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EVALUATING SAMPLING STRATEGIES FOR RAINFALL SIMULATION STUDIES AND SURFACE TRANSPORT OF ANTIBIOTICS FROM SWINE MANURE APPLIED TO FESCUE PLOTS

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EVALUATING SAMPLING STRATEGIES FOR RAINFALL SIMULATION STUDIES AND SURFACE TRANSPORT OF ANTIBIOTICS FROM SWINE MANURE APPLIED TO FESCUE PLOTS

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in Biosystems and Agricultural
Engineering in the College of Engineering at the University of Kentucky

By

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Lexington, Kentucky

Director: Dr. Carmen T. Agouridis, Assistant Professor of
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Lexington, Kentucky

2014

ABSTRACT OF THESIS

EVALUATING SAMPLING STRATEGIES FOR RAINFALL SIMULATION STUDIES AND SURFACE TRANSPORT OF ANTIBIOTICS FROM SWINE MANURE APPLIED TO FESCUE PLOTS

Antibiotics are commonly used in animal agriculture to treat and prevent diseases and promote growth. Unfortunately, large amounts of antibiotics are not metabolized, but instead are excreted in urine and feces. Rainfall simulation studies were used to investigate the transport of the antibiotic oxytetracycline and various constituents in runoff and the ability of alum to reduce pollutant transport. Runoff samples were collected at several points during the simulated storm event from each of four treatments: control (C), manure only (M), manure and antibiotics (MA), and manure, antibiotics and alum (MAA). Flow-weighted composite samples were created and compared to the flow weighted mean concentration (FWMC). Constituents with concentrations well-above the detection limits (*E. coli*, NH_4-N, turbidity, TSS, TOC, and EC) showed a strong correlation between flow-weighted composite samples and FWMC. When constituent concentrations were at or near the detection limits, errors associated with the composite samples were magnified. Oxytetracycline concentrations had the strong correlation to *E. coli*, Cl, TOC, TSS, and turbidity suggesting that a BMP effective at trapping sediment or particulates may work best for reducing oxytetracycline concentrations in runoff. Alum (1%) did not reduce levels of oxytetracycline in runoff. It is recommended that higher doses of alum be tested.

KEYWORDS: Antibiotic, transport, runoff, alum, sampling

Holly Kristina Enlow
Signature

January 6, 2014
Date

EVALUATING SAMPLING STRATEGIES FOR RAINFALL SIMULATION STUDIES AND SURFACE TRANSPORT OF ANTIBIOTICS FROM SWINE MANURE APPLIED TO FESCUE PLOTS

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January 6, 2014

Date

For my grandparents

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CHAPTER 1: INTRODUCTION

1.1 INTRODUCTION

Animal agriculture generates over $154 billion in sales annually in the United States (USDA, 2009). Though the number of livestock and poultry operations in the U.S. has decreased by 80% since the 1950's, production has more than doubled (USEPA, 2013). Livestock operations are now larger in size and are more geographically concentrated in the central portion of the U.S. (Figure 1.1). Over half of swine facilities, for example, are located within one mile of another animal agriculture operation (USDA, 2001). Table 1.1 contains top livestock producing states for each livestock category as well as total livestock production. The greatest numbers of animal units (1 animal unit, AU, equals 454 kg) are associated with beef cattle with Texas, Missouri and Oklahoma producing a combined total of over 9.4 million AUs. Iowa, North Carolina, and Minnesota are the top three swine producing states with nearly 4.8 million AUs. California, Wisconsin, and New York are the leading dairy production states, and North Carolina, Arkansas and Georgia are the leading poultry production states. Considering beef, swine, dairy and poultry together, the largest livestock producing states are Texas, Iowa, Nebraska, California, and Kansas; hence four of the top five states are located in the central U.S.

The USEPA (2013) estimated that livestock and poultry operations in the U.S. generate over 1.1 billion tons of manure on an annual basis. High concentrations of livestock, as seen in Figure 1.1, means producers must manage large amounts of manure oftentimes with insufficient amounts of cropland on which to apply this manure. Ideally, agricultural operations would utilize integrated crops and livestock production methods whereby animal manure serves as a fertilizer for nearby croplands. But because these more concentrated livestock operations generate large quantities of manure, and nutrients in greater volumes than those required by crops, developing a sound and sustainable manure management plan with limited land resources is quite challenging. This is especially true as nutrient management systems shift towards basing manure application rates on phosphorus levels rather than nitrogen levels (Higgins et al., 2013). As noted by Eghball and Power (1999), crops require less phosphorus as compared to nitrogen; hence

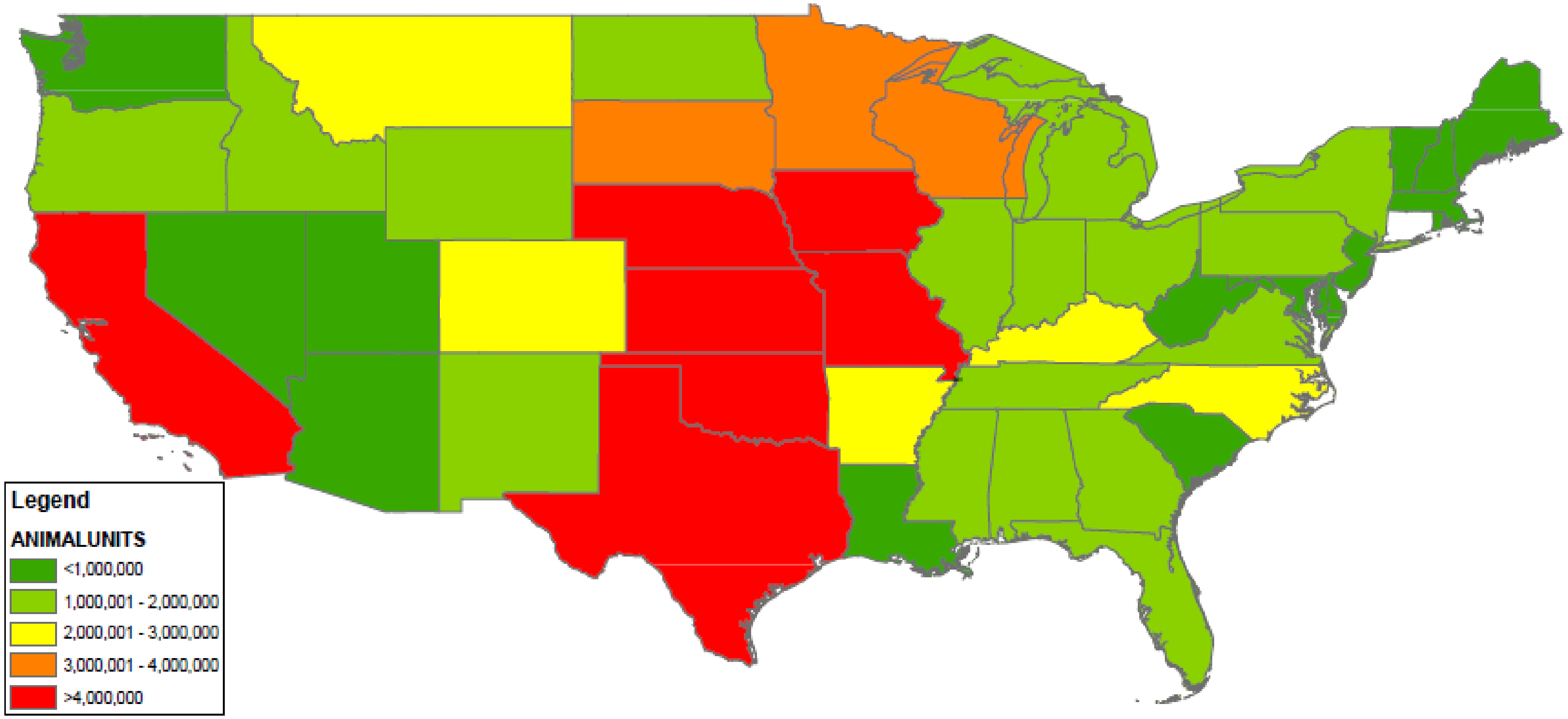


Figure 1.1. Production of Animal Units for All Livestock (Beef, Dairy, Swine, and Poultry) per State (USDA, 2009; USEPA, 2013).

Table 1.1. Top Producing States for Each Category of Livestock and Total Production.

State Rank	Beef		Swine		Dairy		Poultry		Total Livestock	
	State	Total AU[1]	State	Total AU	State	Total AU	State	Total AU	State	Total AU
1	TX	5,259	IA	2,409	CA	2,487	NC	647	TX	11,109
2	MO	2,089	NC	1,382	WI	1,688	AR	642	IA	5,587
3	OK	2,063	MN	999	NY	847	GA	594	NE	5,236
4	NE	1,889	IL	607	PA	748	AL	431	CA	5,235
5	SD	1,649	IN	486	ID	725	TX	367	KS	4,933
6	MT	1,522	NE	462	MN	621	MS	356	OK	4,571
7	KS	1,516	MO	435	TX	546	MN	335	MO	4,179
8	TN	1,179	OK	367	MI	465	CA	282	MN	3,269
9	KY	1,166	KS	256	NM	441	IA	279	WI	3,213
10	AR	947	OH	243	OH	367	MO	260	SD	3,180
11	FL	942	SD	207	WA	329	VA	203	NC	2,704
12	ND	930	PA	160	IA	291	SC	201	CO	2,183
13	IA	904	TX	152	AZ	248	PA	201	MT	2,172
14	CO	735	MI	141	IN	225	IN	198	AR	2,164
15	WY	732	CO	141	VT	189	OH	161	KY	2,143

Source: (USDA, 2009)

[1] AU= 1 animal unit=454 kg of live weight or one beef cattle. Numbers are in 1000’s of AUs.

lesser amount of manure should be applied to the land to help prevent water quality impairment.

Presently, manure management systems for large livestock operations are designed to store manure in lagoons or pit systems before periodic land application. And while contaminants can enter surface and ground waters through spills and leaks from these storage systems, land application of manure is the primary pathway in which pollutants enter our nation’s streams, rivers, lakes and estuaries. Ideally, operators base manure application rates on crop requirements of nitrogen and/or phosphorus (USEPA 2013, NRCS 2012); however, this is not always the case. Nutrients, pathogens and other such constituents not captured in croplands either through plant uptake, soil sorption, or the like are transported via runoff to surface waters or infiltrated into shallow ground waters (NSTC-CENR 2000; Campagnolo et al., 2002). Alexander et al. (2008) concluded that agricultural sources, largely manure, contributed 70% of the nitrogen and 80% of the phosphorus found in the Gulf of Mexico. The authors noted that these nutrients, of which Kentucky is a significant contributor, were largely transported to the Gulf of Mexico via small and midsized streams. Currently, Kentucky currently ranks 15th in the nation in total livestock production and 13th in manure production (USDA, 2009). Additionally, Kentucky has over 148,000 km of stream, many of which are first- or second-order systems (KDOW, 2010).

While much research has been conducted on the environmental impacts of animal agriculture as it relates to nutrients (Moore and Miller, 1994; Edwards et al., 2000; Smith et al., 2001; Penn and Bryant, 2006; Alexander et al., 2008; USEPA, 2013) and pathogens (Khaleel et al., 1980; Mawdsley et al., 1995; Gerba and Smith, 2005; USEPA, 2013), far fewer studies have examined other contaminants such as hormones and antibiotics (Kay et al., 2005; Chee-Sanford et al., 2009; LaShore and Pruden, 2009; Kim et al., 2010; DeLuane and Moore, 2013). Antibiotic use is widespread in animal agriculture as a means of combating diseases and infections in order help ensure the meat supply is safe for human consumption. Unfortunately, it has become more common for livestock operations to use antibiotics as a preventative tool against illness and as a growth additive, particularly in large concentrated animal feeding operations or CAFOs where living conditions are crowded (LaShore and Pruden, 2009). Oftentimes, feed is supplemented with antibiotics to limit nutrient absorption by gastrointestinal microbes thus ensuring more nutrients are available for absorption by the animal in order to achieve more rapid weight gain (Kumar et al., 2005). Davis et al. (2006) estimated that over 11 million kg of veterinary antibiotics were used for nontherapeutic uses in 2002 alone.

Large amounts of these administered antibiotics are not metabolized by livestock but instead are excreted in manure. Tetracyclines, for example, which are one of the most commonly used groups of antibiotics due to their broad spectrum applicability, are excreted a rate of about 70 to 90 % (Kumar et al., 2005). Concentrations of antibiotics in manure can range from 1-10 mg L^{-1} though concentrations as high as 200 mg L^{-1} have been measured (Kumar et al., 2005). A typical daily dose of antibiotics for adult humans ranges from 80 to 6,000 mg depending on the antibiotic and intended treatment (Hirsch et al., 1999). Oxytetracyline is often administered at 1 to 2 g d^{-1} for a typical adult (NLM, 2005). The rate of excretion for other types of antibiotics (non-tetracyclines) ranges from 25-75% (Chee-Sanford et al., 2001). Table 1.2 contains excretion rates for commonly used veterinary antibiotics. The large rate of excretion for tetracycline is of particular concern because it is a widely used antibiotic, and land application of manure has been attributed as the primary means by which veterinary antibiotics are introduced into the terrestrial and subsequently aquatic environments (Baguer et al., 2000).

Because of these and other findings, the U.S. Environmental Protection Agency (USEPA) classifies antibiotics as a contaminant of emerging concern (CEC) (USEPA, 2007). A CEC is one that is now being detected in the environment and/or is at higher than expected levels, meaning it was not previously present in the environment or was present at undetectable or very low levels (USEPA, 2007). The risk that CEC’s pose to human health is often unknown as in the case of antibiotics. Surface and ground waters are not routinely tested for antibiotics; therefore their impact on the health of humans, as well as other biota, is largely unknown. Since antibiotics are designed to combat bacteria, it is expected that the presence of antibiotics in the soil and water would have a negative impact on microorganisms living in these environments (Kay et al., 2005). The primary concern with the wide-spread release of antibiotics into the environment is the development of strains of antibiotic-resistant bacteria. It is feared that the accumulation

Table 1.2. Excretion Rates of Commonly used Veterinary Antibiotics.

Antibiotic	Amount Excreted in Urine and Feces (%)[1]	Animal[1,2]	Common Uses[2]
Tetracycline	80	Beef, dairy, poultry, sheep, swine, humans	Bacterial pneumonia, bacterial enteritis, foot rot, jowl abscesses, mastitis, growth Promotion
Chlortetracycline	75	Beef, dairy, swine, poultry, sheep	Bacterial pneumonia, bacterial enteritis, foot rot, jowl abscesses, mastitis, growth promotion
Oxytetracycline	80	Beef, dairy, poultry, sheep, swine, fish, humans	Bacterial pneumonia, bacterial enteritis, foot rot, jowl abscesses, mastitis, growth promotion
Lincomycin	60	Swine, poultry, humans	Bacterial enteritis, infectious arthritis, dysentery, mycoplasmal pneumonia, growth promotion
Tylosin	50-90	Beef, dairy, swine, sheep	Foot rot, liver abscesses, respiratory disease, infectious arthritis, growth promotion
Erythromycin	50-90	Beef, dairy, poultry, sheep, swine, humans	Foot rot, liver abscesses, respiratory disease, bacterial enteritis, infectious arthritis, growth promotion
Monensin	50-90	Beef, dairy	Liver abscesses, coccidiosis, growth promotion

Source: Kumar (2005)[1] and USEPA (2013)[2]

of antibiotics in human-consumed plants and animals may lead to the introduction of antibiotic-resistant bacteria into the food and water supply hence threatening human health (Kemper, 2007). Likewise, the presence of antibiotics such as penicillin, which is the most commonly reported allergy inducing antibiotic (ACAAI, 2013), in the food and water supply could result in potentially fatal allergic reactions (Kummerer, 2003).

Research into the transport of antibiotics to surface waters via runoff is limited. Arikan et al. (2008) sampled streams in a predominately agricultural watershed and analyzed the water samples for several different types of antibiotics. Chlortetracycline and oxytetracycline were the most commonly identified antibiotics in the samples with concentrations of 0.016 $\mu g\ L^{-1}$. The authors concluded that the antibiotics were transported in runoff from nearby manure-amended fields, as no other potential sources of antibiotics were identified. Dolliver and Gupta (2008a) collected runoff samples from manure amended land over the course of three years. Peak antibiotic concentrations of 0.5, 57.5 and 6.0 µg L^{-1}were found for chlortetracycline, monesin, and tysolin, respectively. Dolliver and Gupta (2008a) found the greatest period for antibiotic losses occurred during the non-growing season after fall application of manure. In another study, Dolliver and Gupta (2008b) measured peak concentrations of 210, 3175, and 2544 µg L^{-1} for chlortetracycline, monensin, and tysolin, respectively, in runoff from unprotected manure stockpiles suggesting that manure storage is also a significant contributor to the presence of antibiotics in the aquatic environment. Kay et al. (2005) spiked liquid hog manure to levels of 18.9 mg L^{-1} for oxytetracycline and 25.6 mg L^{-1} for sulphachloropyridazine and applied this mixture to runoff plots. Runoff samples were analyzed for the two antibiotics with peak concentrations determined to be 32 µg L^{-1} for oxytetracycline and 415.5 µg L^{-1} for sulphachloropyridazine, which corresponds to a mass loss of 0.074% and 0.418%, respectively. This study concluded that overland flow has the potential to transport veterinary antibiotics to surface waters. Davis et al. (2006) applied seven antibiotics at concentrations of 1 mg L^{-1} to plots prior to rainfall simulation. Tetracycline concentrations in runoff were determined to be 0.03 µg L^{-1} which corresponds to a 0.002% antibiotic loss. However, 65% of this loss was associated with sediment suggesting that erosion control practices could help reduce antibiotic transport. Studies have shown antibiotics at levels of 50 µg L^{-1} for tysolin and 34 µg L^{-1} for chlortetracycline can be toxic to some algal species (Halling-Sørensen, 2000). While it is not known at what levels antibiotics have a significant impact on other aquatic life, hormones have been shown to have adverse effects, such as defeminization or demasculinization, on fish at levels as low as 10-120 ng L^{-1} (Durhan et al., 2006).

Only a few studies have examined methods to reduce the transport of runoff to surface waters. Lin (2011) examined three veterinary antibiotics (sulfamethazine, tysolin, and enrofloxacin) and found that vegetated buffer strips reduced antibiotic transport by 40% for sulfamethazine and 75% for both tylosin and enrofloxacin suggesting that vegetated buffer strips can provide reductions in antibiotic transport. It is important to note that the degree of mobility differs between antibiotics. Sulfonamides, such as sulfamethazine, are more mobile in an aqueous phase than antibiotics such as tylosin and enrofloxacin which are more likely to bind to the soil. Tetracyclines also have a stronger affinity for binding to the soil (Tolls, 2001). Other methods from wastewater treatment engineering hold promise in reducing antibiotic transport. Stackelberg et al. (2007) found that conventional water treatment methods such as clarification, disinfection, and granular activated carbon filtration reduced the concentration of several antimicrobials, including erythromycin, linconycine, sulfamethazine, sulfathiazole, and sulfamethoxazole, in water from 0.1 $\mu g\ L^{-1}$ to levels below detection. Aluminum sulfate or alum, which is a flocculating agent commonly used in wastewater treatment to settle out solids, is effective at reducing the transport of phosphorus, hormones, and some metals (Edwards and Daniel, 1992; Moore and Miller, 1994; Moore et al., 1998; Edwards et al., 1999; Smith et al., 2001; Penn and Bryant, 2006; DeLaune and Moore 2013). Smith et al. (2001) used rainfall simulators to observe the surface transport of phosphorus and noted an 84% reduction in phosphorus levels following the addition of alum to swine manure. Alum has also been shown to reduce levels of hormones (17β-estradiol) in runoff from plots amended with poultry litter by up to 40% (Delaune and Moore, 2013). Tetracycline antibiotics bind strongly to manure particles suggesting that a process used to flocculate manure particles might reduce the transport of tetracyclines as well. Based on the results by Smith et al. (2001) and DeLaune and Moore (2013), it is possible that alum, if used as a manure amendment, could reduce concentrations of antibiotics in runoff.

Due to the uncontrollability and unpredictability of weather patterns, investigating the transport of contaminants in overland flow is challenging. Hence, rainfall simulation studies are often used (Miller, 1987; Bushee et al., 1998; Edwards et al., 1999; Edwards et al., 2000; Smith et al., 2001; Sharpley and Kleinman, 2003; Davis et al., 2006; Kim et al., 2010). One challenge with rainfall simulation studies is managing the large number of samples generated as runoff samples are typically collected at several points during the simulated storm event. To reduce analysis expenses and better manage sample analysis time constraints, a single flow-weighted composite sample is created and analyzed in lieu of analyzing each collected sample individually. It is thought that this single composite sample will provide the same constituent values as analyzing multiple samples from a single storm event and computing the flow-weighted mean concentration (FWMC), which is a representation of a constituent's concentration across the entire storm event (Agouridis and Edwards, 2003) though the accuracy of this assumption for rainfall simulation studies has not been tested.

1.2 OBJECTIVES

Research was conducted to examine the error associated with composite sampling and to evaluate techniques antibiotic transport, specifically oxytetracycline, via runoff. Rainfall simulators and fescue plots, located at the University of Kentucky's Maine Chance Research Farm, were used to achieve the following objectives:

1. Determine the error associated creating a single flow-weighted composite sample as it relates to rainfall simulation studies.
2. Evaluate the relationship between oxytetracyline and *E. coli*, NO_3-N, NH_4-N, PO_4, pH, EC, TSS, Cl, TOC and turbidity levels.
3. Evaluate the effect of aluminum sulfate (alum) as a manure amendment on the reduction of oxytetracycline levels in runoff.

1.3 ORGANIZATION OF THESIS

An overview of the research problem and objectives is described in Chapter 1. Chapters 2-4 give detailed descriptions of the work done to accomplish the objectives of this thesis. Chapter 5 discusses conclusions of the research, and Chapter 6 describes potential future work.

CHAPTER 2: COMPARISON OF WATER QUALITY SAMPLING TECHNIQUES FOR RAINFALL SIMULATION STUDIES

2.1 INTRODUCTION

Increases in discharge can affect water quality constituent concentrations differently. While discharge can have an inverse relationship with dissolved pollutants meaning higher discharge rates often result in lower concentrations via dilution, the opposite is often seen with other pollutants such as suspended sediments and total metals (Rickert, 1985). To account for this discharge-associated variation, the flow weighted mean concentration (FWMC) is often used (Hoos et al., 2000; Agouridis and Edwards, 2003). The FWMC is defined as the total mass load of a constituent divided by the total flow volume of an event (Huber, 1993; Cooke et al., 2000) and is presented in Agouridis and Edwards (2003) as

$$\mathrm{FWMC} = \frac{\int c(t)Q(t)dt}{\int Q(t)dt} \cong \frac{\sum_{i=1}^{n}(\frac{c_i + c_{i+1}}{2})(\frac{Q_i + Q_{i+1}}{2})\Delta t}{\sum_{i=1}^{n}(\frac{Q_i + Q_{i+1}}{2})\,\Delta t} \qquad (2.1)$$

In equation 2.1, the variable c represents concentration, Q represents flow, and t represents time. As the FWMC is a single value that represents the concentration of a constituent during a storm event (Agouridis and Edwards, 2003), it is important that this value is computed using samples collected throughout the entire storm event to minimize error (EPA, 1973; Mueller and Stone, 1998). Thus while the FWMC reduces a constituent's concentration for a storm event to a single value, it does not reduce the number of samples one must analyze, and hence the costs one must incur.

Creating a composite sample is one method of reducing laboratory costs while striving to maintain accuracy (i.e. minimizing error). With composite sampling, a single sample is created by mixing defined portions of discrete samples (USEPA, 1982). These defined portions are based on time (time compositing) or flow (flow-weighted compositing). Discrete samples for time compositing are collected at uniform times, such as every 20 minutes throughout the storm event, or at variable time increments considering the first flush phenomenon. With variable time increments, samples are collected at closer time increments at the start of the storm in an effort to capture the rising limb and peak of the hydrograph as compared to later in the storm (Harmel and King, 2005).

A number of studies have evaluated the accuracy of composite sampling techniques when used to collect samples from streams. Aulenbach and Hooper (2005) collected samples from mountain streams near Atlanta, Georgia using different sampling scenarios, including weekly grab samples and flow-weighted and time-weighted samples, for storm events. Alkalinity concentrations in the samples were used to predict the error of a composite sampling strategy to determine stream loads. The study concluded composite sampling produced a bias of ±0.8% which was within the error associated with flow measurements and analytical chemistry. The study also noted that errors with time-weighted and flow-weighted composite sampling were dependent on sampling design. More frequent and a longer time interval for sampling produced more accurate results. Even with some bias, flow-weighted composite sampling produced more accurate results than the other sampling techniques, such as a time-weighted approach, which tended to underestimate stream loads by 0.5% to 7.6%. Harmel et al. (2006) explored the uncertainty in each stage of water quality data collection: streamflow measurement, sample collection, sample storage, and laboratory analysis by compiling water quality data on dissolved N and P, total N and P, and TSS, as well as streamflow measurements from several other studies. When looking at the error for sampling strategies, this Harmel et al. (2006) found an expected error for flow-weighted sampling of -6% to +17% compared to an error associated with grab sampling of ±25% for dissolved constituents and ±50% for sediment. Stone et al. (2000) also examined the differences between flow-composited, time-composited, and grab sampling methods using the constituents nitrate (NO_3), ammonia (NH_4), and total kjeldahl nitrogen (TKN). The authors concluded that flow-weighting puts a greater emphasis on storm events rather than base flow conditions in streams, which could result in an over-prediction of actual stream loadings due to the more intensive monitoring that occurs during storm events. Stone et al. (2000) also noted that if a grab sampling strategy is used, samples must be collected frequently, such as at least twice a month, and at varying flow rates for data to be representative of actual stream loadings.

While flow-weighted compositing may over-predict total stream loading, it is better for characterizing runoff events. Harmel and King (2005) sampled sediment, nitrate, and phosphate concentrations in twenty storm events in two small agricultural watersheds in Texas. The authors determined that flow proportional sampling resulted in an error of ±10% and represented storm loads better than time composited samples. Harmel and King (2005) concluded that flow-weighted composite sampling was the optimal way to balance data accuracy with limited resources.

Rainfall simulation studies are another means of investigation into contaminant transport that is often used because of the ability to control rainfall amounts and patterns. Rainfall simulators have been used to examine nutrient transport (Edwards et al. 1999; Edwards et al., 2000; Smith et al.,2001; Sharpley and Kleinman, 2003), veterinary pharmaceutical transport (Bushee et al., 1998; Davis et al., 2006; Kim et al., 2010), and best management practice effectiveness (Edwards et al. 1999; Smith et al., 2001; Lin et al., 2011). As with storm event sampling in streams, rainfall simulation studies generate large numbers of samples as runoff is collected at several points during the simulated storm event for multiple treatments and replications. To reduce analysis expenses and better manage sample analysis time constraints, such as with *Escherichia coli*, oftentimes a single flow-weighted composite sample is created and analyzed in lieu of analyzing each collected sample individually and then computing the FWMC. It is hypothesized that for rainfall simulation studies a single flow-weighted composite sample will provide the same constituent values as the FWMC.

2.2 MATERIALS AND METHODS

2.2.1 Study Site

The study was performed at the rainfall simulation facility at the University of Kentucky's Maine Chance Research Farm (latitude: 38.1164°N; longitude: 84.4903W). Edwards et al. (2000) thoroughly described the rainfall simulation facility, but briefly, the facility consists of 75 plots with dimensions of 2.4 m by 6.1 m. Thirty of the plots are individual while the remaining 45 are grouped linearly in six sets of five (Appendix A). The five plots in each group can be hydrologically separated or combined to create a longer flow path of 30.5 m. The plots have a 3% slope and are planted in Kentucky 31 fescue. The soil underlying the plots is a Maury silt loam (fine, mixed, mesic Typic Paleudalf) (NRCS, 2013). Each plot is bordered with galvanized steel to ensure that runoff does not leave the plot but is instead directed to a collection system located at the most down-gradient end of the plot.

2.2.2 Plot Selection

In an attempt to minimize the variation associated with possible plot differences, curve numbers (CN) were computed for 48 plots prior to the start of the study, as described in Appendix A. Curve numbers were calculated for each of the thirty individual plots as well as the uppermost and middle plots (sections) of the five plot sets. Adjacent plots in the sets were not used to ensure rainfall from the simulators did not spill over (i.e. edge effect). Based on these results, sixteen plots were identified for use in the study (Table 2.1). Plots used in this study had a mean curve number of 83 ±4.

Table 2.1. Curve Numbers (CN) of Plots Used in the Study.

Plot	CN	Plot	CN
B1	82	C5	89
B4	82	C6	77
B5	88	D1	86
B6	90	D5	84
B10	82	L1	84
C2	89	L3	79
C3	82	N2	77
C4	80	N9	81

2.2.3 Treatments

The treatments consisted of control (C), swine manure (M), swine manure plus an antibiotic (MA), and swine manure plus an antibiotic and alum (MAA). The manure was obtained from a hog facility in Bardstown, Kentucky. The facility did not administer antibiotics to the hogs which were the source of the manure. The manure had a slurry consistency, and it was stored in a pit prior to collection for this study. Collected manure was stored in a 1,040 L polyethylene intermediate bulk container (IBC) tote at the University of Kentucky Biosystems and Agricultural Engineering Department. For mixing and transport to the study site, 55 L of manure was then transferred to a 208 L plastic barrel. One plastic barrel was used for each treatment to prevent cross-contamination.

For the MA and MAA treatments, the antibiotic oxytetracycline (300 mg mL^{-1}), which was sold under the brand name Noromycin 300 LA (Norbrook, Lenexa, KS), was added the manure in each respective barrel to produce a final concentration of 20 mg L^{-1}. For the MAA treatment, liquid alum (Al_2SO_4, 48.5% solution) was added to the manure in the respective barrel to produce a final concentration of 1.2 g L^{-1} (Bushee-Bullock, 1999). Uniform mixing was achieved using a custom mixer attached to a cordless drill. The custom mixer, which was constructed in the Biosystems and Agricultural Engineering Department, was approximately 1.5 m in length and had two 30 cm long fins at a distance of 0 cm and 30 cm from the bottom. Once thoroughly mixed,the manure mixtures (M, MA, or MAA) were applied to the respective plots at an application rate of 3 L m^{-2}, immediately prior to the start of simulated rainfall. This application rate corresponded to 45L per plot. The target application rate was 112 kg N ha^{-1} as recommended by AGR-1 (UK, 2012) and as done in Bushee-Bullock (1999). However, based on laboratory results from the University of Kentucky Regulatory Services, the actual application rate was about 150 kg N ha^{-1}. Treatments, which were part of a larger study into antibiotic transport, were randomly assigned to the sixteen plots (Table 2.2).

Table 2.2. Assignment of Treatments to Plots.

Treatment[1]	**Plots**
C	C2, C6, D1[2], D5
M	B4, B8[3],C5, L1
MA	B6, B10, C3, L3
MAA	B1, B5, C4, N9

[1] C=control; M =manure only; MA=manure plus antibiotics; MAA=manure plus antibiotics and alum
[2] D1 was not used in the *E. coli* analysis due to sampling error.
[3] B8 was not used in the analysis because it did not produce runoff

Three days prior to manure application, each plot was mowed resulting in a sward height of 10 cm. Immediately prior to manure application, soil samples were collected from each plot and manure samples were collected from the barrel for each treatment.

Soil samples were analyzed for P, K, Ca, Mg, Zn, pH, buffer pH, total nitrogen, and soil texture at the University of Kentucky Regulatory Service laboratory while manure samples were analyzed for N, P, K, Ca, Mg, Zn, Cu, Fe, and Mn.

2.2.4 Rainfall

Rainfall simulation began 30 minutes following manure application in order to represent a worst-case scenario. Simulated rainfall was applied to the plots at a rate of 102 mm hr^{-1} and continued until 30 minutes following the start of runoff, after which rainfall was stopped. Appendix A contains the calibration curves for the rainfall simulators used in the study.

2.2.5 Runoff Sample Collection

Runoff samples were collected in 1 L autoclaved polyethylene bottles at intervals of 2, 4, 6, 8, 16, 24 and 30 minutes after the start of runoff. A stopwatch was used to record the time required to fill the sample bottle at each time interval. Using the time (t) required to fill a sample bottle along with the volume (V) of each sample, flow rates (Q) for each time interval were computed.

Composite samples were made by placing a defined volume of sample (SV) from each time interval into a single bottle. The SV for each of the seven time intervals was calculated using equation 2.2.

$$SV = V_{comp} * \frac{Incremental\ Runoff}{Total\ Runoff} = V_{comp} * \frac{\frac{1}{2}(Q_i + Q_{i-1})dt}{\sum_{i=1}^{7}(\frac{1}{2}(Q_i + Q_{i-1})dt)} \tag{2.2}$$

The variable V$_{comp}$ represents the volume of the composite sample. Appendix B contains the data used to create the composite samples. Because of the need to analyze water samples within 24 hours when measuring *E. coli*, two composite samples were created. One was used to analyze *E. coli* levels while the other was used for the remaining constituents. Following collection samples were placed in coolers and transported to the University of Kentucky Biosystems and Agricultural Engineering Department. Samples were stored at 4°C until analyzed.

Flow weighted mean concentration samples were computed for each constituent and each plot using equation 2.1. All of the individual samples (e.g. 2, 4, 6, 8, 16, 24, and 30 minutes after the start of runoff) for simulated rainfall event were used.

2.2.6 Laboratory Analysis

Samples were analyzed for *E. coli*, oxytetracycline, NO_3-N, NH_4-N, orthophosphate (PO_4), pH, electrical conductivity (EC), total suspended solids (TSS), chloride (Cl), total organic carbon (TOC) and turbidity. Chemical analyses for NO_3-N, NH_4-N, PO_4, Cl, and TOC were done at the University of Kentucky's Forestry Department using a Bran-Luebbe Auto-Analyzer III (Norderstedt, Germany) for the analysis of NO_3-N and NH_4-N; a Dionex Ion Chromatograph (Sunnyvale, California) for the analysis of PO_4-P, Cl, and NO_2-N; and a Shimadzu Total Orgainic Carbon Analyzer (Kyoto, Japan) for the analysis of TOC. Analyses of samples for *E. coli*, oxytetracycline, pH, EC, TSS, and turbidity were performed at the Biosystems and Agricultural Engineering Department using an EcoTestr- EC Low (Vernon Hills, Illinois) for analysis of EC; a Thermo Electron Corporation Orion 520A+ (Waltham, Massachusetts) for analysis of pH; a LaMotte 2020 turbidimeter (Chestertown, Maryland) for analysis of turbidity; and a Sequoia Scientific LISST-Portable|XR (Bellevue, Washington) for the analysis of TSS. *E. coli* analyses were performed within 6 hours of sample collection using IDEXX Laboratories Coliert-18 (Westbrook, Maine) (Appendix C). All analyses were conducted per standard methods (APHA et al., 1998). If a constituent concentration was below the detectable limit, a value of half the detectable limit was used (USEPA, 1991).

2.2.6.1 Antibiotic Analysis

Samples were analyzed for antibiotics using a methodology developed in the Biosystems and Agricultural Engineering Department by Dr. Manish Kulshrestha. Appendix D contains a summary of that methodology. The purpose of the methodology was to concentrate the level of antibiotics in the sample for better detection. Briefly, each sample was centrifuged in Thermo Scientific Survall Legend XTR Centrifuge (Waltham, Massachusetts) at 3,500 rpm for 20 minutes. Then, 45 mL of supernatant was pipetted into a 50 mL glass test tube. The test tube was covered with a kimwipe and placed in a SP

Scientific VirTis Wizard 2.0 lyophilzer (Gardiner, New York) until dry (approximately 3 days). The dry residue was dissolved in 900 µL of 50% methanol solution resulting in a 50x concentration. The solution was transferred to microcentrifuge tubes and centrifuged with a Fisher Scientific Marathon 21000 (Waltham, Massachusetts) at 4,000 rpm for 10 minutes. Then, 100 µL of the concentrated solution was spiked with 100 µL of a 2 µg mL^{-1} oxytetracycline solution resulting in a 25x greater concentration of oxytetracycline as compared to the original sample. This step was done in order to develop a more defined peak on the HPLC.

Analytes within a 20 µL sample volume were separated using a Dionex Ultimate 3000 HPLC with an Acclaim 120 (C18) column (Sunnyvale, California) along with an Ultimate 3000RS Variable Wavelength detector (Sunnyvale, California) which was set to a wavelength of 290 nm (Kay et al., 2005). Separation in the HPLC was accomplished using a gradient mobile phase of 0.5% acetic acid in methanol and 0.5% acetic acid in deionized water; a pumping rate of 0.400 mL min^{-1} was used. Four standards (10, 20, 100 and 200 µg mL^{-1}) were used for calibration.

2.2.7 Data Analysis

Constituent concentrations from FWMC (x) and flow-weighted composite (y) samples were compared using linear regression models (y=x) in SigmaPlot 12.0, as described in Agouridis and Edwards (2003). Student's t-tests were performed to test the null hypothesis that the slope equaled one, since the null hypothesis tested in the linear regression model was that the slope equaled zero (Zar, 1999). The linear regression model tested the null hypothesis that the intercept equaled zero. All data were ln transformed to normalize the data.

2.3 RESULTS AND DISCUSSION

Results indicated that constituent concentrations for FWMC and flow-weighted composite samples (slopes) did not differ for ln *E. coli*, ln NO_3-N, ln NH_4-N, ln PO_4, ln pH, ln EC, ln TSS, ln TOC, and ln turbidity (Table 2.3, Figures 2.1-2.9). Strong relationships (R^2 values >0.9) were seen for ln *E. coli*, ln NH_4-N, ln EC, ln TSS, ln TOC and ln turbidity.

Table 2.3. Results of Regressing FWMC vs. Flow-Weighted Composite Concentration.

Constituent[1]	**Slope**	**Intercept**	**R^2**
ln *E. coli*	1.012	-0.383^R	0.995
ln oxytetracycline[2]	0.617^R	1.356^R	0.641
ln NO_3-N	0.792	-1.655^R	0.448
ln NH_4-N	0.898	-0.044	0.962
ln PO_4	0.931	-1.039	0.716
ln pH	1.018	-0.074	0.655
ln EC	0.976	0.174	0.956
ln TSS	1.000	-0.038	0.978
ln Cl^- [3]	1.292^R	-0.924^R	0.964
ln TOC	1.003	0.224	0.911
ln turbidity	1.099	-0.680^R	0.952

[1] Null hypothesis that the slope equaled one and the intercept equaled zero for the linear regression models. The superscript R indicated the null hypothesis was rejected at the $\alpha=0.05$ level of significance.

[2] All values included. If C2 is removed, slope=0.803, intercept=0.635, $R^2=0.688$; null hypotheses, for slope and intercept, accepted.

[3] All values included. If B6 and C5, are removed, slope=1.137, intercept=-0.438, $R^2=0.907$; null hypotheses, for slope and intercept, accepted.

Constituent concentrations for FWMC and flow-weighted composite samples differed for only two of the evaluated constituents: ln oxytetracylcine and ln Cl^-. Figures 2.10 and 2.11 show the results of regressing values of flow-weighted composite sampling against FWMC for these two constituents. For ln oxytetracycline, flow-weighted composite sampling was greater than the FWMC at lower levels (ln oxytetracycline < 2 $\mu g\ L^{-1}$). As seen in Figure 2.10, one plot largely accounted for this variation. For one C plot (C2, refer to Appendix A), four of the seven samples had non-detectable oxytetracycline values, plus, these four samples accounted for nearly 93% of the runoff. By removing this sample from the data set, values for FWMC and flow-weighted composite sampling do not significantly differ (slope=0.803, intercept=0.635, $R^2=0.688$).

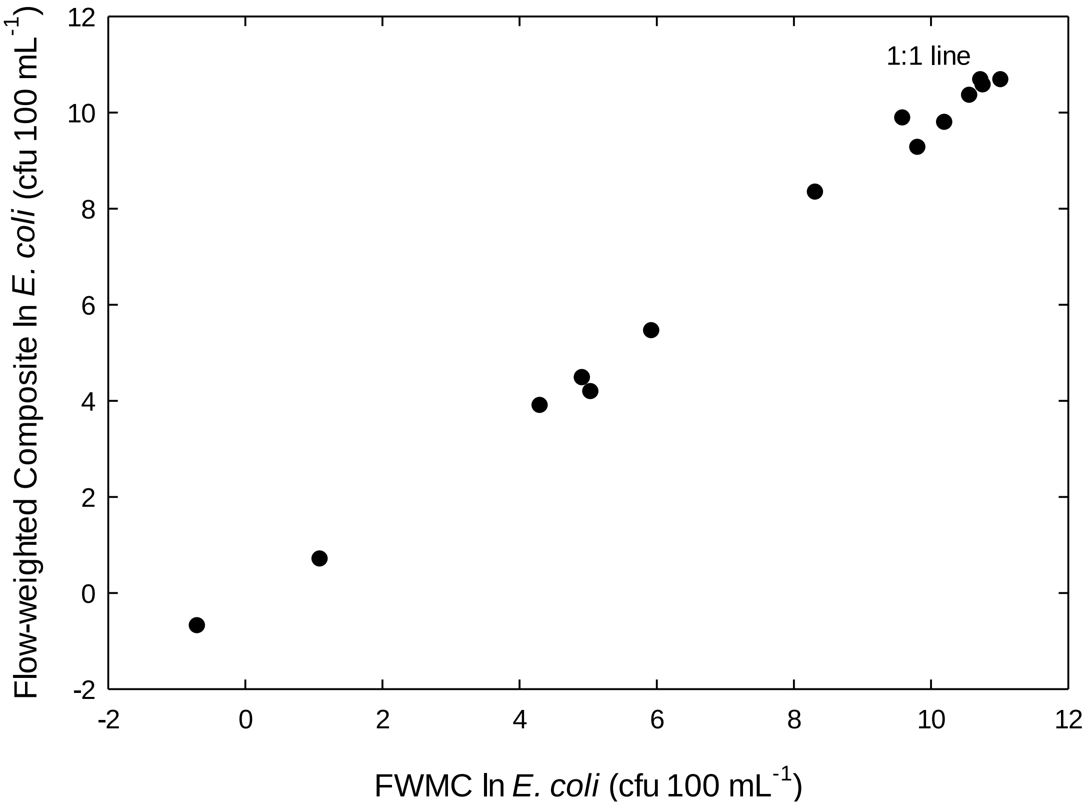


Figure 2.1. Comparison of FWMC and Flow-weighted Composite for ln *E. coli*.

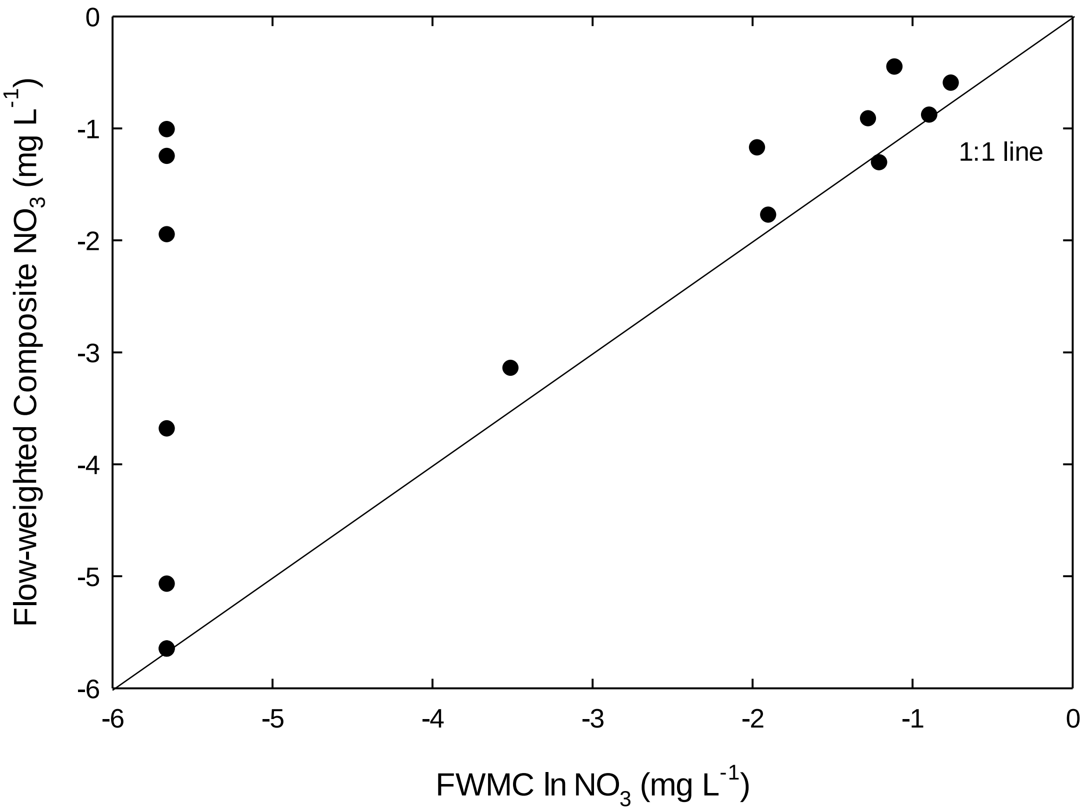


Figure 2.2. Comparison of FWMC and Flow-weighted Composite for ln NO^3.

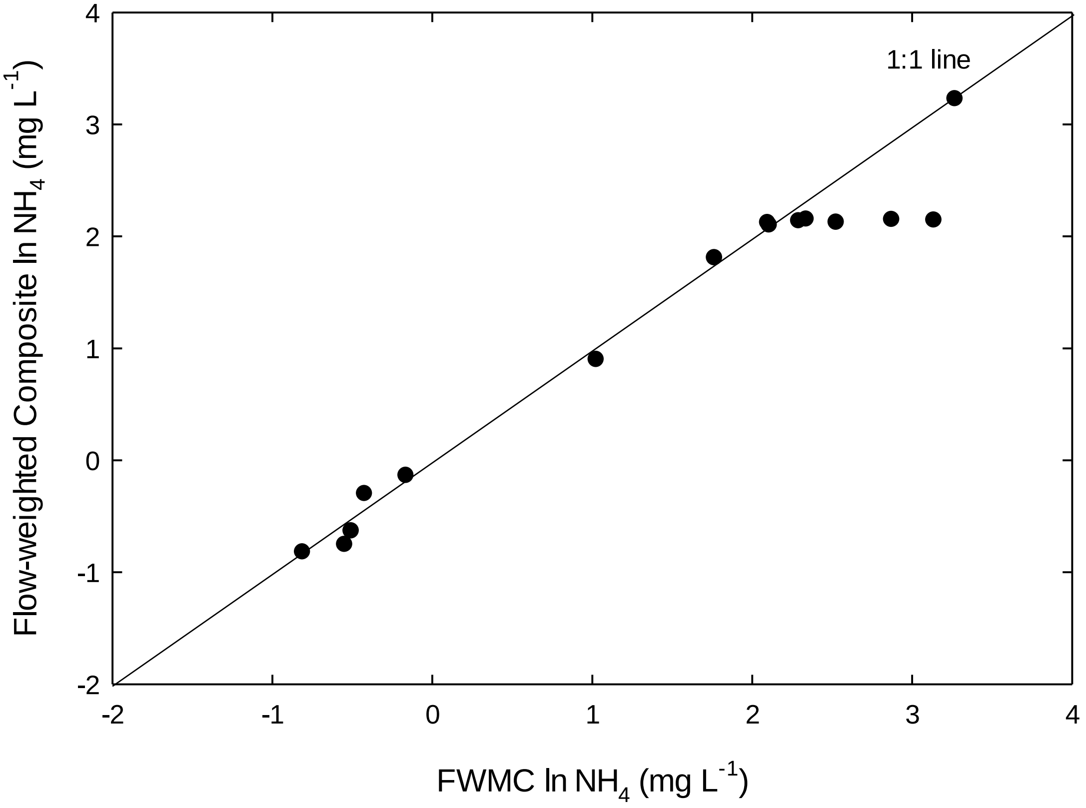


Figure 2.3. Comparison of FWMC and Flow-weighted Composite for ln NH_4.

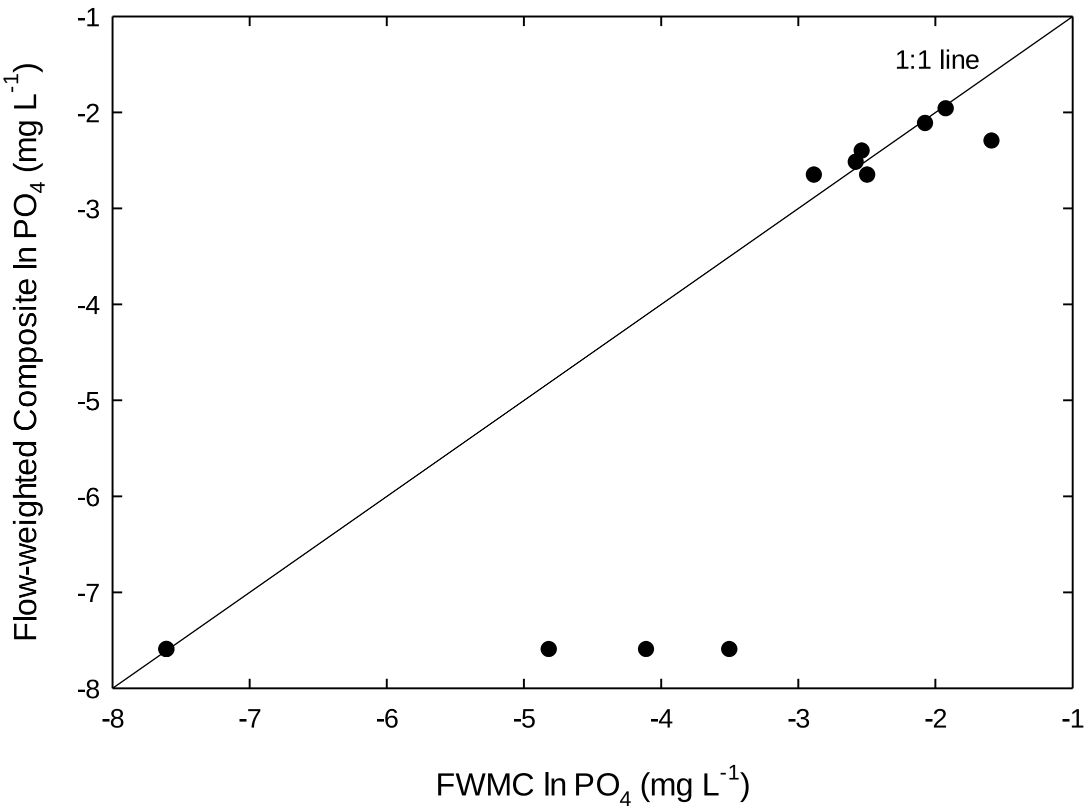


Figure 2.4. Comparison of FWMC and Flow-weighted Composite for ln PO_4.

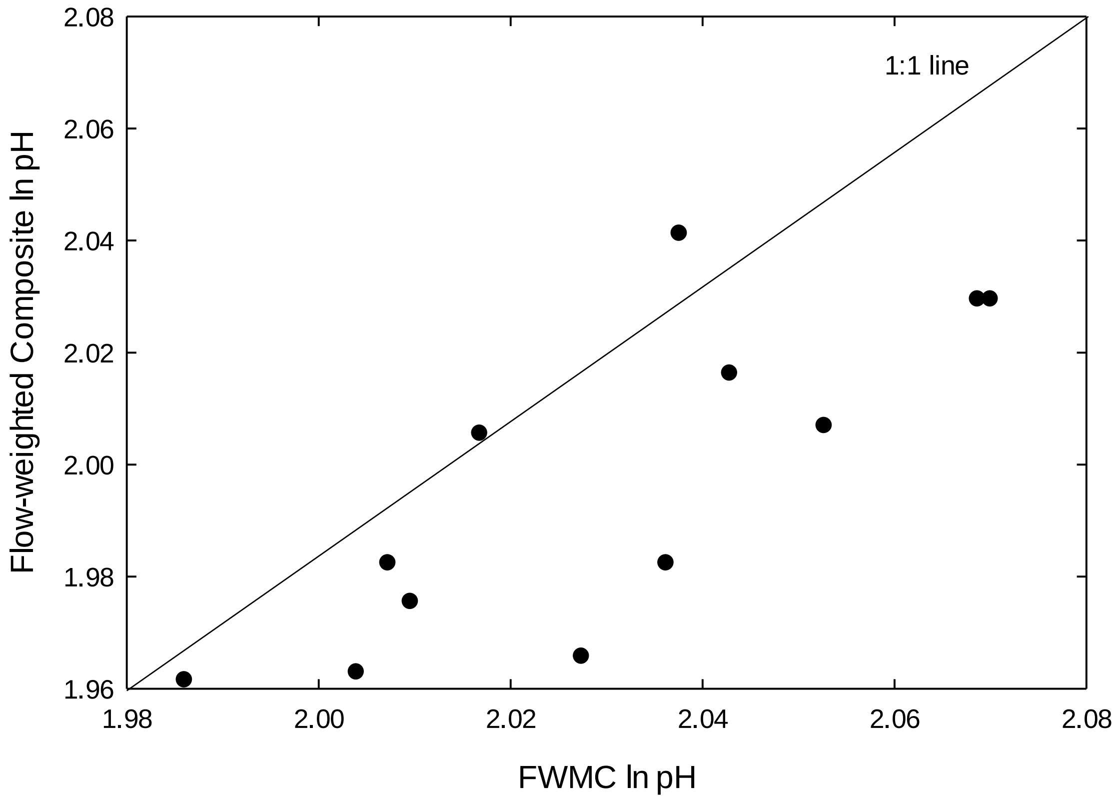


Figure 2.5. Comparison of FWMC and Flow-weighted Composite for ln pH.

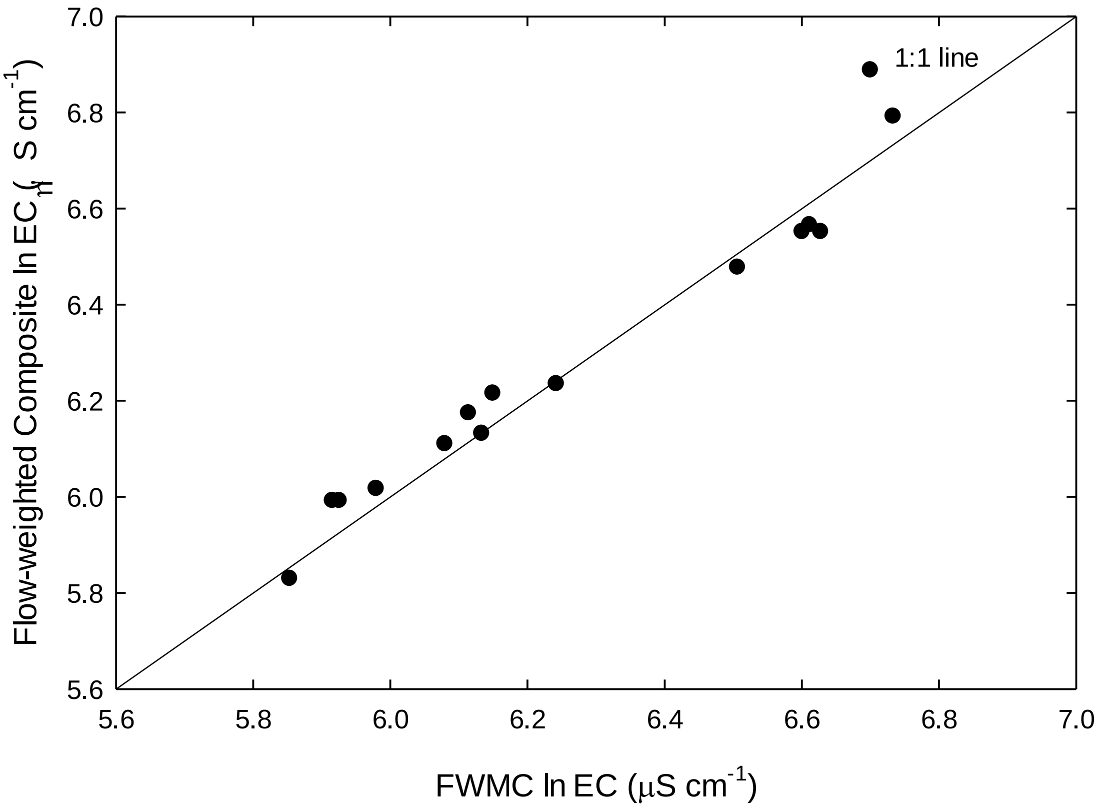


Figure 2.6. Comparison of FWMC and Flow-weighted Composite for ln EC.

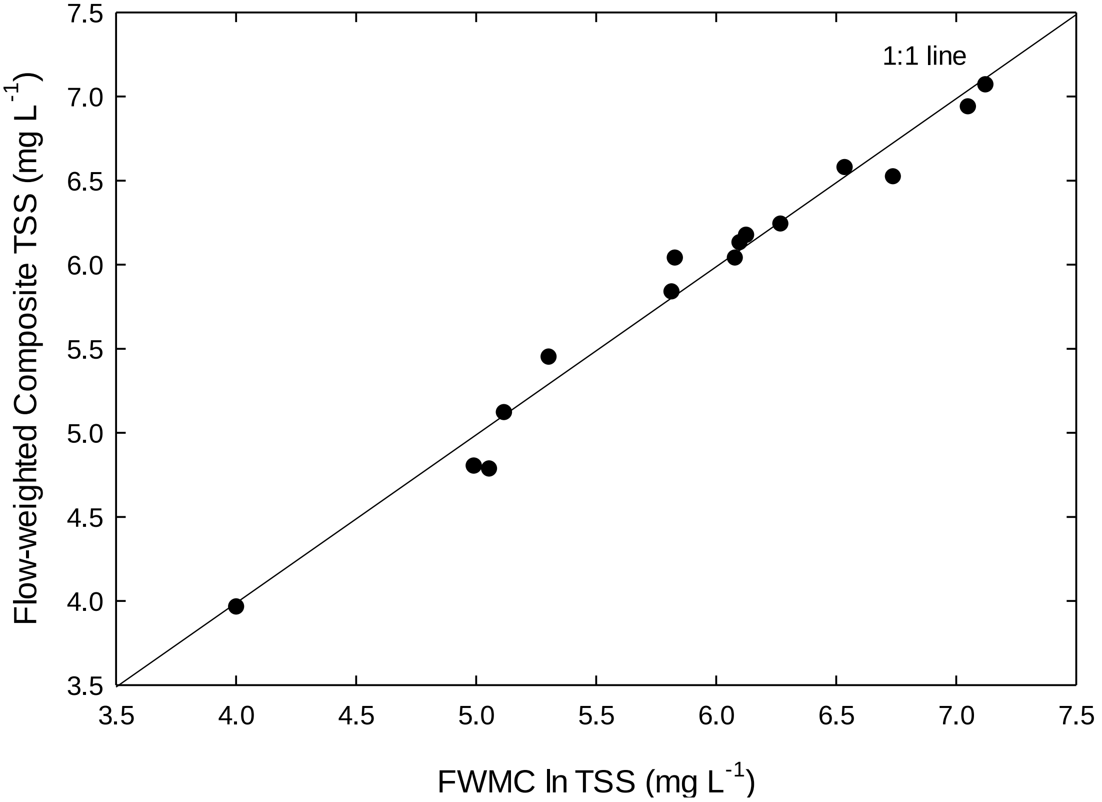


Figure 2.7. Comparison of FWMC and Flow-weighted Composite for ln TSS.

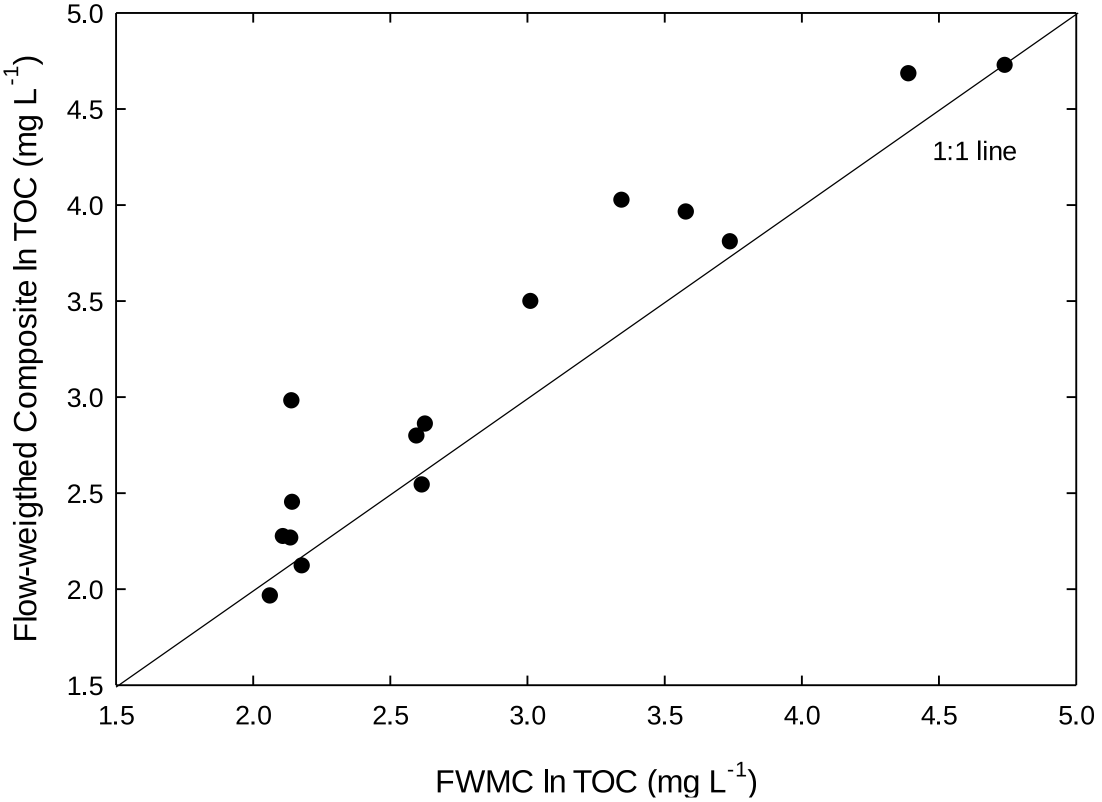


Figure 2.8. Comparison of FWMC and Flow-weighted Composite for ln TOC.

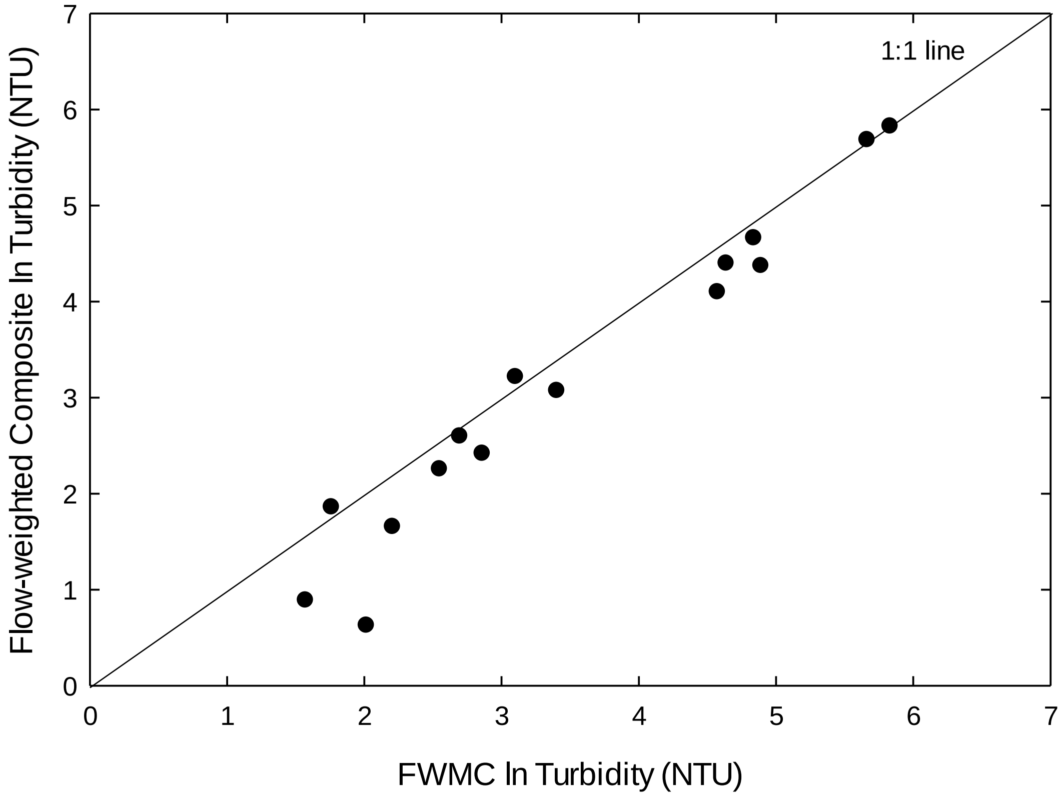


Figure 2.9. Comparison of FWMC and Flow-weighted Composite for ln Turbidity.

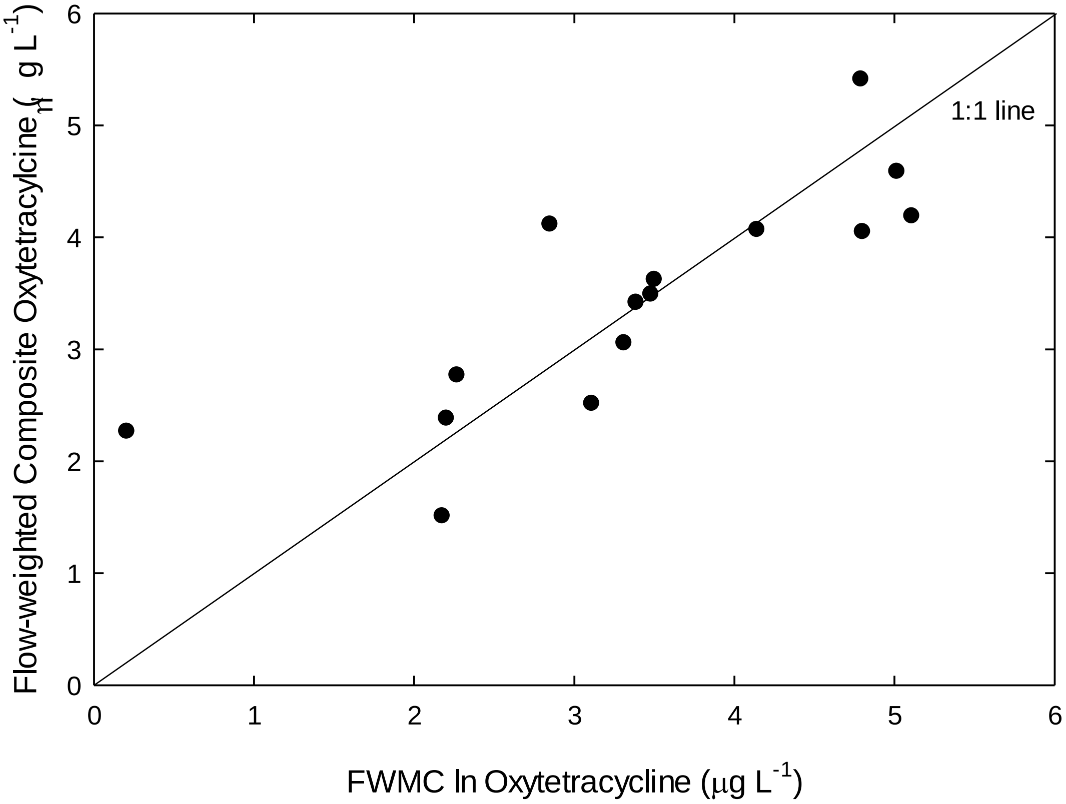


Figure 2.10. Comparison of FWMC and Flow-weighted Composite for ln Oxytetracycline.

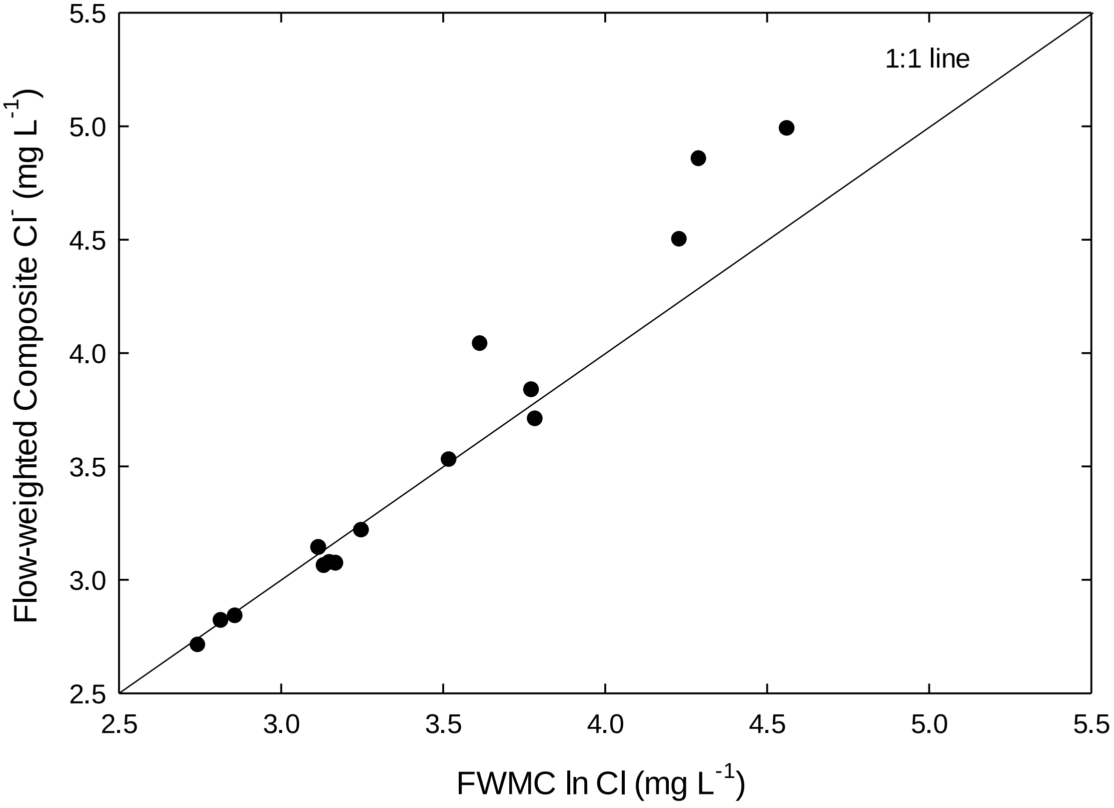


Figure 2.11. Comparison of FWMC and Flow-weighted Composite for ln C1.

The differences between the flow-weighted composite and FWMC values at these low levels (ln oxytetracycline < 2 μg L^{-1}) may be due in part to (1) the error associated with measuring the antibiotic levels, and (2) the magnification of this error when a large number of samples from the runoff event have low to non-detectable oxytetracycline levels, particularly if those samples are associated with relatively high proportions of flow. Measuring antibiotics in water samples is challenging as antibiotics concentrations are often low and numerous factors such as solubility, polarity, pH, presence of chlorine, and organic matter can interfere with extraction and analysis (Lindsey et al., 2001; Yang et al., 2005; Ye et al., 2007). Such factors affect the amount of antibiotic recovered, and hence error. For tetracylcines, Yang et al. (2005) noted recovery rates of about 80% from wastewater treatment plant influent and effluent with decreased recovery (~5%) associated with higher levels of organic matter or TOC. With their methodology, the authors noted accuracies ranging from -9.5 to 13.2% and precisions, relative to standard deviations, of 7.6 to 15.5%; no tetracycline concentration dependence with recovery precision was found. While the accuracy and precision of the antibiotic extraction and analysis methodology used in this study was not specifically examined, results of the calibration curves, where defined amounts of antibiotic were added to deionized water and measured, indicate the procedure works well under these controlled conditions. Challenges were noted with achieving peak separations when samples originated from plots containing manure. Since manure contains many organic contaminants, it was difficult to separate the oxytetracyline peak from the peaks created by the other organic contaminants at low levels. A similar lyophilization technique, as used in this study, was described by Hirsch et al. (1998). The authors found oxytetracyline recoveries average 108% for spiked spring water, but decreased to 49% in the presence of high organic matter.

Also, as seen in equation 2.1, samples for which no (or very little) oxytetracycline was detected do not contribute (or significantly contribute) to the FWMC. For the C plots, 11 samples out of 28 or nearly 36% had oxytetracycline values <2 μg L^{-1}. With the M plots, 11 samples out of 28 or nearly 40% had oxytetracycline values <2 μg L^{-1}. However, these non-detect samples accounted for 31 and 51% of the flow with the C and M plots, respectively. Hence, it is quite possible, at such low levels, that the subsamples collected to form the flow-weighted composites could have low but measureable levels of oxytetracycline, particularly considering the potential error of the antibiotic extraction and analysis methodology used in this study.

With regards to ln Cl, the opposite trend was found with oxytetracycline. FWMC and flow-weighted composite values were quite similar at values <4.0; however at values >4.0, flow-weighted composite sampling over-predicted the FWMC. These higher FWMC values occurred at plots B6 (MA), and C5 (M), which both produced runoff in about six minutes or less (only one other plot produced runoff as quickly, B5 (MAA)). It is possible that the quicker response rates at these two plots influenced Cl transport, but why this would result in differences between FWMC and flow-weighted composite values is not known. If the data points for these two plots are removed, then values for FWMC and flow-weighted composite sampling do not significantly differ (slope=1.137, intercept=-0.438, R^2=0.907).

A similar issue can be seen with NO_3-N and PO_4 (Figures 2.2 and 2.4) as with oxytetracycline. While the FWMC and flow-weighted composite concentrations were statistically the same for these constituents, the relationship was not very strong (R^2=0.382 for NO_3-N and 0.730 for PO_4). Several samples showed concentrations below detectable limits for at least one of these constituents which could account for the error associated with these constituents.

2.4 CONCLUSIONS

A strong correlation was found between FWMC and flow-weighted composite concentrations for the majority of constituents analyzed including ln *E.coli*, ln turbidity, ln TOC, ln TSS, ln NH_4-N, and ln EC. All of these constituents had values well above the detection limits. The parameter ln *E.coli*, which can be quite expensive and time consuming to test for depending on the number of dilutions needed, exhibited the strongest correlation (R^2=0.995). When samples contained constituent concentrations close to the detection limits, larger errors were noted with the flow-weighted composite samples such as seen with PO_4, NO_3-N, and oxytetracycline. These findings suggest that the flow-weighted composite sampling technique is more reliable for higher constituent concentrations as compared to lower ones that are near the detection limits of the analytical procedures. The precision of the oxytetracycline analysis technique used in this study coupled with the low levels of oxytetracycline found in water samples could account for the error in these flow-weighted composite samples. The reason for differences between the FWMC and flow-weighted composite concentrations, which were more prominent at higher Cl concentrations, was not known.

The analysis of water quality constituents can be time-consuming and cost prohibitive. For this study, 112 individual samples and 16 composite samples were analyzed at a cost of approximately $5,125 for the individual samples and $736 for the composite samples. Flow-weighted composites can be a cheaper option for water quality sampling, without sacrificing the quality of data for many constituents. However, when constituent concentrations are close to or below detection limits, errors in flow-weighted composite samples can be magnified, especially at higher flows. Thus in cases where constituent concentrations are low, it is recommended that FWMC are used, if possible, to minimize error.

CHAPTER 3: RELATIONSHIPS BETWEEN OXYTETRACYCLINE AND OTHER WATER QUALITY CONSTITUENTS

3.1 INTRODUCTION

Veterinary antibiotics have a variety of uses in animal agriculture, the main purpose being the treatment of diseases and infections. Oftentimes though, antibiotics are used for sub-therapeutic reasons such as disease prevention and growth promotion via increased feed efficiency. Antibiotics can increase the absorption of feed by inhibiting bacteria, thereby creating a higher feed conversion ratio and more rapid weight gains (Kumar et al., 2005). Because of these benefits, concentrated animal feeding operations (CAFOs) administer antibiotics to livestock through feed, water injections, and/or external applications in order to offset the negative effects that crowded living conditions have on livestock health. While the use of veterinary antibiotics is not well documented, the USEPA (2013) estimates that 60-80% of livestock and poultry routinely receive antibiotics. For example, a 1996 survey conducted by the USDA revealed that 93% of hogs were administered antibiotics at some point during the grower/finisher period with a significant portion (25%) receiving higher than recommended doses (Dewey et al., 1997). Several classes of antibiotics are approved for use in livestock with tetracyclines and ionophores accounting for 70% of all livestock and poultry antimicrobials (USEPA, 2013).

Unfortunately, significant portions of antibiotics administered to livestock are not metabolized by the animal but instead are excreted in urine and feces. Antibiotics are excreted at rates of 30-90%, and the form that is excreted is virtually unchanged from the parent compound (Sarmah et al., 2006). Concentrations of antibiotics have been found in manure ranging from trace amounts to 200 mg L^{-1} (Kumar et al., 2005). One of the most commonly used classes of antibiotics, tetracyclines, are excreted at rates as much as 70-90%. The land application of manure, or even the direct deposition of manure on pasturelands and rangelands via grazing livestock, has thus become the primary pathway in which veterinary antibiotics enter the environment (Baguer et al., 2000). It is estimated that 50% of surface waters contain antibiotics (Koplin et al., 2002). Campagnolo et al. (2002) found antibiotics in 31% of surface and groundwater samples near swine farms and 67% of surface and groundwater samples collected on or adjacent to poultry farms. While sampling streams in an agricultural watershed dominated by poultry production, Arikan et al. (2008) found that tetracyclines (chlortetracycline and oxytetracyline) were the two most commonly identified antibiotics with concentrations of 0.016 µg L^{-1}. The authors concluded the antibiotics were transported via runoff from nearby manure-amended fields, as no other potential sources of antibiotics were identified in the watershed. While it is not known at what levels antibiotics have a significant impact on aquatic life, hormones levels as low as 10-120 ng L^{-1} have been shown to have adverse effects such as defeminization or demasculinization in fish (Durhan et al., 2006).

While many antibiotics are soluble in water, others bind to soil particles and are transported with eroded sediments, while some remain in the soil profile. The method of transport varies with the physical and chemical properties of the antibiotic, such as solubility and attraction to soil particles (Sarmah et al., 2006). The attraction to manure or soil particles is given by the partition coefficient, K_d, which is a ratio of a chemical sorbed to soil to the amount which remains in solution (Brady and Weil, 2008) and can range in values from 0.6 to 6,000 for different antibiotics (Tolls, 2001). Macrolide antibiotics, like tysolin, are highly soluble (5 g L^{-1}) (Chee-Sanford et al., 2009) indicating that such antibiotics are relatively hydrophilic (e.g. water loving). However, tysolin has a K_d value between 8.3-240 (Tolls, 2001), suggesting that mobility may be limited to transport largely by soil. Sulfamethazine, on the other hand, has a lower solubility in water (0.6 g L^{-1}) (Chee-Sanford et al., 2009) coupled with a very low K_d (4.9) (Tolls, 2001) thus making it more susceptible to transport via water. Though tetracycline antibiotics have moderate solubilities (0.6-1.7 g L^{-1}), they have extremely high K_d values (282-2,608) thus making them relatively immobile in water.

Due to the high excretion rates and recorded presence of antibiotics in surface waters, the USEPA (2007) has identified antibiotics as a contaminant of emerging concern (CEC). A CEC is a contaminant that is now being detected in the environment and/or is at higher than expected levels, meaning it was not previously present in the environment or was present at undetectable or very low levels. Plus, as is the case with antibiotics, the impact of their presence in the environment on human health is not well-known as research in this area is quite sparse. In evaluating ecological impacts, Baguer et al. (2000) found that two antibiotics, oxytetracyline and tylosin, exhibited low levels of toxicity to soil fauna and moderate levels of toxicity to aquatic invertebrates. Thus, the potential exists for these organisms, particularly bacteria, to develop a survival response and hence develop a resistance to antibiotics. Mckeon et al. (1995) collected water samples from 44 untreated, rural, private groundwater wells over the course of three years. Bacteria samples were isolated and tested for resistance to several antibiotics. Over 250 coliform and non-coliform bacteria strains were isolated, with 90% showing resistance to at least one antibiotic and 78% demonstrating multiple antibiotic resistances. Of the bacteria isolated, 32% were resistant to tetracyclines. And while antibiotic resistant bacteria have limited viability outside of the gastrointestinal tract of livestock, the potential exists for such bacteria to transfer the genes for antibiotic resistance to other bacteria. Chee-Sanford et al. (2001) examined the dispersion of antibiotic resistant genes in bacteria that originated from a swine waste storage lagoon. The authors found tetracycline resistant genes in bacteria sampled from groundwater wells more 250 m downstream of the swine facility.

One means of reducing antibiotics in waterbodies is via source reduction whereby fewer antibiotics are administered to livestock. For example, the Food and Drug Administration is beginning to work with pharmaceutical companies to voluntarily modify labeling on antibiotics such that those used to treat human infections would not be labeled for use for growth promotion in livestock (Jalonick, 2013). However, since antibiotics are an important component of animal agriculture, a complete ban on their use for growth promotion is unlikely, particularly in the near future. McEwen and Fedorka-Cray (2002) stated that such a ban could have unwanted consequences such as decreased motivation by pharmaceutical companies for new drug development, reduced livestock production efficiencies, and the increased therapeutic uses as the rate of infectious diseases is likely to rise. Another concern is that banning antibiotic use for growth promotion could be extremely costly to the industry. Brorsen et al. (2002) estimated that such a ban would result in an annual loss of $242.5 million for the industry. Thus, with the increased presence of antibiotics in the environment coupled with the unlikelihood of a substantial decrease in antibiotic use in livestock, determining ways to reduce antibiotic transport in the environment has become more critical. The first step in reducing antibiotic transport is gaining a better understanding how antibiotics behave in the natural environment. Such knowledge can aid in the development and/or selection of best management practice (BMP) for reducing antibiotic transport to waterbodies.

Presently, several antibiotics are approved for use in both humans and livestock. Oxytetracycline, because of its use in all types of livestock – it is a registered growth promoter for cattle and swine in the U.S. (Sarmah et al., 2006) - as well as it use in humans, was selected for this study. Oxytetracylcine has solubility in water of 1,000 mg L^{-1} , which is less soluble than other commonly used antibiotics such as tylosin (5 g L^{-1}) or penicillin (4 g L^{-1}) (Chee-Sanford et al., 2009). The objective of this study was to compare the concentrations of oxytetracyline in simulated runoff to those of other constituents to determine whether oxytetracycline behaves more like a dissolved (e.g. NO_3) or particulate (e.g. TSS) constituent. The results of this study could provide insight on which BMPs are likely most suitable for evaluation in reducing antibiotic transport to surface waters.

3.2 MATERIALS AND METHODS

3.2.1 Study Site

Rainfall simulators and fescue runoff plots at the University of Kentucky's Maine Chance Research Farm (latitude: 38.1164°N; longitude: 84.4903W) were used for this study. Plots (2.44 m by 6.10 m) were constructed on Maury silt loam (fine, mixed, mesic Typic Paleufalf) (NRCS, 2013) at a 3% slope as described by Edwards et al. (2000). Plots were planted with Kentucky 31 fescue and bordered with galvanized steel to ensure runoff stayed on the plots during simulation. Each plot was equipped with a gutter that diverted runoff into a PVC pipe that then discharged it into a sump where samples were collected. A wood cover was placed over each gutter during simulation to ensure only runoff was collected.

3.2.2 Plot Selection

Curve numbers (CN) were computed for each plot in May 2013, as described in Appendix A. Twelve plots with similar CNs were selected for this study. These plots and their corresponding CNs are provided in Table 3.1. Plots selected for this study had a mean curve number of 83 ±4.

3.2.3 Experimental Treatments

Manure was obtained from feeder pigs at a private hog farm in Bardstown, Kentucky. The farm had not administered antibiotics to the hogs since their birth. Manure, which had a slurry consistency, was stored in a pit before collection. Manure was transported to and stored in a 1,041 L polyethylene intermediate bulk container (IBC) tote at the University of Kentucky Biosystems and Agricultural Engineering Department. Three treatments were randomly assigned to the plots. The treatments consisted of a control (manure was not applied) (C), manure only (M), and manure and antibiotics (MA). Table 3.2 details which treatments were assigned to which plost. Manure (55 L) was placed in separate 208 L barrels for each treatment the night before each application. Note that one plastic barrel was used for each treatment to prevent cross-contamination.

Table 3.1. Curve Numbers of Plots Used in the Study.

Plot	CN	Plot	CN
B4	82	C6	77
B6	90	D1	86
B10	82	D5	84
C2	89	L1	84
C3	82	L3	79
C5	89	B8	81

Table 3.2. Treatment Associated with Each Plot.

Treatment[1]	Plots
C	C2, C6, D1[2], D5
M	B4, B8[3], C5, L1
MA	B6, B10, C3, L3

[1] C=control, M=manure only, and MA=manure and antibiotics.
[2] D1 was not used in the *E. coli* analysis due to sampling error.
[3] B8 was not used in the analysis because it did not produce runoff

Commercially available, injectable antibiotics (Noromycin 300 LA, Norbrook, Lenexa, KS) were obtained from the local feed store. The antibiotics contained 300 mg of oxytetracycline per mL in suspension. To each manure container, 3.67 mL of oxytetracycline suspension was injected, spiking the manure to an antibiotic concentration of 20 mg L^{-1} of manure. Uniform mixing was achieved using a custom mixer attached to a cordless drill. The custom mixer, which was constructed in the Biosystems and Agricultural Engineering Department, was approximately 1.5 m in length and had two 30 cm long fins at a distance of 0 cm and 30 cm from the bottom. Manure was applied to the M and MA plots, with an application rate of 3 L m^{-2}, immediately prior to the start of simulated rainfall. This application rate corresponded to 45 L per plot, as previously done by Bushee-Bullock (1999). The target application rate was 112 kg N ha^{-1} as recommended by AGR-1 (UK, 2012) and as done in Bushee-Bullock (1999). However, based on laboratory results from the University of Kentucky Regulatory Services, the actual application rate was about 150 kg N ha^{-1}. Plots were mowed to an approximate sward height of 10 cm within three days before manure application. Soil samples and manure samples were collected for each plot immediately before manure application. Soil and manure samples were analyzed at the University of Kentucky Regulatory Service Laboratory (Appendix E). Soil samples were analyzed for P, K, Ca, Mg, Zn, pH, total nitrogen, and soil texture while manure samples were analyzed for N, P, K, Ca, Mg, Zn, Cu, Fe, and Mn. Soil moisture was determined at the Biosystems and Agricultural Engineering Department.

3.2.4 Rainfall

Rainfall simulators were programmed to produce a constant rainfall of 102 mm hr^{-1} (refer to Appendix A for calibration curves). To represent a worst-case scenario, rainfall simulation began 30 minutes after manure application and continued until 30 minutes after runoff began. Time to runoff was recorded for each plot.

3.2.5 Runoff Sample Collection

Runoff samples were collected from the PVC pipe attached to the gutter at intervals of 2, 4, 6, 8, 16, 24 and 30 minutes after runoff began. Two 1-L samples were collected in clean, autoclaved polyethylene bottles at each time interval. Samples were collected for 60 seconds or until the bottle was filled, whichever came first. The time required to fill each bottle and the bottle's weight were recorded. An average tare weight was obtained for all bottles prior to sample collection. These values were used to create two flow-weighted composite samples for each plot. One sample was created for bacterial analysis while the other was used for the remaining constituents. The data collected and volumes of each timed sample were used to create the composite samples (Appendix B).

3.2.6 Laboratory Analysis

All eight samples collected from each plot (seven timed intervals and one composite) were tested for the following constituents: *E. coli*, oxytetracycline, NO_3-N, NH_4-N, PO_4-P, pH, EC, TSS, Cl, TOC and turbidity. Samples were stored at approximately 4°C until analysis. The University of Kentucky's Forestry Department analyzed samples for NO_2-N, NO_3-N, NH_4-N, PO_4-P, Cl, and TOC. A Bran-Luebbe Auto-Analyzer III (Norderstedt, Germany) was used for the analysis of NO_3-N and NH_4-N. A Dionex Ion Chromatograph (Sunnyvale, California) was used for PO_4-P, Cl, and NO_2-N. Total organic carbon (TOC) was analyzed using a Shimadzu Total Orgainic Carbon Analyzer (Kyoto, Japan).

Analysis for *E.coli*, oxytetracycline, pH, EC, turbidity and TSS occurred at the Biosystems and Agricultural Engineering Department. *E.Coli* analysis was conducted within 6 hours of sample collection. Coliert-18 was purchased from IDEXX laboratories (Westbrook, Maine). Each sample from plots receiving manure was diluted three times using sterile deionized (DI) water and buffer water. Instructions for buffer water preparation and dilution can be found in Appendix C. Samples from control plots were only diluted once. All dilutions were analyzed for *E.coli* by dissolving one packet of Coliert-18 and two drops of anti-foaming agent in the sample. The samples were poured into a Quanti-Trays*/2000 and sealed using an IDEXX Quanti-Tray* Sealer. Trays were placed in an incubator at 35°C±0.5°C for 18-22 hrs. A UV-light was used after incubation to determine the number of cells that fluoresced and turned yellow, indicating the presence of *E.coli*. Software provided by IDEXX was used to determine the most probable number of *E.coli* in each sample based on the number of cells that were positive. Averages of the values for each dilution of each sample were computed.

An EcoTestr-EC Low (Vernon Hills, Illinois) was used for EC analysis. A Thermo Electron Corporation Orion 520A+ (Waltham, Massachusetts) was used for pH determination and a LaMotte 2020 turbidimeter (Chestertown, Maryland) was used for turbidity. If the sample was too turbid, a 1/10 dilution was done by adding 1 mL of sample to 9 mL of DI water. TSS analysis was conducted using a Sequoia Scientific LISST-Portable|XR (Bellevue, Washington). Samples (175 mL) were separately added to the mixing chamber and the mixer turned on. If sample was to turbid, 100 ml was removed and the remaining sample was diluted with100 mL of DI water. All analyses were conducted per standard methods (APHA et al., 1998).

3.2.6.1 Antibiotic Analysis

Samples were analyzed for antibiotics using the methodology described in Appendix D that was developed in the Biosystems and Agricultural Engineering Department by Dr. Manish Kulshrestha. Samples were centrifuged at 3,500 rpm for 20 minutes on Thermo Scientific Survall Legend XTR Centrifuge (Waltham, Massachusetts) before adding 45 mL to a 50 ml glass test tube. Each test tube was covered with a kimwipe and placed in SP Scientific VirTis Wizard 2.0 lyophilzer (Gardiner, New York) until dry (approximately 3 days). The remaining residue was dissolved in 900 µL of 50% methanol solution, giving a 50X concentration from the original sample. The solution was centrifuged again at 4,000 rpm for 10 minutes. A known amount of oxytetracycline was added to the solution to give a better defined peak on the HPLC. A 25X concentrated sample was achieved by adding 100µL of 2 µg mL^{-1} oxytetracycline solution to 100 µL of concentrated solution.

High-Pressure Liquid Chromatography (HPLC) was used to separate the analytes. A gradient mobile phase of 0.5% acetic acid in water and 0.5% acetic acid in methanol was used. A Dionex Ultimate 3000 HPLC (Sunnyvale, California) along with an Ultimate 3000RS Variable Wavelength detector set at 290 nm was used for this analysis. The HPLC used a pumping rate of 0.400 mL min^{-1} and injected 20µL of sample through a

Dionex Acclaim 120 (C18) column (Sunnyvale, California). Four standards (10, 20, 100 and 200 µg mL^{-1}) were used for calibration.

3.2.7 Data Analysis

Runoff flow rates from each plot were variable; therefore, constituent concentrations were normalized by flow rate. Differences between treatments were assessed for oxytetracycline using linear mixed models (PROC MIXED) and least square means (LSMEANS) in SAS 9.3. Pearson correlation coefficients (r) between concentrations of oxytetracycline in runoff samples and the other constituents were determined for each treatment using the PROC CORR. The Pearson correlation coefficient measures the linear correlation between two variables and ranges between -1 and 1. Negative values indicate a negative correlation while positive indicate a positive relationship. A significance level of α=0.05 was used fo all analyses.

3.3 RESULTS AND DISCUSSION

3.3.1 Manure and Soil Sampling

Table 3.3 contains the manure sampling results for each treatment. Values for N and P were similar to those reported by ASABE (2005) for flush building. Table 3.4 contains the soil sampling results for each treatment. Values for organic matter (OM) and N differed between C and MA; P did not differ between the treatments. Results for OM were similar to those reported by Edwards et al. (2000) who used plots at the same rainfall facility. However, N and P levels in this study were much lower than those reported by Edwards et al. (2000). Values of N and P in this study ranged between 827 to 1,009 mg kg^{-1} and 37 to 47 mg kg^{-1} for N and P, respectively while Edwards et al. (2000) recorded mean N and P values of 3,547 mg kg^{-1} for N and 169 mg kg^{-1} for P from soils prior to manure and urine application. The differences may be due to the lengthy fallow period (~10 years) experienced by the plots.

Table 3.3. Manure Sampling Results.

Treatment[1]	**C (%)**[2]	**N (%)**	**P (%)**
M	2.0±0.0 a	0.5±0.0 a	0.1±0.0 a
MA	2.0±0.2 a	0.5±0.0 a	0.1±0.0 a

[1] C=control, M=manure only, and MA=manure and antibiotics.
[2] C=carbon, N=nitrogen, P=phosphorus
[3] Within column means followed by the same letter are not significantly different (α=0.05).

Table 3.4. Soil Sampling Results.

Treatment[1]	**OM (%)**[2]	**N (mg kg^{-1})**	**P (mg kg^{-1})**
C	2.8±0.2a[3]	824±52 a	43±12 a
M	3.3±0.2 ab	947±44 ab	53±11 a
MA	3.5±0.4 b	1,009±108 b	47±11 a

[1] C=control, M=manure only, and MA=manure and antibiotics.
[2] OM=organic matter, N=nitrogen, P=phosphorus
[3] Within column means followed by the same letter are not significantly different (α=0.05).

3.3.2 Antibiotic Losses via Runoff

Treatment ($p<0.0001$) and sampling interval ($p=0.0113$) had significant effects on oxytetracyline concentrations while replication ($p=0.4286$) did not. As expected, oxytetracycline concentrations for the MA treatment differed significantly from the M and the C plots (Table 3.5). For all plots, oxytetracycline concentrations were highest at the time intervals 2, 4 and 6 minutes after the start of runoff; concentrations decreased as the simulated storm continued (Figure 3.1). Oxytetracycline, as well as most of the other constituents, exhibited the first flush phenomenon, which refers to a disproportionally high concentration of a constituent in runoff during the first part of a storm (Sansalone and Buchberger, 1997). This first-flush phenomenon is often seen in runoff events with

Table 3.5 Flow Normalized Oxytetracylcine Levels per Treatment.

Treatment[1]	**Mean Concentration (µg·s L^{-2})**[2,3]
C	378.2^{b}
M	421.7^{b}
MA	1897.3^{a}

[1] C=control, M=manure only, and MA=manure and antibiotics.
[2] Treatments with the same superscript indicate the means were not significantly different ($p<0.05$).
[3] Non-normalized means are as follows: C= 15.6 µg L^{-1}, M= 22.5 µg L^{-1}, and MA=129.7 µg L^{-1}.

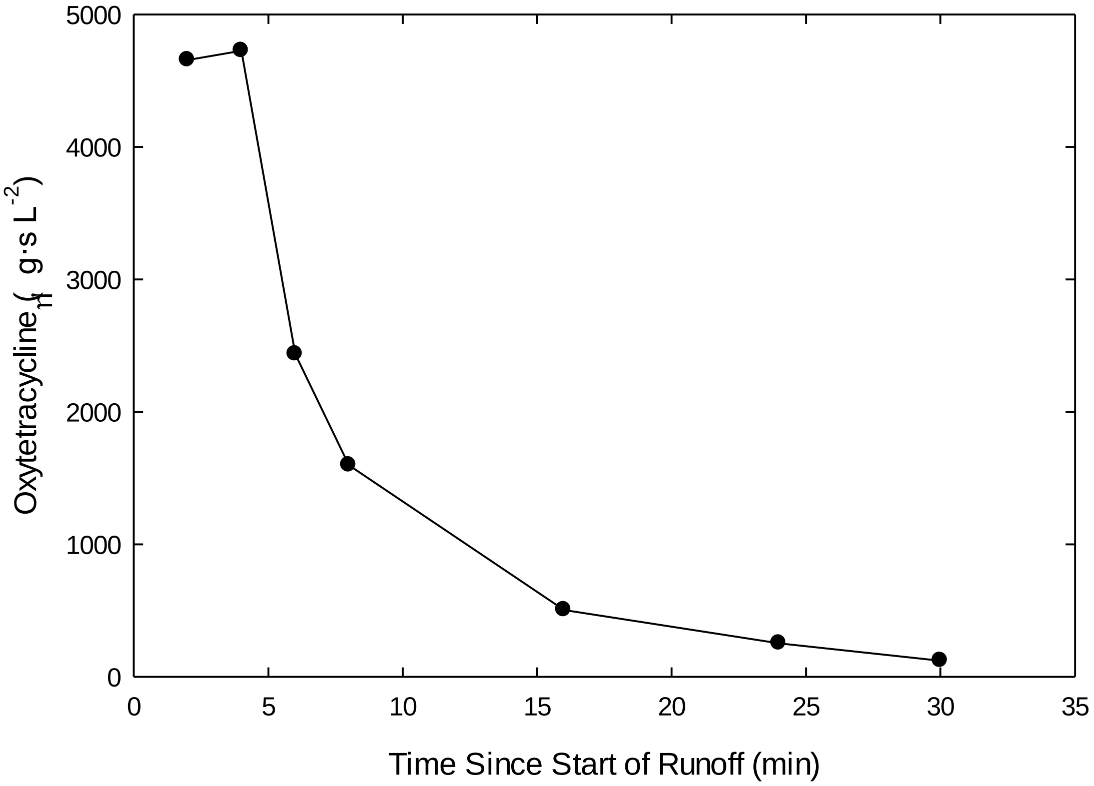


Figure 3.1. Oxytetracycline Concentrations Normalized by Flowrate for a MA Plot (B6).

both suspended and dissolved constituents (Sansalone and Buchberger, 1997; Braskerud, 2002).

While it was expected that the C and M plots would not have detectable oxytetracycline levels, this was not the case. The initial time intervals (2, 4 and 6 minutes) for the C and M plots produced higher than expected means (Figures 3.2 and 3.3). At the 8 minute and subsequent intervals, oxytetracycline levels plateaued, approaching zero or nearly zero for most plots. The higher oxytetracylcine concentrations in the C and M plots, at the beginning of the simulated storm events, were attributed to difficulties in achieving peak separation in the chromatograms produced by the HPLC at these low concentrations. Challenges in achieving peak separations in samples could account for a portion of the oxytetracycline levels as concentrations are computed based on the areas under the curves. Also important to note is that the samples from the M, as well as MA, plots contained manure particles and hence organic matter. The presence of organic matter has been shown to negatively impact oxytetracycline recovery rates (Yang et al., 2005). In the runoff samples, another unknown pollutant was detected near the peak of oxytetracycline thus preventing the return of the measurement line to the datum. This had a greater impact on the samples from the M plots. This interference made quantification of low levels of oxytetracycline difficult. It is possible that concentrating the samples via lyophilization did not allow for the removal of the interfering pollutant. Hirsch et al. (1998) found an average oxytetracyline recovery rate of 108% for spiked spring water when using a similar lyophilization technique; however, recovery rates decreased to 49% in the presence of high organic matter.

The peak (unnormalized) concentrations of oxytetracycline ranged between 62 and 549 μg L^{-1} for the four MA plots. Three of the four peaks (144, 408, and 549 μg L^{-1}) were much higher than those found in other runoff studies. Kay et al. (2005) in the United Kingdom, applied swine manure with an oxytetracycline concentration of 18.85 mg L^{-1} to runoff plots and measured a peak runoff concentration of 71.7 μg L^{-1}. Manure was applied 24 hrs before irrigation, which would have allowed the antibiotics to infiltrate into the soil before the simulated storm event. The plots only received 15 mm of rain over the course of two days, which did not allow for as much runoff to be generated as quickly as in this study. Dolliver and Gupta (2007) measured antibiotic losses

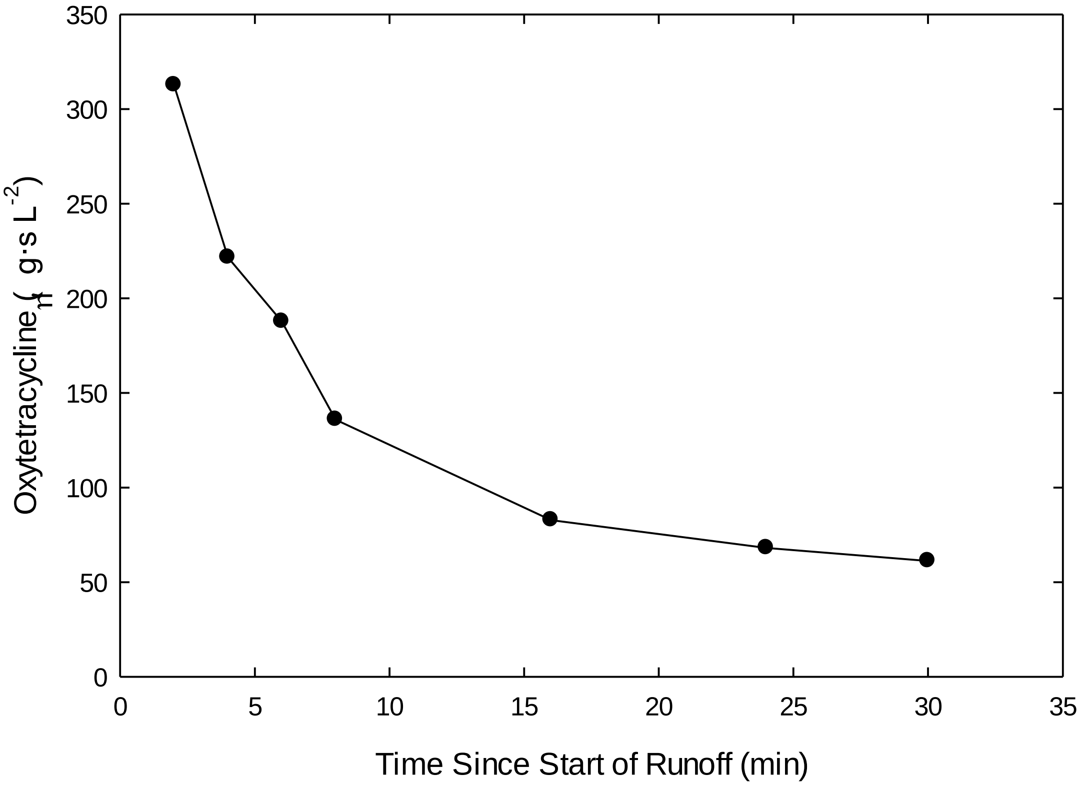


Figure 3.2. Oxytetracycline Concentrations Normalized by Flowrate for a C Plot (C2).

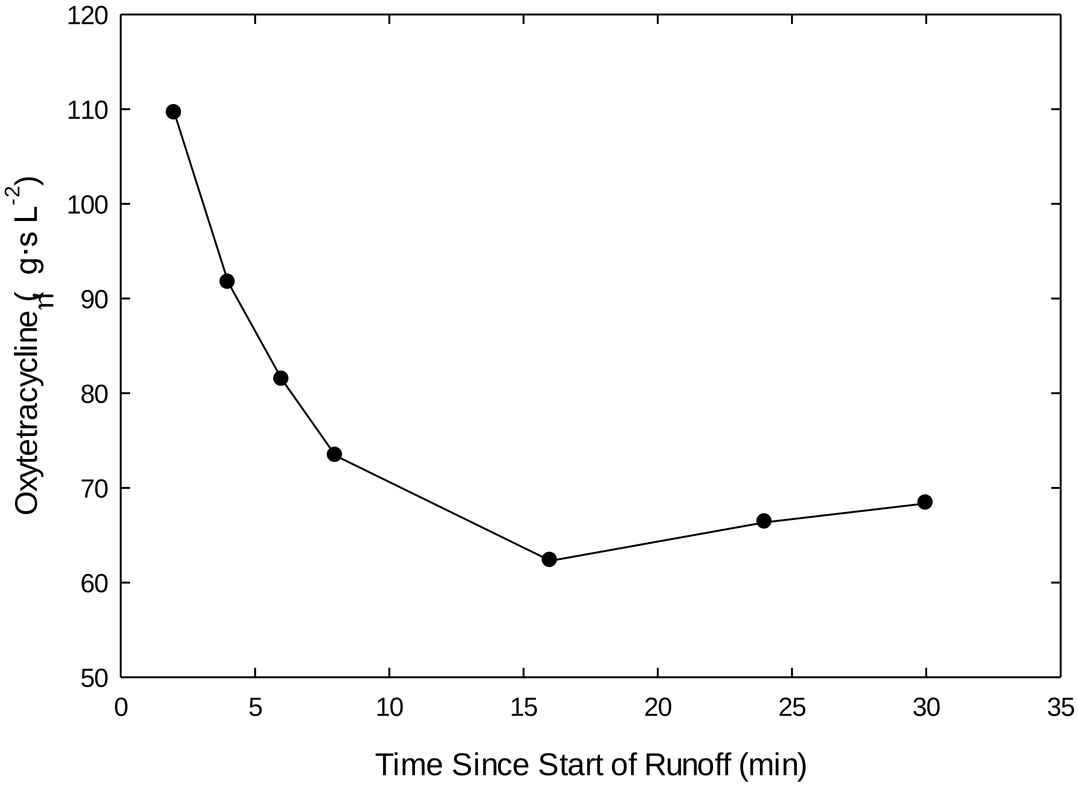


Figure 3.3. Oxytetracycline Concentrations Normalized by Flowrate at M Plot (B4).

from a manure storage facility and found a peak concentration of 210 µg L^{-1} of chlortetracycline. However, the first rainfall event was several days after manure application, which could have allowed for antibiotic degradation or losses due to leaching. Davis et al. (2006) applied seven different antibiotics, including tetracycline and chlortetracycline, to plots at a concentration of 1 mg L^{-1} in water rather than manure. The authors measured concentrations in runoff of 0.03 µg L^{-1} for tetracycline and 0.04 µg L^{-1} for chlortetracycline. Only small amount of solids were found in runoff samples from this study.

The studies by Kay et al. (2005) and Davis et al. (2006) found antibiotic losses via runoff of less than 0.1%; however Davis et al. (2006) noted very little solids in runoff. Greater losses (2.5%) were found by Burkhardt et al. (2005) and Krezig et al. (2005) for sulfonamide antibiotics. The percent losses in this study (Table 3.6) were more consistent with the findings of Burkhardt et al. (2005) and Krezig et al. (2005). As tetracyclines tend to bind to manure or sediment particles (Lee et al., 2007; Chee-Sanford et al., 2009), it is possible that the transport of manure particles in runoff resulted in the greater antibiotic losses found in this study. Note that high levels of TSS were seen in all plots receiving manure treatments (C=215.2 mg L^{-1}, M=932.1 mg L^{-1}, and MA=638.6 mg L^{-1}).

3.3.3 Oxytetracycline and Constituent Correlations

Table 3.7 contains the correlation coefficients (r) for the C, M, and MA treatments. For the C and M treatments, nearly all r values were less than 0.2 indicating no correlation existed between oxytetracycline and the other constituents for these treatments. These results were as expected since no oxytetracycline was added to the C

Table 3.6. Oxytetracyline Losses (%) via Runoff from MA Plots.

Plot	Oxytetracycline Losses (%)
L3	0.03
C3	0.02
B10	4.21
B6	3.5
Mean±Std. Dev.	2.06±1.93

Table 3.7. Pearson Correlation Coefficients (r) between Oxytetracycline Concentrations and Constituent Concentrations for C and M Treatments.[1]

Constituents	**Plots**		
	C	**M**	**MA**
E.coli	-0.199	0.112	0.690[2]
Cl^-	0.026	-0.025	0.717[2]
PO_4	0.273	0.029	0.192
NO_3-N	0.024	-0.065	-0.006
NH_4-N	0.126	-0.144	0.671[2]
TOC	0.180	-0.122	0.593[2]
pH	0.107	0.659[2]	0.298
EC	0.167	0.110	0.586[2]
Turbidity	-0.018	-0.192	0.503[2]
TSS	0.179	0.377[2]	0.515[2]

[1] All concentrations were flow-normalized.

[2] Significant at α=0.05 level.

and M plots. For the M plots, the correlation coefficients between oxytetracycline and pH and TSS were substantially higher. With regards to TSS, this finding could indicate that higher levels of manure particles, and therefore organic matter and pH, could have effected oxytetracycline levels (Yang et al., 2005).

For the MA plots, the majority of the measured constituents were significantly correlated to oxytetracycline (Table 3.5). As seen in Figures 3.4 to 3.6, pH, NO_3-N, and PO_4, respectively, exhibited no relationship with oxytetracycline. With regards to pH, the range of recorded values was small (7.0-7.7), and the presence of manure in the MA plots did not affect pH levels. For NO_3-N, several samples (71%) with levels below the detection limit, an aspect that could have accounted in part for the lack of correlation with oxytetracycline, occurred in the MA plots. PO_4 also had a number of samples with non-detectable values (43%) in the MA plots.

For the MA plots, all other constituents were significantly and positively correlated with oxytetracycline (Table 3.5). In all instances, correlation coefficients were above 0.5. Chloride exhibited the strongest correlation to oxytetracycline (r=0.72), as seen Figure 3.7. The reason for this strong relationship is unclear. The label for

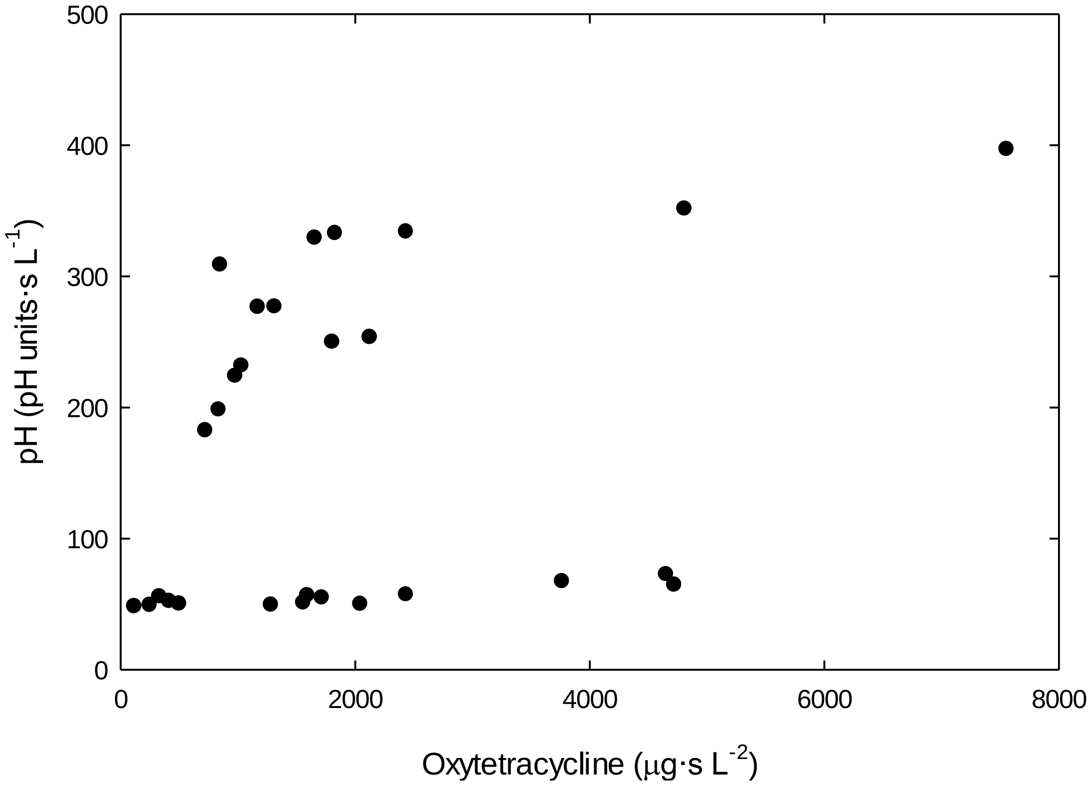


Figure 3.4. Relationship between Oxytetracycline and pH for the MA Plots.

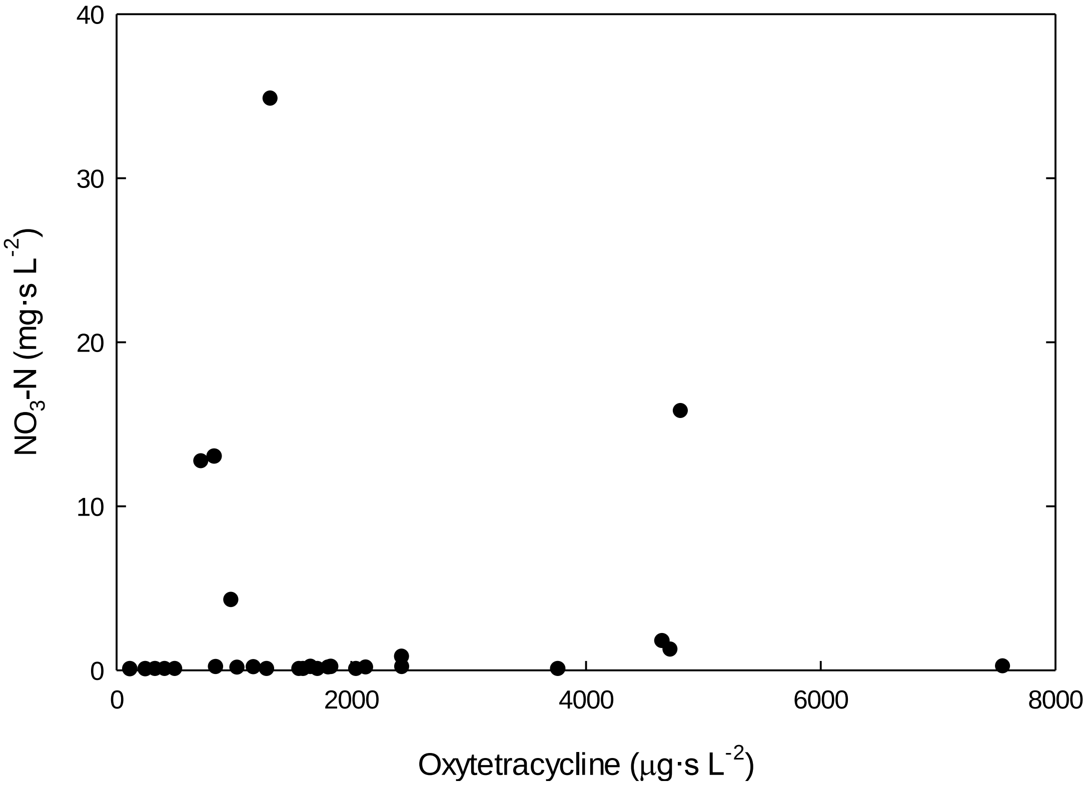


Figure 3.5. Relationship between Oxytetracycline and NO_3-N for the MA Plots.

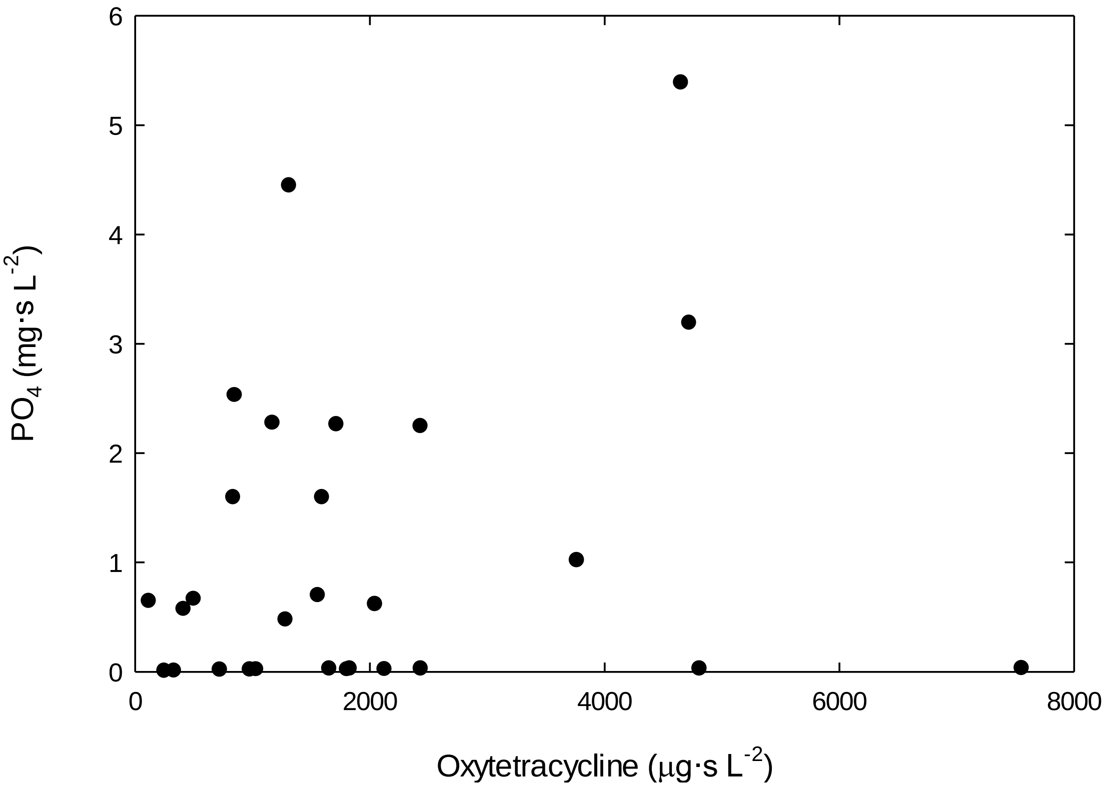


Figure 3.6. Relationship between Oxytetracycline and PO_4 for MA Plots.

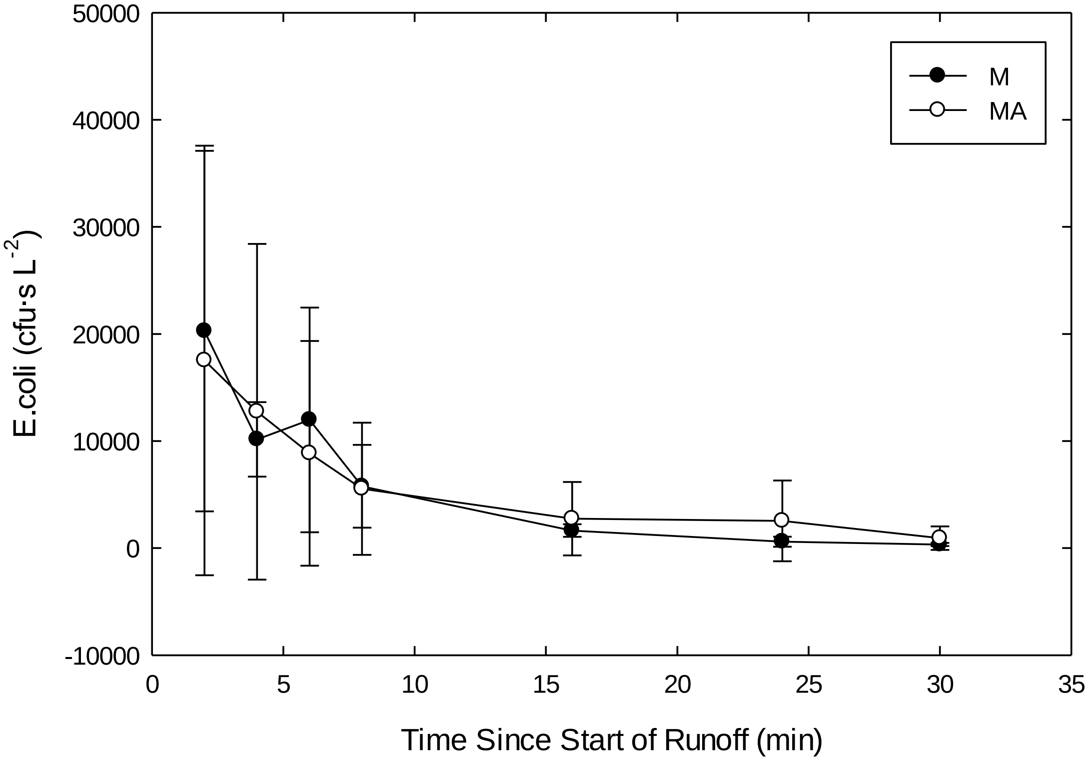


Figure 3.7. E. coli Concentrations for M and MA Plots During the Simulated Storm Events.

Noromycin 300 LA indicates that hydrochloric acid could have been used in production of the antibiotic to adjust the pH. Interestingly, the correlation coefficient between oxytetracycline and *E.coli* indicates a strong positive correlation (r=0.69). Since oxytetracycline is used to kill bacteria such as *E. coli*, it was theorized that higher levels of oxytetracycline in runoff would result in lower levels of *E. coli*; however, this was not the case (Figure 3.7).

The constituents TOC, EC, TSS and NH_4-N also displayed strong positive correlations with oxytetracylcine concentrations (Figures 3.8-3.11). The relationship between TOC and oxytetracycline was expected as both are organic compounds. The relationships between TSS and oxytetracycline and turbidity and oxytetracycline indicate that the antibiotics are correlated with the amount of solids in the samples, a phenomenon noted by Loke et al. (2002). Kulthrestha et al. (2004) noted the likelihood of oxytetracycline to bind to clays with high ammonium content, which could account for the strong correlation between ammonia and oxytetracycline seen in this study.

3.3.4. Best Management Practice Selection

Due to the common usage of antibiotics in livestock coupled with the unlikelihood that significant forms of source reduction will occur in the foreseeable future, other types of BMPs (e.g. structural) are needed to reduce the transport of antibiotics to waterways. The results of this study suggest that BMPs which target particulates or sediments may be more appropriate for reducing the transport of oxytetracycline and potentially other antibiotics. Davis et al. (2006) concluded erosion control practices could aid in the reduction of antibiotics in runoff. A similar sentiment was echoed by Lin et al. (2011) who measured a 75% reduction in the transport of two antibiotics, tysolin and enrofloxacin, with the use of a 4 m vegetative buffer strip. Other BMPs such as composting and chemical additives could also aid in the reduction of off-field transport of antibiotics. Arikan et al. (2007) showed a 99% removal of extractable oxytetracycline in cattle manure after composting for 35 days. Chemical additives are commonly used by the wastewater treatment industry, but their potential for reducing antibiotic transport has received little research. Punamiya et al. (2013) found that aluminum-based water treatment residuals have high potential to bind to tetracycline

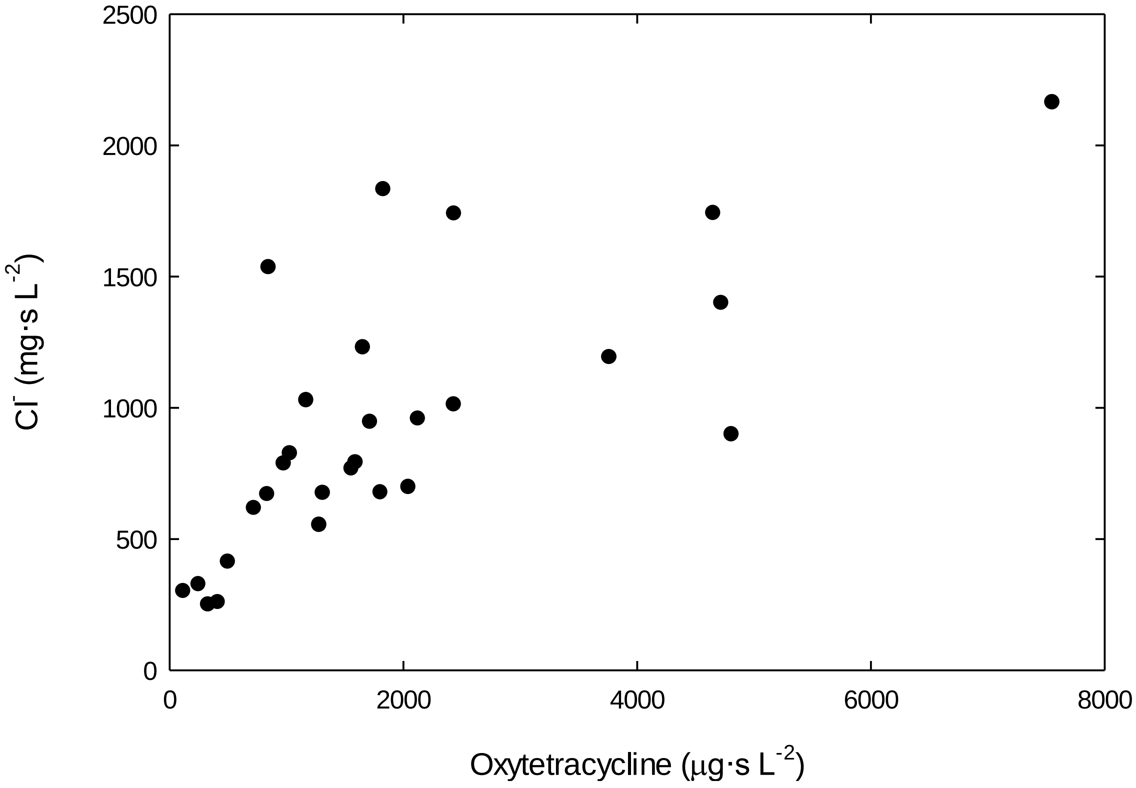


Figure 3.8. Relationship between Oxytetracyline and Cl^- for MA Plots.

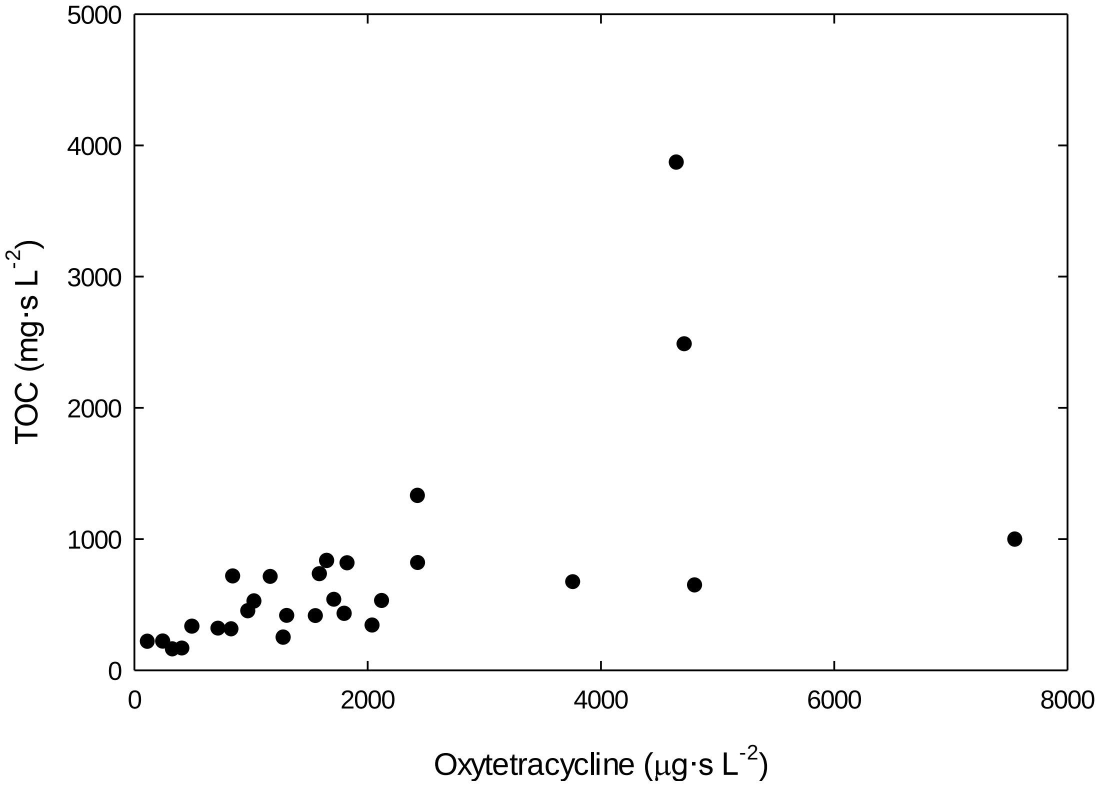


Figure 3.9. Relationship between Oxytetracycline and TOC for MA Plots.

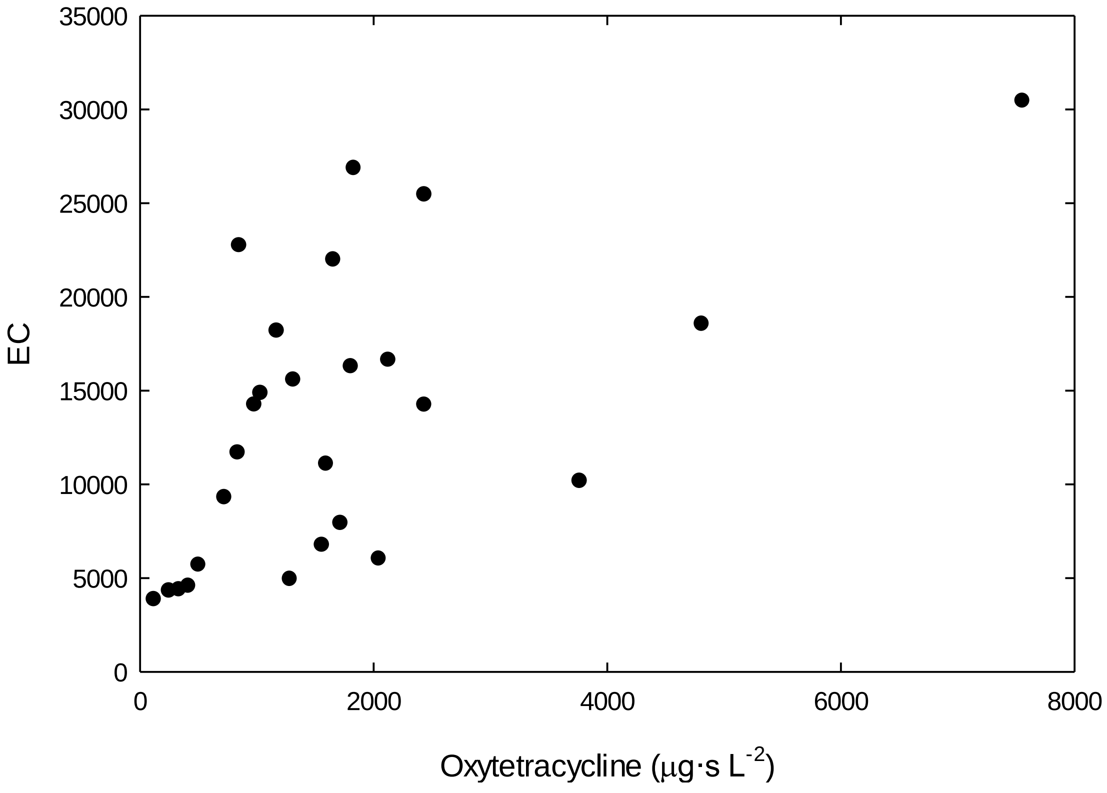


Figure 3.10. Relationship between Oxytetracycline and EC for MA Plots.

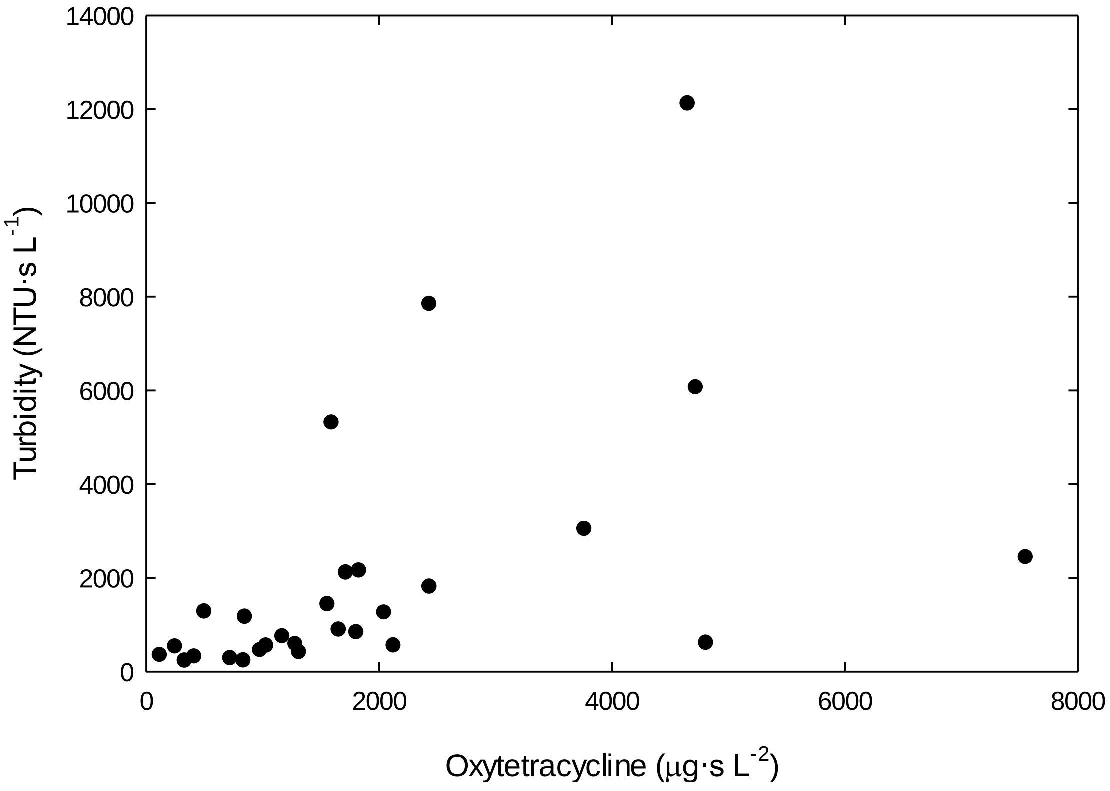


Figure 3.11. Relationship between Oxytetracycline and Turbidity MA Plots.

antibiotics. Another aluminum-based compound, aluminum sulfate or alum, has been used for a number of years as a flocculent to settle out solids and remove organic compounds from wastewater. Alum is also used by the livestock industry, particularly poultry producers, to reduce the transport of phosphorus (DeLuane and Moore, 2013). The strong correlation between oxytetracycline and the constituents TSS, turbidity and TOC suggests a BMP, such as a vegetative buffer strip or alum, may be a viable method for reducing the transport of this antibiotic.

3.4 CONCLUSIONS

Veterinary antibiotics such as oxytetracycline can be transported to waterbodies via overland flow. Studies have shown that oxytetracycline can undergo photodegradation (Doi and Stoskopf, 2000; Xuan et al., 2010), however, for this to occur, the transport rate of oxytetracyline must be reduced so that the exposure time to sunlight is maximized. Results from this study show that the presence of oxytetracycline is strongly correlated to the presence of other water quality constituents such as *E.coli*, Cl, TOC, turbidity, and TSS. The strong correlation between oxytetracycline and measures of suspended sediment, namely turbidity and TSS, coupled with the likelihood of oxytetracycline binding to manure particles suggests that BMPs used to reduce solids in runoff, such as vegetative buffer strips and flocculants, could potentially reduce the transport of oxytetracycline as well.

CHAPTER 4: EFFECT OF ALUM ON SURFACE TRANSPORT OF OXYTETRACYCLINE

4.1 INTRODUCTION

Antibiotics are used in livestock production for both therapeutic and subtherapeutic uses. Subtherapeutic uses are mainly the prevention of the spread of diseases and the promotion of livestock growth through the increased nutrient absorption by the animal and less by gastrointestinal bacteria (LaShore and Pruden, 2009). The USEPA (2013) estimates that 60-80% of livestock and poultry routinely receive antimicrobials through feed, water, injections or external applications. An estimated 93% of grower/finisher hogs were administered antibiotics at some point during the grower/finisher time (USDA, 1996) with 25% of swine facilities administering antibiotics as levels higher than recommended (Dewey et al., 1997). One of the challenges associated with frequently dosing livestock with antibiotics is that livestock can only partially metabolize the antibiotics they are administered. Thus, livestock excrete antibiotics at high rates (30%-90%) and in virtually unchanged forms from the parent compounds (Sarmah et al., 2006). Antibiotics have been found in manures at concentrations ranging from trace levels to as much as 200 mg L^{-1} (Kumar et al., 2005).

Many of the antibiotics approved for use in livestock are used in humans as well. Oxytertracycline, a tetracycline class antibiotic, is approved by the Food and Drug Administration for therapeutic uses in humans as well as livestock, and it is approved for subtherapeutic uses in cattle and swine as a growth promoter. Tetracyclines are one of the largest classes of antibiotics used in livestock and poultry. Over 70% of the antibiotics administered to livestock and poultry are of the classes of tetracyclines and ionophores (USEPA, 2013).

The main concern with such high rates of antibiotic administration to livestock is the potential for the development of antibiotic-resistant strains of bacteria. While sampling private groundwater supplies, McKeon et al. (1995) found that 90% of the over 250 coliforms and non-coliforms samples were resistant to at least one antibiotic with 78% of these coliforms displaying resistance to multiple antibiotics. Chee-Sanford et al. (2001) found tetracycline resistant genes in bacteria in groundwater sampled more than 250 m downstream of a swine waste storage lagoon indicating that such bacteria are readily transported.

Because of the frequent dual usage between humans and livestock coupled with the fact that detection of antiobiotics in the environment is occurring more frequently (USEPA, 2007), the USEPA has classified antibiotic as a contaminant of emerging concern (CEC). A CEC is one that is now being detected in the environment and/or is at higher than expected levels, meaning it was not previously present in the environment or was present at undetectable or very low levels (USEPA, 2007). Land application of manure, which is common with livestock operations, serves as the primary pathway for antibiotics to enter waterbodies (Baguer et al., 2000; Arikan et al., 2008). Approximately 50% of surface waters may contain antibiotics (Koplin et al., 2002). But, as seen with Chee-Sandford et al. (2001), antibiotics are not found in surface waters alone. Campagnolo et al. (2002) also found antibiotics in groundwater near swine farms in Iowa – a state with large amounts of karst (Veni et al., 2001). The authors noted that 31% of ground and surface water samples collected near swine operations and 67% of ground and surface water samples near poultry operations contained antibiotics. Such findings are of particular importance in karstic environment where surface water and ground water connections are strong meaning pollutants applied to the land can readily access aquifers.

Aluminum sulfate, or alum, is a non-toxic flocculent used in the wastewater industry (Tchobanoglous et al., 2003; Mason et al., 2005) and the poultry industry (Moore and Miller, 1994; DeLuane and Moore, 2013). Numerous studies have demonstrated the effectiveness of alum to reduce phosphorus levels in runoff (Moore and Miller, 1994; Edwards and Daniel, 1993; Edwards et al., 1999; Smith et al., 2001; Penn and Bryant, 2006). Essentially, soluble forms of phosphorus are converted to insoluble ones which are then precipitated out. Alum can also reduce concentrations of arsenic (56%), copper (44%), and zinc (36%) (Moore et al., 1998) as well as the hormone 17β-estradiol (DeLaune and Moore, 2013). Only one study has examined the potential use of alum to reduce antibiotic transport. Adams et al. (2002), in a lab-scale study, examined the effectiveness of alum to reduce antibiotic concentrations in river water. The authors examined seven antibiotics, six of which were sulfidimides class of antibiotics; the other was in the carbadox class. Results of the study indicated that alum did not remove a significant amount of antibiotics from the river water. Tetracyclines, however, behave differently than sulfidimides and carbadox classes of antibiotics as tetracyclines have a higher tendency to bind to manure particles (Loke et al., 2002). The tendency of a pollutant to bind to soil particles is described by the sorption coefficient, K_d, which is a ratio of the amount of chemical sorbed to soil to the amount that is still in solution (Brady and Weil, 2008). Higher K_d values mean the pollutant is more likely to bind to soil particles. Sulfidimides, such as those studied by Adams et al. (2002, have low K_d values (0.6-4.9) as compared to tetracyclines which have high K_d values (420-1030) (Tolls, 2001). Punamiva et al. (2013) noted that aluminum water treatment residuals, part of the waste product from the water purification process, can sorb to tetracyclines thus removing them from aqueous solution. The authors found a removal rate of 98% for tetracycline and a 96% for oxytetracycline. Thus, the objective of this study was to examine the effectiveness of alum for reducing concentrations of tetracycline antibiotic (oxytetracycline) in simulated runoff.

4.2 MATERIALS AND METHODS

4.2.1 Study Site

This study was conducted at the rainfall simulation facility at the University of Kentucky’s Maine Chance Research farm (latitude: 38.1164°N; longitude: 84.4903W). The facility consists of three rainfall simulators, which were constructed as described in Miller (1987). Fescue plots measuring 2.44 m by 6.10 m were constructed on Maury silt loam (fine, mixed, mesic Typic Paleufalf) (NRCS, 2013) as described by Edwards et al. (2000) and planted with Kentucky 31 fescue. Each plot was bordered by galvanized steel and was equipped with a gutter at the base that diverted runoff in to a PVC pipe located in a sump. A wood cover was placed over the gutter to ensure that only runoff was collected during the sampling events.

4.2.2 Plot Selection

Rainfall simulators were used to generate curve numbers (CNs) for each plot (Appendix A). Sixteen plots with similar CNs were selected for use in this study (Table 4.1). Plots selected for this study had a mean curve number of 83 ±4.

Table 4.1 Curve Numbers of Plots Used in the Study.

Plot	CN	Plot	CN
B1	82	C5	89
B4	82	C6	77
B5	88	D1	86
B6	90	D5	84
B10	82	L1	84
C2	89	L3	79
C3	82	N2	77
C4	80	N9	81

4.2.3 Experimental Treatments

Liquid swine manure was obtained from a private hog farm in Bardstown, KY. Manure was collected from hogs that had not been administered antibiotics since birth. The manure had a slurry consistency, and it was stored in a pit prior to collection for this study. Collected manure was stored in a 1,040 L polyethylene intermediate bulk container (IBC) tote at the University of Kentucky Biosystems and Agricultural Engineering Department.

Four treatments were randomly assigned to the plots (Table 4.2). Treatments included a control (manure was not applied) (C), manure only (M), manure plus antibiotics (MA), and manure plus antibiotics and alum (MAA). Manure (55L) was placed in separate 208 L barrels which were designated in accordance with treatment to prevent cross-contamination. Manure for each treatment receiving antibiotics was spiked to a concentration of 20 mg L^{-1} by adding 3.67 mL of oxytetracycline to the 55 L of manure. The oxytetracycline (Noromycin 300 LA, Norbrook, Lenexa, KS) was a commercially available, injectable form containing 300 mg of antibiotic per mL in suspension. For the MAA treatment, 1.13 L of liquid alum (48.5% solution) was added to the manure and antibiotics resulting in a 1% alum concentration (Bushee-Bullock, 1999; Smith et al., 2001). Prior to application to the plots, manure was mixed for 5 minutes using a custom built mixer attached to a drill. The custom mixer, which was constructed in the Biosystems and Agricultural Engineering Department, was approximately 1.5 m in length and had two 30 cm long fins at a distance of 0 cm and 30 cm from the bottom.

Table 4.2 Treatment Associate with Each Plot.

Treatment[1]	**Plots**
C	C2, C6, D1[2], D5
M	B4, B8[3] C5, L1
MA	B6, B10, C3, L3
MAA	B1, B5, C4, N9

[1] C=control, M=manure only, MA=manure and antibiotics, and MAA=manure, antibiotics, and alum.
[2] D1 was not used in the *E. coli* analysis due to sampling error.
[3] B8 was not used in the analysis because it did not produce runoff.

With the exception of the C plots, 45 L of manure (3 L m^{-2}) was applied to each plot as done by Bushee-Bullock (1999) immediately prior to the start of the simulated rainfall. The target application rate was 112 kg N ha^{-1}, as recommended by AGR-1, but laboratory results (Appendix E) from University of Kentucky Regulatory Services indicate that the actual application rate was about 150 kg N ha^{-1}. Each MA and MAA plot received 900 mg of oxytetracycline, and each of MAA plot also received 54 g of $Al_2(SO_4)_3$. Plots were mowed within three days prior to manure application. Soil and manure samples were taken for each plot and sent to the University of Kentucky Regulatory Service Laboratory for analysis. A second soil sample from each plot was analyzed for moisture content at the Biosystems and Agricultural Engineering Department. Soil samples were analyzed for P, K, Ca, Mg, Zn, pH, total nitrogen, and soil texture, while manure samples were analyzed for N, P, K, Ca, Mg, Zn, Cu, Fe, and Mn.

4.2.4 Rainfall

Each rainfall simulator was programmed to produce a rainfall intensity of 102 mm hr^{-1} (Appendix A). To represent a worst-case scenario, rainfall simulation was started 30 minutes after manure application and was continued until 30 minutes after runoff began.

4.2.5 Runoff Sample Collection

Runoff samples were collected from the PVC pipe connected to the gutter at time intervals of 2, 4, 6, 8, 16, 24 and 30 minutes after runoff began. Clean, sterile polyethylene bottles were used for sample collection. Samples were collected for 1 min or until the bottle was full. Data pertaining to the time to runoff, time to fill each bottle, and weight of each bottle were recorded (Appendix B). All bottles were weighed before sample collection and an average tare weight was calculated. Two flow-weighted composite samples, one for bacterial analysis and one for the analysis of all other constituents, were created using the weight of each sample along with the respective sample bottle fill times. The data used to create the composite samples are presented in Appendix B.

4.2.6 Laboratory Analysis

For each plot, samples from the seven time intervals along with a composite were analyzed for *E. coli*, oxytetracycline, NO_3-N, NH_4-N, PO_4-P, pH, EC, TSS, Cl, TOC and turbidity. Samples were stored at approximately 4°C until analysis. Analysis for NO_3-N, NH_4-N, PO_4-P, Cl, and TOC occurred at the University of Kentucky's Forestry Department. A Bran-Luebbe Auto-Analyzer III (Norderstedt, Germany) was used for the analysis of NO_3-N and NH_4-N. A Dionex Ion Chromatograph (Sunnyvale, California) was used for PO_4-P, Cl, and NO_2-N. Total Organic Carbon (TOC) was analyzed using a Shimadzu Total Orgainic Carbon Analyzer (Kyoto, Japan).

Samples were analyzed for *E. coli*, oxytetracycline, pH, EC, turbidity, and TSS at the University of Kentucky's Biosystems and Agricultural Engineering Department. *E. coli* analysis was conducted within 6 hours of sample collection using Coliert-18 purchased from IDEXX laboratories (Westbrooke, Maine) (Appendix C). EC was determined using an EcoTestr-EC Low (Vernon Hills, Illinois). A Thermo Electron Corporation Orion 520A+ (Waltham, Massachusetts) was used for pH determination, and a LaMotte 2020 turbidimeter (Chestertown, Maryland) was used for turbidity. TSS analysis was conducted using a Sequoia Scientific LISST-Portable|XR (Bellevue, Washington). All analyses were conducted per standard methods (APHA et al., 1998).

4.2.6.1 Antibiotic Analysis

Samples were analyzed for antibiotics using a methodology developed in the Biosystems and Agricultural Engineering Department by Dr. Manish Kulshrestha. A detailed description of antibiotic analysis can be found in Appendix D. Approximately 50 mL of sample was centrifuged at 3,500 rpm for 20 minutes in a Thermo Scientific Survall Legend XTR Centrifuge (Waltham, Massachusetts), and 45 mL was added to a glass test tube. Kimwipes were uses to cover the test tubes, before placing the samples in a SP Scientific VirTis Wizard 2.0 lyophilzer (Gardiner, New York) until dry. After approximately three days, the test tubes were removed from the lyophilizer and the dry residue was dissolved in 900 µL of 50% methanol solution. This concentrated the original sample 50X. The solution was transferred to microcentrifuge tubes and centrifuged again at 4,000 rpm for 10 minutes with a Fisher Scientific Marathon 21000 (Waltham, Massachusetts). In order to achieve a better defined peak and to ensure the right peak was selected, a known amount of oxytetracycline was added to each sample. A 25X concentrated sample was achieved by adding 100 µL of 2 µg/mL oxytetracycline solution to 100 µL of concentrated solution.

High-pressure liquid chromatography (HPLC) was used to separate the analytes. A Dionex Ultimate 3000 HPLC (Sunnyvale, California) along with an Ultimate 3000RS Variable Wavelength detector set at 290 nm (Kay et al., 2005) was used for this analysis. Using a gradient mobile phase of 0.5% acetic acid in water and 0.5% acetic acid in methanol, samples were ran through a Dionex Acclaim 120 (C18) column (Sunnyvale, California). The HPLC injected 20µL of sample through the column and used a pumping rate of 0.400 mL/min. Four calibration standards ranging from 10µg mL^{-1} to 200µg mL^{-1} were used.

4.2.7 Data Analysis

Runoff flow rates from each plot were variable; therefore, constituent concentrations were normalized by flow rate. Differences between treatments and time intervals were assessed using linear mixed models (PROC MIXED) and least square means (LSMEANS) in SAS 9.3. All data were ln transformed to normalize the data.

4.3 RESULTS AND DISCUSSION

4.3.1 Treatment Effects

Treatments did not significantly affect constituent concentrations for Cl, TOC, pH, EC, turbidity, and TSS, but significant treatment effects were noted for *E. coli*, NO_3-N, NH_4-N, PO_4, and oxytetracycline (Table 4.3). The lack of significance with regards to

Table 4.3. Statistics for Runoff Variables (Mean ± Standard Deviation).

Constituent	**Treatment**[1,2]			
	C	**M**	**MA**	**MAA**
ln *E. coli* (cfu·s L^{-2})	7.9±1.9^{b} [3]	14.4±1.6^{a}	14.5±2.0^{a}	13.1±3.2^{a}
ln Cl (mg·s L^{-2})	6.4±1.4^{a}	6.9±0.7^{a}	6.9±0.6^{a}	6.6±1.3^{a}
ln NO_3-N (mg·s L^{-2})	1.9±1.5^{a}	-1.7±1.9^{b}	-1.5±2.3^{b}	0.5±3.9^{ab}
ln NH_4-N (mg·s L^{-2})	2.9±1.4^{b}	5.7±0.8^{a}	5.2±0.8^{a}	5.2±0.9^{a}
ln PO_4 (mg·s L^{-2})	-4.0±1.8^{b}	-0.8±2.6^{a}	-1.6±2.4^{a}	-1.9±1.9^{a}
ln TOC (mg·s L^{-2})	5.5±1.2^{a}	6.6±1.0^{a}	6.2±0.7^{a}	6.4±1.2^{a}
ln Oxytetracycline (µg·s L-^{2})	5.8±1.0^{b}	5.6±1.1^{b}	7.1±0.9^{a}	7.7±1.0^{a} [2]
ln pH (pH units·s L^{-1})	5.4±1.3^{a}	4.7±0.9^{a}	4.8±0.8^{a}	5.5±1.7^{a}
ln EC (µS·s L^{-1})	9.4±1.1^{a}	9.4±0.7^{a}	9.3±0.6^{a}	9.9±1.3^{a}
ln Turbidity (NTU·s L^{-1})	5.7±1.7^{a}	7.7±1.1^{a}	6.9±1.1^{a}	7.2±0.8^{a}
ln TSS (mg·s L^{-2})	8.5±1.3^{a}	9.4±1.0^{a}	9.1±0.8^{a}	9.6±0.8^{a}

[1] C=control, M=manure only, MA=manure and antibiotics, and MAA=manure, antibiotics, and alum.

[2] Values computed for all time intervals and repetitions. All reported values were normalized by flow and ln transformed.

[3] Different letters within rows indicated means differed statistically at α=0.05.

[4] At α=0.10 means for flow-normalized oxytetracyline were statistically greater for the MAA treatment than for the MA (p=0.062).

the constituents Cl, TOC, EC, turbidity, and TSS was surprising as it was expected that constituent levels would be highest for the M, MA and MAA plots and lowest for the C plots. This lack of significance is due in part to the large amount of variation amongst the plots and across the time intervals. While no significant repetition differences were noted, significant time interval differences were found.

4.3.1.1 Non-significant Constituents

Levels of Cl were the same across all treatments considering all time intervals and repetitions (Figure 4.1). This result was surprising considering that swine manure has been shown to have Cl levels above 1,700 mg L^{-1} (Krapac et al., 2002). Only three of the samples collected during the study had Cl levels above secondary drinking water standards (250 mg L^{-1}), and all of these samples occurred in the M plots within the 2 and 4 minute time intervals. The use of chlorinated water from a municipal water supply as the simulated rain consisted could have influenced Cl levels in runoff to some degree.

The lack of difference between treatments regarding TOC was also surprising given the high levels of TOC in swine manure. Huang et al. (2006) noted that TOC

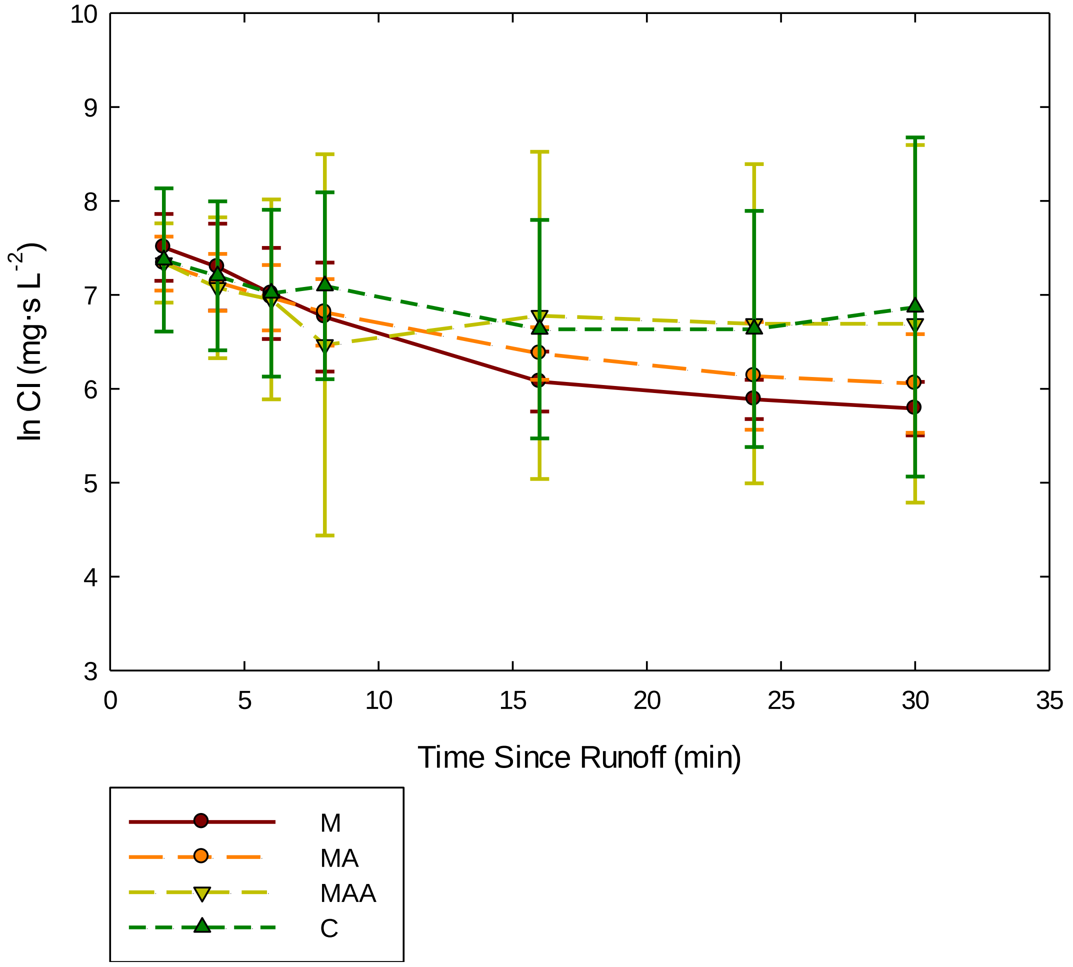


Figure 4.1. Flow Normalized ln Cl Concentrations for Each Treatment. C=Control, M=Manure Only, MA=Manure and Antibiotics, and MAA=Manure, Antibiotics and Alum.

accounted for about 37% of swine manure from a swine facility in Hong Kong. As such, it was expected that the application of swine manure to the non-C plots would increase TOC levels in runoff. Though not significantly different, TOC values for the C plots were less than the M, MA and MAA plots for the first half of the simulated storm event (Figure 4.2).

With regards to pH and EC, it was expected that the addition of manure would increase pH and EC levels; however, this was not the case (Figures 4.3 and 4.4). Edwards and Daniel (1993) recorded a pH from swine manure of 8.4 while Huang et al. (2006) recorded an average value of 8.1. The average pH of runoff from this study was lower for all treatments (7.4-7.6) than those values. The average pH level of 7.3 reported by the municipal water supplier indicated that the rainfall used in this study was a greater influence on runoff pH then the addition of manure, antibiotics and/or alum. And like pH, average EC levels in runoff (429-890 µS cm^{-1}) largely reflected the values consistent with those expected from the municipal water supplier given the limestone geology of the region. Edwards and Daniel (1993) recorded EC values for swine manure of 19,500 µS cm^{-1} while Krapac et al. (2002) recorded EC values over 30,000 µS cm^{-1}

Sediment measures, TSS and turbidity were also not significantly different among treatments though mean values for the C plot time intervals were lowest (Figures 4.5 and 4.6). Mean TSS values for the treatments ranged from 194 to 920 mg L^{-1}, across all repetitions and time intervals, while for turbidity those values ranged from 12 to 381 NTU. These values were notably higher than those reported by Edwards and Daniel (1993) who measured TSS values between 8 and 74 mg L^{-1} for a simulated rainfall study with swine manure. The rainfall intensity used by the authors of 10 cm h^{-1} is the same rate used in this study. The authors found that greater application rates of swine manure resulted in higher levels of TSS in runoff. A similar trend was noted in this study between the C plots and the M, MA and MAA plots though the trend was not significant.

4.3.1.2 Significant Constituents

Levels of *E. coli* were significantly lower for the C plots as compared to the M, MA and MAA plots (Figure 4.7). The addition of manure to these plots increased *E. coli* levels in runoff, as expected. Though the values for the MAA plots were lower than the

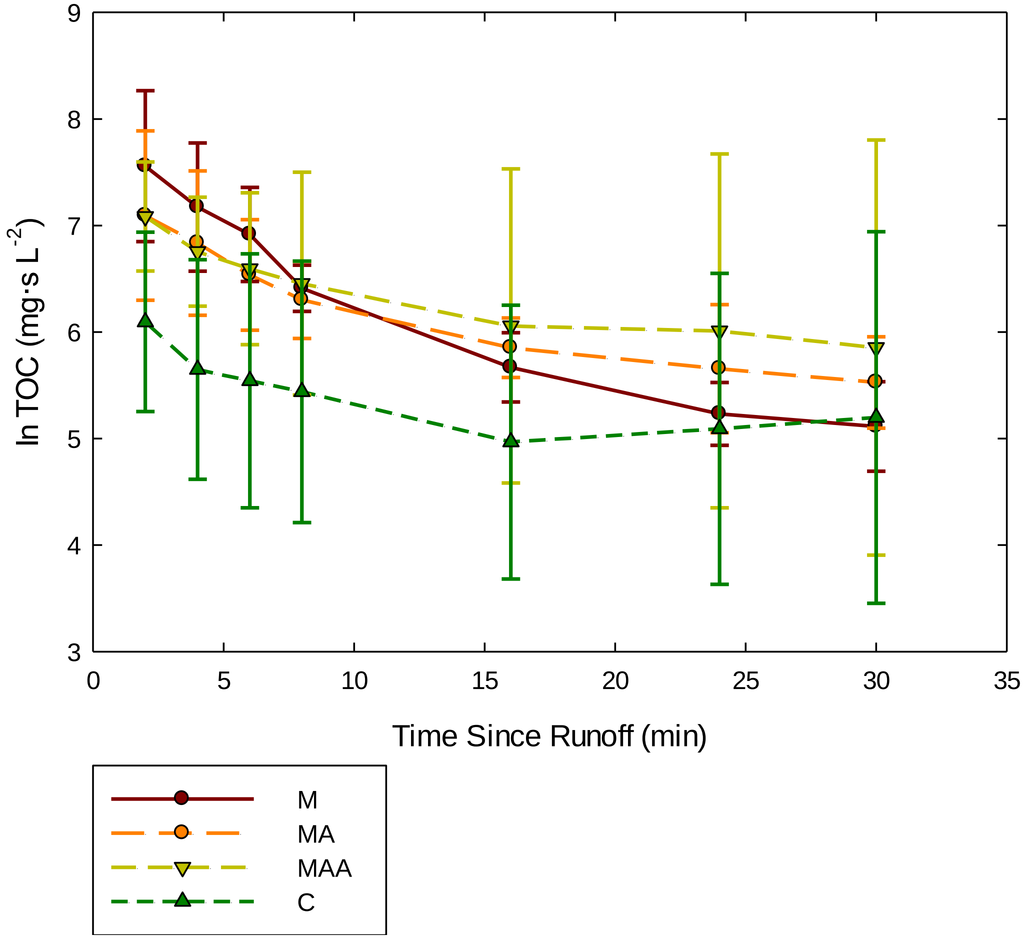


Figure 4.2. Flow Normalized ln TOC Concentrations for Each Treatment. C=Control, M=Manure Only, MA=Manure and Antibiotics, and MAA=Manure, Antibiotics and Alum.

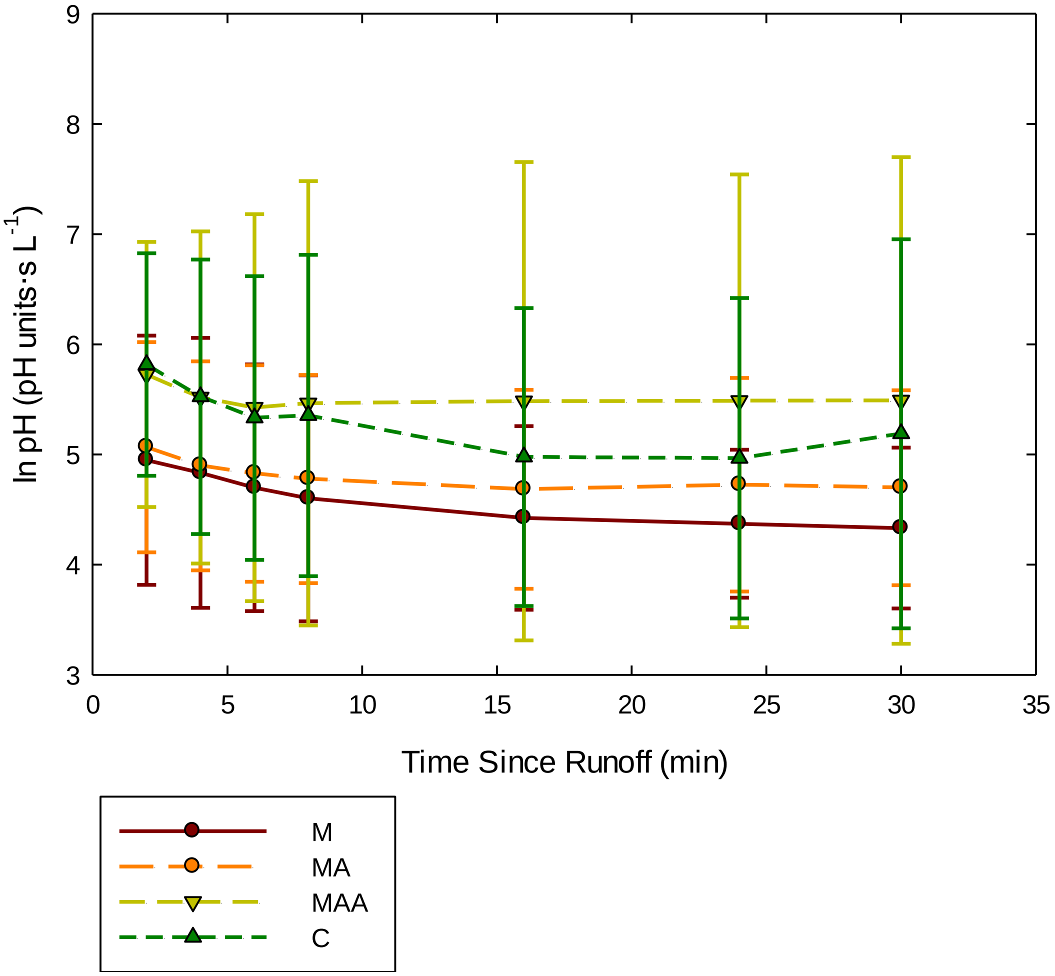


Figure 4.3. Flow Normalized ln pH Concentrations for Each Treatment. C=Control, M=Manure Only, MA=Manure and Antibiotics, and MAA=Manure, Antibiotics and Alum.

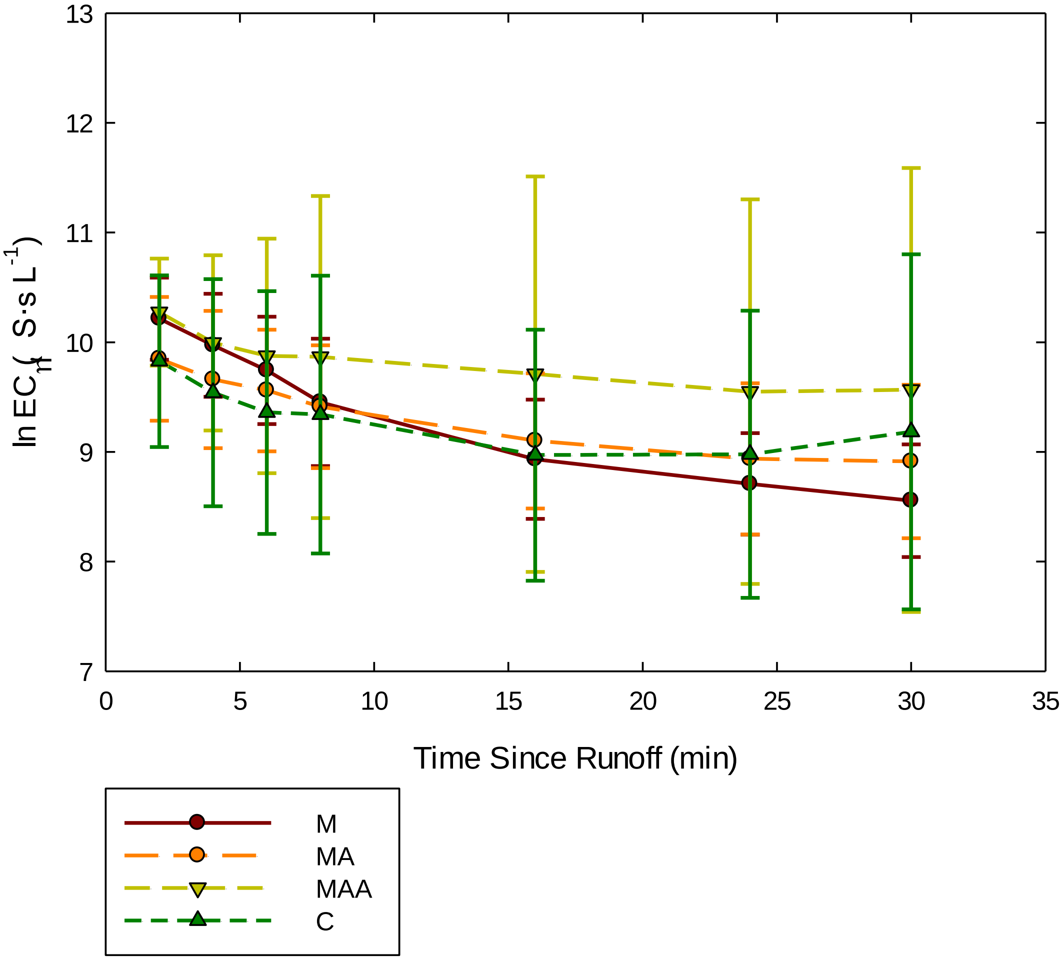


Figure 4.4. Flow Normalized ln EC Concentrations for Each Treatment. C=Control, M=Manure Only, MA=Manure and Antibiotics, and MAA=Manure, Antibiotics and Alum.

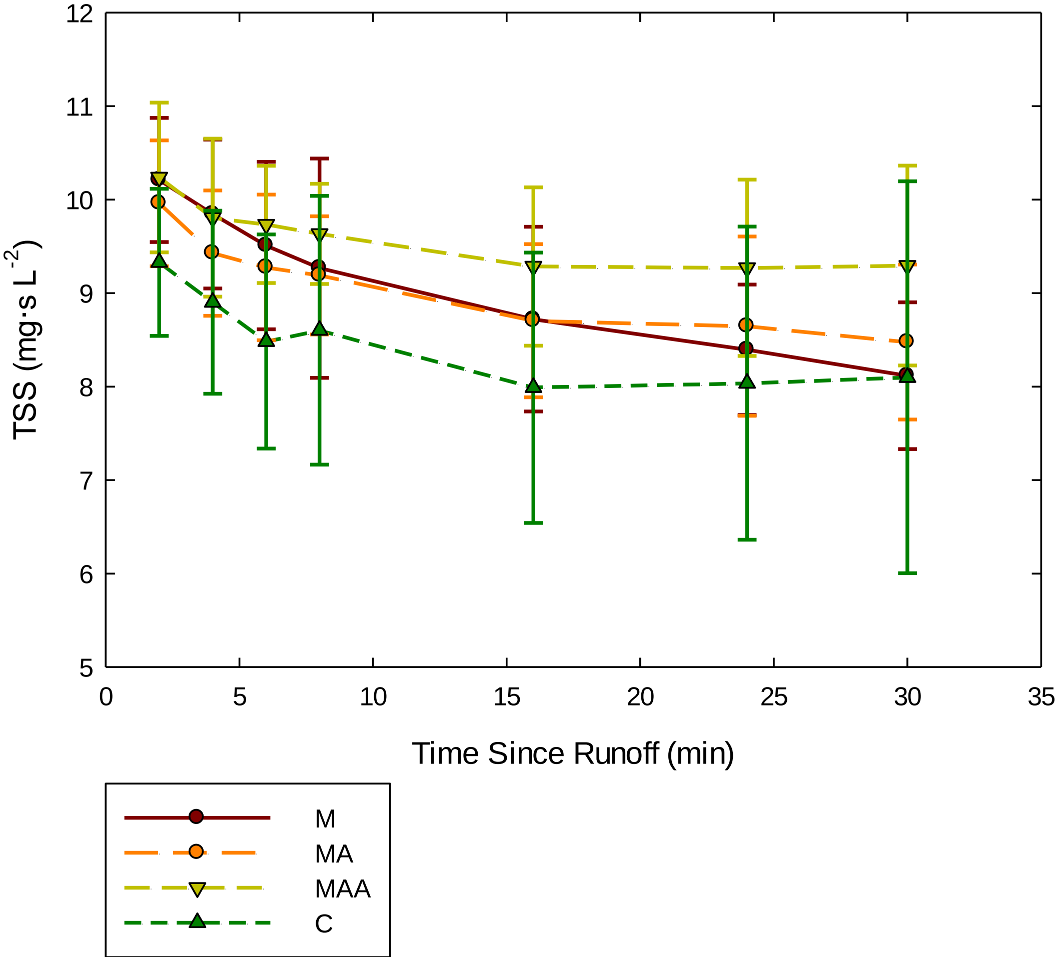


Figure 4.5. Flow Normalized ln TSS Concentrations for Each Treatment. C=Control, M=Manure Only, MA=Manure and Antibiotics, and MAA=Manure, Antibiotics and Alum.

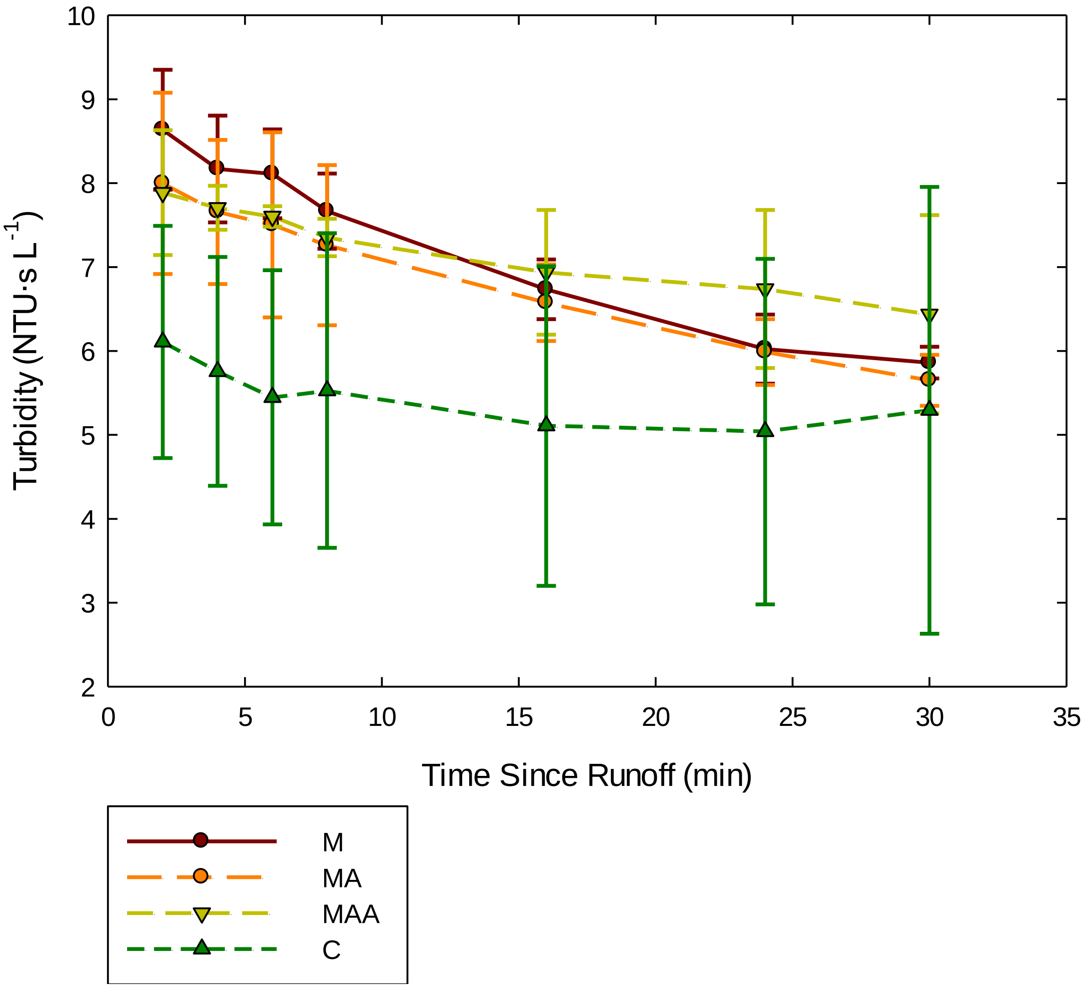


Figure 4.6. Flow Normalized ln Turbidity Concentrations for Each Treatment. C=Control, M=Manure Only, MA=Manure and Antibiotics, and MAA=Manure, Antibiotics and Alum.

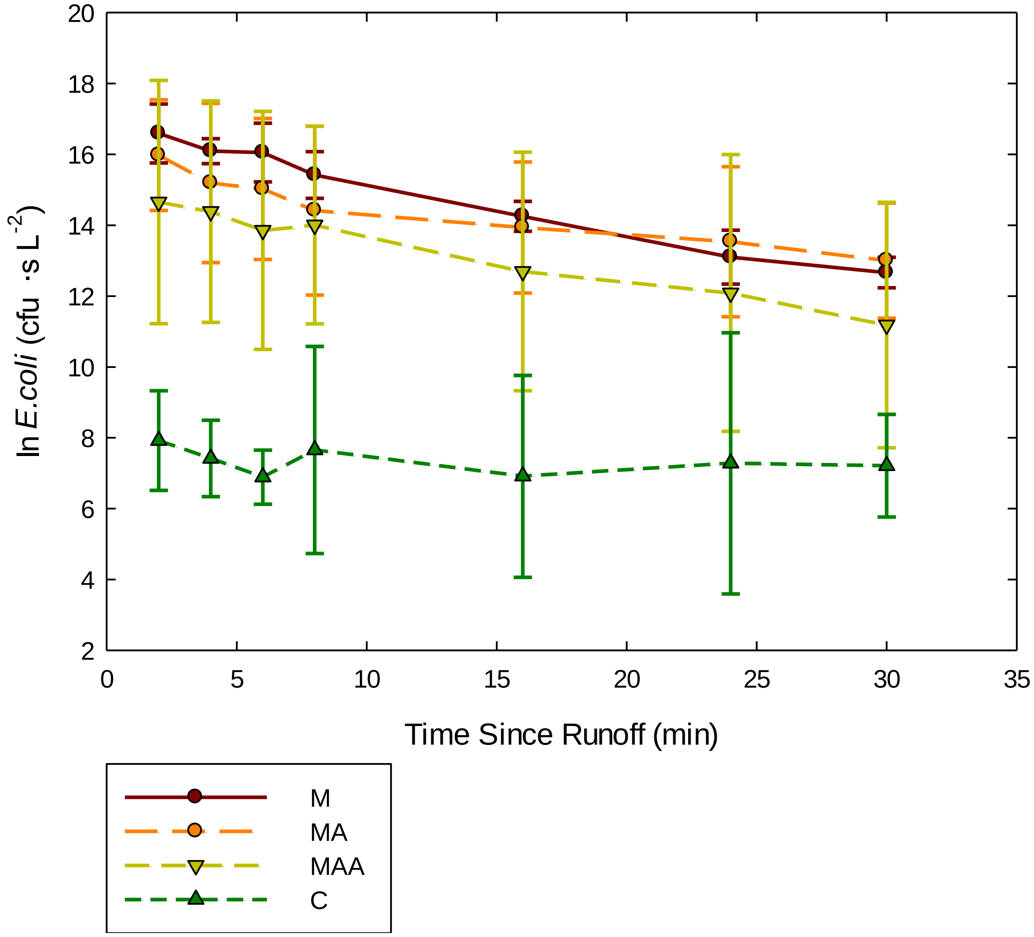


Figure 4.7. Flow Normalized ln *E. coli* Concentrations for Each Treatment. C=Control, M=Manure Only, MA=Manure and Antibiotics, and MAA=Manure, Antibiotics and Alum.

M and MA plots, the addition of alum did not significantly decrease *E. coli* levels. The lack of significant effect of alum on *E. coli* levels is surprising given that alum has been used as a flocculent to remove bacteria from water. Bulson et al. (1984) reported that over 90% of E. *coli* in lake water were removed by the addition of 15 mg L^{-1} of alum. While the study by Bulson et al. (1984) allowed for a longer flocculation time than this study (30 h vs 12-24 h), it was expected that the addition of alum, which was at a much greater concentration (1 g L^{-1}) in this study, would reduce *E. coli* levels.

With regards to NO_3-N, mean levels were highest for the C and MAA plot and lowest for the M and MA plots (Figure 4.8). The reason for these differences is not known. Krupac et al. (2002) noted that the swine manure used in their study had NO_3-N levels less than 0.2 mg L^{-1} with the majority of N in the form of NH_3-N and organic N. Edwards and Daniel (1993) measured low levels (0.1 mg L^{-1}) of NO_3-N in runoff from plots with swine manure. Hence the addition of swine manure to the plots was not expected to increase NO_3-N levels substantially. For NH_4-N, mean concentrations were lowest with the C plots as compared to the M, MA and MAA plots (Figure 4.9). Edwards and Daniel (1993) noted a similar trend between control plots and those to which manure was applied. The addition of manure to the plots in this study significantly increased NH_4-N levels, as expected since the swine manure used in this study contained about 0.5% N (Appendix E). ASABE (2005) indicates that swine manure, such as that used in this study, generally contains 0.14% NH_3-N. Krupac et al. (2002) measured average NH_3-N levels of 4,380 mg L^{-1} in swine manure.

Levels of PO_4 were lowest in the C plots as compared to the M, MA and MAA plots (Figure 4.10). The addition of manure increased PO_4 levels in runoff; however, the addition of alum did not decrease PO_4 levels as expected based upon prior alum research related to poultry litter and phosphorus reductions in runoff (Moore and Miller, 1994; Moore et al., 1999; Smith et al. 2001). The pH of the runoff (7.4-7.6) was within the range of $Al(OH)_3$ floc formations whereby PO_4 is sorbed to the floc (Cooke et al., 1986; Moore and Miller, 1994). It is possible that the alum dosage (1%) was too low to promote significant PO_4-P removal or that the $Al(OH)_3$ flocs were transported with the runoff.

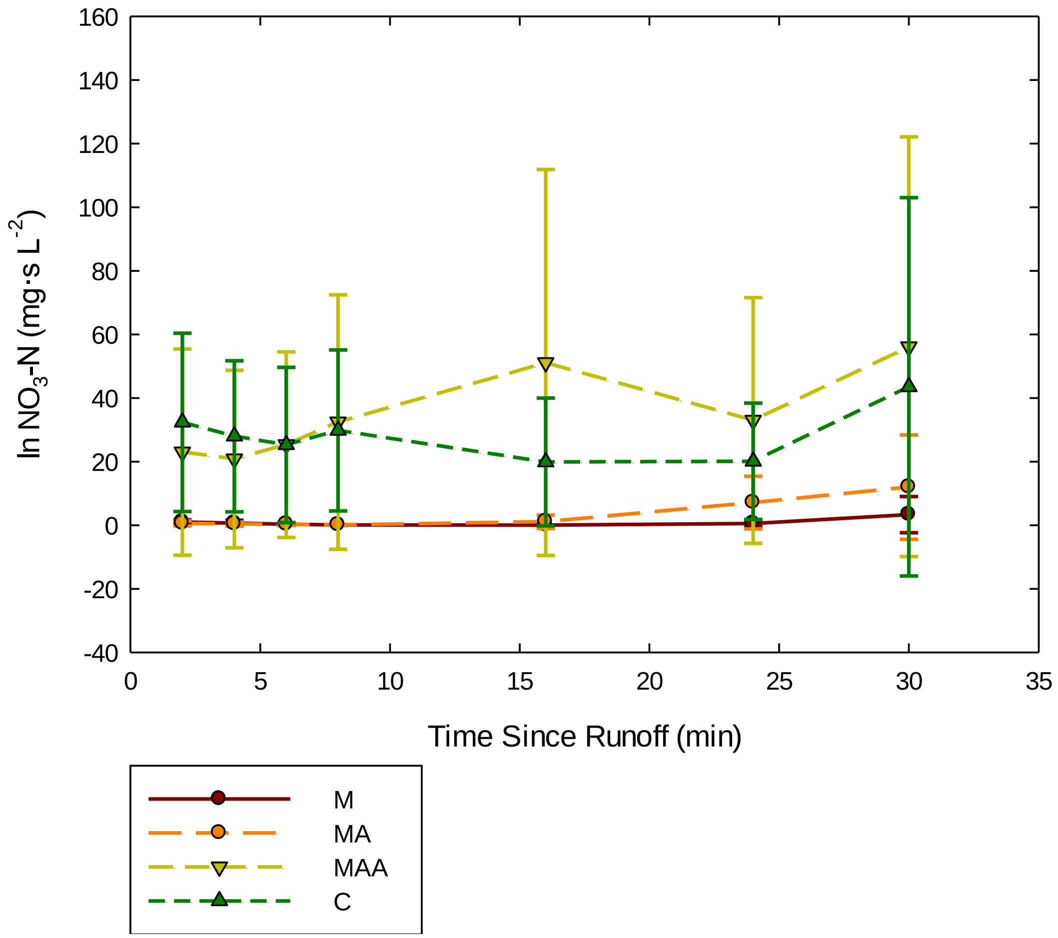


Figure 4.8. Flow Normalized ln NO_3-N Concentrations for Each Treatment. C=Control, M=Manure Only, MA=Manure and Antibiotics, and MAA=Manure, Antibiotics and Alum.

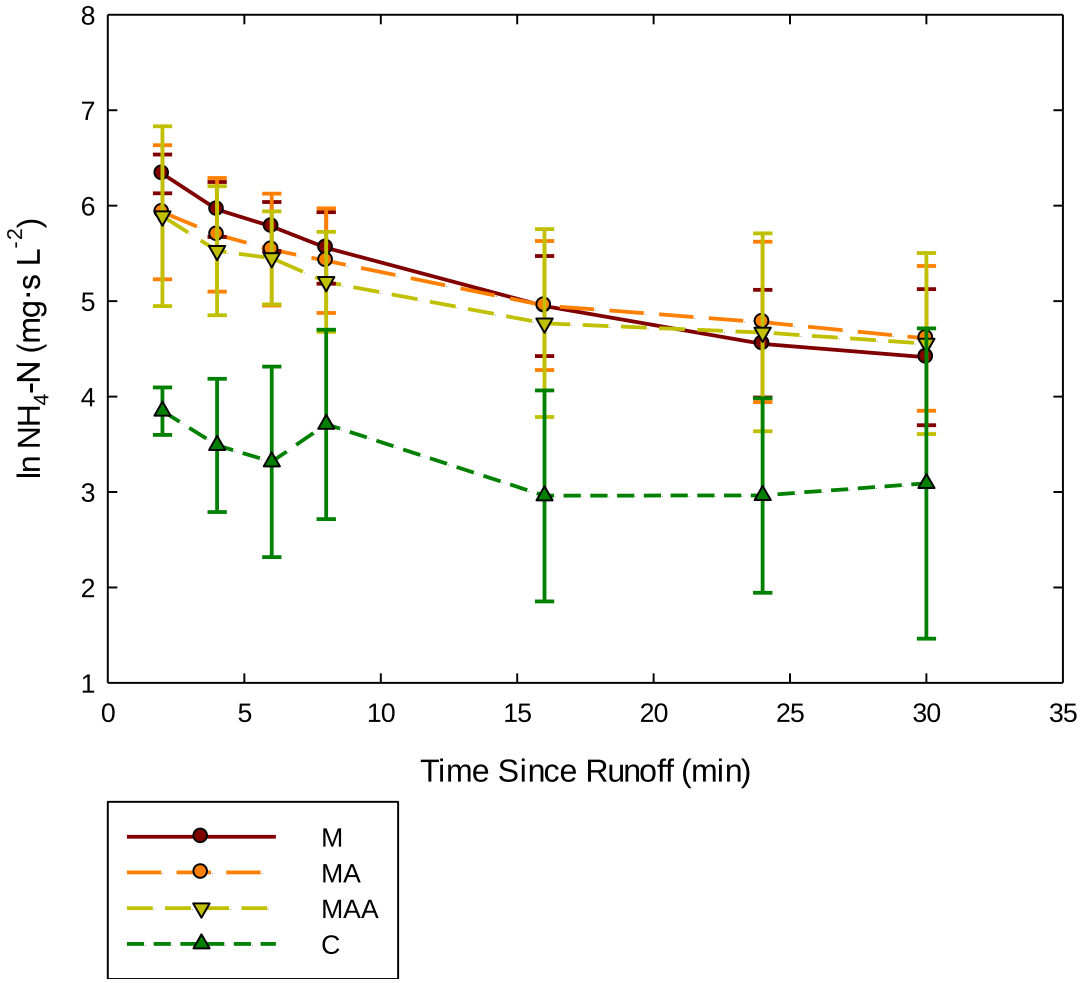


Figure 4.9. Flow Normalized ln NH$_4$-N Concentrations for Each Treatment. C=Control, M=Manure Only, MA=Manure and Antibiotics, and MAA=Manure, Antibiotics and Alum.

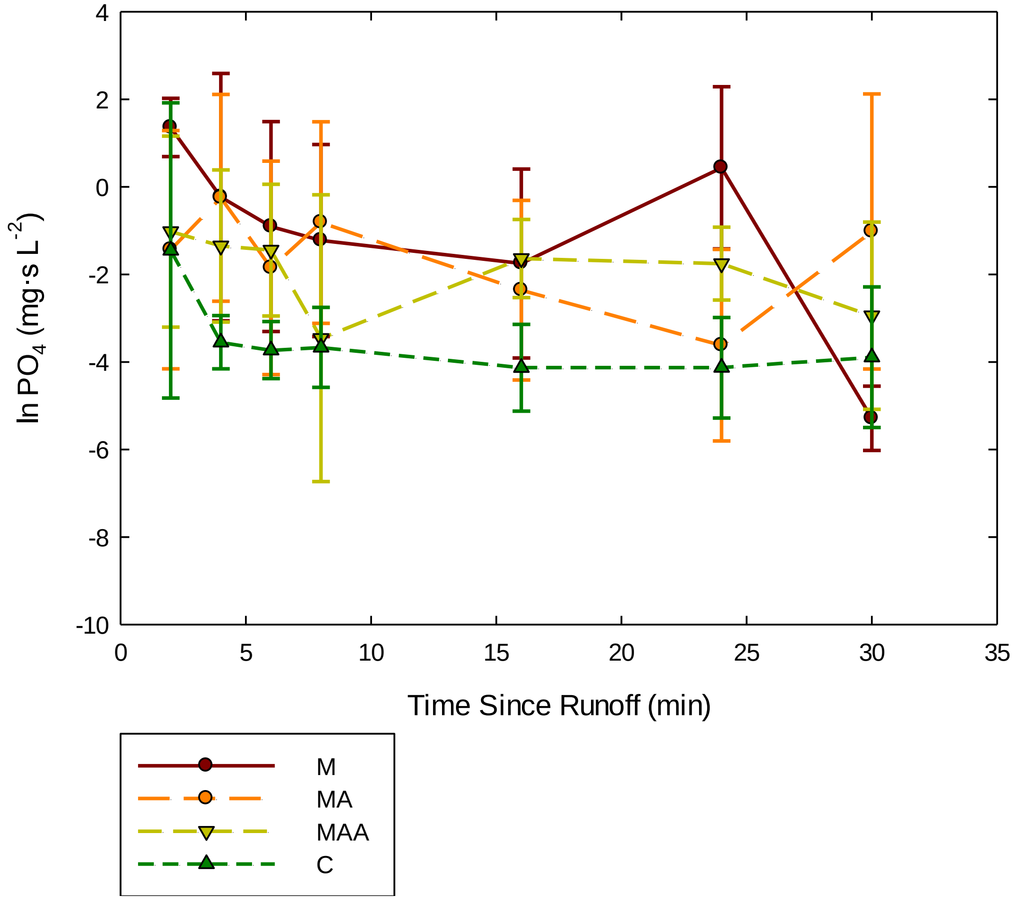


Figure 4.10. Flow Normalized ln PO_4-P Concentrations for Each Treatment. C=Control, M=Manure Only, MA=Manure and Antibiotics, and MAA=Manure, Antibiotics and Alum.

A significant treatment effect was found for oxytetracycline. Both C and M plots had lower concentrations of oxytetracycline as compared to MA and MAA plots (Figure 4.11). While MA and MAA plots did not differ at $\alpha=0.05$, a significant difference was noted at $\alpha=0.10$. At this level of significance, MAA plots had higher concentrations of oxytetracycline than MA plots. It was hypothesized that the alum may have precipitated oxytetracycline, but that the particles were transported in runoff. If such were the case, a second BMP such as a vegetated filter strip would be required. However, the lack of significance, even at $\alpha=0.10$ for the other constituents measured in this study suggests that this was not the case. The ineffectiveness of alum in this study may have been related to the alum dosage. Bushee-Bullock (1999) found no reduction in the transport of the hormone 17β-estradiol at a 1% alum dosage; however, DeLuane and Moore (2013) found reductions in 17β-estradiol using alum doses of 5%, 10% and 20%. On the other hand, other contaminants in the swine manure may inhibit alum regardless of the dosage. Choi et al. (2008) found that organic matter interfered in the removal efficiency of poly-aluminum-chloride in the reduction of oxytetracycline in river water. Removal efficiencies were reduced by 40% when river water samples had high organic matter contents. The high organic matter contents of manure will likely require consideration if alum is to be further explored for oxytetracycline removal.

4.3.2 Time Effects

A significant difference was noted with respect to time interval for all of the examined constituents except NO_3, PO_4, and oxytetracycline (Table 4.4). The first flush phenomenon, whereby a disproportionally high concentration occurs in runoff during the first part of a storm (Sansalone and Buchberger, 1997; Braskerud, 2002), was seen with the majority of examined constituents. The time interval 2 min had the highest concentrations for all constituents. For the most part, constituent concentrations reached a minimum level after either 16 to 24 min since the start of runoff. These results point to the importance of the first 15 minutes of a storm event as this period of time is when the greatest amounts of contaminants are typically transported to waterbodies (Soupir et al., 2006; Kato et al., 2009; Delpla et al., 2011). As such, BMP selection should target reductions of constituent losses during the early parts of the storm (Moore and Miller,

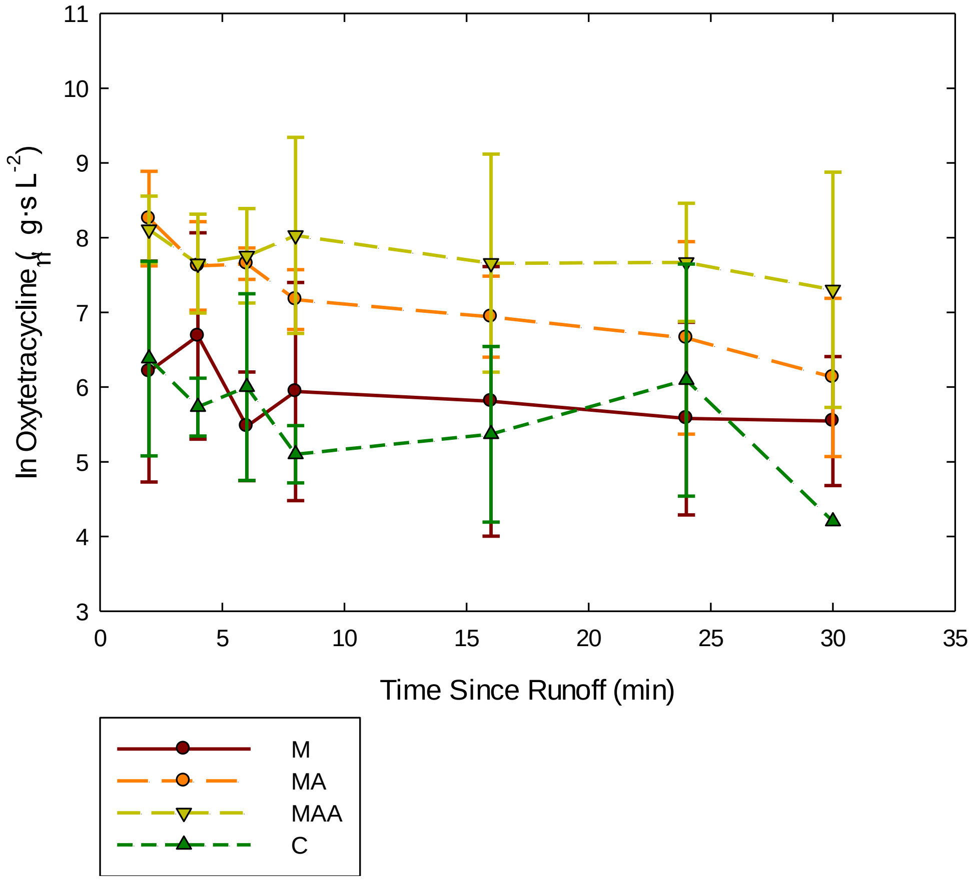


Figure 4.11. Flow Normalized ln Oxytetracycline Concentrations for Each Treatment. C=Control, M=Manure Only, MA=Manure and Antibiotics, and MAA=Manure, Antibiotics and Alum.

Table 4.4 Statistics for Constituent Concentrations at Time Intervals (Mean ± Standard Deviation).

Time Interval (min)	***E. coli***	**Cl**	**NO_3-N**	**NH_4-N**	**PO_4-N**	**TOC**	**Oxytetracycline**	**pH**	**EC**	**Turbidity**	**TSS**
2	13.8 ± 5.1^{a}	7.4 ± 0.8^{a}	0.5 ± 2.6^{a}	5.5 ± 1.4^{a}	-0.8 ± 2.7^{a}	7.1 ± 1.1^{a}	7.1 ± 1.3^{a}	5.4 ± 1.0^{a}	10.1 ± 0.6^{a}	7.8 ± 1.5^{a}	10.0 ± 0.8^{a}
4	13.3 ± 4.9^{b}	7.1 ± 0.9^{b}	-0.2 ± 2.8^{a}	5.2 ± 1.4^{b}	-1.4 ± 2.5^{a}	6.7 ± 1.0^{b}	6.6 ± 1.6^{a}	5.2 ± 1.1^{b}	9.8 ± 0.8^{b}	7.5 ± 1.4^{b}	9.5 ± 0.8^{b}
6	13.0 ± 5.0^{b}	6.9 ± 1.0^{b}	-0.2 ± 2.7^{a}	5.0 ± 1.5^{c}	-2.0 ± 2.3^{a}	6.5 ± 1.0^{c}	6.8 ± 1.2^{a}	5.1 ± 1.2^{c}	9.7 ± 0.9^{c}	7.3 ± 1.6^{c}	9.3 ± 0.9^{c}
8	12.8 ± 4.7^{b}	6.7 ± 1.3^{c}	-0.8 ± 3.2^{a}	4.9 ± 1.5^{c}	-2.3 ± 2.7^{a}	6.3 ± 1.0^{d}	6.5 ± 1.4^{a}	5.1 ± 1.3^{c}	9.6 ± 1.0^{c}	7.1 ± 1.5^{d}	9.2 ± 1.0^{c}
16	12.0 ± 4.6^{c}	6.4 ± 1.1^{c}	-0.7 ± 3.3^{a}	4.4 ± 1.6^{d}	-2.4 ± 2.1^{a}	5.7 ± 1.0^{e}	6.4 ± 1.4^{a}	4.9 ± 1.3^{c}	9.2 ± 1.1^{d}	6.5 ± 1.3^{e}	8.7 ± 1.1^{d}
24	11.5 ± 4.4^{cd}	6.3 ± 1.2^{c}	-0.2 ± 3.5^{a}	4.2 ± 1.7^{d}	-2.4 ± 2.5^{a}	5.6 ± 1.2^{e}	6.4 ± 1.3^{a}	4.9 ± 1.3^{c}	9.1 ± 1.1^{d}	6.0 ± 1.3^{f}	8.7 ± 1.2^{d}
30	11.0 ± 3.9^{d}	6.2 ± 1.3^{c}	0.4 ± 3.5^{a}	4.1 ± 1.6^{d}	-3.4 ± 2.5^{a}	5.5 ± 1.2^{e}	5.9 ± 1.5^{a}	5.0 ± 1.4^{c}	9.1 ± 1.3^{d}	5.9 ± 1.4^{f}	8.6 ± 1.3^{d}

[1] C=control, M=manure only, MA=manure and antibiotics, and MAA=manure, antibiotics, and alum.

[2] Values computed for all treatments and repetitions. All reported values were normalized by flow and log transformed.

[3] Different letters within columns indicated means differed statistically at α=0.05.

1994) and care should be exercised in timing of manure application in relation to anticipated storm events (Edwards and Daniel, 1993).

4.4 CONCLUSIONS

Antibiotics use in livestock is extensive with permitted applications extending beyond therapeutic needs such as disease treatment to subtherapeutic ones such as growth promotion. Such pervasive use of antibiotics coupled with high excretion rates of the substances from livestock has resulted in an increased presence of antibiotics in surface waters and groundwaters, and unfortunately, a greater likelihood of the presence of antibiotic-resistant bacteria in the environment. As land application of manure is one of the main pathways for introducing antibiotics to waterbodies, it is important to identify BMPs for reducing antibiotic transport in runoff. For antibiotics with high sorption coefficients, and hence a greater tendency to bind to soil particles, BMPs that target sediments or constituents with similar tendencies (e.g. phosphorus) may be ideal for antibiotic removal. Prior research examining the effectiveness of alum to reduce phosphorus transport in runoff from poultry litter applied lands suggests that this BMP may serve to reduce oxytetracycline transport in runoff as well. If effective, alum could be added to swine manure prior to land application.

Results of this study, however, demonstrated that the addition of alum (1% concentration) to liquid swine manure did not significantly reduce the concentration of any of the examined constituents (*E. coli*, oxytetracycline, NO_3-N, NH_4-N, PO_4-P, pH, EC, TSS, Cl, TOC and turbidity) in simulated runoff. The reason for the lack of reduction with alum addition could be related to the concentration used, as indicated by results from studies on the use of alum to reduce the hormone 17β-estradiol (Bushee-Bullock, 1999; DeLuane and Moore, 2013). The lack of reduction in PO_4-P transport, for instance, also suggests the alum dosage was too low. For oxytetracycline, the primary constituent of interest in this study, the lack of reduction in runoff could also be linked to the high organic matter contents present in swine manure (Choi et al., 2008). Further research is needed to examine a range of alum doses and organic matter concentrations to better assess its potential as a BMP for reducing antibiotic transport. Consideration should also be given to the use of a secondary BMP (e.g. treatment train), such as a vegetated filter strip, for filtering sediment-bound constituents and flocculants from runoff.

Lastly, constituent concentrations were at their highest in runoff typically during the first 15 minutes of the storm event after which they reached a minimum level. Thus, BMPs should target this first flush (e.g. water quality volume) whereby contaminant transport to waterbodies is the greatest. Likewise, care should be taken in timing manure applications such that they do not precede eminent storm events.

CHAPTER 5: SUMMARY OF CONCLUSIONS

The objectives of this study were to (1) determine the error associated with flow-weighted composite sampling for rainfall simulation studies, (2) evaluate the relationship between the veterinary antibiotic oxytetracycline and other water quality constituents, and (3) evaluate the ability of alum, as a manure amendment, on the reducing antibiotic transport. Rainfall simulation experiments at the University of Kentucky's Maine Chance Research Farm were performed to evaluate these objectives. Runoff samples were collected from fescue plots over the course of a simulated storm (102 mm h^{-1}) and analyzed for the water quality constituents *E. coli*, oxytetracycline, NO_3-N, NH_4-N, PO_4-P, pH, EC, TSS, Cl, TOC and turbidity. All samples at each time interval (2, 4, 6, 8, 16, 24 and 30 min) were analyzed; additionally, flow-weighted composite samples were created using each time interval sample, and the flow-weighted composites were also analyzed. The flow-weighted composite concentrations were compared to the calculated flow-weighted mean concentrations (FWMC). A methodology (Appendix D) was developed to analyze water samples for oxytetracycline in order to compare the concentrations to other water quality constituents to better focus treatment efforts and evaluate the effect of alum on reducing oxytetracycline transport as well as transport of the other constituents.

In Chapter 2, a flow-weighted composite sampling strategy was compared to the standard use of the FWMC. For constituents concentrations well above the detection limits (*E. coli*, turbidity, TOC, TSS, NH_4-N and EC), a strong correlation was seen between FWMC and flow-weighted composite concentrations. This finding indicates that flow-weighted composite sampling more closely represents the FWMC at higher concentrations. Larger errors were noted when constituent concentrations were close to the detection limits (PO_4, NO_3-N, and oxytetracycline). When constituent concentrations were close to the detection limits, larger differences between the flow-weighted composites and FWMCs were noted. For oxytetracycline, it is suspected that the high presence of organic matter in the samples caused some interference with the developed oxytetracycline analysis methodology; as such, refinements to the methodology were suggested. Also, the presence of a number of samples, mainly with the C and M plots, having low detections could also account for some of the error seen with oxytetracycline. For unknown reasons, the flow-weighted composite samples consistently over predicted the FWMCs for Cl.

Chapter 3 evaluated the potential for oxytetracycline to be transported via overland flow and the relationship between oxytetracycline concentrations and the concentrations of other constituents. The goal of this study was to identify best management practices, based on their ability to reduce the transport of constituents with which oxytetracycline was related, for further study in the reduction of antibiotic transport. Oxytetracycline concentrations were found in the $\mu g\ L^{-1}$ range, which is difficult to accurately quantify. Results of the study showed that oxytetracycline concentrations were strongly correlated to *E. coli*, Cl, TOC, turbidity, and TSS. Due to the strong correlation of oxytetracycline to TSS and turbidity and the likelihood of oxytetracycline to bind to manure particles based on the literature, it was determined that a BMP that reduces solids in runoff, such as a flocculants or vegetative buffer strips, may also reduce oxytetracycline transport.

The effectiveness of amending manure with alum was evaluated in Chapter 4. The addition of alum, at a 1% dosage, showed no decrease in the concentrations of any of the examined constituents in runoff. The reason for the lack of reduction with the alum addition could be related to the concentration used. Studies with the hormone 17β-estradiol found reductions using alum but at higher concentrations (5, 10 and 20%). For oxytetracycline, the primary constituent of interest in this study, the lack of reduction in runoff could also be linked to the high organic matter contents present in swine manure. Because oxytetracycline concentrations increased slightly with the addition of alum (α=0.10), it is possible that the alum may resulted in an increase in antibiotic mobility. While oxytetracycline may have formed flocs with the addition of alum, these flocs may not have been large enough to settle out of the runoff. If such is the case, then using a second BMP in addition to alum would be necessary to reduce oxytetracycline transport. Lastly, a first flush phenomenon was seen with higher concentrations of all constituents occurring at the beginning of the simulated storm for all treatments. This result points to the need of a BMP to target the reduction of pollutants during the first part of the storm (e.g. water quality volume).

CHAPTER 6: FUTURE WORK

In order to understand the mobility of oxytetracycline in the environment, a more precise analytical procedure for oxytetracycline is necessary. The recovery rate of the extraction procedure should undergo further exploration to improve the separation between the oxytetracycline peak and the peaks of other organic contaminants using HPLC, particularly for lower levels of oxytetracycline. The use of solid phase extraction for the concentration of oxytetracycline should also be explored and compared to the methodology used in this study. Improved accuracy of the oxytetracylcine analysis methodology will be an important tool in the continued research of antibiotic transport.

Continued research on the behavior of oxytetracycline in the environment and the effectiveness of BMPs on the concentrations of other constituents will assist with the selection of a more effective BMP for oxytetracycline. Higher alum doses should be examined for effectiveness in reducing antibiotic transport, possibly in a bench-scale study first before proceeding to a rainfall simulated study. Also, the effectiveness of vegetative buffer strips (e.g. vegetation heights), either by themselves or forming a treatment train with alum, in reducing the transport of oxytetracycline should be explored.

APPENDIX A: RAINFALL SIMULATOR CALIBRATION AND PLOT CURVE NUMBER DETERMINATION

A.1.1 RAINFALL SIMULATOR CALIBRATION

Each of the three rainfall simulators was calibrated individually using five duty cycles: 10, 30, 50, 75 and 100%. Percentages indicate the percentage of time the valves were open. Sixty-four Tru-Chek rain gauges (Edwards Manufacturing Company, Albert Lea, MN) were evenly distributed underneath the rainfall simulators. Simulators ran for 15 minutes on the 10% duty cycle, 12 minutes on the 30 and 50% duty cycles, and 10 minutes on the 75 and 100% duty cycles. Longer time periods were used at the lower duty cycles to ensure the rain gauges collected a sufficient amount of water to accurately read the gradations on the gauges. Water levels in each rain gauge were recorded at the completion of each duty cycle. For each rainfall simulator and each duty cycle, an average water level was computed using the 64 rain gauges. Based on the average water level or rain fall depth and the duration of rainfall, average intensities were computed. Figures A.1-A.3 show the calibration curves obtained for rainfall simulators 2-4. Note: that rainfall simulator 1 was out of commission, and as such, was not used in this study.

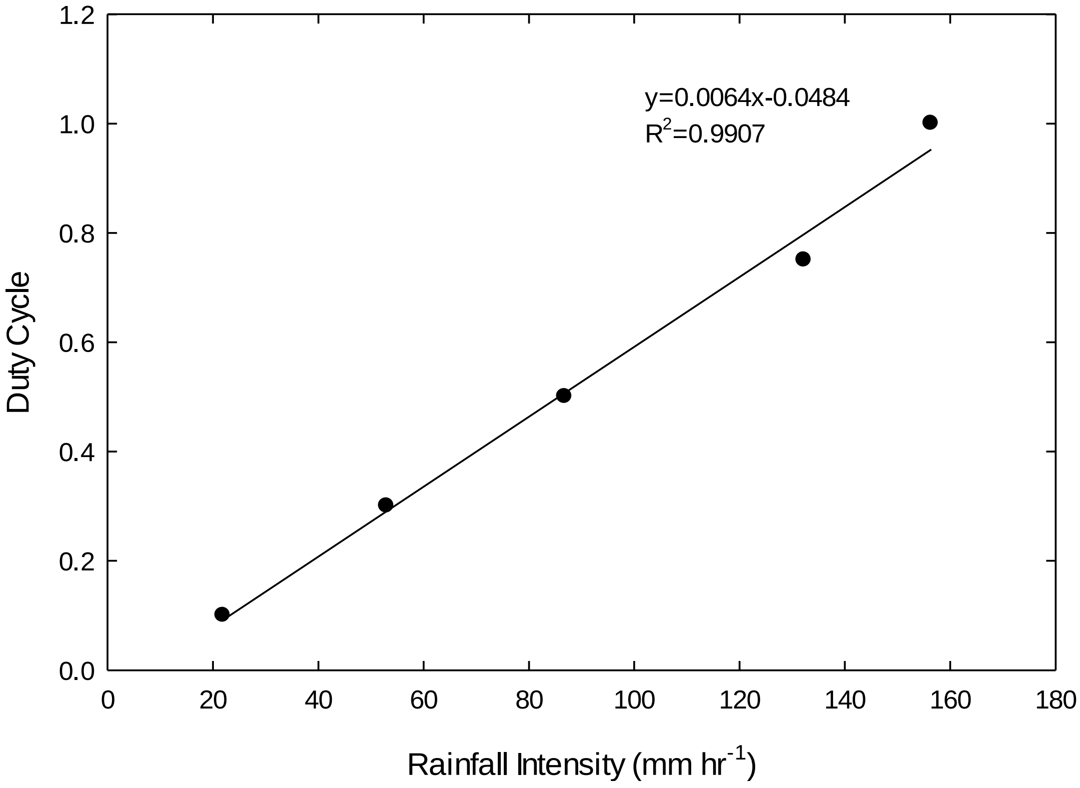


Figure A.1: Simulator 2 Calibration Curve.

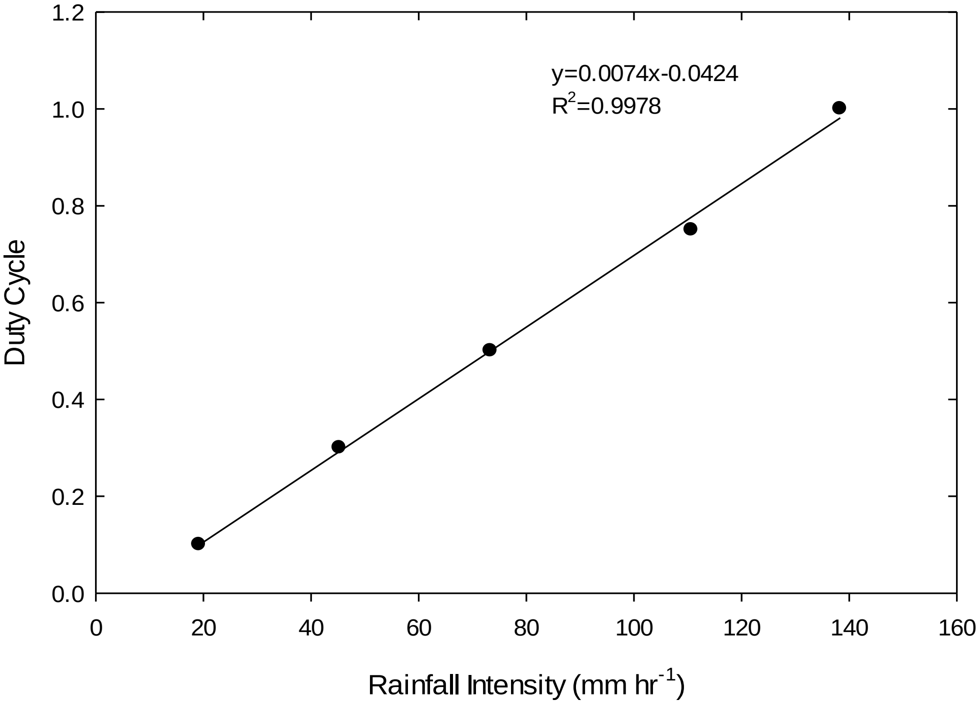


Figure A.2. Simulator 3 Calibration Curve.

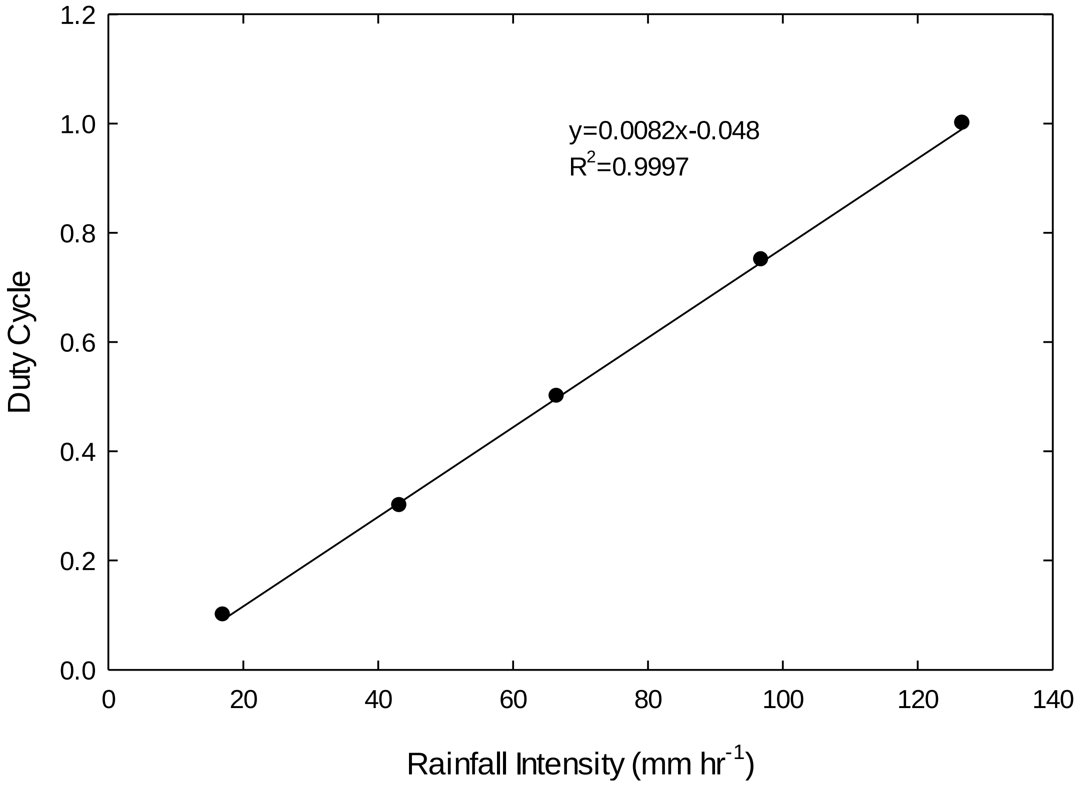


Figure A.3. Simulator 4 Calibration Curve.

A.1.2 PLOT CURVE NUMBER DETERMINATION

Rainfall simulators were programed to a 100% duty cycle, which produced rainfall intensities between 127 and 152.4 mm hr^{-1}. Plots received simulated rainfall until the point of saturation (i.e. start of runoff) at which point the rainfall simulators were turned off. Plots were then allowed to equilibrate for 30 minutes to ensure that runoff, from this saturation, had ceased. After this 30-minute period, rainfall was recommenced, and the time required to produce runoff was recorded. After runoff started, it was collected in 1 L bottles every two minutes until a constant flow rate was reached. Once a constant flow rate was reached, rainfall was stopped; however, runoff was collected at one minute intervals until its cessation. Flow rates were computed by dividing the weight of the collected water (full bottle minus tare weight of bottle) by the time required to fill a bottle.

Hydrographs were developed for each plot. Total runoff, V_{runoff}, was computed by integrating the area under the hydrograph. Runoff depth, Q, was determined using equation A.1 where A_{plot} represents the area of the plot or 14.86 m^2.

$$\mathrm{Q} = \frac{\mathrm{V_{runoff}}}{\mathrm{A_{plot}}} \tag{A.1}$$

Precipitation depth, P, was computed by multiplying rainfall intensity by the total time of the simulated rainfall. The curve number (CN) for each plot was determined using equations A.2 and A.3 where the variable S represents storage.

$$S = 5(P + 2Q - (4Q^2 + 5PQ)^{0.5} \tag{A.2}$$

$$CN = \frac{25{,}400}{S + 254} \tag{A.3}$$

If runoff was not achieved within one hour, the CN was not calculated and the plot was not used in the study. Table A.1 contains the CNs for each plot. Figure A.2 is a map of plot locations, for reference.

Table A.1. Curve Numbers (CN) for All Plots Evaluate for Use in the Study.

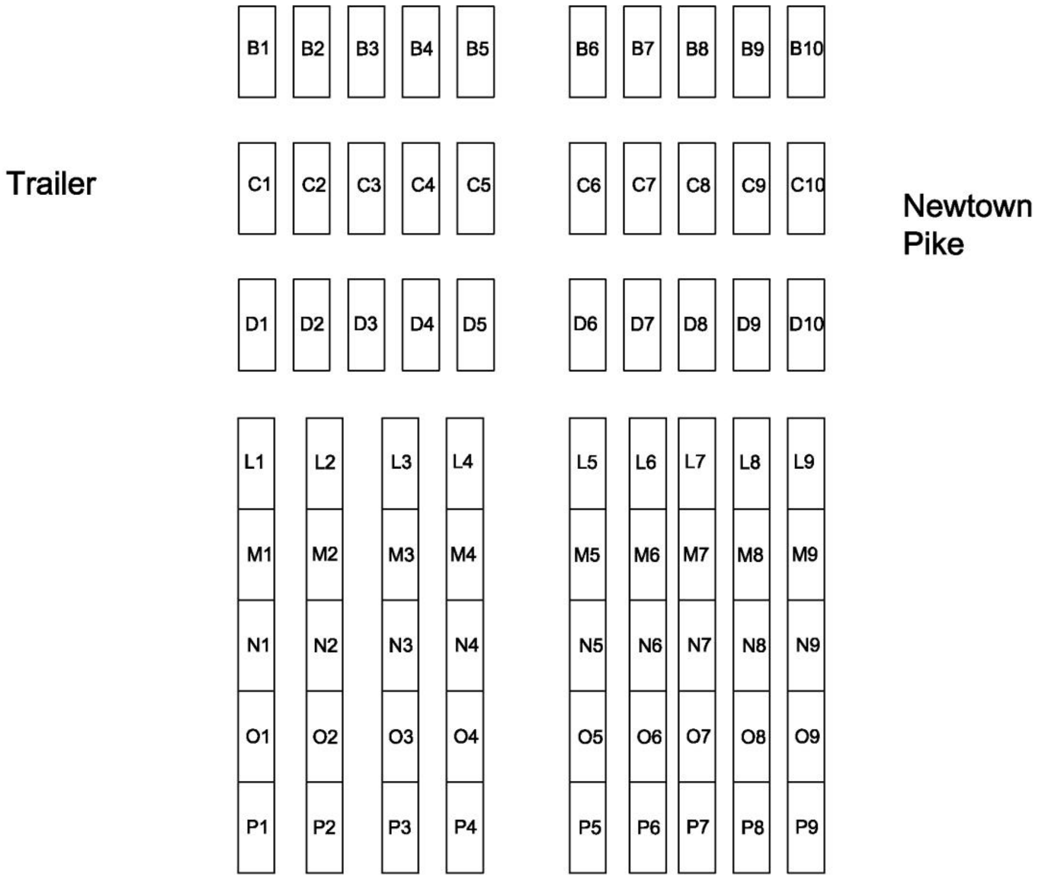
Plot	**Curve Number**
B1	82
B2	96
B3	55
B4	82
B5	88
B6	90
B7	74
B8	81
B9	65
B10	82
C1	90
C2	89
C3	82
C4	80
C5	89
C6	77
C7	68
C8	--[1]
C9	82
C10	78
D1	86
D2	82
D3	--
D4	--
D5	84
D6	--
D7	--
D8	--

Table A.1 continued.

Plot	Curve Number
D9	84
D10	69
L1	84
L2	70
L3	79
L4	--
L5	--
L6	75
L7	--
L8	--
L9	--
N1	77
N2	76
N3	86
N4	--
N5	85
N6	79
N7	77
N8	75
N9	81

[1] No runoff produced after one hour of simulated rain.

Figure A.4. Plot Location Map.



APPENDIX B: FLOW-WEIGHTED COMPOSITE SAMPLE DATA

Date	6/18/2013			
Plot	N2			
Simulator	3			
Bottle Tare Weight	98.7	g		
Time to Runoff	3	min	21	sec
Rainfall Intensity	4	in/hr		

						Composite Volume	
Time since Runoff	Time to fill (s)	Mass (g)	Sample (g)	Flowrate (mL/sec)	Incremental Volume (L)	Bacteria (mL)	Antibiotics (mL)
2	29.72	890.8	792.1	26.7	1.6	3	22
4	29.69	1003.1	904.4	30.5	3.4	6	48
6	27.21	1044.3	945.6	34.8	3.9	7	55
8	26.14	1066.1	967.4	37.0	4.3	7	60
16	23.53	1073.8	975.1	41.4	18.8	31	262
24	20.74	1105.6	1006.9	48.5	21.6	36	301
30	19.45	1107.6	1008.9	51.9	18.1	30	252
				Sum	71.7	120	1000

CN	64	
Total Rainfall	56.5	mm
Runoff Depth	4.8	mm

Date	6/24/2013				
Plot	L1				
Simulator	3				
Bottle Tare Weight	98.7	g			
Time to Runoff	9	min	51	sec	
Rainfall Intensity	4	in/hr			

						Composite Volume	
Time since Runoff (min)	Time to fill (s)	Mass (g)	Sample (g)	Flowrate (mL/sec)	Incremental Volume (L)	Bacteria (mL)	Antibiotics (mL)
2	65.71	890.8	998.2	15.2	0.9	2	16
4	44.94	1003.1	707.2	15.7	1.9	4	33
6	46.38	1044.3	904.7	19.5	2.1	5	38
8	44.73	1066.1	958.0	21.4	2.5	5	44
16	28.21	1073.8	978.4	34.7	13.5	29	240
24	24.07	1105.6	1082.0	45.0	19.1	41	340
30	22.69	1107.6	1031.2	45.4	16.3	35	290
				Sum	56.2	120	1000

CN	57	
Total Rainfall	67.5	mm
Runoff Depth	3.8	mm

Date	6/24/2013	
Plot	L3	
Simulator	2	
Bottle Tare Weight	98.7	
Time to Runoff	27	min
Rainfall Intensity	4	in/hr

						Composite Volume	
Time since Runoff (min)	Time to fill (s)	Mass (g)	Sample (g)	Flowrate (mL/sec)	Incremental Volume (L)	Bacteria (mL)	Antibiotics (mL)
2	45.32	962.4	863.7	19.1	1.1	3	27
4	39.47	966.2	867.5	22.0	2.5	7	58
6	40.49	990.2	891.5	22.0	2.6	7	62
8	38.48	1013.1	914.4	23.8	2.7	8	64
16	30.93	1011.5	912.8	29.5	12.8	36	299
24	39.48	950.9	852.2	21.6	12.3	34	287
30	30.86	932.2	833.5	27.0	8.7	25	204
				Sum	42.8	120	1000

CN	44	
Total Rainfall	96.5	mm
Runoff Depth	2.9	mm

Date	6/24/2013				
Plot	N9				
Simulator	4				
Bottle Tare Weight	98.7				
Time to Runoff	51	min	27	sec	
Rainfall Intensity	4	in/hr			

Time since Runoff (min)	Time to fill (s)	Mass (g)	Sample (g)	Flowrate (mL/sec)	Incremental Volume (L)	Composite Volume Bacteria (mL)	Composite Volume Antibiotics (mL)
2	60.80	575.3	476.6	7.8	0.5	4	23
4	61.65	609.9	511.2	8.3	1.0	7	47
6	59.66	681.0	582.3	9.8	1.1	8	52
8	55.66	706.0	607.3	10.9	1.2	10	60
16	101.59	931.7	833.0	8.2	4.6	35	222
24	92.98	862.4	763.7	8.2	3.9	30	191
30	90.31	972.3	873.6	9.7	3.2	25	156
				Sum	15.5	120	750

CN	31	
Total Rainfall	137.9	mm
Runoff Depth	1.0	mm

Date	6/25/2013			
Plot	C3			
Simulator	3			
Bottle Tare Weight	98.7			
Time to Runoff	43	min	30	sec

						Composite Volume	
Time since Runoff (min)	Time to fill (s)	Mass (g)	Sample (g)	Flowrate (mL/sec)	Incremental Volume (L)	Bacteria (mL)	Antibiotics (mL)
2	43.66	1072.6	973.9	22.3	1.3	3	23
4	35.24	1029.8	931.1	26.4	2.9	6	50
6	32.01	1023.5	924.8	28.9	3.3	7	57
8	29.35	1028.3	929.6	31.7	3.6	7	62
16	27.43	1005.7	907	33.1	15.5	32	265
24	22.21	1008.8	910.1	41.0	17.8	36	303
30	25.68	1068	969.3	37.7	14.2	29	241
				Sum	58.7	120	1000

CN	38	
Total Rainfall	124.5	mm
Runoff Depth	3.9	mm

Date	6/25/2013	
Plot	C4	
Simulator	3	
Bottle Tare Weight	98.7	
Time to Runoff	70	min
Rainfall Intensity	4	in/hr

						Composite Volume	
Time since Runoff (min)	Time to fill (s)	Mass (g)	Sample (g)	Flowrate (mL/sec)	Incremental Volume (L)	Bacteria (mL)	Antibiotics (mL)
2	55.36	674.5	575.8	10.4	0.6	10	62
4	54.83	574.1	475.4	8.7	1.1	18	113
6	53.97	416.1	317.4	5.9	0.9	14	86
8	220.54	823.8	725.1	3.3	0.6	9	54
16	224.74	814.1	715.4	3.2	1.6	25	154
24	171.66	754.1	655.4	3.8	1.7	27	166
30	210.74	653.3	554.6	2.6	1.2	18	115
				Sum	7.6	120	750

CN	25	
Total Rainfall	169.3	mm
Runoff Depth	0.5	mm

Date	6/25/2013	
Plot	D5	
Simulator	2	
Bottle Tare Weight	98.7	
Time to Runoff	35	min
Rainfall Intensity	4	in/hr

						Composite Volume	
Time since Runoff (min)	Time to fill (s)	Mass (g)	Sample (g)	Flowrate (mL/sec)	Incremental Volume (L)	Bacteria (mL)	Antibiotics (mL)
2	55.25	898.5	799.8	14.5	0.9	3	26
4	55.02	915.9	817.2	14.9	1.8	6	53
6	46.55	1037.2	938.5	20.2	2.1	8	63
8	55.13	732.7	634	11.5	1.9	7	57
16	37.18	1012.2	913.5	24.6	8.7	31	260
24	46.56	1010	911.3	19.6	10.6	38	318
30	45.21	1085.4	986.7	21.8	7.5	27	224
				Sum	33.3	120	1000

CN	39	
Total Rainfall	110.1	mm
Runoff Depth	2.2	mm

Date	6/26/2013	
Plot	B1	
Simulator	2	
Bottle Tare Weight	98.7	
Time to Runoff	11	min
Rainfall Intensity	4	in/hr

						Composite Volume	
Time since Runoff (min)	Time to fill (s)	Mass (g)	Sample (g)	Flowrate (mL/sec)	Incremental Volume	Bacteria (mL)	Antibiotics (mL)
2	12.00	1004.8	906.1	75.5	4.5	2	15
4	9.34	1142.8	1044.1	111.8	11.2	5	38
6	6.81	1038.6	939.9	138.0	15.0	6	50
8	5.97	1067.4	968.7	162.3	18.0	7	60
16	5.18	1147.3	1048.6	202.4	87.5	35	292
24	4.99	1047.1	948.4	190.1	94.2	38	315
30	4.66	994.6	895.9	192.3	68.8	28	230
				Sum	299.3	120	1000

CN	75	
Total Rainfall	69.4	mm
Runoff Depth	20.1	mm

Date	6/26/2013	
Plot	B4	
Simulator	2	
Bottle Tare Weight	98.7	
Time to Runoff	10	min
Rainfall Intensity	4	in/hr

						Composite Volume	
Time since Runoff (min)	Time to fill (s)	Mass (g)	Sample (g)	Flowrate (mL/sec)	Incremental Volume (L)	Bacteria (mL)	Antibiotics (mL)
2	14.22	1042.1	943.4	66.3	4.0	3	22
4	11.55	1030.8	932.1	80.7	8.8	6	48
6	10.85	1088.5	989.8	91.2	10.3	7	56
8	9.05	1016.1	917.4	101.4	11.6	8	63
16	8.08	1075.6	976.9	120.9	53.3	35	290
24	8.11	1012.9	914.2	112.7	56.1	37	305
30	8.65	1044.0	945.3	109.3	40.0	26	217
				Sum	184.1	120	1000

CN	69	
Total Rainfall	67.7	mm
Runoff Depth	12.4	mm

Date	6/26/2013	
Plot	B10	
Simulator	4	
Bottle Tare Weight	98.7	
Time to Runoff	11	min
Rainfall Intensity	4	in/hr

						Composite Volume	
Time since Runoff (min)	Time to fill (s)	Mass (g)	Sample (g)	Flowrate (mL/sec)	Incremental Volume (L)	Bacteria (mL)	Antibiotics (mL)
2	8.50	1020.1	921.4	108.4	6.5	3	27
4	6.71	990.9	892.2	133.0	14.5	7	59
6	6.36	1015.1	916.4	144.1	16.6	8	68
8	6.12	996.6	897.9	146.7	17.4	9	71
16	6.71	1096.2	997.5	148.7	70.9	35	290
24	6.76	1051.4	952.7	140.9	69.5	34	284
30	6.75	997.2	898.5	133.1	49.3	24	202
				Sum	244.8	120	1000

CN	72	
Total Rainfall	69.4	mm
Runoff Depth	16.5	mm

Date	6/26/2013	
Plot	C2	
Simulator	3	
Bottle Tare Weight	98.7	
Time to Runoff	30	min
Rainfall Intensity	4	in/hr

						Composite Volume	
Time since Runoff (min)	Time to fill (s)	Mass (g)	Sample (g)	Flowrate (mL/sec)	Incremental Volume (L)	Bacteria (mL)	Antibiotics (mL)
2	38.19	1025.4	926.7	24.3	1.5	1	10
4	28.66	1076.2	977.5	34.1	3.5	3	24
6	22.28	1004.1	905.4	40.6	4.5	4	31
8	17.69	1096.3	997.6	56.4	5.8	5	40
16	10.09	1036.6	937.9	93.0	35.8	30	247
24	8.33	1059.4	960.7	115.3	50.0	41	345
30	7.39	1051.1	952.4	128.9	44.0	36	303
				Sum	145.1	120	1000

CN	51	
Total Rainfall	101.6	mm
Runoff Depth	9.8	mm

Date	6/28/2013	
Plot	D1	
Simulator	3	
Bottle Tare Weight	98.7	
Time to Runoff	10	min
Rainfall Intensity	4	in/hr

						Composite Volume	
Time since Runoff (min)	Time to fill (s)	Mass (g)	Sample (g)	Flowrate (mL/sec)	Incremental Volume (L)	Bacteria (mL)	Antibiotics (mL)
2	11.36	1065.7	967	85.1	5.1	1	12
4	5.95	1050.5	951.8	160.0	14.7	4	35
6	4.80	1103	1004.3	209.2	22.2	6	53
8	4.04	1039.7	941	232.9	26.5	8	63
16	3.54	1036.6	937.9	264.9	119.5	34	285
24	3.54	1129.9	1031.2	291.3	133.5	38	318
30	4.13	1143	1044.3	252.9	97.9	28	234
				Sum	419.4	120	1000

CN	82	
Total Rainfall	67.7	mm
Runoff Depth	28.2	mm

Date	7/2/2013			
Plot	C5			
Simulator	2			
Bottle Tare Weight	98.7			
Time to Runoff	5	min	26	sec
Rainfall Intensity	4	in/hr		

						Composite Volume	
Time since Runoff (min)	Time to fill (s)	Mass (g)	Sample (g)	Flowrate (mL/sec)	Incremental Volume (L)	Bacteria (mL)	Antibiotics (mL)
2	6.97	1103.9	1005.2	144.2	8.7	3	28
4	5.27	1007.7	909.0	172.5	19.0	7	62
6	5.27	1037.2	938.5	178.1	21.0	8	69
8	5.30	1105.4	1006.7	189.9	22.1	9	72
16	5.47	1049.6	950.9	173.8	87.3	34	285
24	5.45	1019.7	921.0	169.0	82.3	32	269
30	4.87	1049.4	950.7	195.2	65.6	26	214
				Sum	305.9	120	1000

CN	80	
Total Rainfall	60.0	mm
Runoff Depth	20.6	mm

Date	7/2/2013				
Plot	B6				
Simulator	4				
Bottle Tare Weight	98.7				
Time to Runoff	6	min	18	sec	
Rainfall Intensity	4	in/hr			

						Composite Volume	
Time since Runoff (min)	Time to fill (s)	Mass (g)	Sample (g)	Flowrate (mL/sec)	Incremental Volume (L)	Bacteria (mL)	Antibiotics (mL)
2	9.08	1043.3	944.6	104.0	6.2	3	25
4	8.18	1048.6	949.9	116.1	13.2	6	53
6	7.64	1088.1	989.4	129.5	14.7	7	60
8	7.54	1094.7	996.0	132.1	15.7	8	64
16	6.41	1069.8	971.1	151.5	68.1	33	276
24	5.95	1012.9	914.2	153.6	73.2	36	297
30	6.47	1110.2	1011.5	156.3	55.8	27	226
				Sum	247.0	120	1000

CN	76	
Total Rainfall	61.5	mm
Runoff Depth	16.6	mm

Date	7/2/2013	
Plot	B5	
Simulator	2	
Bottle Tare Weight	98.7	
Time to Runoff	4	min
Rainfall Intensity	4	in/hr

						Composite Volume	
Time since Runoff (min)	Time to fill (s)	Mass (g)	Sample (g)	Flowrate (mL/sec)	Incremental Volume (L)	Bacteria (mL)	Antibiotics (mL)
2	15.88	1037.6	938.9	59.1	3.5	2	13
4	9.24	1062.5	963.8	104.3	9.8	4	35
6	6.27	1066.7	968.0	154.4	15.5	7	55
8	5.68	1086.0	987.3	173.8	19.7	8	70
16	5.69	1132.9	1034.2	181.8	85.3	36	301
24	6.15	1119.7	1021.0	166.0	83.5	35	295
30	4.82	1057.8	959.1	199.0	65.7	28	232
				Sum	283.1	120	1000

CN	80	
Total Rainfall	57.6	mm
Runoff Depth	19.0	mm

Date	7/2/2013	
Plot	C6	
Simulator	4	
Bottle Tare Weight	98.7	
Time to Runoff	11	min
Rainfall Intensity	4	in/hr

Time since Runoff (min)	Time to fill (s)	Mass (g)	Sample (g)	Flowrate (mL/sec)	Incremental Volume(mL)	Composite Volume Bacteria (mL)	Composite Volume Antibiotics (mL)
2	53.02	569.2	470.5	8.9	0.5	3	27
4	53.92	659.2	560.5	10.4	1.2	7	58
6	54.53	699.3	600.6	11.0	1.3	8	64
8	55.02	733.5	634.8	11.5	1.4	8	68
16	76.41	1110.9	1012.2	13.2	5.9	36	297
24	72.4	1065.4	966.7	13.4	6.4	38	319
30	196.06	1118.0	1019.3	5.2	3.3	20	167
				Sum	20.0	120	1000

CN	50	
Total Rainfall	69.4	mm
Runoff Depth	1.3	mm

APPENDIX C: METHODOLOGY FOR *E.COLI* ANALYSIS

Coliert-18, 120 mL IDEXX sample bottles (pre-sterilized) with sodium thiosulfate (de-chlorinating agent), and Quanti-Tray*/2000 were purchase from IDEXX Laboratories (Westbrook, ME). Dilutions (1:10, 1:100, and 1:1000) were made using **sterilized** deionized water (DI H_2O) and **sterilized** buffer water prepared by the following instructions.

C.1 BUFFER WATER AND DI WATER PREPARATION

C.1.1 Prepare Solutions

1. Add 50g of $MgSO_4 \cdot 7H_2O$ to 1 L flask of DI H_2O.
2. Add 34g of KH_2PO_4 to a second 1 L flask of DI H_2O and adjust pH to 7.2 with 1 N NaOH.

C.1.2 Prepare Buffer Water

3. Add 10 mL of $MgSO_4 \cdot 7H_2O$ solution to a 2 L flask.
4. Add 2.5 mL of KH_2PO_4 solution to the same 2 L flask as in step 3.
5. Fill the remaining volume of the 2 L flask with DI H_2O.
6. Securely place a lid on the 2 L flask. Mix the 2 L flask by inversion. Once mixing is complete, loosen lid (e.g. enough to rattle), but such that it remains on the threads.

C1.3 Prepare DI H_2O

7. Fill a second 2 L flask with DI H_2O. Place a lid on this 2 L flask. The lid should be loose enough to rattle but still catch on threads.

C.1.4 Autoclave

8. Place autoclave tape on each lid (2 L flask of buffer water and 2 L flask of DI H_2O) and check for proper looseness.
9. Autoclave on liquid cycle with the flasks in plastic trays.
10. Wait 10 minutes after cycle is complete before removing flasks.
11. Wait until the flasks are at room temperature before tightening the lids.

C.2 DILUTION BOTTLE PREPARATION

1. Place dilution bottles in plastic trays to prevent tipping. Lids should remain on the sample bottles, but be loosened (e.g. still caught on the threads). Glass or plastic 100-200 mL bottles are recommended.
2. Autoclave dilution bottles on labwares cycle.
3. Allow dilution bottles cool to room temperature before tightening lids.

C.3 DILUTIONS

1. Put 90 mL of buffer water into a sterilized dilution bottle (100-200 mL) to be used for dilution series. When making three dilutions, as done in this study, two sterilized dilution bottles with buffer are needed for each sample. See Figure C.1.
2. Put 90 mL of sterile DI H_2O into the three sterilized dilution bottles to be used for Coliert-18. One bottle for each dilution of each sample is needed for this step, hence three dilutions require three bottles.
3. Add one packet of Coliert-18 to each sterilized dilution bottle containing DI H_2O.
4. Add two drops of anti-foaming agent to each sterilized dilution bottle containing DI H_2O.
5. Put 10 mL of sample into 90 mL of DI H_2O for the 1:10 Dilution. Empty all 100 mL into a Quanti-Tray.
6. Put 10 mL of sample into 90 mL of buffer water (dilution bottle prepared in step 1). This is the 1:10 buffer water and sample mixture.
7. Put 10 mL of the 1:10 buffer water and sample mixture (step 6) into 90 mL of DI H_2O and empty all 100 mL into a Quanti-Tray. This is the 1:100 dilution.
8. Add 10 mL of the 1:10 buffer water and sample mixture (step 6) to 90 mL of buffer water. This is the 1:100 buffer water.
9. Put 10 mL of the 1:100 buffer water and sample mixture (step 8) in 90 mL of DI H_2O for the 1:1000 dilution and empty all 100 mL into a Quanti-Tray.
10. Continue this process for each subsequent dilution needed.

After emptying each dilution into its respective tray, seal using the IDEXX sealer. Place the sealed tray in an incubator for 18-22 hours at 35°C±0.5°C. After the incubation period, use a UV-light to determine which cells tested positive for *E. coli*. Cells must turn yellow and fluoresce to be considered positive. If unsure, use the IDEXX comparator tray to compare samples to the standard.

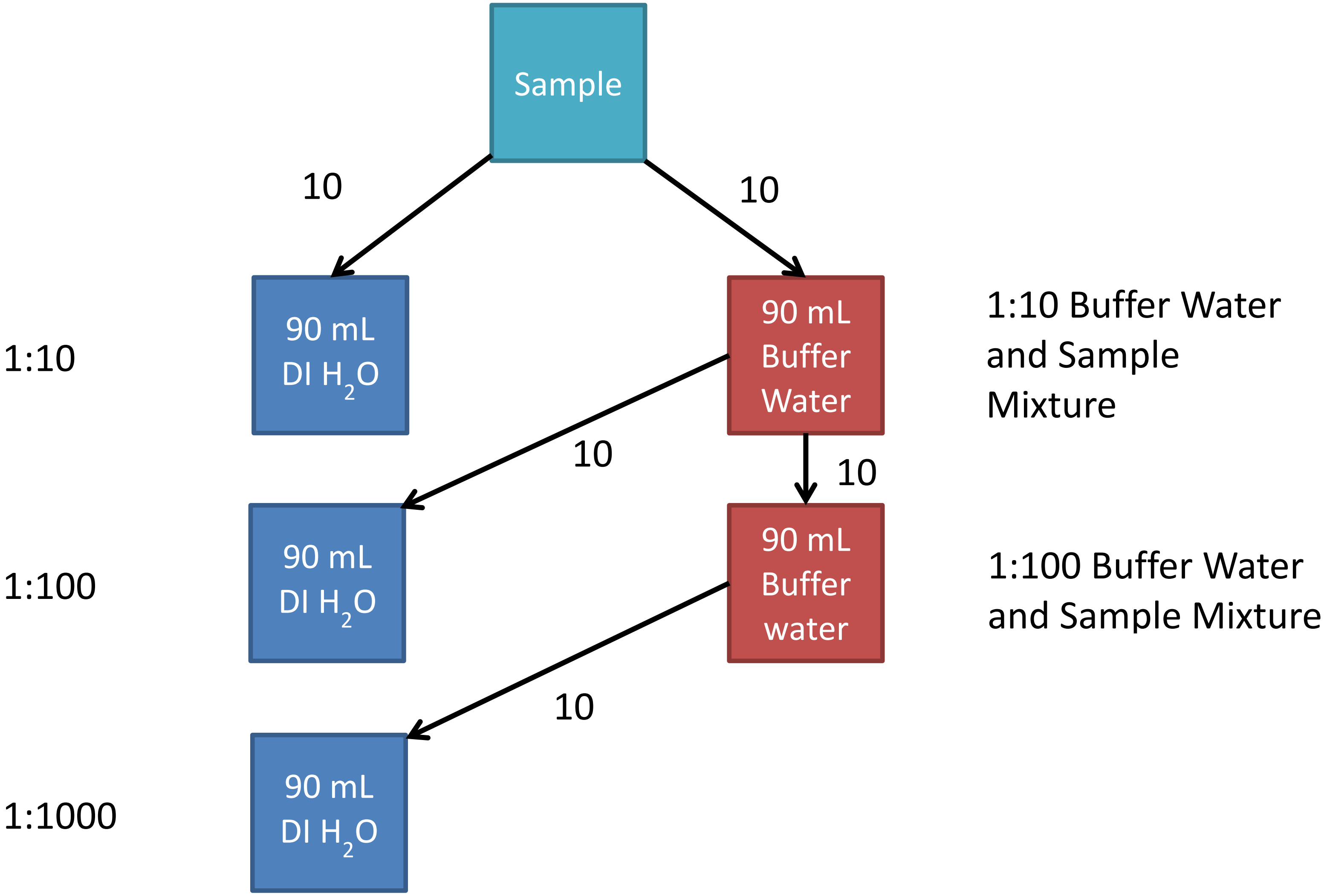


Figure C.1. E. *coli* Dilution Series.

APPENDIX D: METHODOLOGY FOR ANTIBIOTIC EXTRACTION AND ANALYSIS

The first step in antibiotic analysis is sample concentration. This step is necessary due to the low concentrations (mg) typicaly found in water samples. Typically, this concentration step is performed via solid phase extraction. However, the method of solid phase extraction is quite time consuming and often results in low recovery rates of antibiotics (Yang et al., 2005; Zhu et al,. 2001). As such, a new methodology was developed whereby the process of lyophilization (freeze-drying), similar to that described by Hirsh et al. (1998), was used.

D.1 SAMPLE PREPARATION

Approximately, 50 mL of sample was added to a 50 mL centrifuge tube. The sample, in the centrifuge tube, was then centrifuged at 3,500 rpm for 20 minutes. Next, 45 mL of supernatant was pipetted into a 50 mL glass test tube. The test tube was then covered with a kimwipe that was secured in place with tape. <u>Note that filtering sample through a 0.45 µm filter prior to its placement in a glass test tube would likely help refine the chromatogram.</u>

D.2 LYOPHILIZATION

Samples were placed in a freezer (-4°C) for 12 hours prior to placement in a -40°C freezer for about 2 hours to further lower the temperature. An SP Scientific VirTis Wizard 2.0 lyophilzer (Gardiner, New York) was turned on to the freeze cycle until the shelf temperature reached approximately -20°C. Samples were then removed from the -40°C freezer and placed in the lyophilizer. Note that while samples can be frozen in the lyophilzer, a greater potential exists for the test tubes to break due to the rapidness of freezing; hence the reason for using the freezer.

The lyophilizer condenser was turned on and allowed to reach a temperature between -40°C and -60°C. The lyophilizer vacuum was turned on and allowed to reach less than 500 mTorr. Shelf control for the lyophilizer was turned on and began heating the shelf to 10°C. Samples were left in the lyophilizer until dry (approximately 3 days).

D.3 SAMPLE CONCENTRATION

Dry residue in the test tubes was re-dissolved in 900 µL of 50% methanol solution by rinsing the test tube with 300 µL of methanol three times. The solution was then transferred to micro-centrifuge tubes and centrifuged at 4,000 rpm for 10 minutes. Approximately, 600 µL of supernatant was then transferred to HPLC vials.

A solution containing an oxytetratcycline concentration of 2 µg mL^{-1} was made by diluting a 1 mg mL^{-1} oxytetracycline solution. A 300 µL glass insert was then placed in an HPLC vial and 100 µL of concentrated sample was added to 100 µL of the 2 µg mL^{-1} solution. This resulted in a 25X concentration from the original sample and a concentration of at least 1 µg L^{-1} of oxytetracycline.

D.4 HPLC

A Dionex Ultimate 3000 HPLC along with an Ultimate 3000RS Variable Wavelength detector (Sunnyvale, California) set was used for this analysis. The variable wavelength detector was set at a wave length of 290 nm (Kay et al., 2005). A Dionex Acclaim 120 (C18) column with an inner diameter of 3 µm and length of 250 mm was used. The pumping rate was set to 0.400 mL min^{-1} with a gradient mobile phase of 0.5% acetic acid in water and 0.5% acetic acid in methanol. The gradient can be seen in Table D.1. The HPLC injected 20 µL of 25X concentrated sample and ran for 12 minutes per sample.

Table D.1. HPLC Gradient used in Oxytetracycline Analysis.

Time (min)	**HPLC Gradients**[1]	
	A (%)	**B (%)**
1	60	40
4	10	90
7	10	90
9	40	60
10	60	40

[1] A is DI water with 0.5% acetic acid; B is HPLC grade MeOH with 0.5% acetic acid.

APPENDIX E: MANURE AND SOIL SAMPLING RESULTS

Table E.1. Results of Manure Analysis.

Plot[1]	**C (%)**	**N (%)**	**P (%)**	**K (%)**	**Ca (%)**	**Mg (%)**	**Zn (mg L^{-1})**	**Cu (mg L^{-1})**	**Mn (mg L^{-1})**	**Fe (mg L^{-1})**
-----------------------M-----------------------										
B4	1.99	0.49	0.09	0.24	0.11	0.04	34.62	3.46	13.85	103.86
C5	1.95	0.49	0.09	0.23	0.12	0.04	37.80	3.78	15.12	98.28
L1	1.99	0.49	0.08	0.25	0.11	0.03	35.71	3.57	14.28	85.70
-----------------------MA------------------------										
B6	1.72	0.45	0.08	0.25	0.13	0.04	60.67	3.79	15.17	75.84
B10	2.03	0.49	0.09	0.25	0.11	0.04	39.08	3.55	14.21	95.94
C3	2.10	0.49	0.09	0.25	0.11	0.04	36.78	3.68	14.71	114.00
L3	2.06	0.49	0.08	0.25	0.11	0.04	37.07	3.71	14.83	70.43
-----------------------MAA------------------------										
B1	2.07	0.50	0.09	0.29	0.12	0.04	47.01	7.23	14.46	86.78
B5	2.17	0.53	0.10	0.30	0.12	0.04	52.75	12.17	16.23	93.33
C4	1.79	0.48	0.09	0.27	0.12	0.04	44.14	8.03	16.05	112.36
N9	1.94	0.52	0.10	0.30	0.11	0.04	49.04	12.26	16.35	93.98

[1] M=manure only, MA=manure and antibiotics, MAA=manure, antibiotics, and alum.

Table E.2. Results of Soil Analysis.

Plot[1]	P (lb ac^{-1})	K (lb ac^{-1})	pH	Ca (lb ac^{-1})	Mg (lb ac^{-1})	Zn (lb ac^{-1})	Total N (%)	Total C (%)	Sand (%)	Silt (%)	Clay (%)	Moisture (%)
-----------------------C-----------------------												
C2	160	439	5.08	4,147	326	7.8	0.18	1.63	10.04	70.11	19.85	22.6
C6	169	520	4.95	2,989	338	10.8	0.20	1.79	10.01	72.96	17.03	29.9
D1	269	554	5.91	4,895	548	7.8	0.18	1.51	9.91	74.28	15.81	24.2
D5	164	384	5.17	3,913	298	9.0	0.17	1.64	12.14	62.75	25.11	23.8
-----------------------M-----------------------												
B4	233	484	5.03	2,517	597	10.7	0.22	2.03	11.75	73.18	15.07	32.6
C5	184	584	5.28	3,755	317	35.6	0.21	2.00	13.21	69.33	17.46	29.8
L1	282	542	5.26	3,181	699	5.6	0.20	1.77	9.67	73.57	16.76	29.5
-----------------------MA-----------------------												
B6	194	901	5.09	3,024	377	8.7	0.25	2.15	10.82	72.31	16.87	30.6
B10	149	587	5.25	3,297	832	5.0	0.20	1.80	10.37	70.41	19.21	30.3
C3	258	419	5.57	3,809	822	13.8	0.24	2.25	11.01	74.94	14.05	29.1
L3	234	519	5.32	3,540	850	6.4	0.20	1.89	14.25	62.01	23.71	30.0
-----------------------MAA-----------------------												
B1	200	506	5.39	2,958	596	8.0	0.20	1.91	10.67	74.60	14.73	31.3
B5	175	601	5.1	3,427	421	23.6	0.21	1.90	11.67	70.76	17.57	29.0
C4	353	850	5.66	4,579	810	25.8	0.30	3.12	13.75	70.44	15.81	29.5
N9	137	264	5.28	2,972	729	4.2	0.17	1.48	7.95	75.16	16.88	29.0

[1] M=manure only, MA=manure and antibiotics, MAA=manure, antibiotics, and alum.

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