MONENSIN DEGRADATION IN STOCKPILED DAIRY MANURE

A Thesis in
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by
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ABSTRACT

Antibiotics are commonly used in livestock production for growth promotion and parasite control. A significant fraction of the administered antibiotics is excreted in manure rather than being metabolized by the animal. Commonly, livestock manures are stored in lagoons or stockpiled prior to disposal by land application. In recent years, the prevalence of veterinary antibiotics and their fate in the environment has become a topic of concern. The environmental impacts of manure-borne antibiotics have not been fully elucidated. The major concerns are the development of antibiotic resistance and direct toxic effects on organisms in the terrestrial and aquatic environments. The occurrence and fate of monensin in the environment related to dairy manure management has not been fully studied.

Monensin is an ionophore antibiotic used widely in dairy production. Monensin’s classification as a growth amplifier allows it to be considered a marker for agricultural pollution when found in the environment. Therefore, the focus of this research was to quantify the degradation of monensin in stored dairy manure using composting and stockpiling. First, stockpiles were left undisturbed (SP treatments). The second treatment involved turning the piles at day-6 to simulate composting management (CP treatments). Additional turning of piles was initially planned, however, was not done because of pile size and the risk of losing additional pile heat prematurely.

The dairy manure was initially blended with oat straw and wood chips to achieve moisture content and carbon-to-nitrogen ratio of approximately 60% and 28, respectively. These conditions are considered ideal for microbial activity and, in turn, degradation of compounds like monensin. Amended manure was segregated into piles roughly 2.3 m³ (3 yd³) each and the monensin concentration was monitored over a 50-day period. Total monensin (water extractable plus methanol extractable) analysis was conducted using enzyme-linked immunosorbent assay (ELISA) analysis. Pile temperature and organic matter content were also recorded periodically over the experimental period.

A reduction in monensin concentration was observed with time for both treatments. Assuming first-order monensin degradation, the calculated average half-lives were 57.8 d (compost) and 13.6 d (static). Monensin seemed to dissipate by day 10 in the methanol extractable fraction but
the water extractable concentration persisted to day 16. There were only three piles (CP2, CP4, and SP1) that had measurable concentrations of water extractable monensin on the last day of sampling (day 50). Overall, the water extractable form made up 77.9% (compost) and 83.8% (static) of total monensin.

The original intent of this study was not to quantify runoff. But due to heavy rainfall and elevated MC, runoff was observed from both treatments. Monensin was detected in runoff from both treatments. Runoff from the compost treatments averaged 4.04 ng mL\(^{-1}\), while static treatments averaged 3.98 ng mL\(^{-1}\). These values were in the lower range of concentrations reported in literature for runoff containing monensin. Approximately 142 mm of rainfall occurred between sampling days 16 – 50. The runoff volume from the compost was 1,852 L and 1,701 L from the static treatments. A mass balance estimate revealed that runoff losses accounted for < 2 % of monensin remaining at day 16 in both the compost and static treatments. Therefore, even though runoff did occur, the majority of the monensin lost from the piles between days 16 and 50 can be attributed to degradation.

Previous studies have used spiked manure samples which resulted in higher and more easily measured monensin levels. In contrast, this study used manure with monensin at levels actually excreted from dairy cattle. The finding of a longer half-life for the compost treatment was unexpected. It is likely a reflection of the difficulties experienced in measuring monensin at extremely low, but environmentally relevant, levels. These results suggest monensin may be an environmental concern in the aqueous phase. Monensin levels can be degraded using manure management before land application, but piles should be covered to prevent runoff generation. Understanding the degradation behavior of monensin in dairy manure will contribute to our understanding of its fate in environmental systems and could provide insights into practical manure management methods that can be used in dairy production.
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Chapter 1

Background and Justification

Agriculture plays a large contributing role in non-point pollution of both the terrestrial and aquatic environments and this is no different in Pennsylvania. Dairy production is the number one agricultural industry in Pennsylvania and the state ranks fifth nationally in total milk production. As with other livestock types, manure handling, storage and application can be troublesome in production operations. Thus, proper manure management is one of the major issues currently facing the dairy industry.

The use of pharmaceuticals, specifically antibiotics in veterinary and human medicine, and their subsequent effects on the environment are now emerging issues. Antibiotics are poorly absorbed in the body of dairy animals resulting in the majority (40% – 90%) of the administered compound being excreted in urine or feces, which end up in manure (Kumar et al., 2005). Antibiotic concentrations found in the manure range from trace levels to > 200 mg kg\(^{-1}\), with typical concentrations ranging anywhere from 1 mg kg\(^{-1}\) – 10 mg kg\(^{-1}\) (Kumar et al., 2005). As in many countries, manure in the U.S. is often land applied as a source of nutrients for crops and as a means of disposal.

Manure is rich in nitrogen (N), phosphorus (P), potassium (K) and micronutrients, making it a valuable resource for crop production. However, manure that is improperly managed poses a burden to the farming operation and can be problematic to aquatic environments. In the U.S., land application of manure is the most widespread method of disposal with storage in lagoons or pits being other viable options. It is estimated that in dairy production, more than 90% of manure is stockpiled as a temporary management technique (Meyer, 1997). According to the 2007 Agricultural Census, there were over 22 million acres of farmland treated by manure, a slight decrease from the 2002 Agricultural Census (USDA-NASS, 2007). The occurrence of soil, sediment, or crop contamination and pollution of surface and groundwater are possible with land application as a disposal method. For example, cases exist in which manure contaminated with antibiotics from Concentrated Animal Farming Operations (CAFOs) have reached both surface and groundwaters (CDC, 1998). In 2002, a reconnaissance study conducted on rivers across the United States found that approximately 80% of streams contained antibiotics and
hormones (Koplin et al., 2002). With such a large area of agricultural land used for manure application, the risk of antibiotics or other pharmaceuticals entering the environment is large.

The exposure routes of these pharmaceuticals vary depending on their source. Pharmaceuticals that are present in agricultural areas typically arise from two sources: veterinary pharmaceuticals administered to livestock and pharmaceuticals introduced to lands via application of biosolids from wastewater treatment plants (WWTP). Several of the larger municipalities such as Chicago, Houston, Washington D.C., and Milwaukee have biosolids land application programs.

The antibiotics used in animal production are excreted in manure. By definition, manure is fresh feces and urine (ASAE Standards 2005). The manure is then directly applied to agricultural fields or held in a storage tank until application. Once applied the antibiotics in the manure will either degrade or persist in the soil, run off in surface water, leach into groundwater, or be taken up by crops. Once applied to the soil, most antibiotics become unstable, but this depends on the antibiotic itself as well how long it takes for the antibiotic to degrade and binding capabilities of the antibiotic and the soil. According to Venglovsky et al., (2009), “Antibiotics that bind strongly to soil and have shorter half-lives can be completely degraded within the soil and are not usually detected in ground water, surface water, or plants. For those antibiotics that bind strongly to soil yet have long half-lives, there is also a concern that these drugs could be taken up by plants.”

The foremost issue regarding the use of antibiotics in animal production is the development of resistant bacterial strains, which represents a health risk to both humans and animals. According to the Center for Disease Control (CDC), bacteria become resistant to antibiotics through several mechanisms: sharing of genetic information, ability to neutralize an antibiotic, and ability to change the antibiotic attack site by mutation (CRS, 2010).

The antibiotic of concern in this study is monensin ($C_{36}H_{62}O_{11}$). Monensin is a sub-therapeutic antibiotic used predominately in dairy production. Studies have shown monensin’s potential toxicity to several species of livestock. Though monensin is used strictly in agriculture, the occurrence of bacterial resistance is still a concern in the veterinary field. Monensin presents no health risk as far as bacterial resistance to humans, however monensin is very toxic to humans if ingested (ELANCO Animal Health, 2010).
There exist several routes by which humans can possibly be exposed to antibiotic residues. The first route involves the consumption of crops that have accumulated antibiotic residues from soil amended with contaminated manure. The next route occurs through the consumption of livestock that have accumulated antibiotic residues through the food chain. Last, but not least, the consumption of contaminated surface or groundwater by humans is another concern. Even though strenuous monitoring of food from treated livestock is required, the health impacts of the aforementioned exposure routes have not been quantified. If these foods are ingested, there could possibly be some allergic or toxic reactions to a particular antibiotic. This especially may be the case when a person is using one antibiotic therapeutically and ingest another antibiotic through residues within food and water.

This project is primarily concerned with the fate of sub-therapeutic pharmaceuticals. Since land application is used as a disposal method for manure, one means of reducing the environmental impact of manure-borne antibiotics is to treat the manure prior to land application. One practical treatment solution is composting. This proposed research will focus on the degradation of monensin through composting compared to stockpiling as the conventional manure management option. Currently only a few studies (Cessna et al., 2011; Ramaswamy et al., 2010; Dolliver et al. 2008a, 2008b; Storteboom et al. 2007) have investigated antibiotic degradation of livestock manures during composting. This research will focus on the degradation of monensin in dairy manure.
Chapter 2

Literature Review

The occurrence and fate of sub-therapeutic antibiotics in the environment and their potential environmental risk has become a topic of concern in recent years. Antibiotics are used predominately as growth promoters in livestock production. Most of the antibiotics are administered to healthy livestock. These antibiotics are not fully absorbed by livestock and are excreted into manure. Animal manure application to agricultural lands is a common manure management practice. The concern lies with the introduction of antibiotics into the environment by different pathways could have adverse effects on the terrestrial and aquatic environments. The occurrence, transport and fate of several of these antibiotics have been documented, but further research is required. According to Kumar et al. (2005), Barsaraba et al. (1999) stated: “ionophore antibiotics such as monensin, that are specifically used in agriculture have been documented as causing adverse reactions when used in conjunction with other antibiotics.” Finding monensin in the environment can be directly linked as an indicator of agricultural pollution. A study to evaluate the performance of composting versus stockpiling of dairy manure to determine conditions necessary for monensin degradation could provide insights into composting as a practical method for mitigating the adverse effects of monensin in the environment.

2.1 Background

2.1.1 Use of Antibiotics in Dairy Production

With the increasing world population, diminishing agricultural lands, and the escalating demand for food, livestock production has become more intensive. This intensive production has led to the increased use of antibiotics as a common practice with livestock production. In the United States, 12.6 thousand metric tons of antibiotics were sold for animal use in 2007 (Watanabe et al., 2010). Antibiotics are drugs that are used to block, inhibit the growth of, or kill bacteria. Antibiotics are a category of antimicrobials and are derived from naturally occurring microorganisms. These microorganisms have the ability to produce natural compounds, which interfere with or kill competing microbes. Most antibiotics
mimic these actions by different pathways. The cause for concern with antibiotic use in livestock production is the potential for antibiotic resistance. This resistance could occur through resistant bacteria being transferred through the environment or by ingestion of livestock. The use of these antibiotics and their effect on the environment has become an emerging issue that still requires much research.

According to McAllister et al. (2001), Foley et al. (1946) stated: “antibiotics have been used in livestock production for over 50 years” and reported 1946 as the first recorded use of antibiotics in dairy cattle for treatment of mastitis. Antibiotic use is classified under three treatment categories in dairy production; therapeutic, sub-therapeutic and prophylactic. Therapeutic treatment addresses an existing condition, sub-therapeutic is used to amplify production, and prophylactic treatment is used during times of high disease risk. Most antibiotics in dairy production are administered therapeutically but the focus of this paper will be on ionophores, which are administered as sub-therapeutic treatments. The most accepted theory on antibiotic resistance is that sub-therapeutic (long-term, low dose) use of antibiotics will more likely stimulate antibiotic resistance (McAllister et al., 2001). Since low doses are used, this allows more bacteria to withstand the initial antibiotic application. This greatly increases the chances of continued bacterial growth due to the low antibiotic concentrations. Also, the effectiveness of an antibiotic is reliant on the physiological characteristics of the target bacteria. For example, using an antibiotic on a bacterial infection for which it is not intended may induce antibiotic resistance by that target bacteria.

In dairy production, several common families of antibiotics are prevalent. These families include ionophores (monensin, lasalocid, salinomycin), macrolides (erythromycin and tylosin) tetracyclines and the more commonly known penicillins (penicillin G, ampicillin and cloxacillin). Ionophores, macrolides and tetracyclines all derive from the coagulase-negative staphylococci family of pathogens, which are associated with mastitis. The penicillins derive from the *Penicillium spp.* As mentioned, mastitis was the first disease treated by antibiotics in dairy production. Mastitis is still a common disease today in most dairies. This further supports that no antibiotic is completely effective in killing bacteria because of the complexity of the bacterial communities in agricultural settings. Antibiotics can only mediate most diseases and the survival of most bacteria in the environment will continue.

- “Mastitis was the most common disease in cows for which antibiotics were used. Cows with mastitis were treated with antibiotics by about 85 percent of operations.”
- “About 60 percent of operations used antibiotics to treat pre weaned heifers for disease, primarily respiratory disorders and diarrhea or other digestive problems.”
- The use of ionophores by U.S. dairy operations remained the same from 2002 to 2007 at 45 percent.

The NAHMS report consisted of data from 17 of the nation’s major dairy production states including Pennsylvania. These 17 states represent 79.5% of all U.S. dairy operations and 82.5% of all dairy cows in the U.S. Table 2.1 lists some of the other diseases common in dairy production and the causative bacteria.
Antibiotics in the dairy industry are largely used for growth promotion and to increase feed efficiency. These antibiotics are administered through direct injection or through the livestock feed. The latter method is the most common and efficient method when treating large groups of livestock. The literature shows that antibiotics are not absorbed efficiently by livestock. As quoted by Dolliver et al. (2008) “Most antibiotics are partially metabolized by animals” (Kumar et al. 2005). It has been reported

<table>
<thead>
<tr>
<th>Condition</th>
<th>Causative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Common</strong></td>
<td></td>
</tr>
<tr>
<td>Bovine Respiratory Disease</td>
<td>Pasteurella haemolytica</td>
</tr>
<tr>
<td>(Pneumonia)</td>
<td>Pasteurella multocida</td>
</tr>
<tr>
<td></td>
<td>Haemophilus somnus</td>
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<tr>
<td></td>
<td>Mycoplasma bovis</td>
</tr>
<tr>
<td>Enteric Disease</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>(Diarrhea)</td>
<td>Clostridium perfringes</td>
</tr>
<tr>
<td></td>
<td>Salmonella spp.</td>
</tr>
<tr>
<td>Mastitis</td>
<td>Staphylococcus aureus</td>
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<tr>
<td></td>
<td>Streptococcus agalactiae</td>
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<tr>
<td></td>
<td>Streptococcus spp. (environment)</td>
</tr>
<tr>
<td></td>
<td>Klebsiella / E. coli / Enterobacter</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas spp.</td>
</tr>
<tr>
<td></td>
<td>Actinomyces pyogenes</td>
</tr>
<tr>
<td>Foot Rot</td>
<td>Fusobacterium necrophorum</td>
</tr>
<tr>
<td></td>
<td>Bacteroides nodosus</td>
</tr>
<tr>
<td>Metritis</td>
<td>Actinomyces pyogenes</td>
</tr>
<tr>
<td>(Uterine infection)</td>
<td>Fusobacterium necrophorum</td>
</tr>
<tr>
<td></td>
<td>Bacteroides spp.</td>
</tr>
<tr>
<td>Ocular (Pink eye)</td>
<td>Moraxella bovis</td>
</tr>
<tr>
<td><strong>Less common</strong></td>
<td></td>
</tr>
<tr>
<td>Lumpy Jaw</td>
<td>Actinomyces bovis</td>
</tr>
<tr>
<td>Listeriosis</td>
<td>Listeria spp.</td>
</tr>
<tr>
<td>Anaplasmosis</td>
<td>Anaplasma marginale</td>
</tr>
<tr>
<td>Tetanus, blackleg</td>
<td>Clostridium spp.</td>
</tr>
<tr>
<td>Wooden tongue</td>
<td>Actinobacillus lignieresii</td>
</tr>
</tbody>
</table>

2.1.2 Sources of Dairy Production Antibiotics and Their Exposure Routes

Antibiotics in the dairy industry are largely used for growth promotion and to increase feed efficiency. These antibiotics are administered through direct injection or through the livestock feed. The latter method is the most common and efficient method when treating large groups of livestock. The literature shows that antibiotics are not absorbed efficiently by livestock. As quoted by Dolliver et al. (2008) “Most antibiotics are partially metabolized by animals” (Kumar et al. 2005). It has been reported
that roughly 75% of antibiotics administered to livestock are not absorbed and are excreted in urine and feces (Chee-Sanford et al., 2009).

Manure consist of urine, feces, bedding, spilled feed, water and sometimes soil. Metabolites or antibiotic residues reach terrestrial and aquatic environments by direct or indirect means. These residues are directly applied on the land by excretion onto pastures by livestock and are directly applied with manure application onto agricultural lands. According to the pharmacokinetics and pharmacodynamic parameters of the livestock and the characteristics of the antibiotics or metabolites, the excretion and spread in the environment occurs through different exposure pathways (Kemper, 2008). The antibiotics or metabolites in the manure will either degrade in the soil, remain in the soil profile, run off with surface water, leach into groundwater, or be taken up by crops. Figure 2.1 shows a typical exposure pathway flow chart for antibiotics used in livestock production.

*Figure 2.1: Potential exposure pathways for antibiotics used in animal production (Source: Kemper, 2007).*
2.1.3 Regulation of Antibiotics in Livestock Production

There are limited U.S. regulations on antibiotic use in livestock production compared to other countries. The Center for Veterinary Medicine (CVM), a center under the Food and Drug Administration (FDA) umbrella is in charge of regulating the manufacturing and distribution of drugs for all animals, including livestock. The CVM has authority to approve new animal treatments based on a similar criteria used in the approval process for human medicines. In 1996 the Centers for Disease Control (CDC) began a collaborative effort with the FDA and the U.S. Department of Agriculture (USDA) to collect antimicrobial resistance data. This collaborative effort was named the National Antimicrobial Resistance Monitoring System (NARMS) and is aimed at monitoring resistance among food borne bacteria isolated from the human population. The most recent NARMS report released in 2006 and shows an increase in resistance for several bacteria. Even though this collaborative effort exists, currently there are no regulations pertaining to the presence of antibiotics or similar compounds such as hormones in manure (Kumar et al., 2005). The FDA currently approves antimicrobials for disease prevention, disease treatment, disease control, and for growth promotion/feed efficiency. In the U.S. antibiotic use in humans takes precedence over livestock use, human health is the number one priority. The antibiotics that cause resistance to the ones that are used as a “last resort” option in human medicine are starting to come under the greatest scrutiny (McAllister et al., 2001).

In the European Union (EU), some countries like Sweden have banned the use of all sub-therapeutic antibiotics in livestock production. The EU has established several antibiotic resistance monitoring programs as well. In 2006 the EU instituted an outright ban on antibiotics used in livestock as growth promoters. Currently there is an ongoing battle in the U.S. between those who support and those who want to ban antibiotic use in livestock production.

2.2 Scope of Ionophore Antibiotic Problem

Ionophores are classified as carboxylic polyether antibiotics. Ionophores are a part of a subgroup of anticoccodials that are exclusively used for livestock production (i.e. cattle, swine, poultry and small ruminants). In 2009 the Food and Drug Administration’s (FDA) Center for veterinary medicine (CVM) released a report on Antimicrobials sold or distributed for use in food producing animals. In 2009, approximately 3,740,627 kg (8,246,671 lbs) of Ionophores were sold and distributed in the U.S. (FDA-CVM, 2010). The 2009 annual totals represent all approved uses of all dosage forms (injectable, oral, medicated feed, etc) of ionophores actively marketed for food producing species. Ionophores in this
study include (Laidlomycin, Lasalocid, Monensin, Narasin, Salinomycin, and Semduramicin. The FDA has approved 23 different anticoccodial compounds, which are used as prophylactics for preventing outbreaks of coccidiosis (Hansen et al., 2009). Ionophores are used worldwide as feed additives for growth promotion and improved feed efficiency. The most common ionophores used in livestock production are monensin (Rumenson ®), lasalocid (Bovatec ®), laidlomycin (Cattlyst ®) and salinomycin (Sacox ®). Ionophores allow sodium and potassium to pass through cell membranes in exchange for protons, resulting in the disruption of the ion gradient, which causes cell death.

Ionophores have not been thoroughly studied and their widespread use warrants an in-depth investigation to determine their occurrence and fate in the environment. Berton et al. (1982) stated, “The practice of feeding livestock ionophores takes on a particular significance in that it ultimately introduces powerful pharmacological agents into man’s food supply.” From a few studies, ionophores are shown to be emerging contaminants that prevail in soil, surface water, ground water, manure, and sediments. Ionophores have the tendency to exhibit higher toxicities than those of other antibiotics. The LD50 in adult rats for lasalocid and monensin are 100 and 35 mg kg⁻¹, respectively. These values approach that of the well-known poison potassium cyanide, which has a LD50 of 10 mg kg⁻¹ (Sassman and Lee, 2007). As documented by Sassman and Lee (2007), a number of other antibiotics, such as erythromycin and sulfamethazine, when interacting with ionophores, enhance ionophore toxicity.

2.3 Occurrence, Transport, and Fate of Monensin in the Environment

2.3.1 Monensin

Monensin is a polyether ionophore used strictly in animal production. Monensin use accounts for roughly 13% of the sub-therapeutic livestock pharmaceutical usage in the U.S. (Dolliver et al., 2007). According to Purevjav, 2011; monensin is the most widely used ionophore with sales over $ 100 million. The use of ionophores such as monensin has been estimated to have a positive cost-to-benefit ratio, saving the cattle industry approximately $ 1 billion per year (Callaway et al., 2003).

The Streptomyces cinnamonensis microorganism produces monensin. The fermentation of Streptomyces cinnamonensis generates antibiotic factors, i.e.(homologues). Factors have the same
functional group but differ in composition. Monensin generates four factors: A, B, C, and D. Monensin A is the major antibiotic factor of monensin, occurring >90 percent of the time. Monensin has a unique structural makeup, which includes; a free carboxylic acid group, cyclic ethers, and several oxygen containing functions. Its’ unique structural characteristics enable monensin to form cyclic compounds with cations and allows for free movement across biological membranes. It is that free movement which allows monensin to inactivate gram-positive microorganisms by interfering with the ion transport mechanism of microorganisms in the gastrointestinal tract thereby leading to cell death. The free movement across the biological membranes are facilitated by monensin attaching to the bacteria and protozoa (McGuffey et al. 2001). As a result, monensin provides a more hospitable environment for certain microorganisms against bacteria and protozoa. The metabolism of those microorganisms then benefits the cattle, resulting in increased performance in production efficiency.

Utilized mainly as a growth promoter, monensin is also used for the treatment and prevention of coccidiosis. Coccidiosis is a disease caused by parasites in the intestinal tract of livestock with diarrhea being the main symptom. In some cases coccidiosis can be asymptomatic, and in the worst-case scenario can cause death in young livestock. Research has shown that the biological actions of monensin in cattle cause increased efficiency of energy metabolism, increased milk production, reduction of digestive disorders, changes in gas production, and improved protein metabolism. Monensin increases energy metabolism efficiency by diverting hydrogen to other end products. These end products are then able to attract more digestible energy away from fermented organic matter (OM) in the gastrointestinal tract and results in efficient use of feed energy (McGuffey et al., 2001). Monensin has also been proven to increase milk production and decrease milk fat (Odongo et al. 2007, Tedeschi et al. 2003, Phipps et al. 2000). Monensin has the ability to diminish the effects of digestive disorders (bloat, ketosis, etc) in cattle because they are able to effect the end products of fermentation, bacteria, and eating behavior (McGuffey et al., 2001).

Methane is an unavoidable byproduct of OM fermentation in the rumen and ruminants. Research studies indicate monensin has the ability to reduce methane (CH4) in dairy cattle (Odongo et al., 2007; Muller et al., 2006; Tedeschi et al., 2003; and Schelling, 1984). This is accomplished by adjusting the acetate:propionate ratio towards propionate, which decreases methane production (Odongo et al., 2007; Muller et al., 2006; Tedeschi et al., 2003). Protein metabolism is very important in dairy cattle. Monensin allows for decreased protein metabolism, which results in the increased ability for nitrogen (N)
retention in the rumen (Tedeschi et al., 2003; McGuffey et al., 2001; and Schelling, 1984). As a result, there are less N emissions and N found in fecal matter.

Monensin is used in poultry, beef, dairy and small ruminant production. Monensin has been used commercially throughout the world for over 30 years. Monensin was first approved for use on broilers in the U.S. in 1971. Then in 1975, monensin gained approval by the FDA for use in beef cattle production. The FDA recently (2004) approved monensin for use in U.S. dairy production as a feed additive. According to Dolliver et al (2008a), “monensin is administered generally in a dry feed additive mixture at dosages ranging from 5 to 400 grams per ton of feed” (Miller Publishing Company, 2006). Since monensin is orally fed, it is excreted at very high percentage of intake.

2.3.2 Properties of Monensin

Monensin tends to be hydrophobic in nature, which increases its ability to form lipophilic complexes with alkali metals in the soil. Despite this hydrophobic property, monensin has been found in ground water, surface water and river sediments. Carlson and Marbury (2006) concluded that monensin was immobile, since it was not detected below 25 cm in the soil profile after manure application with a 1 mg kg\(^{-1}\) concentration of monensin along with 78 mm of precipitation. But, according to an environmental assessment of Rumensin® (Elanco Products Company, 1989), when monensin is introduced into the environment it has the ability to persist and reach aquifers, due to the slow kinetics of degradation processes like hydrolysis and photolysis.

The sorption coefficient (\(K_d\)) for monensin varies, but all values in the literature (Sassman and Lee, 2007) fall in the range of 0.915 L kg\(^{-1}\) to 78.6 L kg\(^{-1}\). That study was done for various soils at an aqueous phase concentration of 0.05 µmol L\(^{-1}\). The \(K_d\) value reported by Elanco was 9.3 L kg\(^{-1}\) for sandy loam soils. The reported values of the octanol water-partitioning coefficient (log \(K_{ow}\)) of monensin ranges from about 2.8 to 4.2, reflecting its hydrophobic behavior. The water solubility of monensin ranges between 0.85 mg L\(^{-1}\) and 63 mg L\(^{-1}\) at pH between 9 and 7, respectively (Elanco Products Company, 1989).

Several studies have reported on the half-life of monensin in the environment. The half-life of monensin ranges from 2 to 13.5 d in the soil and in fresh manure (Elanco Products Company, 1989; Sassman and Lee, 2007; and Carlson and Marbury, 2006). Dolliver et al. (2008a) concluded that the half-life of monensin in controlled and composted manure treatments ranged from 11 to 23 d, respectively at
an initial concentration of 53 g of monensin in 12 m³ of turkey manure. From the literature, there is no consensus regarding the physiochemical behavior of monensin in the environment, but the half-life studies seem very consistent. Further research is therefore warranted to document the half-life of monensin in composted samples.

2.3.3 Human Health Concerns of Monensin

The potential danger of antibiotics to human and animal health has come to the forefront and cannot be ignored. Due to monensin’s different mode of action in livestock compared to humans, it has never been used as an antibiotic in human healthcare, but accidental ingestion has been reported. According to Sekar and Wu (2006), “monensin residues in foodstuffs and environments may have adverse health effects on human beings because ionophores can exhibit acute pharmacological effects on the cardiovascular systems.” Monensin can be deadly to humans, a boy died of renal failure and myoglobinuria from accidental ingesting monensin (Sekar and Wu, 2006). Confirmed reports indicate that the toxicity of monensin to workers who manufacture, handle, or are regularly exposed to monensin on a continual basis. Current literature on moneninsn use in humans is sparse. The aforementioned cases are the only documented ones thus far.

2.3.4 Occurrence, Transport, and Fate of Monensin and its Impact on the Environment

Few research studies have evaluated the occurrence, transport, and fate of monensin in agricultural environments. As quoted by Watanabe et al. (2008) “monensin is a high priority for detailed risk assessment based on high usage and high toxicity and with an unassessed potential to reach the environment” (Capelton et al., 2008). From the studies that have been conducted, monensin has been discovered in ground water, surface water and sediments (Section 2.2.2). The prevalence of monensin in surface water is due to runoff and leaching through the soil profile (Figure 2.3). When the soil infiltration rate is exceeded by rainfall then overland flow impacts the way water-soluble chemicals migrate.
Figure 2.2: Anticipated exposure routes of monensin (ionophore antibiotic)
(Source: Hansen et al., 2009).

Watanabe et al., (2008) provided some key findings concerning monensin and ground water that can be further investigated. This research study was conducted at two dairies located in the Central Valley of California. The two dairies averaged about 1400 lactating cows, 1300 heifers and 360 dry cows. All of the water used from the dairies was collected into lagoons and later recycled and used to flush freestalls. The hydrology of this area allowed for surface irrigation for agricultural purposes. The ground water levels in this area were very shallow, only 2 m to 5 m in depth at the study site. They indicated that:

- Monensin persisted in high concentrations in the manure transport and manure storage systems. Monensin concentrations in the lagoon water ranged from 3.91 μg L⁻¹ to 16.24 μg L⁻¹ and between 1.89 μg L⁻¹ to 3.91μg L⁻¹ in the flush lanes of the dairies.

- Monensin was detected in the ground water beneath the dairy production areas of the farm. The monensin was detected in one shallow monitoring well at Dairy 1 and in three shallows wells at Dairy 2. The concentration of monensin ranged from 0.04 μg L⁻¹ to 0.39 μg L⁻¹. This demonstrates that monensin has the ability to reach shallow alluvial ground water.
Monensin was not detected in all ground water; the fields in which lagoon manure water was applied had no detection of monensin in the ground water. This result could be due to any number of factors such as aerobic surface conditions or the complexity of the soil biota.

Monensin is hydrophobic in nature, but there has been research conducted showing that monensin is present in runoff from the soil surface. A study was conducted by Davis et al. (2006) evaluating the potential for agricultural runoff to the aquatic environment, specifically surface waters. A solution containing seven antibiotics including monensin, was applied to the study plot 1 h before a rainfall simulation (60 mm h⁻¹). The runoff samples were collected on a continuous basis, and the results showed monensin was the greatest concentration of the seven antibiotics in runoff (1.20 µg L⁻¹) and the second highest concentration in sediment (10.5 µg kg⁻¹). This resulted in monensin having the highest absolute loss of all the antibiotics tested (Davis et al., 2006). Even though monensin concentrations were high in sediment, its relative loss due to sediment transport was <10 % (Davis et al., 2006). This study showed the potential of monensin to persist in the aqueous environment in small volumes but in high concentrations.

A study conducted by Kim and Carlson (2006) investigated the occurrence of three antibiotics (monensin, salinomycin and narasin) in a mixed-landscape watershed containing pristine urban and agricultural lands in Northern Colorado. Five sampling sites were selected, two of which were directly in the agricultural area. The agricultural area was downstream of the pristine and urban land areas. Monensin was mainly found at two sample sites in the area of the watershed influenced by agriculture. Concentrations of monensin were the highest out of all the other antibiotics for in both water and sediment and were located at sample site five. Monensin concentrations in water were 0.036 µg L⁻¹ and 31.5 µg kg⁻¹ in sediment. Kim and Carlson (2006) state, “a significant difference of measured concentration among different sampling events indicates that flow conditions and seasonal variability may effect the concentration of the three ionophore antibiotics.”

Kim and Carlson (2006) used the concept of a pseudo-partitioning coefficient to describe the relationship between the sediment matrix and the ionophore antibiotic. The sediment concentrations along with the overlying water concentrations were used to calculate the average pseudo-partitioning coefficients. At the higher flow conditions, the monensin concentration in the sediment was calculated to be three orders of magnitude greater than in the river (Kim and Carlson, 2006). Low stream flow
exhibited higher sediment partitioning, possibly due to the ionophore antibiotics sorbing to both sediment and suspended solids as they settle during low flow. For monensin, the pseudo-partitioning coefficient ranged from 680 L kg\(^{-1}\) to 10,248 L kg\(^{-1}\). Antibiotics have the ability to accumulate in sediment, which could have adverse impact on the aquatic biota of water bodies. When assessing antibiotics, sediment needs to be included. Monensin concentrations from previous studies are reported in Table 2.2.
Table 2.2: Reported monensin concentrations from various studies.

<table>
<thead>
<tr>
<th>Publication</th>
<th>Analyte</th>
<th>Water</th>
<th>Soil or Manure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kim &amp; Carlson (2006)</td>
<td>Monensin</td>
<td>0.036 μg/L #</td>
<td>31.5 μg/kg ¶</td>
</tr>
<tr>
<td>Davis et al. (2006)</td>
<td>Monensin</td>
<td>1.20 μg/L +</td>
<td>10.5 μg/kg ¶</td>
</tr>
<tr>
<td>Lissemore et al. (2006)</td>
<td>Monensin</td>
<td>6.2 - 1172 ng/L #</td>
<td>-----</td>
</tr>
<tr>
<td>Watanabe et al (2008)</td>
<td>Monensin</td>
<td>0.04 – 0.39 μg/L ¥</td>
<td>-----</td>
</tr>
<tr>
<td>Dolliver &amp; Gupta (2008a)</td>
<td>Monensin</td>
<td>18 – 25 mg/kg *</td>
<td>62 – 120 mg/kg *</td>
</tr>
<tr>
<td>Song et al. (2010)</td>
<td>Monensin</td>
<td>1 – 189 ng/L ◊</td>
<td></td>
</tr>
<tr>
<td>Forrest et al. (2011)</td>
<td>Monensin</td>
<td>2 – 75 ng/L ∆</td>
<td>0.004 – 0.50 μg/kg †</td>
</tr>
</tbody>
</table>

# Surface Water
+ Runoff
¥ Ground water
¶ Sediment
† Surface Soil (top 10 cm)
*Water Extractable sample *Spiked* (expressed on dry weight basis)
◊ Stagnant water (ditches)
∆ Drainage water (drainage tile channels)
Results of Forrest et al. (2011) continue to provide evidence of the occurrence of monensin in the terrestrial environment. This particular study investigated the occurrence and concentrations of antibiotics commonly used in the livestock industry of Alberta, Canada. Twenty-three watersheds were studied over a one-year period, with a total of 247 water samples taken. From these samples, approximately 51% of them were positive for antibiotics. Monensin was detected 34% of the time, more than any other antibiotic. Concentrations of monensin ranged from 2 ng L\(^{-1}\) to 843 ng L\(^{-1}\). This study concluded that manure production and regional cattle densities were correlated to the frequency of monensin detection, while monensin concentrations were correlated to a number of water quality parameters.

There are a few studies that have reported the effect of monensin on plant growth. Hillis et al. (2007) stated that algae and aquatic plants are sensitive to monensin and that monensin has a detrimental effect to zooplankton at a concentration of 500 µg L\(^{-1}\). Mollenhauer et al. (1986) evaluated the effects of monensin on plant growth using rye grass seedlings. The experiment analyzed rye grass seed germination in seed pouches, soil, and ultrastructure analysis in petri dishes. Rye grass seeds were exposed to an aqueous solution of monensin at concentrations of 10\(^{-5}\) and 10\(^{-4}\) M in all three germination methods. The analysis of the seed pouches determined germination was slightly affected by the 10\(^{-5}\) M monensin. Monensin at 10\(^{-4}\) M significantly affected germination yielding 8%. The root and shoot growth rates were similarly affected by the monensin concentrations of 10\(^{-4}\), resulting in no root growth and reduced shoot growth. Similar results of reduction in grow rates of the roots and shoots in seedlings planted in soil were observed. The ultrastructure analysis revealed monensin changes the cell structure of rye grass seedlings at a concentration of 10\(^{-5}\) M and cell death occurs at 10\(^{-4}\) M in the root tip and increases with time until 24 h, when approximately 20% of the cell population is affected. Mollenhauer et al. (1986) concluded germination and root growth of rye grass seedlings is visibly affected by a minimum concentration of 10\(^{-5}\) M monensin.

A study conducted by Hoagland (1996) further investigated the effects monensin has on plant growth. This study examined the effects monensin has in various concentrations on the growth of several species of plants (major agricultural crops and weeds). The plants used in the study include: cotton, okra, hemp sesbania, sicklepod, jimsonweed, johnsongrass, velvetleaf, spurred anoda, and prickly sida. The plants ranged in age from 10 to 14 d and were treated with monensin by foliar and root zone application. The foliar treatments used monensin concentrations of 10\(^{-4}\) to 10\(^{-5}\) M, while the root zone treatments were conducted using 10\(^{-4}\) to 10\(^{-6}\) M concentration. Growth effects and herbicidal injury were observed over a
3 d period. For foliar application, $10^{-5}$ M monensin did not cause substantial damage to the plants. Monensin at the concentration of $10^{-4}$ M caused visible severe discoloration to all of the plants except cotton and okra. Plant growth was also affected at a $10^{-5}$ M concentration with cotton being the least affected of all the plants. At this concentration, the fresh weight accumulation showed cotton was not affected but the weight reduction of the other plants ranged from 18% (okra) to 75% (hemp sesbania).

Root application of monensin caused discoloration of the plants at a $10^{-5}$ M concentration, with cotton and okra displaying minimum visual injury. When monensin was applied at the $10^{-4}$ M concentration, plant death occurred amongst all plant species between 24 to 65 h. Hoagland (1996) concluded cotton and okra are monensin tolerant crops and leguminous weeds (sicklepod and hemp sesbania) are very sensitive to foliar and root application of monensin. The phytotoxicity of monensin generally occurs due to root adsorption rather than foliar adsorption which is limited. As a result the assumption can be made that monensin could have an effect on legumes, but this would have to be further investigated. To clarify the biological and environmental effects of monensin and other ionophores on humans, livestock and the biota in terrestrial and aquatic environments, long-term studies are needed.

2.3.5 Monensin Degradation Chemistry

In 1984 Donoho conducted the most extensive study on monensin metabolism, using radiolabeled monensin in food producing species to determine metabolism mechanisms. Donoho’s work, is heavily cited and serves as the basis for monensin metabolism research. Building on Donoho’s 1984 work, the most recent metabolism research has looked at toxic interactions between monensin and other antibiotics (Roder, 2011 and Szucs et al. 2003.) Donoho (1984) observed that up to 90% of administered monensin is excreted in manure. Of that 90%, less than 10% was excreted as the parent compound and the rest was made up of monensin metabolites. Monensin is rapidly absorbed and extensively metabolized by the liver (Donoho, 1984). These metabolites in the excrement are due to animal metabolism. According to Donoho, (1984), data has suggested there are more than 50 metabolites in the feces of monensin-dosed animals. There are several analytical methods that have been used to identify monensin metabolites, including: high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC/MS) and other forms of MS. Figure 2.3 illustrates the chemical structure of monensin, factors, and selected metabolites. “Due to the large number and low concentrations of monensin metabolites produced by animals, a great amount of effort is needed to catalog and characterize them (Donoho, 1984).” Metabolite M-1 of monensin has been evaluated in several biological studies and was shown to be 20 times less active than monensin. “As a result, the first step in monensin metabolism seems to eliminate
the biological activity in the M-1 metabolite (Donoho, 1984). To date, no other studies have apparently been conducted specifically investigating the excretion rate of monensin with manure.

Figure 2.3: Chemical structure of monensin, factors and metabolites (Source: Dolliver et al. 2008b).

<table>
<thead>
<tr>
<th>Compound</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monensin</td>
<td>-CH₂CH₃</td>
<td>-OCH₃</td>
<td>-COOH</td>
<td>-CH₃</td>
<td></td>
</tr>
<tr>
<td>Monensin B</td>
<td>-CH₃</td>
<td>-OCH₃</td>
<td>-COOH</td>
<td>-CH₃</td>
<td></td>
</tr>
<tr>
<td>Monensin C</td>
<td>-CH₂CH₃</td>
<td>-OCH₃</td>
<td>-COOH</td>
<td>-CH₂CH₃</td>
<td></td>
</tr>
<tr>
<td>Metabolite-1</td>
<td>-CH₂CH₃</td>
<td>-OH</td>
<td>-COOH</td>
<td>-CH₃</td>
<td></td>
</tr>
<tr>
<td>Metabolite-2</td>
<td>-CH₂CH₃</td>
<td>-OH</td>
<td>-COOH</td>
<td>-CH₃</td>
<td>-OH (ring E)</td>
</tr>
<tr>
<td>Metabolite-6</td>
<td>-CH₂CH₃</td>
<td>=O</td>
<td>-H</td>
<td>-CH₃</td>
<td></td>
</tr>
</tbody>
</table>

Studies have shown the fundamental metabolic pathways of monensin are o-demethylation and oxidation, i.e. hydroxylation (Gupta, 2011, Sassman and Lee, 2007, and Donoho, 1984). Within the last 15 years, it has been discovered that monensin is interfered with by cytochrome P450. Specifically, cytochrome P450 3A plays an important role with the oxidative metabolism of monensin (Nebbia et al. 1999). These findings were reached while conducting experiments on rats, further research is being
conducted to determine if P450 3A plays the same role in food producing species such as cattle. Nebbia et al. (2001) conducted a study investigating the oxidative metabolism of monensin and P450 3A function in the liver of animals. It was concluded that monensin’s metabolism in the liver is species dependent. Total monensin metabolism was highest in cattle, followed by rats, chicks, pigs, and horses. It was discovered that the \( o \)-demethylation of monensin is highly restricted in vitro by chemicals competing and binding with P450 isoenzymes that are essential in its oxidative biotransformation. It was also predicted that a similar mechanism is likely to occur in a living organism when target organism are exposed to ionophores and other drugs (Nebbia et al. 2001). These interactions are responsible for inhibiting P450 and enhancing ionophore concentrations and thereby promoting ionophore toxicity.

### 2.4 Remediation of Antibiotics through Composting

Due to the amount of sub-therapeutic antibiotics used in livestock production and their potential adverse effects on humans and the environment, means of reducing their impact are needed. A practical solution is to eliminate them from the manure before they are applied to agricultural lands. A manure management approach that could be used is composting. Composting is a controlled aerobic process that biologically decomposes organic matter into a stable product called compost. As an on-farm manure management practice, the widespread use of composting makes it practical as well as economical.

Composting has been recognized as a viable means of degrading antibiotic-containing manure. Findings from Watanabe et al., (2008) showed that monensin attenuation is greater under aerobic conditions than anoxic conditions, i.e. composting vs. stockpiling. Studies have been conducted by Cessna et al. (2011); Ramaswamy et al. (2010), Dolliver et al., (2008a, 2008b), Dolliver and Gupta (2008a, 2008b), and Storteboom et al. (2007) in which composting or some type of manure management practice was used to reduce or eliminate antibiotics from manure. Four of these studies specifically examined the use of composting to reduce or eliminate ionophore antibiotics with three focusing on monensin. A study by Cessna et al. (2011) investigated the effects on the composting process by veterinary antimicrobials (antibiotics). This study took place over a 2 yr period. The study assessed the use of open air windrow composting on beef manure contaminated with chlortetracycline, sulfamethazine, and tylosin. The objectives of this study included monitoring the dissipation of the antimicrobials and assessing the effect of those antimicrobials on the composting process. Before the construction of the windrows the chlortetracycline had changed its form to iso-chlortetracycline and can exist as enol/keto-
chlortetracycline in solution. The dissipation of iso-chlortetracycline, sulfamethazine, and tylosin were modeled using first-order kinetics, while enol/keto-chlortetracycline was modeled using exponential equations.

The data revealed no significant difference in the initial concentration of antimicrobials in 2005 compared to 2006, though the duration of antimicrobial administration differed, 204 d (2005) compared to 13 d (2006). It was determined that the dissipation rates were affected by the year. In 2006, iso-chlortetracycline and tylosin dissipated at a faster rate compared to that of 2005 due to warmer and drier conditions of the windrows. In 2005, sulfamethazine dissipated the fastest, but no significant data exist to correlate this to the cooler and wet conditions of the windrow in 2005. The time for 50% dissipation (DT$_{50}$) varied for each of the antimicrobials. The DT$_{50}$ (2005) for chlortetracycline ranged from 9.8 to 26.5 d compared to 5.5 to 16.1 d in (2006). The DT$_{50}$ of sulfamethazine jumped from 26.8 d (2005) to 237 d in (2006). For tylosin, the DT$_{50}$ decreased from 43.5 d (2005) to 20.3 d (2006). This study found evidence, which supports antimicrobials having an effect on the compost process based upon temperature and nutrient properties. Further research is needed to verify these effects in a more controlled setting with the use of controlled composting and stockpiling as management options.

Ramaswamy et al. (2010) conducted a study explored the use of composting poultry manure contaminated with salinomycin before land application. Salinomycin is an ionophore antibiotic similar to monensin and is also used as a feed additive. Similar to monensin, salinomycin is hydrophobic in nature and is more likely to persist in sediment. In a previous study it was reported that salinomycin was found at concentrations in sediment 500 times higher than concentrations in water (Kim and Carlson, 2006). This study assessed open heap conditions (control) for comparison. The experiment was conducted in open laboratory conditions where temperature, moisture content, pH and other variables were measured. The concentration of salinomycin in the compost decreased from 22 mg kg$^{-1}$ to $2 \times 10^{-5}$ µg kg$^{-1}$ during the 38 d compost. The control yielded a decrease of 27.5 mg kg$^{-1}$ to 0.024 mg kg$^{-1}$. The rapid degradation of salinomycin in compost exhibited a half-life of 1.3 d and a removal rate of 0.5358 d$^{-1}$. The half-life of the control was 4 d with a removal rate of 0.1731 d$^{-1}$. From this study, Ramaswamy et al. concluded that composting significantly reduced the concentration of salinomycin, which would result in safer manure for land application.

One study examined the ineffectiveness of stockpiling as a manure management practice. The study conducted by Dolliver et al. (2008a) used three different manure management treatments (control
treatment, managed composting and vessel composting) to compost turkey manure. The C:N ratio of the turkey manure was 13:3 (including bedding). The bedding used was a combination of sunflower hulls and aspen shavings at a 50:50 blend ratio. Sampling for the controlled and managed compost piles were taken on the same day intervals whereas the vessel composting used another schedule. Composite sampling was used for each of the three manure treatments. Each composite sample consisted of 3 subsamples.

Antibiotic analysis was performed using enzyme-linked immunosorbent assay (ELISA) method. In this study the concentration of antibiotics recovered from the manure was not quantified and was assumed to remain constant overtime (Dolliver et al. 2008a). Dolliver and colleagues measured the degradation of antibiotics by the decrease of total extractable antibiotic concentration overtime. The study found that thermophilic temperatures (>40°C) were achieved within four days in all of the treatments. The total extractable concentration of monensin increased after the start of the study but gradually declined over time with reductions ranging from 54% to 76% with an average half-life of 17 d. According to Dolliver et al. (2008a), the water extractable antibiotic concentrations may represent the most available type of degradation for microorganisms. The water extractable concentration of monensin was lower on the final day of the study compared to the initial start of the study. As quoted by Dolliver et al. (2008a), “The reduction in the proportion of antibiotics that were water extractable during storage and composting may be due to changes in manure physical/chemical characteristics, adsorption processes, or a reduction in bioavailability.”

Dolliver and Gupta (2008a) also investigated antibiotic losses from unprotected manure stockpiles and concluded that unprotected manure piles can contribute to antibiotic contamination of aquatic environments. Due to a lack of information existing on antibiotic losses from onsite manure management practices, Dolliver and Gupta (2008a) decided to investigate this and were able to quantify antibiotic losses in beef manure. Composting and manure storage techniques often occur outside which can lead to the runoff and leaching of pollutants (Dolliver and Gupta, 2008a). Antibiotic analysis was conducted using the (ELISA) method as in Dolliver and Guptas’ previous study. The antibiotic concentrations in the manure pile runoff increased with the increasing amount of antibiotic in the manure. Monensin had the highest concentration in runoff (2,715 µg L⁻¹) of any of the antibiotics. Dolliver and Gupta concluded that manure storage in protected facilities (covered) could possibly reduce or eliminate contaminants from manure in aquatic environments.
The study conducted by Storteboom et al. (2007) confirmed the general finding of the other three studies; namely, some form of manure management is effective in degrading concentrations of antibiotics in livestock manure. Two studies were conducted during this experiment, the study of interest was the pilot study. The pilot study investigated the response of antibiotics and antibiotic resistant genes (ARG) to high intensity management (HIM) compared to low intensity management (LIM). “It was hypothesized that HIM of animal manures may stimulate the degradation of antibiotics and minimize the spread of ARG into the environment” (Storteboom et al., 2007). HIM was defined as amending, watering and turning of the windrow and LIM was defined as no amending, watering or turning of the window pile. Horse manure was used for this study and was subjected to high and low intensity managements in three replicate piles. The HIM manure was amended with leaves and alfalfa. One control pile was created for each manure management. Monensin was applied to the manure piles in the form of solid pulverized feed by sprinkling, watering and mixing into the piles. Monensin was found to have a half-life of 14.7 d in the HIM manure compared to 30.1 d in the LIM manure, but the final concentrations of monensin were not statistically different between the two management options.

2.5 Gaps in Knowledge

Forrest et al. (2011), conclude that, “despite the limited knowledge of the effects of pharmaceutical residues in the environment, agricultural producers can use proactive practices to reduce their presence and potential impacts.” As a result, providing a viable means for remediating monensin and other forms of antibiotic pharmaceuticals from dairy manure is a very important aspect that warrants further research. Currently, researchers have documented composting as being a proactive practice than can remediate antibiotic residues. To date, there have been no studies on monensin degradation during composting of dairy manure. This proposed research would assess the potential of composting as a biological treatment option for dairy producers, for treatment of manure containing antibiotic residues i.e. monensin. Investigating the usefulness of composting compared to stockpiling manure could have positive environmental and management benefits for the dairy producer.
Chapter 3

Goals, Objectives, Hypothesis

3.1 Research Goals

As an ionophore antibiotic, monensin is primarily used to amplify production. In dairy production, monensin is the most widely administered antibiotic. In the United States, monensin is the only antibiotic permitted as a feed additive for lactating cows (Watanabe et al., 2008). The occurrence and fate of monensin in the environment through dairy manure management has not been fully studied, even though monensin is predominately used in U.S. dairy production. Studies (Dolliver et al., 2008a and Dolliver and Gupta, 2008a) using turkey and beef manure as a media to introduce antibiotics onto land have concluded monensin is prevalent in both terrestrial and aquatic environments. Monensin’s classification as a sub-therapeutic antibiotic allows it to be considered a marker for agricultural pollution when found in the environment (Forrest et al. 2011). The application of dairy manure to agricultural lands is a common management practice. However, the monensin introduced to the terrestrial environment could persist and have adverse effects over time. Methods to degrade monensin prior to land application are needed.

The goal of this investigation is to compare monensin degradation for composting versus stockpiling of dairy manure. Determination of the degradation behavior of monensin in dairy manure will contribute to our understanding of its fate in environmental systems and potential remediation strategies.
3.2 Objectives

The specific objectives of this research are to:

- Determine C:N ratio and moisture content (MC) of dairy manure and develop a procedure for adjusting both to optimize composting conditions.
- Determine the monensin concentration in dairy manure as provided by a dairy producer.
- Quantify the rate of monensin degradation in stockpiled dairy manure.
- Evaluate the effect of composting on the degradation rate of monensin in dairy manure.

3.3 Hypothesis

Composting of dairy manure will accelerate the degradation of monensin relative to stockpiling of dairy manure.

Supporting Argument:

Monensin breaks down in aerobic environments more quickly than anoxic environments (Watanbe et al., 2008). Since composting is a controlled aerobic process, the composting will accelerate the degradation rate of monensin. Aerobic conditions and a balanced C: N ratio from amendments (sawdust, straw, etc) should accelerate degradation of monensin relative to stockpiling. If so, composting could be used as a dairy manure management technique.
Chapter 4

Methodology

4.1 Site Description

The research was conducted at Penn State’s Organic Materials Processing and Education Center (OMPEC) from June 2011 through September 2011. The OMPEC is a 5.26 ha site located at University Park created to compost cafeteria waste, landscaping debris, and livestock manure. The compost site for this research was a 93 m² asphalt surface containing a compost structure 4.2 m (wide) x 14.6 m (length) x 1.22 m (height). The compost structure was made of 32 (0.61 x 0.61 x 1.83 m) concrete blocks. Figure 4.2 shows the layout and placement of treatments at the site. The locations of the treatments within the 8 bays were randomized.

Fig 4.1: Schematic of compost site for composting and stockpiling of dairy manure.
4.2 Summary of Dairy Operation

The manure used in this study was acquired from a local dairy producer near Pine Grove Mills, PA. This dairy facility, consist of several hundred head of cattle and approximately 182 ha of agricultural land. Approximately 146 ha are used for manure application, which accounts for 80% of the total land use. The manure was taken from a freestall barn containing 30 continuously confined Holstein heifers about 6 months of age and ranging in weight from 272 kg to 295 kg (600 to 650 lbs). The freestall barn (Fig. 4.2) consist a typical bedded pack located in 6 bays, free stalls and a free stall alley. The bedded pack consisted of manure and a mixture of corn stalk bedding used. The bedded pack is cleaned out every three months and the freestall alleys are cleaned out every 5 d.

Typically application of solid manure occurs in March and September. In the summer, solid manure is taken to the edge of a field and left in a static pile until land application in the fall. In the summer (June to August), liquid manure from the heifer barn is applied to roughly 10.1 ha of permanent hay pasture.

![Fig 4.2: Schematic of freestall Holstein heifer barn.](image)
4.3 Construction of Compost & Static Piles

The site for the compost bays was thoroughly washed down and cleared of any existing debris on the pavement. The concrete blocks were then set in place using the 3-4-5 triangle method. The back wall of the bays were put in place first, then the lateral walls were placed. The bays were then labeled to distinguish between compost and static piles. The placement and order of the bays were randomized.

4.3.1 Raw Materials

The raw materials included bedded pack manure, oat straw, and wood chips. The oat straw was acquired from Penn State Farm Services and the wood chips were available on site at the OMEP. These raw materials were selected because of their relative availability and their ability to meet optimal composting requirements for moisture content and C:N ratio. The bedded packed manure had a moisture content of 75.3% and a C:N of 14.4. A blending recipe for the compost was formulated using book values (Rynk, 1992) and values used at the OMPEC for each of the raw materials. The target recipe was roughly: 1lb manure: 0.10 lb wood chips: 0.25 lb of oat straw all on a wet basis. In reality, ratios were: 1lb manure: 0.10 lb wood chips: 0.10 lb oat straw. The moisture content of this blend was approximately 62.7% and the C:N ratio was 27.7 as determined by Penn State’s Agricultural Services Laboratory (AASL). A C:N ratio of 25 – 30 is considered ideal for composting (Rynk, 1992).

The total delivered manure weighed 11.59 tons (23,180 lbs). Before mixing, the oat straw bales were loosened for easy access by the frontend skid loader. The mixing of the raw materials occurred in three different batches (Table 4.1). Some of the proposed mixtures were estimated by eye and loosely following the target recipe.
Table 4.1: Batch mixing recipes

<table>
<thead>
<tr>
<th>Batch</th>
<th>Material</th>
<th>Proposed Mixture (lbs)</th>
<th>Actual Mixture (lbs)</th>
</tr>
</thead>
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<tr>
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<td>Manure</td>
<td>3300</td>
<td>3000</td>
</tr>
<tr>
<td></td>
<td>Oak Straw</td>
<td>280</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td>Wood chips</td>
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<td>320</td>
</tr>
<tr>
<td>2</td>
<td>Manure</td>
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<td>4910</td>
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<tr>
<td></td>
<td>Oak Straw</td>
<td>500</td>
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</tr>
<tr>
<td></td>
<td>Wood chips</td>
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<td>570</td>
</tr>
<tr>
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</tr>
<tr>
<td></td>
<td>Oak Straw</td>
<td>500</td>
<td>510</td>
</tr>
<tr>
<td></td>
<td>Wood chips</td>
<td>500</td>
<td>490</td>
</tr>
<tr>
<td>Total</td>
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<td>13,000</td>
<td>12,900</td>
</tr>
<tr>
<td></td>
<td>Oak Straw</td>
<td>1280</td>
<td>1200</td>
</tr>
<tr>
<td></td>
<td>Wood chips</td>
<td>1300</td>
<td>1380</td>
</tr>
</tbody>
</table>

The batches of materials were mixed using a Kuhn Knight Vertical 5055 Mixer. The vertical mixer was cleaned and cleared of all debris within the mixer to prevent any contamination during mixing. Each batch recipe was mixed for about 30 min and checked visually to ensure sufficient mixing. Once mixed, each batch was laid out in a 25-foot windrow, one on top of the other for further mixing, making all three batches homogenous. Once in a windrow, a front-end loader was used to deposit four bucket-loads of material into each bay. The capacity of the bucket on the front-end loader is approximately 0.57 m$^3$ (0.75 yd$^3$), resulting in each bay having 3 yd$^3$ of material.
4.3.2 Treatments

Once in place, each manure pile measured approximately 2.1 m (width) x 1.5 m (length) x 0.91 m (height). The static piles (control) remained undisturbed during the 50-day study period, except for the minor disturbance associated with sampling. In the beginning, this study set out to evaluate composting. The compost piles were to be turned based on temperature (< 40°C), in actuality the compost piles were only turned once, on day 6 during the 50-day study. The piles were turned only once, because of the concern they would not reheat properly due to the small size of the piles. Also, the use of manual thermometers to roughly gauge pile temperature were inaccurate. After examining the temperature profiles of the compost piles, some piles would have only required one to three additional turns to fully stimulate composting. As a result, due to the experimental design, the experiment actually investigated static pile treatment (no disturbance vs. limited disturbance) and its effect on monensin degradation. The
compost piles were turned with the use of a front-end loader Fig. 4.7. Sampling and retrieval of temperature contributed to additional pile disturbance. This experiment was conducted in an outside environment, resulting in the same climatic conditions for both treatments.

4.4. Installation of Temperature Probes

Thermochron temperature logger iButtons (model DS1921G) were used to monitor temperature for this experiment. These iButtons are highly resistant to environmental hazards and are a self-sufficient system. The DS1921G operating range is -40°C to +85°C and has an accuracy of ± 1°C.

Each iButton was cataloged and labeled. Once cataloged, the iButtons were programmed using the One Wire Viewer software. Using the software interface, temperature scale (°C), frequency of measurement (30 min), and synchronization of time for each iButton were set. Before installation in the piles, the iButtons were encased in waterproof capsules for protection from the elements, and tagged with polypropylene twine to allow for easy retrieval of the sensors. The iButton sensors were removed for compost turning based on temperature and when the experiment was concluded. An auger was used to penetrate each pile for proper placement of the iButton sensors. The iButton sensors were placed at a depth of 12 – 18 inches at the bottom, left, right, back and the core of each pile, as shown in Fig. 4.8.
4.5 Sample Technique and Storage

A composite sample was taken from each compost and static pile. The composite sample was made up of 5 subsamples taken from the bottom, left, right, back and the core of each pile. Sampling occurred 3 d per wk with samples every 2 days, yielding 24 composite samples per week for 4 weeks.

Composite samples consist of subsamples from (core, bottom, back, right & left of each pile) taken at a depth of 12 – 18 inches, as shown in Fig. 4.5. As degradation of the piles occurred over time, the sampling depth changed with the shrinking of the piles. Through several trials with different sampling probes, it was determined that a soil screw auger was best for sampling. The corkscrew action of the auger allowed for easy retrieval of the bulky raw material in the compost. Once subsamples were collected from each pile, they were combined, labeled, and placed in air tight Whirl-Pak plastic bags for storage. Samples were stored in a refrigerator at a temperature of ≤4°C to prevent degradation before analysis.

To prevent cross-contamination, the sampling probe was cleaned between the sampling of each bay. The cleaning process consists of soaking the sampling probe in a 18.9 L bucket of water and manually clearing off any excess material still attached to the probe. Once samples were collected for the
first four bays, the 18.9 L bucket was then emptied and cleaned out. The 18.9 L bucket was then refilled for the sampling of the last four bays. When sampling was completed, the probe was cleaned and stored until the next sampling day.

4.5.1 Sample Preparation from Field

Before monensin, moisture, and organic matter analysis of the samples, the subsamples from each pile were combined into one composite sample. That composite sample was considered representative of the entire pile.

A Hobart Buffalo chopper was used to break down each subsample. The samples were then homogenized for 15 min using the Buffalo chopper food processor to a more manageable composite sample. Any clumps of compost or any fibrous materials were chopped into smaller pieces.

To prevent cross-contamination of samples, the blades and the bowl of the food processor were disassembled and cleaned between the processing of each pile sample. When homogeneity was reached, each composite sample is separated into a smaller sample, representative of each pile. The samples were stored in refrigeration until all analyses could occur.

4.6 Sample Analysis

The moisture content was determined by weighing before and after oven drying at 105°C for 24 hr. The organic matter (OM) analysis was provided by AASL. The %OM is defined as OM lost on ignition.

4.6.1 Monensin Analysis

The enzyme-linked immunosorbent assay (ELISA) is a test used in immunology and other scientific fields to detect antibodies and antigens. The ELISA uses enzymes that react with antibodies to form colored products. The development of color in an ELISA test indicates a positive result. The most commonly used ELISA method is called the indirect ELISA; an antibody test used to determine whether a certain type of antibody is present in a sample, and at what concentration. ELISA has been used previously to quantify monensin in manure (Dolliver and Gupta, 2008a; and Dolliver et al., 2008a, 2008b).
A commercially available kit from Immuno-Diagnostic Reagents (Vista, CA) was used to perform the monensin analysis. This kit consisted of a 96-well plate coated with monensin antibodies. The marketed use of the kit is intended for residue analysis in foods, but Dolliver et al. (2008a,b) adapted the kit for use on complex samples. Dolliver et al. (2008b) evaluated the application of this commercially available ELISA kit on environmental samples and concluded that it was highly sensitive, yielding a 1.5 \( \mu g \ L^{-1} \) detection limit and a 3.0 \( \mu g \ L^{-1} \) quantification limit. ELISA analysis was performed in accordance with manufacturer recommendations. Quantification was performed using a microplate reader at a wavelength of 405 nm. All samples were run in triplicate. Table 4.2 displays the linear equations determined for each ELISA kit based upon average absorbance. The average absorbance values were determined by the standards (0, 1, 5, and 25 ng mL\(^{-1}\)) provided in each kit. From the average absorbance, a range was determined for each kit which was used to quantify monensin in the samples. The \( R^2 \) value describes how closely the linear equations model the relationship between the absorbance and standards of the ELISA kit.

Table 4.2: Summary of linear equations for ELISA kits

<table>
<thead>
<tr>
<th>Plate Identification</th>
<th>( R^2 )</th>
<th>Linear Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kit 1</td>
<td>0.95147</td>
<td>( y = -6.229x + 13.13 )</td>
</tr>
<tr>
<td>Kit 2</td>
<td>0.91587</td>
<td>( y = -4.7737x + 9.8408 )</td>
</tr>
<tr>
<td>Kit 3</td>
<td>0.92397</td>
<td>( y = -5.8111x + 9.0905 )</td>
</tr>
<tr>
<td>Kit 4</td>
<td>0.91174</td>
<td>( y = 4.1892x + 7.5479 )</td>
</tr>
<tr>
<td>Kit 5</td>
<td>0.90219</td>
<td>( y = -4.619x + 7.8446 )</td>
</tr>
<tr>
<td>Kit 6</td>
<td>1</td>
<td>( y = -126.85x + 58.066 )</td>
</tr>
<tr>
<td>Kit 7</td>
<td>0.95455</td>
<td>( y = -4.869x + 8.3446 )</td>
</tr>
</tbody>
</table>
4.6.2 Sample Preparation for Monensin Analysis

The compost and static pile samples were prepared for analysis using an extraction technique of Dolliver et al. (2008a), who determined both water and methanol extractable antibiotic concentrations. The complexity of samples, such as compost or manure, create a greater chance of inaccuracy during analysis. This extraction process allowed for a cleaner sample to be analyzed due to the sensitivity of the ELISA. The extraction technique used by Dolliver et al. (2008a) is described below. Antibiotic degradation was interpreted as a decrease of total-extractable (water and methanol-extractable) antibiotic over time. The water extractable analysis represented the monensin that was more soluble. The methanol extractable analysis represented the monensin that was tightly sorbed to the compost.

**Water Extractable Antibiotics**

Five grams of manure was mixed with 10mL of Nanopure® water and vortexed for one min. The sample was then shaken end over end for 15 min at 4˚C and centrifuged at 2000g for 20 min. The supernatant was then collected. An additional 10 mL of Nanopure® water was added, supernatants were combined and then centrifuged at 2000 g for 20 min. The supernatant was then filtered through a non-sorptive 0.45µm filter.

**Total Extractable Antibiotics**

The remaining solids were mixed with 10 mL of an 80:20 (v/v) methanol:water solution. The sample was vortexed, shaken and centrifuged (2000g for 20 min) with the supernatants collected. This step was repeated with an additional 5 ml of the methanol:water solution.

4.7 Data Compilation & Analysis

4.7.1 Data Recording & Processing

At the end of the experiment, the iButtons were collected and bagged for each pile. Each waterproof capsule was cleaned of excess material before the iButton is processed. Once cleaned, each iButton was crossed referenced with the iButton list. The data from each iButton was uploaded with the One Wire Viewer software. The software interface stops the recording of new data and provides a
temperature/time plot and a histogram. The data was saved and then imported into Microsoft Excel. The mean average temperature was calculated for each day, for every pile, producing a representative temperature profile. All degradation, moisture content, organic matter, precipitation, and temperature data was collected and recorded in Microsoft Excel.

4.7.2 Statistical Analysis

The temperature profiles for each treatment were averaged to produce mean temperatures for each treatment. The mean temperatures of both treatments were analyzed to assess trends related to ambient temperature and other variables. The data was then evaluated using a general regression and correlation model procedure using Minitab version 16. The significance level used for statistical analysis was $\alpha = 0.05$. The difference in the mean temperatures of the treatments was evaluated using a two sample t-test. The differences discussed in the text were significant at $\alpha = 0.05$. 
Chapter 5

Results and Discussion

5.1 Monensin in Dairy Manure

Samples were taken on April 27th and June 7th, 2011 to quantify the presence of monensin in the manure provided by the local dairy producer. The two samples had total monensin concentrations of 0.83 and 1.06 mg kg$^{-1}$. On average, the water extractable monensin made up, 52.4% of the total monensin concentration with the remaining 47.6% being in the methanol extractable form. These concentrations of monensin are representative of what was observed during the actual experiment. These amounts are similar to the reported typical concentrations of antibiotics (1 mg kg$^{-1}$ – 10 mg kg$^{-1}$) in livestock manures (Kumar et al., 2005).

5.2 Temperature

Temperature is a critical factor affecting microbial degradation of organic contaminants and the composting process. The average temperature profiles of the compost piles (CPs) and static piles (SPs) indicate thermophilic conditions (>40°C) were reached on day 0 for compost and the following day for static piles. Thermophilic conditions were not sustained during the duration of the experiment, compost and static piles averaged 25 d and 11 d, respectively. Compost piles averaged 40.2°C on day 0 and increased to 49.4°C on day 1. In comparison, the temperature of the static piles averaged 37.5°C and 46.3°C for d 0 and d 1, respectively. The peak maximum temperature of the CPs shown in Table 5.3 occurred with a three-day period: (2 – 4 d; July 21 – 23) where the average ambient temperature was the highest during the entire study. These temperatures were 29.4°C, 32.3°C, and 28.8°C, respectively. The SPs showed a similar pattern lasting four days, with the period starting a day earlier on (d 1; July 20th) at a temperature of 26.6°C.
CP 3 reached a maximum peak temperature of 55.6°C on day 2 and was the highest of all treatments. A temperature of 51.4°C from SP 4 was the highest maximum peak temperature for any SPs. Temperature variation patterns showed the average minimum temperature increase occurred on day 4 in 7 out of 8 piles. The average maximum temperature increase occurred on day 0 in 6 out of 8 piles, shown in Table 5.1 and 5.2. Change in temperature is defined as \( \Delta T_n = T_n - T_{n-1} \). The minimum temperature increase, showed a steep decline of temperature before day 10 of composting bottoming out at 36.6°C. The compost piles then showed a gradual incline peaking a second time at 50.3°C on day 15 and declined gradually to an average temperature of 32.1°C at the conclusion of the experiment. The static piles reflect the same pattern as the compost piles with the same steep decline of temperature before the day 10. The temperature gradually declined in the SPs during the rest of the experiment ranging from 28.2°C to 44.1°C.
Table 5.1: Compost Pile Temperature Variations

<table>
<thead>
<tr>
<th>Temperature Variation</th>
<th>CP 1</th>
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<th>CP 3</th>
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<td>Minimum</td>
<td>Maximum</td>
<td>Minimum</td>
<td>Maximum</td>
<td>Minimum</td>
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<tr>
<td>Temperature Increase (°C)</td>
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<td>6.2</td>
<td>-10.5</td>
<td>7.4</td>
<td>-11.3</td>
</tr>
<tr>
<td>Day of Rate Temp Increase</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Peak Temperature (°C)</td>
<td>30.7</td>
<td>47.7</td>
<td>31.3</td>
<td>49.7</td>
<td>33.3</td>
</tr>
</tbody>
</table>

Table 5.2: Static Pile Temperature Variations

<table>
<thead>
<tr>
<th>Temperature Variation</th>
<th>SP 1</th>
<th>SP 2</th>
<th>SP 3</th>
<th>SP 4</th>
<th>SP Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
<td>Minimum</td>
<td>Maximum</td>
<td>Minimum</td>
</tr>
<tr>
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<td>-7.1</td>
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<td>-7</td>
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<td>19</td>
<td>0</td>
<td>4</td>
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<td>45.7</td>
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<td>46.8</td>
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The peaks and valleys from both treatment profiles roughly follow that of the ambient temperature, which indicates a potential relationship between ambient and pile temperature, shown in Fig. 5.1. Temperature profiles of individual piles are given in appendix A. The mean compost temperatures were positively correlated to ambient temperatures (P = 0.000). A similar positive correlation was shown in the static temperatures (P = 0.000). As ambient temperature climbed, the mean temperatures of the treatments climbed and vice versa. The mean temperatures of the compost piles were higher during the duration of the study, averaging 3°C hotter than the static piles, illustrated in Fig. 5.1. The mean temperature of the compost treatment was significantly greater than the mean temperature of the static treatment (P = 0.001). But, that statistical significance seems to be heavily influenced by the first 25 days of temperature measurements. The last 25 days of the study indicate no statistical difference between treatment temperatures (P = 0.853).

Figure 5.1: Temperature profiles of compost and static treatments
The average moisture content (MC) of both treatments was comparable on day 0; 62.1% (wet basis) for compost and 62.4% (wet basis) for static treatment. The average MC drastically fell on day 2 to 54.8% and 53.5% for the compost and static piles, respectively. This was likely due to evaporation of water from the piles as a result of the release of heat energy caused by active microbial decomposition of organic material. The reverse trend occurred on day 8 where the mean moisture content began to climb back to its day 0 levels for both treatments. This increase corresponds with a period of significant rainfall over a nine-day span, when approximately 8.89 mm of rainfall occurred at the site. On average, the compost treatments had slightly higher moisture content values compared to the static treatments, but differences were not statistically significant. On average, the moisture content measurements for the static pile treatments were within the optimal range (50% - 60%) for microbial degradation of organic matter (Rynk, 1992). At day 50, all of the moisture content measurements had peaked at over 76% (wet basis) in both treatments (Tables 5.3 and 5.4). There was no statistical significance found between the moisture contents of the compost and static treatments during the study.

### Table 5.3. Compost treatment moisture content and cumulative rainfall totals.

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<th></th>
<th>CP 3</th>
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<tr>
<td>Day</td>
<td>MC%</td>
<td>Cum. Rainfall (mm)</td>
<td>Day</td>
<td>MC%</td>
<td>Cum. Rainfall (mm)</td>
<td>Day</td>
<td>MC%</td>
<td>Cum. Rainfall (mm)</td>
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Table 5.4 Static treatment moisture content and cumulative rainfall totals.

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<td>14</td>
<td>60.7</td>
<td>20.6</td>
</tr>
<tr>
<td>16</td>
<td>57.3</td>
<td>35.3</td>
<td>16</td>
<td>49.4</td>
<td>35.3</td>
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<td>63.1</td>
<td>35.3</td>
<td>16</td>
<td>43.3</td>
<td>35.3</td>
</tr>
<tr>
<td>50</td>
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<td>177.3</td>
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<td>78.1</td>
<td>177.3</td>
<td>50</td>
<td>77.0</td>
<td>177.3</td>
<td>50</td>
<td>83.7</td>
<td>177.3</td>
</tr>
</tbody>
</table>

A study by Yoshida et al., (2010) concluded that higher soil moisture content (80 – 100% field capacity) translated to a higher rate of monensin degradation. High moisture content was observed in both treatments during the study, but the difference was the use of soil in Yoshida et al. study. Higher soil MC resulted in degradation on monensin in their study, but high MC values (> 65%) generally suggest that compost treatments are anaerobic in nature. The On Farm Composting Handbook suggests the commonly accepted range for aerobic composting was 40% – 65% (Rynk, 1992). (EPA, 1995), determined 50% - 60% as the optimum range. Anaerobic conditions may have occurred in both treatments toward the end of the study, based on rigid interpretation of the accepted reported ranges for aerobic MC (40% - 65%), but this was not verified using other methods. Additionally, the experimental set up did not allow for the verification of Yoshida et al., 2010 findings of high %MC and its effect on monensin degradation.

The compost treatments in this experiment became waterlogged during each of the first three cumulative rainfall events. During the experiment, significant runoff was observed from each compost treatment during the heavy rainfall events. Due to the amount of rainfall and the high moisture content, eventually both treatments became waterlogged and runoff occurred. Since the intent of this experiment was not to quantify runoff from the treatments, the actual runoff volume for each treatment at that time was unknown. But towards the end of the experiment some runoff samples were collected to get a sense of the monensin concentration that may have still existed. The analysis showed that concentrations were within the range observed during the experiment. The average runoff (n=3) from the compost treatments was approximately 4.04 ng mL⁻¹, while the static treatments averaged 3.98 ng mL⁻¹. From the small
number of runoff samples collected, the CP 4 yielded the highest single measurement: 4.67 ng mL\(^{-1}\). The runoff values from this experiment were higher than what was reported in Davis et al. (2006); 1.20 µg L\(^{-1}\) but significantly lower than the range observed by (Dolliver and Gupta, 2008a) of 951 µg L\(^{-1}\) to 2715 µg L\(^{-1}\), (Dolliver and Gupta, 2008b) of 57.5 µg L\(^{-1}\) and Forrest et al., (2011); 2 ng mL\(^{-1}\) to 843 ng mL\(^{-1}\). The total cumulative rainfall that occurred at the site was 177.3 mm, with August having the highest cumulative rainfall of 142.7 mm during the experiment.

The organic matter (OM) content remained very high for both treatments during the experiment, illustrated in Tables 5.5 and 5.6. The compost treatments averaged a 6% drop in OM compared to only a 4.3% drop for static treatments. Yoshida et al., (2010), found that the higher OM content of soils resulted in a shorter half-life for monensin. But, the amount of OM in soils (3 – 9%) is very different from that of compost (> 80%). The static treatments maintained the higher OM content in the experiment and yielded the shortest half-lives but no statistical significant data was observed to validate the findings of Yoshida et al, (2010). The OM content exhibited some degradation over the duration of the experiment except for the SP 2 treatment. Note, the average fraction remaining for compost treatments was 94% and 95.7% for static treatments.

### Table 5.5: Organic matter content of compost treatments. Note, the average fraction remaining for compost treatments was 94%.

<table>
<thead>
<tr>
<th>Day</th>
<th>CP1 OM</th>
<th>Fraction Remaining</th>
<th>CP2 OM</th>
<th>Fraction Remaining</th>
<th>CP3 OM</th>
<th>Fraction Remaining</th>
<th>CP4 OM</th>
<th>Fraction Remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>86.7</td>
<td>100</td>
<td>0</td>
<td>85.7</td>
<td>0</td>
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<td>0</td>
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</tr>
<tr>
<td>2</td>
<td>86.4</td>
<td></td>
<td>2</td>
<td>85.3</td>
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<td>4</td>
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</tr>
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<td>78.5</td>
<td></td>
<td>6</td>
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<td>6</td>
<td>84.3</td>
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<tr>
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<td>94.9</td>
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<td>82</td>
<td>95.7</td>
<td>50</td>
<td>78.2</td>
<td>91.0</td>
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</table>
Table 5.6: Organic matter content of static treatments. Note, the average fraction remaining for static treatments was 95.7%.

<table>
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<tr>
<th></th>
<th>SP1</th>
<th>SP2</th>
<th>SP3</th>
<th>SP4</th>
</tr>
</thead>
<tbody>
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<td>Fraction</td>
<td>Day</td>
<td>OM%</td>
</tr>
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<tr>
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<td>85.5</td>
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</tr>
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<td>85.1</td>
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<tr>
<td>50</td>
<td>77.3</td>
<td>92.4</td>
<td>50</td>
<td>85.3</td>
</tr>
</tbody>
</table>

5.4 Monensin Degradation

Studies by Yoshida et al., (2010), Dolliver et al., (2008a), Watanabe et al., (2008), Sassman and Lee (2007), and Carlson and Marbury, (2006) have shown that monensin degradation exhibits first-order kinetics. As a result, the data in this experiment was analyzed using a first-order decay process: \( C = C_0 e^{-kt} \) where \( C \) is the monensin concentration at time \( t \), \( C_0 \) is the initial monensin concentration, and \( k \) is the monensin degradation rate constant. With the use of this model, the half-life was calculated as:
\[
t_{1/2} = \frac{\ln(2)}{k}
\]
The average rate constants and half-lives from both treatments are shown in Tables 5.7.

The decay rate of was higher and half-life four times shorter for the static treatment in comparison to the compost treatment. The \( R^2 \) values for model fit of both treatments and the exponential degradation are illustrated in Fig. 5.2 and 5.3.

Table 5.7: First order average rate constant and half-life of total extractable monensin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CP</th>
<th>SP</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-k) (d(^{-1}))</td>
<td>0.012</td>
<td>0.051</td>
</tr>
<tr>
<td>( t_{1/2} ) (d)</td>
<td>57.8</td>
<td>13.6</td>
</tr>
</tbody>
</table>
The half-lives for the compost treatment was 57.8 d and for the static, 13.6 d. This may be due to the variability of each treatment pile and within the data set. In other studies, variability was controlled by the spiking of the manure and composting indoors. Each pile had different initial concentrations of monensin, mimicking what would be observed in the field and not in a lab setting. The half-life for the static treatment may have been shorter than that of the compost treatment due to pile management. Even though the compost treatments were supposed to model controlled compost, in actuality the resembled more of a static treatment, which can be attributed to only one turn of those piles on day 6. So the turning of the compost pile actually disturbed the static nature of it, compared to what occurred with the actual static treatment, possibly affecting the degradation. The longer half-life of the compost treatment was unexpected and is contrary to what was found in the literature. Rate constant and half-life of the static treatment were similar to what was observed in Dolliver et al., (2008a). The half-life of monensin for the compost treatment was comparable to previously reported values: 4.1 – 23 d Watanabe et al., (2008); 14.7 – 30.1 d Storteboom et al., (2007); and 17 d (Dolliver et al., 2008). In both treatments, the data point on day 50 disproportionately influenced the mathematical analysis of monensin degradation.

**Figure 5.2**: Average total extractable monensin concentration of compost piles versus time.
Examinining the four replications for each treatment (Appendix C) a clear pattern is evident where the methanol extraction monensin concentrations declines rapidly and were no longer detected past the 10th d of sampling. In comparison, water extractable monensin concentrations were detected to the 50th d of the study. This may be due to the reported sorption coefficient ($K_d$) of 9.3 L kg$^{-1}$ (Elanco Products Company, 1989), which is in the range also reported by (Sassman and Lee, 2007, Davis et al., 2006, and Kumar et al., 2005). Song et al., (2010) reported monensin $K_d$ values ranging from 1.4 L kg$^{-1}$ – 8.3 L kg$^{-1}$. This low $K_d$ value indicates that monensin is not tightly adsorbed thus promoting mobility. In comparison, other antibiotics such as chlortetracycline, with high $K_d$ values (1280 – 2386 L kg$^{-1}$), are less mobile. In this case, the mobility occurred in the aqueous phase, indicating monensin’s propensity to reach the aqueous environment. This was further validated by Song et al., (2010) who concluded, “monensin sorbed by soils is predictably released into the surface water during rainfall events.”
Another thought is that the microbes are attracted to the water interface of the compost where the monensin is adsorbed. This allows the microbes to readily degrade the monensin that is not strongly adsorbed to the surface of the compost. This degradation would also model an interfacial kinetic expression and follow a similar logarithmic trend (Dunn, 1968). As a result the monensin that is not degraded may be found in the aqueous phase due to its low $K_d$ value. The water extractable monensin concentrations encompassed approximately 77.9% (compost treatments) and 83.8% (static treatments) of the total monensin concentrations for the entire study, this is summarized in Table 5.8. From analysis of both treatments, it was concluded that the water extractable monensin concentrations were most significant and warrant further study.

Table 5.8: Monensin concentration unit totals by extraction method. Note, water-extractable monensin concentrations make up 77.9% (compost) and 83.8% (static) of total-extractable monensin.

<table>
<thead>
<tr>
<th>Pile</th>
<th>CP1</th>
<th>CP2</th>
<th>CP3</th>
<th>CP4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ext-Type</td>
<td>Water-Ext</td>
<td>Meth-Ext</td>
<td>Total-Ext</td>
<td>Water-Ext</td>
</tr>
<tr>
<td>Total</td>
<td>2.41</td>
<td>0.71</td>
<td>3.12</td>
<td>2.42</td>
</tr>
<tr>
<td>Percentage</td>
<td>77.2</td>
<td>22.8</td>
<td>100.0</td>
<td>84.9</td>
</tr>
</tbody>
</table>

Between sampling day 16 – 50, there were 142 mm of rainfall. Due to that significant amount of rainfall it is possible that monensin could have been removed via runoff instead of through degradation. Mass balance calculations, shown in Appendix D, were completed to quantify the loss of monensin via runoff. Monensin removal was modeled as follows: Removal = degradation + runoff losses (no volatilization). To start, the total monensin lost between day 16 – 50 was calculated, which provided a benchmark for the mass of monensin that was present. A water balance was calculated to determine the impact of precipitation on the piles, where: Precipitation = $\Delta$MC + Runoff losses + Evaporation. In this water balance, evaporation was neglected due to the high MC of the piles and the heavy rainfall over the 34 day period. The volume of runoff was calculated based on MC and precipitation, which was defined as: Runoff losses = Precipitation - $\Delta$MC. From the runoff volume calculation, the mass of monensin lost in the runoff was then calculated assuming 4.04 ng mL$^{-1}$ (compost) and 3.98 ng mL$^{-1}$ (static) for monensin concentrations collected from runoff samples.
The total mass of monensin lost from day 16 – 50 only accounted for 21% (compost) and 22% (static) of the total mass of monensin from day 0. According to the mass balance, approximately 1,852 L of runoff volume came from the compost treatments and 1,701 L from the static treatments. Those values are conservative (on the high side) due to evaporation being neglected in the water balance equation. The total mass of monensin measured from the runoff, between day 16 – 50 accounted for 7.48 mg from the compost and 6.6 mg from the static treatments respectively. The total monensin lost due to runoff accounted for < 2% of the total monensin available from day 16 – 50. So it can be concluded that little of the monensin during that 34-day period was attributable to removal via runoff. Thus it was concluded that monensin removal from the piles was predominantly due to degradation. Volatilization was eliminated as a loss mechanism because of monensins’ non-volatile nature.
Chapter 6

Conclusions

6.1 Implications of this study

This study mimicked physiological processes in which metabolites are secreted in manure by dairy cattle, unlike previous studies were manures were spiked. The results of this study showed that compost and static treatments have the ability to degrade monensin in dairy manure before land application and can be used as manure management options by dairy producers. When composting in an outdoor setting, piles should be covered to prevent runoff. The average half-lives of the treatments were 57.8 d (compost) and 13.6 d (static) respectively. Half-lives reported in the literature range from 4.1 to 30.1 days (Watanabe et al. 2008 and Storteboom et al. 2007). Specifically, static treatments required less maintenance time, and would allow dairy producers to be more flexible with time often committed to manure management. Neither treatment completely degraded monensin over the 50 d period but concentrations were greatly reduced.

Methanol-extractable monensin, that adsorbed to the organic material dissipated after d 10 of sampling. This may indicate that bound monensin is degraded quickly or is rapidly transformed to a water soluble form. Water-extractable monensin was more prevalent than methanol-extractable monensin. This study has shown that, even though monensin was degraded to lower concentrations it can still persist in the aqueous phase. Water-extractable monensin was detected up to d 16. Water-extractable monensin was also detected in three out of eight piles on the last day of sampling (d 50). This may be evidence proving monensin poses an environmental concern in aquatic environments. This concern was further substantiated by the detection of monensin in runoff from both treatments, although this study was not designed to evaluate runoff. Monensin concentrations in runoff averaged 4.04 ng mL$^{-1}$ (compost n=3) and 3.98 ng mL$^{-1}$ (static n=3).

Due to the excessive precipitation (142 mm) towards the end of the study, a mass balance was used to determine if degradation or dissipation due to washout of the monensin was occurring. Monensin removal was considered to be losses due to degradation and runoff losses with (no volatilization). A water balance was also used to determine the impact of precipitation on the piles where: Precipitation = $\Delta$MC + runoff losses + evaporation. This gave a conservative estimate of precipitation because
evaporation was neglected. Approximately 83.7% of the precipitation that fell on the compost treatment resulted in runoff and 76.8% for the static treatment. The total mass of monensin lost due to runoff accounted for <2% of the total mass of monensin from day 16 – 50. This showed that degradation was the driving mechanism that had the most influence on the fate of monensin in this study. Dissipation into runoff of monensin had little to no impact of the fate of monensin in this study.

The $K_d$ of monensin is a likely indicator of its potential to exist in the aqueous phase and should be further investigated in compost. The concentrations of monensin observed in this study were below the reported levels that have toxic effects on humans and aquatic life. To date, there have been no studies to investigate the effects of low concentrations of antibiotics including monensin, in the environment over a long period of time.

The effects of these low concentrations are unknown and warrant investigation. Over the 50 d duration of this study, the temperature (pile and ambient), moisture content, organic matter, and rainfall were all monitored and no correlation was found in respect to monensin degradation. A more comprehensive study should be conducted to investigate the influence of those variables on monensin degradation. With the large use of monensin in the dairy industry and its frequent detection in aqueous environments, it is imperative that more monitoring studies are started to gain a stronger understanding of this issue.
Appendices
Appendix A

Figure A-1: Compost pile #1 temperature profile

Figure A-2: Compost pile #2 temperature profile
Figure A-3: Compost pile #3 temperature profile

Figure A-4: Compost pile #4 temperature profile
Figure A-5: Static pile #1 temperature profile

Figure A-6: Static pile #2 temperature profile
Figure A-7: Static pile #3 temperature profile

Figure A-8: Static pile #4 temperature profile
Figure B-1: Compost treatment organic matter content profile. Note: 100% refers to the initial (day 0) O.M. content.
Figure B-2: Static treatment organic matter content profile. Note: 100% refers to the initial (day 0) O.M. content.
Appendix C

Table C-1: Compost treatment monensin concentrations (mg kg\(^{-1}\)) by extraction technique. Note: W\% indicates the fraction of water extractable monensin.

<table>
<thead>
<tr>
<th>Day</th>
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<th>Meth</th>
<th>Total</th>
<th>W%</th>
<th>Day</th>
<th>Water</th>
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<th>W%</th>
<th>Day</th>
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<th>Water</th>
<th>Meth</th>
<th>Total</th>
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<td>ND</td>
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<td>ND</td>
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Table. C-2: Static treatment monensin concentrations (mg kg$^{-1}$) by extraction technique. Note: W% indicates the fraction of water extractable monensin.

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<th></th>
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<td>Total</td>
<td>W%</td>
<td>Day</td>
<td>Water</td>
<td>Meth</td>
<td>Total</td>
<td>W%</td>
<td>Day</td>
<td>Water</td>
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<td>0.104</td>
<td>0.187</td>
<td>44.1</td>
<td>4</td>
<td>0.383</td>
<td>0.099</td>
<td>0.202</td>
<td>90.1</td>
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<td>0.731</td>
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<td>100.</td>
<td>6</td>
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<td>0.306</td>
<td>0.638</td>
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<td>6</td>
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<td>0.119</td>
<td>0.526</td>
<td>77.4</td>
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<td>69.2</td>
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<td>10</td>
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<td>ND</td>
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<td>ND</td>
<td>ND</td>
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<td>0.0.</td>
<td>50</td>
<td>ND</td>
<td>ND</td>
<td>0.000</td>
<td>0.0.</td>
<td>50</td>
<td>ND</td>
</tr>
<tr>
<td>Total</td>
<td>3.69</td>
<td>0.45</td>
<td>4.14</td>
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<td>Total</td>
<td>2.48</td>
<td>0.80</td>
<td>3.28</td>
<td>75.5</td>
<td>Total</td>
<td>3.76</td>
<td>0.69</td>
<td>4.45</td>
<td>84.5</td>
<td>Total</td>
<td>2.63</td>
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</table>
Appendix D
Mass Balance of Total Monensin Lost
Day 16 – Day 50

Introduction:
Due to the excessive rainfall during the period of the experiment, runoff losses occurred from the piles of both treatments. To determine the extent to which loss of monensin in runoff contributed to its removal from the piles, the following analysis was conducted. Since a goal of the research was to evaluate the degradation of monensin under compost and static conditions, it was important to verify if runoff losses were significant enough to result in substantial overestimation of removal due to microbial degradation.

Total amount of compost:

Givens:

<table>
<thead>
<tr>
<th>CP</th>
<th>SP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0: 2,925.7 kg Mass of material</td>
<td>Day 0: 2,925.7 kg Mass of material</td>
</tr>
</tbody>
</table>

Monensin Conc. Totals

<table>
<thead>
<tr>
<th>CP</th>
<th>SP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pile Height:
*Assumption: The width and the length of the piles stayed the same, and only the height changed. The fractional reduction in pile height is assumed to be equivalent to the fractional loss of compost mass.

<table>
<thead>
<tr>
<th>CP</th>
<th>SP</th>
</tr>
</thead>
<tbody>
<tr>
<td>58% loss in height</td>
<td>33% loss in height</td>
</tr>
</tbody>
</table>

Estimate of mass of material from Day$_{16–50}$

<table>
<thead>
<tr>
<th>CP</th>
<th>SP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The total mass of monensin lost from the compost treatment from day 16 – 50, makes up approximately 21% of the total mass of monensin from day 0.

The total mass of monensin lost from the static treatment from day 16 – 50, makes up approximately 22% of the total mass of monensin from day 0.
Mass balance of Monensin Lost in Runoff
Day 16 – Day 50

Compost Piles (CP)

Day 16

\[ MC_{16} = 61.5\% \]
\[ 38.5\% \text{ solids} \]

Precipitation

1. MC
2. Runoff
3. Evap.°

Day 50

\[ MC_{50} = 79.7\% \]
\[ 20.3\% \text{ solids} \]

Evaporation

Water Balance

This is a conservative assumption because evaporation is being neglected.

\[ \text{volume/bay} \]

Runoff Losses

\[ 5.59 \text{ inch} \]

\[ 3.9 \text{ m}^2 \]

\[ \text{final water} \]
Mass of Monensin from Runoff Losses

from compost.
**Static Piles (SP)**

**Water Balance**

This is a conservative assumption because evaporation is being neglected.

1. MC
2. Runoff
3. Evap.

**Runoff Losses**

5.59 inch

3.9 m$^2$
Mass of Monensin from Runoff Losses

Concluding Remarks:
For both CP and SP treatments, the calculated amounts of monensin lost in runoff were only 1 – 2% of the total monensin present at day 16. Several assumptions have been made in this analysis. The assumption of no evaporation from the piles is clearly an unrealistic assumption. However, this assumption resulted in a greater calculated runoff volume than actually occurred. If evaporation was accounted for, the percentage losses of monensin would be lower than calculated. This reasoning lends credibility to the conclusion that monensin disappearance from the piles between days 16 and 50 was predominately due to degradation.
References


EPA. 1995. Composting: decision maker’s guide to solid waste management. Volume II (EPA 530-R-95-023), USA.


