Investigating the drivers of chemical pollution: A spatial and historical analysis of emerging organic contaminants in aquatic and engineered systems

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Abstract

Chemical ingredients from pharmaceuticals, household items, and personal care products are commonly detected in the environment. There are numerous pathways for chemicals to enter the environment, including incomplete removal at wastewater treatment plants and runoff from urban streets and agricultural fields. Pollutants can undergo various transformation processes in the environment to form new (and potentially more toxic) substances. This dissertation evaluated the source, potential risks, and/or persistence of two groups of chemicals, hydroxylated polybrominated diphenyl ethers (OH-BDEs) and antibiotics, in natural and man-made systems using spatial and historical trends. Profiles in dated sediment cores were used to determine historical trends of chemical pollution. OH-BDEs are of particular interest because they have both anthropogenic (transformation products of brominated flame retardants) and natural sources (production by microbiota in marine systems). Also, select OH-BDE congeners photodecompose into dioxins, one of the most toxic classes of chemical pollutants. This work demonstrated that wastewater effluent had little impact on OH-BDE levels. Natural production appeared to be the dominant source of OH-BDEs and brominated dioxins in the studied systems, but the abundance of OH-BDEs was likely indirectly enhanced by anthropogenic activities. Antibiotics are one of the greatest inventions of the 20th century, but their presence may be encouraging the rise and spread of antibiotic resistance genes (ARGs). Antibiotics are used for human chemotherapy, as well as by the agricultural industry as preventative treatments and growth promoters. Here, the historical trends of antibiotics were investigated in four Minnesota lakes, and the spatial distribution of

antibiotics in a small lake and the Minnesota and Mississippi rivers were also measured. Wastewater effluent appears to be the primary source of antibiotics in the studied lakes, with lesser inputs from agricultural activities and natural production. The spatial distribution of antibiotics in the small and large-scale systems captured both human and animal-uses, but differing transport processes likely influenced the observed trends in the small and large-scale systems. The overall result of this dissertation demonstrated that presence of select OH-BDE congeners and antibiotics in the studied systems was enhanced (either directly or indirectly) by human activities. Increasing our understanding about anthropogenic sources of emerging contaminants will be beneficial in implementing future efforts to reduce the human chemical footprint.

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Dedication

To my family and friends,
for your constant supply of love and support

"Unless someone like you
cares a whole awful lot,
nothing is going to get better.
It's not."

-Theodor Seuss Geisel

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Chapter 1: Introduction

1.1 Chemicals in the Environment

The modern human lifestyle leaves a chemical footprint in the environment. A vast number of chemical ingredients from popular consumer products have been detected in various environmental compartments including the atmosphere, soils, sediments, surface waters, ground water, and biota.[1–11] The physicochemical properties of chemicals, in large part, dictate in which environmental matrices specific substances will be found.[12] Advancements in analytical methods and instrumentation have enabled scientists to detect chemicals at low levels in the environment. Thus, a new field of research emerged focused on detecting contaminants of emerging concern (CECs) in the environment and investigating their impact on human and aquatic life. CECs derive from a variety of household uses and products, such as brominated flame retardants, laundry detergents, pesticides, insect repellants, and estrogens.[8,13,14] These substances may pose a risk to human and ecosystem health because little is known about their potential adverse effects.[5,10,15,16]

Pharmaceuticals and personal care products (PPCPs), a subgroup of CECs, are of notable concern because of their inherent ability to have a biological effect.[16–18] PPCPs originate from consumer products that improve quality of life (e.g., toothpaste,[19] fragrances,[20] hand soap,[21] and plastics[22]), as well as medications that treat or prevent human and animal illness (e.g., antidepressants,[23] anti-inflammatories,[24] antibiotics,[25] and birth control pills[26]). Many of these substances are endocrine disruptors that, at certain

dosages, disrupt hormone chemical signaling.[10,15,27] These interferences can cause cancer, reproductive issues, and developmental disabilities.[15,27]

Pathways are the routes that transport pollutants from their point-of-use to the environment. Exposure pathways are often dictated by usage patterns. Pesticides are often land applied to agricultural fields, and thus they are commonly present in soils and in water bodies that receive runoff from agricultural fields.[7] In households, consumer products that are flushed down the drains of sinks, showers, bathtubs, and laundry machines (such as hand and body soaps, laundry detergents, sunscreen, cosmetics, toothpaste, and fragrances)[8,19–21] have a pipeline to the environment through wastewater treatment plants (WWTP). There are also multiple paths for some chemicals. For example, the insect repellent DEET is typically used outdoors and therefore directly exposes surface waters and soils to contamination.[28] It is also present in wastewater effluents because it is washed from the skin during bathing.[8,16]

Pharmaceuticals are not completely metabolized after ingestion or use.[5,29] A portion is excreted in its original form in urine and/or feces and sent to the WWTP.[5,29] WWTPs were designed for the removal of macropollutants (nutrients and bacteria) and almost all were built prior to discovering the huge influx of micropollutants. The effectiveness of conventional wastewater treatment technologies against PPCPs is highly variable[8,30,31] and often PPCPs leave treatment plants via sludge or effluents.[5,8,30,32]

In addition to concern over the presence of specific CECs and PPCPs in the environment, many researchers have investigated the occurrence and toxicity of their transformation products.[5,33–36] Organic chemicals may undergo photodegradation, reduction/oxidation reactions, microbial degradation, hydrolysis, and/or

substitution/elimination reactions in the environment.[4,12,37–41] Research efforts have also focused on product identification and characterization, because transformation or degradation of the original compound does not indicate a decrease in potency or toxicity.[5,33–36] To determine overall toxicity and effects on aquatic systems, a comprehensive assessment should also include transformation products and any of their adverse effects.

Even though concern about the presence of micropollutants in the environment has been expressed by the scientific community, many are not regulated by government agencies. The US Environmental Protection Agency (EPA) comprised a list of 126 chemicals that have high priority for developing effluent limitation guidelines called the Clean Water Act Priority Pollutant List.[42] In 2007, the EPA released The Contaminant Candidate List 3,[43] which lists pollutants that are likely to be found in drinking water but are not currently regulated and may need to be in the future.

1.2 Historical Trends of Pollutants in Sediment Cores

Until recently, the presence of many of micropollutants, such as antibiotics and estrogens, in the environment was largely undetected. Recent advancements in analytical methods and instrumentation have now enabled scientists to detect and quantify pollutants at part per trillion levels (ng/L, ng/kg or pg/g). Historical records of environmental exposure for many pollutants, however, do not exist because the level of sensitivity available now was not available a decade (or longer) ago. It is possible, however, to investigate chemical pollution over the past century by analyzing sediment cores for pollutants of interest. Sediment is an assortment of particles that deposit at the

bottom of a water body. A portion of the dissolved pollutant sorbs to suspended particles as the material sinks to the lake bed. If the bottom of the lake does not undergo severe mixing, stratified layers of pollutant-laden sediment will accumulate, as shown in Figure 1.1. The depths of a sediment core are converted to the age of the sediment using radiometric dating, i.e. lead-210 (^{210}Pb) and cesium-137 (^{137}Cs) methods.[44–46] A century-long monitoring study can be performed by measuring the concentration of a chemical at various depths throughout a sediment core. This method is most effective for chemicals

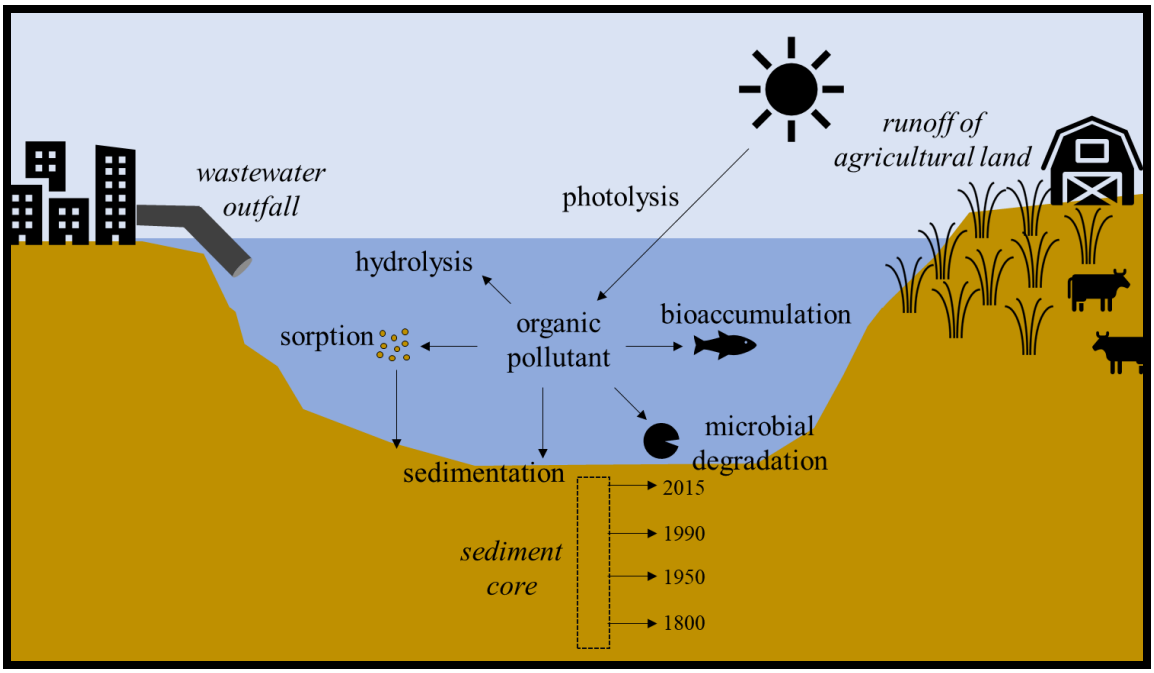


Figure 1.1. Schematic of anthropogenic inputs of an organic pollutant and several example fate processes. Sediment cores record the presence of pollutants in a water body as a function of time.

that are hydrophobic and readily sorb. Organic substances may undergo other fate and transport processes besides sedimentation, and therefore sediment cores capture the relative abundance of pollutants in water bodies. Historical trends of CECs in sediment cores may provide insight into when a pollutant first appeared in the environment, its usage patterns, the degree of pollution, and potential anthropogenic sources.

1.3 Triclosan: Source, Occurrence, and Effects

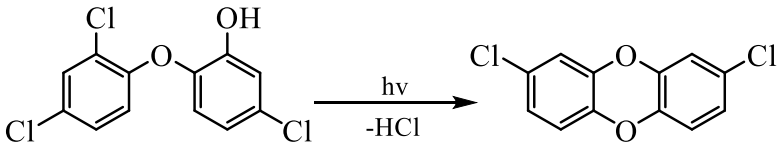
Triclosan (2,4,4'-trichloro-2'-hydroxydiphenylether) is a common antibacterial agent used in many household items (toothpaste, plastics, and detergents), and is best known as the active ingredient in antibacterial liquid hand soap. As of 2001, 76% of liquid hand soaps contained triclosan.[47] Triclosan is a synthetic compound first patented in 1964. Because hand soap is washed down the drain, triclosan has a direct route to WWTPs. In the United States, an estimated 300 tons of triclosan is sent to WWTPs every year.[48] Conventional activated sludge treatment removes more the 90% of dissolved triclosan, but its high influx results in its frequent detection in wastewater effluents.[32,49–51] Triclosan was detected in every sample of treated wastewater effluent from ten different states throughout the United States, with a maximum concentration of 1.6 µg/L and median concentration 0.25 µg/L.[50] At a WWTP in Ontario, Canada, concentrations of triclosan in influent ranged from 0.01 to 4.01 µg/L and in effluent from 0.01 to 0.324 µg/L.[32] High levels of triclosan in the influent (max 23.9 µg/L) and effluent (max 6.88 µg/L) were also observed at a WWTP in Greece.[51]

Wastewater is the primary source of triclosan detected in surface water[1,2] and sediment[44,52] of water bodies receiving wastewater effluents. Runoff from agricultural fields applied with wastewater sludge contributes negligible inputs.[53] From 1999-2000, a national reconnaissance of contaminants in surface waters of streams was conducted in the United States;[1] triclosan was one of the most frequently detected compounds (57.6%) with levels up to 2.3 µg/L.[1] Ferrey and coworkers[2] conducted a survey of 50 lakes in Minnesota and detected triclosan in 14% of samples with detected concentrations ranging from < 4.0 to 11.8 ng/L. Sediment accumulation rates of triclosan in eight Minnesota

lakes corresponded to the degree of wastewater impact and usage patterns of consumer products.[44] In the Chesapeake Bay, triclosan concentrations ranged from 400 to 800 ng/g in a sediment core collected near a wastewater outfall.[52]

Widespread presence of triclosan has also been observed in humans. In 2008, The United States National Health and Nutrition Examination Survey detected triclosan in 74.6% of the 2517 collected urine samples at concentrations varying from 2.4 to 3790 µg/L.[54] As a potential endocrine disruptor, this antibacterial agent may affect steroid metabolism and xenobiotic detoxification.[55] The widespread presence of triclosan in aquatic systems is of great concern for ecosystem health, because it can be highly toxic to algae and capable of altering reproduction and development in fish.[56–58]

In surface waters, triclosan reacts photochemically to form 2,8-dichlorodibenzo-p-dioxin, Figure 1.2.[59,60] Anger et al.[44] reported that triclosan loadings from treated wastewater was the primary source of its dioxin photoproduct in lacustrine systems since 1965. Dioxins are known to be a toxic and carcinogenic class of compounds,[61] thus the ubiquitous presence of triclosan is of great for human and ecosystem health. On January 1, 2017, Minnesota became the first state to ban the sale of consumer products that contain triclosan. The Food and Drug Administration recently announced a ban on triclosan used in hand and body soaps that will go in effect in September 2017.



Triclosan 2,8-DiCDD

Figure 1.2. Photochemical transformation of triclosan into 2,8-dichlorodibenzo-p-dioxin (2,8-DiCDD).

1.4 Hydroxylated Polybrominated Diphenyl Ethers: Sources, Occurrence, and Effects

Hydroxylated polybrominated diphenyl ethers (OH-BDEs) are transformation products of anthropogenically-derived brominated flame retardants and are also natural products in marine systems. A general structure is given in Figure 1.3. Natural production of OH-BDEs does not occur in freshwater environments due to lack of available bromine. Marine bacteria produce OH-BDEs by coupling bromophenols and/or bromocatechols,[62] and this can also occur enzymatically in red algae.[63] Red algae and cyanobacteria associated with marine sponges are potential OH-BDE producers, although genetic evidence is lacking. [64–67] Another possibility is that marine bacteria that interact with red algae and cyanobacteria are solely responsible for OH-BDE production.[62,68]

Since the 1970s, polybrominated diphenyl ethers (PBDEs) have been used as flame retardants in clothing, furniture, and electronics. PBDEs are non-covalently bonded additives and therefore leach from consumer products. Anthropogenic sources include manufacturing facilities,[69] sewage/wastewater effluent,[69,70] and atmospheric deposition.[71] Brominated flame retardants are frequently detected in the environment, due to their mass production and widespread use.[13,69,70,72,73] In the San Francisco Estuary, the total PBDE levels ranged from 3 to 514 pg/L in surface waters and non-detect to 212 ng/g in sediments.[72] These chemicals were also persistent in the marine biota with levels ranging from 9 to 64 ng/g dry weight (dw) in oysters, 13 to 47 ng/g dw in mussels, and 85 to 106 ng/g dw in clams.[72] Soils from one of the largest industrial regions in China measured ΣPBDEs (congeners 28, 47, 66, 100, 99, 154, 153, 138, 183) levels from 0.13 to 3.81 ng/g.[73] As of 2004, the total PBDE human exposure had increased approximately 100-

fold over 30 years and the total PBDE level for people living in the United States was about 35 ng/g lipid.[13] California was the first state in the United States to ban Penta-BDEs (which contains BDE-47, BDE-99, BDE-100, and BDE-154 – the most widespread and bioaccumulative congeners) and Octa-BDE in 2003. The reservoir of previously released PBDEs and debromination of deca-BDEs, however, are a continued source of these CECs.

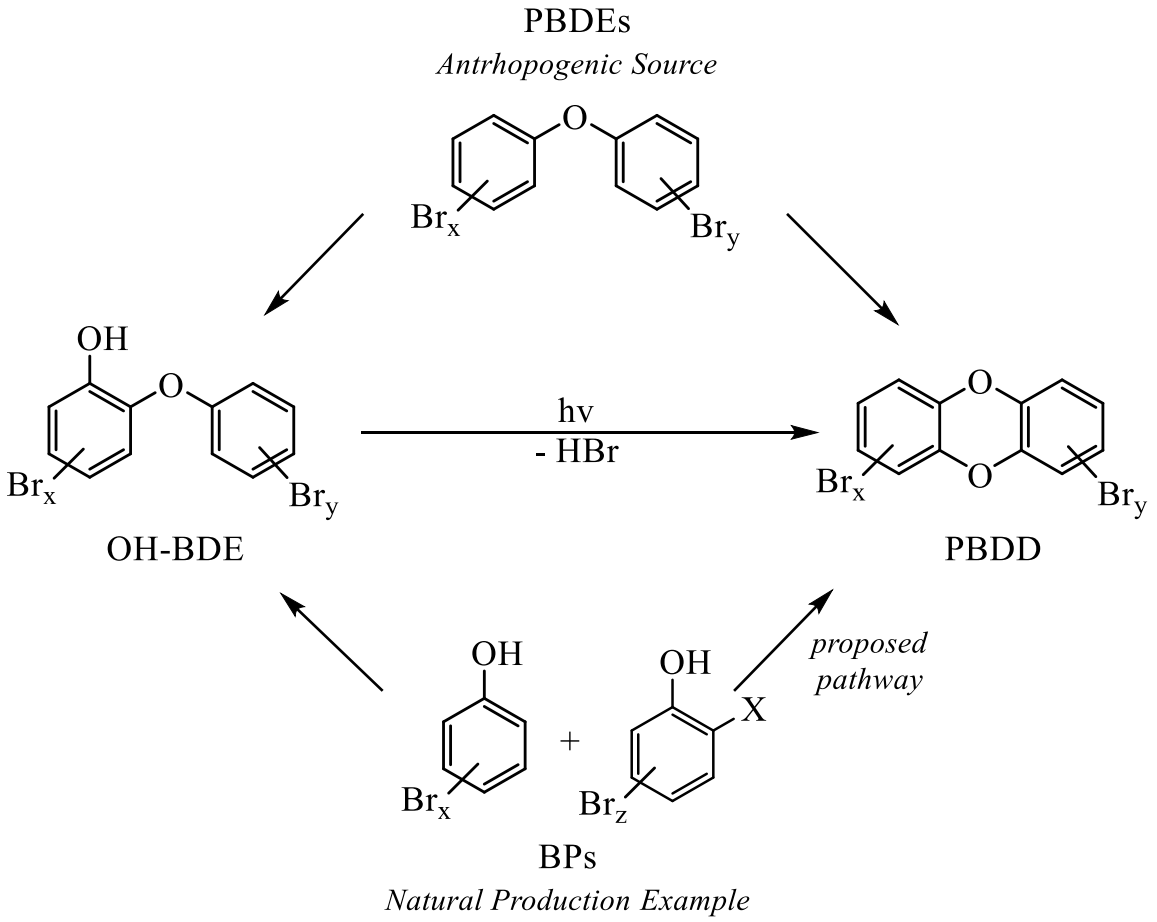


Figure 1.3. Transformation of anthropogenically derived polybrominated diphenyl ethers (PBDEs) and natural production in marine systems from bromophenols are sources of hydroxylated (OH-) BDEs. Photodegradation of a OH-BDE produces a polybrominated dibenzo-p-dioxin (PBDD) unique to each OH-BDE. PBDDs are also formed from natural production and incineration of PBDE-containing materials.

Oxidation of PBDEs is an anthropogenic source of OH-BDEs detected in the environment. Transformation of PBDEs to OH-BDEs occurs through abiotic and biotic processes such as metabolic oxidation,[74] reaction with hydroxyl radicals in the atmosphere,[75] and potentially during oxidative treatments during wastewater and sewage treatment.[76,77] Photochemical synthesis from brominated phenols[76] and oxidation from PBDEs in surface waters have also been reported to occur.[78] The origin of the OH-BDEs can be determined by the position of the hydroxyl group. The hydroxyl group of naturally produced OH-BDEs is only in the *ortho*- position respective to the ether bridge, whereas anthropogenically derived OH-BDEs have the hydroxyl group in the *ortho*-, *meta*- or *para*- position.[79,80]

OH-BDEs have been detected in a variety of environmental matrices.[75,81–84] Ueno and coworkers[75] calculated total OH-BDE fluxes of 3.5 to 190 ng/m^2 in snow and 15 to 170 ng/m^2/day in rain. In inland South Korea, total OH-BDEs varied from 15 to 230 ng/g in surface soil, 43 to 120 pg/g in pine needles, and 13 to 30 pg/L in surface waters.[83] Levels in surface water (2.2 to 70 pg/L) were elevated near sewage treatment plant outfall in Ontario, Canada.[75] Sediment levels of 6-OH-BDE 47 in the East China Sea ranged from 11.4 to 129.1 pg/g dw and increased seaward.[81] Furthermore, the spatial distribution of 6-OH-BDE 47 was unlike those of the anthropogenically-derived chemicals also quantified, suggesting natural production was the primary source of 6-OH-BDE 47.[81] Total OH-BDE levels in marine sediment from Liaodong Bay (3.2 to 116 ng/g dw)[82] where generally higher than maximum levels of 3-OH-BDE-47 (11.9 ng/g), 5-OH-BDE 47 (9.2 ng/g), 2'-OH-BDE-68 (5.1 ng/g), and 6-OH-BDE 47 (4.1 ng/g) in freshwater

sediment from Lake Taihu.[84] Two unidentified tri-brominated OH-BDEs were detected in wastewater/sewage treatment plant effluent at roughly low μg/L to high ng/L levels.[6]

OH-BDEs have also been detected in numerous marine organisms including salmon,[85] whales,[86] sharks,[87] bald eagles,[88] and polar bears.[89] Low levels (0.01 to 0.1 ng/g lipid) of OH-BDEs were quantified in the blubber of beluga whales,[86] and higher levels (up to 8 ng/g lipid) were quantified in bull shark livers.[87] Analysis of the top three marine predators revealed that albatross had the highest liver concentration of OH-BDEs (0.54 ± 0.38 ng/g, wet wt.), followed by tuna (0.025 ± 0.08 ng/g wet wt.), and polar bears (0.012 ± 0.009 ng/g wet wt.). Levels in red algae (*Ceramium tenuicorne)* from the Baltic Sea reached 150 ng/g dw and were most likely of natural orgin.[64] OH-BDEs have been detected in human plasma,[90,91] thus raising concern about potential adverse effects.

OH-BDEs are equivalent or more potent endocrine disruptors than their precursor PBDEs.[92–94] 6-OH-BDE 47 had a higher ability to inhibit estrogenic functions than its PBDE precursor.[94] The neurotoxic potential is higher for OH-BDEs than PBDEs, and the occurrence of OH-BDE metabolites of PBDEs may be of greater concern during brain development.[95] Also, OH-BDEs binding to receptors and transport proteins in the thyroid may dampen the production of thyroid hormone.[95] All 18 OH-BDE congeners tested by Legradi et al[96] were found to disrupt the aerobic metabolic pathway to produce energy, mitochondrial oxidative phosphorylation. This pathway is dependent on the electron transport chain converting adenosine diphosphate (ADP) to adenosine triphosphate (ATP). OH-BDEs also have indirect estrogenic effects in rats[97] and inhibit hormone transport in gulls.[98]

OH-BDE congeners with bromine *ortho*- to an ether linkage and an *ortho*- Br- on the adjacent phenyl can photodegrade into polybrominated dibenzo-*p*-dioxins (PBDDs) in surface water.[99,100] Studies have shown PBDDs have the same or greater toxicity as their chlorinated counterparts which cause wasting syndrome, thymus atrophy, and liver toxicity.[101–104] The similar biological effects for chlorinated and brominated dioxins are due to a shared mechanism the mediates toxicity: a binding affinity to the aryl hydrocarbon (or dioxin) receptor.[104] Incineration of PBDE-containing waste at municipal incinerators and coal-fired power plants is a well-documented anthropogenic source of PBDD, as well as polybrominated dibenzofurans (PBDFs).[105–107] In Taiwan, the mean total emission factor for PBDD/Fs calculated for municipal solid waste incinerators were 7.0 – 9.5 μg TEQ/ton-waste and for a coal-fired power plant was 0.00482 ± 0.00512 μg TEQ/ton-coal.[106] Natural production is another source of PBDD abundance in marine systems.[108–111] Loftstand and coworkers[110] reported mean ΣPBDD concentrations of 2.9 to 340 ng/g extractable organic matter (EOM) in mussels, 18 ng/g EOM in brown algae, and 7.7 ng/g EOM in cyanobacteria.

1.5 Antibiotics and Antibiotic Resistance Genes

The beginning of the antibiotic-era began in the early 1930s with the synthesis of sulfphanilamide, the original sulfonamide antibiotic.[112] Sulfphanilamide revolutionized the health care system by being the first medicine to exhibit broad-spectrum activity against common Gram-positive and Gram-negative pathogens.[112] The accidental discovery of penicillin became the second line of defense for microbial illnesses and galvanized the search for other natural products to be used as chemotherapy.[113] Several

other naturally produced antibiotics were discovered through the 1970s including chlortetracycline, oxytetracycline, erythromycin, and lincomycin.[113] The term antibiotic describes a substance that kills or stops the growth of bacteria. The discovery of antibiotics is perhaps one of the greatest achievements of the 20th century.

Today, antibiotics are in high demand for human and animal chemotherapy. Estimates of annual global antibiotic consumption lies between 100,000 and 200,000 tons.[4] The agricultural industry uses antibiotics as a preventative treatment, as well as a growth promotor. Sales data of antibiotic purchases can be used to estimate total antibiotic consumption, but this method has limitations.[114,115] Human-use antibiotic sales reported by the FDA are primarily comprised of sales to outpatient retail pharmacies, and therefore it is not an estimate of direct use.[114] Determining the livestock most responsible for antibiotic consumption is complicated by 1) multi-animal use; 2) off-label use, and 3) consumption by both food-producing animals (e.g., cattle and swine) and nonfood-producing animals (e.g., cats and dogs).[115] Furthermore, direct comparison of antibiotic sales for animal versus human-use may be skewed due to: 1) larger population of animals versus humans, 2) differences in physical characteristics of humans compared to animals, and 3) human-use drugs can be used for animal treatments.[115] Therefore, other methods are needed to assess the dominant source of antibiotic pollution.

The estimated total sales of antibiotics in the United States was 17,900 tons per year.[114–116] Figure 1.4 displays the core structure of several major antibiotic groups, as well as structures for a few popular antibiotics. Major antibiotics classes sold for veterinary uses in the United States were tetracyclines (41%), ionophores (31%), β-lactamases (7%), and macrolides (4%).[115] For human treatments, penicillin-type drugs

accounted for the greatest portion (44%) of total sales followed by cephalosporins (15%), sulfonamides and trimethoprim (15%), fluoroquinolones (8%), macrolides (5%), and tetracyclines (3.5%).[114]

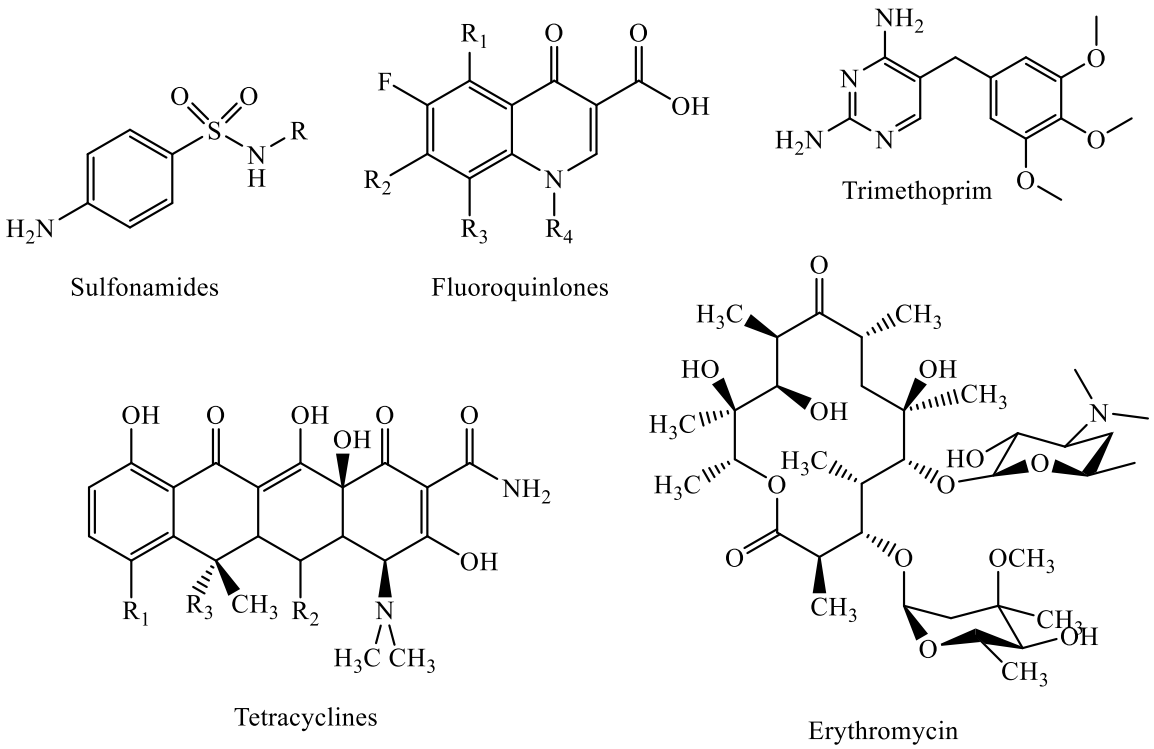


Figure 1.4. Core structures of sulfonamides, fluoroquinolones, and tetracyclines classes, as well as trimethoprim and example from the macrolide group (erythromycin).

Antibiotics are not completely metabolized, and therefore a portion is excreted in urine or feces in its original, bioactive form. Fractions of antibiotic excreted vary among the antibiotic classes: tetracyclines from 0.1 to 0.6; macrolides from 0.08 to 0.2; quinolones from 0.07 to 0.7; and sulfonamides and trimethoprim from 0.58 to 0.9.[117] Antibiotics are frequently detected in wastewater effluents at high ng/L to low µg/L levels, indicating incomplete removal by conventional wastewater treatment.[4,50,118–121] Effluent levels at a Wisconsin WWTP varied from 0.27 to 0.36 µg/L for sulfonamides, 0.42 to 10.9 µg/L for tetracyclines, 0.25 to 0.33 µg/L for fluoroquinolones, and 0.72 to

1.1 μg/L for macrolides.[120] Water bodies receiving wastewater outfalls often have elevated levels of human-use antibiotics in surface waters and sediments near the discharge point.[4,122–128] Agricultural antibiotics predominantly enter aquatic systems via runoff from agricultural fields that have been fertilized with manure contaminated by antibiotics.[123,127,129,130]

The highest levels of antibiotics in aquatic systems are often found downstream of metropolitan (industrial and municipal wastes) and agricultural and aquaculture (feedlots and fish ponds) areas.[122,124,127,128] Although β-lactamases (which includes penicillin) are one of the most popular antibiotic classes, they are rarely detected in the environment because they readily undergo hydrolysis.[4] Livestock waste appeared to be the primary source of antibiotics in Lake Taihu, China.[131] Carbadox, an agricultural antibiotic, was detected in 28% of the 50 surveyed Minnesota lakes with levels up to 121 ng/L.[2] The other antibiotics detected in Minnesota were found at lower frequencies: ciprofloxacin (2%, max 19.4 ng/L), ofloxacin (2%, max 8.94 ng/L), sulfonamides (2-10%, max 2.48 to 134 ng/L), and trimethoprim (4%, max 6.22 ng/L).[2] A spatial analysis of three major rivers in China revealed agricultural inputs as the primary contributor of antibiotic pollution in sediments with high levels of oxytetracycline (652 ng/g), tetracycline (135 ng/g), norfloxacin (5775 ng/g), ofloxacin (653 ng/g), and ciprofloxacin (1287 ng/g).[124] Antibiotics used for human applications (ciprofloxacin and ofloxacin) were most abundant in the urban regions of the Haihe River, whereas, three veterinary sulfonamides were frequently detected (100%) in rural areas near numerous livestock and aquaculture farms.[132]

Select antibiotics, especially tetracyclines and fluoroquinolones which strongly adsorb onto particles, accumulate in sediment.[28] Tetracyclines and fluoroquinolones complex with Ca^{2+} and Mg^{2+}, and thus sediment is often an important reservoir for them.[130,132–136] For example, fluoroquinolones (non-detect to 174 ng/g dw) and tetracyclines (non-detect to 39.6 ng/g dw) were the dominant antibiotics in the sediment of Lake Taihu.[131] Sorption behavior, however, varies from compound to compound and is often influenced by sediment composition, i.e. particle size and organic content.[124,130,137] Humic substances may alter surface properties and availability of sorption sites to either inhibit or promote antibiotic sorption.[4] The sorption capacity for sulfonamides has been seen to increase for decreasing particle sizes.[137]

The widespread occurrence of antibiotics is of particular concern, because these substances are designed to be biologically active at low concentrations.[4] One study showed that microbial communities shifted after exposure to ciprofloxacin, a common fluoroquinolone antibiotic.[138] Antibiotics may also hinder the growth and biomass of algae and benthic invertebrates, which are the foundation of the aquatic food-chain.[139125,140–142] Some antibiotics even retain a degree of bioactivity while sorbed.[143–146] Studies investigating the effects of antibiotics on aquatic microbial environments are limited and the implications of their presence are not yet fully understood.

Of most notable concern is that antibiotics may select for and promote the dispersion of antibiotic resistance genes (ARGs).[3,147–150] ARGs are the genetic code that allow bacteria to withstand the effects of antibiotics. Antibiotic resistance is inherited (i.e., cell division) or acquired from other bacteria by transformation, conjugation, and/or transduction mechanisms.[149] Antibiotic resistance, like some antibiotics, is naturally

occurring but only with associations for select antibiotics.[151] In recent years, there has been a rise in antibiotic resistance that is thought to be induced by the overuse and misuse of antibiotic therapies.[3,147–150] The rate of developing new antibiotics has substantially slowed over recent years, thus limiting the available treatment options for antibiotic resistant diseases.[152] It has been estimated that antibiotic resistance causes more than 700,000 deaths annually.[153] The World Health Organization noted that the proliferation and rising abundance of antibiotic resistance bacteria is one of the major threats to public health in the 21st century.[154] ARGs detected in environment originate from wastewater and sewage treatment plants,[155–157] hospital waste,[149] and livestock lagoons.[143,158,159]

Heavy metal pollution is also known to promote ARG occurrence by cross-resistance (the metal and antibiotic resistance genes are present on the same mobile genetic element) or co-resistance (the same mechanism provides resistance to both antibiotics and metals).[149]

1.6 Scope of Dissertation

The advancement of knowledge and production of new materials, goods, and pharmaceuticals has had an unintended consequence: chemical pollution. Given the vast number of chemical ingredients present in consumer products, produced by and for industry, and used in modern medicine, it is important to determine the origin and drivers of substances that are biologically active and (pseudo)persistent. The main objective of this dissertation was to determine the origin and/or persistence of specific chemical pollutants in natural and man-made systems by evaluating their spatial distribution and/or historical trends.

In Chapter 2, the abundance of five OH-BDEs and their dioxin photoproducts in water and sediment of freshwater and coastal systems along with the anthropogenic wastewater-marker compound triclosan and its photoproduct dioxin, 2,8-dichlorodibenzo-*p*-dioxin, was investigated. Historical records and spatial trends were used to determine the dominant source of OH-BDE pollution in the San Francisco Estuary. Appendix A is a companion study that determined the levels of OH-BDEs in wastewater effluents.

The objective of Chapter 3 was to quantify the accumulation rates of antibiotics used by humans and animals, spanning several major antibiotic classes (sulfonamides, tetracyclines, fluoroquinolones, and macrolides), in Minnesota lake-sediment cores. The goal was to determine temporal trends, the major anthropogenic source to lacustrine systems, and the importance of natural production.

In Chapter 4, a high-resolution profile of antibiotics across short distances in a lake (that receives direct inputs) was compared to a comprehensive, broad spatial pattern of antibiotic occurrence in river systems (that incorporate multiple land uses). Drivers of ARG abundance (metals and antibiotics) were also evaluated in both the small and large-scale systems.

Removal efficiencies of human-use antibiotics from the liquid phase during conventional wastewater treatment was assessed in Chapter 5. Future work will compare the antibiotic residuals throughout the wastewater treatment plant to ARG abundance.

Chapter 2: Quantification of Hydroxylated Polybrominated Diphenyl Ethers (OH-BDEs), Triclosan, and Related Compounds in Freshwater and Coastal Systems

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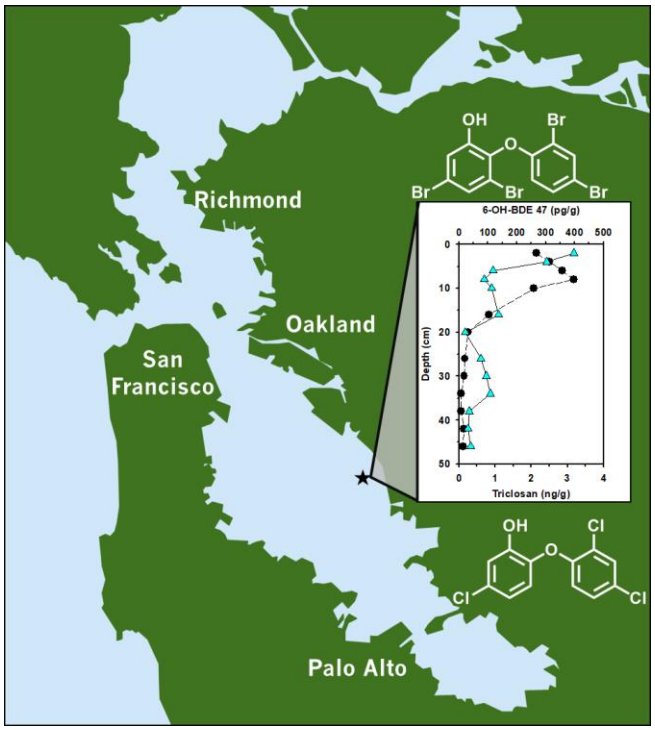

2.1 Summary

Hydroxylated polybrominated diphenyl ethers (OH-BDEs) are a new class of contaminants of emerging concern, but the relative roles of natural and anthropogenic sources remain uncertain. Polybrominated diphenyl ethers (PBDEs) are used as brominated flame retardants, and they are a potential source of OH-BDEs via oxidative transformations. OH-BDEs are also natural products in marine systems. In this study, OH-BDEs were measured in water and sediment of freshwater and coastal systems along with the anthropogenic wastewater-marker compound triclosan and its photoproduct dioxin, 2,8-dichlorodibenzo-*p*-dioxin. The 6-OH-BDE 47 congener and its brominated dioxin (1,3,7-tribromodibenzo-*p*-dioxin) photoproduct were the only OH-BDE and brominated dioxin detected in surface sediments from San Francisco Bay, the anthropogenically impacted coastal site, where levels increased along a north-south gradient. Triclosan, 6-OH-BDE 47, 6-OH-BDE 90, 6-OH-BDE 99, and (only once) 6'-OH-BDE 100 were detected in two sediment cores from San Francisco Bay. The occurrence of 6-OH-BDE 47 and 1,3,7-tribromodibenzo-*p*-dioxin sediments in Point Reyes National Seashore, a marine system with limited anthropogenic impact, was generally lower than in San Francisco Bay surface sediments. OH-BDEs were not detected in freshwater lakes. The spatial and temporal trends of triclosan, 2,8-dichlorodibenzo-*p*-dioxin, OH-BDEs, and brominated dioxins observed in this study suggest that the dominant source of OH-BDEs in these systems is likely natural production, but their occurrence may be enhanced in San Francisco Bay by anthropogenic activities.

2.2 Introduction

Polybrominated diphenyl ethers (PBDEs) have been used as flame retardants in textiles, polyurethane foam furniture padding, and electronics since the 1970s. Mass produced to serve as non-covalently-bonded additives, PBDEs frequently enter the environment by leaching from products and are detected worldwide. Manufacturing facilities,[69] sewage/wastewater effluent,[69,70] and atmospheric deposition[71] are all known sources of PBDE pollution. San Francisco Bay is a global hotspot for PBDE contamination, likely a result of California's early adoption of stringent flammability standards. In 2002, the San Francisco Regional Monitoring Program for Trace Substances (RMP) began monitoring PBDEs in water, surface sediments, and bivalves.[72] Since the ban of commercial mixtures in 2003 of Penta-BDE (which contains BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154 – the most widespread and bioaccumulative congeners) and Octa-BDE, PBDE levels in the estuary have declined in fish, bivalves, bird eggs, and sediment.[160] The reservoir of previously released PBDEs and the debromination of deca-BDEs, however, are a continuing source of these less-substituted congeners of greatest concern.

Hydroxylated PBDEs (OH-BDEs) are abiotic and biotic transformation products of PBDEs,[74–76,78–80,161] and they are also natural products in marine systems.[62,64,65,162,163] The position of the hydroxyl group (OH-) is potentially indicative of the source of OH-BDE congeners. OH-BDEs produced via oxidation of PBDEs may have the OH- in the *ortho*-, *meta*-, or *para*- position relative to the ether bridge, whereas the metabolically produced OH-BDEs have the OH- primarily in the *ortho*-position.[79,80] Studies have shown OH-BDE formation via metabolic oxidation of PBDEs in rats,[74] PBDE oxidation

in the atmosphere by OH radicals,[75] photochemical formation from brominated phenols,[76] potentially during oxidation stages in wastewater and sewage treatment,[77,161] and recently photochemically from PBDEs in aqueous solutions.[78] Recent evidence suggests that the natural production of OH-BDEs occurs by the coupling of simple bromophenols by both marine bacteria[62] and an enzyme isolated from red algae.[63] Although strong genetic evidence is lacking, studies suggest that red algae and cyanobacteria associated with marine sponges are potential OH-BDE producers independently and/or through associations with bacteria.[62,64–68,163]

OH-BDEs have been detected in higher trophic levels, such as Baltic salmon,[85] polar bears,[89] bald eagles,[88] and human plasma.[90] The highest reported level was 150 ng/g dry weight (dw) in red algae from the Baltic Sea.[64] In marine sediments, the mean concentration of 6-OH-BDE 47 was 22 ± 2.3 pg/g dw in Liaodong Bay, China[82] and levels ranged from 11.4 to 128 pg/g dw in the East China Sea.[81] In fresh waters, observed OH-BDEs levels ranged from 34 − 390 pg/L in South Korean rivers[83] and 2.2 – 70 pg/L in Lake Ontario and the Detroit River.[75] A recent study reported ΣOH-BDEs fluxes of 15 to 170 pg/m^2/day in rain and 3.5 to 190 pg/m^2/day in snow.[75] Furthermore, tetra- (6-OH-BDE 28 and 47) and penta-brominated (6-OH-BDE 90 and 99) OH-BDEs have been detected in wastewater effluents, generally at 1-10 ng/L levels.[77,161]

OH-BDEs are either equivalent or more potent endocrine disruptors and neurotoxins than the precursor PBDEs.[92,93] Studies investigating the toxic effects of OH-BDEs have reported uncoupling of oxidative phosphorylation in zebrafish,[96,164] indirect estrogenic effects in rats,[97] disruption of thyroid function and neurological development via prenatal exposure in humans,[165] and effects on hormone transport in gulls.[98] Also,

OH-BDE congeners can form polybrominated dibenzo-*p*-dioxins (PBDDs) as photoproducts in natural waters.[99,100] The phototransformation occurs only in OH-BDE congeners with a bromine *ortho* to ether linkage and an *ortho* OH- on the adjacent phenyl ring. PBDDs also have anthropogenic sources such as formation by incineration of brominated flame retardants,[105–107,166,167] and they too are also natural products in marine environments.[64,108–111] Studies have shown PBDDs have the same or greater toxicity than their chlorinated analogues, polychlorinated dibenzo-*p*-dioxins (PCDDs).[101–103,168]

Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) is an antibacterial agent in various consumer products, best known for its use in hand soaps and toothpaste. Triclosan is chemically similar to OH-TriBDE, except that triclosan is chlorinated, not brominated, and forms 2,8-dichlorodibenzo-p-dioxin (2,8-DiCDD) via photolysis in aquatic systems.[59,60] Triclosan was first produced in the 1960s,[169] and the vast majority of triclosan-containing products are washed down the drain. Triclosan removal efficiencies in wastewater treatment plants (WWTPs) are >90% with conventional activated sludge treatment.[49] Even with high removal efficiencies, triclosan is frequently detected in wastewater effluents,[32,51,170,171] which is the primary source of this pollutant in surface waters[53] and sediments[44,172,173] downstream from WWTPs. Negligible loadings come via runoff from wastewater sludge applied to agricultural fields.[53] A 30-state survey of wastewater-impacted streams and rivers detected triclosan in 57.6% of the sampled locations and reported a median and maximum concentration of 140 ng/L and 2.3 µg/L, respectively.[1] Triclosan accumulation rates in eight Minnesota lakes mirrored increased usage in consumer products, and overall levels were a function of the magnitude of wastewater input relative to lake area.[44] Triclosan may inhibit growth of various coastal

microalgae and cyanobacteria and has toxic effects on freshwater and marine invertebrates and fish.[55–58]

The objective of this research was to ascertain the importance of biosynthetic and anthropogenic OH-BDEs as brominated dioxin sources using the close structural analogue, triclosan, as an anthropogenic marker compound to assess the role of wastewater as a potential source. PBDEs may have large inputs from wastewater effluent, industry, the atmosphere, and other sources, whereas wastewater effluent is the primary source of triclosan. Because the onset of production and use of triclosan and PBDEs followed a similar timeline, we hypothesized that co-occurrence of triclosan and PBDEs/OH-BDEs could indicate a common anthropogenic source for the compounds. In this study we 1) measured OH-BDE congeners and triclosan in sediment and surface waters and 2) measured the levels of OH-BDE-derived brominated dioxins in surface sediments and correlated them with triclosan, triclosan-derived dioxin, and PBDEs levels/trends. Sediments from WWTP-impacted freshwater lakes (Lake Pepin, Lake St. Croix, and East Gemini Lake, MN), a relatively pristine marine environment (Point Reyes National Seashore, CA), and a WWTP-impacted estuary (San Francisco Bay, CA) were collected for this study. The OH-BDEs investigated in this study were selected because they: (1) were all capable of forming dioxins via photolysis, and (2) had different sources (anthropogenic and/or natural). Of the target OH-BDE congeners investigated, some have known natural and anthropogenic origins (6-OH-BDE 47, 6-OH-BDE 90, and 6-OH-BDE 99), whereas others are not known to be natural products (6'-OH-BDE 100 and 6'-OH-BDE 118). Only three brominated dioxins were included in this study due to commercial availability limitations. The photoproducts 1,3,7-TriBDD, 1,2,4,8-TeBDD,

and 2,3,7,8-TeBDD (the most toxic PBDD) of 6-OH-BDE 47, 6-OH-BDE 99, and 6'-OH-BDE 118, respectively, were measured. The OH-BDE levels were compared with PBDE, PBDD, triclosan, and 2,8-DiCDD levels/trends.

2.3 Materials and Methods

San Francisco Bay surface waters were collected by the San Francisco Estuary Institute (SFEI) and Applied Marine Sciences during a regularly scheduled RMP water sampling cruise aboard the vessel *RV Turning Tide*. A water sample was collected from RMP station LSB055W (GPS coord: 37.48458, -122.11815) on July 31, 2013, and from station BG30 (38.02041, -121.80537) on August 8, 2013. Water samples were collected into cleaned amber glass 4-L jugs, and stored on wet ice (~4 °C) in a dark cooler while on board the vessel. Samples were shipped on liquid ice packs to the University of Minnesota where they were filtered with pre-combusted glass fiber filters, acidified to pH 3, and stored at 4 °C.

San Francisco Bay surface sediments were collected between August 22 and August 31, 2011 on the *RV Endeavor* at locations shown in Figure 2.1 (GPS coordinates located below). The sampling scheme was designed as a spatially distributed unbiased representative sampling of the habitat resource. Surface sediments were collected using a Van Veen grab, with a composite of the top 5 cm of sediment from each site. Sediment cores of 50-60 cm in length were collected from RMP sites in Central Bay (Station CB001S, GPS 37.87645, -122.36132) and South Bay (Station SB002S, GPS 37.61025, -122.16757). The sediment cores were collected using a piston corer equipped with a 70-cm polycarbonate core barrel and operated from the water surface by Mg-alloy drive

rods. Cores were extruded while on board the vessel and sectioned at 2- or 4-cm intervals. Push-cores were collected at low tide in shallow waters of the Limantour Estero at three sites (A: GPS 38.031225, -122.903838; B: GPS 38.031725, -122.90855; C: GPS 38.032036, -122.91358) at Point Reyes National Seashore on August 20, 2011 and extruded at 5 or 6-cm intervals. All sediment samples were placed into glass sample jars with foil-lined lids, frozen in the field on dry ice, and transported to the University of Minnesota. The cores from Lake Pepin, East Lake Gemini and Lake St. Croix were previously collected in 2010 (July – September) by Anger et al.[44] using a piston corer as described above.

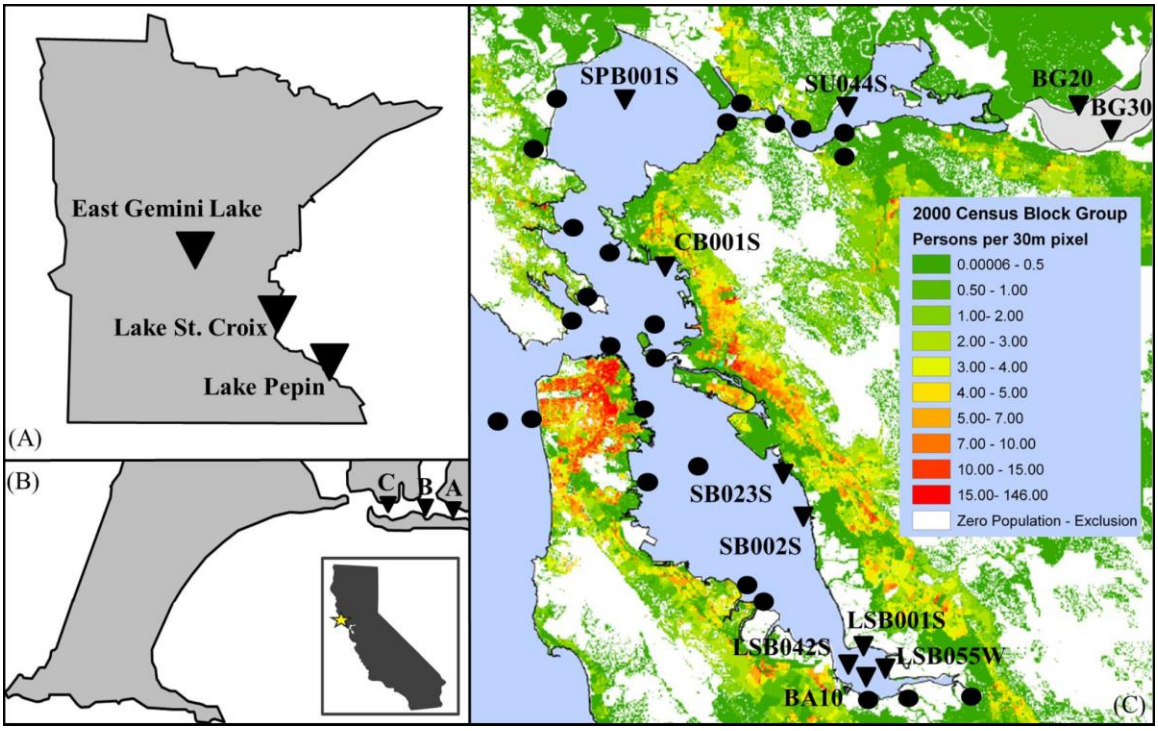

Figure 2.1. Maps of Minnesota (A) and California (B and C) sampling locations. (A) East Gemini Lake, Lake St. Croix, and Lake Pepin in Minnesota; (B) Point Reyes National Seashore, CA, and (C) 2000 census population density for the San Francisco Bay region generated by Dasymetric (ArcGIS10x) software courtesy of the U.S. Geological Survey with wastewater outfalls (black circle) and surface sediments, cores, and surface waters collection sites (black triangles).

The structures of the target analytes are shown in Figure 2.2. 6-OH-BDE 47, 6-OH-BDE 99, 6'-OH-BDE 100, and 6'-OH-BDE 118 were synthesized and purified as described previously.[99,174] The synthesis of 6-OH-BDE 90 was performed according to Hensley et al.[77] Note that the impurity of 6'-OH-BDE 100 was most likely due to a structural rearrangement.[99] Triclosan (TCS, >97%) was purchased from Sigma Aldrich. The $^{13}C_{12}$-triclosan ($^{13}C_{12}$-TCS) (50 µg/mL in methanol, >99%), $^{13}C_{12}$-6-OH-BDE 47 (50 µg/mL in methanol, >99%), and $^{13}C_{12}$-6'-OH-BDE 100 (50 µg/mL in toluene, >99%), were purchased from Wellington Laboratories. The dioxins 1,3,7-TriBDD (10 µg/mL in toluene), 1,2,4,7/1,2,4,8-TeBDD-mixed (10 µg/mL in toluene), 2,3,7,8-TeBDD (1 mg), and 2,8-DiCDD (50 µg/mL in isooctane) and were purchased from AccuStandard, as well

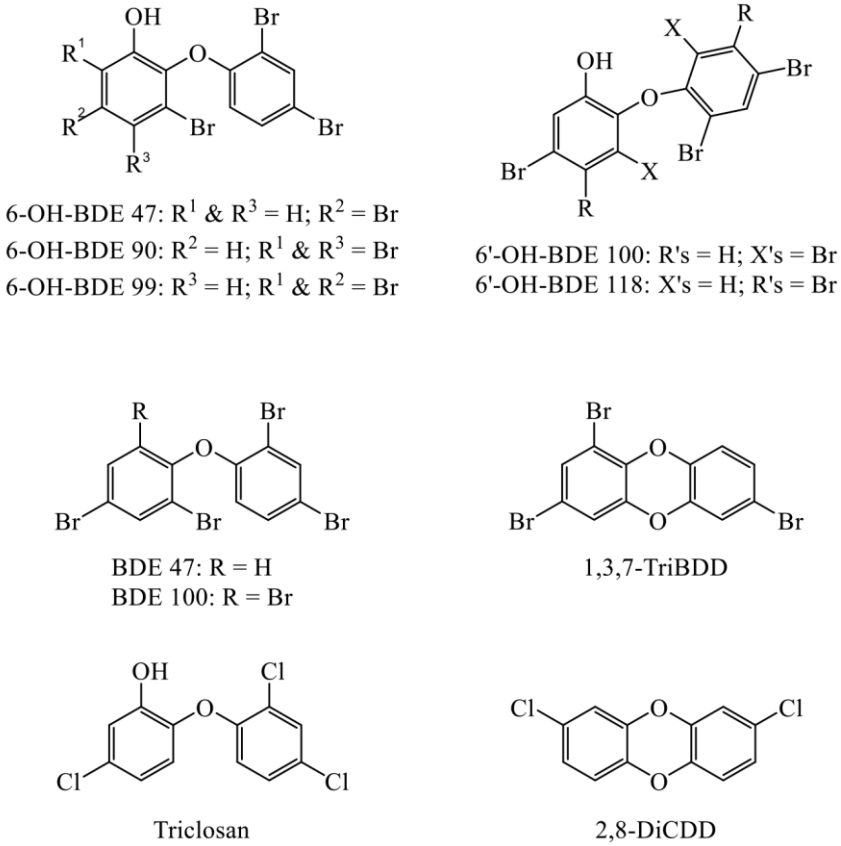

Figure 2.2. Chemical structures of OH-BDEs, PBDEs, triclosan, and polyhalogenated dibenzo-p-dioxins (PXDDs).

as the brominated and chlorinated surrogates $^{13}C_{12}$-2,3,7,8-TeBDD (99%, 5 µg/mL in nonane) and $^{13}C_{12}$-2,3-DiCDD (99%; 50 µg/mL), respectively. Sand (S25516A) and sulfuric acid were from Fisher Scientific. Ammonium acetate was from Mallinckrodt. Ultrapure water (18.2 MΩ-cm) was generated using a Millipore Simplicity UV purification system. All organic solvents used were HPLC grade, expect for methyl-*tert*-butyl ether (MTBE) which was ACS grade (>99%). Ultra-high purity and industrial-grade nitrogen were purchased from Matheson.

Radiometric Dating. The Central and South Bay cores from San Francisco Bay were dated by ^{210}Pb using isotope-dilution, alpha spectrometry methods and the constant flux:constant sedimentation (cf:cs) model.[45,175] The Central Bay core was also analyzed for ^{137}Cs by gamma spectrometry to provide a supplemental dating marker to validate the ^{210}Pb chronology. The Lake Pepin core was dated by stratigraphic correlation of whole-core magnetic susceptibility profiles with a radiometrically-dated master core collected previously from the same location.[46]

Surface Water and Sediment Extraction Methods. The solid phase extraction method and silica column clean-up for isolating OH-BDEs in surface waters was adapted from Buth et al [62] and a detailed explanation can be found in Appendix B. Subsamples of sediments were analyzed for moisture content and loss-on-ignition. Samples were weighed after being heated for 12 hours at 105 °C, 4 hours at 550 °C, and 2 hours at 1000 °C to determine water, organic, and carbonate content, respectively. Sediments (~12 g dw) were freeze dried for 3 – 5 days and stored at -20 °C until extraction. The accelerated solvent extraction (ASE) method for OH-BDEs in sediments was adapted from Anger et al,[44] and a detailed explanation can be found in Appendix B.

Between 1 and 20 g (dw) of each sediment sample were extracted separately from OH-BDE analysis to be analyzed for 2,8-DiCDD and the targeted PBDD congeners. For all cores, samples were spiked with nineteen $^{13}C_{12}$-labeled di- through octa-CDD/F isomers and with $^{13}C_{12}$-labeled 2,3,7,8-TeBDD as isotope dilution surrogates. The core samples were then analyzed using an expanded version of U.S. EPA Method 1613B.[176] The extraction and high-resolution gas chromatography-high-resolution mass spectrometry (HRGC-HRMS) analysis are described in Appendix B..

LC-MS/MS Method. Extracts were analyzed with a Waters nanoAcquity capillary high performance liquid chromatograph (LC) equipped with a Thermo Scientific TSQ Ultra AM MS-Q^3 tandem mass spectrometer (MS/MS) using a negative electrospray ionization (ESI) source. The analytical method was adapted from Feo et al.[177] The stationary phase was a Thermo Hypersil Gold column (150 × 0.5 mm, 3µm) heated at a constant 30 °C. The injection volume was 8 µL. The mobile phase was a binary gradient with (A) 3:2 15 mM ammonium acetate:MeOH and (B) acetonitrile with a flowrate of 15 µL/min. An initial 25% B ramped up to 40% B by 5 minutes, 46% B by 10 min, 48% B by 23 min, and 80% B at 25 min. Until 27 min, B remained at 80% and then ramped down to 25% B for a 10 min re-equilibration. A single reaction monitoring (SRM) transition was used for chemical quantification, in addition to another SRM transition to confirm the identity of the chemical (B.1 Table). Instrument blanks (50:50 H_2O:acetonitrile) were run every 7 or 8 samples to evaluate contamination via sample injections.

The mass spectrometer was infused with $^{13}C_{12}$-TCS (30 mg/L in 50:50 H_2O:acetonitrile) at the beginning of each analysis to optimize MS/MS parameters which

varied slightly between runs due to the high sensitivity of the instrument. Typical optimized values were: collision energy: 11; scan time: 0.15 s; Q_1/Q_3: 0.7; spray voltage: 2700 V; sheath gas pressure: 11 psi; capillary temperature: 300 °C; and collision pressure: 0.9 mTorr. Also, it was necessary to run a sediment extract two or three times at the beginning of each sequence to acquire consistent analyte signals.

Additional experimental and analytical details including cleaning protocols and calculation of absolute and relative recoveries and analyte concentrations using response factors are in Appendix B.

2.4 Results

Analytical Method Performance. The LC-MS/MS method for triclosan and OH-BDEs quantification separated the analytes of interest. Typical chromatograms for standards and samples can be seen in Figure B.1. It was determined that 6'-OH-BDE 100, labeled and unlabeled, transformed into another unknown OH-PentaBDE (retention time of 15.30 min in Figure B.1). This transformation was enhanced during the sediment extraction (using accelerated solvent extraction) and the transformation peak was slightly less retained than the ($^{13}C_{12}$-)6'-OH-BDE 100 during LC-MS/MS analysis. The sum of these two peak areas (($^{13}C_{12}$-)6'-OH-BDE 100 and its transformation product) was used to account for the total presence of ($^{13}C_{12}$-)6'-OH-BDE 100. It should be noted that matrix effects in the samples caused shifts in retention times among samples, thus whenever possible internal standards were used to corroborate a peak's identity. Not all analytes, however, had commercially available isotope labeled congeners. The difference of retention times between internal standard and analyte, therefore, was used to confirm

the peak's identity when no internal standard was available. Furthermore, other studies quantified penta- and tetrabrominated OH-BDEs that were not included in this study (e.g. 2'-OH-BDE 68). It is possible that an unknown OH-BDE co-eluted in environmental samples, but it unlikely considering the separation achieved in the work from which our method was derived.[177]

Linear calibration curves ranged from 2 – 500 μg/L for OH-BDEs and 1 – 400 μg/L for triclosan and were of high quality ($R^2 > 0.98$). The limits of detection (LOD) and quantification (LOQ) were calculated from the method blanks. The area in the blanks at the same retention times as the analytes was integrated and multiplied by 3 or 10 for the LOD and LOQ, respectively. Because 6'-OH-BDE 100 was detected in a single sample and 6'-OH-BDE 118 in no samples, the lowest concentration of the calibration curve was used to calculate an alternative LOQ for these two chemicals. Due to the variability of instrument's sensitivity, LOQs ranged from 16 – 27 pg/g and 0.04 – 0.07 ng/L for triclosan in sediment and water, respectively, and 2 – 28 pg/g and 0.004 – 0.17 ng/L for OH-BDEs in sediment and water, respectively (Table 2.1). LODs ranged from 5 – 8 pg/g and 0.01 – 0.02 ng/L for triclosan in sediment and water, respectively, and 0.6 – 6.4 pg/g and 0.001 – 0.05 ng/L for OH-BDEs in sediment and water, respectively (see Table 2.1).

Table 2.1. Limits of detection and quantification for triclosan and OH-BDEs in water (ng/L) and sediment (pg/g) samples.

Chemicals	**LODs**		**LOQs**	
	Water (ng/L)	*Sediment (pg/g)*	*Water (ng/L)*	*Sediment (pg/g)*
Triclosan	0.01 - 0.02	5 - 8	0.04 - 0.07	16 - 27
6-OH-BDE 47	0.03 - 0.05	0.6 - 2.4	0.10 - 0. 17	2 - 8
6-OH-BDE 90	0.002 - 0.005	0.9 - 6.4	0.007 - 0.015	3 - 21
6'-OH-BDE 99	0.001 - 0.014	0.6 - 5.4	0.004 - 0.046	2 - 18
6'-OH-BDE 100*	N/A	N/A	0.1	28
6'-OH-BDE 118*	N/A	N/A	0.08	8

*Not detected in sample, LOQ was determined by lowest concentration of the calibration curve

The sediment and water concentrations above LOQ were calculated using isotope dilution analysis and were recovery corrected. The absolute recoveries were calculated for the isotope labeled compound (Table 2.2), and details are located in Appendix B. The relative recoveries for triclosan and OH-BDEs ranged from 44 – 133 % in sediment and 70 – 134 % in water samples, respectively (see Table 2.3). Note that lower recoveries increase the uncertainty in reported concentrations, but should not alter observed trends for each analyte. See Table B.2 for the absolute and relative recoveries for $^{13}C_{12}$-PXDDs and PXDDs, respectively. The dry density and percent organic, carbonate, and inorganic for every core interval and surface sediment was determined, and results are located in Table B.3 and Figure B.2.

Table 2.2. Absolute recovery (%) of isotope labeled compounds in sediment and water matrices in n number of samples.

Site	$^{13}C_{12}$-TCS	$^{13}C_{12}$-6-OH-BDE 47	$^{13}C_{12}$-6'-OH-BDE 100	n
Surface Water	74 ± 24	54 ± 19	43 ± 13	17
South Bay Core	50 ± 21	42 ± 21	41 ± 16	17
Central Bay Core	78 ± 18	45 ± 11	36 ± 13	17
Surface Sediments	24 ± 12	21 ± 8	11 ± 4	10
Point Reyes National Seashore	71 ± 51	62 ± 31	41 ± 24	13

Table 2.3. Relative recovery (%) of analytes in sediment and water.

Chemical	Sediment	Water
Triclosan	133 ± 52	134 ± 12
6-OH-BDE 47	99 ± 8	104 ± 5
6-OH-BDE 90	72 ± 27	100 ± 24
6-OH-BDE 99	82 ± 33	93 ± 20
6'-OH-BDE 100	55 ± 9	117 ± 12
6'-OH-BDE 118	44 ± 21	70 ± 10

Contaminant Levels in Surface Water and Surface Sediment Samples. 6-OH-BDE 90 levels were elevated in the southern surface waters (LSB055W, 40 pg/L) relative to the northern surface water (BG30, < 12 pg/L), see Table 2.4. The other naturally produced OH-BDEs, 6-OH-BDE 47 and 6-OH-BDE 99, were not detected in the BG30 sample, but were detected (< 129 pg/L and < 19 pg/L, respectively) in the LSB055W sample. The anthropogenic OH-BDEs, 6'-OH-BDE 100 and 6'-OH-BDE 118, were not detected in any water sample. Triclosan concentrations were elevated in LSB055W (68 ± 26 ng/L) compared to the outlet of the San Joaquin River (BG30, 17 ± 9 ng/L). The salinity near the Sacramento and San Joaquin River outlets was low, 0.1 and 0.2 psu respectively, due to the freshwater input of the rivers. The salinity was fairly uniform

(25.2 ± 1.9 psu) in the Central, South, and Lower South bays. Salinity measurements were taken a month after the sediments were collected, and most salinity values in Table 2.5 were taken at nearby collection points (see Table B.4 for GPS coordinates).

Table 2.4. Concentrations (ng/L) of triclosan and OH-BDEs in surface waters.

Surface Water Levels (ng/L)		
Chemical	*BG30*	*LSB055W*
Triclosan	17 ± 9	68 ± 26
6-OH-BDE 47	ND	< 0.129
6-OH-BDE 90	< 0.012 [a]	0.040 [b]
6-OH-BDE 99	ND	< 0.019 [a]

[a] One replicate > LOD and < LOQ, with other replicates < LOD
[b] One replicate >LOQ, two replicates >LOD and <LOQ, and one replicate <LOD
ND denotes concentration < LOD

6-OH-BDE 47 and 1,3,7-TriBDD were the only OH-BDE and brominated dioxin, respectively, detected in San Francisco Bay surface sediments. Sediments near the northern rivers outlets had low to non-detected levels of 6-OH-BDE 47 and 1,3,7-TriBDD. Concentrations of 6-OH-BDE 47 (< 8.1 – 263.8 pg/g) and 1,3,7-TriBDD (3 – 15 pg/g) varied throughout the rest of the estuary with higher levels in the South and Lower South Bay (see Table 5). 6-OH-BDE 47 levels were higher than 1,3,7-TriBDD (2 – 36×) in San Francisco Bay surface sediments.

The relevant precursor PBDEs of anthropogenic 6-OH-BDE 47 are BDE 47 and BDE 100 (∑PBDE(47 +100)). The major formation pathways of 6-OH-BDE 47 is addition of –OH to the ring (BDE 47) and replacement of a –Br by –OH (BDE 100). The SFEI monitors approximately 50 PBDEs congeners in San Francisco Bay sediments and the entire data set is available at http://www.sfei.org/rmp/wqt.[178] The levels of BDE 47 and 100 shown in Table 5 originated from this data set. There were low to negligible

Table 2.5. Concentrations of triclosan, PBDEs, 6-OH-BDE 47, and PXDDs in San Francisco Bay sediments and salinity in surface waters.

Site Name	Sample ID[a]	Latitude	Longitude	Triclosan (ng/g)	2,8-DiCDD (pg/g)	BDE 47[b] (pg/g)	BDE 100[b] (pg/g)	6-OH-BDE 47[c] (pg/g)	1,3,7-TriBDD[d] (pg/g)	Salinity[b] (psu)
Sacramento River	BG 20	38.0583	-121.81407	0.17	12	46	12	ND	ND	0.1
Sacramento River	BG 30	38.02285	-121.80845	0.11	8	ND	ND	ND	1.5	0.2
Suisun Bay	SU044S	38.07597	-122.05687	0.21	15	30	ND	< 8.1	3	5.4[f]
San Pablo Bay	SPB001S	38.07262	-122.38622	2.03	38	318	57	< 8.1	1.8	18.3[f]
Central Bay	CB001S	37.87645	-122.36132	4.32	58	513	93	23.0	ND	28.4[f]
South Bay	SB023S	38.10478	-122.39208	2.45	110	137	23	82.5	11	26.6[f]
South Bay	SB002S	38.01615	-122.34122	2.30	150	226	40	263.8	15	24.3[f]
Lower South Bay	LSB001S	37.49168	-122.09868	6.00	N/A	590	105	16.5	N/A	24[f]
Lower South Bay	LSB042S	37.47168	-122.09555	5.47	160	273	35	188.2	7.2	23.9[f]
Coyote River	BA10	37.46812	-122.06385	4.64	120	524	106	12.8	6.4	24.1[f]

[a] Sample IDs are those used by SFEI for these sampling locations in their Regional Monitoring Program
[b] San Francisco Estuary Institute[178]
[c] OH-BDEs with concentrations < LOD are not shown, includes: 6-OH-BDE 90, 6-OH-BDE 99, 6'-OH-BDE 100, and 6'-OH-BDE 118
[d] PBDDs with concentrations < LOD are not shown, includes: 1,2,4,7/1,2,4,8-TeBDD, and 2,3,7,8-TeBDD
[e] Concentration > LOD and < LOQ
[f] Measured at nearby sites, see S4 Table.
ND denotes concentration < LOD
N/A denotes a sample that was not analyzed for a specific compound

levels of triclosan, BDE 47, and BDE 100 in surface sediments near the Sacramento and San Joaquin rivers in the northern part of the estuary, but higher and relatively uniform concentrations (2-6 ng/g for triclosan, 137-590 pg/g for BDE 47, and 23-106 pg/g for BDE 100) across the Central, South, and Lower South bays (see Table 2.5). A significant and positive correlation was seen between ∑PBDE(47 +100) and triclosan ($p = 0.001$, $R^2 = 0.75$; Figure B.3). There was no significant correlation between 6-OH-BDE 47 and either ∑PBDE(47 +100) ($p = 0.89$, $R^2 = 0.002$) or triclosan ($p = 0.50$, $R^2 = 0.057$) in the surface sediments (Figure B.3). Furthermore, both 6-OH-BDE 47 and triclosan had positive and significant correlations with their respective photochemically produced dioxins (Figure B.3).

Previous work by Anger et al[44] and Buth et al[172] showed increasing levels of triclosan and 2,8-DiCDD in Minnesota lake sediments, including Lake Pepin, East Lake Gemini, and Lake St. Croix, since the mid-1960s. The same Lake Pepin samples analyzed by Anger et al[44] were re-analyzed using this study's LC-MS/MS method, and no OH-BDEs were detected. Yet, 1,3,7-TriBDD was detected in three Lake Pepin sediments in core intervals dated to 2009, 2005, and 1997 at 2, 2.1, and 1.2 pg/g, respectively. No brominated dioxins were detected in core sediments dated to 1992-1944 in Lake Pepin, nor in any sediments from Lake St Croix and East Lake Gemini. Thus, wastewater effluent is a known source of anthropogenic chemicals in these freshwater lakes, yet no OH-BDEs, which must arise from anthropogenic sources in these freshwaters, were detected in these sediments.

At the relatively pristine marine site (Point Reyes National Seashore), 6-OH-BDE 47 and 1,3,7-TriBDD concentrations ranged from non-detected to 36.3 pg/g and non-

detected to 2.4 pg/g, respectively. No other OH-BDEs or brominated dioxins were detected in these marine sediments. Low levels of the anthropogenic marker triclosan (0.02 – 0.55 ng/g) and 2,8-DiCDD (7 – 10 pg/g) were detected in these cores (Table 2.6), suggesting that the measured OH-BDEs at Point Reyes National Seashore originate from biological production and not from anthropogenic PBDEs.

Table 2.6. Concentration of triclosan, 2,8-DiCDD, 6-OH-BDE 47, and 1,3,7-TriBDD in three sediment cores (A, B, & C) at Point Reyes National Seashore.

Depth (cm)	Triclosan (ng/g)			2,8-DiCDD (pg/g)			6-OH-BDE 47[c] (pg/g)			1,3,7-TriBDD[d] (pg/g)		
	A	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*
0 - 5	0.12	0.21	0.55	7.5	5.4	7.1	<8.1[b]	<8.1	14.2	2.4	0.99	1.2
5 - 10	0.02	0.19	0.23	8.2	9.1	7.3	9.4	ND	21.9	2.4	ND	1.7
10 – 15[a]	0.17	0.2	0.31	10	7	8.8	<8.1	<8.1	36.3	3	ND	1.8

ND denotes analyte levels below LOD
[a] Final depth for core 'C' is 16 cm
[b] Concentration > LOD and < LOQ
[c] OH-BDEs with concentrations < LOD are not shown, includes: 6-OH-BDE 90, 6-OH-BDE 99, 6'-OH-BDE 100, and 6'-OH-BDE 118
[d] PBDDs with concentrations < LOD are not shown, includes: 1,2,4,7/1,2,4,8-TeBDD, and 2,3,7,8-TeBDD

Temporal Trends for 6-OH-BDE 47 and Triclosan. Lead-210 activities were low throughout both San Francisco Bay cores, showing an irregular down-core decline to steady background (supported) values below 40 cm in Central Bay and 20 cm in South Bay. Given the uncertainty in these activity profiles, an approximate chronology was determined by assuming a constant sediment flux fitted to the data by least-squares regression (the cf:cs model) (Figure B.4). Although dating uncertainty is high (± 10 years at 1950 in Central Bay; ± 22 years at 1964 in South Bay), the resulting correspondence with the known history of triclosan use and discharge (see below) provides confirmation that the dating is reasonably correct. The analysis of ^{137}Cs as a supplemental dating

marker was uninformative, because no radiocesium was detected below the uppermost core interval in Central Bay (0-2 cm). We attribute the absence of measurable ^{137}Cs in more recent sediments to diffusional losses and/or dilution from high rates of sediment mixing.

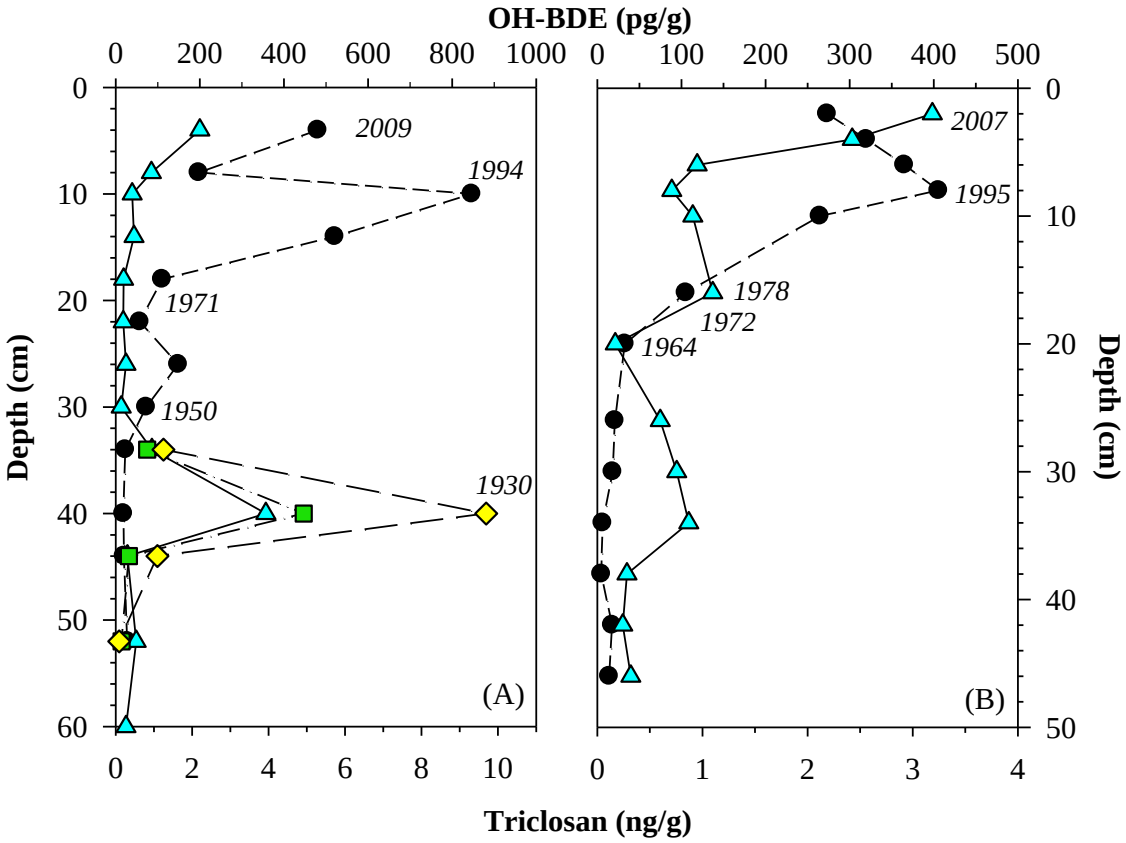

Figure 2.3. Concentration profile of OH-BDEs and triclosan in sediment cores. (A) Central Bay, CA and (B) South Bay, CA. Profiles are given for 6-OH-BDE 47 (blue triangle), 6-OH-BDE 90 (yellow diamond), 6-OH-BDE 99 (green square), and triclosan (black circle). Italicized dates are approximate years determined by ^{210}Pb.

Triclosan levels rose in Central Bay around 1960, and a maximum concentration (9.31 ng/g) was reached in ca. 1994 (Figure 2.3). 6'-OH-BDE 100 (1.2 ng/g) was also measured in ca. 1994 Central Bay sediment, and this was the only sample in which an anthropogenic-only-sourced OH-BDE was detected. The detection of 6'-OH-BDE 100

was determined by the presence of its un-identified, less retained transformation product during LC-MS/MS analysis. 6-OH-BDE 47 was detected throughout the sediment record with maximum concentrations in 1930 (356 pg/g) and the surface sediment (200 pg/g). Around the 1930s, 6-OH-BDE 90 and 6-OH-BDE 99 were detected at high concentrations (447 and 881 pg/g, respectively), in addition to an unknown compound, most likely another OH-PentaBDE (Figure B.1). Two additional unknown compounds, potentially OH-PentaBDEs, were detected in the two most recent sediments of Central Bay. Sediments before 1964 had low levels of triclosan (mean 0.11 ng/g, median 0.13 ng/g) in the South Bay core, and rose over time with more recent sediments having 2-3 ng/g. The occurrence of 6-OH-BDE 47 fluctuated throughout the South Bay core (concentrations ranged from 21 to 398 pg/g) with rising levels beginning in 1995 that continued to present day.

2.5 Discussion

Spatial Trend in Impacted vs Pristine Marine Systems. Triclosan and ∑PBDE(47 +100) were used as anthropogenic markers in this study. Triclosan and ∑PBDE(47 +100) were used as anthropogenic markers in this study. Wastewater effluent is the main source of triclosan, but PBDEs have numerous pathways including: manufacturing facilities,[69] sewage/wastewater effluent,[69,70] and atmospheric deposition.[71] The positive correlation between ∑PBDE(47 +100) and triclosan levels in San Francisco Bay suggests that these anthropogenic chemicals originate from urban sources, like wastewater effluent, and past studies have measured PBDEs in local wastewater.[70]

Lower levels of the target chemicals at the BG20 and BG30 sites are most likely due to the generally lower population density, smaller and fewer nearby WWTP outfalls (Figure 2.1), and larger inputs of freshwater from major rivers. Natural production of OH-BDEs likely does not occur in surface waters near the outlets of the Sacramento (BG20 site) and San Joaquin River (BG30 site) because of the low salinity and bromide levels. Suisun and San Pablo Bay are also well flushed from large freshwater inflows in addition to tidal mixing,[179] and are less urbanized than the central and southern bays, so lower levels of most chemical pollutants are generally expected and found.[72,180] Thus, low levels of triclosan, ∑PBDE(47 +100), biosynthetic or anthropogenic OH-BDEs, and PXDDs were detected in surface waters or sediments in the northern bays due to the low bromide levels, less urbanization, and well-flushed bays. The South and Lower South bays are not as well-flushed relative to their development density, have high salinity and bromide concentrations, and thus are more susceptible to both pollution from anthropogenic discharge and conditions favoring the accumulation of natural OH-BDEs.

Metabolites of PBDEs might be expected to have a similar distribution to PBDEs but generally at lower concentrations.[181–184] The generally uniform distribution of ∑PBDE(47 +100) (around 4- to 5-fold difference between minimum and maximum concentrations) in the southern bays was not observed for 6-OH-BDE 47 and 1,3,7-TriBDD, whose concentrations varied among sampling sites by up to 15-fold. Such uncorrelated spatial trends for these groups of chemicals were also noted along the Swedish coastline.[110] No signification correlation was seen between 6-OH-BDE 47 and ∑PBDE(47 +100), suggesting that these chemicals have different origins, most likely natural production and wastewater effluent/atmospheric deposition, respectively.[110] The

lack of correlation could also be due to a variation in PBDE degradation pathways across the sampling sites in San Francisco Bay or the co-elution of an unknown OH-BDE with 6-OH-BDE 47 during LC-MS/MS analysis. Lower OH-BDE concentrations compared to PBDEs is indicative of PBDE transformation. Several studies investigating the oxidation mechanism of PBDE measured lower concentrations of OH-BDEs compared to the parent compound, owing to a slow oxidation reaction rate.[181–184] This pattern was found at most sites with 6-OH-BDE 47 lower that BDE 47, aside from SB002S and LSB042S, where the 6-OH-BDE 47 was nearly the same concentration or higher than the parent BDE 47.

On the other hand, the OH-BDEs hypothesized to be primarily anthropogenic (6'-OH-BDE 100 and 6'-OH-BDE 118), i.e. not known natural products, were not detected in San Francisco Bay (with the exception of the single detection of 6'-OH-BDE 100). The presence of 6'-OH-BDE 100 also corresponded to the maximum concentration of triclosan in the Central Bay core suggesting that at this time there may have been higher loadings of anthropogenic inputs. The overall absence of these two OH-BDEs supports the hypothesis that the dominant source of OH-BDEs in these locations is natural production. One limitation of this study is the relatively lower analytical sensitivity (1.5 to 25-fold) for 6'-OH-BDE 100 and 6'-OH-BDE 118 compared to the other OH-BDEs. Another possibility is that higher brominated PBDEs may have had slower oxidation rates, and their OH-BDE products were not detected due to the relatively higher LODs. There is a possibility, therefore, that a portion of the 6-OH-BDE 47 measured in San Francisco Bay are metabolites of BDE 47, given the previous arguments. The natural production of 6-OH-BDE 47, however, is likely more important.

Anthropogenic activities may also *indirectly* influence the natural production of OH-BDEs. The elevated nutrient load and temperature from anthropogenically impacted waters may cause a flourish in marine microbial activity near large urban areas, such as San Francisco Bay. In contrast, Point Reyes National Seashore is a lightly developed coastal ecosystem with little to no urban anthropogenic influences, which is reflected in the low levels of triclosan and 2,8-DiCDD. There is some agricultural activity (mostly ranching) in surrounding watersheds, but monitoring of nutrients in creeks draining to Point Reyes show no consistent trends of higher concentrations in watersheds with agricultural uses.[185] 6-OH-BDE 47 and 1,3,7-TriBDD levels in the national park, therefore, are representative concentrations for natural production that are slightly or negligibly altered by human activities. Slight variations in 6-OH-BDE 47 levels were observed in the samples taken from Point Reyes National Seashore (only one of the three sets of samples had levels above 10 pg/g). These concentrations, however, are smaller than or near the lowest levels measured pre-1970s sediments in the South Bay (21 – 108 pg/g) and Central Bay (13 – 86 pg/g, excluding the ca. 1930 spike) cores. Therefore, the overall higher 6-OH-BDE 47 concentrations observed in the urbanized San Francisco estuary suggest that enhanced natural production (or, less likely, degradation of anthropogenic PBDEs) is occurring in the estuary

Freshwater vs Coastal Systems. Lake Pepin is a natural impoundment of the upper Mississippi River, located downstream of several WWTPs and the large metropolitan centers of Minneapolis and St. Paul, MN. Buth et al[172] documented the historical accumulation of triclosan and its triclosan-derived dioxin in Lake Pepin since the 1960s. Although no previous studies have investigated PBDE levels in Lake Pepin,

PBDEs are ubiquitous and it is highly likely that PBDEs are present in Lake Pepin sediments given the notable presence of triclosan. Because natural production of OH-BDEs cannot occur in freshwater, any OH-BDEs present in Lake Pepin are likely derived from PBDEs. No OH-BDEs were detected and there were only low levels of 1,3,7-TriBDD in a few samples. The presence of 1,3,7-TriBDD without detected 6-OH-BDE 47 could be due to a lower detection limit for the PBDD, or the brominated dioxin was a combustion product of brominated flame retardants and atmospherically deposited from regional sources. Other researchers have found chlorinated dioxins in freshwater sediments, but no brominated dioxins, which indicate different sources,[109] or at least different relative magnitudes of their sources. Chlorine is naturally more abundant, with measured PCDD formation even from combustion of wood and other natural fuels, whereas literature on combustion formed PBDDs primarily documents production from co-combustion of anthropogenic wastes, which generally include PBDEs. This is not to say, however, that there is no PBDD production from combustion of natural fuels, given potentially trace levels of bromines in many materials. Some PBDD formation from combustion is possible and perhaps even likely, but may be too low to measure using current analytical methods.

Temporal Trends. Although radiometric dating of the two coastal cores from San Francisco Bay yielded results with substantial uncertainties, the resulting chronologies agree well with the expected temporal trends of triclosan, providing important confirmation of the historical trend of OH-BDE production. As seen in ^{210}Pb, the profile of triclosan may have been affected by fluctuating sedimentation rates which caused the drop in Central Bay after 1994. The noticeable rising levels after the 1960s, however,

confirm the overall usage trends for this anthropogenic chemical. The presence of triclosan near the LOD in pre-1960s sediments is likely due to contamination during the collection, extraction, and/or sample clean-up process. It is extremely difficult to maintain a triclosan-free laboratory environment due to the ubiquitous presence of triclosan. Levels of triclosan pre-1960 are much lower than post-1960s, and thus samples were not significantly contaminated.

Based on the presence of OH-BDEs throughout the cores, it is likely that these compounds were naturally produced in Central and South Bay during the last century. 6-OH-BDE 47 is present throughout both cores, even though PBDEs were not used in consumer products until the 1970s. The accumulation of OH-BDEs in the environment, however, is likely influenced by other anthropogenic drivers. Around the 1930s, concentrations of the biosynthesized 6-OH-BDE 47, 6-OH-BDE 90, and 6-OH-BDE 99 in Central Bay spiked upward, and an unidentified, probably biosynthetic, OH-PentaBDE was detected. Increasing population and the discharge of raw sewage into San Francisco Bay may have contributed to enhanced natural production.[186] It was roughly estimated that in 1910 approximately half of the total population in California was disposing of untreated sewage by discharging into estuaries, tidal bays, etc.[187] It was not until the early 1950s that San Jose, San Francisco, Oakland and other communities surrounding the bay built primary wastewater treatment plants to combat and resolve the pollution of the bay.[186] The drought that occurred from 1928-1934 may have also influenced OH-BDEs production in the system. With the exception of Central Bay in the 1930s, which was more heavily developed at the time, the levels of 6-OH-BDE 47 pre-1970s in the rest of the bay were similar to those measured in Point Reyes National Seashore, which is

consistent with the expectation that enhanced biosynthesis would occur in the more developed areas.

The increasing levels of 6-OH-BDE 47 after the 1970s are likely a result of increased natural production. That is not to say that the transformation from PBDEs via abiotic (i.e. photolysis and oxidation by OH radicals) and biotic processes (i.e. metabolic oxidation) cannot account for a percentage of the more recent rise and fall in levels in San Francisco Bay, but our work suggests that biogenic production enhanced by wastewater discharge, another anthropogenic activity, or potentially even climate change are more likely contributors given: (1) the lack of synthetic PBDEs during an earlier ca. 1930s spike in OH-BDEs; (2) no OH-BDEs were detected in the anthropogenically impacted freshwater lakes; and (3) lower OH-BDE levels were measured in the relatively pristine marine system than in surface sediments from the anthropogenically impacted bay. Rising levels of PBDDs in mussels over the past decade was attributed to eutrophication and climate change enhancing biosynthetic production.[109] Thus, the OH-BDE producers, e.g. marine bacteria, may flourish in waters with high nutrient levels and temperatures, and these conditions are believed to enhance the natural production of OH-BDEs and PBDDs.

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Chapter 3: Sedimentary Record of Antibiotic Accumulation in Minnesota Lakes

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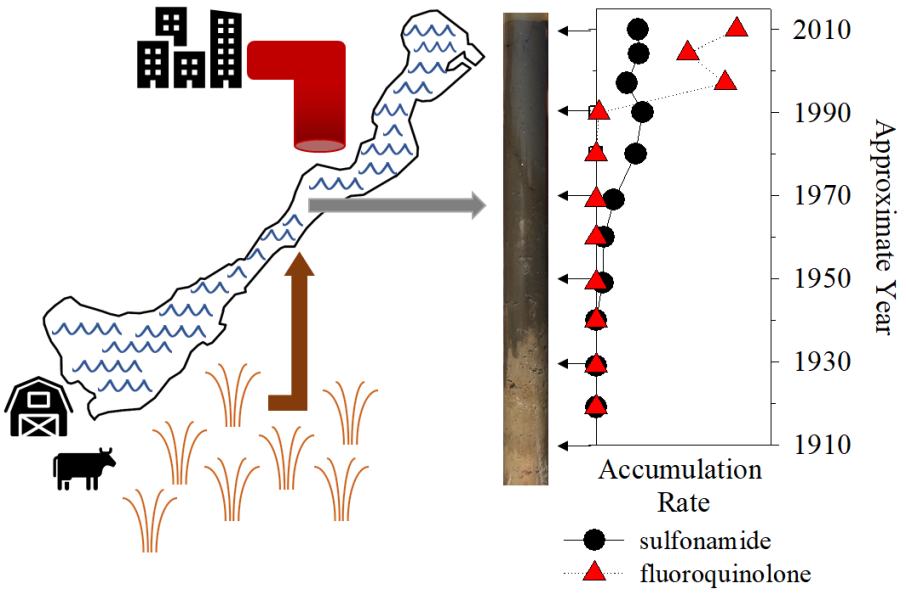

3.1 Summary

The widespread detection of antibiotics in the environment is concerning because antibiotics are designed to be effective at small doses. The objective of this work was to quantify the accumulation rates of antibiotics used by humans and animals, spanning several major antibiotic classes (sulfonamides, tetracyclines, fluoroquinolones, and macrolides), in Minnesota lake-sediment cores. Our goal was to determine temporal trends, the major anthropogenic source to lacustrine systems, and the importance of natural production. A historical record of usage trends for ten human and/or animal-use antibiotics (four sulfonamides, three fluoroquinolones, one macrolide, trimethoprim, and lincomycin) was faithfully captured in the sediment cores. Ten other antibiotics were not detected. Ofloxacin, trimethoprim, sulfapyridine, and sulfamethazine were detected in all of the anthropogenically-impacted studied lakes with maximum fluxes reaching 20.5, 1.2, 3.3, and 1.0 ng cm^{-2} yr^{-1}, respectively. Natural production of lincomycin may have occurred in one lake at fluxes ranging from 0.4 to 1.8 ng cm^{-2} yr^{-1}. Wastewater effluent appears to be the primary source of antibiotics in the studied lakes, with lesser inputs from agricultural activities.

3.2 Introduction

The health care system was revolutionized with the discovery of antibiotics in the 1930s. The ability to treat and prevent microbial infections resulted in antibiotics being one of the greatest inventions of the 20th century. The effectiveness of antibiotics has led to their mass production and widespread use. In 2011 and 2012, an estimated 17,900 tons of antibacterials were sold and distributed by retail and non-retail channels in the United States for use in humans and animals.[114–116] Given the large quantities of antibiotics used, it is important to understand their potential impact in aquatic systems.

Only a fraction of the antibiotics administered is metabolized by humans and animals; up to 90% of the dose is excreted in urine and feces.[188] Wastewater treatment plant (WWTP) effluents are point sources of human-use antibiotics to aquatic systems due to incomplete removal by conventional treatment technologies.[50,118] Concentrations of antibiotics in municipal wastewater are typically in the low µg/L range, and receiving water levels range from low to high ng/L.[1,2,4,122,123,125,127,128] The agriculture industry uses antibiotics to treat and prevent microbial illnesses and as growth promoters in livestock. Agricultural practices contribute to antibiotic pollution in water bodies by surface runoff from fields to which manure contaminated by antibiotics is applied.[123,127,129]

Antibiotics have also been detected downstream of wastewater outfalls in sediment.[4,122–126] Compounds, such as tetracyclines and fluoroquinolones, that strongly adsorb onto particles, accumulate in sediment.[189,190] Similar to surface water, the highest observed levels of antibiotics in sediments were downstream of metropolitan (industrial and municipal wastes) and agricultural and aquaculture areas (feedlots and fish ponds).[123–125] In addition to their mass production, some antibiotics are naturally

produced in the environment, such as select tetracyclines, penicillin, erythromycin, tylosin, and lincomycin.[191,192]

Different from other classes of contaminants of emerging concern, antibiotics are designed to have an effect on microorganisms.[4] Ecosystem health may be influenced by antibiotics by hindering the growth of algae and benthic invertebrates.[125,141,142] Use of antibiotics may increase the occurrence of antibiotic resistance in bacteria, which poses a risk to human and veterinary health by reducing the ability of antibiotics to treat microbial illnesses.[3,147] Lethal concentrations cause a specific immediate response, but prolonged sub-inhibitory levels of antibiotics can also select for and promote the dispersion of antibiotic resistant genes (ARGs).[147,148] Recent studies have shown conflicting findings as to whether environmental samples show correlations between antibiotic and ARG levels.[25,158,193,194]

The objective of this work is to quantify the current and historical levels of selected human- and animal-use antibiotics in lake sediment cores. Measuring levels of antibiotics in dated sediments is a useful tool to reconstruct chemical pollution of water bodies over time. Antibiotic levels in surface waters and surface sediments due to anthropogenic inputs have been well studied.[123,124,127,129] This study, however, aims to assess the trends of environmental levels of antibiotics throughout the past century due to anthropogenic inputs. Also investigated is whether synthetic or naturally produced antibiotics are more persistent in the environment, what the dominant source of antibiotic pollution is in the targeted lakes, and whether the degree of anthropogenic impact is reflected in the historical trends. The potential pressure of antibiotics selecting for ARGs provides motivation for further understanding in the abundance and persistence of

antibiotics in the environment. Thus, historical levels of 20 antibiotics (including those from the fluoroquinolone, tetracycline, sulfonamides, and macrolide classifications) were quantified in sediment cores from four Minnesota lakes.

3.3 Materials and Methods

Sediment Core Collection. Sediment cores were collected in August and September 2014 from four Minnesota lakes (Figure C.1). Lake Pepin (GPS coordinates: 44.499750, -92.294170) and the Duluth Harbor of Lake Superior (46.732783, -92.065333) were selected because they have large watersheds and receive multiple waste inputs. Lake Winona (45.87501, -95.40402) has a small watershed with one municipal wastewater discharge, and Little Wilson Lake in the Superior National Forest (47.656138, -91.067905) lacked any major waste inputs and served as a control site.

The cores were collected via a piston corer equipped with a polycarbonate tube and deployed into the sediment from the surface using Mg-alloy rods. The sediment cores were extruded vertically top-down and sectioned on site into 2 – 4 cm intervals, except for Lake Pepin. The outer circumference of the core was removed to prevent carryover of younger to older sediment via smearing during extrusion. Sections were stored in cleaned glass jars, homogenized, and a subsample was taken for radiometric dating. Samples were cooled to 4 °C in the field and were subsequently kept at -20 °C for long term storage. Because a magnetic susceptibility profile of Lake Pepin was used to determine the deposition date of core sections (see below), this core was sectioned in the laboratory.

Loss-on-ignition tests on homogenized samples were used to determine water, organic carbon, carbonate, and inorganic content of sediment by weighing the sediment after heating for 12 hours at 105 °C, 4 hours at 550 °C, and 2 hours at 1000 °C, respectively. An aliquot of sediment (approx. 10 g dry wet) at select intervals was freeze-dried and then stored at -20 °C until extraction for antibiotic analysis.

The antibiotics selected for this study include six sulfonamides (sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamethazine, sulfamethoxazole, and sulfapyridine), three macrolides (erythromycin, roxithromycin, and tylosin), four tetracyclines (chlortetracycline, doxycycline, oxytetracycline, and tetracycline), four fluoroquinolones (ciprofloxacin, enrofloxacin, norfloxacin, ofloxacin), and three non-categorized antibiotics (carbadox, lincomycin, and trimethoprim). The β-lactams amoxicillin, penicillin G, and penicillin V were originally included in the analysis suite, but degraded during the extraction process. β-lactams are known to undergo hydrolysis readily[195] and are infrequently detected in surface waters and wastewater effluents.[4,196]

Chemicals sources and purities are in Supporting Information (SI). The antibiotics chosen for this study include those that: 1) are natural products; 2) had human and/or animal uses; 3) were part of several major classifications; and 4) had been previously detected in sediment samples. The compounds, their abbreviations, and uses are given in Table 3.1. Degradation products of chlortetracycline (epi-chlortetracycline, iso-chlortetracycline, and epi-iso-chlortetracycline) and erythromycin (erythromycin-H_2O) were also included and summed into their respective parent compound concentrations.

Table 3.1. List of antibiotics included in the study separated into classifications with their respective abbreviations and general uses. Also noted is whether antibiotic is naturally produced and if it is on the World's Health Organization 19th list of essential medications.[106]

Antibiotic	Acronym	Natural Production	General Uses[197]	List of Essential Medications
		Sulfonamides		
sulfachlorpyridazine	SCP	no	swine, calves, dogs	no
sulfadiazine	SDZ	no	horses, humans	yes
sulfadimethoxine	SDM	no	fish, poultry	no
sulfamethazine	SMZ	no	swine, cattle	no
sulfamethoxazole	SMX	no	human	yes
sulfapyridine	SPD	no	human	no
		Macrolides		
erythromycin	EMC	yes	humans, poultry, swine	yes
roxithromycin	RXC	no	humans	no
tylosin	TYL	yes	chicken, swine, cattle	no
		Tetracyclines		
chlortetracycline	CTC	yes	swine, poultry, cattle, sheep, ducks	no
doxycycline	DXC	no	human, dogs	yes
oxyetracycline	OTC	yes	poultry, fish, swine, cattle, sheep	no
tetracycline	TCC	yes	human, dogs, cattle	yes
		Fluoroquinolones		
ciprofloxacin	CFC	no	human, swine, chickens	yes
enrofloxacin	EFC	no	cattle, swine, poultry, dogs, cats	no
norfloxacin	NFC	no	human, poultry	no
ofloxacin	OFC	no	poultry, human	yes
		Non-Categorized		
carbadox	CBX	no	swine	no
trimethoprim	TMP	no	human, dogs, horses	yes
lincomycin	LMC	yes	poultry, swine	no

Radiometric Dating. Sediment cores were dated by lead-210 (^{210}Pb) methods, as described previously.[44–46] Briefly, ^{210}Pb was quantified by alpha spectrometry of its daughter isotope polonium (^{210}Po), with dates and sediment accumulation rates calculated according to the constant rate of supply model.[45,46] Core-specific rates of sediment accumulation were corrected for sediment focusing based on the inventory of ^{210}Pb in the core to derive mean whole-lake accumulation rates (see Anger et al[44] SI for details).

Analyte concentrations were converted to accumulation rates (fluxes) by multiplying by the focus-corrected sediment accumulation rate for each analyzed interval. The Lake Pepin core was dated by matching the magnetic profile to that of cores collected previously.[44]

Extraction and Analysis. Proper analytical cleaning procedures were followed to prevent sample contamination. Details are in the SI. Internal standards (clinafloxacin, $^{13}C_2$-erythromycin, $^{13}C_2$-erythromycin-H_2O, simeton, and $^{13}C_6$-sulfamethoxazole, 100 ng) and surrogates (demeclocycline, nalidixic acid, and $^{13}C_6$-sulfamethazine, 20 ng) were spiked onto sediment in a methanol solution prior to extraction. Two sediment extraction methods were used: accelerated solvent extraction (ASE) and ultrasound assisted extraction (UAE). The following is a brief description of both extraction methods with a more detailed description provided in the SI. The ASE method was optimized to extract antibiotics from 0.5 or 1 g of sediment with 50:50 methanol:50mM pH 7 phosphate buffer at 100 °C, heated for 5 min followed by 2 cycles of 5 min static periods. Less sediment was used for samples with higher organic content (Lake Winona and Little Wilson Lake) to facilitate the clean up using solid phase extraction (SPE). The UAE method was adapted from Wallace and Aga.[198] Sediment (0.5 g) was mixed with Ottawa sand (2.5 g) and suspended in 10 mL of 20:30:50 acetonitrile:methanol: 0.1 M ethylenediaminetetraacetic acid (EDTA)/0.08 M disodium phosphate/0.06 M citrate buffer (pH 4) solution, vortexed (30 sec), placed in an ultrasound bath (40 kHz, 10 min), and centrifuged (3300 rpm, 10 min). The UAE was repeated two additional times per sample, and extracts were combined.

Organic solvents were removed from sediment extracts using a rotary evaporator in a 35 °C water bath. ASE aqueous extracts were spiked with 250 µL of 20:80 formic acid: 10% sodium chloride/0.5% EDTA solution. The Little Wilson Lake ASE extracts were diluted to 500 mL with ultrapure water before loaded onto the solid phase extraction (SPE) cartridge due to higher organic content. UAE aqueous extracts were diluted to 400 mL and adjusted to pH 4 with phosphoric acid.

An SPE method adapted from Meyer et al.[197] was used to remove interferences from the extracts and concentrate the sample. Two different sorbents were used for SPE, Oasis HLB (6cc, 200 mg, 30 µm) and Oasis MCX (6 cc, 150 mg, 30 µm) cartridges. Both cartridges were cleaned with 10 mL of methanol and ultrapure water. Samples were loaded in tandem with HLB on top of MCX under vacuum that did not exceed 15 mm Hg. Cartridges were then disassembled and the HLB was washed with 40:60 methanol:water (6 mL) and MCX with water (3 mL). Cartridges were eluted with MCX on top of HLB. Methanol (3 mL) was added to HLB prior to placing MCX on top. Methanol (5 mL, ×2) was then added to MCX and eluted through both cartridges. MCX was also eluted separately with 3 mL of 5% ammonium acetate in methanol that was combined with the methanol eluent. Cartridges were eluted on the manifold into 15-mL centrifuge tubes. Vacuum pressure was used to start the elution, then subsequently eluted by gravity. Eluents were blown down to dryness with industrial grade nitrogen in a 40 °C water bath. Samples were resuspended in 20 mM ammonium acetate (200 µL) and any particles were removed with a syringe filter (GHP, 0.4 µm) prior to liquid chromatography tandem mass spectrometry analysis. Additional details are in the SI.

Several quality assurance and control measures were taken to assure the precision of reported antibiotic concentrations. One duplicate per core was extracted to monitor reproducibility. Extraction efficiency was monitored in triplicate from Ottawa sand and from each core with pre-1900s sediment. Method blanks were extracted at least every eight samples to monitor and correct for any carryover contamination. Method blanks were comprised of either Ottawa sand or pre-1900s sediment and were spiked with surrogates and internal standards and subjected to the entire extraction process.

ASE samples were analyzed on an Agilent 1100 high pressure liquid chromatograph (HPLC) equipped with a Thermo TSQ Vantage triple quadrupole tandem mass spectrometer (MS/MS) in positive electrospray ionization mode. Separation was performed on a Phenomenex Kinetex F5 (1.7 µm, 100 Å, 50 × 2.1 mm) column with a SecurityGuard ULTRA guard column. Flow rate was maintained at 250 µL/min, temperature was set to 50 °C, and 8 µL was injected onto column. The HPLC-MS/MS was shared among several researchers, so the system was flushed with a 50:50 10 mM EDTA:methanol solution for 30 minutes prior to each analysis to remove metals from the system and improve peak shapes of tetracyclines and fluoroquinolones. A gradient elution of mobile phases 0.1% formic acid in ultrapure water and 0.1% formic acid in acetonitrile was developed, see Table S3, and flow was diverted to waste from 0 to 1 and 7.5 to 25 min. Due to the number of analytes included in the study, each sample was analyzed by three HPLC-MS/MS methods that monitored for: (1) sulfonamides and surrogates; (2) tetracyclines and fluoroquinolones; and (3) others and macrolides.

A Thermo Dionex ultimate 3000 RSLCnano system replaced the Agilent 1100 HPLC prior to analysis of the UAE sediment extracts. A Waters XSelect CSH C18 (3.5

μm, 130 Å, 50 × 2.1 mm) column was used. Separation of antibiotics was achieved with a flow rate of 0.5 mL/min, 8 μL injection volume, and temperature at 35 °C. From 0 to 1.5 min and 5.5 to 20 min, flow was diverted to waste. Two gradient elution methods consisting of 0.1% formic acid in water and 0.1% formic acid in methanol were developed, see Table S4. UAE samples were also analyzed by three methods: (1) sulfonamides, $^{13}C_6$-sulfamethazine, and others; (2) tetracyclines, fluoroquinolones, demeclocycline, and nalidixic acid; and (3) macrolides.

Analytes were detected and quantified using single reaction monitoring (SRM) transitions, (Table S5). An additional SRM was monitored for each analyte to confirm the identity of quantified peak. The mass spectrometer sensitivity varied between analyses, and thus parameters were optimized with the infusion of 5μM simeton in 50:50 20 mM ammonium acetate:acetonitrile (or methanol for UAE analysis) prior to each analysis. Typical values for mass spectrometer parameters were: scan time 0.02 sec; scan width: 0.15; Q_1/Q_3: 0.7; spray voltage: 3300 V; sheath gas pressure: 18 psi; capillary temperature: 300 °C; collision pressure: 1.5 mTorr; declustering voltage: -9 V; and tube lens: 95.

Limits of quantification (LOQs) were calculated from 10× the peak area of an analyte's retention time in the method blank minus the mass calculated from the method blank. Limits of detection (LODs) were calculated from 3× the peak area in the method blank at the retention time of an analyte. Antibiotic accumulation rates above LOQ were calculated from recovery corrected sediment concentrations using isotope dilute methodology and were sediment focusing corrected to determine antibiotic accumulation

on a whole-lake scale, see SI for equations. Reported LOQs and LODs were recovery and sediment focus corrected for each lake.

3.4 Results

Loss-On-Ignition Results and Dating. Organic, carbonate, and inorganic content of Little Wilson Lake, Duluth Harbor, Lake Pepin, and Lake Winona sediment cores and percent water of sample determined by loss-on-ignition are in Figure C.2 and Tables C.6-C9. Little Wilson Lake had the highest organic content (39.0 ± 1.1%) followed by Lake Winona (19.4 ± 2.0%), Lake Pepin (12.3 ± 1.6%), and Duluth Harbor (10.7 ± 1.1%). Results from ^{210}Pb dating are similar to those for cores taken previously from the same lakes and core-sites (Table C.10-C.12).[32, 34] Mean dry-mass accumulation rates (DMAR) range from 0.04 g cm^{-2} yr^{-1} (Little Wilson) to 0.43 g cm^{-2} yr^{-1} (Lake Pepin), and increase by 5-7× from c. 1860 to present day in the Pepin, Winona, and Duluth Harbor cores. DMAR are relatively constant over time in Little Wilson.

Analytical Method Performance. Two liquid chromatography tandem mass spectrometry methods were developed. Both methods/stationary phases gave linear calibration curves ranging from 0.5 to 450 µg/L and were of good quality for all analytes ($R^2 > 0.95$). See Figures C.3 – C.7 for representative chromatograms.

LODs for antibiotics via ASE ranged from 0.06 to 3.74 ng/g for macrolides, 0.08 to 0.68 ng/g for sulfonamides, 0.5 to 22.6 ng/g for tetracyclines, 0.03 to 19.75 ng/g for fluoroquinolones, and 0.05 to 1.03 ng/g for non-categorized antibiotics. ASE produced LOQs that varied from 0.06 to 11.22 ng/g for macrolides, 0.28 to 2.03 ng/g for sulfonamides, 1.6 to 55.7 ng/g for tetracyclines, 0.08 to 59.24 ng/g for fluoroquinolones,

and 0.13 to 3.43 ng/g for non-categorized antibiotics. Table S13 contains specific antibiotic LOD and LOQ values. ASE extraction efficiencies varied among antibiotic classes and between cores. Relative recoveries varied from 85 to 277 % for macrolides, 70 to 224 % for sulfonamides, 1 to 122 % for tetracyclines, 3 to 102 % for fluoroquinolones, and 12 to 82% for non-categorized. It is important to note that, while low extraction efficiencies lead to an increase in uncertainty in measured antibiotic concentrations, the observed temporal trends should not have been affected. Absolute and relative recoveries for internal standards, surrogates, and antibiotics via the ASE method are in Table S14.

The UAE method produced LODs and LOQs ranges of 0.02 to 1.56 ng/g and 0.05 ng/g to 4.68 ng/g, respectively, with non-categorized antibiotics and sulfonamides generally having the lowest detection limits followed by fluoroquinolones and tetracyclines. Relative recoveries varied from 60 to 106 % for sulfonamides, 34 to 123 % for tetracyclines, 36 to 53% for fluoroquinolones, and 23 to 157 % for non-categorized compounds. Table S15 and Table S16 contain analyte specific absolute and relative recoveries and LODs and LOQs, respectively, for UAE extracts.

Antibiotics in Minnesota Lakes. The depth profiles of detected antibiotics in Lake Pepin, Lake Winona, and Duluth Harbor are shown in Figure 3.1 in terms of whole-lake (focusing corrected) accumulation rates (ng cm^{-2} yr^{-1}). Figure C.8 shows recovery corrected sediment concentrations (in ng antibiotic/g sediment) throughout sediment cores for the detected antibiotics. The reported accumulation rates in Figure 3.1 were determined using ASE, except for trimethoprim in Lake Winona which were determined by UAE. Contamination by trimethoprim during the ASE extraction of Lake Winona,

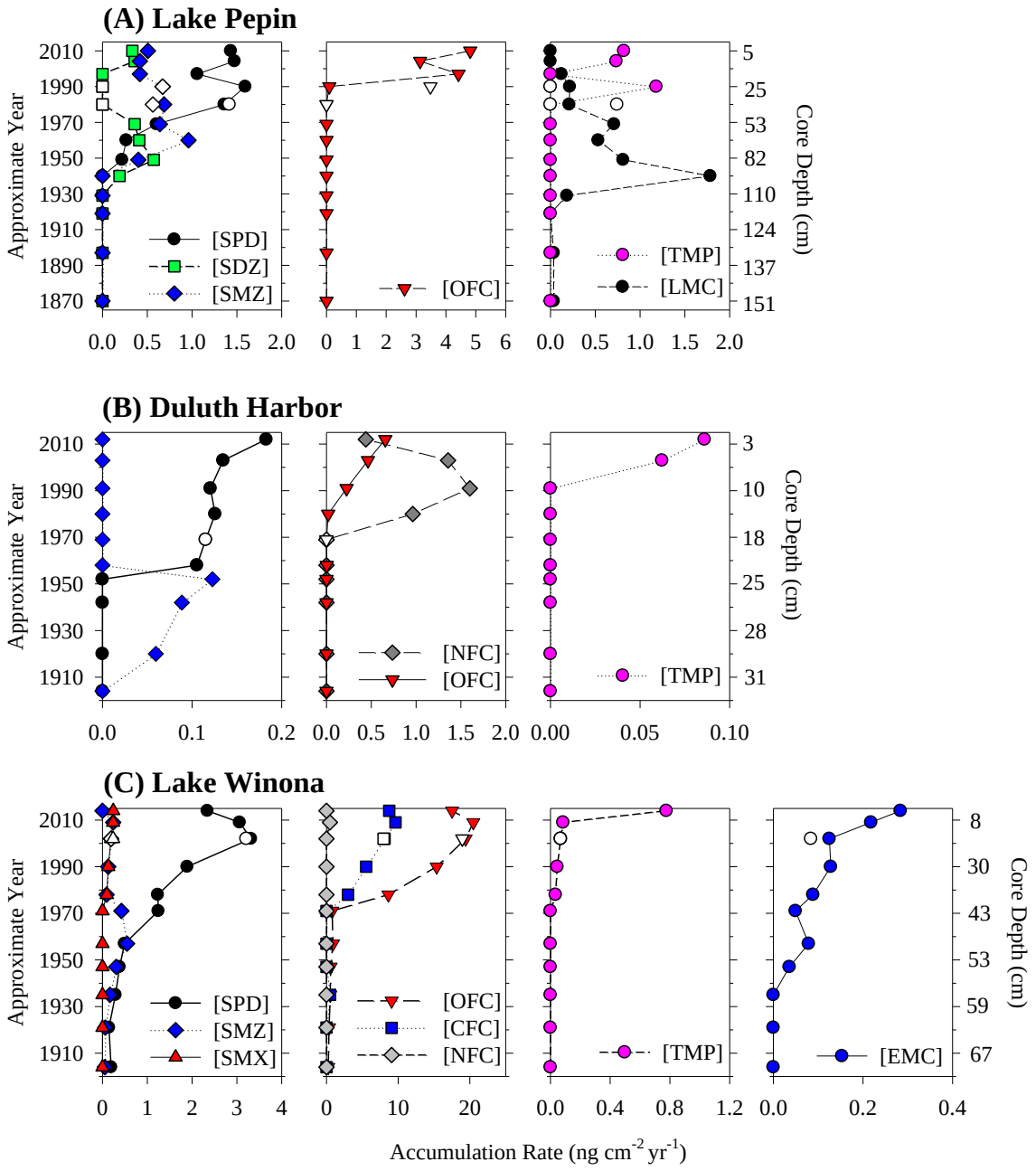

Figure 3.1. Focus-corrected accumulation rates (ng cm^{-2} yr^{-1}) of sulfapyridine (SPD), sulfadiazine (SDZ), sulfamethazine (SMZ), sulfamethoxazole (SMX), ofloxacin (OFC), ciprofloxacin (CFC), norfloxacin (NFC), trimethoprim (TMP), lincomycin (LMC), and erythromycin (EMC) in sediment cores from: (A) Lake Pepin; (B) Duluth Harbor; and (C) Lake Winona. White symbols indicate replicates. Concentrations in ng/g are given in Figure C.8.

which was resolved when the UAE extraction was performed, resulted in no discernable trend. Other accumulation rates determined by UAE are not shown because they are highly similar to those determined by ASE, see Figure 3.2 and C.9. No antibiotics were measured in the control lake, Little Wilson Lake, except for single detection of ciprofloxacin and norfloxacin in 1916 and 1990 sediment, respectively. Their presence is likely due to carry over contamination during ASE extraction given their single occurrences.

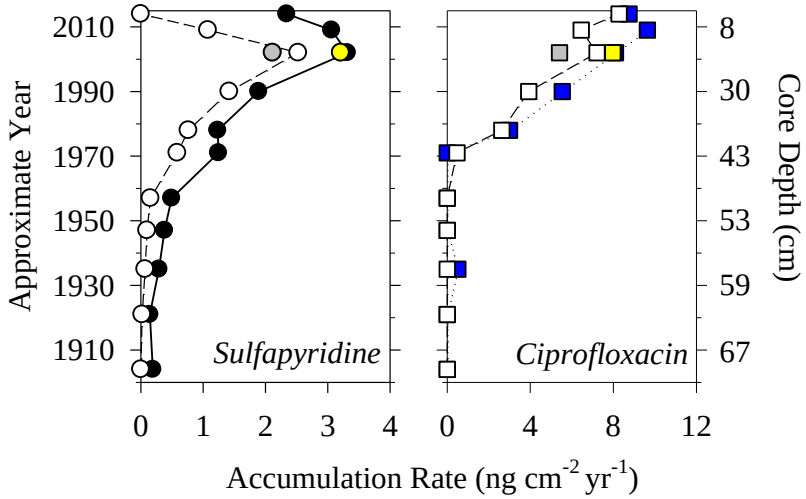

Figure 3.2. Focus-corrected accumulation rates (ng cm^{-2} yr^{-1}) of sulfapyridine and ciprofloxacin in Lake Winona. White symbols represent accumulation rates determined by ultrasound assisted extraction (UAE) method. Colored symbols are accumulation rates quantified by accelerated solvent extraction (ASE) method. Grey and yellow symbols are UAE and ASE replicates, respectively. Additional comparisons are in Figure C.9.

Sulfonamides. Sulfonamides are a group of antibiotics that were first synthesized in the late 1930s. Of the six sulfonamides included in this study, only sulfapyridine and sulfamethazine were detected in all three anthropogenically-impacted lakes. Sulfapyridine is a human-use antibiotic that received FDA approval in 1939, but marketing was discontinued in 1990. It was first detected in Lake Pepin and Duluth

Harbor ca. 1950. In Lake Winona, accumulation rates of sulfapyridine ranged from 0.2 to 0.5 ng cm^{-2} yr^{-1} prior to 1960, but increased to 1.2 ng cm^{-2} yr^{-1} around 1970. Sulfapyridine had the highest accumulation rates of the sulfonamides, with the highest fluxes observed in Lake Winona (3.3 ng cm^{-2} yr^{-1} ca. 2000) followed by Lake Pepin (1.6 ng cm^{-2} yr^{-1} in 1990) and then Duluth Harbor (0.18 ng cm^{-2} yr^{-1} in 2010). Sulfamethazine, which is used to promote growth and prevent diseases in animals, reached accumulation rates of 0.12, 0.96, and 0.54 ng cm^{-2} yr^{-1} in Duluth Harbor, Lake Pepin and Lake Winona, respectively. The first occurrence of sulfamethazine in Lake Pepin corresponded with its 1949 FDA approval. Sulfamethazine was present throughout the Lake Winona core, and appeared from 1920 to 1950 in Duluth Harbor.

Sulfadiazine is used for both human and agricultural treatments. After its first appeared in Lake Pepin near its 1941 FDA approval, fluxes varied from non-detect to 0.57 ng cm^{-2} yr^{-1} to the present day. A human-use only drug, sulfamethoxazole was only detected in Lake Winona. It first appeared about 1980 (corresponding to the year of FDA approval) at 0.09 ng cm^{-2} yr^{-1} and increased to a present-day flux of 0.24 ng cm^{-2} yr^{-1}. Two agricultural sulfa drugs, sulfachlorpyridazine and sulfadimethoxine, were not detected.

Fluoroquinolones. The only fluoroquinolone present in all three wastewater-impacted lakes was ofloxacin, a synthetic antibiotic used by poultry and humans. Ofloxacin levels generally increase near the 1990 FDA approval. In Lake Winona, ofloxacin was detected throughout the core, but accumulation rates increased dramatically in the 1980s from 0.8 to 8.6 ng cm^{-2} yr^{-1}, reaching a maximum flux of 20.5 ng cm^{-2} yr^{-1} in the 2010s before decreasing to 17.5 ng cm^{-2} yr^{-1} at present day. Lower

fluxes of ofloxacin were found in both Duluth Harbor (less than 0.7 ng cm^{-2} yr^{-1}) and Lake Pepin (less than 5 ng cm^{-2} yr^{-1}) after initial appearance in 1980 and 1990, respectively. Norfloxacin, a human-use drug, received FDA approval in 1986. It was detected once in Lake Winona around 2010, but was found in Duluth Harbor from 1980 to the present day. Ciprofloxacin, a fluoroquinolone generally consumed by humans, swine, and chickens, was approved by the FDA in 1987. Aside from an unexplained detection in 1935, accumulation rates in Lake Winona rose from 3.0 ng cm^{-2} yr^{-1} in 1980 to a present-day flux of 8.7 ng cm^{-2} yr^{-1}. Enrofloxacin, used by the agricultural industry, was not detected in any sample. Enrofloxacin is known to photo-transform into ciprofloxacin in surface waters and may contribute to ciprofloxacin accumulation.[199]

Macrolides. Of the three macrolides included in this study, only erythromycin was detected. In addition to mass production for human and animal use since 1972, erythromycin is also naturally produced. The presence of the erythromycin, however, has a level of uncertainty. The purity of isotopically labeled erythromycin, which was used as an internal standard, was 90%. Therefore, roughly 10 ng of unlabeled erythromycin was added to each sample. Erythromycin was typically detected in all samples, but most often the method blank would subtract off the contamination, e.g., no mass above the method blank was quantified in Little Wilson. For both Duluth Harbor and Lake Pepin, the appearance of erythromycin above the method blank was sporadic and likely due to the addition of the internal standard. Unlike the other cores, erythromycin was present in Lake Winona from 1950 to 2015 with fluxes ranging from 0.04 to 0.28 ng cm^{-2} yr^{-1}. Thus, it is likely that the detection of erythromycin Lake Winona derives from anthropogenic inputs rather than the addition of isotopically labeled internal standard.

The other macrolides included in this study, tylosin and roxithromycin, are not prescribed to humans and were not detected in any of the study lakes.

Tetracyclines. None of the tetracyclines included in this study were detected in the sediment cores. Even with the improved extraction efficiency with the UAE, no tetracyclines were detected in Lake Winona, the most heavily WWTP-impacted lake included in this study.

Non-categorized. Trimethoprim was first approved as a mixture with sulfamethoxazole in 1973 and was detected in all three wastewater-impacted lakes. The detection of trimethoprim in Lake Pepin and Duluth in 1990 was delayed several years relative to FDA approval, but trimethoprim appeared in Lake Winona around 1980, only a few years after receiving approval. Accumulations in Lake Pepin (0.7 to 1.2 ng cm^{-2} yr^{-1}) and Lake Winona (0.03 to 0.78 ng cm^{-2} yr^{-1}) were about 10-fold higher than Duluth Harbor (0.06 to 0.09 ng cm^{-2} yr^{-1}). Lincomycin is a naturally occurring antibiotic that is also mass produced for human and animal treatments. It was detected throughout the Lake Pepin sediment record with accumulation rates ranging from 0.04 to 1.8 ng cm^{-2} yr^{-1}. The occurrence of lincomycin did not appear to be affected by the 1964 FDA approval. Carbadox, primarily used by swine, was not detected in any of the lake sediment cores.

3.5 Discussion

Method Comparison. ASE was the initial extraction method used to extract and quantify antibiotic concentrations in the sediment. It produced low recoveries for select fluoroquinolones and tetracyclines. Lake Pepin and Lake Winona sediment cores were, therefore, re-extracted with the UAE method in an attempt to achieve higher recoveries.

Lake Pepin and Lake Winona sediment cores were chosen because they were the most heavily impacted by wastewater and therefore the most likely to accumulate antibiotics.

A new stationary phase, Waters XSelect CSH C18, was used for LC-MS/MS analysis of UAE extracts due to the broad peaks of tetracyclines with Phenomenex Kinetex F5 column. The peak broadening was thought to have been caused by interactions between the positive charge on tetracyclines and the negatively charged silanol on the particle core, thus a column with a positively charged surface was selected.

The UAE method was equal to or more efficient than ASE as an extraction method for all four tetracyclines and three out of the four fluoroquinolones. Tetracyclines and fluoroquinolones generally had similar or better detection limits using the UAE method when compared to ASE detection limits. UAE is also a viable option for extraction of sulfonamides, lincomycin, carbadox, and trimethoprim with sufficient, comparable or better recoveries than ASE and similar detection limits between the two extraction methods. Even with different extraction methods and stationary phases for LC-MS/MS analysis, the reported antibiotic accumulation rates were similar between the two methods in Lake Winona, (Figures 3.2 and C.9). Both extraction methods were reproducible given the similar accumulation rates between replicates.

Benefits of using the UAE method include not requiring an expensive instrument to maintain or the rigorous and time-consuming step of cleaning the ASE stainless-steel cells. Also, the chance of carry over contamination is reduced, because a new centrifuge tube is used to extract each sample. A major disadvantage of UAE is that it does not appear to be suitable for all types of sediment. A precipitate formed in the Lake Pepin UAE extract that resulted in non-detects of tetracyclines and fluoroquinolones, even in

the samples spiked with antibiotics. ASE appears to be a more robust and preferable extraction method, even with low recoveries for some tetracyclines and fluoroquinolones. The Waters XSelect CSH C18 column was successful in producing narrow tetracycline peaks (see Figure C.3) and in separating all the antibiotics included in this study. Also, this column did not require the flushing of a EDTA:methanol solution prior to analysis to maintain tetracycline and fluoroquinolone peak shapes which was necessary for the Phenomenex column. Thus, our research suggests that future analyses should use the ASE extraction coupled with a Waters XSelect CSH C18 column for the most robust extraction and analysis of antibiotics, although further testing is needed to confirm these observations.

Historical Trends. The sediment cores were successful in capturing historical trends in select antibiotic usage. In general, the appearances of antibiotics in the sediment cores were consistent with the initial FDA approval dates, which further validates results of the radiometric dating. In some profiles, the presence of an antibiotic was delayed from the approval date, such as sulfapyridine and trimethoprim. This suggests that either the popularity of the drug increased years after its FDA approval, there was a delay between FDA approval and administering to patients, the analytical method was not sensitive enough, or degradation occurred in the sediment.

On the other hand, some of the synthetic antibiotics in the Lake Winona core were present prior to their FDA approval. Their occurrence may be due to smearing from the topmost sediment of the core down during the collection process. The outer circumference of core was removed to reduce contamination during the collection process, but perhaps not enough was removed. It is also possible that some compounds

have limited downward mobility in the sediment bed.[200] This is more likely for compounds with lower sorption capacity, such as sulfonamides, relative to analytes that sorb more readily, e.g. tetracyclines and fluoroquinolones.[130,189,190] Furthermore, Tamtam et al [200] saw limited to non-existent mobility of sulfonamides and tetracyclines in their sediment record. Therefore, it is more likely that the collection process is the source of the contamination. This does not hinder interpretations regarding overall trends, as accumulation rates increased notably around drug approval dates. The presence of sulfamethazine in Duluth Harbor from 1920 to 1950 occurred within a core length of 8 cm. Its presence prior to FDA approval may be attributed to sediment mixing, perhaps related to nearby harbor dredging activities. It is unclear why this anomalous detection only occurred for this compound, however.

Quantifying historical levels of the ten detected antibiotics also indicates that these pharmaceuticals are relatively persistent in the sediment matrix. Even low levels of antibiotics in the environment are concerning, because studies have shown that sub-therapeutic levels may promote greater variety of antibiotic resistance over time.[147,148,201] The effect of antibiotics in sediments on the bacterial community is not fully understood and therefore needs further investigation.[4,149,202]

Usage Trends. Antibiotic profiles in sediment cores may provide insight into which drugs are most frequently prescribed. The World Health Organization's (WHO) list of essential medications indicate which pharmaceuticals are needed for a basic human health-care system. Six of the eight antibiotics that were on the WHO list and included in this study (sulfadiazine, sulfamethoxazole-trimethoprim, erythromycin, ciprofloxacin, and ofloxacin) were detected in at least one of the lakes. The WHO list may serve as a

catalog of frequently used drugs for which the fate and transport in the environment need to be more fully understood. The significance of tracking the fate and transport of heavily used drugs is demonstrated by the widespread presence of sulfapyridine after it fell into disuse. The accumulation of sulfapyridine is likely due to the consumption of sulfasalazine, a drug used to treat and prevent ulcerative colitis and treat rheumatoid arthritis and Crohn's disease.[112] Sulfasalazine is on the list of essential medications and was approved in 1950. Sulfapyridine is a metabolite of sulfasalazine. Thus, the accumulation of sulfapyridine in these lakes since 1950 is likely due to both direct use and the metabolism of sulfasalazine.

Comparing historical antibiotic accumulation rates may indicate which antibiotics are most prevalent and/or persistent. In the studied lakes, fluoroquinolone fluxes were greater than any other antibiotic class. According to a recent US study, fluoroquinolones were the most commonly prescribed in US hospitals.[203] It is also well known that fluoroquinolones sorb readily to sediment.[189,190] The high observed fluxes are likely due to both sorption and usage trends.

It was somewhat surprising to not detect tetracyclines in the sediment cores given their high affinity for solids. Because some tetracyclines are naturally produced, unlike sulfonamides and fluoroquinolones, it is possible that tetracyclines are more susceptible to transformations and therefore are detected less frequently. All tetracyclines have been shown to biodegrade,[204–206] and oxytetracycline can undergo hydroloysis.[4] Tetracyclines are also photochemically labile in surface waters,[207,208] as well as while sorbed to minerals.[209]

In the popular drug combination of trimethoprim and sulfamethoxazole, trimethoprim accumulated at higher concentrations and rates than sulfamethoxazole. These two synthetic, human-use drugs have been typically prescribed in a 1:5 trimethoprim:sulfamethoxazole ratio since 1973. The lack of sulfamethoxazole may be due to other fate processes that are less significant for trimethoprim, such as sulfamethoxazole being more photolabile.[210] This was also demonstrated by a recent study that detected both sulfamethoxazole and trimethoprim in surface water, but only trimethoprim was found in sediment beds.[128] Zhou et al.[124] also did not detect sulfamethoxazole in the presence of trimethoprim in river sediments. Other studies have reported higher levels and frequency of trimethoprim in sediment compared to sulfamethoxazole.[131,211] The sedimentary record suggests the trimethoprim is more persistent in sediment and therefore may be of greater concern.

Natural vs Synthetic. Of the antibiotics known to be natural products (erythromycin, tylosin, lincomycin, chlortetracycline, tetracycline, and oxytetracycline), two were detected in the sediment cores. Erythromycin was present in Lake Winona prior to its FDA approval; but as previously mentioned, a limitation of this study was the addition of erythromycin while spiking in the isotopically labeled erythromycin. In the other cores, none or sporadic samples had levels of erythromycin above the method blank. Lake Winona was the only core that had concentrations above the method blank throughout most of the core. It is possible that erythromycin was naturally produced in Lake Winona, but unlikely. On the other hand, the dominant source of the lincomycin accumulating in Lake Pepin may be natural production. This antibiotic was detected pre-FDA approval and accumulation did not noticeably change after approval. It is unclear

why the peak at around 1940 is present, but it is possible water conditions at the time were conducive to its natural production. Lincomycin is primarily used by the agriculture industry (e.g. poultry and swine), unless a patient has an infection resistant to penicillin. The limited detection of naturally produced antibiotics overall suggests that, as would be expected, they are more susceptible to degradation than synthetic antibiotics.

Human vs Agricultural Activity. Wastewater effluent appeared to be the primary source of antibiotic pollution in the anthropogenically-impacted lakes. Antibiotics that were partially or completely used for human treatments were predominately detected. It is likely that a portion of the antibiotics accumulating in Lake Winona and Lake Pepin are derived from animal use, given the extent of agricultural activity in their watersheds. The frequent detection of antibiotics on the WHO list of essential medications also indicates wastewater effluent as the primary source.

The degree of antibiotic pollution also appears to reflect the degree of wastewater impact. In general, the highest fluxes and greatest number of antibiotics were measured in Lake Winona, followed by Lake Pepin, and Duluth Harbor in decreasing order. Antibiotics were not detected at the control site, Little Wilson Lake. The Minnesota Pollution Control Agency reported that from 2000 to 2008, approximately 63% of the average inflow to Lake Winona was wastewater effluent from Alexandria Lakes Area Sanitation District WWTP (3.75 MGD).[212] It was not surprising, therefore, that antibiotic pollution was greatest in Lake Winona because it was also the most heavily impacted by treated wastewater.

The Duluth Harbor core was expected to record more antibiotic pollution than in Lake Pepin, because the highest levels of human-use antibiotics are generally seen in

surface waters and sediments near wastewater outfalls.[123,127,128] The Duluth Harbor core was collected 5 km from the outfall of Western Lake Superior Sanitary District WWTP (40 MGD) and less than 1 km from Superior, Wisconsin WWTP (5 MGD). Lake Pepin is a natural impoundment of the Mississippi River on the Minnesota-Wisconsin border and downstream of the convergence of the Minnesota and Mississippi River. In addition to receiving upstream inputs from the Metropolitan WWTP (170 MGD) and other smaller municipalities, Lake Pepin receives direct wastewater discharges from Red Wing, WI (3 MGD) and Lake City, MN (1.8 MGD) 22 km upstream and 6 km downstream, respectively, of the collection site. As shown in Figure 3.1, higher fluxes were observed in Lake Pepin than in Duluth Harbor, a likely consequence of Lake Pepin's large watershed (half of Minnesota) and extremely high sediment load, which may carry sorbed antibiotics. Thus, the accumulation rates of antibiotic in Lake Pepin may incorporate antibiotic usage along the Minnesota and upper Mississippi Rivers.

The ability of antibiotics to migrate downstream likely explains the presence of sulfamethazine in Lake Pepin. The presence of the animal-use medicine in Lake Pepin may be due to upstream agricultural activity along the Minnesota and Mississippi rivers. The detection of sulfamethazine in Lake Winona indicates that agricultural usage in the surrounding watershed was captured by the sediment record as well. Approximately 30% of its watershed is cultivated crop land where manure may be applied.

It is also interesting to note that carbadox, which is only administered to swine, was not detected in any of the sediment samples. This is in contrast to a recent study that found carbadox in 28% of the 50 Minnesota lakes and streams sampled.[2] The extraction/analysis method (LOD range from 0.09 to 1.24 ng/g of sediment) and

experimental sorption capacity to organic matter (log Koc 3.96 ± 0.18 L/kg OC) appears sufficient for accumulation and detection of the antibiotic.[213] The absence of carbadox could be due to fate processes in the water column or benthic sediments that degraded the antibiotic. Another possibility is that the detection of carbadox in the previous study is a false positive, because the ASE method used in this work saw a large peak at a similar retention time to carbadox in the quantification SRM that was not present in the confirmation SRM and was therefore not quantified. The unknown peak in the quantification SRM designated for carbadox was sufficiently large enough a peak in the confirmation SRM should have been present.

Environmental Implications. This research suggests that wastewater impacted lakes capture the trends of antibiotic usage predominantly in human medicine. Human-use antibiotics present in lakes originate largely from WWTP effluent and therefore have a direct point-source route into these systems. Agricultural/veterinary antibiotic trends are not as readily captured in these lake systems, even when their watersheds contain animal feeding operations where antibiotics are used or cropland where antibiotic-contaminated manure may be spread. Only in Lake Winona, which has a relatively small watershed, were antibiotics used in agriculture routinely detected. While a recent study estimated that a majority of the antibiotics consumed in the US are for agricultural activities,[3] land application of agricultural antibiotics may limit their transport and accumulation into the studied lakes. Although, lakes appear to be suitable locations to evaluate historical loading trends of antibiotics used in human medicine via their direct input from WWTPs, our study suggests that accumulation rates and impacts due to antibiotic use in agriculture requires sampling in soils or waterways near agricultural activities (rather than in lakes

that integrate signals from large watersheds) due to the non-point source route of agricultural antibiotics to the environment.

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Chapter 4: Small and Large-Scale Distribution of Four Classes of Antibiotics: Association with Metals and Antibiotic-Resistance Genes

4.1 Introduction

Antibiotics are commonly detected in aquatic systems that are impacted by human and animal waste. Many antibiotics are not completely metabolized after being administered, and therefore some antibiotics are excreted in their original form.[188] Human-use antibiotics are released into the environment due to incomplete removal at wastewater treatment plants (WWTPs),[118,120,123,214] whereas animal-use antibiotics predominately enter via runoff from agricultural fields that have had antibiotic contaminated manure applied.[124,127,129] Antibiotics are an essential component of the human health care system, as well as the agriculture industry which uses antibiotics to prevent illnesses and promote growth in livestock.[4,215,216]

In 2012, the estimated annual sales of antibiotics in the United States was 17,900 tons.[114–116] Major antibiotics classes sold for veterinary uses in the United States were tetracyclines (41%), ionophores (31%), β-lactamases (7%), and macrolides (4%).[115] Penicillins accounted for the greatest portion (at 44%) of human-use antibacterial sales followed by cephalosporins (15%), sulfonamides and trimethoprim (15%), fluoroquinolones (8%), macrolides (5%), and tetracyclines (3.5%).[114]

Antibiotics have been detected in surface waters, sediments, soils, wastewater effluents, and drinking water.[4,125,126,128,130,196,217,218] Antibiotics typically have elevated concentrations and higher detection frequencies near anthropogenic sources.[122,124,127,128] In New Jersey, maximum concentrations of ciprofloxacin (0.077 μg/L), erythromycin–

H_2O (0.085 μg/L), sulfamethoxazole (0.25 μg/L) and trimethoprim (0.14 μg/L) were observed downstream of WWTP outfalls in the river water.[128] Spatial distribution of tetracyclines and sulfonamides along the Cache la Poudre River in Colorado coincided with their veterinary and human-uses.[127] Following anthropogenic inputs, several antibiotics (including ciprofloxacin, erythromycin, erythromycin-H_2O, ofloxacin, sulfachloropyridazine, sulfadimethoxine, sulfamethoxazole, tetracycline, oxyterracycline, and trimethoprim) have been shown to be persistent and able to be transported downstream.[132,214,219,220] Seasonal variations (such as high versus low flow and temperature) may influence the occurrence of antibiotics,[126,132,221] but this is not always observed.[123]

Sorption to sediment is another important fate process that influences the distribution of antibiotics. Sorption behavior of antibiotics, however, is complex and varies from compound to compound. The ability to sorb is influenced by sediment characteristics and likely depends on particle sizes and the organic content of sediment.[124,130,137] For example, the sorption coefficient (K_d) for ciprofloxacin was correlated positively with clay content and negatively with pH.[146] Sorption of sulfonamides to particle-size fractions was shown to increase from sand to clay to fine silt content and was also influenced by pH.[137] Humic substances may either inhibit or promote the sorption of antibiotics to sediment by altering the surface properties and site availability.[4] The sediment total organic carbon (TOC) was related to sediment concentrations of sulfonamides, tetracyclines, fluoroquinolones, and macrolides in three major rivers in northern China during the wet season.[124] Tetracyclines and fluoroquinolones have a high affinity for minerals because they complex with Ca^{2+} and

Mg^{2+}.[130,134–136] Thus, sediment is often an important reservoir for tetracyclines and fluoroquinolones.[132,133]

The presence and persistence of antibiotics in the environment is of concern because some antibiotics retain a degree of bioactivity while sorbed and could have adverse effects on the bacterial community.[143–146] The growth of benthic invertebrates and algae may be altered by antibiotics, and antibiotic pollution may result in food web disruption.[125,140–142] Anthropogenic inputs of antibiotics could also reduce the ability to treat and prevent microbial illnesses in humans. Antibiotics, even at sub-inhibitory levels, may select for and promote the dispersion of antibiotic resistance genes (ARGs).[3,147,148] ARGs are naturally occurring genetic elements that allow bacteria to withstand the effects of antibiotics.[151] Several studies, have found significant correlations between the occurrence of antibiotics and ARGs in aquatic systems.[25,116,145,193,211] Wastewater and sewage treatment plants,[155,156] hospital waste,[149] and livestock lagoons[143,158] are known anthropogenic sources that discharge ARGs into the environment.

Another important factor in ARG occurrence is metal pollution. Heavy metals co-select for ARGs by cross-resistance (the metal and antibiotic resistance genes are present on the same mobile genetic element) or co-resistance (the same mechanism provides resistance to both antibiotics and metals).[149] Metals have also been found to strongly correlate with ARG abundance in freshwater lake sediments,[222] as well as municipal solid waste leachates.[223]

The objective of this study was to compare the antibiotic profile across short distances in a lake (that receives direct inputs) to a comprehensive spatial pattern of antibiotic occurrence in river systems (that incorporates multiple land uses).[149] Sediment

samples were collected from the studied systems, which includes a lake with a small watershed (Lake Winona) that receives runoff from agricultural lands and a direct input of treated wastewater and two major river systems in Minnesota (Mississippi and Minnesota rivers) that transect multiple land uses. The twenty antibiotics selected for this study include several from the major classes and have a mixture of human and/or animal-uses. Concentrations of heavy metals that co-select ARGs were also quantified. The levels of antibiotics and heavy metals were correlated with levels of metal, antibiotic, and antibiotic resistance-associated resistance genes. This studied aimed at determining if either metals or antibiotics were associated with the appearance of resistance genes across long (i.e. river) or short (i.e. lake) distances.

4.2 Materials and Methods

Sample Collection. Sediment grab samples from the Mississippi and Minnesota River were collected from a canoe or small boat from the surface with an Ekman dredge (Figure 4.1 and Table D.1). Samples (except for the St. Peter and Lake Pepin) were collected upstream of dams because these are locations where sediments generally deposit. Surface sediments from Lake Winona, Alexandria, MN were collected with a gravity corer equipped with a polycarbonate tube (7 cm diameter) from the surface with alloy rods (Figure 4.1 and Table D.2). Samples were homogenized, aliquots for gene and metal analyses were taken, and sediment was stored in cleaned glass jars on ice during transport. Triplicate sediment samples of approximately 0.5 g were placed in sterile microcentrifuge tubes. Sediment was stored long-term at -20 °C. Sediment from Lake Pepin and one sample in Lake Winona (noted by *) was collected via piston coring (see

Chapter 3 for details) and antibiotic, metal, and target gene concentrations were measured in the top 4 cm of the sediment core.

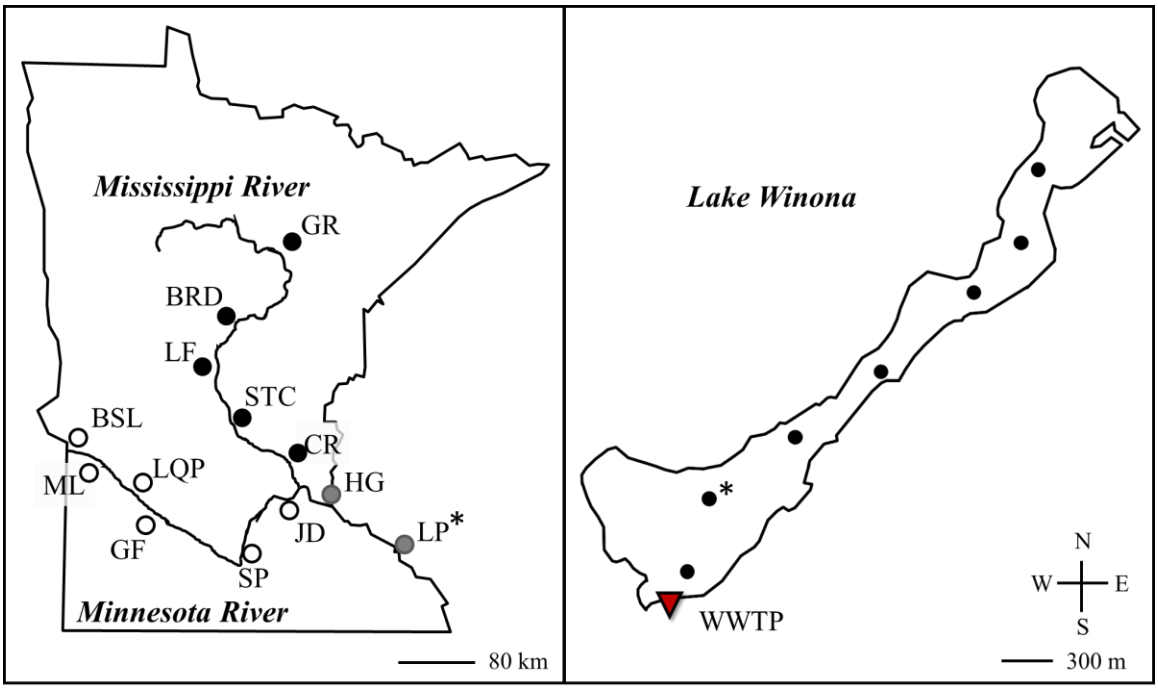

Figure 4.1. Left: river sediment grab sampling locations: along the Minnesota River (white fill) at Big Stone Lake (BSL), Marsh Lake (ML), La qui Parle (LQP), Granite Falls (GF), St. Peter (SP), and Jordan (JD); within the Mississippi River (black fill) at Grand Rapids (GR), Brainerd (BRD), Little Falls (LF), St. Cloud (STC), and Coon Rapids (CR); and after the Minnesota joined the Mississippi River (gray fill) in Hastings (HG) and Lake Pepin (LP). Right: an outline of Lake Winona in Alexandria, MN with surface sediment sampling locations represented with black circles and the Alexandria Lake Area Sanitary District wastewater treatment plant (WWTP) discharge with a triangle. Samples that were collected with a piston corer are denoted by an asterisk.

Chemicals. The twenty antibiotics selected for this study span several of the major classifications (sulfonamides, tetracyclines, fluoroquinolones, and macrolides) and several major degradation products, see Table 4.1. Many of the selected antibiotics were often detected in sediment in previous studies.[25,122,124,126,128,217,224,225] A mixture of human and/or animal use antibiotics were included, as well as several antibiotics that are natural products.

Table 4.1. List of antibiotics included in the study and their acronyms and general uses. Also noted is whether the antibiotic is naturally produced.

Antibiotic	Acronym	Natural Product	General Uses[197]
		Sulfonamides	
sulfachlorpyridazine	SCP	no	swine, calves, dogs
sulfadiazine	SDZ	no	horses, humans
sulfadimethoxine	SDM	no	fish, poultry
sulfamethazine	SMZ	no	swine, cattle
sulfamethoxazole	SMX	no	human
sulfapyridine	SPD	no	human
		Macrolides	
erythromycin[a]	EMC	yes	humans, poultry, swine
roxithromycin	RXC	no	humans
tylosin	TYL	yes	chicken, swine, cattle
		Tetracyclines	
chlortetracycline[b]	CTC	yes	swine, poultry, cattle, sheep, ducks
doxycycline	DXC	no	human, dogs
oxyetracycline	OTC	yes	poultry, fish, swine, cattle, sheep
tetracycline	TCC	yes	human, dogs, cattle
		Fluoroquinolones	
ciprofloxacin	CFC	no	human, swine, chickens
enrofloxacin	EFC	no	cattle, swine, poultry, dogs, cats
norfloxacin	NFC	no	human, poultry
ofloxacin	OFC	no	poultry, human
		Non-Categorized	
carbadox	CBX	no	swine
trimethoprim	TMP	no	human, dogs, horses
lincomycin	LMC	yes	poultry, swine

[a] includes the presence of erythromycin-H_2O

[b] includes the presence of epi-chlortetracycline, iso-tetracycline, and epi-iso-tetracycline

Analytical Methods. Particle size distributions of sediment samples were determined by the hydrometer method. Briefly, approximately 45-50 g of oven dried sample (when available) were dispersed in 2.5% sodium hexametaphosphate (100 mL of 5% SHMP and 100 mL of distilled water) by shaking for 16 hours on a rotary benchtop shaker at 30 rpm. The resulting dispersed slurry was transferred completely into a 1000 mL settling column and filled to volume with 800 mL of distilled water. A weighted brass plunger was used to completely mix and distribute the particles throughout the column, at which point the beginning of settling time was recorded. A hydrometer reading (corrected by a factor of 0.36 for every °C above 20) was taken at 40 seconds, 4 hours, and 8 hours. For these samples, which contained appreciable organic matter that remained undigested (no pretreatment with H_2O_2 to remove organic matter), the 4 hour hydrometer reading was used to determine the clay fraction,[226] while the 40 second reading gave the sand fraction. The silt fraction was determined by difference.

Antibiotics were extracted from the sediment using accelerated solvent extraction, and the analytes were detected and quantified by liquid chromatography tandem mass spectrometry with a Phenomenex Kinetex F5 column. A detailed description is given in Chapter 3. Extraction efficiencies of compounds of interest were determined by spiking 100 ng of each antibiotic in a methanolic solution onto the sediment and measuring the recovered mass from the extraction process. In Lake Winona, triplicate spike and recovery analyses were performed on sediment that was deposited in Lake Winona pre-1900s. This sediment was collected via piston coring for the study described in Chapter 3. Due the high variability in sediment composition amongst the river sediments, relative recovery of antibiotics was assessed at each sample site.

Method blanks were run every eight samples to monitor for carry over contamination during the extraction process. Method blanks consisted of Ottawa sand spiked with surrogates and internal standards and were processed in an identical manner to the river sediments. Limits of detection (LODs) for each antibiotic were 3× the peak area near the analyte retention time in method blank. Limits of quantification (LOQs) were 10× the peak area in method blank near the analyte retention time minus the mass determined in the method blank. Sediment concentrations, LODs, and LOQs were determined by internal standard dilution methodology and were recovery corrected.

Loosely bound (i.e. bioavailable) concentrations of 14 metals (arsenic, cadmium, chromium, cobalt, copper, gadolinium, lead, manganese, molybdenum, nickel, selenium, tin, vanadium, and zinc) were measured by Rick Knurr of the Geochemistry Lab in the Department of Earth Science at the University of Minnesota. A detailed description of extraction and analysis is in Appendix D and was previously published.[227] Briefly, metals were extracted with 0.2 N HCl at 80 °C for 30 minutes and quantified with inductively coupled plasma mass spectrometry (ICP-MS).[228,229]

Levels of 16S rRNA genes (a surrogate for biomass) and 45 metal, antibiotic, and antibiotic resistance-associated genes in the Lake Winona, Minnesota River, and upper Mississippi River sediments (Table 4.2) were also quantified. Details on DNA extraction and purification and gene quantification using microfluidic quantitative polymerase chain reaction (MF-qPCR) are in Appendix E and were also published in a previous work.[227]

Table 4.2. List of genes corresponding to their resistance function. "Other" category includes biomass surrogate (16S rRNA), kanamycin, rifampicin, esterase, and streptomycin resistance.

Resistance/Function	Genes
aminoglycoside	*aacD, aadA5*
β-lactamase	*ampC*, bla_{KPC}, bla_{NDM1}, bla_{NPS}, blA_{OXA},
chloramphenicol	*catB8, cmlB, floR*
erythromycin	*ermB, ermF*
integrons	*intI1, intI2, intI3*
macrolides	*mefE, mphBM*
metal	*cadA, chrA, copA, merA, nikA, rcnA*
multidrug efflux	*acrD, mexB*
quaternary ammonium	*qacF, qacG*
quinolones	*qnrA, qnrB*
sulfonamide	*sul1, sul2, sul3*
tetracycline	*tet*(A), *tet*(L), *tet*(M), *tet*(S), *tet*(W), *tet*X
trimethoprim	*dfr13*
vancomycin	*vanA, vanB*
other	16S rRNA, *aadD, arr2, ereB, strB*

4.3 Results and Discussion

Sediment Characterization. Lake Winona sediment primarily consisted of carbonate (41.9 to 58.6%), followed by inorganic (23.3 to 36.9%) and organic components (18.1 to 26.2%), see Table D.4. Organic, carbonate, and inorganic content of river sediment fluctuated throughout the studied river systems, as shown in Table D.5. Organic content varied from 0.6 to 9.4% in the Minnesota River and 0.7 to 26.8% in the Mississippi River. Textural analysis revealed that sediments from Big Stone Lake, St. Cloud, and Coon Rapids were 90% or more sand and the rest of the sediment samples had sand content that ranged from 22 to 68%, see Table 4.3.

Table 4.3. Particle size distribution of river sediment samples organized by sand (50 – 200 µm), silt (2 – 50 µm), and clay (less than 2 µm) content.

Textural Analysis			
Sample Site	*Sand %*	*Silt %*	*Clay %*
Minnesota River			
Big Stone Lake	99	<1	<1
Marsh Lake	22	48	30
Lac Qui Parle	68	19	13
Granite Falls	52	32	16
St. Peter	56	28	16
Jordan	68	22	10
Mississippi River			
Grand Rapids†	--	--	--
Brainerd	26	54	19
Little Falls	51	24	26
St. Cloud	99	<1	<1
Coon Rapids	90	2	8
Minnesota & Mississippi River			
Hastings	22	56	22
Lake Pepin†	--	--	--

† textural analysis was not performed due to insufficient sample volume

Analytical Method Performance. Relative recoveries of antibiotics from Lake Winona sediment were generally highest for macrolides (99 – 218%), followed by sulfonamides (83 – 120%), fluoroquinolones (23 – 38%), tetracyclines (5 – 71%), and non-categorized antibiotics (6 – 24%), see Table D.6. LODs ranged from 0.01 to 0.85 ng/g for sulfonamides, 1.11 to 4.07 ng/g for tetracyclines, 0.01 to 2.06 ng/g for fluoroquinolones, 0.05 to 0.45 ng/g for macrolides, and 0.02 to 0.42 ng/g for non-categorized antibiotics. LOQs varied from 0.04 to 2.54 ng/g for sulfonamides, 3.32 to 12.20 ng/g for tetracyclines, 0.3 to 6.18 ng/g for fluoroquinolones, 0.15 to 1.35 ng/g for macrolides, and 0.06 to 0.76 ng/g for non-categorized antibiotics in Lake Winona.

Due to the variability of the river sediment organic content and particle size distribution, the extraction efficiency was also highly variable (Tables 4.4 and 4.5), and resulted in a wide distribution of LODs and LOQs. It should be noted that a major limitation of this study was the varying recoveries between the river sediment samples and occasional very low recovery. Therefore, the presence of the antibiotics may have been missed due to the sample matrix.

Table 4.4. Limit of detection (LOD) and quantification (LOQ) in ng/g for antibiotics in Minnesota and Mississippi River sediment extracts.

	Limit of Detection [ng/g]				**Limit of Quantification [ng/g]**			
Analyte	**Mean**	**Median**	**Max**	**Min**	**Mean**	**Median**	**Max**	**Min**
				Sulfonamides				
Sulfapyridine	0.007	0.008	0.012	0.002	0.035	0.036	0.037	0.029
Sulfadiazine	0.020	0.023	0.035	0.005	0.103	0.105	0.107	0.087
Sulfamethoxazole	0.126	0.138	0.145	0.049	0.431	0.440	0.449	0.362
Sulfamethazine	0.009	0.010	0.014	0.002	0.028	0.028	0.029	0.023
Sulfachloropyridazine	0.138	0.146	0.155	0.048	0.432	0.442	0.451	0.363
Sulfadimethoxine	0.006	0.007	0.009	0.001	0.028	0.029	0.029	0.024
				Fluoroquinolones				
Norfloxacin	3.31	1.20	18.94	0.27	8.97	3.25	51.39	0.73
Ciprofloxacin	11.62	5.34	39.73	0.83	35.74	16.44	122.20	2.54
Enrofloxacin	0.39	0.19	1.60	0.04	0.48	0.24	1.97	0.05
Ofloxacin	0.08	0.05	0.20	0.02	0.26	0.18	0.67	0.07
				Tetracyclines				
Tetracyclines	9.17	5.94	29.14	1.88	27.82	18.00	88.37	5.70
Doxycycline	9.42	6.68	26.51	2.74	29.07	20.63	81.85	8.46
Oxytetracycline	179.6	113.6	676.7	16.19	558.9	353.7	2106	50.38
Chlortetracycline	4.25	3.20	9.82	1.21	13.73	10.36	31.76	3.91
				Macrolides				
Erythromycin	0.45	0.49	0.59	0.23	1.36	1.46	1.76	0.68
Roxithromycin	0.45	0.37	0.85	0.15	1.36	1.11	2.56	0.44
Tylosin	0.95	1.00	1.82	0.39	3.07	3.23	5.84	1.27
				Non-Categorized				
Carbadox	1.25	0.65	5.66	0.36	6.42	3.36	29.08	1.86
Trimethoprim	0.20	0.19	0.27	0.12	0.43	0.42	0.58	0.26
Lincomycin	0.08	0.04	0.49	0.02	0.26	0.11	1.53	0.06

Table 4.5. Absolute recoveries of internal standards and relative recoveries of surrogates and antibiotics in Minnesota and Mississippi River sediment extracts.

	Absolute and Relative Recovery (%)			
Analyte	**Mean**	**Median**	**Max**	**Min**
Sulfonamides				
Sulfapyridine	178%	107%	497%	73%
Sulfadiazine	170%	102%	471%	70%
Sulfamethoxazole	97%	82%	234%	77%
Sulfamethazine	212%	131%	753%	93%
Sulfachloropyridazine	107%	93%	281%	76%
Sulfadimethoxine	191%	110%	743%	96%
$^{13}C_6$-Sulfamethazine[a]	141%	109%	603%	68%
$^{13}C_6$-Sulfamethoxazole[b]	37%	30%	82%	4%
Fluoroquinolones				
Norfloxacin	31%	26%	114%	2%
Ciprofloxacin	12%	9%	54%	1%
Enrofloxacin	25%	20%	95%	2%
Ofloxacin	35%	34%	91%	9%
Nalidixic Acid[a]	215%	206%	305%	146%
Clinafloxacin[b]	48%	45%	120%	2%
Tetracyclines				
Tetracyclines	10%	9%	29%	2%
Doxycycline	10%	8%	22%	2%
Oxytetracycline	4%	2%	16%	0%
Chlortetracycline	115%	89%	253%	31%
Demeclocycline[a]	22%	18%	60%	0%
Macrolides				
Erythromycin	73%	64%	138%	54%
Roxithromycin	61%	57%	141%	20%
Tylosin	155%	131%	305%	73%
$^{13}C_2$-Erythromycin[b]	27%	24%	48%	12%
Non-categorized				
Carbadox	47%	47%	84%	6%
Trimethoprim	90%	89%	130%	66%
Lincomycin	110%	89%	212%	9%
Simeton[b]	32%	33%	24%	41%

[a] denotes surrogate
[b] denotes internal standard

Description of Studied Systems. Lake Winona is 185 acres in size, approximately 1.6 miles long, and extends linearly from southwest to northeast (Figure 4.1). The Alexandria Lake Area Sanitary District (ALASD) WWTP discharges treated effluent into the south end of Lake Winona. The ALASD WWTP services about 24,000 people with an average plant flow of 2.9 million gallons per day. The watershed contains one bovine feedlot, and about a third of the landscape is used for fields of corn, hay forage, soy bean, legumes, grain, seeds, and wheat.

According to the Minnesota Department of Natural Resources, agricultural activity is generally higher in the watersheds of collection sites within the Minnesota River (Figure 4.2).[230] Cultivated land area increased eastward along the Minnesota River and several feedlots for bovines, pigs, birds, horses and/or other livestock are within every watershed. Watersheds north of the river junction in the Mississippi River had notably less agricultural activity than south of the junction.

Population density was used as a surrogate for degree of treated wastewater impact. As shown in Figure 4.2, population density generally increases north to south and west to east across the state. Because the Mississippi River is more developed, it was thought to receive a larger input of wastewater-derived, human-use antibiotics. WWTPs on these rivers generally discharge effluent downstream of dams, and therefore also downstream of the sediment collection sites. Thus, antibiotic levels in the Minnesota and Mississippi River did not represent direct inputs of wastewater effluent, but rather residuals after an integration of fate and transport mechanisms.

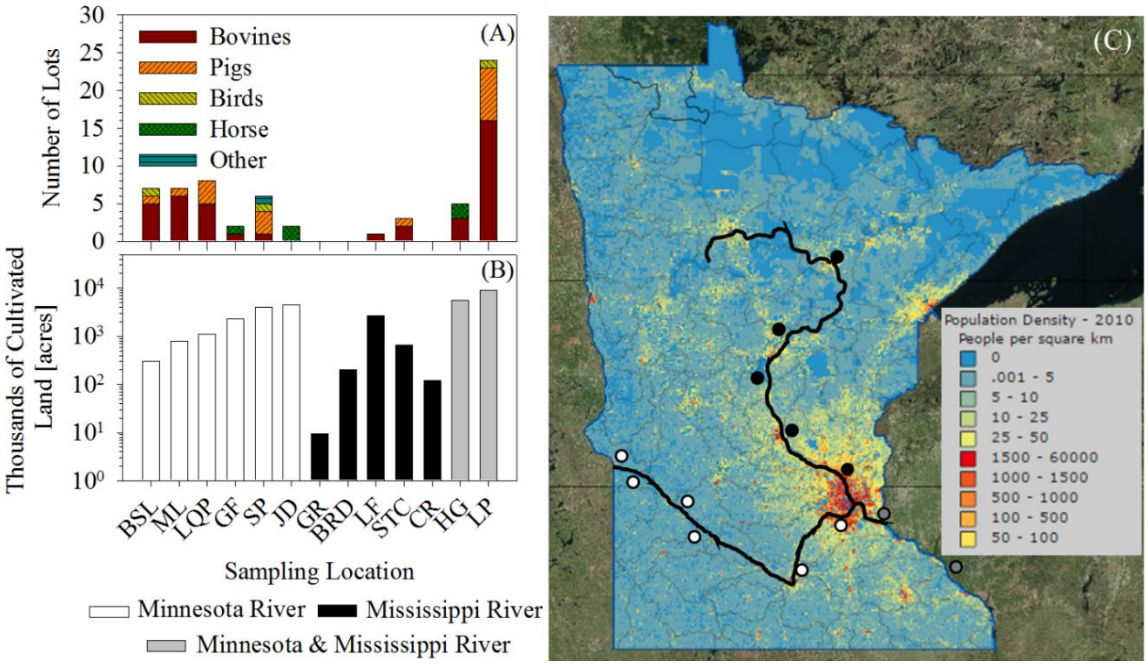

Figure 4.2. Agricultural activity represented by (A) number of animal feedlots and (B) thousands of cultivated land [acres] in the catchments of Big Stone Lake (BSL), Marsh Lake (ML), La qui Parle (LQP), Granite Falls (GF), St. Peter (SP), Jordan (JD), Grand Rapids (GR), Brainerd (BRD), Little Falls (LF), St. Cloud (STC), Coon Rapids (CR), Hastings (HG), and Lake Pepin (LP). Panel (C) is a screenshot of 2010 census of Minnesota population density with outline of Minnesota and Mississippi River and approximate location of collection sites. Data was collected and organized by the Minnesota Department of Natural Resources at *http://arcgis.dnr.state.mn.us/ewr/whaf/Explore/#* and is displayed with permission. Minnesota River sampling locations are displayed west to east, Mississippi River sampling locations are arranged north to south, and Hastings (HG) and Lake Pepin (LP) represent sampling points after Minnesota River joined Mississippi River.

Also important to note is that stream flows in the Minnesota River are lower than the Mississippi River at some locations (Table 4.6). Lower stream flows may lead to less dilution of anthropogenic inputs, thus the Minnesota River may be more susceptible to antibiotic pollution.

Table 4.6. Long-term median stream flow (ft^3/s) in the Minnesota and Mississippi River Basin at monitored Minnesota cities recorded by the United States Geological Survey.[231]

Station Site	Closest Sediment Collection Site	Long-Term Median Flow (ft^3/s)
Minnesota River Basin		
Ortonville	Big Stone Lake & Marsh Lake	88
Near Lac qui Parle	Lac qui Parle	909
Montevideo	–	970
Granite Falls	Granite Falls	3,150
Morton	–	3,550
Mankato	St. Peter	4,680
Near Jordan	Jordan	6,770
Fort Snelling State Park	–	9,920
Mississippi River Basin (above Minnesota River)		
Grand Rapids	Grand Rapids	1,019
Aitkin	–	3,750
Brainerd	Brainerd	4,470
Royalton	Little Falls	5,900
St. Cloud	St. Cloud	8,580
Brooklyn Park	Coon Rapids	10,500
Mississippi River Basin (below Minnesota River)		
St. Paul	–	18,400
Hastings	Hastings	28,100

Lake Winona: Small Scale System. Of the 20 antibiotics included in this study, 13 antibiotics were detected in at least one of the surface sediments from Lake Winona (Figure 4.3). Tetracycline and chlortetracycline were detected in one sample each, their presence is noted in the following paragraphs and not in Figure 4.3. Sulfapyridine, sulfamethoxazole, ciprofloxacin, and ofloxacin had the highest detection frequency (100%). Levels of ciprofloxacin and ofloxacin were the highest among the detected antibiotics in Lake Winona.

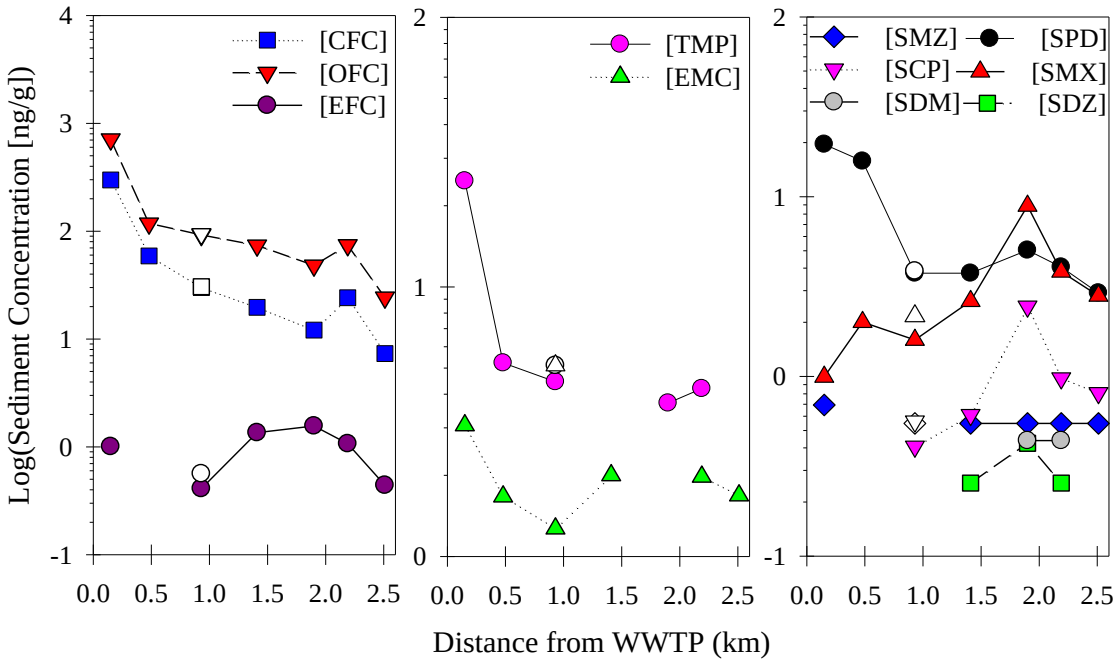

Figure 4.3. Log_{10} transformed surface sediment concentrations (ng/g) of sulfapyridine (SPD), sulfamethoxazole (SMX), sulfadiazine (SDZ), sulfadimethoxine (SDM), sulfachlorpyridazine (SCP), sulfamethazine (SMZ), ofloxacin (OFC), ciprofloxacin (CFC), enrofloxacin (EFC), and trimethoprim (TMP) in Lake Winona corresponding to distance from wastewater treatment plant (WWTP) outfall. White symbols represent replicates.

Similar spatial trends were observed for several antibiotics: sulfapyridine, ofloxacin, ciprofloxacin, tetracycline and trimethoprim. The maximum concentration of sulfapyridine (19.6 ng/g), ofloxacin (711 ng/g), ciprofloxacin (298.3 ng/g), tetracycline (2.7 ng/g), and trimethoprim (2.7 ng/g) was quantified in the sample closest to the WWTP outfall. Their levels dropped within 0.48 km of the WWTP. Concentrations ranged throughout the rest of the lake from 2.9 to 5.0 ng/g for sulfapyridine, 7 to 59 ng/g for ciprofloxacin, 24 to 118 ng/g for ofloxacin, and non-detect to 5.2 ng/g for trimethoprim. Tetracycline was only detected in the site closest to wastewater outfall.

Sulfapyridine, ciprofloxacin, and trimethoprim are predominately used for human chemotherapy and given their spatial distribution is it likely that they derived from

wastewater effluent. Elevated levels of human-use antibiotics in sediments near wastewater outfalls has been observed by previous studies.[122,123,127] Tetracycline and ofloxacin are used for both human and veterinary treatments, but their similar spatial distribution to the three human-use only antibiotics suggests that their dominant source is also wastewater effluent.

A different spatial distribution was observed for sulfamethoxazole, another pharmaceutical used exclusively for human treatments, from the other wastewater-derived antibiotics. The highest concentration (10.4 ng/g) was measured 1.9 km away from the WWTP and levels varied from 1.12 to 4.04 ng/g throughout the rest of the lake. Sulfamethoxazole is frequently paired with trimethoprim for human-use. These two drugs, however, had very different spatial distributions which indicates differing fate and transport processes. Gibs et al[128] found sulfamethoxazole to be more mobile in the water column than trimethoprim in a river system, presumably because trimethoprim sorbed more readily to sediment. Back-transformation of sulfamethoxazole metabolites to its original compound has been shown to occur in surface waters via photolysis [232] and in sediment by bacteria[233], and these processes may lead to the elevated concentrations away from the outfall.

Several veterinary antibiotics (sulfadimethoxine, sulfamethazine, sulfachlorpyridazine, enrofloxacin, and chlortetracycline) were also detected in Lake Winona. These antibiotics were generally detected at lower concentrations than human-use antibiotics and were more prevalent in the northeastern region of Lake Winona. Chlortetracycline was measured once (5.9 ng/g) 1.9 km from WWTP, and a relatively uniform distribution of enrofloxacin was observed (non-detect to 1.56 ng/g) throughout

Lake Winona. Concentrations reached 2.45 ng/g, 0.44 ng/g, and 0.69 ng/g for sulfachloropyridzadine, sulfadimethoxine, and sulfamethazine 1.9 km away from the WWTP. The presence of several animal-use antibiotics is likely the result of the landscape in the watershed of Lake Winona, which is approximately 30% cultivated land. The occurrence of enrofloxacin and sulfamethazine near the WWTP may also be attributed to the bovine feedlot in the southwest corner of the lake. Other studies have also found veterinary antibiotics near agricultural land.[124,127,129]

Lower levels of animal-use antibiotics indicate that agricultural pressures contribute less to the overall antibiotic burden in the studied lake, which is reasonable because 63% of the inflow to Lake Winona is wastewater effluent. Agricultural antibiotic pollution also may have been lower because they enter via diffuse, non-point sources, whereas wastewater effluent is a point source into Lake Winona.

For some antibiotics that have both human and animal-uses, it was difficult to determine their primary source. The profile of sulfadiazine is similar to both sulfamethoxazole and the agricultural sulfa drugs, and therefore its presence may be due to either human or animal use. The dominant source of erythromycin is also difficult to determine. Uniform distribution of erythromycin was observed throughout Lake Winona with concentrations varying from non-detect to 3.07 ng/g. In addition to mass production for human, poultry, swine, and other animal-uses, erythromycin is naturally produced.[191] Thus, the accumulation of erythromycin may be due to a combination of wastewater effluent, runoff from agricultural fields and feedlots, and/or natural production.

The spatial trends of heavy metals were also investigated because metal pollution has been associated with ARG abundance via co-resistance and cross-resistance.[234] All

the metals included in the study were detected in Lake Winona surface sediments (Figures D.1 and D.2). Metal concentrations ranged from 100s ng/g to less than 1 pg/g. Metals visually appeared to have increasing concentrations (Mo, Co, As, Ni, and Pb), decreasing concentrations (V, Cr, Sn, and Gd), or uniform/no apparent trend (Mn, Cu, Cd, Se, and Zn) moving west to east across Lake Winona. The natural abundance of heavy metals may have been elevated by anthropogenic inputs from agricultural activities (fertilizers, manures, fungicides, pesticides, and herbicides), mining, industry (iron and steel), or domestic and industrial wastewaters.[235] Given the proximity of Lake Winona to agricultural activities and the large input of treated wastewater, anthropogenic inputs of metals likely occurred in Lake Winona.

Concentrations of 16S rRNA, the surrogate for biomass, ranged from 10^9 to 10^{11} copies/g throughout Lake Winona sediments (Figure 4.4). Of the 45 metal, antibiotic, and antibiotic resistance-associated genes included in this study, 16 were detected above the quantification limits in more than half the surface sediments (Figure D.3 and D.4).

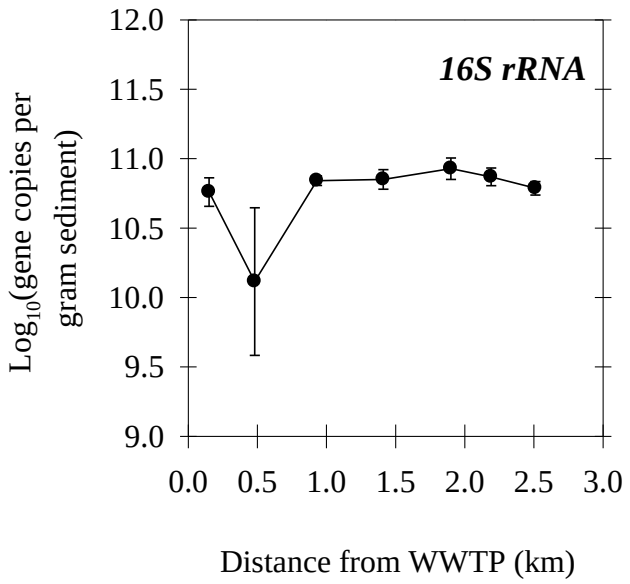

Figure 4.4. Concentration of 16S rRNA copies per gram of Lake Winona surface sediment samples with respect to distance from the wastewater treatment plant (WWTP) outfall.

Resistance to β-lactamases (*bla*$_{OXA}$ and *bla*$_{SHV}$), streptomycin (*strB*), macrolides (*mefE*), sulfonamides (*sul1*, *sul2*, and *sul3*), tetracyclines (*tet*(A)), and metals (*cadA, copA nikA*, and *merA*) were frequently detected, as were genetic codes for integrons (*intI1* and *intI3*) and multi-drug effluxes (*acrD* and *mexB*). Gene concentrations ranged from 10^{-3} to 10^{-7} copies per 16S rRNA gene copies.

Pearson correlations were performed between metals, antibiotics, and target genes in lake surface sediments on a log-log scale. Statistical significance was defined as $\alpha < 0.05$. Non-detect concentrations were excluded from the correlations, because $\log_{10}(0)$ is undefined. Correlations were only performed on antibiotics, metals, and genes that were present in more than half of the sediment samples and data sets that had at least five common data points.

Several significant associations were observed between target genes and both metals and antibiotics in Lake Winona surface sediments, see Table D.7 – D.10 for p-

values and Pearson coefficients. Concentrations of target genes were expressed as gene copies per bacterial biomass (16S rRNA). The abundance of a sulfonamide resistance gene (*sul1*) was significantly correlated to three antibiotics (sulfapyridine, ciprofloxacin, and ofloxacin), and tin, as well as mercury resistance (Figure 4.5 and 4.6). Mercury resistance (*merA*) was also associated with ciprofloxacin, ofloxacin, and tin (Figure 4.6).

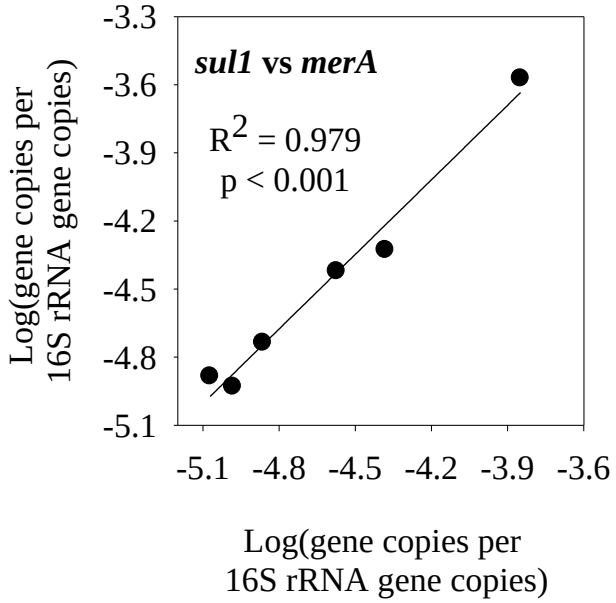

Figure 4.5. Pearson correlation between *sul1* and *merA* [$\log_{10}$(gene copies per 16S rRNA gene copies)] in Lake Winona surface sediments. Linear trendline, R^2 value, and p-value are displayed.

The numerous positive correlations of *sul1* and *merA* with several wastewater-derived antibiotics suggest that these ARGs entered via wastewater effluent. Previous studies have shown WWTP effluent to be a source of antibiotic, metal, and antibiotic-associated resistance genes.[156,236] The relationship may also be due to the sorbed human-use antibiotics selecting for and promoting the occurrence of resistance genes *in situ*. The association between the abundance of antibiotics and their respective resistance genes has also been previously shown.[25,116,193,211] The influence of heavy metals on ARG abundance

must also be considered because heavy metals co-select for ARGs by cross-resistance or co-resistance.[237,238] Thus, tin may have attributed to the prevalence of *sul1* and *merA*.

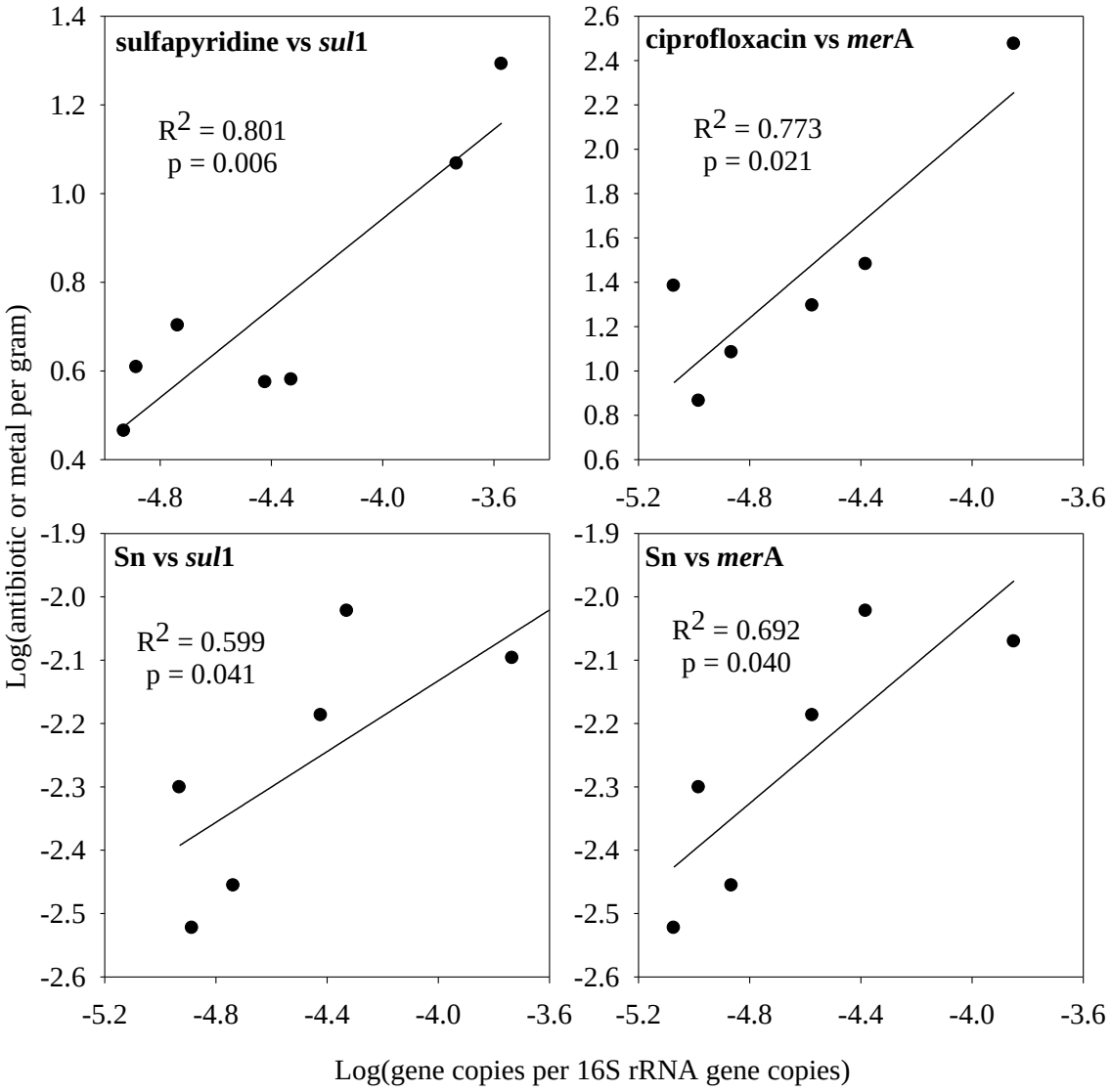

Figure 4.6. Pearson correlations among metals (log_{10}(ng/g)), antibiotics (log_{10}(ng/g)), and resistance genes (log_{10}(gene copies per 16S rRNA gene copies)) in Lake Winona surface sediments. Linear trendline, R^2 value, and p-value are displayed.

Minnesota and Mississippi River: Large Scale Systems. To determine whether the relationships observed across short distances were applicable to large distances as well, profiles of antibiotics, metals, and ARGs in rivers were investigated. Nine of the twenty targeted antibiotics were detected at least once in the Minnesota and Mississippi River (Figure 4.7). Not shown in Figure 4.7 are the levels of oxytetracycline, sulfadimethoxine, and sulfadiazine that were detected infrequently in the river sediment, and their occurrence is discussed below. Similar spatial trends were observed when antibiotic concentrations are expressed as antibiotic mass per gram of sediment, per gram clay, or per gram clay and silt (Figures D.5 and D.6). Sulfapyridine, trimethoprim, sulfamethazine, and erythromycin were the most frequently detected antibiotics (62% of samples) and typically at low levels (near or less than 1 ng/g).

Two human-use only antibiotics (sulfapyridine and trimethoprim) were detected in both river systems, with similar or greater detection frequencies in the Minnesota River. In the Minnesota River, sulfapyridine and trimethoprim levels ranged from non-detect to 0.08 ng/g and non-detect to 0.61 ng/g, respectively. Sulfapyridine was not detected in the Mississippi River until St. Cloud (0.04 ng/g), and higher levels (0.14 to 1.91 ng/g) were measured downstream of the confluence. Detected levels of trimethoprim were lower in Mississippi River above the confluence (0.39 to 0.58 ng/g) than below (0.89 to 1.43 ng/g). The high sand content may have resulted in the non-detects of trimethoprim in St. Cloud. Another human-use antibiotic (sulfamethoxazole) was detected twice, but only in the Minnesota River and at concentrations less than 1 ng/g.

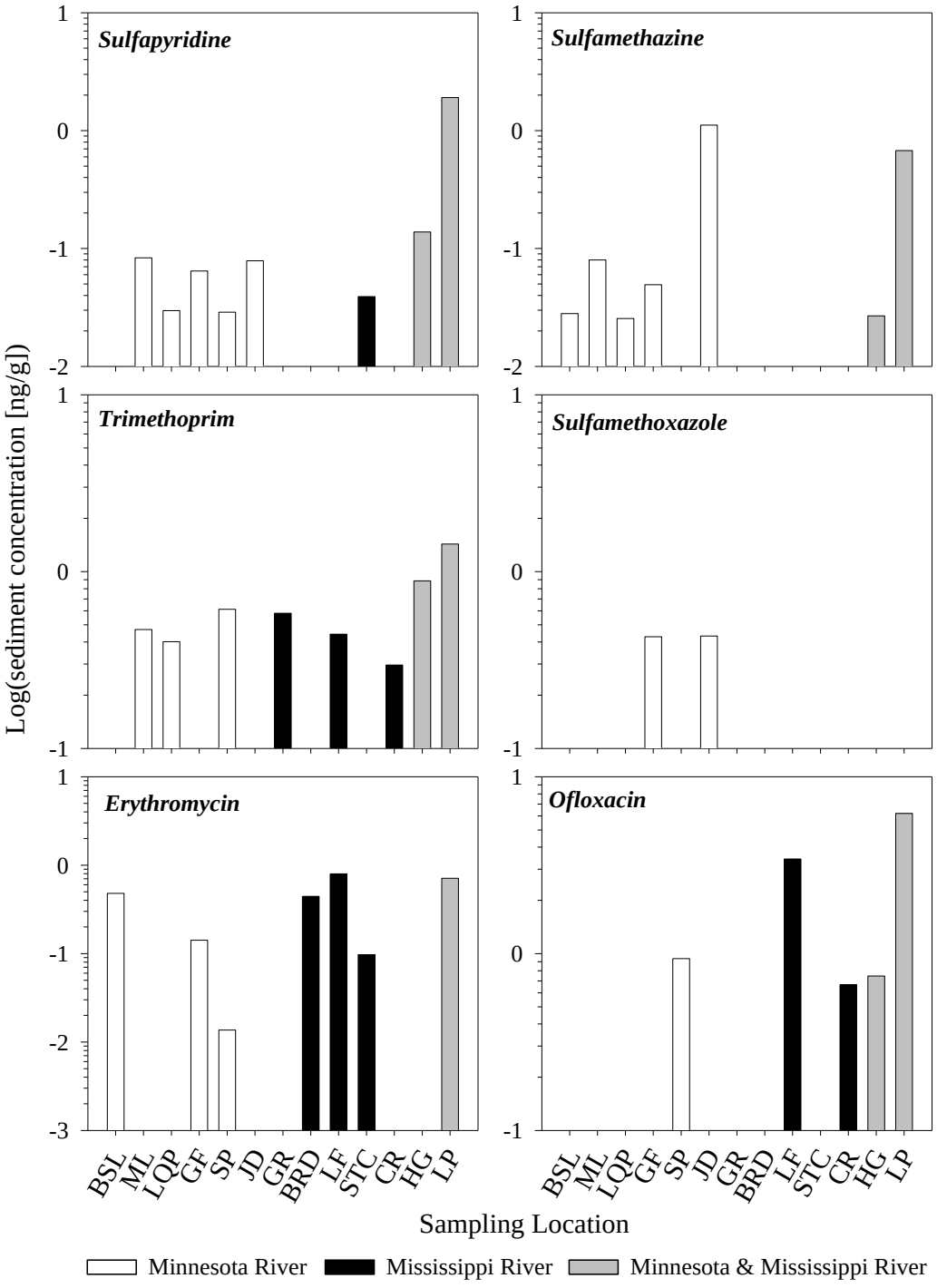

Figure 4.7. Log_{10} transformed antibiotic concentrations (ng/g) in river surface sediments. Minnesota River sampling locations (white bars) are displayed west to east, Mississippi River sampling locations (black bars) are arranged north to south, and gray bars represent sampling points after Minnesota River joined the Mississippi River. Sampling locations abbreviations are in Table 4.6.

The greatest input of treated wastewater occurs after the Minnesota River joins the Mississippi. The Minneapolis and St. Paul area is the largest metropolitan region in Minnesota and these communities discharge their treated wastewater into the Mississippi River south of confluence. Above the confluence, the Mississippi River generally flows through slightly larger, more densely populated urban areas, and therefore it is more likely to be impacted by WWTP effluent than the Minnesota River. The higher frequency of wastewater-derived, human-use antibiotics in the Minnesota River was an unexpected result. The elevated occurrence of wastewater-derived antibiotics in the Minnesota River may be due to lower flow rates in the Minnesota River,[231] and therefore antibiotic inputs may have been more diluted in the Mississippi River. Textural analysis also revealed that sand content was 90% or greater for two sites further downstream on the Mississippi River (St. Cloud and Coon Rapids), thus sorption of antibiotics to sediment might have been diminished due to larger particle sizes.[137]

Ofloxacin, sulfadiazine, and erythromycin were detected in the river sediment and are used by both human and animals. Prior to the rivers joining, ofloxacin was detected twice in the Mississippi River (3.4 ng/g in Little Falls and 0.67 ng/g in St. Cloud) and once in the Minnesota River (0.94 ng/g in St. Peter). After the rivers joined, ofloxacin was present in both Hastings and Lake Pepin samples at 0.74 and 6.20 ng/g, respectively. Erythromycin was also detected in both rivers, but with no apparent trend and with similar concentrations in the Minnesota (0.01 to 0.48 ng/g) and Mississippi River (0.097 to 0.796 ng/g). Sulfadiazine was only detected in Lake Pepin (0.45 ng/g). The source of these three antibiotics (ofloxacin, sulfadiazine, and erythromycin) is attributed to multiple

anthropogenic activities, and further analysis and sampling would need to be performed to determine dominant sources.

Three antibiotics (sulfamethazine, sulfadimethoxine, and oxytetracycline) that are primarily used by animals were predominately detected in the agriculturally-impacted Minnesota River. Oxytetracycline (50.3 ng/g) was detected at Big Stone Lake and sulfadimethoxine was present at Marsh Lake and Jordan at 0.06 and 0.01 ng/g, respectively. Sulfamethazine is primarily given to swine and cattle, and was often detected (0.03 to 1.1 ng/g) in the Minnesota River. Sulfamethazine was only present in the Mississippi River below the Minnesota River junction, and then it was detected in both Hastings and Lake Pepin at 0.027 and 0.677 ng/g, respectively.

In addition to acres of cultivated land, animal feedlots are also located within the catchments of the Minnesota River sample sites. Sulfadimethoxine is commonly given to poultry. The occurrence of sulfadimethoxine in the Minnesota River did not correspond to poultry feedlots, thus its appearance is likely due to runoff from agricultural fields. The presence of oxytetracycline and sulfamethazine is likely attributed to runoff from row-crop fields and feedlots of livestock that use them. Previous studies have reported agricultural fields as non-point sources of veterinary antibiotics.[123–125,129] Also of interest is that sulfamethazine was only present in the Mississippi River after the Minnesota River joined and previous work reported that sulfamethazine has been accumulating in Lake Pepin since the 1950s (Chapter 3).

Lake Pepin is a natural catchment separating Minnesota and Wisconsin and approximately 75-90% of the sediment load to the Mississippi originates from the Minnesota River Basin.[239,240] In 2006, it was estimated that approximately 78% of the

Minnesota River Basin landscape was covered by row-crop agricultural fields.[241] In recent years, higher river flow rates have been observed in the Minnesota River and are thought to be caused by the expansion of the agricultural artificial drainage network.[242–244] The source of the elevated levels of sediment in Minnesota River is primarily attributed to the erosion of non-field, near-channel sources (i.e. stream banks, ravines, and bluffs) which has been enhanced by the higher flow rates.[242–244] The occurrence of sulfamethazine in the Mississippi River only after the Minnesota River joined suggests that animal-use antibiotics may be transported from the agriculturally-developed river to then be deposited in the Mississippi River. Therefore, in addition to potential inputs of sulfamethazine from agriculture activities within Lake Pepin watershed, a portion may have originated from the Minnesota River basin.

The greater frequency of wastewater-derived antibiotics in the Minnesota River may also be an indirect effect of agricultural pressures. Elevated levels of suspended sediment (thought to be caused by intense agricultural activity) is a severe water quality issue that affects the Minnesota River.[243] High turbidity in the water column may promote sorption of wastewater-derived pollutants to particles in the river water column. Thus, intense agricultural activity may indirectly facilitate the accumulation and transport thof human-use antibiotics.

Antibiotic pollution is generally greater downstream in Lake Pepin than near the headwaters of the rivers. Elevated levels in Lake Pepin may be due to migration of antibiotics from upstream inputs or the higher degree of anthropogenic activities in the watershed of Lake Pepin. Another possibility is that the sediment in Lake Pepin sorbs antibiotics more readily. Lake Pepin is a natural impoundment in the Mississippi River,

and therefore fine particles that were suspended in and transported down the rivers would have naturally deposited in Lake Pepin. Lake Pepin receives treated wastewater effluent from Red Wing, WI (3 MGD) and Lake City, MN (1.8 MGD), as well as runoff from over 9 million acres of cultivated land and 24 feedlots. Thus, the elevated levels of antibiotics in Lake Pepin may be the result of direct anthropogenic inputs of antibiotics readily sorbing to the small particles transported from the Minnesota River.

Data for and a detailed discussion about metal levels in the Minnesota and Mississippi River has been previously published.[227] Briefly, Sandberg[227] reported that there was no statistical differences in metal concentrations in the Minnesota and upper Mississippi rivers, except for tin. Metal pollution was sometimes greater downstream of the river junction in Hastings and Lake Pepin than in the Minnesota River (for Cr, Cu, Zn, Cd, Sn, and Pb) and the upper Mississippi River (for Mo, Cd, Sn, and Pb).[227]

The occurrence of metal, antibiotic, and antibiotic resistance-associated genes was also quantified and discussed previously.[227] Sandberg[227] found that bacterial biomass (quantified by copies of 16S rRNA genes) ranged from 10^8 to 10^{11} copies/g in the river sediments and 9 genes (*bla*$_{SHV}$, *cadA*, *floR*, *intI1*, *mexB*, *nikA*, *sul1*, *sul3*, and *tet*(A)) were quantified in more than half of the sediment samples at levels ranging between 10^{-2} to 10^{-7} copies per 16S rRNA gene copies. Gene concentrations were generally lower in Lake Pepin and Little Falls than the rest of the sediment samples, expect for *nikA* which was highest in Lake Pepin.

Pearson correlations were also performed between target genes and both metals and antibiotics in the river sediments on a log-log scale with the same parameters set the lake samples. No significant correlations were observed for gene abundance in the river

sediments with either metals or antibiotics. Except for the Lake Pepin outlier, the remaining samples appeared to be randomly grouped together with no clear trends between the occurrence of the target genes with either antibiotic or metal pollution. There were many significant correlations among the target genes (Table D.11 and D.12) and a few representative correlations are presented in Figure 4.8.

An increase in antibiotic pollution along the Minnesota and Mississippi River did not result in a significant increase of antibiotic or metal resistance. Levels of metal and antibiotic resistance genes, however, were often strongly correlated with other resistance genes. The presence of *intI1* (a mobile genetic element that can transfer multiple resistance genes among bacteria[245–247]) corresponded with the abundance of other ARGs in the studied rivers. This finding further supports recent studies that suggest *intI1* could be a potential indicator of ARG abundance in natural and man-made environments.[145,248]

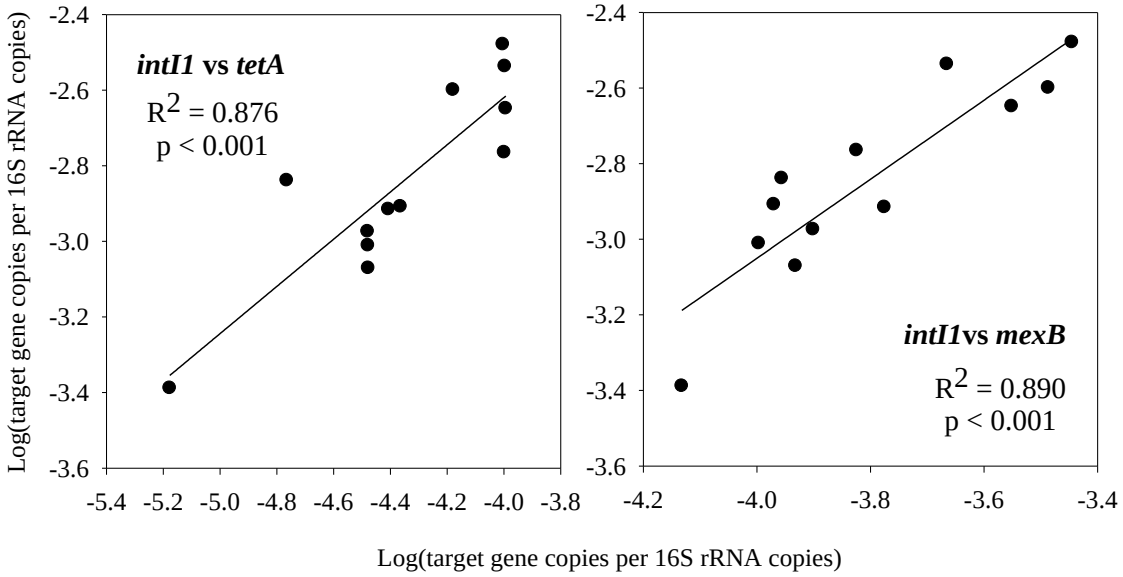

Figure 4.8. Representative Pearson correlations between target genes (log_{10}(gene copies per 16S rRNA gene copies)) that were significant (p-value < 0.05) in river surface sediments. Linear trendline, R^2 value, and p-value are displayed.

Environmental Implications. Dynamics between antibiotic and ARG abundance were captured over short distances that were diminished over long distances. One possibility for this observation is that differences in transport mechanisms or persistence between antibiotics and ARGs have a greater influence on the resulting spatial distribution across larger distances. If antibiotics and ARGs originated from the same source, any relationship observed near the source would be disrupted due to different transport and fate processes as distance increased. Furthermore, it appears the presence of metals and antibiotics were not the driver of ARG abundance over large spatial scales. A mobile genetic component (*intI*1), however, maybe a good predictor of ARG abundance in the large-scale systems. This relationship may be the result of similar migration patterns from their anthropogenic inputs or they may have comparable *in situ* proliferation mechanisms.

The dominant fate and transport processes driving the spatial distribution of antibiotics also likely differs across short and long distances. High-resolution spatial trends are heavily influenced by numerous fate and transport processes, such as sorption, photolysis, microbial activity, etc. Whereas, a comprehensive spatial pattern of antibiotic occurrence in a river system may be primarily driven by the ability of antibiotics to sorb to the sediment and the transport of sediment downstream. Understanding the mechanisms that drive the migration of antibiotics from their anthropogenic source across small and large-scales in riverine systems is necessary for predicting their transport within environmental systems and any impacts of antibiotics and ARGs.

Chapter 5: Assessment of Removal of Antibiotics at Various Locations Throughout a Wastewater Treatment Plant

5.1 Introduction

The presence of human-use antibiotics in surface waters is a result of incomplete removal at wastewater treatment plants (WWTPs).[118,120,123,214] The occurrence of these micropollutants in the environment is of concern due to their selection for antibiotic resistant bacteria.[3,147,148] Antibiotics are one of the greatest inventions of the 20th century, but their overuse and misuse is accelerating the abundance of antibiotic resistant bacteria. The rise and spread of antibiotic resistant bacteria is one of the major threats to public health in the 21st century according to the World Health Organization.[154]

Manufacturing, consumption, and disposal are known sources of antibiotics to the environment.[4] Conventional wastewater treatment plants are effective at removing macropollutants (organic matter, nutrients, and bacteria), but many were not designed for removal of micropollutants. WWTP effluent, therefore, is a source of antibiotics and antibiotic resistance genes (ARGs).[121,155,156] Across the globe, researchers have investigated the efficiency of antibiotic removal via conventional wastewater treatment operations, primarily by comparing influent and effluent concentrations of analytes.[119,121,249–251] Several studies have also investigated the role of treatment operation (type, solid retention time, and hydraulic retention time), temperature, and compound specific properties on the removal of antibiotics from waste streams.[252,253] A consensus has yet to be reached on the effectiveness of conventional unit operations due to conflicting observations within a single WWTP, as well as comparing multiple WWTPs.

More research is needed to predict and potentially control the removal of antibiotics through conventional wastewater treatment.

The objective of this study was to quantify antibiotics levels in the liquid phase after each treatment step in a WWTP located in Minnesota. Seasonal variations in removal efficiencies were also assessed. Wastewater was extracted to allow measurement of three sulfonamides (sulfapyridine, sulfamethoxazole, and sulfadiazine), two tetracyclines (tetracycline and doxycycline), two macrolides (roxithromycin, erythromycin and its major degradation product, erythromycin-H_2O), three fluoroquinolones (norfloxacin, ofloxacin, ciprofloxacin), trimethoprim, and lincomycin. Future work will investigate the relationship between antibiotic residuals in the liquid phase with the abundance of ARGs.

5.2 Materials and Methods

Wastewater Treatment Plant. The Minnesota WWTP includes conventional treatment processes, seasonal chlorine disinfection, and a Water Reclamation Facility, see Figure 5.1. The WWTP is designed to treat 9.38 million gallons per day from domestic, commercial, and industrial sources. Bar screens remove large objects from influent, followed by grit removal via a centrifugal basin. Conventional water treatment processes (in order of the treatment train) include: ferric chloride ($FeCl_3$) addition for phosphorus removal, primary clarifier, aeration basin, secondary clarifier, and seasonal chlorine disinfection. During the summer months, treated wastewater effluent is disinfected with sodium hypochlorite (NaOCl) to a residual range between 0.5 to 1 mg/L Cl^-. Sodium bisulfite ($NaHSO_3$) is then added to reduce the chlorine residual to less than 0.3 mg/L Cl^-

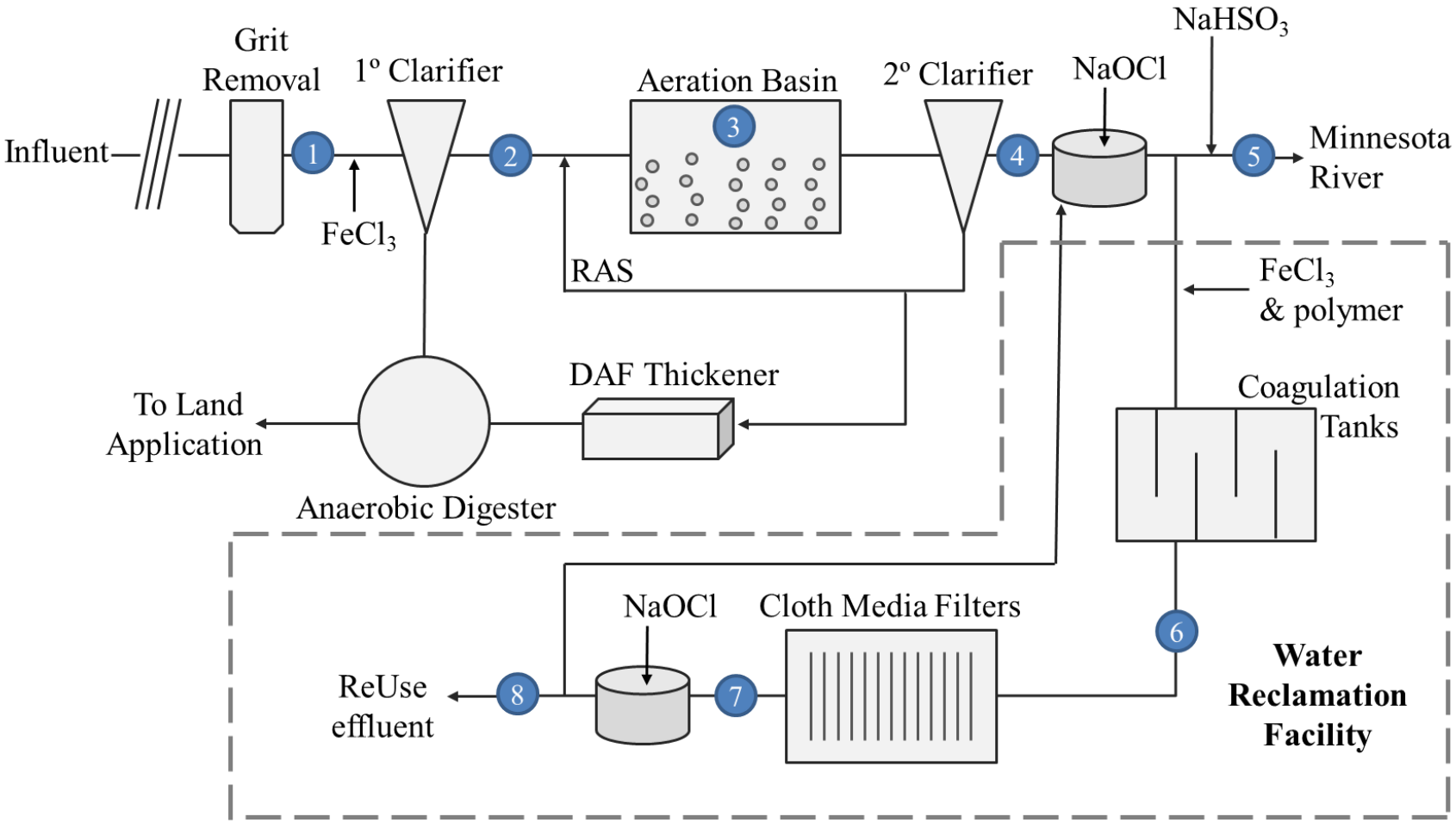

Figure 5.1. Schematic of treatment steps at the wastewater treatment plant. Sampling points were: (1) influent, (2) effluent of primary clarifier, (3) aeration basin, (4) effluent of secondary clarifier, (5) effluent discharged to receiving river, (6) after tertiary chemical treatment, (7) after filtration, and (8) reuse effluent. RAS = returned activated sludge; DAF = dissolved air floatation

before discharging into the Minnesota River. The anaerobic digester receives sludge from the primary clarifier and dissolved air floatation thickener (DAF) thickener. After further treatment, the sludge is land applied.

A portion of the secondary clarifier effluent goes to the Water Reclamation Center for additional phosphorus removal, as well as further processing to meet California Title 22 Standards for Water Reuse.[254] Phosphorus removal to less than 0.9 mg/L is achieved by addition $FeCl_3$ and an anionic polymer. Cloth media filters remove (10 µm) particles after coagulation to reduce turbidity to less than 2.0 NTU. Finally, a chlorine residual between 5 and 25 mg/L is maintained in reuse effluent.

Sample Collection. Water samples (approximately 4 L) were collected in glass jars from eight locations throughout the WWTP, see Figure 5.1. Glass jars were cleaned by triple rinsing with a dilute Alconox solution and deionized water, and then baked at 550 °C for four hours. Samples were collected approximately every three months to capture any seasonal trends. Wastewater was collected on June 20, 2016 (summer), September 27, 2016 (fall), January 9, 2017 (winter), and March 4, 2017 (spring). Wastewater samples were stored on ice during transport back to laboratory. Within 24 hours, samples were filtered (pre-combusted glass fiber filter, 0.45µm), the pH recorded and adjusted to 3 with concentrated sulfuric acid (H_2SO_4), and stored at 4 °C.

Solid Phase Extraction. Antibiotics were extracted from wastewater using a solid phase extraction (SPE) method adapted from Meyer et al.[197] Wastewater was extracted within seven days of sampling. Three replicates were prepared for each sample by spiking surrogates (100 ng, nalidixic acid, $^{13}C_6$-sulfamethazine, and demeclocycline) and 0.1 mg/mL of ethylenediaminetetraacetic acid solution (EDTA, 5 mL) into 500 mL splits

of the collected wastewater. The samples collected on June 20, 2016 were also spiked with internal standards (100 ng, clinafloxacin, simetone, $^{13}C_2$-erythromycin, and $^{13}C_6$-sulfamethoxazole). One replicate was spiked with all the compounds of interest (100 ng) to assess extraction efficiency. Samples were equilibrated overnight in the dark. Method blanks (500 mL) were ultrapure water (pH 3 H_2SO_4) spiked with 100 ng of surrogates. Two or three method blanks underwent the entire extraction process per sampling event to monitor for any cross contamination.

Two SPE cartridges, Oasis HLB (6cc, 200 mg, 30 µm) and Oasis MCX (6 cc, 150 mg, 30 µm), were used to extract antibiotics from wastewater and remove interferences from the sample matrix. Excess ultrapure water was adjusted to pH 3 with concentrated H_2SO_4 to be used throughout SPE process. Cartridges were conditioned separately with 3 mL ultrapure water, 3 mL methanol, 3 mL ultrapure water, and 3 mL pH 3 water. The MCX cartridge was loaded onto vacuum manifold first with 5 mL of pH 3 water. An SPE adapter was used to connect the bottom on the HLB cartridge to the top of the MCX. Fluoropolymer tubing (Saint-Gobain Chemofluor) and another SPE adapter was used to transfer sample from flask to HLB cartridge. Fluoropolymer tubing was cleaned with methanol (LC-MS grade) and pH 3 water. SPE adapters were soaked in nitric acid bath (2%) overnight and then rinsed with ultrapure water. Samples were loaded onto cartridges in tandem (HLB on top of MCX) under vacuum that never exceed 15 mm Hg. Cartridges were then disassembled, cleaned with water (3 mL), and dried under vacuum for approximately 1 minute. The HLB cartridge was then loaded onto the vacuum manifold with methanol (3 mL, LC-MS grade). The MCX cartridge was placed on top of the HLB. The cartridges were eluted in tandem with 10 mL of methanol (LC-MS grade).

A vacuum pulse was used to start the elution process. The MCX cartridge was then eluted separately with 5% ammonium acetate in methanol (3 mL), which was combined with the methanol eluent. Internal standards (100 ng, clinafloxacin, simetone, $^{13}C_2$-erythromycin, and $^{13}C_6$-sulfamethoxazole) in a methanol solution were spiked into SPE eluents for fall, winter, and spring samples. Eluents were blown down to dryness in a 40 °C water bath under a gentle stream of nitrogen. Samples were resuspended in ammonium acetate solution (20 mM, LC-MS grade, 200 µL). Any particles were removed with a syringe filter (GHP, 0.4 µm) prior to liquid chromatography tandem mass spectrometry analysis.

HPLC-MS/MS Analysis. Two high performance liquid chromatography (HPLC) methods were used for analysis of water samples, see Table 5.1. For HPLC Method 1, the column was flushed with a 50:50 10 mM EDTA:methanol solution and 50:50 water:methanol for 30 minutes each prior to each analysis. This removed any metals and improved peak shapes of tetracyclines and fluoroquinolones.

Analytes were detected and quantified with single reaction monitoring (SRM) transitions in Table E.3 with Thermo Vantage triple quadrupole tandem mass spectrometer (MS/MS) in positive ESI mode. Each analyte had an additional SRM to confirm the identity of quantified peak. Three HPLC-MS/MS methods were run per sample to have sufficient instrument responses across a peak. Mass spectrometer parameters were optimized with the infusion of 5µM simeton in 50:50 20 mM ammonium acetate:acetonitrile prior to each analysis, due to the sensitivity of the instrument. Typical values were: scan time 0.02 sec; scan width: 0.15; Q_1/Q_3: 0.7; spray voltage: 3300 V; sheath gas pressure: 18 psi; capillary temperature: 300 °C; collision pressure: 1.5 mTorr; declustering voltage: -9 V; and tube lens: 95.

Table 5.1. High pressure liquid chromatography method details.

	High Performance Liquid Chromatography	
Parameters	**Method 1**	**Method 2**
Samples	summer and fall	winter and spring
Instrument	Agilent 1100	Thermo Dionex ultimate 3000 RSLCnano
Column	Phenomenex Kinetex F5	Waters XSelect CSH C18
Particle Size (µm)	1.7	3.5
Porosity (Å)	100	130
Dimensions (mm)	50 × 2.1	50 × 2.1
Guard Column	SecurityGuard ULTRA	–
Flow rate (µL/min)	250	500
Temperature (°C)	50	35
Injection Vol. (µL)	8	8
Mobile Phases	A) 0.1% formic acid in ultrapure water B) 0.1% formic acid in acetonitrile gradient elution, see Table E.1	A) 0.1% formic acid in ultrapure water B) 0.1% formic acid in methanol gradient elution, see Table E.2
Flow diverted to waste (min)	0 – 1 and 7.5 – 25	0 – 1.5 and 5.5 – 20
Methods:	1.) SAs and surrogates 2.) TCs and FQs 3.) NC and MCs	1.) SAs, NCs, and $^{13}C_6$-sulfamethazine 2.) TCs, FQs, demeclocycline, and nalidixic acid 3.) MCs

SAs = sulfonamides; TCs = tetracyclines; FQs = fluoroquinolones; NC = non-categorized; MCs = macrolides

Limits of quantification (LOQs) and detection (LODs) were calculated from 10× and 3×, respectively, the peak area near the retention time of an analyte in the method blank. Antibiotic concentrations above LOQ were recovery corrected and calculated via internal standard dilution methodology. Reported LOQs and LODs were not recovery corrected.

5.3 Results and Discussion

pH and disinfectant levels. The pH of all samples did not vary significantly throughout the year, and average values ranged from 7.34 to 7.88 in the WWTP, see Table E.4. Summer and fall wastewater effluent underwent chlorine disinfection prior to being discharged into the Minnesota River, see Table 5.2. A high flow event occurred just before collecting the summer and fall samples. The high flow event in the fall caused a malfunction within the WWTP and a chlorine residual greater than the allowed 0.3 mg/L was measured in the effluent. A chlorine residual between 5.2 to 7.5 mg/L as Cl^- was maintained for reuse effluent throughout the year.

Table 5.2. Chlorine residual in final effluent discharged to Minnesota River and reuse effluent.

	Chlorine Residual (mg/L)			
Sampling Location	**Summer**	**Fall**	**Winter**	**Spring**
Final Effluent	0	1.77	0	0.01
Reuse Effluent	7.5	5.4	5.2	7.2

Analytical Performance. Average LODs and LOQs for the targeted antibiotics ranged from 0.05 to 1.58 ng/L and 0.18 to 5.32 ng/L and varied from compound to compound, see Table 5.3. Reported LODs and LOQs were not recovery corrected because relative recovery varied greatly from sample to sample, see Table E.5. If the

concentration of an antibiotic was less than LOQ and greater than LOD, then the concentration was reported as the recovery corrected LOQ.

Relative recovery varied greatly for some antibiotics throughout the wastewater samples, see Table E.5. Trimethoprim, lincomycin, sulfonamides, doxycycline, fluoroquinolones, and surrogates had low to no recoveries in the reuse effluent (ID #8), likely due to the high chlorine residual which may have oxidized the spiked-in analytes. Excluding the reuse effluent, macrolides recoveries varied from 11 to 393%; trimethoprim recoveries varied from 5 to 161%; lincomycin recoveries varied from 4 to 218%, sulfonamides recoveries varied from 0 to 139%, tetracyclines recoveries varied from 10 to 248%, and fluoroquinolones varied from 8 to 150%. Average surrogate recoveries varied from 0 to 111% for $^{13}C_6$-sulfamethazine, 0 to 175% for demecycline, and 24 to 109% for nalidixic acid, Figure E.1. and Tables E.6–E.8.

Table 5.3. Limits of detection (LODs) and quantification (LOQs) in units of ng/L for analytes.

	LODs		**LOQs**	
antibiotic	**mean**	**stdev[a]**	**mean**	**stdev**
Erythromycin	0.18	0.02	0.58	0.05
Roxithromycin	0.21	0.10	0.70	0.32
Trimethoprim	0.29	0.13	0.97	0.45
Lincomycin	0.10	0.12	0.35	0.40
Sulfapyridine	0.11	0.04	0.36	0.15
Sulfadiazine	0.05	0.06	0.18	0.19
Sulfamethoxazole	0.17	0.18	0.58	0.59
Tetracycline	1.58	2.73	5.32	9.06
Doxycycline	1.39	2.15	4.67	7.11
Norfloxacin	0.87	0.77	2.88	2.60
Ciprofloxacin	0.55	0.38	1.83	1.26
Ofloxacin	0.29	0.17	0.94	0.56

[a] standard deviation

Recovery of sulfonamides from influent, primary clarifier effluent, aeration basin, and secondary clarifier effluent was frequently poor and not consistent among seasons. The Minnesota WWTP also treats industrial and commercial waste, and therefore unknown substances may have interfered with the sulfonamide extraction.

The wide distribution of analyte recoveries was partially due to the addition to internal standards before and after SPE. Analyte recoveries were generally higher in the summer wastewater samples. This was due to internal standards undergoing SPE, whereas internal standards were added to SPE eluent in fall, winter, and spring samples. Thus, clinafloxacin, simetone, $^{13}C_2$-erythromycin, and $^{13}C_6$-sulfamethoxazole were serving as internals standards and surrogates and their recoveries were a combination of matrix effects and extraction efficiency. The decision to add internal standards to the SPE eluent was motivated by wanting to separate matrix effects from extraction efficiencies. Thus, surrogates were used to monitor extraction efficiency and internal standards the matrix interferences during HPLC-MS/MS analysis for fall, winter, and spring samples.

Another limitation of the study was that some concentrations were measured above the calibration range. This may have resulted in underestimating antibiotic concentrations in wastewater. The concentration trends, however, should not have been lost, and reported concentrations are within the range of antibiotic concentrations measured in other studies (see below).

Antibiotic Occurrence. A total of eight antibiotics were detected in this study, including one macrolide (erythromycin and its degradation product, erythromycin-H_2O), two sulfonamides (sulfapyridine and sulfamethoxazole), trimethoprim, one tetracycline (doxycycline), and two fluoroquinolones (ofloxacin and ciprofloxacin). Except for

sulfapyrdine, all detected antibiotics were on the World Health Organization 19th list of essential medications.[255] This list selects pharmaceuticals that are needed for a basic health-care system based on their effectiveness, safety, and cost–effectiveness. Sulfapyridine is a metabolite of sulfasalazine, which is also on the list of essential medications, and is no longer prescribed by physicians. Thus, the high frequency of sulfapyridine is likely due to use of sulfasalazine. Two of the four antibiotics not detected (tetracycline and sulfadiazine) are on the list of essential medications, and the other two non-detect antibiotic (roxithromycin and norfloxacin) were not. Usage patterns within the Minnesota community may result in their absence.

Erythromycin, trimethoprim, and ofloxacin were detected at the highest frequency (100%) during conventional wastewater processes and chlorine disinfection, i.e. sampling locations 1 to 5. Ciprofloxacin and sulfapyridine were the next most frequently detected antibiotics at 90%, followed by doxycycline (70%), sulfamethoxazole (65%), and lincomycin (50%).

Seasonal trends were not observed for erythromycin, trimethoprim, or ofloxacin at the WWTP, Figure 5.2. Lincomycin appeared only in fall and winter samples. Ciprofloxacin was not detected in the chlorine disinfection effluent. The occurrence of doxycycline throughout the WWTP varied from season to season. Also, no apparent seasonal trends were observed for the detection frequency of sulfapyridine and sulfamethoxazole.

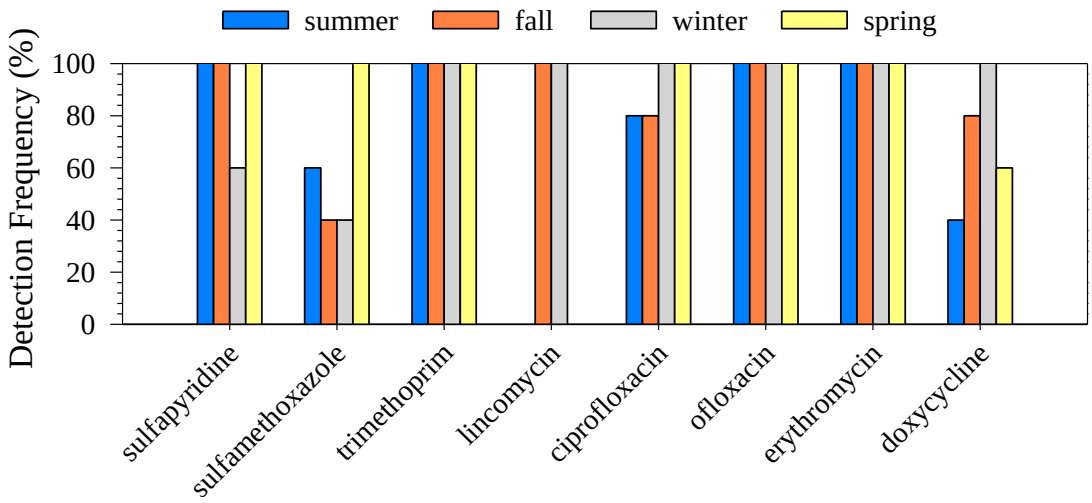

Figure 5.2. Detection frequency of antibiotics during conventional wastewater treatment, sampling locations 1 to 5 in Figure 5.1.

Figures E.2 to E.6 show the concentrations of antibiotics in the aqueous phase at eight locations in the studied WWTP. Table 5.4 provides a summary of antibiotic levels during conventional wastewater treatment. Tukey's tests were performed on influent and effluent concentrations of antibiotics to evaluate seasonal trends. Significant differences were defined as $\alpha < 0.05$. Influent concentrations in the fall were statistically greater for trimethoprim and doxycycline than in any other season. Influent levels of fluoroquinolones (ciprofloxacin and ofloxacin) were statistically higher in spring and inputs of sulfamethoxazole were statistically greater in winter. Levels of doxycycline, trimethoprim, ciprofloxacin, ofloxacin, and sulfamethoxazole were statistically greater in winter and/or spring effluents. No seasonal trends were exhibited for erythromycin, lincomycin, and sulfapyridine in influent or effluent samples.

As previously discussed, the poor recoveries of all the sulfonamides (even the sulfonamide surrogate) in the influent resulted in their non-detection, and thereby

Table 5.4. Total concentration of major classes and proportion removed due to treatment steps. Sample location IDs corresponding to Figure 5.1 are bolded.

	Influent (**1**)	Primary Clarifier Effluent (**2**)		Activated Sludge (**3**)		Secondary Clarifier Effluent (**4**)			Post Disinfection (**5**)		Overall
	sub-total (ng/L)	sub-total (ng/L)	**%PR**[a]	sub-total (ng/L)	%R[b]	sub-total (ng/L)	%R	**%SR**[c]	sub-total (ng/L)	%PR	**%TR**[d]
Fluoroquinolones											
Summer	679	389	**43%**	789	-103%	394	50%	**-1%**	85	78%	**87%**
Fall	744	831	**-12%**[e]	755	9%	615	18%	**26%**	80	87%	**89%**
Winter	1381	824	**40%**	325	61%	393	-21%	**52%**	626	-59%	**55%**
Spring	5769	3886	**33%**	1308	66%	558	57%	**86%**	2730	-390%	**53%**
Tetracyclines											
Summer	148	61	**59%**	ND	100%	ND	–	**100%**	ND	–	**100%**
Fall	693	91	**87%**	23	75%	9	60%	**90%**	ND	100%	**100%**
Winter	53	36	**31%**	12	68%	14	-17%	**63%**	4	67%	**91%**
Spring	117	82	**30%**	28	66%	ND	100%	**100%**	ND	–	**100%**
Sulfonamides											
Summer	201[f]	98[f]	**51%**	174	-78%	782	–	–	278	64%	--
Fall	119[f]	298[f]	**-151%**	105[f]	65%	497	–	–	271	45%	--
Winter	1484	ND[f]	–	ND[f]	–	2547[f]	–	–	742	71%	**50%**
Spring	965	1204[f]	**-25%**	534[f]	56%	969[f]	-81%	**19%**	563	42%	**42%**
Macrolides											
Summer	16	9	**45%**	17	-91%	16	4%	**-83%**	30	-84%	**-86%**
Fall	21	28	**-31%**	39	-39%	37	5%	**-32%**	21	42%	**0%**
Winter	69	92	**-34%**	169	-83%	279	-66%	**-203%**	236	16%	**-242%**
Spring	61	41	**32%**	49	-19%	76	-56%	**-86%**	57	25%	**5%**

Table 5.4. Continued.

Trimethoprim											
Summer	501	272	**46%**	249	8%	82	67%	**70%**	69	16%	**86%**
Fall	301	727	**-142%**	114	84%	73	35%	**90%**	31	58%	**90%**
Winter	406	763	**-88%**	224	71%	90	60%	**88%**	294	-226%	**28%**
Spring	1306	1487	**-14%**	589	60%	614	-4%	**59%**	1148	-87%	**12%**
Lincomycin											
Summer	ND	ND	**–**	ND	**–**	ND	**–**	**–**	ND	**–**	**–**
Fall	2	4	**-100%**	1	65%	2	-83%	**37%**	0.5	81%	**75%**
Winter	11	6	**45%**	8	-31%	11	-42%	**-86%**	8	31%	**29%**
Spring	ND	ND	**–**	ND	**–**	ND	**–**	**–**	ND	**–**	**–**
Overall											
Summer	1544	828	**46%**	1229	-48%	1274	-4%	**-54%**	462	64%	**70%**
Fall	1879	1978	**-5%**	1036	48%	1233	-19%	**38%**	404	67%	**79%**
Winter	3404	1722	**49%**	738	57%	3334	-352%	**-94%**	1911	43%	**44%**
Spring	8218	6700	**18%**	2508	63%	2218	12%	**67%**	4499	-103%	**45%**

Total concentrations are the sums of the mean values for each antibiotic within specified class
[a] proportion removed from primary treatment
[b] proportion removed from previous sample
[c] proportion removed from secondary treatment
[d] total removal from influent to effluent
[e] negative removal efficiency indicates concentrations increased
[f] matrix effects hindered detecting or/and quantifying antibiotic, i.e. either low (≤ 6%) or no recovery
ND = below limit of detection

decreased their detection frequency. Their presence in downstream treatment steps, indicates that they were likely present in the influent, either as the compounds themselves or as conjugates. Nevertheless, it is difficult to draw conclusions about their detection frequencies given their poor recoveries.

Tetracyclines. Influent concentrations of doxycycline ranged from 53 to 693 ng/L and similar levels were measured in WWTP influent from Australia (65 ng/L)[119] and Colorado (104 ng/L).[249] Primary and secondary treatment decreased doxycycline levels from the liquid phase by 30 to 87% and 63 to 100%, respectively. Sorption to solids is an important removal mechanism for tetracyclines in man-made and natural systems, which is attributed to their complexation with metals.[130,134,135] Therefore, removal by sorption is likely the dominant elimination mechanism for doxycycline during conventional wastewater treatment.[256] Doxycycline had the highest overall removal rates (>90%) of all the antibiotics included in this study resulting in low levels (non-detect to 4.5 ng/L) discharged into the Minnesota River. Effective removal of doxycycline by conventional treatment is frequently observed.[249,257,258] That said, levels ranging from 40 to 10,900 ng/L are still quantified in wastewater effluents across the globe.[118–120,249]

Fluoroquinolones. Levels of ciprofloxacin in the influent increased from summer (259 ng/L) to fall (443 ng/L), winter (827 ng/L), and spring (2,911 ng/L). Ciprofloxacin has been detected in influents worldwide from 3800 ng/L (median concentration) in Australia,[119] 639 to 1307 ng/L in Spain,[121] and 0.82 to 147 ng/L in China.[250] Input levels of ofloxacin at the Minnesota WWTP (201 to 2755 ng/L) were comparable to those observed by Dong et al.[250] (15.7 to 5411 ng/L) and Rodriguez-Mozaz et al.[121] (582 to 1,565 ng/L).

The primary clarifier removed approximately 40% of dissolved fluoroquinolones, except for in fall when levels increased by 12%. A range of removal efficiencies of fluoroquinolones during primary treatment has been previously observed.[119] Variability in removal efficiencies (-1 to 86%) of fluoroquinolones was also observed during secondary treatment, which was an unexpected observation. The highest removal rates of fluoroquinolones (46 to 88%) typically occurs during activated sludge via sorption to sludge.[119,120,259]

Ciprofloxacin and ofloxacin are frequently detected in WWTP effluents, ranging from 95 to 700 ng/L.[118–120,250,259,260] Effluent concentrations of fluoroquinolones at the Minnesota WWTP were lower during periods of chlorine disinfection (80 to 85 ng/L) than those without (626 to 2730 ng/L). Chlorine disinfection improved the overall removal rates from 53 to 55% in winter and spring to 87 to 89% in summer and fall. Although low overall elimination rates from conventional wastewater treatment (less than 50%) are not uncommon,[260–262] some WWTPs are able to achieve higher removal rates (79 to 100%).[121,257,263]

Sulfonamides. Influent concentrations of sulfapyridine varied little throughout the year (145 to 219 ng/L) and were comparable to Swiss WWTP influents (60 to 150 ng/L).[251] Sulfamethoxazole was only detected in winter (1266 ng/L) and spring (821 ng/L) influents, because of low extraction recoveries in summer and fall sample. Sulfamethoxazole is frequently detected in raw sewage (0.4 to 1090 ng/L)[118,119,121,249,251] and the presence of sulfamethoxazole in downstream treatment processes suggests that the antibiotic was likely present in the summer and fall influents, perhaps as a conjugate.

Primary clarification removal of sulfonamides varied seasonally (51 to -151%), biological treatment reduced sulfonamides levels decreased by 19%, and overall removal performance in the winter and spring was approximately 46%. Contradictory removal rates of sulfonamides are found in the literature with both effective[121,249,264] and poor[119,265,266] removal efficiencies reported. For example, Karthikeyan et al.[120] reported sulfamethoxazole degradation varied between -23% to 100%, whereas Carballa et al.[267] reported a 67% reduction. Variability in removal performance may be due to back-transformation of metabolites to original form during treatment steps.[251,268,269] Nevertheless, sulfonamides are frequently detected in surface waters[4], and thus incomplete removal at WWTPs is likely common.

Trimethoprim. The highest influent concentration of trimethoprim (1,306 ng/L) in this study was greater than those measured in New Mexico (180 to 1000 ng/L[266]), Australia (median 340 ng/L[119]), Switzerland (210 to 440 ng/L[251]), China (2.3 to 813 ng/L[250]), and Spain (88 to 180 ng/L[121]). Secondary treatment was more efficient and consistent (50 to 90%) at decreasing trimethoprim levels than primary treatment (-13 to 30%). Several other studies have also reported high removal rates (50 to 100%) of trimethoprim in aeration basins,[119,120] but this is not always observed.[251] Trimethoprim levels in the effluent varied substantially throughout the year from 31 to 1148 ng/L. The maximum effluent concentration of trimethoprim was also higher than those quantified in WWTP effluents from Wisconsin (300 ng/L),[120] Australia (70 ng/L),[119] China (108 ng/L),[250] and Spain (180 ng/L).[121] Chlorine disinfection was extremely effective at reducing trimethoprim levels in treated wastewater. Overall removal rates of trimethoprim ranged from 86 to 90% with chlorine disinfection and 12 to 28% without.

Macrolides. Influent levels of erythromycin were similar throughout the year (15.9 to 68.9 ng/L) and in agreement with previous studies.[250,251] Conventional wastewater treatment and chlorine disinfection was not effective at removing erythromycin from the waste stream. The portion of macrolides (32 to -34%) removed by primary clarification varied seasonally and higher levels of the erythromycin were consistently observed post-secondary treatment. De-conjugation of metabolites is likely not responsible for inconsistent biological removal rates.[270] Erythromycin is typically excreted in feces, thus release from partially enclosed fecal matter during biological treatment may be the source of erythromycin.[251,270] Levels of erythromycin discharged to the Minnesota River (21 to 236 ng/L) were similar or greater than influent levels. Poor removal of erythromycin during conventional wastewater treatment has resulted in its frequent detection in WWTP effluents. [118–120,250,251,260,271] Chlorine disinfection was likely not effective against erythromycin occurrence because this antibiotic does not have functions groups that react with free chlorine, i.e. reduced nitrogen or sulfur function groups or aromatic moieties.[272]

Lincomycin. Low levels of lincomycin were measured in fall (1.9 ng/L) and winter (11.2 ng/L) influents and neither primary or secondary treatment reliably decreased lincomycin levels. Other studies have noted that conventional treatment technologies are not very effective against lincomycin.[119,120] Chlorine disinfection increased overall removal efficiency of lincomycin by 46% from conventional treatment alone. Lincomycin levels (0.5 – 7.9 ng/L) in wastewater effluents were lower than previous studies.[118–120,250,260]

Water Reclamation Facility. Wastewater underwent additional treatments for phosphorus and turbidity removal and chlorine disinfection to meet reuse standards. The addition of chemical additives (for phosphorus removal) and filtration were generally not effective at reducing antibiotic levels. The chlorine residual (greater than 5 mg/L) maintained in the reuse effluent completely eliminated the occurrence of antibiotics, except for erythromycin, Figure E.2 – E.6. Even antibiotics spiked into the reuse effluent for spike and recovery analysis were degraded during the overnight equilibration. Chlorine disinfection at the Water Reclamation Facility was the most effective treatment technology for removing erythromycin, but levels between non-detect and 4 ng/L were still present in reuse water. Thus, dosing with elevated levels of free chlorine in treated wastewater is an effective removal mechanism for antibiotics. Whether such treatment is practical beyond reuse applications will require further study.

Chapter 6: Conclusions and Recommendations

This dissertation contributes to the body of knowledge investigating the chemical footprint of human activities on the environment. Out of the vast number of chemicals present in aquatic systems, it is important to identify which chemicals are of greatest concern based on their abundance, persistence, transformation products, and toxicity. Here, the historical trends and the spatial profiles of two classes of chemicals, hydroxylated polybrominated diphenyl ethers (OH-BDEs) and antibiotics, were used to investigate their environmental implications.

Sedimentary records of OH-BDEs and antibiotics were successful and reliable in capturing chemical pollution within a water body. Given that the presence of many chemicals in the environment was unknown until recently, sediment cores can be a useful tool for investigating historical trends. Future work should include quantifying historical records for other hydrophobic, readily sorbing pollutants that are persistent in aquatic systems.

Wastewater effluent does not appear to be a significant source of OH-BDEs in wastewater-impacted water bodies. Natural production appears to be the primary source of OH-BDEs and brominated dioxins, although natural production may be enhanced indirectly by anthropogenic activities. To further investigate the source of these substances, radiocarbon (^{14}C) analysis of OH-BDEs and brominated dioxins would give insight into whether or not these chemicals were synthetic or natural products in the studied systems.

Conventional wastewater treatment was generally not reliable or effective at removing antibiotics from raw sewage. The historical record of human-use antibiotics in

wastewater-impacted lakes also indicates there has been ongoing, incomplete removal at wastewater treatment plants. Chlorine disinfection maybe useful in reducing antibiotic pollution, although whether this is a feasible and practical treatment step requires further research. A greater understanding about the treatment parameters that influence micropollutant removal will be key in any future efforts to reduce antibiotic inputs to the environment. Future work should also include comparisons of antibiotic resistance gene (ARG) abundance to antibiotic residuals.

Usage patterns for select human-use antibiotics were duly recorded in sediment cores from wastewater-impacted lakes. The sedimentary records reflected the degree of wastewater impact, as well as indicated that synthetic antibiotics were highly persistent. Future assessments of accumulation rates and impacts of antibiotics used by the agricultural industry will require sampling in soils or waterways near agricultural activities (rather than in lakes that integrate signals from large watersheds). Future work can also investigate whether sediment cores capture any relationship between antibiotics and ARGs accumulation.

Finally, the spatial profiles of antibiotics in small versus large-scale systems were likely derived from different fate and transport processes. The drivers of ARG abundance were also lost as distance from anthropogenic sources increased. A mobile genetic component (*intI*1) was the best predictor of ARG abundance in the large-scale systems, rather than a chemical signature. Future work should investigate the pressures that select for ARGs at locations far from anthropogenic sources, as well as the mechanisms that facilitate the migration of antibiotics from anthropogenic sources.

The overall result of this dissertation demonstrated that presence of select OH-BDE congeners and antibiotics in the studied systems has been enhanced, either directly or indirectly, by human activities. By understanding the contributions from and impacts of human activities on chemical pollution, we are better equipped in future efforts to reduce and/or stop the human chemical footprint in the environment.

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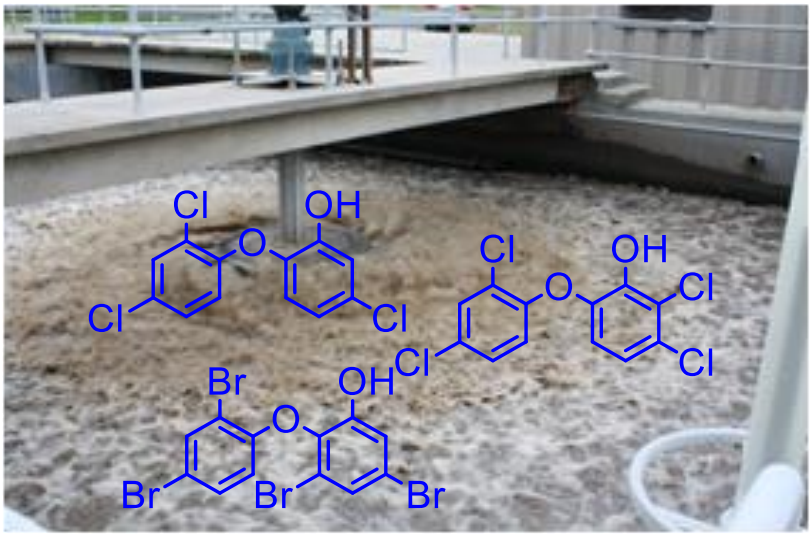
Appendix A: Triclosan, chlorinated triclosan derivatives, and hydroxylated polybrominated diphenyl ethers (OH-BDEs) in wastewater effluents

[‡]Hensley, R. H.; [‡]**Kerrigan, J. F.**; Pang, H.; Erickson, P. R.; Grandbois, M.; McNeill, K.; Arnold, W. A. Triclosan, Chlorinated Triclosan Derivatives, and Hydroxylated Polybrominated Diphenyl Ethers (OH-BDEs) in Wastewater Effluents. *Environ. Sci.: Water Res. Technol.* **2015**. 1, 316–325

([‡]*contributions of these two authors should be considered equal)*



A.1 Summary

Various halohydroxydiphenyl ethers, including triclosan, chlorinated triclosan derivatives (CTDs), and hydroxylated polybrominated diphenyl ethers (OH-BDEs), are present in aquatic systems. While it is well established that wastewater effluents are a source of triclosan and CTDs, the evidence for OH-BDEs being in wastewater is limited. In this work, pre- and post-disinfection effluent samples were taken from four activated sludge plants, two using chlorine and two using ultraviolet (UV) disinfection. Triclosan levels ranged from 36-465 ng L^{-1} and CTD levels were non-detect to 27 ng L^{-1}. While CTDs were generally higher in the plants using chlorine, they were also present in the UV plants, likely due to chlorine residual in the drinking water. Of the five target OH-BDE congeners (selected because they produce dioxins upon photolysis), three were detected. When detected the levels were generally 1-10 ng L^{-1}, but some samples had levels as high as 100 ng L^{-1}. Three different analytical methods were used to quantify OH-BDEs, and the levels were comparable using the different methods. Results were inconclusive as to the effect of disinfection method on OH-BDE levels. This study confirms that wastewater is a source of selected OH-BDEs to surface waters, but the overall loading is likely small. Further experiments and analyses are required to determine if the OH-BDEs are formed during the wastewater treatment process.

A.2 Introduction

Halohydroxydiphenyl ethers are a class of emerging contaminants that contains both the antimicrobial agent triclosan and hydroxylated polybrominated diphenyl ethers (OH-BDEs). Triclosan (2,4,4'-trichloro-2'-hydroxydiphenylether) contained in personal care products, such as antibacterial liquid handsoap and toothpaste, is flushed into sewer systems and enters wastewater treatment plants (WWTPs).[1] While much of the incoming triclosan is removed via biodegradation and sorption to biosolids during the treatment process,[2-10] triclosan concentrations have been detected in the effluent of WWTPs around the world ranging from 0.04 – 18.6 nM (0.011-5.4 μg L^{-1}).[6, 9, 11-15] From measurements of wastewater effluent, it has been estimated that approximately 11 metric tons of triclosan per year flows into the surface waters of the US.[6, 11, 12]

Three chlorinated triclosan derivatives (CTDs) are known to form from the chlorination of triclosan: 4,5-chloro-2-(2,4-dichlorophenoxy)phenol (4-Cl-TCS), 5,6-chloro-2-(2,4-dichlorophenoxy)phenol (6-Cl-TCS), and 4,5,6-chloro-2-(2,4-dichlorophenoxy)phenol (4,6-Cl-TCS).[6, 11, 16] Chlorination of wastewater can increase concentrations of total CTDs in WWTP effluent up to 30% of the concentration of triclosan.[11] WWTPs with UV disinfection do not see this effect, although CTDs may still be present at low concentrations from reactions with bleach or residual chlorine in tap water during transport to the WWTPs.[6, 11, 16] A recent report demonstrated that both CTDs and brominated triclosan derivatives are present in biosolids samples from WWTPs.[17] Additionally, CTDs have been detected in several sediment cores from wastewater impacted lakes, indicated that CTDs are present in wastewater impacted surface waters.[18]

Since the 1970's brominated flame retardants have been used in polyurethane foams, textiles, carpets, and electronics to prevent fires and the spread of fire.[19, 20] Due to the extent that PBDEs are found in environmental matrices, transformation products of PBDEs have also become a concern. OH-BDEs derived from the transformation of PBDEs are suggested to arise from metabolism of PBDEs by animals, oxidation of PBDEs by hydroxyl radicals in the atmosphere, and biological processing during wastewater treatment.[21-29] OH-BDEs are of environmental concern because they have been shown to be endocrine disruptors and neurotoxins with potency equivalent to or greater than PBDEs[30, 31]. Toxic effects that have been reported include uncoupling of oxidative phosphorylation,[32] indirect estrogenic effects in rats[33], and effects on hormone transport.[34] In addition, OH-BDEs are produced by marine organisms.[28, 35-38] Whether natural or anthropogenic sources are more important contributors to environmental levels of OH-BDEs continues to be an open question.[28, 29, 39]

Reports of OH-BDEs in wastewater systems are sparse. While looking for triclosan in wastewater from a WWTP on the Detroit River, one study reported other peaks near the internal standard, 2'-OH-BDE-28, with the same mass fragmentation pattern, but the compounds were not identified.[40] 6-OH-BDE-47 and 5-OH-BDE-47 were recently detected at ~1 pg L^{-1} in wastewater effluent.[1] Six OH-BDE congeners were found in sewage sludge samples, with 6-OH-BDE-47 and 2'-OH-BDE-68 comprising the majority of the OH-BDE mass,[42] and 6-OH-BDE-47 has been found in water impacted by sewage from a seafood processing facility.[43]

Photodegradation of triclosan and CTDs has been shown to produce specific dioxin congeners with a yield of 0.5 - 2.5% with a potential upper limit of 3%.[44-46]

Friedman et al.[47] measured an efflux of 2,7/8-DCDD from Newark Bay to the surrounding atmosphere, which was attributed to the photolysis of triclosan, and studies of lake sediment cores have shown that the levels of triclosan, CTDs, and their photoproduced dioxins correlate temporally.[18, 48] OH-BDEs also undergo photolysis to form PBDDs.[49-51] Because the toxicity of PBDDs is equal or greater to their chlorinated analogues,[52, 53] potential sources of PBDDs to the environment need to be understood.

The focus of this research was to measure triclosan, CTDs, and OH-BDEs in wastewater effluents and to compare the levels in systems using different modes of disinfection. The OH-BDEs chosen for study are among those capable of forming dioxins upon photolysis (i.e., with OH and Br substituents *ortho* to the ether linkage on opposing rings). Because data from different sampling campaigns were combined, this also gave the opportunity to compare different analytical methods.

A.3 Materials and Methods

Chemicals. Triclosan (>97%) was purchased from Sigma Aldrich. Isotopically labeled triclosan ($^{13}C_{12}$-triclosan, >99%) was purchased from Wellington Laboratories as a solution in methanol. Three CTDs (4-Cl-TCS, 6-Cl-TCS, and 4,6-Cl-TCS) and 6-hydroxy-2,2',4,4'-tetrabromodiphenyl ether (6-OH-BDE-47) were synthesized for previous studies.[44, 49] The 6-OH-BDE-47, 6-OH-BDE-99, 6'-OH-BDE-100 and 6'-OH-BDE-118 were from stocks synthesized as previously described.[51] 6-OH-BDE-90 was synthesized as described in the Supplementary Data.

Stock solutions of each compound were prepared gravimetrically in methanol. Sulfuric acid (ACS grade, BDH) silica gel (60 Å, BDH), ammonium acetate

(Mallinckrodt AR), methanol (HPLC grade, > 99%, Sigma-Aldrich), methyl t-butyl ether (MTBE, >99.0% Sigma-Aldrich), and ethyl acetate (>99.5%, Macron Chemicals) were purchased from commercial suppliers. Ultrapure water (18.2 MΩ-cm) was obtained from a Millipore Simplicity UV purification system. A Thermo-Orion Ross Ultra Semi-Micro pH meter was used to make pH measurements.

Collection and Preparation of Samples. Time-composited samples (24-hour) from three WWTPs, Metropolitan Wastewater Treatment Plant (MWP; 251 MGD; chlorine disinfection), Palo Alto Regional Water Quality Control Plant (PAWP; 21 MGD; UV disinfection) and Saint John's University Wastewater Treatment Plant (SJWP; 0.23 MGD; UV disinfection)) were collected. Additionally, grab samples from a fourth WWTP (Western Lake Superior Sanitation District, WLSSD; 40 MGD; chlorination when fecal coliforms exceed 100 MPN/100 mL) were collected. Further details about the WWTPs and their disinfection practices are in the Supplementary Information. At MWP, PAWP, and SJWP, pre- and post-disinfection effluent, offset to represent the same wastewater stream, were collected in solvent rinsed glass containers. At WLSSD, samples were collected using a small watercraft at the discharge point in the St. Louis River. Samples were transported on ice. Samples were filtered within a day of arrival through pre-combusted glass fiber filters (47 mm; Fisher Scientific). The pH of each sample was recorded and then adjusted to 3-4 with sulfuric acid. At pH < 4, all of the analytes will be > 98% in their hydrophobic, neutral forms allowing high recovery from solid-phase extraction.[44, 54] Samples were then stored in the dark at 4 °C until further processing, which was usually carried out within 72 hours.

Solid Phase Extraction. A previously developed method[11] was slightly modified for analysis of the compounds of interest. Three or four 500 mL replicates were prepared in Erlenmeyer flasks by spiking 0.5 nM $^{13}C_{12}$-triclosan as a surrogate for the compounds of interest. Another 500 mL sample of wastewater or deionized water was prepared in the same manner, but was also spiked with 1.5 nM triclosan and 0.3 nM of the other analytes (CTDs and OH-BDEs) to verify that the other compounds partition as triclosan does throughout the extraction method. All the flasks were then shaken and stored overnight in the dark to allow for equilibration of the analytes.

Solid phase extraction (SPE) cartridges (Oasis HLB) were loaded onto a vacuum manifold and preconditioned with consecutive 5 mL aliquots of MTBE, methanol, and pH 3 ultrapure water. Wastewater replicates were loaded onto the SPE cartridges at a flow rate of 15 mL/min. Samples spiked with all analytes were processed after the replicates to minimize cross contamination. After loading the samples, cartridges were flushed with 3 consecutive aliquots of 50:50 methanol:H_2O (v/v) under slight vacuum (~5 g/min) and dried under vacuum for at least 15 min. Cartridges were eluted with 10 mL of methanol and 5 mL of 90:10 MTBE:methanol (v/v). Eluents were then blown down with a gentle stream of nitrogen to ~500 µL.

Silica Column Clean-up. The eluent from the SPE step was loaded onto a silica column (comprised of glass wool, a thin layer of sand, 2 g silica gel, and a second thin layer of sand in a 6 mL plastic Luer tip syringe), as were three 1 mL aliquots of ethyl acetate used to rinse the centrifuge tube containing the SPE eluent.[11] After the rinses were loaded, the column was eluted with ~11 mL of ethyl acetate. The collected ethyl acetate was blown down with nitrogen to ~300 µL. This final extracts were transferred to amber

glass vials with 350 µL conical inserts. The extract was allowed to dry overnight in the vial and resuspended in 40-50 µL of 50:50 acetonitrile:H_2O (v/v). Spiked samples were diluted 5-10 times to lessen suppression effects of $^{13}C_{12}$-triclosan.

Mass Spectrometry Methods. Extracts were analyzed by HPLC and tandem mass spectrometry (LC-MS/MS). A previously published method for triclosan and CTDs using electrospray ionization (ESI), was initially used for analysis of processed samples[11], but of the OH-BDEs, only 6-OH-BDE-47 could be detected via this method. Thus, two additional methods, one using atmospheric pressure chemical ionization (APCI; based on ref. [55]) and one using ESI[56] were developed. A comparison of the chromatography and mass spectrometer settings is given in Table A.1. SRM transitions for triclosan, the CTDs, and OH-BDEs are in the Supplementary Information. Calibration curves using more than five points were constructed by plotting the analyte peak area to internal standard peak area ratio (y-axis) versus the analyte concentration (x-axis). Triclosan concentrations in standards ranged from 0.001 – 4.3 mg L^{-1}, while the concentrations of CTDs and 6-OH-BDE-47 ranged from 0.0003 – 1.5 mg L^{-1}. In most cases, two calibrations curves were plotted for each analyte, one for low ranges and one for high ranges. The concentrations of the spiked samples determined the endpoints of the high range calibration curve, while the concentration of the unspiked and blank samples determined the endpoints of the low range calibration curve. At higher concentrations, the $^{13}C_{12}$-triclosan signal became suppressed by triclosan (i.e., ion suppression), thus changing the slope of the calibration curve.

A.4 Results and Discussion

Chromatography and Limits of Detection and Quantification. Each LC method effectively separated the analytes and provided satisfactory peak shapes without processing through peak fitting. Example chromatograms for ESI Method 1 are in the Supplementary Information. The separation for the OH-BDEs via the APCI Method and ESI Method 2 are shown in Figure A.1. Note that in the APCI method, the 6'-OH-BDE-100 and 6'-OH-BDE-118 were detected with the 500.6 → 79 transition rather than the expected 578.6 → 79 transition.

For ESI Method 1 analyses with only one method blank (early stage of the sampling campaign), the limit of quantitation (LOQ) for each analyte was defined as 10 times the analyte concentration determined in a single method blank. Later multiple method blanks were used, and the LOQ was the concentration determined in the method blanks plus 10 times the standard deviation of the method blanks. The limit of detection (LOD) was calculated as 3 times the method blank or the average method blank plus 3 times the standard deviation of the method blanks. Using multiple method blanks allowed for lower LODs and LOQs as the standard deviation of the analyte concentrations in the method blanks were much lower than the analyte concentrations in the method blanks. The reported limits with only one method blank (Table A.S2) are, therefore, conservative.

For the APCI Method and ESI Method 2, the LOQ and LOD were obtained by different means, due to insufficient sample volume for the additional analyses. The analytes were quantified if: (1) the analyte was above 80% of the lowest calibration point (the LOQ), and (2) the analyte was above a 10 signal-to-noise ratio within the water

matrix. The LODs are 0.3 × LOQs, and the analyte must have been above a 3 signal-to noise ratio within the water matrix.

Most calibration curves were of high quality, with $R^2 > 0.93$. A detailed summary of LOQ and LOD information for each method is located in the Supplementary Information. Because these were based on the method blank (i.e., MilliQ water put through the extraction process), these values varied depending on the date the analyses were run. Briefly, for ESI Method 1 the LOQs ranged from 2.3 – 29 ng L^{-1} for triclosan, 0.003 – 2.8 ng L^{-1} for the CTDs, and 0.22 – 3 ng L^{-1} for 6-OH-BDE-47. The OH-BDE LOQs for the APCI method ranged from 0.10 – 3.23 ng L^{-1} for 6-OH-BDE-47, 6-OH-BDE-90, and 6-OH-BDE-99. In this method, no limits are reported for 6'-OH-BDE-100 and 6'-OH-BDE-118 because they were not detected in the method blank (nor in any samples), but the lowest calibration point was 2.9 ng L^{-1}. For ESI Method 2, the LOQs ranged from 0.46 – 1.09 ng L^{-1} for 6-OH-BDE-47, 6-OH-BDE-90, 6-OH-BDE-99, and 6'-OH-BDE-118. No limits were reported for 6'-OH-BDE-100 for this method because it was not detected in any of the water samples or method blanks, but the lowest calibration point was 1.74 ng L^{-1}. The chromatographic peak area for every reported concentration was greater than 10 times the peak area of the corresponding instrument and method blanks.

Recoveries. The absolute recovery of $^{13}C_{12}$-triclosan was 59 ± 31 % in ESI Method I, 36 ± 28 % ESI Method II, and 67 ± 34 % in the APCI Method (average ± standard deviation), see Table A.S3 – A.S5 for more detailed results as a function of data/location. Accurate results are confirmed by the relative recovery of each analyte, rather than the absolute recovery, based on isotope dilution methodology. Spiked samples

were used to determine the relative recovery as compared with triclosan. The average relative recoveries of all spiked samples for each analyte are shown in Table A.2. Equations used to calculate the absolute and relative recoveries is located in the Supporting Information. All reported concentrations are recovery corrected values using the relative recoveries for wastewater samples processed at the same time (see Tables A.S3 – A.S5).

Triclosan and CTD concentrations in wastewater. Triclosan and the CTDs were detected in all wastewater samples analyzed, except for 4-Cl-TCS in the SJWP samples in April 2012, as compiled in Table A.3. Triclosan concentrations varied from 36 – 465 ng L^{-1}. Concentrations of total CTDs ranged from below the LOD to 27.2 ng L^{-1}.

The results for MWP are comparable to those previously reported by Buth et al.[11]. The chlorination of the effluent in the September samples leads to production of CTDs. In the October samples, the triclosan and CTD levels are similar (and CTDs are elevated in the prechlorination sample), which is inconsistent with the September sample and previous findings at MWP.[11] This suggests either that is a balance between CTD removal and formation at this time period or that the sample timing offset was not correct. In the November 2011 sample, seasonal chlorination had ceased, and only small levels of CTDs were detected, which are ascribed to influent CTDs that had persisted through the treatment process. The WLSSD plant only chlorinates occasionally, but the presence of CTDs in the collected samples, along with the ratio of CTDs to triclosan in the final effluent, which were similar to those at the MWP, indicated that the chlorination was active at WLSSD during the sample collection.

In the two plants using UV disinfection, PAWP and SJWP, the triclosan and CTD levels are essentially constant through the disinfection step, indicating that the UV dose was not enough to cause significant triclosan transformation. The higher levels of CTDs in the PAWP samples is explained by the fact that this plant serves a community that has residual chlorine in their drinking water, while SJWP serves a community that does not. This is consistent with the findings of formation of CTDs upon chlorination in tap water by Rule et al.[16] and previous detections of CTDs in influents to wastewater treatment plants attributed to reaction with residual chlorine.[6, 11] The concentrations of CTDs in PAWP final effluent are still less than chlorinated MWP effluent and similar to WLSSD and non-chlorinated MWP effluents. In our prior study [11], CTDs were not detected in the SJWP effluents, but in the LOQs in the current study were, in general, 2-10 times lower than those in our prior study.

OH-BDEs in Wastewater Effluent. While triclosan is expected to be in wastewater given its use in soap and toothpaste, it is less obvious whether OH-BDEs should also be found in wastewater samples. There are some precedents that indicate OH-BDEs might be expected. The 6-OH-BDE-47 has been previously detected in wastewater,[1] several OH-BDEs have been detected in biosolids,[42] and elevated levels of OH-BDEs were detected near a WWTP.[26]

In the present work, an APCI method and two ESI Methods were used to determine the concentration of 6-OH-BDE-47 in the samples. A comparison of the results are found in Table A.4**,** which highlights the samples from this study that had detectable amounts of 6-OH-BDE-47 in at least one replicate extract. A grab sample from MWP had by far the highest amounts of 6-OH-BDE-47 (analyzed by ESI Method 1), while lower

levels were detected in the composite samples. The ~17 ng L^{-1} concentration for 6-OH-BDE-47 detected in the April 2012 grab sample of MWP wastewater is the highest wastewater concentration reported to date. From comparison of pre and post disinfection extracts, 6-OH-BDE-47 may be susceptible to UV light (>71% removal; PAWP July 2011). The differing analyte concentrations were near or below the LOQ, and these slight differences are likely due to the high uncertainty at these low concentrations. It is unclear why there is a higher frequency and abundance of 6-OH-BDE-47 in MWP effluents compared to PAWP or SJWP, but it could be due to the larger population served.

An APCI and additional ESI method were developed to analyze pentabrominated OH-BDEs, which were not measureable with ESI Method 1. The highest concentration of 6-OH-BDE-90 (109 ng L^{-1}) was detected using the APCI method at SJWP. The differences in the concentrations, shown in Table A.5, for the two analytical methods are likely caused by (1) the 10-fold dilution of samples prior to the ESI Method 2 analysis (but not APCI); and (2) the variation of sensitivity between analyses. The differences may have also been reduced if a $^{13}C_{12}$-OH-BDE was used as the surrogate/internal standard rather than $^{13}C_{12}$-triclosan. The samples underwent a 10-fold dilution to increase the sample volume in order to undergo ESI Method 2 analysis. Thus, analytes with low levels prior to the dilution went undetected by the ESI Method 2. A comparison of samples with sufficient analyte levels by the two analytical methods showed similar results. At MWP, 6-OH-BDE-90 levels were statistically unaffected ($p > 0.05$) by chlorination in September 2011. Levels of 6-OH-BDE-90, however, increased ($p \approx 0 < 0.05$) during UV disinfection at PAWP on July 2011. On the other hand, UV disinfection

at SJWP removed 99.6% ($p = 0.005$) of the 6-OH-BDE-90 from the effluent stream in January 2012.

The highest concentration of 6-OH-BDE-99 detected was approximately 24 ng L^{-1} at PAWP using both the APCI Method and ESI Method 2. Overall, both analytical methods determined similar concentrations (seen in Table A.6), but again slight differences were observed due to varying sensitivities between analyses. Similar trends were observed between 6-OH-BDE-90 and 6-OH-BDE-99. The July 2011 UV disinfection at PAWP resulted in an increase of 6-OH-BDE-99 using the APCI method and the ESI Method 2. A different trend was seen in Jan 2012 at PAWP. 6-OH-BDE-99 increased after UV disinfection according to the APCI Method, but ESI Method 2 showed a slight decrease. Similar to 6-OH-BDE-90, UV disinfection at SJWP removed the 6-OH-BDE-99 (88.9% removal ratio) in Jan. 2012. But one month later, disinfection appeared to slightly increase the levels of 6-OH-BDE-99. Therefore, these results are inconclusive in determining whether UV disinfection is effective at removing OH-BDEs, while showing clearly that chlorination is ineffective at removing 6-OH-BDE-90 and 6-OH-BDE-99.

6-OH-BDE-100 was not detected in any of the effluents. 6'-OH-BDE-118, however, was detected in only one sample using the ESI Method 2 at MWP on September 2011 in the post chlorination effluent at 1.77 ng L^{-1} (other replicate was in between LOD and LOQ). An unknown peak at an earlier retention time with the same quantification and confirmation ions as the pentabrominated (Br_5-) OH-BDEs was also detected in the same sample as 6'-OH-BDE-118. The unknown compound may be

another Br_5-OH-BDE or could be a dihydroxylated polybrominated biphenyl,[51] which also has the same formula as Br_5-OH-BDEs.

This study confirms that wastewater can be a source of selected OH-BDEs to surface waters. Further experiments and analyses are required to determine if the OH-BDEs are formed during the wastewater treatment process. Whether wastewater is the most important source of OH-BDEs, and consequently PBDDs via photolysis, in freshwater environments remains to be seen. Natural production of OH-BDEs in freshwater is unlikely, owing to lack of bromide ions available needed for construction of these compounds. Photolysis of brominated phenols contributes to formation OH-BDEs in the fresh waters. Sustained levels of 2'-OH-BDE-68 were formed from photolysis of 2,4-dibromophenol.[57] Like PBDEs, brominated phenols are present in dust[58] and may also be present in wastewater. If brominated phenols are present in wastewater, they could be contributing to levels of OH-BDEs and PBDDs in fresh water environments.

A.5 Conclusions

The method of wastewater disinfection affects levels of CTDs in the final effluent. Chlorination can significantly increase all three CTDs. Even in the case where CTDs did not increase after chlorination, CTDs are still detected in higher amounts than other non-chlorinating plants. UV disinfection has little, if any, effect on triclosan and CTDs in wastewater.

Overall, the concentrations of OH-BDEs are of similar levels as CTDs. Although the loadings to surface water are small, the confirmation of 6-OH-BDE-47, 6-OH-BDE-90, and 6-OH-BDE-99 in WWTP effluent is of concern for the same reasons as triclosan

and CTDs. PBDDs may form from OH-BDEs via photolysis. The presence of 6-OH-BDE-47, 6-OH-BDE-90, and 6-OH-BDE-99, which are not directly manufactured, in wastewater provides evidence that these compounds are formed via metabolism of PBDEs, which are also present in wastewater. Whether the OH-BDEs are present in the influent (as the result of human metabolism) or bacterial metabolism from the activated sludge is unknown.

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Table A.1. Comparison of the three analytical methods used. The APCI method and ESI Method 2 were used to quantify pentabrominated OH-BDEs

	ESI Method 1	*APCI Method*	*ESI Method 2*
		Chromatography	
HPLC	Agilent 1100 Series	Agilent 1100 Series	Waters nanoAcquity
Column Type	Phenomenex Synergi RP-Max	Phenomenex Synergi Polar-RP	Thermo Hypersil Gold
Size (mm × mm)	150 × 0.5	150 × 2	100 × 0.32
Particle Sizes (µm)	4	4	1.9
Pore Size (Å)	80	80, 100	
Injection Volume (µL)	8	20	8
Mobile Phase A	10mM NH_4OAc Buffer	2mM NH_4OAc Buffer (10% MeOH)	5mM NH_4OAc (40% MeOH)
Mobile Phase B	CH_3CN	MeOH	CH_3CN
Flow Rate (µL/min)	10	200	10
Gradient	50 % A for 10 min 100% B by 20 min 50% A by 23 min 50% A until 35 min	55% B for 3 min 86% B by 15 min 86% B from 15-27 min 55% B for 29-36 min	25% B Initial; 40% B by 5 min 55% B by 25 min 80% B from 27 – 30 min 25% B from 32 – 45 min
Divergence to Waste	First and last 10 min	First 10 min	First 5 min
		Mass Spectrometer	
Triple quadrupole MS	Thermo Scientific TSQ Vantage	Thermo Electron Quantum Discovery Max	Thermo Scientific TSQ Ultra AM
Source	Negative Mode ESI	Negative Mode APCI	Negative Mode ESI
		6-OH-BDE-47 Precursor and Product Ions	
First SRM (Quantification)	500.7 → 79	500.6 → 79[a]	500.6 → 79
Second SRM (Confirmation)	498.7 → 79	502.6 → 81[a]	502.6 → 81
		OH-pentaBDE Precursor and Product Ions	
First SRM (Quantification)	--	578.6 → 79[b]	578.6 → 79
Second SRM (Confirmation)	--	580.6 → 81[b]	580.6 → 81
		$^{13}C_{12}$-Triclosan Precursor and Product Ions	
SRM	299 → 35.1	299 → 35	299 → 35.2
		Tuning Parameters	
Tuning Compound	$^{13}C_{12}$-Triclosan	2'-OH-BDE-118	$^{13}C_{12}$-Triclosan
Spray Voltage (V)	2800	--	3200
Sheath Gas Pressure (psi)	45	20	35

Table A.1. Continued.

Capillary Temperature (°C)	250	250	300
Collision Energy	10	10	12
Skimmer Offset (V)	8	10	5
Collision Gas Pressure (mTorr)	0.8	2	0.9
Q1	0.1	0.05	0.7
Q3	0.1	0.05	0.7
Discharge Current (kV)	--	25	--
Vaporizer Temperature (°C)	--	250	--
Scan Time (s)	0.13	--	0.15

[a]Includes 6'-OH-BDE -100 and 6'-OH-BDE-118
[b]Does not include 6'-OH-BDE-100 and 6'-OH-BDE-118

Table A.2. The absolute recovery of $^{13}C_{12}$-triclosan and relative recoveries of analytes of interest to $^{13}C_{12}$- triclosan for three LC-MS/MS methods

	Absolute Recovery (%)		
Compound	ESI Method I	APCI Method	ESI Method II
$^{13}C_{12}$-Triclosan	59 ± 31	67 ± 34	36 ± 28
	Relative Recovery (%)		
Triclosan	93 ± 18	-	-
4-Cl-TCS	84 ± 18	-	-
6-Cl-TCS	75 ± 31	-	-
4,6-Cl-TCS	59 ± 15	-	-
6-OH-BDE-47	54 ± 15	66 ± 12	52 ± 0
6-OH-BDE-90	-	54 ± 14	25 ± 2
6-OH-BDE-99	-	48 ± 13	24 ± 2
6'-OH-BDE-100	-	73 ± 32	96 ± 7
6'-OH-BDE-118	-	48 ± 10	17 ± 7

Table A.3. Triclosan and CTD concentrations in wastewater effluent samples before and after disinfection.

Wastewater Sample	**n**	**Concentration ± SD ng L^{-1}**			
Metropolitan plant (MWP)		***Triclosan***	***4-Cl-TCS***	***6-Cl-TCS***	***4,6-Cl-TCS***
Pre-chlorination effluent, September 2011	**3**	**239 ± 42**	**0.5 ± 0.1**	**1.9 ± 0.1**	**1.9 ± 0.2**
Post-chlorination effluent, September 2011	**4**	**425 ± 51**	**4 ± 0.7**	**9.8 ± 1.2**	**13.4 ± 1.6**
Pre-chlorination effluent, October 2011	**4**	**112 ± 4**	**0.73 ± 0.02**	**8.5 ± 0.7**	**11.3 ± 0.4**
Post-chlorination effluent, October 2011	**4**	**144 ± 12**	**1.9 ± 0.2**	**7.9 ± 0.1**	**11.7 ± 1.1**
Final effluent with no chlorination, November 2011	**4**	**465 ± 90**	**< 0.9[c]**	**< 2.4[c]**	**< 2.8[c]**
Western Lake Superior Sanitary District (WLSSD)					
Grab Sample, June 2011	4	**94 ± 20**	**0.3 ± 0.1**	**3.0 ± 0.6**	**4.7 ± 0.6**
Grab Sample, April 2012	4	**108 ± 4**	**0.16 ± 0.03**	**1.9 ± 0.1**	**3.9 ± 0.3**
Palo Alto Regional Water Quality Control Plant (PAWP)					
Pre UV effluent, July 2011	4	**390 ± 32**	**1.2 ± 0.2[a]**	**4.2 ± 0.3**	**7.8 ± 0.9**
Post UV effluent, July 2011	4	**313 ± 72**	**1.2 ± 0.3[b]**	**3.1 ± 0.7[b]**	**5.8 ± 0.8**
Pre UV effluent, January 2012	4	**51 ± 11**	**0.3 ± 0.1**	**2 ± 0.4**	**4.1 ± 0.9**
Post UV effluent, January 2012	4	**58 ± 4**	**0.42 ± 0.04**	**2.4 ± 0.1**	**4.3 ± 0.4**
St. John's University (SJWP)					
Pre UV effluent, January 2012	4	**48 ± 3**	**0.27 ± 0.04**	**0.6 ± 0.1**	**1.4 ± 0.2**
Post UV effluent, January 2012	4	**48 ± 7**	**0.18 ± 0.04**	**0.5 ± 0.1**	**1.2 ± 0.3**
Pre UV effluent, February 2012	3	**57 ± 2**	**< 0.2[c]**	**< 0.2[c]**	**0.7 ± 0.1**
Post UV effluent, February 2012	3	**36 ± 1**	**ND**	**< 0.2[c]**	**1.1 ± 0.2**

* LODs and LOQs of analytes for each sample analyzed are summarized in the Appendix; ND - not detected (< LOD)

If a replicate is > LOD but < LOQ, the LOQ is shown.

[a] One replicate between LOD and LOQ, while other replicates above LOQ

[b] Two replicates between LOD and LOQ, while other replicate(s) above LOQ

[c] All replicates between LOD and LOQ

Table A.4. 6-OH-BDE-47 concentrations in wastewater effluent samples before and after disinfection.

Wastewater Sample	**6-OH-BDE-47 Concentration ± SD ng L^{-1}**					
Metropolitan plant (MWP)	**n**	*ESI Method 1*	**n**	*APCI Method*	**n**	*ESI Method 2*
Pre-chlorination effluent, September 2011	**3**	< 1.8[a]	**2**	0.72[a]	**1**	< 0.46[c]
Post-chlorination effluent, September 2011	**4**	3.4 ± 2.2[b]	**2**	< 3.04	**2**	0.50[a]
Final effluent with no chlorination, November 2011	**4**	< 0.4[c]	**3**	ND	**2**	ND
Effluent grab sample, April 2012	**3**	16.9 ± 3.1		N/A		N/A
Western Lake Superior Sanitary District (WLSSD)						
Grab Sample, June 2011	**3**	1.8 ± 0.2		N/A		N/A
Palo Alto Regional Water Quality Control Plant (PAWP)						
Pre UV effluent, July 2011	**4**	1.4 ± 0.3	**3**	0.83[e]	**3**	< 0.46[c]
Post UV effluent, July 2011	**4**	< 0.4[d]	**3**	ND	**3**	ND
Pre UV effluent, January 2012	**4**	ND	**3**	ND	**2**	ND
Post UV effluent, January 2012	**4**	ND	**3**	ND	**1**	ND
St. John's University (SJWP)						
Pre UV effluent, January 2012	**4**	ND	**3**	< 0.48[d]		N/A
Post UV effluent, January 2012	**4**	ND	**3**	ND		N/A
Pre UV effluent, February 2012	**3**	ND	**3**	ND		N/A
Post UV effluent, February 2012	**3**	ND	**3**	< 0.96[d]		N/A

* LODs and LOQs of analytes for each sample analyzed are summarized in the Appendix; ND - not detected (< LOD)
If a replicate is > LOD but < LOQ, the LOQ is shown.
N/A was used when not enough sample was left to be analyzed

[a] One replicate between LOD and LOQ, while other replicates above LOQ

[b] Two replicates between LOD and LOQ, while other replicate(s) above LOQ

[c] All replicates between LOD and LOQ

[d] One replicate between LOD and LOQ, while other replicates below LOD

Table A.5. 6-OH-BDE-90 concentrations in wastewater effluent samples before and after disinfection.

Wastewater Sample	**6-OH-BDE-90 Concentration ± SD ng L^{-1}**			
Metropolitan plant (MWP)	**n**	*APCI Method*	**n**	*ESI Method 2*
Pre-chlorination effluent, September 2011	**2**	2.3 ± 1.2	**1**	ND
Post-chlorination effluent, September 2011	**2**	4.8 ± 1.2	**2**	ND
Final effluent with no chlorination, November 2011	**3**	1.6 ± 0.2	**2**	< 0.7[d]
Palo Alto Regional Water Quality Control Plant (PAWP)				
Pre UV effluent, July 2011	**3**	3.7 ± 0.2	**3**	0.79 ± 0.04[a]
Post UV effluent, July 2011	**3**	39.4 ± 1.4	**3**	24.1 ± 6.3
Pre UV effluent, January 2012	**3**	ND	**2**	< 0.7[c]
Post UV effluent, January 2012	**3**	ND	**1**	< 0.7[c]
St. John's University (SJWP)				
Pre UV effluent, January 2012	**3**	109.4 ± 33.1		N/A
Post UV effluent, January 2012	**3**	0.5 ± 0.2[b]		N/A

* LODs and LOQs of analytes for each sample analyzed are summarized in the Appendix; ND - not detected (< LOD)

If a replicate is > LOD but < LOQ, the LOQ is shown.

N/A was used when not enough sample was left to be analyzed

[a] One replicate between LOD and LOQ, while other replicates above LOQ

[b] Two replicates between LOD and LOQ, while other replicate(s) above LOQ

[c] All replicates between LOD and LOQ

[d] One replicate between LOD and LOQ, while other replicates below LOD

Table A.6. 6-OH-BDE-99 concentrations in wastewater effluent samples before and after disinfection.

Wastewater Sample	**6-OH-BDE-99 Concentration ± SD ng L^{-1}**			
Metropolitan plant (MWP)	**n**	*APCI Method*	**n**	*ESI Method 2*
Pre-chlorination effluent, September 2011	**2**	< 0.8[c]	**1**	< 0.6[c]
Post-chlorination effluent, September 2011	**2**	< 3.2[c]	**2**	1.8 ± 1.6
Final effluent with no chlorination, November 2011	**3**	1.4 ± 0.1	**2**	< 0.6[d]
Palo Alto Regional Water Quality Control Plant (PAWP)				
Pre UV effluent, July 2011	**3**	16.1 ± 0.2	**3**	11.9 ± 2.5
Post UV effluent, July 2011	**3**	24.9 ± 0.8	**3**	23.7 ± 9.0
Pre UV effluent, January 2012	**3**	ND	**2**	1.1[a]
Post UV effluent, January 2012	**3**	4.2 ± 4.2	**1**	< 0.6[c]
St. John's University (SJWP)				
Pre UV effluent, January 2012	**3**	4.5 ± 2.1		N/A
Post UV effluent, January 2012	**3**	< 0.5[d]		N/A
Pre UV effluent, February 2012	**3**	ND		N/A
Post UV effluent, February 2012	**3**	1.5 ± 0.9		N/A

* LODs and LOQs of analytes for each sample analyzed are summarized in the Appendix; ND - not detected (< LOD)

If a replicate is > LOD but < LOQ, the LOQ is shown.

N/A was used when not enough sample volume was left to be analyzed

[a] One replicate between LOD and LOQ, while other replicates above LOQ

[b] Two replicates between LOD and LOQ, while other replicate(s) above LOQ

[c] All replicates between LOD and LOQ

[d] One replicate between LOD and LOQ, while other replicates below LOD

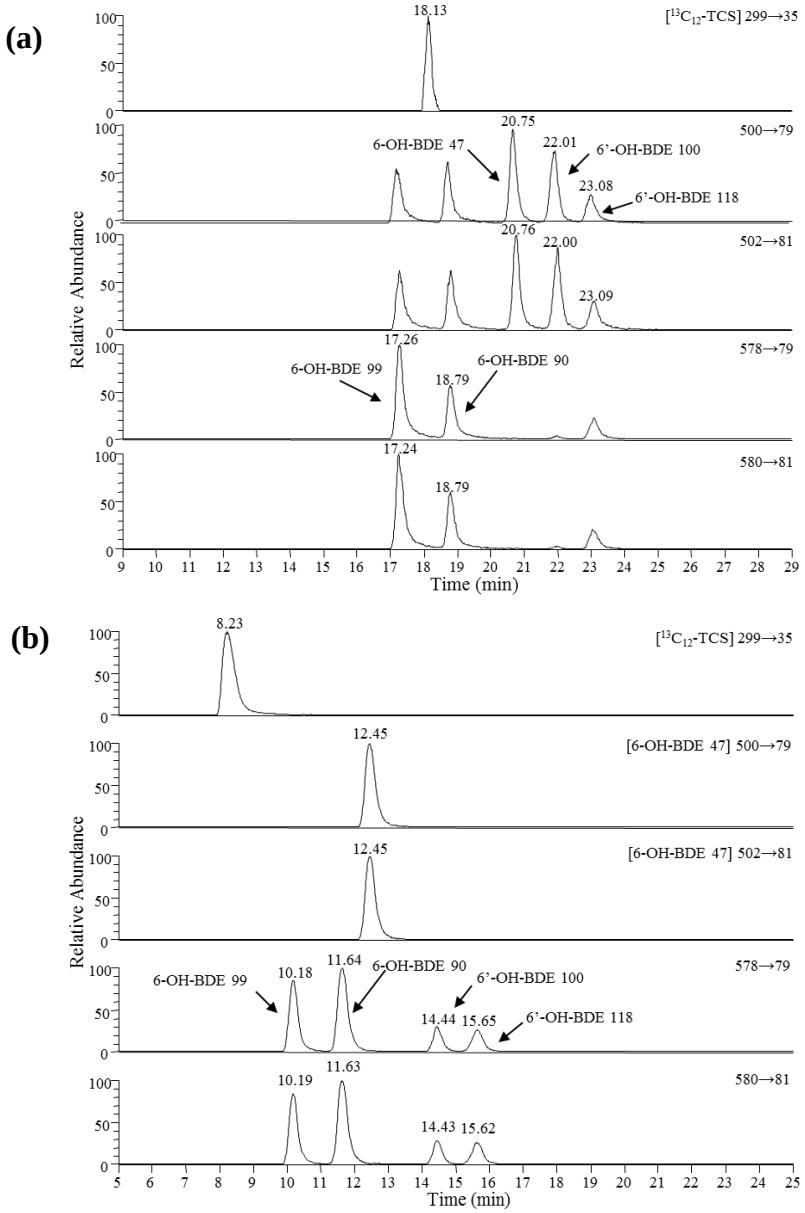

Figure A.1. Representative chromatogram of the OH-BDEs, using the (a) APCI-LC-MS/MS method and (b) ESI-LC-MS/MS method 2. Details of these methods are provided in Table A.1.

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Electronic Supplementary Information for
Triclosan, Chlorinated Triclosan Derivatives, and Hydroxylated Polybrominated Diphenyl Ethers (OH-BDEs) in Wastewater Effluents

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Environmental Science: Water Research & Technology

Synthesis of 6-OH-BDE-90

The 6-OH-BDE-90 congener was synthesized and purified using methods developed by Hofsløkken and Skattebøl (1999) for step 2 and Marsh et al (2003) for steps 1 and 3-5. The relevant reactions are shown in Figure A.S1.

(1): The synthesis of 2,2',4,4'-tetrabromodiphenyliodonium chloride (**A**) from 1,3-dibromobenzene was previously described in the supporting information of Steen et al. (2009).

(2): Bromophenol (2.3 mL), MgCl (2.86 g) and triethylamine (10.5 mL) were added to a flask containing 100 mL of anhydrous ACN under nitrogen. Next, paraformaldehyde (3.7 g) was added and the mixture was refluxed for 3.5 hrs. The reaction mixture was cooled, and 5% HCl was added until an acidic pH was reached. The mixture was washed three times with diethyl ether, and the organic phases were combined and dried with Na_2SO_4. The solvent was removed *in vacuo*, where eventually two layers formed again. The bottom, dark layer was separated and pumped to dryness, yielding a dark solid. This crude product was cleaned by silica gel flash column chromatography (90% hexane 10% dioxane) and finally by sublimation yielding yellow crystals of 3-bromosalicylaldehyde (**B**; 3 g, 70%). ^{1}H NMR (400 MHZ) δ = 11.612 (s, 1H, CHO), 9.864 (s, 1H, OH), 7.786 (d, 7.8 Hz, 1H, CH) 7.552 (d, 7.8 Hz, 1H, CH) 6.952 (t, 7.8 Hz 1H, CH)

(3): Sodium hydroxide (12 mmol) and **B** (10 mmol) were dissolved in dioxane (16 mL) and water (30 mL). To this yellow mixture, **A** (12 mmol) was added. The mixture was heated to 80 ^{o}C and allowed to stir (90 min). The solution was then cooled to room

temperature and diluted with water (60 mL) and methylene chloride (60 mL). The layers were allowed to separate and the aqueous layer was extracted with methylene chloride (100 mL, 3×). The organic fractions were combined, washed with aqueous sodium hydroxide (1 M, 100 mL, 2×), water (100 mL, 2×) and dried over sodium sulfate. The solvents were removed under vacuum. Flash chromatography (3:2 hexanes: CH_2Cl_2; R_f= 0.375) gave the desired product 3-bromo-2-(2,4-dibromophenoxy) benzaldehyde (**C**) in Figure A.S1 (1.7952 g, 65%) as a white solid. ^{1}H NMR (500 NMR, $CDCl_3$) δ = 10.136 (s, 1H, CHO), 7.97 (dd, 1H, CH)

(4): **C** (2.6830 mmol) and monopotassium phosphate (53.2 mmol, 7.24 g) were suspended in methylene chloride (25 mL). In another flask, hydrogen peroxide (30%) and methylene chloride (2 mL) were cooled to 0 ^{o}C. Trifluoroacetic acid anhydride was added dropwise (20.04 mmol). The mixture stirred for 1 h at 0 ^{o}C. The aldehyde mixture was cooled and added dropwise to the peroxide solution. The mixture was stirred for 2.25 hours, after which brine (32 mL) and aqueous Na_2SO_3 (20 %) were added to quench the reaction. The solution stirred overnight. The layers were separated and the aqueous layer was extracted with methylene chloride (30 mL, 3×) The organic layers were removed under vacuum. The residue was re-dissolved in methanol (32 mL) with two drops of concentrated hydrochloric acid. Solvents were removed *in vacuo*. Flash chromatography (5:2 CH_2Cl_2:hexanes; R_f= 0.5) gave the desired product 3-bromo-2-(2,4-dibromophenoxy)phenol (**D)** in Figure A.S1, (0.611 g, 58%) as a clear and colorless oil.

(5): **D** (0.8379 mmol) and calcium carbonate (6.62 mmol, 0.662 g) were suspended in methylene chloride (100 mL) and methanol (20 mL). Benzyltrimethylammonium tribromide (14.66 mmol, 3.991 g) was added in small increments over a time span of two

hours. The solution then stirred for an additional two hours. The reaction mixture was filtered and the supernant was added to aqueous Na_2SO_3 (5%, 80 mL). The layers separated and the aqueous layer was extracted with methylene chloride (50 mL, 3×) and dried over sodium sulfate. Solvents were removed with under vacuum. Flash chromatography 6-OH-BDE-90 (**E**) in Figure A.S1 (4:1 CH_2Cl_2: hexanes, R_f= 0.5) gave desired product (0.815 g, 32%) as a yellow solid.

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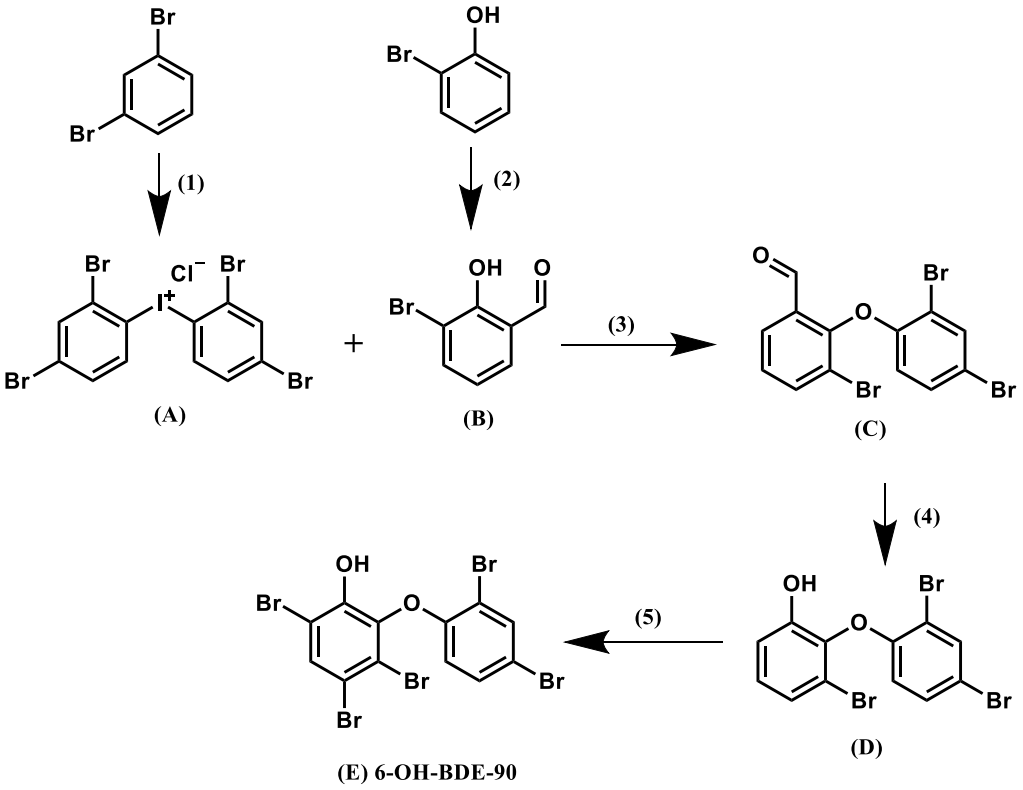

Figure A.S1. The synthesis procedure of 6-OH-BDE-90. (A) 2,2',4,4'-tetrabromodiphenyliodonium chloride; (B) 3-bromosalicyaldehyde (C) 3-bromo-2-(2,4-dibromophenoxy) benzaldehyde; (D) 3-bromo-2-(2,4-dibromophenoxy)phenol.

Wastewater treatment plants

Metropolitan Wastewater Treatment Plant (MWP) in St. Paul, MN has a capacity of 251 million gallons/day (MGD) serving 1.8 million people. MWP chlorinates their effluent from April through October with a dosage of 1.25 µg/L Cl as Cl_2 for > 30 min, aiming for a residual of 0.20 µg/L Cl as Cl_2. Effluent is dechlorinated with sodium bisulfite at 0.95 µg/L. MWP discharges directly into the Mississippi River. Composite samples from MWP were obtained on three separate dates. On two dates in the fall, pre- and post-chlorination samples were obtained, and one non-chlorinated effluent sample

was obtained during the winter. A pre-chlorination grab sample was also obtained to determine the effects of ozonation on the analytes.

Two activated sludge WWTPs, Palo Alto Regional Water Quality Control Plant (PAWP) and Saint John's University Wastewater Treatment Plant (SJWP), were each sampled (24-hour composite, pre- and post-UV) during two dates to measure the effect of UV disinfection on triclosan and CTDs in wastewater. PAWP treats on average 21.8 MGD serving 220,000 people with ~5% industry wastewater. PAWP disinfects year round using a system of Trojan UV 3000 Plus assemblies with an energy output of 35 mW-s cm^{-2}. The average ultraviolet transmittance of the wastewater is 62% with a contact time of 3.8 seconds. PAWP discharges effluent directly into the southern San Francisco Bay.

St. John's University uses groundwater for their potable water supply and does not chlorinate prior to use. The SJWP treats the used water serving a population of 2600 during the academic year and 1200 in summer. SJWP is licensed to process a maximum flow of 0.23 MGD and treats about 0.16 MGD on an average day. After filtration through sand, secondary effluent is disinfected using a Package Treatment UV-3000 system containing six modules. Each module has four 162.6-cm lamps which provide 190 µW cm^{-2} at 1 m with radiation centered at 254 nm. The contact time in the disinfection tank is between 2 and 4 minutes depending on flow conditions. SJWP discharges into East Lake Gemini which then drains into the North Fork of the Watab River and eventually into the Mississippi River.

Effluent grab samples from Western Lake Superior Sanitation District (WLSSD) were collected by boat on two occasions. Approximately half of the wastewater that

WLSSD treats originates from industries. WLSSD filters the secondary effluent through mixed media beds and disinfects with chlorine, but is only required to chlorinate when a fecal coliform analysis of treatment plant intake exceeds 100 MPN/100 mL. The chlorine dosages were not available for the dates that sampling took place. WLSSD discharges into the St. Louis Bay which flows into Lake Superior.

Table A.S1. Quantification and confirmation SRM transitions for analyte detection for ESI Method I and II and APCI method.

Analyte	Precursor ion m/z	Product ion m/z	Purpose[a]
	ESI Method I		
Triclosan	287	35.1	Q
	289	37.1	C
4-Cl-TCS	321	35.1	Q
	323	37.1	C
6-Cl-TCS	321	35.1	Q
	323	37.1	C
4,6-Cl-TCS	355	35.1	Q
	357	37.1	C
6-OH-BDE-47	500.7	79	Q
	498.7	79	C
$^{13}C_{12}$-Triclosan	299	35.1	Q
	ESI Method II		
6-OH-BDE-47	500	79	Q
	502	81	C
6-OH-BDE-90	578	79	Q
	580	81	C
6-OH-BDE-99	578	79	Q
	580	81	C
6'-OH-BDE-100	578	79	Q
	580	81	C
6'-OH-BDE-118	578	79	Q
	580	81	C
$^{13}C_{12}$-Triclosan	299	35	Q
	APCI Method		
6-OH-BDE-47	500	79	Q
	502	81	C
6-OH-BDE-90	578	79	Q
	580	81	C
6-OH-BDE-99	578	79	Q
	580	81	C
6'-OH-BDE-100	500	79	Q
	502	81	C
6'-OH-BDE-118	500	79	Q
	502	81	C
$^{13}C_{12}$-Triclosan	299	35	Q

[a]Quantification SRM denoted as "Q" and confirmation SRM denoted as "C"

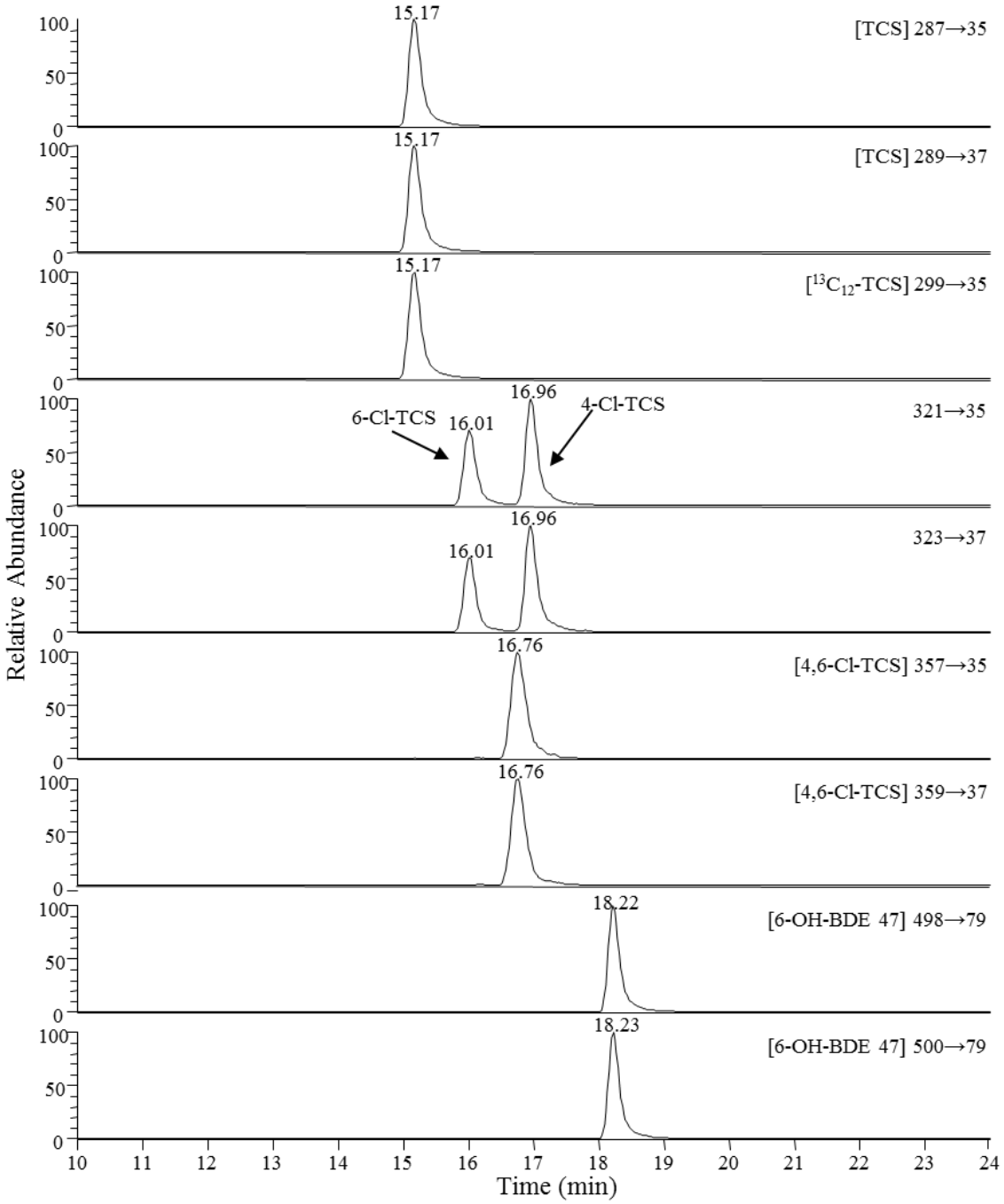

Figure A.S2. ESI Method I Standard Chromatograph. Representative of a standards (normalized to the highest peak) for ESI Method I. Retention times (RT) are noted for known analytes. The SRM transitions are indicated to the right of each chromatogram.

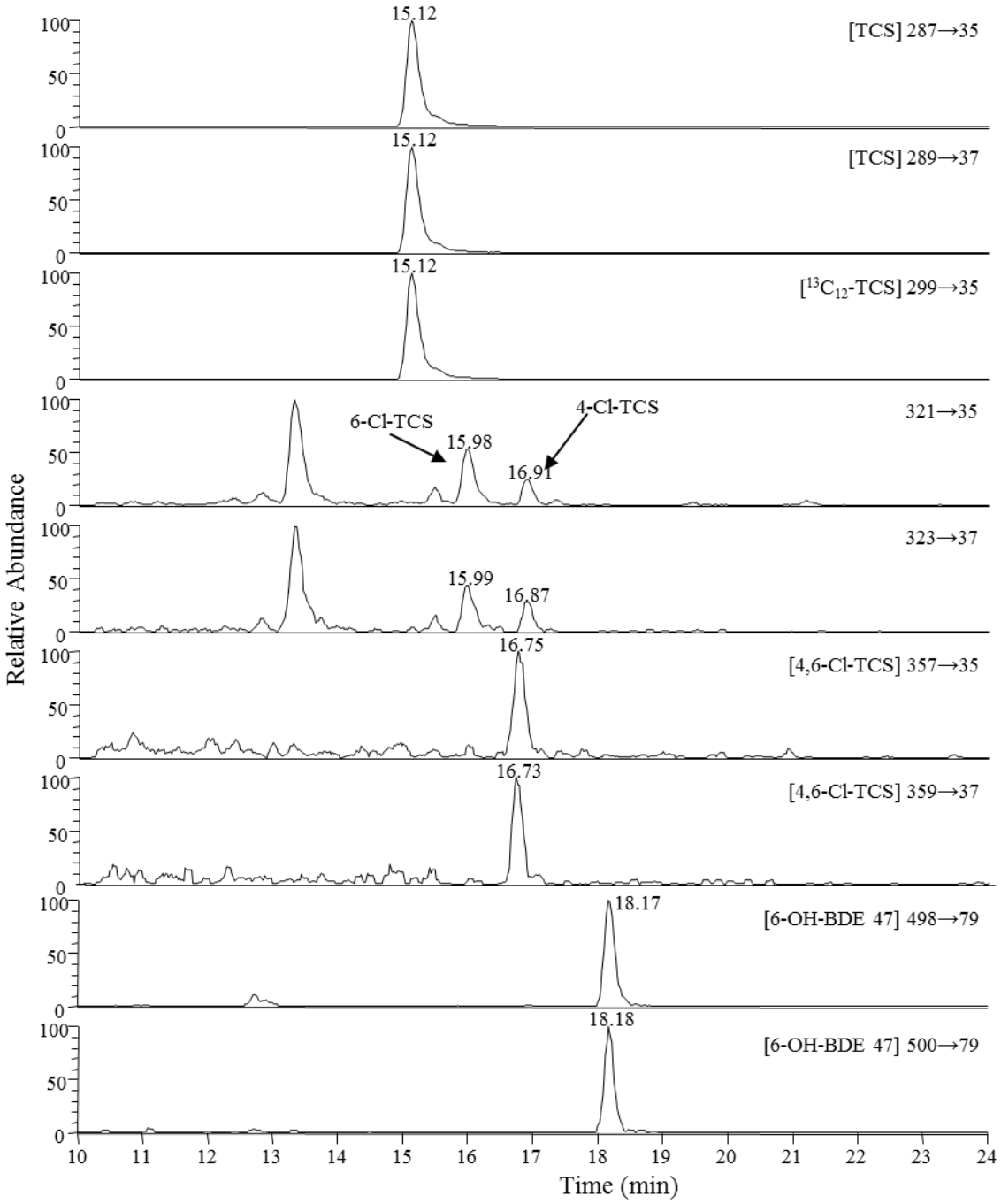

Figure A.S3. ESI Method I chromatograph of PAWP pre-UV sample

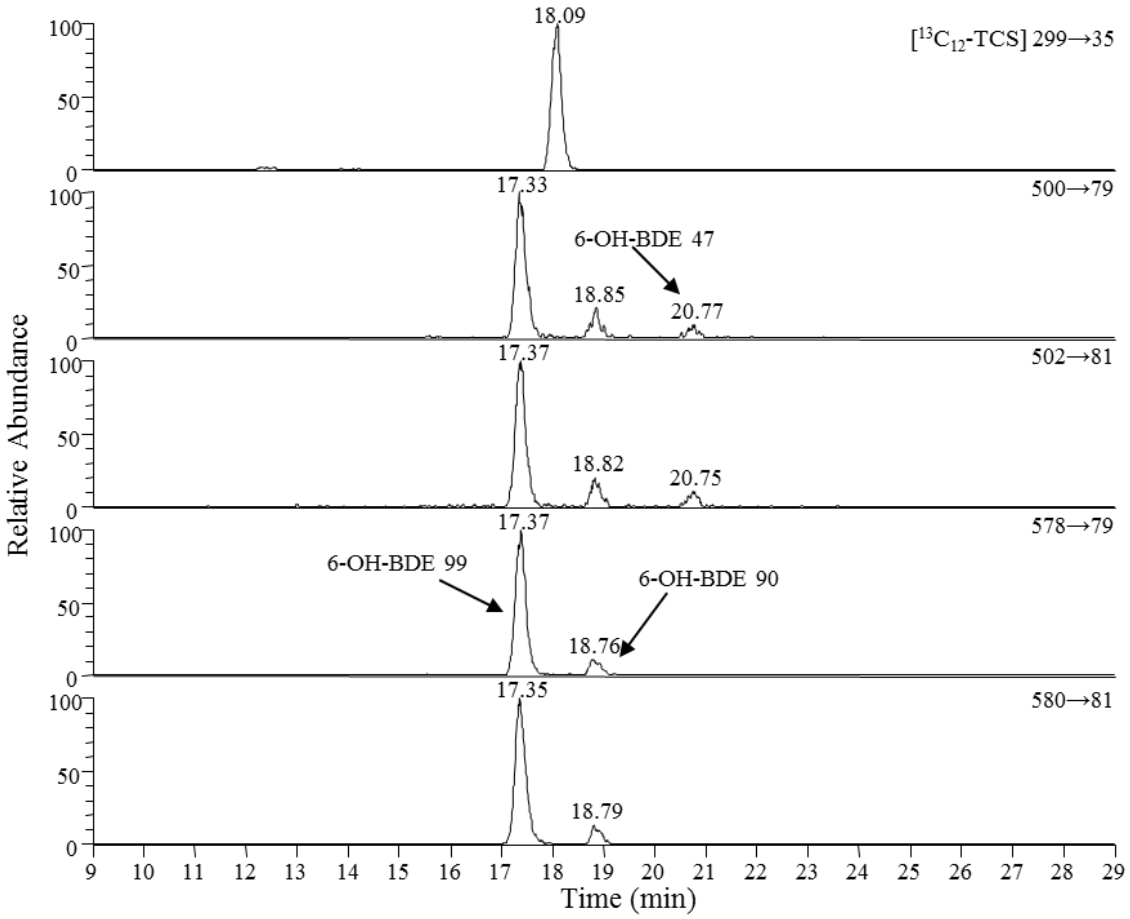

Figure A.S4. APCI Method chromatograph of PAWP pre-UV sample

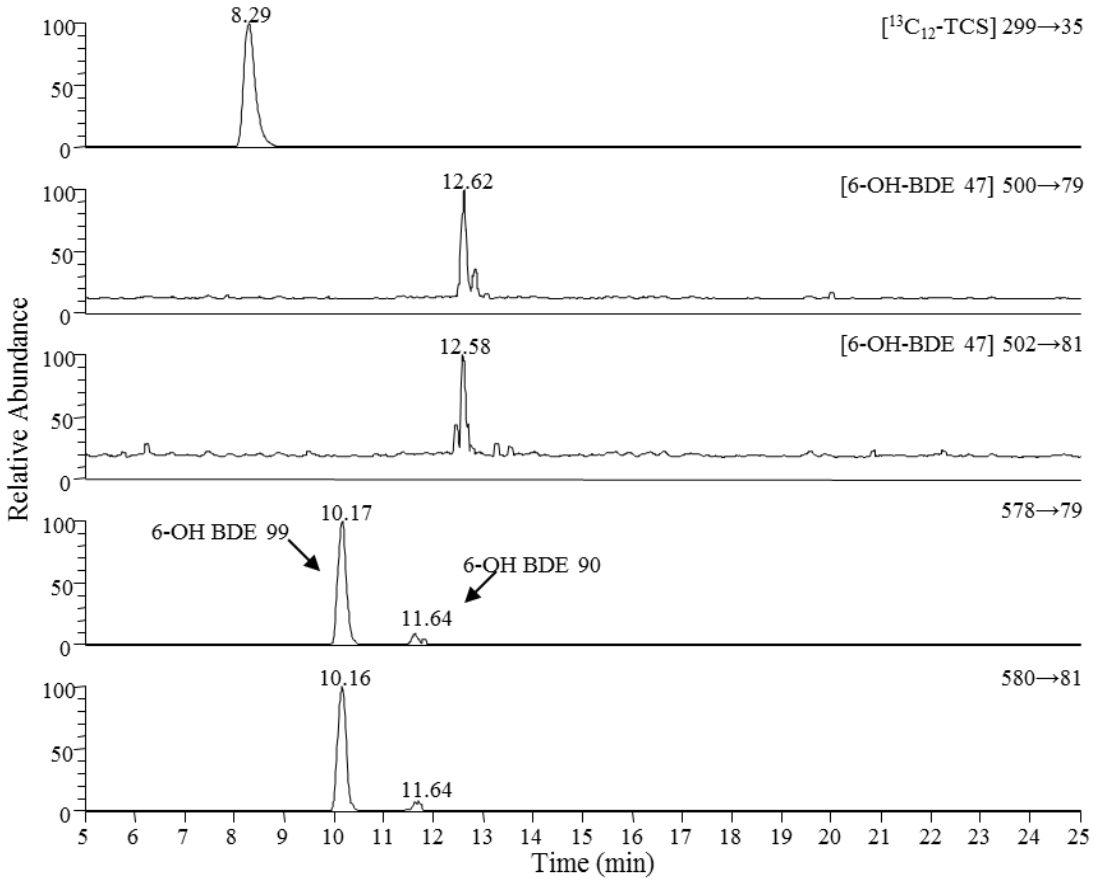

Figure A.S5. ESI Method II chromatograph of PAWP pre-UV sample

Table A.S2. LOQs for ESI Method I, APCI Method, and ESI Method II

	LOQ* Ranges (ng/L)		
	ESI Method I[a]	APCI Method[b]	ESI Method II[b,c]
Triclosan	3.9 - 29.1	-	-
4-Cl-TCS	0.02 - 0.24	-	-
6-Cl-TCS	0.1 - 2.4	-	-
4,6-Cl-TCS	0.1 - 2.6	-	-
6-OH-BDE-47	0.2 - 1.8	0.2 - 3.0	0.5
6-OH-BD- 90	-	0.1 - 2.7	0.6
6-OH-BDE-99	-	0.1 - 3.2	0.7
6'-OH-BDE-100	-	2.9[d]	1.74[d]
6'-OH-BDE-118	-	2.9[d]	1.09

[*]LODs may be calculated from LOQ by multiplying by 0.3

[a]LOQs defined as 10 times the analyte concentration determined in the method blank

[b]LOQs defined as 80% of lowest concentration on calibration curve

[c]No range because all samples were analyzed in a single run

[d]Analyte not detected; LOQ defined as lowest concentration on calibration curve

Table A.S3. The relative recoveries of analytes of interest to $^{13}C_{12}$-triclosan for ESI Method I and absolute recovery of $^{13}C_{12}$-triclosan for *n* number of replicates.

ESI Method I

Wastewater Sample		**Relative Recovery (%)**						**Absolute Recovery (%)**
Metropolitan plant (MWP)	n	Triclosan	4-Cl-TCS	6-Cl-TCS	4,6-Cl-TCS	6-OH-BDE 47	n	$^{13}C_{12}$-Triclosan
September 2011	2	103 ± 48	96 ± 55	109 ± 72	72 ± 40	54 ± 38	7	37 ± 8
October 2011	2	99 ± 3	81 ± 1	80 ± 4	56 ± 1	50 ± 4	8	78 ± 5
November 2011	1	71.2	75.2	60.2	52.9	39.5	4	46 ± 4
April 2012	2	105 ± 1	83 ± 24	102 ± 28	76 ± 19	65 ± 5	6	99 ± 7
Western Lake Superior Sanitary District (WLSSD)								
June 2011	1	93.73	80.80	47.82	50.55	64.96	4	73 ± 8
April 2012	1	110.57	108.53	104.26	79.64	71.22	4	79 ± 7
Palo Alto Regional Water Quality Control Plant (PAWP)								
July 2011	2	71 ± 4	93 ± 1	90 ± 2	67 ± 0	56 ± 2	8	33 ± 7
January 2012	2	94 ± 5	80 ± 11	58 ± 9	54 ± 10	47 ± 9	8	36 ± 3
St. John's University (SJWP)								
January 2012	2	99 ± 3	70 ± 20	49 ± 15	52 ± 25	51 ± 27	8	74 ± 18
February 2012	2	106 ± 3	93 ± 3	102 ± 7	67 ± 5	66 ± 2	6	119 ± 2

Table A.S4. The relative recoveries of analytes of interest to $^{13}C_{12}$-triclosan for ESI Method II and absolute recovery of $^{13}C_{12}$-triclosan for *n* number of replicates.

ESI Method II

Wastewater Sample		**Absolute Recovery (%)**
Metropolitan plant (MWP)	n	$^{13}C_{12}$-Triclosan
September 2011	3	53 ± 56
October 2011	1	39
November 2011	2	16 ± 7
Palo Alto Regional Water Quality Control Plant (PAWP)		
July 2011	6	41 ± 8
January 2012	3	47 ± 27
Compound		**Relative Recovery (%)**
6-OH-BDE-47	2	52 ± 0
6-OH-BDE-90	2	25 ± 2
6-OH-BDE-99	2	24 ± 2
6'-OH-BDE-100	2	96 ± 7
6'-OH-BDE-118	2	17 ± 7

Table A.S5. The relative recoveries of analytes of interest to $^{13}C_{12}$-triclosan for APCI Method and absolute recovery of $^{13}C_{12}$-triclosan for *n* number of replicates.

APCI Method

Wastewater Sample		**Absolute Recovery (%)**
Metropolitan plant (MWP)	n	$^{13}C_{12}$-Triclosan
September 2011	4	60 ± 19
November 2011	3	68 ± 6
Palo Alto Regional Water Quality Control Plant (PAWP)		
July 2011	6	65 ± 11
January 2012	6	22 ± 15
St. John's University (SJWP)		
January 2012	3	64 ± 32
February 2012	3	135 ± 24
Compound		**Relative Recovery (%)**
6-OH-BDE-47	3	66 ± 12
6-OH-BDE-90	3	54 ± 14
6-OH-BDE-99	3	48 ± 13
6'-OH-BDE-100	3	73 ± 32
6'-OH-BDE-118	3	48 ± 10

Equations Used to Calculate Environmental Concentrations and Absolute and Relative Recoveries

The water concentrations above LOQ were calculated using isotope dilution analysis, an example is shown below for chemical 'X' using peak areas (PA) in standards (std) and samples. The response factor (RF) was calculated using:

$$RF = \frac{1}{[^{13}C_{12}X_{std}] \cdot m}$$

where $^{13}C_{12}X_{std}$ is the concentration of isotope labeled compound in standards and *m* is the calibration slope defined by:

$$m = \frac{X^{PA}_{sample}}{^{13}C_{12}X^{PA}_{std} \cdot [X_{std}]}$$

The concentration (µM) of X was calculated using:

$$X_{sample}(\mu M) = RF \times \frac{X^{PA}_{sample}}{^{13}C_{12}X^{PA}_{sample}} \times {}^{13}C_{12}X_{sample}(\mu M)$$

where $^{13}C_{12}X_{sample}$ is the amount of isotope labeled chemical spiked in the sample matrix. The relative recovery for each compound was calculated using the following equation:

$$RelativeRecovery = \frac{X_{spiked,sample} - X_{sample,avg}}{X_{spiked}} \times 100$$

were $X_{spiked,sample}$ is the concentration in the spiked sample after SPE and silica column clean-up, $X_{sample,avg}$ is the average concentration in the sample, and X_{spiked} is the concentration in the sample after being spiked. Water concentrations were corrected for relative recovery. The absolute recovery (AbsRec) of $^{13}C_{12}$-triclosan was calculated using:

$$AbsRec = \frac{^{13}C_{12}X^{PA}_{sample}}{^{13}C_{12}X^{PA}_{std}(avg)} \times \frac{[^{13}C_{12}X_{std}]}{[^{13}C_{12}X_{sample}]} \times 100$$

where $^{13}C_{12}X^{PA}_{std}$ (avg) is the average peak area of isotope labeled compound in standards.

Appendix B: Supporting Information for Chapter 2

B.1 Materials and Methods

Cleaning Protocols

All glassware and tools were tripled rinsed with diluted Alconox, tap, and deionized (DI) water. Glassware, silica gel, glass wool, sand, and disposable pipettes were baked (550 ^{o}C, 4 hours). Items that would melt at 550 ^{o}C were triple rinsed with ethyl acetate, methanol (MeOH), and acetonitrile (ACN) after the series of water triple rinses. Gas-tight, glass syringes were cleaned by drawing up five aliquots of ethyl acetate, MeOH, and ACN. Each syringe had a designated task – one for each isotope labeled chemical, the 50:50 H_2O:ACN mixture, and transferring extracts. A syringe was cleaned with acetone after each extract transfer.

The solid phase extraction (SPE) sample transfer lines were cleaned with ethyl acetate (20 mL), MeOH (20 mL), and ACN (20 mL). The stainless steel cells for the Accelerated Solvent Extractor (ASE 350; Dionex) underwent a rigorous cleaning procedure. The endcaps were tripled rinsed with tap and DI water, ethyl acetate, MeOH, and ACN. Note that detergents were not used on the endcaps because they degrade the seal. Next, the endcaps were disassembled and sonicated (15 min) in an acetone bath. After being reassembled, they were again triple rinsed with ethyl acetate, MeOH, and ACN. The bodies of the cells underwent the same cleaning as other non-baked glassware.

Accelerated Solvent Extraction (ASE)

The ASE cells (22 mL) were prepared with two glass fiber filters on bottom followed by a thin sand layer, freeze-dried sediment, thin sand layer, and 1 glass fiber filter. Additional sand was mixed in with the sediment using a disposable glass pipette if sediment volume was not sufficient to fill the cell body. The ASE method used was: temperature: 100 ^{o}C, pressure: 1500 psi; cell heat time: 5 min; cell static time: 5 min; rinse volume: 100 %; purge time: 100 s; extraction cycles: 2; and solvent: dichloromethane. Approximately 20% of the extract was blown down to dryness with nitrogen and re-suspended (~500 µL) in the same mixture as the SPE eluent (55:45 MeOH:MTBE). The sediment extract was cleaned using the silica column method described above.

Equations Used to Calculate Analyte Concentrations in Environmental Samples

The sediment and water concentrations above LOQ were calculated using isotope dilution analysis, an example is shown below for chemical 'X' using peak areas (PA) in standards (std) and samples. Units for sediments and water were g/L and µM, respectively. First, the response factor (RF) was calculated using:

$$RF = \frac{1}{[^{13}C_{12}X_{std}] \cdot m} \qquad \textbf{(B-1)}$$

where $^{13}C_{12}X_{std}$ is the concentration of isotope labeled compound in standards and m is the calibration slope described with the following equation:

$$m = \frac{X^{PA}_{sample}}{^{13}C_{12}X^{PA}_{std} \cdot [X_{std}]} \qquad \textbf{(B-2)}$$

The mass of X in sediments or concentration in sediments were calculated using:

$$X_{sample}(g\ or\ \mu M) = RF \times \frac{X^{PA}_{sample}}{{}^{13}C_{12}X^{PA}_{sample}} \times {}^{13}C_{12}X_{sample}(g\ or\ \mu M) \qquad \textbf{(B-3)}$$

where $^{13}C_{12}X_{sample}$ is the amount of isotope labeled chemical spiked in the sample matrix. The relative recovery for each compound was calculated using the following equation:

$$RelativeRecovery = \frac{X_{spiked,sample} - X_{sample,avg}}{X_{spiked}} \times 100 \qquad \textbf{(B-4)}$$

The sediment concentration was calculated using the normalized amount of sediment analyzed in the extract. Sediment and water concentrations were relative recovery corrected. The internal standard for triclosan and 6-OH-BDE 47 and 6'-OH-BDE 100 was its isotope labeled counterpart. $^{13}C_{12}$-6'-OH-BDE 100 was the internal standard for all of the OH-PentaBDEs. The absolute recovery (AbsRec) of internal standards was calculated using:

$$AbsRec = \frac{{}^{13}C_{12}X^{PA}_{sample}}{{}^{13}C_{12}X^{PA}_{std}(avg)} \times \frac{[{}^{13}C_{12}X_{std}]}{[{}^{13}C_{12}X_{sample}]} \times 100 \qquad \textbf{(B-5)}$$

where $^{13}C_{12}X^{PA}_{std}$ (avg) is the average peak area of isotope labeled compound in standards.

Polychlorinated dibenzo-p-dioxin (PCDD), Polychlorinated dibenzofuran (PCDF) and Polybrominated dibenzo-p-dioxin (PBDD) Extraction Method.

Once spiked with labeled recovery surrogates, each sample was extracted with toluene for at least 18 hours using a Soxhlet/Dean Stark apparatus. Extracts were subsequently spiked with $^{37}Cl_4$-2,3,7,8-TCDD to measure the efficiency of sample cleanup. Soxhlet/Dean Stark extracts were concentrated using a Snyder column, back-

extracted with concentrated H_2SO_4 and NaOH, and eluted through multi-layer silica columns (2 g neutral silica, 4 g acidic silica, and 2 g basic silica) with hexane. Eluates were then added to 4 g activated aluminum oxide (Al_2O_3) columns and eluted with 60:40 DCM:hexane (v/v). After solvent exchange into hexane, Al_2O_3 column eluates were cleaned up via carbon chromatography, where samples were passed through 0.5 g of 18% activated carbon mixed with Celite. These columns were preconditioned with 5 mL of toluene, 2 mL of 75:20:5 DCM:MeOH:toluene (v/v/v), 2 mL of 50:50 DCM:cyclohexane (v/v), and 5 mL of hexane. Sample extracts were added to the column and flushed in the forward direction with 2 mL of 50:50 DCM:cyclohexane (v/v) and 2 mL of 75:20:5 DCM:MeOH:toluene (v/v/v) to remove potential interfering compounds. Finally, analytes were washed off the column in the reverse direction with 10 mL of toluene. The toluene was then concentrated, spiked with $^{13}C_{12}$-1,2,3,4-TCDD and 13C12-1,2,3,7,8,9-HxCDD as recovery standards, and concentrated to a final volume of 20 μL.

HRGC-HRMS Analysis, Analyte Quantification, and QA/QC

PCDD/F and PBDD analyses were performed using high-resolution gas chromatography-high-resolution mass spectrometry (HRGC-HRMS). Aliquots of final extracts (1 μL) were injected into an HP 5890 gas chromatograph with a split/splitless injector and a 60 meter DB-5MS capillary column (0.25 mm ID x 0.25 μm film). An additional analysis substituting a 15 meter RTX-1614 column (0.25 mm ID x 0.10 μm film) was used for the determination of PBDDs. The gas chromatograph was coupled to a Waters Autospec Ultima high-resolution mass spectrometer operated in selected ion monitoring (SIM) mode (positive electron impact, > 10,000 resolution, 32 eV, 280 °C).

Acquisition windows were set to include all tetra- through octa-CDD/F isomers. Windows for di- and tri-CDD/F isomers were centered around the di- and tri-CDD congeners in this study. Therefore, total DCDD and TriCDD values presented should be considered an estimate, as the first and last DCDD and TriCDD eluters may have been outside the established acquisition windows for these isomers. The PBDD analyses included four congeners, 1,3,7-triBDD, 1,2,4,7/1,2,4,8-tetra-BDD and 2,3,7,8-tetra-BDD. Standards for HRGC-HRMS analysis were prepared using a U.S. EPA Method 1613B calibration set (tetra- through octa-CDD/F isomers). A secondary calibration set for di- and tri-CDD/Fs was prepared at similar levels to tetra-CDD/F in the Method 1613B. From these calibration sets, five-point calibration curves were constructed for each PCDD/F congener. Standards for PBDD analysis were prepared at similar levels to tetra-CDD in the Method 1613B using individual native and labeled PBDD standards. A five-point calibration curve was also constructed for each PBDD congener. PBDD results were reported down to the calibration curve based quantitation limits.

B.2 Results

Table B.1. Selected reaction monitoring transitions (SRM) for chemical quantification (Q) and confirmation (C).

Analyte	SRM (m/z)	Purpose
Triclosan	287 → 35.2	Q
	289 → 37.2	C
6-OH-BDE 47	500.6 → 79	Q
	502.6 → 81	C
OH-PentaBDEs	578.6 → 79	Q
	580.6 → 81	C
$^{13}C_{12}$-TCS	299 → 35.2	Q
$^{13}C_{12}$-6-OH-BDE 47	512.6 → 79	Q
$^{13}C_{12}$-6'-OH-BDE 100	590.6 → 79	Q

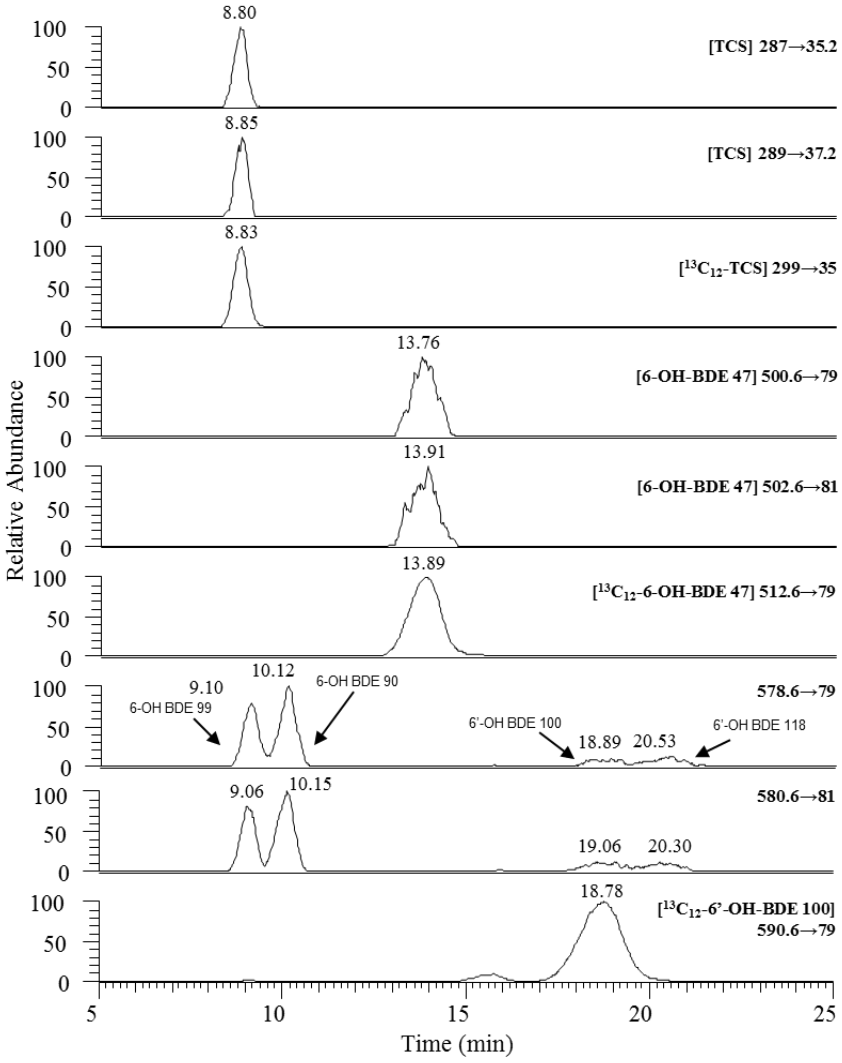

Figure B.1. Representative chromatogram for standard using LC-MS/MS method displaying SRM transition and retention times.

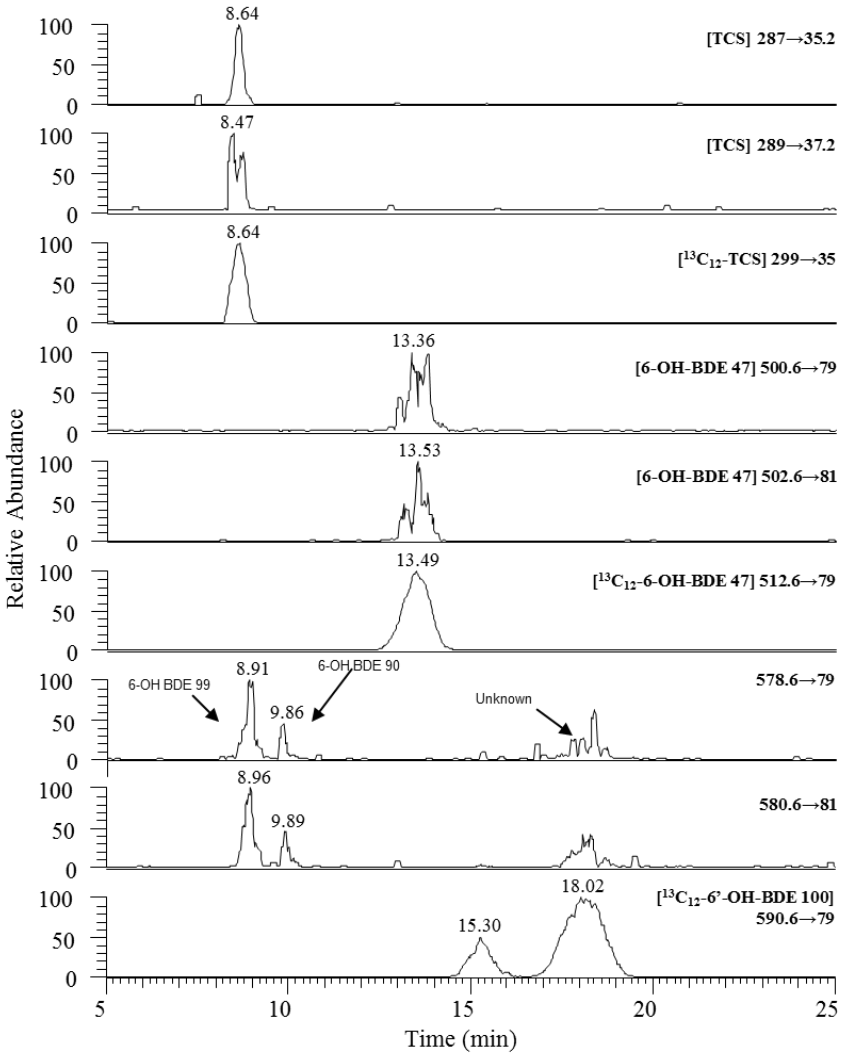

Figure B.2. Chromatogram for Central Bay 38 – 40 cm sediment displaying SRM and retention times. Note that peak at 18.02 min in 578.6 → 79 and 580.6 → 81 frames are unknown and not 6'-OH-BDE 100, because this sample was pre-BDE production.

Table B.2. Absolute and relative recovery for $^{13}C_{12}$-PXDDs and PXDDs, respectively, in sediments.

PXDD Recovery

	Absolute Recovery (%)		
Sediment	**$^{13}C_{12}$-2,3,7,8-TeBDD**	**$^{13}C_{12}$-2,8-DiCDD**	**n [a]**
San Francisco Bay Surface Sediments	72 ± 13	55 ± 7	8
Point Reyes National Seashore	80 ± 15	43 ± 7	9
Analyte	**Relative Recovery (%)**	**%RPD [b]**	**n**
1,3,7-TriBDD	79 - 82	3.7	2
1,2,4,7/1,2,4,8-TeBDD	95 - 99	4.1	2
2,3,7,8-TeBDD	104 - 106	1.9	2
2,8-DiCDD	113 – 120	6	2

[a] n = number of samples
[b] RPD = relative percent difference between matrix spike replicates

Table B.3. Loss-on-ignition results for San Francisco Bay surface sediments and Point Reyes National Seashore cores.

Sample ID	Top Depth (cm)	Bottom Depth (cm)	Dry Density (g/cc)	% Organic	% Carbonate	% Inorganic
BG20	0	5	1.29	1.32	1.95	96.7
BG30	0	5	0.85	5.76	4.23	90.0
SU044S	0	5	1.05	2.54	3.33	94.1
SPB001S	0	5	0.61	5.99	6.84	87.2
CB001S	0	5	0.42	6.71	7.94	85.4
SB023S	0	5	0.77	4.69	42.63	52.7
SB002S	0	5	0.70	5.08	7.33	87.6
LSB001S	0	5	N/A	N/A	N/A	N/A
LSB042S	0	5	0.54	5.49	7.88	86.6
BA10	0	5	0.76	4.32	12.32	83.4
Point Reyes A	0	5	1.43	1.17	1.93	96.9
	5	10	1.39	1.67	2.15	96.2
	10	15	1.46	1.56	2.01	96.4
Point Reyes B	0	5	1.42	1.53	1.91	96.6
	5	10	1.15	2.66	2.75	94.6
	10	15	1.53	1.35	1.73	96.9
Point Reyes C	0	5	1.38	1.20	1.96	96.8
	5	10	1.25	1.92	2.22	95.9
	10	16	1.28	1.95	2.31	95.7

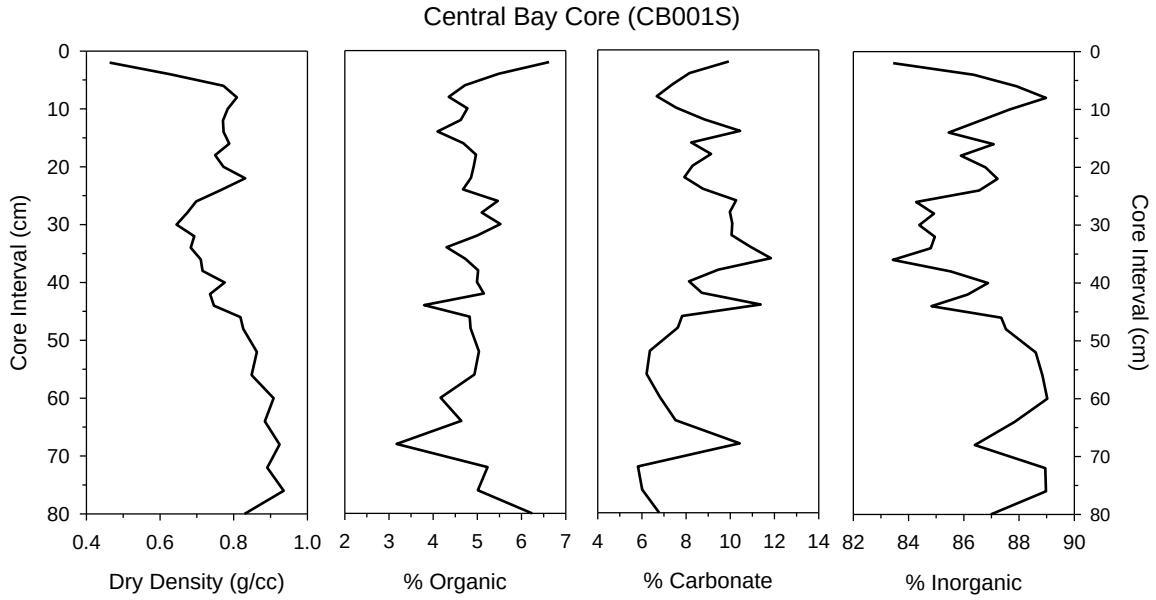

Figure B.3. Loss-on-ignition results for Central Bay (CB001S) core.

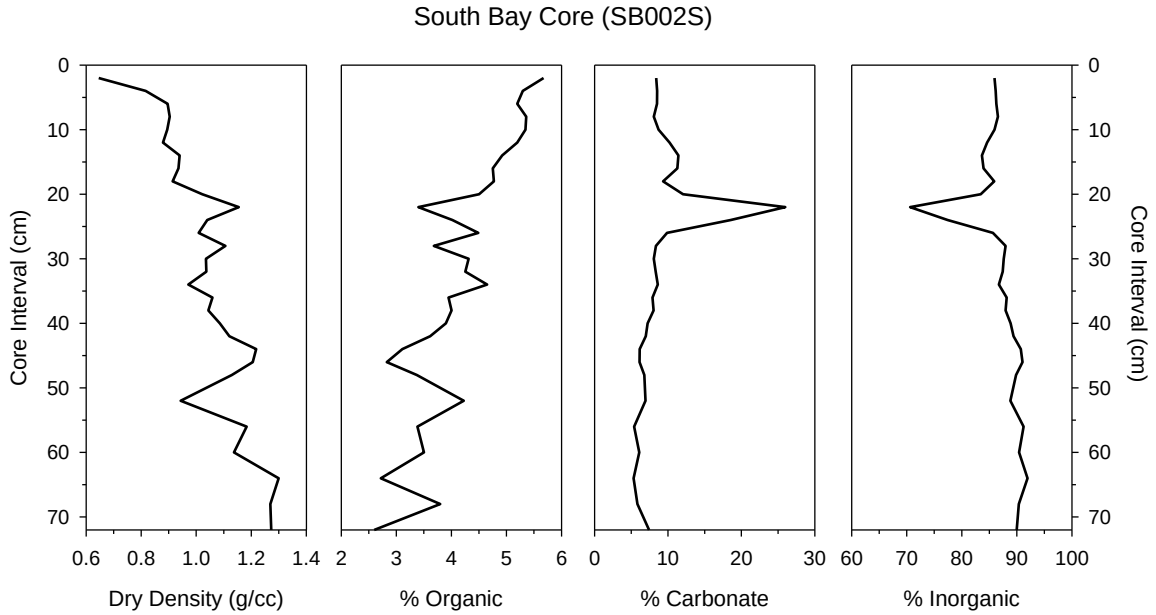

Figure B.4. Loss-on-ignition results for South Bay (SB002S) core.

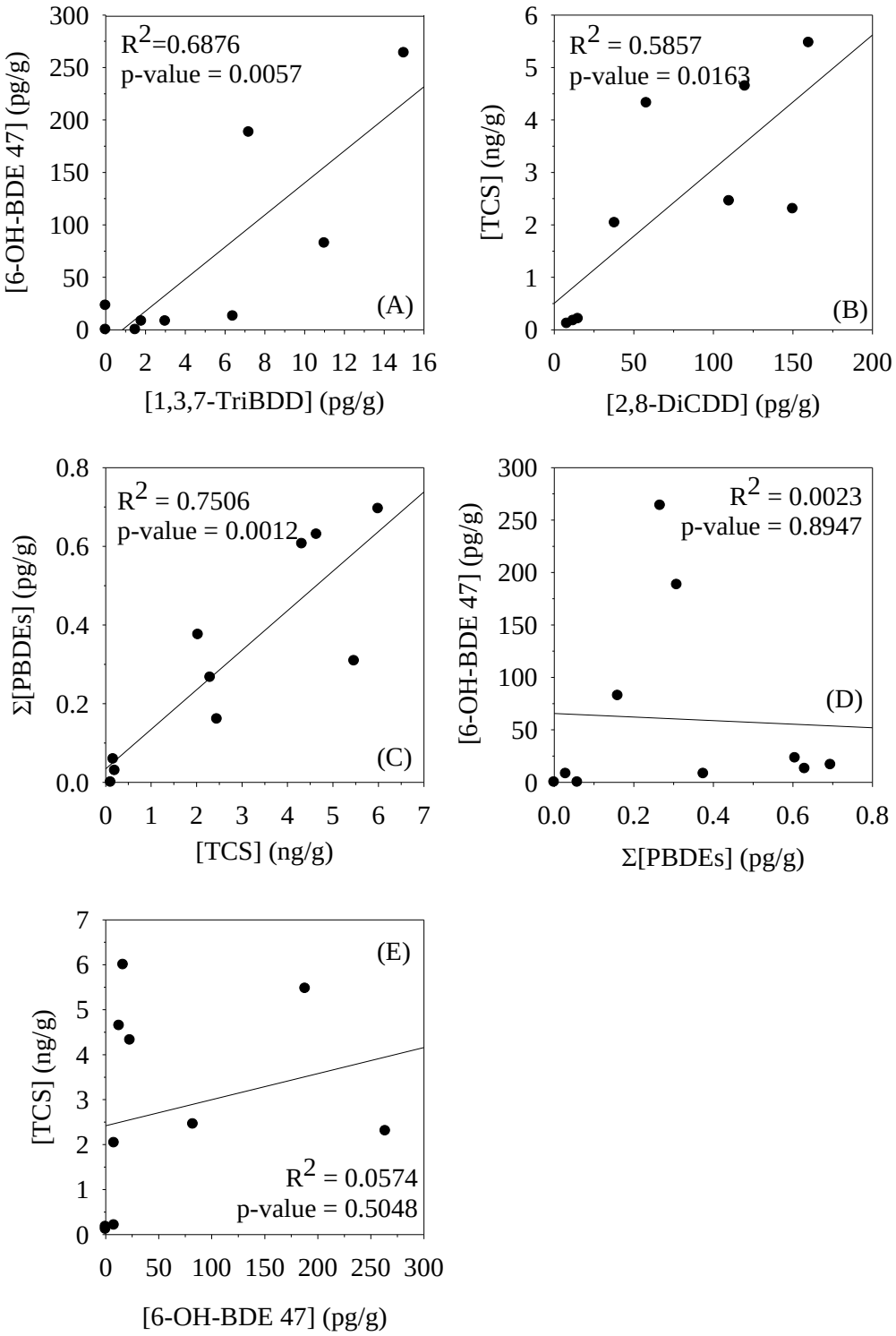

Figure B.5. Graph displaying correlation between (A) 6-OH-BDE 47 and 1,3,7-TriBDD, (B) triclosan and its dioxin (2,8-DiCDD), (C) BDE 47 and 100 (∑PBDEs) and triclosan (TCS), (D) 6-OH-BDE 47 and ∑PBDEs, and (E) triclosan and 6-OH-BDE 47 in San Francisco Bay surface sediments.

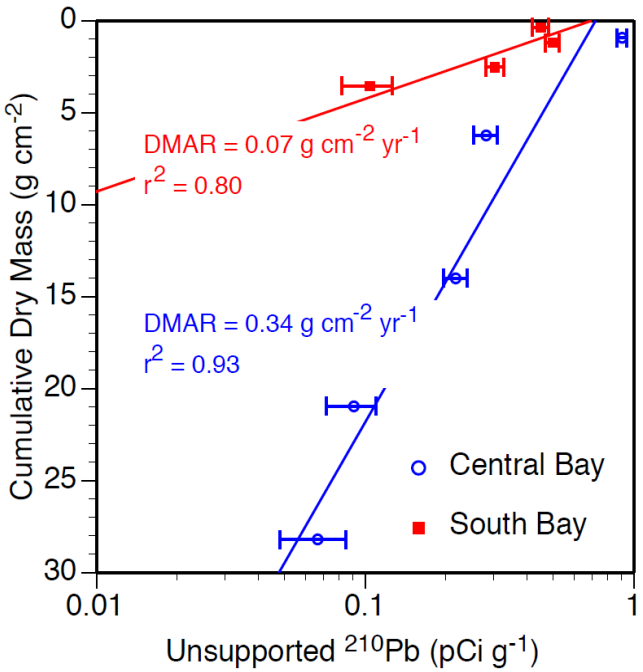

Figure B.6. An approximate chronology was determined for the San Francisco Bay cores by assuming a constant sediment flux (DMAR; dry mass accumulation rate) fitted to the data by least-squares regression (the cf:cs model).

Table B.4. Latitude and longitude of salinity measurements for surface water sampling sites near the sediment sampling sites.

	Water			Sediment
Site Name	**Sample ID**	**Latitude**	**Longitude**	**Sample ID**
Suisun Bay	SU041W	38.09582	-122.063	SU044S
San Pablo Bay	SPB033W	38.08548	-122.38517	SPB001S
Central Bay	CB033W	37.87172	-122.36898	CB001S
South Bay	SB063W	37.6937	-122.22285	SB023S
South Bay	SB062W	37.54092	-122.1682	SB002S
Lower South Bay	LSB053W	37.49253	-122.09265	LSB001S
Lower South Bay	LSB052W	37.47805	-122.09085	LSB042S
Lower South Bay	LSB054W	37.46833	-122.06432	BA10

Appendix C: Supporting Information for Chapter 3

C.1 Materials and Methods

Sediment cores were collected in August and September 2014 from four Minnesota lakes, Figure C.1.

Figure C.1. Map of collection sites of sediment cores from lakes around Minnesota.

Chemical Sources and Purity

Ultrapure water (18.2 MΩ-cm) was produced by Millipore Simplicity UV purification system and ethylenediaminetetraacetic acid disodium salt dihydrate (> 99%) was purchased from Avocado Research Chemicals. Ottawa sand, sodium phosphate monobasic monohydrate (ACS grade), formic acid (ACS grade and Optima® LC-MS), methanol (HPLC grade and Optima® LC/MS), water (Optima® LC/MS), acetone (HPLC grade), acetonitrile (HPLC grade), and ethyl acetate (HPLC grade) were bought from Fisher scientific. Sodium phosphate dibasic, anhydrous (ACS grade), and acetonitrile

(Ultra LC/MS) were obtained from J.T. Baker. Mallinckrodt Chemicals supplied citric acid monohydrate (ACS grade) and sodium chloride (ACS grade), and sodium hydroxide (ACS grade) came from Macron Chemicals. Industrial and ultrapure nitrogen (99.999%) were manufactured by Matheson Gas.

Penicillin V potassium salt was obtained from Alfa Aesar. $^{13}C_2$-erythromycin (~ 90%) and $^{13}C_6$-sulfamethazine (99%) were purchased from Cambridge Isotopes. Sulfachloropyridazine (99.6%), ammonium acetate (LC-MS grade), and clinafloxacin (97.6%) were supplied by Fluka. Erythromycin A dihydrate (VETRANAL), ammonium hydroxide solution (28 – 30%, ACS grade), sulfuric acid (> 95%, ACS grade), amoxicillin, sulfapyridine (>99%), sulfadimethoxine (98.5%), roxithromycin (90%), ofloxacin, oxytetracycline hydrochloride, carbadox, demeclocycline (>90%), $^{13}C_6$-sulfamethoxazole (>99.5%), and simeton (>99.5%) were obtained from Sigma-Aldrich. Chem-Impex supplied trimethoprim (100%), lincomycin HCl (99.2%), norfloxacin (99.5%), sulfamethoxazole (99%), penicillin G sodium, chlortetracycline hydrochloride, tetracycline hydrochloride, and nalidixic acid (>99%). Ciprofloxacin (>98%), enrfloxacin (>98%), sulfadiazine (>98%), and sulfamethazine (>98%) were bought from TCI. Tylosin was purchased from Wako and doxycycline hyclate from Millipore.

Cleaning Procedure

All glassware used was cleaned with triple rinses of dilute Alconox solution, tap water, and deionized water before being baked at 550 °C for greater than 5 hours to remove organic matter. Labware that could not sustain 550 °C was triple rinsed with the dilute Alconox solution, tap water, deionized water, acetonitrile, ethyl acetate, and methanol.

Accelerated Solvent Extraction.

The stainless steel accelerated solvent extraction (ASE) cells were rigorously cleaned to prevent cross-contamination. The endcaps with triple rinsed with tap water, deionized water, acetonitrile, ethyl acetate, and methanol and then disassembled. The frit, cap insert, and snap fitting were placed in water bath and subsequently an acetone bath and sonicated 10 minutes each. The cell endcap was triple rinsed with acetonitrile, ethyl acetate, and methanol while the frit, cap insert, and snap fitting were being sonicated. After being reassembled they were again triple rinsed with acetonitrile, ethyl acetate, and methanol. Note that Alconox could not be used on the endcaps due to the frit. The cylindrical body for the ASE cell was cleaned in the same fashion as the non-glass labware.

Internal standards (clinafloxacin, $^{13}C_2$-erythromycin, $^{13}C_2$-erythromycin-H_2O, simeton, and $^{13}C_6$-sulfomethoxazole, 100 ng) and surrogates (demeclocycline, nalidixic acid, and $^{13}C_6$-sulfamethazine, 20 ng) were spiked onto sediment in a methanol solution prior to ASE extraction. The ASE cells were assembled in the following order from bottom to top: 2 glass fiber filters, a thin layer of Ottawa sand, 0.5 or 1 g of sediment, sufficient volume of Ottawa sand to fill chamber, and 1 glass fiber filter. The optimized ASE method is given in Table C.1.

Table C.1. Parameters and respective values for accelerated solvent extraction (ASE) method.

ASE METHOD	
SOLVENT	50:50 methanol: 50mM pH 7 phosphate buffer
TEMPERATURE	100 °C
HEAT TIME	5 min
STATIC TIME	5 min
CYCLES	2
RINSE VOLUME	150%

Sediment from Lake Winona and Little Wilson Lake had higher organic content compared to Duluth Harbor and Lake Pepin. Less sediment (0.5 g) was extracted for the lakes with higher organic content to facilitate the solid phase extraction (SPE) clean up step. Methanol was removed from the ASE extract using a rotary evaporator in a 35 °C water bath prior to clean-up.

Ultrasound Assisted Extraction

The ultrasound assisted extraction (UAE) method was adapted from Wallace and Aga.[198] Sediment (0.5 g) was mixed with Ottawa sand (2.5 g) in a 15-mL plastic centrifuge tube. Centrifuge tubes were cleaned by soaking in 2% nitric acid (8 hours) then rinsed with ultrapure water and air-dried. The mixture was suspended in 10 mL of 20:30:50 acetonitrile: methanol: 0.1 M ethylenediaminetetraacetic acid (EDTA)/0.08 M disodium phosphate/0.06 M citrate buffer (pH 4, v/v/v) solution, vortexed (30 sec), placed in an ultrasound bath (40 kHz, 10 min), and subsequently centrifuged (3300 rpm, 10 min). The UAE was repeated two additional times per sample and extracts were

combined. The organic solvents were removed from the UAE extract using a rotary evaporator in a 35 °C water bath prior to clean-up.

Solid Phase Extraction

The solid phase extraction (SPE) method was adapted from Meyer 2007[197] to remove interferences from the extract and concentrate the sample. The Little Wilson Lake extracts had to be diluted to 500 mL with ultrapure water. ASE aqueous extracts were spiked with 250 µL of 20:80 formic acid: 10% sodium chloride/0.5% EDTA solution. UAE aqueous extracts were diluted to 400 mL with ultrapure water and pH adjusted to 4 with phosphoric acid. Fluoropolymer tubing (Saint-Gobain Chemofluor) and an SPE adapter was used to transfer the Little Wilson Lake extract and UAE extracts to the SPE cartridges. Tubing was never reused and cleaned with methanol (LC-MS grade) and water prior to use.

Liquid Chromatography Tandem Mass Spectrometry Analysis

Two liquid chromatography methods were used to analyze the ASE and UAE extracts, see Table S2. The gradient elution program for the ASE extracts is located in Table S3 and for the UAE extracts in Table S4. Due to the number of analytes included in the study, each ASE sample was analyzed by three HPLC-MS/MS methods that monitored for: (1) sulfonamides and surrogates; (2) tetracyclines and fluoroquinolones; and (3) others and macrolides. UAE samples were also analyzed by three methods: (1) sulfonamides, $^{13}C_6$-sulfamethazine, and others; (2) tetracyclines, fluoroquinolones, demeclocycline, and nalidixic acid; and (3) macrolides.

Table C.2. Liquid chromatography separation methods for samples extracted via accelerated solvent and ultrasound assisted extraction methods.

Liquid Chromatography Method		
	Method 1	*Method 2*
Extraction Method	Accelerated Solvent	Ultrasound Assisted
Instrument	Apilent 1100 HPLC	Thermo Dionex Ultimate 3000 RSI nano
Stationary Phase	Phenomenex Kinetex F5 (1.7 µm, 100 Å, 50 × 2.1 mm)	Waters Xselect CSH C18 (3.5 µm, 130 Å, 50 × 2.1 mm)
Flow rate	250 µL/min	500 µL/min
Temperature	50 °C	35 °C
Injection Volume	8 µL	8 µL
Flow Diverted To Waste	0 – 1 min 7 – 25 min	0 – 1.5 min 5.5 – 20 min
Mobile Phase A	0.1 % formic acid in water	0.1 % formic acid in water
Mobile Phase B	0.1 % formic acid in acetonitirle	0.1 % formic acid in methanol

Table C.3. Gradient elution of 0.1% formic acid in acetonitrile (% B) with respect to time (min) on Phenomenex Kinetex F5 column for ASE extracts.

Time (min)	*% B*
0	5
1	5
5	100
7	100
7.5	5
25	5

Table C.4. Gradient elution of 0.1% formic acid in methanol (% B) with respect to time (min) on Waters XSelect CSH C18 column that separated sulfonamides, macrolides, and others via method 1 and fluoroquinolones and tetracyclines via method 2 in UAE extracts.

Method 1		Method 2	
Time (min)	*% B*	*Time (min)*	*% B*
0.0	0	0.0	0
5.5	100	0.5	0
7.5	100	4.0	40
8.0	0	7.0	100
20.0	0	9.0	100
--	--	10.0	0
--	--	20.0	0

Analytes were detected and quantified with the single reaction monitoring (SRM) transitions in Table S5 with Thermo Vantage triple quadrupole tandem mass spectrometer in positive ESI mode. Confirmation SRMs were used to corroborate the identity of quantified peak. Mass spectrometry sensitivity varied between analyses, thus parameters were optimized with the infusion of 5µM simeton in 50:50 20 mM ammonium acetate:acetonitrile solution prior to each analysis. Typical values for mass spectrometer parameters were: scan time 0.02 sec; scan width: 0.15; Q_1/Q_3: 0.7; spray voltage: 3300 V; sheath gas pressure: 18 psi; capillary temperature: 300 °C; collision pressure: 1.5 mTorr; declustering voltage: -9 V; and tube lens: 95.

Table C.5. Single reaction monitoring quantification and confirmation transitions and collision energy (CE) for analytes.

Analyte	Parent Ion (m/z)	Product Ion (m/z)	CE (V)	Quantification or Confirmation
Sulfonamides				
sulfapyridine	250.10	156.00	17	quantification
	250.10	108.05	25	confirmation
sulfadiazine	251.05	156.00	15	quantification
	251.05	108.05	24	confirmation
sulfamethoxazole	254.05	92.10	29	quantification
	254.05	108.00	24	confirmation
sulfamethazine	279.05	186.00	17	quantification
	279.05	156.00	20	confirmation
sulfachloropyridazine	285.00	156.06	15	quantification
	285.00	92.05	35	confirmation
sulfadimethoxine	311.10	156.06	21	quantification
	311.10	92.05	35	confirmation
$^{13}C_6$-sulfamethoxazole	260.05	98.10	32	quantification
(internal standard)	260.05	114.10	27	confirmation
$^{13}C_6$-sulfamethazine	285.05	186.00	22	quantification
(*surrogate)*	285.05	123.00	20	confirmation
Fluoroquinolones				
norfloxacin	320.10	276.10	17	quantification
	320.10	302.10	21	confirmation
ciprofloxacin	332.10	231.05	35	quantification
	332.10	314.10	21	confirmation
enrofloxacin	360.10	245.10	25	quantification
	360.10	316.15	19	confirmation
ofloxacin	362.10	261.10	28	quantification
	362.10	318.10	19	confirmation
clinafloxacin	366.10	348.00	20	confirmation
(*internal standard)*	366.10	305.00	22	quantification
nalidixic acid	233.15	187.00	27	confirmation
(*surrogate*)	233.15	104.05	40	quantification
Tetracyclines				
tetracycline	445.10	410.10	19	quantification
	445.10	427.05	11	confirmation
doxycycline	445.10	321.05	31	quantification
	445.10	428.15	18	confirmation
oxytetracycline	461.10	426.10	17	quantification
	461.10	443.10	12	confirmation

Table C.5. Continued.

chlortetracycline	479.05	462.10	20	quantification
& degradation products	479.05	444.10	17	confirmation
	481.05	464.10	20	quantification
	481.05	446.10	30	confirmation
demeclocycline	465.10	448.05	20	quantification
(*surrogate*)	465.10	430.05	17	confirmation
Macrolides				
erythromycin	734.4	158.15	35	quantification
	734.4	576.35	15	confirmation
erythromycin-H_2O	716.45	158.15	35	quantification
	716.45	558.35	15	confirmation
roxithromycin	837.45	158.10	35	quantification
	837.45	679.45	20	confirmation
tylosin	916.45	174.10	40	quantification
	916.45	772.45	30	confirmation
$^{13}C_2$-erythromycin	736.40	160.15	35	quantification
	736.40	578.35	20	confirmation
	718.40	160.15	35	quantification
$^{13}C_2$-erythromycin-H_2O	718.40	560.35	20	confirmation
Non-categorized				
carbadox	263.10	130.05	22	quantification
	263.10	231.05	13	confirmation
trimethoprim	291.10	230.10	23	quantification
	291.10	123.05	24	confirmation
lincomycin	407.30	126.10	35	quantification
	407.30	359.20	18	confirmation
simeton	198.20	68.10	33	quantification
(*internal standard)*	198.20	100.10	27	confirmation

Equations Used to Calculate Analyte Concentrations in Environmental Samples

The response factor (RF) for 'X' antibiotic was calculated using the slope of the calibration curve (m) and concentration of internal standard [IS]:

$$RF = \frac{1}{[IS] \cdot m} \quad \textbf{(C-1)}$$

$$m = \frac{X_{std}^{PA}}{IS_{std}^{PA} \cdot [X_{std}]} \quad \textbf{(C-2)}$$

where the slope (m) was determined with the ratio of peak area (PA) of 'X' to internal standard in standards (std) . The mass (g) of 'X' in sediment sample was calculated using:

$$X_{sample}(g) = RF \times \frac{X_{sample}^{PA}}{IS_{sample}^{PA}} \times IS_{sample}(g) - X_{blank} \quad \textbf{(C-3)}$$

where IS_{sample} is the mass of internal standard spiked onto the sediment and X_{blank} is the mass in the method blank. The sediment concentrations were normalized by the mass of sediment extracted. Triplet relative recoveries were determined from Ottawa sand and sediments from deep within the core for each analyte. Relative recovery (RR) was calculated with the following:

$$RR = \frac{X_{spiked,sample} - X_{sample,avg}}{X_{spiked}} \times 100 \quad \textbf{(C-4)}$$

Antibiotic accumulation rates were derived from multiplying sediment concentrations by sediment accumulation rates (SedAccum) and correcting for focusing factor (FF).

$$X_{sediment}\left(\frac{g}{cm^2 \cdot yr}\right) = \frac{X_{sample}}{sediment\ (g) \cdot RR \cdot FF} \times SedAccum\left(\frac{g}{cm^2 \cdot yr}\right) \quad \textbf{(C-5)}$$

The focusing factor and sediment accumulation rates were calculated from lead-210 depositions as previously described by Anger et al.[44] Sediment focusing corrected gives

insight into whole-lake antibiotic accumulation rates. The absolute recovery (AbsRec) of internal standards was calculated using:

$$AbsRec = \frac{IS^{PA}_{sample}}{IS^{PA}_{std}(avg)} \times \frac{[IS_{std}]}{[IS_{sample}]} \times 100 \qquad \textit{(C-6)}$$

where IS^{PA}_{std} (avg) is the average peak area of internal standards in standards.

C.2 Results

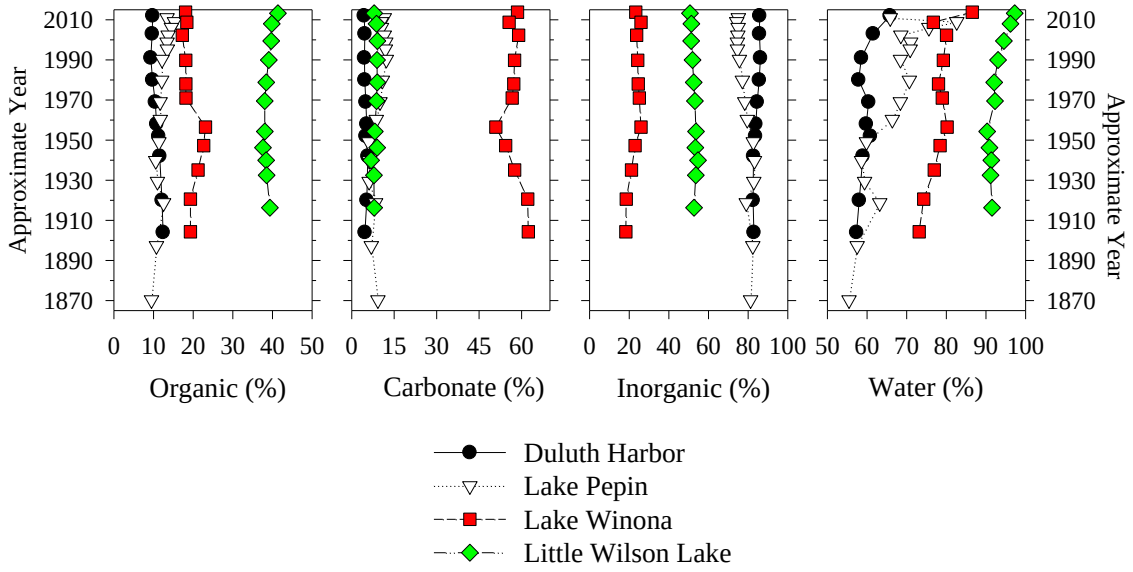

Figure C.2. Percent organic, carbonate, and inorganic of Duluth Harbor, Lake Pepin, Lake Winona, and Little Wilson Lake sediment cores and percent water of sample determined by loss-on-ignition.

Table C.6. Percent organic, carbonate, and inorganic of Duluth Harbor sediment core and percent water of sample determined by loss-on-ignition.

Duluth Harbor

Interval Top (cm)	*Interval Base (cm)*	*Year*	*Organic %*	*Carbonate %*	*Inorganic %*	*Water %*
0	2	2012	9.8	4.4	85.8	65.8
4	6	2003	9.7	4.6	85.7	61.6
8	10	1991	9.3	4.5	86.2	58.6
12	14	1980	9.8	4.6	85.6	57.9
16	18	1969	10.4	5.0	84.6	60.4
20	22	1958	10.8	5.3	83.9	59.8
22	24	1952	11.3	5.0	83.7	60.8
24	26	1942	11.6	5.7	82.7	59.0
28	30	1920	12.1	5.3	82.5	58.0
30	32	1904	12.4	4.7	82.9	57.4

Table C.7. Percent organic, carbonate, and inorganic of Little Wilson Lake sediment core and percent water of sample determined by loss-on-ignition.

Little Wilson Lake

Interval Top (cm)	*Interval Base (cm)*	*Year*	*Organic %*	*Carbonate %*	*Inorganic %*	*Water %*
0	2	2013	41.4	7.9	50.7	97.3
6	8	2008	39.8	8.8	51.4	96.2
12	14	1999	39.7	9.1	51.3	94.5
16	18	1990	39.1	8.9	52.0	93.1
20	22	1979	38.4	9.0	52.6	92.1
24	26	1970	38.1	8.8	53.2	92.3
32	34	1954	38.1	8.2	53.7	90.3
36	38	1946	37.6	9.0	53.4	90.9
40	42	1940	38.4	6.9	54.7	91.4
44	46	1932	38.5	7.9	53.6	91.2
52	54	1916	39.4	7.9	52.7	91.5

Table C.8. Percent organic, carbonate, and inorganic of Lake Pepin sediment core and percent water of sample determined by loss-on-ignition.

Lake Pepin

Interval Top (cm)	*Interval Base (cm)*	*Year*	*Organic %*	*Carbonate %*	*Inorganic %*	*Water %*
0	4	2011	13.4	11.5	75.1	84.5
4	6	2009	15.3	10.5	74.3	82.6
6	8	2006	14.5	10.5	75.0	75.6
8	10	2002	13.7	11.5	74.8	68.5
10	12	1999	13.4	12.0	74.6	71.0
12	14	1995	13.5	11.9	74.6	70.9
20	24	1990	12.2	12.2	75.6	68.4
36	40	1980	12.1	10.8	77.1	70.7
52	56	1969	11.7	10.0	78.3	68.4
64	68	1960	11.7	8.7	79.6	66.4
80	84	1949	11.4	6.0	82.6	59.9
92	96	1940	10.6	6.2	83.2	58.6
104	108	1929	11.0	6.2	82.9	59.4
116	120	1919	12.5	8.4	79.1	63.2
128	136	1897	10.7	6.9	82.4	57.5
144	152	1870	9.5	9.3	81.3	55.5

Table C.9. Percent organic, carbonate, and inorganic of Lake Winona sediment core and percent water of sample determined by loss-on-ignition.

Lake Winona

Interval Top (cm)	*Interval Base (cm)*	*Year*	*Organic %*	*Carbonate %*	*Inorganic %*	*Water %*
0	2	2014	18.1	58.6	23.3	86.6
8	10	2009	18.4	55.6	26.0	76.7
16	18	2002	17.3	59.0	23.8	80.1
28	30	1990	18.1	57.5	24.3	79.3
36	38	1978	18.2	57.3	24.6	78.0
40	42	1971	18.2	56.7	25.1	79.0
48	50	1957	23.1	50.9	26.0	80.2
52	54	1947	22.7	54.4	23.0	78.4
56	58	1935	21.2	57.6	21.2	77.0
60	62	1921	19.3	62.2	18.5	74.3
64	66	1904	19.3	62.4	18.3	73.2

Table C.10. Lead-210 dating for Duluth Harbor sediment core.

Top of Interval (cm)	Base of Interval (cm)	Cum. Dry Mass (g/cm2)	Unsup. Activity (pCi/g)	Error of Unsup. Act. (±s.d.)	Cum. Act. below Int. (pCi/cm2)	Age: Base of Int. (yr)	Error of Age (±s.d.)	Date A.D.	Sediment Accum. (g/cm2 yr)	Error of Sed. Accum. (±s.d.)
0	2	0.8139	2.7010	0.1048	21.4013	3.14	1.61	2011.5	0.2592	0.01323
2	4	1.6541	2.6013	0.1470	19.2158	6.60	1.69	2008.0	0.2429	0.01603
4	6	2.5916	2.9068	0.1046	16.4908	11.51	1.86	2003.1	0.1909	0.01047
6	8	3.5564	3.0174	0.1394	13.5797	17.75	2.10	1996.9	0.1547	0.01023
8	10	4.5859	2.1382	0.1031	11.3784	23.43	2.39	1991.2	0.1813	0.01363
10	12	5.5916	1.7351	0.1130	9.6334	28.77	2.71	1985.9	0.1881	0.01754
12	14	6.6391	1.4573	0.0658	8.1069	34.31	3.14	1980.3	0.1891	0.01775
16	18	8.6117	1.1237	0.0862	5.7290	45.46	4.16	1969.2	0.1734	0.02335
18	20	9.5510	0.9239	0.0611	4.8612	50.74	4.84	1963.9	0.1781	0.02645
20	22	10.5316	0.8682	0.0585	4.0098	56.92	5.80	1957.7	0.1586	0.02738
22	24	11.4795	0.7289	0.0568	3.3189	62.99	6.95	1951.6	0.1561	0.03236
24	26	12.4803	0.8006	0.0562	2.5177	71.87	9.09	1942.8	0.1128	0.02854
26	28	13.5329	0.6235	0.0566	1.8614	81.56	12.21	1933.1	0.1085	0.03646
28	30	14.5577	0.6239	0.0710	1.2220	95.08	18.45	1919.5	0.0758	0.03618
30	32	15.6019	0.4508	0.0550	0.7513	110.70	29.88	1903.9	0.0668	0.04961
32	34	16.7217	0.3046	0.0543	0.4102	130.13	54.49	1884.5	0.0576	0.07371

Supported Pb-210:	0.8291 ± 0.0488 pCi/g
Number of Supported Samples:	5

Cum. Unsup. Pb-210:	23.5996 pCi/cm2
Unsup. Pb-210 Flux:	0.7541 pCi/cm2 yr

Table C.11. Lead-210 dating for Little Wilson Lake sediment core.

Top of Interval (cm)	Base of Interval (cm)	Cum. Dry Mass (g/cm2)	Unsup. Activity (pCi/g)	Error of Unsup. Act. (±s.d.)	Cum. Act. below Int. (pCi/cm2)	Age: Base of Int. (yr)	Error of Age (±s.d.)	Date A.D.	Sediment Accum. (g/cm2 yr)	Error of Sed. Accum. (±s.d.)
0	2	0.0546	25.4609	0.4857	31.3287	1.39	1.09	2013.2	0.0392	0.00119
6	8	0.2622	21.6343	0.7292	26.5960	6.65	1.16	2008.0	0.0395	0.00166
12	14	0.5573	21.0115	0.3793	20.3363	15.27	1.36	1999.4	0.0318	0.00120
16	18	0.8208	19.2861	0.6899	15.1486	24.73	1.67	1989.9	0.0266	0.00144
20	22	1.1288	12.9451	0.4243	10.7322	35.80	1.75	1978.8	0.0282	0.00153
24	26	1.4411	7.4394	0.2469	8.0300	45.11	1.52	1969.5	0.0360	0.00178
28	30	1.7761	4.1510	0.2053	6.4035	52.38	1.23	1962.2	0.0507	0.00278
32	34	2.1550	3.5489	0.1083	5.0052	60.29	1.39	1954.3	0.0469	0.00209
36	38	2.5281	2.8277	0.1382	3.8849	68.43	1.57	1946.2	0.0456	0.00278
40	42	2.8806	1.6898	0.0825	3.1964	74.69	1.53	1939.9	0.0616	0.00378
44	46	3.2342	1.9357	0.0796	2.5341	82.15	1.81	1932.5	0.0435	0.00268
48	50	3.5808	1.5568	0.0524	1.9625	90.36	2.18	1924.3	0.0419	0.00283
52	54	3.9217	1.1752	0.0676	1.5306	98.34	2.62	1916.3	0.0432	0.00395
56	58	4.2597	1.1999	0.0654	1.1271	108.17	3.47	1906.5	0.0318	0.00348
60	64	4.7805	0.7735	0.0467	0.6822	124.29	5.28	1890.3	0.0326	0.00481
68	72	5.5141	0.3299	0.0470	0.3657	144.31	6.49	1870.3	0.0401	0.00857
76	80	6.2184	0.2273	0.0471	0.1875	165.77	11.67	1848.9	0.0307	0.01082

Supported Pb-210:	1.0607 ± 0.0196 pCi/g
Number of Supported Samples:	3

Cum. Unsup. Pb-210:	32.7191 pCi/cm2
Unsup. Pb-210 Flux:	1.0463 pCi/cm2 yr

Table C.12. Lead-210 dating for Lake Winona sediment core.

Top of Interval (cm)	Base of Interval (cm)	Cum. Dry Mass (g/cm2)	Unsup. Activity (pCi/g)	Error of Unsup. Act. (±s.d.)	Cum. Act. below Int. (pCi/cm2)	Age: Base of Int. (yr)	Error of Age (±s.d.)	Date A.D.	Sediment Accum. (g/cm2 yr)	Error of Sed. Accum. (±s.d.)
0	2	0.2732	3.2177	0.1351	32.1361	0.87	0.63	2013.8	0.3152	0.01376
8	10	1.8833	2.8175	0.1209	27.3804	6.01	0.61	2008.7	0.3100	0.01361
16	18	3.6277	2.8633	0.0966	22.4162	12.43	0.67	2002.3	0.2501	0.00907
24	26	5.2343	3.0162	0.0740	17.6637	20.09	0.74	1994.6	0.1884	0.00561
28	30	6.0725	2.9341	0.0836	15.1878	24.94	0.81	1989.8	0.1678	0.00575
32	34	6.9469	2.4476	0.0791	12.9466	30.06	0.87	1984.6	0.1715	0.00659
36	38	7.8454	2.7532	0.0694	10.5429	36.66	1.00	1978.0	0.1262	0.00449
40	42	8.7196	2.2644	0.0642	8.4601	43.73	1.12	1971.0	0.1229	0.00498
44	46	9.5535	1.9598	0.0739	6.7637	50.91	1.31	1963.8	0.1138	0.00580
48	50	10.3643	1.6169	0.0569	5.3858	58.23	1.55	1956.5	0.1099	0.00603
52	54	11.3189	1.3542	0.0618	4.0359	67.49	1.95	1947.2	0.1004	0.00695
56	58	12.3717	1.1537	0.0532	2.7711	79.57	2.70	1935.1	0.0828	0.00713
60	62	13.4289	0.8926	0.0479	1.7619	94.11	3.98	1920.6	0.0693	0.00830
64	66	14.5179	0.5728	0.0402	1.0593	110.45	6.02	1904.3	0.0658	0.01157
66	68	15.0851	0.6065	0.0411	0.7153	123.06	8.84	1891.6	0.0450	0.01054
68	72	16.2849	0.3928	0.0369	0.2440	157.60	25.23	1857.1	0.0347	0.01681
72	76	17.3066	0.1221	0.0330	0.1192	180.60	50.83	1834.1	0.0444	0.05088

Supported Pb-210:	0.2217 ± 0.0311 pCi/g
Number of Supported Samples:	2

Cum. Unsup. Pb-210:	33.0153 pCi/cm2
Unsup. Pb-210 Flux:	1.061 pCi/cm2 yr

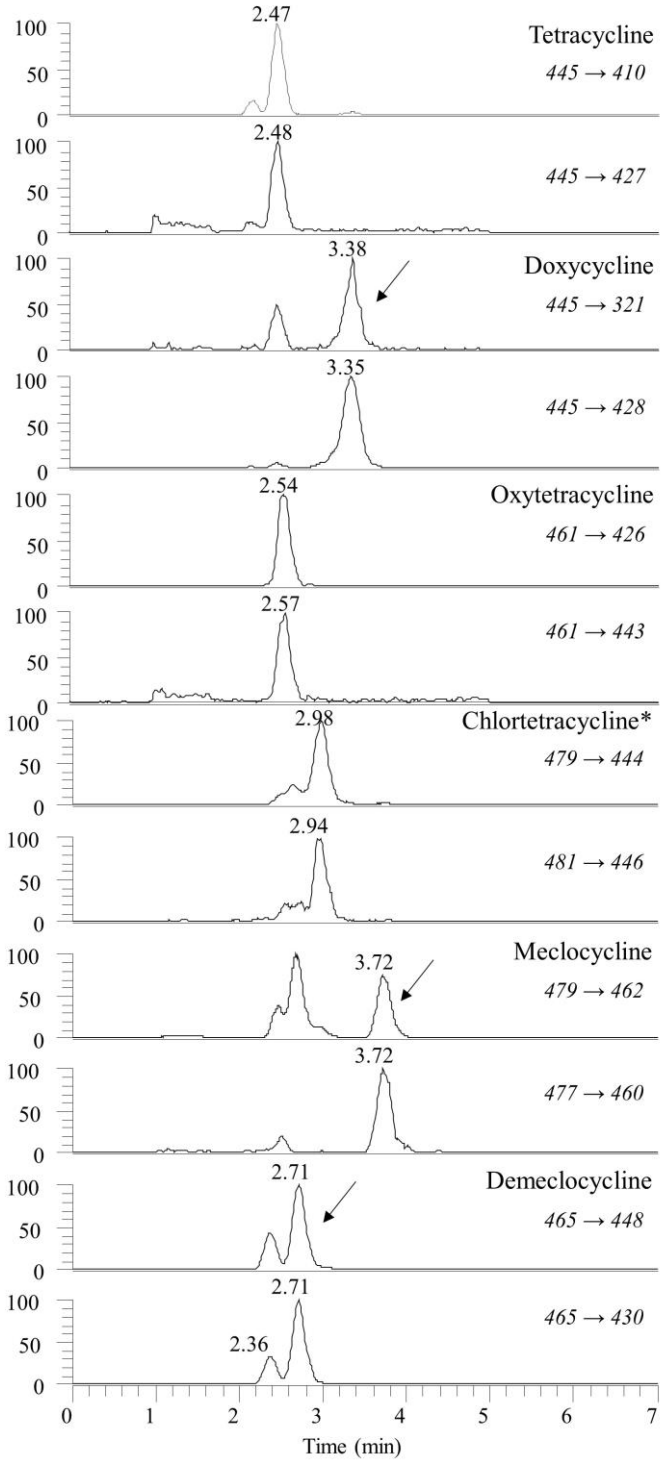

Figure C.3. LC-MS/MS chromatogram of tetracyclines including single reaction monitoring transitions in representative standard. Antibiotic retention times are displayed above respective peaks. Separation achieved with Waters XSelect CSH C18 column.

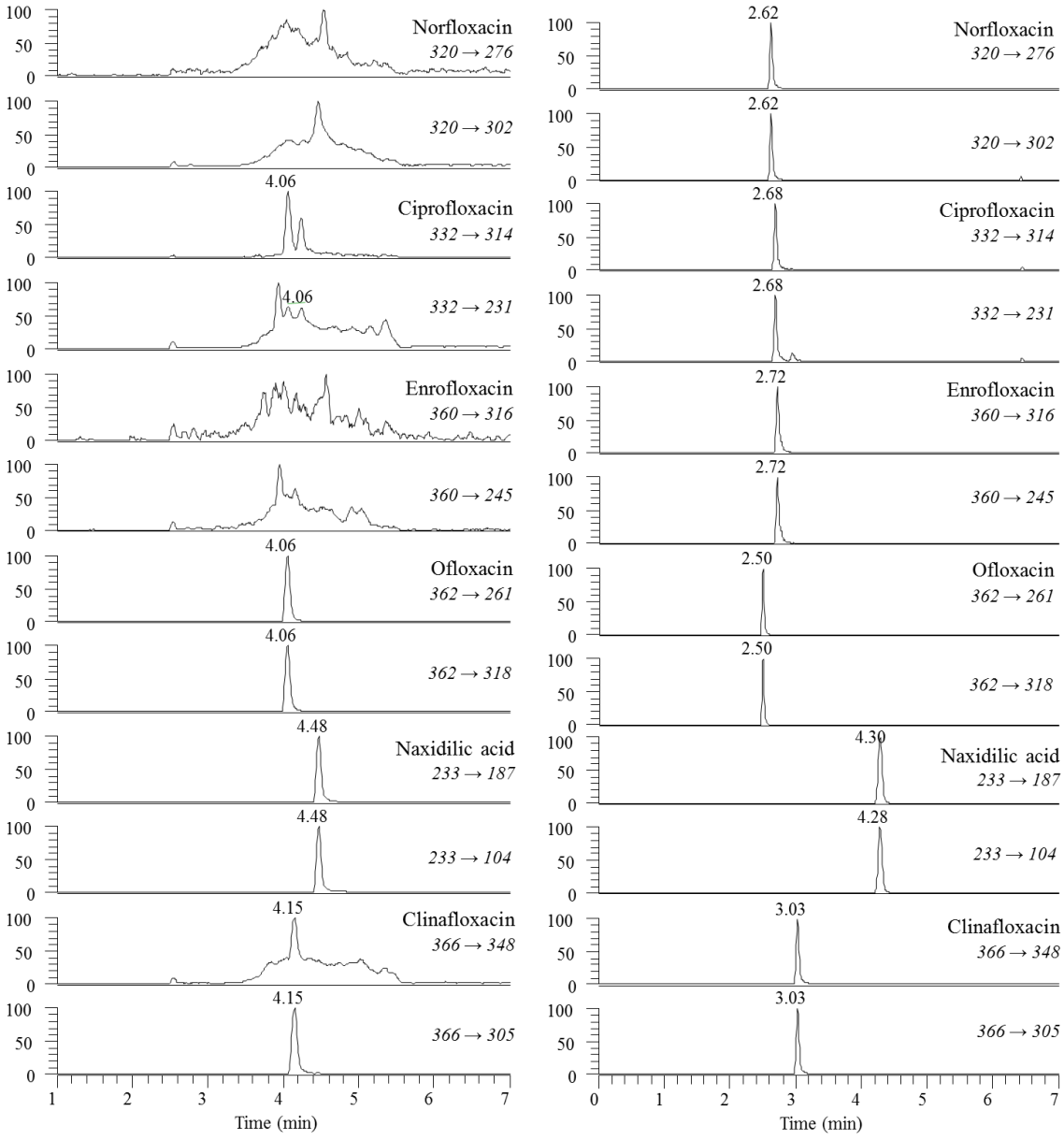

Figure C.4. LC-MS/MS chromatograms of fluoroquinolones including single reaction monitoring transitions in representative sediment sample (left) and standard (right). Antibiotic retention times are displayed above respective peaks. Separation was achieved with Phenomenex Kinetex F5 column.

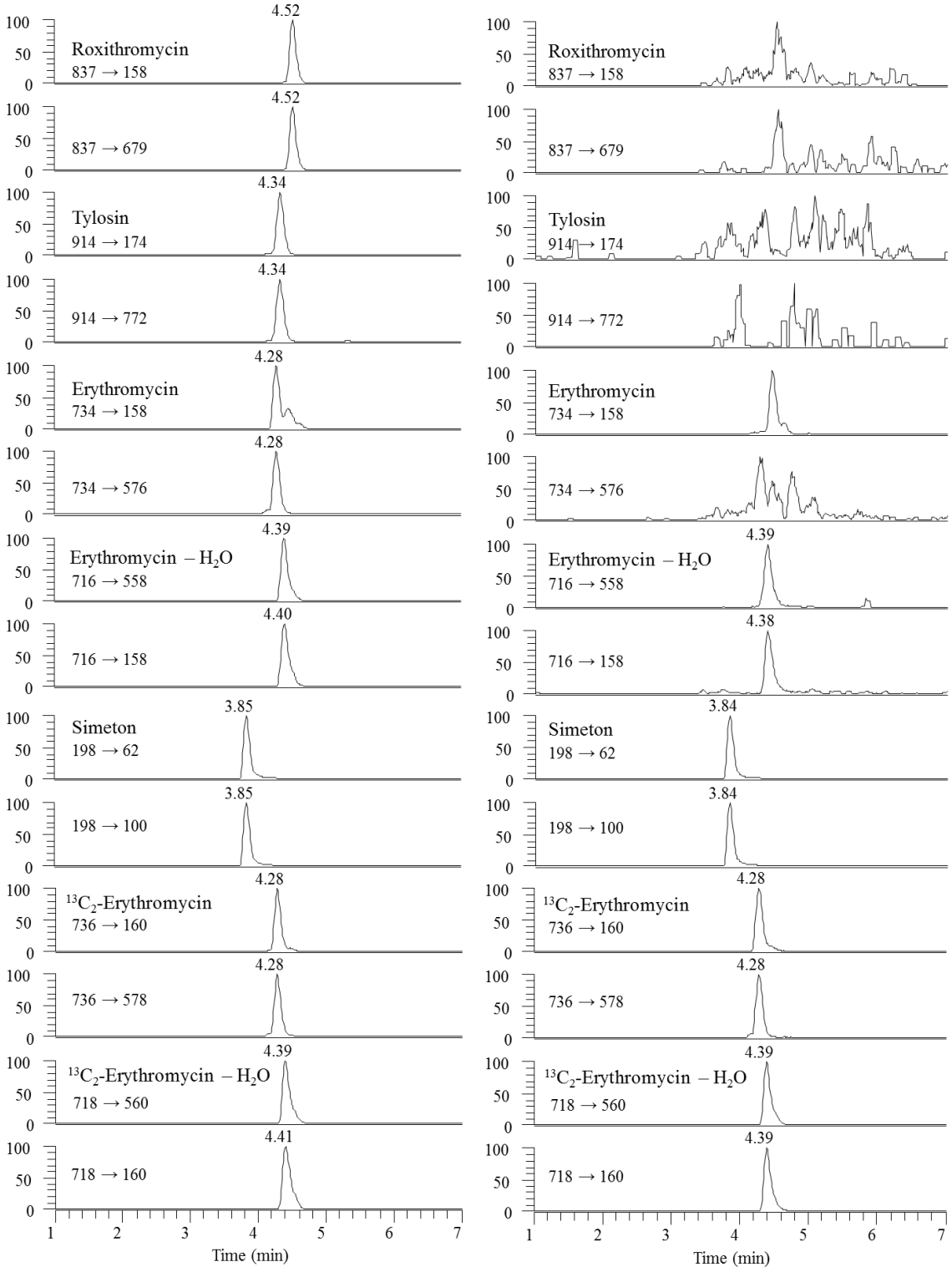

Figure C.5. LC-MS/MS chromatograms of macrolides including single reaction monitoring transitions in representative standard (left) and sediment sample (right). Antibiotic retention times are displayed above respective peaks. Separation was achieved with Phenomenex Kinetex F5 column.

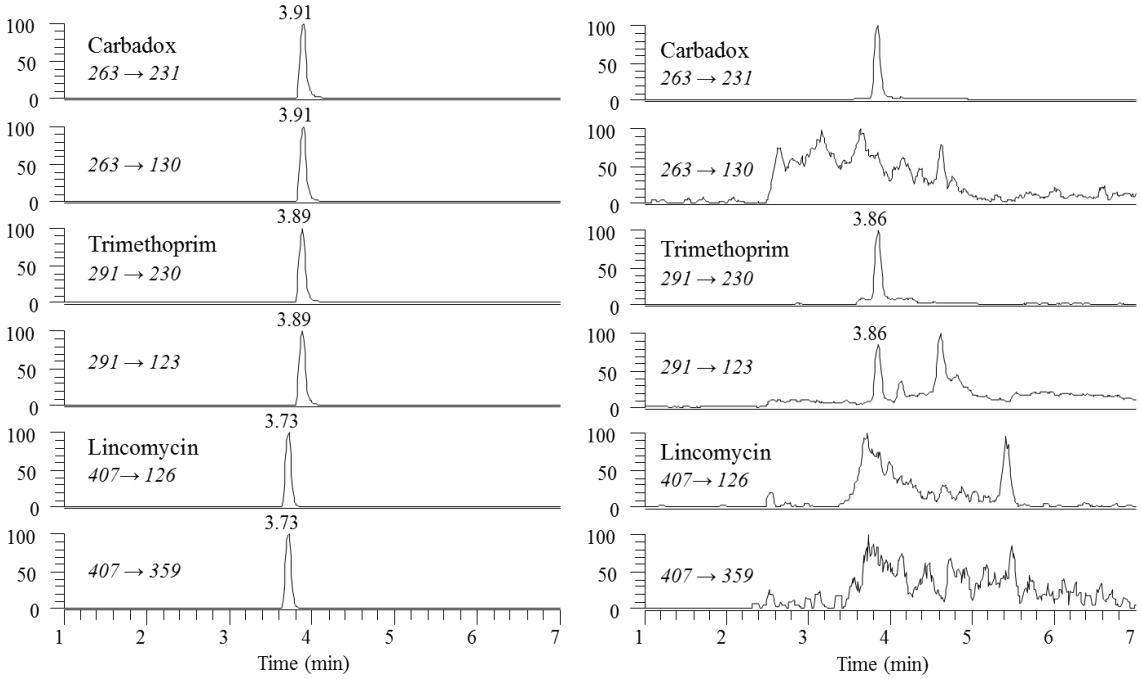

Figure C.6. LC-MS/MS chromatograms of carbadox, trimethoprim, and lincomycin including single reaction monitoring transitions in representative standard (left) and sediment sample (right). Antibiotic retention times are displayed above respective peaks. Separation was achieved with Phenomenex Kinetex F5 column.

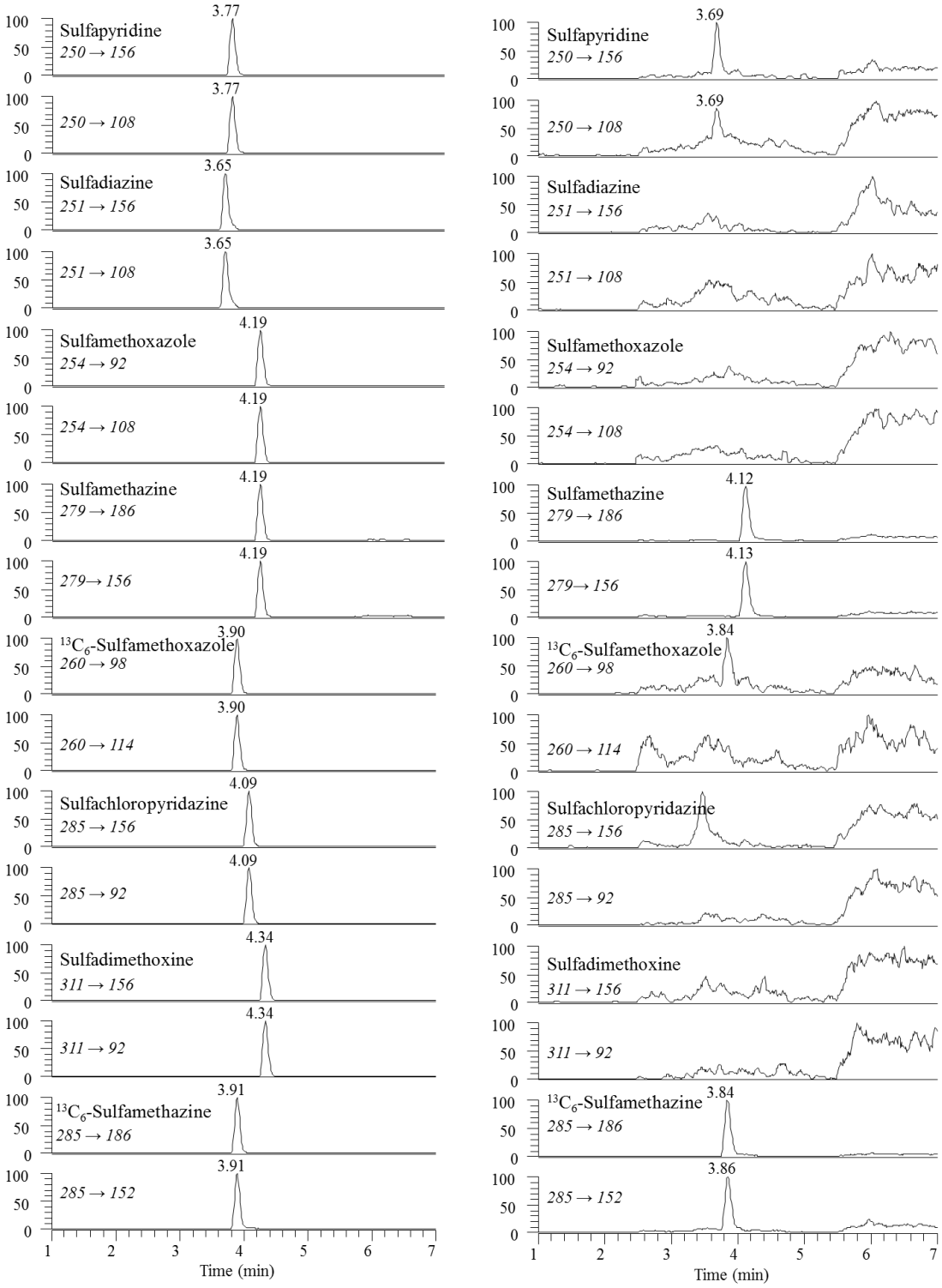

Figure C.7. LC-MS/MS chromatograms of sulfonamides including single reaction monitoring transitions in representative standard (left) and sediment sample (right). Antibiotic retention times are displayed above respective peaks. Separation was achieved with Phenomenex Kinetex F5 column.

Table C.13. Average and standard deviation for antibiotic limit of detections (LODs) and quantification (LOQs) in four sediment cores extracted by the ASE method.

LOQs and LODs for ASE Method

Antibiotic	LOD [ng/g]	LOQ [ng/g]
Sulfonamides		
Sulfapyridine	0.18 ± 0.07	0.54 ± 0.22
Sulfadiazine	0.23 ± 0.16	0.70 ± 0.48
Sulfamethoxazole	0.35 ± 0.23	1.04 ± 0.68
Sulfamethazine	0.20 ± 0.09	0.59 ± 0.26
Sulfachloropyridazine	0.45 ± 0.22	1.34 ± 0.65
Sulfadimethoxine	0.31 ± 0.05	0.94 ± 0.15
Tetracyclines		
Tetracycline	2.1 ± 1.9	5.3 ± 5.8
Doxycycline	3.7 ± 4.0	11.0 ± 11.9
Oxytetracycline	8.1 ± 9.8	21.3 ± 23.3
Chlortetracycline	4.5 ± 3.9	13.7 ± 11.5
Fluoroquinolones		
Norfloxacin	5.7 ± 9.1	17.1 ± 27.3
Ciprofloxacin	7.5 ± 8.2	22.4 ± 24.7
Enrofloxacin	0.92 ± 0.36	2.77 ± 1.08
Ofloxacin	0.11 ± 0.10	0.32 ± 0.29
Macrolides		
Erythromycin	0.59 ± 0.41	1.90 ± 1.38
Roxithromycin	0.15 ± 0.06	0.49 ± 0.20
Tylosin	1.0 ± 1.8	2.9 ± 5.5
Others		
Carbadox	0.59 ± 0.41	1.86 ± 1.28
Trimethoprim	0.19 ± 0.12	0.55 ± 0.37
Lincomvcin	0.18 ± 0.23	0.53 ± 0.68

Table C.14. Absolute recovery internal standards and relative recovery of surrogates and target antibiotics from *n* number of sediment and Ottawa sand samples via the ASE method.

Absolute & Relative Recovery (%) for ASE Method

Antibiotic	Ottawa Sand	*n*	Duluth Harbor	*n*	Lake Pepin	*n*	Lake Winona	*n*	Little Wilson Lake	*n*
					Sulfonamides					
Sulfapyridine	105 ± 31	*11*	160 ± 4	*3*	185 ± 9	*3*	184 ± 19	*3*	70 ± 18	*3*
Sulfadiazine	68 ± 45	*11*	201 ± 6	*3*	210 ± 16	*3*	224 ± 15	*3*	78 ± 28	*3*
Sulfamethoxazole	105 ± 7	*11*	93 ± 1	*3*	144 ± 4	*3*	110 ± 6	*3*	98 ± 5	*3*
Sulfamethazine	130 ± 50	*11*	156 ± 13	*3*	156 ± 9	*3*	178 ± 11	*3*	144 ± 30	*3*
Sulfachloropyridazine	97 ± 21	*11*	93 ± 2	*3*	109 ± 7	*3*	137 ± 8	*3*	152 ± 22	*3*
Sulfadimethoxine	140 ± 49	*11*	121 ± 9	*3*	108 ± 7	*3*	153 ± 12	*3*	78 ± 13	*3*
$^{13}C_6$-Sulfamethazine[a]	180 ± 50	*15*	157 ± 14	*15*	147 ± 12	*19*	139 ± 25	*17*	177 ± 72	*17*
$^{13}C_6$-Sulfamethoxazole[b]	71 ± 39	*15*	11 ± 2	*15*	17 ± 10	*19*	45 ± 5	*17*	18 ± 4	*17*
					Tetracyclines					
Tetracyclines	30 ± 31	*11*	9 ± 1	*3*	8 ± 1	*3*	19 ± 5	*3*	12 ± 3	*3*
Doxycycline	74 ± 40	*11*	14 ± 2	*3*	12 ± 3	*3*	18 ± 4	*3*	13 ± 1	*3*
Oxytetracycline	5 ± 5	*11*	4 ± 1	*3*	5 ± 1	*3*	5 ± 1	*3*	1±1	*3*
Chlortetracycline	54 ± 55	*11*	121 ± 28	*3*	122 ± 13	*3*	71 ± 11	*3*	108 ± 40	*3*
Demeclocycline[a]	50 ± 33	*15*	16 ± 4	*15*	18 ± 15		212 ± 97	*17*	39 ± 8	*17*
					Fluoroquinolones					
Norfloxacin	66 ± 47	*11*	5 ± 1	*3*	9 ± 2	*3*	24 ± 4	*3*	29 ± 23	*3*
Ciprofloxacin	48 ± 29	*11*	3 ± 0	*3*	6 ± 1	*3*	18 ± 3	*3*	10 ± 1	*3*
Enrofloxacin	102 ± 55	*11*	15 ± 3	*3*	17 ± 2	*3*	49 ± 6	*3*	12 ± 3	*3*
Ofloxacin	68 ± 56	*11*	18 ± 3	*3*	29 ± 3	*3*	59 ± 4	*3*	18 ± 4	*3*
Nalidixic Acid[a]	123 ± 55	*15*	38 ± 7	*15*	51 ± 9	*19*	54 ± 6	*17*	43 ± 43	*17*
Clinafloxacin[b]	29 ± 25	*15*	2 ± 0	*15*	10 ± 3	*19*	23 ± 6	*17*	6 ± 1	*17*
					Macrolides					
Erythromycin	131 ± 49	*11*	151 ± 5	*3*	96 ± 8	*3*	125 ± 7	*3*	142 ± 16	*3*
Roxithromycin	126 ± 62	*11*	141 ± 27	*3*	85 ± 6	*3*	161 ± 4	*3*	146 ± 21	*3*
Tylosin	277 ± 258	*11*	360 ± 41	*3*	94 ± 19	*3*	169 ± 44	*3*	225 ± 22	*3*
$^{13}C_2$-Erythromycin[b]	21 ± 18	*15*	11 ± 2	*15*	24 ± 6	*19*	55 ± 9	*17*	12 ± 3	*17*

Table C.14. Continued.

Others										
Carbadox	8 ± 7	*11*	19 ± 3	*3*	12 ± 2	*3*	15 ± 8	*3*	39 ± 29	*3*
Trimethoprim	78 ± 68	*11*	48 ± 4	*3*	49 ± 0	*3*	68 ± 11	*3*	82 ± 6	*3*
Lincomvcin	79 ± 55	*11*	58 ± 39	*3*	57 ± 52	*3*	72 ± 52	*3*	--	*3*
Simeton[b]	38 ± 11	*15*	48 ± 5	*15*	53 ± 9	*19*	54 ± 8	*17*	37 ± 3	*17*

[a] denotes surrogate
[b] denotes internal standard

Table C.15. Absolute recoveries of internal standards and relative recoveries of surrogates and target antibiotics from *n* number of sediment and Ottawa sand samples via the UAE method.

Relative and Absolute Recovery (%) for UAE Method

Antibiotic	Lake Pepin	*n*	Lake Winona	*n*
	Sulfonamides			
Sulfapyridine	60 ± 4	*3*	87 ± 2	*3*
Sulfadiazine	100 ± 5	*3*	92 ± 2	*3*
Sulfamethoxazole	106 ± 1	*3*	103 ± 4	*3*
Sulfamethazine	69 ± 5	*3*	93 ± 2	*3*
Sulfachloropyridazine	96 ± 4	*3*	100 ± 5	*3*
Sulfadimethoxine	71 ± 5	*3*	71 ± 5	*3*
$^{13}C_6$-Sulfamethazine[a]	69 ± 8	*19*	87 ± 6	*17*
$^{13}C_6$-Sulfamethoxazole[b]	43 ± 6	*19*	55 ± 5	*17*
	Tetracyclines			
Tetracycline	--		34 ± 3	*3*
Doxycycline	--		45 ± 6	*3*
Oxytetracycline	--		66 ± 2	*3*
Chlortetracycline	--		123 ± 16	*3*
Demeclocycline[a]	--		49 ± 15	*17*
	Fluoroquinolones			
Norfloxacin	--		46 ± 5	*3*
Ciprofloxacin	--		44 ± 5	*3*
Enrofloxacin	--		36 ± 3	*3*
Ofloxacin	--		53 ± 1	*3*
Nalidixic Acid[a]	--		80 ± 8	*17*
Clinafloxacin[b]	--		23 ± 4	*17*
	Non-categorized			
Carbadox	157 ± 12	*3*	45 ± 3	*3*
Trimethoprim	128 ± 7	*3*	59 ± 8	*3*
Lincomvcin	143 ± 30	*3*	23 ± 9	*3*
Simeton[b]	34 ± 10	*19*	57 ± 6	*17*

[a] denotes surrogate
[b] denotes internal standard

Table C.16. Antibiotic limits of detection (LODs) and quantification (LOQs) in Lake Winona and Lake Pepin extracted by the UAE method.

LOQs & LODs for USE Method

	Lake Pepin		Lake Winona	
	LOQ [ng/g]	LOD [ng/g]	LOQ [ng/g]	LOD [ng/g]
Sulfonamides				
Sulfapyridine	0.33	0.10	0.17	0.05
Sulfadiazine	0.13	0.04	0.05	0.02
Sulfamethoxazole	0.93	0.31	0.21	0.07
Sulfamethazine	1.28	0.43	1.52	0.51
Sulfachloropyridazine	0.28	0.09	0.10	0.03
Sulfadimethoxine	4.68	1.56	2.48	0.83
Others				
Carbadox	0.47	0.18	0.66	0.26
Trimethoprim	0.80	0.31	0.58	0.23
Lincomvcin	0.08	0.03	0.15	0.06
Tetracyclines				
Tetracycline	--	--	1.96	0.66
Doxycycline	--	--	2.82	0.95
Oxytetracycline	--	--	2.33	0.79
Chlortetracycline	--	--	2.90	0.98
Fluoroquinolones				
Norfloxacin	--	--	2.67	0.90
Ciprofloxacin	--	--	1.67	0.56
Enrofloxacin	--	--	2.22	0.75
Ofloxacin	--	--	1.52	0.51

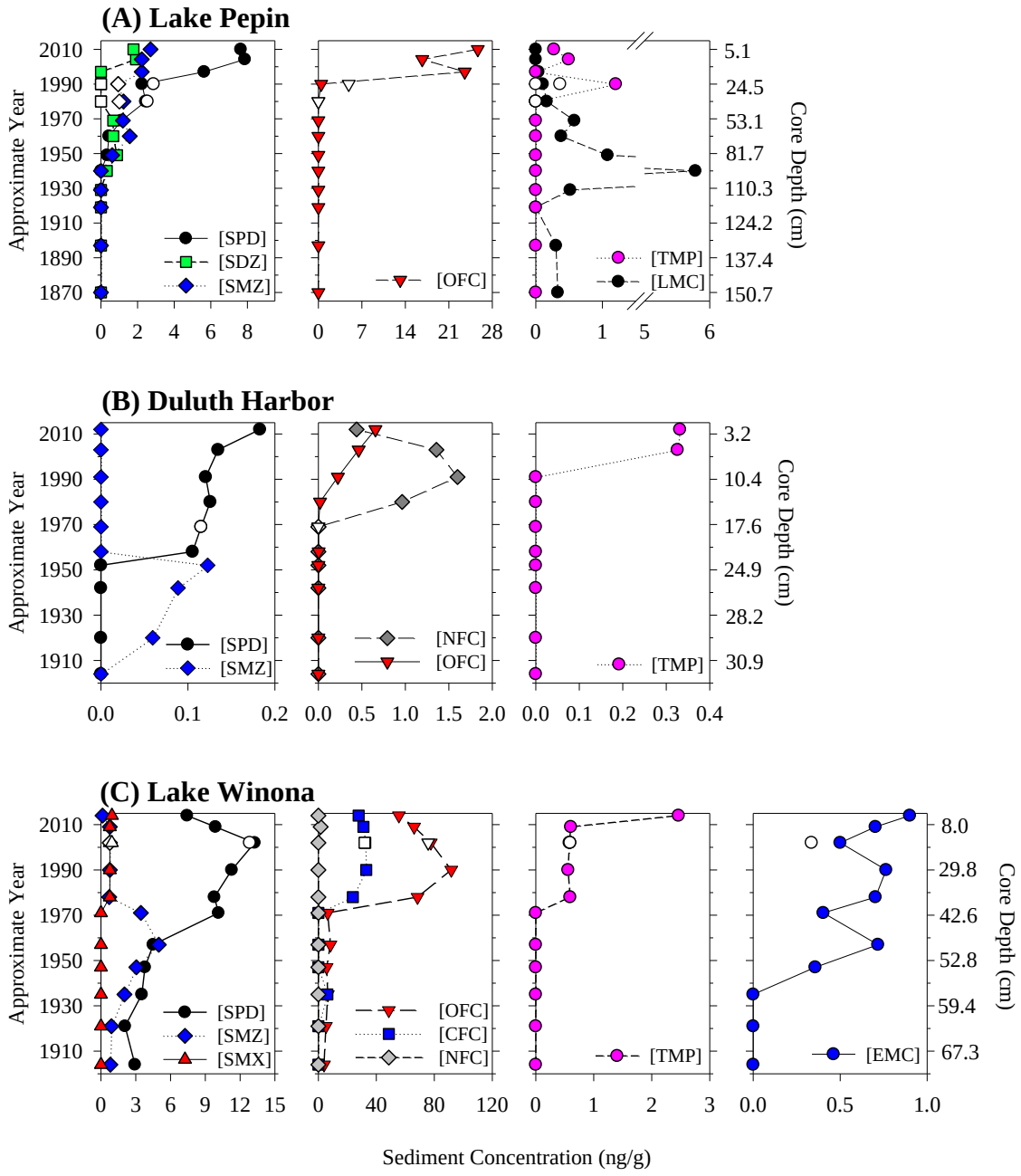

Figure C.8. Focus-corrected sediment concentrations (ng/g) of sulfapyridine (SPD), sulfadiazine (SDZ), sulfamethazine (SMZ), sulfamethoxazole (SMZ), ofloxacin (OFC), ciprofloxacin (CFC), norfloxacin (NFC), trimethoprim (TMP), lincomycin (LMC), and erythromycin (EMC) in sediment cores from: (A) Lake Pepin; (B) Duluth Harbor; and (C) Lake Winona. Open symbols indicate replicates.

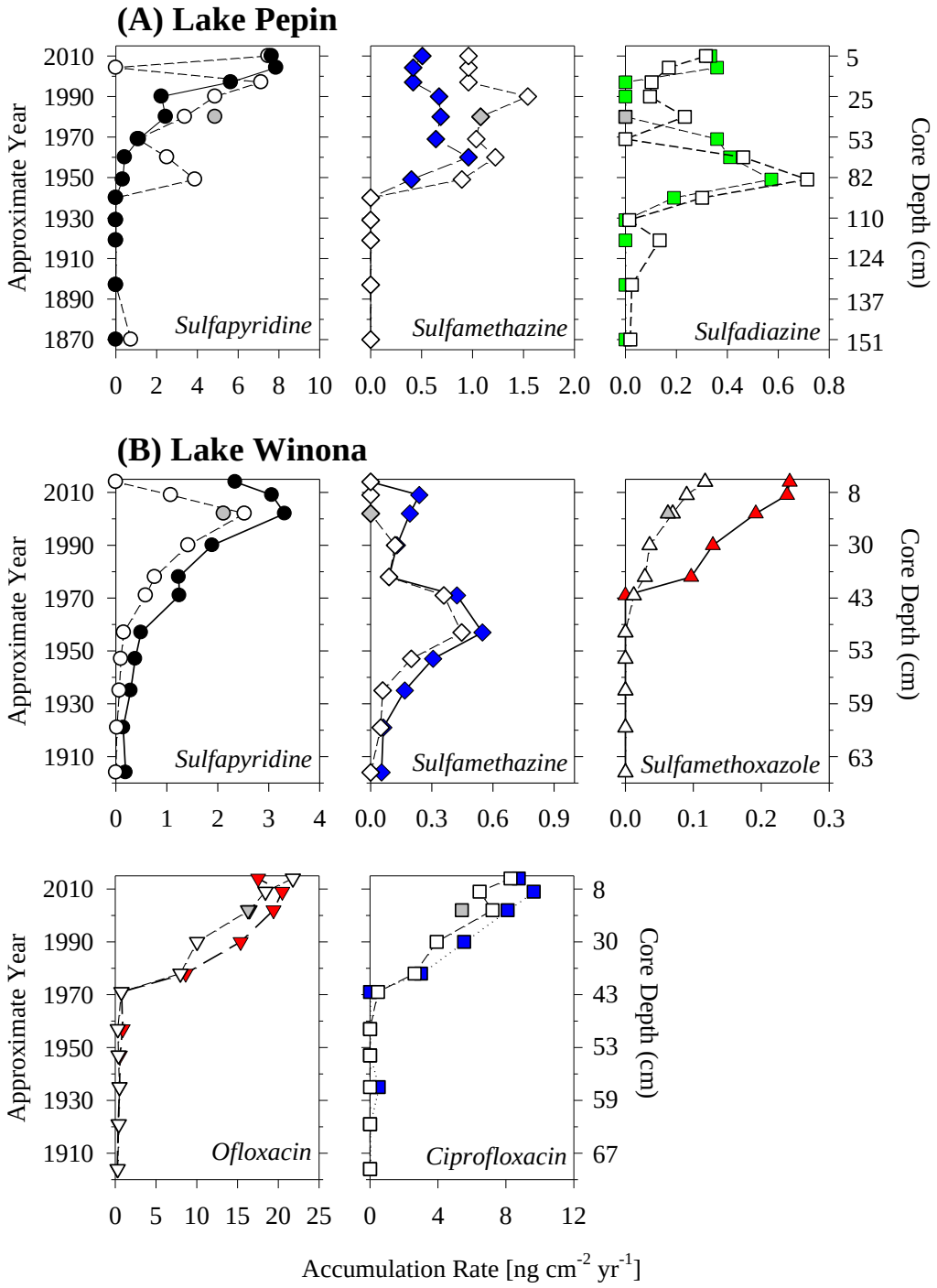

Figure C.9. Focus-corrected accumulation rates (ng cm^{-2} yr^{-1}) of detected antibiotics in Lake Pepin and Winona. White symbols represent accumulation rates determined by ultrasound assisted extraction (UAE) method. Grey symbols are USE replicates. Colored symbols are accumulation rates quantified by accelerated solvent extraction (ASE) method.

Appendix D: Supporting Information for Chapter 4

D.1 Materials and Methods

Table D.1. Global positioning system (GPS) coordinates of river surface sediment collection sites and their abbreviations.

River Sampling Site	Abbreviation	GPS Location
Big Stone Lake	BSL	45.303492, -96.453089
Marsh Lake	ML	45.171606, -96.094239
Lac qui Parle	LQP	45.022186, -95.868581
Granite Falls	GF	44.812499, -95.535147
St. Peter	SP	44.324499, -93.953020
Jordan	JD	44.692811, -93.641017
Grand Rapids	GR	47.231792, -93.530150
Brainerd	BRD	46.378194, -94.183337
Little Falls	LF	45.975469, -94.368498
St. Cloud	STC	45.548207, -94.147166
Coon Rapids	CR	45.144222, -93.312308
Hastings	HG	44.762600, -92.873418
Lake Pepin	LP	44.499750, -92.294170

Table D.2. Global positioning system (GPS) coordinates of Lake Winona surface sediment collection sites relative to the outfall of to the Alexandria Lake Area Sanitary District wastewater treatment plant (WWTP).

Distance from WWTP (km)	GPS Location
0.15	45.87219, -95.40524
0.48	45.87501, -95.40402
0.93	45.87740, -95.39924
1.41	45.88030, -95.39468
1.9	45.88293, -95.38963
2.19	45.88477, -95.38699
2.51	45.88752, -95.38596

Metal, Antibiotic, and Antibiotic-Associated Resistance Gene Quantification
Reproduced from Dr. Kyle Sandberg's Dissertation[227]

Metal Quantification.

Fourteen metals were quantified in the sediment samples: vanadium, chromium, manganese, cobalt, nickel, copper, zinc, arsenic, selenium, molybdenum, cadmium, tin, gadolinium, and lead. Samples were freeze- dried and crushed into a fine powder (diameter < 0.15 mm) using a clean mortar and pestle. Samples were partially digested to limit quantification to those metals that are loosely bound to the sediment and bioavailable to bacteria. This digestion used 0.5 g of dried and powdered sediment which was leached into 20 mL of 0.5 N HCl in Teflon vials at 80 °C for 30 minutes. Metals were quantified using a Thermo Scientific XSeries2 ICP-MS fitted with a hexapole collision/reaction cell. Unknowns were quantified by comparing intensities of the unknowns to a curve prepared by 4 multi-analyzed standards from SPEX industries that were diluted accordingly. Elements of mass less than 39 were analyzed at standard mass resolution with no reactive or collision gasses. Elements of mass 39 or greater were analyzed at standard mass resolution using Helium/Hydrogen collision reaction mode (CCT) with kinetic energy discrimination (KED). All elements had a dwell time of 15 ms with 30 sweeps; 5 replicates were used to determine means and standard deviations. An ESI PC3 FAST system with sample loops was used for sample introduction and to reduce oxide formation and carryover between samples. ^{155}In was used as an internal standard to compensate for matrix effects and signal drift.

DNA Extraction and Purification.

Prior to DNA extraction, samples were mixed with 500 μL of CLS-TS buffer (MP Biomedicals LLC; Solon, OH) and placed in Lysis Matrix E bead beating tubes (MP Biomedicals). Bacterial cells were lysed by placing each tube in a BIO 101 Thermo Savant Fast-Prep FP120 Cell Disruptor (Qbiogene, Inc., Carlsbad, CA) for 30 seconds. DNA was extracted and purified using a FastDNA Spin Kit for Soil (MP Biomedicals) following the manufacturer's instructions. Extracted DNA was stored at -20 °C.

Microfluidic qPCR.

Microfluidic quantitative polymerase chain reaction (MF-qPCR) was used in order to quantify the 16S rRNA gene as well as 45 antibiotic resistance, metal resistance, and antibiotic resistance-associated genes. Fluidigm Biomark Gene Expression 48.48 IFC or 192.24 gene expression chips (Fluidigm; South San Francisco, CA) were run according to the protocols developed by Fluidigm. An MX IFC controller (Fluidigm; South San Francisco, CA) was used to load the samples and reagents onto the chip and a Biomark HD was used to analyze the chip. The chip was run following the following thermal protocol: 95 °C for 60 seconds, 40 cycles of 96 °C for 5 seconds and 60 °C for 20 seconds, followed by 3 seconds at 60 °C and slow heating to 95 °C at a rate of 1 °C per 3 seconds. Following MF-qPCR, melt curves were analyzed to ensure that non-specific amplification was not present.

Due to the small volumes of template DNA used for MF-qPCR, a preamplification step was needed in order to amplify the DNA into a quantifiable range.

This preamplification used the same primers that were used for the MF-qPCR and a low number of PCR cycles. A standard curve, which also underwent the preamplification step, was prepared using serial 10-fold dilutions of a mixture of DNA standards for all genes of interest. Reaction volumes were 25 µL and consisted of: 12.5 µL EvaGreen, 6.25 µL mixture of 50 nM of each primer, and 0.625 µL of DNA template. The thermal protocol used was as follows: initial denaturation at 95 °C for 10 minutes followed by 17 cycles of a 15 second denaturation at 95 °C and anneal and extension for 4 minutes at 60 °C. Preamplification was performed on a Bio-Rad (Hercules, CA) CFX Connect Real-Time System. Preamplification products were diluted 10-fold with DNase and RNase free water and stored at -20 °C.

The 16S rRNA gene was quantified using conventional qPCR as the concentrations in the samples were too high to quantify using MF-qPCR. In addition, *intI1* for the Lake Pepin samples were run using conventional qPCR as the standard curve for the 192.24 MF-qPCR chip did not amplify well. For conventional qPCR, a Bio-Rad (Bio-Rad; Hercules, CA) CFX Connect Real-Time System was used. Reaction volumes were 25 µL and consisted of: 12.5 µL of EvaGreen MasterMix (Bio-Rad; Hercules, CA), 25 µg of bovine serum albumin, optimized quantities of forward and reverse primers, and approximately 1 ng of template DNA. The thermal protocol used was: 2 minutes initial denaturation at 95 °C followed by 40 cycles of denaturation at 95 °C for 15 seconds and a one-minute annealing/extending step at 60 °C. Standard curves were prepared by performing a serial 10-fold dilution of a DNA solution with known concentration, the slopes of which were used to calculate amplification efficiency (Table D.3).

Amplification curves were inspected to ensure that no inhibition had taken place and melt curves were inspected to ensure that non-specific amplification did not occur.

Table D.3. Amplification details for all target genes in this study reproduced from Kyle Sandberg's dissertation. These values correspond to conditions during quantification of all samples.

Gene	*Amplification Efficiency*	*Quantification Limit (lower), log(copies/µL of DNA extract)*
16S rRNA	0.973	5.04
aacA	1.150	3.59
aacA5	1.003	3.63
aadD	1.056	1.63
acrD	1.095	2.54
ampC	0.985	3.65
arr2	1.090	3.49
blaKPC	0.933	2.54
blaNDM-1	0.830	2.78
blaNPS	1.005	2.54
blaOXA	0.966	2.60
blaSHV	0.843	3.20
blaVIM	1.035	2.65
cadA	1.077	2.54
catB8	0.946	1.56
chrA	0.780	2.57
cmlB	1.063	2.66
copA	1.002	3.56
ctxm32	1.079	1.65
dfr13	1.081	2.62
ereB	0.873	2.59
floR	0.757	2.62
imp13	1.071	1.62
intI1	0.940	3.55
intI2	0.959	2.58
intI3	1.023	2.54
mefE	0.905	2.59
merA	0.806	4.56
mexB	0.794	2.56
nikA	1.083	1.56
qacF	0.985	3.70
qnrA	1.051	3.70
qnrB	1.026	2.62

D.2 Results

Table D.4. Percent organic, carbonate, and inorganic of Lake Winona surface sediment relative to distance (km) from wastewater treatment plant (WWTP) effluent and percent water of sample determined by loss-on-ignition.

Lake Winona Surface Sediment Loss-On-Ignition Results

Distance from WWTP (km)	*Organic*	*Carbonate*	*Inorganic*	*Water*
0.15	26.6	46.1	27.3	85.0
0.48	18.1	58.6	23.3	86.6
0.93	19.0	55.1	26.0	86.4
1.41	19.8	49.1	31.1	88.3
1.90	20.1	46.9	33.0	90.7
2.19	20.3	46.3	33.4	87.6
2.51	21.2	41.9	36.9	86.5

Table D.5. Percent organic, carbonate, and inorganic of Minnesota and Mississippi River surface sediment and percent water of sample determined by loss-on-ignition.

Minnesota and Mississippi River Loss-On-Ignition Results

Sample Site	*Organic*	*Carbonate*	*Inorganic*	*Water*
Big Stone Lake	0.6	7.0	92.3	23.6
Marsh Lake	9.4	14.4	76.2	55.5
Lac Qui Parle	3.4	12.6	84.0	33.2
Granite Falls	4.8	14.8	80.4	39.1
St. Peter	3.8	13.2	83.0	39.5
Jordan	1.9	9.8	88.3	30.4
Grand Rapids	26.8	9.2	63.9	84.2
Brainerd	8.4	8.0	83.6	63.8
Little Falls	17.4	8.5	74.2	75.1
St. Cloud	0.7	3.1	96.2	18.1
Coon Rapids	1.8	3.7	94.5	27.0
Hastings	7.8	9.4	82.8	51.6
Lake Pepin	13.4	11.5	75.1	84.5

Table D.6. Limits of detection (LODs) and quantification (LOQs) in ng/g for antibiotics in Lake Winona surface sediment extractions. Also displayed are absolute recoveries of internal standards and relative recoveries of surrogates and target antibiotics.

Limits of Detection and Recovery in Lake Winona

Analytes	LOD [ng/g]	LOQ [ng/g]	Recovery (%)
Sulfonamides			
Sulfapyridine	0.85	2.54	110 ± 16
Sulfadiazine	0.09	0.26	120 ± 24
Sulfamethoxazole	0.12	0.36	94 ± 0
Sulfamethazine	0.18	0.55	91 ± 9
Sulfachloropyridazine	0.01	0.04	112 ± 5
Sulfadimethoxine	0.15	0.44	83 ± 4
$^{13}C_6$-Sulfamethazine[a]	-	-	56 ± 5
$^{13}C_6$-Sulfamethoxazole[b]	-	-	52 ± 14
Tetracyclines			
Tetracyclines	1.43	4.29	19 ± 5
Doxycycline	1.11	3.32	18 ± 4
Oxytetracycline	4.07	12.20	5 ± 1
Chlortetracycline	1.92	5.76	71 ± 11
Demeclocycline[a]	-	-	10 ± 11
Fluoroquinolones			
Norfloxacin	1.46	4.37	23 ± 3
Ciprofloxacin	2.06	6.18	26 ± 5
Enrofloxacin	0.10	0.30	38 ± 12
Ofloxacin	0.47	0.80	33 ± 5
Nalidixic Acid[a]	-	-	54 ± 6
Clinafloxacin[b]	-	-	18 ± 5
Macrolides			
Erythromycin	0.45	1.35	99 ± 20
Roxithromycin	0.30	0.89	128 ± 19
Tylosin	0.05	0.15	218 ± 24
$^{13}C_2$-Erythromycin[b]	-	-	27 ± 20
Non-categorized			
Carbadox	0.42	0.76	13 ± 2
Trimethoprim	0.04	0.06	24 ± 5
Lincomvcin	0.09	0.15	6 ± 6
Simeton[b]	-	-	67 ± 3

[a] surrogate
[b] internal standard

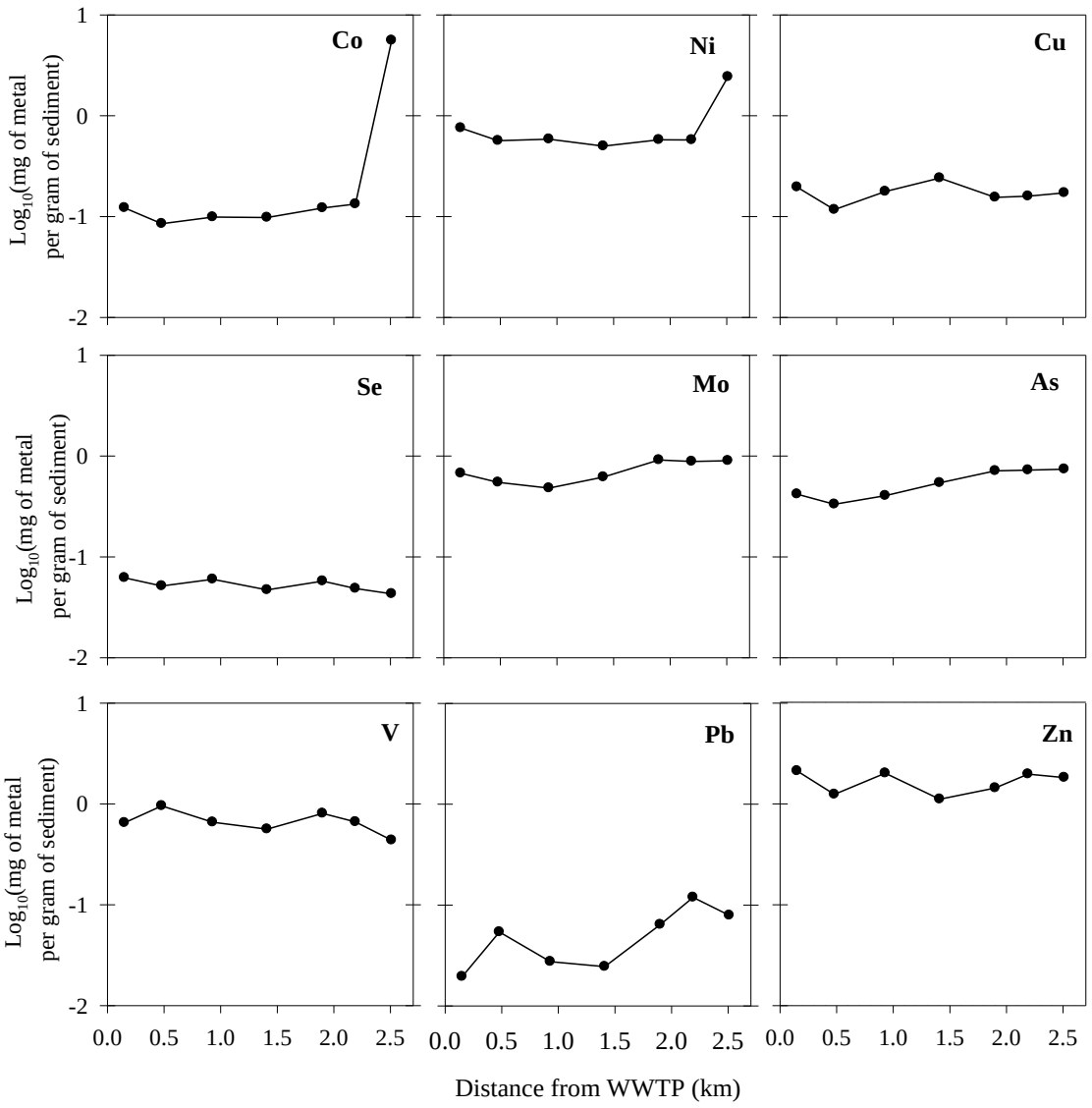

Figure D.1. Concentration of metals in Lake Winona surface sediment relative to discharge of wastewater treatment plant (km).

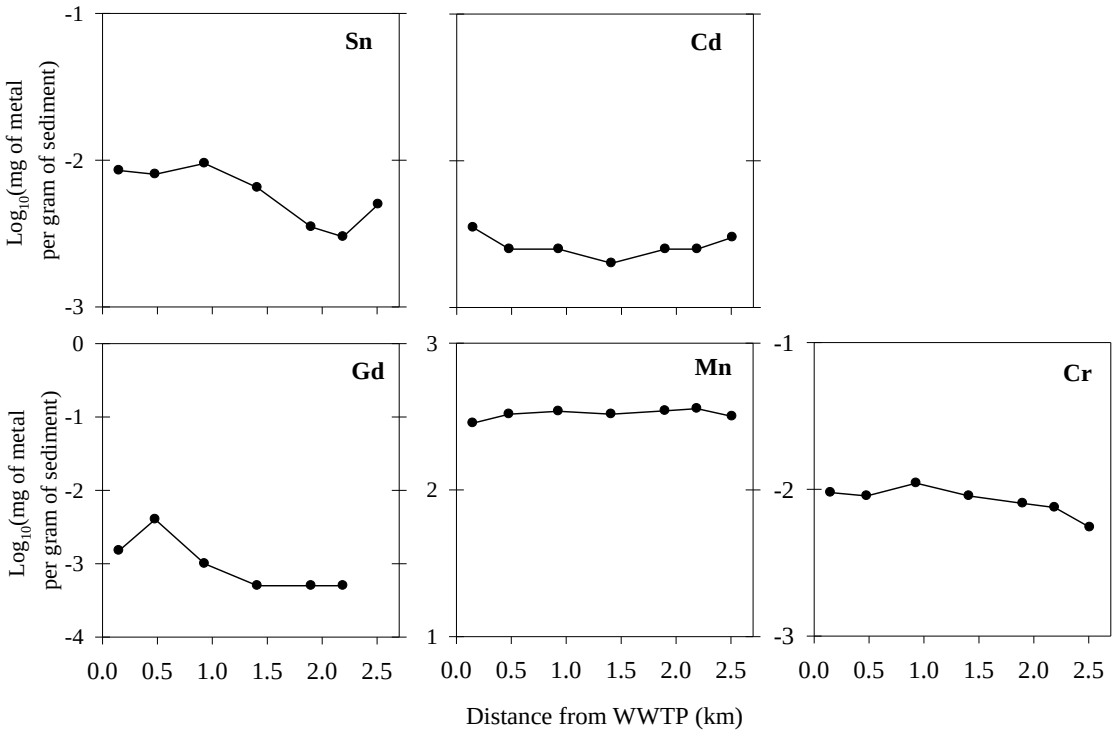

Figure D.2. Concentration of metals in Lake Winona surface sediment from the outfall of wastewater treatment plant (WWTP).

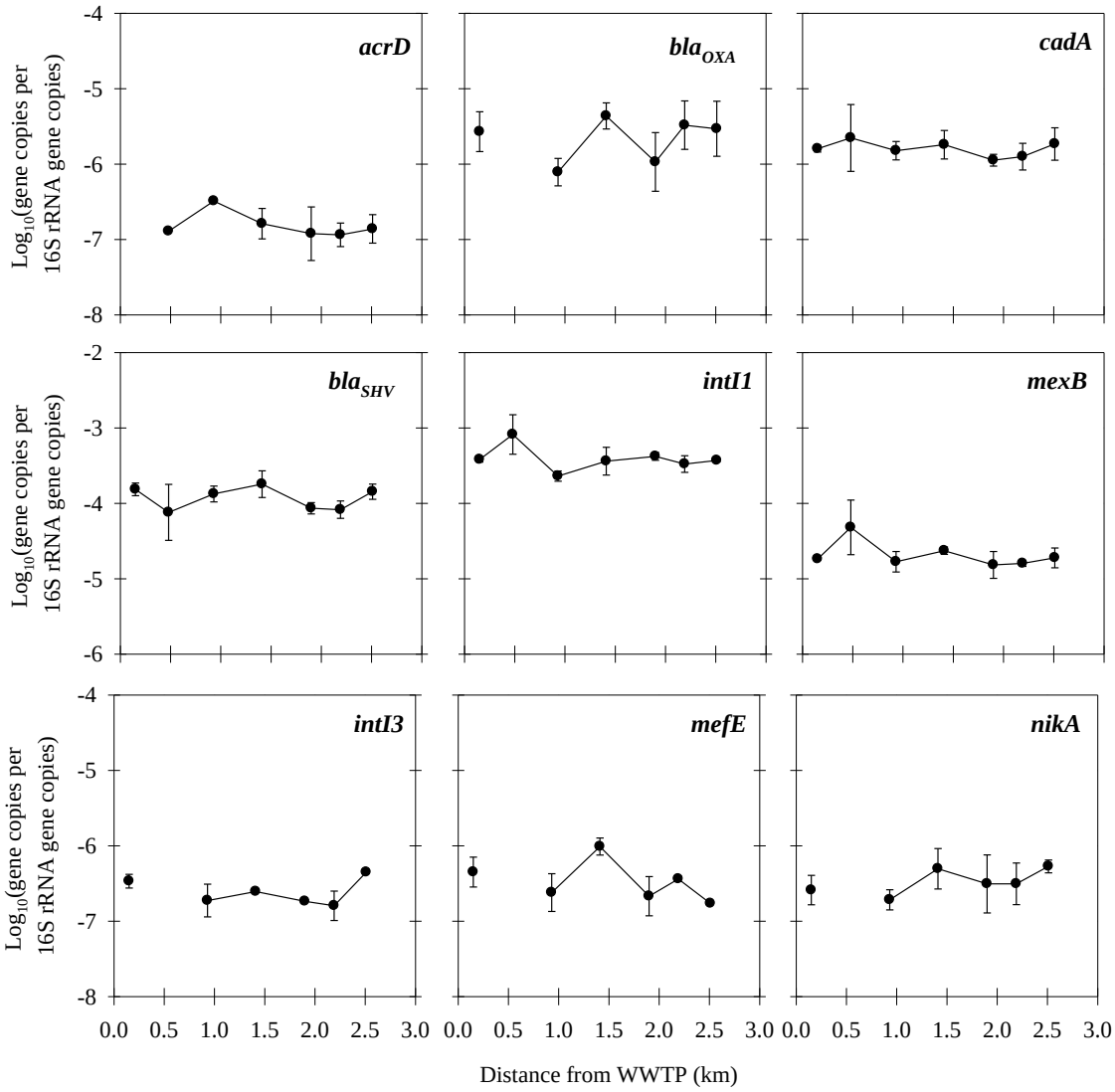

Figure D.3. Concentrations of resistance genes that were quantified in more than half of the Lake Winona surface sediment samples with respect to the wastewater treatment plant (WWTP) outfall.

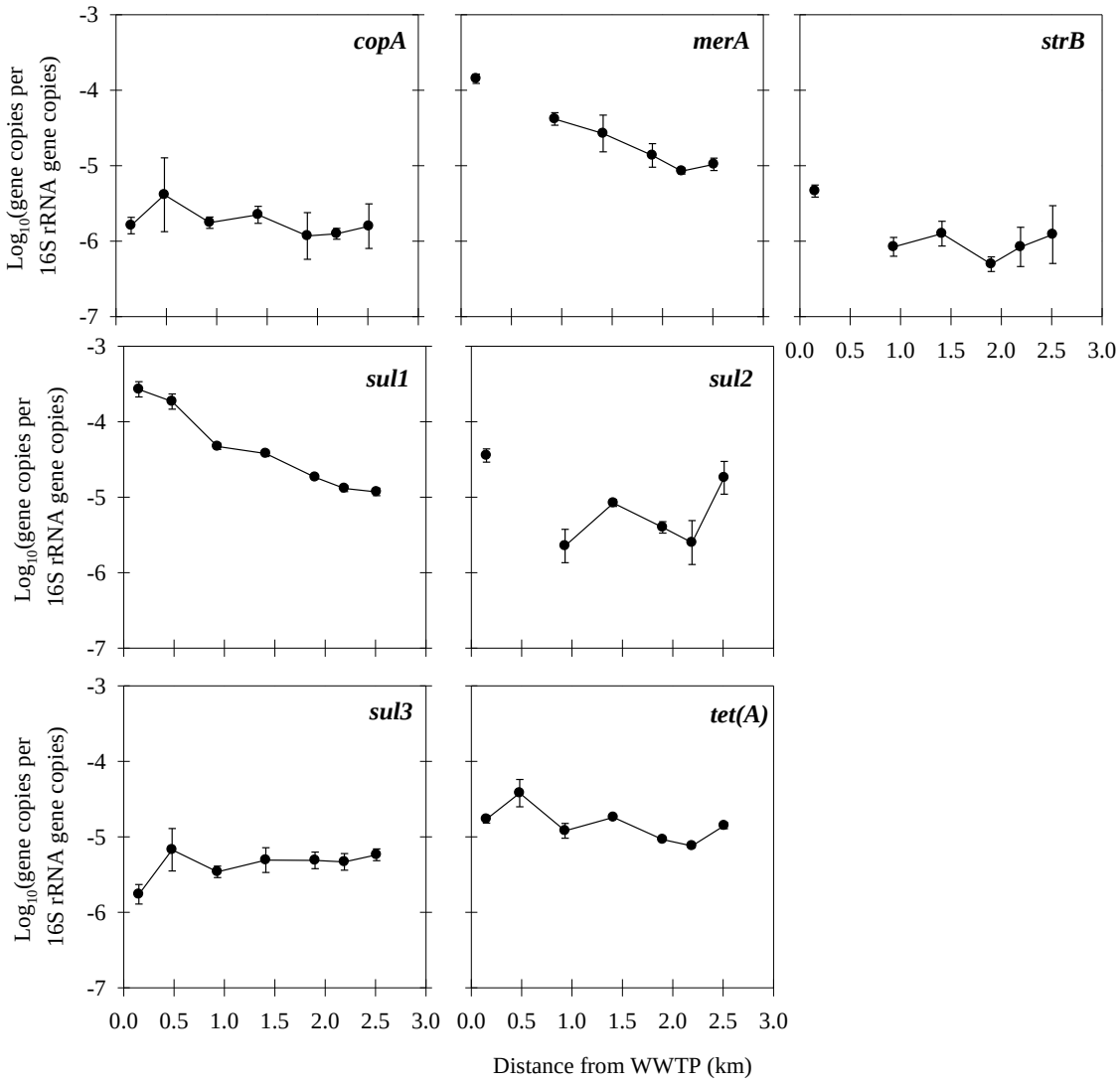

Figure D.4. Concentrations of resistance genes that were quantified in more than half of the Lake Winona surface sediment samples with respect to the wastewater treatment plant (WWTP) outfall.

Table D.7. P-values generated from Pearson correlations between metals and antibiotics [$\log_{10}$(ng/g)] with target genes [$\log_{10}$(gene copies per 16S rRNA gene copies)] in Lake Winona. Shaded regions indicate p-values less than 0.05.

	acrD	***bla$_{OXA}$***	***bla$_{SHV}$***	***cadA***	***copA***	***intI1***	***intI3***	***mefE***	***merA***	***mexB***	***nikA***	***strB***	***sul1***	***sul2***	***sul3***	***tetA***
V	0.767	0.263	0.061	0.869	0.399	0.184	0.075	0.974	0.748	0.308	0.139	0.581	0.276	0.329	0.842	0.509
Cr	0.183	0.406	0.860	0.988	0.509	0.787	0.350	0.410	0.127	0.751	0.093	0.761	0.116	0.615	0.299	0.620
Mn	0.977	0.535	0.203	0.314	0.695	0.703	0.055	0.716	0.100	0.742	0.860	***0.010***	0.124	***0.004***	0.155	0.286
Co	0.752	0.644	0.579	0.663	0.624	0.833	0.098	0.272	0.424	0.698	0.208	0.947	0.260	0.409	0.586	0.807
Ni	0.820	0.704	0.538	0.582	0.688	0.887	0.051	0.252	0.659	0.750	0.328	0.717	0.471	0.277	0.818	0.945
Cu	0.503	0.308	***0.008***	0.819	0.464	0.121	0.547	***0.036***	0.321	0.282	0.545	0.307	0.813	0.420	0.304	0.575
Zn	0.558	0.627	0.852	0.412	0.186	0.181	0.846	0.295	0.642	0.146	0.224	0.519	0.901	0.891	0.140	0.246
As	0.334	0.586	0.952	0.174	***0.047***	0.470	0.945	0.537	***0.013***	0.116	0.180	0.266	***0.006***	0.763	0.576	0.051
Se	0.332	0.158	0.899	0.425	0.780	0.727	0.545	0.910	0.120	0.654	***0.022***	0.698	0.187	0.893	0.079	0.904
Mo	0.095	0.546	0.636	0.240	0.094	0.991	0.821	0.505	0.167	0.265	0.304	0.658	0.112	0.803	0.722	0.171
Cd	0.811	0.949	0.840	0.990	0.597	0.997	0.231	0.388	0.407	0.657	0.659	0.178	0.493	0.173	0.159	0.997
Sn	0.097	0.717	0.295	0.121	0.136	0.938	0.493	0.660	***0.040***	0.323	0.489	0.234	***0.041***	0.500	0.404	0.094
Gd	0.913	0.730	0.667	0.085	0.066	0.164	0.222	0.847	***0.029***	0.067	0.220	0.139	***0.033***	0.312	0.997	***0.040***
Pb	0.104	0.868	0.087	0.551	0.567	0.676	0.672	0.299	***0.014***	0.865	0.568	0.220	0.094	0.452	0.122	0.397
SPD	0.604	0.925	0.785	0.604	0.430	0.235	0.698	0.646	0.054	0.378	0.414	0.076	***0.006***	0.240	0.219	0.222
SMX	0.421	0.618	0.465	0.075	0.148	0.714	0.345	0.515	0.076	0.253	0.653	***0.025***	***0.033***	0.272	0.342	0.099
SMZ	***0.014***	0.127	0.994	0.945	0.703	***0.012***	0.424	0.563	0.964	0.708	0.296	0.356	0.796	0.152	0.791	0.732
SCP	0.179	0.765	0.171	0.159	0.105	0.161	0.716	0.578	0.312	0.323	0.915	0.129	0.351	0.925	0.503	0.325
TMP	0.136	0.557	0.113	0.823	0.859	0.820	***0.026***	0.232	0.115	0.788	0.836	***0.014***	0.244	***0.046***	***0.025***	0.812
EMC	0.155	0.720	0.389	0.377	0.436	0.415	0.973	0.648	***0.026***	0.339	0.227	0.130	0.306	0.579	< ***0.001***	0.686
CFC	0.588	0.895	0.760	0.763	0.660	0.819	0.855	0.457	***0.021***	0.744	0.265	0.053	***0.012***	0.400	***0.034***	0.468
EFC	0.340	0.677	0.582	0.321	0.695	0.217	0.390	0.258	0.994	0.982	0.869	0.868	0.842	0.889	0.944	0.792
OFC	0.468	0.909	0.625	0.956	0.863	0.985	0.906	0.405	***0.018***	0.970	0.269	0.066	***0.030***	0.419	***0.012***	0.639

SPD = sulfapyridine; SMX = sulfamethoxazole; SMZ = sulfamethazine; SCP = sulfachloropyridazine; TMP = trimethoprim; EMC = erythromycin; CFC = ciprofloxacin; EFC = enrofloxacin; OFC = ofloxacin

Table D.8. Pearson coefficients generated from Pearson correlations between metals and antibiotics [$\log_{10}$(ng/g) with target genes [$\log_{10}$(gene copies per 16S rRNA gene copies)] in Lake Winona. Shaded regions indicate p-values less than 0.05.

	acrD	***bla$_{OXA}$***	***bla$_{SHV}$***	***cadA***	***copA***	***intI1***	***intI3***	***mefE***	***merA***	***mexB***	***nikA***	***strB***	***sul1***	***sul2***	***sul3***	***tetA***
V	-0.157	-0.546	-0.733	-0.078	0.381	0.567	-0.767	0.017	0.169	0.453	-0.678	-0.287	0.480	-0.486	0.093	0.303
Cr	0.626	-0.421	0.083	-0.007	0.303	-0.126	-0.467	0.418	0.693	0.148	-0.739	0.161	0.647	-0.263	-0.460	0.230
Mn	0.015	-0.321	-0.548	-0.447	-0.183	-0.177	-0.802	-0.192	-0.729	-0.154	-0.094	***-0.919***	-0.637	***-0.950***	0.600	-0.471
Co	-0.167	0.242	0.256	0.202	-0.227	-0.099	0.732	-0.537	-0.406	-0.181	0.600	0.035	-0.494	0.418	0.252	-0.114
Ni	-0.121	0.200	0.283	0.255	-0.187	-0.067	0.810	-0.556	-0.232	-0.149	0.486	0.191	-0.329	0.532	0.108	-0.032
Cu	0.345	0.504	***0.887***	-0.107	-0.334	-0.640	0.312	***0.841***	0.493	-0.474	0.314	0.505	-0.111	0.410	-0.456	-0.259
Zn	0.304	-0.254	0.087	-0.371	-0.566	-0.571	0.103	-0.516	0.243	-0.609	-0.583	0.333	-0.058	0.073	-0.617	-0.507
As	-0.481	0.284	-0.028	-0.578	***-0.761***	-0.330	-0.036	-0.320	***-0.906***	-0.647	0.630	-0.543	***-0.897***	-0.159	0.258	-0.753
Se	0.483	-0.655	-0.060	-0.362	-0.131	-0.163	-0.314	-0.060	0.702	-0.208	***-0.875***	0.204	0.564	-0.071	-0.702	-0.057
Mo	-0.737	0.313	-0.220	-0.512	-0.678	-0.006	0.120	-0.344	-0.644	-0.489	0.508	-0.232	-0.653	0.132	0.166	-0.581
Cd	-0.127	-0.034	0.095	0.006	-0.245	0.002	0.576	-0.436	0.420	-0.207	-0.231	0.632	0.314	0.638	-0.594	-0.002
Sn	0.734	-0.191	0.463	0.641	0.622	0.036	0.353	0.231	***0.832***	0.440	-0.356	0.573	***0.774***	0.347	-0.378	0.679
Gd	0.068	-0.214	-0.226	0.751	0.782	0.649	0.664	-0.120	***0.915***	0.781	-0.666	0.757	***0.848***	0.573	0.002	***0.832***
Pb	-0.723	0.088	-0.689	-0.275	-0.264	0.195	-0.222	-0.512	***-0.904***	-0.080	0.297	-0.588	-0.678	-0.384	0.639	-0.383
SPD	-0.271	0.050	-0.128	0.240	0.358	0.517	0.204	0.240	0.803	0.397	-0.414	0.766	***0.895***	0.568	-0.532	0.529
SMX	-0.409	-0.260	-0.333	-0.707	-0.607	-0.171	-0.472	-0.336	-0.765	-0.500	0.236	***-0.867***	***-0.793***	-0.537	0.425	-0.671
SMZ	***-0.785***	0.693	-0.357	0.443	0.497	***0.916***	0.406	0.300	0.024	0.673	0.514	0.462	0.433	0.662	0.205	0.613
SCP	-0.708	-0.169	-0.584	-0.501	-0.338	0.426	-0.172	-0.310	-0.255	-0.133	0.038	-0.269	-0.123	0.017	0.102	-0.220
TMP	0.146	-0.274	-0.151	-0.251	-0.122	-0.002	***-0.125***	-0.014	0.690	-0.130	-0.766	***0.517***	0.648	***0.180***	***-0.792***	-0.007
EMC	0.568	0.314	0.546	0.312	0.143	-0.318	0.279	0.432	***0.700***	-0.002	-0.297	0.811	0.527	0.386	***-0.694***	0.188
CFC	0.282	0.070	0.143	0.141	0.205	0.107	0.097	0.380	***0.879***	0.152	-0.544	0.806	***0.863***	0.426	***-0.792***	0.331
EFC	-0.555	0.219	-0.290	-0.319	-0.023	0.378	-0.434	0.551	0.004	0.091	0.088	-0.088	0.138	-0.074	0.009	-0.008
OFC	0.372	0.061	0.227	0.026	0.081	-0.009	0.063	0.422	***0.887***	0.017	-0.540	0.783	***0.803***	0.410	***-0.864***	0.218

SPD = sulfapyridine; SMX = sulfamethoxazole; SMZ = sulfamethazine; SCP = sulfachloropyridazine; TMP = trimethoprim; EMC = erythromycin; CFC = ciprofloxacin; EFC = enrofloxacin; OFC = ofloxacin

Table D.9. P-values generated from Pearson correlations among target genes [$\log_{10}$(gene copies per 16S rRNA gene copies)] in Lake Winona. Shaded regions indicate p-values less than 0.05.

	acrD	***blaOXA***	***blaSHV***	***cadA***	***copA***	***intI1***	***intI3***	***mefE***	***merA***	***mexB***	***nikA***	***strB***	***sul1***	***sul2***	***sul3***
blaOXA	0.349	--	--	--	--	--	--	--	--	--	--	--	--	--	--
blaSHV	0.297	0.517	--	--	--	--	--	--	--	--	--	--	--	--	--
cadA	0.859	0.324	0.587	--	--	--	--	--	--	--	--	--	--	--	--
copA	0.872	0.545	0.871	***0.010***	--	--	--	--	--	--	--	--	--	--	--
intI1	0.194	0.351	0.241	0.242	0.102	--	--	--	--	--	--	--	--	--	--
intI3	0.900	0.424	0.158	0.074	0.495	0.508	--	--	--	--	--	--	--	--	--
mefE	0.976	0.208	0.348	0.557	0.192	0.823	0.868	--	--	--	--	--	--	--	--
merA	***0.044***	0.840	0.231	0.572	0.386	0.774	0.623	0.493	--	--	--	--	--	--	--
mexB	0.763	0.169	0.593	***0.022***	***<0.001***	***0.022***	0.321	0.119	0.651	--	--	--	--	--	--
nikA	0.295	0.113	0.601	0.341	0.657	0.219	0.308	0.703	0.324	0.208	--	--	--	--	--
strB	0.787	0.366	0.185	0.255	0.437	0.768	0.180	0.451	0.075	0.407	0.957	--	--	--	--
sul1	0.638	0.964	0.843	0.265	0.144	0.348	0.660	0.417	***<0.001***	0.197	0.348	0.054	--	--	--
sul2	0.620	0.317	0.196	0.199	0.579	0.274	***0.016***	0.755	0.324	0.341	0.457	***0.042***	0.299	--	--
sul3	0.096	0.849	0.309	0.588	0.458	0.331	0.774	0.687	***0.010***	0.324	0.188	0.069	0.269	0.387	--
tetA	0.991	0.498	0.907	***0.006***	***0.001***	0.068	0.117	0.337	0.169	***0.003***	0.575	0.126	0.065	0.094	0.720

Table D.10. Pearson coefficients generated from Pearson correlations among target genes [$\log_{10}$(gene copies per 16S rRNA gene copies)] in Lake Winona. Shaded regions indicate p-values less than 0.05.

	acrD	***bla$_{OXA}$***	***bla$_{SHV}$***	***cadA***	***copA***	***intI1***	***intI3***	***mefE***	***merA***	***mexB***	***nikA***	***strB***	***sul1***	***sul2***	***sul3***
bla$_{OXA}$	-0.539	--	--	--	--	--	--	--	--	--	--	--	--	--	--
bla$_{SHV}$	0.514	0.335	--	--	--	--	--	--	--	--	--	--	--	--	--
cadA	0.095	0.490	0.251	--	--	--	--	--	--	--	--	--	--	--	--
copA	0.086	0.313	-0.076	***0.873***	--	--	--	--	--	--	--	--	--	--	--
intI1	-0.615	0.467	-0.511	0.510	0.667	--	--	--	--	--	--	--	--	--	--
intI3	-0.079	0.407	0.655	0.769	0.351	0.341	--	--	--	--	--	--	--	--	--
mefE	-0.019	0.600	0.469	0.305	0.617	0.119	-0.088	--	--	--	--	--	--	--	--
merA	***0.889***	-0.107	0.576	0.294	0.437	-0.152	0.257	0.353	--	--	--	--	--	--	--
mexB	-0.160	0.642	-0.247	***0.826***	***0.968***	***0.825***	0.493	0.704	0.237	--	--	--	--	--	--
nikA	-0.590	0.712	0.273	0.475	0.233	0.589	0.504	0.201	-0.490	0.600	--	--	--	--	--
strB	0.168	0.454	0.624	0.553	0.396	0.156	0.631	0.385	0.768	0.420	-0.029	--	--	--	--
sul1	0.247	-0.024	0.093	0.489	0.613	0.420	0.231	0.412	***0.990***	0.554	-0.469	0.803	--	--	--
sul2	-0.303	0.496	0.613	0.610	0.289	0.535	***0.896***	0.165	0.490	0.475	0.381	***0.828***	0.512	--	--
sul3	-0.734	0.101	-0.452	0.251	0.339	0.434	-0.152	-0.212	***-0.918***	0.439	0.621	-0.777	-0.486	-0.437	--
tetA	0.006	0.349	0.055	***0.901***	***0.943***	0.719	0.706	0.479	0.642	***0.926***	0.292	0.694	0.726	0.738	0.167

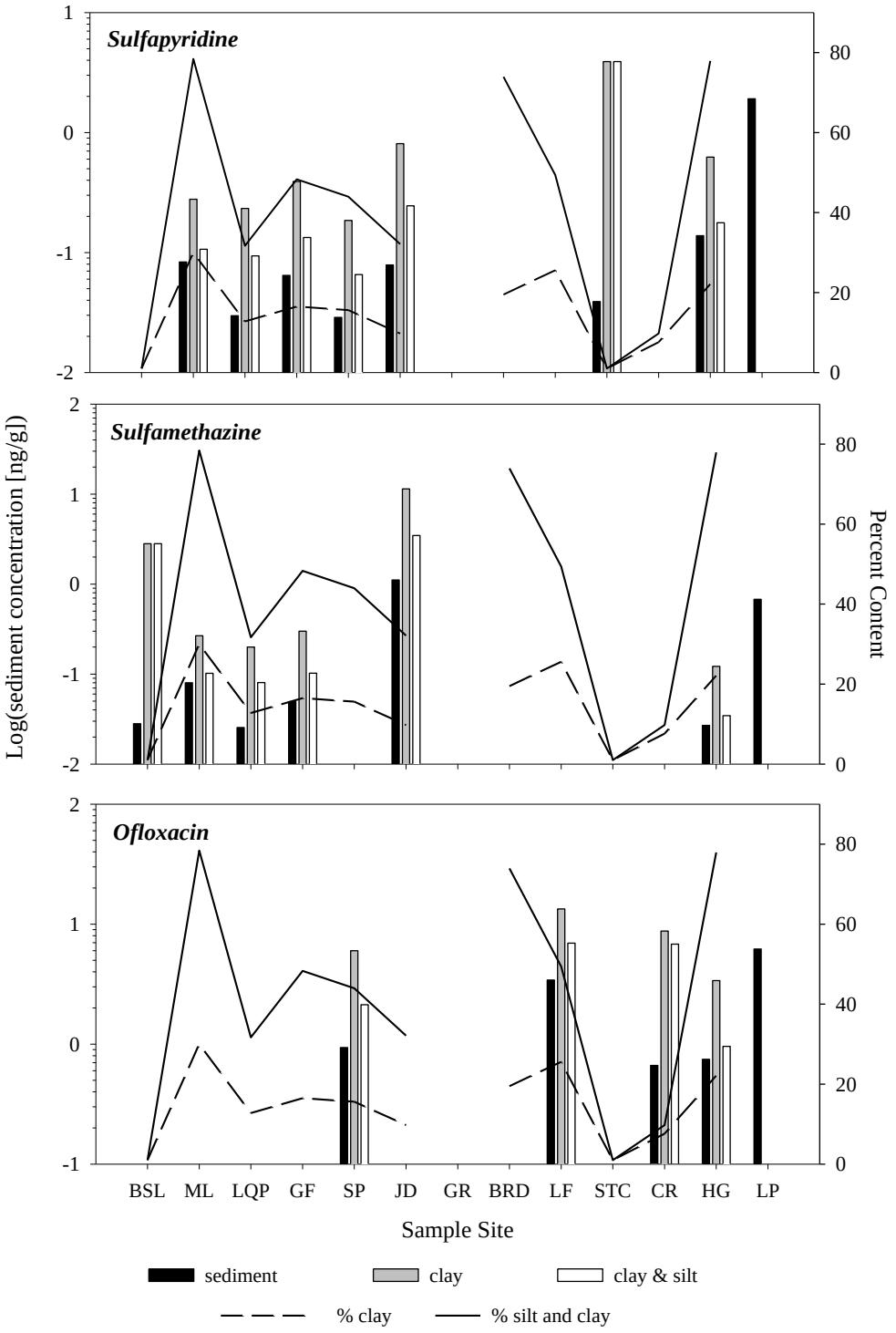

Figure D.5. Log_{10} transformed antibiotic sediment concentration expressed as per gram of sediment (black bars), per gram clay (gray bars), and per gram clay and silt (white bars) with percent clay (dashed line) and percent silt and clay (solid line) of sediment samples.

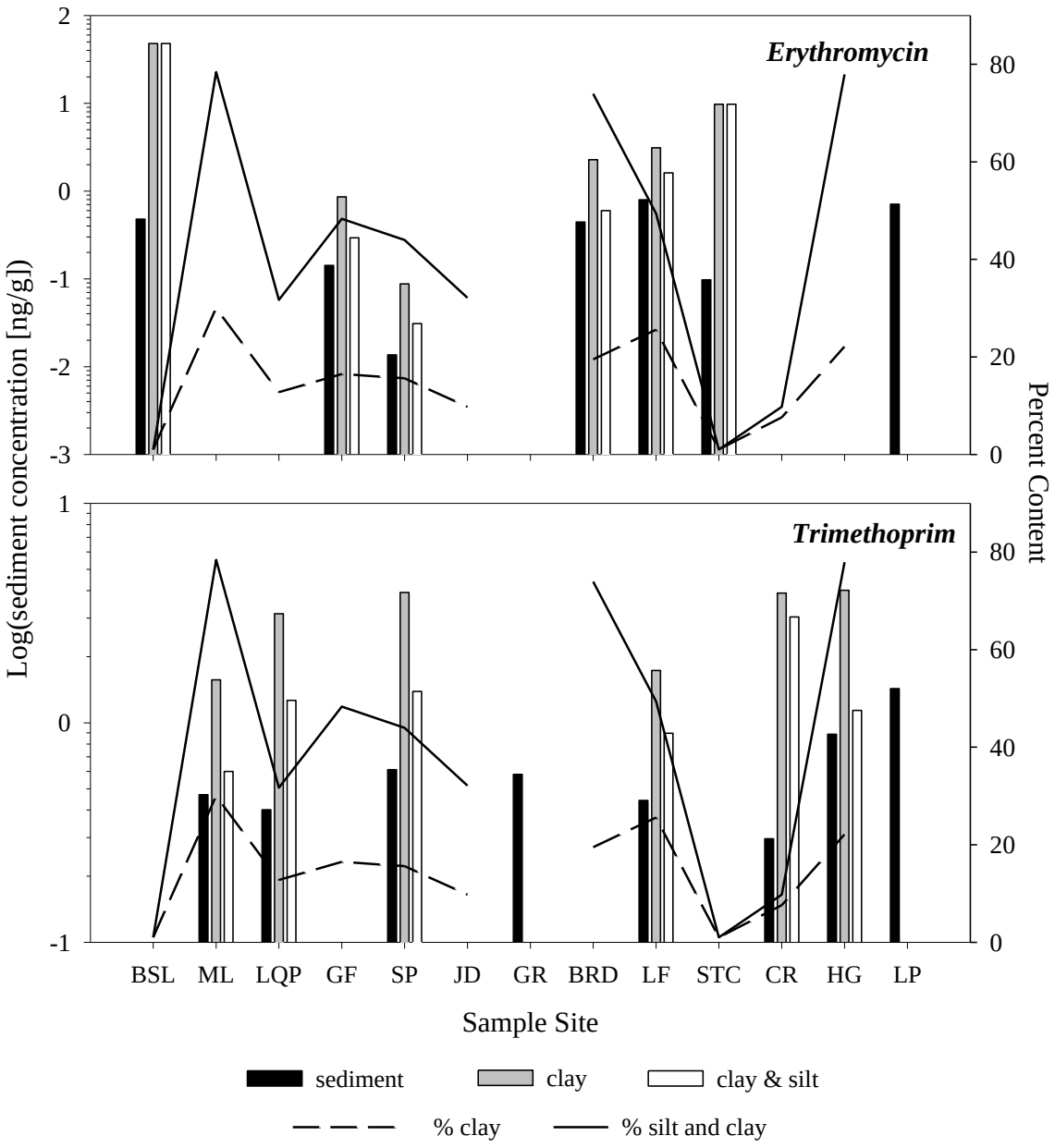

Figure D.6. Log_{10} transformed antibiotic sediment concentration expressed as per gram of sediment (black bars), per gram clay (gray bars), and clay and silt (white bars) with percent clay (dashed line) and percent silt and clay (solid line) of sediment samples.

Table D.11. P-values generated from Pearson correlations among target genes [$\log_{10}$(gene copies per 16S rRNA gene copies)] in river sediments. Shaded regions indicate p-values less than 0.05.

	bla$_{SHV}$	*cadA*	*floR*	*intI1*	*mexB*	*nikA*	*sul1*	*sul3*
cadA	***< 0.001***	--	--	--	--	--	--	--
floR	***0.002***	***0.003***	--	--	--	--	--	--
intI1	***0.001***	***< 0.001***	***0.002***	--	--	--	--	--
mexB	***0.001***	***0.001***	***0.001***	***< 0.001***	--	--	--	--
nikA	***0.016***	0.079	***0.018***	***0.049***	0.074	--	--	--
sul1	***0.001***	***0.000***	0.189	***0.002***	***0.016***	0.442	--	--
sul3	0.101	***0.006***	***0.022***	***0.011***	***0.010***	0.302	0.669	--
tetA	***< 0.001***	***0.006***	***0.007***	***< 0.001***	***0.002***	0.081	***0.018***	0.154

Table D.12. Pearson coefficients generated from Pearson correlations among target genes [$\log_{10}$(gene copies per 16S rRNA gene copies)] in river sediments. Shaded regions indicate p-values less than 0.05.

	bla$_{SHV}$	*cadA*	*floR*	*intI1*	*mexB*	*nikA*	*sul1*	*sul3*
cadA	***0.934***	--	--	--	--	--	--	--
floR	***0.820***	***0.802***	--	--	--	--	--	--
intI1	***0.814***	***0.912***	***0.818***	--	--	--	--	--
mexB	***0.814***	***0.827***	***0.849***	***0.890***	--	--	--	--
nikA	***0.700***	0.551	***0.723***	***0.605***	0.559	--	--	--
sul1	***0.898***	***0.918***	0.562	***0.884***	***0.807***	0.350	--	--
sul3	0.496	***0.742***	***0.678***	***0.701***	***0.705***	0.343	0.181	--
tetA	***0.884***	***0.742***	***0.761***	***0.876***	***0.801***	0.548	***0.797***	0.438

Appendix E: Supporting Information for Chapter 5

E.1 Materials and Methods

Table E.1. Gradient elution of 0.1% formic acid in acetonitrile (% B) with respect to time (min) on Phenomenex Kinetex F5 column for summer and fall samples.

Time (min)	% B
0	5
1	5
5	100
7	100
7.5	5
25	5

Table E.2. Gradient elution of 0.1% formic acid in methanol (% B) with respect to time (min) on Waters XSelect CSH C18 column that separated sulfonamides, macrolide, and others via method 1 and fluoroquinolones and tetracyclines via method 2 for winter and spring samples.

Method 1		Method 2	
Time (min)	% B	Time (min)	% B
0.0	0	0.0	0
5.5	100	0.5	0
7.5	100	4.0	40
8.0	0	7.0	100
20.0	0	9.0	100
--	--	10.0	0
--	--	20.0	0

Table E.3. Single reaction monitoring quantification and confirmation transitions and collision energy (CE) for analytes.

Analyte	Parent Ion (m/z)	Product Ion (m/z)	CE (V)	Quantification or Confirmation
		Sulfonamides		
sulfapyridine	250.10	156.00	17	quantification
	250.10	108.05	25	confirmation
sulfadiazine	251.05	156.00	15	quantification
	251.05	108.05	24	confirmation
sulfamethoxazole	254.05	92.10	29	quantification
	254.05	108.00	24	confirmation
$^{13}C_6$-sulfamethoxazole	260.05	98.10	32	quantification
(internal standard)	260.05	114.10	27	confirmation
$^{13}C_6$-sulfamethazine	285.05	186.00	22	quantification
(*surrogate)*	285.05	123.00	20	confirmation
		Fluoroquinolones		
norfloxacin	320.10	276.10	17	quantification
	320.10	302.10	21	confirmation
ciprofloxacin	332.10	231.05	35	quantification
	332.10	314.10	21	confirmation
ofloxacin	362.10	261.10	28	quantification
	362.10	318.10	19	confirmation
clinafloxacin	366.10	348.00	20	confirmation
(*internal standard)*	366.10	305.00	22	quantification
nalidixic acid	233.15	187.00	27	confirmation
(*surrogate*)	233.15	104.05	40	quantification
		Tetracyclines		
tetracycline	445.10	410.10	19	quantification
	445.10	427.05	11	confirmation
doxycycline	445.10	321.05	31	quantification
	445.10	428.15	18	confirmation
demeclocycline	465.10	448.05	20	quantification
(*surrogate*)	465.10	430.05	17	confirmation
		Macrolides		
erythromycin	734.4	158.15	35	quantification
	734.4	576.35	15	confirmation
erythromycin-H_2O	716.45	158.15	35	quantification
	716.45	558.35	15	confirmation
roxithromycin	837.45	158.10	35	quantification
	837.45	679.45	20	confirmation

Table E.3. Continued.

$^{13}C_2$-erythromycin	736.40	160.15	35	quantification
	736.40	578.35	20	confirmation
$^{13}C_2$-erythromycin-H_2O	718.40	160.15	35	quantification
	718.40	560.35	20	confirmation
Non-categorized				
trimethoprim	291.10	230.10	23	quantification
	291.10	123.05	24	confirmation
lincomycin	407.30	126.10	35	quantification
	407.30	359.20	18	confirmation
simeton	198.20	68.10	33	quantification
(*internal standard)*	198.20	100.10	27	confirmation

E.1 Results

Table E.4. Average ± standard deviation of pH levels throughout WWTP.

Sampling Location	**ID**	**pH**
Influent	1	7.88 ± 0.10
Primary Clarifier Effluent	2	7.88 ± 0.11
Aeration Basin	3	7.34 ± 0.17
Secondary Clarifier Effluent	4	7.66 ± 0.03
Final Effluent	5	7.62 ± 0.15
Post-Tertiary Treatment	6	7.45 ± 0.11
Post-Filtration	7	7.60 ± 0.16
Reuse Effluent	8	7.54 ± 0.21

Table E.5. Relative recovery of antibiotics from in: (1) influent; (2) primary clarifier effluent; (3) activated sludge; (4) secondary clarifier effluent; (5) effluent; (6) post-tertiary treatment; (7) post filtration; and (8) reuse effluent.

	Relative Recovery (%)											
ID	**EMC**	**RXC**	**TMP**	**LMC**	**SPD**	**SDZ**	**SMX**	**TCC**	**DXC**	**NFC**	**CFC**	**OFC**
						Summer						
1	223	253	32	155	3	– [a]	0	97	95	49	13	65
2	238	185	75	212	9	–	0	154	423	65	92	123
3	181	140	42	144	53	–	102	12	36	28	26	28
4	244	228	161	218	97	–	60	167	139	62	59	97
5	157	160	74	182	179	–	90	107	102	55	46	83
6	171	177	29	189	125	–	115	162	151	61	172	101
7	238	175	45	180	106	–	161	159	134	64	187	113
8	393	102	0	0	0	–	0	167	0	0	0	0
						Fall						
1	11	– [a]	83	184	6	6	0	215	32	86	116	85
2	11	–	42	159	2	4	0	248	171	84	111	77
3	11	6	25	175	5	6	0	237	73	90	31	28
4	12	9	34	104	84	120	20	219	94	93	45	59
5	14	13	40	66	137	139	30	198	63	60	37	84
6	10	9	41	99	89	86	13	146	73	89	51	73
7	12	10	36	117	148	138	25	199	104	103	80	90
8	10	10	0	0	0	0	0	0	0	22	14	50
						Winter						
1	215	187	16	14	20	13	19	133	109	150	0	148
2	245	158	11	13	1	1	1	143	77	38	51	107
3	167	78	10	6	0	0	0	150	78	103	121	117
4	134	76	13	4	4	4	1	139	66	94	102	76
5	125	70	7	4	20	20	25	106	48	79	68	27
6	112	57	8	9	19	19	9	101	40	68	56	43
7	125	67	5	11	8	21	8	103	39	72	67	37
8	70	37	0	0	0	0	0	0	2	33	29	28
						Spring						
1	201	30	40	29	45	15	23	34	12	48	48	14
2	209	42	32	20	1	0	7	86	32	47	60	11
3	132	63	46	14	5	0	7	72	35	39	46	22
4	85	51	39	8	10	10	5	66	34	38	48	37
5	107	42	27	9	66	49	39	34	10	16	9	8
6	112	53	54	5	15	13	10	80	32	34	35	29
7	95	38	48	17	16	16	7	73	38	39	47	21
8	91	50	0	0	0	0	0	0	0	1	2	12

EMC = erythromycin, RXC = roxithromycin; TMP = trimethoprim; LMC = lincomycin; SPD = sulfapyridine; SDZ = sulfadiazine; SMX = sulfamethoxazole; TCC = tetracycline; DXC = doxycycline; NFC = norfloxacin; CFC = ciprofloxacin; OFC = ofloxacin

[a] peak shift during HPLC-MS/MS analysis resulted in non-detection

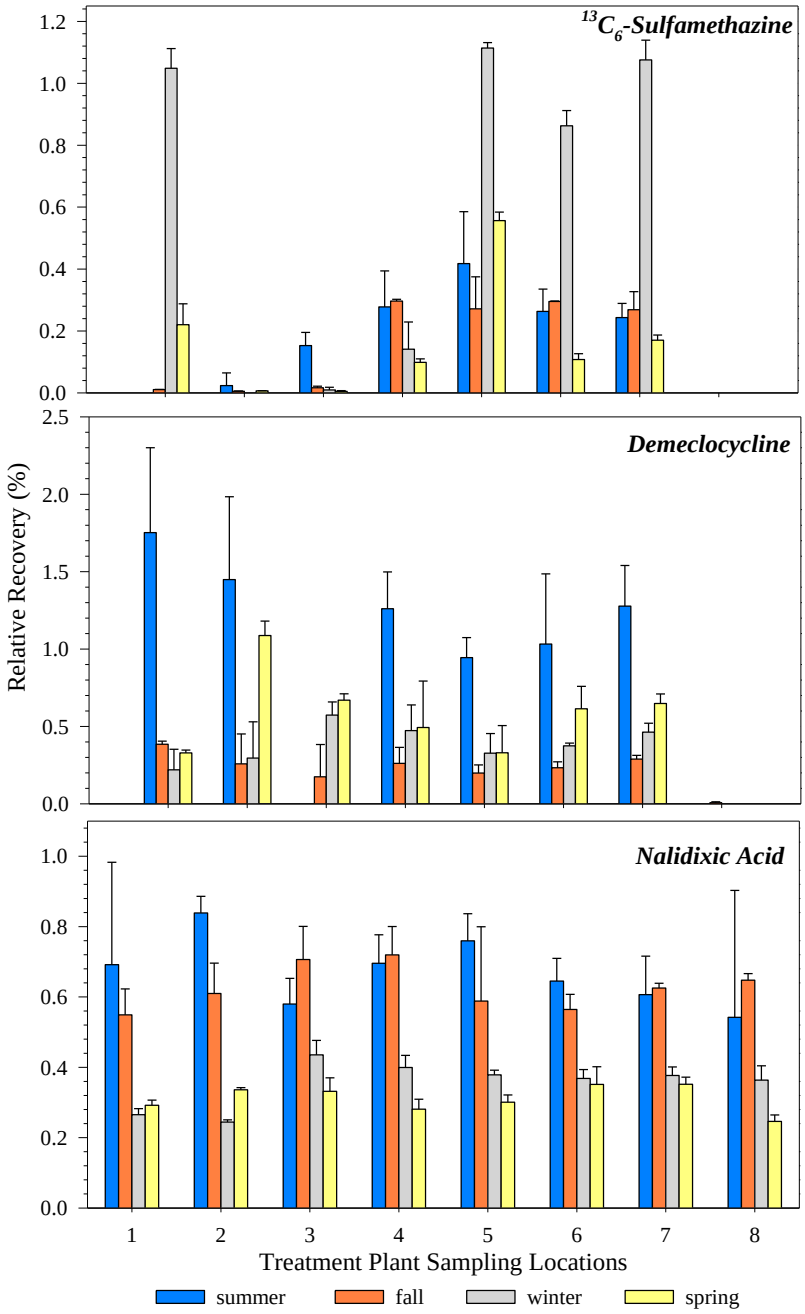

Figure E.1. Relative recovery (%) of surrogates in: (1) influent; (2) primary clarifier effluent; (3) activated sludge; (4) secondary clarifier effluent; (5) effluent; (6) post-tertiary treatment; (7) post filtration; and (8) reuse effluent. Samples were collected during summer (blue), fall (orange), winter (grey), and spring (yellow). Bars represent standard deviation of replicates.

Table E.6. Relative recovery (average and standard deviation) of $^{13}C_6$-sulfamethazine from triplicate samples of: (1) influent; (2) primary clarifier effluent; (3) activated sludge; (4) secondary clarifier effluent; (5) effluent; (6) post-tertiary treatment; (7) post filtration; and (8) reuse effluent.

	$^{13}C_6$-Sulfamethazine Relative Recovery (%)							
Sampling	**Summer**		**Fall**		**Winter**		**Spring**	
Location	**avg**	**stdev**	**avg**	**stdev**	**avg**	**stdev**	**avg**	**stdev**
1	0.0	0.0	1.1	0.0	104.9	6.4	22.0	6.7
2	2.4	4.1	0.5	0.1	0.0	0.0	0.6	0.1
3	15.3	4.3	1.6	0.5	0.9	0.8	0.5	0.2
4	27.8	11.6	29.7	0.6	14.1	8.8	9.9	1.1
5	41.8	16.8	27.2	10.3	111.4	1.7	55.6	2.8
6	26.3	7.2	29.5	0.2	86.3	4.9	10.8	1.9
7	24.3	4.5	26.8	5.9	107.6	6.4	17.0	1.7
8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table E.7. Relative recovery (average and standard deviation) of demecycline from triplicate samples of: (1) influent; (2) primary clarifier effluent; (3) activated sludge; (4) secondary clarifier effluent; (5) effluent; (6) post-tertiary treatment; (7) post filtration; and (8) reuse effluent.

	Demecycline Relative Recovery (%)							
Sampling	**Summer**		**Fall**		**Winter**		**Spring**	
Location	**avg**	**stdev**	**avg**	**stdev**	**avg**	**stdev**	**avg**	**stdev**
1	175.2	54.8	38.4	2.0	21.9	13.3	29.2	1.5
2	144.8	53.6	25.8	19.4	29.5	23.4	33.6	0.6
3	0.0	0.0	17.4	20.8	57.3	8.5	33.2	3.8
4	126.0	23.8	26.1	10.3	47.3	16.6	28.1	2.8
5	94.4	12.9	19.9	5.2	32.7	12.6	30.1	2.1
6	103.2	45.4	23.3	3.9	37.5	1.7	22.2	19.3
7	127.7	26.2	28.9	2.4	46.3	5.8	35.2	2.0
8	0.0	0.0	0.7	0.6	0.0	0.0	24.6	1.8

Table E.8. Relative recovery (average and standard deviation) of nalidixic acid from triplicate samples of: (1) influent; (2) primary clarifier effluent; (3) activated sludge; (4) secondary clarifier effluent; (5) effluent; (6) post-tertiary treatment; (7) post filtration; and (8) reuse effluent.

	Nalidixic Acid Relative Recovery (%)							
Sampling	**Summer**		**Fall**		**Winter**		**Spring**	
Location	**avg**	**stdev**	**avg**	**stdev**	**avg**	**stdev**	**avg**	**stdev**
1	69.2	29.1	54.9	7.4	26.5	1.7	32.8	1.9
2	83.9	4.7	61.0	8.6	24.4	0.6	108.7	9.4
3	58.0	7.3	70.6	9.4	43.5	4.1	67.0	4.1
4	69.6	8.1	72.0	8.1	40.0	3.4	49.3	30.0
5	76.0	7.7	58.8	21.1	37.9	1.3	32.9	17.6
6	64.5	6.5	56.4	4.3	36.8	2.5	61.3	14.5
7	60.7	10.9	62.5	1.3	37.7	2.4	64.8	6.2
8	54.2	36.1	64.8	1.9	36.4	4.1	0.0	0.0

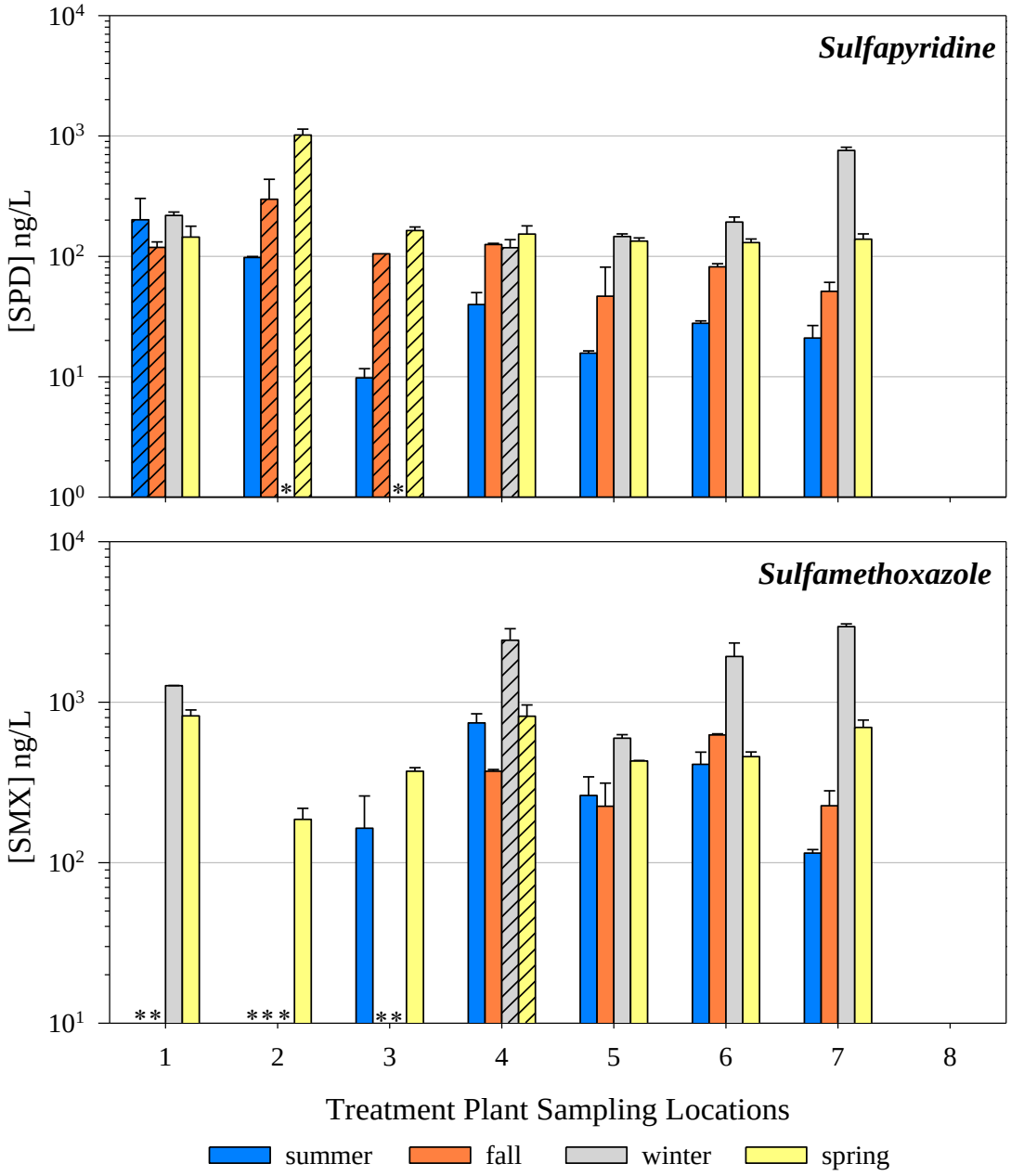

Figure E.2. Water concentration (ng/L) on $\log_{10}$-scale of detected sulfonamides in: (1) influent; (2) primary clarifier effluent; (3) activated sludge; (4) secondary clarifier effluent; (5) effluent; (6) post-tertiary treatment; (7) post filtration; and (8) reuse effluent. Samples were collected during summer (blue), fall (orange), winter (grey), and spring (yellow). Bars represent standard deviation of replicates. Bars with diagonal pattern are concentrations with high uncertainties due to poor recoveries (≤ 6%). Asterisks denote samples with non-detected analytes due to no recoveries.

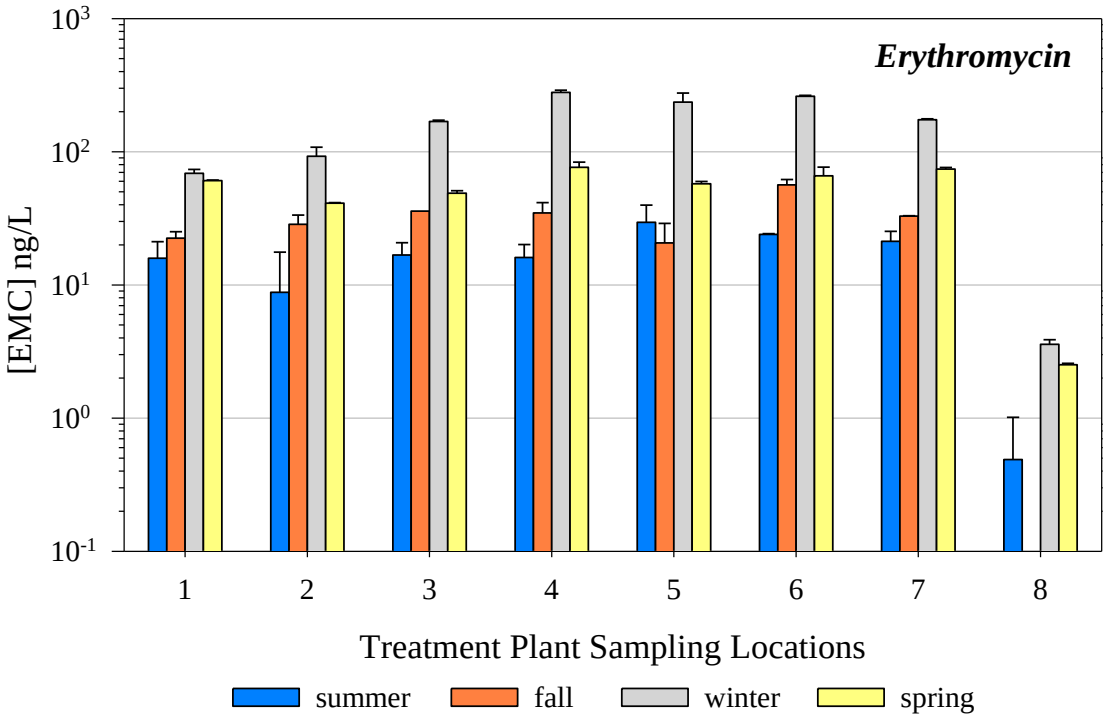

Figure E.3. Water concentration (ng/L) on $\log_{10}$-scale of detected macrolides in: (1) influent; (2) primary clarifier effluent; (3) activated sludge; (4) secondary clarifier effluent; (5) effluent; (6) post-tertiary treatment; (7) post filtration; and (8) reuse effluent. Samples were collected during summer (blue), fall (orange), winter (grey), and spring (yellow). Bars represent standard deviation of replicates.

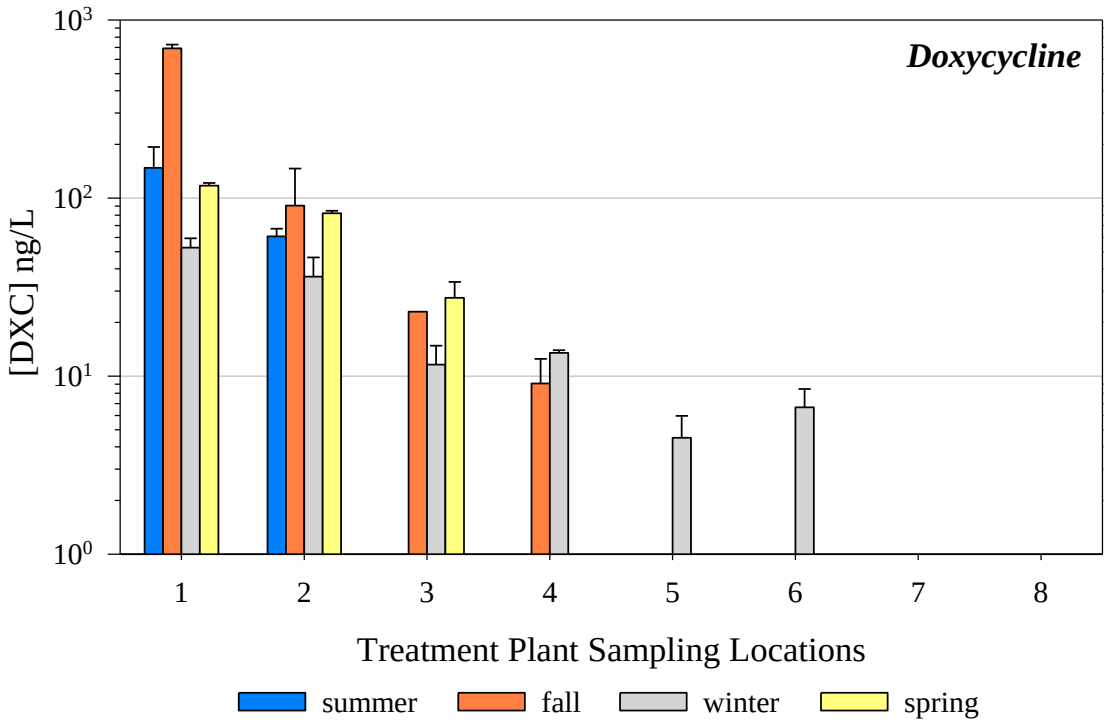

Figure E.4. Water concentration (ng/L) on $\log_{10}$-scale of detected tetracyclines in: (1) influent; (2) primary clarifier effluent; (3) activated sludge; (4) secondary clarifier effluent; (5) effluent; (6) post-tertiary treatment; (7) post filtration; and (8) reuse effluent. Samples were collected during summer (blue), fall (orange), winter (grey), and spring (yellow). Bars represent standard deviation of replicates.

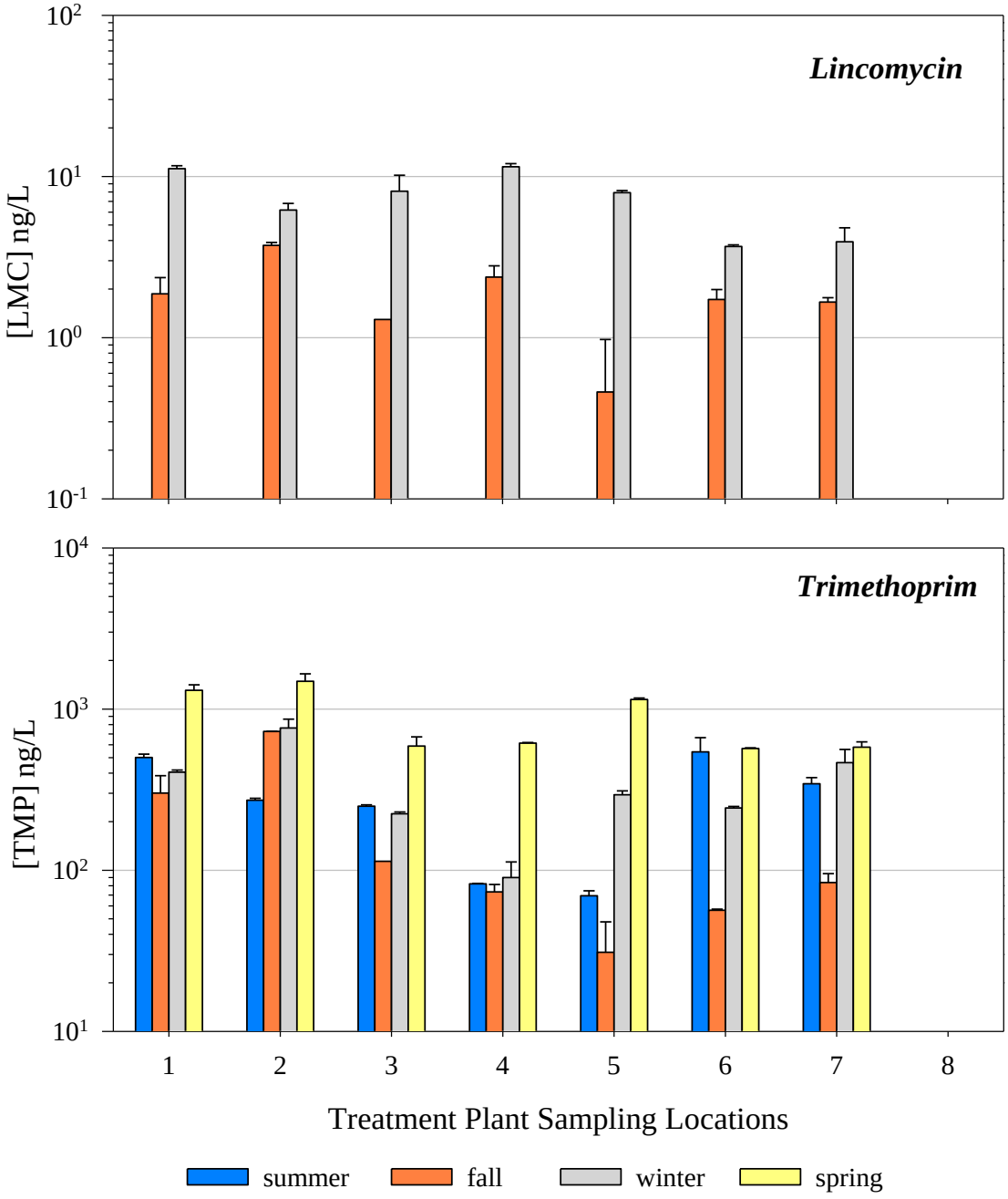

Figure E.5. Water concentration (ng/L) on $\log_{10}$-scale of detected non-categorized antibiotics in: (1) influent; (2) primary clarifier effluent; (3) activated sludge; (4) secondary clarifier effluent; (5) effluent; (6) post-tertiary treatment; (7) post filtration; and (8) reuse effluent. Samples were collected during summer (blue), fall (orange), winter (grey), and spring (yellow). Bars represent standard deviation of replicates.

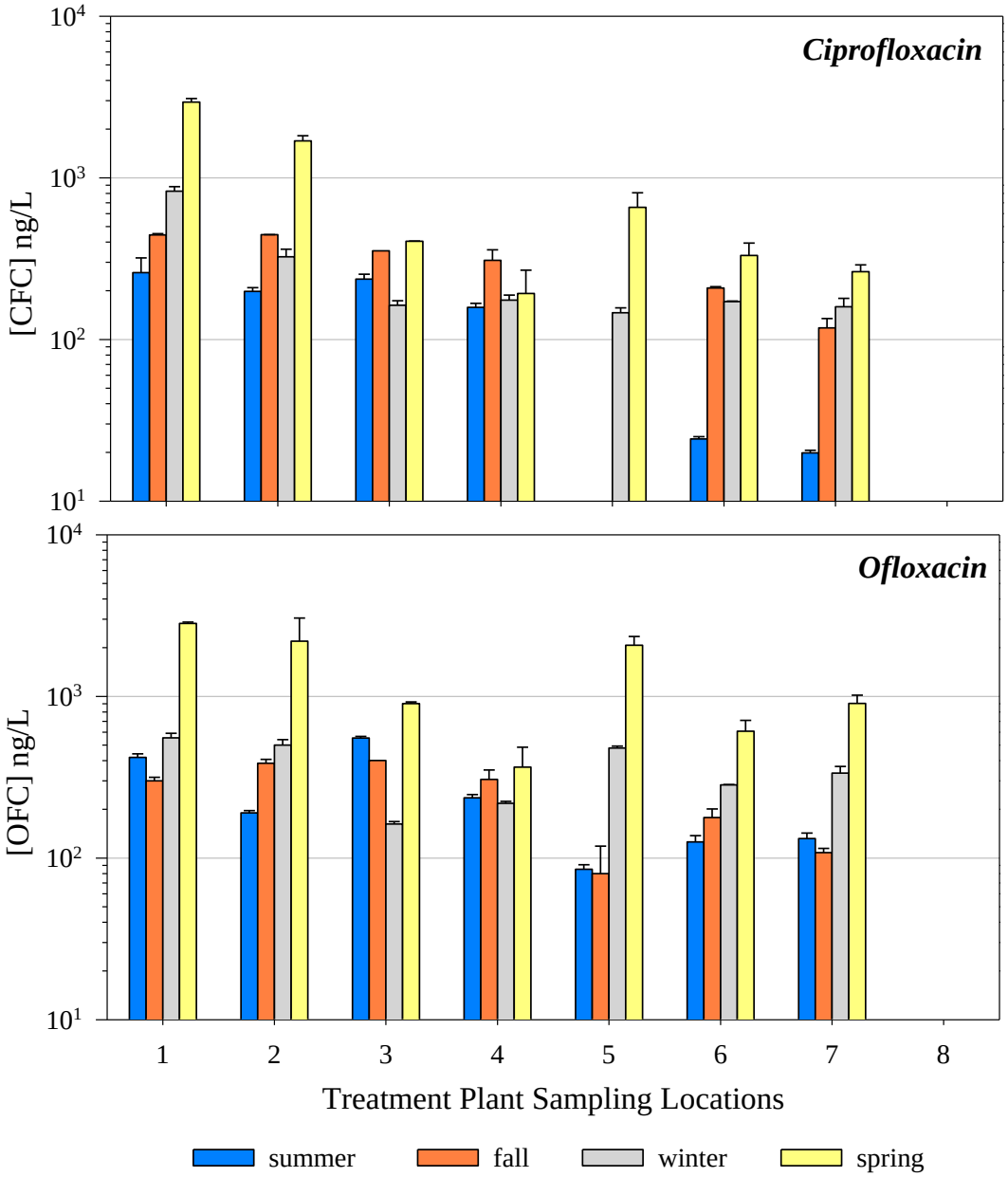

Figure E.6. Water concentration (ng/L) on $\log_{10}$-scale of detected fluoroquinolones in: (1) influent; (2) primary clarifier effluent; (3) aeration basin; (4) secondary clarifier effluent; (5) effluent; (6) post-tertiary treatment; (7) post-filtration; and (8) reuse effluent. Samples were collected during summer (blue), fall (orange), winter (grey), and spring (yellow). Bars represent standard deviation of replicates.