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Monitoring of antibiotics and Atrazine in tile drained Landscapes.

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Monitoring of antibiotics and Atrazine in tile drained Landscapes.

by

Maurice Taylor Washington

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Agricultural Engineering

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The student author and the program of study committee are solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University

Ames, Iowa

2017

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DEDICATION

This dissertation is dedicated to my family, specifically to my grandfather who is still watching over me today. From a sixth-grade education, to working on the railroad for 40 plus years, providing for the entire family, and sending mommy to college. You were the epitome of what a man should be. You always told me to get my lesson done, and I thank you for emphasizing the importance of education. This is for you! Thank you for all your sacrifices, I miss you.

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NOMENCLATURE

AEC	Agricultural Emerging Contaminants
ASE	Accelerated solvent extractor
AU	Animal Unit
ATZ	Atrazine
CEC	Cation exchange capacity
CP	Chisel plow
HLB	Hydrophilic Lipophilic Balance
IB	Inner band
LOD/LOQ	Limit of detection/Limit of quantification
MB	Manure band
MIC	Minimum inhibitory concentration
mg	milligram
mL	milliliter
MRM	Multiple reaction monitoring
ng	nanogram
NT	No-till
PLE	Pressure liquid extraction
POCIS	Polar organic chemical integrative sampler
PTFE	Polytetrafluoroethylene
SAX	Strong Anion Exchange
SFIR	South Fork of the Iowa River

SMZ	Sulfamethazine
SOM	Soil organic matter
SPE	Solid Phase Extraction
TET	Tetracycline
TWA	Time weighted average
TYL	Tylosin

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ABSTRACT

Antibiotics are used at subtherapeutic levels in swine production for growth promotion, disease treatment, and disease prevention. These antibiotics are not fully metabolized and at subtherapeutic concentrations are thought to be linked to antibiotic resistance. These antibiotics enter the environment through the land application of swine manure. In the Iowa, most agricultural fields are drained using subsurface drainage, lowering the water table and removing excess water from the rootzone. With this movement of water, antibiotics have the potential to enter the subsurface soil and be transported to surface water by drainage systems. The studies described in this dissertation include; monitoring of tylosin and sulfamethazine in a tile drained agricultural watershed using Polar Organic Integrative Sampler (POCIS), the sediment concentrations of tylosin, sulfamethazine, and atrazine, in a tile drained watershed, and investigating the persistence and transport of atrazine and veterinary antibiotics to a tile drain system following swine manure injection.

A reconnaissance study of the South Fork watershed (SFIR) of the Iowa River, was conducted from 2013 – 2015. All analytes were detected, and detection frequencies ranged from 69 – 100% showing the persistence in the watershed. Antibiotics at sub-inhibitory concentrations were detected at a higher frequency using POCIS when compared to grab samples. We observed statistically significant seasonal trends for SMZ and ATZ concentrations during growing and harvest seasons. Time weighted average (TWA) concentrations quantified from the POCIS were 1.87 ng L⁻¹ (SMZ), 0.30 ng L⁻¹ (TYL), and 754.2 ng L⁻¹ (ATZ), in the watershed. SMZ and TYL concentrations were lower than the minimum inhibitory concentrations (MIC) for *E. coli*. All analytes were detected in tile drain

effluent, confirming tile drainage as a pathway for antibiotic transport. SMZ, TYL, and ATZ were detected in instream SFIR sediments, detection frequencies ranged from 42 – 84%. Statistical analysis revealed annual and seasonal significance for sediment TYL concentrations. On an annual basis TYL concentrations were statistically significant in 2013 and 2014. Seasonal significant concentrations occurred during the growing and harvest seasons, which coincide with the heaviest precipitation periods in the watershed, contributing to the transport of TYL via runoff. On a field scale, TYL, SMZ, TET, and ATZ residue concentrations were detected in fields with history of swine manure application. TYL and TET soils residues were concentrated at the 0 – 30 cm soil depth, while ATZ was concentrated from 0 – 60 cm. The detection of TYL and SMZ in tile drainage water indicates their ability to leach from the surface soil where manure slurry was injected. ATZ residues in tile drainage had a detection frequency of 100%, in the absence of application.

CHAPTER 1. GENERAL INTRODUCTION

1.1 Introduction

The state of Iowa is located in the heart of the corn belt and plays an integral role in agricultural production of the United States and the world. Of the 36 million acres of land in Iowa, approximately 30.7 million acres are used for farmland, with the total value of agricultural products sold accounting for 30.8 billion dollars, ranking second in the U.S. and 50th worldwide. Currently, Iowa is the top U.S. producer of corn grain (13.7 million acres harvested), soybean (9.3 million acres harvested) and hogs (20.5 million hogs produced) (USDA-NASS, 2012).

While agricultural production clearly has positive impact on the economy of Iowa and the U.S., it also contributes to the degradation of the natural environment. The crux of the matter, is the lack of balance between agricultural production and environmental stewardship. With the world's population projected to increase to 9.6 billion by 2050, pressure to increase agricultural production, will only increase, along with demands for reduced environmental impact.

Iowa's agricultural system and many of those like it in the corn belt, are dominated by intensive corn and soybean row cropping supported by artificial subsurface drainage, along with a landscape inundated with confined animal feeding operations (CAFOs).

The basis behind this type of agricultural system is the use of agrochemicals. These chemicals have been used to make agricultural production more efficient and thereby more profitable. But with that said, the environmental impact of these agrochemicals has not been fully investigated or considered. Herbicides are used in corn production while antibiotics are used in livestock production. These agrochemicals are classified as agricultural emerging contaminants (AECs) because they originate from agricultural production.

1.2 Agricultural Emerging Contaminants (AECs)

AECs are any agricultural naturally occurring or synthetic compound, or any microorganism detected in the environment, which is not routinely monitored, and has the potential to cause a known or perceived health risk to humans or the environment. Recently, AECs as veterinary antibiotics and their subsequent effects on the environment are now emerging issues. Antibiotics are used in livestock production and incorporated in their feed and water. They are used therapeutically to treat disease, sub-therapeutically for disease prevention, and for increased feed efficiency/growth promotion. In swine, approximately 70 – 80% of pig starters and grower feeds contain antibiotics, while 40 – 60% of finisher and sow feeds contain them (Cromwell, 2002). Antibiotics are poorly absorbed in the body of livestock animals resulting in the majority (70% – 90%) of the administered compound being excreted in urine or feces, which end up in manure and urine (Masse et al., 2014 and Kumar et al., 2005). Antibiotic concentrations found in the manure range from trace levels to $> 200 \text{ mg kg}^{-1}$, with typical concentrations ranging anywhere from 1 mg kg^{-1} – 10 mg kg^{-1} (Kumar et al. 2005). As in many countries, manure in the U.S. is often land applied as a source of nutrients for crops and as a means of disposal.

1.3 Occurrence, Transport and Fate of AECs

Manure is rich in nitrogen (N), phosphorus (P), potassium (K) and micronutrients, making it a valuable resource for crop production and the largest byproduct of agricultural production. In the U.S., land application of manure is the most widespread method of distribution while the storage in lagoons or pits are other viable options. Per the 2012 Agricultural Census, there were over 22 million acres of farmland treated by manure Agricultural Census (USDA-NASS, 2012). However, manure that is improperly managed poses a burden to the farming operation and can be problematic to aquatic environments. The increase of livestock production efficiency through CAFOs has caused substantial concentrations of manure to exist (Risse et al. 2006). According to Gollehon et

al. (2001), the majority of livestock production farms have enough land to apply their manure at agronomic rates, but the ones who don't account for over half of the manure N and P in the U.S. This excess availability of manure has been the cause for potential environmental concern. Both overapplication and agronomic application of manure can cause contamination to move in the aquatic environment (Burkholder et al. 2007).

Transport and fate of AECs are influenced by several factors including the physiochemical characteristics of the compound, weather, soil properties, and land management. AECs are transported into the aquatic environment via runoff, infiltration, leaching, and artificial drainage. During intense and heavy precipitation events, overland flow can also transport AECs into surface waters. AECs have been detected in surface water, ground water, plants, soil, dust, and sediments from manure amended fields (Bassil et al. 2013; Gao et al. 2012; Mojica and Aga, 2011; Burkholder et al. 2007; Dolliver et al. 2007; and Lissemore et al. 2006). Many antibiotic compounds are hydrophobic in nature, but when they adsorb on to sediment they can be transported to surface water due to runoff. Antibiotics that adsorb onto sediment have the potential to bioaccumulate in the aquatic environment (Gao et al. 2012). Despite the widespread use of antibiotics, they are not currently regulated in the environment.

1.4 Scope of the Problem

The presence of antibiotic residues and their metabolites in manure, and the potential for those residues to enter the environment, are cause for concern. Many of these antibiotics aren't fully metabolized and are excreted as the parent compound into manure. AECs act biocidal, and have the ability to impact target organisms and affect non-target organisms at the same time. According to (Gao et al. 2012), antibiotics adsorbed on sediments are still biologically active and have the potential to impact microbial functions in aquatic environments. This thought is further supported by Mojica and Aga (2011) and Burkholder et al. (2007), who indicate antibiotics affect

the microbial communities' structure, activity, and function in the terrestrial and aquatic environments.

The most important issue regarding antibiotics is the increased emergence and spread of antibiotic-resistant bacteria and the potential impact on human health. The use of antibiotics in livestock production and the large-scale human uses of antibiotics have created a thriving environment for antibiotic resistant genes (ARG). In agriculture, manure is a reservoir for resistant bacteria and antibiotic compounds, and it is thought its application to agricultural soils greatly increases ARGs and the selection of resistant bacteria in microbial populations (Heur et al. 2011). These bioactive compounds in manure exhibit hormesis behavior at doses below cell inhibition concentrations, i.e., low doses (Hughes and Andersson, 2012; Allen et al. 2010). Low exposure concentrations of antibiotics, similar to that found in surface waters, may potentially stimulate ARGs. The bacteria that are susceptible to antibiotics are inhibited, thereby creating favorable conditions for resistant strains of bacteria. These resistant strains aren't confined to specific environments, and have the ability to be transported via food, animals, humans, water etc. The transport mechanisms for these ARGs are physical (wind, runoff, leaching), anthropogenic, and biological (animals) in nature.

As these resistant strains become more prevalent, antibiotics will and have become an ineffective option for physicians and veterinarians. Work in the Netherlands has shown ARG levels have drastically increased, over approximately a 70-year span dating from 1940 to 2008 in five long-term soil series (Knapp et al., 2010). According to Khachatourians (1998), "microbial resistance to antibiotics is on the rise, in part because of inappropriate use of antibiotics in human medicine but also because of practices in the agricultural industry. These uses promote the selection of antibiotic resistance in bacterial populations. The resistant bacteria from agricultural

environments may be transmitted to human pathogens, which may cause disease that cannot be treated by conventional antibiotics.”

1.5 Justification of Work

The fate and transport of antibiotics in the environment is not fully understood, but the groundwork for this research has begun to develop. From what we do know, antibiotic compounds have the ability to partition on to the solid phase, into the dissolved phase, or in the colloidal phase, which impacts their fate and transport in the environment. The first nationwide reconnaissance study of the pharmaceuticals and other emerging contaminants was conducted from 1999 – 2000 (Kolpin et al., 2002). This study determined that approximately 80% of the 139 streams monitored contained one or more emerging contaminants, including 31 different antibiotics. Several of the stream sites were located in the state of Iowa. Two of the antibiotics detected in the study were tylosin and sulfamethazine. Both of these antibiotics are used in swine production for growth promotion and disease prevention. Numerous studies have documented the prevalence of these antibiotics in surface waters (Ou et al. 2014; Kim et al. 2011; Song et al. 2010; MacLeod et al. 2009).

Currently, Iowa is the number-one producer of swine. Swine tend to have a higher frequency of carrying bacteria with ARG, and this directly correlates with the amount of antibiotics used in the swine industry (Heur et al., 2011). Work by Campagnolo et al. (2002) showed the ability of antibiotics to be transported from swine farms to proximal surface and ground water. The prevalence of antibiotic resistant bacteria was further documented on swine farms by Chander et al. (2007). Evidence suggests that ARG are potentially stimulated by low doses of antibiotics. These low doses are often used for non-therapeutic purposes, including in livestock feed, increasing feed efficiency and growth promotion, and end up in the environment via manure. Much uncertainty still exists about the environmental and health impacts of antibiotics, residues, and ARGs.

In order to investigate the potential relationship between antibiotic resistance and low doses/concentrations, one must be able to quantify and assess this impact through monitoring. Pruden et al. (2013) suggest that strategic monitoring is a management option for providing baseline data on antibiotics, residues, and ARGs. The monitoring of the dissolved phase of antibiotics is undertaken by passive sampling technology.

1.6 Organization of Dissertation

This dissertation filled several existing gaps in knowledge, by 1) assessing the ability to quantify low concentrations of antibiotics using passive sampling technology for monitoring 2) identifying tile drainage as a transport mechanism for antibiotics, 3) identifying sediment as an important sink and source for antibiotics, and 4) finding a statistical approach to deal with censored data from antibiotic monitoring studies for contaminants at low concentrations and determining their subsurface transport mechanisms in tile drained landscapes. Chapter two is an overall literature review, while the rest of the chapters are described below.

1.7 Research Goals and Hypothesis

1.7.1 Chapter 3. Monitoring of Tylosin and Sulfamethazine in a Tile Drained agricultural watershed using POCIS.

Goal:

- Conduct a reconnaissance study of the SFIR, to establish the baseline water quality levels in respect to sulfamethazine (SMZ) and tylosin (TYL), and determine their distribution in the watershed using POCIS technology.

Objectives:

- Use POCIS samplers to determine the time weighted average (TWA) of AECs.
- Determine the frequency of detections and concentrations of selected AECs in the SFIR.
- Investigate the influence of temporal and spatial variation on the fate and transport of AECs.

- Assess the impact of surface flow versus subsurface drainage conditions on antibiotic concentrations.

Hypothesis:

- The POCIS samplers will detect at low concentrations and provide TWA concentrations of selected AECs based upon each constituents sampling rates.
- AECs will be detected at a high frequency due to the cropping system and the number of livestock confinements in the watershed.
- The fate and transport of AECs will exhibit behavior similar to the “1st flush phenomenon” with the highest concentrations and transport occurring during the early planting/growing season, subsequently decreasing by harvest time.
- Tile flow will be a significant contributor to AEC concentrations.

1.7.2 Chapter 4. Stream Sediment Concentrations of Tylosin, Sulfamethazine, and Atrazine in a Tile Drained Watershed.

Goal:

- Quantify antibiotic and pesticide residue concentrations in sediment of the SFIR.

Objectives:

- Establish the baseline stream sediment concentrations for tylosin, sulfamethazine, and atrazine.
- Determine the frequency of detections in the sediment matrix of selected AECs in the SFIR.
- Investigate temporal variation of the presence of these analytes and determined the impact of hydrological flow conditions on their distribution in sediment.

Hypothesis:

- Sediment acts as a sink for contaminants, the detection and concentration of the analytes will be highly influenced by their physiochemical properties and interactions in the environment.

- The detection frequency of TYL and ATZ in sediment will be high because of their affinity to adsorb to the solid phase while SMZ has more hydrophilic tendencies.
- Temporal variation will most likely be impacted by precipitation and runoff, while high instream flow conditions can be influential on sediment concentrations.

1.7.3 Chapter 5. Investigating persistence and transport of atrazine and veterinary antibiotics to a tile drain system following swine manure injection.

Goal:

- To investigate the subsurface transport and attenuation behavior of antibiotics and atrazine under tile drained field conditions after swine manure injection.

Objectives:

- Use POCIS to monitor AECs from tile flow.
- Quantify AEC concentrations and determine detection frequency from soil samples and tile effluent and assess their mobility in subsurface.

Hypothesis:

- The POCIS will allow for the detection of these AECs at low concentrations by concentrating the analytes on the sampler.
- The physiochemical properties of the AECs and the change of soil texture, a decrease of aerobic microorganisms and an increase of anaerobic microorganisms, and a change in soil organic matter as depth increases will be influential on detection frequency. Based on these interactions, we will be able to identify the transport mechanisms.

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CHAPTER 2. LITERATURE REVIEW

2.1 Hydrologic Impacts of Subsurface Drainage in Central Iowa

The Swamp and overflow land Acts of 1849, 1850, and 1860 transferred the titles of swamp and overflow lands to 15 individual states, including Iowa. Under the act, states were allowed to reclaim those lands within their boundaries by installing drainage and levees for the development of wetlands for agricultural purposes (Ikenberry et al., 2014). Thus, this reclaimed land led to the influx of European Americans due to the availability of farmable lands. The subsequent increase of artificial drainage has changed the hydrology of the Midwest United States. Tile drains and other forms of artificial drainage have had an enormous economic impact on Midwestern agriculture. Subsurface drainage improves agricultural production by creating consistent yields, allowing timelier field operations, regulating water table to reduce crop stress, and the reduction of soil compaction. Also, having subsurface drainage installed increases the sale value of the land.

From the latest Agricultural census (2012), approximately 19.6 million ha are under tile drainage while 16.9 million ha use some form on subsurface drainage (field ditches) in the United States, totaling 434,245 farms (USDA-NASS, 2014). The state of Iowa accounts for 26% of the total ha under tile drainage, 5.1 million ha more than any other state. Agricultural tile drainage is an integral part of Iowa's landscape (Schilling and Helmers, 2008).

The intake of water by tile drains occur through three main mechanisms: surface intakes, drainage of soil below the water table during saturated conditions, and macropore/preferential flow under saturated conditions (Morrison, 2014). Vertical surface intakes coupled with subsurface tile drains, short circuit water from the agricultural landscape directly into drainage channels or streams. As a result, surface waters are drained in a timely manner allowing agricultural lands to be ready for cultivation. Subsurface drainage doesn't increase the storage capacity of soils, instead it transform

how the fraction of soil water and surface water is stored and released in respect to time.

Consequently, artificial drainage serves as the main conduit for the transport of dissolved forms of nutrients and chemicals (Ikenberry et al., 2014; Edwards et al., 2009; Larsbo et al., 2009; Lapen et al., 2008; Schilling and Helmers, 2008; Green et al., 2006; and Baker et al., 2004). Therefore, serving as a key transport mechanism and creating unintended ramifications on water quality. Research has shown the ability of tile drains to specifically transport nutrients and AECs into surface waters (Frey et al., 2015; Qi et al., 2011; Kay et al., 2003; Campagnolo et al., 2002; Gentry et al., 2000; and Kladvko et al., 1991). Work conducted by Schilling et al., 2012 indicated tile drainage is a key mechanism which impacts fundamental watershed characteristics and should be evaluated when investigating pollutant delivery from agricultural environments.

Artificial drainage has been documented to impact runoff, baseflow, and peakflow. According to Schilling and Libra (2003), subsurface artificial drainage in agricultural settings of Iowa have increased baseflow of rivers over the past 50 years. Hydrologic changes have occurred in these landscapes because of European settlers and land management improvements dating back to the days of the Soil conservation service (SCS) in the 1930s. The land management improvements such as soil conservation practices enhanced the infiltration capacity of the landscape thereby increasing baseflow and low flows of streams (Schilling and Libra, 2003). “Baseflows are directly related to the shift in the proportion of precipitation that is not evaporated or transpired, but rapidly conveyed into subsurface drain flow,” (Blann et al., 2009). Schilling and Libra, 2003; suggest that the hydrologic discharge trends may be linked to the extensive use of artificial drainage. Along with the artificial drainage, the increased production of soybeans from 1940 to now on previously untilled land or other cover cropped land increased baseflow by eliminating the ability to limit annual evapotranspiration (ET), (Schilling and Libra, 2003). Today, subsurface

drainage makes up the majority of baseflow from agricultural landscapes in the midwest (Blann et al., 2009).

Topographical depressions, known as prairie pot holes also impact tile drainage hydrologically. The prairie pothole region (PPR) in Iowa, coincides with the Des Moines Lobe. Potholes are topographical depressions and or wetlands. These potholes serve as a drainage pool for accumulating water from the landscape often forming ponds (Johnson et al., 2008). These depressional stores also reduce the chance of runoff and increase infiltration (Sloan, 2013). Surface intakes connected to subsurface drains are often used to drain these potholes. These surface intakes provide a direct conduit for surface water to enter the tile drain system (Tomer et al., 2010). The hydrology of these potholes is influenced by spatial and temporal variability of climate, soil, and landscape characteristics (LaBaugh et al., 1998, van der Kamp and Hayashi, 1998). Research on the hydrologic impact of tile drainage is limited but continues to develop, the overall conclusion is that the impact is very complex and situationally dependent (Sloan, 2013).

Researchers have determined that macropore flow is a major influence on water and chemical transport (Sloan, 2013; Tomer et al., 2010; Larsbo et al., 2009; and Kumar et al., 1998). Kay et al., 2004 specifically indicated that preferential flow to tile drains is a transport mechanism for antibiotics. Field studies have shown that macropores directly connected to tile drains have the ability to immediately transport surface solutes (Larsbo et al., 2009). It is generally accepted that surface storage, soil type, and the availability of macropores influence peak flow in subsurface drainage (Sloan, 2013). At a field scale, subsurface drainage reduces the surface storage capacity, thereby increasing peak flows, but this can be variable and site specific (Blann et al., 2009). Particularly, the formation of macropores enables water to flow rapidly through the subsurface. (Robinson and Rycroft, 1999) suggest that the installation of tile drainage promotes the formation of macropores by drying the soil enough to create well defined subsurface flow paths. Preferential

transport of chemicals through macropores is governed by soil type, management conditions, rainfall intensities, and the physiochemical characteristics of the compound (Shipitalo et al., 2000). Specifically, the amount of chemical that's transported is determined by the amount of overland flow, amount of chemical present in overland flow, and the overall flow capacity of the macropores (Kumar et al., 1998). The preferential flow of chemicals through macropores is a bigger concern in no-till management systems where minimal surface soil disturbance occurs, leaving the macropores intact (Kumar et al., 1998).

2.2 Hydrology of the South Fork Watershed (SFIR)

The natural hydrology of the SFIR has been substantially modified in support of agriculture. Artificial drainage has tremendously changed the hydrology of the SFIR. Approximately 80% of the SFIR is tile drained. Majority of the flow in the SFIR originates from subsurface drainage flow. Flooding in the SFIR is typically caused by rains in the spring over frozen or saturated soils, summer thunderstorms, and by fall rains after harvest (McCarthy et al., 2012).

A study conducted by J. Roth and P. Capel, 2012, investigated the hydrology of drained topographical depressions in the SFIR. During wet conditions, normally representative of May and June in the SFIR, sustained ponding of water was contributed to groundwater inflow and overland flow. Where overland flow was the initial contributor to the formation of ponds. Once groundwater inflow decreased below the soil surface, the pools receded. Vertical surface drainage accounted for the primary loss of water from the potholes, while evaporation was a negligible outflow. Dry conditions, from late June to August resulted in shorter pond durations. Overland flow was the main contributor to the late season ponding, while precipitation and groundwater decreased. Similar to the wet conditions, vertical surface drainage served as the primary loss mechanism for water, but infiltration was a factor as well.

Much of the earlier drainage practices (19th century) focused on straightening streams and increasing the flow by which streams could remove water from agricultural landscapes to surface waters (Blann et al., 2009). These earlier drainage practices would unknowingly have a severe impact on the hydrology and water quality of agricultural watersheds. The natural meandering pattern of streams are due to the energy dissipation of flowing streams, thereby producing recognizable patterns of transport and deposition of sediment (Yan et al., 2010). As a result, the stream channelization became widespread, resulting in stream bank erosion and the accumulation of sediment. Yan et al., 2010 study investigated channel movement and post settlement alluvium (PSA) in the SFW. Comparison of aerial analysis between 1939 and 2002 showed reduction in the channel lengths of Tipton Creek (TC), South Fork (SF), and Beaver Creek (BC) basins. Stream channelization caused approximately 9.2×10^6 Mg of PSA to be stored in the alluvial valleys of TC and SF combined. This PSA roughly represents 156.6 Mg ha^{-1} of soil eroded from the uplands of the watershed since the settlement of the European farms. The accumulation of PSA in the watershed caused a loss of water storage capability on the magnitude of $5.09 \times 10^6 \text{ m}^3$.

2.3 Atrazine in the South Fork (SFIR)

Much of the water quality work in the SFIR involves nutrients and herbicide monitoring. A handful of studies have documented the presence of herbicides and their metabolites in the surface waters of the SFW (Coupe et al., 2012; Kalkhoff et al., 2012; Kolpin et al., 2010; Meyer et al., 2007; Thurman et al., 1992). These herbicides and their metabolites have been also been documented in the groundwater of the SFIR and in wastewater treatment plants influent/effluent (Kolpin et al., 2002).

An early reconnaissance study on herbicides and their metabolites was conducted the late 1980's by Thurman et al. 1991. The study examined the effect of herbicides and their metabolites on the regional surface water quality of the Midwest. A large number of sampling sites were in

Iowa, including several along the Iowa River. This work showed a seasonal distribution pattern of triazine herbicides in the pre planting, post planting, and harvest stages of planting. High concentrations of herbicides were flushed from cropland then transported to surface water as pulses, caused by the late spring and early summer rainfall (Thurman et al., 1992). The pulse effect was shown to occur on a regional scale throughout the corn belt of the Midwest. Several of the herbicides exceeded the Environmental Protection Agency (EPAs) maximum contaminant level (MCL) for drinking water during post planting. The post planting samples yielded the greatest detection of herbicides and herbicides were detected at most sites in the study. Atrazine was the most prevalent herbicide detected in the reconnaissance study; 91% (pre planting); 98% (post planting); and 76% (harvest). Storm water runoff data indicated background concentrations of triazines was less than $1\mu\text{g L}^{-1}$, while a sharp increase in herbicide concentrations ranging from 30 – 40 $\mu\text{g L}^{-1}$ during post planting. The temporal trends of herbicide detection indicate that some of the compounds persisted from year to year in both soil and water.

Monitoring the transport of atrazine and similar pesticides is very difficult, due to their ability to exist in three different states; sorbed, liquid, and gas (Gish et al., 2011; Majewski and Capel, 1995; Taylor and Spencer, 1990). The overall movement of triazine herbicides involves transport through the soil profile, across the soil surface, into the atmosphere, and into the aquatic environment. Transport includes, but isn't limited to: plant uptake, surface runoff, volatilization, and upward/downward transport in the soil profile (LeBaron et al., 2008). There are three generally recognized transport mechanisms for atrazine; surface runoff, leaching, and volatilization. In addition, with the use of artificial drainage in much of the agricultural landscapes in the Midwest, the use of tile drains to expedite the movement of water from soil surface can be considered another transport mechanism. Surface runoff and leaching of atrazine have been heavily studied, revealing

that surface runoff is the dominant transport mechanism of atrazine. But, there have been few studies investigating the volatilization of atrazine.

Atrazine and other triazine herbicides fate in soil and the terrestrial environment are regulated by degradation, sorption, and transport behavior. Abiotic and microbial processes carry out degradation in soil. These processes degrade the parent compound and transform it into degradation products; desethyl atrazine (DEA), deisopropyl atrazine (DIPA), hydroxy atrazine (HA), and other residues. Atrazine sorption on to soil particles is the dominant mechanism affecting retention in soil, thereby impacting transformation and transport. Literature shows pH, organic matter, clay content, temperature, water content, concentration, and incubation time impact atrazine sorption behavior (Park et al., 2003). Atrazine sorption has been shown to be positively correlated with organic matter and have a strong relationship to adsorption of all soil constituents.

2.4 Passive Sampling Monitoring Technology

2.4.1 Background

Water quality monitoring of antibiotics and other emerging contaminants can be difficult due to their physiochemical properties and their interactions in the environment. Traditional active sampling techniques include discrete grab samples and the use of automatic samplers have been used to sample environmental concentrations of emerging contaminants. These sampling techniques often require preconditioning large volumes of water to detect these contaminants with current analytical methods (Söderström et al., 2009; Alvarez et al., 2009; Alvarez et al., 2005). The biggest shortcoming of discrete grab sampling, is that it only provides a snap shot or an instantaneous estimate of environmental levels, neglecting episodic events and overestimating concentrations (Thomatou et al., 2010; Vrana et al., 2005). Grabs are collected periodically at predetermined time intervals and provide the extent, frequency, and variables of water quality (Novtony, 2003).

Typically, the increased frequency of grab samples is a method used to capture pollutant

concentrations varying over time. This method is often time consuming and impractical. To capture episodic pollutant events, automatic samplers are often used, but can be expensive and time consuming to use. Another WQ monitoring approach used is biological monitoring. Biological monitoring consist of sampling fish, macroinvertebrates, or benthic macroinvertebrates. The analysis of the tissues from these test species provide information on environmental relevant concentrations of the contaminants and the equilibrium level of the contaminant. The analysis for this method can be complex. The use of these sampling methods can be expensive and time consuming (Söderström et al., 2009; Alvarez et al., 2007; Vrana et al., 2005).

The development of passive sampler technology such as the Polar Organic Chemical Integrative samplers (POCIS) has provided a better alternative for sampling polar organic contaminants such as tylosin, sulfamethazine, and atrazine. Passive samplers avoid many of the problems associated with the typical methods used in water quality monitoring programs due to their ability to collect target analytes in-situ while not impacting the bulk solution (Vrana et al., 2005). The advantage of using passive samplers to monitor pollutants in aquatic environments is the ability to monitor a broad range of chemicals with different physiochemical properties, non-mechanical or passive operation, sampling of large volumes of water, the ease of deployment and sample processing (Vrana et al., 2005). The POCIS provides time integrative sampling, enabling estimation of time weighted averages (TWA) concentrations, captures episodic events, and improves the detection limit by concentrating sequestered analytes of interest.

2.4.2 History

Over the past 20 years, passive samplers have been developed and used to combat many of the issues of the aforementioned monitoring techniques. Passive samplers can collect target analytes in-situ without disturbing the bulk solution (Vrana et al. 2005). They have shown the ability to measure a wide range of organic and inorganic contaminants in dissolved form. Passive

samplers have been used in air quality monitoring since the 1970s. These principles were taken and adapted, and then applied to WQ monitoring where the first passive sampler for organic micro-pollutants was used in 1987. The science and research behind the use of these samplers is still developing, but large strides have been made in recent years. It is thought, passive samplers can complement or either replace grab samples. Passive samplers produce time integrated data, allowing a time weighted average (TWA) concentration to be determined over an extended sampling duration.

2.4.3 Polar Organic Chemical Integrative Sampler (POCIS)

In the past 5 – 10 years, passive sampler technology has been used for water quality monitoring. One of the most commonly used passive sampler's is the polar organic chemical integrative sampler (POCIS). POCIS are manufactured to monitor and sample hydrophilic compounds such as macrolide antibiotics. To date, tylosin has not been one of the antibiotics that the POCIS has been calibrated to sample. A study to evaluate the performance of the POCIS sampling of tylosin could provide insights into the sampling rate of tylosin and provide greater understanding of the POCIS in monitoring tylosin in the aquatic environment.

The POCIS sampler uses passive diffusion, i.e. (movement from an area of high concentration to low concentration) which follows first order kinetics. According to (Molin et al. 2012 and Li et al. 2010), the POCIS has three different phases of pollutant accumulation Figure 2.1. These three phases are a function of time and include: linear, pseudo-linear, and equilibrium. The linear phase of the POCIS is usually used to determine TWA concentrations of contaminants. The POCIS sampler takes a long period of time to reach equilibrium which results in the accumulated contaminant remaining in the device long after deployment (Alvarez, 2004). The other two phases can be used for qualitative data such as the quantity of a particular contaminant. The linear phase

uptake allows the POCIS to act as an infinite sink for the accumulation of chemicals, where a constant exposure concentration is assumed and linear accumulation occurs with respect to time.

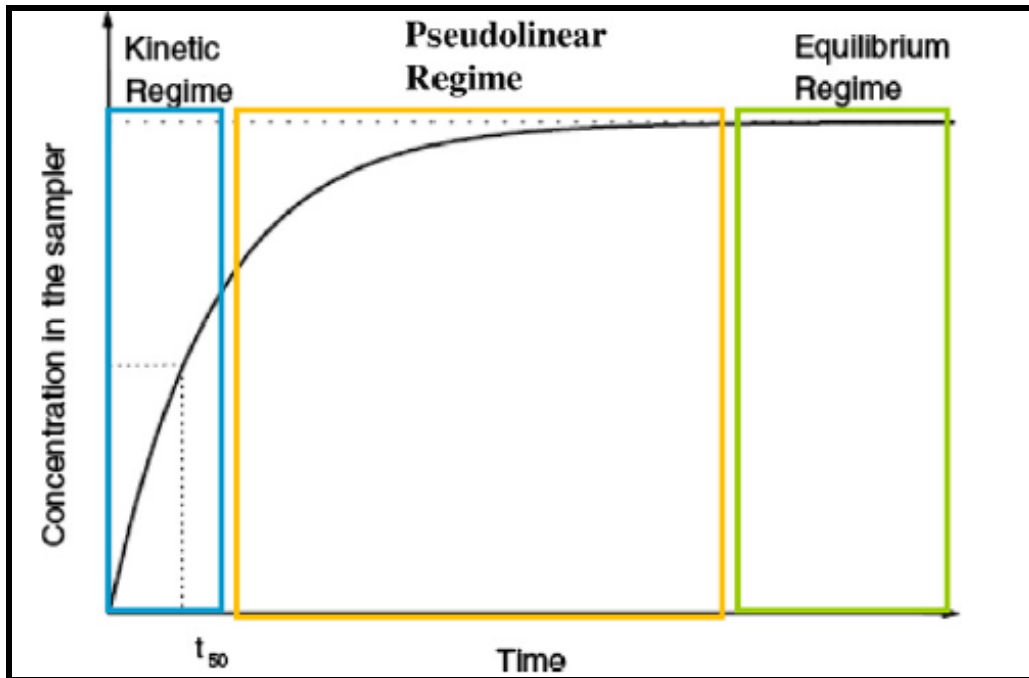


Figure 2.1 Accumulation phases of the POCIS (Source: Morin et al. 2012).

2.4.4 History and Development

The POCIS was developed by (Alvarez et al., 1999) as a part of the environmental contaminants research conducted by US EPA and the USGS. It was designed to mimic respiratory exposure of aquatic organisms to dissolved chemicals. Thus, it acts as an abiotic device, allowing the estimation of biologically relative TWA concentrations. Currently, there are two commercially available forms of the POCIS; pesticide and pharmaceutical form. The pesticide-POCIS configuration sorbent receiving material is made up of, a triphasic admixture of Isolute® ENV+ polystyrene divinylbenzene, Ambersorb® 1500 or 572 carbon, and X3 Biobeads, and the Oasis hydrophilic-lipophilic balance (HLB) sorbent is the pharmaceutical-POCIS (Fauvelle et al., 2012; Alvarez et al., 2004). This sorbent receiving material is covered by polyethersulfone diffusion

membranes with 100nm pore size and is secured with two stainless steel rings (Figure 2.) The semi permeable membrane of the POCIS allows dissolved polar organic chemicals to pass through to the sorbent phase, while inhibiting the flow of particulates and microorganisms with the cross-sectional area greater than 100 nm (Greenwood et al., 2007). Originally, the pesticide POCIS was designed to sample pesticides and hormones. But, it was discovered that the pesticide POCIS had difficulty sampling additional chemical compounds such as pharmaceuticals with multiple functional groups (Alvarez, 2012). The pharmaceutical POCIS was then developed and has the ability to detect a wider scope of chemical compounds.

The POCIS is designed to sample contaminants in the aqueous environment that are hydrophilic in nature and have a $\log K_{ow} < 4$ (Alvarez, 2012; Morin et al. 2012; and Alvarez et al. 2004). But studies have also documented that the POCIS sampler is also able to sample contaminants with a $\log K_{ow} > 4$, such as azithromycin and alkylated phenols. The potential of analytes to degrade while in transport or during storage in traditional matrices, is minimized with passive samplers because of their ability to isolate the analytes on a sorbent material (Kot-Wasik et al., 2007). The advantage of using the POCIS is its ability to sequester contaminants from episodic events and the ability to capture contaminants are trace levels. Carlson et al., 2013 tested the stability of some agrochemicals and pharmaceuticals captured on the POCIS after being stored at - 20° C for approximately 20 months and determined the average loss was about 11%. The loss during storage was smaller than the variability associated with the overall use of the POCIS.

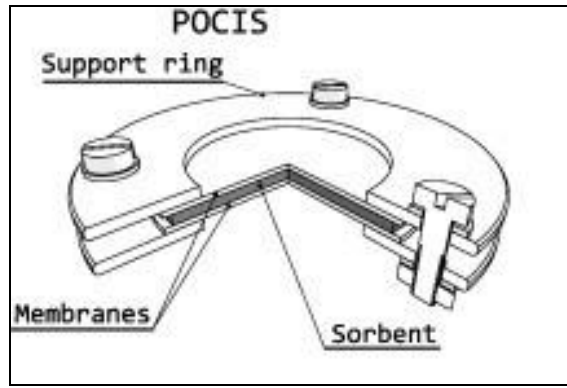


Figure 2.2 POCIS Schematic, Kot-Wasik et al., 2007

The accumulation of the POCIS in the kinematic/integrative phase, is thought to be proportional to the concentration in the bulk solution while the elimination rate is proportional to the concentration on the sorbent material (Fauvelle et al., 2012; Mazzella et al., 2007; Vrana et al., 2005; Alvarez et al., 2004). The equation expressing accumulation was first applied to the POCIS by Alvarez et al., 1999.

$$C_{\text{POCIS}} = C_w K_{\text{sw}} (1 - e^{-k_e t}) \quad (1)$$

C_{POCIS} is the concentration ($\mu\text{g g}^{-1}$) of the analyte on the sorbent at time t , C_w is the TWA concentration ($\mu\text{g L}^{-1}$) of the analyte in water, K_{sw} is the membrane water partition constant (L g^{-1}). The elimination rate constant k_e , is negligible during the kinematic phase, where the POCIS acts as an infinite sink, simplifying equation (1)

$$C_{\text{POCIS}} = C_w k_u t \quad (2)$$

where k_u is defined as the rate of uptake ($\text{L g}^{-1} \text{d}^{-1}$). To link concentrations quantified by in the POCIS to its concentration in the sampling sorbent, the sampling rate (R_s) is used. The R_s , is simply the volume of water cleared by the POCIS with respect to time for a given molecule (Morin et al., 2012). Rearranging equation (2) and introducing the mass of sorbent M_{POCIS} , while replacing k_u with R_s , produces an equivalent relationship.

$$C_{\text{POCIS}} = \frac{C_w R_{st}}{M_{\text{POCIS}}} (3)$$

2.4.5 POCIS Calibration and R_s Values

To obtain quantitative information (TWA concentrations) from the POCIS, it must be calibrated for each chemical of interest. Without calibration, the POCIS will only provide qualitative information and serves as a screening tool. According to Morin et al., 2012, “The calibration links the quantity of a compound accumulated in the tool to its sampling rate (R_s).” Calibration of the POCIS occurs in the kinematic accumulation phase. Typical calibration consist of laboratory or in-situ calibration methods. Laboratory calibration allows for a more controlled system, with selection of desired molecules and constant concentration. Disadvantages of laboratory calibration is that it can be time consuming, require a lot of resources, and performance-reference compounds (PRCs) could be required to correct laboratory derived R_s values. PRCs are used to correct R_s values for environmental conditions, in which in-situ calibrations account for. The PRC approach was first documented by Huckins et al, (2002) and Booij et al, (1998 with their respective work using the semipermeable membrane device (SMPD) passive sampler. Mazella et al, (2007) later modified this approach for use on pharmaceutical POCIS (Oasis-HLB). But, due to the complex hydrophilic and lipophilic interactions which occur on the Oasis HLB, it’s uncertain that the validity of the application of PRC approach (Harman et al., 2011; Mazella, 2012). Three accepted methods to produce laboratory R_s are; static renewal, static depletion, and continuous flow calibration. Static renewal calibration involves a closed system where molecule spiking occurs at desired time points, and static depletion involves just an initial molecule spike at the beginning of the calibration. In situ calibration allows the POCIS to be exposed to site specific environmental parameters and conditions. In theory, this makes R_s values more accurate and realistic, but in practice, the use on in situ calibration is time consuming and requires a lot of resources.

Sampling rates are very difficult to obtain and can vary for individual compounds based on temperature, water flow/turbulence, UV-light, biofouling, type of POCIS, and their physiochemical properties, i.e. pH and salinity (Morin et al., 2012; Söderström et al., 2009). The influence of these factors on Rs can vary (Table 2.1). In addition, Morin et al., 2012 suggest Rs can possibly be influenced by duration of exposure, calibration technique, calculation method, and the level of pollutant concentration exposed to the POCIS.

Table 2.1 Factors which influence the Rs of POCIS.

<i>Factor</i>	<i>Effect on Rs</i>	<i>Source</i>
Temperature	Increase of temperature increases Rs for pharmaceuticals.	Morin et al., 2012; Söderström et al., 2009; Togola and Budzinski, 2007.
Water flow/turbulence/agitation	Increase of flow increases Rs by four to nine fold.	Morin et al., 2012; Alvarez, 2004.
Biofouling	Limited impact on Rs.	Alvarez et al., 2004; Alvarez et al., 1999.
POCIS configuration	Rs vary based on use of pesticide or pharmaceutical POCIS using the same calibration method.	Hernando et al., (Morin paper)
pH	High Rs for acidic pharmaceuticals at low pH. Rs for neutral compounds is not impacted by pH. Rs for basic compounds are high at high pH.	Morin et al., 2012; Li et al.,
Salinity	Rs varies, depending on the functional group of the compound.	Togola and Budzinski, 2007

Once calibrated, Rs are generally calculated by rearranging equation (3) and solving for Rs.

$$Rs = \frac{C_{POCIS}M_{POCIS}}{C_{wt}} \quad (4)$$

Several other methods have also been used to calculate Rs. One uses the analyte concentration remaining after a desired time duration (17, 21), where C_i is the initial analyte concentration ($\mu\text{g L}^{-1}$), C_t the analyte concentration ($\mu\text{g L}^{-1}$) at time t , and V_T is the volume of the calibration vessel. It's assumed, the analyte loss due to degradation is negligible (Morin et al., 2012).

$$Rs = \frac{C_i - C_t}{C_i} \times \frac{V_T}{t} \quad (5)$$

Another approach, uses the slope of the decreasing analyte concentration with respect to time and a positive control is used to consider degradation of the analyte (Morin et al., 2012; MacLeod et al., 2007):

$$R_s = k_u V_T \quad (6)$$

With the different approaches used to calibrate and to quantify the R_s , different values can be derived for the same analyte. The following R_s values for tylosin, sulfamethazine, and atrazine are displayed below Table 2.2.

Table 2.2 R_s values from literature.

<i>Analyte</i>	<i>R_s</i>	<i>Source</i>
<i>Tylosin</i>	1.33 ± 0.151	Bartelt-Hunt et al., 2011
<i>Sulfamethazine</i>	0.114 ± 0.029	MacLeod et al., 2007
	0.049 ± 0.040	MacLeod et al., 2007
<i>Atrazine</i>	.18	
	$.243 \pm 0.003$	Bartelt-Hunt et al., 2011
	$.290 \pm 0.003$	Bartelt-Hunt et al., 2011
	$.239 \pm 0.008$	Mazzella et al., 2007
	$.189 \pm 0.006$	Morin et al., 2013
	$.228 \pm 0.041$	Lissalde et al., 2011

2.4.6 POCIS Application in the environment

The POCIS is a dynamic monitoring tool, which can detect ultra-concentrations of the dissolved phase of chemicals. The POCIS has three general designated uses: screening of pollutants, determination of TWA concentrations, and toxicity bioassay analysis. The screening capability of the POCIS allows for the determination of the source and concentration gradient of chemicals. The application of screening and TWA determination allows for the evaluation of spatial and temporal distribution in aquatic environments (Morin et al., 2012; Söderström et al., 2009). Morin et al., 2012 has noted the application of the POCIS has detected and quantified and estimated 300 chemicals. These chemicals include: antibiotics, pesticides (fungicides, insecticides, and herbicides), antidepressants, hormones, and plasticizers, just to name a few. The POCIS is an

extensive tool that has been used in many aquatic environments including: waste water treatment plants (WWTP) (Bailly et al., 2013; Harman et al., 2011; Li et al., 2010; Di Carro et al., 2010; Zhang et al., 2008), Rivers/Streams/Creeks (Carlson et al., 2013; MacLeod et al., 2007; Alvarez et al., 2005; Alvarez et al., 2004), Estuaries (Dougherty et al., 2010; Togola and Budzinski, 2007), Lakes (Thomatau et al., 2010; Kohoutek et al., 2010; Writer et al., 2010; Liedtke et al., 2009), and Seas/Bays/Harbors (Harman et al., 2010; Harman et al., 2009).

2.5 References

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**CHAPTER 3. MONITORING TYLOSIN AND SULFAMETHAZINE IN A
TILE-DRAINED AGRICULTURAL WATERSHED USING
POLAR ORGANIC CHEMICAL INTEGRATIVE SAMPLER (POCIS)**

A paper submitted to Science of the Total Environment

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3.1 Abstract

This study evaluated the influence of temporal variation on the occurrence, fate, and transport of tylosin (TYL) and sulfamethazine (SMZ); antibiotics commonly used in swine production. Atrazine (ATZ) was used as a reference analyte to indicate the agricultural origin of the antibiotics. We also assessed the impact of season and hydrology on antibiotic concentrations. A reconnaissance study of the South Fork watershed (SFIR) of the Iowa River, was conducted from 2013 – 2015. Tile drain effluent and surface water were monitored using polar organic integrative sampler (POCIS) technology. Approximately 169 animal feeding operations (AFOs) exist in SFIR, with 153 of them being swine facilities. All analytes were detected, and detection frequencies ranged from 69 – 100% showing the persistence in the watershed. Antibiotics at sub-inhibitory concentrations were detected at a higher frequency using POCIS when compared to grab samples. We observed statistically significant seasonal trends for SMZ and ATZ concentrations during growing and

harvest seasons. Time weighted average (TWA) concentrations quantified from the POCIS were 1.87 ng L⁻¹ (SMZ), 0.30 ng L⁻¹ (TYL), and 754.2 ng L⁻¹ (ATZ), in the watershed. SMZ and TYL concentrations were lower than the minimum inhibitory concentrations (MIC) for *E. coli*. All analytes were detected in tile drain effluent, confirming tile drainage as a pathway for antibiotic transport. Our results identify the episodic occurrence of antibiotics, and highlights the importance identifying seasonal fate and occurrence of these analytes.

Keywords: Tile drainage; POCIS; Antibiotics; Tylosin; Sulfamethazine; Atrazine

3.2 Introduction

Antibiotics have been used in livestock production since the early 1950's for growth promotion (subtherapeutic), disease prevention (prophylactic), and disease treatment (therapeutic use). In 2013, the total dispersal of approved antibiotics for food producing livestock was approximately 14.9 million kilograms, in which 99.3% of that total dispersal was used, domestically in the United States (FDA, 2015). In a five-year span between 2009 –2013, the domestic sale and distribution of antibiotic active ingredients for agricultural use increased approximately 17%, while those classified as medically important increased 20%. (FDA, 2015).

Subtherapeutic use of antibiotics in animal feed and water for growth promotion is a concern due to their ability to select resistant bacteria in the gastrointestinal tract of livestock (Chee-Sanford et al., 2009). These antibiotics are not fully metabolized in livestock and are excreted as the parent compound or as a metabolite (Kim et al., 2011; Joy et al., 2013; Stone et al., 2011; Kemper, 2008). Antibiotics enter the environment via land application of manure or lagoon treated water (Kim and Carlson, 2007). Once delivered into the terrestrial environment, their potential to induce antibiotic resistance is a cause for concern. Recently, the U.S Federal Drug Administration (FDA) introduced a strategy to combat antibiotic resistance, with the issuance of “Guidance for Industry” (GFI) documents #209 (FDA, 2012) and #213 (FDA, 2013) and the Veterinary feed directive (VFD). The

VFD requires the supervision of a licensed veterinarian for the use of drugs in or on animal feed. Currently, all antibiotics ranked under GFI #152 (FDA, 2003) are classified as medically important to human health, and include the macrolide antibiotic tylosin and the sulfonamide antibiotic sulfamethazine.

To investigate the potential relationship between antibiotic resistance and low environmental concentrations, monitoring strategies are needed to detect these low concentrations. Pruden et al., 2013; suggests that strategic monitoring is needed to provide baseline data on antibiotics, residues, and antibiotic resistance genes (ARGs). Since the first national reconnaissance pharmaceutical water quality study (Kolpin et al., 2002) the investigation of the occurrence, fate, and transport of emerging contaminants has become more prevalent. From this study and others, antibiotics have been detected in surface water (Fairbairn et al., 2015; Ou et al., 2015; Gao et al., 2012), ground water (Barber et al., 2008; Campagnolo et al., 2002; Watanabe et al., 2010), soil (Joy et al., 2013 and Kurdwadkar et al., 2011), sediment (Gao et al., 2012; Ok et al., 2011; Kim and Carlson, 2007), and crops (Carter et al., 2014; Bassil et al., 2013; Wu et al., 2011; Jones-Lepp et al., 2010; Dolliver et al., 2007).

Water quality monitoring of antibiotics and other emerging contaminants is difficult due to their diverse physiochemical properties and their interactions in the environment. Traditional environmental sampling techniques including discrete grab samples and automatic samplers have been used for emerging contaminants. These sampling techniques often require extracting large volumes of water to detect these contaminants (Soderstrom et al., 2009 and Alvarez et al., 2005). The greatest shortcoming of discrete grab sampling, is that it only provides a snapshot of environmental levels, neglecting episodic events and overestimating concentrations. The use of these sampling methods can be expensive and time-consuming (Soderstrom et al., 2009; Alvarez et al., 2007). The development of passive sampler technology such as the Polar Organic Chemical

Integrative Samplers (POCIS) has potentially provided a better alternative for sampling polar organic contaminants such as tylosin, sulfamethazine, and atrazine

The POCIS is a dynamic monitoring tool, which has the ability to detect ultra-low concentrations of the dissolved phase of chemicals. The POCIS has three general designated uses: screening of pollutants, determination of TWA concentrations, and toxicity bioassay analysis. The screening capability of the POCIS allows for the determination of the source and concentration gradient of chemicals. The application of screening and TWA determination allows for the evaluation of spatial and temporal distribution in aquatic environments (Morin et al., 2012; Söderström et al., 2009). The ability of the POCIS to screen pollutants was also shown in a study conducted by Kolpin et al. (2013); where, POCIS were used to determine the exposure of chemical contaminants to smallmouth bass in the Potomac River basin. Among the chemical contaminants tylosin, sulfamethazine, and atrazine detection frequencies were 0, 40, and 100 percent respectively. Recently, Jaimes-Correa et al. (2015) used the POCIS to determine the seasonal occurrence of 12 different antibiotics, including tylosin and sulfamethazine, and a beta agonist in a predominantly agricultural watershed in Nebraska. The tylosin and sulfamethazine, did not show any spatial or temporal variation in the watershed. Morin et al. (2012) has noted the application of the POCIS to the detection and quantification an estimated 300 chemicals. The POCIS is an extensive tool that has been used in many aquatic environments including: rivers, streams, creeks, estuaries, lakes, seas, bays, and harbors.

We conducted a reconnaissance study of the SFIR, to establish the baseline water quality levels in respect to sulfamethazine (SMZ) and tylosin (TYL), and determine their distribution in the watershed using POCIS technology. Our objectives were to investigate the influence of temporal and spatial variation on the occurrence, fate, and transport of tylosin and sulfamethazine; determine the frequency of detection, and assess the impact of tile drainage vs. surface water on antibiotic

loads and concentrations. Tylosin and sulfamethazine were chosen because they are used in swine production and we had previously detected tylosin in agricultural drainage water (Garder et al., 2014). Atrazine was included as a reference compound as it has often been detected in agricultural watersheds.

3.3 Materials & Methods

3.3.1 Watershed Description

The South Fork watershed (SFIR) is a predominantly agricultural watershed, which encompasses approximately 78,000 ha (193,000 acres). The greater part of SFIR is located in Hamilton and Hardin counties in north central Iowa, with the most northern part located in Wright and Franklin counties. Three major drainage areas make up the SFIR; Tipton Creek tributary in the southwest, South Fork of the Iowa River in the center, and the Beaver Creek tributary in the southeast. The headwaters of the South Fork of the Iowa River originate from three subsurface drains located in Hamilton County. From the headwaters, the South Fork flows in a northeasterly direction until entering Hardin County where it flows in a southeasterly direction meeting the Iowa river south of Eldora (McCarthy et al., 2012).

The SFIR is dominated by agricultural land covering approximately 96% of the watershed. There is a large concentration of animal production facilities along with intense row cropping. There are approximately 169 animal feeding operations (AFOs) in the watershed, with 153 of them being swine facilities (Figure 1), accounting for 91% of AFOs (Iowa DNR, 2014). Swine seem to have a higher frequency of bacteria with antibiotic resistant genes (ARG), which directly correlates with the amount of antibiotics used by the swine industry compared to cattle or sheep (Heur et al., 2011). Swine manure produced from treated pigs, has been shown to enhance the spread of antibiotic resistance in soil bacterial communities (Heur et al., 2010). Campagnolo et al. (2002) showed that antibiotics are transported from swine farms to proximal surface and ground water.

The prevalence of antibiotic resistant bacteria was further documented in swine herds by (Chander et al., 2007; Mathew et al., 2001). According to Tomer et al. (2008a), the estimated swine population of the watershed is 601,193 (Beaver Creek: 75,379, South Fork: 301,628, and Tipton Creek: 224,186). The resulting swine densities are 4.14 (Beaver Creek), 7.9 (South Fork), and 11.29 (Tipton Creek) swine ha⁻¹. More recently Hamilton and Hardin counties were estimated to have a swine inventory of 1.37 million (USDA-NASS, 2012). Previous work shows that the SFIR contains persistent populations of *E coli* and *Enterococcus* (Tomer et al., 2008a), and genes associated with zoonotic pathogens (Givens et al., 2016), suggesting that transport of antibiotics within this watershed is likely. Finally, three small towns with a combined human population of less than 500 have sewage treatment facilities the discharge within the watershed, with potential use of SMZ and TYL in humans or companion animals.

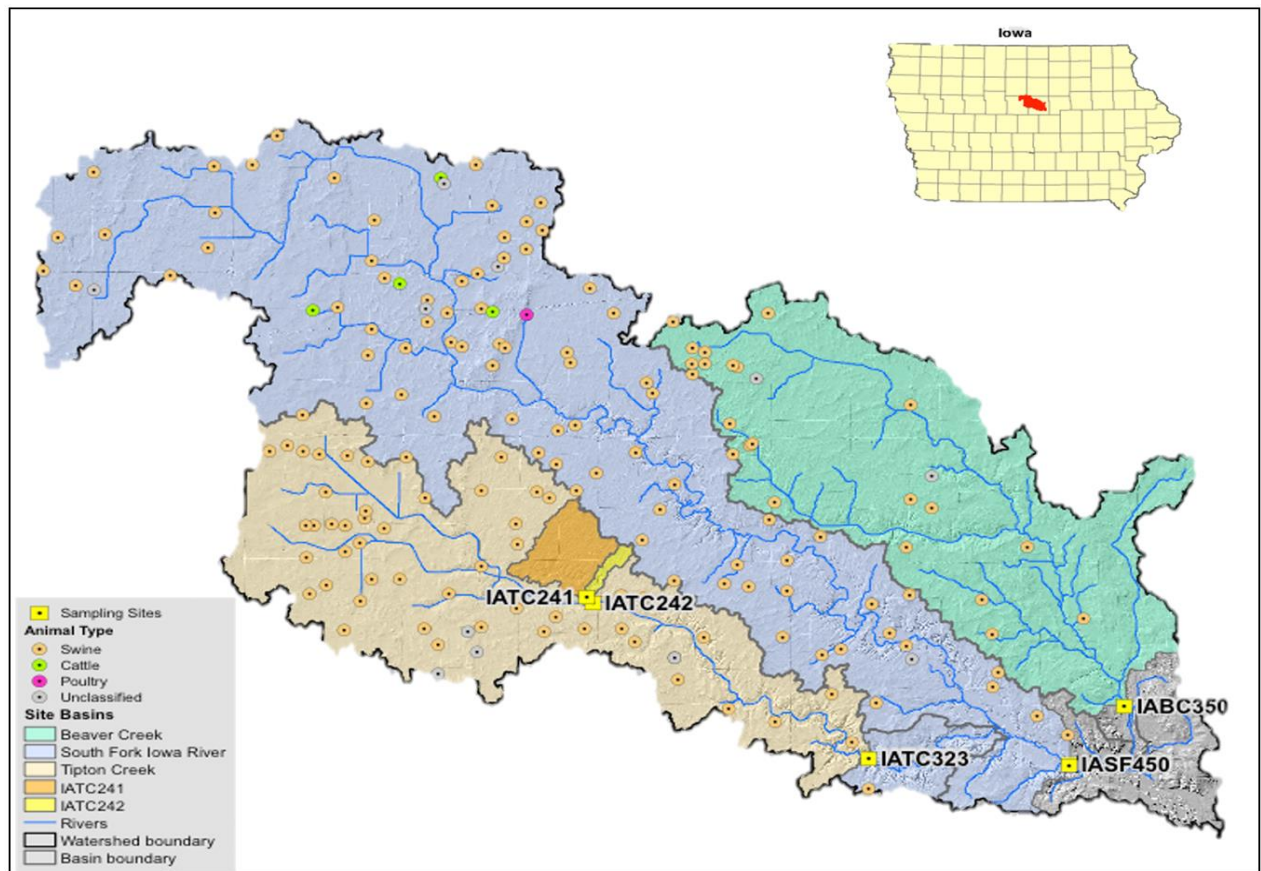


Figure 3.1. *Map of the animal feeding operations (AFO) and sampling site locations in the South Fork watershed of the Iowa River (SFIR). The AFO's are categorized by swine, cattle, poultry, and unclassified. The map inset shows the extent of the SFIR watershed boundary in central Iowa.*

Historically corn and soybeans are the crops grown in the watershed, and that remains the trend today (Tomer et al., 2008b). Greater than 85% of the agricultural land is used for the production of corn and soybeans, map shown in supplementary information (SI). Planting occurs in April to May and harvesting occurs from September to October. The manure produced from the CAFOs in the SFIR is the main source of nutrient application. Inorganic fertilizers and a broad band of herbicides are also used for increased crop production (McCarthy et al., 2012).

Approximately 54% of watershed consist of hydric soils (Tomer and James, 2004). These hydric soils include; Clarion, Nicollet, Webster, Harps, and Okobojo soil classifications. Due to these hydric soils, artificial drainage has changed the hydrology of the SFIR. Approximately 80% of the SFIR is tile drained (Green et al., 2006). Vertical surface drains, coupled with subsurface tile drains, route water from the agricultural landscape directly into drainage channels or streams. As a result, the water table is lowered which ensures agricultural lands are ready for cultivation and the root zone is not saturated. Consequently, artificial drainage expedites the transport of dissolved forms of nutrients and chemicals, including AEC, to surface waters, thereby negatively impacting water quality (Frey et al., 2015; Qi et al., 2011; Kay et al., 2003; Campagnolo et al., 2002; Gentry et al., 2000; and Kladvko et al., 1991). Work conducted by Schilling et al. (2012) indicates that tile drainage is a key mechanism impacting fundamental watershed characteristics and should be evaluated when investigating pollutant delivery from agricultural environments.

3.3.2 Sampling Sites

Five field sites in the central to southern part of the SFIR were monitored, including IATC-241, IATC-242, IATC-323 (Tipton Creek tributary); IASF-450 (South Fork tributary); and IABC-

350 (Beaver Creek tributary) (Figure 3.1). These stations were selected because of the ongoing collection of hydrology and water quality data by USDA-ARS. Sites IATC-241 and 242 are tile drain discharge points, while the other three sites are in-stream stations. The drainage area of the sampling sites is shown in (Table 3.1). The mean precipitation at the sampling sites in SFIR was (849.4 ± 104.4 mm year⁻¹), increasing from 2013 – 2015.

Table 3.1. *Sampling field sites in the SFIR watershed and their animal unit's (AU's), number of confined animal feeding operations (CAFOs), and sub basin drainage area.*

Site ID	Sub Basin Area (Hectares)	Animal Unit (AU)	CAFO Count
IATC-241	1,043	0	0*
IATC-242	150	0	0*
IATC-323	17,178	94,916	55
IASF-450	39,798	138,437	91
IABC-350	18,118	25,737	23

*No CAFOs are in the drainage areas of IATC-241 and 242, but swine manure injection occurs (Kevin Cole, personal communication)

3.3.3 Water Samples

USDA-ARS operates tipping bucket rain gauges (Texas Electronics TE525), high-accuracy stage recordings (PS-2 pressure sensor and high-accuracy stage OTT CBS bubbler recorder), thermocouples for air and stream temperature (Type-T thermocouple), flow meters (WaterLog H-355 bubbler), Teledyne ISCO 6712 samplers, and data loggers (Campbell Scientific CR1000) at each sampling site. Samples were collected in 2013, 2014, and 2015 from April to November, to include planting, growing, and harvest seasons for corn and soybean. Sampling frequency was initially monthly, but was increased to bi-monthly in 2014 and 2015, to capture more episodic events.

To monitor the AEC concentrations in water, duplicate grab samples were collected from the tile outlets and in streams at the corresponding sites. Grab samples were collected in 0.5 liter amber glass jars with PTFE-lined caps. Grab samples were kept on ice in the field and stored at 4°

C, at USDA-ARS NLAE (National Laboratory of Agriculture and Environment) prior to analysis. Tiles maintained flow throughout the majority of the sampling season.

All POCIS (Environmental Sampling Technologies Laboratory) were stored frozen prior to their anticipated field deployment. Prior to deployment, POCIS were prepared by soaking them in milli-Q water for approximately day. The presoaking of the POCIS helps to reduce the rapid uptake of analytes, i.e. the burst effect, which occurs during the first few days of sampling. Once preconditioned with milli-Q water, POCIS were sealed back in their cans until deployment. POCIS were deployed at four of the five sampling sites, (IATC-241, IATC-323, IASF-450, and IABC-350). Due to the elevated height of the (IATC-242) tile drain outlet, a POCIS sampler couldn't be successfully installed and submerged in the flow path of the tile discharge, and thus only grab samples were collected at this site. To protect the POCIS during deployment, they were housed in stainless steel perforated protective canisters (Alvarez, 2010). Depending on the location and physical characteristics of the site, the POCIS canisters were mounted or suspended in the waterbody, and anchored with wire cable to the shore. Due to the high flow at IATC-241, the POCIS canister was located to the side of the tile drain to prevent POCIS from being punctured by high-velocity flows and debris.

3.4 Sample Analysis

3.4.1 Extraction Procedure

POCIS extraction procedure was adapted from the protocols used by Alvarez et al. (2004) and Mazzella et al. (2007). POCIS was disassembled and hydrophilic-lipophilic balance (HLB) sorbent material was washed with 20 mL of acetonitrile-isopropyl alcohol (50:50, v:v) into a 60 mL SPE reservoir, fitted with a 20 μ m frit. A second 20 μ m frit was placed on top of the transferred solvent, before elution. The washing solvent was collected and then combined with 100 mL of acetonitrile-isopropyl alcohol to elute the sorbent material. The washing solvent was not discarded

because testing showed significant amounts of constituents were found in the solution. Once the 120 mL of solvent was eluted, 250 μL of simetone dissolved in MeOH was added at a concentration of 42 ng mL^{-1} as an internal standard. The combined extract and wash was then evaporated down to 0.2 – 0.3 mL using a nitrogen evaporator. After evaporation, the residual solvent was reconstituted to 2 mL using 10 mM ammonium acetate and allowed to reach equilibrium for approximately 30 minutes. After equilibrium, samples were filtered using a 13 mm 0.2 μm syringe pore filter and submitted for analysis.

In addition, POCIS residues from the SPE reservoirs were placed in 100 mL beakers and filled with 60 mL of solvent. Each residue sample soaked for 24 hours, extract and wash were collected, and 125 μL of internal standard was added. Extract and wash were evaporated down to 0.2 mL, reconstituted with ammonium acetate to 2 mL, filtered using a 13 mm 0.2 μm syringe pore filter and submitted for analysis. The POCIS + POCIS residue concentrations were summed after analysis, providing the total mass concentration accumulated on the POCIS. A lab spike and lab blank were processed with each set of POCIS samples. The spike was used to determine POCIS extraction efficiency. POCIS extraction yielded 108% (ATZ), 82% (SMZ), and 81% (TYL) extraction efficiencies.

Grab samples were first processed by filtering 250 mL of sample through 0.45 μm filter, eliminating particulate matter. Oasis HLB cartridges were preconditioned with 2 mL of MeOH, and drawn down, followed by 2 mL of Milli-Q water. Samples were then eluted through Oasis HLB solid phase extraction (SPE) cartridges with acetonitrile-isopropyl alcohol. Simetone was used as an internal standard for the grab samples and extracts were evaporated down, reconstituted, filtered, and submitted for analysis.

3.4.2 AEC Analysis

Analysis was performed using an ABSciex 5500 QTrap mass spectrometer with an Agilent 1260 Infinity LC. Separation took place on a Phenomenex-Gemini - 3 μm C18 110 A column, 50 x 2.0 mm, at a flow rate of 0.5 ml/min. Mobile phase A was 0.1% formic acid in water and B was 0.1% formic acid in methanol. The LC gradient begins at 98% A and holds for 0.3 minutes, then ramps to 20% A in 7.27 minutes, then rapidly increases to 1% A by 7.37 minutes and is held for 3.53 minutes. The column is re-equilibrated back to the initial conditions, for a total run time of 15 minutes. Compounds were monitored using multiple reaction monitoring (MRM), with 3 stages collected for each. The most abundant transition was used for quantitation, and the second and third product ions were used for ion ratio confirmation. Acceptance criteria for the ions were based on the European Standard, which uses a larger acceptance range for smaller ion ratios as follows: the ratio is between 0-10% when the acceptable percent difference is 50, if the ratio is 10-20% the acceptable difference is 30%, a ratio range of 20-50% must agree with a percent difference of 25, and a ratio above 50% has an acceptable percent difference of 20 (European Standard EN 1662, 2008). The precursor and product ion masses and optimized mass spectrometer conditions for the determinations of SMZ, TYL, and ATZ are shown in (SI).

All sample extracts were analyzed for SMZ, TYL and ATZ. The instrumental limit of detection (LOD) and limit of quantification (LOQ) were determined for each analyte for water and POCIS (Table 3.2). Instrumental LOD and LOQ is the smallest signal above background noise that an instrument can detect or quantify reliably. The LOD and LOQ for POCIS samplers are back-calculated based on the analytical protocol and on the sampling rate, R_s (Poulier et al., 2015).

Table 3.2. Instrumental and matrix limits of detection (LOD) and limit of quantification (LOQ) for atrazine (ATZ), sulfamethazine (SMZ) and tylosin A (TYL).

Analyte	INSTRUMENTAL		GRABS		POCIS	
	Limit of Detection (ng mL ⁻¹)	Limit of Quantification (ng mL ⁻¹)	Limit of Detection (ng L ⁻¹)	Limit of Quantification (ng L ⁻¹)	Limit of Detection (ng L ⁻¹)	Limit of Quantification (ng L ⁻¹)
SMZ	0.041	0.04	0.000328	0.00032	0.000163	0.000159
TYL	0.044	0.04	0.000352	0.00032	0.00000965	0.000009
ATZ	0.027	0.03	0.000216	0.00024	0.00009574	0.000106

3.4.3 POCIS Time-Weighted Average Concentrations and Calibration

Time-weighted average (TWA) concentrations of river and drainage water were calculated using experimentally determined POCIS uptake rates (R_s , L d⁻¹), sampling duration (t), the analyte mass accumulated (M_s , g), and the concentrations were quantified from POCIS extracts (C_s , ng L⁻¹) by mass spectrometry. The TWA was determined by the following equation:

$$\text{TWA} = \frac{C_s M_s}{R_s t} \text{ (eq. 1)}$$

POCIS uptake rates for each target compound were calculated from lab calibration experiments using the following equation:

$$R_s = \frac{C_i - C_t}{C_i} \times \frac{V_T}{t} \text{ (eq. 2)}$$

Where, (C_i and C_t , ng L⁻¹), initial concentration and concentration at time, t. V_T is the total volume of water at the time of calibration.

R_s values were determined by using a static depletion laboratory calibration method (Morin et al., 2012). Duplicate two-liter solutions containing ATZ, SMZ, and TYL at 60 ng mL⁻¹, were prepared and a single POCIS was added to each container. Negative and positive controls were also prepared. The negative control consisted of ultra-pure water with a POCIS, whereas the positive control was spiked ultra-pure water with ATZ, SMZ, TYL at 60 ng mL⁻¹. The positive control

accounted for the natural degradation of the analytes. Duplicate water samples were taken each day for 21 days and the solution concentrations were determined as described previously. To protect against photodegradation and evaporation, the calibration experiment was conducted in the dark and each vessel was fully covered. Sampling rates (R_s) were quantified for SMZ (0.084 L d⁻¹), TYL (1.52 L d⁻¹), and ATZ (0.094 L d⁻¹), respectively.

3.4.4 Statistical Analysis

Due to the number of samples with non-detectable concentrations, SMZ ($n=70$ of 290) and TYL ($n=136$ of 290) assumptions of normality are not met and data are considered censored. Censored observations (non-detects) are defined as low-level concentrations that measure between 0 and the detection/reporting limit of laboratory analytical equipment (Heisel, 2012). Tobit censored regression analysis was used to account for censoring of the dependent variable, y , where y is the analyte concentration, such that $y = \text{site} + \text{season} + \text{year}$. These measurements are considered imprecise and are commonly reported as an analytical threshold, less than some value. The detection limit for each analyte was back-calculated, removing the less than notation and then input into the Tobit model, acting as a threshold limit for the censored observations in each data set. The Tobit model was used to determine differences in analyte concentration, based on site, season, and year. Seasons were defined as: Pre-Planting (March – May); Growing (June – August); and Harvest (September – November). Pearson product-moment correlation coefficient was determined for each analyte model. Additionally, interactions between site, season, and year were analyzed. Significant differences for all comparisons were evaluated at $p < 0.05$. Statistical analysis was performed using SAS 9.4.

3.5 Results & Discussion

3.5.1 Occurrence of AECs

POCIS TWA concentrations were determined for four sampling sites in the SFIR watershed from May – November (2013), April – November (2014), and March – November (2015). TYL, SMZ, and ATZ were detected at all sampling sites in every year. Detailed seasonal occurrence and concentration data for each sampling site is provided in (Table 3.3). From 2013 – 2015, the detection frequencies for SMZ and TYL were 83% and 70%, respectively. ATZ, which is ubiquitous throughout the Midwest (Van Metre et al., 2015; Kolpin et al., 2010; Battaglin et al., 2005), was detected in 100% of the samples in the SFIR watershed. The detection rates of these analytes are comparable to other studies using POCIS samplers in agricultural settings (Table 3.4). Jaimes-Correa et al. (2015) reported concentrations of SMZ fairly close to those observed in the SFIR, while TYL was an order of magnitude lower than SFIR concentrations. The detailed annual and seasonal occurrence of each analyte is available in (SI).

The physiochemical properties of SMZ indicate it is loosely sorbed in the soil matrix, allowing for it to be highly mobile in the aqueous phase (Wegst-Uhrich et al., 2014; Carstens et al., 2013; Boxall et al., 2002). Degradation behavior of SMZ, shows an initial rapid degradation followed by a slowdown period, reducing its dissipation in soil (Lertpaitoonpan et al., 2015). These properties show the ability of SMZ to be relatively persistent in the environment. In each of the sample years, SMZ was detected above seventy percent; 93% (2013), 72% (2014), and 84% (2015). In comparison, TYL physiochemical properties indicate that it is more likely to be tightly sorbed and degrade very quickly in the soil matrix and not as available for transport (Wegst-Uhrich et al., 2014; Blackwell et al., 2007; Lee et al., 2007). Contrary to these properties, TYL had a detection frequency of 70% in the SFIR. TYL was persistent throughout the sample seasons with detection frequencies of: 93% (2013), 47% (2014), and 69% (2015).

Table 3.3 Summary of seasonal mean, median, and maximum POCIS analyte concentrations in SFIR from 2013 – 2015, based on sampling site.

Site	Analyte	Season	Mean (ng L ⁻¹)	Median (ng L ⁻¹)	Max (ng L ⁻¹)	% Non -Detects
IATC-241	SMZ	Preplant	0.92	0.74	2.10	0.0
		Growing	6.54	1.95	44.08	0.0
		Harvest	1.83	0.87	14.12	3.7
	TYL	Preplant	0.18	0.02	1.51	30.0
		Growing	0.07	0.03	0.27	11.8
		Harvest	0.18	0.03	0.77	57.7
	ATZ	Preplant	368	128	1949	0.0
		Growing	288	239	939	0.0
		Harvest	128	115	458	0.0
IATC-323	SMZ	Preplant	0.6	0.6	1.2	10.5
		Growing	6.2	0.9	32.7	12.0
		Harvest	1.0	0.7	2.8	31.0
	TYL	Preplant	0.19	0.03	1.46	44.4
		Growing	0.87	0.10	6.92	48.0
		Harvest	0.23	0.06	1.33	48.3
	ATZ	Preplant	1704	458	15357	0.0
		Growing	882	387	4880	0.0
		Harvest	146	122	538	0.0
IABC-350	SMZ	Preplant	0.29	0.24	0.52	62.5
		Growing	0.72	0.40	1.99	45.5
		Harvest	0.39	0.28	1.00	48.3
	TYL	Preplant	0.21	0.03	0.89	57.9
		Growing	0.10	0.06	0.61	43.5
		Harvest	0.19	0.07	1.00	58.6
	ATZ	Preplant	2152	563	10817	0.0
		Growing	983	452	4956	0.0
		Harvest	102	91	386	0.0
IASF-450	SMZ	Preplant	0.41	0.35	0.65	36.8
		Growing	5.30	0.70	66.92	8.0
		Harvest	1.08	0.50	6.46	25.9
	TYL	Preplant	0.24	0.04	1.46	47.4
		Growing	3.45	0.08	22.80	52.0
		Harvest	0.75	0.07	2.35	66.7
	ATZ	Preplant	1968	789	13041	0.0
		Growing	1054	354	7518	0.0
		Harvest	170	76	692	0.0

Table 3.4 Comparison of atrazine (ATZ), sulfamethazine (SMZ) and tylosin (TYL) concentrations in SFIR watershed to concentrations in other agricultural watersheds using POCIS samplers.

Site Name	Area	Study Duration	Land Cover	Analyte	Detection Freq	Mean Conc	Source
The River Trec, France	200 km ²	Apr. - Jun. 2013	Corn, Wheat, Rapeseed, Arboriculture, Vegetables	ATZ	100%	6 - 29 ng L ⁻¹	Poulier et al., 2014
Auvézère River, France	900 km ²	Jan. - Sept. 2002	Ag. Lands (73%) Grasslands (50%) Cereal Crops (28%)	ATZ DEA	45 - 60% 90 - 100%	6 - 8 ng L ⁻¹	Poulier et al., 2015
Shell Creek Watershed, Nebraska USA	1,200 km ²	Sept. - Nov. 2008 Jun. - Oct. 2009	Cultivated land cover, 1550 Farms (Swine, Cattle, Poultry)	SMZ TYL	94.5% -	1.3 ng L ⁻¹ 0.034 ng L ⁻¹	Jaimés-Correa et al., 2015
South Nation Watershed, Canada	3,915 km ²	May - Jul. 2010	Corn-Soybean Tile Drainage	ATZ	100%	6 - 256 ng L ⁻¹	Dalton et al., 2014
Yangtze Estuary, China	30,000 km ²	Oct. - Dec. 2013	Aquaculture Fisheries	SMZ	100%	40.7 ng L ⁻¹	Shi et al., 2014
South Fork of the Iowa River, Buckeye Iowa USA	264.7 km ²	Jun. - Aug. 2013	Cultivated Crops (90.4%) Subsurface Drainage (88.7%)	ATZ	-	610.4 ng L ⁻¹	Van Metre et al., 2017
South Fork of the Iowa River, New Providence, Iowa USA	582.4 km ²	Jun. - Aug. 2013	Cultivated Crops (85.7%) Subsurface Drainage (84.8%)	ATZ	-	211.2 ng L ⁻¹	Van Metre et al., 2017
South Fork Watershed of the Iowa River, Iowa USA	781 km ²	May - Nov. 2013 Apr. - Nov. 2014 Mar. - Nov. 2015	Ag. Lands (96%) Corn-Soybean, Tile Drainage (80%), 169 AFOs	SMZ TYL ATZ	83% 70% 100%	1.87 ng L ⁻¹ 0.30 ng L ⁻¹ 754.2 ng L ⁻¹	Current Study

3.5.2 Tobit censored regression analysis

From the parameter estimates of the Tobit model, the sigma parameter measures the estimated standard error of the regression, which is then compared to the standard deviation of the dependent variable, y , indicating if there is statistical significance in the model parameter estimates. Based on the sigma values, the model fit for the Tobit was statistically significant for all analytes

(SMZ, TYL, and ATZ) for POCIS and grabs (Table 3.5). To further quantify model fit, the Pearson product-moment correlation coefficient was estimated for predicted concentrations versus actual concentrations, which results showed statistical significance except for the POCIS tylosin model ($p = 0.7469$). Model fit was further improved for each analyte by including interactions, which were all statistically significant.

Table 3.5 Comparison summary of the Tobit model fit based on sigma values.

Analyte	POCIS Samples			Grab Samples*		
	Standard Deviation	σ	Sigma P-value	Standard Deviation	σ	Sigma P-value
SMZ	6.417	6.853	<0.0001	0.0031	0.0037	<0.0001
TYL	1.896	2.642	<0.0001	0.0221	0.0302	<0.0001
ATZ	1842.7	1521.8	<0.0001	0.4359	0.3902	<0.0001

* Grab samples include a total of 5 sites, not 4 like the POCIS

3.6 Temporal Variation

3.6.1 POCIS Samples

A pattern of temporal variation was observed for SMZ, TYL and ATZ in the SFIR, on an annual and seasonal scale. SMZ exhibited significant differences in concentration ($p < 0.05$) between 2013 – 2015. SMZ was significantly higher ($p = 0.0033$) in 2014 with a TWA 2.83 ng L^{-1} , while there was no statistical difference between 2013 and 2015. TWA of TYL, 1.54 ng L^{-1} was significantly higher in 2013 than in the subsequent years of the study. ATZ showed a strong annual variation in 2013 ($p < 0.0001$) and 2014 ($p = 0.0170$), significantly higher in 2013 ($2,227.9 \text{ ng L}^{-1}$) compared to 2014 (478.6 ng L^{-1}). POCIS monitoring of SMZ and TYL by Jaimes-Correa et al., 2015 did not report significant temporal variation by the same antibiotics.

Next, we examined the impact of seasonality. From the results of the Tobit model, a pattern of seasonality was found only for SMZ and ATZ in the SFIR from 2013 – 2015, shown in (Figure 3.2). The growing season for SMZ was statistically significant ($p < 0.0001$). Peak SMZ

concentrations occurred during this time period and accounted for the highest detection frequency of SMZ, at 92%. There was no significant difference between harvest and preplant concentrations. ATZ seasonality was significant during growing and harvest season, with ($p < 0.0001$) for both. Growing and harvest seasonality could be linked to high base flow conditions. Base flow accounted for 54% of total flow during the growing season and 72% of total flow during harvest season. Base flow was separated from the hydrograph using an algorithm developed by (Arnold and Allen, 1999; Around et al., 1995). The seasonal pulses of these veterinary antibiotics that occurred during the growing and harvest season in the SFIR, is consistent with studies that indicate similar patterns of occurrence and detection during summer months (Jaimes-Correa et al., 2015; Kim and Carlson, 2007; Lissemore et al., 2006). High atrazine TWA concentrations occurred predominantly during preplant in the month of May. This relationship was not significant, but it coincides with the period when ATZ is typically expected to be high due to previous heavy use of herbicides and periods of heavy precipitation, resulting in the first flush phenomenon (Thurman et al., 1991; Graziano et al., 2006). Overall, there was a decreasing trend in TWA concentrations for ATZ, from preplant to harvest. The high detection frequency of ATZ throughout this study, is most likely due to its slow degradation and high persistence in the watershed. TYL, did not exhibit a trend of seasonality, which may be due to its tendency to be tightly sorbed and unavailable in the aqueous phase. There was also a decrease in detection of TYL from 2013 – 2015, 93.1% (2013), 47% (2014), and 69% (2015).

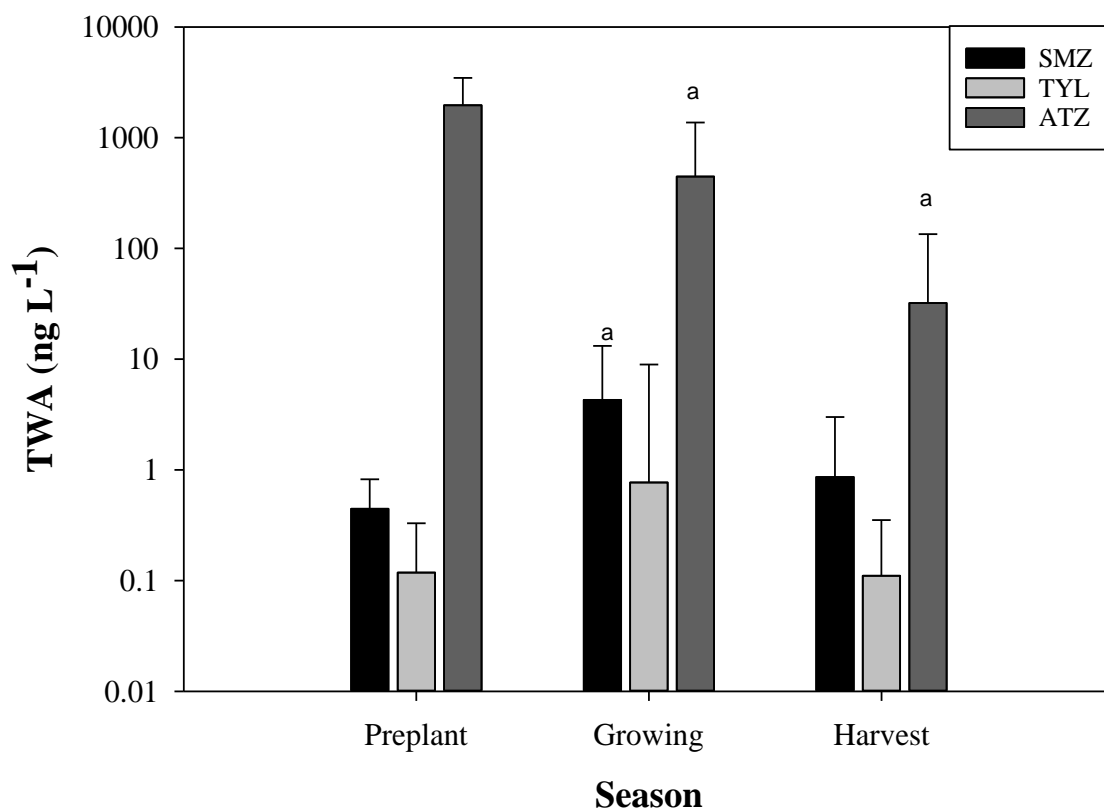


Figure 3.2 Bar graph of the POCIS TWA concentrations across the SFIR watershed, based on season from 2013 - 2015. The error bars indicate the standard deviation and seasonal significance for analyte concentrations ($p < 0.05$) is indicated by the letter (a) above the bar.

3.6.2 Grab Samples

A similar seasonal trend was verified by grab samples (Figure 3.3), where growing and harvest seasons were statistically significant terms in the tobit regression for SMZ, TYL, and ATZ concentrations. In addition, concentrations for all analytes were significant for sampling years 2013 and 2014 for grab samples (SI).

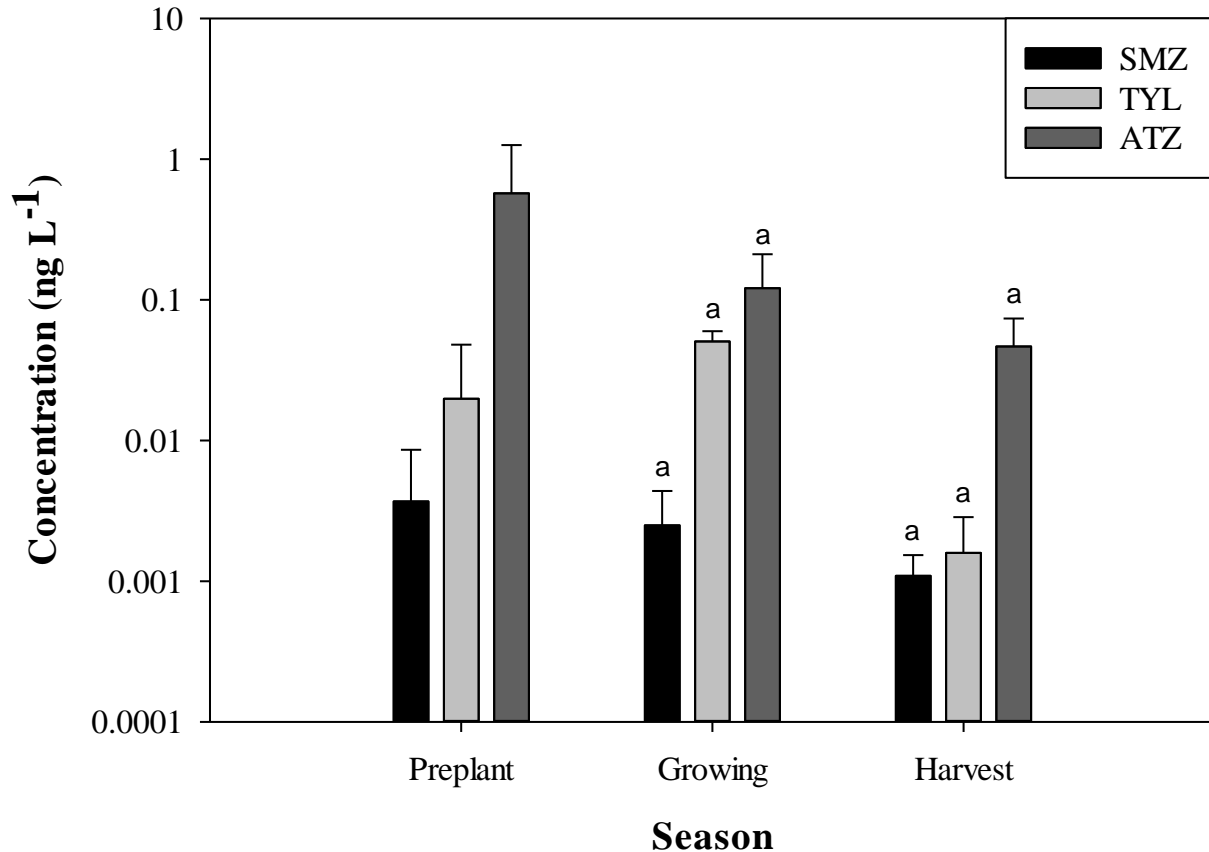


Figure 3.3 Bar graph of the Grab sample concentrations across the SFIR watershed, based on season from 2013 - 2015. The error bars indicate the standard deviation and seasonal significance for analyte concentrations ($p < 0.05$) is indicated by the letter (a) above the bar.

3.7 Impact of Tile Drainage and Hydrology

In this study, site IATC-241 provided the only direct measurement of tile drain effluent using the POCIS sampler. The other three sites monitored all have indirect contributions from tile drain outflows into surface water upstream of those sites, but may also be affected by in-stream processes after drainage enters the stream channel. Tile drain mean TWA concentrations were 3.0 ng L⁻¹ (SMZ) and 0.14 ng L⁻¹ (TYL), with detection frequencies of 100% and 81%, respectively (Table 3.4). Maximum TWA concentrations were higher for SMZ at 44.1 ng L⁻¹ than for TYL at 1.51 ng L⁻¹. SMZ was more prevalent than TYL from the tile drain. In comparison, studies that

monitored SMZ and TYL in other Iowa agricultural settings (Cain et al., 2004; Garder et al., 2014), found concentrations an order of magnitude higher than concentrations from IATC-241. The baseflow contribution of IATC-241 was approximately 64% of the total flow from 2013 – 2015. The percentage contribution of base flow increased with season as total flow decreased. IATC-241 produced high concentrations and high frequencies of detections for SMZ and TYL, but monitoring site was not a significant ($p > 0.05$), SMZ ($p = 0.0621$) and TYL ($p = 0.7204$) contributor to the Tobit regression model. The remaining subsurface sites did not contribute statistical significance to the regression, except for IABC-350 for SMZ.

Even though the tile-drainage sites (IATC241 and IATC242) do not contribute to the Tobit model, the detection of SMZ and TYL demonstrates the ability of tile drains to transport antibiotics from land-applied manures into the subsurface environment, then to surface waters. Furthermore, the increase of baseflow percentage as the season transpires highlights the importance of monitoring subsurface drainage, due to the capability to transport antibiotics. This is consistent with results by Kay et al. (2004), who first demonstrated the transport of antibiotics through tile drains.

3.8 Comparison between POCIS and Grab Samples

Comparing POCIS results to those for grab samples is difficult due to the duration of the sampling period between the two methods of sampling (Morin et al., 2012). The biggest shortcoming of discrete grab sampling is that it provides only a snapshot or an instantaneous estimate of environmental levels, neglecting episodic events and overestimating concentrations (Thomatou et al., 2010; Vrana et al., 2005). The POCIS provides time integrative sampling by capturing episodic events, thereby providing a more complete picture. The most noticeable difference observed between sampling methods was detection frequency. ATZ had a detection frequency of 100% for both methods, but SMZ and TYL had higher detection frequencies for POCIS, at 82% and 68% respectively. In comparison, SFIR grab samples detected SMZ at 59%

and TYL at 60%. The higher detection frequencies for POCIS samples could be explained by its lower LOD/LOQ compared to that of the grab samples. The POCIS improves the LOD by concentrating sequestered analytes of interest. Estimated POCIS concentrations were lower for SMZ and TYL, compared to grab samples (Figure 3.4). A similar relationship was observed by Jones-Lepp et al. (2012).

3.9 Conclusion

Baseline knowledge on concentrations, occurrence, transport, and temporal behavior of SMZ, TYL, and ATZ in a swine dominated watershed are presented. This study suggests SMZ, TYL, and ATZ were all ubiquitous in

SFIR with detection frequencies of 68 – 100%. We demonstrated application of POCIS to monitor and detect antibiotics at sub-inhibitory concentrations in tile drained landscapes. The detection of SMZ & TYL was higher with POCIS samples than grab samples. The POCIS technology resulted in a lower percentage of censored data for all analytes, compared to grab samples.

While the half-life of these antibiotics are relatively short term, they have shown the ability to be persistent throughout the year in the SFIR, releasing from the terrestrial environment in an episodic nature caused by seasonal pulses. At the single tile drain site monitored by POCIS, IATC-241, a high occurrence of SMZ and TYL was observed across the duration of the study. More

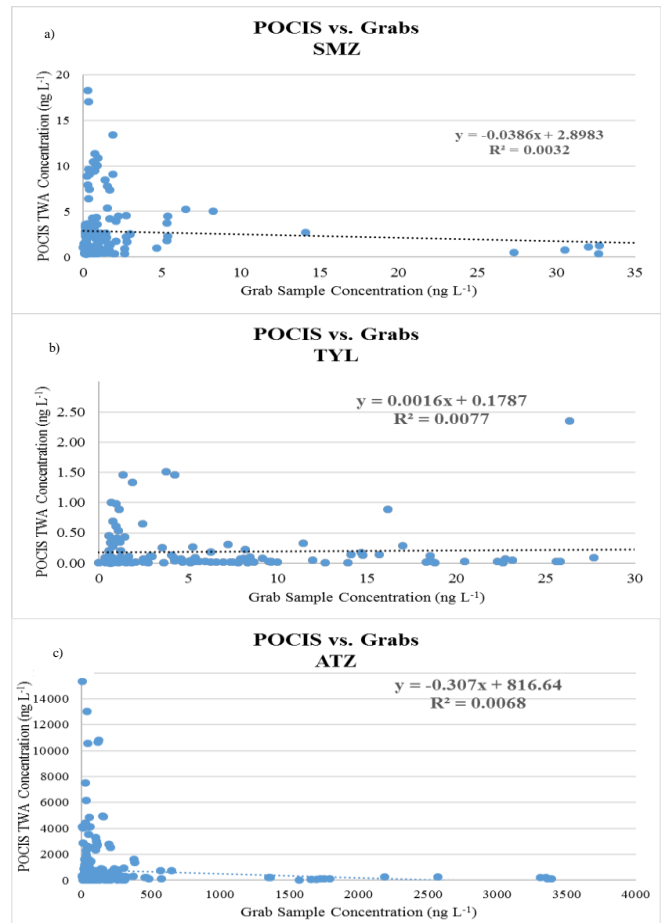


Figure 3.4 Scatterplot comparison of POCIS and grab sample concentrations in the SFIR watershed for (a) SMZ, (b) TYL, and (c) ATZ.

importantly, this study verifies the role of tile drainage in the transport TYL in an agricultural watershed. TYL is thought to be less available in the aqueous phase, and more likely sorbed to sediment or soil, suggesting that runoff is the main mechanism for transport.

TWA concentrations for SMZ and TYL were an order of magnitude lower (ng L^{-1}) than the minimum inhibitory concentrations (MIC) for *E. coli*. Still, the prevalence of these antibiotics at sub-inhibitory concentrations could be a cause for concern, due to the potential selective pressure from these antibiotics on the retention of resistance genes (Anderson and Hughes, 2012; Gullberg et al., 2014). The fate and transport of these analytes are impacted by their time of application, hydrological conditions of the watershed, and seasonality. SMZ and ATZ concentrations were found to be statistically significant during growing and harvest seasons, consistent with other studies which indicated similar trends during summer months. By identifying the seasonal fate and occurrence of these analytes, we can be proactive by focusing on the environmental conditions (precipitation, runoff, erosion) and land management techniques (timing of manure application, surface and subsurface drainage) which influence their persistence in the environment, thereby by reducing their potential environmental impact. Management options which have been proven to reduce the transport of antibiotics in the environment, include controlled tile drainage systems (surface water) (Frey et al., 2015) and vegetative buffer strips (surface runoff).

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CHAPTER 4. STREAM SEDIMENT CONCENTRATIONS OF TYLOSIN, SULFAMETHAZINE, AND ATRAZINE IN A TILE DRAINED WATERSHED

This paper to be submitted to *Science of the Total Environment*

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4.1 Abstract

Sediment serves as a matrix that is important in understanding the fate and transport of environmental contaminants. Agricultural practices are influential in the creation and transport of sediment instream. Instream sediments can act as a sink and a source for environmental contaminants, and with the large volume of sediment that is lost from agricultural fields, it is a matrix which requires more investigation. The instream sediments were sampled in the South Fork watershed of the Iowa river (SFIR) to determine the extent of tylosin (TYL), sulfamethazine (SMZ), and atrazine (ATZ) occurrence. Sediment in SFIR watershed consisted of sand (88%) > loamy sand (9%) > sandy loam (4%). Statistical analysis revealed annual and seasonal significance for TYL concentrations. On an annual basis TYL concentrations were statistically significant in 2013 and 2014. Seasonal significant concentrations occurred during the growing and harvest seasons, which coincide with the heaviest precipitation periods in the watershed, contributing to the transport of TYL via runoff. The impact of flow conditions was only observed with TYL where high flow and moist flow conditions were statistically significant. Detection frequencies for analytes were slightly lower in sediment (SMZ: 42%, TYL: 60%, ATZ: 84%) than water samples (SMZ:

54%, TYL: 60%, ATZ: 100%). Mean sediment concentrations were higher for TYL and SMZ on a volumetric basis compared to water samples. Sediment-water pseudo partition coefficients were normalized to the organic carbon content of the sediment, K_{oc} values were higher than the calculated K_d values, indicating these antibiotics are less mobile than indicated by their K_d . Since these analytes have a lower tendency to be mobile, focusing on the fate and transport in the sediment phase is very important.

Keywords: Sediment; Antibiotics; Tylosin; Sulfamethazine; Atrazine

4.2 Introduction

Antibiotics are often used by the animal production industry as feed additives, to promote growth/feed efficiency and to prevent and treat disease. Often, these antibiotics are not fully metabolized, entering the terrestrial and aquatic environment as the parent compound or as metabolites. Once in the environment, they maintain their biocidal activity and have the potential to impact target and non-target organisms in soil and water environments. The persistence of antibiotics in the environment is influenced by their wide range of physiochemical properties, weather, land management and interactions in the environment (Wegst-Uhrich et al., 2014; Mojica and Aga, 2011; Henderson et al., 2009).

Water quality monitoring is often focused on the dissolved or suspended phase of a pollutant. While this is key, we must investigate the impact of pollutants which partition into the solid phase, such as sediment (Fairbairn et al., 2015; Kolok et al., 2014; Kim and Carlson, 2007; Pei et al., 2006). Sediments have the ability to accumulate chemical compounds, thereby acting as sink and an eventual source (Kolok et al., 2014). Much of the previous work with regards to sediment focuses on the transport of phosphorus (P). Benthic sediments are known for being influential in the transport and dynamics of P, acting as a sink

and source, with the ability to interact with the water column (Ahiablame et al., 2010). The role of sediment in the spread of antibiotic resistance warrants more investigation, especially in the benthic zones in water bodies (Kim and Carlson, 2007; Pei et al. 2006). Antibiotics sorbed onto sediment have the potential to bioaccumulate in the aquatic environment (Gao et al., 2012), and since many antibiotic compounds are hydrophobic in nature, they can be transported to surface water in the sediment-associated phase.

Common sources of sediment in agriculture include streambank erosion, surface runoff of croplands (rill and sheet erosion), and unsealed rural gravel roads (Lamba et al., 2015; Williamson et al., 2014). In addition, surface and subsurface drainage has been documented as a source/transport mechanism for agricultural sediments in drain effluent (Stull, 2014; Maalim and Melesse, 2013; Palmer, 2012; Stone and Krishnappan, 1998). We do not know the specific pathways of sediment which are attributed to antibiotic transport, but these potential source pathways could provide different sediment matrices that antibiotics could adsorb to. The composite effects of streambank instability from sediment accretion caused by historical erosion and the alteration of hydrology via channelization and implementation of surface/subsurface drainage has transformed naturally meandering streams to incised and widened channels (Noonan, 2016). Actively eroding streambanks are a large contributor of suspended sediments found in streams (Williamson et al., 2015). Surface tile inlets, used to drain closed depressions serve as a key mechanism for sediment and other pollutants to enter subsurface waters by way of tile drainage as well as macropore flow (Blann et al., 2009; Deasy et al., 2009).

In the South Fork watershed of the Iowa River (SFIR), the installation of drainage ditches in the early 20th century altered the natural meanders of the SFIR. Subsurface drainage outfalls connected to drainage channels in the lower portion of the watershed created new routes for drainage (Merten et al., 2016; Simon and Klimetz, 2008). As a result, the increased bank heights and drainage volume contributed to destabilizing the channel banks, eroding sediment away, thereby widening the channels, causing the reestablishment of new meanders (Merten et al., 2016; Baowen et al., 2010). The destabilized channel material could be the potential source of instream sediments sorbed with antibiotics. These new meanders, are less sinuous than naturally occurring meanders. A good indication of channel straightening in the SFIR is shown in (Figure 4.1).

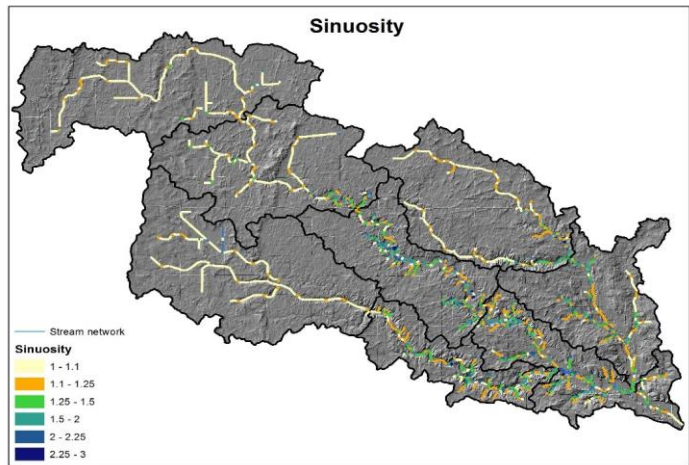


Figure 4.1 Sinuosity of the SFIR watershed

According to Simon and Klimetz (2008), approximately 50% of streambanks in the SFIR contribute to the sediment load of the watershed. Other work suggests stream channels in the SFIR contribute approximately 80% to sediment loads, while sheet and rill erosion account for 20% (Tomer et al., 2010; Wilson et al., 2008).

The delivery of sediment to surface waters via streambank erosion, surface runoff, and tile drainage have the potential to provide a reservoir of antibiotic resistance in these agricultural water systems. The purpose of this research was to quantify antibiotic and pesticide residue concentrations in sediment of the SFIR, well known for its swine production. The objectives of this research were to establish the baseline stream sediment

tylosin and sulfamethazine concentrations, determine their distribution, and frequency of detections. In addition, we investigated the temporal variation of the presence of these analytes and determined the impact of hydrological flow conditions on their distribution in sediment, and compared sediment-water concentrations on a volumetric basis. Assessing the occurrence, fate, and subsequent transport of these analytes is critical to understanding their impact on sediment and its role as a sink and source.

4.3 Materials & Methods

4.3.1 Watershed Description

SFIR is an agriculture dominated watershed, with 80% of the landscape under tile drainage, accounting for 90 – 95% of all flows (Tomer et al., 2008a). The SFIR is composed of three drainage areas; Tipton Creek tributary in the southwest, the South Fork of the Iowa River in the center, and the Beaver Creek tributary in the southeast. Land use for the SFIR includes corn (*Zea mays*)-soybean (*Glycine max* (L.) Merr.) rotation or corn-corn and corn-corn-soybean rotations covering approximately 85% of the watershed, with the remainder consisting of pasture or Conservation Reserve Program (CRP) land, forest or wetland, and development or roads (Tomer et al., 2008b). An estimated 153 swine animal feeding operations (AFOs) exist in the SFIR with a swine inventory of 1.39 million. A detailed watershed description is discussed by Washington et al. (2017).

4.3.2 Sampling Sites

Five field sites in the central to southern part of the SFIR were monitored: IATC-241, IATC-242, IATC-323 (Tipton Creek tributary); IASF-450 (South Fork tributary); and IABC-350 (Beaver Creek tributary) (Figure 4.2). All five sites are currently monitored for hydrology and water quality data by USDA-ARS. At sites IATC-241 and IATC-242 direct

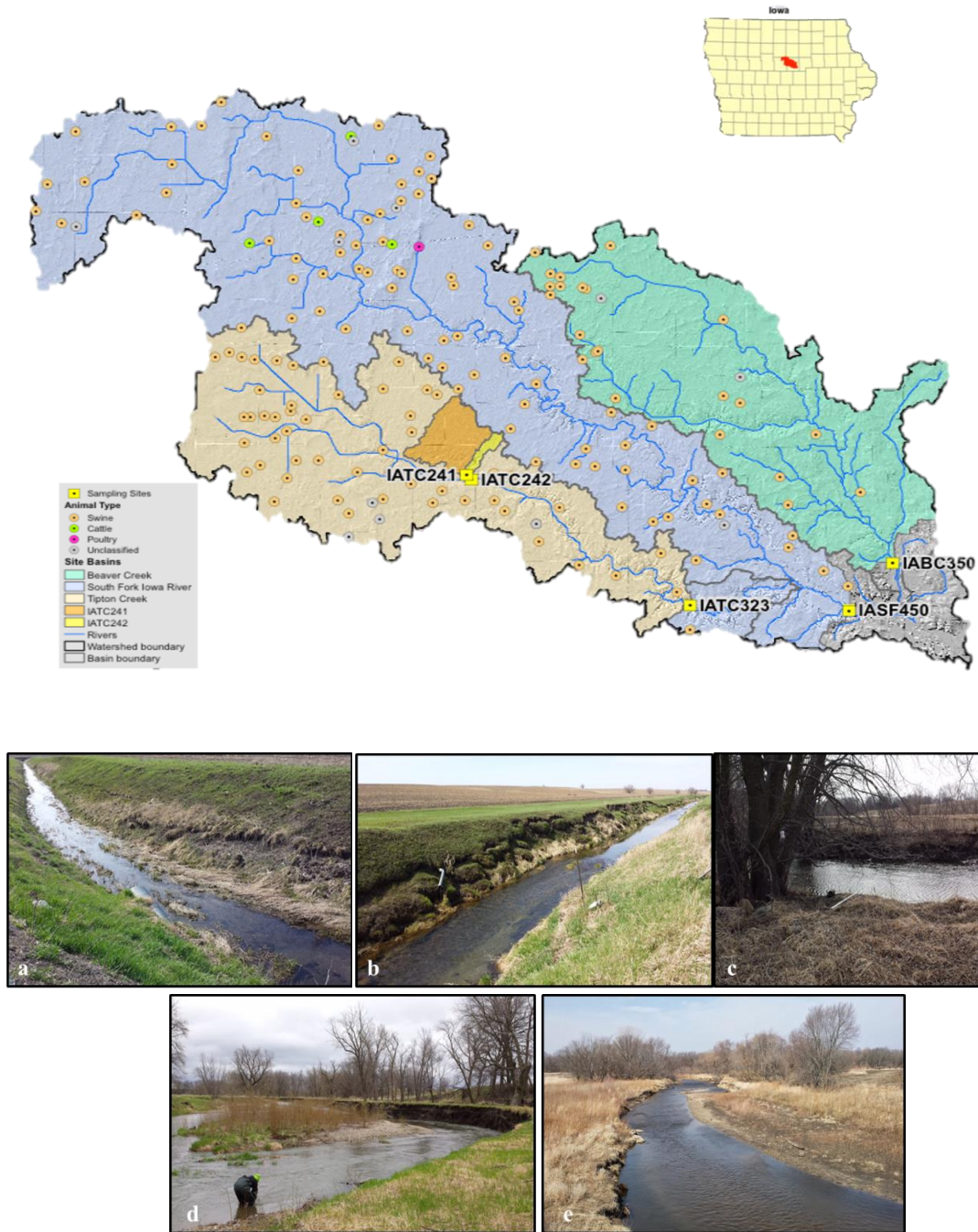


Figure 4.2 Sampling sites in the SFIR: a) IATC-241 channelized agricultural drainage ditch with tile drain outlet, b) IATC-242 channelized agricultural drainage ditch with tile drain outlets. c) IATC-323 meandering stream site d) IABC-350 meandering stream site with eroding banks before channel reconstruction, e) IASF-450 meandering stream site with eroding banks.

tile drain discharge flows into channelized agricultural drainage ditches, an example of the historical channelization that has occurred in the watershed. The instream sites, IATC-323, IASF-450, and IABC-350, all exhibit stream bank erosion which is prevalent throughout the watershed. These three sites all have indirect tile drainage access upstream and downstream of the sites. Bridge access with open gravel surfaces cross each of these sites and potentially provide an additional source of sediment to these streams. In 2014 during the later portion of the sampling season (July – September) further channelization occurred when site IABC-350, was straightened into a trapezoidal channel.

4.3.3 Sample Collection

Samples were collected from 2013 - 2015 (April – November), 2014 (April – November), and 2015 (March – November). Sampling frequency was initially monthly, but was increased to bi-monthly in 2014 and 2015, to gather more sediment data. Sediment samples were collected using a 125 cm³ plastic cap, which was firmly pushed into the sediment bed, roughly 0.05 meters until the sampling cap was full. A spatula was used to seal and secure the sediment from the opening at the bottom of the cap. Each sample was bagged and preserved on ice and stored at -20°C. Sediment samples at tile drain discharges (IATC-241 and IATC-242) were collected near the tile outlet, approximately in a 0.28 m² area. The instream sediment samples were collected in a 0.28 m² proximity to the water grab samples, when accessible. Duplicate water grab samples were collected from the tile outlets and from the stream sites. Grab samples were collected in 0.5-liter amber glass jars with PTFE-lined caps, kept on ice in the field and then stored at 4° C, at USDA-ARS NLAE (National Laboratory of Agriculture and Environment) prior to analysis.

4.4 Sample Analysis

4.4.1 Extraction Procedure

Sediment samples were prepared for extraction based upon a protocol described by Garder et al., 2014. Sediments were weighed (15 g) into Teflon centrifuge tubes. Samples were extracted with acetonitrile: 0.1 M ammonium acetate (85:15, v:v) by shaking on a reciprocating shaker and left overnight at room temperature and then centrifuged. Supernatant from the centrifuged sediment was then concentrated by evaporating the acetonitrile with nitrogen gas. The concentrated supernatant was then extracted using Blackwell, (2004) solid phase extraction (SPE) protocol. Sediment extracts were first passed through a strong anion exchange (SAX) column then through an Oasis HLB SPE cartridges. The SAX column helps to eliminate matrix effects in the extract. Once eluted, 0.1 mL of simetone was added to the sample as an internal standard. The eluted material was then evaporated to 0.3 mL volume using nitrogen gas and then reconstituted to a 2 mL volume using 10mM ammonium acetate. The analytes were re-dissolved in the ammonium acetate after approximately 30 minutes, and samples were filtered and submitted for analysis. Grab samples were processed using the procedure by Washington et al., 2017.

4.4.2 Textural Analysis

Representative sediment samples were selected for textural analysis. Samples were selected for each monitoring site, based on year and season. Each sample year was represented among the sample seasons for analysis. Approximately 150 grams of wet sample was weighed out, and left over night to air dry. Once sediment samples completely dried, they were processed through a 2mm sieve and bagged. Sediment samples were sent to Midwest Laboratories, Inc. for textural analysis.

4.4.3 Analyte Analysis

Analysis was performed using an ABSciex 5500 QTrap mass spectrometer with an Agilent 1260 Infinity LC on all environmental samples. All sample extracts were analyzed for SMZ, TYL and ATZ. The instrumental limit of detection (LOD) and limit of quantification (LOQ) were determined for each analyte. Based on these instrumental values, LOD/LOQ values were back calculated for sediment and grab samples (Table 4.1). Compounds were monitored using multiple reaction monitoring (MRM), with 3 stages collected for each (European Standard EN 1662, 2008). A more detailed analytical analysis is provided by Washington et al., 2017.

Table. 4.1 Instrumental and Matrix LOD and LOQ for selected analytes

ANALYTE	INSTRUMENTAL		GRABS		SEDIMENT	
	Limit of Detection (ng L ⁻¹)	Limit of Quantification (ng L ⁻¹)	Limit of Detection (ng mL ⁻¹)	Limit of Quantification (ng mL ⁻¹)	Limit of Detection (ng g ⁻¹)	Limit of Quantification (ng g ⁻¹)
SMZ	0.041	0.04	0.000328	0.00032	0.0055	0.0053
TYL	0.044	0.04	0.000352	0.00032	0.0058	0.0053
ATZ	0.027	0.03	0.000216	0.00024	0.0036	0.0040

4.4.4 Sediment-Water Pseudo Partition Calculations

Discrete sediment and water sample concentrations were used to calculate observed sediment-water partition coefficients (K_d). The K_d (L kg⁻¹) is the ratio between the concentration of a sorbed compound in sediment (C_s) to the concentration of the dissolved compound in water (C_w). K_d is a direct measure of the sorption behavior with respect to the solid phase, where

$$K_d = \frac{C_s}{C_w} \quad (1).$$

To account for the organic carbon content of the sediment, the K_d was normalized to calculate the K_{oc} , as:

$$K_{oc} = \frac{K_d}{f_{oc}} \quad (2)$$

where f_{oc} is the fraction of organic carbon in the sediment.

4.5 Statistical Analysis

A Tobit censored regression analysis was used to account for the censoring of antibiotic concentrations. Censored observations (non-detects) are defined as low-level concentrations that measure between 0 and the detection/reporting limit of laboratory analytical equipment (Heisel, 2012). The Tobit regression was modeled as, $y = \text{site} + \text{season} + \text{year} + \text{flow regime}$, where y the analyte concentration is the censored dependent variable. The regression model was used to determine the differences in analyte concentration based on site, season, year, and flow conditions.

Along with the Tobit model the Pearson product-moment correlation coefficient was determined for each analyte model. Additionally, interactions between site, season, year, and flow regime were analyzed. Significant differences for all comparisons were evaluated at $p < 0.05$. Statistical analysis was performed using SAS 9.4.

4.6 Results and Discussion

4.6.1 Sediment characteristics

Textural analysis indicates 87% of sampled sediments can be classified as sand, followed by 9% loamy sand, and 4% sandy loam. Sand particles ranged from 54 – 96%, with a median of 96%. Textural triangles for each sample site show there is not much variation in texture from site to site (Figure 4.3). The sandy composition of these sediments can partly be contributed to the glacial deposits from the Des Moines lobe, consisting of sand and gravel (McCarthy, 2012). The vast majority of these deposits are located in proximity to the SFIR and its tributaries in the southeastern portion of the watershed where our sampling sites are

located. The composition of sediment governs the ability of compounds to adsorb, specifically influencing the strength and degree of sorption (Kümmerer, 2009).

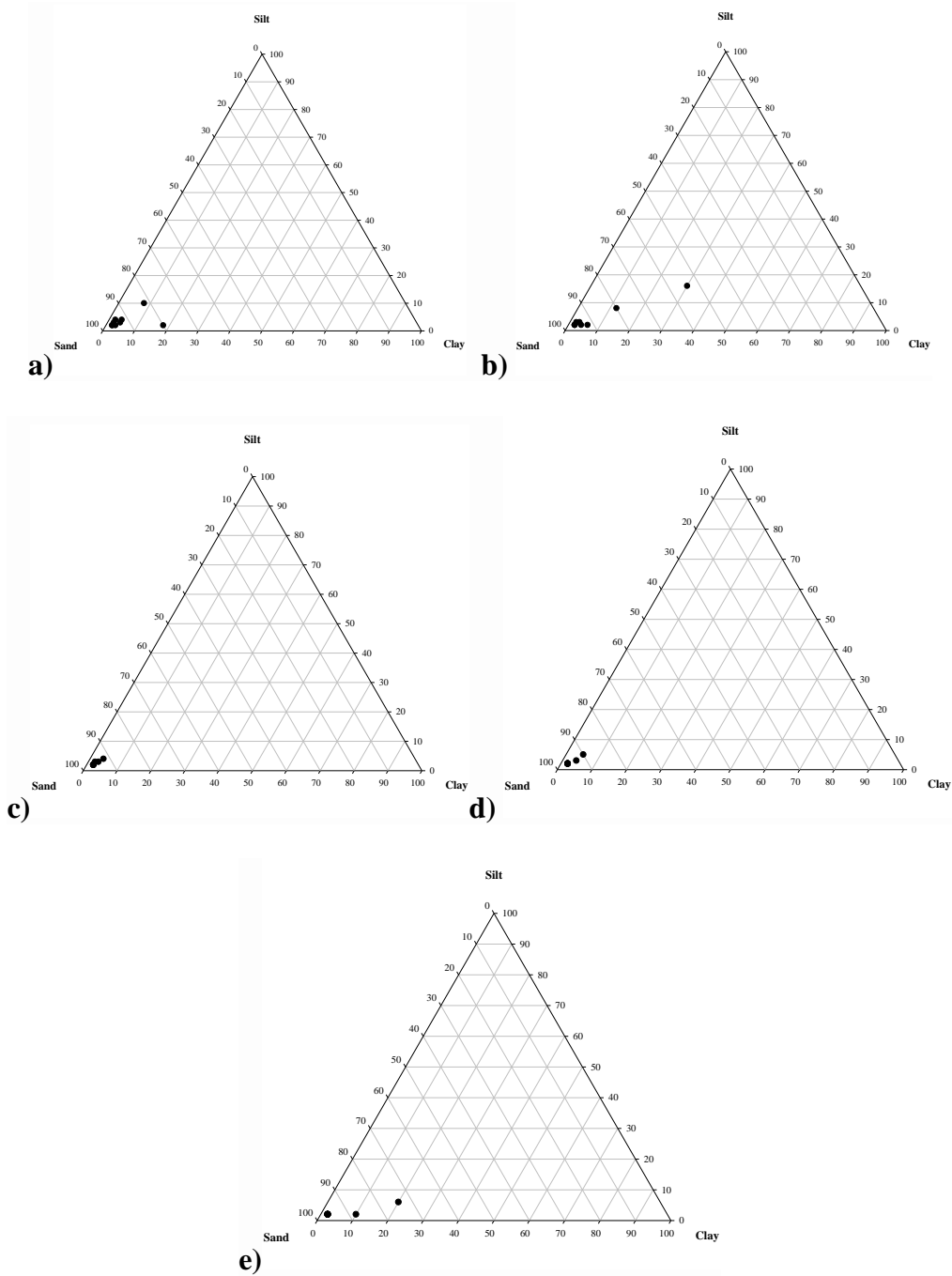


Figure 4.3 Textural analysis diagrams giving sand, silt, and clay composition for select/representative sediment samples at each sampling site a) IATC-241 b) IATC-242 c)

IATC-323 d) IABC-350 and e) IASF-450. These representative samples cover from 2013 – 2015, as well as preplant, growing, and harvest seasons.

4.6.2 Occurrence and concentration of analytes in sediment and water

Analyte concentrations and detection frequencies were determined for five sampling sites in the SFIR watershed from May – November (2013), April – November (2014), and March – November (2015) (Table 4.2). TYL, SMZ, and ATZ were detected at all sampling sites in both sediment and grab samples. ATZ and TYL were detected most frequently in the sediment matrix with detection frequencies of 84% and 60% respectively, while SMZ was detected at a much lower rate of 42%. All analytes were readily detected in water samples, and followed the same order of detection as the sediment samples (Washington et al., 2017). ATZ had a detection frequency of 100% while TYL and SMZ were detected at 60% and 54%. The physiochemical behavior of SMZ indicates it is loosely sorbed and is more available in the dissolved phase (Wegst-Uhrich et al., 2014). In comparison, TYL is more tightly sorbed, therefore it is more likely to be available in the solid phase (Wegst-Uhrich et al., 2014; Blackwell et al., 2007). We observed a higher detection frequency for SMZ in the dissolved phase, which is consistent with its physiochemical behavior, which indicates it is poorly adsorbed and more likely to exist in solution. TYL was detected at the same moderate rate in both matrices, which was not expected based on its physiochemical behavior. The moderate availability of TYL in the dissolved phase could be due to its desorption from sediment, back into the water column. This moderate to high detection of analytes in the SFIR confirms the use of these compounds in the watershed.

A mean bulk density of 1.05 g cm^{-3} was calculated for sediment, allowing us to compare sediment and water concentrations on a volumetric basis. Mean sediment

concentrations were higher than water concentrations for all analytes in the SFIR. ATZ and TYL concentrations had the same concentration, 0.36 ng cm^{-3} , while SMZ had a much lower concentration of 0.031 ng mL^{-1} . ATZ, SMZ, and TYL had mean concentrations of 0.1606, 0.0014, and $0.0067 \text{ ng mL}^{-1}$ respectively. Both matrices are expressed in units that are part per billion (ppb). In comparison to other studies which have investigated antibiotics in sediment and water, mean concentrations of SMZ and TYL in the SFIR were lower (Table 4.2). ATZ was the only analyte which was comparable in both matrices. Higher antibiotic sediment concentrations indicate their ability to accumulate and have the potential to release back into water column. Therefore, sediment can act as a sink and a source for these antibiotics.

Site Name	Area	Study Duration	Land Cover	Analyte	Sediment Detection Freq	Sediment Mean Conc	Water Detection Freq	Water Mean Conc	Source
Cache la Poudre Watershed, Colorado USA	8459 km ²	May 2003 - Feb. 2005	Pristine, Urban, & Agricultural Areas	SMZ TYL	25% 53%	4.7 ng g ⁻¹ 3.0 ng g ⁻¹	60% 5%	0.11 ng mL ⁻¹ 0.05 ng mL ⁻¹	Kim and Carlson, 2007
Haihe River Basin, China	318,000 km ²	Dec. 2008 Sept. 2010	Aquaculture, Animal Husbandry	SMZ	100% 100%	2.9 ng g ⁻¹ 4.4 ng g ⁻¹	- 89%	- 0.027 ng mL ⁻¹	Gao et al., 2012
South Fork Watershed of the Zumbro River, Minnesota USA	809 km ²	May. - Jun. (2011) Aug. - Sept. (2011) Oct. - Nov. (2011) Jan. - Feb. (2012) Mar. (2012) May. - Jun. (2012) Aug. - Sept. (2012) Oct. - Nov. (2012)	Ag. Lands (62%), Poultry (101K), Swine (73K), Cattle (23K)	ATZ	22%	0.13 ng g ⁻¹	100%	0.04 ng mL ⁻¹	Fairbairn et al., 2015
Hongcheon, Kangwon Province, Korea	-	-	Swine Manure Composting Facility	SMZ TYL	- -	38.6 - 70.32 ng g ⁻¹ 12.32 - 13.38 ng g ⁻¹	- -	0.31 - 9.12 ng mL ⁻¹ 0.23 - 2.19 ng mL ⁻¹	Ok et al., 2011
South Fork Watershed of the Iowa River, Iowa USA	781 km ²	May - Nov. 2013 Apr. - Nov. 2014 Mar. - Nov. 2015	Ag. Lands (96%) Corn-Soybean, Tile Drainage (80%), 169 AFOs	SMZ TYL ATZ	42% 60% 84%	0.04 ng g ⁻¹ 0.10 ng g ⁻¹ 0.04 ng g ⁻¹	54% 60% 100%	0.0014 ng mL ⁻¹ 0.0067 ng mL ⁻¹ 0.1606 ng mL ⁻¹	Current Study

Table 4.2 Comparison of Tobit model atrazine (ATZ), sulfamethazine (SMZ) and tylosin (TYL) estimated concentrations in the

SFIR watershed of sediment and water to concentrations in other agricultural watershed studies.

4.6.3 Seasonal and Flow Patterns

To assess the impact of seasonal variation and long-term stream flow conditions on analyte concentrations, we used concentration duration curves to provide a graphical representation. This approach allows for the assessment of the cumulative distribution function, showing the percentage of stream discharge that is equaled or exceeded, i.e. hydrological conditions and identification of source/delivery relationships of the watershed (USEPA, 2007). For example, duration curves for site IABC-350 display the distribution of TYL, SMZ, and ATZ mean concentrations based on flow regime and season for sediment and water matrices (Figure 5). Flow regimes were defined as: high flows (0-10%), moist conditions (10 - 40%), mid-range flows (40-60%), dry conditions (60-90%), and low flows (90-100%). Seasons were defined as preplant (March – May), growing (June – August), and harvest (September – November). Examining site IABC-350, the dominant flow regime was moist condition (MC). During MC, 45% of all antibiotic detections in sediment and 44% of all water detections for all analytes occurred, making MC the dominant flow condition in which detections were observed in the watershed. Antibiotics were not detected in sediment or water during low flow events, this is typically representative of point sources, therefore indicating the most likely origin of these analytes in the IABC-350 sub basin are from nonpoint sources. Typically point sources such as municipal waste water treatment plants (WWTTPs) are the dominant influence on WQ during low flow periods when groundwater is the main source of water. According to USEPA, 2007, “Instream channel processes tend to dominate the sediment regime at high flows, while sediment delivered from surface erosion may be of greater concern under mid-range flows.” Duration curves for both matrices for the remaining sites can be found in the supplementary information (SI).

4.6.4 Tobit censored regression analysis

From the parameter estimates of the Tobit model, the sigma parameter measures the estimated standard error of the regression, which is then compared to the standard deviation of the dependent variable, y = analyte concentration, indicating if there is statistical significance in the model parameter estimates. Based on the sigma values, the model fit for the Tobit was statistically significant for all analytes (TYL, SMZ and ATZ) for sediment, as shown in (Table 4.3).

Table 4.3 Comparison summary of the Tobit model fit based on sigma values for sediment and grab samples.

Analyte	Sediment Samples			Grab Samples		
	Standard Deviation	σ	P-value	Standard Deviation	σ	P-value
SMZ	0.03459	0.33884	<0.0001	0.0031	0.0037	<0.0001
TYL	0.01855	0.24098	<0.0001	0.0221	0.0302	<0.0001
ATZ	0.009850	0.17684	<0.0001	0.0140	0.3902	<0.0001

Using the Tobit model, a statistically significant annual relationship for TYL and ATZ in the sediment matrix, was determined. TYL and ATZ concentrations were found to be statistically significant for 2013 and 2014 (SI). There was no annual temporal significance for SMZ. In comparison, TYL, SMZ, and ATZ water concentrations were all statistically significant for 2013 and 2014 (Washington et al., 2017). The annual precipitation for the SFIR is approximately 750 mm (USDA-ARS, 2016). The mean annual precipitation at our sampling sites in SFIR were 736 mm (2013), increasing to 870 mm (2014) and 941.9 mm (2015). Even though mean annual precipitation for 2013 was slightly lower than the watershed average, we believe the statistical significance of this year is related to the drought conditions of 2012, where mean annual precipitation for our sampling sites was 517 mm.

Due to this lower than average precipitation, a reservoir affect could have occurred, where antibiotic residues were stored in soil and not flushed out by normal precipitation events. Therefore, when normal precipitation occurred in 2013, antibiotic residues remaining from the previous year were carried over and available for transport in addition to the new applied concentrations. This same phenomenon has also been observed with nitrate in tile drained systems (Ikenberry et al., 2014; Murphy et al., 2013; Sawyer, 2013).

4.6.5 Sediment

The Tobit model identified some statistically significant trends with respect to sediment concentrations during different seasons and flow conditions. Statistically significant trends were shown for TYL during growing ($p = 0.0087$) and harvest ($p = 0.0021$) seasons (Figure 4.5), while high flow ($p = 0.0072$) and moist flow ($p = 0.0006$) conditions were statistically significant. SMZ concentrations were shown to be statistically significant during the harvest season. No seasonal trends were observed for ATZ. Also, no statistically significant relationships were shown for flow conditions for SMZ or ATZ. In the SFIR, 60% of the normal annual precipitation falls from May through August in short and intense rainfall events (Stott et al., 2011). We documented this same trend during the duration of our study and found 63% of the precipitation at our sampling sites occurred during the growing and harvest seasons combined, which align with that same time period. In addition, 59% of the estimated total runoff in the watershed occurred during the growing and harvest seasons from 2013 – 2015. This indicates, that increased precipitation during these months likely contributed to the transport of tylosin via runoff. A similar seasonal trend was observed in grab samples in the SFIR (Washington et al., 2017). Growing and harvest season concentrations were statistically lower for all analytes. Since this trend is also seen in TYL

and SMZ sediment, it can be concluded that sediment acts as an reservoir for these antibiotics.

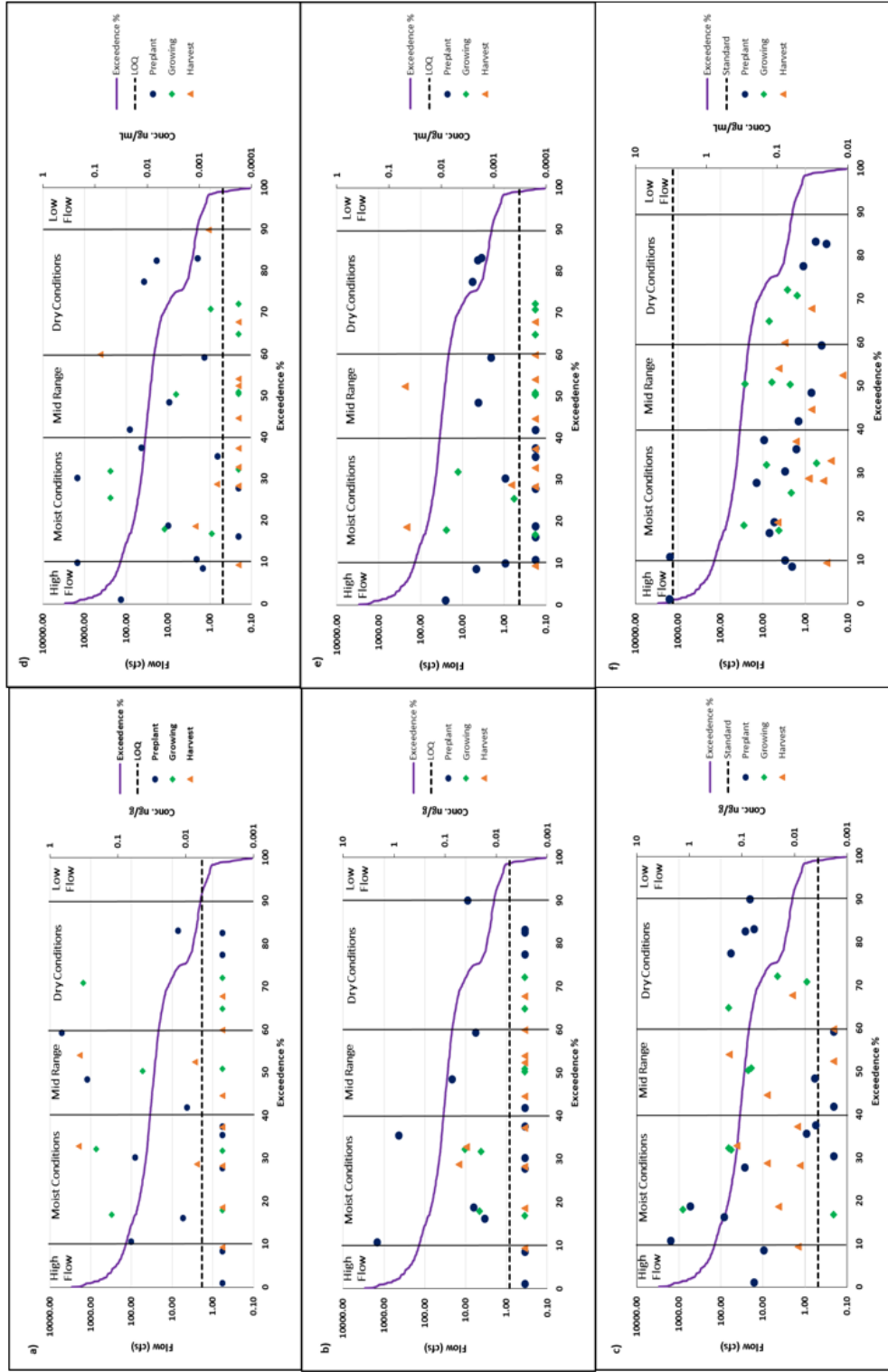


Figure 4.4 Mean Water Quality Duration Curve comparisons for sediment a) TYL, b) SMZ, c) ATZ and grab samples: d)

TYL, e) SMZ, f) ATZ concentrations at IABC-350 in the SFIR watershed. The duration curves show analyte detects and

non-detects based on LOD for the antibiotics and for atrazine in sediment, while the WQ standard was used for ATZ in

water. These duration curves also show flow regime and seasonal distribution of the analytes.

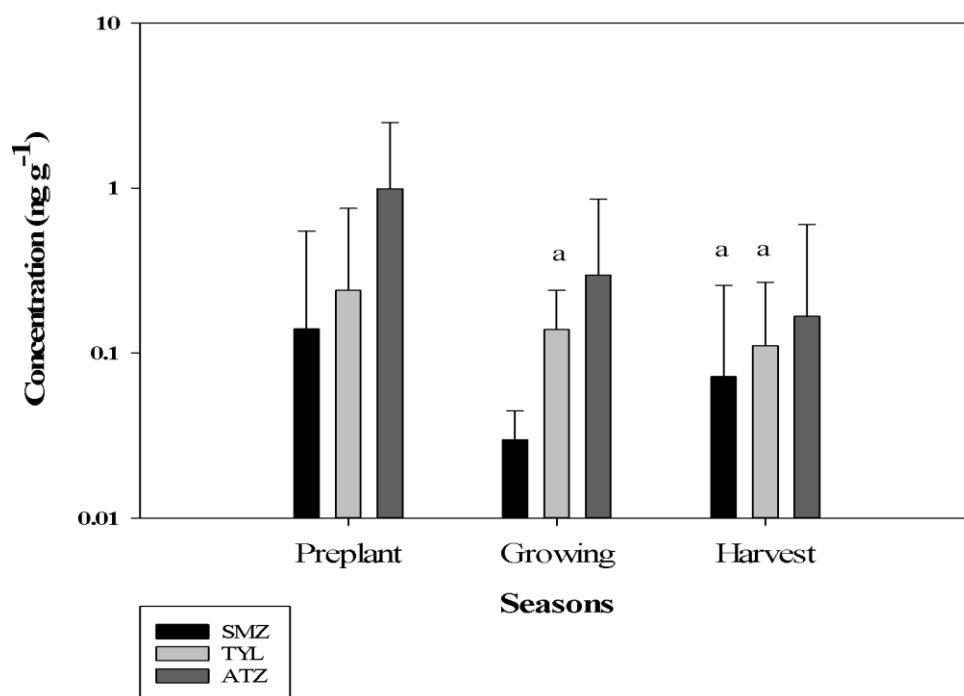


Figure 4.5 Sediment concentrations across the SFIR watershed, based on season from 2013 - 2015. The error bars indicate the standard deviation, seasonal significance ($p < 0.05$) in the Tobit model for analyte concentrations is indicated by the letter (a) above the bar.

4.6.6 Sediment and Water Distribution

Interactions of compounds between the sediment and water matrices are pivotal to understanding their fate and transport in the environment. The sediment-water pseudo-partition coefficient, K_d ($L\ kg^{-1}$) was the calculated ratio for all individual measured discrete sediment and overlying stream grab samples (Kim and Carlson, 2007). We calculated pseudo-partition coefficients because the stream and sediment are not at equilibrium, so it is not a true partitioning coefficient (Kim and Carlson, 2007). The K_d values were fairly variable, TYL had the highest variability ranging from 0.1 – 1,916 $L\ kg^{-1}$, followed by a range of 2.6 – 194.2 $L\ kg^{-1}$ for SMZ, and 0.02 – 194.6 $L\ kg^{-1}$ for ATZ (Table 4.4). TYL exhibited a wider K_d range, which could indicate the constant influence of adsorption and desorption kinetics of TYL on instream sediments. Mean K_d values are much closer to the literature values.

To account for the difference in organic carbon content of the SFIR sediment, we normalized the K_d to the organic carbon content of the sediment K_{oc} . The K_{oc} values show a slightly different pattern than the K_d values, $SMZ < ATZ < TYL$ (Table 4.4). K_{oc} values for all analytes were higher than K_d values, which was the same trend demonstrated in other studies which investigated the fate of antibiotics. Wegst-Urich et al. (2014) suggested that these analytes were less mobile than their K_d values indicated, highlighting the importance of organic carbon content in sediment-water interactions. Hydrophobic partitioning is one of the dominant mechanisms which governs the sorption of organic compounds to organic carbon, by normalizing the K_d value you can account for variation that can occur with the partition coefficient. In comparison, our maximum K_d and K_{oc} values are much higher than the literature values reported in (Table 4.4). Of these values, the only comparable study was by Davis et al., 2006, which examined sediment on a field scale, where the other studies primarily examined soil. In addition, the values from these laboratories studies may be different due to higher non relevant environmental concentrations, the defined duration of equilibration, and possibly greater clay content of soils used in the experiment. The K_d and K_{oc} values in our study, provide a more accurate frame of reference for the fate and behavior of these analytes because they are calculated from environmentally relevant concentrations observed in the SFIR, compared to laboratory derived values.

Sorption of SMZ has been shown to be strongly governed by organic C content in sediment (Carstens et al., 2013). Sorption of TYL, is regulated by mainly clay, organic matter content, and pH (Wang and Wang, 2015; Wegst-Urich et al., 2014). When the pH of a system decreases, TYL sorption increases in sandy loam sediments (Wegst-Urich et al., 2014). ATZ sorption has been correlated to organic C content, organic matter, and clay content (LeBaron, et al., 2008). The impact of sediment organic C on the sorption of TYL, SMZ, and ATZ is shown in (Figure 4.7). The transport of sediment has the ability to improve or deplete the organic carbon content of soil

depending on the total organic content (TOC) of the sediment (Saint-Laurent et al., 2016). The impact of sediment on TOC is important to the fate and transport of organic compounds such as antibiotics.

Table 4.4 *The range of pseudo-partition coefficients, K_d ($L\ kg^{-1}$) based on the detected concentrations from water and sediment samples in the SFIR from 2013 – 2015, mean values are in bold. The K_d was also normalized to the organic carbon content of the sediment, K_{oc} for comparison to other studies with similar sediment and soil textures.*

Analyte	Sediment Type	Kd (Mean)	Koc (Mean)	Literature Sediment/Soil Type	Literature Kd	Literature Koc	Source
TYL	Sand	0.1 - 1,916 (140.6)	0.2 - 16,513 (594.8)	Sand	8.3 - 128	553 - 7990	Rabølle and Spliid, 2000
				Sand	91	-	Kim and Carlson, 2007
				Sand (Sediment)	1061	-	Davis et al., 2006
SMZ	Sand	2.6 - 194.2 (43.7)	3.7 - 693.4 (144.4)	Sandy Loam	1.2 - 3.1	125 - 208	Langhammer, 1989
				Sandy Loam/Loam	0.23 - 3.91	30.4 - 639	Lertpaitoonpan et al., 2009
				Sand (Sediment)	2	-	Davis et al., 2006
ATZ	Sand	0.02 - 194.6 (10.4)	0.1 - 917.4 (32.4)	Sandy Clay	1.88 - 6.09	150.5 - 184.2	Aquino et al., 2013

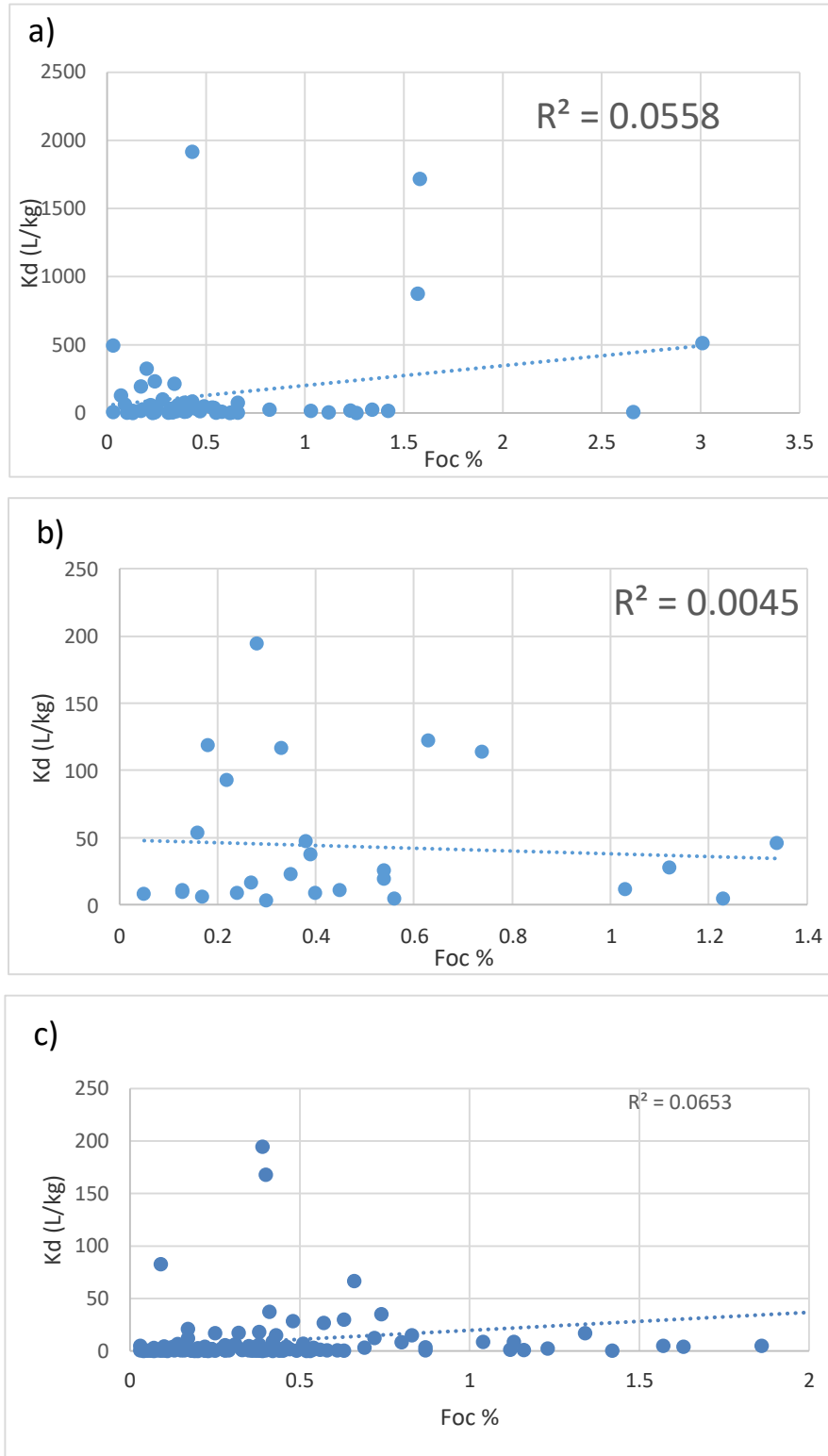


Figure 4.6 The impact of the organic carbon in sediment on the adsorption of a) TYL, b) SMZ, and c) ATZ on an annual basis 2013 – 2015.

4.7 Conclusion

The adsorption of TYL, SMZ, and ATZ in the sediment matrix is important for understanding their environmental fate and transport. The occurrence of TYL, and ATZ were all ubiquitous in SFIR, and exhibited detection frequencies consistent with values seen in literature. Annual trends were observed for TYL and ATZ, while seasonal trends were observed in the SFIR for TYL and SMZ. TYL had a moderately high detection rate of 60% in sediment and yielded the highest concentration amongst the analytes at 0.10 ng g^{-1} . TYL concentrations were shown to have statistical significance in the Tobit regression during growing and harvest seasons, which coincides with a period in which 60% of the total precipitation occurs in the SFIR. Flow conditions indicate TYL sediment concentrations are statistically significant during high flow and moist flow conditions in the watershed. TYL was strongly adsorbed to sediment and its K_d values were in agreement with the literature. The K_d values for TYL and ATZ were found to be weakly correlated to sediment organic C in SFIR sediments. High K_{oc} values indicate these analytes are less mobile than their K_d values indicate, which suggest the importance of understanding their sediment-water interactions, specifically the ability of sediment to act as a sink and a source. Thus, these analyte residues have the potential to remain bioavailable in these sediments, which could pose harm to aquatic organisms and become a source of antibiotic resistance.

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**CHAPTER 5. INVESTIGATING PERSISTENCE AND TRANSPORT OF
ATRAZINE AND VETERINARY ANTIBIOTICS TO A TILE DRAIN SYSTEM
FOLLOWING SWINE MANURE INJECTION**

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J. Morrow.

5.1 Abstract

The injection of swine manure reduces the potential of antibiotic surface runoff but it may facilitate their subsurface instead, making it a cause for concern in the terrestrial and aquatic environment. Tylosin (TYL), sulfamethazine (SMZ), tetracycline (TET), and atrazine (ATZ) concentrations were detected in fields with a history of swine manure application. TYL and TET residues were concentrated at the 0 – 30 cm soil depth, while ATZ was concentrated from 0 – 60 cm. We also observed background concentrations of TET and SMZ before swine manure application. The detection of TYL and SMZ in tile drainage water demonstrates their ability to leach from the surface soil where manure slurry was injected. ATZ residues in tile drainage had a detection frequency of 100%. The detection frequency of these analytes was higher in tile drainage compared to soil samples, but the soil samples had higher concentrations, which would indicate the ability of the soil to act as a source and sink for these analytes. The leaching of these analytes is most likely facilitated by macropore flow and colloidal attachment especially for TYL which is tightly

sorbed. Results of this study show the potential for tile drainage to transport antibiotics from fields receiving manure to surface waters through tile drainage.

Keywords: Tile drainage; POCIS; Leaching, Persistence, Antibiotics; Atrazine

5.2 Introduction

The application of livestock manure to agricultural fields serves as the dominant source of entry for antibiotics into the terrestrial environment. The injection of manure into surface soils is beneficial because it reduces concentrations of antibiotics that are transported by surface runoff and it increases the capture of nutrients for crop production (Kulesza et al., 2016; Chee-Sanford et al., 2012). The disadvantage of manure injection, is that it concentrates antibiotics at surface soil depths which could then potentially be vertically transported through the soil profile to tile drainage or to groundwater. Preferential flow, facilitated by macropores is a major influence on water and chemical transport in subsurface soils (Sloan, 2013; Tomer et al., 2010; Larsbo et al., 2009; and Kumar et al., 1998). Preferential transport of chemicals through macropores is governed by soil type, management conditions, rainfall intensities, and the physiochemical characteristics of the compound (Shipitalo et al., 2000). In addition, swine manure amended soil has been documented to significantly decrease the degradation of ATZ in soil aiding in its persistence (Nordenholt et al., 2014). Long term injection of swine manure could lead to the accumulation of antibiotics in subsurface environments. Soils, which receive manure can potentially act as a sink and source for antibiotics in environmental compartments (Spielmeyer et al., 2017).

The purpose of this research was to quantify antibiotic and pesticide residue concentrations to investigate their subsurface transport and attenuation behavior under tile drained field conditions following swine manure injection. The objectives of this research

was to quantify antibiotic and atrazine concentrations and determine their detection frequencies in surface soil, subsurface soil, and tile drain effluent thereby assessing their mobility in the subsurface. Assessing the impact of injected swine manure on different environmental compartments is important for determining its impact on soil health and water quality.

5.3 Materials and Methods

5.3.1 Site Description

Field data were collected from Iowa State University Northeast Research and Demonstration farm, located near Nashua, Iowa. A portion of the demonstration farm consists of 36 0.4-ha (1 ac) plots used for water quality research. Installation of subsurface drainage for these plots occurred in the fall of 1979 (Malone et al., 2014). Ten-centimeter diameter tile lines run through the center of each plot at a depth of 120 cm with a drainage spacing of 29 m. Border drains were installed to prevent cross flow between individual plots. Each tile drained plot is connected to a sump which houses flow measurement and drainage volume instrumentation, and allows for the collection of water samples. Four water quality plots were selected based on two plot systems, consisting of two tillage practices. Plots 1 and 7 (chisel plow); 2 and 16 (no-till) (Figure 1). All plots are in a corn (*Zea mays*)-soybean (*Glycine max* [L.] Merr.) rotation with fall manure application. Liquid swine manure was injected into the soil on November 14, 2013 at an application rate equivalent to 169 kg N ha⁻¹, roughly 32,644 L ha⁻¹ with injection bands on 76-cm spacing. Manure for this application was obtained from a nearby commercial swine production facility. Details on the commercial swine production facility are provided by Garder et al (2014). The predominant soils series at these four plots are Readlyn, Floyd, and Kenyon, all hydric and poorly drained

(Figure 1). Historical pesticide usage on conventional corn hybrids before the year 2000 included, the herbicide (acetochlor) sprayed during planting. Post emergence management included spraying 0.84 kg ha⁻¹ of ATZ (facility manager, personal communication, 2015). After 2000, ATZ was no longer applied.

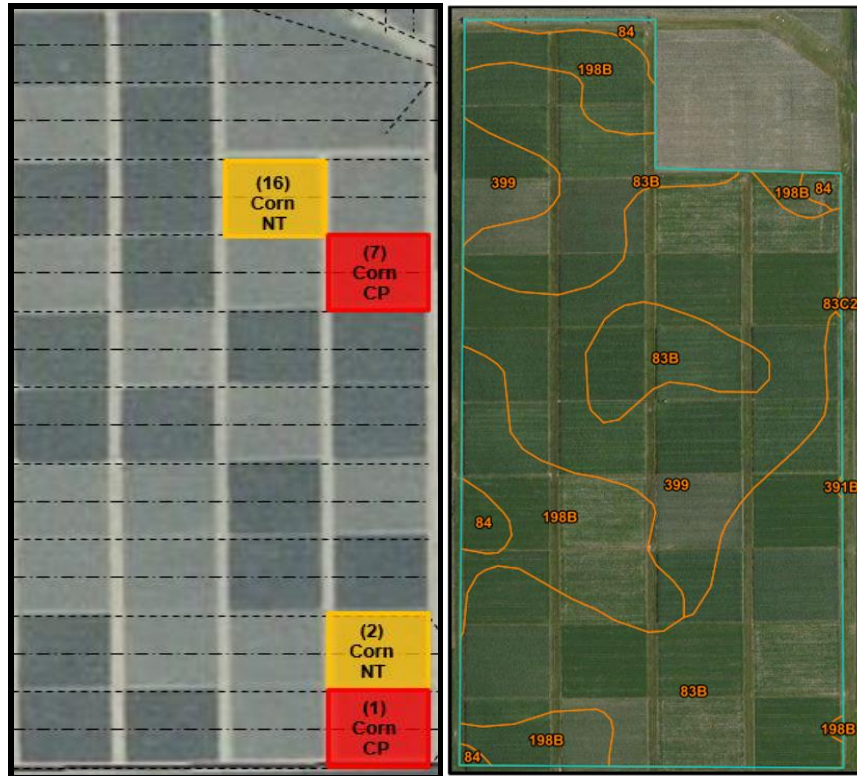


Figure 5.1 Selected Nashua experimental field plots with schematic of tile drain lines and Web soil survey soil map. (83B: Kenyon Loam 2 – 5%, 83C2: Kenyon Loam 5 – 9%, 84: Clyde Silty Clay Loam 0 – 3%, 198B: Floyd Loam 1- 4%, 391B: Clyde-Floyd complex 1 – 4%, 399: Readlyn Loam 1 – 3% slope).

5.3.2 Water Quality Sampling

POCIS and tile water effluent sampling

Polar Organic Chemical Integrative Sampler (POCIS) and grab samples of tile drainage water were collected every two weeks from April 13, 2014 to July 29, 2014, when tile flow stopped at the four sample plots. Duplicate POCIS were installed in the sump of each field plot. POCIS canisters were fabricated from schedule 40, four-inch diameter PVC pipe. Each canister was fitted with two 4-inch round drain grates on opposite ends and pilot holes were drilled in each canister to allow the free flow of water. POCIS were secured within each canister using stainless steel threaded rods, with nuts and washers through the round drain grates (Figure 5.2). POCIS canisters were then anchored outside of each sump.

Grab water samples were collected from the outflow of the tile drain in the sump for each plot. Sampling occurred at two week intervals from April 13 – July 29, 2014. A one-liter flow measurement bottle attached to an extendable pole was used to reach the outflow of the tile drain. Flow rate was measured by recording the amount of time (seconds) it took to fill the 1 liter flow measurement bottle. Flow volume was recorded from flow meters (Gardner et al., 2014). Once the flow measurement bottle was filled, the sample was split and transferred into two 250 mL amber glass sample bottles. Samples were placed on ice and transported to USDA-NLAE and stored at 4° C until analysis.



Figure 5.2 Fabricated POCIS canister, and installation of POCIS at Nashua tile sump.

5.3.3 Manure and Soil Sampling

One-liter liquid swine manure samples were collected from the manure applicator at each field plot, transported on ice and frozen until analysis. On November 5, 2013, 3 composite pre-manure application soil samples (0-15 cm) were taken for each plot. Each composite sample consisted of three subsamples taken along a transect parallel to the drainage line in each plot, providing background levels of analytes. Subsequently, 15-cm soil samples were collected on day 0, day 5 after manure application and then sampled once more on day 212 in the spring. Shortly after day 5, soil temperatures declined and froze. All subsequent 15-cm soil composite samples consisted of 3 samples from manure injection band and 3 inter-band samples from each plot. In addition, 120-cm soil cores were taken on day 1 after manure application on November 15, 2013 using a Giddings soil coring machine. Four cores were taken per plot; 2 from manure band and 2 from the inter-band.

5.3.4 Soil Preparation

Deep soil cores were sectioned off at depths of 0 – 30, 30 – 60, and 60 – 120 cm. Replicates of each core were combined at their corresponding depths for each field plot. Sectioned cores were then air dried and gently crushed, passed through an 8 mm sieve and then through a 2 mm sieve. Sub samples were taken for textural analysis, background AECs, organic C, and pH. These samples were stored at 4°C until analysis. The pH, CEC, OM, OC, and texture results are shown in (Table 5.1).

Table 5.1 Soil management and properties by depth.

PLOT	Treatment	Depth cm	pH	CEC meq/100 g	OM %	Org C %	Texture		
							Sand %	Silt%	Clay%
1	Chisel Plow	0 - 30	6.3	16.9	3.2	1.51	38	42	20
		30 - 60	6.3	12.8	1.5	0.38	47	31	22
		60 - 120	6.6	11.4	0.9	0.2	55	20	25
2	No-Till	0 - 30	6.6	14.4	3.8	1.91	42	41	17
		30 - 60	6.5	14.2	2.1	0.83	40	38	22
		60 - 120	6.7	10.6	0.8	0.22	48	26	26
7	Chisel Plow	0 - 30	5.9	17.7	3.8	2.11	46	38	16
		30 - 60	6.2	16.5	2.5	1.89	42	41	17
		60 - 120	6.8	13.9	1.1	0.26	46	28	26
16	No-Till	0 - 30	6.1	16.6	3.5	1.78	44	40	16
		30 - 60	6.1	16.5	2	1.27	42	34	24
		60 - 120	6.9	10.8	0.8	0.21	60	20	20
	Average	0 – 30	6.2	16.4	3.6	1.83	43	40	17
		30 – 60	6.3	15.0	2.0	1.10	43	36	21
		60 - 120	6.8	11.7	0.7	0.22	52	24	24

5.3.5 Extraction of antibiotics and atrazine

The extraction of tylosin, tetracycline, sulfamethazine, and atrazine from soil samples was conducted using pressure liquid extraction (PLE) following the protocol used by Jacobson et al. (2004). PLE was carried out using an accelerated solvent extractor (ASE) 200 from Dionex. The ASE 200 used 33 mL pressure resistant stainless steel extraction cells lined with glass fiber filters provided by Dionex. Before extraction, all soil samples were sieved to 2 mm and then combined with diatomaceous earth at a 1:1 ratio into each extraction

cell. Diatomaceous earth allowed for a constant extract volume and efficient solvent contact within the pore spaces of the soil particles (O'Connor et al., 2007). The PLE was programmed for two extraction cycles, set at 1500 psi using 30 mL of methanol-0.2 M citric acid (50:50, v:v) followed by another 30 ml of methanol-0.2 M citric acid (50:50, v:v) extraction solvent, collected in the same sample vial. The final volume of extract was approximately 50 mL.

PLE is a highly efficient extraction process, but has the ability to co-extract undesirable matrix components (O'Connor et al., 2007). This requires additional steps in the extraction, including cleaning up the PLE extract using a SAX (strong anion exchange) column in tandem with an Oasis HLB cartridge and dilution of the sample. The SAX column removes humic material from the extract while the HLB cartridge retains the TYL and SMZ. The PLE extracts are then diluted with Milli-Q water until the methanol content was < 10%. Once completed, TYL and SMZ are eluted from the HLB cartridge. Simentone was added to the final extract as an internal standard, followed by evaporation, and reconstitution with ammonium acetate. Samples were then filtered and submitted for analysis.

Manure Extraction

Antibiotics were extracted from manure using the protocol developed by Kolz et al., 2005 and modified by Gardner, 2014. All glass and plastic ware used for antibiotic manure extractions was pre-cleaned by presoaking in acidified methanol, followed by soap and solvent wash. Approximately 30 grams of liquid manure were weighed into 50 mL Polytetrafluoroethylene (PTFE) centrifuge tubes and centrifuged at 12,500 x g and the aqueous supernatant was collected. The samples were then extracted twice more using two solvent solutions; acetonitrile-ammonium acetate (85:15, v:v) and acetonitrile-isopropyl alcohol (95:5, v:v). The solvent extracts were combined and evaporated down to 5 mL using

a Nitrogen evaporator. The aqueous supernatant was then combined with the solvent extract and centrifuged at 12,500 x g for 30 minutes. The combined sample was filter twice, first through a 0.7 μ m filter and then through a 0.45 μ m filter. The clean filtrate was then diluted with 80 ml of milli-q water and passed through Oasis MAX (maximum anion exchange) cartridge in tandem with an Oasis HLB cartridge using SPE. The final extract was eluted with methanol and 0.1 mL of simetone, internal standard was added. Sample was then evaporated down to 0.3 mL and reconstituted to 2 mL with 10 mM ammonium acetate, filtered with a 13 mm, 0.2 μ m pore filter and submitted for analysis.

POCIS Extraction

POCIS extraction procedure was adapted from the protocols used by Alvarez et al., 2004 and Mazzella et al., 2007. POCIS was disassembled and sorbent material was washed with 20 mL of acetonitrile-isopropyl Alcohol (50:50, v:v) into a 60 mL SPE reservoir, fitted with a 20 μ m frit. A second 20 μ m frit was placed on top of the transferred solvent, before elution. The washing solvent was collected and then combined with 100 mL of acetonitrile-isopropyl alcohol to elute the sorbent material. The washing solvent wasn't discarded because testing showed significant amounts of constituents were found in the solution. Once the 120 mL of solvent was eluted, 1.0 mL of 5% Na-EDTA was added along with .250 μ L of simetone as an internal standard. The combined extract and wash was then evaporated down to 0.2 – 0.3 mL. Extract and wash were reconstituted to 5 mL using 10 mM ammonium acetate and allowed to reach equilibrium for approximately 30 minutes. After equilibrium, samples were filtered and submitted for analysis. Extraction efficiency test were performed on each POCIS sample. POCIS residues from the SPE reservoirs were placed in 100 mL beakers and filled with 60 mL of solvent. Each residue sample soaked for 24 hours, extract

and wash was collected, and .125 µl of internal standard was added. Extract and wash were evaporated down to 0.2 mL, reconstituted and to 2 mL and submitted for analysis as described above. We assume POCIS plus the POCIS residue to be the total extracted, so extraction efficiency would be the difference between the two. POCIS samples were quantified based on sampling rates developed by Washington et al. (2017).

Tile Water Extraction

Tile samples were first processed by filtering 250 mL of sample through 0.45 µm nylon filter, eliminating particulate matter. The extraction procedure was adapted from Lindsey et al. (2001) and further modified by Yang and Carlson (2003). After filtering, 1.0 mL of 5% Na-EDTA was added to the 250 mL water sample. Na-EDTA was used as a metal complexing agent to bind the divalent cations in the sample which interfere with the extraction process. Samples were then eluted through Oasis HLB solid phase extraction (SPE) cartridges with acetonitrile-isopropyl alcohol. Simetone was used as an internal standard for the grab samples and extracts were evaporated down, reconstituted, filtered and submitted for analysis.

5.3.6 Analysis of Analytes

Analysis was performed using an ABSciex 5500 QTrap mass spectrometer with an Agilent 1260 Infinity LC on all environmental samples. All sample extracts were analyzed for SMZ, TET and ATZ. The instrumental limit of detection (LOD) and limit of quantification (LOQ) were determined for each analyte for soil, manure, POCIS and grab samples (Table 5.1). Compounds were monitored using multiple reaction monitoring (MRM), with 3 stages collected for each (European Standard EN 1662, 2008). A more detailed analytical analysis is provided by Washington et al., 2017.

5.4 Statistical Analysis

Statistical analysis was conducted using a two-stage approach. The non-parametric Mann-Whitney rank sum test was used to compare analyte concentrations between no-till and chisel plow managements for tile drainage, surface soil, and subsurface soil samples. Across all matrices, median analyte concentrations were not statistically significantly different between managements, therefore no-till and chisel plow data were combined for each analyte. Next, a Tobit censored regression analysis approach was used to account for censored data (non-detects) as described by Washington et al. (2017). The rank sum approach heavily depends on the ability to accurately rank all measurements, but censored observations (non-detects) generally prevent a complete ranking because they are treated as tied values (USEPA, 2009). The Tobit model identifies the regression coefficients that

Table 5.2 Instrumental and Matrix LOD LOQ for and selected analytes.

Analyte	Instrumental		Grabs		POCIS		Sediment	
	Limit of Detection (ng mL ⁻¹)	Limit of Quantification (ng mL ⁻¹)	Limit of Detection (ng L ⁻¹)	Limit of Quantification (ng L ⁻¹)	Limit of Detection (ng L ⁻¹)	Limit of Quantification (ng L ⁻¹)	Limit of Detection (ng g ⁻¹)	Limit of Quantification (ng g ⁻¹)
SMZ	0.041	0.04	0.000328	0.00032	0.000163	0.000159	0.0055	0.0053
TYL	0.044	0.04	0.000352	0.00032	0.00000965	0.000009	0.0059	0.0053
TET	0.313	0.31	0.002504	0.00248	-	-	0.0209	0.0207
ATZ	0.027	0.03	0.000216	0.00024	0.0000957	0.000106	0.0036	0.0040

contribute significantly to the over-all model that describes analyte concentrations and accounts for censoring of the dependent variable, y (analyte concentration), where:

- 1) $y = \text{management} + \text{band} + \text{date}$ (15 cm surface soil)
- 2) $y = \text{soil depth} + \text{management}$ (deep core soil)
- 3) $y = \text{date} + \text{management}$ (tile drain effluent)

Significant differences for all comparisons were evaluated at $p < 0.05$, and statistical analysis was performed using SAS 9.4.

5.5 Results and Discussion

5.5.1 Occurrence of analyte concentrations in soil and tile drain effluent

Analytes were detected in surface soil, deep core soil, and tile drain effluent. The combined detection frequency and median concentrations for analytes are shown in Figure 5.3, including interband surface soil samples. Based upon initial statistical analysis using the Mann-Whitney rank sum test there was no statistically significant difference between analyte concentrations under NT and CP management ($p > 0.05$) in surface soil, subsurface soil, and tile drain effluent. All analytes were detected in surface soil samples. The detection frequency indicated $\text{ATZ} > \text{TYL} = \text{TET} > \text{SMZ}$ in surface soils. Median concentrations ranged from $0.330 - 5.113 \text{ ng g}^{-1}$ for analytes in surface soils. After combining the data for the two tillage managements, higher detection frequencies of TYL, TET, and SMZ were obtained in the manure band (MB) compared to inter band IB samples. ATZ was the only analyte with a higher detection frequency in IB (97%) samples compared to MB (67%). Due to this, we focused primarily on our MB (0 – 120 cm) core soil samples during analysis. In these 120-cmcore samples only TYL, TET, and ATZ were detected. Examining the combined managements, we determined the detection frequency of all analytes was as

follows ATZ > TYL > TET > SMZ. In tile drain effluent, ATZ was ubiquitous, followed by the detection of TYL and SMZ. TET was not detected in the tile drain effluent.

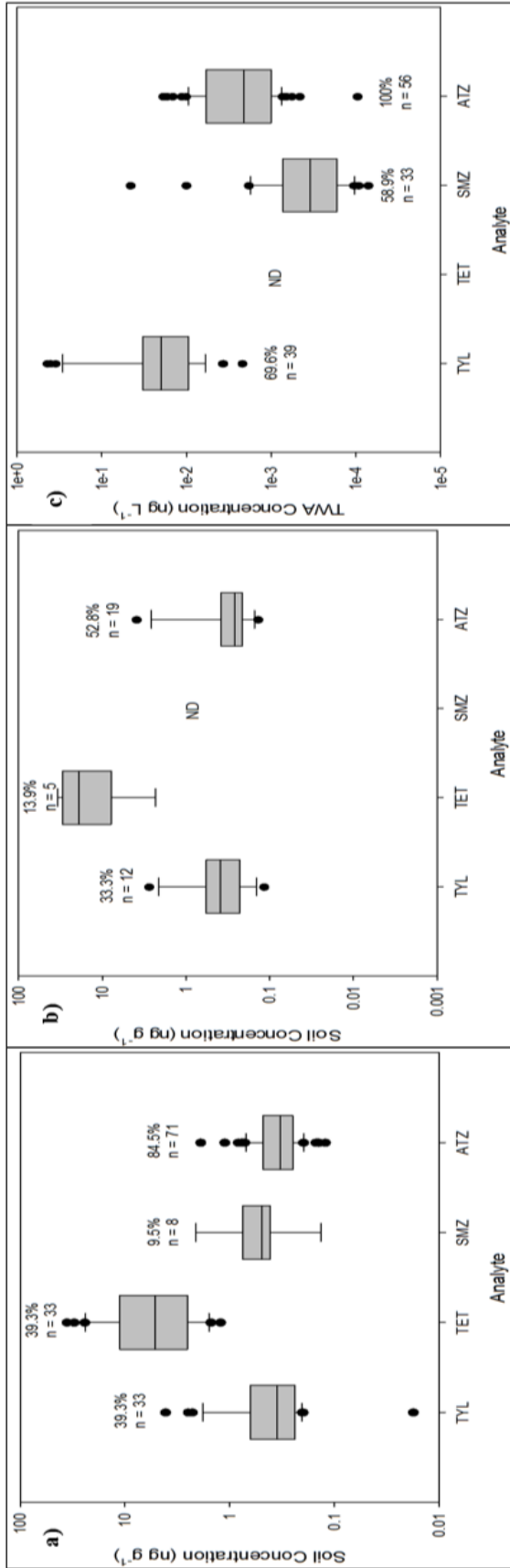


Figure 5.3 Median concentrations and detection frequencies of manure band tylosin (TYL), tetracycline (TET), sulfamethazine (SMZ) and atrazine (ATZ) in **a)** surface soil (0-15 cm depth), **b)** deep core soil (0 – 120 cm depth), and **c)** tile drain water.

5.5.2 *Properties of soils and manures*

Soils from the selected Nashua field plots are classified predominantly as Readlyn, Kenyon, and Floyd loams (Figure 5.1). Based on the CEC content of these soils, their clay mineralogy reveals the dominant clay species is kaolinite, which is consistent with our CEC results (Dr. Thompson, personal communication, 2016). The CEC, OM, and OC all decrease with soil depth. The pH varies slightly and increases with depth (Table 5.1). The mean pH for the soils is slightly acidic at 6.4.

Swine manure slurry was applied at mean rate of 169 kg N ha⁻¹. Mean slurry concentrations for TYL, TET, and SMZ were 0.286 ng g⁻¹, 4.169 ng g⁻¹, and 0.029 ng g⁻¹, on a dry weight basis, respectively. Swine manure TYL in manure has shown the ability to degrade during slurry storage or once applied to agricultural fields (Kolz et al., 2005; Kay et al., 2004, 2005; Aust et al., 2008; Sassman et al., 2007). Kolz et al. (2005) attributes the dissipation of TYL from swine manure to abiotic and biotic degradation, and to irreversible degradation processes.

5.5.3 *Tobit censored regression analysis*

From the parameter estimates of the Tobit model, the sigma parameter measures the estimated standard error of the regression, which is then compared to the standard deviation of the dependent variable, y , indicating if there is statistical significance in the model parameter estimates (Washington et al., 2017). Based on the sigma values, the model fit for the Tobit was statistically significant for all analytes (TYL, TET, SMZ and ATZ) for surface soil, deep core soil samples and POCIS samples (Appendix 5). To further quantify model fit, the Pearson product-moment correlation coefficient was estimated for predicted concentrations versus actual concentrations, which showed statistical significance except for

the surface soil SMZ model. The model fit was further improved for each analyte with the modeling of interactions, which provided a more detail assessment of model variables.

5.5.4 Transport of analyte concentrations in soil and tile drain effluent

Tylosin in soil and tile drain effluent

The highest concentration of TYL occurred at the 0 – 30 cm soil depth (Figure 5.4), where its detection frequency was 75%. Detection of TYL at subsequent depths only accounted for 8.3% respectively. Tobit analysis determined there was a statistical significance for TYL soil concentrations at a 0 – 30 cm depth ($p = 0.05$). Below 30 cm depth, TYL was only detected twice and concentration decreased with depth. According to Sassman et al. (2007) the strong adsorption behavior of TYL in soil, will mitigate its mobility, trapping it in the top soil. At the Nashua, IA field sites, Garder et al. (2014) detected TYL in 15 cm surface soil samples and reported two-year mean concentrations ranging from 0.57 – 1.17 ng g⁻¹. Others have detected none or minute concentrations of TYL in surface or subsurface soil depths (Aust et al., 2008; Kay et al., 2004, 2005). Due to the low concentrations observed at the Nashua site, Garder et al. (2014) concluded, that TYL has little to no risk of accumulating in soil. Kay et al. (2004) found no TYL in surface or subsurface soils in tile drained clay soils. Lysimeter work (Kay et al., 2005) indicated TYL did not leach into tile drainage. This research has been supported by several other studies which indicate no detection of TYL in tile drain effluent (Frey et al., 2015; Kay et al., 2004). Contrary to these results, TYL was found in leachate from a column leaching study by Hu et al. (2009). The limitation of that study, is that soil cores were only at a depth of 30 cm, which is the depth in which previous studies indicate TYL should be present. Nevertheless, the Hu et al. (2009) study concluded that the conditions which make TYL susceptible to

leaching include soils with high sand content, low OM, and low CEC. TYL was detected in 69.6% of tile drain effluent samples in our study in Nashua soils. The low OM and CEC of the 60 – 120 cm soil (Table 5.1), suggests further leaching of TYL is likely, once it travels through the top 30-cm of soil. More recently, TYL was detected in tile drain effluent at Nashua at the plot scale by Garder et al. (2014) and at the watershed scale (Washington et al., 2017).

Tetracycline in Soil and Tile Drain Effluent

TET had the highest median concentrations amongst all analytes, with a combined detection frequency of 13.9%. TET primarily was detected at a 0 – 30 cm soil depth with one detection each in the subsequent depth increments (Figure 4). The Tobit analysis indicates TET concentrations are statistically significant at the 0 – 30 cm soil depth, ($p = 0.0423$). Our results are in agreement with the literature indicating TET tends to stay in surface soil depths of 0 – 30 cm but can infiltrate down to 40 cm (Sollicec et al., 2016). Sorption behavior of TET, indicates it is strongly bounded to the solid phase (Kim et al., 2010; Avisar et al., 2009). TET adsorption is driven by organic matter content, cation exchange capacity (CEC), and pH. This affinity to strongly adsorb to soil explains why TET was primarily detected in 0 – 30 cm depth soils where swine manure injection occurred. It has a moderately low potential to leach in the subsurface (Pan and Chu, 2017; Avisar et al., 2009). Even with its low potential to leach, TET has been documented in tile drain effluent (Sollicec et al., 2016; Frey et al., 2015). However, in our study, there was no detection of TET in tile drainage effluent.

Sulfamethazine in soil and tile drain effluent

There was no detection of SMZ, at any depth in the Nashua 0 – 120 cm soil cores. The physiochemical properties of SMZ indicate a weak sorption behavior and it is very water soluble and mobile in soil. Due to this mobility, SMZ has the potential to move deep into the soil profile and leaching has been demonstrated in several studies (Srinivasan and Sarmah, 2014; Unold et al., 2009). The lack of SMZ detection could be related to the low concentrations of SMZ in the swine manure. Degradation processes could also account for not detecting any SMZ in the soil. Research by Fan et al. (2011) showed 33 – 70% of SMZ was degraded within a 6 hr time-period during their transport experiment. The soil pH is influential in SMZ fate and transport in the environment (Aust et al., 2008; Lertpaitoonpan et al., 2009; Wegst-Urich et al., 2014). In the Nashua soils, the range of pH of the soils increase with depth to pH 6.2 – 6.8. These pH values were below the pKa of 7.4 for the anionic form of SMZ, and as a result SMZ should be in the neutral form and more tightly adsorbed to the soil than in the surface soil, limiting its mobility into the deeper soil depths. The non-detection of SMZ in soil cores could be due to the low initial concentration in the swine manure or the variability of sampling along the manure band.

Atrazine in soil and Tile Drain Effluent

ATZ was detected throughout the soil profile, showing its mobility in soil. The detection frequency of ATZ decreases with depth, where 60 -120 cm < 30 – 60 cm < 0 – 30 cm (Figure 4). Tobit analysis indicated both the 0 – 30 and 30 – 60 cm depths were statistically significant for the model of ATZ concentrations, with $p = 0.0033$ and $p = 0.0031$, respectively. Soil concentrations ranged from 0.135 – 3.816 ng g⁻¹. ATZ was leached through the subsurface into tile drainage and had a detection frequency of 100% in tile drain

effluent in our study, with concentrations ranging from 0.00085 - 0.321 ng L⁻¹. ATZ has shown the ability to persist in soil and water long after application. At the time of sampling, ATZ had not been used at Nashua in over 10 years. A study by Vonberg et al. (2014) showed ATZ residues were detected in surface soil, subsurface soil, and groundwater after a 21-year ban. Earlier research by Buhler et al. (1993), detected ATZ residues in tile drainage after long-term use showing its ability to persist for years. The fate and mobility of ATZ is governed by degradation and sorption processes, furthermore preferential flow in macropores may cause rapid transport of pesticides in subsurface soils (Arias-Estevez et al., 2008; Fox et al., 2004).

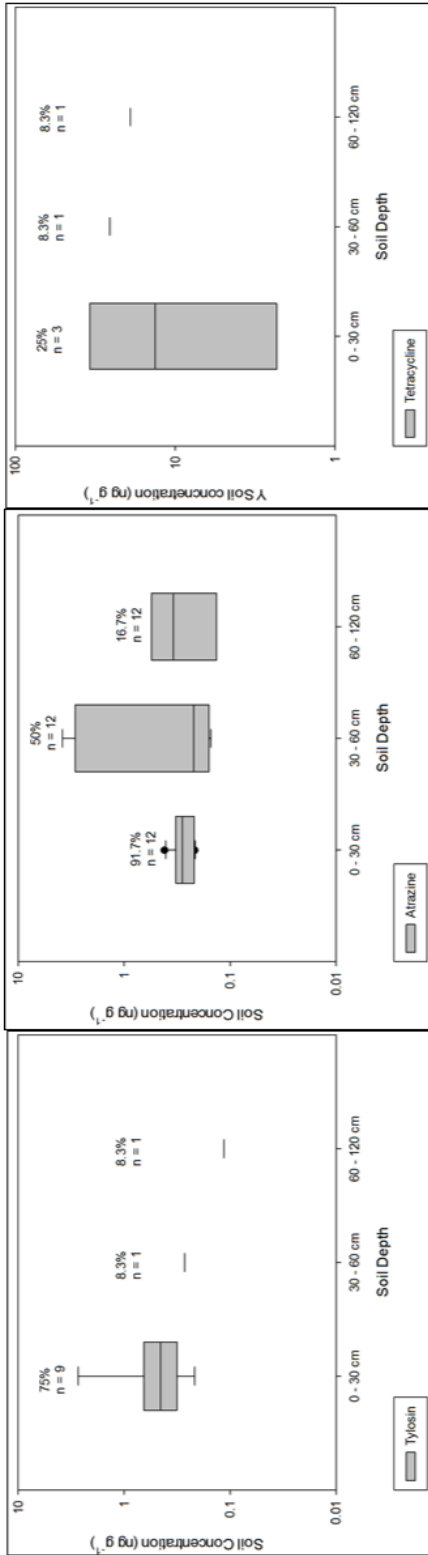


Figure 5.4 Analyte concentration ranges and their detection frequency at each soil depth.

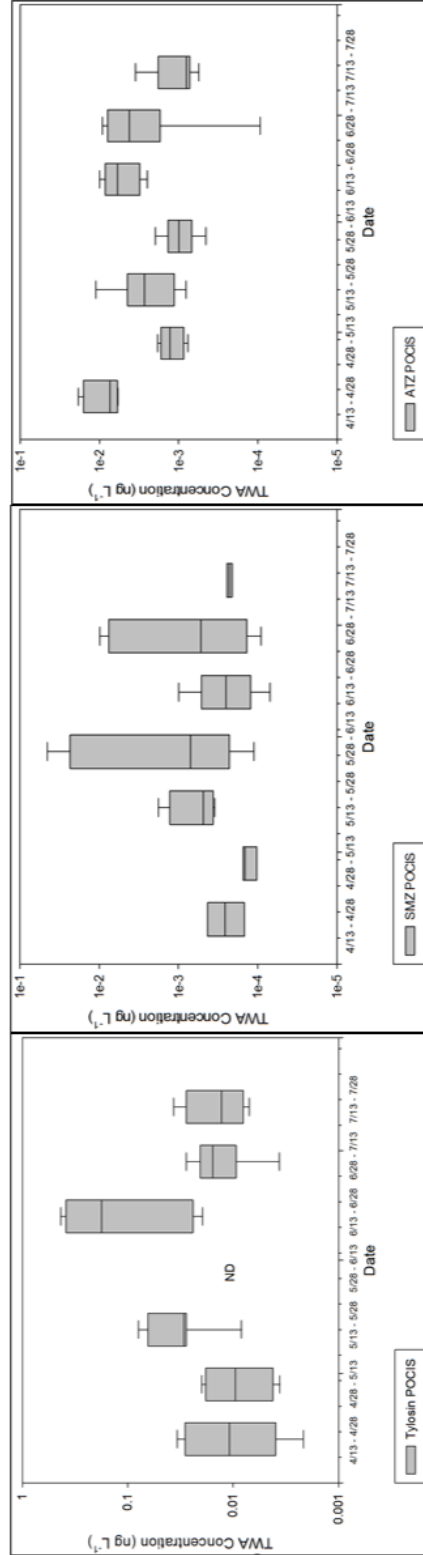


Figure 5.5 Tile drainage effluent concentrations of analytes over the course of the sampling season.

5.5.5 Dissipation of analyte concentrations in soil and temporal trends in tile drain effluent

From our soil data, all analytes were concentrated in the 0 – 30 cm depth, except for SMZ which was not detected. All analytes except for TET were then detected in tile drain effluent. The transport of these analytes, which were primarily at the 0 – 30 cm depth could be explained by leaching or by colloidal facilitated transport through macropores. Colloidal facilitated transport is connected to manure, which serves as a reservoir for suspended colloidal material and plays an important role in facilitating the transport of contaminants in the soil profile (Zou and Zheng, 2013; Unold et al., 2009). Based, on this, we investigated the fate of these analytes by examining their change in surface soils (15 cm) and tile drain effluent. Box plots of analytes demonstrate their dissipation pattern with respect to time (Figure 5.5 – 5.6).

The estimated mean surface soil (0 – 15 cm) concentration from the Tobit model for TYL was 0.266 ng g^{-1} . Surface soil concentrations for day 155 were found to be statistically greater by the Tobit model ($p < 0.0001$) (Figure 6). This coincides with the period after the soil thawed from winter. The inactivity of biological degradation processes from winter into late spring are the likely cause of high TYL soil concentrations. Tile drain effluent had lower mean concentration, 0.04 ng L^{-1} . TYL showed an overall steady trend in concentration, where median values were between $0.01 - 1 \text{ ng g}^{-1}$ over the four-month sampling season (Figure 5.5). There was an increase in concentration the two-week period of 6/13 – 6/28. The Tobit model showed this period was statistically significant for TYL tile effluent concentrations ($p = <0.0001$). This period coincides with the highest cumulative precipitation total of the season, 202.4 mm (Appendix 3).

TET surface soil concentrations were statistically significant for pre manure application soil samples (11-5-13), one day after manure application (11-15-13), and during the spring (4-

17-14), 212 days after manure application ($p < 0.0001$). Detection of pre-manure TET, indicates its persistence from the previous manure application, indicating an ability to accumulate in surface soils. The highest TET concentrations were measured in surface soils one day after slurry manure application, followed by no detection five days after. Concentrations in spring show slight degradation from levels measured one day after application (Figure 6). In our study, TET was tightly bound in the 0 – 30 cm soil depth and was not detected in tile drain effluent, indicating no leaching occurred.

A total of 4 samples of SMZ were detected in the surface soil samples accounting for 5.5% detection. Tobit analysis indicates statistical significant concentrations for pre-manure application soil samples, day 1 and day 5 after manure application ($p < 0.0001$). SMZ surface soil concentrations remained relatively the same throughout the sampling season (Figure 6). Tile drain effluent concentration of SMZ show relatively no trend, remaining between 0.001 – 0.0001 ng L⁻¹. SMZ tile effluent concentrations were statistically significant during 5/28 – 6/13 two-week period ($p = 0.0483$). This period coincides with a cumulative precipitation of 16.7 mm, but is followed by the start of a larger 202.4 mm precipitation period.

Background surface soil ATZ concentrations are higher than concentrations measured during manure application period and into the spring. In a study by Nordenholt et al. (2016), swine manure application was shown to decrease the degradation of ATZ, due to the altering of the soil microbial community structure. ATZ shows a slight decrease in concentration during this time. ATZ tile effluent follows the trend of TYL where statistically significant concentrations occur during the 202.4 mm precipitation ($p < 0.0001$). No discernable trend is observed with ATZ tile effluent degradation, but concentrations are between 0.01 – 0.0001 ng L⁻¹ (Figure 5.5).

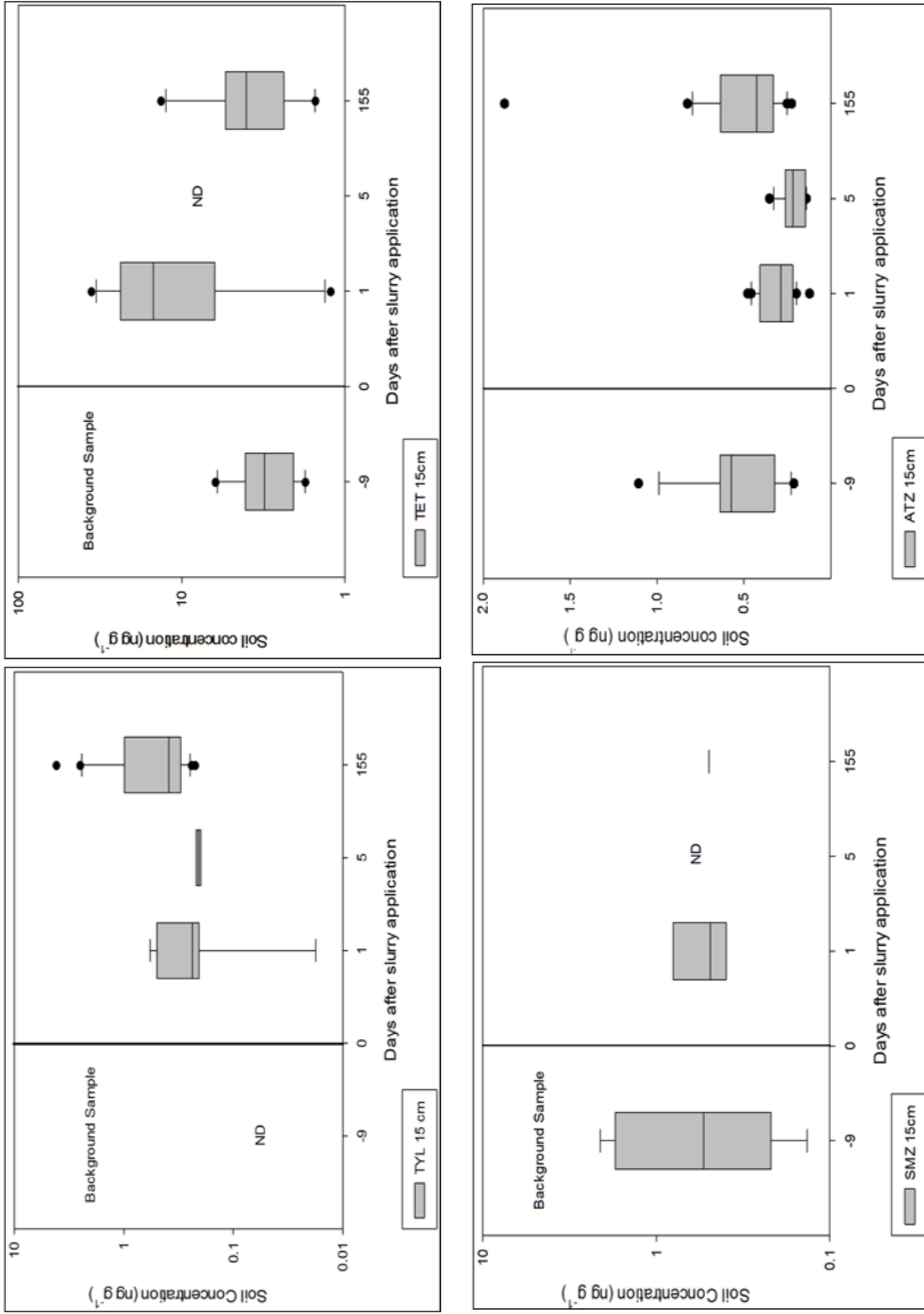


Figure 5.6 Surface soil analyte concentrations days after slurry application.

5.6 Conclusion

TYL, SMZ, TET, and ATZ residue concentrations were detected in fields with history of swine manure application. TYL and TET soils residues were concentrated at the 0 – 30 cm soil depth, while ATZ was concentrated from 0 – 60 cm. The detection of these analytes in tile drainage water indicate their ability to leach from the surface soil where manure slurry was injected. The leaching of these analytes is most likely facilitated by macropore flow and colloidal attachment especially for TYL, which was tightly sorbed. Precipitation patterns also showed the potential to aid in the transport of TYL, SMZ, and ATZ. Results of this study also show the potential for tile drains to transport antibiotics from the terrestrial to the aquatic environment. This study does not take into consideration the full impact of long term swine slurry application on the occurrence, fate and transport of these antibiotics, only providing a snapshot based upon one sampling season. These soils have received repeated applications of swine manure and the detection of atrazine long after atrazine was applied illustrates the potential for longer term processes to possibly govern antibiotic transport and persistence as well as atrazine. Thus, the findings of this study point out the difficulty in understanding the behavior of these analytes in the environment.

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CHAPTER 6. GENERAL CONCLUSIONS

6.1 Review of Central Themes

The central theme of this dissertation was the monitoring of antibiotics and atrazine in agricultural fields under tile drainage, using passive sampling technology. The fate and transport of antibiotics is a rising concern due to their subtherapeutic use which has potentially been linked to the development of antibiotic resistant genes (ARGs) in the terrestrial and aquatic environments. Having the ability to monitor these compounds at these subtherapeutic levels and understanding their physiochemical properties is vital to providing insight of their behavior in the environment and to providing relevant environmental data for further antibiotic resistance research.

This dissertation describes three studies undertaken to improve our understanding of the fate and transport of these analytes under tile drained conditions. The first two studies used a reconnaissance approach to monitor the SFIR. The first study was focused on the use of POCIS and grab samples to quantify the dissolved phase concentrations of analytes. The second study focused on quantifying the sorbed phase of these analytes on instream sediments. TYL and SMZ were detected at frequencies from 70 to 83% in water and 42 to 60% in sediment at four locations during preplant, growing, and harvest seasons. Time weighted antibiotic concentrations were less than 2 ng L^{-1} and were substantially less than atrazine concentrations. Direct sampling of subsurface drainage water showed that antibiotics are leaching through the soil profile. TYL sediment concentrations were significant during growing and harvest seasons, coinciding with a period that accounted for

60% of the total precipitation occurring in the SFIR and flow conditions were influential to TYL sediment concentrations.

The third and final study focused on the behavior of antibiotics and atrazine in tile drainage on a field plot scale. This study showed, TYL, TET, SMZ, and ATZ were all found in field plots. TYL and TET were tightly bound and concentrated in surface soils. Sampling of subsurface drainage showed detections of TYL, SMZ, and ATZ. The detection of TYL and SMZ shows they leached through the soil profile, similar to results observed in the first study. ATZ was detected in soil and subsurface drainage water, showing its persistence in the absence of application, where its use was discontinued at the field site. These findings show the role of subsurface drainage in the transport of antibiotics and atrazine to surface waters. In addition, antibiotic and atrazine residues sorbed to sediment, identify it as an important matrix in the fate and transport of antibiotics and atrazine.

6.2 Implications of Research

The injection of swine manure in surface soils provides entry of antibiotics into the environment, reducing the amount that exposed to the surface environment thereby reducing surface transport. But, the adverse implication of manure injection is that these antibiotics can still be transported into subsurface drainage and therefore into surface water. The identification of tile drains as a route of transport for antibiotics, Our findings indicate antibiotic residues were primarily concentrated at a 0 – 30 cm depth. The detection of antibiotic residues in subsurface drainage, highlighted the ability of these compounds to leach through the soil profile. This study also provides baseline WQ data on antibiotics for the SFIR which could help with future antibiotic fate and transport research and inform future policy and regulations. Annual and seasonal trends were observed for the analyte

concentrations. By identifying these temporal trends, we can focus on the environmental conditions and land management techniques which influence their persistence in the environment.

6.3 Recommendations for Future Research

In this dissertation, we were able to successfully monitor analytes in the dissolved phase using POCIS samplers in surface and drainage waters. We also identified leaching as a key transport mechanism for antibiotics in subsurface soils and tile drainage as a key transport mechanism into subsurface water. However, there are still some limitations of this research in regards to monitoring and understanding the occurrence, fate and transport of antibiotics. Here are some recommendations for future research:

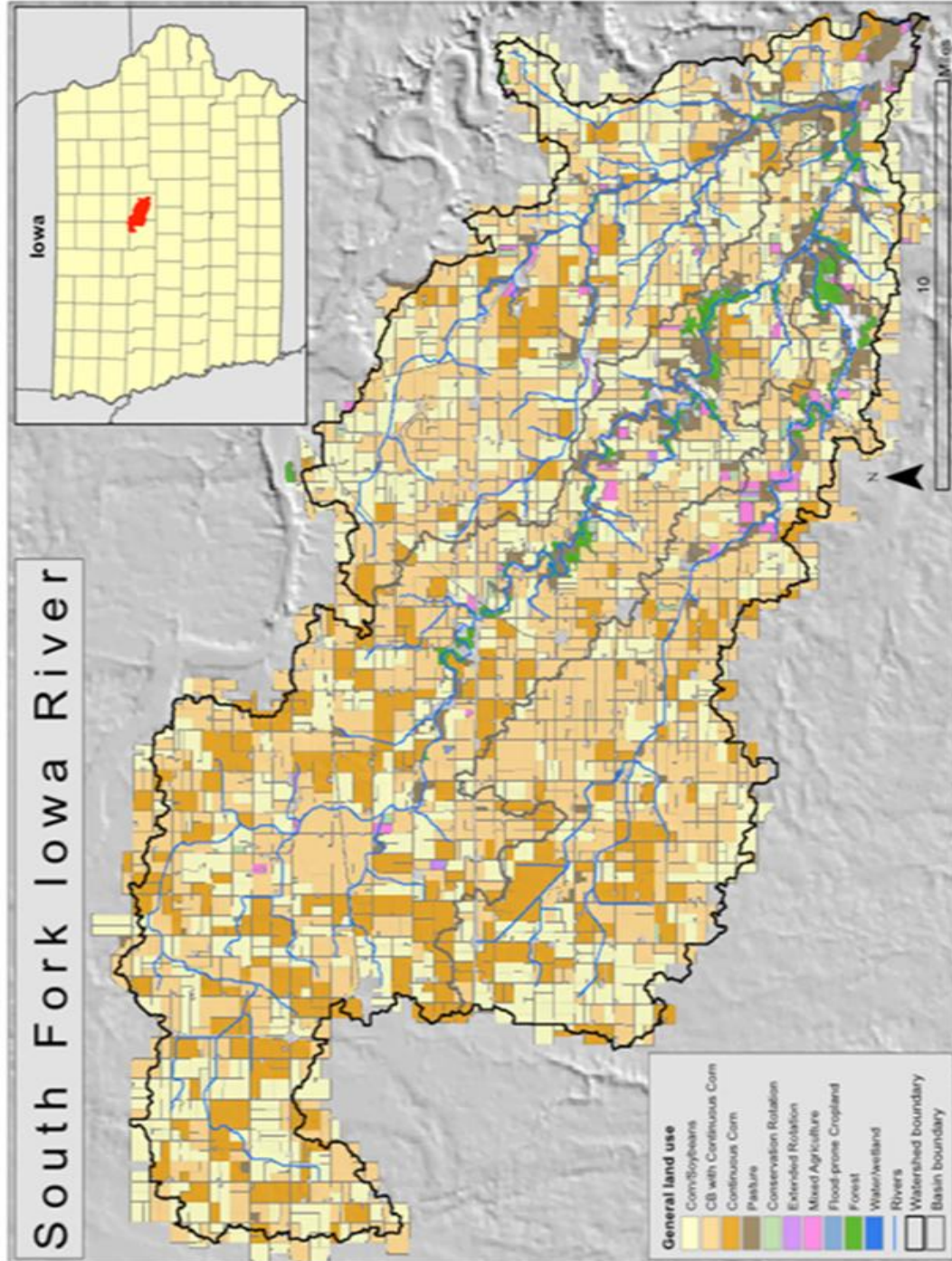
- POCIS samplers proved to be an effective tool in monitoring and detecting analytes at environmental relevant concentrations. The POCIS had lower detection capability and higher detection frequencies for all analytes. POCIS samplers provided TWA concentrations for analytes over a selected 2 week time duration. I would recommend the use of automatic samplers in tandem with the POCIS, which would allow for more specific identification of precipitation events. This approach would provide time weighted and flow weighted monitoring information.
- POCIS provide semi-quantitative data because of the variability in sampling rates. I would conduct an in-situ calibration experiment to derive sampling rates custom for the SFIR.
- We provided background information on many sources of agricultural sediment. Identifying the particle size distribution of these sediments and its

impact on sorption to antibiotics would helpful in understanding their fate and transport

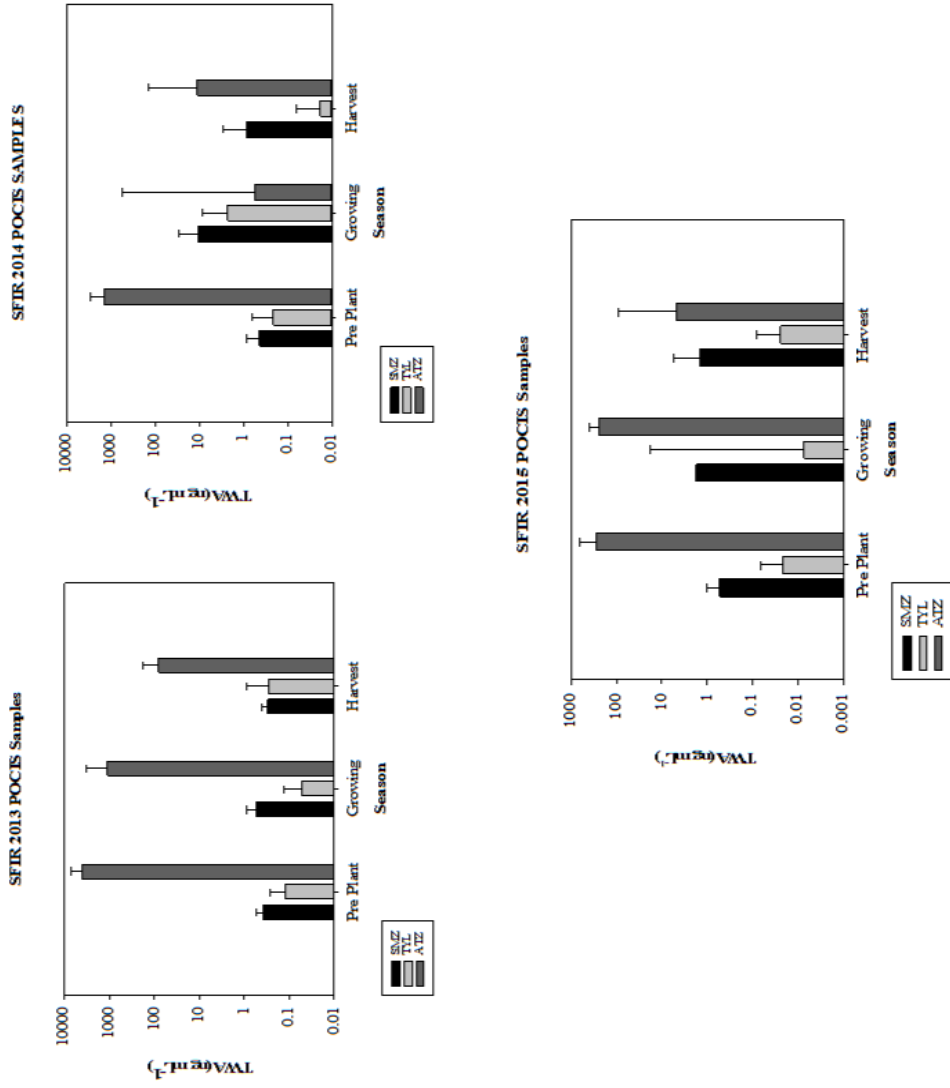
- Our field study did not take in consideration the long-term impact of swine manure application on the fate and transport of antibiotics and atrazine. A long-term study lysimeter study and soil sampling would be informative.
- When monitoring emerging contaminants, we often deal with censored data points. Further investigating the Tobit Model and other statistical techniques would provide insight on additional models that can be used for statistical analysis.

APPENDIX A

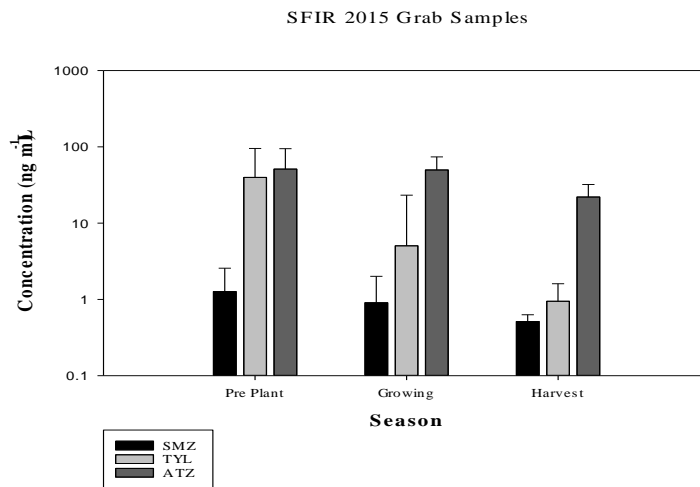
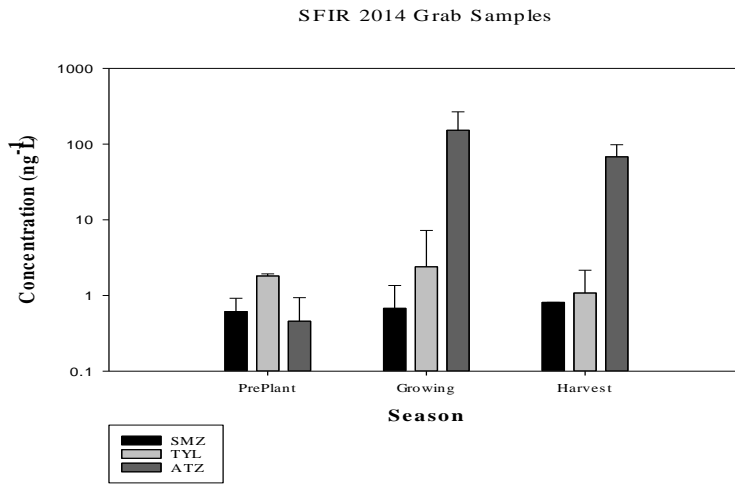
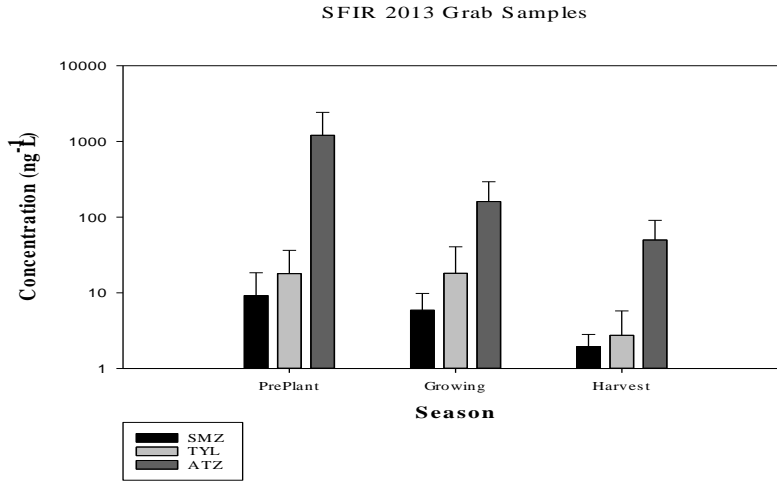
CHAPTER. 3 SUPPLEMENTAL DATA



SI Figure 1. Map of Land use of the South Fork Iowa River watershed of the Iowa River (SFIR). Color shading denotes land use. The map inset shows the extent of the SFIR watershed boundary in central Iowa.



SI Figure 2. Annual SFIR POCIS TWA concentrations (2013 – 2015). Error bars are presented as the standard deviation of the sample.



SI Figure 3. Annual SFIR Grab sample concentrations (2013 – 2015). Error bars are presented as the standard deviation of the sample.

SI Table 1. SFIR watershed POCIS detection frequencies for 2013 – 2015.

SFIR 2013 POCIS Detection Frequencies

Preplant Season (n = 6)				Growing Season (n = 6)				Harvest Season (n = 6)			
Site	SMZ	ATZ	TYL	Site	SMZ	ATZ	TYL	Site	SMZ	ATZ	TYL
241	100	100	100	241	100	100	100	241	100	100	100
323	100	100	100	323	100	100	83.3	323	83.3	100	100
350	100	100	100	350	100	100	66.7	350	83.3	100	100
450	100	100	100	450	100	100	66.7	450	50	100	100

SFIR 2014 POCIS Detection Frequencies

Preplant Season (n = 8)				Growing Season (n = 10)				Harvest Season (n = 10)			
Site	SMZ	ATZ	TYL	Site	SMZ	ATZ	TYL	Site	SMZ	ATZ	TYL
241	100	100	50	241	100	100	100	241	100	100	10
323	75	100	62.5	323	100	100	50	323	83.3333	100	41.6667
350	12.5	100	50	350	50	100	75	350	50	100	16.6667
450	50	100	50	450	90	100	50	450	50	100	8.33333
Detection Frequencies (%)				*241 only 2 reps* Detection Frequencies (%)				Detection Frequencies (%)			

SFIR 2015 POCIS Detection Frequencies

Preplant Season (n = 10)				Growing Season (n = 10)				Harvest Season (n = 12)			
Site	SMZ	ATZ	TYL	Site	SMZ	ATZ	TYL	Site	SMZ	ATZ	TYL
241	100	100	90	241	100	100	100	241	100	100	80
323	100	100	50	323	90	100	88.9	323	91.7	100	50.0
350	40	100	60	350	70	100	70.0	350	50	100	58.3
450	70	100	50	450	100	100	60.0	450	100	100	70.0
Detection Frequencies (%)											

SI Table 2. Tobit Regression Model Parameter Estimates for POCIS

Parameter	SMZ Censored at 1.63E-5 ng L ⁻¹	P-value	TYL Censored at 9.65E-6 ng L ⁻¹	P-value	ATZ No Censoring	P-value
Intercept	-2.13051 (1.170291)	0.0687	-1.183496 (0.505903)	0.0193	1346.842198 (249.7384)	<.0001
Site						
241	2.196570 (1.177351)	0.0621	-0.182371 (0.509552)	0.7204	-731.383906 (258.88809)	0.0047
323	0.077784 (1.149740)	0.9461	-0.146444 (0.490626)	0.7653	-125.310228 (248.567905)	0.6142
350	-4.713769 (1.251878)	0.0002	-0.694056 (0.504736)	0.1691	4.066627 (250.314315)	0.987
450	0 .	.	0 .	.	0 .	.
Year						
2013	-0.725826 (1.169477)	0.5348	1.539259 (0.463497)	0.0009	2227.911049 (247.060386)	<.0001
2014	2.826082 (0.961719)	0.0033	0.553909 (0.409644)	0.1763	478.630123 (200.501239)	0.017
2015	0 .	.	0 .	.	0 .	.
Season						
Growing	5.60128 (1.122245)	<.0001	0.682676 (0.450448)	0.1296	-1053.512058 (234.646882)	<.0001
Harvest	0.916164 (1.084954)	0.3984	-0.561636 (0.450558)	0.2126	-1653.381540 (222.730018)	<.0001
Preplant	0 .	.	0 .	.	0 .	.
Sigma	6.853898 (0.328730)	<.0001	2.642441 (0.154783)	<.0001	1521.803376 (63.189924)	<.0001
Log Likelihood	-775.54633		-437.78315		2537	
Significance = P(<0.05)						

SI Table 3. Optimized conditions for mass spectrometer AEC quantification.

Compound	Precursor Mass (m/z)	Product Ions (m/z)	Confirmation Ratio	Retention Time	Period
Sulfamethazine	279.1	186		4.35	1
		124.1	51		
		156	32		
Simeton (IS)	198.1	68		4.35	1
Tylosin A	916.5	174.1		6.40	2
		772.4	61		
		88.1	18		
Atrazine	216.1	174		7.20	2
		68	33		
		62	12		

APPENDIX B

CHAPTER. 4 SUPPLEMENTAL DATA

SI Table 1. Tobit Regression Model Parameter Estimates for Sediment

Parameter	SMZ Censored at 0.005467 ng g ⁻¹	P-value	TYL Censored at 0.005867 ng g ⁻¹	P-value	ATZ Censored at 0.0036 ng g ⁻¹	P-value
Intercept	-0.034612	0.7831	0.511104	<.0001	0.008794	0.8744
Standard Err	(0.125757)		(0.085506)		(0.055613)	
Site						
241	-0.033855 (0.113116)	0.7647	0.326306 (-0.217369)	0.1879	0.001363 (0.048819)	0.9777
242	-0.156813 (0.111607)	0.1600	0.284110 (0.224035)	0.6073	-0.015530 (0.043129)	0.7188
323	-0.024406 (0.102469)	0.8117	-0.176716 (0.219824)	0.2174	0.013151 (0.042778)	0.7585
350	0.038842 (0.100238)	0.6984	-0.113385 (0.217292)	0.0768	0.079286 (0.041853)	0.0582
450	0 .	.	0 .	.	0 .	.
Year						
2013	0.019922 (0.114298)	0.8616	0.759632 (0.197431)	<.0001	2227.911049 (247.060386)	0.6553
2014	-0.033773 (0.080432)	0.6746	0.455345 (0.154496)	<.0001	478.630123 (200.501239)	0.0418
2015	0 .	.	0 .	.	0 .	.
Season						
Growing	-0.041729 (0.087726)	0.6343	-0.478573 (0.179260)	0.0087	-1053.512058 (234.646882)	0.0577
Harvest	-0.177834 (0.089386)	0.0466	-0.568558 (0.173966)	0.0021	-1653.381540 (222.730018)	0.0907
Preplant	0 .	.	0 .	.	0 .	.
Flow						
DC	-0.215499 (0.087726)	0.1081	-0.129679 (0.090917)	0.1538	0.010566 (0.050832)	0.8353
HF	-0.216871 (0.143605)	0.1310	-0.227807 (0.084789)	0.0072	-0.025170 (0.057548)	0.6618
LF	-2.087499 0	.	-0.107970 (0.211143)	0.6091	0.005712 (0.118110)	0.9614
MC	0.000925 (0.088013)	0.9916	-0.207264 (0.060293)	0.0006	0.056868 (0.038498)	0.1396
MR	0 .	.	0 .	.	0 .	.
Sigma	0.338840 (0.034588)	<.0001	0.240984 (0.018546)	<.0001	0.176839 (0.009850)	<.0001
Log Likelihood	-62.07848		-32.06233		48.37043	

Significance = P(<0.05)

SI Table 2. Tobit Regression Model Parameter Estimates for water grab samples

Parameter	SMZ Censored at 0.000328 ng L ⁻¹	P-value	TYL Censored at 0.000352 ng L ⁻¹	P-value	ATZ Censored at 0.0036 ng L ⁻¹	P-value
Intercept	-0.002050	0.0039	0.006494	0.1976	0.337574	<.0001
Standard Err	(0.000710)		(0.005041)		(0.058649)	
Site						
241	0.001762 (0.000703)	0.0121	-0.000885 (0.005607)	0.8745	-0.184543 (0.062498)	0.0031
242	0.000105 (0.000761)	0.8902	-0.000195 (0.005698)	0.9727	-0.207604 (0.063404)	0.0011
323	0.000372 (0.000721)	0.6062	-0.002939 (0.005659)	0.6035	-0.104467 (0.062498)	0.0946
350	-0.000974 (0.000773)	0.2076	0.007993 (0.005501)	0.1462	-0.015592 (0.062496)	0.8030
450	0 .	.	0 .	.	0 .	.
Year						
2013	0.008441 (0.000636)	<.0001	0.021796 (0.004640)	<.0001	0.322131 (0.054676)	<.0001
2014	0.001145 (0.000561)	0.0415	-0.013318 (0.004203)	0.0015	0.174124 (0.044402)	<.0001
2015	0 .	.	0 .	.	0 .	.
Season						
Growing	-0.001580 (0.000595)	0.0079	-0.013512 (0.004398)	0.0021	-0.237832 (0.051652)	<.0001
Harvest	-0.002454 (0.000573)	<.0001	-0.028943 (0.004577)	<.0001	-0.322520 (0.049624)	<.0001
Preplant	0 .	.	0 .	.	0 .	.
Sigma	0.003691 (0.000203)	<.0001	0.030196 (0.001570)	<.0001	0.390230 (0.014045)	<.0001
Log Likelihood	633.00836		319.08267		-184.47646	

Significance = P(<0.05)

APPENDIX C

CHAPTER. 5 SUPPLEMENTAL DATA

SI Table 1. Tobit Regression Model Parameter Estimates for POCIS

Parameter	SMZ Censored at 1.63E-5 ng L ⁻¹	P-value	TYL Censored at 9.65E-6 ng L ⁻¹	P-value	ATZ No Censoring	P-value
Intercept	-0.006056	0.1924	0.019599	0.5203	1346.842198	0.4838
Standard Err	(0.004646)		(0.030483)		(249.7384)	
Date						
4/13 - 4/28	-0.000387 (0.005931)	0.9479	-0.047489 (0.042710)	0.2662	-731.383906 (258.88809)	0.7697
4/28 - 5/13	-0.058484 (0)		-0.050412 (0.042868)		0.2396	
5/13 - 5/28	0.008439 (0.005323)	0.1129	0.021899 (0.039639)	0.5806	4.066627 (250.314315)	0.504
5/28 - 6/13	0.010636 (0.005386)		0.0483		-568459 (0)	
6/13 - 6/28	0.006102 (0.005372)	0.256	0.182400 (0.039639)	<.0001	2227.911049 (247.060386)	<.0001
6/28 - 7/15	0.003178 (0.005678)		0.5757		-0.011398 (0.040093)	
7/15 - 7/29	0 .	.	0 .	.	0 .	.
Mgmt						
Chisel Plow	-0.003226 (0.002959)	0.2757	-0.005535 (0.023967)	0.8174	-1053.512058 (234.646882)	0.9533
No Till	0 .		.		0 .	
Sigma	0.008807 (0.001289)	<.0001	0.079278 (0.009022)	<.0001	0.065541 (0.006194)	<.0001
Log Likelihood	71.7918		38.54812		73.14351	

Significance = P(<0.05)

SI Table 2. Tobit Regression Model Parameter Estimates for Surface soils (15 cm)

Parameter	SMZ Censored at 0.0164 ng g ⁻¹	P-value	TYL Censored at 0.0176 ng g ⁻¹	P-value	TET Censored at 0.1252 ng g ⁻¹	P-value	ATZ Censored at 0.0036 ng g ⁻¹	P-value
Intercept	-8.909778	<.0001	-0.521213	0.1076	-43.580032	<.0001	-0.008924	0.9000
Standard Err	(0.464332)		(0.323931)		(1.184368)		(0.071003)	
Date								
11/5/2013	8.234270 (0.503562)	<.0001	-4.652405 (0)	.	44.935640 (1.686599)	<.0001	0.517035 (0.095032)	<.0001
11/15/2013	8.204611 (0.478131)	<.0001	0.567132 (0.339639)	0.0950	59.568778 (1.650972)	<.0001	0.268228 (0.073578)	0.0003
11/19/2013	0 .	.	0 .	.	0 .	.	0 .	.
4/17/2014	7.285800 (0.664338)	<.0001	1.783979 (0.335134)	<.0001	46.121589 (1.669212)	<.0001	0.506690 0.073555	<.0001
Mgmt								
Chisel Plow	-1.006264 (0.852904)	0.2381	-0.359874 (0.222472)	0.1057	478.630123 (200.501239)	0.5701	0.069699 (0.053650)	0.1939
No Till	0 .	.	0 .	.	0 .	.	0 .	.
Band								
Background	0 .	.	0 .	.	0 .	.	0 .	.
Interband	-12.545901 0	.	-0.737365 (0.232683)	0.0015	-26.496413 (4.276617)	<.0001	-0.015625 (0.058823)	0.7905
Manureband	0 .	.	0 .	.	0 .	.	0 .	.
Sigma	1.388477 (0.487589)	0.0044	0.748030 (0.095963)	<.0001	6.775095 (0.844842)	<.0001	0.065541 (0.006194)	<.0001
Log Likelihood	-18.96571		-48.55679		-115.35602		-9.06841	

Significance = P(<0.05)

SI Table 3. Tobit Regression Model Parameter Estimates for Surface & Subsurface soils (0 - 120 cm)

Parameter	TYL Censored at 0.0176 ng g⁻¹	P-value	TET Censored at 0.1252 ng g⁻¹	P-value	ATZ Censored at 0.0036 ng g⁻¹	P-value
Intercept	-0.967739	0.0119	-33.826458	0.0159	-0.565896	0.0678
Standard Err	(0.384906)		(14.029001)		(0.309825)	
Depth						
0 - 30 cm	0.670102 (0.356014)	0.05	25.435890 (12.525779)	0.0423	0.959999 (0.36372)	0.0033
30 - 60 cm	-4.393490 (0)		.		3.074964 (15.220586)	
60 - 120 cm	0 .	.	0 .	.	0 .	.
Mgmt						
Chisel Plow	0.327716 (0.246831)	0.1843	9.106083 (7.486454)	0.2239	-0.270993 (0.191831)	0.1578
No Till	0 .	.	0 .	.	0 .	.
Sigma	0.747286 (0.130217)	<.0001	22.019803 (4.241080)	<.0001	0.681693 (0.077214)	<.0001
Log Likelihood	-39.63169		-98.5796		-53.59742	

Significance = P(<0.05)