FACILITATED TRANSPORT OF ANTIBIOTICS BY BIOCHAR UNDER RAINFALL SIMULATIONS

by

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ABSTRACT

Antibiotic treatments in livestock operations are widely used in order to maintain a healthy herd and a healthy environment for livestock. From an agronomic perspective, the spreading of manure onto agricultural fields is beneficial to the soil as a renewable source of fertilizer by increasing organic matter and providing nutrient inputs for crops. However, the use of antibiotics can be excessive, resulting in manures containing residual antibiotics contaminating soils and waterways. Thus, there is a need to improve existing or develop new management practices to minimize the losses of antibiotics from manure entering waterways and groundwater. Biochar is a carbon-rich material produced from the oxygen-free pyrolysis of biomass. Generally, biochars have high surface area and sorb organic compounds and trace metals; thus, it is reasonable to hypothesize that biochars sorb antibiotics. The main goal of this research was to investigate if incorporated biochar to soil facilitates the transport of antibiotics under simulated rainstorm events. The specific objectives were to investigate the losses of surface-applied antibiotics to soils with different (1) application rates of biochar and rainfall intensities, and (2) if the losses were antibiotic type-dependent. The antibiotics, lincomycin, monensin, and tylosin were chosen because of their different chemical properties and to represent a wide range of family of antibiotics used in livestock in the U.S. Midwest. Softwood-derived biochar, at rates 0, 1, and 2% (w/w), was incorporated into a mollisol, followed by the spraying of a mix solution of antibiotics (target concentration of 1000 $\mu g\ L^{-1}$ per antibiotic) on the soil surface to mimic surface-applied manure practices. Simulated rainfall consisted of a 45-min rainstorm event of either 50 or 100 mm hr^{-1} to represent “normal” and “worst-case scenario” rainstorm events in the U.S. Midwest, respectively. Surface runoff and drainage samples were

collected to quantify the losses of antibiotics associated with the biochar, sediment, and water lost during the rainstorm events.

Runoff losses of biochar accounted for < 0.3% of total applied to the soil. Of the total antibiotics applied, <0.05% was accounted in the biochar (loss via surface runoff), indicating that antibiotic loss via facilitated transport by biochar was minimal. Of the three antibiotics used in this study, surface losses associated with biochar follow the order tylosin > monensin > lincomycin. Drainage antibiotic losses were as follows: monensin > lincomycin > tylosin; conversely, antibiotic losses from water runoff were of the order lincomycin > monensin > tylosin. Lastly, antibiotic losses in sediment were as follows: tylosin > lincomycin > monensin. Tylosin had the lowest recoveries in drainage and water runoff due to its strong adsorption capabilities to soil. Monensin had the highest losses in drainage but the lowest in sediment, instead partitioning to soil and biochar in the boxes. Lincomycin had the highest recovery in water runoff but the lowest recoveries in soil and biochar from boxes, explained by its hydrophilic properties making it mobile in the soil. The 100 mm hr^{-1} rainfall intensity increased the losses of lincomycin by 30% and of monensin by 50%, relative to the 50 mm hr^{-1}; however, tylosin losses were not affected by the rainfall intensity. The results of this study suggest that the incorporated biochar to soils had a very limited affect of antibiotic losses; conversely, the type of antibiotic and the rainfall rate did influence the losses of antibiotics. Biochar additions increased monensin loss in drainage and water runoff, lincomycin losses in water runoff, and tylosin losses in sediment runoff but had no effect for any antibiotic loss from biochar runoff. Under the conditions of the study, biochar had little influence on the runoff and drainage losses of antibiotics; thus, the incorporation of biochar to soil may not negatively affect the losses of antibiotics.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

Antibiotic treatments in livestock operations are widely used in order to maintain a healthy herd and a healthy environment for livestock. From an agronomic perspective, the spreading of manure onto agricultural fields is beneficial to the soil as a renewable source of fertilizer by increasing organic matter and providing nutrient inputs for crops. However, the use of antibiotics can be excessive resulting in manures containing residual antibiotics contaminating soils and waterways. A report by the FDA in 2009 found that 80% of the total antibiotic use in the United States was for livestock, with the remaining 20% being used for human health (FDA, 2009). Residual antibiotics in manure have resulted in measurable concentrations in waterways that negatively impact aquatic species (Halling-Sørensen, 2000); it can also affect human health and our ability to use soil bacterium as a means to produce new antibiotics (Kumar et al., 2005; Casanova and Sobsey, 2016). One mitigation method is to keep antibiotics in the soil until they degrade to minimize antibiotic contaminants entering waterways and in-ground water supplies. Biochar is pyrolyzed biomass produced in an oxygen-absent/depleted environment. Depending on how the biochar is produced (e.g., temperature), it can have large surface area, and is a carbon-rich material that when applied to soils may improve soil properties including an increase in cation exchange capacity, organic matter content, and water-holding capacity (Schmidt and Noack, 2000; Lehmann, 2007; Abrol et al., 2016; Tag et al., 2016; Liu et al., 2017). Use of biochar as a soil amendment could also be used as a mitigation method to reduce bioavailability of hydrophobic organic contaminants such as antibiotics due to its high surface area, porosity, hydrophobicity, and aromaticity (Ahmed et al., 2016).

1.2 Reasons for Antibiotic Mitigation

Livestock production in the United States generates about 132 million metric tons of dry weight manure annually (USDA-ERS, 2005); this manure is applied to about 6.4 million hectares of cropland, representing about 5 percent of the total cropland in the United States (USDA-ERS 2009). Between 2000 and 2010 the global consumption of antibiotics dramatically increased from 50 billion to 70 billion standard units, with a standard unit defined by IMS Health MIDAS (IMS Health, Danbury, CT, USA) as a "measure of volume based broadly on the smallest identifiable dose given to a patient, dependent on the pharmaceutical form," such as a pill, capsule, or ampoule; 70 to 80 percent of those standard units applied to the livestock industry (Gelband et al., 2015). Unlike human biosolids, it is not common to treat animal waste before it is applied to fields (Franklin et al. 2016). Most of the antibiotics given to the livestock are poorly metabolized. Therefore, the antibiotics may pass through the animal system into their feces and urine whether in its original or metabolite form (Boxall et al., 2003; Kumar et al., 2005; Zhang et al., 2016). Reports on how much of an antibiotic passes through an animal varies with the antibiotic used and the type of animal, but usually 30-90% of the antibiotic passes through into the urine and feces (Zhang and Zhang, 2011). It has been reported in one study that up to 100% of tylosin administered to the cattle passed through into the manure (Kutcha and Cessna, 2009b). Once antibiotics enter the soil via manure applications, these antibiotics may increase the chance for antibiotic-resistant genes (ARGs) to form within soil bacteria (Udikovic-Kolic et al., 2014). Even at low concentration of antibiotics, these ARGs can pose a threat to the public and to animals in the environment (Kemper, 2008). Prior research has shown that manure-derived ARGs present in the upper layers of soil have an increased chance of passing into the food chain, e.g. vegetables grown in these soils may uptake the ARGs and subsequently consumed by humans (Chen et al., 2017; Zhang et al., 2017). Just as troubling, ARGs can

amplify during bacterial replication and be passed to the following generations from vertical gene transfer, the "transfer of genetic material from mother to daughter cells through asexual reproduction" (Martinez et al., 2007; Franklin et al., 2016). This transformation can also lead to the "development and spread of single, cross-, and even multiple resistance in pathogens either directly or indirectly" (Kemper, 2008). Antibiotics will not always stay in the upper layers of soil, but rather can accumulate and be transported via rainfall and erosion into surface waters or ground water (Alder et al., 2001; Dominguez et al., 2014). Antibiotics that have high water solubility and low log K_{ow}, log K_{ow} defined as the octanol/water partition coefficient, (<1) such as lincomycin and sulfadimenthoxine can be mobile within a soil profile (Dominguez et al., 2014) relative to those antibiotics with a high log K_{ow} (e.g. monensin), strong affinities for clay (e.g. tylosin) or chelate strongly to soil surfaces (e.g. oxytetracyclines) that can be found in water ways due to sorption with dissolved organic carbon (Carlson and Mabury, 2007; Dominguez et al., 2014). Once ARGs reach water systems they can enter into the aquatic organism food chain at toxic levels. For algae and *Daphnia magna* (a crustacean), antibiotic concentrations in water become toxic between 5 and 100mg L^{-1} (Halling-Sørensen, 2000; Wollenberger et al., 2000), or in fish populations where fluoroquinolone, sulfonamide, tetracycline, and lincosamide classes bio-accumulated in organs and muscles of nine species of fish whose habitats were close to point sources (Zhao et al., 2015). With an increasing population comes an increased demand for animal products, resulting in an upward trajectory of antibiotic residues in animal manures (Le et al., 2018) thus becoming an increasing problem for the environment.

1.3 Sources of Antibiotics

1.3.1 Confined Animal Feeding Operations

Confined animal feeding operations (CAFOs) are a major source of manure-derived antibiotic contamination in the environment, whether from leaching from storage lagoons (Kumar et al., 2005; Watanabe et al., 2008; Chee-Sanford et al., 2009) or manure containing antibiotics being applied as fertilizer for agricultural purposes (Chee-Sanford et al., 2009; Popova et al., 2013; Ray et al., 2017). ARGs of tetracyclines have been found in groundwater near lagoons from CAFOs and transported as far as 250 meters away downstream in groundwater flow (Koiki et al., 2007; Chee-Sanford et al., 2001). CAFOs can produce up to an estimated 450 metric tons of waste per year, which are at least three times more than human waste generated in the United States alone (Braunig, 2005). The United States Environmental Protection Agency (EPA) considers small- and medium-sized CAFOs to be a nonpoint source of pollution and therefore are not required to have National Pollutant Discharge Elimination System (NPDES) permits, which regulates CAFO discharges into the environment (U.S. EPA, 2012). Most CAFOs in the United States fall into the small- and medium-sized category, leaving a large portion of CAFO operations to not needing NPDES permits and with limited regulation unlike large CAFOs that are required to have NPDES permits (Kolbe, 2013). Often, CAFOs store the manure in lagoons where the manure sits untreated until it is applied to the fields (Brauning, 2005). Besides lagoons, uncovered composting manure piles (Ray et al., 2017) and runoff from feedlot pens (Sura et al., 2015) can also be sources of pollution if not appropriately contained. Pollution from these point surfaces not only affect the areas immediately surrounding them, but also downstream where elevated levels of ARGs can exist (Sapkota et al., 2007, Storteboom et al., 2010).

1.3.2 Farm Management

There are multiple ways in which manure is handled and applied. Manure can be stored and allowed to compost; these processes may reduce the concentration of antibiotics in the manure before it is applied to the fields. After 30 days of composting manure, the dissipation degree (>85%) was dependent on (1) the type of antimicrobial compound dependent on physiochemical properties (molecular weight, pKa, half-life), (2) the origin of the compound in the manure i.e., excreted with manure or fortified manure, or (3) management of composting (static or turned), (4) and time of composting (Amarakoon et al., 2016; Ray et al., 2017). Tylosin degradation was small in a 3-day composting operation (Ray et al., 2017), but degradation increased from 85 to 99% in a 30-day static composting (Amarakoon et al., 2016). In some instances, ARGs can increase in concentration during thermophilic compost (compost introduced with heat-loving bacteria) (Xie et al., 2016). However, it should be noted that composting is not seen as a method to completely remove antibiotics from manure (Dolliver and Gupta, 2008; Ray et al., 2017). In terms of applying the manure three application methods are used: broadcast, incorporation, and injection. Incorporation, usually through a chisel plow, and injection have been shown to most effectively reduce the amount of antibiotics found in surface runoff water and surface sediment runoff compared to broadcasting (Joy et al., 2013; Le et al., 2018). This is likely due to increased soil porosity due to tillage and the ability for farmers to incorporate the manure farther down in the soil profile, limiting antibiotic loss due to runoff (Dolliver and Gupta, 2008). Even in studies where broadcast, incorporation, and injection applications were used, it had little significance on the concentrations of antibiotic loss in leachates and leaching losses; however, broadcast applications still yielded higher total antibiotic loss than for incorporation and injection applications in runoff (Dolliver and Gupta 2008; Joy et al., 2013).

1.4 Antibiotics of Emerging Concern

The three antibiotics used for this study, lincomycin, monensin, and tylosin, were selected for their chemical properties, behavior in the environment, and widespread use in livestock production. These antibiotics vary in size, hydrophobicity, charge, and mechanisms of sorption to soil and organic matter surfaces. In our soil pH of 6.48 these compounds are expected to act as follows: lincomycin as cationic because of its pKa of 7.6 and with hydrophilic properties will move within the aqueous phase in the soil, monensin as anionic due to its pKa of 4.2 and with being very hydrophobic we expect it to sorb to organic matter, and tylosin as cationic with a pKa (7.7) similar to lincomycin; however, due to its slight hydrophobic properties (K_{ow}=1.6) but very high water solubility (5000 mg L^{-1}) it may be mobile in the soil. Lincomycin is the smallest compound of the three with a molecular weight of 406.5 g mol^{-1}, followed by monensin at 670.9 g mol^{-1} and the largest being tylosin at 916.1 g mol^{-1}. Solubility for each compound are 3.0 x 10^{-3}, 927, and 5000 mg L^{-1} for monensin, lincomycin, and tylosin, respectively. In addition to tylosin having the largest solubility in water, it is the only polar compound of the three (see Table 2.1 in Materials and Methods for full chemical properties).

1.4.1 Lincomycin

Lincomycin, discovered in the 1950s from the actinomycete *Streptomyces lincolnensis* is a lincosamide antibimicrobial, narrow spectrum compound used only against Gram-positive bacteria (MacLoed et al., 1964). It is used to prevent and control post-weaning diarrhea in weanling pigs (Kutcha and Cessna, 2009a) and common infections in other livestock (Pyörälä et al., 2014). The mode of action in animals is by hindering the microbial protein synthesis and activation processes within Gram-positive cocci (Spizek and Rezanka, 2017).

1.4.1.1 Chemical and Physical Properties

The structure of lincomycin consists of a moiety of the proline amino acid linked via an amide with a sulfur-containing octose (Table 1) (Spizek and Rezanka, 2017; Wang et al., 2012). The proline moiety and the amide in the lincomycin are weak bases that can accept a proton (Williams et al., 2014). The reported pKa value (7.6) of lincomycin suggests that this antibiotic is the positively charged under most environmental conditions (Wang et al., 2012). The positively charged molecule of lincomycin can sorb on clay surfaces at pH 3.2- 7, but the sorption decreases at pH values higher than 7 (Wang et al., 2009) as suggested by the sorption coefficient values (K_d), K_d > 2000 at pH 3.2- 7 and <550 at pH 7.5- 8.5 (Wang et al., 2009). Lincomycin is slightly water soluble (927 mg L^{-1} at 25°C) and has a small log K_{ow} (0.2) (U.S. EPA 2010).

1.4.1.2 Lincomycin Usage in Livestock

Lincomycin is most commonly mixed into the feed of livestock, but in swine it can be injected intramuscularly at 1mL solution per 10 kg of body weight for up to seven days (North American Compendiums, 2001). Lincomycin recovered in swine manure from total administration can be anywhere from 1.2% to 32% (Kutcha and Cessna, 2009a, b). However, up to 80% of lincomycin orally administered lincomycin to swine could be excreted in uncharacterized metabolites of lincomycin (Hornish et al., 1987). Lincomycin can be stable in liquid manure and dissipate slowly. Therefore loss of lincomycin mass into animal waste can be partially contributed by metabolic loss within the animal (Kutcha and Cessna, 2009b).

1.4.1.3 Lincomycin in the Environment

Lincomycin is often found in the environment in both water and soil. Li et al. (2013) found lincomycin in all water and soil samples taken from sources near a swine production farm; water samples ranged from 0.018 to 9.29 ng ml^{-1} and soil samples ranging from 0.025 to 0.97 ng ml^{-1}. In a greenhouse study done by Dominguez et al. (2014) a similar outcome occurred where lincomycin was found in most leachate samples with swine-slurry amended soil at concentrations ranging from 28 to 30 ng ml^{-1} however no lincomycin was found in the soil possibly due to irreversible binding to the soil inorganic particles or organic matter. Conversely, Kutcha and Cessna (2009c) found lincomycin in all soil samples injected with swine manure in the upper 5cm ranging from 46.3 to 117 µg L^{-1}. Lincomycin has shown to be recalcitrant in the environment as it has been found in water samples from wetlands and closed basin depressions filled by snowmelt runoff the spring after a fall application of swine manure to agriculture fields (Kutcha and Cessna, 2009b). In this study, neither the wetlands or closed basin depressions had manure applied to them, suggesting that lincomycin moves down and through soil profiles and, eventually, into flowing waterways. However, the concentration of lincomycin decreases with time; under rainfall simulations to fields with manure fall-applied, the concentration of lincomycin in the fall was 12 times higher (1.2 µg L^{-1}) than in the following spring.

1.4.2 Monensin

Monensin, a weak acid, is characterized as the anionic antibiotic under the conditions of our study due to the soil pH (6.5) being higher than its pKa of 4.2 but would be a neutral compound in pH environments less than 4.2. Monensin is under the ionophore family of antibiotics and is used as an antibiotic and a growth promoter in cattle (Lowicki and Huczyski, 2013) and was the first ionophore to be approved by the U.S. Food and Drug Administration

(FDA). To date over 100 ionophore antibiotics have been discovered (Pressman and Fahlm, 1982); however there are only seven derivatives that are approved for commercial use, including monensin (Novilla et al., 2017). Unlike lincomycin and tylosin that are approved for human and livestock treatment monensin is only approved for use in livestock (US General Accounting Office, 1999).

1.4.2.1 Chemical and Physical Properties

Ionophores are characterized as a "class of compounds making complexes with cations and then transporting it as a lipid-soluble complex across (a) lipid bilayer" (Łowicki and Huczyński, 2013). Monensin is a weak organic acid with a pKa value of 4.2 and a molecular weight of 670.9 g/mol, making it anionic under the conditions used in this study (pH = 6.5). Of the three compounds, monensin has the lowest aqueous solubility (3.0×10^{-3} mg L^{-1}) and the largest log K_{ow} value of 5.4 (see Table 1). Monensin is typically in a "pseudocyclic conformation due to the presence of bifurcated intramolecular hydrogen bonds formed between carboxyl group on one side of the molecule and two hydroxyl groups on the opposite side" (Łowicki and Huczyński, 2013). Monensin is structurally comprised of eleven oxygen atoms, which six oxygens are concentrated in the pseudocyclic ring and spread across the outer edges are alkyl groups allowing monensin to pass through biological membranes (Łowicki and Huczyński, 2013; Mollenhauer et al., 1990). This structure allows for the complexation of cations that include Mg^{2+}, Ca^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, Mn^{2+}, and Ni^{2+} (Łowicki and Huczyński, 2013). Monensin works by blocking intracellular transport of Golgi apparatus proteins thus affecting the growth of selected cells, inhibiting the transfer of products produced within the Golgi apparatus, and also alters the sequence of formations and their growth on external cell structures (Łowicki and Huczyński, 2013; Mollenhauer et al. 1990).

1.4.2.2 Monensin Usage in Livestock

Monensin is widely used in dairy cows and beef cattle (Song et al., 2010) and is used to prevent coccidiosis, as well as a growth promoter and aid in increasing milk production (Dungan et al, 2017). According to Watanabe et al. (2008) monensin by weight is the most used antibiotic used in dairy operations and it accounts for roughly 13% of total sub-therapeutic antibiotic usage in livestock in the United States (Dolliver et al., 2008). Dosage varies by the operation; however one recommended dosage of monensin in a study was 300 mg per animal per day, with a majority of the antibiotic passing through and found at 86.9 μg kg^{-1} in the surrounding soils (Netthisinghe 2018).

1.4.2.3 Monensin in Previous Studies

Monensin has been found often in the environment, whether in soil or water. Although monensin is frequently detected in soil, usually low concentrations are observed for this compound. Monensin has been reported to have a K_{oc} larger than 1000 (Sassman and Lee, 2007), thus soils with high organic carbon may retain this compound. Netthisinghe et al. (2013) frequently recovered monensin in a feedlot that was adjacent to the application site, and sites further away from the application site showed less frequent recovery. Song et al. (2010) reported frequent detection of amprolium and monensin in manure-amended fields in both spring and fall samples with average monensin recoveries of 0.095 μg L^{-1} in the spring and 0.01 μg L^{-1} in the fall. Monensin has been found in a variety of waterways including rivers and creeks (Kurwadkar et al., 2013; Dungan et al., 2017), stagnant water (Dolliver and Gupta, 2008; Song et al., 2010), and irrigation returns from livestock wastewater (Dungan et al., 2017). Monensin was the most commonly detected antibiotic in an Idaho watershed, being reported 23 times during the sampling campaign in waterways and at all eight irrigation return sites (Dungan et al., 2017).

Song et al. (2010) found monensin at concentrations from 0.005 to 0.189 μg L^{-1} in stagnant water samples but found lower concentrations in drainage-water presumably due to dilution through the drainage tile system. Dolliver and Gupta (2008) fall-applied beef manure with monensin concentrations of 242 to 922 g ha^{-1} to cropland and reported monensin in 8% of the leachate samples, with the highest concentration reported at 40.9 μg L^{-1} and in 20% of the runoff samples with the highest concentration at 57.5 μg L^{-1}, all during the non-growing season. Although often detected in water samples, in proportion to the initial mass of monensin applied recovery is often low, e.g. from <0.08 to 2% in runoff (Davis et al., 2006; Dolliver and Gupta, 2008) and <0.005% in leachate from water percolation (Dolliver and Gupta, 2008).

1.4.3 Tylosin

Tylosin is a macrolide antibiotic representing a mixture of four tylosin derivatives: tylosin A, B, C, and D and (Lewicki et al., 2009; Hu and Coats 2009). Tylosin, introduced to the market in 1961 (Arsic et al., 2018), is used in livestock to treat mycoplasma and respiratory infections (Ishikawa et al., 2018), as well as treat liver abscesses caused by *Fusobacterium necrophorum* and *Arcanobacterium pyogenes* (US FDA, 2018).

1.4.3.1 Chemical and Physical Properties

Tylosin, synthetized by the soil microorganism *Streptomyces fradiae* (Lewicki et al., 2009; Hu and Coats 2009), is formed from four structurally similar components: tylosin A, tylosin B, tylosin C, and tylosin D with tylosin A being the main component used in treatments, usually 80% of the tylosin mixture (Arsic et al., 2018; Lewicki et al., 2009). Tylosin is a large molecule with the main structure of Tylosin A consisting of a 16-membered polyketide macrocyclic ring called tylactone (Schonfeld et al., 2012), and "with an amino sugar

(mycaminose) attached to the lactone ring at position 5 via a β-glycosidic linkage" (Sassman et al., 2007). Tylosin is a weak base with reported pKa values ranging from 7.1 to 7.73 (Kan and Petz, 2000; Wollenberger et al., 2000; Qiang and Adams, 2004; McFarland et al., 1997); thus, at pH below its pKa, tylosin is positively charged in the N of the aminosugar moiety. The log K_{ow} value of 1.6 and water solubility value of 5000 mg L^{-1} indicates tylosin has polar properties but is more likely to partition to octanol than water (Zhang et al., 2016). Tylosin works as an antibiotic by binding to the 23S rRNA of the bacterial ribosomal 50S subunit, inhibiting protein synthesis (Hamill et al., 1961), allowing tylosin to be active against Gram-positive bacteria, select Gram-negative bacteria, and mycoplasma (Lewicki et al., 2009).

1.4.3.2 Tylosin Usage In Livestock

Macrolides in general are first in line to treat bovine respiratory disease (Zaheer et al., 2013), along with being used as a growth promoter (Song et al., 2010). Tylosin is mostly used to treat chronic respiratory diseases for poultry, cattle, and swine (Lewicki et al., 2009). In a United States survey, 84% of US feedlots disclosed that about 42% of cattle received tylosin in their feed, and over two-thirds of the cattle were administered with injectable macrolides (USDA, 1999). Tylosin is usually administered via feed, and according to the FDA in 2018 farmers administering tylosin via feed are to receive no less than 60mg and no more than 90mg per head per day to cattle to ensure proper treatment.

1.4.3.3 Tylosin in Previous Studies

Tylosin has been shown to have a greater propensity to bind to soil and manure than be found in solution. For example, in one leachate study from manure beef plots, 1.0 μg L^{-1} of tylosin was recovered versus 40.9 μg L^{-1} for monensin in the same plots (Dolliver and Gupta,

2008); however initial concentrations of monensin were higher (242 to 922 g ha^{-1}) than tylosin (7-15 g ha^{-1}). The same study found a similar pattern with surface runoff, finding about 30 times more monensin (57.5 μg L^{-1}) than tylosin. Mode of application of tylosin in manure to soils is important when discussing recovery of this compound. Surface application tends to lead to higher recoveries in water samples relative to subsurface injection. Le et al. (2018) found that when antibiotic-spiked manure was injected into the soil subsurface, tylosin recovery was 13 times lower on Day 0 in surface runoff versus surface application, and also 28 times lower on Day 7 in surface runoff. The same study found the same pattern for surface runoff sediment and significantly more so, with a tylosin reduction of 46 times in the subsurface application relative to surface application. Field management can also impact the movement of tylosin through a soil profile; Dolliver and Gupta (2008) reported higher concentrations of tylosin in no-till plots relative to chisel tillage due to the increased presence of macropores in the no-till plots. Whether tylosin was applied within manure or not is also a driving factor in how it persists in soil. The half-life of tylosin is shorter when in manure-amended conditions versus no manure (Carlson and Mabury, 2006), with manure-amended conditions saw a tylosin half-life of 4.5 days and non-manure conditions 6.1 days. This is most likely due to the presence of microbial activity in the manure relative to the soil alone (Carlson and Mabury 2006); however it should be noted that low concentrations of tylosin can persist for long periods of time in manure once they reach a "stable" phase and can later be released into the environment (Chen et al., 2018).

1.5 Biochar

1.5.1 Origin of Biochar

The term biochar is a more recent management practice in the United States, however its origins can be traced back to in the Amazon pre-Columbian era when biochar was used as a soil

additive to incorporate nutrients into the dystrophic Terra Preta soils (Glaser et al., 2002). This addition of biochar allowed for total carbon storage to be as high as 250 Mg C ha^{-1} relative to the typical values of around 100 Mg C ha^{-1} for Amazonian soils of the same parent material (Glaser et al. 2001). This stability of carbon comes from the polycyclic aromatic structure of biochar, which renders a recalcitrant character and thus persistence in the environment (Glaser et al. 2001). This stability is thought to have stemmed from the low-temperature smoldering of domestic fires by natives rather than slash-and-burn techniques that deposited little black carbon to the soil (Glaser et al. 2001, 2002) and would degrade quickly in the Amazonian temperatures and humidity. The structure of biochar provides a high amount of exchange sites often leading to an increase of CEC in a soil; the high porosity and surface area in biochar "can potentially stabilize other sources of organic carbon in soil through adsorption processes" (Hernandez-Soriano et al. 2015). Biochar neutralized the acidic nature of the Amazonian soils through the incorporation of ash, while improving soil properties (Lehmann et al. 2006).

1.5.2 Chemical and Physical Properties

1.5.2.1 Physical Properties

Biochar physical properties include high surface area and porosity. Production temperature and the source of biomass are important to the structure and functionality of biochar for mitigation of various organic contaminants (Tan et al., 2015; Tag et al., 2016). The optimal temperature range of pyrolysis to achieve high surface area and low ash content is 400-550°C (Lehmann 2007) but from 100°C to 700°C (Chen et al., 2008) pyrolysis temperatures have been used to produce biochar. Typically, biochars produced at less than 400°C have lower porosity, pore volume, CEC, pH, and surface area; therefore, it is not suitable media to improve soil fertility (Lehmann 2007; Ahmed et al., 2016). As pyrolysis temperature increases, the above

attributes will increase up to a point until ash produced from the pyrolysis increases (Ahmed et al., 2016; Tag et al., 2016). The ash potentially fills or blocks pores, preventing access of adsorbates and therefore decreasing the surface area (Tag et al., 2016). Also, higher temperatures increase the oxidation-resistant carbon in biochars therefore increasing the recalcitrance of biochar (Tag et al., 2016). Biochars produced at lower temperatures have more active sites and stable carbon-oxygen complexes (Kumar et al., 2011) which makes the biochar more suitable for soil amendment. In addition to pyrolysis temperatures, the speed of pyrolysis influence the type of biochar produced. Slow pyrolysis (<10 °Cs-1) is used most often rather than fast (>10 °Cs-1) (Bridgwater et al., 1999). as slow pyrolysis produces higher biochar yields and the biomass pretreatment cost is 50% that of fast pyrolysis (Lehmann and Joseph 2009).

Surface area and porosity are critical attributes in the biochar ability to sorb and store organic compounds. During pyrolysis, the emission of volatile gases created within the "combustion zone" surfaces of cellulose, hemicellulose, and lignin generate pore structures (Kim at al., 2010; Ahmad et al., 2012); thus, increasing surface area. Surface area, determined by BET, varies depending on biomass source; surface area has been reported as low as 1.2 m^2 g^{-1} for *citrus aurantium* (orange) pomace (Tag et al., 2016) to high for wood biomass at 317 m^2 g^{-1} (Brewer et al., 2014). Pore size distribution and structure characterization are difficult to quantify as pore size ranges "over at least five orders of magnitude, from sub-nanometer slit-shaped pores…. to pores on the order of tens of micrometers" (Brewer et al., 2014). Pore sizes greater than 50 μm are accountable for most of the biochar's porosity (Brewer et al., 2014).

Many materials have been used as a source for biochar including animal feedings, crop residues, agricultural materials, food wastes, woody materials, animal litters, and solid wastes (Mitchell et al., 2015; Ahmed et al., 2016). Softwood and grass biochars have the most suitable

physicochemical properties for biochar (increased porosity, surface area, cation exchange capacity) with food waste and agricultural materials also seen as ideal (Ahmed et al., 2016; Brewer et al., 2014; Song et al., 2019). These feedstock sources have high H:C and O:C ratios, leading to increased carboxyl, carbonyl, and oxygenated groups which are ideal for sorption of organic compounds (Song et al., 2019). Solid wastes can have higher mean ash and nitrogen content, especially as temperature increases, leading to a decrease in pore availability for organic compounds (Ahmad et al., 2014; Ahmed et al., 2016).

1.5.2.2 Chemical Properties

Biochar chemical properties are characterized by cation exchange capacity (CEC), hydrophobicity, nutrient holding capacity, and recalcitrant molecular structure. The chemical structure of biochar is dominated by stable condensed aromatic structures, along with more easily degradable aliphatic and oxidized carbon structures (Schmidt and Noack 2000; Preston and Schmidt 2006). Because of this, biochar is one of the most recalcitrant forms of pyrolyzed carbon, having the ability to resist chemical oxidation along with both microbial and physical breakdown in a soil environments for long periods of time (Lehmann 2007; Schmidt and Noack, 2000; Tag et al., 2016). Pyrolysis yields a reduction in oxygen functioning groups even at low temperatures thus decreasing polar groups on the surface of biochars, which in turn increases the hydrophobic character to biochar, a key recalcitrance characteristic in environmental decay (Chen et al., 2008; Zimmerman 2010). Furthermore, biochar exists as a particulate form in soils, which further allows it to be physically protected by minerals and within aggregates (Brodowski et al., 2006; Lehmann 2007). However biochar stability can varies with the source of biomass, production temperature, and the speed of pyrolysis (Baldock and Skjemstad 2000; Harvey et al., 2012; Schmidt and Noack, 2000; Singh et al., 2010; Tag et al., 2016) along with environmental

factors such as photochemical abiotic oxidation and microbial decomposition (Goldberg, 1985). Depending on pyrolysis temperatures, biochars can have variable quantities of labile C, which contain mainly of aliphatic compounds and O-functioning groups that can increase degradation (Harvey et al., 2012; Singh et al., 2016). An increase in pyrolysis temperature can lead to a decrease in hydrogen and oxygen content, indicated as the H/C and the O/C ratios (Ahmed et al., 2016, Tag et al., 2016). The H/C ratio identifies aromaticity in biochars, or their ability to remain stable in soil environments (Tag et al., 2016) while the O/C ratios indicates the presence of hydroxyl, carboxylate, and carbonyl groups which lead to higher cation exchange capacities (Ahmed et al., 2016; Tan et al., 2015). However, CEC of freshly produced biochars is lower than aged biochars in soil environments (Cheng et al., 2006; Lehmann 2007; Cheng et al., 2008;). The CEC is seen as the dominant mechanism of biochars to organic compounds (Inyang et al., 2014); however, other adsorption mechanisms usually contribute such as π- π electron donor-acceptor interactions, pore-filling, and hydrophobic effects seen with atrazine in swine manure- and oak (*Quercus*)- derived biochars (Tan et al., 2015; Penn et al., 2018). These latter mechanisms usually occur at pH conditions above or below a compounds' pKa value when cation exchange capacity cannot be the driving sorption mechanism (Liu et al., 2016).

1.5.3 Mitigation Use of Biochar

The use of biochar to mitigate organic and inorganic compounds within soil matrices is complex since it depends on the biochar characteristics, the environmental conditions, and chemical properties of the compounds. The sorption of sulfamethoxazole (an antibiotic) by biochar was influenced by water pH (up to 83.6% of the total) and pyrolysis temperature (higher at 600°C than at 500°C) (Zheng et al., 2013). Animal manure containing antibiotics is used to produce biochar. Soil incorporation of pyrolyzed (400-450°C) composted swine manure

destroyed most of the antibiotics within the manure, reducing the antibiotic resistant genes (ARGs) and mobile genetic elements (MGEs) introduced to the soil (Zhou et al., 2019). In rainwater leachate, broiler-litter derived biochar reduced Cu concentrations while pecan shell-derived steam-activated carbon was most effective to remove Ni and Cd (Uchimiya et al., 2010). In a canal bank soil heavily contaminated with Cd and Zn, hardwood-derived biochar significant reduced Zn and Cd concentrations after a 60-days (Beesley et al., 2010). In soils amended with woodchip- derived biochar, Zn concentrations were reduced by 54% (Netthisinghe et al., 2018) On a sandy ultisol soil, biochar effectively reduced the negative effects of aluminum toxicity and may have also contributed to manganese (Mn) detoxification in an oxisol "due to retention of Mn on DOM (dissolved organic matter) molecules through chelation" (Buntan et al., 2015).

1.6 Sorption of Antibiotics to Biochar

1.6.1 Biochar and Lincomycin

Information on lincomycin sorption by biochars is limited. Liu et al. (2016) reported that the sorption of lincomycin by manure-derived biochar was two-phased; first, a rapid sorption (external sorption of surface sites), then a slow process (slow pore diffusion mechanism). From 24 to 34% of lincomycin was removed within two days and up to 99% after 180 days. Additionally, these authors reported as biochar particle size decreased, sorption of lincomycin decreased suggesting that with smaller biochar size particles, the pore diffusion mechanism is less important and/or the higher surface plays a key role on the sorption of lincomycin. In addition, pH of the solution is determinant on the extent of lincomycin sorption; higher lincomycin sorption by biochar is observed at pH levels lower than lincomycin pKa (7.6), indicating that a electrostatic attraction mechanism between the negatively charged biochar and positively charged lincomycin (Tan et al., 2015; Liu et al., 2016).

1.6.2 Biochar and Monensin

There is lack of information on the sorption to monensin by biochar. Netthisinghe et al. (2018) found that when biochar was added as an amendment to soil in a field study, the soil monensin level decreased (2.1 to 1.1 ng g^{-1}) suggesting sorption of monensin to the surface of biochar. Further discussion of monensin and its relationship with soil organic matter will continue in the "Soil and Monensin" section of this review.

1.6.3 Biochar and Tylosin

Tylosin has a strong capacity to bind with biochar due in part to its ability to bind to organic matter through different mechanisms; however, these mechanisms are pH- dependent (Jeong et al., 2012). The tylosin molecule has a macrolide ring which is an effective π-electron acceptor because of its electron-withdrawing amidogen group; add the heterocyclic group that contains oxygen, it decreases its π-electron density and increases electron negativity resulting in a substituted macrolide ring and aromatic heterocyclic group (Chen et al., 2015). This allows an additional π-π EDA interaction with the carbon-rich biochars in alkaline conditions; however, in environments where the pH is high, at or above its pKa of 7.7, this weak interaction of cation-π bonding or π-π stacking means a decrease in tylosin sorption to biochar (Guo et al., 2016b). At pH below 7, tylosin is positively charged and sorbs either by H-bonding, cation exchange, or both (Guo et al., 2016a; Guo et al., 2016b). Tylosin contains moieties that are hydrogen acceptors or donors allowing for the hydrogen bonding (Guo et al., 2016a). Tylosin sorption by cation exchange was demonstrated when K^+ concentration increased, while the tylosin sorption by black carbon decreased, indicating the competition for exchange sites by K^+ and tylosin (Guo et al., 2016b). Tylosin also exhibits fast and slow sorption mechanisms; in a study biochars,

tylosin sorption was fast by biochar-amended soils and then continued the slow sorption for 240 hours (Jeong et al., 2012).

1.7 Sorption of Antibiotics to Soil

1.7.1 Overview

Sorption of antibiotics to soil is highly dependent on the chemical properties of the antibiotics themselves and the physicochemical properties of soil (Wang and Wang, 2015). The pH has a significant effect on sorption to soils along with soil organic matter and soil minerals/clay content (Holten Lützhøft et al., 2000; Thiele-Bruhn, 2003; Bewick, 1979). The octanol/water coefficients of ionizing compounds will change depending upon the soil solution pH around the compound's pKa (Holten Lützhøft et al., 2000) and therefore determining if antibiotics sorb by electrostatic forces (Sassman et al., 2007; Zhang et al., 2011; Wang et al., 2012) or other mechanisms such as hydrogen bonding (Tolls, 2001) and van der Waals forces (Zhang et al., 2011).

1.7.2 Lincomycin Sorption to Soils

Lincomycin sorption to soil is dominated by cation exchange in pH solutions below or near its pKa of 7.6 as it becomes cationic in nature (Wang et al., 2012). This was further proven by Wang et al. (2009) when lincomycin was able to displace K^+ on clay surfaces. However, at lower pH, negative charged moieties in soil organic matter (SOM) becomes protonated into neutral species and therefore reducing the amount of negatively charged sites that lincomycin could sorb to with electrostatic interactions (Wang et al., 2012). Lincomycin tends to exists more in the aqueous phase (Kutcha and Cessna, 2009b) than in soil; however, the low recoveries of lincomycin from soil extractions may be due to an irreversible bond to soil surfaces (clay

minerals and organic matter) as seen in Dominguez et al. (2014): only 30% of applied lincomycin was recovered in soil with a clay content of 2% and low organic matter. The extent of lincomycin sorption to clay minerals is influenced by the clay type; smectites (2:1 expandable clays) sorb more lincomycin than the 1:1 non-expandable kaolinites and SOM (Want et al., 2009; Wang et al., 2012). Soils with higher content of smectites will have the highest sorption of lincomycin.

1.7.3 Monensin Sorption to Soils

Monensin, with a log K_{ow} value of 5.4, is practically insoluble in water and therefore adsorbs to soil and sediment particles (Kurwadkar et al., 2013). In a column study by, monensin was not detected in any effluent and only found in pore water samples in the upper portions of the column, suggesting that monensin has a strong affinity to soil and has low mobility (D'Alessio et al., 2019). Similar trends are reported in other agriculture soils (Carlson and Mabury, 2006; Sassman and Lee, 2007), and can be explained by the hydrophobicity of monensin. However, monensin can easily partition from soil particles during rainfall events and end up in surface runoff (Song et al., 2010; Sun et al., 2013). This phenomenon is likely due to the facilitate transport of monensin by the dissolved organic carbon from; the facilitated transport of organic compounds by dissolved organic carbon had been observed (Chiou et al., 1986; Lissemore et al., 2006). Unlike lincomycin, there are no definitive sorption mechanisms of monensin to soil but likely mechanisms include hydrophobic partitioning, cation exchange, and hydrogen bonding (Tolls, 2001, Watanabe et al., 2008).

1.7.4 Tylosin Sorption to Soils

Tylosin is a large, polar, water soluble, weakly base (pKb = 7.1) molecule; due to its size tylosin is not able to sorb into micropores within soil and cannot easily enter smectite interlayers making the dominant sorption mechanisms cation exchange and hydrophobic bonding on soil surfaces (Sassman et al., 2007; Zhang et al., 2011; Wegst-Uhrich et al., 2014). Zhang et al. (2011) observed that there was correlation between tylosin distribution coefficient and soil properties, further indicating cation exchange and van der Waals forces were the mechanisms for soil sorption. At pH less than its pKb, tylosin becomes positively charged and therefore strongly attracted to the negatively charged sites on clays (Tolls 2001; Zhang et al., 2011). Tylosin can irreversibly bind to soil, as observed by the little to no desorption hysteresis that occurs in isotherms, leading to speculation that the covalent bonds between tylosin and the negative sites on clays are less prevalent than the hydrophobic interactions and ion exchange (Zhang et al., 2011). Further, extraction of tylosin from soils using methanol proved to yield high recovery proving hydrophobic forces at play (Sassman et al., 2007). Field studies have shown that the higher the organic matter and clay content present in soil, the less tylosin is able to be recovered in both leachate and soil samples due to the high affinity for tylosin to adsorb to organic matter (Sassman et al., 2007; Zhou 2008; Hu and Coats, 2009; Jeong et al., 2012). If tylosin is recovered in water samples, it will more likely be due to facilitated transport by soil and manure during an erosion event (Hu and Coats, 2009).

1.8 Half-Life of Antibiotics

1.8.1 Lincomycin

Lincomycin is highly persistent in the environment depending on field and weather conditions. Kutcha and Cessna (2009b) reported little to no degradation of lincomycin in soil and

snow over the winter in Canada and subsequently was detected in surface water samples produced by snowmelt and rainfall simulations on those sites. Ephemeral wetlands that collect said snowmelt water runoff then reported a half-life of 30 days for lincomycin. Lincomycin has also been shown to persist in manure storage piles, rapidly decaying at first and then slowly decaying from there, still being present after five months of storage time (Kutcha and Cessna, 2009a). However, degradation in the soil is shown to be faster with and without manure amendment. Kutcha et al. (2009) observed first-order dissipation and half-lives of 19.4 in 2004 and 17.4 in 2005 in manure-amended cropland. In soils not amended with manure half-lives were reported as 3.4, 3.5, and 3.7 days for loam, sandy loam, and sandy soils, respectively. Microbial changes can also affect degradation, showed by more rapid mass recovery loss through the column versus anoxic and aerobic soil conditions (D'Alessio et al., 2019)

1.8.2 Monensin

Monensin has been shown to have relatively short half-lives in both the environment and in the lab. Monensin has not been seen to persist in the environment for longer than a month with or without manure amendment (Donaho, 1984), with the longest half-life being observed by Watanabe et al. (2008) at 23 days in a field amended with manure. Half-lives have been reported as low as two days (Sassman and Lee, 2007) in a laboratory setting with and without manure application. The same study observed that even at varying organic matter content (1.7-4.4%) with no manure addition, monensin half-lives were still two days. Similar results were seen in a study by Carlson and Mabury (2006) where half-lives in a field were reported to be 3.3 and 3.8 days with and without manure application, respectively. Laboratory degradation was also observed in this study, reporting a half-life of 13.5 days in manure-amended soil. Aerobic activity is seen to be a factor in monensin degradation (Watanabe et al., 2008) along with abiotic

factors (Sassman and Lee, 2007; Yoshida et al., 2010) as observed by Yoshida et al. (2010) where a 150% increase of organic matter in soil yielded a 5.5 fold decrease in half-life and a 20% increase in soil water content saw an increase of 33% in degradation time.

1.8.3 Tylosin

Tylosin has also been shown to have a relatively short half-life depending on its environment, though not as short as monensin. Tylosin has had a reported half-life of up to 46 days in a manure-amended loam soil (Halling-Sørensen et al., 2005) however, other half-lives reported in the literature show more rapid degradation. Carlson and Mabury (2006) observed in laboratory studies tylosin half-life was 4.4 days with more than 80% degraded after 17 days and in field studies reported half-lives as 4.5 and 6.1 days in manure-amended and non-amended soils, respectively. Similar values were seen with Halling-Sørensen et al. (2005) with raw manure amendment in laboratory studies reported a half-life of 3.7 days within phase one (3-29 days) of their study. Hu and Coats (2007) measured the half-life of tylosin in sterile and non-sterile soil finding there were no significant degradation patterns between the two soils, resulting in reported half-lives of 7 days, suggesting that tylosin degrades primarily by abiotic transformation. Halling-Sørensen et al. (2005) observed that tylosin degradation was the same under anaerobic and aerobic conditions, further confirming abiotic transformations driving degradation. Lastly, tylosin has been observed not to translocate to lower depths within a soil profile as reported by Carlson and Mabury (2006) where they did not detect tylosin in the 25-35cm depth of their field samples suggesting that tylosin will degrade before reaching these lower depths and subsequently groundwater.

1.9 Groundwater Contamination

Groundwater contamination of antibiotics from applications of manure is a threat to water quality near agricultural fields and can persist for long periods, as seen by Kivits et al. (2018) where ground water samples found traces of sulfamethoxazole and sulfamethazine that were up to 40 years old. For monensin, it was found in groundwater samples that were recharged by lagoon water near a dairy production farm in California with concentrations being lower than that found in lagoon water possibly due to anoxic conditions in the groundwater environment (Watanabe et al., 2008). This same study found monensin in 3 of 8 shallow monitoring wells with concentrations ranging from 0.04 to 0.39 ug L^{-1}, however this may be due to direct leakage from surface runoff as observed by the turbidity in the area. Song et al. (2010) found monensin in half of its 109 drainage tile channels and surface ditches water samples at an average concentration of 0.19 μg L^{-1}, providing examples of monensin infiltrating through the soil profile and then draining further into waterways. For lincomycin, Kutcha et al. (2009) found the antibiotic often in groundwater. Lincomycin was found at concentrations <0.005 μg L^{-1} in 25 of 30 samples taken from two different fields. Further, lincomycin was found at higher concentrations (0.036 μg L^{-1}) from ground water samples taken ground water depressions though this is most likely due to preferential flow of snowmelt runoff water.

1.10 Objectives for the Study

The objectives of this research were to (1) quantify the sorption of antibiotics to biochar under erosion conditions, (2) investigate if adding varying rates of biochar to the soil reduced mobilization of antibiotics into the environment by surface runoff and drainage and (3) use two different rainfall intensities of 50 mm/hr and 100 mm/hr to represent “normal” and “worst case

scenario" rainfall events in the U.S. Midwest, respectively, and its affects on antibiotic and biochar runoff losses.

CHAPTER 2. MATERIALS AND METHODS

2.1 Chemicals

Lincomycin (lincomycin hydrochloride), monensin (monensin sodium salt hydrate), and tylosin (tylosin tartrate) were all purchased from Sigma-Alrich (St. Louis, MO, USA). All solvents used in this experiment, unless specified, were Optima® grade including water, methanol, acetonitrile, and formic acid (Fisher Scientific, Waltham MA, USA). Stock solutions of lincomycin and tylosin (2 mg ml $^{-1}$ of each antibiotic) were prepared in Optima grade water and monensin in Optima grade methanol. Distilled water used in the laboratory was prepared using the Thermo Scientific Barnstead Nanopure filtration system (Waltham, MA, USA).

2.2 Biochar

Biochar was kindly provided by Dr. Kevin Gibson (Purdue University), who purchased the biochar from Diacarbon Energy Inc. (Burnaby, Canada). It was produced by slow pyrolysis at 450 °C from a mixture of softwoods including spruce (*Picea*), pine (*Pinus*), and fir (*Abies*). Biochar was sieved to 4mm, with particle sizes of fine dust to 4mm being used for rainfall simulations. Carbon content was analyzed at 55 ± 1.52% and nitrogen at 0.17 ± 0.08%. Surface area was determined to be 123 m^2 g^{-1} using BET (Micromeritics Instrument Corp., Georgia, USA) provided from Dr. Cliff Johnston of Purdue University. The BET was a 3 Flex Surface Characterization Analyzer version 4.05.

2.3 Soil

The soil, a Chalmers silty clay loam (Fine silty, mixed, superactive, mesic Typic Endoaquoll) was collected from United States Department of Agriculture, Agriculture Research

Service (USDA-ARS) plots located at the Purdue Agronomy Research Center for Education (ACRE) in West Lafayette, IN from a fallow plot following soybean harvest that was under corn-soybean rotation under no-till for 12 years. Some properties of the soil are listed in Table 2. About one cubic meter of the top 15 cm soil layer was collected using a backhoe in November of 2015. Soil was allowed to air dry inside the laboratory and was ground through a Royer large-quantity grinder (Oshkosh, WI, USA); the ground soil was sieved through 5mm sieves by hand. Since biochar is incorporated into the soil, it was not important in this project to keep any large soil aggregate structures and the homogeneity of the ground soil minimized preferential flow due to large-pores.

2.4 Water-Extractable Cations

Water-extractable cations in the soil were determined by shaking 2 grams of soil with 20 mL of Nanopure water on an oscillating shaker for 10 minutes; then syringe-filtered using 0.45 μm nylon filters and analyzed by ion chromatography (IC). The IC consisted of a Reagent-Free™ Dionex ICS-2100 system (ICS) with an IonPac® CS12A 4 x 250mm analytical column and an IonPac® CG12A 4 x 50mm guard column (Fisher Scientific, Waltham MA, USA) for the separation of Ca^{+2}, Mg^{+2}, K^{+}, and NH_4^{+}. Methansulfonic acid was used to separate cations in solution. System flow rate was 1 mL/min, eluent generator of 20 mM, and suppressor type of CSRS 4mm with a current of 59 mA. The ICS-2100 is a system with a run time of 20 minutes for cation analysis. External standards ranging from 0.5 to 20 mg/L were used for quantification. Retention time for potassium was 6.56 minutes, calcium 13.37 minutes, and magnesium 10.39 minutes.

2.5 Soil and Biochar Isotherms

Isotherms were kindly completed by Dr. Cheng-Hua Liu of Michigan State University and his laboratory. For both adsorption isotherms, all three antibiotics were combined into one solution and adjusted to various concentrations ranging from 100 to 1000 μg L^{-1}. Samples were shaken end-over-end at 30 rpm for 24 hours at room temperature. Biochar was sieved to <0.75 μm and the initial pH of the biochar isotherms was 6 with a final pH of 9, ionized with 0.02 M of KCl. Soil was sieved to <2mm and the initial pH of the soil isotherms was 6 with a final pH of about 8, with an electrolyte background of 0.02 M KCl.

2.6 Rainfall Simulations

In this study, a recirculating trough rainfall simulator with nine 80/100 Veejet nozzles (Wheaton, IL, USA) was used to conduct the rainfall simulations to the treatments contained in the stainless steel boxes (100cm x 20xm x 5cm) with a spout at the end and nine nozzles attached underneath. Each box consisted of 2cm of washed gravel sieved to >8mm, landscape fabric (Jobes WeedBlock®**,** Waco, TX), and a 3-cm layer of a treatment packed evenly in 1cm layers. The boxes with the treatments, laid in a metal frame, were three feet high off the ground and positioned at a five-degree angle to best simulate a field slope while also providing enough angle for runoff to occur. To minimize any cross contamination from the raindrop impact, metal sheets were placed between each box and slightly angled inwards so any splashed water could drip back into the box. Each run had three replications of each treatment with each box side by side.

2.7 Rainfall Setup

Antibiotics were sprayed via spray bottle in a 50/50 (v/v) methanol/water solution onto the soil boxes before being pre-wet with the rainfall simulator. Each box had its own 1-liter

solution of 1mg L^{-1} concentration applied to it. The solution, once applied to the box, was allowed to sit for 20 minutes before pre-wetting began. Each box had to be pre-wet to saturate the soil and allow surface runoff. Pre-wetting was done by the rainfall simulator and would take 40 minutes or an hour, with pre-wetting at 50 mm/hr taking one hour and pre-wetting at 100 mm/hr taking 40 minutes. Once water runoff began for each box water and soil/biochar runoff were collected every five minutes over a 45 minute run. In order to capture drainage, Teflon-lined tubes were attached to the metal nozzles located underneath the boxes. These tubes directly drained water drainage to one PDFE three gallon bucket placed approximately 2 feet underneath the box. The buckets were placed high enough off the ground that there was little to no potential of water splashing from the ground into the buckets, and there was little to no space between the boxes for additional rain to fall into the buckets.

2.8 Runoff Collection

Rainfall experiments for both rainfall intensities were run for 45 minutes not including the pre-wetting. Within each five minute sampling period, soil sediment runoff was captured for 3.5 minutes in 1 L plastic bottles while the water runoff was collected in 60ml plastic bottles for the remainder of the five minutes. Water runoff collection could take the rest of the minute and a half or just one minute, depending how much fast the flow was during the run. Usually the first 2 collections, the five and ten minute marks, took closer to 1.5 minutes to fill a bottle, while minutes 15-45 only took one minute to fill a bottle.

2.9 Density Separation of Biochar in Sediment Runoff

In sediment runoff collections, soil and biochar were both present and needed to be separated for analysis. Sediment samples were first vacuum-filtered using a nylon 0.45-micron

filter, then trapped sediment on the filters were covered and allowed to dry overnight. Once dried, sediment was removed from the filters and transferred to 60mL HDPE bottles. Biochar is lighter in density than soil, so by using a density separation technique we can separate all particulate sizes of biochar more effectively from soil versus physical separation (i.e. forceps). Sodium polytungstate (SPT) (Fisher Scientific, Waltham MA, USA) was suspended into a 1.45 g cm^{-3} solution with Nanopure water using Sometu (Germany) manufacturer's instructions. SPT solution was added to each sample, vortexed for 10 seconds, then sit for a minimum of one hour. A pipette was used to extract biochar suspended on the surface of the solution. All biochar removed was vacuum filtered through a glass fiber filter, allowed to dry, then removed from the filter and stored in 30ml amber HDPE bottles at 4°C until ready for analysis. Sediment samples were refiltered again to separate it from the SPT solution via vacuum filtration with 0.45-micron filters, allowed to dry over night, then removed from the filter paper and stored in 30ml amber HDPE bottles at 4°C until ready for analysis.

2.10 Freeze-Dry Protocol

Soil samples acquired from the boxes post-rainfall were processed through a freeze dryer to speed up the drying process with limited light exposure to decrease any chance of compound degradation. Fifty grams of soil sample taken from the treatment boxes after rainfall simulations were placed into a specialized glass beaker, sealed with a fast-freeze flask top, and placed onto rollers within a 100% ethanol bath at -40°C until sample was completely frozen through. Samples were then moved to freeze dryer, vacuum-sealed, and allowed to dry overnight. Once samples were dry they were removed from the dryer and stored at 4°C until ready for analysis.

2.11 Pressurized Solvent Extraction of Soil and Biochar

Soil and biochar samples were extracted using the Dionex Accelerated Solvent Extractor 200 (Sunnyvale, CA, USA) following the method by Chuang et al. (2015). About 1.0 gram of soil or .05 grams of biochar sample were mixed with about 14 grams of sand (Fisher Scientific, Illinois, USA) and placed in 11ml stainless steel cells with glass fiber filters (Thermo Scientific, Illinois, USA) positioned on each end of the cell. The ASE program consisted of five minutes of heating (or reheat), static extraction for 5 minutes, flush volume of 60% and purge with nitrogen for 120 seconds. The temperature was set at 80°C under 1500 psi with two cycles. The solvent mixture consisted of 65:15:20 (v/v/v) acetonitrile: water: methanol; this solvent mixture yields high recoveries (<93%) for the three compounds used in this study (Chuang et al., 2015). The solutions obtained from the ASE were syringe-filtered through a 0.45 micron nylon filter and then stored in the refrigerator in amber glass vials for further analysis.

2.12 Solid Phase Extraction

Solid phase extraction (SPE) was conducted for all runoff and drainage samples using Waters Oasis HLB 6cc (150mg) cartridges. All water samples had to go through solid phase extraction to suspend antibiotics in methanol, as monensin is only soluble in organic solvents and can only be analyzed in its dissolved state; lincomycin and tylosin are slightly soluble in organic solvents. Extraction was carried out following the procedure recommended by the manufacturer: each cartridge was conditioned with 5ml of methanol followed by 5ml water equilibration. Then, 5mL of sample was loaded into the conditioned cartridge and allowed to flow through the cartridge, followed by a wash of 5% methanol in water to remove salts and unwanted compounds. The sorbed antibiotics on the SPE cartridges were eluted with 5mL of methanol in amber bottles and stored at 4°C until further analysis. During the SPE process, all flow rates were set to 1 drop

per second, and every solution/sample was administered in 2.5ml increments, as the cartridges could not hold more than 3ml of solution/sample at a time. Flow rate was set to 1 drop per second, and every sample done in 2.5ml increments as the cartridges could not hold more than 3ml of solution at a time.

2.13 Analysis for Antibiotics

Samples from the water runoff and drainage, and those obtained from the ASE and SPE extraction techniques were analyzed for the antibiotics used in this study by liquid chromatography with mass detection following the method by Chuang et al. (2015) with some modifications. In this study, a Waters Acquity ultra performance liquid chromatography (ULPC) (IVD) system and a Waters Acquity C18 column (2.1mm x 100mm, particle size 1.7µm, Milford, MA, USA) were used to separate the targeted pharmaceuticals using a binary mobile phase consisted of 0.3% formic acid in water (phase A) and 0.3% formic acid in a acetonitrile/methanol (1/1, v/v) mixture (phase B). Isocratic elution (8% phase A) for two minutes with variable flow rate was used as followed: 0.6 ml min^{-1} for 0 to 0.5 min, and 0.8 ml min^{-1} at 0.75 min. A triple quadrupole mass spectrometer detector (MS-MS) (Milford, MA, USA) was used identification and quantification of the compounds using the multiple reaction monitoring (MRM) mode. The MS-MS conditions consisted of Turbo IonSpray source of the mass spectrometer was operated in positive mode with ion spray voltage at 1000 V and temperature at 500°C. Curtain gas pressure was 20 psi, collision gas pressure was 6 psi, and ion source gas pressure was 60 psi. The runoff and drainage water samples were diluted 1:1 with water. Water runoff and drainage samples were already suspended in methanol from SPE and had to be reconstituted with water in order to analyze lincomycin and tylosin. The retention time for lincomycin was 0.42 min, for monensin was 0.89 min, and tylosin was 0.47 min. External standards ranging from .0075 µg L^{-1} to 30 µg

L^{-1} were used to prepare calibration curves. The limit of quantification, using the EPA method (US EPA, 2016), was 0.01 μg L^{-1} for all compounds.

2.14 Sorption Capacity of Materials

Sorption capacity experiments were conducted on materials that would have prolonged contact with antibiotics during the experiment. Sorption capacity experiments were split into two categories: 24 hour and 45 minute adsorb/desorb shakes. We conducted 24-hour sorption experiments to measure the sorption of antibiotics to materials over longer periods of time and the 45-minute sorption experiments to simulate the total time of contact materials would have to antibiotics over the course of the rainfall simulation. See Table 2.3 for list of all materials tested.

Each material had five variables tested: the treatment (antibiotic solution (AS) + material), the control (nanowater + material), treatment control (water + AS), blank (water), and 24-hour treatment (AS + material). The AS stock solution was set to an initial concentration of 100 μg L^{-1} in a 1:1 H2O:MeOH solution. A 1:1 H2O:MeOH solution was needed for monensin to be totally soluble in solution with tylosin and lincomycin. Final concentrations were diluted from the 1:1 H2O:MeOH stock solution for each sorption experiment.

45-Minute Sorption Capacity Experiments

Samples were placed on a lateral shaker and allowed to absorb for 45 minutes. After the allotted time, an aliquot of the solution was taken, syringe filtered through a 0.22 micron filter, and then stored at 4°C until ready for analysis. Any solution left in the bottles after an aliquot was taken was poured out and safely disposed of. A 0.01M calcium chloride solution was then added to the samples for desorption. Samples were allowed to shake for another 45 minutes, then

a second aliquot was taken of the solution, syringe filtered through a 0.22 micron filter, and again stored at 4°C until ready for analysis.

24-Hour Sorption Capacity Experiments

Sorption experiments were conducted exactly as above however samples were placed on a lateral shaker for 24 hours for both adsorption and desorption. A 10ml subsample was taken at the beginning of the 24 hours to confirm the concentration of the solution before beginning isotherms. Samples were shaken in amber bottles to avoid any degradation of antibiotics via light.

2.15 Sequential Filtering

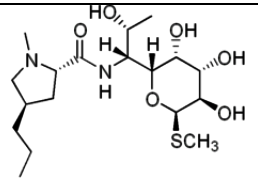
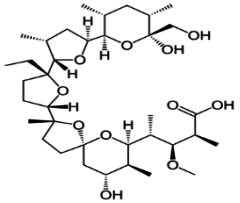
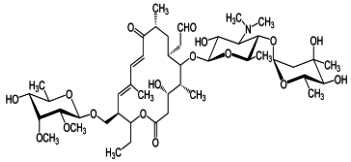
ater runoff and drainage samples were subjected to sequential filtering to determine if antibiotic detection was due to colloidal particles within the water matrix. Samples were syringe filtered through PTFE 0.45, 0.22, and 0.1 μm filters with 25 mm diameters (Sterlitech Corporation Kent, WA, USA) into. Water samples were first filtered through the 0.45 μm filter, then a subsample of what passed through 0.45 was filtered through the 0.22 μm filter and lastly a subsample of what passed through 0.22 μm was filtered through 0.1 μm filters. All samples were analyzed by the UPLC and detailed in the Methods and Materials section.

2.16 Statistical Analysis

Data analysis was completed using the statistical software package SAS version 9.4 (SAS Institute, Cary, NC). Data was subject to the Levene's homogeneity of variance test before running ANOVAs to determine transformation necessity. Transformation was needed for almost all antibiotics for each runoff variable (sediment, biochar, and water) in this study. Sediment runoff transformations were as followed: lincomycin (LINC)^{5}, monensin (MON)^{6}, and tylosin

SQRT(TYL); water runoff transformations: $(LINC)^3$, no monensin transformation, and ln(TYL+.000001); lastly biochar runoff transformations: none for lincomycin, 1/(MON+.0001), and 1/(TYL). Transformations were not needed for bulk biochar and bulk soil samples; however since there was no lincomycin recovered from the bulk biochar samples in both 1% and 2% biochar rates at 50mm/hr, a t-test was used instead of 2-factor ANOVA for analysis of 1% and 2% biochar rates at 100mm/hr. Transformation was not needed for lincomycin and monensin drainage data but tylosin was transformed by SQRT(TYL). PROC GLM was conducted on all six variables testing within biochar rates, rainfall rates, and biochar rate x rainfall rate for means and standard deviations. All statistical analysis were conducted on the transformed data, but raw means are presented for ease of interpretation

Table 2.1 Chemical and physical properties of lincomycin, monensin, and tylosin

Antibiotic	Antibiotic Class	Molecular Weight (g mol^{-1})	pKa	pKb	log K_{ow}	Solubility in Water mg L^{-1}	Molecular Structure
Lincomycin	Lincosamide	406.5	7.6[a]		0.2[b]	927.0[c]	
Monensin	Ionophore	670.9	4.2[d]		5.4[e]	3.00E-03[c]	
Tylosin	Macrolide	916.1	7.7[f]	7.1[g]	1.6[f]	5,000[a]	

[a] Merck Index
b American Chemical Society
c US EPA
d Sassman and Lee (2007)
e US EPA 2009
f McFarland et al. (1997)
g Wollenberg et al. (2000)

Table 2.2 Soil Properties

Soil Depth cm	% Carbon	Mehlich III mg/kg	Potassium mg/kg	Magnesium mg/kg	Calcium mg/kg	Ammonium mg/kg	pH	Cation Exchange Capacity cmol/kg
0-15	2.5[a]	38[b]	13.1[c]	16.7[c]	54.8[c]	3.2[c]	6.48[d]	20.5[e]

[a] Analysis by LECO (total carbon analyzer)
[b] Provided by A&L Great Lakes Laboratories
[c] Water-extractable cations (see below for further details)
[d] pH determined by National Soil Erosion Research Laboratory using a 1:1 ratio of soil:water
[e] Cation exchange capacity was determined by A&L Great Lakes Laboratories using Ca, Mg, and K plus the buffer pH

Table 2.3 Concentrations of sorption capacity experiment

Material	Concentration of solution ($\mu g\ L^{-1}$)
Landscape Fabric	25
Gravel	25
250ml Amber Bottles	1
60ml PTFE bottles	1
20ml Amber Glass Vials	0.5
1 Liter PTFE Bottles	1

CHAPTER 3. RESULTS AND DISCUSSIONS

3.1 Isotherm Discussion

Sorption isotherms of lincomycin, monensin, and tylosin on the biochar and soil used in this study are shown in Figures 3.1 and 3.2, respectively. All three of the solutes were present and the overall sorption behavior was approximately linear. Based on the slope of the three isotherms, tylosin had the highest sorption affinity, followed by monensin and then lincomycin.

3.1.1 Soil Isotherm Results

Tylosin was shown to have the greatest sorption to soil of the three antibiotics, followed by monensin and lincomycin. Wang et al. (2009) reported a reduction of lincomycin sorption onto soils from pH 7.5 to 8.7 due to lincomycin shifting from its cationic state to its neutral state and therefore was not able to bind to negatively charged clay sites. Our final solution pH~8 would have reduced the cationic state of lincomycin and therefore a reduction of sorption to the soil. Additionally, with 0.02M KCl being present in the solution as an electrolyte background, it may have inhibited the ability for lincomycin to sorb to the soil. Conversely, Williams et al. (2014) reported an increase of lincomycin sorption to a silt loam soil compared to two clay loams in adsorption isotherms that had background electrolytes of NaCl and $CaCl_2$ even though the soil pH (7.8) was above the pKa of lincomycin (7.6); this was most likely due to the neutral state of lincomycin adsorbing to organic matter though with specificity. However, in a study similar to ours Wang et al. (2012) conducted isotherms with 0.02 M KCl solution and found a 79.2% reduction of lincomycin to a mollisol compared to no KCl in solution at pH 6.5, proving that K+ is competitive for sorption sites. The soil in our study had low clay content of 9% and high water soluble K^+, Ca^{2+}, and Mg^{2+}; for every 1 gram of soil there was 131, 548, and 167 μg of K^+, Ca^{2+},

and Mg^{2+}, respectively. According to the Le Châtelier's principle, the cation exchange capacity would favor K^+ at reversible exchange sites on clays leaving lincomycin in its soluble ionic form but excluded from clay exchange sites (Williams et al., 2014). Therefore the additional K^+ in our isotherms combined with reduced availability of sorption sites of clays due to high amounts of water-extractable cations may account for the low sorption of lincomycin to our soil. There may have also been a competition factor amongst the three compounds for clay sites. Lincomycin is the smallest molecule of the three with a molecular weight of 406.5 g mol^{-1}, which is almost half the size of tylosin (molecular weight of 916.1 g mol^{-1}); with exchangeable clay sites already limited with K^+ in solution, the larger molecule tylosin would outcompete lincomycin. Monensin, with a pKa of 4.2, is highly unlikely to adsorb to soil via electrostatic forces especially at pH environments higher than 7 (Watanabe et al., 2008), which is the case for our isotherm. Monensin also has a log K_{ow} of 5.4, suggesting that this compound is a hydrophobic and likely to partitioning with organic matter (Sassman and Lee, 2007; Watanabe et al., 2008), explaining the higher sorption of monensin relatively to lincomycin. Tylosin sorption to soil was observed at the greatest quantities in the isotherms; this may be due to the strong affinity of tylosin to bind to clays via electrostatic attraction (pKa=7.7) and its ability to partition through hydrophobic forces onto organic matter (Sassman et al., 2007; Zhang et al., 2011). The initial pH of 6 in our isotherms supports tylosin sorption by cation exchange. Interestingly, the pH of the isotherms shifted from an initial pH of 6 to a final pH 8 and it is unclear how this change would have occurred. However, tylosin is known to adsorb to soils very quickly: Srinivasan et al. (2014) reported >95% of tylosin adsorbed to a silty clay loam in 0-4 hours and Allaire et al. (2005) reported complete sorption of tylosin to a sandy loam in 5 minutes and to a heavy clay soil in 3 hours. Tylosin therefore may have adsorbed very quickly to soil when the pH was 6 before the

solution shifted to a final pH 8. At pH~8 tylosin exists in its neutral species and therefore cationic exchange may not be the driving sorption mechanism. Tylosin can form a cation bridge between its carboxyl groups and with surface-associated cations (Sassman et al., 2007) and with K^+ in solution and a high amount of water-extractable cations on the soil surface (Table 2) its possible that even if K^+ were blocking adsorption sites on the clays tylosin could bridge with these cations. It still must be considered that even though tylosin has the ability to bind to clays via cation bridging, if the final pH of the solution is 8 then tylosin was still most likely in its neutral form and not able to bind to these cations; it may be a combination of cation bridging, hydrogen bonding, and van der Waals forces that gives tylosin the ability to bind to clays at higher pH (Sassman et al., 2007; Zhang et al., 2011).

3.1.2 Biochar Isotherm Results

Isotherms for biochar reveal similar trends to the soil isotherms yielding high sorption of tylosin followed by monensin and then lincomycin. While high sorption of tylosin is reported by rice straw, charcoal, and motor vehicle fly ash (Guo et al., 2016a), it was unusual to observe low sorption of lincomycin, which has the ability to bind to biochar by cation exchange and hydrogen bonding (Liu et al., 2016; Wang et al., 2012). However, manure-derived biochars removed only from 25 to 34% lincomycin of the total after 2 days; whereas >92% removal was observed after 180 days; yet, no sorption equilibrium was observed, suggesting that lincomycin sorption by biochar is determined by both fast sorption on surface and slow pore diffusion (Liu et al., 2016). Conversely, tylosin sorption equilibrium was reached within 24 h using rice straw charcoal (Guo et al., 2016a). Thus, the low sorption of lincomycin by the biochar used in this study may be explained by several factors, including (1) the moderately low surface area of the biochar (123 $m^2\ g^{-1}$), (2) the hydrophobic properties of biochar (Tan et al., 2015) and the hydrophilic character

of lincomycin, (3) a final isotherm pH of 9 being above the pKa of lincomycin (7.6), and (4) the background electrolyte KCl at 0.02M blocking sorption sites. Higher sorption of lincomycin to manure-derived biochars was observed at solution pH < 7.3 relatively to pH > 9.9; this behavior was attributed to the cationic character of lincomycin at these pH levels lower than its pKa (Liu et al., 2016). Electrostatic forces were the main sorption mechanism in the above study since as ionic strength increased, lincomycin sorption decreased at pH 6.1-7.5, but at pH 9.9-10.3 lincomycin sorption was relatively unchanged. Competition between Na+ and lincomycin was observed at lower pH, therefore, proving electrostatic forces as the primary sorption mechanism; weaker sorption mechanisms such as hydrogen bonding and van der Waals may explain lincomycin sorption to biochars at higher pH (Liu et al., 2016). With a final pH of 9 in our biochar isotherms, this may explain why so little sorption was observed. What may be the biggest factor in low lincomycin sorption is the presence of the background electrolyte KCl in the isotherms. Wang et al. (2012) reported that lincomycin is competitive with K^+ for clay sorption sites, and that when KCl is present in solution (0.02M, the same as our isotherm) that lincomycin sorption to soil was greatly reduced. Even at lincomycin concentrations of 1000 μg L^{-1} with 0.02 M of KCl in solution, that meant for every 1 lincomycin molecule there were 8,130 molecules of K^+. This introduced a big competitive factor between the limited exchange sites on biochar and thus produced low sorption in our isotherms. It is possible that even if the final pH of the solution were lower and allowed lincomycin to be in its cationic state, that the disproportionate amount of K^+ to lincomycin still would have been too great of a competition for sorption sites and we still would have seen low sorption of lincomycin to biochar.

High adsorption of tylosin was expected due to its affinity for soil organic matter (Jeong et al., 2012; Guo et al., 2016) by being able to adsorb to organic matter surfaces via hydrophobic

interactions (Sassman et al., 2007) as biochar is inherently a hydrophobic substance. Cation exchange would not have been a factor for tylosin adsorption due to the high final pH of 9; with a pKa of 7.7 and pKb of 7.1, tylosin would have been in its neutral state at pH 9 and therefore not able to bind to cation exchange sites. Guo et al. (2016) reported that in an alkaline environment, it is also possible for tylosin to adsorb to black carbon via π- π electron donor-acceptor interactions between the π-rich surface of black carbon and the protonated amidogen of tylosin. However, this interaction becomes weaker as pH increases past the pKa of tylosin so it best to assume that hydrophobic interactions were the primary sorption mechanism of tylosin to biochar in high pH environments.

Little research, to the best of our knowledge, has been done on the ability for monensin to adsorb to biochar. However, based on the physiochemical properties of monensin it makes sense that monensin had the second-highest sorption to biochar of the three compounds. Monensin is very hydrophobic with a log K_{ow} of 5.4 and therefore will bind easily to the hydrophobic surface of biochar (Tan et al., 2015). While monensin and tylosin both can bind to organic matter surfaces via hydrophobic interactions, it is peculiar that monensin would not have just as high of sorption to biochar as tylosin. It is possible due to the size of tylosin, molecular weight of 916.1 g mol^{-1}, versus the size of monensin, molecular weight of 670.9 g mol^{-1}, that there was a competition factor between the two compounds for sorption sites; factor in that the surface area was a modest 123 m^2 g^{-1} and this might have intensified competition. More research needs to be done on the specific relationship between monensin and biochar to fully understand the sorption mechanisms.

3.2 Rainfall Simulations

3.2.1 Water Runoff

The total recoveries of lincomycin, monensin, and tylosin in water runoff for 50 mm/hr rainfall rates were 0.47%- 1.12%, 0%- 0.17%, and 0%- 0.01%, respectively. At the 100 mm/hr rainfall rate, lincomycin, monensin, and tylosin recoveries were 0.87%- 0.99%, 0.08%- 0.28%, and 0.02%- 0.03%, respectively. For all three antibiotics, the 100 mm/hr rainfalls significantly increased loss relative to 50 mm/hr. In addition, for lincomycin and monensin the biochar rate affected loss but with no clear pattern (Table 3.1). For lincomycin, with 1% biochar in the soil, the highest loss of this compound was observed, followed by 2% and 0%; for monensin, 2% biochar rate produced the highest loss followed by 1% = 0% rates (Table 3.1). Only in the case of tylosin did the interaction of biochar rate and rainfall rate provided significant differences ($p < .05$) (Table 3.1). The average water flow at every five-minute time interval for 50 and 100 mm/hr was 356 and 519 ml, respectively, explaining the differences between antibiotic losses at different rainfall rates.

Unlike Davis et al. (2006) who reported significant differences in antibiotic loss depending on different times during a rainfall event, we observed no significant differences of antibiotic losses at any one time during the 45-minute rainfall. Lincomycin loss was evenly distributed over time ranging between 0.7 and 1.5 μg for the entire rainfall. Monensin during the 100 mm/hr, the highest load loss at the beginning of the rainfall and tapered off though no significant differences from the 5-minute to the 45-minute sampling were observed; monensin at 50mm/hr did not observe the same pattern, much like lincomycin having a consistent loss at each sample time. Tylosin recovery was low, and therefore, no pattern was observed.

Previous studies (Kutcha and Cessna, 2009b; Kutcha et al., 2009; Li et al., 2013) observed high quantities of lincomycin in surface water runoff, whether from rainfall simulations or samples collected downstream of a point source; which is consistent with this study where lincomycin had the highest loss via runoff among the three antibiotics. The above results are attributed to the low Kow (0.2) of lincomycin and high water solubility (932 mg L^{-1}), indicating high mobility in soil and tendency to remain in solution. The pH of our soil was 6.48, a pH unit lower than the pKa of lincomycin (pKa = 7.6), which should provide conditions for lincomycin to sorb to the soil (D'Alessio et al., 2019). However, the timing of the simulated rainfall (15 minutes after application) and volume (7 L) after the application of the antibiotics to the soil, may contribute to lincomycin losses via runoff and lack of sorption to soil particles (Kulesza et al., 2016) as suggested by the sorption kinetic study by Liu et al. (2016). Monensin had recoveries less than 0.30% for all treatments and may be explained by its high K_{ow} (5.4), suggesting the higher affinity for organic matter, via hydrophobic interactions, rather than in water (Carlson and Mabury, 2007; Sun et al., 2013). Conversely, Davis et al. (2006) reported that monensin had the highest average concentration in water runoff, 1.2 μg L^{-1}, at 60 mm/hr rainfall intensity versus six other antibiotics. Tylosin runoff was recovered at the lowest percentage, within the range of 0%- 0.03%, consistent with previous studies of tylosin low mobility in soils (Carlson and Mabury, 2006; Sassman et al., 2007; Hu and Coats, 2009; Zhang et al., 2011) and subsequently low concentrations found in surface water runoff. Although tylosin has high water solubility (5 mg/ml) and a Kow of 1.6, due to its pKa (7.7) tylosin can strongly bind to soil via electrostatic attraction in soils with pH <7 and due to its large size and inability to access micropores, by interacting with soil surface exchange sites via its aliphatic amine group (Sassman et al., 2007). This is exampled by Davis et al. (2006) who measured an average 0.09

μg L^{-1} of tylosin during a one-hour rainfall event at 60 mm/hr intensity, resulting in a 0.02% of total recovery and Dolliver and Gupta (2008) who reported recoveries between <0.05% and 5% relative to total tylosin applied from surface runoff of swine and beef manure-amended plots, with manure applications ranging from 65,500- 131,000 L ha^{-1} and 54- 90 mg ha^{-1}, respectively, over a three year period.

3.2.2 Sediment Runoff

Tylosin had the largest recoveries of all the antibiotics for sediment runoff with relative recoveries of 2-4% in 50 mm/hr rainfalls and 6-13% in 100 mm/hr rainfalls. Total recoveries at 50 mm/hr for each of the antibiotics in sediment runoff are as follows: 0.04%- 0.11% for lincomycin, <0.005%- 0.05% for monensin, and 0.14%- 0.40% for tylosin; and for 100 mm/hr for each antibiotic: 0.15%- 0.34% for lincomycin, 0.09%- 0.15% for monensin, and 0.68%- 1.48% for tylosin. For all antibiotics there was a significantly greater loss at rainfall rate 100 mm/hr and only for tylosin did biochar rate affect sediment loss. Biochar rates 0% and 2% were equally more statistically significant ($p < 0.05$) and higher than the 1% biochar rate (Table 3.2), which presents no clear pattern between biochar rate and tylosin loss via sediment runoff. Loss of antibiotics over time was fairly constant over time with no spike in loss except for lincomycin at 0% and 2% biochar rates between 25 and 40 minutes into the rainfalls (Fig. 3.3). However, this observed spike is due to an analysis error that occurred for all sediment samples at time intervals 5, 25, and 45; those samples were analyzed through the UPLC with a preliminary method that was different from the method used to analyze all other samples in the project. Due to very low sediment capture at 50 mm/hr and possible degradation issues, by the time the error was caught these samples could not be run again.

High recoveries of tylosin from sediment would be expected because its K_{ow} (1.6) and pKa (7.7). Davis et al. (2006) found tylosin had highest relative loss associated with sediment runoff at 77%, however for all antibiotics observed in the study less than 0.1% of the total relative applied was recovered. This trend was also observed in a rainfall simulation where 0.13% relative loss in sediment of the 209 mg $plot^{-1}$ applied was recovered and the antibiotics were applied to the soil without manure amendment (Kim et al., 2010). The relative loss values for our study (2%- 13%) fall between the two studies mentioned, however our total recovery is higher than that of what has been previously reported. Both of these previous studies were done on the same soil, a sandy clay loam, that had a pH of 7.9; our soil pH was 6.48, lower than the tylosin pKa value of 7.7, and therefore can be assumed that tylosin was in its cationic form in our soil and more suited to bind to cation exchange sites.

Lincomycin had the second highest recovery of the three antibiotics in this study with losses between 0.04%- 0.34% between the two rainfall rates. To the best of our knowledge, no previous rainfall simulation experiment has been done with lincomycin to compare sediment runoff values; however, lincosamide antibiotics have been used. Pirlimycin is a lincosamide antibiotic that is similar in size to lincomycin with a molecular weight of 411 g mol^{-1} but has a higher pKa of 8.5. A study by Kulesza et al. (2016) conducted rainfall simulations on soil amended with dairy manure containing pirlimycin and recovered 0.06% of the initially applied dose in sediment runoff immediately after manure application. This recovery agrees with our 50 mm/hr rainfall rate data (.04%- 0.11%); but lower than all recoveries we found with 100 mm/hr (0.15%- 0.34%). Compared to lincomycin recovered in water runoff, sediment runoff recovery was much lower due to lincomycin being very hydrophilic.

Monensin had the lowest recovery of all the antibiotics in sediment runoff samples with all detections below <0.16% recovery. This was not expected as monensin had the second highest sorption capacity to soils of the three antibiotics in our isotherms (Fig. 3.1). However, low relative recoveries of monensin in sediment runoff from rainfall simulations have been observed in previous studies. Davis et al. (2006) reported monensin to have the highest loss in both water and sediment runoff, but relative loss in sediment runoff was the lowest of all antibiotics studied at 9%; similar to our study, <0.1% of total applied monensin was lost in the one-hour extreme rainfall event. Kim et al. (2010) also had low relative loss in sediment at 0.03% when antibiotics were applied directly to soil with no manure amendment. Further, it is possible we had such low recoveries in the sediment runoff because of the high monensin recovery in drainage and water runoff. Monensin will strongly adsorb to organic matter (Carlson and Mabury, 2006; Yoshida et al., 2010) and thus be more likely to be bound to DOC in our water samples (Sun et al., 2013). It is possible there was little organic matter in our sediment runoff especially considering the average sediment mass loss for 50 mm/hr and 100 mm/hr runs during the 45-minute span were 21.2 grams and 51.1 grams, respectively, which is just a 0.003% and 0.007% loss of soil from the boxes, respectively. Additionally, there was no apparent correlation between addition of biochar and decreased monensin recovery in sediment but with higher monensin recoveries for both rainfall rates in drainage, water runoff, soil in boxes, and biochar in boxes (Table 3.3) it is possible monensin adsorbed to DOC within the water matrix and organic matter within the soil rather than be lost via sediment runoff.

3.2.3 Drainage

Monensin accounted for the highest recovery of all three antibiotics in drainage, followed by lincomycin and then tylosin. The highest recovery of monensin was 11% for treatment 2%

biochar at 50 mm/hr versus the highest recovery of lincomycin was 2.0% for treatment 1% biochar at 100 mm/hr and for tylosin the highest recovery was 0.2% for treatment 2% biochar, 100 mm/hr. Average drainage volume for 50 mm/hr rainfalls was 6400 ± 894 ml per 45 minutes and for 100 mm/hr rainfalls 5350 ± 1258 ml per 45 minutes; drainage volumes were not significantly different when it was expected that drainage would be higher at 100 mm/hr (Fig. 3.4). The similar drainage volumes may be attributed to the increase in water runoff at 100 mm/hr (519 ml $5min^{-1}$) compared to 50 mm/hr (356 ml $5min^{-1}$). Although monensin loss was significantly higher at 0% biochar, 100 mm/hr (Fig. 3.5) this did not hold true for 1% and 2% biochar rates within the same rainfall rates; however it must be noted that recovery of monensin at treatment 0% biochar, 50 mm/hr was incredibly low compared to the other 5 treatments in this experiment (Table 3.3).

Monensin was predicted to be most frequently found in drainage; its pKa of 4.2 is lower than the pH of our soil (6.48) so it would have had anionic properties and therefore not be attracted to negatively charged clay sites in the soil. However, previous studies show little to no movement of monensin within the environment, whether down a soil profile up to 35 cm (Carlson and Mabury, 2006) or <0.005% detected in leachate relative to manure application rates (Dolliver and Gupta, 2008). Monensin has a K_{ow} of 5.4 and therefore more of an affinity to stay in the soil matrix than in water. To explain the quantity of monensin detected in our drainage, it can be assumed that monensin was bound to dissolved organic carbon (DOC). Drainage water samples were subjected to sequential filtering using filter sizes 0.45 μm, 0.22 μm, and 0.1 μm to determine monensin sorption to colloidal particles and found a pattern of decreasing monensin concentration with decreasing filter size. It must be noted that sorption capacity for antibiotics by the nylon filters was not tested in this study. Regardless, it is possible that the patterned losses of

monensin through each filter was due to DOC and not filter entrapment. Lissemore et al. (2006) calculated that the Spearman rank correlation coefficient for their study showed a moderate correlation of monensin to DOC, and Sassman and Lee (2007) observed that monensin can be recovered in both DOC and in water but has a larger propensity to be bound with suspended solids in effluent. Additionally, the depth of our soil was only 3cm therefore our simulation may overestimate how far down monensin would move through a soil profile in the environment.

Lincomycin was the second most frequently found antibiotic in drainage with loads ranging from 2.46- 20.0 μg and subsequently 0.25- 2.00% total recovery. Due to its hydrophilic properties and K_{ow} of 0.2 it would be expected there would be some movement of lincomycin through the soil profile. A column study by D'Alessio et al. (2019) found lincomycin in all effluent samples and a rainfall simulation study by Kutcha and Cessna (2009) found lincomycin in groundwater samples after liquid manure application in low concentrations of <0.005 to 0.15 μg L^{-1}. With our soil pH below 7 and the pKa of lincomycin being 7.6, lincomycin has an increased likelihood to sorb to soil (D'Alessio et al., 2019) and may explain the low recoveries found in this study. Sequential filtering, detailed above, also showed a pattern with decreasing lincomycin concentration in correlation with decreasing filter size but again sorption capacity of antibiotics by the filter paper themselves was not tested and therefore could account for some of the losses observed. However, lincomycin recovered in drainage would have most likely been dissolved in water and not associated with DOC as the Spearman rank correlation coefficient calculated by Lissemore et al. (2006) found that there was no correlation between DOC and lincomycin in water samples collected from a Canadian watershed.

Tylosin was found in the least quantities in drainage with total recoveries for every treatment at less than 0.2%. This aligns with previous studies of low tylosin recovery in leachates

and groundwater. Hu and Coats (2009) and Dolliver and Gupta (2008) both found very low recoveries of tylosin in leachate from manure-amended soils at <0.02% and <0.005%, respectively. Even with soils containing 3% clay content, tylosin was not found in lower soil depths of 25-35cm due to tylosin having a strong affinity to sorb to clays (Carlson and Mabury, 2006). With the clay content of our soil being 9% and our isotherms showing almost complete sorption of tylosin to our soil, our results fit well with the predicted movement of tylosin.

3.2.4 Biochar Runoff

Antibiotics recovered from biochar were quite low, at less than 0.05% for all treatments. Following the trend of sediment and water runoff, 100 mm/hr rainfalls had significantly higher recoveries in biochar runoff than 50 mm/hr but only for lincomycin and monensin; tylosin showed no statistically significant differences between rainfall rates (Table 3.4). The lowest recoveries came from lincomycin at <0.005% for both biochar rates during 50 mm/hr rainfalls while the highest came from monensin at 0.049% for treatment 2% biochar, 100 mm/hr.

The low recoveries from each of the antibiotics do not support the premise that the biochar surface area is a key factor in the sorption on compounds. With a surface area of 123 m^2 g^{-1}, higher sorption of all of the antibiotics was expected, however pH of biochars are traditionally alkaline (above pH of 7) and due to the main sorption mechanisms of lincomycin and tylosin being electrostatic attraction, the high pH of biochar may inhibit sorption. Monensin, however, can still bind via hydrophobic interactions on the biochar surface and not relying on biochar pH. Under slow pyrolysis, softwood biochars produced at 450°C had surface areas of <25 m^2 g^{-1} (Kloss et al., 2012; Brewer et al., 2014) and 317 and 159 m^2 g^{-1} and at 700°C and 900°C, respectively (Brewer et al., 2014; Jeong et al., 2012), however the sorption capacity of these biochars was not measured. The surface area of our biochar compared to those pyrolyzed at

the same temperature was higher therefore we would have expected higher sorption of our antibiotics. However, Kloss et al. (2012) reported CEC of a spruce-derived biochar to be from 52.2 to 73.5 $mmol_c\ kg^{-1}$ which is low compared to poplar-derived biochar in the same study 107.6 to 144 $mmol_c\ kg^{-1}$; spruce is one of the woods in our softwood mixture biochar and could explain that even with higher surface area, the CEC may have been low. It is also possible that there was some permanent sorption to biochar (Jeong et al., 2012) or that the biochar itself had weak adsorption capacities. The latter was tested in a 24-hr sorption study of 1 mg L^{-1} atrazine (a herbicide) solution by our biochar following the method by Gonzalez et al. (2016). Of the total added, our biochar only removed 4.8% of total atrazine added, compared to 99% removal atrazine by a hardwood-derived biochar pyrolyzed at 450 °C (Gonzalez, et al., 2016) and surface area of 324 $m^2\ g^{-1}$. Atrazine is thought to sorb by biochar via hydrogen bonding and hydrophobic and pi interactions (Gonzalez, et al., 2016; Penn et al, et al., 2018). Although the surface area of our biochar is about 38% of the hardwood biochar, the low removal of atrazine by the softwood biochar used in our study was not expected. Sorption isotherms of antibiotics by biochar (Fig. 3.2) in our study however do not necessarily show low sorption except for lincomycin. Tylosin had the highest sorption of roughly 430 μg g^{-1} followed by monensin at roughly 260 μg g^{-1} and lastly by lincomycin at less than 30 μg g^{-1} when all three compounds had an initial concentration of 1000 μg kg^{-1}. Liu et al. (2016) showed two-phase kinetics of lincomycin to four different types of biochar leading to believe that more lincomycin would have adsorbed to ours. However, the limitation of biochar surface area and the short amount of time between application and rainfall might have influenced sorption. Tylosin and monensin had higher recoveries compared to lincomycin, at least at 50 mm/hr, due to their ability to sorb to surfaces via hydrophobic interactions (Sassman et al., 2007; Sassman and Lee, 2007; Carlson and Mabury, 2006) and also

followed the trend of our isotherms both having higher sorption capacities to the biochar. However, at 100 mm/hr for all antibiotics and both biochar rates the recoveries had no clear distinction between each other and didn't follow the trend observed in the isotherms.

When looking at relative recoveries (Fig. 3.6, 3.7, and 3.8) biochar runoff accounts for less than 1% for all treatments. This is most likely due to the very low biochar runoff quantity that accrued during the rainfalls. The highest sum of biochar runoff over a treatment was 0.51 grams for 2% biochar at 100 mm/hr, only 0.29% of biochar applied for that treatment (156 grams initially added) with the lowest collected sum being 0.15 grams for 1% biochar at 50 mm/hr (78 grams initially added), a 0.27% loss via runoff (Table 3.5); however, the lowest percentage lost was 0.10% for 1% biochar at 100 mm/hr. In our study, trace amounts of antibiotics were adsorbing to the biochar and less than 0.4% of the biochar applied was being loss during rainfall.

Biochar is buoyant, inherently hydrophobic, and normally has a density of less than 1 g cm^{-3} (Wang et al., 2013; Brewer et al., 2014) due to high porosity and pore volume created during pyrolysis (Schmidt et al., 2000; Ahmad et al., 2012; Ahmed et al., 2016). Temperature can have significant impacts on cation exchange capacities and surface area; the higher the temperature, up to a certain point otherwise ash can fill pores (Tag et al., 2016), the more pore structures form and create exchange sites. Additionally, grass and woody biochars tend to possess higher surface area than their manure and food waste counterparts (Ahmed et al., 2016) making them more mobile and susceptible to erosion. Further, the addition of biochar should decrease the bulk density of the soil and allow for more infiltration and less runoff of sediment and possibly biochar itself (Jeong et al., 2012; Andrenelli et al., 2016; Hamidreza et al., 2016; Ahmed et al., 2017). Abrol et al. (2016) showed this with a 2% biochar application by weight onto a non-calcareous soil, where infiltration increased but reduced sediment loss. With two

different rainfall rates we would also expect more specific patterns: a 50 mm/hr rainfall would have higher sediment loss and lower drainage volume than 100 mm/hr due to slower infiltration of water through the soil and a lower volume of rainfall water applied. Although BD did decrease with the addition of biochar in our study, the water, sediment, and biochar losses did not necessary follow the expected pattern. Sediment loss at 100 mm/hr was significantly higher than at 50 mm/hr (Fig. 3.9) and the addition of biochar at 50 mm/hr significantly increased sediment loss versus the control. However at 100 mm/hr the 0% and 2% biochar rates were not significantly different but both were higher than the 1% biochar rate. Water runoff also had higher volumes of runoff at 100 mm/hr versus 50 mm/hr at 500- 535 ml/ 5 minutes and 339- 365 ml/5 minutes, respectively, with no significant differences within the rainfall rates. For biochar runoff loss, only at 2% was 100 mm/hr significantly higher than at 50 mm/hr and 1% biochar rate was significantly higher at 100 mm/hr than at 50 mm/hr but by a very small margin. The high biochar runoff loss at 2% biochar, 100 mm/hr (Fig. 3.10) is likely due to the high kinetic energy of the rainfall loosening more biochar and forcing biochar from below the surface up to the surface due to biochar's low density allowing it to be easily displaced by denser soil particles and creating a "diffusion" affect (Wang et al., 2013).

What is most interesting is that drainage at every treatment was not statistically significant from each other (Fig. 3.4). At the very least, a 2% biochar rate should have seen an increase in drainage due to decreased BD (Abrol et al., 2016) at both rainfall rates but this was not the case; high standard deviations for 1% and 2% biochar rates at 100 mm/hr show high variance within the repetitions. It is possible with the soil depth only being 3cm and a total box depth of 5cm, there may have been complete saturation within the soil plus pooling affect within

the gravel layer that forced more water to be lost by runoff in 100 mm/hr than typically seen in a rainfall simulation.

3.2.5 Soil and Biochar from Boxes

Recovery from soil left in the boxes after the rainfall events were the highest as was expected. Monensin had the highest recoveries across all treatments, followed by tylosin and then lincomycin. As with other recoveries in this study, 100 mm/hr rainfall rates had higher recoveries for lincomycin and monensin versus 50 mm/hr. However with tylosin the recoveries were almost the same for both rainfall rates: 8.72% - 12.9% and 9.7- 11% recoveries for 50 mm/hr for 100 mm/hr, respectively. Biochar rate had no significant affect on soil recovery for all antibiotics.

Although monensin had the highest percentage recovery from soil in this study, tylosin by far had the highest rates of relative recovery, with relative recovery being the ratio of recovered antibiotics to total antibiotics applied. Among the total tylosin recovered across treatments, 96%- 98% and 85%-93% for 50 mm/hr and 100 mm/hr, respectively, were recovered from the soil boxes alone. This trend in tylosin aligns with previous studies showing tylosin being moderately mobile to immobile in soils depending on soil type (Laak et al., 2006; Zhang et al., 2011). Tylosin has a pKb of 7.1 (Wollenberger et al., 2000) making it a weak base and cationic in our soil pH. Tylosin has a strong affinity for clay edge sites but due to its size cannot enter the interlayers and therefore is surface area dependent for sorption onto edge sites (Sassman et al., 2007). Although our clay content was 9% it may have been enough for tylosin to stay in the soil boxes. Additionally, tylosin can bind to organic matter via hydrophobic bonds and possibly bind irreversibly by covalent bonds though this is a minor mechanism compared to hydrophobic and electrostatic interactions (Hu and Coats, 2009; Zhang et al., 2011). Compared

to other smaller and polar compounds, tylosin is more immobile (Zhang et al., 2011) so combined with all other traits it is almost always more likely to be in soil and sediments and not a cause for concern unless subjected to an erosion event where it could be transported partitioned to DOC and sediments (Sassman et al., 2007; Hu and Coats, 2009; Kim and Carlson, 2007).

Relative recoveries of lincomycin in the soil boxes were 28%- 86% and 67%- 75% in 50 and 100 mm/hr rainfall rates, respectively. The addition of biochar significantly reduced recoveries of lincomycin in the 50 mm/hr rainfalls and significantly increased drainage but the same pattern did not hold true for 100 mm/hr instead observing consistent recoveries across those treatments for both soil in boxes and drainage (Fig. 3.6). Not only did drainage increase during 50 mm/hr rainfalls but water runoff as well. This is most likely explained by two factors: bulk density (BD) decreasing with the addition of biochar and the affinity of lincomycin to exist in the water matrix. The targeted BD of soil for the controls was 1.3 g cm^{-3}; laboratory BD experiments confirmed that our mollisol had a BD of 1.27 g cm^{-3} and 1% and 2% biochar rates in our soil produced bulk densities of 1.07 g cm^{-3} and 1.14 g cm^{-3}, respectively. This changing of soil composition would explain the increase in drainage and surface water runoff. However, it is peculiar we did not observe the same trend in the 100 mm/hr rainfalls. While the increase in rainfall rate did increase the load loss of lincomycin in both drainage and water runoff, on a relative loss basis it seems the addition of biochar had no significant affect in changing the proportion of where lincomycin was recovered. However it must be noted that for each treatment, total recoveries were never more than 13% and were even as low as 2.8% for treatment 2% biochar, 50 mm/hr (Table 3.6). With up to 93% of lincomycin unaccounted for in this study it is possible there was permanent sorption to biochar and soil or lincomycin degraded to some degree before analysis could be completed. However, it is more likely our ASE extraction

solution was not able to successfully remove all of the lincomycin from the soil. After soil and biochar extractions were run, we noticed these low outputs and ran lincomycin extraction on just Ottawa sand alone through the ASE. Only 25% was recovered from our outputs suggesting that we may have not extracted up to 75% of lincomycin bound to soils. In the future it may be worth extracting soils with a $CaCl_2$ solution as Ca^{2+} can easily displace lincomycin from clay sites due to ionic competition (Wang et al., 2012).

Monensin observed relative recoveries in the soil of 52%- 73% and 49%- 69% for 50 and 100 mm/hr rainfall rates, respectively. Comparing the relative recoveries of 1% biochar addition for both rainfall rates, there was significantly higher recovery of monensin in drainage at 50 mm/hr and conversely there were significantly higher recoveries in the soil for 100 mm/hr. As discussed previously, the BD at 1% biochar rate was lower (1.07 g cm^{-3}) than that of 2% (1.14 g cm^{-3}) therefore the higher drainage at 50 mm/hr makes sense due to the loosening of the soil. Although relative monensin recovery for 1% biochar at 100 mm/hr was only 20%, there was an 11% relative recovery of biochar left in the boxes for the same treatment, which was significantly higher than at 50 mm/hr (Fig. 3.7). This addition of biochar increased the soil organic matter of the soil, which monensin has strong affinity to bind to by hydrophobic interactions (Carlson and Mabury, 2006; Sassman and Lee, 2007; Ahmed et al., 2015). Therefore even with lower BD and a greater chance for monensin to pass through into drainage, it instead adsorbed onto soil and biochar surfaces. Relative biochar recovery for 2% biochar, 100 mm/hr was about the same as its 1% counterpart at 13%, however relative recovery in drainage was higher (36%) and lower in the soil (49%) which with the lower BD at 2% biochar (1.14 g cm^{-3}) aligns more with where we hypothesized the monensin would be.

Recoveries from biochar samples from the boxes after the rainfall were very low for lincomycin and tylosin ranging from 0% to 0.028% and 0% to 0.026% for lincomycin and tylosin, respectively, across all treatments. Conversely, monensin had the highest recoveries across all treatments ranging from 0.6%- 1.8% and 3.4% to 3.9% for 50 and 100 mm/hr, respectively. We contribute the lack of recoveries from lincomycin and tylosin due to the moderate surface area (m^2 g^{-1}) of our biochar and the biochar's low sorption capacity. The soil pH was below pKa for both lincomycin and tylosin and therefore electrostatic attraction between the cationic state of the antibiotics and the negative charge of the biochar would have dominated sorption (Liu et al., 2016; Jeong et al., 2012). In general organic compounds can adsorb to biochars by electrostatic attraction, hydrogen bonding, hydrophobic interactions, or pore filling or a combination of those listed (Tan et al., 2015). However, with a lack of large surface area usually seen in biochar this means there are less pores available for pore filling and edges for electrostatic interactions. Therefore weaker bonds such as hydrogen bonds and hydrophobic interactions would dominate. Monensin is a very hydrophobic compound and strongly adsorbs to soil organic matter (Carlson and Mabury, 2006; Ahmed et al., 2015). Between organic matter found in our mollisol and the addition of biochar the high recoveries observed were to be expected. Additionally, monensin can easily detach during an erosion event by partitioning to DOC (Doydora et al., 2015) so very small biochar particles could have led to elevated levels of monensin seen in drainage as "dust-sized" particles were included in this study.

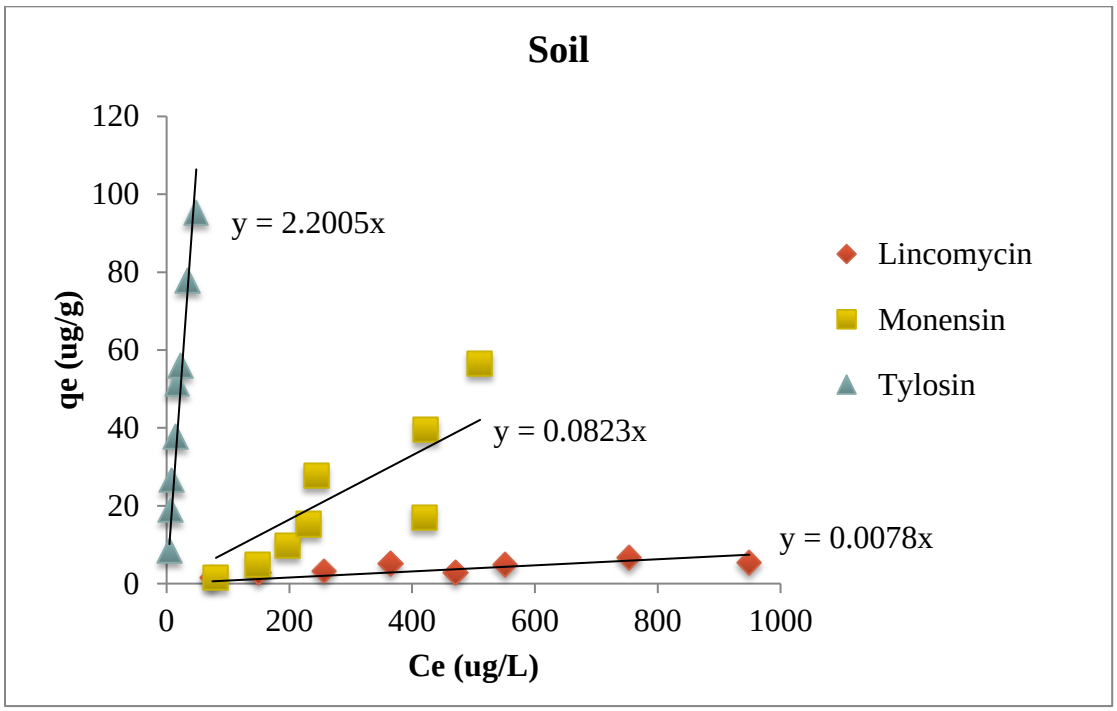


Figure 3.1 Sorption isotherm of lincomycin, monensin, and tylosin in a Chalmers soil at 25°C after 24 hours; dosage of 10 g L-1

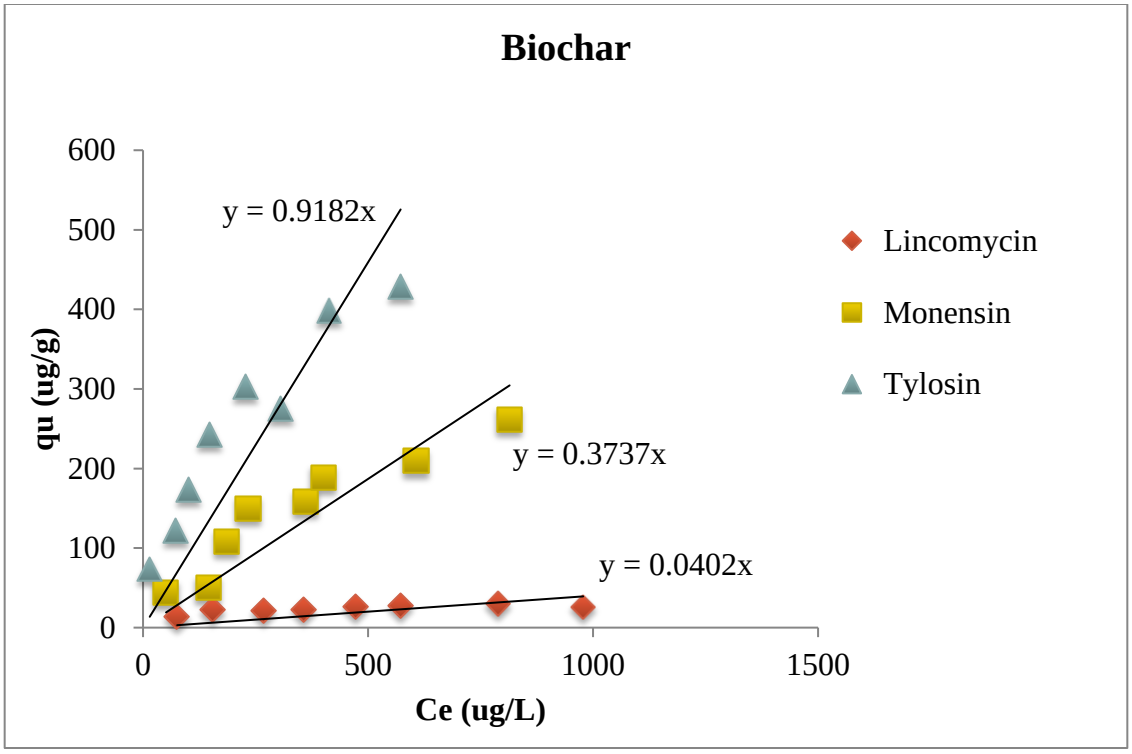


Figure 3.2 Sorption isotherm of lincomycin, monensin, and tylosin in a Chalmers soil at 25°C after 24 hours; dosage of 1 g L^{-1}

Table 3.1 Means and Standard Deviations of Rainfall Rate, Biochar Rate, and Rainfall Rate x Biochar Rate Interaction in Water Runoff (μg)

Rainfall Rate	N	Lincomycin		Monensin		Tylosin	
		Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
50	81	0.596[b]	0.366	0.073[b]	0.31	0.005	0.008
100	80*	1.031[a]	0.436	0.189[a]	0.163	0.032	0.024

Percent Biochar	N	Lincomycin		Monensin		Tylosin	
		Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
0	54	0.662[b]	0.417	0.042[b]	0.067	0.013	0.014
1	54	0.937[a]	0.461	0.103[b]	0.112	0.016	0.02
2	53*	0.837[ab]	0.455	0.250[a]	0.398	0.025	0.029

Rainfall Rate	Percent Biochar	N	Lincomycin		Monensin		Tylosin	
			Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
50	0	27	0.35	0.253	0	0	0.00[d]	0
50	1	27	0.857	0.429	0.027	0.044	0.00[d]	0.001
50	2	27	0.581	0.175	0.192	0.521	0.015[c]	0.008
100	0	27	0.975	0.297	0.083	0.075	0.026[ab]	0.007
100	1	27	1.018	0.486	0.179	0.108	0.032[a]	0.015
100	2	26*	1.102	0.504	0.31	0.197	0.036[bc]	0.038

*One sample was missing hence differences in sample numbers

Table 3.2 Means and Standard Deviations of Rainfall Rate, Biochar Rate, and Rainfall Rate x Biochar Rate Interaction in Sediment Runoff (μg)

Rainfall Rate	Lincomycin			Monensin			Tylosin	
	N	Mean	Std Dev	N	Mean	Std Dev	Mean	Std Dev
50	65[δ]	0.072[b]	0.076	64	0.018[b]	0.022	0.250[b]	0.222
100	81	0.197[a]	0.141	81	0.089[a]	0.051	0.861[a]	0.526

Percent Biochar	Lincomycin			Monensin			Tylosin	
	N	Mean	Std Dev	N	Mean	Std Dev	Mean	Std Dev
0	45[δ]	0.196	0.136	45	0.046	0.049	0.646[a]	0.461
1	48[δ]	0.08	0.088	47	0.045	0.051	0.365[b]	0.352
2	53	0.151	0.14	53	0.08	0.054	0.745[a]	0.616

Rainfall Rate	Percent Biochar	Lincomycin			Monensin			Tylosin	
		N	Mean	Std Dev	N	Mean	Std Dev	Mean	Std Dev
50	**0**	18[δ]	0.094	0.102	18	0.0004	0.0006	0.25	0.211
50	**1**	21[δ]	0.037	0.042	20	0.005	0.005	0.151	0.178
50	**2**	26[δ]	0.086	0.068	26	0.04	0.017	0.327	0.236
100	**0**	27	0.264	0.112	27	0.076	0.042	0.911	0.385
100	**1**	27	0.113	0.1	27	0.075	0.049	0.525	0.367
100	**2**	27	0.214	0.163	27	0.118	0.05	1.149	0.602

[δ]Certain samples were run with a different UPLC method versus the rest of the data. Due to limited quantities in our runoff samples, these samples could not be run again through the ASE and therefore are excluded from analysis

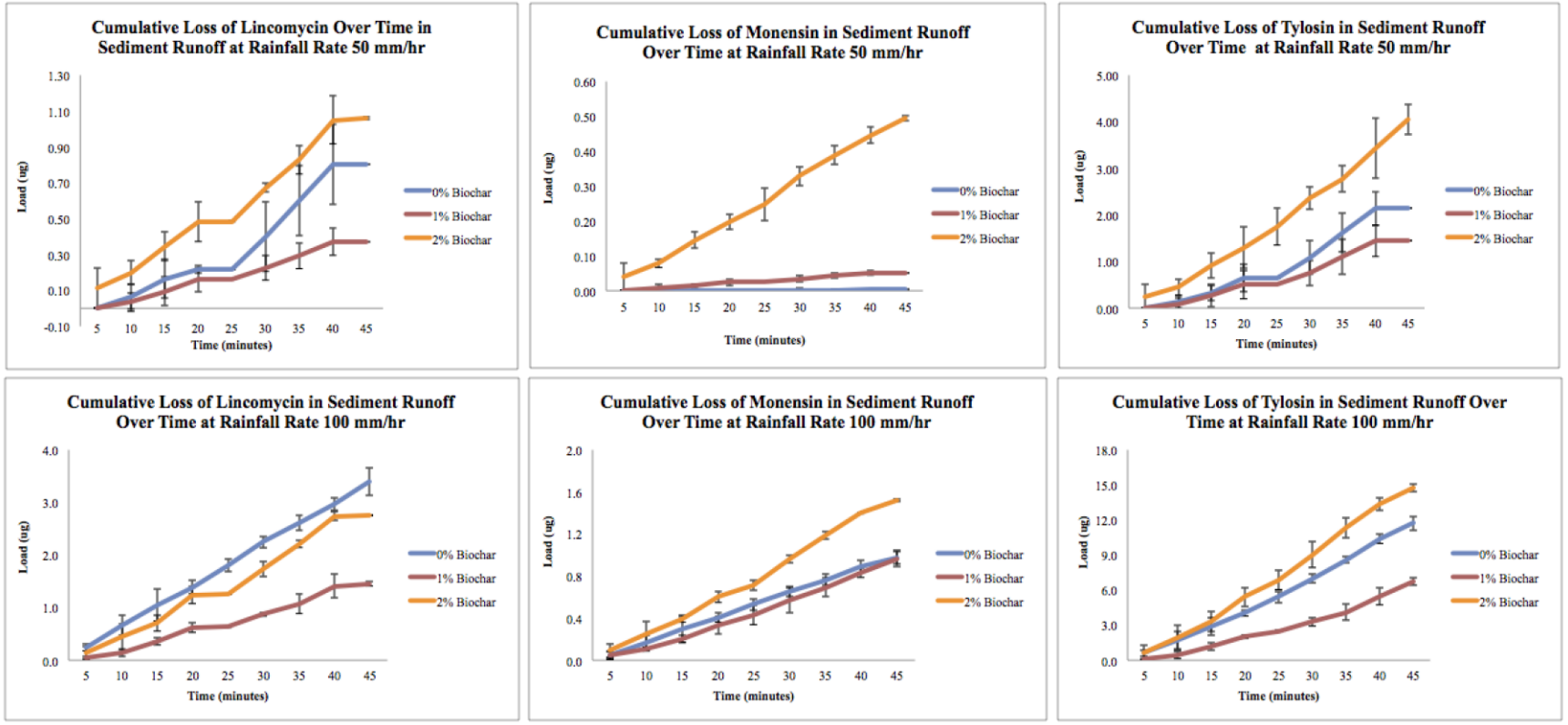


Figure 3.3 Average load per antibiotic in both 50 and 100 mm/hr rainfall rates; Note for 50 mm/hr at time 5, 25, and 45 minutes we only have data for 2% biochar rate. This is due to a different UPLC method used to analyze 0% and 1% biochar rate samples before we changed to the UPLC method we used for all other analysis. Due to minimal recovery of sediment we could not do re-extractions.

Table 3.3 Percent Total Recoveries of Monensin by Treatment

	Sediment Runoff	Water Runoff	Biochar Runoff	Soil in Boxes	Drainage	Biochar in Boxes	% Recovery
0% Biochar 50mm/hr	0.00	0.00		0.56	0.20		0.76
1% Biochar 50mm/hr	0.01	0.03	0.02	8.88	7.70	0.57	17.2
2% Biochar 50mm/hr	0.05	0.17	0.02	17.3	7.91	1.78	27.3
0% Biochar 100mm/hr	0.10	0.08		14.3	9.59		24.0
1% Biochar 100mm/hr	0.10	0.16	0.02	21.9	6.23	3.40	31.9
2% Biochar 100mm/hr	0.15	0.28	0.05	15.2	11.0	3.98	30.7

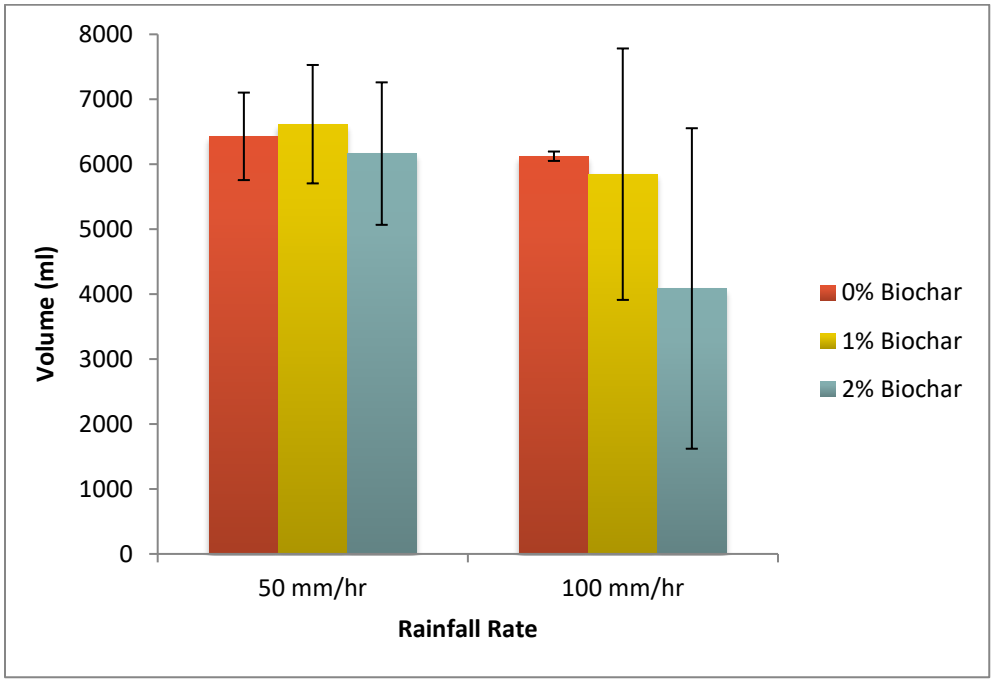


Figure 3.4 Average drainage volume across a 45-minute simulation per treatment

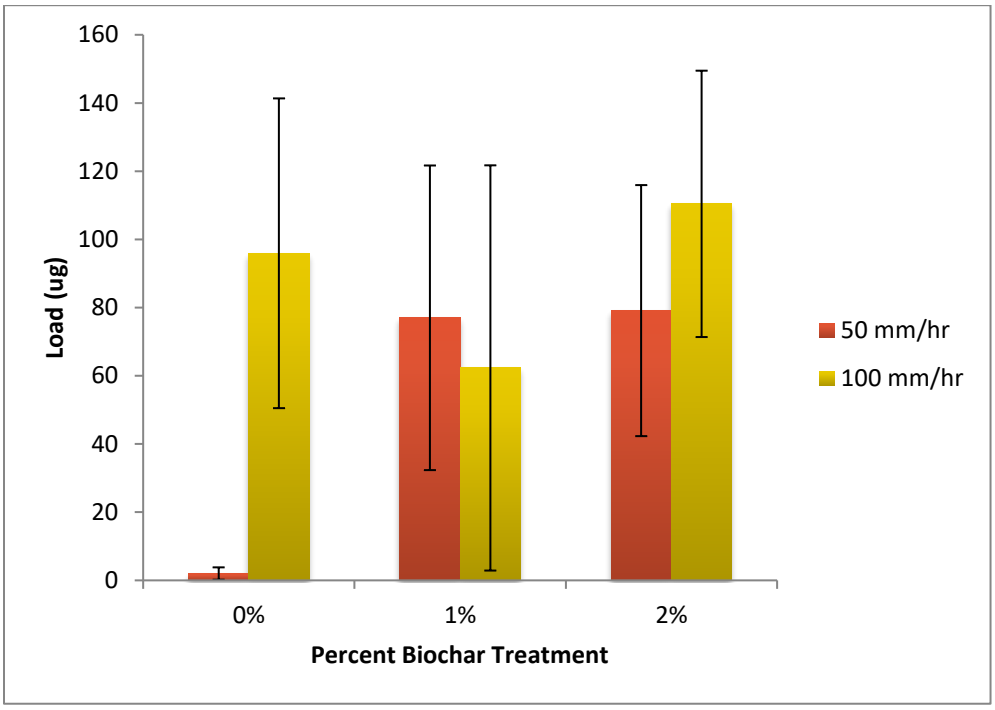


Figure 3.5 Average monensin load loss in drainage at 50 and 100 mm/hr rainfall rates

Table 3.4 Means and Standard Deviations of Rainfall Rate, Biochar Rate, and Rainfall Rate x Biochar Rate Interaction in Biochar Runoff (μg)

Rainfall Rate	N	Lincomycin		Monensin		Tylosin	
		Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
50	81	0.002[b]	0.002	0.001[a]	0.001	0.011	0.009
100	80*	0.018[a]	0.029	0.028[b]	0.018	0.019	0.021

Percent Biochar	N	Lincomycin		Monensin		Tylosin	
		Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
1	54	0.008	0.013	0.009	0.01	0.02	0.022
2	53*	0.012	0.028	0.02	0.023	0.009	0.004

Rainfall Rate	Percent Biochar	N	Lincomycin		Monensin		Tylosin	
			Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
50	1	27	0.003	0.003	0.001	0.002	0.012	0.013
50	2	27	0.002	0.001	0.001	0	0.009	0.003
100	1	27	0.013	0.017	0.017	0.008	0.028	0.027
100	2	26*	0.022	0.038	0.038	0.019	0.01	0.005

*One sample was missing hence differences in sample numbers

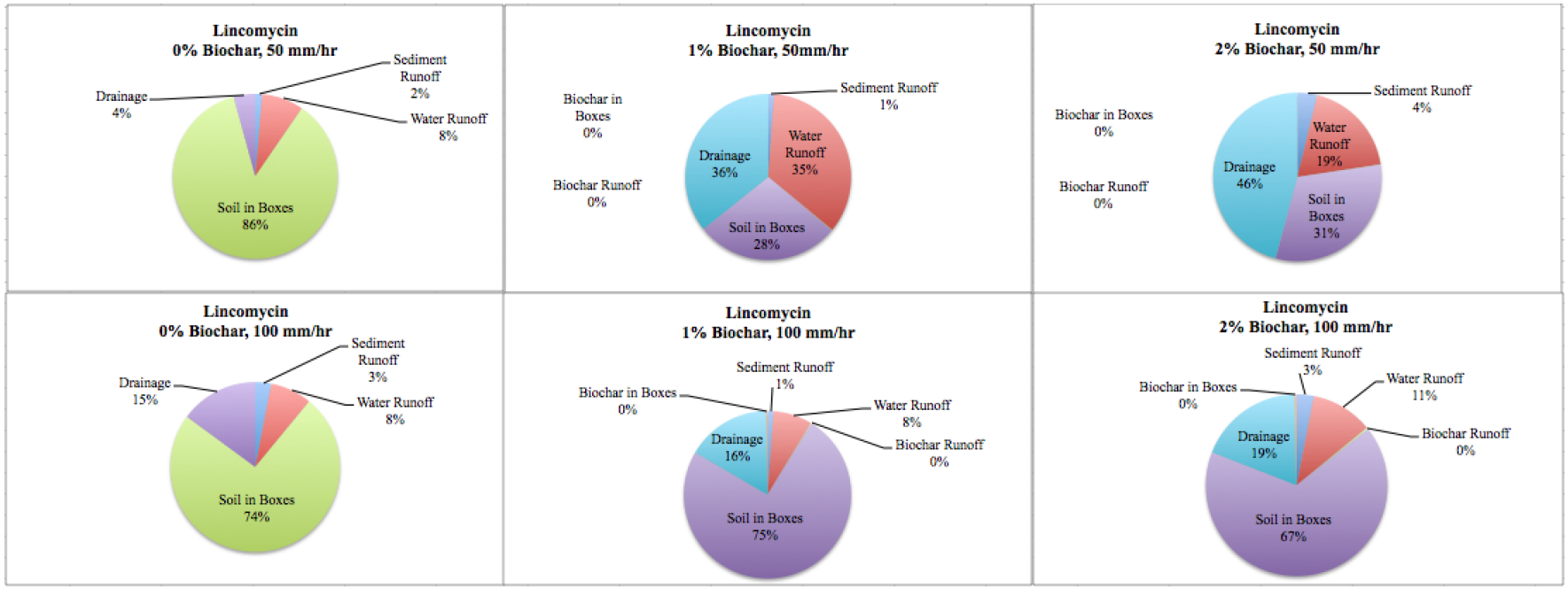


Figure 3.6 Charts show of the total relative recoveries for each treatment where lincomycin was detected in sediment runoff, water runoff, biochar runoff, drainage, soil from boxes, and biochar from boxes.

Note: Labels with lines attached that read “0%” are not actually 0%, but rather are low enough relative recoveries they appear close to zero on the charts

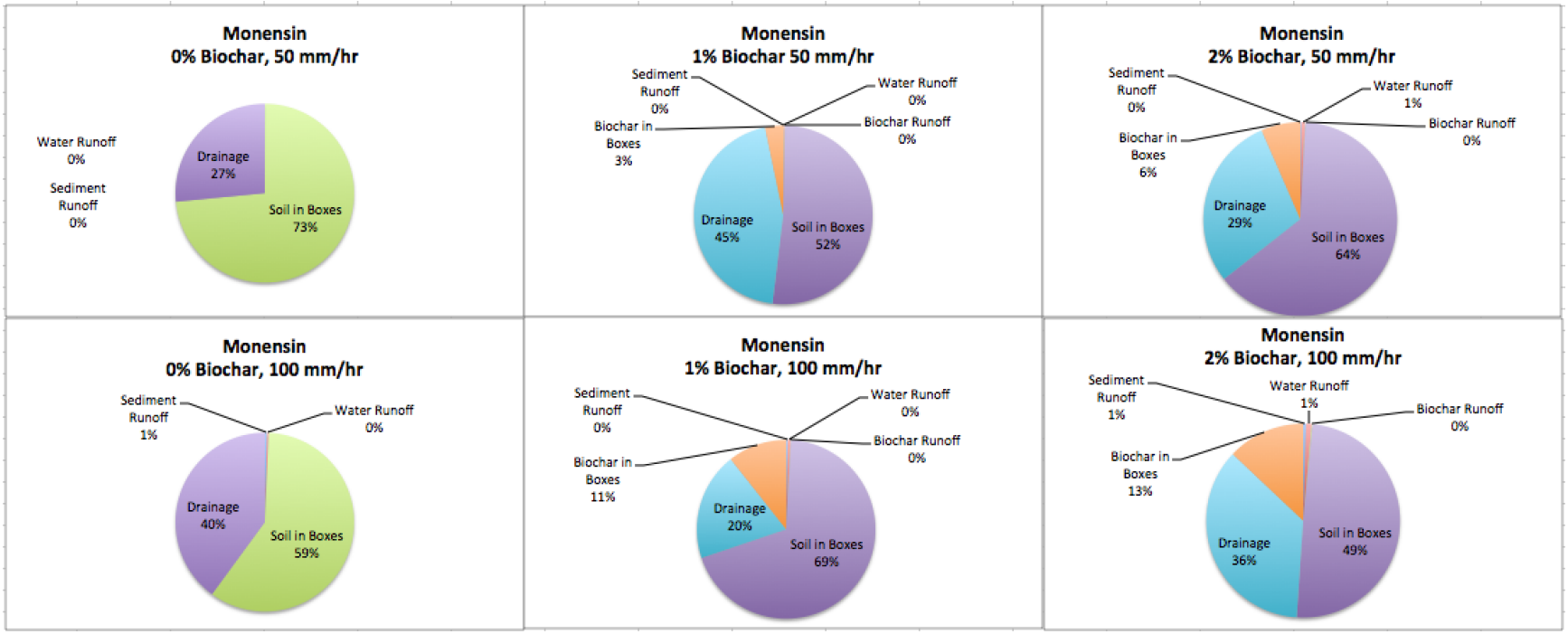


Figure 3.7 Charts show of the total relative recoveries for each treatment where monensin was detected in sediment runoff, water runoff, biochar runoff, drainage, soil from boxes, and biochar from boxes.

Note: Labels with lines attached that read “0%” are not actually 0%, but rather are low enough relative recoveries they appear close to zero on the charts

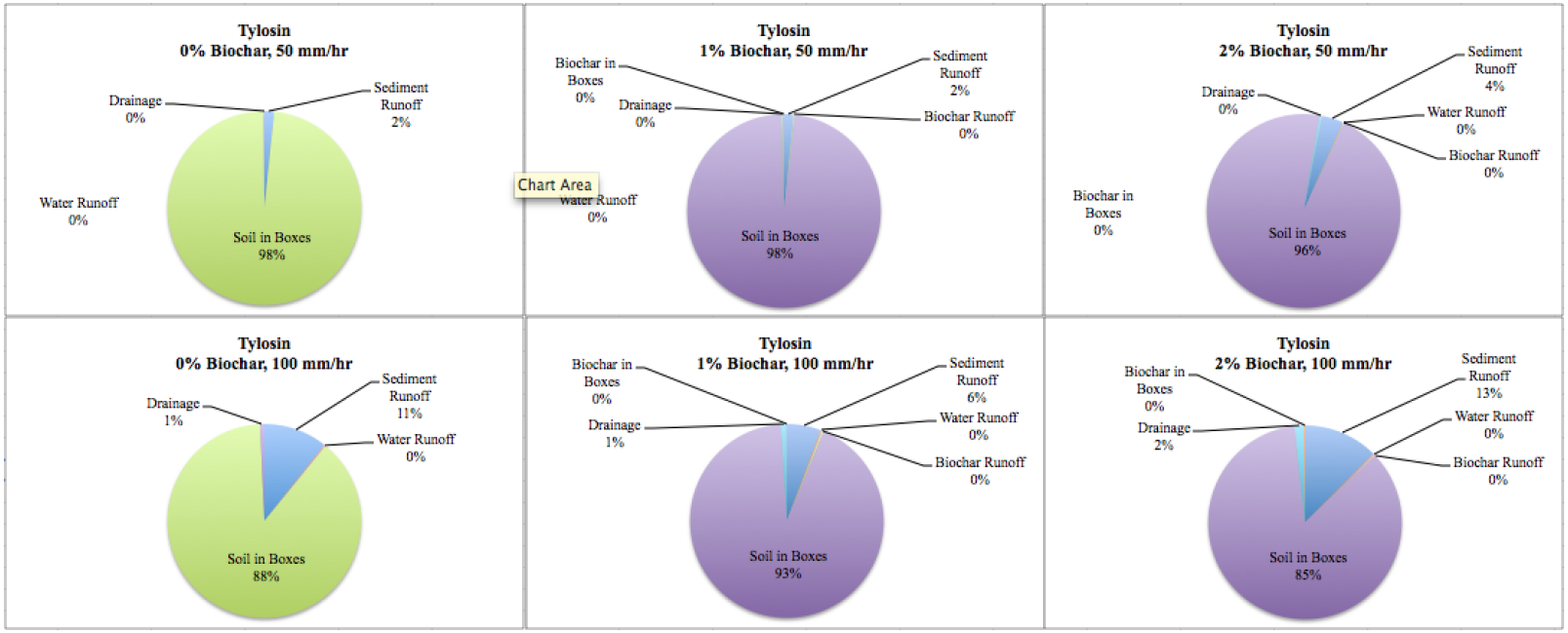


Figure 3.8 Charts show of the total relative recoveries for each treatment where tylosin was detected in sediment runoff, water runoff, biochar runoff, drainage, soil from boxes, and biochar from boxes.

Note: Labels with lines attached that read "0%" are not actually 0%, but rather are low enough relative recoveries they appear close to zero on the charts

Table 3.5 Total Sum of Biochar Runoff for each Treatment

	Grams Lost*	Biochar Applied (grams)	Percent Loss
1% Biochar, 50 mm/hr	0.15	56	0.27%
2% Biochar, 50 mm/hr	0.19	56	0.34%
1% Biochar, 100 mm/hr	0.18	178	0.10%
2% Biochar, 100 mm/hr	0.51	178	0.29%

*Grams Lost was calculated as a sum of the reps across the 45-minute rainfall simulations

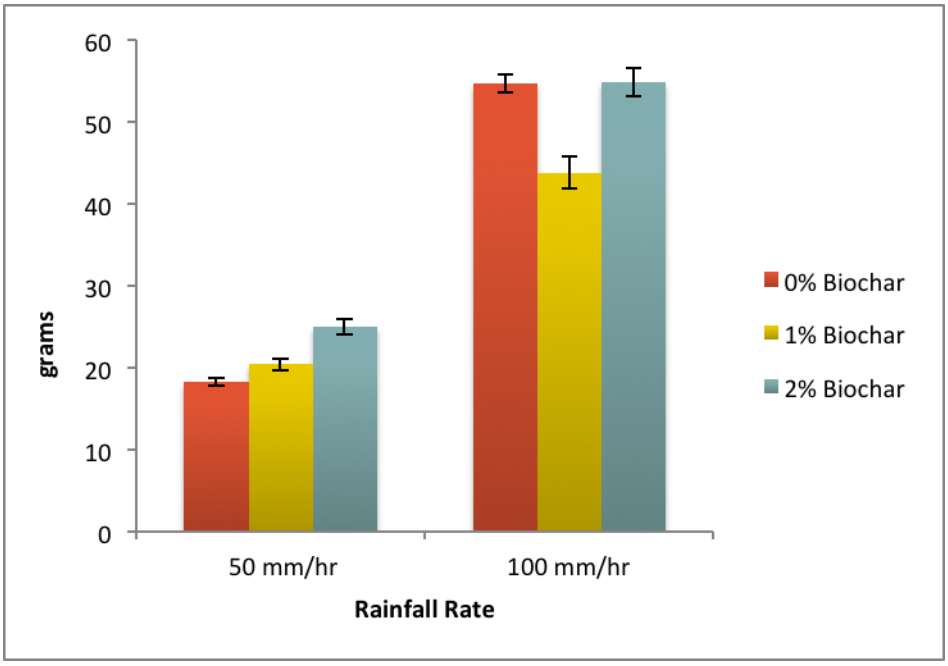


Figure 3.9 Sum of sediment loss across a 45-minute simulation between rainfall rates

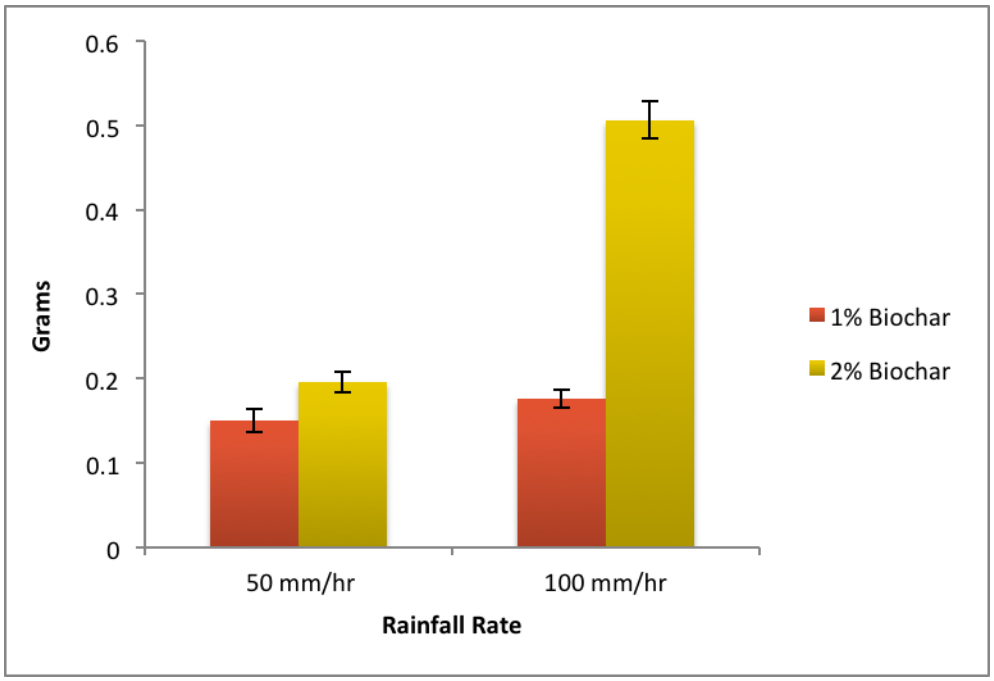


Figure 3.10 Sum of biochar runoff loss across a 45-minute simulation between rainfall rates

Table 3.6 Percent Total Recoveries of Lincomycin by Treatment

	Sediment Runoff	Water Runoff	Biochar Runoff	Soil in Boxes	Drainage	Biochar in Boxes	% Recovery
0% Biochar 50mm/hr	0.08	0.48		5.02	0.25		5.82
1% Biochar 50mm/hr	0.04	1.13	0.0	0.91	1.16	0.0	3.23
2% Biochar 50mm/hr	0.11	0.52	0.0	0.88	1.27	0.0	2.78
0% Biochar 100mm/hr	0.34	0.88		8.22	1.65		11.1
1% Biochar 100mm/hr	0.15	0.92	0.02	9.10	2.00	0.03	12.2
2% Biochar 100mm/hr	0.28	0.99	0.03	5.97	1.69	0.03	8.98

CHAPTER 4. CONCLUSION

The biochar used in this study, having a moderate surface area of 123 m^2 g^{-1}, had low sorption capacities for all antibiotics used and may not be the best indicator of how the antibiotics could have adsorbed to biochar in the environment. Recoveries for each antibiotic for all treatments were quite low which could imply permanent adsorption to soil (Jeong et al., 2012). Additionally, the soil used in this study was a mollisol, a soil characterized by high amounts of organic matter the antibiotics may have had more of a propensity to adsorb to than the biochar. Regardless, the loss of biochar via runoff was very low at less than 0.4% of what was initially added for all treatments and combined with the fact that the addition of biochar did not decrease sediment loss or water runoff relative to the controls shows that adding biochar had little effect of potential antibiotic mitigation under the conditions of this study. For all treatments, rainfall rate had more of an effect on surface and antibiotic losses than biochar rates, with 100 mm/hr rainfall producing greater losses than 50 mm/hr. Biochar may serve a better purpose in localized areas of high drainage where it can filter contaminants before entering waterways, such as blind inlets. Incorporating biochar into the soil on a field scale leaves biochar susceptible to erosion and could act as an additional pathway for antibiotics to enter waterways especially if the antibiotics do not have enough time to degrade within the soil before an erosion event. However, with such small losses of biochar in our study even if a rainfall event occurred the antibiotic loss due to facilitated transport to biochar may be negligible.

CHAPTER 5. FUTURE WORK

The experiment was complex in that there were a few unknowns, such as how tylosin and lincomycin would compete in the same mixture and little to no research has been done previously on how monensin interacts with biochar. Additionally, the relationship between lincomycin, monensin, and tylosin and their environment, including clays, organic matter, and water needs to be investigated more thoroughly. The use of a prairie soil with moderate organic matter and low clay content may not have been the most ideal choice when studying the effects of biochar in soil. Biochar classically has been applied to soils that are "poor" in soil health whether by having low nutrient holding capacity, water holding capacity, and/or low organic matter. Adding an organic matter source to a soil that already had "good" soil health may not have been beneficial in seeing the full potential biochar has in retaining contaminants that enter the soil. Additionally, by not using manure in this experiment we excluded material of application plus an additional organic matter source for the antibiotics to sorb to. By applying antibiotics directly to the soil it is possible the loss of antibiotics was overestimated compared to what would typically happen on a farm. The antibiotics may have also not had enough time to reach equilibrium onto the soil and biochar surface as there was only a time difference of fifteen minutes between when the antibiotics were applied and when the rainfall simulation began. Previous rainfall simulation studies (Davis et al., 2006; Kim et al., 2010) applied antibiotics at least one hour before starting the simulation. If we had extended the time between applying the antibiotics and the simulation there may have more time for the antibiotics to sorb to the soil or even to the biochar.

In future studies, sorption capacity and isotherm experiments for each individual antibiotic with biochar and soil should be conducted before rainfall simulations begin to assess

the sorption of each antibiotic to soil and biochar without a competition factor between antibiotics. The isotherms for this experiment were a mixture of all three antibiotics and therefore competition between lincomycin and tylosin for sorption sites was observed. No sorption capacity or kinetic experiments were conducted for the antibiotics individually in this experiment. Additionally, isotherm studies with a mix of just lincomycin and tylosin would have been beneficial to confirm competition between two cationic compounds for sorption sites on soil and biochar.

Biochar particle size in this experiment was less than 4mm, leaving in dust-sized particles that have greater surface area than larger particle sizes and those particles could have been bound to soil during analysis. Narrowing the size spectrum of biochar would give a better understanding of what size of biochar the antibiotics were adsorbing to and eliminating dust-sized particles would have reduced the possibility of these particles being analyzed with soil.

Lastly, extraction procedures for lincomycin and an additional analysis of DOC in water may improve the explanation of where antibiotics were located within the experiment. The solvent mixture used in the ASE (65:15:20 acetonitrile:water:methanol) should have extracted most of lincomycin from the soil since lincomycin is a polar molecule; however, a mixture of using ASE and a $CaCl_2$ desorption shake may have been the best method to extract all lincomycin from exchange sites, granted there being little to no permanent sorption to soil. $CaCl_2$ is known to replace or block lincomycin on cation exchange sites (Wang et al., 2009; Wang et al., 2012) and could be an additional effective method to remove lincomycin from cation exchange sites. Additionally, measuring the DOC in both water runoff and in drainage and analyzing if monensin was adsorbed to this DOC will be necessary. Previous research (Sassman and Lee, 2007) noted that if monensin is detected in water it is most likely bound to DOC, however, we

did not directly measure monensin sorption to DOC and its possible that monensin was in fact dissolved in water especially considering the high recovery of monensin found in drainage.

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APPENDIX

Table A1. Means and Standard Deviations of Rainfall Rate, Biochar Rate, and Rainfall Rate x Biochar Rate Interaction in Soil from Boxes (μg)

Rainfall Rate	N	Lincomycin		Monensin		Tylosin	
		Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
50	9	22.67[b]	28.32	89.17[b]	98.54	107.6	27.82
100	9	77.63[a]	20.54	171.5[a]	60.93	102.6	19.4

Percent Biochar	N	Lincomycin		Monensin		Tylosin	
		Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
0	6	66.18	32.07	74.05	75.47	112.9	23.02
1	6	50.02	46.24	154.3	85.84	98.58	25.32
2	6	34.25	29.98	162.6	93.64	103.8	23.8

Rainfall Rate	Percent Biochar	N	Lincomycin		Monensin		Tylosin	
			Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
50	0	3	50.15	38.75	5.558	0.842	128.76	11.04
50	1	3	9.088	1.282	88.76	43.08	87.18	28.04
50	2	3	8.768	2.164	173.18	126.13	106.94	29.81
100	0	3	82.21	17.29	142.55	12.81	97.03	21.16
100	1	3	90.96	17.77	219.9	60.55	109.99	20.64
100	2	3	59.73	17.15	151.93	75.34	100.73	22.33

Table A2. Means and Standard Deviations of Rainfall Rate, Biochar Rate, and Rainfall Rate x Biochar Rate Interaction in Drainage (μg)

Rainfall Rate	N	Lincomycin		Monensin		Tylosin	
		Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
50	9	8.922[b]	7.587	52.71	47.8	0.297[b]	0.241
100	9	17.56[a]	8.718	87.17	51.36	1.591[a]	0.826

Percent Biochar	N	Lincomycin		Monensin		Tylosin	
		Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
0	6	10.29	9.453	38.01[b]	48.81	0.64	0.573
1	6	17.43	9.434	101.3[a]	54.02	1.304	1.169
2	6	12	8.314	70.51[ab]	35.23	0.889	0.852

Rainfall Rate	Percent Biochar	N	Lincomycin		Monensin		Tylosin	
			Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
50	0	3	2.467	1.891	2.008	1.809	0.148	0.139
50	1	3	11.56	6.176	77.01	44.67	0.306	0.318
50	2	3	12.74	9.681	79.12	36.81	0.438	0.219
100	0	3	18.12	6.005	74.02	45.42	1.132	0.275
100	1	3	23.3	8.995	125.6	59.42	2.301	0.577
100	2	3	11.25	8.797	61.9	39.05	1.34	1.076

Table A3. Means and Standard Deviations of Rainfall Rate, Biochar Rate, and Rainfall Rate x Biochar Rate Interaction in Biochar from Boxes (µg)

		Lincomycin		Monensin		Tylosin	
Rainfall Rate	**N**	**Mean**	**Std Dev**	**Mean**	**Std Dev**	**Mean**	**Std Dev**
50	81	ND		11.75	9.068	0.072	0.176
100	80	ND		36.9	16.92	0.145	0.31

		Lincomycin		Monensin		Tylosin	
Percent Biochar	**N**	**Mean**	**Std Dev**	**Mean**	**Std Dev**	**Mean**	**Std Dev**
1	54	0.263	0.302	19.83[b]	18.92	0.088	0.172
2	53	0.283	0.272	28.82[a]	18.49	0.129	0.316

			Lincomycin		Monensin		Tylosin	
Rainfall Rate	**Percent Biochar**	**N**	**Mean**	**Std Dev**	**Mean**	**Std Dev**	**Mean**	**Std Dev**
50	1	27	ND		5.657	0.246	0.144	0.249
50	2	27	ND		17.84	9.705	0	0
100	1	27	ND		34	17.09	0.033	0.057
100	2	26	ND		39.79	19.98	0.258	0.447

ND: Not detected; No lincomycin was recovered in 50 mm/hr biochar samples therefore a t-test was used instead of 2-way ANOVA (see table 9.4)

Table A4. Lincomycin T-Test for Rainfall Rate 100 mm/hr (µg)

Percent Biochar	N	LSMEAN	95% CI
1	3	0.263	(-0.488, 1.014)
2	3	0.283	(-0.393, 0.959)

Table A5. Percent Total Recoveries of Tylosin by Treatment

	Sediment Runoff	Water Runoff	Biochar Runoff	Soil in Boxes	Drainage	Biochar in Boxes	% Recovery
0% Biochar 50mm/hr	0.21	0.0		12.9	0.02		13.1
1% Biochar 50mm/hr	0.14	0.0	0.02	8.72	0.03	0.01	8.9
2% Biochar 50mm/hr	0.41	0.01	0.01	10.7	0.04	0.0	11.2
0% Biochar 100mm/hr	1.17	0.02		9.70	0.08		10.9
1% Biochar 100mm/hr	0.68	0.03	0.04	11.0	0.12	0.0	11.9
2% Biochar 100mm/hr	1.48	0.03	0.01	10.1	0.19	0.03	11.8

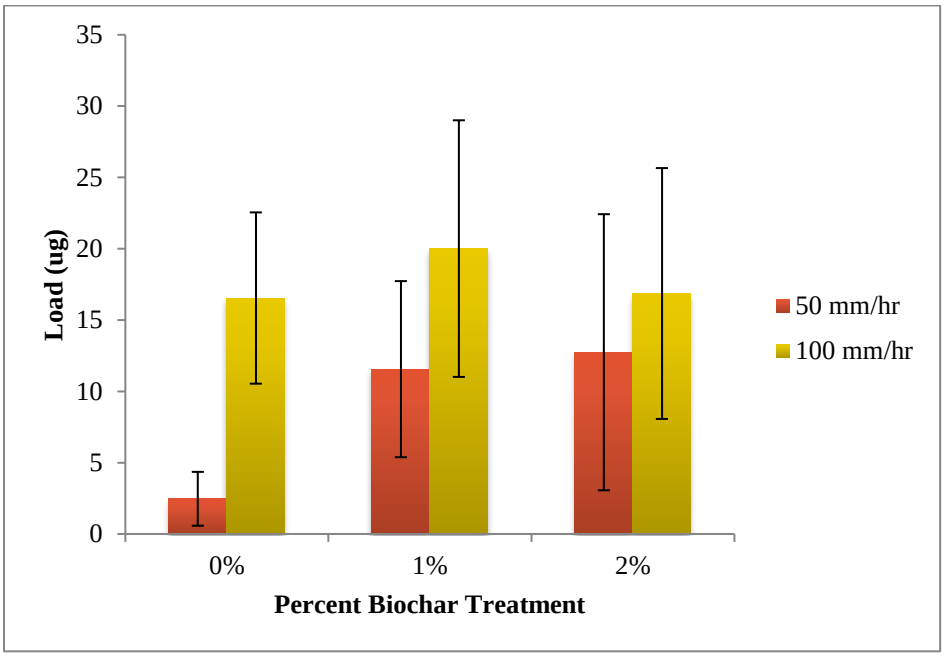

Figure A1. Average lincomycin load in drainage at 50 and 100 mm/hr rainfall rate

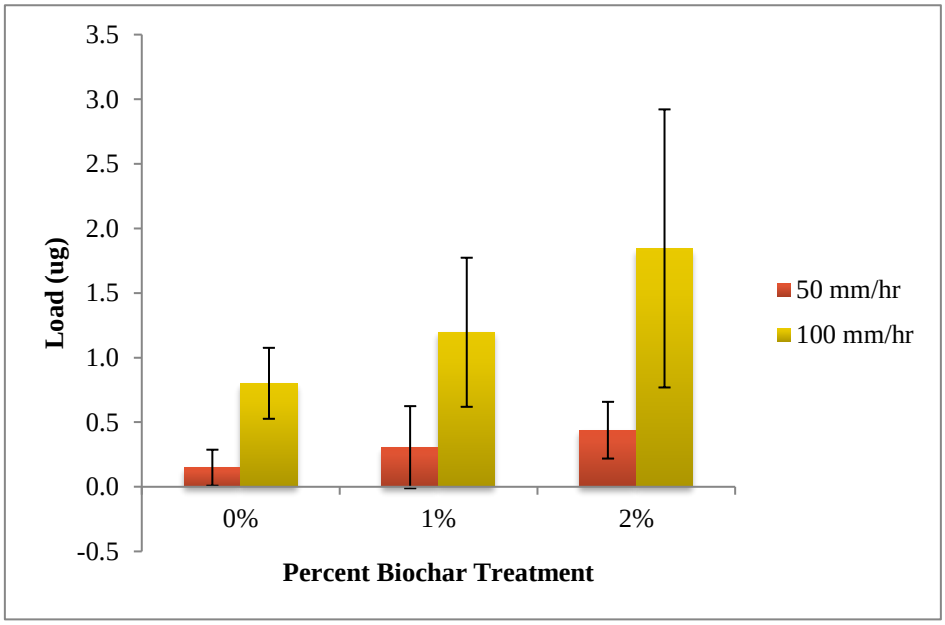

Figure A1. Average tylosin load in drainage at 50 and 100 mm/hr rainfall rates

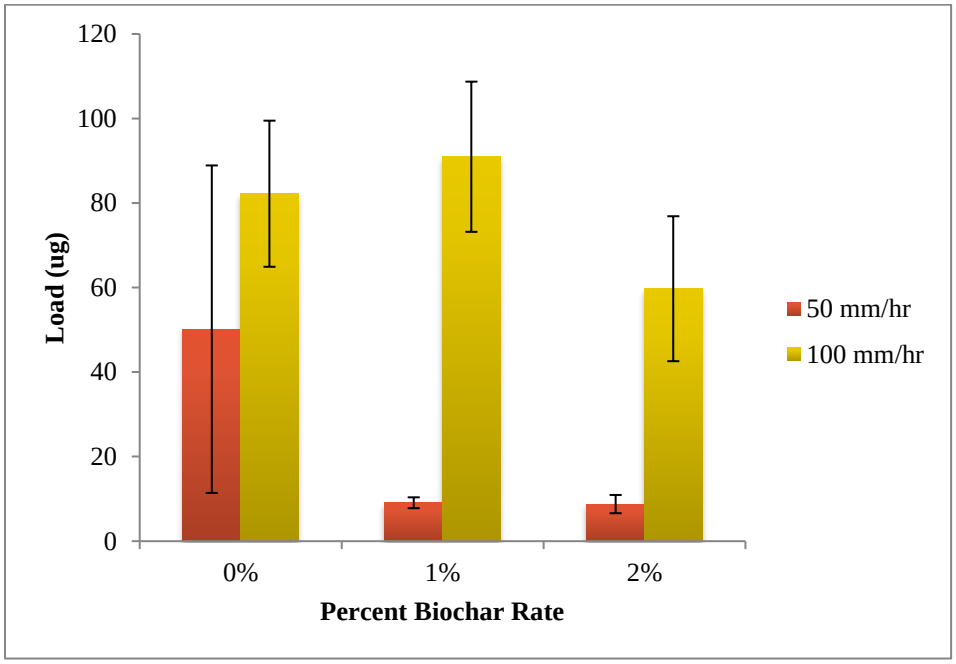

Figure A3. Average lincomycin load in soil from boxes at 50 and 100 mm/hr rainfall rates

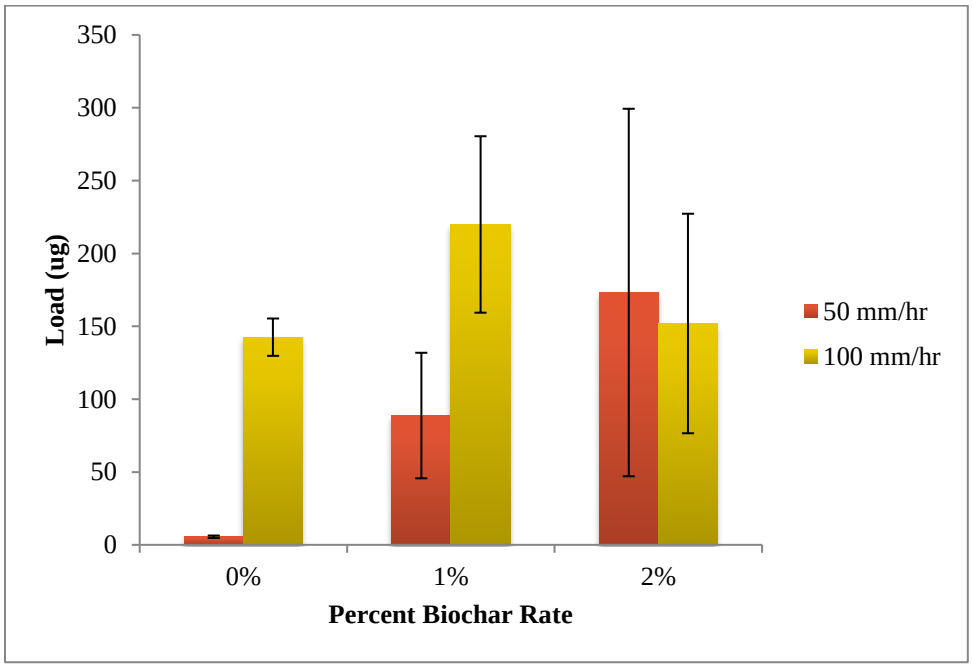

Figure A4. Average monensin load in soil from boxes at 50 and 100 mm/hr rainfall rates

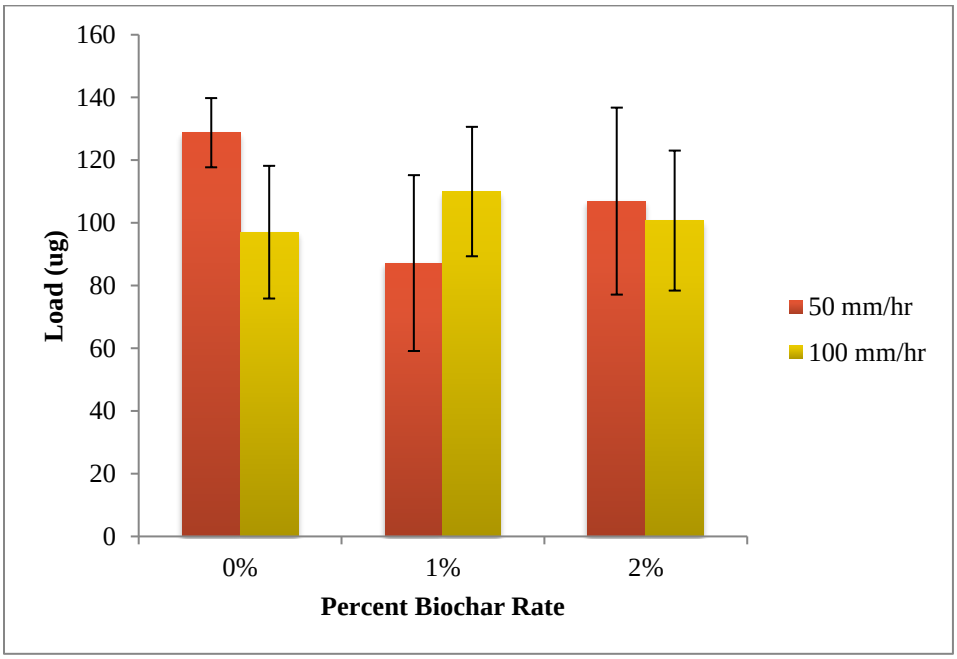

Figure A2. Average tylosin load in soil from boxes at 50 and 100 mm/hr rainfall rates

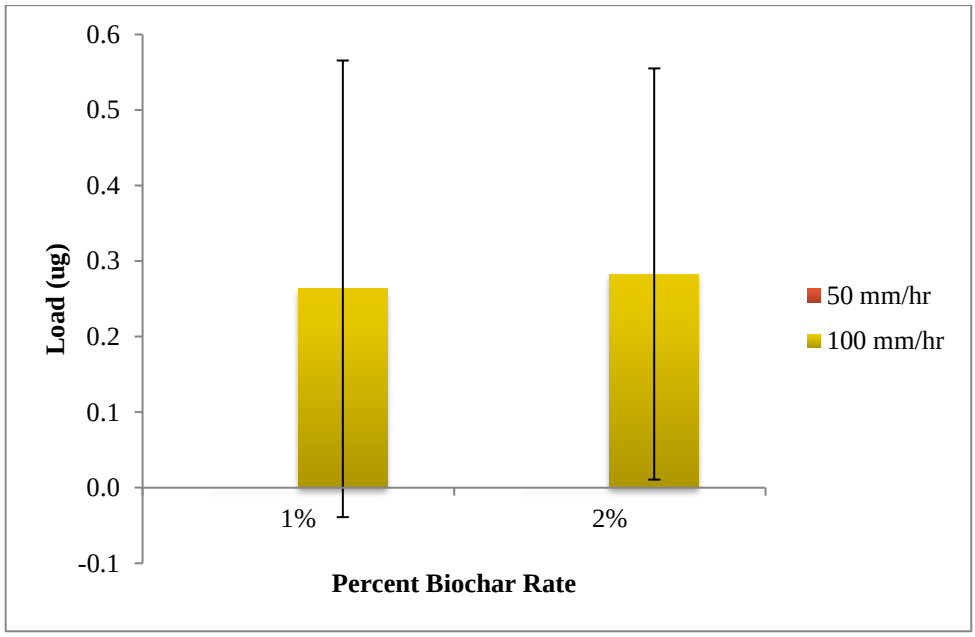

Figure A3. Average lincomycin load in biochar from boxes at 50 and 100 mm/hr rainfall rates

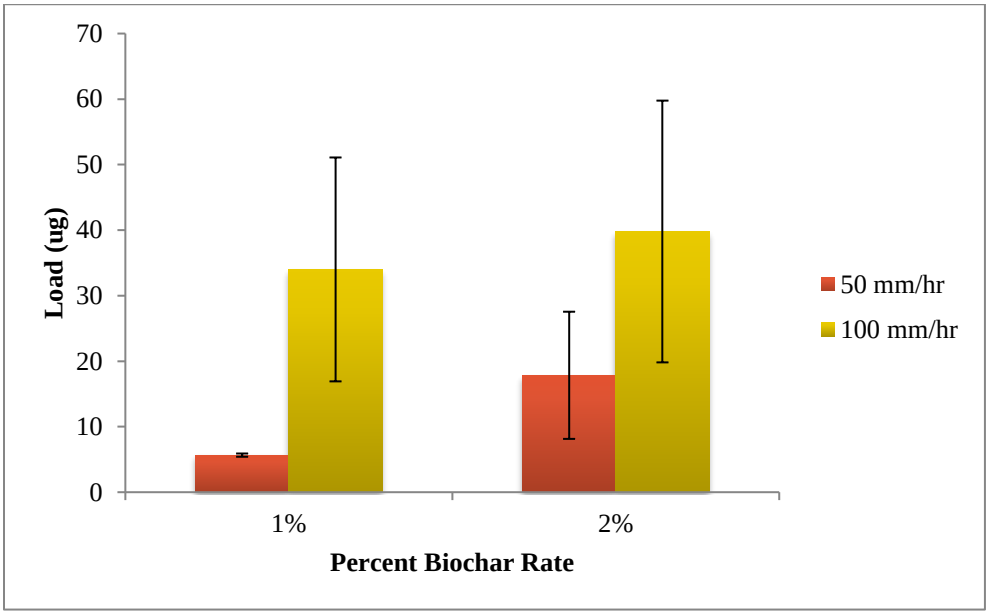

Figure A4. Average monensin load in biochar from boxes at 50 and 100 mm/hr rainfall rates

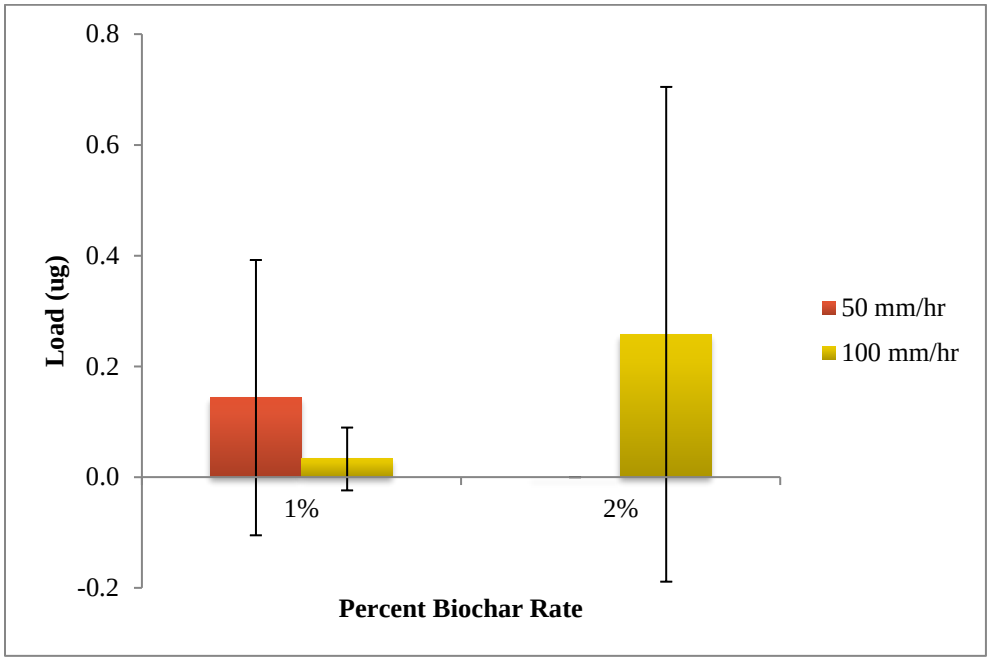

Figure A5. Average tylosin load in biochar from boxes at 50 and 100 mm/hr rainfall rates

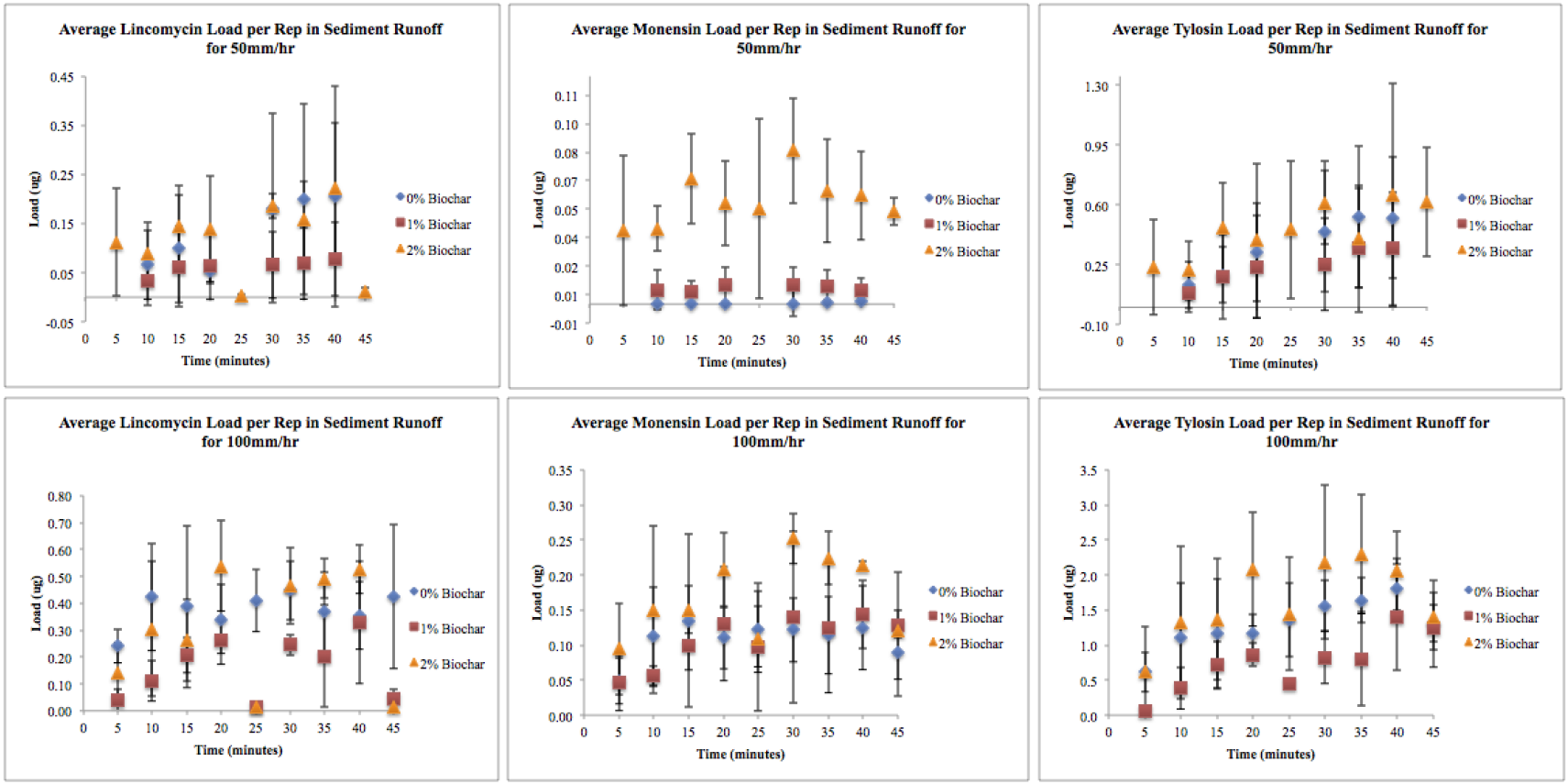

Figure A9. Cumulative losses of lincomycin, monensin, and tylosin in surface sediment runoff over the course of the 45-minute rainfall. Samples were collected every five minutes.

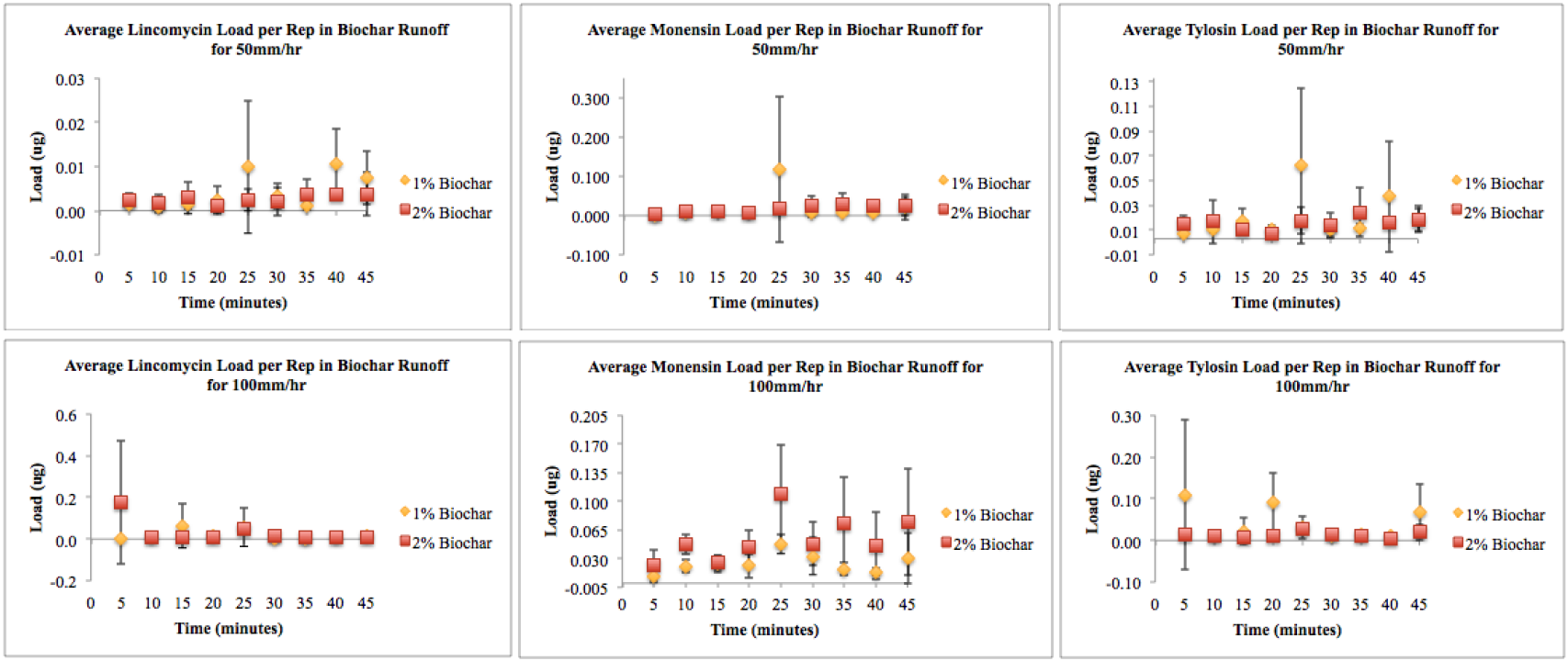

Figure A10. Average load per antibiotic in both 50 and 100 mm/hr rainfall rates for surface biochar runoff, shown with standard deviations.

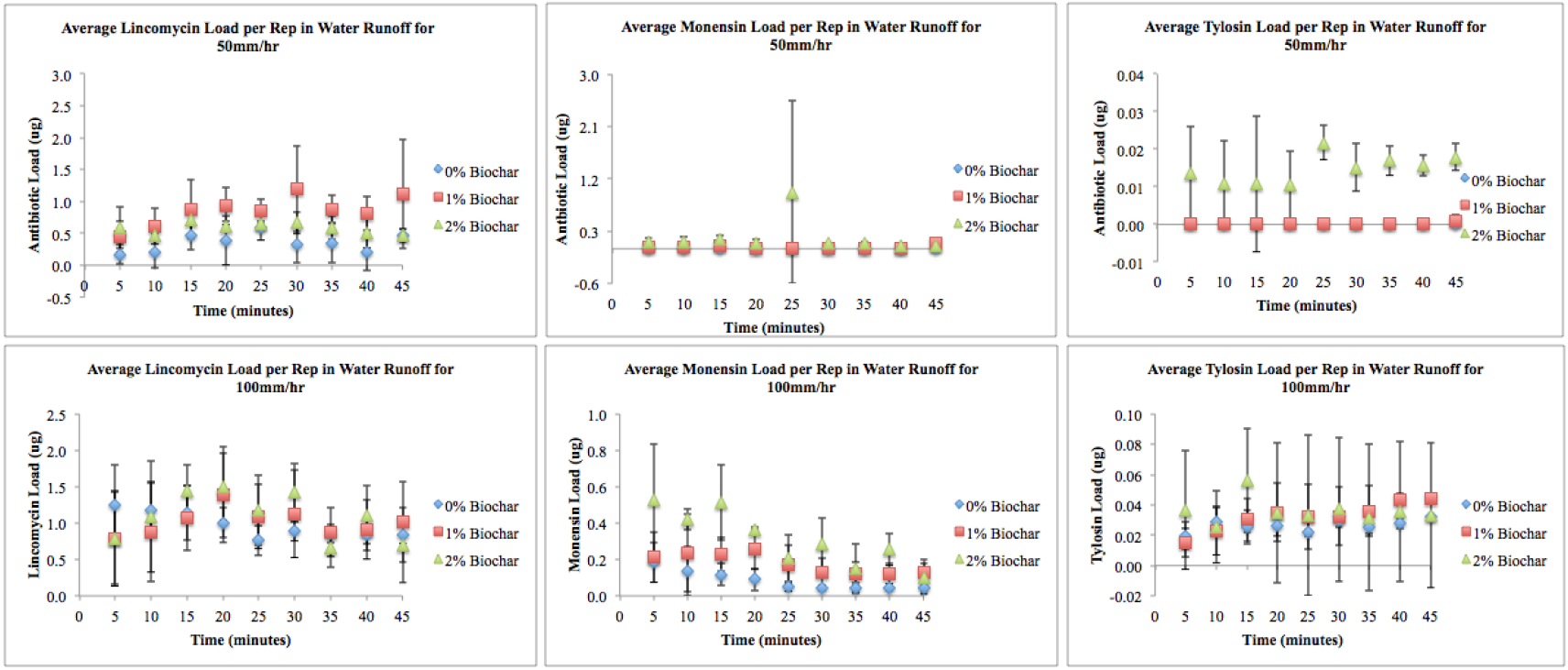

Figure A11. Average load per antibiotic in both 50 and 100 mm/hr rainfall rates for surface water runoff, shown with standard deviations.

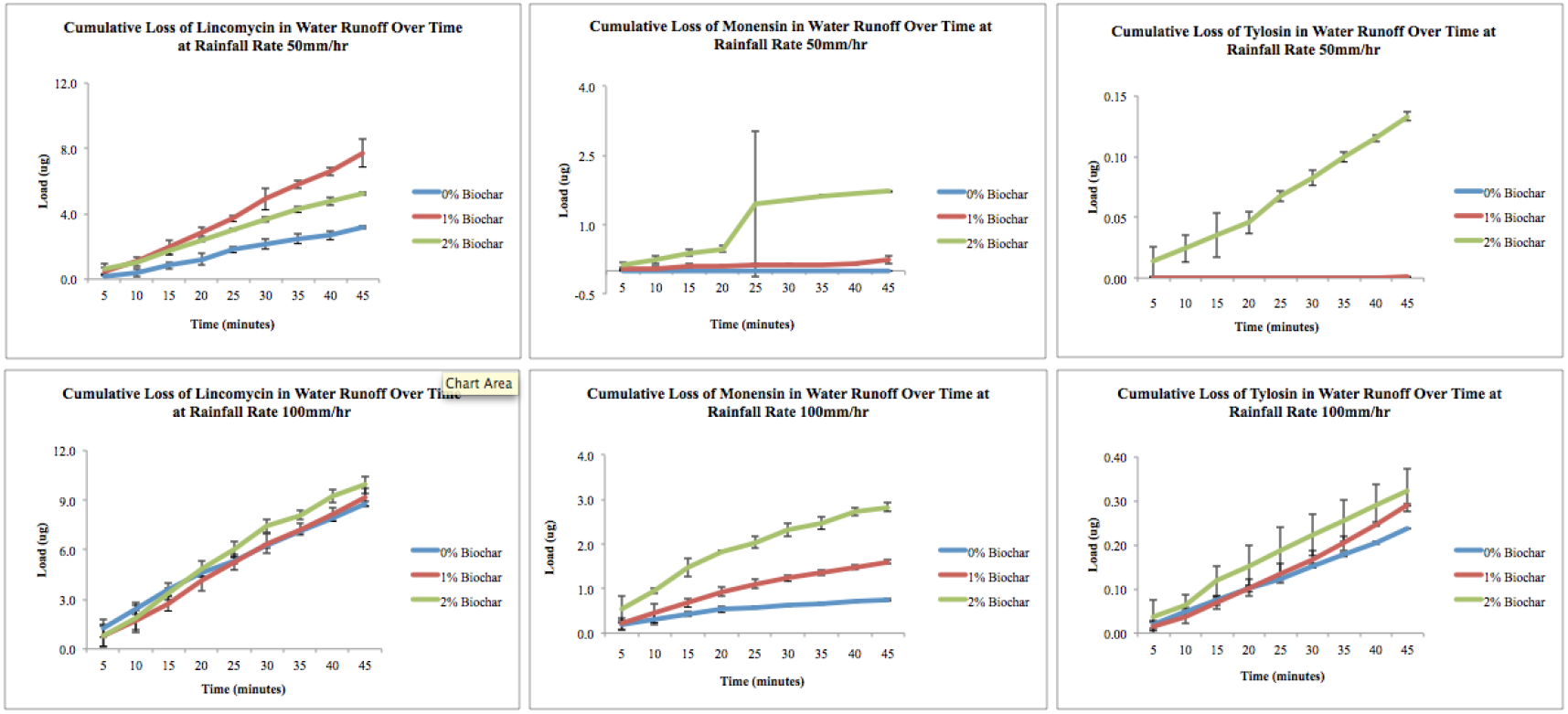

Figure A12. Cumulative losses of lincomycin, monensin, and tylosin in surface water runoff over the course of the 45-minute rainfall. Samples were collected every five minutes.

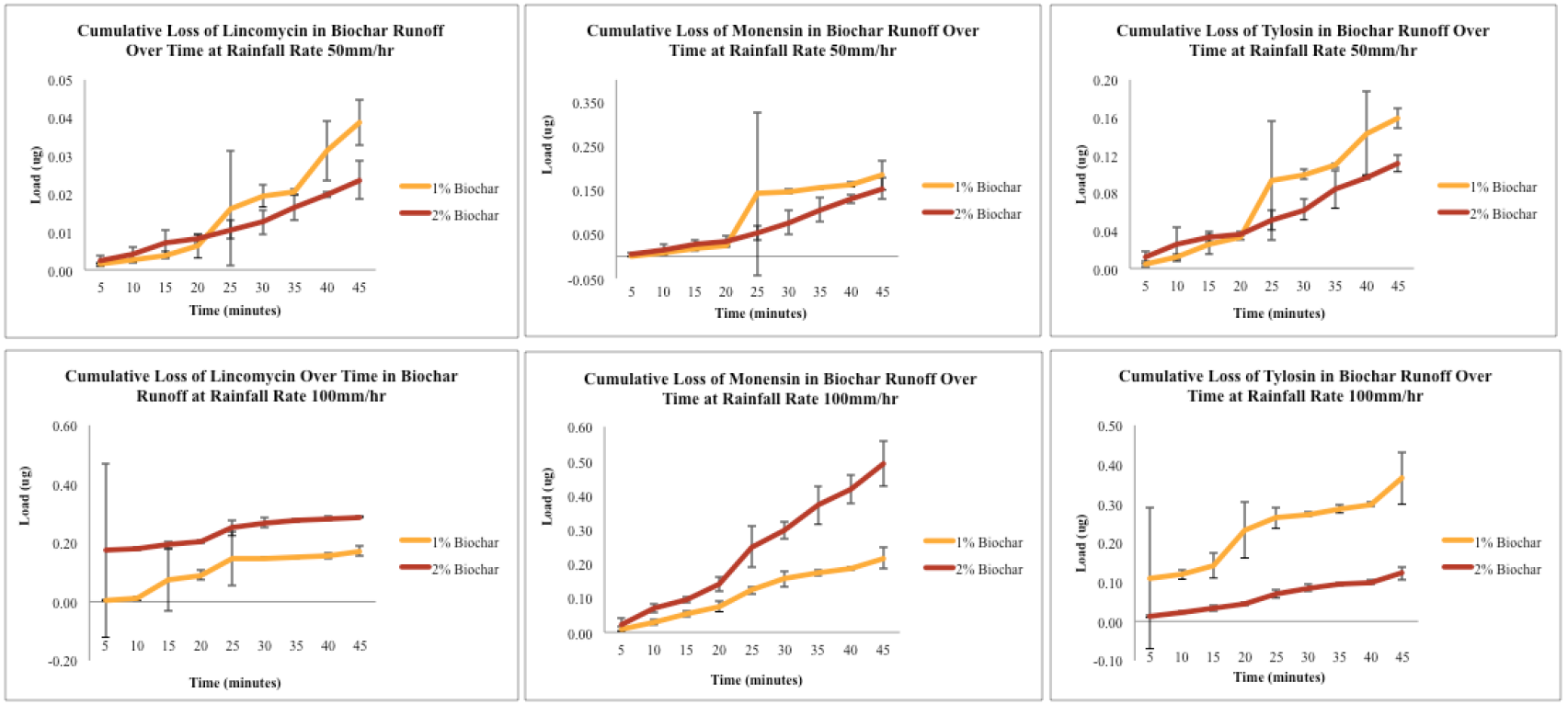

Figure A13. Cumulative losses of lincomycin, monensin, and tylosin in surface biochar runoff over the course of the 45-minute rainfall. Samples were collected every five minutes.

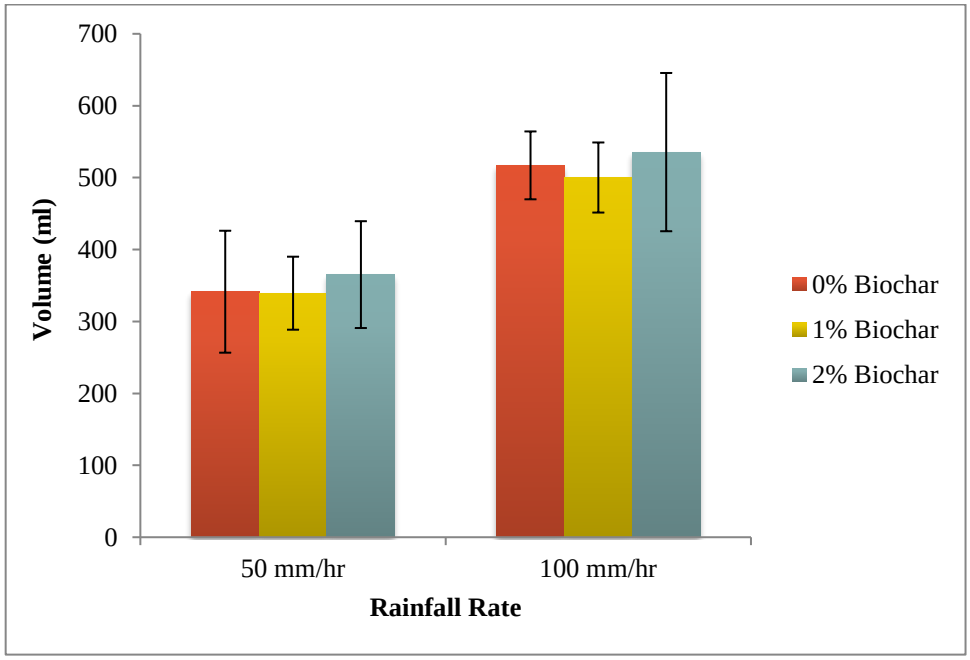

Figure A14. Sum of water runoff loss across a 45-minute simulation between rainfall rates

Figure A15. Infiltration Setup

Figure A16. Runoff Setup