.8
10.29.12	WC	Fall	1.1	<MRL	0.16	0.57	<MRL	1.0	1.4	<MRL	<MRL	40	27	<MRL	<MRL	27				0.61	1.3		1.7
6.3.11	WWTP	ES	4.7	<MRL	<MRL	0.32	<MRL	<MRL	<MRL	4.1	58	27	14	69	<MRL	37	3.1				1.3		
6.10.11	WWTP	ES	0.4	<MRL	<MRL	0.34	0.032	<MRL	<MRL	7.0	16	60	53	45	0.80	35	1.8			0.82			
8.23.11	WWTP	LS	2.6	<MRL	<MRL	0.22	0.11	<MRL	<MRL	2.0	<MRL	29	160	85	<MRL	90	3.1			0.14		0.112	
8.30.11	WWTP	LS	<MRL	<MRL	<MRL	0.32	<MRL	<MRL	1.7	2.6	<MRL	40	8.4	80	<MRL	15					1.6		2.0
11.23.11	WWTP	Fall	0.60	2.3	<MRL	0.42	0.063	<MRL	<MRL	6.1	<MRL	22	23	59	0.69	13	2.0				1.3	0.028	
11.30.11	WWTP	Fall	0.21	<MRL	<MRL	0.47	0.085	<MRL	<MRL	5.9	<MRL	17	17	49	<MRL	<MRL	1.5				1.4	0.24	
1.30.12	WWTP	W	0.46	1.0	<MRL	0.41	<MRL	<MRL	<MRL	3.8	<MRL	9.6	15	88	0.67	21	2.1						
3.23.12	WWTP	Sp	0.16	<MRL	<MRL	0.68	0.11	0.24	<MRL	4.8	1.4	16	59	96	3.1	44	1.5			1.1		0.06	1.9
5.3.12	WWTP	ES	0.55	<MRL	<MRL	0.36	0.036	<MRL	<MRL	2.6	35	42	54	73	0.72	110	2.3			0.82		-0.31	
5.10.12	WWTP	ES	2.3	2.8	<MRL	0.50	<MRL	<MRL	<MRL	5.7	98	56	14	82	0.73	110	2.6	1.5			1.6		
5.17.12	WWTP	ES	0.34	1.3	<MRL	0.46	<MRL	0.28	2.3	3.9	10	38	81	99	<MRL	100	1.9	2.1		0.76			1.4
8.27.12	WWTP	LS	1.7	0.22	<MRL	0.46	<MRL	<MRL	<MRL	5.9	<MRL	17	120	100	0.54	23	2.5			0.59			
9.4.12	WWTP	LS	1.5	3.4	0.067	0.40	<MRL	<MRL	<MRL	5.9	<MRL	19	47	110	<MRL	78	2.4			0.93			
10.22.12	WWTP	Fall	1.5	0.77	<MRL	0.40	0.045	<MRL	<MRL	3.2	<MRL	25	61	150	<MRL	47	2.7			0.82		-0.53	
10.29.12	WWTP	Fall	0.85	0.22	<MRL	0.20	0.027	<MRL	1.8	2.1	1.2	21	71	130	<MRL	23	2.6			0.44		-0.7	1.9

Table A-9. Mean characteristics of sediment (n=8) and water samples (n=41) collected from the study sites (Chapter 5). TOC=total organic carbon; SFZR=South Fork of Zumbro.

Parameter	Bear Creek	Willow Creek	SFZR-Golf Course	SFZR-WWTP
Sediment TOC (%)	0.55	0.26	0.50	0.53
Sediment pH	8.3	8.2	8.5	8.0
Sand/Silt/Clay (%)	83.8/<2.0/16.3	83.8/<2.0/15.0	82.5/<2.0/16.2	82.5/<2.0/16.3
In-stream pH - mean (range)	8.2 (7.3-8.6)	7.3 (6.8-8.2)	8.0 (7.5-8.3)	7.0 (6.6-7.3)

Table A-10. Detection frequencies of CECs in grab sediment and water samples by season and by site (Chapter 5).

	Acetaminophen		Acetochlor		Atrazine		Caffeine		Carbamazepine		Daidzein		DEET	
	Sed.	Water	Sed.	Water	Sed.	Water	Sed.	Water	Sed.	Water	Sed.	Water	Sed.	Water
Bear Creek (n=15)	100%	80%	93%	53%	6.7%	100%	100%	100%	0%	67%	27%	33%	20%	87%
SFZR-Golf Course (n=15)	87%	87%	73%	40%	27%	100%	100%	93%	0%	13%	13%	20%	40%	93%
Willow Creek (n=15)	80%	87%	6.7%	33%	47%	100%	100%	100%	0%	0%	33%	27%	40%	80%
SZFR-WWTP (n=15)	93%	100%	53%	47%	6.7%	100%	100%	100%	53%	100%	13%	47%	20%	93%
Winter (n=4)	75%	100%	75%	25%	25%	100%	100%	100%	0%	50%	0%	75%	25%	100%
Spring (n=4)	100%	50%	50%	75%	0%	100%	100%	100%	25%	25%	25%	100%	0%	100%
Early Summer (n=20)	95%	100%	60%	95%	15%	100%	100%	95%	10%	35%	20%	35%	30%	90%
Late Summer (n=16)	75%	88%	44%	6.3%	31%	100%	100%	100%	6.3%	50%	25%	25%	31%	88%
Fall (n=16)	100%	81%	63%	13%	25%	100%	100%	100%	25%	56%	25%	6.3%	38%	81%

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Table A-11. Literature and estimated values for K_d (Chapter 5).

Compound	log K_d (Estimation Methods) (displayed in Fig. 4)	log K_d (Literature) (displayed in Fig. 4)	K_d	K_{oc}	Reference
Acetaminophen		0.085	1.2	270	[124] (Tamiya river sediment, TOC: 0.87%)
		1.8	59	13000	[124] (Akui River, TOC: 0.075%)
		0.12	1.3	290	[128] (TOC: 1.7%)
		0.17	1.5	330	[256] (river sediment, TOC: 0.72%)
		-0.12	0.77	170	[124] (Tatara river, TOC: 1.7%)
	-1.0		0.09	20	[290] (K_{ow} method)
	-0.69		0.20	45	[290] (MCI method)
			0.41	92	[307]
Acetochlor	0.12		1.3	290	[290] (K_{ow} method)
		-0.20	0.63	140	[308] (TOC: 2.46%)
	0.13		1.3	300	[290] (MCI method)
		1.1	12	2700	[287] (TOC: ~5%)
	-0.027		0.94	210	[290]
		-0.26	0.55	120	[308] (TOC 3.46%)
		-0.16	0.69	150	[308] (TOC 0.89%)
Atrazine		-0.03	0.93	210	[309] (TOC: 0.79%)
		-0.066	0.86	190	[309] (TOC: 1.1%)
	-0.20		0.64	140	[290] (K_{ow} method)
		-0.12	0.76	170	[262] (2.6-4.1% OC)
		-0.39	0.41	91	[263] (TOC: 2.5%)
		-0.018	1.0	230	[264] (TOC: 0.69%)
		-0.45	0.35	78	[264] (TOC: 0.51%)
Caffeine	-1.4		0.043	9.5	[290] (K_{ow} method)
	-1.3		0.045	10	[290] (MCI method)
		1.8	66	15000	[128] (TOC: 1.7%)
Carbamazepine	-0.12		0.76	170	[290] (K_{ow} method)
	0.78		6.0	1300	[290] (MCI method)
		-0.35	0.45	100	[124] (Tatara River, TOC: 1.7%))
		0.053	1.1	250	[310] (TOC: 0.74%)

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		0.09			[311]
		-0.20	0.63	140	[124] K_{oc} (Tamiya River, TOC: 0.87%))
		-0.22	0.60	130	[250] (TOC: 0.37%)
		0.15	1.4	320	[310] (TOC: 4.36%)
		1.1	12	2700	[255] (TOC: 0.2%)
		0.11	1.3		[217]
		-0.31	0.50	110	[124] (Akui River, TOC: 0.075%)
		0.5	3.2	700	[255] (TOC 0.2%, water pH 7.1)
Daidzein	0.57		3.7	830	[290] (K_{ow} method)
	1.0		10	2300	[290] (MCI method)
		0.94	8.8	1900	[261] (exp't. using NOM)
DEET		1.3	21	2400	[312] (TOC 0.89%) high value
		0.0043	1.0	32	[312] (TOC 0.89%) low value
	-0.30		0.50	110	[290] (MCI method)
	-0.49		0.33	72	[290] (K_{ow} method)

Table A-12. Concentrations of CECs in 35 water samples (ng/L) collected in 2012 (Chapter 6). SFZR = South Fork of the Zumbro River. "<MRL" indicated the CEC was not quantified above the method reporting limit in the sample.

Date	Site	Aceta minop hen	Aceto chlor	Atra zine	Caffein e	Carbam azepine	Carbar yl	Cotini ne	Daidzei n	DEE T	Erythro mycin	Metola chlor	Sulfame thoxazol e	4-Nonylphen ol	Meco prop	Trimetho prim	Tylosin
Mar 12	Effluent	10	<MRL	8.1	46	220	<MRL	7.5	<MRL	120	1300	<MRL	1200	460	4.6	53	17
May 3	Effluent	11	5.1	22	48	210	7.9	12	<MRL	380	1100	<MRL	460	600	20	70	10
May 10	Effluent	19	<MRL	15	35	170	<MRL	14	<MRL	260	890	<MRL	980	500	42	140	11
May 17	Effluent	9	<MRL	13	18	170	11	7.6	<MRL	210	580	<MRL	260	530	72	120	9.2
Sept 4	Effluent	12	<MRL	10	16	260	<MRL	10	<MRL	170	1200	<MRL	760	2000	4.0	11	10
Oct 22	Effluent	8.6	10	10	17	520	<MRL	6.3	<MRL	100	1100	<MRL	150	2800	6.3	8	10
Oct 29	Effluent	7.0	<MRL	11	12	440	<MRL	<MRL	<MRL	42	1200	<MRL	790	5200	1.5	8.3	8.9
Mar 12	SFZR-US1	1.0	3.1	29	140	<MRL	<MRL	4.5	2.1	13	<MRL	8.5	<MRL	<MRL	2.4	<MRL	<MRL
May 3	SFZR-US1	4.9	82	86	110	<MRL	<MRL	4.4	0.9	11	<MRL	98	<MRL	<MRL	23	7.2	<MRL
May 10	SFZR-US1	4.4	240	160	23	<MRL	<MRL	<MRL	<MRL	19	<MRL	55	<MRL	<MRL	4.4	5.1	<MRL
May 17	SFZR-US1	3.6	18	53	28	<MRL	<MRL	<MRL	<MRL	9	<MRL	26	<MRL	<MRL	1.4	6.9	<MRL
Sept 4	SFZR-US1	4.1	1.2	29	29	<MRL	<MRL	<MRL	<MRL	15	<MRL	6.5	<MRL	<MRL	1.3	25	<MRL
Oct 22	SFZR-US1	1.4	0.9	34	90	0.35	<MRL	2.4	<MRL	27	<MRL	11	<MRL	<MRL	13	12	<MRL
Oct 29	SFZR-US1	0.7	2.1	26	71	0.18	<MRL	<MRL	<MRL	14	<MRL	22	<MRL	92	25	8.6	<MRL
Mar 12	Bear Creek	4.0	13	32	10	<MRL	<MRL	<MRL	1.4	19	<MRL	54	<MRL	<MRL	<MRL	<MRL	<MRL
May 3	Bear Creek	4.0	44	56	28	<MRL	<MRL	2.5	1.4	13	<MRL	71	<MRL	<MRL	11	7.0	<MRL
May 10	Bear Creek	3.9	25	46	17	<MRL	<MRL	<MRL	0.9	19	<MRL	38	<MRL	<MRL	4.0	<MRL	<MRL
May 17	Bear Creek	4.3	14	57	15	<MRL	<MRL	<MRL	1.4	13	<MRL	31	<MRL	<MRL	0.5	4.6	<MRL
Sept 4	Bear Creek	3.1	<MRL	15	250	0.83	<MRL	<MRL	1.3	55	<MRL	12	<MRL	<MRL	<MRL	8.7	<MRL
Oct 22	Bear Creek	1.8	<MRL	18	110	0.81	<MRL	3.9	<MRL	29	<MRL	8.4	<MRL	<MRL	4.5	5.5	<MRL
Oct 29	Bear Creek	<MRL	0.8	19	130	0.77	<MRL	<MRL	<MRL	10	<MRL	17	<MRL	<MRL	<MRL	5.0	<MRL
Mar 12	SFZR-US2	<MRL	8.0	21	17	<MRL	<MRL	<MRL	0.7	19	<MRL	11	<MRL	<MRL	<MRL	<MRL	<MRL
May 3	SFZR-US2	4.3	17	39	15	<MRL	<MRL	<MRL	1.1	7	<MRL	6.7	<MRL	<MRL	0.6	11	<MRL
May 10	SFZR-US2	4.4	150	160	11	<MRL	<MRL	<MRL	<MRL	15	<MRL	45	<MRL	<MRL	<MRL	4.6	<MRL
May 17	SFZR-US2	3.9	20	74	13	<MRL	<MRL	<MRL	<MRL	14	<MRL	23	<MRL	<MRL	<MRL	5.2	<MRL
Sept 4	SFZR-US2	2.5	<MRL	42	6.2	<MRL	<MRL	<MRL	<MRL	22	<MRL	6.4	<MRL	<MRL	1.1	8.7	<MRL
Oct 22	SFZR-US2	1.2	<MRL	45	100	<MRL	<MRL	2.2	<MRL	15	<MRL	4.4	<MRL	<MRL	6.9	8.5	<MRL
Oct 29	SFZR-US2	<MRL	<MRL	390	17	<MRL	<MRL	<MRL	<MRL	10	<MRL	4.7	<MRL	<MRL	<MRL	7.5	<MRL
Mar 12	SFZR-DS	4.8	1.4	16	59	96	<MRL	4.3	3.1	44	260	3.0	430	120	2.5	27	15
May 3	SFZR-DS	2.6	35	42	54	73	2.7	<MRL	0.72	110	200	41	120	75	14	39.0	4.0
May 10	SFZR-DS	6.0	92	63	28	96	0.54	5.4	1.2	110	390	22	580	390	19	68.0	3.6

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May 17	SFZR-DS	3.9	11	38	81	99	5.0	5.5	<MRL	100	430	14	180	350	30	48.0	3.2
Sept 4	SFZR-DS	5.9	<MRL	19	47	110	<MRL	3.9	<MRL	78	600	<MRL	460	1100	2.7	17.0	3.4
Oct 22	SFZR-DS	3.2	<MRL	25	61	150	<MRL	3.4	<MRL	47	220	7.0	59	630	12	11.0	3.6
Oct 29	SFZR-DS	2.1	1.2	21	71	130	<MRL	<MRL	<MRL	23	390	13	270	2200	19	7.6	3.5

Table A-13. Results of statistical comparisons (Chapter 6). Wilcoxon Signed-Rank test results were used to assess the accuracy of the predicted loadings at SFZR-DS by comparing them with measured loadings at SFZR-DS. Predictions that differ significantly from measured loadings are indicated by p-values less than 0.05 in the Wilcoxon Signed-Rank column. Analysis of variance (ANOVA) on ranks was completed to assess differences among loadings due to sampling location. When ANOVA models showed significant variation due to location, the protected Least Significant Difference procedure was used to determine which sites differed from each other. Sites that do not differ significantly in terms of loading of a given CEC share common letters in the row for that CEC. Note that ANOVA was conducted to compare loading in effluent with aggregated upstream areas (i.e., SFZR-DS vs. SFZR-US1 vs. the sum of Bear Creek plus SFZR-US2) and to compare loading in effluent against the individual upstream sites (i.e., SFZR-DS vs. Bear Creek vs. SFZR-US2). The pairwise comparisons given under each ANOVA indicate significant differences related to the associated ANOVA models.

Compound of Interest	Wilcoxon Signed-Rank Test of Pred. vs. Obs. Loadings	ANOVA on Ranks of Loadings of WWTP-Effluent, SFZR-US1, and Sum (Bear Creek + SFZR-US2)				ANOVA on Ranks of Loadings of WWTP-Effluent, Bear Creek, and SFZR-US2				ANOVA on Ranks of Concentrations of WWTP-Effluent, SFZR-DS, and SFZR-US1			
		p-value of group effect	WWTP Effluent	SFZR-US1	Bear Creek + SFZR-US2	p-value of group effect	WWTP Effluent	Bear Creek	SFZR-US2	p-value of group effect	WWTP Effluent	SFZR-DS	SFZR-US1
Acetaminophen	0.176	0.687	A	A	A	**0.034***	A	B	B	**<0.001***	A	B	B
Acetochlor	**0.018***	**0.021***	B	A	A	0.179	A	A	A	0.071	B	B	A
Atrazine	**0.028***	**<0.001***	B	A	A	0.096	B	AB	A	**<0.001***	B	AB	A
Caffeine	0.866	**<0.001***	B	A	A	0.481	A	A	A	**0.018***	B	A	A
Carbamazepine	0.128	**<0.001***	A	B	B	**<0.001***	A	B	C	**<0.001***	A	B	C
Carbaryl	0.109	0.119	A	A	A	0.119	A	A	A	0.217	A	A	A
Cotinine	0.917	**0.046***	A	AB	B	**0.001***	A	B	B	**0.005***	A	B	B
Daidzein	0.109	**0.021***	B	AB	A	**0.014***	B	A	AB	0.185	A	A	A
DEET	0.176	**0.007***	A	B	B	**<0.001***	A	B	B	**<0.001***	A	B	C
Erythromycin	0.499	**<0.001***	A	B	B	**<0.001***	A	B	B	**<0.001***	A	B	C
Metolachlor	**0.018***	**<0.001***	B	A	A	**<0.001***	B	A	A	**<0.001***	B	A	A
Sulfamethoxazole	**0.028***	**<0.001***	A	B	B	**<0.001***	A	B	B	**<0.001***	A	B	C
4-Nonylphenol	0.176	**<0.001***	A	B	B	**<0.001***	A	B	B	**<0.001***	A	A	B
Mecoprop	0.237	0.059	AB	A	B	**0.006***	A	B	B	0.554	A	A	A
Trimethoprim	0.176	0.247	A	A	A	**0.014***	A	B	AB	**0.021***	A	A	B
Tylosin	**0.043***	**<0.001***	A	B	B	**<0.001***	A	B	B	**<0.001***	A	B	C