CHARACTERIZING THE PLANT-ASSISTED ATTENUATION OF ANTIBIOTICS IN MUNICIPAL BIOSOLIDS

by

Theresa Oluwatosin Adesanya

A Thesis
Submitted to the Faculty of Graduate Studies of
The University of Manitoba
in partial fulfilment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

Department of Soil Science
University of Manitoba
Winnipeg, Manitoba

ABSTRACT

Theresa Adesanya, Ph.D., The University of Manitoba, May 2021. Characterizing the plant-assisted attenuation of antibiotics in municipal biosolids. Advisors: Dr. Annemieke Farenhorst and Dr. Francis Zvomuya

Biosolids contain contaminants, including antibiotics, which are ingested by humans and excreted intact or as metabolites. Land application of biosolids could be a pathway for antibiotic release into the environment. The presence of antibiotics in the environment could lead to the promotion of bacterial resistance in microorganisms, with dire consequences for human health due to the severity of antibiotic resistant infections. Phytoremediation may reduce antibiotic concentrations in biosolids and wastewaters prior to land application.

Three main studies were conducted to (i) characterize the dissipation of sulfamethoxazole and trimethoprim in biosolids; (ii) evaluate the extent and kinetics of sulfamethoxazole sorption by cattail and switchgrass roots; and (iii) evaluate the uptake and translocation of ciprofloxacin and sulfamethoxazole by cattail and switchgrass.

Sulfamethoxazole dissipated rapidly under both saturated (99% dissipation) and unsaturated (96% dissipation) conditions, indicating a lower persistence of sulfamethoxazole in biosolids, while trimethoprim was more persistent under unsaturated (25% dissipation) compared to saturated conditions (95% dissipation). Switchgrass roots were more effective than cattail roots in removing sulfamethoxazole from aqueous solutions through sorption. Percentage sulfamethoxazole removal at the three temperatures tested (5, 15, and 25 °C) was 26 – 33% for cattail and 60 – 77% for switchgrass. Phytoextraction of antibiotics was lower for switchgrass (9.8% for both ciprofloxacin and sulfamethoxazole) than for cattail (~34% for ciprofloxacin and

~20% for sulfamethoxazole). Both plant species concentrated antibiotics in the roots, with poor translocation to the aboveground biomass. Harvesting of both aboveground biomass and roots of cattail would be more effective in the phytoextraction of ciprofloxacin compared to switchgrass. Overall, our results indicate that reducing conditions favoured trimethoprim dissipation, which was otherwise persistent under aerobic/unsaturated conditions. Phytoremediation of sulfamethoxazole and ciprofloxacin through phytoextraction and rhizofiltration could attenuate these antibiotics from wastewater and aqueous systems.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my amazing supervisors Dr. Zvomuya and Dr. Farenhorst for their invaluable assistance, guidance, encouragement, and support. I am grateful to have had supervisors who wanted the best for me and encouraged me to be better. I am also grateful to all members of my advisory committee, Dr. Tim McAllister, and Dr. Claudia Sheedy (deceased) for their constant support and contributions towards my Ph.D. program. I am also thankful to Dr. Inoka Amarakoon who graciously agreed to join my advisory committee at the later stages following the passing of Dr. Sheedy. I appreciate funding support from the Natural Sciences and Engineering Research Council (NSERC) CREATE-H_2O Program, the NSERC Discovery Grant Program, and the University of Manitoba Graduate Fellowship (UMGF).

I would also like to express my gratitude to Rob Ellis for his technical help and continued willingness to help with my numerous requests. Thanks Rob! Special thanks to Dr. Chris Metcalfe and Dr. Tamanna Sultana for their contributions towards the second chapter of my thesis. I am also thankful to Brenda Seaborn for technical assistance during antibiotic extraction. My appreciation also goes to Dr. Ike Agomoh for his support, encouragement, and statistical help. Special thanks to Dr. Nicholson Jeke, Dr. Mayowa Adelekun, Dr. Ahmed Lasisi, Dr. Sirajum Munira, Cherish Ofeoritse and Dr. Marsha Serville for their help and support. To all faculty, staff, and students of the Department of Soil Science, thank you for making the Department of Soil Science a place to call home. I am also grateful to my amazing friend, Toluwanimi Bankole, for her constant help with sample processing, countless presentation rehearsals, and encouragement to complete my program. Your support went a long way in making this possible. Thank you.

To my mum (Abolaji Adesanya) and siblings, I extend my heartfelt appreciation. Thank you for your continual prayers, support, and encouragement. To my late dad, Mr. Olayinka Adesanya,

who left close to the end of my Ph.D., your unwavering belief in me helped me push through tough times of my program. I will always love, cherish, and remember you. Thank you for all you did for me and for seeing the best in me.

I am most grateful to God Almighty for the strength and grace to finally make it to the end of my Ph.D. program.

FOREWORD

This thesis was prepared in manuscript format in accordance with the thesis guidelines of the Department of Soil Science, University of Manitoba. The thesis consists of five chapters. Chapter 1 is the general introduction while Chapter 2 evaluated the behaviour of sulfamethoxazole and trimethoprim under conditions mimicking temporary storage of biosolids. Chapter 3 examined the sorption of sulfamethoxazole (rhizofiltration) as a mechanism of attenuation and Chapter 4 focused on the mechanisms of attenuation of ciprofloxacin and sulfamethoxazole by phytoextraction. Chapter 5 is the overall synthesis. Chapter 2 has been published in the Journal of Environmental Science and Health, Part B. Chapter 3 has been published in Chemosphere while Chapter 4 is being prepared for submission to Chemosphere.

Publication based on Chapter 2:

Adesanya, T., F. Zvomuya, T. Sultana, C. Metcalfe, and A. Farenhorst. 2020. Dissipation of sulfamethoxazole and trimethoprim during temporary storage of biosolids: A microcosm study. Chemosphere: 128729. doi: 10.1016/j.chemosphere.2020.128729.

Publication based on Chapter 3:

Adesanya, T., F. Zvomuya, and A. Farenhorst. 2020. Sulfamethoxazole sorption by cattail and switchgrass roots. Journal of Environmental Science and Health, Part B 0: 1–11. doi: 10.1080/03601234.2020.1807263

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. GENERAL INTRODUCTION

1.1 Pharmaceuticals in the Environment

Pharmaceutical compounds, used to prevent and/or treat diseases, are often excreted as parent molecules and metabolites from the human body. As such, the primary dispersal route of pharmaceuticals into the broader environment remains wastewater disposal (Göbel et al., 2005a; Clarke and Smith, 2011; Carvalho et al., 2014). Antibiotics are an important group of pharmaceuticals, in part because of their wide-spread use by humans and in veterinary medicine (Hirsch et al., 1999). Antibiotics have been detected in 80% of the 139 streams sampled across 30 states in the United States (Kolpin et al., 2002). In Canada, several antibiotics, including amoxicillin, ciprofloxacin, sulfamethoxazole, and trimethoprim, were detected in surface water in Wascana Creek (Waiser et al., 2011). Yargeau et al. (2007) reported sulfamethoxazole concentrations of up to 578 ng L^{-1} in surface water samples collected from the Yamaska River Basin in Quebec, Canada. Other studies have detected antibiotics in stream water (Lissemore et al., 2006), groundwater (Fram and Belitz, 2011), and soils amended with biosolids (Golet et al., 2002) and manure (Zhang et al., 2016).

The presence of antimicrobials in the environment, over and above microbial production due to human and/or animal use, is a major concern because of their promotion of bacterial resistance, negative impacts on microbial communities, disruption of microbial structures, and potential negative effects on humans (Daughton and Ternes, 1999; Kolpin et al., 2002). Wastewater-related sources of antibiotics in the environment range from residential facilities to municipal wastewater treatment plants (WWTPs).

Conventional WWTPs are not designed for the complete removal of organic compounds such as antibiotics and other pharmaceuticals (Fatta-Kassinos et al., 2011). This incomplete elimination of antibiotics results in the discharge of treated wastewater containing pharmaceuticals into the environment. For example, Brown et al. (2006) detected at least one antibiotic in 58% of the effluent samples collected from municipal wastewater treatment plants, residential buildings, hospitals, and dairies. Antibiotics detected in the municipal wastewater effluent samples included sulfamethoxazole, trimethoprim, and ciprofloxacin (Brown et al. 2006). Fatta-Kassinos et al. (2011) detected 19 pharmaceuticals in effluents from three WWTPs in Cyprus. Similarly, Hirsch et al. (1999) reported frequent detection of erythromycin, trimethoprim and sulfamethoxazole in effluents from WWTPs and river water samples, with average concentrations ranging from 0.66 – 6 $\mu g\ L^{-1}$.

Antibiotics can be strongly sorbed by solids during wastewater treatment, which may render them less available for microbial degradation (Wu et al., 2009). Hence, several studies have reported the presence of antibiotics in biosolids. For example, Kinney et al. (2006) reported that pharmaceuticals were among the common organic contaminants detected in biosolids. Six of the nine biosolids tested in their study contained at least one of the antibiotics erythromycin-H_2O, sulfamethoxazole, and trimethoprim (Kinney et al. 2006). Golet et al. (2002) detected ciprofloxacin and norfloxacin in sewage sludges from several WWTPs in Switzerland, with concentrations ranging from 1.40 to 2.42 $mg\ kg^{-1}$ (dry wt.). Ding et al. (2011) detected antibiotics in biosolids sampled from six cities in Michigan, with concentrations ranging from 2.6 $\mu g\ kg^{-1}$ for lincomycin to 743.6 $\mu g\ kg^{-1}$ for oxytetracycline. Land application of biosolids could therefore lead to the dispersal of the antibiotics to the environment (Jjemba, 2002; Göbel et al., 2005a). As such, there is a need to develop cost-effective approaches to remove antibiotics from biosolids before they are land-applied, as an eco-service benefit that safeguards human health and ensures a healthy environment.

1.2 Antibiotics Effects

Although many antibiotics dissipate rapidly in the environment, their continuous and widespread use causes their pseudo-persistence (Brooks et al., 2006). The increasing use of antibiotics in human and veterinary treatments has increased the frequency of their detection in the environment (Grenni et al., 2018). The broader detection of antibiotics in a range of environmental compartments is likely contributing to the development of resistance in bacteria, including potential pathogens causing diseases in humans, and a proliferation of antibiotic resistance genes and bacteria in the environment is a great cause for concern to human society (Daughton and Ternes, 1999). The World Health Organization (WHO) described antibiotic resistance as one of the greatest threats to global health and food security; therefore, addressing and tackling antibiotic resistance has become a priority for WHO (World Health Organization, 2020).

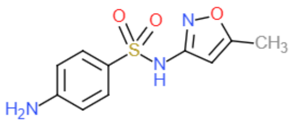
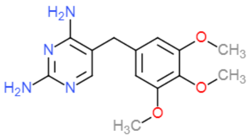
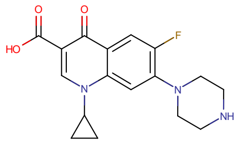
Several studies have found antibiotic resistance even at low antibiotic concentrations (Hughes and Andersson, 2012; Huang et al., 2016). Antibiotics can also bioaccumulate in plants grown on antibiotic-contaminated soils and in aquatic organisms; this raises concerns about potential health problems when ingested (Ebele et al., 2017). Another consequence of antibiotics in the environment is the alteration of microbial community structure and function (Grenni et al., 2018). For example, Hou et al. (2015) reported that as the concentration of sulfamethazine increased in the sediment slurry used in their study, the abundance of denitrifying genes decreased, suggesting inhibition of the growth of these denitrifiers by the antibiotic (Hou et al., 2015). Several studies have also indicated potential changes in fundamental nutrient cycling processes due to antibiotic effects on beneficial microbial biofilms and communities (Lawrence et al., 2005; Rico et al., 2014).

Klaver and Matthews (1994) reported reduced nitrification in an aquatic microcosm containing 12.5-75 mg L^{-1} oxytetracycline. They observed a reduction in the conversion of ammonia to nitrate at low rates of oxytetracycline and a near complete disruption of nitrification at higher rates (50 and 75 mg L^{-1}). In a study by Stone et al. (2011), tylosin inhibited CO_2 production while a mixture of chlortetracycline and tylosin inhibited nitrification in deeper column layers, resulting in the accumulation of ammonium. Liu et al. (2009) also observed a decrease in microbial respiration in soils spiked with sulfonamides and trimethoprim during the first 4 d following antibiotic addition.

1.3 Ciprofloxacin, Sulfamethoxazole and Trimethoprim and their Occurrence in Wastewater

Ciprofloxacin, sulfamethoxazole and trimethoprim were selected for this study because of their frequent detection in wastewater. The common occurrence of the three antibiotics in wastewater gives them a pseudo-persistence in the environment. The environmental fate and transport of antibiotics is dependent on a number of factors, such as the physico-chemical properties of the antibiotic, including its potential to bioaccumulate or sorb to matrices (e.g., sediment and soil), and the characteristics of the compartment in which the antibiotic resides, such as organic carbon content, moisture content and oxygen level (Hellström, 2004; Stone et al., 2011). Physico-chemical properties of the three antibiotics are presented in Table 1.1.

Table 1.1: Physico-chemical characteristics of sulfamethaxazole, trimethoprim and ciprofloxacin

	Sulfamethoxazole	Trimethoprim	Ciprofloxacin
Structure			
Class	Sulfonamides	Diaminopyrimidines	Fluoroquinolones
Molecular weight (g mol^{-1})	253.28	290.32	331.35
Molecular formula	$C_{10}H_{11}N_3O_3S$	$C_{14}H_{18}N_4O_3$	$C_{17}H_{18}FN_3O_3$
Solubility at 25 °C (mg L^{-1})	438[a]	400[b]	86[e]
Log K_{ow}	0.89[b]	0.91[b]	0.28[f]
pK_a	pKa_1=1.8; pKa_2=5.6[c]	7.12[b]	pKa_1=6.15, pKa_2=8.66[e]
K_{oc} (L kg^{-1})	219[d]	724[d]	6100[g]

Data from: [a] Fioritto et al. (2007), [b] Lin and Gan (2011), [c] Lin et al. (1997), [d] Barron et al. (2009),[e]Yu et al. (1994), [f] Albero et al. (2018), and [g] Nowara et al. (1997)

1.3.1 Sulfamethoxazole

Sulfamethoxazole is a member of the sulfonamide class of antimicrobials, which are commonly used to treat humans and livestock (Miao et al., 2004). Sulfonamides are the most prescribed antimicrobial class for humans (Miao et al. 2004). They are hydrophilic and hence relatively mobile in water. Sulfamethoxazole inhibits the production of dihydrofolic acid, which is necessary for bacterial growth and survival (Isidori et al., 2005). The dominant form of SMX is SMX^+ (cationic) at pH below pKa_1, SMX^0 (neutral) at pH between pKa_1 and pKa_2, and SMX^- (anionic) at pH above pKa_2 (Hu et al., 2019). It is one of the antibiotics frequently detected in treated wastewater and biosolids (Brown et al., 2006; Kim et al., 2007; Sabourin et al., 2009) and has been detected at concentrations of up to 0.17 μg L^{-1} in portable groundwater sources in California (Fram and Belitz, 2011). Sulfamethoxazole has a log K_{ow} of 0.89 and a water solubility of 438 mg L^{-1} at 25 °C (Fioritto et al., 2007; Herklotz et al., 2010). It can be sorbed by sludge particles during wastewater treatment, resulting in its presence in biosolids (Carballa et al., 2004). Carballa et al. (2004) concluded that sorption by primary and secondary sludge as well as biological degradation were the two mechanisms of sulfamethoxazole removal in WWTPs. In contrast, Wu et al. (2009) observed weak sorption of sulfamethoxazole by biosolids used in their study and concluded that sorption was a less important mechanism of sulfamethoxazole removal during wastewater treatment.

1.3.2 Ciprofloxacin

Ciprofloxacin belongs to the fluoroquinolone class of antibiotics (Cardoza et al., 2005) and is transferred to the environment through the discharge of treated wastewater (Hirsch 1999). It has a log K_{ow} of 0.28 but has been reported to have a strong tendency to sorb onto sediments (Belden et al., 2007; Wu et al., 2009; Guerra et al., 2014). Antibacterial fluoroquinolones are commonly used to treat respiratory and urinary tract infections (Lewis et al., 2012). Ciprofloxacin is also a primary product of the degradation of enrofloxacin, which is an antibiotic used in agriculture and aquaculture (Cardoza et al., 2005). A significant proportion (60%) of ingested fluoroquinolones is excreted unchanged (that is, as the parent molecule) (Hirsch et al., 1999; Lewis et al., 2012), leading to the presence of ciprofloxacin in biosolids and wastewater. The dominant forms of ciprofloxacin are cationic at pH below 6.15 (pKa_1), zwitterionic at pH between 6.15 (pKa_1) and 8.6 (pKa_2), and anionic at pH above 8.6 (Aristilde and Sposito, 2013). Golet et al. (2002) detected ciprofloxacin and norfloxacin and their metabolites in biosolids and in agricultural soil that had received biosolids application. McClellan and Halden (2010) observed that ciprofloxacin was the antibiotic detected at the highest concentration (mean = 6858 µg kg^{-1}) in biosolids sampled from WWTPs across 32 states in the USA (McClellan and Halden, 2010). Ciprofloxacin has been reported to photodegrade under sunlight in quartz glass reactors (Sponza and Koyuncuoglu, 2019) and in water in Teflon-lined mesocosms (Cardoza et al. 2005). Although Lin et al. (2018) observed the photodegradation of ciprofloxacin in both solid-phase (kaolinite) and aqueous-phase systems, the authors reported that the photolysis half-life of ciprofloxacin in kaolinite under simulated sunlight was 2-25 times longer than ciprofloxacin half-life in deionized water (20 min). Ciprofloxacin can also be sorbed by solids, such as activated carbon (Carabineiro et al., 2011; Genç and Dogan, 2015), quartz sand (Xu et al., 2014), and organic matter (Belden et al., 2007).

1.3.3 Trimethoprim

Trimethoprim belongs to the diaminopyrimidines family and is commonly used to treat urinary and respiratory tract infections in humans (Wu et al., 2011). It is also widely used in livestock as a growth promoter and for the prevention and treatment of diseases (Renew and Huang, 2004; Sarmah et al., 2006). Trimethoprim is commonly co-administered with sulfamethoxazole (Renew and Huang, 2004). It restricts dihydrofolate reductase, thus interfering with the synthesis of bacterial folate (Göbel et al., 2005b). It has a log K_{ow} of 0.91 (Lin and Gan, 2011). Trimethoprim exists mostly as a cation at pH below its pKa (7.12), and neutral form at pH greater than 7.12 (Zhang et al., 2014). Trimethoprim is persistent in biosolids and is frequently detected in effluents from WWTPs (Liu et al., 2018). Göbel et al. (2005b) detected trimethoprim at a concentration of 41 µg kg^{-1}(dry wt.) in activated sludge samples from a WWTP in Switzerland. Trimethoprim was one of the antibiotics detected in biosolids from WWTPs at concentrations ranging from 0.7 - 22 µg kg^{-1} (Kinney et al., 2006).

1.4 Constructed Wetlands and Phytoremediation

Constructed wetlands may be an effective approach for the in situ removal of contaminants from biosolids, including through phytoremediation. They are designed to treat wastewater via natural processes associated with wetland microbial communities, sediments, and vegetation (Braskerud, 2002; Vymazal, 2007). Relative to conventional WWTPs, constructed wetlands require lower costs for construction and maintenance; they also require less energy and are less operationally demanding (Cameron et al., 2003; Hijosa-Valsero et al., 2011; Wu et al., 2015). Constructed wetlands are suitable for wastewater treatment in small communities, including communities located in remote areas (Wu et al., 2015). The use of constructed wetlands for phytoremediation of biosolids could reduce the risk of nutrients, metals, and pharmaceuticals being transported to the broader environment (Jeke et al., 2015). Constructed wetlands could also reduce contaminant concentrations in biosolids, making them safer for agricultural land application. Constructed wetlands have also been used to reduce concentrations of nutrients (Braskerud, 2002), pharmaceuticals (Matamoros and Bayona, 2006; Conkle et al., 2008), pesticides (Blankenberg et al., 2006), and pathogens (Karim et al., 2004) in wastewater.

Phytoremediation is the use of plants to remove organic and inorganic contaminants from the environment (Raskin et al., 1994). Phytoremediation has gained increasing popularity in recent years mainly because it is more cost-effective than other remediation methods (Pandey et al., 2016). The removal of contaminants by plants occurs through phytoextraction (plant uptake), rhizofiltration (sorption to plant roots), phytodegradation, phytostabilization, and phytovolatilization (Salt et al., 1998). Wetland-based and terrestrial phytoremediation have been proven to be reliable approaches to remove contaminants and nutrients from wastewater and biosolids (Kivaisi, 2001; Vymazal, 2007; Jeke et al., 2015) and have been demonstrated to be effective in the in situ treatment of biosolids in end-of-life municipal lagoons (Jeke et al., 2015). Phytoremediation may be useful in removing pharmaceuticals from wastewater, biosolids or sewage sludge prior to land application.

1.4.1 Mechanisms of Antibiotic Removal during Storage and Wetland-based Phytoremediation

1.4.1.1 Antibiotic dissipation

Biosolids produced from wastewater treatment can be stored temporarily for stabilization or for further reduction of pathogens present in the biosolids (Ahmed and Sorensen, 1995). Dewatered biosolids may be stockpiled on or adjacent to fields (Ahmed and Sorensen, 1995), while saturated biosolids are typically stored in tanks or lagoons prior to dewatering (Chae et al., 2018). There is a potential for further treatment and dissipation of antibiotics in biosolids during storage in either stockpiles or storage tanks. Antibiotic dissipation could be influenced by storage conditions such as exposure to sunlight or aeration, and antibiotic physicochemical properties (Chenxi et al., 2008; Zhang et al., 2017). For example, Berendsen et al. (2018) investigated the dissipation of antibiotics in five different types of manure over a 24-d storage period. The authors reported rapid dissipation of sulfamethoxazole during storage, with DT_{50} values ranging from 1.6-5.7 d. Chenxi et al (2008) examined the dissipation of six antibiotics in biosolids and found that ciprofloxacin persisted while other antibiotics (tetracycline, doxycycline, clindamycin, erythromycin, and clarithromycin) completely dissipated during the 77-d storage period. The authors attributed the persistence of ciprofloxacin to its low biodegradability. Chenxi et al. (2008) also reported that the antibiotics were more persistent under anaerobic than aerobic conditions.

1.4.1.2 Sorption

Sorption of antibiotics by sediments has been an effective method for removal of antibiotics from aqueous media such as wastewater (Maheshwari et al., 2013). In a study investigating mechanisms of antibiotic removal in constructed wetlands, Choi et al. (2016) observed higher removal efficiencies for sulfonamides compared with the tetracyclines tested in their study. The authors attributed the antibiotic removal to sorption by soil particles due to the higher pK_a of the sulfonamides.

Plant roots could provide surfaces for sorption of inorganic and organic chemicals and thus reduce their mobility and bioavailability in soil (Laperche et al., 1997; Sas-Nowosielska et al., 2008). For example, Song et al. (2018) observed sorption of fluoride ions by reed roots while investigating the mechanism of uptake of fluoride ions by reed residues. In a study on the removal of crystal violet dye from aqueous solution, Kulkarni et al. (2017) reported that water hyacinth roots provided surfaces for sorption of the dye and could serve as a low-cost sorbent for the removal of the dye from aqueous solution. Plant roots were also effective in removing metals such as copper, cadmium, chromium, nickel, lead, and zinc from aqueous solution through sorption (Dushenkov et al., 1995). Adnan and Thanasupsin (2016) reported that the floating aquatic plant, *Salvinia cucullate*, removed an androgenic steroid hormone (17α-methyltestosterone) through sorption to plant roots. Sorption of a contaminant by media such as soil and plant roots is influenced by the physicochemical properties of the sorbent and sorbate (Wang and Wang, 2015; Banerjee and Chattopadhyaya, 2017). Other factors that could play an important role in the sorption of organic chemicals by roots or soil include temperature, initial concentration of the compound, and contact time (Albero et al., 2018). While sorption by wetland sediments has been demonstrated to be effective in removing organic contaminants (Carvalho et al., 2013; Cardinal et al., 2014), the role of plant roots in the attenuation of antibiotics has not been adequately demonstrated in the literature.

1.4.1.3 Uptake by plants

Phytoextraction is the plant removal of contaminants from soil by concentrating the compounds in harvestable plant biomass (Salt et al., 1998). Ideal plants for phytoextraction would be those that produce high biomass, have high tolerance for the contaminants, and are easy to harvest (Chen et al., 2011). Phytoextraction has been effective in removing nutrients from biosolids (Jeke et al., 2015, Jeke et al., 2017), pesticides from runoff (Blankenberg et al., 2006), and pathogens from wastewater and sediments (Karim et al., 2004). Uptake of antibiotics by food crops has been reported in some studies (Chitescu et al., 2013; Hawker et al., 2013). For example, Chitescu et al. (2013) investigated the uptake of sulfamethoxazole, oxytetracycline and ketoconazole from soil by watercress and reported sulfamethoxazole concentrations ranging from 4 - 7.5 µg kg^{-1} in the plant. Dodgen et al. (2013) reported an uptake of 51% of the initial spike of naproxen by collard plants from hydroponic solutions. In recent years, there has been increasing interest in the use of non-food crops for the phytoextraction of organic and inorganic chemicals from soil, water and biosolids. For example, Panja et al. (2019) evaluated the potential of vertiver grass for plant-based treatment of wastewater and observed that the grass removed 80% of ciprofloxacin present in the aqueous media. Liu et al. (2013) reported that the native wetland plant, *Phragmites australis* accumulated (ng g^{-1} dry wt.) 13,834 ciprofloxacin, 6,901 oxytetracycline, and 2,047 sulfamethazine from a hydroponic solution spiked with these antibiotics. Plant uptake of antibiotics is affected by antibiotic properties, such as hydrophobicity, molecular size, and dissociation potential, antibiotic concentration, and plant species (Carvalho et al., 2014).

1.4.2 Selected plant species for phytoremediation

1.4.2.1 Cattail

Cattail (*Typha spp*) is found in wetland habitats across North America. This aquatic perennial plant has its roots and basal parts submerged under water and its shoots and reproductive parts above the water surface (Nilratnisakorn et al., 2007). Cattail is commonly used for phytoremediation (Doucette et al., 2005) because of its high biomass yield and tolerance to trace element contaminants (Ling et al., 2011; Xu et al., 2011). Cattail can colonize an area and grow rapidly (Ye et al., 2001). Studies have demonstrated the ability of cattail to remove nutrients, trace elements, and pharmaceuticals from contaminated soil, wastewater, and biosolids (Jeke et al., 2015; Zhang et al., 2016). However, little is known about the active uptake of antibiotics from hydroponics or wastewater using cattail. Similarly, there is a dearth of published research on the sorption of antibiotics by cattail roots. Because of its ability to remove contaminants from wastewater, cattail has a potential to extract and accumulate antibiotics from biosolids during phytoremediation.

1.4.2.2 Switchgrass

Switchgrass (*Panicum virgatum*) is a perennial C4 plant which is naturally occurring in North America. It is commonly used as a forage crop and has been used to generate bioenergy (Parrish and Fike, 2005). This hardy plant has the ability to adapt to extreme weather conditions, grow in acidic soils, and produce high biomass yields, which makes it a suitable plant for phytoremediation (McLaughlin and Walsh, 1998; Parrish and Fike, 2005; Chen et al., 2011). Chen et al. (2011) reported that switchgrass could tolerate high levels of cadmium in soil and could grow in contaminated soils. Other studies have shown the effectiveness of switchgrass to remove excess nutrients such as N and P (Kovar and Claassen, 2009; Jeke et al., 2015), trace metals (Reed et al., 2002; Jeke et al., 2015) from soils, manure or wastewater and biosolids. Murphy and Coats (2011) observed that switchgrass could take up and biodegrade atrazine in its leaf biomass, as evidenced by the metabolites deethylatrazine, didealkylatrazine and deisopropylatrazine, which were detected in the leaves.

1.5 Objectives

To our knowledge, there is limited information on the mechanisms involved in antibiotic uptake and translocation in plants, and their dissipation during storage (e.g., in stockpiles and lagoons or storage tanks). Similarly, the sorption of antibiotics by cattail and switchgrass roots has not been investigated. Therefore, the overall objective of this thesis was to identify and characterize the mechanisms involved in the phytoremediation of biosolids and wastewater. Specific objectives of this study were to (i) characterize the dissipation of sulfamethoxazole and trimethoprim in biosolids (Chapter 2); (ii) evaluate the extent and kinetics of sulfamethoxazole sorption by cattail and switchgrass roots (Chapter 3); and (iii) evaluate the uptake and translocation of ciprofloxacin and sulfamethoxazole by cattail and switchgrass (Chapter 4). Results from the research will provide insights into the effective use of phytoremediation to attenuate antibiotics in environmental media.

1.6 Thesis Outline

The general layout of this thesis follows the thesis guidelines of the Department of Soil Science, University of Manitoba. The individual research chapters (Chapters 2 through 4) were prepared in manuscript format and are as follows:

Chapter 2: Dissipation of sulfamethoxazole and trimethoprim during temporary storage of biosolids: a microcosm study;

Chapter 3: Root sorption as a mechanism for sulfamethoxazole attenuation by cattail (*Typha latifolia* L.) and switchgrass (*Panicum virgatum* L.); and

Chapter 4: Phytoextraction of ^{14}C-labeled sulfamethoxazole and ciprofloxacin by cattail and switchgrass.

1.7 References

Adnan, F., and S.P. Thanasupsin. 2016. Kinetic studies using a linear regression analysis for a sorption phenomenon of 17a-methyltestosterone by Salvinia cucullata in an active plant reactor. Environ. Eng. Res. **21**: 384–392. doi: 10.4491/eer.2016.019.

Ahmed, A.U., and D.L. Sorensen. 1995. Kinetics of pathogen destruction during storage of dewatered biosolids. Water Environ. Res. **67**: 143–150. doi:10.2175/106143095x131286

Albero, B., J.L. Tadeo, M. Escario, E. Miguel, and R.A. Pérez. 2018. Persistence and availability of veterinary antibiotics in soil and soil-manure systems. Sci. Total Environ. **643**: 1562–1570. doi: 10.1016/j.scitotenv.2018.06.314.

Aristilde, L., and G. Sposito. 2013. Complexes of the antimicrobial ciprofloxacin with soil, peat, and aquatic humic substances. Environ. Toxicol. Chem. 32: 1467–1478. doi: https://doi.org/10.1002/etc.2214

Banerjee, S., and M.C. Chattopadhyaya. 2017. Adsorption characteristics for the removal of a toxic dye, tartrazine from aqueous solutions by a low-cost agricultural by-product. Arab. J. Chem. **10**: S1629–S1638. doi: 10.1016/j.arabjc.2013.06.005.

Barron, L., J. Havel, M. Purcell, M. Szpak, B. Kelleher, and B. Paull. 2009. Predicting sorption of pharmaceuticals and personal care products onto soil and digested sludge using artificial neural networks. Analyst **134**: 663–670. doi: 10.1039/B817822D.

Belden, J.B., J.D. Maul, and M.J. Lydy. 2007. Partitioning and photodegradation of ciprofloxacin in aqueous systems in the presence of organic matter. Chemosphere **66**: 1390–1395. doi: 10.1016/j.chemosphere.2006.09.032.

Berendsen, B.J.A., J. Lahr, C. Nibbeling, L.J.M. Jansen, I.E.A. Bongers, E.L. Wipfler, and M.G.M van de Schans. 2018. The persistence of a broad range of antibiotics during calve, pig and broiler manure storage. Chemosphere **204**: 267–276. doi: 10.1016/j.chemosphere.2018.04.042.

Blankenberg, A.-G.B., B. Braskerud, and K. Haarstad. 2006. Pesticide retention in two small constructed wetlands: treating non-point source pollution from agriculture runoff. Int. J. Environ. Anal. Chem. **86**: 225–231. doi: 10.1080/03067310500247470.

Braskerud, B.C. 2002. Factors affecting nitrogen retention in small constructed wetlands treating agricultural non-point source pollution. Ecol. Eng. **18**: 351–370. doi:10.1016/s0925-8574(02)00014-9

Brooks, B.W., T.M. Riley, and R.D. Taylor. 2006. Water quality of effluent-dominated ecosystems: Ecotoxicological, hydrological, and management considerations. Hydrobiol. Dordr. **556**: 365–379. doi: http://dx.doi.org.uml.idm.oclc.org/10.1007/s10750-004-0189-7.

Brown, K.D., J. Kulis, B. Thomson, T.H. Chapman, and D.B. Mawhinney. 2006. Occurrence of antibiotics in hospital, residential, and dairy effluent, municipal wastewater, and the Rio Grande in New Mexico. Sci. Total Environ. **366**: 772–783. doi: 10.1016/j.scitotenv.2005.10.007.

Cameron, K., C. Madramootoo, A. Crolla, and C. Kinsley. 2003. Pollutant removal from municipal sewage lagoon effluents with a free-surface wetland. Water Res. **37**: 2803–2812. doi: 10.1016/S0043-1354(03)00135-0.

Carabineiro, S.A.C., T. Thavorn-Amornsri, M.F.R. Pereira, and J.L. Figueiredo. 2011. Adsorption of ciprofloxacin on surface-modified carbon materials. Water Res. **45**: 4583–4591. doi: 10.1016/j.watres.2011.06.008.

Carballa, M., F. Omil, J.M. Lema, M. Llompart, C. García-Jares, I. Rodriguez, M. Gomez, and T. Ternes. 2004. Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. Water Res. **38**: 2918–2926. doi: 10.1016/j.watres.2004.03.029.

Cardinal, P., J.C. Anderson, J.C. Carlson, J.E. Low, J.K. Challis, S.A Beattie, C.N. Bartel, A.D. Elliot, O.F. Montero, S.L. Lokesh, A.F. Favreau, T.A. Kozlova, C.W. Knapp, M.L. Hanson, and C.S. Wong. 2014. Macrophytes may not contribute significantly to removal of nutrients, pharmaceuticals, and antibiotic resistance in model surface constructed wetlands. Sci. Total Environ. **482–483**: 294–304. doi: 10.1016/j.scitotenv.2014.02.095.

Cardoza, L.A., C.W. Knapp, C.K. Larive, J.B. Belden, M. Lydy, and D.W. Graham. 2005. Factors affecting the fate of ciprofloxacin in aquatic field systems. Water. Air. Soil Pollut. **161**: 383–398. doi: 10.1007/s11270-005-5550-6.

Carvalho, P.N., M.C.P. Basto, C.M.R. Almeida, and H. Brix. 2014. A review of plant–pharmaceutical interactions: from uptake and effects in crop plants to phytoremediation in constructed wetlands. Environ. Sci. Pollut. Res. **21**: 11729–11763. doi: 10.1007/s11356-014-2550-3.

Chae, M., L. Xia, C. Zhu, and D.C. Bressler. 2018. Accelerating settling rates of biosolids lagoons through thermal hydrolysis. J. Environ. Manag. **220**: 227–232. doi: 10.1016/j.jenvman.2018.05.044.

Chen, B.-C., H.-Y. Lai, D.-Y. Lee, and K.-W. Juang. 2011. Using chemical fractionation to evaluate the phytoextraction of cadmium by switchgrass from Cd-contaminated soils. Ecotoxicology **20**: 409–418. doi: 10.1007/s10646-010-0592-y.

Chen, J., X.-D. Wei, Y.-S. Liu, G.-G. Ying, S.-S. Liu, L.Y. He, H.C. Su, L.X. Hu, F.R. Chen, and Y.Q. Yang. 2016. Removal of antibiotics and antibiotic resistance genes from domestic sewage by constructed wetlands: Optimization of wetland substrates and hydraulic loading. Sci. Total Environ. **565**: 240–248. doi: 10.1016/j.scitotenv.2016.04.176.

Chenxi, W., A.L. Spongberg, and J.D. Witter. 2008. Determination of the persistence of pharmaceuticals in biosolids using liquid-chromatography tandem mass spectrometry. Chemosphere **73**: 511–518. doi: 10.1016/j.chemosphere.2008.06.026.

Chitescu, C.L., A.I. Nicolau, and A.A.M. Stolker. 2013. Uptake of oxytetracycline, sulfamethoxazole and ketoconazole from fertilised soils by plants. Food Addit. Contam. Part A **30**: 1138–1146. doi: 10.1080/19440049.2012.725479.

Choi, Y.-J., L.-H. Kim, and K.-D. Zoh. 2016. Removal characteristics and mechanism of antibiotics using constructed wetlands. Ecol. Eng. **91**: 85–92. doi: 10.1016/j.ecoleng.2016.01.058.

Clarke, B.O., and S.R. Smith. 2011. Review of ‘emerging’ organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. Environ. Int. **37**: 226–247. doi: 10.1016/j.envint.2010.06.004.

Conkle, J.L., J.R. White, and C.D. Metcalfe. 2008. Reduction of pharmaceutically active compounds by a lagoon wetland wastewater treatment system in Southeast Louisiana. Chemosphere **73**: 1741–1748. doi: 10.1016/j.chemosphere.2008.09.020.

Daughton, C.G., and T.A. Ternes. 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ. Health Perspect. **107**: 907. doi: 10.2307/3434573.

Ding, Y., W. Zhang, C. Gu, I. Xagoraraki, and H. Li. 2011. Determination of pharmaceuticals in biosolids using accelerated solvent extraction and liquid chromatography/tandem mass spectrometry. J. Chromatogr. A **1218**: 10–16. doi: 10.1016/j.chroma.2010.10.112.

Dodgen, L.K., J. Li, D. Parker, and J.J. Gan. 2013. Uptake and accumulation of four PPCP/EDCs in two leafy vegetables. Environ. Pollut. **182**: 150–156. doi: 10.1016/j.envpol.2013.06.038.

Doucette, W.J., J.K. Chard, B.J. Moore, W.J. Staudt, and J.V. Headley. 2005. Uptake of sulfolane and diisopropanolamine (DIPA) by cattails (*Typha latifolia*). Microchem. J. **81**: 41–49. doi: 10.1016/j.microc.2005.01.015.

Dushenkov, V., P.B.A.N Kumar, H. Motto, and I Raskin. 1995. Rhizofiltration: The use of plants to remove heavy metals from aqueous streams. Environ. Sci. Technol. **29**: 1239–1245. doi: 10.1021/es00005a015.

Ebele, A.J., M. Abou-Elwafa Abdallah, and S. Harrad. 2017. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. Emerg. Contam. **3**: 1–16. doi: 10.1016/j.emcon.2016.12.004.

Fatta-Kassinos, D., I.K. Kalavrouziotis, P.H. Koukoulakis, and M.I. Vasquez. 2011. The risks associated with wastewater reuse and xenobiotics in the agroecological environment. Sci. Total Environ. **409**: 3555–3563. doi: 10.1016/j.scitotenv.2010.03.036.

Fioritto, A.F., S.N. Bhattachar, and J.A. Wesley. 2007. Solubility measurement of polymorphic compounds via the pH-metric titration technique. Int. J. Pharm. **330**: 105–113. doi: 10.1016/j.ijpharm.2006.09.003.

Fram, M.S., and K. Belitz. 2011. Occurrence and concentrations of pharmaceutical compounds in groundwater used for public drinking-water supply in California. Sci. Total Environ. **409**: 3409–3417. doi: 10.1016/j.scitotenv.2011.05.053.

Genç, N., and E.C. Dogan. 2015. Adsorption kinetics of the antibiotic ciprofloxacin on bentonite, activated carbon, zeolite, and pumice. Desalin. Water Treat. **53**: 785–793. doi: 10.1080/19443994.2013.842504.

Göbel, A., A. Thomsen, C.S. McArdell, A. Joss, and W. Giger. 2005b. Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment. Environ. Sci. Technol. **39**: 3981–3989. doi: 10.1021/es048550a.

Göbel, A., A. Thomsen, C.S. McArdell, A.C. Alder, W. Giger, N. Theib, D. Loffler, and T.A. Ternes. 2005a. Extraction and determination of sulfonamides, macrolides, and trimethoprim in sewage sludge. J. Chromatogr. A **1085**: 179–189. doi: 10.1016/j.chroma.2005.05.051.

Golet, E.M., A. Strehler, A.C. Alder, and W. Giger. 2002. Determination of fluoroquinolone antibacterial agents in sewage sludge and sludge-treated soil using accelerated solvent extraction followed by solid-phase extraction. Anal. Chem. **74**: 5455–5462. doi: 10.1021/ac025762m.

Grenni, P., V. Ancona, and A. Barra Caracciolo. 2018. Ecological effects of antibiotics on natural ecosystems: a review. Microchem. J. **136**: 25–39. doi: 10.1016/j.microc.2017.02.006.

Guerra, P., M. Kim, A. Shah, M. Alaee, and S.A. Smyth. 2014. Occurrence and fate of antibiotic, analgesic/anti-inflammatory, and antifungal compounds in five wastewater treatment processes. Sci. Total Environ. **473–474**: 235–243. doi: 10.1016/j.scitotenv.2013.12.008.

Hawker, D.W., R. Cropp, and M. Boonsaner. 2013. Uptake of zwitterionic antibiotics by rice (*Oryza sativa* L.) in contaminated soil. J. Hazard. Mater. **263**: 458–466. doi: 10.1016/j.jhazmat.2013.09.066.

Hellström, A. 2004. Uptake of organic pollutants in plants. Upps. Swed. Dep. Environ. Assess. Swed. Univ. Agric. Sci. **1**: 7–8.

Herklotz, P.A., P. Gurung, B. Vanden Heuvel, and C.A. Kinney. 2010. Uptake of human pharmaceuticals by plants grown under hydroponic conditions. Chemosphere **78**: 1416–1421. doi: 10.1016/j.chemosphere.2009.12.048.

Hijosa-Valsero, M., G. Fink, M.P. Schlüsener, R. Sidrach-Cardona, J. Martín-Villacorta,T. Ternes, and E. Becares. 2011. Removal of antibiotics from urban wastewater by constructed wetland optimization. Chemosphere **83**: 713–719. doi: 10.1016/j.chemosphere.2011.02.004.

Hirsch, R., T. Ternes, K. Haberer, and K.L. Kratz. 1999. Occurrence of antibiotics in the aquatic environment. Sci. Total Environ. **225**: 109–118. doi: 10.1016/S0048-9697(98)00337-4.

Hou, L., G. Yin, M. Liu, J. Zhou, Y. Zheng, J. Gao, H. Zong, Y. Yang, L. Gao, and C. Tong 2015. Effects of sulfamethazine on denitrification and the associated N_2O release in estuarine and coastal sediments. Environ. Sci. Technol. **49**: 326–333. doi: 10.1021/es504433r.

Hu, S., Y. Zhang, G. Shen, H. Zhang, Z. Yuan, and W. Zhang. 2019. Adsorption/desorption behavior and mechanisms of sulfadiazine and sulfamethoxazole in agricultural soil systems. Soil Tillage Res. 186: 233–241. doi: 10.1016/j.still.2018.10.026.

Huang, T., Y. Xu, J. Zeng, D.-H. Zhao, L. Li, X.P. Liao, Y.H. Liu, and J.Sun. 2016. Low-concentration ciprofloxacin selects plasmid-mediated quinolone resistance encoding genes and affects bacterial taxa in soil containing manure. Front. Microbiol. **7**. doi: 10.3389/fmicb.2016.01730.

Hughes, D., and D.I. Andersson. 2012. Selection of resistance at lethal and non-lethal antibiotic concentrations. Curr. Opin. Microbiol. **15**: 555–560. doi: 10.1016/j.mib.2012.07.005.

Isidori, M., M. Lavorgna, A. Nardelli, L. Pascarella, and A. Parrella. 2005. Toxic and genotoxic evaluation of six antibiotics on non-target organisms. Sci. Total Environ. **346**: 87–98. doi: 10.1016/j.scitotenv.2004.11.017.

Jeke, N.N., A.O. Hassan, and F. Zvomuya. 2017. Phytoremediation of biosolids from an end-of-life municipal lagoon using cattail (*Typha latifolia* L.) and switchgrass (*Panicum virgatum* L.). Int. J. Phytoremediation **19**: 270–280. doi: 10.1080/15226514.2016.1225279.

Jeke, N.N., F. Zvomuya, N. Cicek, L. Ross, and P. Badiou. 2015. Biomass, nutrient, and trace element accumulation and partitioning in cattail (*Typha latifolia* L.) during wetland phytoremediation of municipal biosolids. J. Environ. Qual. **44**: 1541. doi: 10.2134/jeq2015.02.0064.

Jjemba, P.K. 2002. The potential impact of veterinary and human therapeutic agents in manure and biosolids on plants grown on arable land: a review. Agric. Ecosyst. Environ. **93**: 267–278. doi:10.1016/s0167-8809(01)00350-4

Karim, M.R., F.D. Manshadi, M.M. Karpiscak, and C.P. Gerba. 2004. The persistence and removal of enteric pathogens in constructed wetlands. Water Res. **38**: 1831–1837. doi: 10.1016/j.watres.2003.12.029.

Kim, S.D., J. Cho, I.S. Kim, B.J. Vanderford, and S.A. Snyder. 2007. Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters. Water Res. **41**: 1013–1021. doi: 10.1016/j.watres.2006.06.034.

Kinney, C.A., E.T. Furlong, S.D. Zaugg, M.R. Burkhardt, S.L. Werner, J.D. Cahill, and G.R. Jorgensen. 2006. Survey of organic wastewater contaminants in biosolids destined for land application. Environ. Sci. Technol. **40**: 7207–7215. doi: 10.1021/es0603406.

Kivaisi, A.K. 2001. The potential for constructed wetlands for wastewater treatment and reuse in developing countries: a review. Ecol. Eng. **16**: 545–560. doi:10.1016/s0925-8574(00)00113-0

Klaver, A.L., and R.A. Matthews. 1994. Effects of oxytetracycline on nitrification in a model aquatic system. Aquaculture **123**: 237–247. doi: 10.1016/0044-8486(94)90062-0

Kolpin, D.W., E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.R. Barber, and H. T. Buxton. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999−2000: A national reconnaissance. Environ. Sci. Technol. **36**: 1202–1211. doi: 10.1021/es011055j.

Kovar, J.L., and N. Claassen. 2009. Growth and phosphorus uptake of three riparian grass species. Agron. J. **101**: 1060. doi: 10.2134/agronj2008.0088x.

Kulkarni, M.R., T. Revanth, A. Acharya, and P. Bhat. 2017. Removal of crystal violet dye from aqueous solution using water hyacinth: Equilibrium, kinetics and thermodynamics study. Resour. Effic. Technol. **3**: 71–77. doi: 10.1016/j.reffit.2017.01.009.

Laperche, V., T.J. Logan, P. Gaddam, and S.J. Traina. 1997. Effect of apatite amendments on plant uptake of lead from contaminated soil. Environ. Sci. Technol. **31**: 2745–2753. doi: 10.1021/es961011o.

Lawrence, J.R., G.D.W. Swerhone, L.I. Wassenaar, and T.R. Neu. 2005. Effects of selected pharmaceuticals on riverine biofilm communities. Can. J. Microbiol. **51**: 655–669. doi: 10.1139/w05-047.

Lewis, G., A. Juhasz, and E. Smith. 2012. Detection of antibacterial-like activity on a silica surface: fluoroquinolones and their environmental metabolites. Environ. Sci. Pollut. Res. **19**: 2795–2801. doi: 10.1007/s11356-012-0781-8.

Lin C.E, C.C Chang, and W.C Lin. 1997. Migration behavior and separation of sulfonamides in capillary zone electrophoresis III. Citrate buffer as a background electrolyte. J. Chromatogr. A. **768**: 105–112. doi: 10.1016/S0021-9673(97)00010-1.

Lin, K., and J. Gan. 2011. Sorption and degradation of wastewater-associated non-steroidal anti-inflammatory drugs and antibiotics in soils. Chemosphere **83**: 240–246. doi: 10.1016/j.chemosphere.2010.12.083.

Lin, Y.-C., K.-W. Hsiao, and A.Y.-C. Lin. 2018. Photolytic degradation of ciprofloxacin in solid and aqueous environments: kinetics, phototransformation pathways, and byproducts. Environ. Sci. Pollut. Res. **25**: 2303–2312. doi: 10.1007/s11356-017-0666-y.

Ling, S., C. Joseph, and H. Eng. 2011. Biosorption of lead contaminated wastewater using cattails (*Typha angustifolia*) leaves: kinetic studies. J. Serbian Chem. Soc. **76**: 1037–1047. doi: 10.2298/JSC100628084L.

Lissemore, L., C. Hao, P. Yang, P.K. Sibley, S. Mabury, and K.R. Solomon. 2006. An exposure assessment for selected pharmaceuticals within a watershed in Southern Ontario. Chemosphere **64**: 717–729. doi: 10.1016/j.chemosphere.2005.11.015.

Liu, F., G.G. Ying, R. Tao, J.L. Zhao, J.F. Yang, and L.F. Zhao. 2009. Effects of six selected antibiotics on plant growth and soil microbial and enzymatic activities. Environ. Pollut. **157**: 1636–1642. doi: 10.1016/j.envpol.2008.12.021.

Liu, L., Y. Liu, C. Liu, Z. Wang, J. Dong, G.F. Zhu, and X. Huang. 2013. Potential effect and accumulation of veterinary antibiotics in *Phragmites australis* under hydroponic conditions. Ecol. Eng. **53**: 138–143. doi: 10.1016/j.ecoleng.2012.12.033.

Liu, Q., M. Li, X. Liu, Q. Zhang, R. Liu, Z. Wang, X. Shi, J. Quan, X. Shen, and F. Zhang. 2018. Removal of sulfamethoxazole and trimethoprim from reclaimed water and the biodegradation mechanism. Front. Environ. Sci. Eng. **12**: 6. doi: 10.1007/s11783-018-1048-5.

Maheshwari, M., R.K. Vyas, and M. Sharma. 2013. Kinetics, equilibrium and thermodynamics of ciprofloxacin hydrochloride removal by adsorption on coal fly ash and activated alumina. Desalin. Water Treat. **51**: 7241–7254. doi: 10.1080/19443994.2013.775076.

Matamoros, V., and J.M. Bayona. 2006. Elimination of pharmaceuticals and personal care products in subsurface flow constructed wetlands. Environ. Sci. Technol. **40**: 5811–5816. doi: 10.1021/es0607741.

McClellan, K., and R.U. Halden. 2010. Pharmaceuticals and personal care products in archived U.S. biosolids from the 2001 EPA national sewage sludge survey. Water Res. **44**: 658–668. doi: 10.1016/j.watres.2009.12.032.

McLaughlin, S.B., and M.E. Walsh. 1998. Evaluating environmental consequences of producing herbaceous crops for bioenergy. Biomass Bioenerg. **14**: 317–324. doi:10.1016/s0961-9534(97)10066-6

Miao, X.-S., F. Bishay, M. Chen, and C.D. Metcalfe. 2004. Occurrence of antimicrobials in the final effluents of wastewater treatment plants in Canada. Environ. Sci. Technol. **38**: 3533–3541. doi: 10.1021/es030653q.

Murphy, I.J., and J.R. Coats. 2011. The capacity of switchgrass (*Panicum virgatum*) to degrade atrazine in a phytoremediation setting. Environ. Toxicol. Chem. **30**: 715–722. doi: 10.1002/etc.437.

Nilratnisakorn, S., P. Thiravetyan, and W. Nakbanpote. 2007. Synthetic reactive dye wastewater treatment by narrow-leaved cattails (*Typha angustifolia Linn*.): effects of dye, salinity and metals. Sci. Total Environ. **384**: 67–76. doi: 10.1016/j.scitotenv.2007.06.027.

Nowara, A., J. Burhenne, and M. Spiteller. 1997. Binding of fluoroquinolone carboxylic acid derivatives to clay minerals. J. Agric. Food Chem. **45**: 1459–1463. doi: 10.1021/jf960215l.

Pandey, V.C., O. Bajpai, and N. Singh. 2016. Energy crops in sustainable phytoremediation. Renew. Sustain. Energy Rev. **54**: 58–73. doi: 10.1016/j.rser.2015.09.078.

Panja, S., D. Sarkar, K. Li, and R. Datta. 2019. Uptake and transformation of ciprofloxacin by vetiver grass (*Chrysopogon zizanioides*). Int. Biodeterior. Biodegrad. **142**: 200–210. doi: 10.1016/j.ibiod.2019.05.023.

Parrish, D.J., and J.H. Fike. 2005. The biology and agronomy of switchgrass for biofuels. Crit. Rev. Plant Sci. **24**: 423–459. doi: 10.1080/07352680500316433.

Raskin, I., N. Kumar, S. Dushenkov, and D.E. Salt. 1994. Bioconcentration of heavy metals by plants. Curr. Opin. Biotechnol. **5**: 285–290.

Reed, R.L., M.A. Sanderson, V.G. Allen, and R.E. Zartman. 2002. Cadmium application and pH effects on growth and cadmium accumulation in switchgrass. Commun Soil Sci. Plant Anal. **33**: 1187–1203. doi: 10.1081/CSS-120003881.

Renew, J.E., and C.-H. Huang. 2004. Simultaneous determination of fluoroquinolone, sulfonamide, and trimethoprim antibiotics in wastewater using tandem solid phase extraction and liquid chromatography–electrospray mass spectrometry. J. Chromatogr. A **1042**: 113–121. doi: 10.1016/j.chroma.2004.05.056.

Rico, A., M.R. Dimitrov, R.P.A. Van Wijngaarden, K. Satapornvanit, H. Smidt, and P.J. Van den Brink. 2014. Effects of the antibiotic enrofloxacin on the ecology of tropical eutrophic freshwater microcosms. Aquat. Toxicol. **147**: 92–104. doi: 10.1016/j.aquatox.2013.12.008.

Sabourin, L., A. Beck, P.W. Duenk, S. Kleywegt, D.R. Lapen, H. Li, C. Metcalfe, M. Payne, and E. Topp 2009. Runoff of pharmaceuticals and personal care products following application of dewatered municipal biosolids to an agricultural field. Sci. Total Environ. **407**: 4596–4604. doi: 10.1016/j.scitotenv.2009.04.027.

Salt, D.E., R.D. Smith, and D.S. Raskin. 1998. Phytoremediation. Annu. Rev. Plant Physiol. Plant Mol. Biol. **49**: 643–668. doi: 10.1146/annurev.arplant.49.1.643

Sarmah, A.K., M.T. Meyer, and A.B.A. Boxall. 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere **65**: 725–759. doi: 10.1016/j.chemosphere.2006.03.026.

Sas-Nowosielska, A., R. Galimska-Stypa, R. Kucharski, U. Zielonka, E. Małkowski, and L. Grey. 2008. Remediation aspect of microbial changes of plant rhizosphere in mercury contaminated soil. Environ. Monit. Assess. **137**: 101–109. doi: 10.1007/s10661-007-9732-0.

Song, R., S. Yang, H. Xu, Z. Wang, Y. Chen, and Y. Wang. 2018. Adsorption behavior and mechanism for the uptake of fluoride ions by reed residues. Int. J. Environ. Res. Public. Health **15**. doi: 10.3390/ijerph15010101.

Stone, J.J., E.K. Dreis, C.D. Lupo, and S.A. Clay. 2011. Land application of tylosin and chlortetracycline swine manure: impacts to soil nutrients and soil microbial community structure. J. Environ. Sci. Health Part B **46**: 752–762. doi: 10.1080/03601234.2011.603988

T Sponza, D., and P. Koyuncuoglu. 2019. Photodegradation of ciprofloxacin and ofloxacin antibiotics and their photo-metabolites with sunlight. Clin. Microbiol. Infect. Dis. **4**. doi: 10.15761/CMID.1000149.

Vymazal, J. 2007. Removal of nutrients in various types of constructed wetlands. Sci. Total Environ. **380**: 48–65. doi: 10.1016/j.scitotenv.2006.09.014.

Waiser, M.J., D. Humphries, V. Tumber, and J. Holm. 2011. Effluent-dominated streams. Part 2: Presence and possible effects of pharmaceuticals and personal care products in Wascana Creek, Saskatchewan, Canada. Environ. Toxicol. Chem. **30**: 508–519. doi: 10.1002/etc.398.

Wang, S., and H. Wang. 2015. Adsorption behavior of antibiotic in soil environment: a critical review. Front. Environ. Sci. Eng. **9**: 565–574. doi: 10.1007/s11783-015-0801-2.

World Health Organization. 2020. Antibiotic resistance [Fact sheet]. https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance.

Wu, C., J.D. Witter, A.L. Spongberg, and K.P. Czajkowski. 2009. Occurrence of selected pharmaceuticals in an agricultural landscape, western Lake Erie basin. Water Res. **43**: 3407–3416. doi: 10.1016/j.watres.2009.05.014.

Wu, H., J. Zhang, H.H. Ngo, W. Guo, Z. Hu, S. Liang, J. Fan, and H. Liu. 2015. A review on the sustainability of constructed wetlands for wastewater treatment: design and operation. Bioresour. Technol. **175**: 594–601. doi: 10.1016/j.biortech.2014.10.068.

Wu, Y., D. Chen, and R. Kookana. 2011. Aqueous photodegradation of selected antibiotics under different conditions. Energy Procedia **11**: 2098–2103. doi: 10.1016/j.egypro.2011.10.511.

Xu, W., W. Shi, F. Yan, B. Zhang, and J. Liang. 2011. Mechanisms of cadmium detoxification in cattail (*Typha angustifolia L*.). Aquat. Bot. **94**: 37–43. doi: 10.1016/j.aquabot.2010.11.002.

Xu, X., J. He, Y. Li, Z. Fang, and S. Xu. 2014. Adsorption and transport of ciprofloxacin in quartz sand at different pH and ionic strength. Open J. Soil Sci. **4**: 407–416. doi: 10.4236/ojss.2014.412041.

Yargeau, V., A. Lopata, and C. Metcalfe. 2007. Pharmaceuticals in the Yamaska River, Quebec, Canada. Water Ouality Res. J. Can. **42**: 231–239. doi: 10.2166/wqrj.2007.026

Ye, Z.H., S.N. Whiting, Z.-Q. Lin, C.M. Lytle, J.H. Qian, and N. Terry. 2001. Removal and distribution of iron, manganese, cobalt, and nickel within a Pennsylvania constructed wetland treating coal combustion by-product leachate. J. Environ. Qual. **30**: 1464–1473. doi: 10.2134/jeq2001.3041464x.

Yu, X., G.L. Zipp, and G.W.R. Davidson III. 1994. The effect of temperature and ph on the solubility of quinolone compounds: estimation of heat of fusion. Pharm. Res. **11**: 522–527. doi: 10.1023/A:1018910431216.

Zhang, D., J. Luo, Z.M.P. Lee, R.M. Gersberg, Y. Liu, S.K. Tan, and W.J. Ng. 2016. Ibuprofen removal in horizontal subsurface flow constructed wetlands: treatment performance and fungal community dynamics. Environ. Technol. **37**: 1467–1479. doi: 10.1080/09593330.2015.1119201.

Zhang, Y., S. Hu, H. Zhang, G. Shen, Z. Yuan, and W. Zhang. 2017. Degradation kinetics and mechanism of sulfadiazine and sulfamethoxazole in an agricultural soil system with manure application. Sci. Total Environ. **607–608**: 1348–1356. doi: 10.1016/j.scitotenv.2017.07.083.

Zhang, Y., S. Lin, C. Dai, L. Shi, and X. Zhou. 2014. Sorption-desorption and transport of trimethoprim and sulfonamide antibiotics in agricultural soil: effect of soil type, dissolved organic matter, and pH. Environ Sci. and Pollut. Res. Int. 21: 5827–35. doi: http://dx.doi.org.uml.idm.oclc.org/10.1007/s11356-014-2493-8.

2. DISSIPATION OF SULFAMETHOXAZOLE AND TRIMETHOPRIM DURING TEMPORARY STORAGE OF BIOSOLIDS: A MICROCOSM STUDY

2.1 Abstract

Little is known about the dissipation rate of microcontaminants in biosolids during storage and stabilization in stockpiles (unsaturated) or storage lagoons/tanks (saturated). The objective of this study was to characterize the dissipation in biosolids of two antibiotics, sulfamethoxazole (SMX) and trimethoprim (TMP), in microcosms under saturated and unsaturated conditions that simulated biosolids that are stockpiled on land or deposited in lagoons/tanks, respectively. The laboratory experiment was conducted at 22°C using biosolids spiked at an initial nominal concentration of 10 mg kg^{-1} for both antibiotics. Biosolids were sampled in triplicate at seven sampling times over a 42-d period. Concentrations of SMX and TMP in extracts prepared from biosolids were quantified using liquid chromatography with tandem mass spectrometry. Dissipation followed first-order kinetics, with the time to 50% dissipation (DT_{50}) of SMX significantly shorter in the unsaturated microcosms (2.8 d) than in the saturated microcosms (4.4 d), while the DT_{50} for TMP was significantly shorter in microcosms under saturated conditions (10 d) relative to unsaturated conditions (116 d). These results indicate that the reducing conditions that develop in biosolids deposited in lagoons or placed in storage tanks might be effective for enhancing the microbial degradation of antibiotics that are otherwise persistent under aerobic conditions (i.e., TMP), while also being effective for removing other antibiotics, including those that dissipate relatively readily under aerobic conditions (i.e., SMX).

2.2 Introduction

Biosolids are organic by-products of municipal sludge treatment (Kinney et al., 2006; CCME, 2012) and are rich in nutrients such as N, P, and Ca. Therefore, application of biosolids to agricultural lands results in the beneficial recycling of nutrients (Christie et al., 2001; White et al., 2010). However, there are concerns that biosolids may contain contaminants such as pharmaceuticals and personal care products, persistent organic compounds, heavy metals and pathogens, among other harmful agents (Elliott and O'Connor, 2007). Many classes of pharmaceuticals are present at trace levels in domestic wastewater and are not removed effectively during treatment (Ortiz de Garcia et al., 2014; Luo et al., 2014). One of the major concerns regarding the release of pharmaceuticals into the environment is the development of antimicrobial resistance (AMR) by microorganisms due to chronic exposure to the antimicrobial compounds present in wastewater and biosolids (Singer et al., 2016).

Miao et al. (2004) detected a range of antibiotics in treated wastewater collected from 8 municipal wastewater treatment plants (WWTPs) located in five Canadian cities. In Canada and in the Laurentian Great Lakes, sulfamethoxazole (SMX) and trimethoprim (TMP) have been frequently detected in surface waters impacted by discharges from WWTPs (Blair et al., 2013; Couperus et al., 2016). These two antibiotics often co-occur in environmental matrices because they are frequently co-prescribed for the treatment of bacterial infections. There are some antibiotic formulations that contain both SMX and TMP. In WWTPs, SMX and TMP also partition from wastewater to biosolids (Göbel et al., 2005; Nielson and Bandosz, 2016). In regions where biosolids are applied to agricultural fields, SMX and TMP have been detected in amended soils from which they can be transported into tile drains or surface runoff during rain events (Sabourin et al., 2009; Edwards et al., 2009).

Biosolids produced from wastewater treatment are often stored temporarily for stabilization and reduction of pathogens, but also because inclement weather conditions may prevent immediate land application (Ahmed and Sorensen, 1995). Dewatered or dry biosolids may be stockpiled on or adjacent to fields (Ahmed and Sorensen, 1995), while saturated biosolids are stored in tanks or lagoons prior to dewatering (Chae et al., 2018). Biosolids held in lagoons generally have a high moisture content as they usually have water ponded on the biosolids layer, especially during the spring melt or periods of heavy rainfall (Jeke et al., 2015).

There is a potential for dissipation of contaminants in biosolids during storage. For example, in a study examining the dissipation of antibiotics in five different types of manure over a 24-d temporary storage period, Berendsen et al. (2018) reported rapid dissipation of SMX, with 50% elimination (DT_{50}) values ranging from 1.6 to 5.7 d. The dissipation of antibiotics during storage could be influenced by the storage method (e.g., stockpiling vs. storage tanks/lagoons), since dissipation can be affected by the physical and chemical properties of the biosolids/substrate (Lin and Gan, 2011; Zhang et al., 2017). Biosolids deposited in lagoons are saturated with water and the oxygen supply in the lagoons may be limited, resulting in reducing conditions that favor the proliferation of anaerobic microorganisms. Previous studies have demonstrated that the redox status can influence the biodegradation of organic contaminants in environmental media (Shrout and Parkin, 2006; Ali et al., 2013). For example, Ali et al. (2013) observed lower dissipation rates for the veterinary antibiotic, tylosin in animal manure under low oxygen conditions.

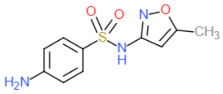
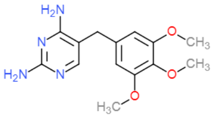
Although a few studies have investigated the dissipation of antibiotics in animal manure during storage (Kuchta and Cessna, 2009; Berendsen et al., 2018; Liu et al., 2019), there is little information on the dissipation of antibiotics during storage of biosolids prior to land application. In this study, we used a microcosm approach to simulate biosolids storage under saturated and unsaturated conditions and, therefore, mimic conditions in stockpiles on land and storage tanks/lagoons, respectively. We evaluated the dissipation of two antibiotics, SMX and TMP, that are usually co-prescribed clinically and are commonly detected in municipal wastewater and biosolids.

2.3 Materials and Methods

2.3.1 Chemicals and Reagents

Analytical grade SMX (≥ 98% purity) and TMP (≥ 98% purity) were purchased from Sigma-Aldrich (Oakville, ON, Canada). High purity (HPLC grade) methanol, acetic acid, acetone, and acetonitrile were purchased from Fisher Scientific (Ottawa, ON, Canada). Hydromatrix® was purchased from Agilent Technologies (Mississauga, ON, Canada). Selected physicochemical properties of the two antibiotics are listed in Table 2.1.

Table 2.1 Physical and chemical properties and structures of sulfamethoxazole and trimethoprim.

Antibiotic	Antibiotic class	Mol. wt. (g mol^{-1})	Structure	log K_{ow}	pK_a	Solubility (mg L^{-1})
Sulfamethoxazole	Sulfonamide	253.3		[a] 0.89	1.6[b], 5.7[c]	610[a]
Trimethoprim	Diaminopyrimidines	290		[a] 0.91	7.12[c]	400[a]

Data from: [a]Lin and Gan (2011), [b]Liu et al. (2020) and [c]Kim et al. (2007)

2.3.2 Biosolids Characterization

The biosolids were collected from an end-of-life municipal sewage lagoon located in Niverville, MB, Canada (49°35’42.7” N, 97°02’50.3” W). The lagoon had ceased operation in 2008, hence there were no residual levels of SMX and TMP in the biosolids, as confirmed by laboratory analysis of the biosolids. The biosolids were air-dried, ground, and sieved (< 2 mm). The pH was measured in a 1:2 biosolids/water suspension using an Accumet AB15 pH meter

(Fisher Scientific, Waltham, MA). The water holding capacity of the biosolids was measured using the method described by Cassel and Nielsen (1986). Other selected biosolids parameters were measured by an analytical services company, ALS Laboratories (Winnipeg, MB, Canada), using the methods briefly described below. Exchangeable cations in the biosolids were extracted using ammonium acetate and the cation content in the extracts was determined colorimetrically (Hendershot et al., 2008). Total nitrogen in a combusted biosolids sample was analyzed using a Vario Macro combustion analyzer (Elementar Americas Inc., NY, USA), as described by Rutherford et al. (2008). Biosolids nitrate-N concentration was measured using an auto-analyzer (Technicon AA II, Technicon Instrument Corp., Tarrytown, NY, USA) following extraction with 2 M KCl (Keeney and Nelson, 1982). Total phosphorus, Fe and Mn were measured using a 770 series ICP-MS (Agilent technologies, CA, USA) following digestion of the biosolids with HNO_3 and HCl (Martin et al., 1991). Biosolids samples were combusted, and total carbon was determined by measuring reduced CO_2 gas in a Vario Macro combustion analyzer (Elementar Americas Inc., NY, USA) equipped with a thermal conductivity detector. Carbonates in the biosolids were neutralized with acetic acid, and inorganic carbon was measured by comparing the pH of the resulting solution against a standard curve relating solution pH to the weight of carbonates (Goh and Mermut, 2008). Total organic carbon was calculated as the difference between the concentrations of total carbon and inorganic carbon (Skjemstad and Baldock, 2008).

2.3.3 Microcosms

Biosolids (100 g dry wt.) were packed into plastic containers (7.7 cm diam. × 7.5 cm ht.) to a bulk density of 0.74 Mg m^{-3}. Each container had four 2-mm diameter holes drilled on the lids to allow for gas exchange. The experiment was laid out as a completely randomized design with a 2 × 2 factorial treatment structure consisting of the two antibiotics (SMX and TMP) and the two treatment systems (saturated and unsaturated), with three replicates per treatment. Each of the resulting 12 experimental units consisted of 7 microcosms. Microcosms were removed from the experiment for analysis of biosolids at the intervals indicated below over the 42-d experiment.

The antibiotics (SMX and TMP) were added to a subsample of biosolids targeted for each microcosm by spiking 1 mL of a 1,000 mg L^{-1} solution in methanol, after which the subsample was thoroughly mixed within biosolids in the microcosms to achieve a nominal concentration of 10 mg kg^{-1}. This nominal concentration, which is higher than typical levels of SMX and TMP in biosolids (Sabourin et al., 2009; Edwards et al., 2009), was selected to ensure detectable concentrations over the duration of the experiment. For the saturated microcosms, reverse osmosis (RO) water was added to each container to attain saturation of the biosolids plus a 2.5-cm surface water layer to mimic ponding conditions in biosolids stored in storage tanks or deposited in lagoons. In the unsaturated microcosms, RO water was added to achieve 60% maximum water holding capacity of the biosolids. The microcosms were all placed in an incubator set at 22°C, in the dark. Each unsaturated microcosm was weighed weekly and water was added to replace any moisture lost via evaporation in the microcosm, or to maintain the 2.5-cm surface water depth in the saturated microcosms. Triplicate microcosms were removed from the incubator on Days 0, 1, 3, 7, 14, 28 and 42. Biosolids in each microcosm were retrieved, thoroughly mixed, and stored at -20°C until preparation for analysis. At each sampling time, biosolids pH was measured in a 1:2 biosolids:water suspension, as described above.

Another experiment was conducted to measure changes in redox potential (Eh) in saturated and unsaturated microcosms under the same experimental conditions as described above. Redox potential was measured using a platinum electrode permanently placed at a biosolids depth of ~2 cm in the saturated and unsaturated microcosms. The Eh was measured on Days 1, 3, 7, 14, 28, 35, and 42 by inserting a Ag/AgCl reference electrode into the biosolids layer at each sampling time and connecting both the reference and platinum electrodes to a Mastercraft 0052-0060-2 voltmeter (Canadian Tire, Toronto, ON). Voltmeter readings were corrected to values obtainable from a standard hydrogen electrode (Amarawansha et al., 2015).

2.3.4 Analysis of antibiotics

Biosolids were freeze-dried overnight and then extracted by pressurized liquid extraction (PLE) using a Dionex Model 300 Accelerated Solvent Extractor (ASE) (Fisher Scientific, Ottawa, ON). Briefly, approximately 1 g of dried biosolids was thoroughly mixed with 4 g of Hydromatrix® medium and packed into a 34-mL ASE cell lined with a 42.5-mm glass fibre filter. The mixture was then spiked with 100 µL of a 1 mg kg^{-1} solution of stable isotope-labeled surrogates of SMX and TMP (i.e., $^{13}C_3$-SMX; $^{13}C_6$-TMP). The packed cell was extracted by PLE using the following ASE conditions: heat, 5 min; static, 5 min; flush, 60%; purge, 60 s; cycles, 3; solvent, 3:7 Acetone/MilliQ water; pressure, 1500 psi; and temperature, 80°C. Procedural blanks were extracted simultaneously with the biosolids samples.

The extracts from PLE were evaporated to dryness using a rotary evaporator, dissolved in 250 mL of Milli-Q water, and adjusted to a pH of 7.5. The resulting extract was then cleaned up via solid phase extraction (SPE) using Oasis HLB 500 mg extraction cartridges (60 µm, Waters, Milford, MA, USA). The cartridge was preconditioned with 6 mL acetone, 6 mL methanol, and 6 mL Milli-Q water (pH 7.5), after which the diluted biosolids extracts were passed through the cartridges at a rate of 5 mL min^{-1}. After all the extracts had passed through the manifold, the bottles were rinsed with 10 mL Milli-Q water to ensure complete removal of extract from the bottles and allowed to pass through the cartridges. Thereafter, cartridges were aspirated to dryness and eluted with 3 × 3 mL of methanol. The eluate (9 mL) was evaporated to near dryness in a CentriVap

Vacuum Concentrator (Labconco Corp., MO) and reconstituted to a volume of 0.4 mL with HPLC-grade methanol. Extracts were stored at -20°C until quantification.

Antibiotic concentrations were determined by liquid chromatography with tandem mass spectroscopy (LC-MS/MS) with an electrospray ionization (ESI) source using an Agilent 1100 HPLC coupled to a 5500 QTrap mass spectrometer (Applied Biosystems Sciex, Concord, ON, Canada) operated in positive ion mode. Samples were separated chromatographically using a Kinetex 2.6 µm C18 column (50 × 4.6 mm) (Phenomenex, Torrance, CA, USA). The LC binary mobile phases for gradient elution were: Solvent A: Milli-Q water (100%) with 0.1% acetic acid; and Solvent B: acetonitrile (100%) with 0.1% acetic acid. Mass spectrometry (MS) detection was performed using Multiple Reaction Monitoring (MRM). The antibiotics and their stable isotope surrogates were detected by MRM by monitoring the mass transitions listed in Table 2.2. For quantitation, an internal standard method with an eight-point calibration graph covering the range of anticipated analyte concentration in the biosolids samples was used. Quantitation of the internal standards (SMX-$^{13}C_3$ and TMP-$^{13}C_6$) was used to correct for analyte recovery and matrix effects. The limits of detection (LOD) and limits of quantitation (LOQ) were determined by spiking the biosolids with the analytes (n = 5) at near the lowest concentration in the calibration standard and measuring the variation in responses. The LODs and LOQs were, respectively, 0.045 and 0.152 µg kg^{-1} for TMP, and 0.027 and 0.092 µg kg^{-1} for SMX.

Table 2.2 Ions monitored for MRM analysis of target antibiotics and their stable isotope surrogates.

Compounds	Mass transition (m/z) monitored
Sulfamethoxazole	254.1>156.0
Sulfamethoxazole-$^{13}C_6$	260.0>162.0

Trimethoprim	291.1>123.2
Trimethoprim-$^{13}C_3$	294.1>126.2

2.3.5 Model fitting and statistical analysis

Nonlinear modeling techniques, using PROC NLIN in SAS 9.4 (SAS Institute, 2013), were used to identify the kinetic models that best fit the data for the dissipation of SMX and TMP in biosolids. Antibiotic dissipation data were fitted to a first-order model, a first-order double exponential model, an availability-adjusted first-order model, a mixed zero- and first-order, a bi-exponential model, a first-order two compartment model, and a 3/2 order model. The model with the lowest Akaike Information Criterion (AIC) was selected as the best fit. In all cases, the dissipation of both antibiotics was best described by the first-order kinetic model:

$$C_t = C_0 e^{-kt} \qquad (1)$$

where C_0 is the initial antibiotic concentration (mg kg^{-1}), C_t is the antibiotic concentration (mg kg^{-1}) at time t (d), and k is the first order rate constant (d^{-1}).

The dissipation half-life (DT_{50}) was calculated as:

$$DT_{50} = \frac{\ln 2}{k} \qquad (2)$$

The dissipation percentage was calculated as

$$\%\ \text{dissipated} = \frac{C_0 - C_t}{C_0} \times 100 \qquad (3)$$

The first-order kinetic model was fitted to each replicate and treatment effects on the resultant parameter estimates were evaluated via analysis of variance using PROC GLIMMIX in SAS 9.4, with treatment system and antibiotic compound as fixed effects. The gamma distribution was specified for k and DT_{50}, while percent dissipation data followed a beta distribution. Treatment differences were assessed at $\alpha = 0.05$ using the Tukey multiple comparison procedure.

2.4 Results and Discussion

2.4.1 Biosolids properties and redox potential

The biosolids had a pH of 7.36 and an exchangeable cation content of 41 $\text{cmol}_c\ \text{kg}^{-1}$ and contained 22,000 mg kg^{-1} iron, 500 mg kg^{-1} manganese, 3400 mg kg^{-1} total nitrogen, 366 mg kg^{-1} nitrate, 900 mg kg^{-1} total phosphorus, 42 g kg^{-1} organic carbon, and 55 g kg^{-1} total carbon at the start of the experiment. The pH in the microcosms changed slightly over the 42-d period, with the pH being significantly greater for biosolids in the saturated microcosms (mean 7.43) than the unsaturated microcosms (mean 7.38). However, this small difference in pH was not expected to significantly affect antibiotic dissipation. Both SMX and TMP are weak bases, with pKa values of 5.7 and 7.12, respectively (Table 2.1). Therefore, TMP would be present primarily in the neutral form and SMX in the anionic form at the pH values recorded in the microcosms. This might be an important consideration when comparing the dissipation rates observed in the present study to previous studies.

In the separate experiment that examined changes in redox potential, Eh decreased rapidly over time in biosolids under saturated conditions, indicating the development of anaerobic conditions (Figure 2.1). For SMX, Eh values declined from 262 mV on Day 1 to -129 mV on Day 42. For TMP, Eh values declined from 252 mV on Day 1 to -74 mV on Day 42. In contrast, in the unsaturated microcosms, the Eh remained above 300 mV throughout the experiment, indicative of aerobic conditions. According to Reddy et al. (2000), Eh values of 300 to -50 mV indicate moderately reducing conditions. The Eh of the saturated microcosms decreased to below -50 mV between Days 28 and 42, indicating that the biosolids in the saturated microcosms evolved to reducing conditions, with nitrate, manganese, and iron as the likely electron acceptors and facultative microbes being the active microorganisms (Pezeshki and DeLaune, 2012). The numerical differences in Eh of the treatments with SMX and TMP in saturated microcosms were likely due to the relative antibacterial activity of these compounds. Since bacterial communities can be impacted by antibiotics, this can influence redox potential (Hunting and Kampfraath, 2013; Reese et al., 2018). We speculate that SMX and TMP select for differing bacterial communities, which produced varying effects on redox conditions.

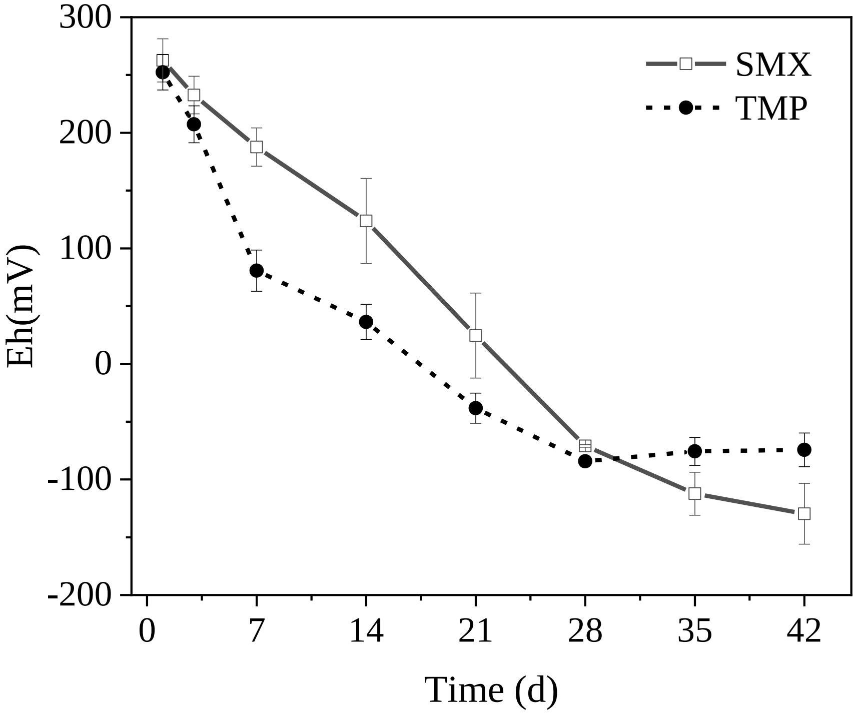


Figure 2.1 Changes in redox potential (Eh) over time in saturated microcosms spiked with sulfamethoxazole (SMX) and trimethoprim (TMP). Error bars represent standard errors of the mean (SE) for n = 3 replicates in each treatment.

2.4.2 Dissipation kinetics

There was a significant antibiotic × system interaction for the percentage of antibiotics dissipated (Table 2.3). Total SMX and TMP dissipation after 42 d ranged from 25 to 99% (Figure 2.2). Total SMX dissipation after 42 d was slightly, but significantly greater in saturated (99%) than unsaturated (96%) microcosms (Figure 2.2). Total TMP dissipation after 42 d was significantly greater in saturated (95%) than unsaturated (25%) microcosms. Dissipation of antibiotics can be influenced by many factors, such as environmental conditions (e.g., redox potential) and the physicochemical properties of both antibiotics and media (Liu et al., 2010; Lin and Gan, 2011; Zhang et al., 2017). There was a larger difference in dissipation between saturated and unsaturated microcosms for TMP than for SMX but both antibiotics showed greater total dissipation under saturated conditions. For three manure-amended soils, Wu et al. (2012) also reported a relatively large difference in total TMP dissipation between saturated (75-90% at 20 d) and unsaturated (39-74% at 100 d) conditions. Thus, the difference in antibiotic removal from saturated vs. unsaturated microcosms was likely due to the difference in redox status between the two treatments. As the biosolids in the saturated microcosms were completely saturated with water, oxygen was depleted over time and a decrease in Eh was evident. Under such anaerobic conditions, various facultative and anaerobic microorganisms are present in the media and can convert compounds to their reduced form through anaerobic respiration (Pezeshki and DeLaune, 2012).

Table 2.3 Percent of antibiotics dissipated in unsaturated and saturated microcosms after 42 d.

Treatment	Percentage dissipated (%)

Antibiotic	
Sulfamethoxazole	98 ± 0.2
Trimethoprim	73 ± 0.9
System	
Unsaturated	74 ± 0.1
Saturated	98 ± 0.2
	P value
Antibiotic	< 0.0001
System	< 0.0001
Antibiotic × system	< 0.0001

Data are least-square means (±SE, n = 3).

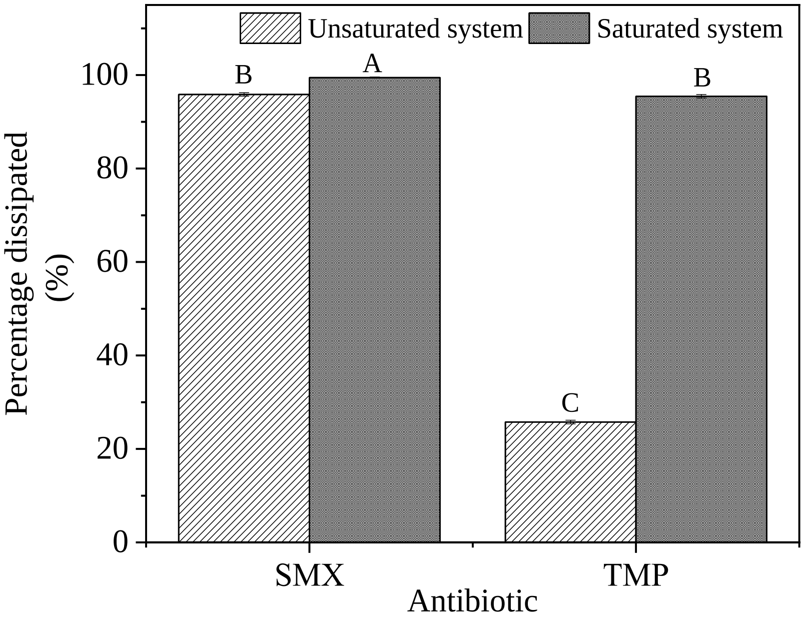


Figure 2.2 Mean (n = 3) percentage of sulfamethoxazole (SMX) and trimethoprim (TMP) dissipated in saturated and unsaturated microcosms. Error bars represent standard errors of the mean (SE).

The dissipation of SMX (Figure 2.3) and TMP (Figure 2.4) followed first-order kinetics for both saturated (aerobic) and unsaturated (anaerobic) microcosms. There was a significant antibiotic × system interaction for the first-order rate constant (Table 2.4). The first-order rate constant for TMP was significantly greater in saturated (0.07 h^{-1}) than unsaturated (0.006 h^{-1}) systems (Figure 2.5a) with the DT_{50} in the unsaturated microcosms (116 d) being an order of magnitude greater than the DT_{50} in the saturated microcosms (10 d) (Fig. 2.5b). In contrast, the first-order rate constant for SMX dissipation was significantly smaller under saturated (0.16 h^{-1}) than unsaturated (0.26 h^{-1}) conditions (Figure 2.5a) and hence the DT_{50} for SMX was smaller in unsaturated (2.7 d) than saturated (4.4 d) microcosms (Figure 2.5b). Consistent with our study but using an agricultural clay loam soil (0-20 cm), Liu et al. (2010) also reported relatively fast dissipation rates for SMX (0.42 to 0.10 h^{-1}). They indicated that SMX has a low sorption coefficient in soil and hence is readily bioavailable for microbial degradation. The DT_{50} for SMX in the study by Liu et al. (2010) was smaller under aerobic (2 d) than anaerobic (7 d) conditions but signified a rapid dissipation of SMX under both anaerobic and aerobic conditions. For manure-amended soils, Wu et al. (2012) reported much larger differences, but also with a faster SMX dissipation under unsaturated (DT_{50} 2.3 - 2.6 d) than saturated (DT_{50} 38.5 - 55 d) conditions.

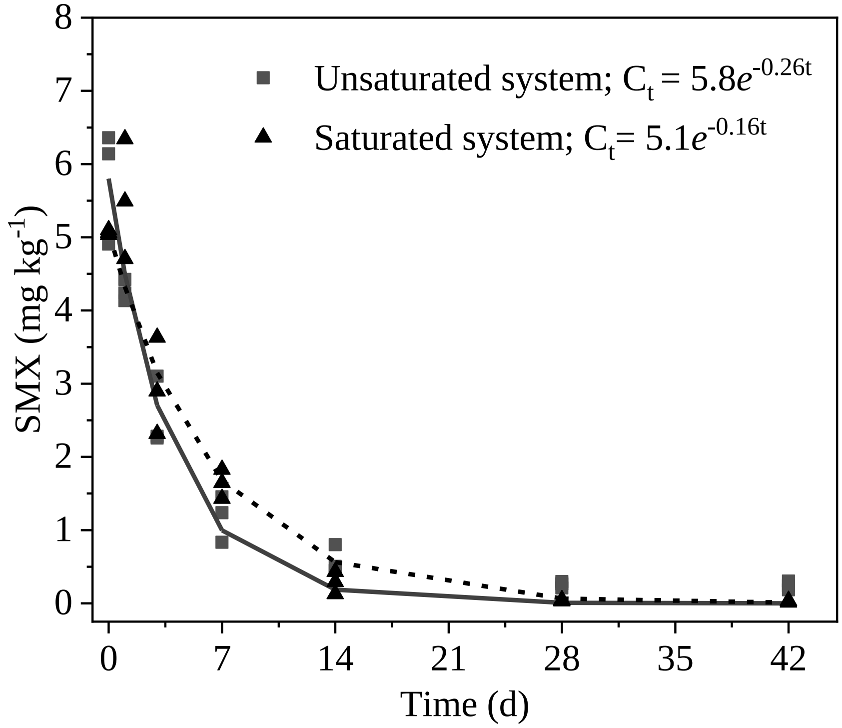


Figure 2.3 Concentrations (mg kg^{-1}) of sulfamethoxazole (SMX) in triplicate microcosms under unsaturated and saturated conditions. The first-order equations describing the dissipation data are also shown in the figure.

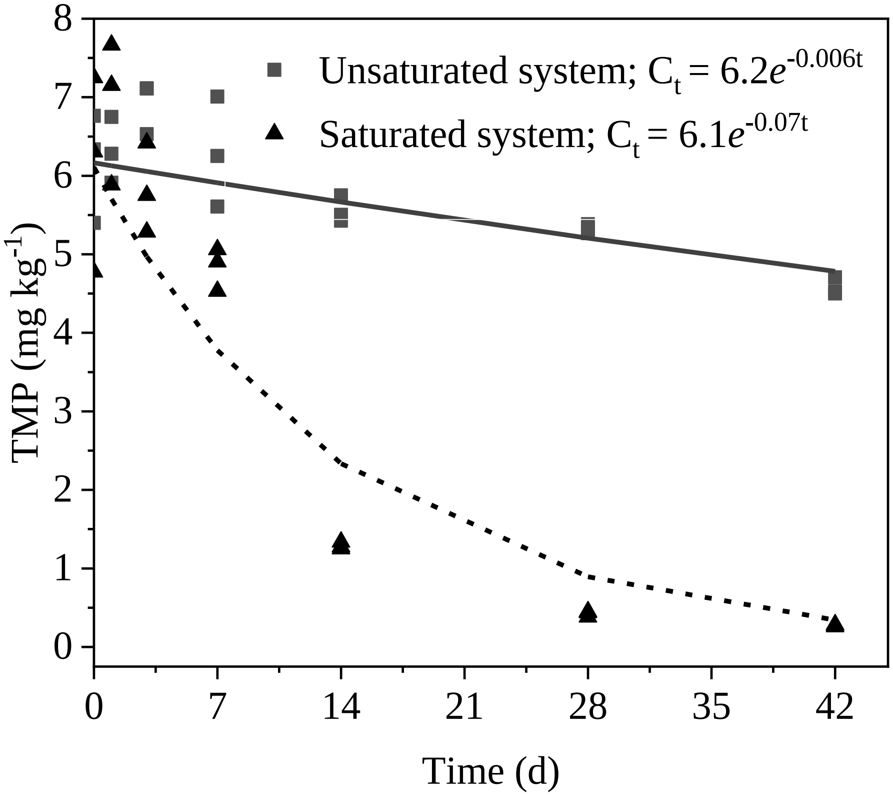


Figure 2.4 Concentrations (mg kg^{-1}) of trimethoprim (TMP) in triplicate microcosms under unsaturated and saturated conditions. The first-order equations describing the dissipation data are also shown in the figure.

Table 2.4 First-order parameters for dissipation of sulfamethoxazole (SMX) and trimethoprim (TMP) in biosolids in microcosms.

Treatment	k (h^{-1})	DT_{50} (d)
Antibiotic		
Sulfamethoxazole	0.20 ± 0.009	3.5±0.16
Trimethoprim	0.02 ± 0.001	34.2±1.6
System		
Unsaturated	0.04 ± 0.002	17.9 ± 0.83
Saturated	0.10 ± 0.005	6.7 ± 0.31
	P value	
Antibiotic	< 0.0001	< 0.0001
System	< 0.0001	< 0.0001
Antibiotic × system	< 0.0001	< 0.0001

Data are least-square means (±SE, n = 3). Means within a column followed by the same letters are not significantly different according to the Tukey multiple comparison procedure ($\alpha = 0.05$); k is the first order rate constant; DT_{50} is the time to 50% dissipation.

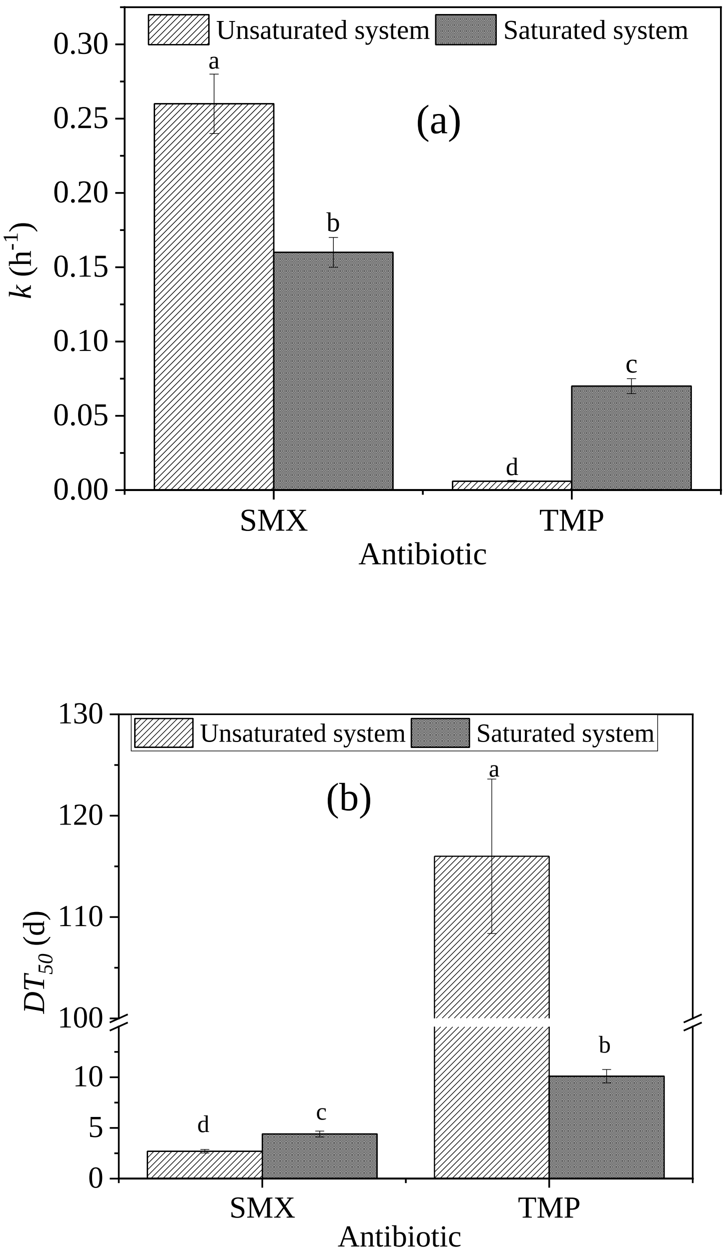


Figure 2.5 Rate constants (a) and half-lives (DT50) (b) of sulfamethoxazole (SMX) and trimethoprim (TMP) in saturated and unsaturated microcosms. Error bars represent standard errors of the mean (SE).

First-order rate constants in both saturated and unsaturated microcosms were significantly greater for SMX than for TMP, indicating slower rates of degradation of TMP, especially in unsaturated microcosms. TMP has a higher sorption coefficient than SMX and, therefore, has a lower bioavailability for microbial degradation (Lin and Gan, 2011). The faster TMP dissipation that we observed for TMP under saturated vs. unsaturated conditions is consistent with several previous studies. For example, Wu et al. (2012) reported DT_{50} values ranging from 6.6 to 9.2 d for TMP in three manure-amended soils under saturated (anaerobic) conditions, whereas DT_{50} ranged from 53.7 to 138.6 d under unsaturated (aerobic) conditions. The TMP half-lives observed under aerobic conditions were remarkably similar for manure-amended soils in their study (6.6 to 9.2 d) and biosolids in our study (10 d), given the differences in matrices and other factors, such as the initial TMP concentration (i.e., 2 mg kg^{-1} in Wu et al. (2012) vs. 10 mg kg^{-1} in our study). Greater antibiotic concentrations could more strongly reduce microbial activity, thus leading to a longer half-life (Xu et al., 2009). In other studies, Chen et al. (2017) observed rapid TMP degradation in a laboratory simulation of swine wastewater treatment systems and reported DT_{50} values of 2.6 and 24.8 d in anaerobic and aerobic biodegradation systems, respectively. In a study of TMP in sewage sludge that had been subjected to aerobic treatment, Pérez et al. (2005) observed rapid degradation of TMP in the nitrification stage of a secondary wastewater treatment plant. The rapid dissipation was attributed to the capabilities of microorganisms involved in nitrification to degrade TMP, while these microorganisms were absent in other steps of the wastewater treatment process (Pérez et al. 2005). Mohring et al. (2009) also observed rapid degradation of TMP within 8 d of the start of an anaerobic fermentation study. Liu et al. (2010) were the only researchers to conclude that TMP was less persistent under aerobic (DT_{50} of 4 d) than anaerobic (DT_{50} of 11 d) conditions.

Perhaps the substrate used by these authors contained a microbial community that preferred aerobic conditions.

Abiotic degradation pathways could have contributed to the dissipation of SMX and TMP in our saturated and unsaturated microcosms (Cardinal et al., 2014). However, previous studies have shown that degradation by abiotic mechanisms contributes little to the dissipation of SMX (Liu et al., 2010; Wu et al., 2012). Sulfamethoxazole cannot be readily hydrolysed and TMP does not contain any hydrolysable groups (Loftin et al., 2008). Baena-Nogueras et al. (2017) also observed negligible abiotic hydrolysis of TMP and SMX. In the present study, it is unlikely that photolysis was a mechanism for the dissipation of SMX and TMP since the microcosms were incubated in the dark. Giang et al. (2015) concluded that photodegradation was not a significant dissipation mechanism for TMP as they observed similar rates of TMP degradation in systems with and without light. Adsorption/desorption processes could also have contributed to the differences in dissipation in saturated and unsaturated microcosms. For example, flooding can cause strongly sorbed organic contaminants to be desorbed from sediments into flood water, thus increasing contaminant bioavailability (Yao et al., 2007). Therefore, the greater degradation of TMP in our microcosms under saturated conditions may have resulted from the increased bioavailability of TMP due to desorption from biosolids into water over time. Desorption has been reported to enhance the degradation of antibiotics (Xu et al., 2011).

2.5 Conclusions

The rapid dissipation of SMX from biosolids under saturated and unsaturated storage conditions indicates a relatively low risk of persistence in soils amended with biosolids. The difference in dissipation of SMX, although greater under saturated conditions, was small (96 vs

99%), thus the extra step of constructing a storage lagoon instead of stockpiling might not be cost-effective for such a small difference in dissipation percentage. Trimethoprim was more persistent under aerobic, unsaturated conditions and was rapidly lost from biosolids under anaerobic, saturated conditions. Therefore, we conclude that temporary storage of biosolids under saturated conditions, as in lagoons/storage tanks, may be preferable relative to stockpiling for removing TMP and other antibiotics from biosolids prior to land application. Further studies are needed to better understand the dissipation mechanism of TMP under anaerobic (saturated) conditions and identify the transformation products of SMX and TMP under saturated and unsaturated conditions, as well as their impact on biosolids microbiota during storage of biosolids.

2.6 References

Ahmed, A.U., and D.L. Sorensen. 1995. Kinetics of pathogen destruction during storage of dewatered biosolids. Water Environ. Res. **67**: 143–150. doi:10.2175/106143095x131286

Ali, M., J.J. Wang, R.D. DeLaune, D.C. Seo, S.K. Dodla, and A.B. Hernandez. 2013. Effect of redox potential and pH status on degradation and adsorption behavior of tylosin in dairy lagoon sediment suspension. Chemosphere **91**: 1583–1589. doi: 10.1016/j.chemosphere.2012.12.050.

Amarawansha, E. A. G.S., D. Kumaragamage, D. Flaten, F. Zvomuya, and M. Tenuta. 2015. Phosphorus mobilization from manure-amended and unamended alkaline soils to overlying water during simulated flooding. J. Environ. Qual. **44**: 1252–1262. doi: 10.2134/jeq2014.10.0457.

Baena-Nogueras, R.M., E. Gonzlez-Mazo, and P.A. Lara-Martin. 2017. Degradation kinetics of pharmaceuticals and personal care products in surface waters: photolysis vs biodegradation. Sci. Total Environ. **590–591**:643–654. doi: 10.1016/j.scitotenv.2017.03.015.

Berendsen, B.J.A., J. Lahr, C. Nibbeling, L.J.M. Jansen, I.E.A. Bongers, E.L. Wipfler, and M.G.M van de Schans. 2018. The persistence of a broad range of antibiotics during calve, pig and broiler manure storage. Chemosphere **204**:267–276. doi: 10.1016/j.chemosphere.2018.04.042.

Blair B.D., J.P. Crago, C.J. Hedman, and R.D. Klaper. 2013. Pharmaceuticals and personal care products found in the Great Lakes above concentrations of environmental concern. Chemosphere **93**: 2116-2123. doi: 10.1016/j.chemosphere.2013.07.057

Cardinal, P., J.C. Anderson, J.C. Carlson, J.E. Low, J.K. Challis, S.A Beattie, C.N. Bartel, A.D. Elliot, O.F. Montero, S.L. Lokesh, A.F. Favreau, T.A. Kozlova, C.W. Knapp, M.L. Hanson, and C.S. Wong. 2014. Macrophytes may not contribute significantly to removal of nutrients, pharmaceuticals, and antibiotic resistance in model surface constructed wetlands. Sci. Total Environ. **482–483**: 294-304. doi: 10.1016/j.scitotenv.2014.02.095.

Cassel, D.K. and Nielsen, D.R., 1986. Field capacity and available water capacity. p. 901–926. *In* A. Klute (ed.) Methods of soil analysis. American Society of Agronomy, Madison, Wisconsin, USA.

CCME. 2012. Guidance document for the beneficial use of municipal biosolids, municipal sludge and treated septage. Canadian Council of Ministers of the Environment, Winnipeg, MB, Canada.

Chae, M., L. Xia, C. Zhu, and D.C. Bressler. 2018. Accelerating settling rates of biosolids lagoons through thermal hydrolysis. J. Environ. Manage. **220**:227–232. doi: 10.1016/j.jenvman.2018.05.044.

Chen, J., D. Zhang, H. Zhang, S. Ghosh, and B. Pan. 2017. Fast and slow adsorption of carbamazepine on biochar as affected by carbon structure and mineral composition. Sci. Total Environ. **579**: 598–605. doi: 10.1016/j.scitotenv.2016.11.052.

Christie, P., D.L. Easson, J.R. Picton, and S.C.P. Love. 2001. Agronomic value of alkaline-stabilized sewage biosolids for spring barley. Agron. J. **93**: 144–151. doi: 10.2134/agronj2001.931144x.

Couperus M.P., S. Pagsuyoin, L.M. Bragg, and M. Servos. 2016. Occurrence, distribution and sources of antimicrobials in a mixed-use watershed. Sci. Total Environ. **541**:1581- 1591. doi: 10.1016/j.scitotenv.2015.09.086.

Edwards, M., E. Topp, C.D. Metcalfe, H. Li, N. Gottschall, P. Bolton, W. Curnoe, M. Payne, A. Beck, S. Kleywegt, and D.R. Lapen. 2009. Pharmaceuticals and personal care products in tile drainage following surface spreading and injection of dewatered municipal biosolids. Sci. Total Environ. 407:4220-4230. http://dx.doi.org/10.1016/j.scitotenv.2009.02.028

Elliot, H.A., O'Connor, and G.A., 2007. Phosphorus management for sustainable biosolids recycling in the United States. Soil Biol. Biochem **39:** 1318-1327. https://doi.org/ 10.1016/j.soilbio.2006.12.007.

Giang, C.N.D., Z. Sebesvari, F. Renaud, I. Rosendahl, Q.H. Minh, and W. Amelung. 2015. Occurrence and dissipation of the antibiotics sulfamethoxazole, sulfadiazine, trimethoprim, and enrofloxacin in the Mekong Delta, Vietnam. PLoS One San Franc. **10**: e0131855. doi: http://dx.doi.org.uml.idm.oclc.org/10.1371/journal.pone.0131855.

Göbel, A., A. Thomsen, C.S. McArdell, A. Joss, and W. Giger. 2005. Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment. Environ. Sci. Technol. **39**: 3981–3989. doi: 10.1021/es048550a.

Goh, T.B. and A.R. Mermut. 2008. Carbonates. p. 215-223. *In* M. R. Carter and E. G. Gregorich (eds.) Soil sampling and methods of analysis. 2nd ed. CRC Press Inc., Boca Raton, FL.

Hendershot, W.H., H. Lalande, and M. Duquette. 2008. Ion exchange and exchangeable cations. p. 197-206. *In* M.R. Carter and E.G. Gregorich (eds.) Soil sampling and methods of analysis, 2nd ed. CRC Press Inc., Boca Raton, FL.

Hunting, E.R., and A.A. Kampfraath. 2013. Contribution of bacteria to redox potential (Eh) measurements in sediments. Int. J. Environ. Sci. Technol. **10**: 55–62. doi: 10.1007/s13762-012-0080-4.

Jeke, N.N., F. Zvomuya, and L. Ross. 2015. Moisture effects on nitrogen availability in municipal biosolids from end-of-life municipal lagoons. J. Environ. Qual. **44**: 1883-1891. doi: 10.2134/jeq2015.02.0084.

Keeney, D. R, and D.W. Nelson. 1982. Nitrogen-inorganic forms. p. 643-698. *In* A.L. Page, R.H Miller, D.R. Keeney (eds.) Methods of soil analysis. Part 2: Chemical and microbiological properties. 2nd ed. American Society of Agronomy, Madison, WI.

Kinney, C.A., E.T. Furlong, S.D. Zaugg, M.R. Burkhardt, S.L. Werner, J.D. Cahill, and G.R. Jorgensen. 2006. Survey of organic wastewater contaminants in biosolids destined for land application. Environ. Sci. Technol. **40**: 7207–7215. doi: 10.1021/es0603406.

Kuchta, S.L., and A.J. Cessna. 2009. Fate of lincomycin in snowmelt runoff from manure-amended pasture. Chemosphere **76**: 439–446. doi: 10.1016/j.chemosphere.2009.03.069.

Lin, K., and J. Gan. 2011. Sorption and degradation of wastewater-associated non-steroidal anti-inflammatory drugs and antibiotics in soils. Chemosphere **83**: 240–246. doi: 10.1016/j.chemosphere.2010.12.083.

Liu, F., G.-G. Ying, J.-F. Yang, L.-J. Zhou, R. Tao, L. Wang. L.-J. Zhang, and P.-A. Peng. 2010. Dissipation of sulfamethoxazole, trimethoprim and tylosin in a soil under aerobic and anoxic conditions. Environ. Chem**. 7**: 370. doi: 10.1071/EN09160.

Liu, H., X. Huang, X. Yu, C. Pu, Y. Sun, and W. Luo. 2019. Dissipation and persistence of sulfonamides, quinolones and tetracyclines in anaerobically digested biosolids and compost during short-term storage under natural conditions. Sci. Total Environ. **684**: 58–66. doi: 10.1016/j.scitotenv.2019.05.341.

Liu, Y., X. Liu, S. Lu, B. Zhao, Z. Wang, B. Xi, and W. Guo. 2020. Adsorption and biodegradation of sulfamethoxazole and ofloxacin on zeolite: Influence of particle diameter and redox potential. Chem. Eng. J. **384**: 123346. doi: 10.1016/j.cej.2019.123346.

Loftin, K.A., C.D. Adams, M.T. Meyer, and R. Surampalli. 2008. Effects of ionic strength, temperature, and pH on degradation of selected antibiotics. J. Environ. Qual. **37**: 378–86. doi: 10.2134/jeq2007.0230

Luo, Y., Guo, W., Ngo, H. H., Nghiem, L.D., and Hai, F.I. 2014. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci. Total Environ. **473-474** : 619-641. doi: 10.1016/j.scitotenv.2013.12.065

Ma, J., Y. Cui, A. Li, W. Zhang, J. Liang, S. Wang, and L. Zhang. 2020. Evaluation of the fate of nutrients, antibiotics, and antibiotic resistance genes in sludge treatment wetlands. Sci. Total Environ. **712**: 136370. doi: 10.1016/j.scitotenv.2019.136370.

Martin T.D., J.T. Creed, and S.E. Long. 1991. Sample preparation for spectrochemical determination of total recoverable metals. Method 200.2. *In* Methods for the determination of metals in environmental samples. USEPA, Washington D.C., USA.

Miao X-S, F. Bishay, M. Chen, and C.D. Metcalfe. 2004. Occurrence of antimicrobials in the final effluents of wastewater treatment plants in Canada. Environ. Sci. Technol. **38**:3533-3541. doi: 10.1021/es030653q

Mohring, S.A.I., I. Strzysch, M.R. Fernandes, T.K. Kiffmeyer, J. Tuerk, and G. Hamscher. 2009. Degradation and elimination of various sulfonamides during anaerobic fermentation: a promising step on the way to sustainable pharmacy? Environ. Sci. Technol. **43**: 2569–2574. doi : 10.1021/es802042d.

Nielsen, L., and T.J. Bandosz. 2016. Analysis of sulfamethoxazole and trimethoprim adsorption on sewage sludge and fish waste derived adsorbents. Micropor. Mesopor. Mat. **220**: 58–72. doi: 10.1016/j.micromeso.2015.08.025.

Ortiz de Garcia, S.A., G.P. Pinto, P.A. Garcia-Encina, and R. Irusta-Mata. 2014. Ecotoxicity and environmental risk assessment of pharmaceuticals and personal care products in aquatic environments and wastewater treatment plants. Ecotoxicology **23**:1517-1533. doi: 10.1007/s10646-014-1293-8

Pérez, S., P. Eichhorn, and D.S. Aga. 2005. Evaluating the biodegradability of sulfamethazine, sulfamethoxazole, sulfathiazole, and trimethoprim at different stages of sewage treatment. Environ. Toxicol. Chem. **24**: 1361–7. doi: 10.1897/04-211r.1

Pezeshki, S.R., and R.D. DeLaune. 2012. Soil oxidation-reduction in wetlands and its impact on plant functioning. Biology **1**: 196–221. doi: 10.3390/biology1020196.

Reddy, K. R., E. M. D'Angelo, and W. G. Harris. 2000. Biogeochemistry of wetlands. p. G89–G119. *In* M. E. Sumner(ed.) Handbook of Soil Science. CRC Press, Boca Raton, FL.

Reese, A.T., E.H. Cho, B. Klitzman, S.P. Nichols, N.A. Wisniewski, M.M. Villa, H.K. Durand, S. Jiang, F.S. Midani, S.N. Nimmagadda, T.M. O'Connell, J.P. Wright, M.A. Deshusses, and L.A. David. 2018. Antibiotic-induced changes in the microbiota disrupt redox dynamics in the gut. eLife **7**. doi: 10.7554/eLife.35987.

Rutherford, P. M., W. B McGill, J. M. Arocena, and C. T. Figueiredo. 2008. Total nitrogen. p. 239-250. *In* M. R. Carter and E. G. Gregorich, (eds.) Soil sampling and methods of analysis. 2nd ed. CRC Press Inc., Boca Raton, FL

Sabourin, L., A. Beck, P.W. Duenk, S. Kleywegt, D.R. Lapen, H. Li, C.D. Metcalfe, M. Payne, and E. Topp. 2009. Runoff of pharmaceuticals and personal care products following application of dewatered municipal biosolids to an agricultural field. Sci. Total Environ. **407**: 4596–4604. doi: 10.1016/j.scitotenv.2009.04.027.

SAS Institute. 2013. SAS/STAT 9.4 User's Guide. SAS Institute Inc., Cary, NC.

Shrout, J.D., and G.F. Parkin. 2006. Influence of electron donor, oxygen, and redox potential on bacterial perchlorate degradation. Water Res. **40**: 1191–1199. doi: 10.1016/j.watres.2006.01.035.

Singer AC, Shaw H, Rhodes V, Hart, and A. 2016. Review of antimicrobial resistance in the environment and its relation to environmental regulators. Front. Microbiol. **7**:1-22. doi: 10.3389/fmicb.2016.01728

Skjemstad, J. O. and J.A. Baldock. 2008. Total and Organic Carbon. p 225-237. *In* M. R. Carter and E. G. Gregorich (ed.) Soil sampling and methods of analysis. 2nd ed. CRC Press Inc., Boca Raton, FL.

White, J.W., F.J. Coale, J.T. Sims, and A.L. Shober. 2010. Phosphorus runoff from wastewater treatment biosolids and poultry litter applied to agricultural soils. J. Environ. Qual. **39**: 314–323. doi: 10.2134/jeq2009.0106.

Wu, Y., M. Williams, L. Smith, D. Chen, and R. Kookana. 2012. Dissipation of sulfamethoxazole and trimethoprim antibiotics from manure-amended soils. J. Environ. Sci. Health Part B **47**: 240–249. doi: 10.1080/03601234.2012.636580.

Xu, B., D. Mao, Y. Luo, and L. Xu. 2011. Sulfamethoxazole biodegradation and biotransformation in the water–sediment system of a natural river. Bioresour. Technol. **102**: 7069–7076. doi: 10.1016/j.biortech.2011.04.086.

Xu, J., L. Wu, and A.C. Chang. 2009. Degradation and adsorption of selected pharmaceuticals and personal care products (PPCPs) in agricultural soils. Chemosphere **77**: 1299–1305. doi: 10.1016/j.chemosphere.2009.09.063.

Yao, F., G. Yu, Y. Bian, X. Yang, F. Wang, and X. Jiang. 2007. Bioavailability to grains of rice of aged and fresh DDD and DDE in soils. Chemosphere **68**: 78–84. doi: 10.1016/j.chemosphere.2006.12.035.

Zhang, Y., S. Hu, H. Zhang, G. Shen, Z. Yuan, and W. Zhang. 2017. Degradation kinetics and mechanism of sulfadiazine and sulfamethoxazole in an agricultural soil system with manure. Sci. Total Environ. **607–608**: 1348–1356. doi: 10.1016/j.scitotenv.2017.07.083.

3. ROOT SORPTION AS A MECHANISM FOR SULFAMETHOXAZOLE ATTENUATION BY CATTAIL (*Typha latifolia* L.) AND SWITCHGRASS (*Panicum virgatum* L.)

3.1 Abstract

Sorption by roots is one of several mechanisms by which plant-assisted attenuation of antibiotics in environmental media can be achieved. The objectives of this study were to (1) evaluate the sorption of sulfamethoxazole (SMX) by cattail (*Typha latifolia* L.) and switchgrass (*Panicum virgatum* L.) roots, (2) determine the kinetics of SMX sorption by cattail and switchgrass roots, and (3) characterize the temperature-dependency of SMX sorption. A batch sorption experiment was conducted to measure sulfamethoxazole sorption by roots of the two plant species using five initial antibiotic concentrations (2.5, 5, 10, 15, and 20 µg L^{-1}) and eight sampling times (0, 0.5, 1, 2, 4, 8, 12, and 24 h). Another batch experiment was conducted at three temperatures (5, 15, and 25 °C) to determine the effect of temperature on sorption kinetics. Sulfamethoxazole sorption followed pseudo-second-order kinetics for both plant species, with the rate constant (k_2) decreasing as temperature increased. The rate constant followed the order: 5 °C = 15 °C > 25 °C for cattail and 5 °C >15 °C = 25 °C for switchgrass. Percentage SMX removal at the three temperatures tested was 26 – 33% for cattail and 60 – 77% for switchgrass. Results from this study show that switchgrass roots are more effective than cattail roots in the removal of sulfamethoxazole. Therefore, the use of switchgrass in systems designed for phytoremediation of contaminants might also help attenuate sulfamethoxazole in environmental media.

3.2 Introduction

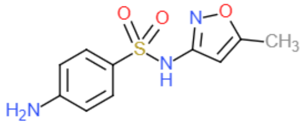
Pharmaceutical compounds, which are used to treat diseases in humans, are often excreted as parent compounds or metabolites from the human body. Conventional wastewater treatment plants (WWTPs) are not designed for removal of pharmaceuticals and other organic compounds from wastewater and therefore have limited capabilities to eliminate such compounds. Pharmaceuticals and their transformation products are therefore transferred to the broader environment by disposal of wastewater from WWTPs (Clarke and Smith, 2011; Fatta-Kassinos et al., 2011; Carvalho et al., 2014). For example, sulfamethoxazole (SMX) and roxithromycin, an erythromycin degradation product, were frequently detected in the discharge of treated wastewater from WWTPs (Hirsch et al., 1999).

Antibiotics are an important group of pharmaceuticals due to their widespread use by humans and in veterinary medicine (Hirsch et al., 1999). Consequently, they are widely dispersed in the environment. For example, at least one antibiotic was detected in 58% of samples from municipal wastewater effluent and effluent from residential facilities, hospitals, and livestock operations (Brown et al., 2006). The presence of antibiotics in the environment is a major concern because of their promotion of bacterial resistance, negative impacts on microbial communities, disruption of native microbial structures, and potential negative effects on humans (Daughton and Ternes, 1999; Kolpin et al., 2002).

Sulfamethoxazole is a member of the sulfonamide class of antibiotics, which are among the most prescribed antibiotics for humans and livestock (Miao et al., 2004). Sulfamethoxazole inhibits the production of dihydrofolic acid, which is essential for the growth and survival of bacteria (Isidori et al., 2005). It is detected at a wide range of concentrations in surface waters, treated wastewater, and biosolids (Sabourin et al., 2009; Rostamian and Behnejad, 2016). It has also been detected at concentrations of 0.17 µg L^{-1} in groundwater sources of public drinking water in California (Fram and Belitz, 2011).

The physico-chemical properties of SMX are presented in Table 3.1. Sulfamethoxazole has a moderate solubility and can be adsorbed by sludge particles during wastewater treatment, resulting in its presence in biosolids (Carballa et al., 2004). In a study involving WWTPs in Michigan, USA, SMX was detected in biosolids at concentrations of 4.8 to 35.9 µg kg^{-1} (Ding et al., 2011). Another study showed that sorption was a less important mechanism of SMX removal during wastewater treatment because of weak sorption of SMX by biosolids (Wu et al., 2009). Sulfamethoxazole was detected at concentrations of up to 2 µg L^{-1} in effluent samples from a sewage treatment plant in Germany (Hirsch et al., 1999). Low sorption coefficients, hence high bioavailability, have been reported for SMX in soil and biosolids (Ji et al., 2011). Given that biosolids and wastewater contain levels of SMX that contribute to the detection of antibiotics in the environment, there is a need to develop cost-effective approaches to remove antibiotics from biosolids and wastewater effluents before recyclying back into the environment, for example, prior to the application of biosolids on land.

Table 3.1 Physico-chemical properties of sulfamethoxazole. [*]

Properties	Value
Molecular weight	253.279 g mol^{-1}
Molecular formula	$C_{10}H_{11}N_3O_3S$
Structure	
pK_a	$pKa_1 = 1.8$; $pKa_2 = 5.6$
Log K_{ow}	0.89
Solubility	610 mg L^{-1} at 37 °C

[*] Modified after Gao and Pedersen (2005) and Yalkowsky et al. (2010)

Phytoremediation is the use of plants to remove organic and inorganic contaminants from the environment. It is a remediation strategy that has gained much popularity in recent times, in part because it is less expensive than other clean-up methods, and offers other advantages such as eco-friendliness, aesthetics, and a high degree of self-sustenance (Wang et al., 2011; Pandey et al., 2016). Phytoremediation can be useful in removing pharmaceuticals from biosolids, sewage sludge, wastewater, and streams (Wang et al., 2011). Previous studies have demonstrated the absorption and uptake of SMX by plant roots (Ahmed et al., 2015; Franklin et al., 2016). Kurade et al., (2019) reported that uptake of SMX by water spinach (*Ipomea aquatica*) contributed significantly (~17%) to the removal of SMX from aqueous solutions. Biodegradation of SMX is the major removal mechanism, contributing ~82% to the removal of SMX. Similarly, SMX was taken up by cabbage and Wisconsin Fast plant roots grown under hydroponic conditions (Herklotz et al., 2010).

The diffusion of organic compounds into plant cells and their subsequent translocation within plant cells is influenced by the structure of the cell membrane; the hydrophobicity, pK_a, solubility, and concentration of the chemical; and the pH of the medium (Kodešová et al., 2019). For example, in their study on the plant uptake of SMX from fertilized soils, Chitescu et al. (2013) concluded that the absorption and transport of SMX in grass and watercress plants was through passive transport as most of the SMX in their study (pH = 5.5) existed in neutral molecular forms. Plant uptake of neutral molecules has been reported to be greater than the uptake of charged (anionic or cationic) molecules (Chitescu et al., 2013; Malchi et al., 2014; Kodešová et al., 2019). Diffusion of anionic molecules through the cell membrane can be reduced by repulsion between the negative charges on both the compounds and the roots, while uptake of cationic molecules can be hindered by their adsorption to negatively charged cell walls (Malchi et al., 2014; Dodgen et al., 2015; Kodešová et al., 2019). Michelini et al. (2012), in a preliminary study on the use of *Salix fragilis* L. for phytoremediation purposes, reported an accumulation of sulfadimethoxine by roots in the soil-plant system. The authors detected low concentrations of sulfadimethoxine in the aerial parts of the plant and attributed this to inhibited transpiration, as suggested by their soil moisture data. The inhibited transpiration could have affected sulfadimethoxine uptake and translocation because transpiration plays a significant role in the uptake of pharmaceutical compounds (Dodgen et al., 2015).

While SMX can be absorbed and translocated to plant tissues, plant roots can also provide surfaces for sorption of contaminants and thus reduce contaminant mobility and bioavailability (Sas-Nowosielska et al., 2008; Zheng et al., 2017). This process, known as rhizofiltration, can be effective in removing trace element contaminants such as cadmium, lead, and copper from aqueous solution (Dushenkov et al., 1995). Sorption of a contaminant by media such as soil or plant roots is influenced by the physicochemical properties of the sorbent and the sorbate (Wang and Wang, 2015; Banerjee and Chattopadhyaya, 2017). Other factors that could play an important role in the sorption of organic chemicals by roots or soil include temperature, initial concentration of the compound, and contact time (Albero et al., 2018). The removal of nutrients and organic contaminants by plants also occurs through phytoextraction (plant uptake), phytodegradation, phytostabilization, and phytovolatilization (Salt et al., 1998).

Wetland-based and terrestrial phytoremediation approaches have been proven to be effective at removing nutrients (Jeke et al., 2015) and organic contaminants (Dzantor et al., 2000) from wastewater and biosolids. Cattail and switchgrass, due to their high biomass, rapid growth, and, in the case of cattail, ubiquity in wetland systems in North America, can remove contaminants from wetland or terrestrial environments (Jeke et al., 2015; Dzantor et al., 2000). However, the role of these plant species in the attenuation of antibiotics via rhizofiltration is not clear. A better understanding of rhizofiltration will aid the development of effective phytoremediation schemes using cattail and switchgrass. Therefore, the objectives of this study were to (1) evaluate the sorption of SMX by cattail and switchgrass roots, (2) determine the kinetics of SMX sorption by cattail and switchgrass roots, and (3) characterize the temperature-dependency of SMX sorption. We hypothesized that (1) plant roots would sorb SMX from aqueous solutions, (2) the rate of sorption of SMX would differ between switchgrass and cattail roots, and (3) temperature would influence the sorption rate and sorption capacity of SMX by plant roots.

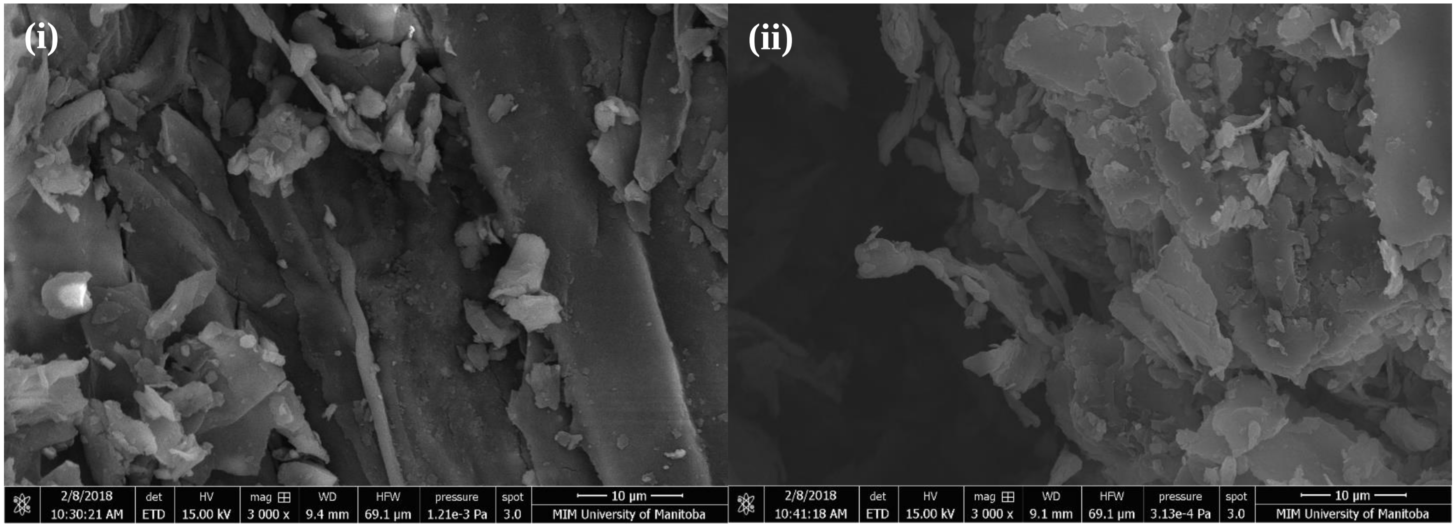
3.3 Materials and Methods

3.3.1 Adsorbent (roots) preparation and characterization

Cattail and switchgrass were grown in biosolids media under a controlled environment with a 16-h photoperiod and day/night temperatures of 22/15 °C. After a 2-mo growing period, plant roots were harvested and thoroughly rinsed with reverse osmosis (RO) water to remove biosolids and then dried at 60 °C for one week. Dried roots were ground to a fine powder using a SPEX 8000D ball mill (Metuchen, NJ, USA). The structure of the roots and surface topography were obtained at different magnifications using a FEI Quanta 650 Field Emission Scanning Electron Microscope (SEM) (Figure 3.1a). Prior to placing samples in the SEM, powdered root samples were placed on aluminum stubs using carbon tape and coated with a 10-15 nm thin layer of Gold-Palladium with a Denton Desk II Sputter coater.

Functional groups of root tissue were determined with a Fourier transform infrared (FTIR) spectrophotometer (Nicolet 6700, Thermo Electron Scientific Instruments Corp., USA). The FTIR spectra of cattail and switchgrass root tissues are shown in Figure 3.1b.

(a)


(i)
2/8/2018
10:30:21 AM
det
ETD
HV
15.00 kV
mag
3 000 x
WD
9.4 mm
HFW
69.1 µm
pressure
1.21e-3 Pa
spot
3.0
10 µm
MIM University of Manitoba
(ii)
2/8/2018
10:41:18 AM
det
ETD
HV
15.00 kV
mag
3 000 x
WD
9.1 mm
HFW
69.1 µm
pressure
3.13e-4 Pa
spot
3.0
10 µm
MIM University of Manitoba

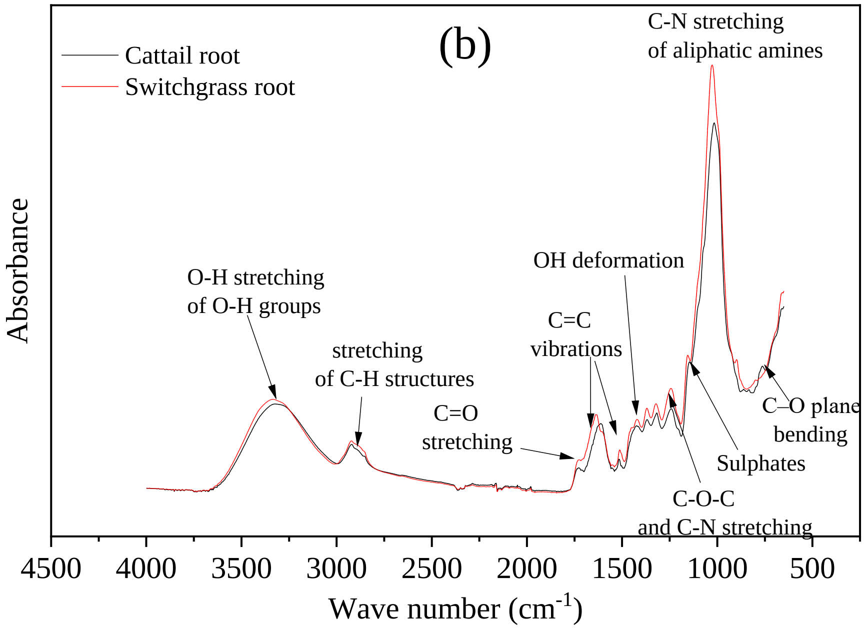


Figure 3.1(a) SEM image of (i) cattail roots and (ii) switchgrass roots at 3000× magnification, (b) FTIR image of cattail and switchgrass roots.

3.3.2 Chemicals and solution preparation

Chemicals and reagents used in the study were: ^{14}C ring-labeled sulfamethoxazole (> 99% radiochemical purity; specific activity 50 µCi) from American Radiolabeled Chemicals Inc. (St. Louis, MO, USA); analytical grade sulfamethoxazole (≥ 98% purity) from Sigma-Aldrich Co. (Oakville, ON); and methanol (HPLC grade) and calcium chloride dihydrate (>99% purity) from Fisher scientific (Nepean, ON).

Sulfamethoxazole sorption isotherms and sorption kinetics were determined using the Tier-2 parallel batch equilibration technique following the OECD 106 guideline (OECD, 2000). A stock solution of SMX in methanol was prepared in an amber bottle and used to prepare a 20 µg L^{-1} SMX solution containing 1.33×10^5 Bq L^{-1} of ^{14}C-labeled SMX in 0.01 M $CaCl_2$, while ensuring that the concentration of methanol in the Teflon tube was less than 0.1% to minimize co-solvent effects on sorption (OECD, 2000). The sorption of SMX by cattail and switchgrass roots was determined at SMX concentrations of 2.5, 5, 10, 15, and 20 µg L^{-1}. Initial antibiotic solution pH was 6.1; the pH was not adjusted during the experiment.

3.3.3 Sorption isotherms

The sorption experiment was laid out in a randomized complete block design (RCBD) with a factorial treatment structure (2 plants × 5 initial SMX concentrations) and three replications per treatment. Aliquots (10 mL) of the SMX solutions were added to 50-mL Teflon centrifuge tubes containing 0.1-g samples of cattail and switchgrass root tissue to give a 1:100 plant mass to solution volume ratio (predetermined in a preliminary test). Blanks (antibiotic solution without roots) were included to determine SMX sorption to test vessels. Sorption of SMX to Teflon tubes was negligible throughout the sorption experiment.

Samples were shaken on a rotary shaker for 24 h (equilibration time) at 15 °C, followed by centrifugation at 10,000 rpm for 10 min, with the reaction temperature maintained at 15 °C during centrifugation. A 1-mL aliquot was added to each of two 6-mL scintillation vials, followed by 3 mL 30% Scintisafe scintillation cocktail (Fisher Scientific, Fair Lawn, NJ). The vials were kept in the dark for 24 h before radioactivity was measured, to reduce false positives due to photoluminescence or chemiluminescence. Radioactivity was quantified using a liquid scintillation counter (Tri-Carb 4910 TR, PerkinElmer Inc., MA, USA) and a counting time of 10 min.

3.3.4 Sorption kinetics

The sorption kinetics experiment was laid out as a randomized complete block design with a factorial treatment structure (2 plants × 5 initial SMX concentrations × 7 sampling times) and three replications per treatment. A batch equilibration study was conducted as described above and samples were collected after reaction times of 0, 0.5, 1, 2, 4, 8, 12, and 24 h for determination of sorption kinetics. To determine the effect of temperature on sorption kinetics, another sorption experiment was conducted at 5, 15, and 25 °C. The experimental layout was a RCBD with a split-plot treatment structure in which temperature was the main effect and plant root type was the subplot. Temperature was replicated twice. The batch study was conducted as described above with eight incubation times (0, 0.5, 1, 2, 4, 8, 12, and 24 h) following the Tier 2 parallel method (OECD, 2000). Reaction temperature was held constant during centrifugation.

After each reaction time in both experiments, a 1-mL aliquot was collected from each sample and pretreated as described above for the quantification of radioactivity in solution.

3.3.5 Models and Calculations

3.3.5.1 Sorption and sorption isotherms

The amount of antibiotics adsorbed at time *t* (q_t, µg g^{-1}) was calculated as

$$q_t = \frac{(C_i - C_e) \times V}{m} \qquad [1]$$

where C_i is the initial solute concentration (µg mL^{-1}); C_e is the equilibrium concentration (µg mL^{-1}); *m* is the mass of the adsorbent (plant roots, µg) and *v* is the volume of the solution (mL). Percent removal of SMX from solution was calculated as:

$$\%\ \text{removal} = \frac{(C_i - C_f) \times 100}{C_i} \qquad [2]$$

where c_f is the final SMX concentration in the solution (µg mL^{-1}).

The Langmuir and Freundlich sorption models, which were deemed most eligible based on scatter plots for the data, were compared for their fit to the data. The Langmuir model describes monolayer sorption onto a homogenous adsorbent surface (i.e., equal sorption energy for all sites), where sorbate molecules are adsorbed at a fixed number of sites, with each site holding one molecule. Additional assumptions are that there is no interaction between sorbed molecules on neighboring sites and that the sorption system is in dynamic equilibrium, with equal adsorption and desorption rates. The mathematical expression of the Langmuir model is:

$$q_e = \frac{K_L q_m C_e}{1 + K_L C_e} \qquad [3]$$

where q_e is the amount of SMX adsorbed per gram of plant roots (µg g^{-1}); K_L is the Langmuir sorption constant or the affinity constant (mL µg^{-1}), which is a measure of the strength with which a sorbate molecule is attracted onto the sorbent surface; C_e is the equilibrium concentration of the adsorbate (µg mL^{-1}); and q_m is the maximum monolayer sorption capacity of the adsorbent (µg g^{-1}).

The Freundlich model describes sorption onto a heterogenous surface (Banerjee and Chattopadhyaya, 2017). It is an empirical model which accommodates multilayer sorption and assumes that the sorption energy decreases exponentially with increasing surface coverage and depends on the physicochemical properties of sorption sites. The Freundlich model is expressed as:

$$q_e = K_f C_e^{\frac{1}{n}} \qquad [4]$$

where K_f is the Freundlich constant ((µg g^{-1}) (ml µg^{-1}) $^{1/n}$) and n is the Freundlich coefficient (or heterogeneity factor) related to the sorption intensity and adsorbent surface heterogeneity (Matouq et al., 2015).

3.3.5.2 Sorption kinetics

Kinetic models commonly used in sorption studies with antibiotics, pseudo-first order and pseudo-second order, were tested for their fit to the data. The pseudo-first order model is

$$q_t = q_e(1 - e^{-k_1 t}) \qquad [5]$$

where q_e is the sorption capacity (µg g^{-1}) of adsorbent at equilibrium; q_t is the amount of adsorbate sorbed (µg g^{-1}) at time t (h); and k_1 (h^{-1}) is the pseudo-first order rate constant.

The pseudo-second order equation (Ho and Mckay, 1998) estimates q_t as:

$$q_t = \frac{k_2 q_e^2 t}{1 + k_2 q_e t} \qquad [6]$$

where $k_2 q_e^2$ is the initial sorption rate (µg g^{-1} h^{-1}); q_e is the sorption capacity (µg g^{-1}) of adsorbent at equilibrium; and k_2 (g µg^{-1} h^{-1}) is the pseudo-second order rate constant.

3.3.5.3 Sorption thermodynamics

Gibbs free energy ($\triangle G°$, J mol^{-1}) was calculated as:

$$\triangle G° = -RT\ln(K_e) \quad [7]$$

where

$$K_e = \frac{C_{ae}}{C_e} \quad [8]$$

is the equilibrium constant; C_{ae} and C_e are the equilibrium SMX concentration on plant roots and in the solution, respectively; R is the universal gas constant (8.314 J K^{-1} mol^{-1}); and T is the absolute temperature (K).

The thermodynamic parameters, change in enthalpy ($\Delta H°$, J mol^{-1}) and change in entropy ($\Delta S°$, J mol^{-1} K^{-1}), were determined after combining the Gibbs free energy isotherm equation (Eq. 7) and the Gibbs free energy equation:

$$\Delta G° = \Delta H° \text{-} T\Delta S° \quad [9]$$

to obtain the linear form of the van't Hoff equation:

$$ln\ K_e = -\frac{\Delta H°}{RT} + \frac{\Delta S°}{R} \quad [10]$$

Plotting ln K_e as a linear function of $1/T$ yielded a linear regression with $-\Delta H°/R$ as the slope and $\Delta S°/R$ as the intercept, which was then multiplied by R to give $\Delta H°$ and $\Delta S°$ (Guo et al., 2009).

Activation energy was determined from the linear form of the Arrhenius equation:

$$k_2 = Ae^{-E_a/RT} \quad [11]$$

$$ln\, k_2 = ln\, A - {E_a}/{RT} \quad [12]$$

Where k_2 is the pseudo-second order rate constant, A is the frequency or pre-exponential factor (g μg^{-1} h^{-1}), E_a is the activation energy of sorption (J mol^{-1}), R is the universal gas constant (8.314 J K^{-1} mol^{-1}), and T is the absolute temperature (K).

Plotting ln k_2 against $1/T$ yields a straight line with slope $-E_a/R$, which is then multiplied by $-R$ to give E_a.

3.3.6 Statistical analysis

3.3.6.1 Model fitting and selection

The sorption and kinetic models described above were fitted to normally-distributed data using PROC NLIN in SAS 9.4 (SAS Institute, 2013). Model fits were compared using the corrected Akaike Information Criterion (AIC_c), and the model with the lowest AIC_c was selected as the best fit. The requisite assumption of normality of distribution of the residuals was tested using the Shapiro-Wilk W Statistic from PROC UNIVARIATE in SAS 9.4 prior to fitting the models to the data.

3.3.6.2 Analysis of variance

Models selected as described above were fitted to data from each replicate, and the resultant model parameters were subjected to analysis of variance using the GLIMMIX procedure of SAS 9.4 to determine the main and interaction effects of initial SMX concentration, plant root type, and temperature on sorption kinetics. Analysis of variance was also conducted on the isotherm parameters, with plant root type as the fixed effect. The gamma distribution was specified for rate constants (k_2 and h) while data for the amount of SMX sorbed at equilibrium (q_e), Freundlich constants (K_f and n), and thermodynamic parameters ($\Delta G^{\circ}, \Delta H^{\circ}$, and ΔS°), followed the Gaussian distribution. Data for the percentage of SMX sorbed followed a beta distribution while data for activation energy (E_a) followed a lognormal distribution. All distributions other than Gaussian were specified using the "DIST =" function in the MODEL statement of PROC GLIMMIX. Treatment differences were assessed at $\alpha = 0.05$ using the Tukey multiple comparison procedure.

3.4 Results and discussion

3.4.1 Root characterization

The SEM micrograph of the plant roots at 3000X magnification (Fig. 3.1a) shows a heterogeneous surface for both cattail and switchgrass roots, providing possible surfaces for antibiotic sorption. The FTIR spectra show similar components in both switchgrass and cattail roots (Fig. 3.1b), with few different peaks. Cattail root tissue showed peaks at 1728, 862, 833, and 762 cm^{-1}, respectively, while these peaks were absent in the spectra for switchgrass root tissue. This might have contributed to the observed differences in SMX sorption between the two plant roots, as described below.

The appearance of a broad band in the FTIR spectrum at 3335 and 3326 cm^{-1} is generally ascribed to OH-stretching of hydrogen bonded O-H groups (Palacio et al., 2014). The peak at 2920 cm^{-1} suggests stretching of aliphatic C–H structures (Cocozza et al., 2003). The band at 1728 cm^{-1}, which was present only in the spectra for cattail roots, represents C=O stretching of carbonyl groups (Cocozza et al., 2003). Peaks at 1612 and 1638 cm^{-1} are assigned to aromatic C=C stretching and/or asymmetric C–O stretching in COO^{-} functional groups (Cocozza et al., 2003). Peaks at 1515 and 1513 cm^{-1} correspond to aromatic C=C vibrations (Palacio et al., 2014). Absorption bands at 1423, 1369, and 1321 cm^{-1} are attributed to symmetric C–O stretching from COO– and/or OH deformation (COOH), C-H and/or OH deformations of phenol and aliphatic groups, and CH in-plane bending in cellulose I and cellulose II, respectively (Artz et al., 2008; Shi et al., 2012; Palacio et al., 2014). The peak at 1243 cm^{-1} is attributed to C-O-C and C-N stretching (Palacio et al., 2014), while peaks 1155 cm^{-1} and 1027 cm^{-1} represent sulphates and C-N stretching of aliphatic amines, respectively (Smidt et al., 2011; Sravan Kumar et al., 2015). Peak 834 cm^{-1} corresponds to aromatic C-H out of plane attributed to lignin (Palacio et al., 2014), 898 cm^{-1} to out of phase ring stretching (Artz et al., 2008), and 762 cm^{-1} to C–O plane bending-carbonates (Smidt et al., 2011) and were only present in cattail root spectra.

3.4.2 Effect of initial antibiotic concentration and contact time

Initial antibiotic concentration, pH, and contact time are important factors in the removal of adsorbate species from solutions (Banerjee and Chattopadhyaya, 2017).

The sorption of SMX by cattail and switchgrass roots was rapid at the initial stages, then slowed down with time as sorption approached equilibrium. This was likely due to the higher driving force and availability of active sorption sites at initial stages of sorption. As contact time increased, the availability of these active sites and the driving force decreased (Li et al., 2010), thus the sorption rate became slower. This sorption pattern for SMX has been reported in other studies (Rostamian and Behnejad, 2016; Liu et al., 2017).

As the initial concentration of SMX increased, the time it took to reach sorption equilibrium also increased (Figure 3.2). At lower SMX concentrations (2.5-10 µg L^{-1}), there was no significant difference in the amount of antibiotic sorbed by cattail roots between the beginning of the sorption study (time 0) and at 24 h. As SMX concentration increased to 15 and 20 µg L^{-1}, equilibrium time for SMX sorption by cattail increased to 30 min and 2 h, respectively. For switchgrass, at the lowest concentration of SMX, there was no significant change in the amount of SMX sorbed with time elapsed since the start of the experiment. However, as SMX concentration increased, equilibrium time increased to 30 min for an initial SMX concentration of 5 µg L^{-1} and 8 h for initial SMX concentrations of 10, 15, and 20 µg L^{-1}. This suggests that an increase in initial SMX concentration led to a slower sorption of SMX by the plant roots. This could be a result of a decrease in the number of active sorption sites on the roots at higher SMX concentration

(Açıkyıldız et al., 2015). Overall, sorption equilibrium time for cattail was less than that for switchgrass. However, q_t for cattail was significantly less than that for switchgrass, except at SMX concentrations of 2.5 and 5 µg L^{-1}. As initial SMX concentration increased, there was an increase in q_t with time (Figure 3.2).

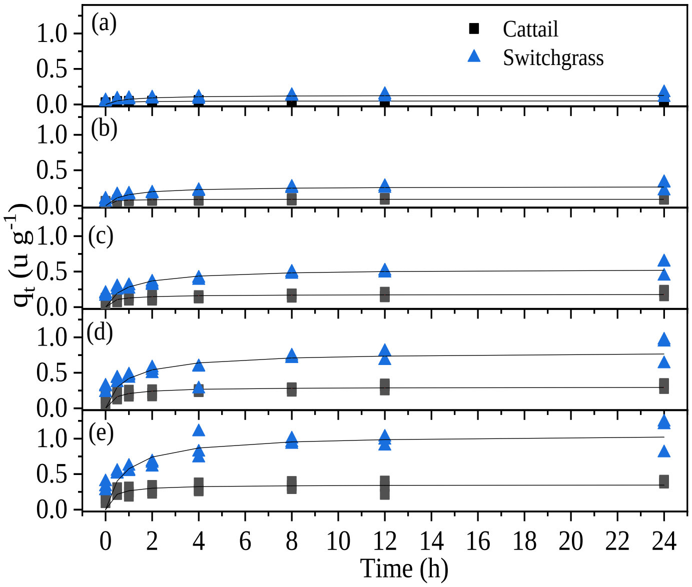


Figure 3.2 Pseudo-second-order kinetics of SMX sorption at different initial antibiotic concentrations (a) 2.5 (b) 5 (c) 10 (d) 15 and (e) 20 µg L^{-1}.

As the solution concentration of SMX increased, more SMX was sorbed by cattail and switchgrass roots. For example, when SMX concentration increased from 2.5 to 20 µg L^{-1}, the sorption capacity of cattail roots increased from 0.05 to 0.39 µg g^{-1} while that of switchgrass roots increased from 0.14 to 1.07 µg g^{-1} (Figure 3.3). Interestingly, there was no significant concentration effect on the percentage of SMX removed from solution for both plant roots (results not presented). Across all initial SMX concentrations, the percentage of SMX removed by switchgrass (56%) was significantly greater than that for cattail (20%). Using fresh *I. aquatica* roots in their study on the uptake and biodegradation of SMX from an aqueous solution, Kurade et al. (2019) observed that root biosorption of SMX accounted for 0.77% of total SMX removal from the solution. It is not clear if the substantially lower sorption percentage relative to that observed in our study was due to differences in plant properties and experimental conditions between the two studies since Kurade et al. (2019) did not report critical variables such as temperature and suspension pH.

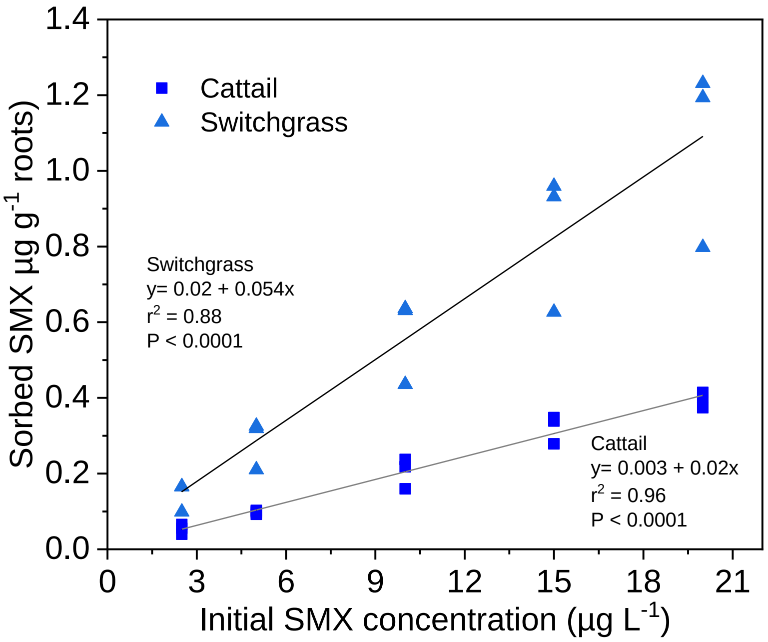


Figure 3.3 Changes in amount of SMX sorbed at equilibrium by cattail and switchgrass roots as a function of SMX concentration.

Surface sorption of organic chemicals is controlled by various mechanisms and processes, including electrostatic, hydrophobic, hydrogen bonding, and π-π interactions (Pan and Xing, 2008). At the solution pH in this study (6.1), SMX existed predominantly in the anionic form (SMX^-), with a smaller proportion of neutral forms (SMX^0) (Zhang et al., 2010; Zheng et al., 2013). Although electrostatic repulsion could occur between negatively charged plant surfaces and the anionic forms of SMX, sorption of SMX^- to negative surfaces has been observed. Iatrou et al. (2017) reported that sorption of SMX by duckweed (*Lemna minor*) biomass contributed to the removal of SMX from water and wastewater, with plant uptake being the major mechanism of removal. Their study was conducted at a pH of 7.0, which would have favored the predominance of the anionic form of SMX in the solution. This suggests that sorption of negatively charged SMX to negatively charged plant root surfaces is possible. The sorption of SMX molecules to negatively charged biochar surfaces at a pH > 6 has been reported, where the anionic form of SMX was dominant (Zheng et al., 2013). It is therefore conceivable that sorption of SMX to root surfaces in our study may have occurred via negative charge-assisted H-bonding (CAHB) (Teixidó et al., 2011; Zheng et al., 2013). Physical sorption through van der Waals interaction may also be a contributing mechanism by which sorption of negatively charged SMX molecules onto the negatively-charged root surfaces occurs (Xu et al., 2018).

3.4.3 Sorption isotherms

The sorption of SMX by switchgrass and cattail roots was best described by the Freundlich isotherm (RMSE = 0.018), which suggests multilayer sorption of SMX onto the heterogenous sorbent (plant root) surfaces (Miraboutalebi et al., 2017). The Freundlich constant (K_f) was greater for switchgrass (71.5 ($\mu g\ g^{-1}$) ($ml\ \mu g^{-1})^{1/n}$) than for cattail (24.5 ($\mu g\ g^{-1}$) ($ml\ \mu g^{-1}$) $^{1/n}$) roots (Table 3.2), indicating greater sorption capacity and greater sorption bond strength for SMX sorption by switchgrass roots (Banerjee and Chattopadhyaya, 2017). The *n* value in our study was above unity for both cattail and switchgrass roots (1.04-1.13) (Table 3.2). However, there was no significant difference in the n values between cattail and switchgrass roots. If n =1, then the sorption is linear, if $n < 1$, then the sorption process is chemical while $n > 1$ is representative of a physical sorption process (Desta, 2013). In our study, n value corresponded to $n > 1$, which indicated a favourable and physical sorption process (Desta, 2013). Similar to our results for cattail, Ahsan et al. (2018) reported a K_f of 29.1 (µg g^{-1}) (ml $µg^{-1})^{1/n}$ and n value of 2.3 for the sorption of SMX by graphite carbon derived from sawdust. In a study investigating the sorption of SMX by giant reed biomass, a K_f of 49.6 (µg g^{-1}) (ml $µg^{-1})^{1/n}$ was reported (Zheng et al., 2013). Greater SMX sorption has been reported for biochar (Zheng et al., 2013), polyethylene microplastics (Xu et al., 2018), and crop-derived ashes (Ji et al., 2011). Although strong sorption of pharmaceuticals by plant roots has been reported to reduce their subsequent uptake by plants, thus affecting effective phytoremediation (Chuang et al., 2019), studies have shown that SMX can still be measurably taken up by plants (Chitescu et al., 2013; Ahmed et al., 2015). Thus, rather than being a limitation to phytoremediation, sorption by plant roots could provide an additional removal mechanism for SMX.

Table 3.2 Effect of plant type on Freundlich parameters K_f and n

Plant	K_f ($\mu g\, g^{-1}$) ($ml\ \mu g^{-1}$) $^{1/n}$	n
Cattail	24.5 ± 11.1^{a}	1.04 ± 0.05
Switchgrass	71.5 ± 11.1^{b}	1.13 ± 0.05
ANOVA	Normal distribution	Normal distribution
Plant	$p < 0.04$	$p = 0.23$

Data are means (±SEM, n = 3). Means followed by the same superscript letters are not significantly different according to the Tukey multiple comparison procedure ($\alpha = 0.05$). K_f is the Freundlich constant and n is the heterogeneity factor.

3.4.4 Sorption kinetics

The pseudo-second order model provided the best fit for the kinetic data (RMSE = 0.074). This model suggests that the sorption of SMX onto the roots was a chemical process and that the sorption capacity of the roots was proportional to the active sorption sites (Ho and Mckay, 1998). This sorption pattern has been reported for SMX in previous studies (Zhao et al., 2016; Rostamian and Behnejad, 2016). In contrast to our results, Iatrou et al. (2017) reported pseudo-first order sorption kinetics for SMX ($k_2 = 0.009\ d^{-1}$) in a study examining plant (*Lemna minor*) removal of antibiotics from water and wastewater.

Sorption kinetics is controlled by several mechanisms, the most limiting of which relate to diffusion, including external diffusion and intra-particle diffusion mechanisms (Guibal et al., 2003). The initial rapid sorption is usually attributed to external diffusion, followed by the slower sorption rate due to the decrease in active sites, which is attributed to intra-particle diffusion processes (Guibal et al., 2003).

The rate constant (k_2) ranged from 1.2 to 10.2 g µg^{-1} h^{-1} for switchgrass and 5.2 to 9.2 g µg^{-1} h^{-1} for cattail. Averaged across initial antibiotic concentrations, k_2 was significantly greater for cattail (23.9 g µg^{-1} h^{-1}) than switchgrass (3.0 g µg^{-1} h^{-1}), although the amount of SMX sorbed was significantly greater for switchgrass, especially at antibiotic concentrations of 10 µg L^{-1} or greater. As the initial SMX concentration increased, k_2 decreased for both plant roots (Table 3.3). However, initial sorption rate (h) increased as initial SMX concentration increased but, averaged across concentrations, did not differ significantly between cattail (0.61 µg g^{-1} h^{-1}) and switchgrass roots (0.57 µg g^{-1} h^{-1}).

The sorption capacities at equilibrium (q_e), estimated from non-linear regression agree with the experimental values from our study ($R^2 = 0.94$). There was a significant concentration × plant type interaction effect on q_e. At lower initial antibiotic concentrations (2.5 and 5 µg L^{-1}), there were no significant differences in q_e between cattail and switchgrass roots (Table 3.3). At the higher initial concentration of 10 µg L^{-1}, switchgrass roots had a significantly greater q_e than cattail roots (Figure 3.3).

Table 3.3 Pseudo-second order model parameters for cattail and switchgrass roots at different initial antibiotic concentrations.

Treatment	k_2	h	q_e
	g µg^{-1} h^{-1}	µg g^{-1} h^{-1}	µg g^{-1}
Plant			
Cattail	23.9 ± 7. 7^{a}	0.61±0.15	0.20 ± 0.02
Switchgrass	3.01 ± 0.9^{b}	0.57±0.14	0.56 ± 0.02
Concentration			
2.5 µg L^{-1}	23.9 ± 8.1^{a}	0.16 ± 0.04^{c}	0.09 ± 0.03
5 µg L^{-1}	15.5 ± 5.4^{ab}	0.42 ± 0.12^{b}	0.19 ± 0.03
10 µg L^{-1}	9.02 ± 3.1^{b}	0.87 ± 0.24^{ab}	0.36 ±0.03
15 µg L^{-1}	3.9 ± 1.3^{c}	0.95 ± 0.25^{a}	0.55 ± 0.03
20 µg L^{-1}	3.4 ± 1.1^{c}	1.25 ± 0.33^{a}	0.70 ±0.03
ANOVA	Gamma distribution	Gamma distribution	Normal distribution
Plant	$p < 0.0001$	$p = 0.68$	$p < 0.0001$
Concentration	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Plant × concentration	$p = 0.12$	$p = 0.49$	$p < 0.0001$

Parameter values are least-square means (±SEM, n = 3). Means followed by the same superscript letters are not significantly different according to the Tukey multiple comparison procedure ($\alpha = 0.05$),k_2 is the pseudo-second order rate constant, h is the initial sorption rate and, q_e is the sorption capacity.

As temperature increased from 5 to 25 °C, the amount of SMX sorbed by switchgrass and cattail roots increased significantly (Figure 3.4a). The percentage of SMX removed from solution also increased with increasing temperature (Figure 3.4b). Percentage SMX removal at the three temperatures (5, 15, and 25 °C) was 26 – 33% for cattail, and 60 – 77% for switchgrass. For both plant species, the greatest sorption was observed at the highest temperature, indicating that an increase in temperature favored the removal of SMX from aqueous solution. The increase in SMX uptake by roots suggests an increase in the equilibrium capacity of the sorbents as the temperature increased, hence an endothermic sorption process. This increase in equilibrium capacity may be due to a faster diffusion process due to a decrease in viscosity of the aqueous solution, an increase in the solubility of the adsorbate, or/and an increase in pore size in the adsorbents as the temperature increased (Banerjee and Chattopadhyaya, 2017).

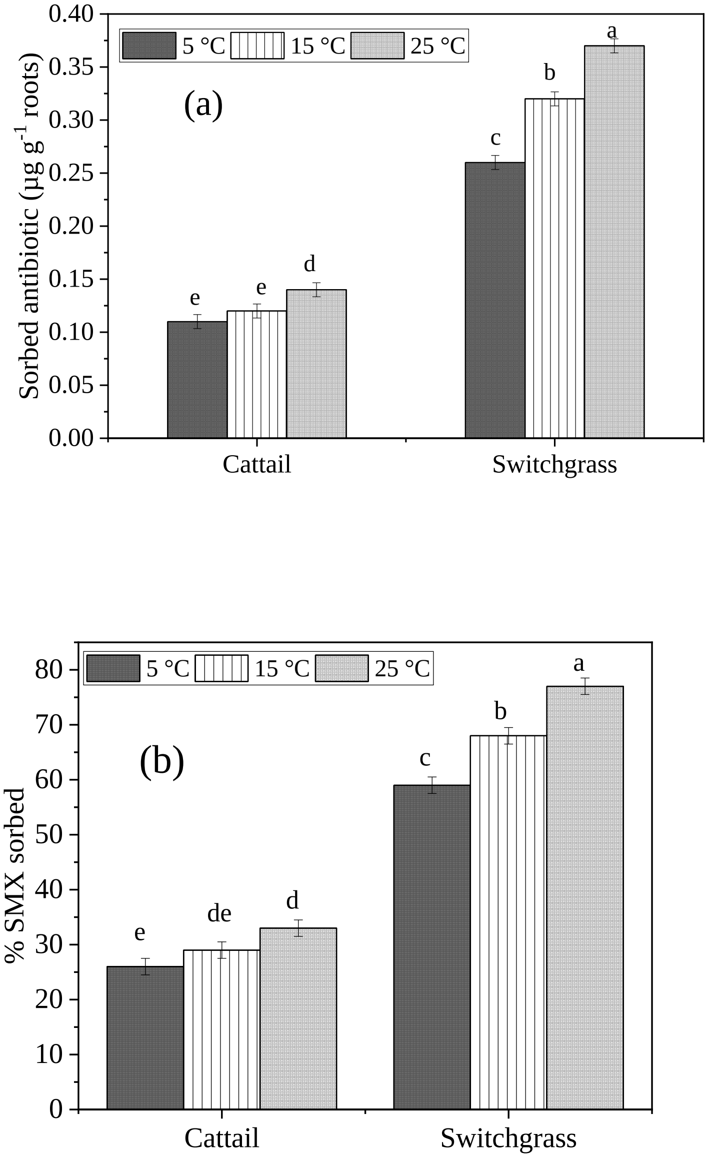


Figure 3.4 Interaction effects of temperature and plant species on (a) SMX sorption and (b) percentage of SMX sorbed by roots at equilibrium.

Temperature increase in our sorption study resulted in a decrease in sorption rate constants for both plant roots. However, this temperature effect varied with plant species (Figure 3.5). For cattail roots, k_2 decreased from 38.8 to 7.9 g µg^{-1} h^{-1} when temperature increased from 5°C to 25°C while it decreased from 5.5 to 1.7 g µg^{-1} h^{-1} for switchgrass. However, the corresponding increase in sorption capacity confirms that the sorption of SMX by cattail and switchgrass roots is an endothermic process.

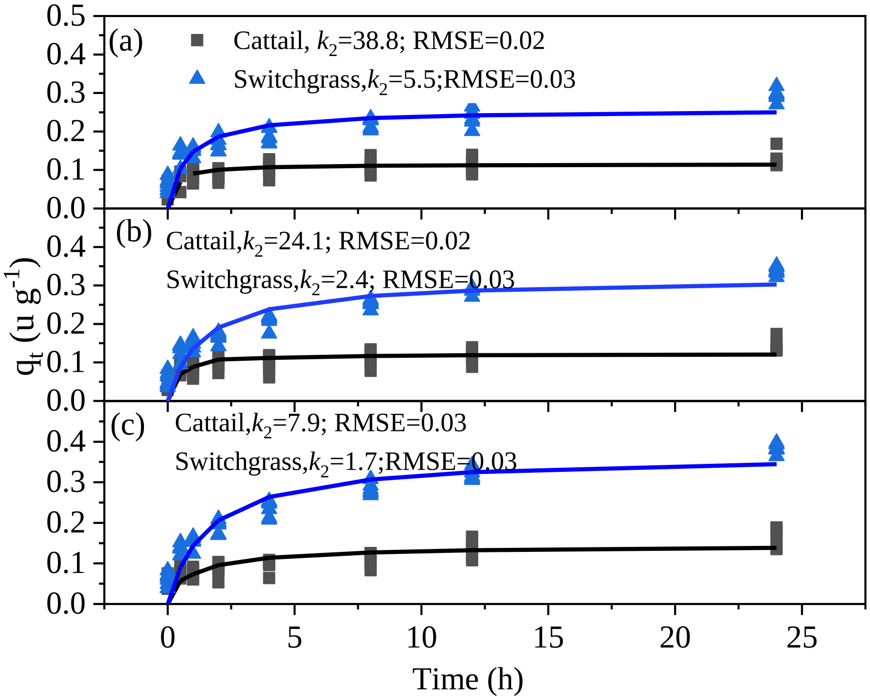


Figure 3.5 Pseudo-second order sorption kinetics of SMX by cattail and switchgrass at ((a) 5 °C, (b) 15 °C, and (c) 25 °C. RMSE, root-mean-square error.

3.4.5 Sorption thermodynamics

As temperature increased, $\Delta G°$ decreased for both plant species (Table 4) indicating that the feasibility of the sorption process improved as temperature increased (Sandoval-Flores et al., 2018). The negative value of $\Delta G°$ confirms that the sorption of SMX by switchgrass roots is a spontaneous and favorable reaction (Kulkarni et al., 2017). The $\Delta G°$ value for cattail was positive, suggesting that sorption of SMX by cattail roots is not a spontaneous and thermodynamically feasible process. The lower the $\Delta G°$, the greater the sorption driving force, and therefore, the greater the sorption capacity (Li et al., 2011). Switchgrass roots had a lower $\Delta G°$ than cattail, indicating a greater affinity of SMX for switchgrass roots than for cattail roots.

The positive $\Delta H°$ (Table 3.4) confirms that the sorption of SMX by cattail and switchgrass roots is an endothermic process. Switchgrass had a significantly greater $\Delta H°$ (28.6 kJ mol^{-1}) than cattail (11.9 kJ mol^{-1}). The value of $\Delta H°$ provides insights into the sorption mechanism involved. A $\Delta H° < 84$ kJ mol^{-1} is typically indicative of a physical sorption process, whereas chemisorption bond strengths occur within a range of 84-420 kJ mol^{-1}, at which a chemical bond is formed between the adsorbate and adsorbent (Errais et al., 2011). Hence, sorption of SMX by cattail and switchgrass roots appears to be a physical process

The positive value of $\Delta S°$ denotes that the degree of chaos increases at the solid-liquid interface and suggests some changes to the structure of both adsorbate and adsorbent during the sorption process (Açıkyıldız et al., 2015). Additionally, a positive $\Delta S°$ indicates the affinity of the roots for SMX (Durnie et al., 1999). The $\Delta S°$ was significantly greater for switchgrass (105.8 J mol^{-1} K^{-1}) than for cattail (33.8 J mol^{-1} K^{-1}), suggesting a greater affinity of SMX for switchgrass roots than for cattail roots.

The E_a for cattail and switchgrass were negative (Figure 3.6), which is consistent with the decrease in pseudo-second order rate constant as temperature increased from 15 to 35 °C. However, cattail (-54.1 kJ mol^{-1}) had significantly lower negative activation energy than switchgrass (-37.4 kJ mol^{-1}). The low values of E_a reported in this study are consistent with those reported for biosorption (Aksu, 2001; Karaoğlu et al., 2010). This suggests that SMX sorption by cattail and switchgrass roots is a physical process which is diffusion-controlled (Sparks, 1989).

Table 3.4 Thermodynamic parameters for the sorption of SMX by cattail and switchgrass roots

Adsorbent	Temp	ΔG° (kJ $\mathrm{mol^{-1}}$)	ΔH° (kJ $\mathrm{mol^{-1}}$)	ΔS° (J $\mathrm{mol^{-1}\,K^{-1}}$)
Cattail	5 °C	2.17 ± 0.17^{a}	11.9 ± 2.1^{a}	33.8 ± 7.4^{a}
	15 °C	2.19 ± 0.17^{ab}		
	25 °C	1.80 ± 0.17^{b}		
Switchgrass	5 °C	-0.84 ± 0.17^{c}	28.6 ± 2.1^{b}	105.8 ± 7.4^{b}
	15 °C	-1.77 ± 0.17^{d}		
	25 °C	-3.0 ± 0.17^{e}		
ANOVA		Normal distribution	Normal distribution	Normal distribution
Plant		$P < 0.0001$	$P = 0.0003$	$p < 0.0001$
Temperature		$p < 0.0001$		
Plant × temperature		$p < 0.0001$		

Least-square means (±SEM) followed by the same superscript letter in a column are not significantly different according to the Tukey multiple comparison procedure ($\alpha = 0.05$); ΔG° is the Gibbs free energy; ΔH° is the change in enthalpy; and ΔS° is the change in entropy.

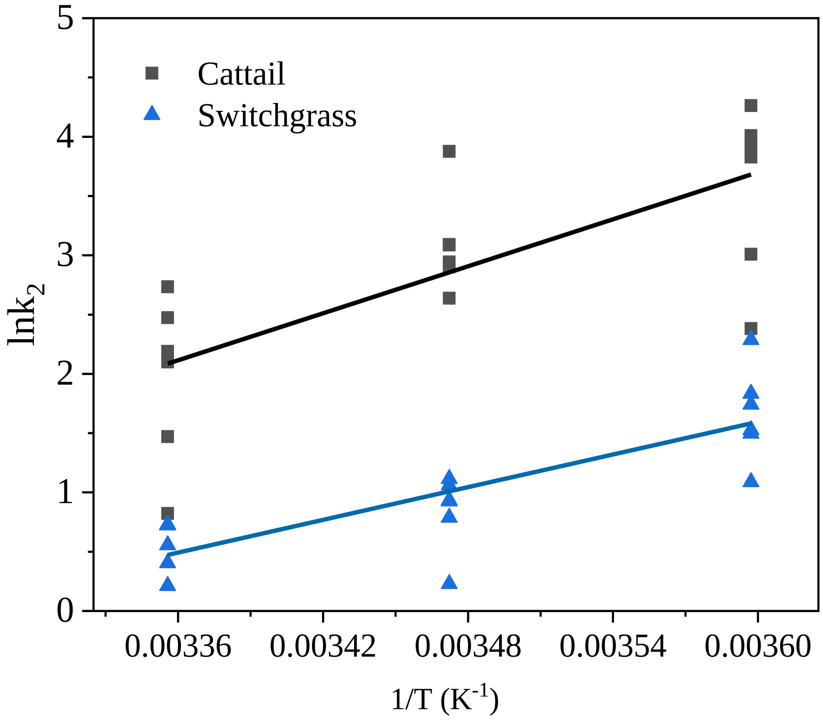


Figure 3.6 Plot of $\ln k_2$ versus $^1/_T$ for cattail and switchgrass

3.5 Conclusion

Results from this study indicate that switchgrass and cattail can sorb SMX in systems designed for in situ phytoremediation of inorganic contaminants (trace metals and nutrients). While few studies have demonstrated the absorption of SMX by plant roots, we were able to demonstrate that sorption on plant tissue surfaces could play a role in the removal of SMX from aqueous systems. The affinity of the roots for SMX and the amount of SMX sorbed at equilibrium were greater for switchgrass than for cattail roots. Initial SMX concentration and temperature affected the rate of SMX sorption by plant roots. As temperature increased, the amount of SMX sorbed by plant roots increased, indicating that higher temperature favored the sorption process. The sorption by cattail and switchgrass roots likely occurred via physisorption through van der Waals interaction and negative charge-assisted H bonding (CAHB). Thermodynamic parameters suggest that sorption of SMX by switchgrass roots was a spontaneous process and endothermic in nature.

Equilibrium sorption data were best described by the Freundlich isotherm. The sorption of SMX by cattail and switchgrass roots followed pseudo-second order kinetics. Extrapolation of our results to actual plant-biosolid-water systems must be done with caution, as sorption behavior might be different due to other competing factors. Nonetheless, this study is an important step towards elucidation of the sorption of SMX by cattail and switchgrass surfaces. We conclude that cattail and switchgrass roots can serve as a sorbent for removal of SMX from aqueous solutions, with switchgrass roots having the greater removal efficiency.

3.6 References

Açıkyıldız, M., A. Gürses, K. Güneş, and D. Yalvaç. 2015. A comparative examination of the adsorption mechanism of an anionic textile dye (RBY 3GL) onto the powdered activated carbon (PAC) using various the isotherm models and kinetics equations with linear and non-linear methods. Appl. Surf. Sci. **354**: 279–284. doi: 10.1016/j.apsusc.2015.07.021.

Ahmed, M.B.M., A.U. Rajapaksha, J.E. Lim, N.T. Vu, I.S. Kim, H.M. Kang, S.S. Lee, and Y.S. Ok. 2015. Distribution and accumulative pattern of tetracyclines and sulfonamides in edible vegetables of cucumber, tomato, and lettuce. J. Agric. Food Chem. **63**: 398–405. doi: 10.1021/jf5034637.

Ahsan, Md. A., Md. T. Islam, C. Hernandez, E. Castro, S.K. Katla, H. Kim, Y. Lin, M.L. Curry, J. Gardea-Torresdey, and J.C. Noveron. 2018. Biomass conversion of saw dust to a functionalized carbonaceous material for the removal of tetracycline, sulfamethoxazole and bisphenol a from water. J. Environ. Chem. Eng**. 6**:4329–4338. https://doi.org/10.1016/j.jece.2018.06.040

Aksu, Z. 2001. Equilibrium and kinetic modelling of cadmium (II) biosorption by C. vulgaris in a batch system: effect of temperature. Sep. Purif. Technol. **21**: 285–294. doi: 10.1016/S1383-5866(00)00212-4.

Albero, B., J.L. Tadeo, M. Escario, E. Miguel, and R.A. Pérez. 2018. Persistence and availability of veterinary antibiotics in soil and soil-manure systems. Sci. Total Environ. **643**: 1562–1570. doi: 10.1016/j.scitotenv.2018.06.314.

Artz, R.R.E., S.J. Chapman, A.H. Jean Robertson, J.M. Potts, F. Laggoun-Défarge, S. Gogo, L. Comont, J.-R. Disnar, and A.J. Francez. 2008. FTIR spectroscopy can be used as a screening tool for organic matter quality in regenerating cutover peatlands. Soil Biol. Biochem. **40**: 515–527. doi: 10.1016/j.soilbio.2007.09.019.

Banerjee, S., and M.C. Chattopadhyaya. 2017. Adsorption characteristics for the removal of a toxic dye, tartrazine from aqueous solutions by a low-cost agricultural by-product. Arab. J. Chem. **10**: S1629–S1638. doi: 10.1016/j.arabjc.2013.06.005.

Brown, K.D., J. Kulis, B. Thomson, T.H. Chapman, and D.B. Mawhinney. 2006. Occurrence of antibiotics in hospital, residential, and dairy effluent, municipal wastewater, and the Rio Grande in New Mexico. Sci. Total Environ. **366**: 772–783. doi: 10.1016/j.scitotenv.2005.10.007.

Carballa, M., F. Omil, J.M. Lema, M. Llompart, C. García-Jares, I. Rodriguez, M. Gomez, and T. Ternes. 2004. Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. Water Res. **38**: 2918–2926. doi: 10.1016/j.watres.2004.03.029.

Carvalho, P.N., M.C.P. Basto, C.M.R. Almeida, and H. Brix. 2014. A review of plant–pharmaceutical interactions: from uptake and effects in crop plants to phytoremediation in constructed wetlands. Environ. Sci. Pollut. Res. **21**: 11729–11763. doi: 10.1007/s11356-014-2550-3.

Chitescu, C.L., A.I. Nicolau, and A.A.M. Stolker. 2013. Uptake of oxytetracycline, sulfamethoxazole and ketoconazole from fertilised soils by plants. Food Addit. Contam. Part A **30**: 1138–1146. doi: 10.1080/19440049.2012.725479.

Chuang, Y.-H., C.-H. Liu, J.B. Sallach, R. Hammerschmidt, W. Zhang, S.A. Boyd, and H. Li. 2019. Mechanistic study on uptake and transport of pharmaceuticals in lettuce from water. Environ. Int. **131**: 104976. doi: 10.1016/j.envint.2019.104976.

Clarke, B.O., and S.R. Smith. 2011. Review of 'emerging' organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. Environ. Int. **37**: 226–247. doi: 10.1016/j.envint.2010.06.004.

Cocozza, C., V. D'Orazio, T.M. Miano, and W. Shotyk. 2003. Characterization of solid and aqueous phases of a peat bog profile using molecular fluorescence spectroscopy, ESR and FT-IR, and comparison with physical properties. Org. Geochem.: **12**.

Daughton, C.G., and T.A. Ternes. 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ. Health Perspect. **107**: 907. doi: 10.2307/3434573.

Desta, M.B. 2013. Batch sorption experiments: Langmuir and Freundlich isotherm studies for the adsorption of textile metal ions onto teff straw (Eragrostis tef) agricultural waste. J. Thermodyn. **2013**: 1-6. doi: 10.1155/2013/375830.

Ding, Y., W. Zhang, C. Gu, I. Xagoraraki, and H. Li. 2011. Determination of pharmaceuticals in biosolids using accelerated solvent extraction and liquid chromatography/tandem mass spectrometry. J. Chromatogr. A **1218**: 10–16. doi: 10.1016/j.chroma.2010.10.112.

Dodgen, L.K., A. Ueda, X. Wu, D.R. Parker, and J. Gan. 2015. Effect of transpiration on plant accumulation and translocation of PPCP/EDCs. Environ. Pollut**.** **198**: 144–153. doi: 10.1016/j.envpol.2015.01.002.

Durnie, W., R.D. Marco, A. Jefferson, and B. Kinsella. 1999. Development of a structure-activity relationship for oil field corrosion inhibitors. J. Electrochem. Soc. **146**: 1751–1756. doi: 10.1149/1.1391837.

Dushenkov, V., P.B.A. Kumar, H. Motto, and I. Raskin. 1995. Rhizofiltration: The use of plants to remove heavy metals from aqueous streams. Environ. Sci. Technol. **29**: 1239–1245. doi: 10.1021/es00005a015.

Dzantor, E.K., T. Chekol, and L.R. Vough. 2000. Feasibility of using forage grasses and legumes for phytoremediation of organic pollutants. J. Environ. Sci. Health Part A **35**: 1645–1661. doi: 10.1080/10934520009377061.

Errais, E., J. Duplay, F. Darragi, I. M'Rabet, A. Aubert, F. Huber, and G. Morvan. 2011. Efficient anionic dye adsorption on natural untreated clay: Kinetic study and thermodynamic parameters. Desalination **275**: 74–81. doi: 10.1016/j.desal.2011.02.031.

Fatta-Kassinos, D., I.K. Kalavrouziotis, P.H. Koukoulakis, and M.I. Vasquez. 2011. The risks associated with wastewater reuse and xenobiotics in the agroecological environment. Sci. Total Environ. **409**: 3555–3563. doi: 10.1016/j.scitotenv.2010.03.036.

Fram, M.S., and K. Belitz. 2011. Occurrence and concentrations of pharmaceutical compounds in groundwater used for public drinking-water supply in California. Sci. Total Environ. **409**: 3409–3417. doi: 10.1016/j.scitotenv.2011.05.053.

Franklin, A.M., C.F. Williams, D.M. Andrews, E.E. Woodward, and J.E. Watson. 2016. Uptake of three antibiotics and an antiepileptic drug by wheat crops spray irrigated with wastewater treatment plant effluent. J. Environ. Qual. **45**: 546. doi: 10.2134/jeq2015.05.0257.

Gao, J., and J.A. Pedersen. 2005. Adsorption of sulfonamide antimicrobial agents to clay minerals. Environ. Sci. Technol. **39**: 9509–9516.

Guibal, E., P. McCarrick, and J.M. Tobin. 2003. Comparison of the Sorption of Anionic Dyes on Activated Carbon and Chitosan Derivatives from Dilute Solutions. Sep. Sci. Technol. **38**: 3049–3073. doi: 10.1081/SS-120022586.

Guo, S., W. Li, L. Zhang, J. Peng, H. Xia, and S. Zhang. 2009. Kinetics and equilibrium adsorption study of lead (II) onto the low-cost adsorbent—Eupatorium denophorum spreng. Process Saf. Environ. Prot. **87**: 343–351. doi: 10.1016/j.psep.2009.06.003.

Herklotz, P.A., P. Gurung, B. Vanden Heuvel, and C.A. Kinney. 2010. Uptake of human pharmaceuticals by plants grown under hydroponic conditions. Chemosphere **78**: 1416–1421. doi: 10.1016/j.chemosphere.2009.12.048.

Hirsch, R., T. Ternes, K. Haberer, and K.-L. Kratz. 1999. Occurrence of antibiotics in the aquatic environment. Sci. Total Environ. **225**: 109–118. doi: 10.1016/S0048-9697(98)00337-4.

Ho, Y.S., and G. Mckay. 1998. Kinetic Models for the Sorption of Dye from Aqueous Solution by Wood. Process Saf. Environ. Prot. **76**: 183–191. doi: 10.1205/095758298529326.

Iatrou, E.I., G. Gatidou, D. Damalas, N.S. Thomaidis, and A.S. Stasinakis. 2017. Fate of antimicrobials in duckweed Lemna minor wastewater treatment systems. J. Hazard. Mater. **330**: 116–126. doi: 10.1016/j.jhazmat.2017.02.005.

Isidori, M., M. Lavorgna, A. Nardelli, L. Pascarella, and A. Parrella. 2005. Toxic and genotoxic evaluation of six antibiotics on non-target organisms. Sci. Total Environ. **346**: 87–98. doi: 10.1016/j.scitotenv.2004.11.017.

Jeke, N.N., F. Zvomuya, N. Cicek, L. Ross, and P. Badiou. 2015. Biomass, nutrient, and trace element accumulation and partitioning in cattail (L.) during wetland phytoremediation of municipal biosolids. J. Environ. Qual. **44**: 1541. doi: 10.2134/jeq2015.02.0064.

Ji, L., Y. Wan, S. Zheng, and D. Zhu. 2011. Adsorption of Tetracycline and Sulfamethoxazole on Crop Residue-Derived Ashes: Implication for the Relative Importance of Black Carbon to Soil Sorption. Environ. Sci. Technol. **45**: 5580–5586. doi: 10.1021/es200483b.

Karaoğlu, M.H., Ş. Zor, and M. Uğurlu. 2010. Biosorption of Cr (III) from solutions using vineyard pruning waste. Chem. Eng. J. **159**: 98–106. doi: 10.1016/j.cej.2010.02.047.

Kodešová, R.; Klement, A.; Golovko, O.; Fér, M.; Nikodem, A.; Kočárek, M.; Grabic, R. 2019. Root uptake of atenolol, sulfamethoxazole and carbamazepine, and their transformation in three soils and four plants. Environ. Sci. Pollut. Res. Int. Heidelb. **26**: 9876–9891. doi: http://dx.doi.org.uml.idm.oclc.org/10.1007/s11356-019-04333-9.

Kolpin, D.W., E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.R. Barber, and H. T. Buxton. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999−2000: A national reconnaissance. Environ. Sci. Technol. **36**: 1202–1211. doi: 10.1021/es011055j.

Kulkarni, M.R., T. Revanth, A. Acharya, and P. Bhat. 2017. Removal of Crystal Violet dye from aqueous solution using water hyacinth: Equilibrium, kinetics and thermodynamics study. Resour. Effic. Technol. **3**: 71–77. doi: 10.1016/j.reffit.2017.01.009.

Kurade, M.B., J.-Q. Xiong, S.P. Govindwar, H.-S. Roh, G.D. Saratale, and B-H. Jeon. 2019. Uptake and biodegradation of emerging contaminant sulfamethoxazole from aqueous phase using *Ipomoea aquatica*. Chemosphere **225**: 696–704. doi: 10.1016/j.chemosphere.2019.03.086.

Li, X., X. Zhou, J. Mu, L. Lu, D. Han, C. Lu, and M. Wang. 2011. Thermodynamics and kinetics of *p* -aminophenol adsorption on poly(aryl ether ketone) containing pendant carboxyl groups. J. Chem. Eng. Data **56**: 4274–4277. doi: 10.1021/je2009297.

Li, Y., Q. Du, X. Wang, P. Zhang, D. Wang, and Y. Xia. 2010. Removal of lead from aqueous solution by activated carbon prepared from Enteromorpha prolifera by zinc chloride activation. J. Hazard. Mater. **183**: 583–589. doi: 10.1016/j.jhazmat.2010.07.063.

Liu, X., S. Lu, Y. Liu, W. Meng, and B. Zheng. 2017. Adsorption of sulfamethoxazole (SMZ) and ciprofloxacin (CIP) by humic acid (HA): characteristics and mechanism. RSC Adv. **7**: 50449–50458. doi: 10.1039/C7RA06231A.

Malchi, T., Y. Maor, G. Tadmor, M. Shenker, and B. Chefetz. 2014. irrigation of root vegetables with treated wastewater: evaluating uptake of pharmaceuticals and the associated human health risks. environ. Sci. Technol. **48**: 9325–9333. doi: 10.1021/es5017894.

Matouq, M., N. Jildeh, M. Qtaishat, M. Hindiyeh, and M.Q. Al Syouf. 2015. The adsorption kinetics and modeling for heavy metals removal from wastewater by Moringa pods. J. Environ. Chem. Eng. **3**: 775–784. doi: 10.1016/j.jece.2015.03.027.

Miao, X.-S., F. Bishay, M. Chen, and C.D. Metcalfe. 2004. Occurrence of antimicrobials in the final effluents of wastewater treatment plants in Canada. Environ. Sci. Technol. **38**: 3533–3541. doi: 10.1021/es030653q.

Michelini, L., F. Meggio, N.L. Rocca, S. Ferro, and R. Ghisi. 2012. Accumulation and effects of sulfadimethoxine in *Salix Fragilis* L. plants: A preliminary study to phytoremediation purposes. Int. J. Phytoremediation **14**: 388–402. doi: 10.1080/15226514.2011.620654.

Miraboutalebi, S.M., S.K. Nikouzad, M. Peydayesh, N. Allahgholi, L. Vafajoo, and G. McKay. 2017. Methylene blue adsorption via maize silk powder: Kinetic, equilibrium, thermodynamic studies and residual error analysis. Process Saf. Environ. Prot. **106**: 191–202. doi: 10.1016/j.psep.2017.01.010.

OECD. 2000. Guideline for the Testing of Chemicals 106: Adsorption–desorption using a batch equilibrium method [Online]. 1–45. 10.1787/20745753 (accessed Aug 15,2016). https://www.oecd-ilibrary.org/environment/test-no-106-adsorption-desorption-using-a-batch-equilibrium-method_9789264069602-en

Palacio, S., M. Aitkenhead, A. Escudero, G. Montserrat-Martí, M. Maestro, and A.H.J. Robertson. 2014. Gypsophile chemistry unveiled: Fourier Transform Infrared (FTIR) Spectroscopy provides new insight into plant adaptations to gypsum soils. PLoS ONE **9**: e107285. doi: 10.1371/journal.pone.0107285.

Pan, B., and B. Xing. 2008. Adsorption mechanisms of organic chemicals on carbon nanotubes. Environ. Sci. Technol. **42**: 9005–9013. doi: 10.1021/es801777n.

Pandey, V.C., O. Bajpai, and N. Singh. 2016. Energy crops in sustainable phytoremediation. Renew. Sustain. Energy Rev. **54**: 58–73. doi: 10.1016/j.rser.2015.09.078.

Rostamian, R., and H. Behnejad. 2016. A comparative adsorption study of sulfamethoxazole onto graphene and graphene oxide nanosheets through equilibrium, kinetic and thermodynamic modeling. Process Saf. Environ. Prot. **102**: 20–29. doi: 10.1016/j.psep.2015.12.011.

Sabourin, L., A. Beck, P.W. Duenk, S. Kleywegt, D.R. Lapen, H. Li, C. Metcalfe, M. Payne, and E. Topp 2009. Runoff of pharmaceuticals and personal care products following application of dewatered municipal biosolids to an agricultural field. Sci. Total Environ. **407**: 4596–4604. doi: 10.1016/j.scitotenv.2009.04.027.

Salt, D.E., R.D. Smith, and D.S. Raskin. 1998. Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol **49**: 643–668.

Sandoval-Flores, G., S. Alvarado-Reyna, L.G. Elvir-Padilla, D.I. Mendoza-Castillo, H.E. Reynel-Avila, and H.E. Bonilla-Petricolet. 2018. Kinetics, thermodynamics, and competitive adsorption of heavy metals from water using orange biomass. Water Environ. Res. **90**: 2114–2125. doi: 10.2175/106143017X15131012188321.

SAS Institute. 2013. SAS/STAT 9.4 User's Guide. SAS Institute Inc., Cary, NC.

Sas-Nowosielska, A., R. Galimska-Stypa, R. Kucharski, U. Zielonka, E. Małkowski, and L. Grey. 2008. Remediation aspect of microbial changes of plant rhizosphere in mercury contaminated soil. Environ. Monit. Assess. **137**: 101–109. doi: 10.1007/s10661-007-9732-0.

Shi, J., D. Xing, and J. Lia. 2012. FTIR studies of the changes in wood chemistry from wood forming tissue under inclined treatment. Energy Procedia **16**: 758–762. doi: 10.1016/j.egypro.2012.01.122.

Smidt, E., K. Böhm, and M. Schwanninger. 2011. The application of FT-IR spectroscopy in waste management. Fourier Transforms - New Anal. Approaches FTIR Strateg. doi: 10.5772/15998.

Sparks, D. L. 1989. Kinetics of soil chemical processes. Pages 4-38. In Sparks, D.L. (Ed.). Application of chemical kinetics to soil chemical reactions, The California Academic Press LLC: California.

Sravan Kumar, S., P. Manoj, and P. Giridhar. 2015. Fourier transform infrared spectroscopy (FTIR) analysis, chlorophyll content and antioxidant properties of native and defatted foliage of green leafy vegetables. J. Food Sci. Technol. **52**: 8131–8139. doi: 10.1007/s13197-015-1959-0.

Teixidó, M., J.J. Pignatello, J.L. Beltrán, M. Granados, and J. Peccia. 2011. Speciation of the ionizable antibiotic sulfamethazine on black carbon (biochar). Environ. Sci. Technol. **45**: 10020–10027. doi: 10.1021/es202487h.

Wang, H., H. Zhang, and G. Cai. 2011. An Application of phytoremediation to river pollution Remediation. Procedia Environ. Sci. **10**: 1904–1907. doi: 10.1016/j.proenv.2011.09.298.

Wang, S., and H. Wang. 2015. Adsorption behavior of antibiotic in soil environment: a critical review. Front. Environ. Sci. Eng. **9**: 565–574. doi: 10.1007/s11783-015-0801-2.

Wu, C., A.L. Spongberg, and J.D. Witter. 2009. Sorption and biodegradation of selected antibiotics in biosolids. J. Environ. Sci. Health Part A **44**: 454–461. doi: 10.1080/10934520902719779.

Xu, B., F. Liu, P.C. Brookes, and J. Xu. 2018. The sorption kinetics and isotherms of sulfamethoxazole with polyethylene microplastics. Mar. Pollut. Bull. **131**: 191–196. doi: 10.1016/j.marpolbul.2018.04.027.

Yalkowsky, S. H., Y. He, and P. Jain. 2010. Handbook of aqueous solubility data; CRC press, Boca Raton FL, pp 675-676

Zhang, D., B. Pan, H. Zhang, P. Ning, and B. Xing. 2010. Contribution of different sulfamethoxazole species to their overall adsorption on functionalized carbon nanotubes. Environ. Sci. Technol. **44**: 3806–3811. doi: 10.1021/es903851q.

Zhao, H., X. Liu, Z. Cao, Y. Zhan, X. Shi, Y. Yang, J. Zhou, and J. Xu. 2016. Adsorption behavior and mechanism of chloramphenicols, sulfonamides, and non-antibiotic pharmaceuticals on multi-walled carbon nanotubes. J. Hazard. Mater. **310**: 235–245. doi: 10.1016/j.jhazmat.2016.02.045.

Zheng, H., Z. Wang, J. Zhao, S. Herbert, and B. Xing. 2013. Sorption of antibiotic sulfamethoxazole varies with biochars produced at different temperatures. Environ. Pollut. **181**: 60–67. doi: 10.1016/j.envpol.2013.05.056.

Zheng, T., N.B. Sutton, P. de Jager, R. Grosshans, S. Munira, and A. Farenhorst. 2017. Glyphosate (ab)sorption by shoots and rhizomes of native versus hybrid cattail (Typha). Bull. Environ. Contam. Toxicol. **99**: 595–600. doi: http://dx.doi.org.uml.idm.oclc.org/10.1007/s00128-017-2167-6.

4. PHYTOEXTRACTION OF SULFAMETHOXAZOLE AND CIPROFLOXACIN BY CATTAIL AND SWITCHGRASS

4.1 Abstract

Cattail (*Typha latifolia L.)* and switchgrass (*Panicum virgatum L.)* can effectively remove inorganic contaminants from soils and biosolids, but their role in the attenuation of organic contaminants, such as antibiotics, is currently poorly understood. Uptake by plants is one of several mechanisms by which plant-assisted attenuation of antibiotics can be achieved. The objectives of this growth room study were to (1) evaluate the uptake of sulfamethoxazole (SMX) and ciprofloxacin (CIP) by switchgrass versus cattail from a hydroponic system and (2) examine the partitioning of antibiotics between plant roots and aboveground biomass (AGB). Plant uptake of the two ^{14}C labeled antibiotics was studied at two environmentally relevant concentrations (5 and 10 µg L^{-1}). Plants were destructively sampled every 3-4 d during the 21-d growth period. Harvested plants were separated into roots and AGB and oxidized using a PerkinElmer Model 307 Sample Oxidizer to quantify the accumulated radioactivity for each ^{14}C labeled antibiotic. Accumulation of SMX and CIP in both plant species was greater in the roots than in the AGB. Translocation factors of the two antibiotics were <1 for both plants, indicating slow movement of the antibiotics from the roots to the shoots. The percentage uptake values of the two antibiotics were significantly greater for cattail (CIP ~34%, SMX ~20%) than for switchgrass (CIP=9.8%, SMX=9.8%). These results indicate greater potential for cattail to phytoextract CIP and SMX and significantly contribute to the attenuation of these antibiotics in systems designed for the phytoremediation of contaminated wastewater.

4.2 Introduction

Pharmaceutical products, including antibiotics, are frequently detected in wastewater (Xu et al., 2007), biosolids (Liu et al., 2019), rivers (Hirsch et al., 1999), and soil (Kinney et al., 2006). The incomplete elimination of antibiotics during wastewater treatment results in increasing levels of these contaminants in the environment (Miao et al., 2004). Although many antibiotics dissipate rapidly in the environment, their continuous and widespread use causes their pseudo-persistence (Brooks et al., 2006). The presence of antibiotics in the environment could lead to the development of antimicrobial resistance in pathogens (Hughes and Andersson, 2012; Huang et al., 2016). Antibiotics could also adversely affect microbial populations and their function in the environment (Carman et al., 2004; Liu et al., 2009).

Sulfamethoxazole (SMX, belonging to the sulfonamide family of antibiotics) and ciprofloxacin (CIP, belonging to the fluoroquinolone family) are commonly prescribed antibiotics for the treatment of urinary tract, enteric, or respiratory infections (Miao et al., 2004; Çalışkan and Göktürk, 2010; Cramariuc et al., 2012; Huang et al., 2016). Sulfamethoxazole works by inhibiting the production of folic acid in the dihydrofolic form, thus inhibiting protein production by bacteria (Bogialli et al., 2003; Jansomboon et al., 2016). By comparison, CIP acts through topiosomerase IV inhibition, thereby blocking bacterial DNA replication (Chatila et al., 2016). These antibiotics have been detected in varying concentrations in biosolids, wastewater, and the environment. For example, CIP was the antibiotic with the highest concentration (mean = 6,800 µg kg^{-1}) in biosolids collected from wastewater treatment plants (WWTPs) across 32 states in the USA (McClellan and Halden, 2010). Ding et al. (2011) reported SMX concentrations ranging from 4.8 to 35.9 µg kg^{-1} in biosolids from four WWTPs across Michigan, USA. Sabourin et al. (2009) reported a mean SMX concentration of 0.003 µg L^{-1} in runoff samples from plots amended with biosolids. Kairigo et al. (2020) detected SMX concentrations ranging from 0.06 to 56.6 µg L^{-1} in river water, sediment, and influent and effluent from WWTPs across Kenya. The authors reported that the highest concentration of SMX recorded (56.6 µg L^{-1}) was detected in surface grab samples collected downstream of effluent discharges, indicating the incomplete removal of SMX during wastewater treatment. However, CIP was detected at lower concentrations (0.2 - 3.0 µg L^{-1}) in the influent, effluent, and water samples (Kairigo et al., 2020).

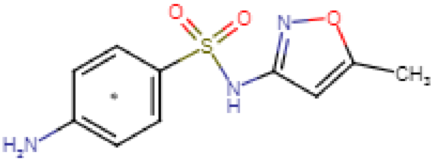
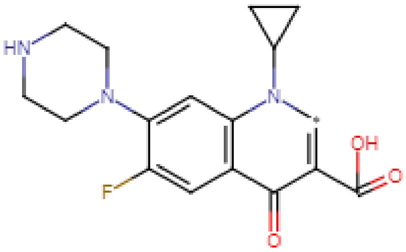
Remediation approaches, such as constructed wetlands and terrestrial phytoremediation, can be used to facilitate the removal of contaminants, such as antibiotics, from wastewater and biosolids before discharge or land application, thus minimizing contaminant transfer to the broader environment. Plant-assisted removal of contaminants during phytoremediation occurs through processes such as passive sorption, phytoextraction (active plant uptake), phytodegradation, phytostabilization, and phytovolatilization (Salt et al., 1998). Phytoremediation, through phytoextraction, has been shown to be effective in removing nutrients from biosolids (Jeke et al., 2015; Jeke et al., 2017), pharmaceuticals in wastewater (Matamoros and Bayona, 2006; Conkle et al., 2008), pesticides in runoff (Blankenberg et al., 2006), and pathogens in wastewater and sediments (Karim et al., 2004). While phytoremediation has been used mostly to clean up soils, sediments, and wastewaters contaminated with nutrients and trace elements, data on the collateral removal of antibiotics during phytoremediation of other contaminants is of interest as antibiotics are likely to co-occur with such other contaminats (Cardinal et al., 2014; Carvalho et al., 2014).

Cattail (*Typha latifolia* L.) and switchgrass (*Panicum virgatum* L.) are suitable for phytoremediation due to their high biomass yields and rapid growth (Jeke et al., 2017). These plants have been demonstrated to be efficient at removing contaminants such as metals, herbicides, and nutrients from contaminated media. For example, Chen et al. (2011) conducted a greenhouse study and reported that switchgrass removed cadmium from contaminated soils with 20-60 mg kg^{-1} cadmium. In a growth chamber study examining atrazine degradation by switchgrass (grown in commercial sand), Murphy and Coats (2011) observed the uptake and detoxification of the herbicide and its metabolites by switchgrass roots and leaves. Bankston et al. (2002) reported that cattail was efficient in degrading trichloroethylene (a halogenated short-chain hydrocarbon) from wetland microcosms. Despite widespread evidence of contaminant removal by cattail and switchgrass from various media, their role in the attenuation of antibiotics in biosolids through phytoextraction remains unexplored. Therefore, the objectives of this study were to (1) evaluate the uptake of CIP and SMX by switchgrass versus cattail from hydroponic microcosms, and (2) examine the partitioning of antibiotics between plant roots and aboveground biomass (AGB).

4.2 Materials and Methods

Cattail and switchgrass (var. Alamo) seedlings (average height 19-cm and in the vegetative stage) pre-germinated in biosolids were transferred into 500-mL mason jars containing a half-strength modified Hoagland solution (Epstein and Bloom, 2005). The nutrient solution was adjusted from a pH of 5 to 6.5 using NaOH and HCl. Nutrients were supplied at the following concentrations (µM): N, 8000; K, 3000; Ca, 2000; P, 1000; S, 500; Mg, 500; Cl, 25; B, 12.5; Mn, 1.0; Zn, 1.0; Cu, 0.25; Mo, 0.25; and Fe, 17.5. Each seedling was rinsed several times in reverse osmosis (R.O.) water prior to transferring into mason jars, which were painted black to prevent algal growth in the nutrient solution. Each mason jar had a Hydrofarm net pot (Hydrofarm LLC, Petaluma, CA) with a non-reactive foam collar to support the plants and contained 300 mL of nutrient solution. All jars and nutrient solutions were replaced on Days 3 and 7 to restore nutrient levels and minimize microbial growth and contamination of the solution (Dodgen et al., 2013). After 10 d of acclimatization, plants were transferred into fresh nutrient solution containing antibiotics (SMX [ring-^{14}C (U)-sulfamethoxazole, attached to the phenyl ring] and CIP [2-^{14}C-ciprofloxacin, (attached on 2 position of the quinolone ring)] (Table 1) to give initial concentrations of 5 µg L^{-1} (3.33 Bq mL^{-1}) and 10 µg L^{-1} (6.67 Bq mL^{-1}). After initial antibiotic treatment, antibiotic-spiked nutrient solutions were replaced on days 3, 7, 10, 14, 17, and 21 as described above. Plants were grown for 21 d. The experiment was laid out in a completely randomized design in which antibiotic (CIP and SMX), antibiotic concentration (5 and 10 µg L^{-1}), and plant species (cattail and switchgrass) were arranged in a 2 × 2 × 2 factorial treatment structure, with all treatments replicated three times. Each of the resulting 24 experimental units consisted of 5 jars.

Table 4.1 Physico-chemical characteristics of the sulfamethoxazole and ciprofloxacin

	Sulfamethoxazole	Ciprofloxacin
Structure		
Class	Sulfonamides	Fluoroquinolones
Molecular weight (g mol^{-1})	253.28	331.35
Molecular formula	$C_{10}H_{11}N_3O_3S$	$C_{17}H_{18}FN_3O_3$
Solubility at 25 °C (mg L^{-1})	438[a]	86[e]
Log K_{ow}	0.89[b]	0.28[f]
pK_a	$pKa_1 = 1.8$; $pKa_2=5.6$[c]	$pKa_1 = 6.15$, $pKa_2 = 8.66$[e]
K_{oc} (L kg^{-1})	219[d]	6100[g]

- Represents location of ^{14}C in antibiotic structure.

Data from: [a] Fioritto et al. (2007), [b] Lin and Gan (2011), [c] Lin et al. (1997), [d] Barron et al. (2009), [e]Yu et al. (1994), and [f] Albero et al. (2018)

4.2.1 Plant Sampling and Analysis

Plants grown in the ^{14}C antibiotic-spiked nutrient solutions were harvested on Days 7, 10, 14, 17, and 21. Triplicate units per treatment were randomly selected, and whole plants removed for quantification of ^{14}C accumulation in tissues. Each whole plant was rinsed several times with R.O. water and the rinsate was collected for radioactivity quantification. The ^{14}C measured in the rinsate was at background levels, indicating that the ^{14}C detected in roots was not residual ^{14}C from droplets of hydroponic solution on plant roots. Plants were separated into roots and AGB, which were placed in pre-weighed beakers and dried for 60 h at 50 °C (Dodgen et al., 2013). Dried tissue was then combusted in a Model 307 sample oxidizer (PerkinElmer Life and Analytical Sciences, Waltham, MA, USA) at a temperature of 1300 °C for 90 s. The evolved $^{14}CO_2$ was trapped in 10 mL Carbo-sorb® E plus 10 mL Permaflour® E^+. Radioactivity was quantified using a Beckman LS 6500 scintillation counter (Beckman Instruments, Fullerton, CA) and a counting time of 10 min.

Cumulative uptake by plants was calculated as:

$$\text{Cumulative uptake } (\mu g\ jar^{-1}) = \left(\frac{R_p}{R_i \times n}\right) \times C_p \times V \times n \qquad (1)$$

Antibiotic concentration in plants was calculated as:

$$Concentration\ of\ \ antibiotic\ (\mu g\ g^{-1}) \ = \frac{((R_p/R_i \times n) \times C_p \times V \times n)}{w} \qquad (2)$$

Percentage uptake was calculated as:

$$\%\ uptake\ = \frac{14C\ activity\ measured\ in\ plant\ tissue}{Initial\ amount\ of\ 14C\ in\ hydroponic\ solution \times n} \qquad (3)$$

where n is the number of times antibiotic spiked nutrient solution was replaced, R_p is the radioactivity in plant tissue, R_i is the initial radioactivity in the hydroponic solution, C_p is the antibiotic concentration in the hydroponic solution ($\mu g\ mL^{-1}$), V is the volume of hydroponic solution (mL), and w is plant weight (g).

Bioconcentration factor (BCF) and translocation factor (TF) were calculated for the cumulative uptake data over the 21-d duration of the study (Eggen et al., 2011; Dodgen et al., 2013):

$$TF = \frac{14C\ activity\ in\ AGB}{14C\ measured\ in\ roots} \qquad (4)$$

$$BCF = \frac{Concentration\ of\ antibiotic\ in\ root\ (ug\ g^{-1})}{Concentration\ in\ hydroponic\ solution\ (\mu g\ L^{-1})} \qquad (5)$$

4.2.2 Statistical analysis

Six growth models were compared using PROC NLIN in SAS 9.4 (SAS Institute, 2013) for their fit to cumulative uptake data: the 2-parameter logistic, the 3-parameter logistic, the 4-parameter logistic, the Beta, the Gompertz, and the Richards model (Archontoulis and Miguez, 2013). The model with the lowest RMSE and Akaike Information Criterion (AIC) was selected as the best fit. The 3-parameter logistic model (Eq. 6) provided the best fit for SMX uptake by both cattail and switchgrass (with the exception of switchgrass roots at the 10 µg L^{-1} concentration), whereas CIP uptake was not adequately described by any of the models tested.

$$y = \frac{y_{asymp}}{1 + e^{-k(x - x_m)}} \qquad (6)$$

where y is antibiotic uptake (µg jar^{-1}), y_{asymp} is maximum antibiotic uptake (µg jar^{-1}), k is a parameter which controls the steepness of the curve, x is time (d), x_m is the time (d) at which antibiotic uptake is maximized. Uptake data were fitted to AGB and roots separately and model parameter estimates were compared using 95% confidence intervals to evaluate treatment effects.

Concentration data for CIP and SMX in cattail and switchgrass were analyzed separately for each antibiotic with PROC GLIMMIX for repeated measures in SAS 9.4 (SAS Institute, 2013) to test for treatment and interaction effects on antibiotic uptake. Analysis of variance was also conducted to test for treatment and interaction effects on BCF, TF, and percentage uptake using

PROC GLIMMIX, with antibiotic type, antibiotic concentration, plant species, and their interactions as fixed effects. The beta distribution was specified for percentage uptake and lognormal distribution for TF. All other variables followed the normal distribution. Treatment differences were assessed at $\alpha = 0.05$ using the Tukey multiple comparison procedure.

4.3 Results and Discussion

4.3.1 Percentage uptake

There was a significant plant × antibiotic × tissue interaction effect on the cumulative percentage of antibiotic taken up by plants from the spiked nutrient solution (Figure 4.1). A greater percentage of CIP (34%) was taken up by cattail roots compared to SMX (20%), indicating that cattail could remove a significantly greater percentage of CIP through phytoextraction. In contrast, antibiotic uptake by switchgrass roots (~10%) did not differ significantly between the two antibiotics.

The percentage of SMX accumulating in the AGB was significantly greater for cattail (~ 3%) than switchgrass (~1%). In comparison, CIP accumulation in the AGB (~1%) did not differ significantly between cattail and switchgrass. The percentage of antibiotic accumulating in the AGB was low for both antibiotics, suggesting that harvesting of AGB during phytoremediation would only remove a small fraction of CIP and SMX. In contrast, harvesting both AGB and roots during phytoremediation could remove ~23% of SMX and ~36% of CIP in cattail and about 11% of both antibiotics in switchgrass. A phytoremediation technique that would be amenable to the harvest of both AGB and roots is a floating bed system. Floating bed systems have been shown to be effective in removing metals (copper, zinc) and nutrients (dissolved reactive phosphorus) from stormwater (Tanner and Headley, 2011; Borne et al., 2013), and nutrients (N and P) and sulfonamide antibiotics (e.g., sulfadiazine, sulfamethazine, and sulfamethoxazole) from wastewater from a swine operation (Xian et al., 2010).

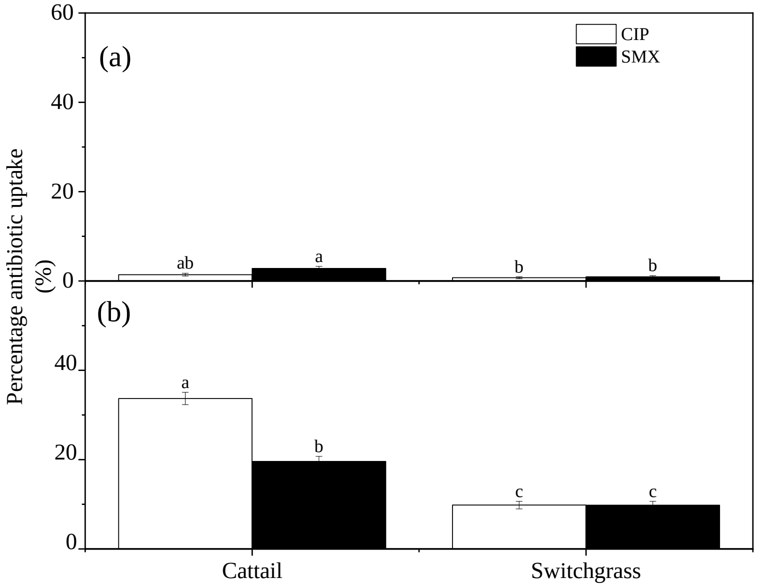


Figure 4.1 Percentage antibiotic uptake in the AGB (a) and roots (b) after 21 d. Error bars represent standard errors of the mean (SEM) (n = 3). Bars with the same letter within the same plant tissue component are not significantly different according to the Tukey multiple comparison procedure (α = 0.05).

4.3.2 Translocation and Bioconcentration Factors

The detection of CIP and SMX in AGB of cattail and switchgrass in our study confirms the translocation of antibiotics from roots to the above ground parts (Fig 4.2a). However, the low TFs (<1) observed indicate restricted translocation of the antibiotics from the roots to the shoots (Pan and Chu, 2017). The TF for SMX was significantly greater for cattail than switchgrass, whereas the TF for CIP did not differ significantly between the two plant species. For cattail, the TF was significantly greater for SMX than CIP. In contrast, there was no significant difference in TF between the two antibiotics for switchgrass (Fig 4.2a). The BCF of cattail and switchgrass roots for CIP and SMX ranged from 0.8 to 1.6 L g^{-1} (Fig 4.2b). For cattail roots, the BCF for CIP (1.58 L g^{-1}) was significantly greater than that for SMX roots (0.8 L g^{-1}). By comparison, switchgrass root BCFs did not differ significantly between SMX (1.13 L g^{-1}) and CIP (0.88 L g^{-1}). Bioconcentration factors < 1 L g^{-1} indicate low bioaccumulation of the compound by the plant (Arnot and Gobas, 2006)**.** Consistent with our results, other studies have shown that BCF varies with plant species. For example, Wu et al. (2013) reported that the translocation of the 16 pharmaceutical compounds in their study (including acetaminophen, atenolol, carbamazepine, caffeine, meprobamate, and trimethoprim) differed among lettuce, cucumber, pepper and spinach. The authors observed that triclosan accumulated more in the roots of lettuce and spinach with a BCF of 0.04 - 0.12 L g^{-1} while BCF in roots of cucumber and pepper was smaller (0.0005 - 0.0042 L g^{-1}). Dodgen et al. (2015) suggested that plant and antibiotic properties greatly influenced tomato, lettuce, and carrot root BCFs for organic compounds, as compounds like atorvastatin, diclofenac, and clofibric acid had smaller BCFs (0.0 – 0.069 L g^{-1}) than perfluorooctanoic acid, diazepam, and diuron (0.004 – 0.72L g^{-1}).

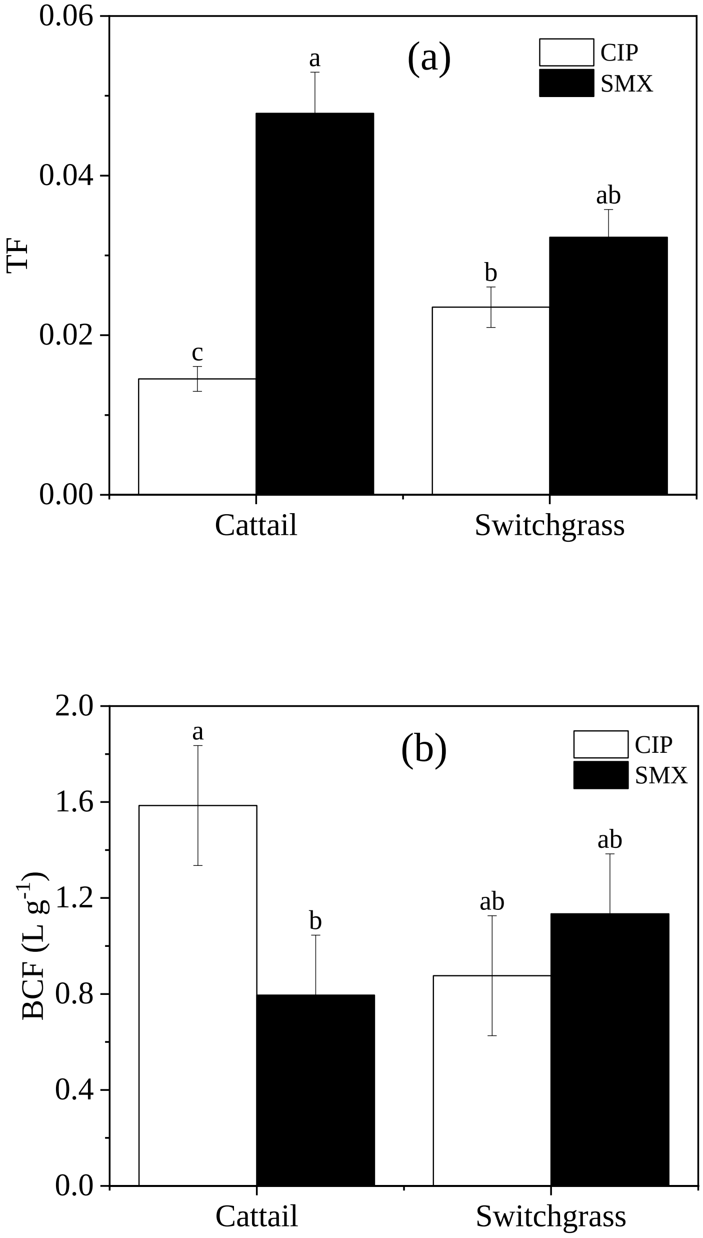


Figure 4.2 Effect of antibiotic type × plant species interaction on (a) translocation factor (TF) and (b) bioconcentration factor (BCF) of cattail and switchgrass. Error bars represent standard errors of the mean (SEM) (n = 3). Bars with the same letter are not significantly different according to the Tukey multiple comparison procedure ($\alpha = 0.05$).

Translocation factors and BCFs of plants are influenced by physicochemical characteristics such as hydrophobicity, dissociation potential of antibiotics, and plant characteristics such as lipid content, metabolism, growth rate, and transpiration rate (Wu et al., 2015; Pan and Chu, 2017; Coleman et al., 1997). For example, the TF of SMX was found to be positively correlated with the mass of nutrient solution transpired (Dodgen et al., 2015), suggesting that transpiration influenced the translocation of SMX from roots to leaves. Wu et al. (2013) also concluded that water movement by transpiration could have influenced the translocation of pharmaceutical compounds after root uptake, as hydrophilic compounds can move within the xylem and may be translocated to the shoots. This could explain the higher accumulation of antibiotics observed for cattail, which exhibited greater transpiration than switchgrass in our study. Cattail, being an aquatic plant, generally shows greater evapotranspiration (Towler et al., 2004) than switchgrass, which is drought-resistant and has better water use efficiency (Wright and Evans, 2008; Liu et al., 2015). Translocation of zwitterions, such as CIP, from roots to AGB can be restricted as membranes have been shown to have low permeability of such chemical species compared to neutral compounds (Sigler et al., 2000). In a study on the uptake of zwitterionic antibiotics by rice, Hawker et al. (2013) observed no translocation of norfloxacin from the roots to AGB. The authors attributed the lack of translocation to low mobility of norfloxacin within the low-permeability rice plant membrane. Yan et al. (2020) reported a TF of 0.02-1.36 for CIP in *Eichhornia crassipes* as initial CIP concentration increased from 10 to 1000 µg L^{-1}. The authors concluded that the high lipid content of the roots of the aquatic plant likely restricted the transfer of CIP from the roots to AGB. The molecular weight of an organic compound may also influence its transport within plant cells (Chuang et al., 2019). Chuang et al. (2019) found that pharmaceuticals with molecular weight < 300 g mol^{-1} were more readily translocated to the shoots of lettuce plants than compounds with larger molecular weights. Similar to Chuang et al. (2019), we observed greater translocation of the antibiotic with the lower molecular weight (SMX = 253 g mol^{-1}) compared to CIP (331g mol^{-1}).

4.3.3 Antibiotic Uptake

4.3.3.1 Ciprofloxacin

The mechanism of uptake of organic xenobiotics is through passive diffusion as there are no specific transporters of these compounds in plant membranes because of their xenobiotic nature (Dietz and Schnoor, 2001; Pilon-Smits, 2005). Plant uptake of CIP by plants could be through passive uptake (diffusion) and water transport (Liu et al., 2013). Ciprofloxacin was detected in AGB and roots of cattail and switchgrass, with concentrations varying with the timing of sampling (Fig. 4.3; Table 4.2). Tissue concentration of CIP in switchgrass increased from Day 7 to 14, after which there was a decrease in the concentration. However, for cattail, there was no significant change in tissue CIP concentration during Days 7 through 21, suggesting a relatively steady CIP accumulation over time. Surprisingly, no decrease in CIP concentration in cattail tissues was observed. If the amount of antibiotic entering plant tissues remains unchanged, an increase in biomass relative to CIP accumulation might result in a dilution effect (Ma et al., 2016), which could explain the decrease in concentration observed for switchgrass. Yan et al. (2020) reported eight transformation products from the degradation of CIP in *Eichhornia crassipes* after 7 d of incubation. Similar to our observations, Yan et al. (2020) reported an initial increase in CIP concentration in *E. crassipes* until Day 2, after which the concentration decreased within the 7-d study period in pots spiked with CIP concentrations of 50-1000 µg L^{-1}. This trend was attributed to the biodegradation of CIP in the *E. crassipes* tissues (Yan et al., 2020). A similar decline in antibiotic concentration in plant tissues after an initial increase was reported by Hawker et al. (2013), who studied the uptake of zwitterionic antibiotics by rice. They attributed the decrease in antibiotic uptake in rice roots to the attainment of maximum antibiotic uptake by the crop and to the response of the plant to the loss of antibiotics from the soil/water compartment due to transformation processes. This was not the case in our study as antibiotics in the solution were replaced every 3-4 d. Although we measured ^{14}C, we believe that if any degradation of the antibiotic occurred in the plant tissue, quantification of ^{14}C using the liquid scintillation counter would include metabolites as well.

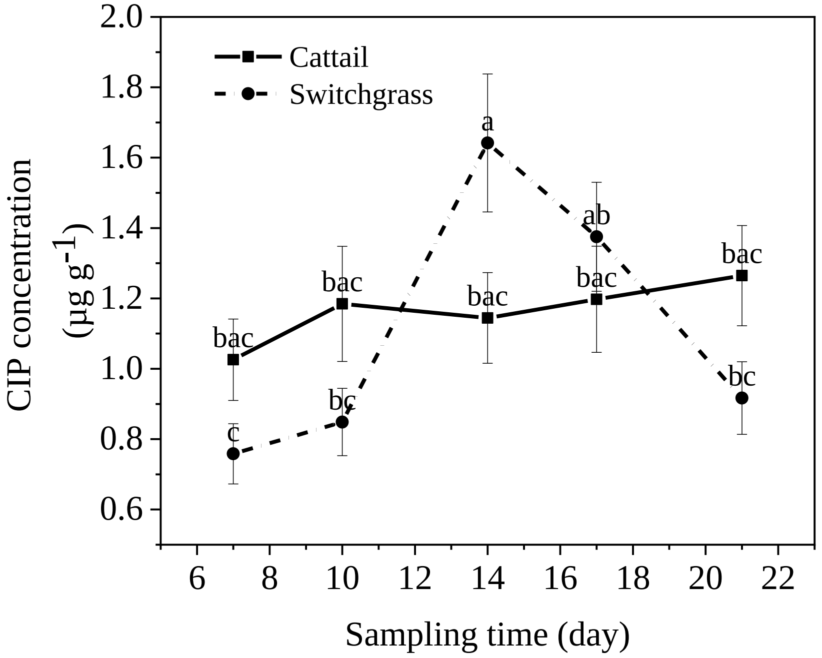


Figure 4.3 Effect of sampling time on CIP accumulation by cattail and switchgrass. Error bars represent standard errors of the mean (SEM) (n = 3). Points on the lines with the same letter are not significantly different according to the Tukey multiple comparison procedure (α = 0.05).

Table 4.2 Treatment effects on plant uptake of SMX and CIP.

Treatment	SMX uptake (µg g^{-1})	CIP uptake (µg g^{-1})
Plant		
Cattail	0.71 (0.04)	1.15 (0.06)
Switchgrass	0.51 (0.04)	1.05 (0.05)
Tissue		
AGB	0.14(0.01)	0.25 (0.01)
BGB	2.67 (0.18)	8.0 (0.42)
Concentration		
5 µg L^{-1}	0.47 (0.03)**b**	0.80 (0.04)
10 µg L^{-1}	0.78 (0.05)**a**	1.51 (0.08)
Day		
7	0.22 (0.03)	0.88 (0.07)
10	0.37 (0.04)	1.0 (0.09)
14	0.68 (0.07)	1.4 (0.11)
17	1.09 (0.11)	1.3 (0.11)
21	1.29 (0.13)	1.1 (0.08)
ANOVA	*P* value	
Plant	0.001	0.22
Concentration	<0.0001	<0.0001
Tissue	<0.0001	<0.0001
Day	<0.0001	0.002
Plant × concentration	0.64	0.01
Day × concentration	0.06	0.05
Tissue × concentration	0.76	0.98
Plant × day	<0.0001	0.007
Plant × tissue	0.31	<0.0001
Day × tissue	<0.0001	0.05
Plant × day × concentration	0.33	0.85
Plant × concentration × tissue	0.89	0.22
Day × concentration × tissue	0.98	0.10
Plant × day × tissue	0.11	0.34
Plant × day × concentration × tissue	0.96	0.85

Data are least-square means ((SEM), n = 3). Means followed by the same letter are not significantly different according to the Tukey multiple comparison procedure (α = 0.05).

The concentration of CIP in plant tissues varied with plant species (Table 4.2, Fig 4.4a). There was significantly more CIP in switchgrass AGB than cattail AGB, whereas cattail roots accumulated significantly more CIP than switchgrass roots. The greater CIP concentration in switchgrass AGB despite the lower CIP concentration in switchgrass roots than cattail roots suggests that switchgrass translocated a greater proportion of CIP than cattail, indicating that plant-specific properties affected CIP uptake. For both cattail and switchgrass, CIP concentration was significantly greater in plant roots than in AGB. Ciprofloxacin can strongly partition to root lipids, which might restrict movement from roots to shoots (Yan et al., 2020). Similar results (roots > AGB) have been reported for uptake of other antibiotics (Wu et al., 2010; Wu et al., 2013; Zhao et al., 2019). Consistent with our findings, Yan et al. (2020) also reported that most of the CIP taken up by a floating macrophyte accumulated in the roots relative to aboveground compartments. The authors attributed the higher concentration of CIP in the roots to the fact that roots are the plant parts in direct contact with the media containing the organic compound (Yan et al., 2020). Eggen et al. (2011) also reported more CIP in the roots of barley and Napoli carrot, relative to AGB. Similarly, Panja et al. (2019) observed greater accumulation of CIP in roots of vertiver grass compared to shoots and leaves.

The effect of initial CIP concentration on CIP uptake was dependent on plant species (Table 2, Fig 4b). At the initial CIP concentration of 5 μg L^{-1}, cattail accumulated significantly more CIP than switchgrass, while at the higher initial concentration (10 μg L^{-1}) there was no significant difference in CIP concentration between cattail and switchgrass. This might be due to a biomass dilution effect occurring at the lower concentration for switchgrass due to the limited accumulation of CIP compared to the uptake at the higher concentration as plants tend to take up higher amounts of antibiotics when exposed to higher concentrations (Azanu et al., 2016). Similar to our study, Ahmed et al. (2015) reported varying effects of initial antibiotic concentration on antibiotic accumulation by the plants used in their study. For example, while there was no significant difference in SMX accumulation by cucumber fruits between initial SMX concentrations of 5 to 10 mg kg^{-1}, Ahmed et al. (2015) reported an increase in SMX concentration in tomato fruits as initial SMX concentration increased.

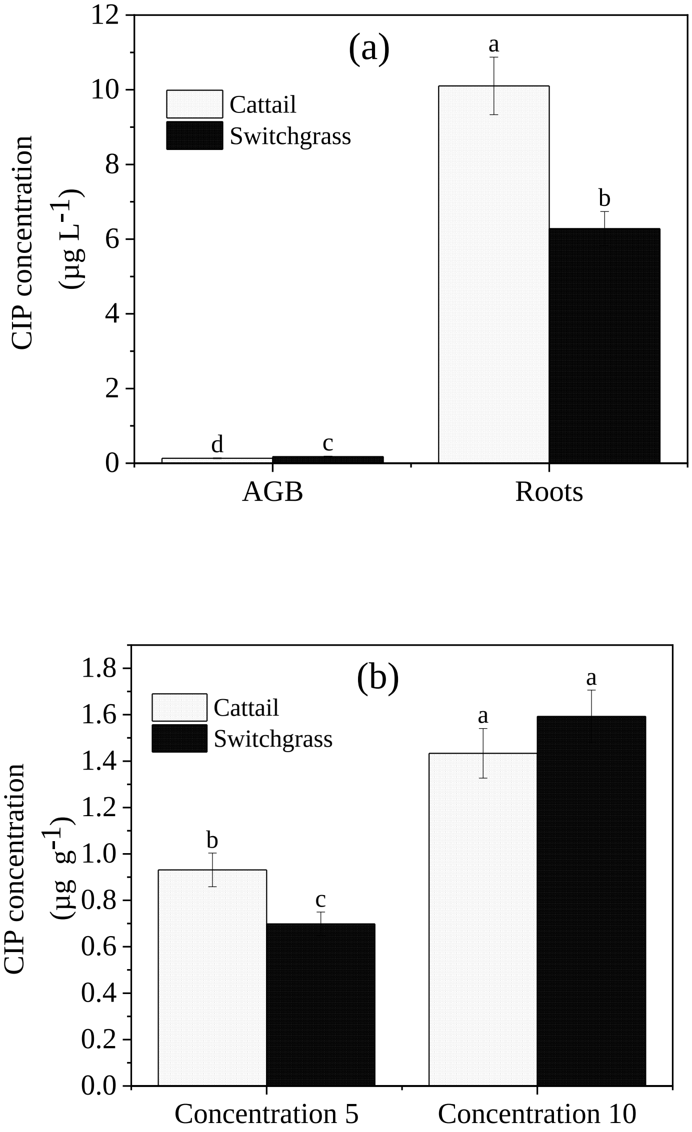


Figure 4.4 Effect of (a) plant species × tissue component (b) plant species × concentration interaction effects on CIP accumulation. Error bars represent standard errors of the mean (SEM)

(n = 3). Bars with the same letter are not significantly different according to the Tukey multiple comparison procedure (α = 0.05).

4.3.3.2 Sulfamethoxazole

None of the six growth models provided a fit for CIP uptake by cattail and switchgrass, but for both plant species, the three-parameter logistic model provided the best fit for cumulative SMX uptake (amount of SMX per jar) by cattail and switchgrass (Fig. 4.5). We observed a period of slow uptake during the first 14 d in both AGB and roots, after which antibiotic uptake increased. The primary mechanism of plant uptake of most organic compounds with molecular weight < 500 g mol^{-1} is through passive diffusion (Kumar and Gupta, 2016). Previous studies have concluded that SMX is absorbed by plant roots via simple diffusion (Kurade et al., 2019) and that the uptake and translocation of SMX by watercress was through passive transport (Chitescu et al., 2013). A number of other studies have inferred that the primary mechanism of uptake for sulfonamides is through water transport and passive diffusion (e.g., Hu et al., 2010; Liu et al., 2013).

For AGB, maximum SMX uptake (y_{asymp}) was significantly greater for cattail (0.24 µg per jar^{-1}) than switchgrass (0.05 µg per jar^{-1}) at an initial SMX concentration of 5 µg L^{-1}. For roots, at the initial SMX concentration of 5 µg L^{-1}, the maximum SMX uptake was numerically (but not significantly) greater for cattail (1.97 µg per jar^{-1}) than switchgrass (0.73 µg per jar^{-1}). The significantly greater maximum SMX uptake by cattail than switchgrass suggests that SMX is better translocated to the AGB parts of cattail than switchgrass, which is consistent with the TF observed for SMX in this study.

At the initial SMX concentration of 10 µg L^{-1}, although numerically greater than switchgrass, the maximum attainable SMX uptake for cattail AGB (0.64 µg per jar^{-1}) was not significantly different from that for switchgrass (0.19 μg per jar^{-1}). The maximum SMX uptake was three (cattail) to four times (switchgrass) greater at the initial SMX concentration of 10 μg L^{-1} than at 5 μg L^{-1}. Thus, regardless of the plant species, greater initial SMX concentrations lead to greater root uptake and translocation from roots to AGB. This suggests that as the initial concentration doubled in the plant solution or growing medium, the amount of SMX that accumulated in plant tissues increased.

The y_{asymp} for cattail roots at concentration 10 μg L^{-1} (4.5 μg per jar^{-1}) was significantly greater than the y_{asymp} for cattail AGB (0.64 μg per jar^{-1}) (Fig. 6). This could be because roots are in direct contact with the medium containing the organic compound, resulting in the greater accumulation in the roots (Yan et al., 2020). A similar uptake pattern (y_{asymp} roots > y_{asymp} AGB) has previously been reported for other antibiotics (Herklotz et al., 2010; Dodgen et al., 2015).

The x_m parameter, which indicates the time that it takes for antibiotic uptake rate to peak, did not differ significantly between plant species and between plant components (Fig. 4.5). At the initial SMX concentration of 5 μg L^{-1}, the maximum SMX uptake rate for cattail occurred 17.7 d after transplanting (DAT) for AGB and 18.3 DAT for roots. For switchgrass, the maximum SMX uptake rate was 18.6 DAT for AGB and 18.0 DAT for roots. At the initial SMX concentration of 10 μg L^{-1}, SMX uptake rate was maximized at 17 DAT for cattail AGB and 16.4 DAT for cattail roots, while for switchgrass, it was 19 DAT for AGB (no model fit for switchgrass roots). Thus, at the lower SMX concentration, we observed that cattail roots reached maximum uptake rate before AGB whereas at the higher concentration, AGB reached maximum uptake rate before cattail roots. The x_m for SMX uptake at the initial concentration of 10 μg L^{-1} did not differ significantly from that at 5 μg L^{-1}.

The rate of SMX uptake (*k*) did not differ significantly between plant species or plant tissues at the initial SMX concentration of 5 µg L^{-1} (cattail AGB, 0.45; cattail roots, 0.40; switchgrass AGB, 0.67; switchgrass roots, 0.68). The *k* mirrors x_m, which indicated similar elapsed times to attain SMX uptake maxima. Similar to what was observed at an initial antibiotic solution of 5 µg L^{-1}, the rate of SMX uptake did not differ among treatments at the initial concentration of 10 µg L^{-1} (cattail AGB, 0.50; cattail roots, 0.57; switchgrass AGB, 0.66).

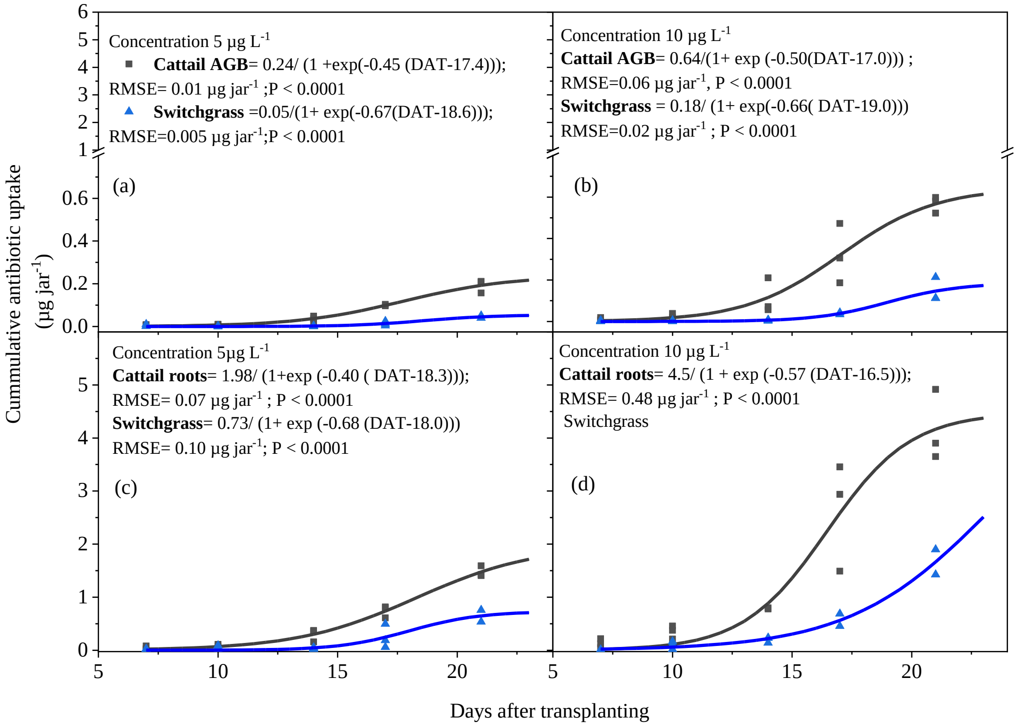


Figure 4.5 SMX accumulation as described by the three-parameter logistic growth model: (a) AGB and (b) roots at initial concentration 5 µg L^{-1}; (c) AGB and (c) roots at initial concentration 10 µg L^{-1}. DAT, days after transplanting; AGB, aboveground biomass.

There was a significant effect of initial SMX concentration on SMX uptake by plants (Table 4.2). As the initial SMX concentration increased from 5 to 10 µg L^{-1}, the concentration in plant tissues almost doubled (Table 4.2). This pattern (uptake at 10 µg L^{-1} > uptake at 5 µg L^{-1}) is consistent with cumulative SMX uptake by plant tissues in this study. Ahmed et al. (2015) reported an increase in the uptake of sulfonamides (sulfamethazine and sulfadimethoxine) by tomato, cucumber, and lettuce from a loamy sand as initial sulfonamide concentration increased. Similarly, in a study by Li et al. (2013), the concentration of sulfamethazine in pakchoi cabbage increased as concentrations spiked in soil increased from 1 mg kg^{-1} to 25 mg kg^{-1}. Liu et al. (2013) observed that the concentration of sulfamethazine, a sulfonamide like SMX, was positively correlated with the initial antibiotic concentration. In contrast to our study, there was no concentration effect on SMX uptake in a study by Chitescu et al. (2013), as the authors observed no significant difference between uptake of SMX by watercress and grass at 5 and 10 mg kg^{-1}.

We also observed a significant sampling time × plant tissue interaction, indicating that the effect of sampling time on SMX uptake by plants varied with the plant tissue compartment. While SMX accumulation by AGB was gradual, for roots, SMX accumulation was more rapid (Fig 4.6a). For the AGB, peak concentration in plant tissue occurred on Day 14, with no significant change in concentration on Day 21. However, for roots, peak accumulation occurred on Day 17 as concentration in plant tissue on Day 17 was not significantly different from that on Day 21. At all sampling times, SMX concentration was significantly greater in the roots than in the AGB, likely due to the repulsion of the negatively charged SMX by plant cell membranes, which restricted its mobility and translocation to AGB (Klement et al., 2020). Consistent with our study, Kodešová et al. (2019) observed greater concentrations of SMX in the roots of potted lettuce, arugula and radish compared to concentrations in the leaves. The authors attributed the lower concentrations of SMX in the leaves to the restricted translocation in plants due to the negative form of SMX occurring at the pH of 5.6 – 8.2 in their study. Liu et al. (2013) also observed greater concentration of sulfamethazine and oxytetracycline in the roots of a wetland plant, *Phragmites australis*, grown under hydroponic conditions. In contrast, Kurade et al. (2019) observed significant SMX bioaccumulation in leaves (16.9% of initial) with no bioaccumulation observed in roots and stems. The authors attributed the lower SMX concentration in plant roots to the translocation of SMX from the roots to the leaves through the stem vacuoles. The greater concentration of antibiotics in roots compared to leaves/shoots in the present study could be because roots are the primary uptake organ and the organ in direct contact with the contaminant (Chen et al., 2017).

The effect of sampling time on SMX uptake also differed with plant species (Fig 4.6b). While uptake by cattail was rapid at earlier stages (between Days 7 and 14), it reached a plateau on Day 14, when peak accumulation probably occurred. In contrast, accumulation of SMX by switchgrass was initially (Day 7-10) slow, after which the concentration continued to increase until the end of the experiment. Also, there was significantly more SMX in cattail than in switchgrass tissues on Days 10 and 14; however, on other days, there was no significant difference in SMX concentration between the two plant species. This suggests that differences in plant species characteristics influence antibiotic uptake and could cause differences in uptake trends between plant species. Other studies have shown differences in the accumulation of antibiotics by different plants (Ahmed et al., 2015; Zhao et al., 2019).

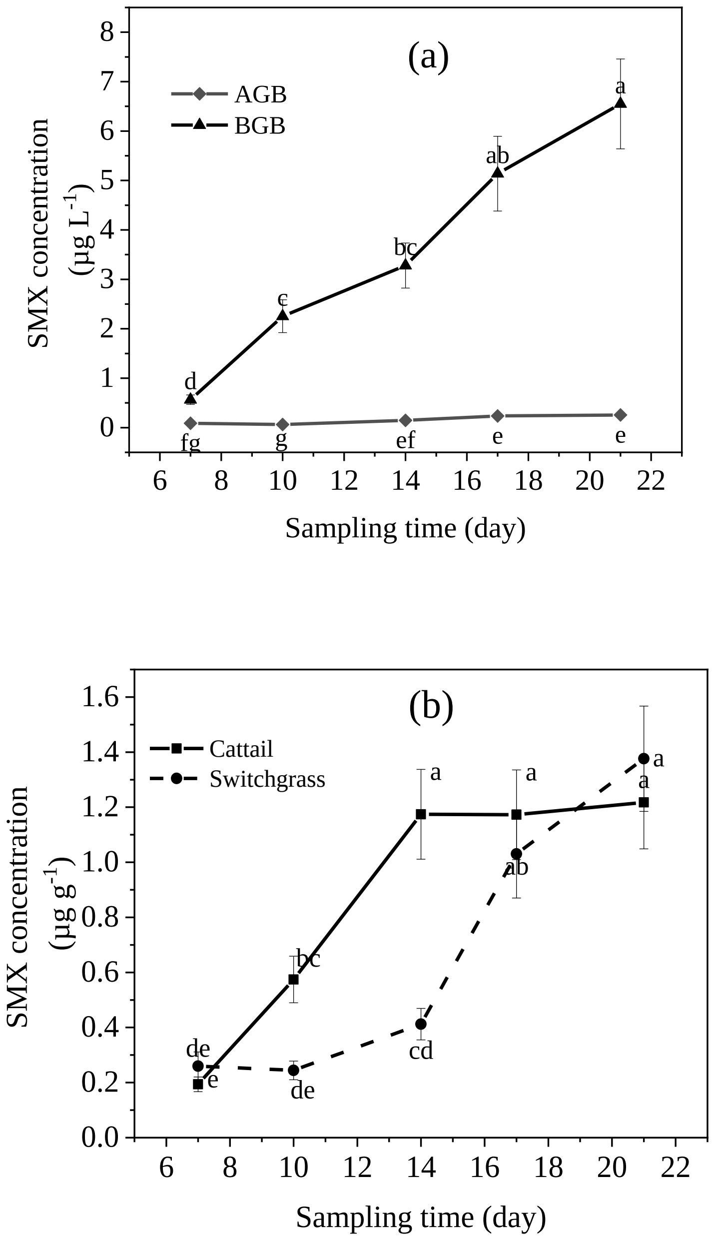


Figure 4.6 Effect of (a) sampling time × tissue (b) sampling time × plant species interaction on SMX accumulation by cattail and switchgrass. Error bars represent standard errors of the mean (SEM) (n = 3). Points on the lines with the same letter are not significantly different according to the Tukey multiple comparison procedure (α = 0.05).

4.4 Conclusion

Cattail and switchgrass were able to accumulate SMX and CIP. Cattail roots accumulated a significantly greater percentage of antibiotics than switchgrass roots. Plant roots preferentially accumulated both antibiotics with minimal translocation to AGB. Therefore, harvesting of both AGB and roots of switchgrass and cattail would be more effective for the removal of CIP and SMX. We conclude that cattail could be more effective for phytoextraction of CIP and SMX from systems designed for in situ phytorememdiation of contaminants. Degradation of antibiotics could occur in plant tissues; therefore, future studies should evaluate transformation products of SMX and CIP in cattail and switchgrass for better understanding of the degradation mechanisms of these antibiotics in the plant tissues.

4.5 References

Ahmed, M.B.M., A.U. Rajapaksha, J.E. Lim, N.T. Vu, I.S. Kim, H.M. Kang, S.S. Lee, and Y.S. Ok. 2015. Distribution and accumulative pattern of tetracyclines and sulfonamides in edible vegetables of cucumber, tomato, and lettuce. J. Agric. Food Chem. **63**: 398–405. doi: 10.1021/jf5034637.

Albero, B., J.L. Tadeo, M. Escario, E. Miguel, and R.A. Pérez. 2018. Persistence and availability of veterinary antibiotics in soil and soil-manure systems. Sci. Total Environ. **643**: 1562–1570. doi: 10.1016/j.scitotenv.2018.06.314.

Archontoulis, S.V., and F.E. Miguez. 2013. Nonlinear regression models and applications in agricultural research. Agron. J. **105:**1–13. doi:10.2134/ agronj2012.0221

Arnot, J.A., and F.A. Gobas. 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environ. Rev. **14**: 257–297. doi: 10.1139/a06-005.

Azanu, D., C. Mortey, G. Darko, J.J. Weisser, B. Styrishave, and R. Abaidoo. 2016. Uptake of antibiotics from irrigation water by plants. Chemosphere **157**: 107–114. doi: 10.1016/j.chemosphere.2016.05.035.

Bankston, J.L., D.L. Sola, A.T. Komor, and D.F. Dwyer. 2002. Degradation of trichloroethylene in wetland microcosms containing broad-leaved cattail and eastern cottonwood. Water Res. **36**: 1539–1546. doi: 10.1016/s0043-1354(01)00368-2

Barron, L., J. Havel, M. Purcell, M. Szpak, B. Kelleher, and B. Paull. 2009. Predicting sorption of pharmaceuticals and personal care products onto soil and digested sludge using artificial neural networks. Analyst **134**: 663–670. doi: 10.1039/B817822D.

Blankenberg, A.-G.B., B. Braskerud, and K. Haarstad. 2006. Pesticide retention in two small constructed wetlands: treating non-point source pollution from agriculture runoff. Int. J. Environ. Anal. Chem. **86**: 225–231. doi: 10.1080/03067310500247470.

Bogialli, S., R. Curini, A. Di Corcia, M. Nazzari, and M.L. Polci. 2003. Rapid confirmatory assay for determining 12 sulfonamide antimicrobials in milk and eggs by matrix solid-phase dispersion and liquid chromatography−mass spectrometry. J. Agric. Food Chem. **51**: 4225–4232. doi: 10.1021/jf0343837.

Borne, K.E., E.A. Fassman, and C.C. Tanner. 2013. Floating treatment wetland retrofit to improve stormwater pond performance for suspended solids, copper and zinc. Ecol. Eng. **54**: 173–182. doi: 10.1016/j.ecoleng.2013.01.031.

Brooks, B.W., T.M. Riley, and R.D. Taylor. 2006. Water quality of effluent-dominated ecosystems: ecotoxicological, hydrological, and management considerations. Hydrobiologia Dordrecht **556**: 365–379. doi: http://dx.doi.org.uml.idm.oclc.org/10.1007/s10750-004-0189-7.

Çalışkan, E., and S. Göktürk. 2010. Adsorption characteristics of sulfamethoxazole and metronidazole on activated carbon. Sep. Sci. Technol. **45**: 244–255. doi: 10.1080/01496390903409419.

Cardinal, P., J.C. Anderson, J.C. Carlson, J.E. Low, J.K. Challis, S.A Beattie, C.N. Bartel, A.D. Elliot, O.F. Montero, S.L. Lokesh, A.F. Favreau, T.A. Kozlova, C.W. Knapp, M.L. Hanson, and C.S. Wong. 2014. Macrophytes may not contribute significantly to removal of nutrients, pharmaceuticals, and antibiotic resistance in model surface constructed wetlands. Sci. Total Environ. **482–483** : 294-304.

Carman, R.J., M.A. Simon, H. Fernández, M.A. Miller, and M.J. Bartholomew. 2004. Ciprofloxacin at low levels disrupts colonization resistance of human fecal microflora growing in chemostats. Regul. Toxicol. Pharm. **40**: 319–326. doi: 10.1016/j.yrtph.2004.08.005.

Carvalho, P.N., M.C.P. Basto, C.M.R. Almeida, and H. Brix. 2014. A review of plant–pharmaceutical interactions: from uptake and effects in crop plants to phytoremediation in constructed wetlands. Environ. Sci. Pollut. Res. **21**: 11729–11763. doi: 10.1007/s11356-014-2550-3.

Chatila, S., M.R. Amparo, L.S. Carvalho, E.D. Penteado, I.N. Tomita, l.J. Santos-Neto, P.C.F. Lima Gomes, and M. Zaiat. 2016. Sulfamethoxazole and ciprofloxacin removal using a horizontal-flow anaerobic immobilized biomass reactor. Environ. Technol. **37**: 847–853. doi: 10.1080/09593330.2015.1088072.

Chen, B.C., H.Y. Lai, D.Y. Lee, and K.W. Juang. 2011. Using chemical fractionation to evaluate the phytoextraction of cadmium by switchgrass from Cd-contaminated soils. Ecotoxicology **20**: 409–418. doi: 10.1007/s10646-010-0592-y.

Chen, H.-R., T. Rairat, S.-H. Loh, Y.-C. Wu, T.W. Vickroy, and C.C Chou. 2017. Assessment of veterinary drugs in plants using pharmacokinetic approaches: the absorption, distribution and elimination of tetracycline and sulfamethoxazole in ephemeral vegetables. PLOS One **12**: e0183087. doi: 10.1371/journal.pone.0183087.

Chitescu, C.L., A.I. Nicolau, and A.A.M. Stolker. 2013. Uptake of oxytetracycline, sulfamethoxazole and ketoconazole from fertilised soils by plants. Food Addit. Contam. Part A **30**: 1138–1146. doi: 10.1080/19440049.2012.725479.

Chuang, Y.H., C.H. Liu, J.B. Sallach, R. Hammerschmidt, W. Zhang, S.A. Boyda, and H. Lia. 2019. Mechanistic study on uptake and transport of pharmaceuticals in lettuce from water. Environ. Int. **131:** 104976. doi: 10.1016/j.envint.2019.104976.

Coleman, J., M. Blake-Kalff, and E. Davies. 1997. Detoxification of xenobiotics by plants: chemical modification and vacuolar compartmentation. Trends Plant Sci. **2**: 144–151. doi: 10.1016/S1360-1385(97)01019-4.

Conkle, J.L., J.R. White, and C.D. Metcalfe. 2008. Reduction of pharmaceutically active compounds by a lagoon wetland wastewater treatment system in Southeast Louisiana. Chemosphere **73**: 1741–1748. doi: 10.1016/j.chemosphere.2008.09.020.

Cramariuc, O., T. Rog, M. Javanainen, L. Monticelli, A.V. Polishchuk, and I. Vattulainen. 2012. Mechanism for translocation of fluoroquinolones across lipid membranes. Biochim. Biophys. Acta BBA - Biomembr. **1818**: 2563–2571. doi: 10.1016/j.bbamem.2012.05.027.

Dietz, A.C., and J.L. Schnoor. 2001. Advances in Phytoremediation. Environ. Health Perspect. **109**: 163–168. doi: 10.2307/3434854.

Ding, Y., W. Zhang, C. Gu, I. Xagoraraki, and H. Li. 2011. Determination of pharmaceuticals in biosolids using accelerated solvent extraction and liquid chromatography/tandem mass spectrometry. J. Chromatogr. A **1218**: 10–16. doi: 10.1016/j.chroma.2010.10.112.

Dodgen, L.K., J. Li, D. Parker, and J.J. Gan. 2013. Uptake and accumulation of four PPCP/EDCs in two leafy vegetables. Environ. Pollut. **182**: 150–156. doi: 10.1016/j.envpol.2013.06.038.

Dodgen, L.K., A. Ueda, X. Wu, D.R. Parker, and J. Gan. 2015. Effect of transpiration on plant accumulation and translocation of PPCP/EDCs. Environ. Pollut. **198**: 144–153. doi: 10.1016/j.envpol.2015.01.002.

Eggen, T., T.N. Asp, K. Grave, and V. Hormazabal. 2011. Uptake and translocation of metformin, ciprofloxacin and narasin in forage- and crop plants. Chemosphere **85**: 26–33. doi: 10.1016/j.chemosphere.2011.06.041.

Epstein, E., and A.J. Bloom. 2005. Mineral nutrition of plants: principles and perspectives. 2nd ed. Sinauer Associates, Sunderland, MA.

Fioritto, A.F., S.N. Bhattachar, and J.A. Wesley. 2007. Solubility measurement of polymorphic compounds via the pH-metric titration technique. Int. J. Pharm. **330**: 105–113. doi: 10.1016/j.ijpharm.2006.09.003.

Hawker, D.W., R. Cropp, and M. Boonsaner. 2013. Uptake of zwitterionic antibiotics by rice (*Oryza sativa* L.) in contaminated soil. J. Hazard. Mater. **263**: 458–466. doi: 10.1016/j.jhazmat.2013.09.066.

Herklotz, P.A., P. Gurung, B. Vanden Heuvel, and C.A. Kinney. 2010. Uptake of human pharmaceuticals by plants grown under hydroponic conditions. Chemosphere **78**: 1416–1421. doi: 10.1016/j.chemosphere.2009.12.048.

Hirsch, R., T. Ternes, K. Haberer, and K.-L. Kratz. 1999. Occurrence of antibiotics in the aquatic environment. Sci. Total Environ**. 225**: 109–118. doi: 10.1016/S0048-9697(98)00337-4.

Hu, X., Q. Zhou, and Y. Luo. 2010. Occurrence and source analysis of typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases, northern China. Environ. Pollut. **158**: 2992–2998. doi: 10.1016/j.envpol.2010.05.023.

Huang, T., Y. Xu, J. Zeng, D.-H. Zhao, L. Li, X.P Liao, Y.H Liu, and J. Sun. 2016. Low-concentration ciprofloxacin selects plasmid-mediated quinolone resistance encoding genes and affects bacterial taxa in soil containing manure. Front. Microbiol. **7**. doi: 10.3389/fmicb.2016.01730.

Hughes, D., and D.I. Andersson. 2012. Selection of resistance at lethal and non-lethal antibiotic concentrations. Curr. Opin. Microbiol. **15**: 555–560. doi: 10.1016/j.mib.2012.07.005.

Jansomboon, W., S.K. Boontanon, N. Boontanon, C. Polprasert, and C. Thi Da. 2016. Monitoring and determination of sulfonamide antibiotics (sulfamethoxydiazine, sulfamethazine, sulfamethoxazole and sulfadiazine) in imported Pangasius catfish products in Thailand using liquid chromatography coupled with tandem mass spectrometry. Food Chem. **212**: 635–640. doi: 10.1016/j.foodchem.2016.06.026.

Jeke, N.N., A.O. Hassan, and F. Zvomuya. 2017. Phytoremediation of biosolids from an end-of-life municipal lagoon using cattail (*Typha latifolia* L.) and switchgrass (*Panicum virgatum* L.). Int. J. Phytoremediat. **19**: 270–280. doi: 10.1080/15226514.2016.1225279.

Jeke, N.N., F. Zvomuya, N. Cicek, L. Ross, and P. Badiou. 2015. Biomass, nutrient, and trace element accumulation and partitioning in cattail (L.) during wetland phytoremediation of municipal biosolids. J. Environ. Qual. **44**: 1541. doi: 10.2134/jeq2015.02.0064.

Kairigo, P., E. Ngumba, L.-R. Sundberg, A. Gachanja, and T. Tuhkanen. 2020. Occurrence of antibiotics and risk of antibiotic resistance evolution in selected Kenyan wastewaters, surface waters and sediments. Sci. Total Environ. **720**: 137580. doi: 10.1016/j.scitotenv.2020.137580.

Karim, M.R., F.D. Manshadi, M.M. Karpiscak, and C.P. Gerba. 2004. The persistence and removal of enteric pathogens in constructed wetlands. Water Res. **38**: 1831–1837. doi: 10.1016/j.watres.2003.12.029.

Kinney, C.A., E.T. Furlong, S.L. Werner, and J.D. Cahill. 2006. Presence and distribution of wastewater-derived pharmaceuticals in soil irrigated with reclaimed water. Environ. Toxicol. Chem **25**: 317–326. doi: 10.1897/05-187R.1.

Klement, A., R. Kodešová, O. Golovko, M. Fér, A. Nikodem, M. Kočárek, and R. Grabic 2020. Uptake, translocation and transformation of three pharmaceuticals in green pea plants. J. Hydrol. Hydromech. **68**: 1–11. doi: 10.2478/johh-2020-0001.

Kodešová, R., A. Klement, O. Golovko, M. Fér, et al. 2019. Root uptake of atenolol, sulfamethoxazole and carbamazepine, and their transformation in three soils and four plants. Environ. Sci. Pollut. Res. Int. Heidelb. **26**: 9876–9891. doi: http://dx.doi.org.uml.idm.oclc.org/10.1007/s11356-019-04333-9.

Kumar, K., and S.C. Gupta. 2016. A framework to predict uptake of trace organic compounds by plants. J. Environ. Qual. **45**: 555–564. doi: 10.2134/jeq2015.06.0261.

Kurade, M.B., J.-Q. Xiong, S.P. Govindwar, H.-S. Roh, G.D. Saratale, and B-H. Jeon. 2019. Uptake and biodegradation of emerging contaminant sulfamethoxazole from aqueous phase using Ipomoea aquatica. Chemosphere **225**: 696–704. doi: 10.1016/j.chemosphere.2019.03.086.

Li, X., H. Yu, S. Xu, and R. Hua. 2013. Uptake of three sulfonamides from contaminated soil by pakchoi cabbage. Ecotoxicol. Environ. Saf. **92**: 297–302. doi: 10.1016/j.ecoenv.2013.03.010.

Lin C.E, C.C Chang, and W.C. Lin. 1997. Migration behavior and separation of sulfonamides in capillary zone electrophoresis. III. Citrate buffer as a background electrolyte. J. Chromatogr. A. **768**: 105–112. doi: 10.1016/S0021-9673(97)00010-1.

Lin, K., and J. Gan. 2011. Sorption and degradation of wastewater-associated non-steroidal anti-inflammatory drugs and antibiotics in soils. Chemosphere **83**: 240–246. doi: 10.1016/j.chemosphere.2010.12.083.

Liu, F., G.G. Ying, R. Tao, J.L. Zhao, J.F. Yang, and L.F. Zhao. 2009. Effects of six selected antibiotics on plant growth and soil microbial and enzymatic activities. Environ. Pollut. **157**: 1636–1642. doi: 10.1016/j.envpol.2008.12.021.

Liu, H., X. Huang, X. Yu, C. Pu, Y. Sun, and W. Luo. 2019. Dissipation and persistence of sulfonamides, quinolones and tetracyclines in anaerobically digested biosolids and compost during short-term storage under natural conditions. Sci. Total Environ. **684**: 58–66. doi: 10.1016/j.scitotenv.2019.05.341.

Liu, L., Y. Liu, C. Liu, Z. Wang, J. Dong, G. Zhu, and X. Huang. 2013. Potential effect and accumulation of veterinary antibiotics in Phragmites australis under hydroponic conditions. Ecol. Eng. **53**: 138–143. doi: 10.1016/j.ecoleng.2012.12.033.

Liu, Y., X. Zhang, H. Tran, L. Shan, J. Kim, K. Childs, E.H. Ervin, T. Frazier, and B. Zhao. 2015. Assessment of drought tolerance of 49 switchgrass (Panicum virgatum) genotypes using physiological and morphological parameters. Biotechnol Biofuels **8**: 1–18. doi: 10.1186/s13068-015-0342-8.

Ma, T., X. Pan, L. Chen, W. Liu, P. Christie, Y Luo, and L. Wu. 2016. Effects of different concentrations and application frequencies of oxytetracycline on soil enzyme activities and microbial community diversity. Eur. J. Soil Biol. **76**: 53–60. doi: 10.1016/j.ejsobi.2016.07.004.

Matamoros, V., and J.M. Bayona. 2006. Elimination of pharmaceuticals and personal care products in subsurface flow constructed wetlands. Environ. Sci. Technol. **40**: 5811–5816. doi: 10.1021/es0607741.

McClellan, K., and R.U. Halden. 2010. Pharmaceuticals and personal care products in archived U.S. biosolids from the 2001 EPA national sewage sludge survey. Water Res. **44**: 658–668. doi: 10.1016/j.watres.2009.12.032.

Miao, X.-S., F. Bishay, M. Chen, and C.D. Metcalfe. 2004. Occurrence of antimicrobials in the final effluents of wastewater treatment plants in Canada. Environ. Sci. Technol. **38**: 3533–3541. doi: 10.1021/es030653q.

Murphy, I.J., and J.R. Coats. 2011. The capacity of switchgrass (*Panicum virgatum*) to degrade atrazine in a phytoremediation setting. Environ. Toxicol. Chem. **30**: 715–722. doi: 10.1002/etc.437.

Pan, M., and L.M. Chu. 2017. Fate of antibiotics in soil and their uptake by edible crops. Sci. Total Environ. **599–600**: 500–512. doi: 10.1016/j.scitotenv.2017.04.214.

Panja, S., D. Sarkar, K. Li, and R. Datta. 2019. Uptake and transformation of ciprofloxacin by vetiver grass (Chrysopogon zizanioides). Int. Biodeterior. Biodegrad. **142**: 200–210. doi: 10.1016/j.ibiod.2019.05.023.

Pilon-Smits, E. 2005. Phytoremediation. Annu Rev Plant Biol **56**: 15–39. doi: 10.1146/annurev.arplant.56.032604.144214

Sabourin, L., A. Beck, P.W. Duenk, S. Kleywegt, D.R. Lapen, H. Li, C.D. Metcalfe, M. Payne, and E. Topp. 2009. Runoff of pharmaceuticals and personal care products following application of dewatered municipal biosolids to an agricultural field. Sci. Total Environ. **407 :** 4596–4604.

Salt, D.E., R.D. Smith, and D.S. Raskin. 1998. Phytoremediation. Annu. Rev. Plant Physiol. Plant Mol. Biol **49**: 643–668.

SAS Institute. 2013. SAS/STAT 9.4 User's Guide. SAS Institute Inc., Cary, NC.

Sigler, A., P. Schubert, W. Hillen, and M. Niederweis. 2000. Permeation of tetracyclines through membranes of liposomes and *Escherichia coli*. Eur. J. Biochem. **267**: 527–534. doi: 10.1046/j.1432-1327.2000.01026.x

Tanner, C.C., and T.R. Headley. 2011. Components of floating emergent macrophyte treatment wetlands influencing removal of stormwater pollutants. Ecol. Eng. **37**: 474–486. doi: 10.1016/j.ecoleng.2010.12.012.

Towler, B.W., J.E. Cahoon, and O.R. Stein. 2004. Evapotranspiration crop coefficients for cattail and bulrush. J. Hydrol. Eng. **9**: 235–239. doi: 10.1061/(ASCE)1084-0699(2004)9:3(235).

Wright, J.H., and D.A. Evans. 2008. New research on biofuels. Nova Science Publishers, New York.

Wu, C., A.L. Spongberg, J.D. Witter, M. Fang, and K.P. Czajkowski. 2010. Uptake of pharmaceutical and personal care products by soybean plants from soils applied with biosolids and irrigated with contaminated Water. Environ. Sci. Technol. **44**: 6157–6161. doi: 10.1021/es1011115.

Wu, X., F. Ernst, J.L. Conkle, and J. Gan. 2013. Comparative uptake and translocation of pharmaceutical and personal care products (PPCPs) by common vegetables. Environ. Int. 60: 15–**22**. doi: 10.1016/j.envint.2013.07.015.

Wu, X., L.K. Dodgen, J.L. Conkle, and J. Gan. 2015. Plant uptake of pharmaceutical and personal care products from recycled water and biosolids: a review. Sci. Total Environ. **536**: 655–666. doi: 10.1016/j.scitotenv.2015.07.129.

Xian, Q., L. Hu, H. Chen, Z. Chang, and H. Zou. 2010. Removal of nutrients and veterinary antibiotics from swine wastewater by a constructed macrophyte floating bed system. J. Environ. Manage **91**: 2657–2661. doi: 10.1016/j.jenvman.2010.07.036.

Xu, W., G. Zhang, X. Li, S. Zou, P. Li, Z. Hu and J. Li. 2007. Occurrence and elimination of antibiotics at four sewage treatment plants in the Pearl River Delta (PRD), South China. Water Res. **41**: 4526–4534. doi: 10.1016/j.watres.2007.06.023.

Yan, Y., Y. Pengmao, X. Xu, L. Zhang, G. Wang, Q. Jin, and L. Chen. 2020. Migration of antibiotic ciprofloxacin during phytoremediation of contaminated water and identification of transformation products. Aquat. Toxicol. **219**: 105374. doi: 10.1016/j.aquatox.2019.105374.

Yu, X., G.L. Zipp, and G.W.R. Davidson III. 1994. The effect of temperature and ph on the solubility of quinolone compounds: estimation of heat of fusion. Pharm. Res. **11**: 522–527. doi: 10.1023/A:1018910431216.

Zhao, F., L. Yang, L. Chen, S. Li, and L. Sun. 2019. Bioaccumulation of antibiotics in crops under long-term manure application: occurrence, biomass response and human exposure. Chemosphere **219**: 882–895. doi: 10.1016/j.chemosphere.2018.12.076.

5. OVERALL SYNTHESIS

5.1. Summary of Findings and Contributions to Knowledge

Antibiotics enter wastewaters and receiving water bodies mainly because antibiotics administered to humans are excreted intact or as metabolites (Clarke and Smith, 2011; Carvalho et al., 2014). Biosolids are organic by-products of municipal sludge treatment and are beneficial soil amendments rich in plant nutrients and organic matter (Kinney et al., 2006). Therefore, application of biosolids to agricultural lands results in beneficial recycling of nutrients (Christie et al., 2001; White et al., 2010). However, biosolids may contain contaminants, such as pharmaceuticals, metals, and pathogens (Ahmed and Sorensen, 1995). A major concern relating to the release of pharmaceuticals into the environment is the development of antimicrobial resistance (AMR) by microorganisms due to chronic exposure to the antimicrobial compounds present in wastewater and biosolids (Singer et al., 2016). This calls for the development of cost-effective techniques, such as phytoremediation, to reduce antibiotic concentrations in biosolids and wastewaters prior to land application. Elucidation of the processes and mechanisms controlling the plant-assisted attenuation of three antibiotics commonly detected in wastewater and/or biosolids was the focus of this thesis. The behaviour of sulfamethoxazole (SMX) and trimethoprim (TMP) under conditions mimicking temporary storage of biosolids was examined in Chapter 2 of this thesis. Chapter 3 examined the sorption of SMX (rhizofiltration) as a mechanism of attenuation and Chapter 4 focused on the mechanisms of attenuation of ciprofloxacin (CIP) and SMX by phytoextraction.

The dissipation study reported in Chapter 2 demonstrated the feasibility of SMX and TMP dissipation during temporary storage of biosolids and the influence of storage method on dissipation of these antibiotics. This study is one of a handful that have examined SMX and TMP dissipation under both saturated and unsaturated conditions, mimicking storage in stockpiles and lagoons. Sulfamethoxazole dissipated rapidly under both saturated (99% dissipation) and unsaturated (96% dissipation) conditions. The time to 50% dissipation (DT_{50}) for SMX under both conditions was short (2.8 d under saturated conditions and 4.4 d under unsaturated conditions), indicating a lower persistence of SMX in biosolids (Chapter 2). In contrast, trimethoprim was more persistent under unsaturated conditions (25% dissipation over 42 d) compared with saturated conditions (95% dissipation), with the dissipation rate being an order of magnitude greater for saturated biosolids (0.07 d^{-1}) than unsaturated biosolids (0.006 d^{-1}). The DT_{50} of TMP under saturated conditions was 10 d vs. 116 d under unsaturated storage conditions. The rapid dissipation of TMP under saturated conditions was explained by the reducing/anaerobic conditions that developed in saturated microcosms. Thus, temporary storage of biosolids in lagoons or storage tanks may be effective in reducing the concentration of antibiotics that are persistent under aerobic conditions.

The use of plants for contamination clean-up (phytoremediation) is a proven technology for the management of contaminated sites (Jeke et al., 2015; Schwab et al., 1998). Rhizofiltration (sorption by plant roots) is one mechanism for the removal of contaminants from wastewater or biosolids during phytoremediation (Salt et al., 1998). It has been utilized for the removal of metals (Dushenkov et al., 1995; Aafi et al., 2012) and organic contaminants (Schwab et al., 1998). Little is known about the sorption of antibiotics by plant roots and its contribution to the attenuation of antibiotics during phytoremediation. This thesis research is one of a handful of studies that have examined the sorption of a commonly detected antibiotic, SMX, by cattail and switchgrass roots (Chapter 3). Chapter 3 focused on characterizing the sorption, sorption kinetics, and temperature effects on SMX sorption by cattail and switchgrass roots. Sulfamethoxazole sorption by cattail and switchgrass roots was best described by the Freundlich model, with the sorption capacity (K_f) significantly greater for switchgrass (71.5 (µg g^{-1}) (ml µg^{-1})$^{1/n}$) than for cattail (24.5 (µg g^{-1}) (ml µg^{-1}) $^{1/n}$) roots. The sorption of SMX by roots of both plant species was an endothermic process, with sorption increasing as temperature increased from 5 to 25 °C. Percentage SMX removal at the three temperatures (5, 15, and 25 °C) was 26 – 33% for cattail and 60 – 77% for switchgrass. Sulfamethoxazole sorption also followed pseudo-second-order kinetics for both plant species, with the rate constant (k_2) decreasing as temperature increased. The results showed that, compared to cattail roots, sorption of SMX by switchgrass roots was a feasible and favorable process. Thus, switchgrass roots are more effective than cattail roots in removing SMX from aqueous solutions.

Phytoextraction (uptake by plants) is one of the mechanisms of phytoremediation (Cunningham et al., 1995). The phytoextraction of CIP and SMX by cattail and switchgrass was reported in Chapter 4, including an examination of the partitioning of CIP and SMX between the aboveground biomass (AGB) and belowground biomass (BGB). Total uptake of the antibiotics by switchgrass was low (9.8% for both CIP and SMX) compared with cattail (~34% for CIP and ~20% for SMX). Both antibiotics were concentrated in roots, with restricted translocation to AGB (translocation factors < 1). Harvesting of both AGB and BGB of cattail would be more effective in the phytoextraction of CIP and SMX compared to switchgrass.

Phytoextraction of SMX by switchgrass might not be an effective removal mechanism (Chapter 4); however, sorption by plant roots can contribute significantly to SMX removal from wastewater and/or biosolids, as demonstrated in Chapter 3. Thus, switchgrass could contribute to the attenuation of SMX during phytoremediation through rhizofiltration.

5.2 Implications of the Research

The observed persistence of TMP under unsaturated conditions mimicking storage in stockpiles is critical information for policy-makers and waste treatment specialists, as well as those involved in land remediation. A key finding of this Ph.D. research was that the DT_{50} of TMP under unsaturated conditions was 116 d, which is many folds longer than the DT_{50} under saturated conditions (10 d). Thus, specialists can be aware that saturated conditions could promote the degradation of antibiotic parent molecules (active ingredients). Specifically, information generated by this study on the dissipation of SMX and TMP during temporary storage under saturated and unsaturated conditions (Chapter 2) can be used by policy-makers to formulate recommendations for temporary storage of biosolids in lagoons prior to application as a soil amendment, especially in regions where biosolids are applied to agricultural fields. This recommendation would be useful in limiting the transport of antibiotics to the environment and in reducing the development of antimicrobial resistance.

The sorption parameters generated will be useful in calculating the maximum SMX sorption capacity and binding strength of plant roots in phytoremediation systems with cattail and switchgrass (Chapter 3). This information will assist remediation specialists in estimating SMX removal during phytoremediation. Pseudo-second-order parameters generated from this study will be useful in determining whether rhizofiltration of SMX would proceed fast enough to make an impact on attenuation in phytoremediation systems with cattail and switchgrass. The sorption information generated, especially at a range of temperatures (5, 15, and 25 °C), is critical in the estimation of SMX sorption by cattail and switchgrass roots during different seasons in the Canadian prairies.

Results from this research will inform policymakers and remediation specialists on the best strategies for utilizing plants to enhance antibiotic attenuation in contaminated media (Chapters 3 and 4), for example, attenuation via rhizofiltration with switchgrass or via phytoextraction with cattail for compounds amenable to the respective mechanisms. This research generated information for a better understanding of the potential of phytoremediation to remove antibiotics from wastewaters, sewage sludge, and biosolids. The study also provided important information on the uptake of SMX and CIP by plants over time and demonstrated the antibiotic accumulation potential of cattail and switchgrass. The research further confirms that antibiotics are concentrated in the roots during phytoremediation and informs policy-makers and remediation specialists to develop strategies (e.g., floating beds) to remove the majority of the contaminants during plant harvesting. In the floating bed system, the whole plant could be removed to maximize phytoextraction by removing both roots and shoots. Also, since the antibiotics are concentrated within the roots, part of the root biomass could be harvested, with enough roots left for regrowth of perennial plants (e.g., cattail). The observed greater efficiency of cattail for phytoextraction of CIP and SMX is an important finding that will assist remediation specialists select the best plant for use in phytoremediation systems. It also further strengthens the use of cattail for phytoremediation processes as cattail is also able to extract antibiotics during phytoremediation of other contaminants.

5.3 Recommendations

Results from this research showed the dissipation of TMP under saturated conditions, which was attributed to the development of anaerobic conditions in saturated microcosms. However, further studies are needed to elucidate the processes involved in TMP dissipation under saturated conditions. This extended knowledge can be used to more specifically design mitigation strategies for antibiotics such as TMP that dissipate under anaerobic conditions. Future studies should also identify the transformation products of SMX and TMP formed during the temporary storage of biosolids and to evaluate their toxicity to microbial organisms, and their influence on the development of antimicrobial resistance. Temperature is an important factor which can influence antibiotic dissipation (Cycoń et al., 2019). We conducted our dissipation study at a single temperature (22 °C); studies on the influence of temperature on SMX and TMP dissipation are needed to provide an understanding of the dissipation of these antibiotics under different climatic conditions.

Biosolids generally contain a mixture of antibiotics (Kinney et al., 2006; Sabourin et al., 2012). Therefore, a study evaluating the dissipation of antibiotics present as a mixture in biosolids is recommended to provide insights into antibiotic dissipation behavior in the presence of other antibiotics, representing real life situations.

Models can be useful in prediction and estimation of the effectiveness of a plant species for removal of antibiotics during remediation. Further studies are needed to acquire enough data to be able to fit kinetic models which would be beneficial to remediation specialists when estimating time to peak accumulation and harvest strategies to remove a majority of the contaminants. Studies on the sorption of other common antibiotics by cattail and switchgrass would further add to the understanding of the role of these plants in the attenuation of antibiotics during phytoremediation.

The sorption of one antibiotic, SMX, by cattail and switchgrass roots was evaluated in this research. Future studies should examine the sorption characteristics of these plant roots for other antibiotics used in this study (CIP and TMP) to determine the contribution of rhizofiltration to the attenuation of these antibiotics. As mentioned earlier, antibiotics rarely occur in isolation in the environment and are, in fact, usually present in mixtures. Therefore, a sorption study on the sorption behavior of SMX in the presence of other antibiotics would be critical in understanding rhizofiltration of SMX in phytoremediation systems. Root characterization techniques, such as X-ray photoelectron spectroscopy, Fourier-transform infrared spectroscopy, and scanning electron microscopy need to be utilized to evaluate the changes in root morphology and chemistry before and after SMX sorption so that the sorption process can be adequately defined as either physical or chemical.

Uptake of SMX and CIP from hydroponic solutions was reported in this study (Chapter 4). However, I recommend that field studies be carried out to evaluate uptake of these antibiotics from wastewaters and biosolids, as uptake behavior may be different in non-hydroponic systems (e.g., soils) due to other factors, such as organic matter and competing chemicals (nutrients or other inorganic chemicals, other organic chemicals), including contaminants that may be competing for uptake or sorption by plant roots. This study did not evaluate the uptake of SMX and CIP metabolites. Future uptake studies should include the metabolites as transformation products of antibiotics usually exist with parent (active ingredient) molecules in soils and biosolids (Sarmah et al., 2006; Rosendahl et al., 2011). As antibiotics could be degraded in plant tissues (Huynh and Reinhold, 2019), future studies should examine the degradation of SMX and CIP in cattail and switchgrass plant components to provide a better understanding of the attenuation mechanisms in these plants.

Overall, studies presented in this thesis are a significant contribution to literature on the environmental fate of SMX, CIP and TMP, as well as the role of plants (cattail and switchgrass) in the attenuation of antibiotics from wastewaters. These contributions will assist policy-makers and remediation specialists in making decisions that will further mitigate the dispersal of antibiotics in the environment. The findings will also inspire future fundamental studies governing the fate and use of antibiotics in society.

5.4 References

Aafi, N.E., F. Brhada, M. Dary, A.F. Maltouf, and E. Pajuelo. 2012. Rhizostabilization of metals in soils using *Lupinus luteus* inoculated with the metal resistant rhizobacterium *Serratia sp*. Msmc541. Int. J. Phytorem. **14**: 261–274. doi: 10.1080/15226514.2011.604693.

Ahmed, A.U., and D.L. Sorensen. 1995. Kinetics of pathogen destruction during storage of dewatered biosolids. Water Environ. Res. **67**: 143–150.

Carvalho, P.N., M.C.P. Basto, C.M.R. Almeida, and H. Brix. 2014. A review of plant–pharmaceutical interactions: from uptake and effects in crop plants to phytoremediation in constructed wetlands. Environ. Sci. Pollut. Res. **21**: 11729–11763. doi: 10.1007/s11356-014-2550-3.

Christie, P., D.L. Easson, J.R. Picton, and S.C.P. Love. 2001. Agronomic value of alkaline-stabilized sewage biosolids for spring barley. Agron. J. **93**: 144–151.

Clarke, B.O., and S.R. Smith. 2011. Review of 'emerging' organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. Environ. Int. **37**: 226–247. doi: 10.1016/j.envint.2010.06.004.

Cunningham, S.D., W.R. Berti, and J.W. Huang. 1995. Phytoremediation of contaminated soils. Trends Biotechnol. **13**: 393–397. doi: 10.1016/S0167-7799(00)88987-8.

Dushenkov, V., P.B.A. N. Kumar, H. Motto, and I. Raskin. 1995. Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. Environ. Sci. Technol**. 29**: 1239–1245. doi: 10.1021/es00005a015.

Huynh, K., and D. Reinhold. 2019. Metabolism of sulfamethoxazole by the model plant *Arabidopsis thaliana*. Environ. Sci. Technol. **53**: 4901–4911. doi: 10.1021/acs.est.8b06657.

Jeke, N.N., F. Zvomuya, N. Cicek, L. Ross, and P. Badiou. 2015. Biomass, nutrient, and trace element accumulation and partitioning in cattail (L.) during wetland phytoremediation of municipal biosolids. J. Environ. Qual. **44**: 1541. doi: 10.2134/jeq2015.02.0064.

Kinney, C.A., E.T. Furlong, S.D. Zaugg, M.R. Burkhardt, S.L. Werner, J.D. Cahill, and G.R. Jorgensen. 2006. Survey of organic wastewater contaminants in biosolids destined for land application. Environ. Sci. Technol. **40**:7207–7215.

Rosendahl, I., J. Siemens, J. Groeneweg, E. Linzbach, V. Laabs, C. Herrmann, H. Vereecken, and W. Amelung. 2011. Dissipation and sequestration of the veterinary antibiotic sulfadiazine and its metabolites under field conditions. Environ. Sci. Technol. **45**: 5216–5222. doi: 10.1021/es200326t.

Sabourin, L., P. Duenk, S. Bonte-Gelok, M. Payne, D.R. Lapen, and E. Topp. 2012. Uptake of pharmaceuticals, hormones and parabens into vegetables grown in soil fertilized with municipal biosolids. Sci. Total Environ. **431**: 233–236. doi: 10.1016/j.scitotenv.2012.05.017.

Salt, D.E., R.D. Smith, and D.S. Raskin. 1998. Phytoremediation. Annu. Rev. Plant Physiol. Plant Mol. Biol. **49**: 643–668.

Sarmah, A.K., M.T. Meyer, and A.B.A. Boxall. 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere **65**: 725–759. doi: 10.1016/j.chemosphere.2006.03.026.

Schwab, A.P., A.A. Al-Assi, and M.K. Banks. 1998. Adsorption of naphthalene onto plant roots. J. Environ. Qual. **27**: 220–224. doi: 10.2134/jeq1998.00472425002700010031x.

Singer AC, Shaw H, Rhodes V, Hart, and A. 2016. Review of antimicrobial resistance in the environment and its relation to environmental regulators. Front. Microbiol. **7**:1-22. doi: 10.3389/fmicb.2016.01728

White, J.W., F.J. Coale, J.T. Sims, and A.L. Shober. 2010. Phosphorus runoff from waste water treatment biosolids and poultry litter applied to agricultural soils. J. Environ. Qual**. 39**: 314–323. doi: 10.2134/jeq2009.0106.