Sorption characteristics of veterinary ionophore antibiotics monensin and lasalocid to soil clay constituents kaolinite, illite and montmorillonite

Kathie Lanette Swan

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A Thesis

Entitled

Sorption Characteristics of Veterinary Ionophore Antibiotics
Monensin and Lasalocid to Soil Clay Constituents
Kaolinite, Illite and Montmorillonite

by

Kathie Lanette Swan

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the Master of Science Degree in Geology.

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Dr. Alison Spongberg, Committee Chair

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The University of Toledo
December, 2012
An Abstract of
Sorption Characteristics of Veterinary Ionophore Antibiotics
Monensin and Lasalocid and Soil Clay Constituents
Kaolinite, Illite and Montmorillonite
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Monensin and Lasalocid are veterinary ionophore antibiotics used for therapeutic and non-therapeutic treatment of livestock. Manure containing un-metabolized compounds is in turn used as beneficial amendment for agricultural crops, thus, these compounds may be available for transport through soils. These antibiotics have been detected in run-off, surface water, and groundwater and may be toxic to soil and aquatic biota. This research focuses on adsorption mechanisms of Monensin (MON) and Lasalocid (LAS) to clay constituents common in agricultural soils. Methods were developed to extract these compounds from water, soil, and sludge and for subsequent analysis using liquid chromatography tandem mass spectrometry. Batch equilibrium experiments indicated that Monensin and Lasalocid have unique adsorption mechanisms that are highly pH-dependent. Predicted order of adsorption strength and desorption capabilities were not observed.
Illite (ILL) had optimal adsorption conditions for and showed strong affinity for both LAS and MON. Desorption was very high in the competitive study. Kaolinite (KAO) showed moderate adsorption and desorption was nearly twice as high when the compounds were paired. Montmorillonite (MTM) displayed low adsorption and desorption was higher in the non-competitive study. More retention of LAS by MTM was apparent in the competitive study. Manure (MAN) used in this study did not show a strong affinity for the MON and LAS complexes. Desorption from this manure was low, particularly for LAS in the competitive study. Ottawa sand (OTS) showed little adsorption as was expected. Desorption was generally moderate, except for MON which was high, particularly in the non-competitive study. Soil (GLRI) adsorption was generally high and there was a one order of magnitude difference between the non-competitive and competitive studies.

Adsorption was rapid and partially irreversible unto all sorbents indicating that portions of these compounds may remain in soils, possibly toxic to biota until degradation occurs. Remaining adsorbed compound has limited availability for transport via runoff to surface waters, leaching to groundwater, transformation, and/or bioaccumulation. More research regarding the fate of Monensin and Lasalocid is necessary to fully understand their behavior in soil, particularly in Northwest Ohio which is a large agricultural region spatially proximate to the Great Lakes.
Acknowledgements

With God all things are possible (Matthew 19:26). I would like to first thank God, without whom there would be nothing magnificent to awe, question, study, or discover.

To my parents, none better, I am blessed and glad I’ve got you. Kudos, my daughters, you always say I provide you with inspiration, but it’s you two, who inspire, lift, and give me courage. I love you both and will never forget the support you’ve given me and all you’ve taught me. When life hands you lemons, make that pretty sour pucker face and kiss me….hugs ah endorphins!

To my grandbabies, Ming, Lena and Nanny, you are magnificent! Thanks to my family and friends (you all know who you are) for tolerating my moods, absences and incessant chatter about this project. 24/7 Priceless! I would also like to thank Dr. Alison Spongberg for welcoming me into her lab, giving me patient support and encouragement, teaching me to think and speak the science, that cool really is cool and chemistry is not a four letter word. Thanks to my committee members, you are more appreciated than you know. Jason Witter, this wouldn’t have been possible without you. There are no words….thanks man!
Table of Contents

Abstract ........................................................................................................................................ iii

Acknowledgements ...................................................................................................................... v

Table of Contents ........................................................................................................................ vi

List of Tables ................................................................................................................................ ix

List of Figures ............................................................................................................................... x

1. Introduction to Monensin and Lasalocid ............................................................................. 1

1.1 Monensin and Lasalocid ....................................................................................................... 2

1.1.1 Compound Properties ..................................................................................................... 2

1.1.2 Uses of Monensin and Lasalocid ................................................................................ 4

1.2 Environmental Concern ....................................................................................................... 6

1.2.1 Toxicity ............................................................................................................................. 6

1.2.2 Environmental Occurrence ............................................................................................ 7

1.2.3 Fate and Transport .......................................................................................................... 7

1.2.4 Adsorption/Desorption and Degradation ....................................................................... 8

1.2.5 Fate and Transport Determinations ............................................................................... 10

1.3 Analytical Methods ............................................................................................................. 11

1.3.1 Detection, Optimization, and Separation ..................................................................... 11
1.3.2 Extraction..............................................................................................................12

1.4 Objectives and Hypotheses................................................................................13

  1.4.1 Purpose of Study..............................................................................................13

  1.4.2 Objectives........................................................................................................14

  1.4.3 Hypotheses.......................................................................................................15

2. Materials and Methodology..................................................................................16

  2.1 Sorbent Matrices: Clays, Soils, and Manure......................................................17

  2.2 Analytical Methodology......................................................................................19

    2.2.1 Chemicals and Reagents..............................................................................19

    2.2.2 Liquid Chromatography Tandem Mass Spectrometry.................................20

    2.2.3 Matrix Effects...............................................................................................25

    2.2.4 Quantification, Method Linearity and Detection Limits..............................30

  2.3 Experimental Design..........................................................................................32

    2.3.1 Soil to Solution Ratios.................................................................................33

    2.3.2 Direct Method Procedure, Extraction, and Recovery.................................34

    2.3.3 Kinetics........................................................................................................38

    2.3.4 Adsorption....................................................................................................38

    2.3.5 Desorption....................................................................................................39

  2.4 Sorption Isotherms and Distribution Coefficient Calculations..........................40

  2.5 Environmental Validity and Relevance............................................................42

3. Results and Discussion..........................................................................................43

  3.1 Batch Equilibrium Experiments.......................................................................43
3.1.1 Adsorption Kinetics..............................................................44
3.1.2 Adsorption Coefficients..................................................47
3.1.3 Adsorption Isotherms.......................................................49
3.1.4 Desorption Kinetics..........................................................53
3.1.5 Isotherm Shapes..............................................................56
3.2 Adsorption Mechanisms......................................................65
4. Conclusions and Future Research........................................71
  4.1 Objectives and Hypotheses...............................................71
  4.2 Future Research..............................................................76
References.............................................................................78
Appendix A Adsorption Kinetics............................................84
Appendix B Adsorption Isotherm Raw Data.............................86
Appendix C Desorption Kinetics..............................................110
List of Tables

Table 1.1 Properties of Monensin.................................................................2
Table 1.2 Toxicity of Monensin and Lasalocid...........................................6
Table 1.3 Summaries of Analytical Methods for Detection.........................13
Table 2.1 Clay Minerals Reference Data......................................................18
Table 2.2 Instrument Optimization Parameters...........................................22
Table 2.3 Percent Matrix Effects of Aqueous Solutions.............................29
Table 2.4 Percent Matrix Effects of Soil and Extract Solutions....................30
Table 3.1 Adsorption Kinetics by Percent Adsorption.................................44
Table 3.2 Non-Competitive Adsorption Isotherm Parameters.....................48
Table 3.3 Competitive Adsorption Isotherm Parameters............................50
Table 3.4 Non-Competitive Percent Desorption..........................................54
Table 3.5 Competitive Percent Desorption..................................................55
Table 3.6 Adsorbent ZPC, CEC, and pH.....................................................69
List of Figures

Figure 1-1 Structure of Monensin and Lasalocid..........................................................3
Figure 1-2 Structure of Monensin and Lasalocid Complexes.......................................5
Figure 2-1 Gradient Elution Method............................................................................26
Figure 2-2 Matrix Effects of % Acetonitrile..................................................................29
Figure 2-3 Extraction Method Selection......................................................................36
Figure 2-4 Method Recovery......................................................................................37
Figure 3-1 Percent Adsorption with Time.................................................................46
Figure 3-2 Linear Adsorption Isotherm & Freundlich Isotherm ILL..........................52
Figure 3-3 Shapes of Isotherms................................................................................57
Figure 3-4 Isotherm Shapes for Ottawa Sand.............................................................60
Figure 3-5 Isotherm Shapes for Illite..........................................................................61
Figure 3-6 Isotherm Shapes for Montmorillonite.....................................................63
Figure 3-7 Isotherm Shapes for GLRI........................................................................64
Figure 3-8 Isotherm Shapes for Manure....................................................................65
Figure 3.9 Example of Zero Point of Charge.............................................................68
Chapter 1

Introduction to Monensin and Lasalocid

Worldwide production and use of pharmaceuticals for human medical practice and animal husbandry are growing multi-billion dollar enterprises. Current research regarding environmental contamination by pharmaceuticals is focused upon detection, transport, retention and toxicity in surface water, groundwater and soils. Many compounds survive municipal and septic system wastewater treatment processes, and persist interminably in the environment (Williams et al., 2006). Pharmaceutical compounds of the antibiotic class, often beneficial to the user, are becoming recognized as emerging environmental contaminants and have been associated with development of antibiotic-resistant bacteria (Diaz-Cruz et al., 2003; Watanabe et al., 2008; Hussain and Prasher, 2011). Seventy percent of all veterinary antibiotic use in the U.S. is for non-therapeutic treatment of livestock (H.R. 1549/S. 619, 2009; Song et al., 2010).
1.1 Monensin and Lasalocid

1.1.1 Compound Properties

Among the most widely used veterinary antibiotic compounds are two carboxylic polyether ionophores, Monensin (MON) and Lasalocid (LAS) (Hansen et al., 2009a). MON and LAS are anticoccidials biosynthesized by *Streptomyces cinnamonensis* and *Streptomyces lasaliensis* bacteria, respectively. These compounds have relatively high molecular weights and low solubility in water. Chemical properties and structure of MON and LAS are presented in Table 1.1 and Figure 1-1.

<table>
<thead>
<tr>
<th>Table 1.1 Properties of Monensin and Lasalocid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compound</strong></td>
</tr>
<tr>
<td>Chemical Formula</td>
</tr>
<tr>
<td>Molecular Weight</td>
</tr>
<tr>
<td>CAS #</td>
</tr>
<tr>
<td>Solubility H(_2)O-25°C</td>
</tr>
<tr>
<td>Source Bacteria</td>
</tr>
<tr>
<td>pKa</td>
</tr>
<tr>
<td>Log K(_{ow})</td>
</tr>
</tbody>
</table>

pKa- acid dissociation constant, LogK\(_{ow}\)- octanol-water partition coefficient; All source information is from Hansen (2009c) unless otherwise indicated; \* Calculated U.S. EPA, EPI Suite (2012); \dagger Sassman and Lee (2007); \ddagger Russell and Houlihan (2003); € Hoogerheide and Popov (1979).
Differing from other antibiotic classes, ionophores have the ability to form complexes that facilitate their bioactivity. Complexes can form with monovalent and, in the case of LAS, divalent metal cations (Lopes et al., 2002, 2006; Cha et al., 2005). The complex formed by carboxylic ionophores such as MON and LAS is described as pseudo-macrocyclic (Martinek et al., 2000; Huczyński et al., 2006) (Figure 1-2). The complexes are neutral and very stable; interior oxygens are coordinated with the metal cation and the ring is closed by hydrogen bonding of the head and tail ends of the ionophore molecule (Aggarap et al., 1967; Woznicka et al., 1989; Chapman et al., 2010).

![Figure 1-1 Structure of Monensin and Lasalocid free acid](image-url)
The complex is unique, exhibiting a polar interior and a non-polar highly hydrophobic exterior which enables free movement across lipid bi-layers of cells to exchange cations (Ben-Tal et al., 2000; Chapman et al., 2010). This action ultimately results in cell ion imbalance and subsequent cell death within target species (Volmer and Lock, 1998; Hansen et al., 2009b).

1.1.2 Uses of Monensin and Lasalocid

MON and LAS are used to prevent disease and promote growth in cattle and poultry, particularly at confined animal feeding operations (CAFOs). Dosage methods to host species are commonly oral via feed mix, and released to the environment by fecal excretion. Concentrations excreted in fecal deposits, up to 95% un-metabolized (Sassman and Lee, 2007; Hansen et al., 2009b), as well as toxicity to biota are highly species-dependent.
Figure 1-2 Structure of Monensin and Lasalocid coordinated with Sodium Cation.
1.2 Environmental Concern

1.2.1 Toxicity

MON and LAS have been found to be moderately to extremely toxic to terrestrial and aquatic organisms (Sassman and Lee, 2007; Nogueira et al., 2009; Zizek et al., 2011). For instance, LD$_{50}$ rates for oral single dosages range from 1-2 mg kg$^{-1}$ for horses to 122 mg kg$^{-1}$ for rats (Kart and Bilgili, 2008; Watanabe et al., 2008). Comparatively, Material Safety Data Sheets for the commonly used antibiotic, Amoxicillin, report an oral LD$_{50}$ of 15,000 mg kg$^{-1}$ for rats. MON and LAS particularly attack all three types of muscle tissue, resulting in considerable cellular damage to most tissue and organ systems (Kart and Bilgili, 2008). MON and LAS are considered extremely toxic to humans, thus are not used as human pharmaceuticals. However, these compounds may become a potential hazard to humans via persistence in edible plant and animal tissues (Volmer and Lock, 1998; Matabudul et al., 2001; Olejnik et al., 2009). Representative toxicities are presented in Table 1.2.

<table>
<thead>
<tr>
<th>Table 1.2 Toxicity of Monensin and Lasalocid</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD$_{50}$ Oral</td>
</tr>
<tr>
<td>*mg kg$^{-1}$</td>
</tr>
<tr>
<td>Rat</td>
</tr>
<tr>
<td>Monensin</td>
</tr>
<tr>
<td>Lasalocid</td>
</tr>
</tbody>
</table>

*Applies to single dose mg kg$^{-1}$ animal weight. Kart and Bilgili (2008)
1.2.2 Environmental Occurrence

MON and LAS are introduced into the environment indirectly as fecal deposits used as agricultural amendments to soil in the form of solid cattle manure, liquid poultry manure, or manure-rich lagoon wastewater from CAFOs (Dolliver and Gupta, 2008; Watanabe et al., 2008). Upon land application of manure, soils become the primary reservoir for MON and LAS (Kim and Carlson, 2006, Song et al., 2010). Song et al. (2010) detected MON in agricultural soils and found distribution coefficients ranging from 1.4 to 8.3 L kg\(^{-1}\).

1.2.3 Fate and Transport

The fate of pharmaceutical compounds in soil is heavily dependent on adsorption and desorption processes. Adsorption can be described as movement of a chemical species (adsorbate) from the aqueous phase onto the surface of an adsorbent (solid phase or soil). Desorption is the reversal of the adsorption process. Factors affecting adsorption and desorption are the chemical properties of the adsorbate, climate, pH and temperature of the soil environment, physical and chemical properties of inorganic and organic soil constituents, such as cation exchange capacity (CEC), surface area, and charge of soil colloids. One important chemical property of MON and LAS is their ability to exist as an organic anion and/or a neutral complex dependent upon environmental conditions. If pharmaceuticals such as MON and LAS do not degrade prior to leaching into soil, they may be available for bioaccumulation, photo-
transformation, bio-transformation and/or transport to surface water and groundwater during precipitation events (Diaz-Cruz et al., 2003).

Occurrence of MON in other environmental compartments has also been previously reported in literature. Researchers found MON in water samples taken from flush lanes, lagoons and groundwater of two CAFOs (Watanabe et al., 2008). MON has also been detected in surface water (Cha et al., 2005; Thompson et al., 2009; Forrest et al., 2011) and sediment (Kim and Carlson, 2006). MON has exhibited high potential for transport through runoff in simulated agricultural studies (Davis et al., 2006) and has been detected at levels over 100 ng L$^{-1}$ in drainage and stagnant surface water receiving agricultural runoff (Song et al., 2007). Surface water receiving runoff from animal agricultural operations in Ontario, Canada, contained several veterinary pharmaceuticals, including MON at concentrations up to hundreds of ng L$^{-1}$ (Hao et al., 2006). Although there has been some method development for detection of LAS in environmental samples, no reports of application of these methods could be found.

1.2.4 Adsorption/Desorption and Degradation

Limited literature is available regarding adsorption/desorption mechanisms of MON and LAS to soils. Sassman and Lee (2007) conducted controlled experiments with ten soils and reported distribution coefficients up to hundreds of L kg$^{-1}$. Soils varying in organic content and clay fraction (Sassman and Lee, 2007) resulted in high variance of adsorption coefficient ($K_d$) values. Two selected soils for their study had $K_d$ values of 10.8 and 210 L kg$^{-1}$. The two
soils had organic contents of 0.64% and 1.34% and clay fractions of 5% and 21%, respectively. These values concur with the concept that organic content and clay fraction play a large role in adsorption processes for these compounds. However, in this same study, results indicated that a soil with an organic content percentage of 0.52 and a clay fraction of 8% had a $K_d$ value of 44.5 L kg$^{-1}$, and another with 2.2% organics and a clay fraction of 33%, had a $K_d$ of 16.1 L kg$^{-1}$. These results indicate that the clay composition may have a strong influence upon adsorption and that more research is needed.

Degradation rates for MON and LAS also vary in the literature. Sassman and Lee (2007) reported degradation as rapid; a slight increase was observed after manure amendment. For one soil, with and without manure amendment, half life for MON was reported at 2 and 1.6 days and for LAS at 3.6 and 4.3 days, respectively. This suggests that LAS not only takes longer to degrade but also has increased persistence in the presence of manure. Dolliver et al. (2008) reported half lives for MON of 11 and 23d in control and composted manure samples respectively. Carlson and Mabury (2006) reported half lives of 13.5 days for MON.
1.2.5 Fate and Transport Determinations

Sassman and Lee (2007) reported highly pH-dependent moderate adsorption strength for LAS>MON in several soils. Further, degradation of MON was faster than LAS in laboratory controlled conditions of temperature, pH and soil moisture. Forrest et al. (2011) reported high incidence of MON detection from various sites, which they attributed to more widespread use of MON than other ionophores included in the study. Watanabe et al. (2008) reported persistence of MON in soils containing high concentrations with some natural attenuation throughout CAFO manure preparation stations, particularly in anoxic groundwater associated with a storage lagoon. In this study, no MON concentration was detected in groundwater beneath agricultural fields previously amended with lagoon water. Attenuation may be optimal in the aerobic portion of the soil.

Hussain and Prasher (2011) constructed a treatment wetland for mitigation of MON and other ionophores and found various treatments least effective for MON. Song et al. (2010) found that frequency of detection and concentration in runoff from manure amended agricultural fields is affected by seasonality combined with application times and rates. Further study on effects of climate conditions, soil type, landscape, and crop growth are needed to further understanding of MON behavior in the environment.
1.3 Analytical Methods

1.3.1 Detection, Optimization, and Separation

There are no standard methods for detection and separation of MON and LAS. Detection of ionophores has been accomplished using single, triple quadrupole, and ion trap mass spectrometry (Harris et al., 1998; Cha et al., 2005; Hao et al., 2008). The addition of an electrospray ionization (ESI) interface coupled with mass spectrometry (MS) has been found to be an effective analytical tool that permits targeted mass spectrometric analysis of multiple organic residues (Volmer and Lock, 1998; Hao et al., 2006). Commonly, in the literature, the use of a C18 reversed-phase HPLC chromatographic column (stationary phase) and a water/solvent combination (mobile phase) gradient elution prove most effective for detection. A summary of existing analytical methods is presented in Table 1.3.

Since sensitivity is enhanced with the use of ESI, targeted ion signals can vary greatly and possibly be adversely affected by suppression or enhancement from co-eluting constituents, commonly referred to as matrix effects (Matuszewski et al., 2003). Ionophores present a particular challenge with regard to these matrix effects. Appropriate steps are needed to ensure the quality and reproducibility of experimental results when using liquid chromatography tandem mass spectrometry (LC-MS/MS) coupled with ESI.

Developing analytical methods for the detection of ionophores is challenging because of their physiochemical properties. Their unique
characteristics make them particularly sensitive to sample matrices, altering detection limits (DL) as matrix complexity increases. The sodium adducts of MON and LAS have been found to produce the best signal quality for detection and quantitation (Lopes et al., 2002; Schug and McNair, 2002; Lopes et al., 2006).

1.3.2 Extraction

In order to use LC/MS-MS for analysis of samples, it is necessary to extract analytes from solid matrices. Several methods employing various solvents for extraction of MON and LAS from a variety of samples are found in the literature (Hanson, 2009a). Extraction of MON and LAS has been accomplished from feed samples using microwave solvent extraction (Volmer and Lock, 1998). Extractions from egg and animal tissues (Matabudul et al., 2001), and environmental water, slurry and soil (Watanabe et al., 2008; Song et al., 2010) have been accomplished using multi-step methods combining centrifugation and either ultrasonication or solid phase extraction.
Table 1.3 Summaries of Analytical Methods for Detection of Ionophores in Environmental Waters and Soil

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Matrix</th>
<th>Instrumentation</th>
<th>Columns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monensin</td>
<td>River 2</td>
<td>LC-MS², ESI/Ion Trap</td>
<td>X-Terra/MS Genesis</td>
</tr>
<tr>
<td></td>
<td>SW 4,6,7</td>
<td>LC-MS², ESI/Ion Trap</td>
<td>Gemini C18</td>
</tr>
<tr>
<td></td>
<td>Runoff 3</td>
<td>ESI/Ion Trap</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>GW 7</td>
<td>LC-MS², ESI/Ion Trap</td>
<td>Gemini C18</td>
</tr>
<tr>
<td></td>
<td>Lagoon 7</td>
<td>LC-MS², ESI</td>
<td>Gemini C18</td>
</tr>
<tr>
<td></td>
<td>Sediment 4</td>
<td>LC-MS², ESI/Q³</td>
<td>X-Terra C18</td>
</tr>
<tr>
<td></td>
<td>Soil 5,6</td>
<td>LC-MS²</td>
<td>NR</td>
</tr>
<tr>
<td>Lasalocid</td>
<td>SW 6</td>
<td>LC-MS², ESI-Q-trap</td>
<td>Hypersil Gold C18</td>
</tr>
<tr>
<td></td>
<td>Soil 1,3,5</td>
<td>LC-MS², ESI, Ion rap</td>
<td>Allure/X-Terra C18</td>
</tr>
</tbody>
</table>

NR-not reported; SW-surface water; GW-groundwater.
1. Carlson and Mabury (2006); 2. Cha et al. (2005); 3. Davis et al. (2006); 4. Kim and Carlson (2006); 5. Sassman and Lee (2007); 6. Song et al. (2007); (2010); 7. Watanabe et al. (2008); (Hansen et al., 2009a).

1.4 Objectives and Hypotheses

1.4.1 Purpose of Study

Manure from CAFO lagoon holding ponds is often used as a nutrient amendment to agricultural fields. Northwest Ohio is a vast agricultural region occupied by several CAFOs situated in the Lake Erie watershed. Limited information exists on the chemical and physical behavior of MON and LAS in soil, thus their mobility and fate with time after land application is unknown. Open systems, such as soils, are subject to interaction with virtually all activities of humans. Because soils are dynamic, some natural attenuation of certain anthropogenic pollutants can be expected over time. However, due to their
potential for alteration of physical and chemical properties of soil (Yaron et al., 2010; Toth et al., 2011), impacts upon organisms, and increasing potential for ubiquity in the environment, the adsorption/desorption mechanisms which affect fate and transport of MON and LAS should be investigated.

Adsorption strength onto solid soil matrices is commonly but not consistently associated with increasing clay fraction and/or organic content. Data presented by Sassman and Lee (2007) conflict with previously understood mechanisms of sorption behavior. Possibly clay mineralogy rather than percentage may influence adsorptive behavior. In this study, three clays common to Northwestern Ohio soils were selected for tests and comparison to a natural soil and a reference sand. Identifying their role in adsorption and desorption of MON and LAS, may further understanding of anomalies found with regard to their behavior in the soil environment. Prior to this, analytical, extraction, and quantification methods needed to be perfected.

1.4.2 Objectives

The objectives of this study are directly related to testing the listed hypotheses and establishing useful methodology for further study with these compounds.

Objective 1) Develop methodology to detect and quantify MON and LAS using LC-ESI-MS/MS.
Objective 2) Test and quantify the sorptive behavior of MON and LAS to clay components of soil and compare results with those of a reference sand and a natural soil.

Objective 3) Identify possible sorption mechanisms and illustrate differences in competitive sorption behavior and possible effects of increased matrix complexity of natural soils from incorporation of manure.

1.4.3 Hypotheses

**Hypothesis 1** MON and LAS will rapidly and strongly adsorb to clay minerals in the order montmorillonite > illite > kaolinite, and compete with each other for sorption in these selected solid matrices.

**Hypothesis 2** Desorption rates of MON and LAS will proceed in the order kaolinite > illite > montmorillonite and will occur at a slower rate than adsorption.

**Hypothesis 3** The addition of manure will increase sorption strength and further inhibit desorption in the selected solid matrices
Chapter 2

Materials and Methodology

The hypotheses and objectives of this study were addressed using batch equilibrium experimentation. Initially, methods were developed and evaluated for instrumental analysis of Monensin and Lasalocid by LC-MS/MS analysis. Preliminary experiments and method development was required to understand instrumental limitations and their effects on precision and accuracy. The first step was optimization of instrumental and column chromatography parameters for both compounds and instrumental internal standards (ISS). Next, methodology was developed and evaluated to extract Monensin and Lasalocid from the sorbent materials tested which was required for direct method/mass balance analysis. Finally, adsorption and desorption coefficients and characteristics of the target compounds to clay minerals, soil, a reference sand, and manure were evaluated using the developed methods.
2.1 Sorbent Matrices: Clays, Soils, and Manure

American Petroleum Institute reference clays chosen for this study include Illite (ILL) #36 (Morris, IL), Montmorillonite (MTM) #21 (Polkville, MS), and Kaolinite (KAO) #4 (Macon, GA) (A.P.I., 1949) which were purchased from Ward’s Natural Science Inc. (Rochester, NY). The clays were ground using a mortar and pestle, sieved to 2 mm or less, and stored at 25°C in their original container until use.

Hypotheses for the sorptive characteristics of the chosen clays were based upon their crystal structure and related CEC. KAO, a phyllosilicate, is common in soils. Kaolinite has a 1:1 crystal structure prohibitive of swelling capacity and a low CEC of 3-15 cmol kg\(^{-1}\). Illite, particularly common in glacial soils such as those in Northwest Ohio, is a soft phyllosilicate clay formed from alteration of micas and feldspars. Potassium ions occupy the interlayer space of the crystal tetrahedron-octahedron-tetrahedron structure, prohibiting any significant swelling capacity. Illite has a CEC of 10-40 cmol kg\(^{-1}\). Montmorillonite a very common smectite clay, produced from weathering of various rocks, volcanic tuffs, and ash is the only clay in this study with a swelling capacity and a broad range CEC of 8-150 cmol kg\(^{-1}\). Reference data for the clay sorbents are presented in Table 2.1.

A soil (GLRI) was obtained from the University of Toledo Stranahan Arboretum Research Facility. Physical and chemical properties were previously determined in Dr. Spongberg’s lab and by Brookside Laboratories (New
Knoxville, OH). A particle size distribution analysis determined that this soil is 7% sand, 76% silt and 17% clay, which corresponds to a USDA texture of silt loam. Organic material (LOI at 450°C, 4 hrs.) and organic carbon (CHN analysis) are 1.8% and 1.38%, respectively. Soil pHs are 7.8 and 7.3 in 1:1 deionized water (DI) and 1:2 CaCl₂, respectively. CEC was calculated at 22.8 cmol kg⁻¹.

The silicate Ottawa sand (OTS) used as a clean reference material was acquired from Fisher Scientific (Rochester, NY). OTS is composed of well-rounded medium sand sized particles (0.25-0.5 mm) of 99% quartz mineralology and a CEC of 0.6 cmol kg⁻¹ (Zhuang et al., 2003).

<table>
<thead>
<tr>
<th>Table 2.1 Clay Mineral Reference Data</th>
</tr>
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<tbody>
<tr>
<td>Clay Mineral</td>
</tr>
<tr>
<td>Clay Type</td>
</tr>
<tr>
<td>CEC</td>
</tr>
<tr>
<td>Hardness</td>
</tr>
<tr>
<td>Properties</td>
</tr>
<tr>
<td>Location</td>
</tr>
<tr>
<td>pH DI</td>
</tr>
<tr>
<td>pH TS</td>
</tr>
</tbody>
</table>

A.P.I. Reference Clay Minerals (1949); DI- deionized water; TS- test solution after agitation for 24h. CEC- cation exchange capacity measured in cmol kg⁻¹. Parentheses indicate standard deviation of the test set.
Manure (MAN) used in this experiment was taken from a lagoon holding pond at a CAFO facility in Northwest Ohio. Manure had 12.89% solids established by gravimetric analysis. Analysis was completed using four volumes of manure slurry pipetted into clean tin containers previously weighed on a Mettler Toledo AB104 balance (Toledo, OH), at volumes of 0.025 L, 0.005 L, 0.00375 L, and 0.0025 L in triplicate. Samples were weighed, and placed in a drying oven at 105°C for 36 hours. Dried slurry was then weighed for calculation of percent solids using equation Eq. 1. Manure pH was 7.8 and 7.9 in 1:1 deionized water and 1:2 CaCl₂, respectively.

Eq. 1 % solids = \( \frac{((C_i + S_i) - C_f)}{((C_i + S_i) - C_i)} \times 100 \)

Where: C = grams container, S = grams soil, i= concentration initial and f = concentration final

### 2.2 Analytical Methodology

#### 2.2.1 Chemicals and Reagents

Standards of MON as sodium salt and LAS at 100 mg L\(^{-1}\) dissolved in acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO). A stock standard of MON was made at 100 mg L\(^{-1}\) in acetonitrile. All standards were stored at -20°C. Solvents and reagents were purchased from Fisher Chemical (Fair Lawn, NJ). All compounds, solvents, and reagents used in this study were of HPLC or analytical grade.
2.2.2 Liquid Chromatography Tandem Mass Spectrometry

Analytical instrumentation used for compound analysis consisted of a Varian 1200L triple quadrupole tandem mass spectrometer equipped with dual off-axis ESI, a Varian ProStar 210 binary LC pump system, a Varian ProStar 420 auto-sampler (Walnut Creek, CA), and a Phenomenex Thermosphere HPLC column heater/chiller (Torrence, CA). Varian Workstation 6.8 software (Walnut Creek, CA) was used for instrumental control, data acquisition and quantification.

Instrumental conditions for each analyte were optimized using flow injection analysis. A Harvard Apparatus mechanical syringe pump (Cambridge, MA,) was used to deliver continuous flow of 20 µL min⁻¹ of a 1000 µg L⁻¹ stock solution prepared in acetonitrile of each analyte into the mass spectrometer. During flow, automated voltage adjustments were made to the nebulizing needle, spray shield, capillary, and breakdown collision energies. The following gases were used for portions of mass spectrometric analyses: (1) nitrogen, for electrospray ionization (ESI) nebulization, (2) argon for collision-induced fragmentation of ions, and (3) nitrogen as the drying gas.

The first objective of this research was to develop methods to detect and quantify MON and LAS. The mass spectrometer identifies ions by mass to charge (m/z) ratios. Mass spectra were detected and identified through open scanning of spectra in the range of 100-800 m/z with subsequent readjustment of ranges closer to expected parameters of the target ions. The precursor and four
product ions of each compound were acquired in (ESI+) mode and monitored in multiple reaction mode. Initially, the mass spectrometer was programmed to acquire the [M + H+] precursor of each analyte which is commonly used in analysis of pharmaceutical compounds, however spectral intensity was low and detection limits were unacceptable. Since both MON and LAS show high affinity for complexation, the favored sodium adduct ion [M + Na+] was isolated as the precursor ion for MON with an m/z of 693.5. The two most abundant product ions were recorded and used as quantification [m/z 461] and confirmation [m/z 675] ions. The same procedure was applied to LAS. A precursor ion [M + Na+] of 613.2 was detected along with product ions in order of abundance [m/z 377] and [m/z 595]. Parameters for Simatone (SIM), an internal standard used to accommodate for instrument variations, were acquired from previous in-house lab experimentation and validation. Instrumental optimization parameters and quantification ions are presented in Table 2.2. For purposes of reporting, only the first transition (product) ion data will be presented here.
Table 2.2 Instrument Optimization Parameters and Quantification Ions

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Molecular Weight (g)</th>
<th>ESI mode</th>
<th>Precursor ion (m/z)</th>
<th>Product ion (m/z)</th>
<th>Capillary</th>
<th>CE</th>
<th>Needle</th>
<th>Shield</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIM*</td>
<td>197.24</td>
<td>+</td>
<td>198.1</td>
<td>113.9</td>
<td>55</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAS</td>
<td>590.79</td>
<td>+</td>
<td>613.2</td>
<td>377</td>
<td>55</td>
<td>38</td>
<td>5100</td>
<td>275</td>
</tr>
<tr>
<td>LAS</td>
<td>590.79</td>
<td>+</td>
<td>613.2</td>
<td>595</td>
<td>55</td>
<td>26.5</td>
<td>5100</td>
<td>275</td>
</tr>
<tr>
<td>MON</td>
<td>670.87</td>
<td>+</td>
<td>693.3</td>
<td>675.2</td>
<td>105</td>
<td>31.5</td>
<td>4350</td>
<td>325</td>
</tr>
<tr>
<td>MON</td>
<td>670.87</td>
<td>+</td>
<td>693.3</td>
<td>461.1</td>
<td>105</td>
<td>45</td>
<td>4350</td>
<td>325</td>
</tr>
</tbody>
</table>

Capillary, Collision energy (CE), Needle, and Shield are all in voltages.

Liquid chromatography relies on interactions between the mobile (solvents) and stationary (typically C18 material) phases and analytes of interest. Reversed-phase high performance liquid chromatographic (HPLC) columns function as the stationary phase to retain and separate target analytes by chemical properties such as polarity. HPLC columns enable focus of the mass spectrometer detector power at a particular time upon the analyte of choice while also separating interferences.

Three HPLC columns were tested both with and without a Waters guard column (Milford, MA) attached. The columns tested included a Genesis C18 150 X 2.1 HPLC reversed-phase column (Walnut Creek, CA), a Supleco Discovery C18 150 X 4.6 HPLC reversed-phase column (St. Louis, MO), and an Agilent Zorbax Eclipse XDB 150 X 4.6 reversed-phase HPLC column (Santa Clara, CA). The Genesis C18 and Discovery C18 were finally selected for further method refinement based on their wide usage in the literature (Table 1.3), availability in the laboratory, and acceptable performance. Each column was placed in the
column heater/chiller and kept at 25°C during testing. A stock solution of 1000 
µg L⁻¹ of each analyte prepared in acetonitrile was pipetted into 2 mL amber 
glass autosampler vials and injected at 20 µL samples via autosampler. Flow rate 
was maintained at 0.3 mL min⁻¹ while water/solvent combinations and pump 
methods were tested to acquire optimal retention times and detection limits for 
each compound. The following mobile phase solution combinations were tested 
with each column (stationary phase):

Mobile phase 1) Pump A: 0.1% formic acid/Nanopure water; Pump B: 
acetonitrile only

Mobile phase 2) Pump A: 0.03 M ammonium formate/Nanopure water; 
Pump B: acetonitrile only

Additional mobile phase solution combinations were tested with the Discovery 
C18:

Mobile phase 3) Pump A: 0.05% NaCl/Nanopure water; Pump B: 
acetonitrile only.

Mobile phase methods were adjusted to test both isocratic (100% solvent) and 
gradient elution of the target compounds.

Detection limits were high and retention times exceeded 25 minutes with 
the Genesis C18 and Discovery C18 columns testing mobile phase program 1 and 
2 with all solution combinations. The signals produced by the Genesis C18 
contained multiple peaks, an indication of staggered, poor elution, and had high 
detection limits, indicating poor signal intensity and stability. The Genesis C18
column failed to re-equilibrate during the portion of the elution program allocated to returning the column to its initial conditions between sample injections. Column dimensions and/or the size or condition of column packing material may have attributed to the problem. Eluents from the Discovery C18 produced spectra with varying detection limits and shifting retention times, thus signals were deemed unreliable. Mobile phase 3 was tested with the Discovery C18 column and initially produced acceptable retention times and high detection limits, however over time the salts in solution were corrosive to the nebulization needle of the mass spectrometer and the method was abandoned.

After initial testing of the Zorbax Eclipse XDB with mobile phase 1 produced acceptable retention times, additional buffer was added to Pump B to improve separation. A Phenomenex KrudKatcher disposable pre-column (Torrence CA) was coupled to the column. The selected mobile phases A and B solutions consisted of 0.1% formic acid/Nanopure water and 0.1% formic acid/acetonitrile, respectively. The pump method was as follows: 0-2 minutes at 75% B, a 2-4 minute ramp to 100% B, a 4-23 minute hold at 100% B, from 23-25 minutes a return to 75% B, and a 10 minute hold at initial conditions to allow the column to re-equilibrate solvent ratio and pressure (Figure 2-1). The column temperature was maintained at 25°C. Autosampler injection volume was set at 20 µL per each sample injection.
2.2.3 Matrix Effects

Matrix effects are caused by components in a sample (other than the target analyte) which interfere with analytical results. Interference is evidenced by enhanced or suppressed signals and/or shifts in retention time. When working with different sample matrices such as test solutions, each matrix contains different components in solution other than the target analyte. Components, particularly salts in the case of ionophores, may enhance or suppress signal intensity and cause considerable inaccuracy and variability when using an ESI source for mass spectrometric analysis. If retention time is not consistent and timely, analysis of multiple pharmaceutical residues is difficult and not time effective, particularly where compounds such as MON and LAS tend to show instability over time.
Optimization and separation experiments were completed where the target analytes were prepared in pure acetonitrile, which is an optimal aprotic polar solvent and highly compatible with both analytes. USEPA 2008 test guidelines for batch equilibrium adsorption/desorption experiments (USEPA, 2008) call for the use of 0.01 M CaCl₂ solution as a soil solution in the test procedure. Further, calibration curves used to calculate compound concentrations are made from standard stock solutions prepared in solvent.
However, considerable matrix effects became apparent when standard calibration curves were used to quantify data.

Therefore, a series of experiments was designed to assess matrix effects of potential experimental test solutions and the percentage of acetonitrile in samples and soil/solutions at equilibrium. Spiked solutions (250 µg L\(^{-1}\) MON and LAS) were prepared in various combinations and analyzed according to previously described methodology. The solution combinations varied in ratio \(M\) CaCl\(_2\): % NaN\(_3\) in water (v:v), and % acetonitrile, at 3 concentrations (250, 100, and 25 µg L\(^{-1}\) MON and LAS) and were as follows, 50:50, 25:75, and 0:100. Signals from these solutions were compared to signals derived from standards prepared in 100% acetonitrile to assess feasibility of their use as alternate test solutions instead of those recommended by the USEPA protocol. Matrix effects were calculated by equation 2 as applied by Matuszewski et al. (2003).

\[
\text{Eq. 2} \quad \text{ME}\% = \frac{(B/A)}{1} \times 100
\]

Where: \(A\) = Stock solution of analyte prepared in acetonitrile

\(B\) = spiked test solution.

Calculated values > 100% or < 100% represent signal enhancement or suppression, respectively, during LC/MS-MS analysis. For example, a matrix effect of 44% indicates that the quantifiable signal represents only 56% of what would manifest if the compound was in a 100% acetonitrile matrix. Each matrix produced some enhancement or suppression of signals, reduction of detection
limits and expected changes in retention time. Matrix effects for aqueous solutions are presented in Table 2.3.

The effect of the percentage of acetonitrile in test solution was also tested and results are presented in Figure 2-2. Results confirmed that all samples and matrix matched calibrations samples required adjustment to contain a 25% solvent concentration prior to analysis. This adjustment was made directly to aliquots in autosampler vials.

Lastly, matrix effects were evaluated once test solutions reached equilibrium with the soil. To test this, each solid phase was shaken overnight in test solution to achieve equilibrium, spiked at both 100 and 500 µg L⁻¹ with MON and LAS and shaken again for 24 hours. An aliquot of each was analyzed and results were compared with the matrix effects of acetonitrile alone and the test solution matrices. Results are presented in Table 2.4.
Table 2.3 Percent Matrix Effects of Aqueous Solutions

<table>
<thead>
<tr>
<th>Matrix</th>
<th>LAS</th>
<th>MON</th>
<th>SIM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Methanol</td>
<td>90</td>
<td>102</td>
<td>95</td>
</tr>
<tr>
<td>CaCl₂: NaN₃ (0.01 M: 1%)</td>
<td>91</td>
<td>73</td>
<td>65</td>
</tr>
<tr>
<td>CaCl₂ (0.01 M)</td>
<td>104</td>
<td>93</td>
<td>83</td>
</tr>
<tr>
<td>Soil Leachate</td>
<td>44</td>
<td>72</td>
<td>92</td>
</tr>
<tr>
<td>DI H₂O</td>
<td>119</td>
<td>123</td>
<td>79</td>
</tr>
<tr>
<td>CaCl₂ (0.001 M)</td>
<td>139</td>
<td>95</td>
<td>117</td>
</tr>
<tr>
<td>NaN₃ (1%)</td>
<td>102</td>
<td>133</td>
<td>64</td>
</tr>
<tr>
<td>NaN₃ (0.01%)</td>
<td>122</td>
<td>121</td>
<td>80</td>
</tr>
</tbody>
</table>

Figure 2-2 Matrix Effects relative to % Acetonitrile in sample solutions. Error bars represent relative standard deviation n=3.
Table 2.4 Percent Matrix Effects of Soil and Extract Solutions

<table>
<thead>
<tr>
<th></th>
<th>Acetonitrile</th>
<th>CaCl₂</th>
<th>Extract †</th>
<th>MTM: CaCl₂</th>
<th>MTM: Extract †</th>
<th>GLRI: CaCl₂</th>
<th>GLRI: Extract †</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>100 µg L⁻¹</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAS</td>
<td>100</td>
<td>157</td>
<td>61</td>
<td>48</td>
<td>83</td>
<td>63</td>
<td>60</td>
</tr>
<tr>
<td>MON</td>
<td>100</td>
<td>109</td>
<td>53</td>
<td>41</td>
<td>49</td>
<td>40</td>
<td>34</td>
</tr>
<tr>
<td>SIM*</td>
<td>100</td>
<td>63</td>
<td>110</td>
<td>33</td>
<td>52</td>
<td>30</td>
<td>68</td>
</tr>
<tr>
<td><strong>500 µg L⁻¹</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAS</td>
<td>100</td>
<td>100</td>
<td>51</td>
<td>16</td>
<td>36</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td>MON</td>
<td>100</td>
<td>79</td>
<td>50</td>
<td>28</td>
<td>27</td>
<td>27</td>
<td>28</td>
</tr>
<tr>
<td>SIM*</td>
<td>100</td>
<td>51</td>
<td>88</td>
<td>26</td>
<td>31</td>
<td>23</td>
<td>53</td>
</tr>
</tbody>
</table>

† Test solution of CaCl₂ and NaN₃ (0.01 M: 1%) in water; †Extract Solution v:v 20:80 0.03M Ammonium Acetate in water: acetonitrile; *Instrumental Internal Standard concentration kept constant at 250 µg L⁻¹ for each set of standards.

2.2.4 Quantitation, Method Linearity, and Detection Limits

Instrumental and method detection limits establish the lowest concentration levels for which reliable and reproducible results could be quantified and validated. Limits of detection and limits of quantitation are defined as signal to noise (S/N) ratios of >3 and >10, respectively, for the most abundant monitoring ions. Values were established using blank samples of test solution spiked at graduated concentrations from 250 µg L⁻¹ to 1 µg L⁻¹. Linearity of the instrument response was also determined using a similar procedure. Simatone was used as an instrumental internal standard to monitor performance.
variations of the mass spectrometer. Simatone provides a consistent signal which was monitored throughout the experiments. Matrix matched calibrations were used to eradicate enhanced or suppressed signals caused by ESI sensitivity. Data were quantified using calibration curves with a minimum of four points fitted with a polynomial function resulting in $R^2$ values > 0.95. To determine working period variation for sample sets, one concentration was repeatedly sampled over the duration of the analysis phase of each experiment.

For the purposes of these experiments, each calibration sample was matrix matched to the test samples and this data was used to construct an applicable curve to obtain accurate concentration calculations for test samples.

The efficiency of SIM as an internal standard to adjust for matrix effects needed to be determined to further improve reliability of results. The internal standard failed to compensate for signal enhancement or suppression. An internal standard functions by compensating for the compound through mathematical adjustment to improve the linearity of the signal across a range of concentrations. SIM did not adjust in this manner for either compound, but the signal produced was very stable and was determined to be a good control to monitor instrumental variation, that is, reliability and accuracy of instrumental function from sample to sample and analysis to analysis.
2.3 Experimental Design

To address hypothesis 1, which predicts rapid, strong, and competitive adsorption to selected clays, batch equilibrium adsorption/desorption experiments were completed using USEPA 2008 guidelines: Fate, Transport, and Transformation Test Guidelines: OPPTS 835.1230 (EPA, 2008). Sorbent matrices included three clay minerals kaolinite, illite, montmorillonite, GLRI soil, Ottawa sand reference sand (OTS), and manure from a CAFO lagoon holding pond. Test vessels used for each experimental unit consisted of Pyrex® 25 mL round bottom glass centrifuge tubes with Teflon® lined (Lowell, MA) plastic screw-on caps. The aqueous phase used in sorption experiments consisted of 0.01 M calcium chloride (CaCl₂) and 0.01% sodium azide (NaN₃) dissolved in Nanopure water (test solution). CaCl₂ serves as artificial rain, improves centrifugation, and inhibits cation exchange during experiments (USEPA, 2008), while NaN₃ is a bacteriostat which minimizes biotransformation of the compounds during sorption experiments. Sets of control (spiked; no sorbent) and blank samples were utilized to account for cross-contamination, adsorption to experimental set-ups, degradation, and background compound in samples. All samples were stored at 4°C during non-working portions of experiments.
2.3.1 Soil to Solution Ratios

Soil/solution ratios were determined according to the guideline protocol. Briefly, a specific mass of each sorbent matrix, equivalent of 0.05, 0.1, and 0.2 grams was weighed and placed into a glass centrifuge tube, and filled with 9.998 mL of test solution yielding ratios of m:v, 1:200, 1:100 and 1:50. The tubes were placed on an end to end agitator, protected from photolysis, and shaken for 24 hrs at ambient lab temperature of 25°C to achieve equilibrium. A spike of 20 µL of a 100 mg L⁻¹ MON and LAS stock solution was added to each centrifuge tube for a final concentration of 200 µg L⁻¹ and a final volume of 10 mL, then returned to the agitator for an additional 24 hrs. Subsequently, each tube was centrifuged for 5 minutes at 1500 rpm. Each test and control sample was spiked with 150 µL of 0.5 mg L⁻¹ SIM (ISS) in acetonitrile stock solution, increasing the concentration of solvent in the vial to >25% to resolve matrix effects. The samples were then analyzed using LC-MS/MS. From those results a soil/solution ratio was determined that produced at least 50-80% sorption as suggested by the protocol. Applicable soil to solution ratios for the three clays and GLRI soil were determined at 1:200 and for sand at 1:100. Since clay and organic percentage are known to play a large role in sorptive processes, a soil/solution ratio for the manure slurry was calculated at 1:200 utilizing previously determined percent solids data. To achieve the soil/solution ratio of 1:200 and a total volume of 10 mL, 3.9 mL of MAN, (equivalent to 0.05 grams solid) was added to 5 mL of test solution and DI water to accommodate for spikes as needed.
2.3.2 Direct Method Procedures, Extraction, and Recovery

During the soil/solution phase determinations, the direct method of measurement was necessary for sorption experiments. The direct method is a mass balance analysis of the aqueous and solid phase concentrations. This approach is required when compounds are suspected of being unstable during the experimental process or lost to the experimental setup. When loss of the test substance (Sassman and Lee, 2008) was detected during the soil/solution experiments, test guidelines suggested testing the experimental setup (test vessels and caps) as the likely cause. To assess loss, test vessels with soil and test solution were shaken overnight, spiked at 500 µg L⁻¹, and shaken overnight again; an aliquot of each was analyzed. Then, each test vessel and cap was washed with 1 mL of isopropanol, methanol, or acetonitrile from which an aliquot was analyzed. If the experimental set up is not determined to be the cause of detected losses, amount of loss is determined and the direct method (mass balance) is used.

To obtain mass balance, methods were developed to extract the compounds from the solid matrices. First, a procedure to repetitively and consistently decant a large number of samples was necessary. Centrifuge tubes, in triplicate, were weighed. Sorbent and test solution were added at the determined soil/solution ratio and the samples were shaken overnight. Each sample was decanted by repeated (2X) alternation of centrifugation at 1500 rpm for five minutes and pipetting off the remaining aqueous phase in the test tube.
The solids remaining in the test tube were dried by gentle nitrogen stream and the mass was obtained.

Several sorbent extraction methods were tested. After accelerated solvent extraction (ASE) was found to be ineffective, numerous water/solvent combinations were tested followed by overnight agitation (OV) or ultrasonication (USE) and subsequent centrifugation. Attempts to extract compound from the solid matrices using solvent alone coupled with overnight agitation or ultrasonication were unsuccessful. Extraction was accomplished with a 20:80 (v:v) solution of 0.3 M ammonium acetate/water : acetonitrile (extraction solution). Several steps were required to test validity of this method. First, matrix effects of the extract solution were determined (Table 2.3). Next, to establish a working extraction method, sets of MTM clay were shaken overnight in test solution, spiked at 200 µg L⁻¹, and shaken again overnight. An aliquot of the solution was removed for analysis and the remaining solution was decanted. Sorbents were dried by nitrogen stream. One sample set (triplicate) was extracted by overnight agitation with extract solution, another set was ultrasonicated (USE) for 60 minutes with extract solution, and a third set extracted by ASE. An aliquot of each was removed and subsequently analyzed. No significant differences were found for extraction methods and USE was chosen because of time efficiency. Results are presented in Figure 2-3. To determine recovery, sets of MTM clay and OTS with test solution were brought to equilibrium overnight, decanted and dried by nitrogen stream, each sample
was spiked with 50 µL of 10 mg L⁻¹ of MON, immediately extracted with 1 mL of extract solution, of which and aliquot was removed and analyzed. Recovery from MTM, the potential strongest adsorbent and OTS the potential weakest adsorbent were above 90%. Results are presented in Figure 2-4.

Figure 2-3 Extraction Method Selection
Figure 2-4 Method Recovery; OTS-Ottawa Sand, MTM-montmorillonite
2.3.3 Kinetics

Adsorption kinetics was determined at one concentration with the sand, three clay minerals, and soil. Each sorbent was weighed in triplicate according to the soil/solution ratio established by previous tests. For 1:200 and 1:100 samples 0.05 g or 0.1 g of soil were placed in 25 mL glass centrifuge tubes with 9.990 mL of test solution and agitated overnight. Then each sample was spiked with 10 µL of 100 mg L⁻¹ MON or LAS, returned to the agitator, and sampled at time intervals of 2, 4, 8, 16 and 24 hrs. Control samples consisted of test solution only. Blank matrix matched solutions were created for each time interval. Controls and blanks were sampled the same as the soil samples, and all samples were analyzed. Matrix matched calibration curves were constructed from the 8 hour samples. Equation 3 was used to calculate kinetic data.

Eq. 3 \( A_{ti} (\%) = \frac{M_{sads} (t_i)}{M_0} \times 100 \)

Where: \( M_{sads} (t_i) \) = mass in soil at the time \( t_i \)

\( M_0 \) = mass total in test solution at test onset

2.3.4 Adsorption

The remaining experiments were conducted at five concentrations at equilibrium. Each sorbent material was weighed according to the soil solution ratio, placed in a test vessel with the appropriate amount of test solution to accommodate spikes at 250, 200, 100, 50 and 25 µg L⁻¹, and maintain the soil to solution ratio. The span of two orders of magnitude in concentration recommended by the protocol was achievable due to detection and linearity
limits. The samples were agitated overnight to equilibrium, spiked with MON or LAS, and removed from the agitator at the appropriate equilibrium time as established by the kinetic study. After centrifugation for 5 minutes at 1500 rpm, an aliquot of the sample was removed for subsequent analysis. Samples were then decanted and dried by nitrogen stream. Samples were extracted with either 1 or 2 mL of extraction solution by USE for 60 minutes. An aliquot of the extract solution was removed for subsequent analysis.

For the competitive adsorption study (Hypothesis 1), less test solution was used to accommodate for dual spikes at the same concentrations used in experiments where compounds were tested separately. All other test procedures were performed as in the adsorption study.

2.3.5 Desorption

A desorption kinetics test for MON and LAS was conducted to address Hypothesis 2. Protocol desorption contact times were extended to 72 hrs, using the parallel method of desorption testing (USEPA, 2008). Sample sets were made in order to accommodate for removal times of i (initial), 8, 24, 48, and 72 hours and brought to adsorption equilibrium. Samples were spiked at 100 µg L⁻¹, returned to the agitator and allowed to reach equilibrium. After achieving equilibrium, test solution was decanted and fresh test solution (without spike) was added to each test vessel. An aliquot was removed from the first set of samples as an initial time (i) for analysis and the remaining samples were returned to the agitator for removal at appropriate times. At each time, an
aliquot of solution was removed, the remaining solution was decanted, and sorbent was dried for subsequent extraction and analysis per the previously described method.

2.4 Sorption Isotherms and Distribution Coefficient Calculations

Mass balance calculations were used to create isotherms and calculate sorption coefficients. Mass balance assumes no degradation or loss during the experimental process. Therefore both the mass remaining in the liquid phase and the mass adsorbed and extracted from the solid phase was used to determine sorption and desorption values in these experiments. Working at equilibrium and spiking at five or more concentration levels allows construction of isotherm plots, where concentration adsorbed can be plotted against concentration in the aqueous phase at equilibrium. From this, isotherms can be fitted for linear distribution ($K_d$), and Freundlich ($K_l$) coefficients, also linearity constants ($n$) and regression coefficients ($R^2$) can be calculated by Equations 4 and 5.
Eq. 4 \( K_{d_{ads}}(eq) \) (L kg\(^{-1}\)) = \((C_{s_{ads}(eq)})/(C_{aq_{ads}(eq)})\)

Where: \( C_{s_{ads}(eq)} = \) concentration in soil at equilibrium

\( C_{aq_{ads}(eq)} = \) concentration in test solution at equilibrium

Eq. 5 \( C_{s_{ads}(eq)} (\mu g \, g^{-1}) = K_{f_{ads}}(eq) \times C_{aq_{ads}(eq)} \)^{1/n}

\( n = \) linearity constant

Equations 6 and 7 were used to calculate percent desorption and \( K_{des} \) at one concentration.

Eq. 6 \( D_{ti} (\%) = \frac{M_{aq_{ads}}(t_i)}{M_{s_{ads}}(eq)} \times 100 \)

Where: \( M_{s_{ads}} = \) mass in soil at equilibrium

\( M_{aq_{ads}}(eq) = \) mass in test solution at equilibrium

Eq. 7 \( K_{des} (L \, kg^{-1}) = \frac{(M_{s_{ads}}(eq) - M_{aq_{des}}(eq)/M_{aq_{des}}(eq)) \times (V_t/M_{soil})}{M_{soil} = \text{Total mass of soil} \, (g)} \)
2.5 Environmental Validity and Relevance

Field data is often confounded by many environmental variables, such as temperature, precipitation, and external disturbance. These laboratory experiments were designed to simulate the natural setting where contamination could occur, yet hold the external variables constant or at least controlled. Concentrations used in these experiments were higher than or within the same orders of magnitude as environmentally relevant ranges or as limited by detection limits. In the case of LAS where detection limits were high, all efforts were made to reduce matrix effects or higher concentrations were used and interpreted accordingly. To assess environmental relevance, a set of three manure samples and extracts without spike were analyzed for background occurrence of MON and LAS.
Chapter 3

Results and Discussion

3.1 Batch Equilibrium Experiments

The second objective of this research was to describe the sorptive behaviors and differences of MON and LAS to the three selected reference clay minerals, soil, and pure sand. This was accomplished using batch equilibrium experiments.

Results indicate that MON and LAS adsorbed rapidly to the clay adsorbents with ILL > KAO > MTM. Competition between MON and LAS only occurred with the presence of organic matter. Desorption rates were slower than those for adsorption and were not equal for either compounds or adsorbents. Desorption rates followed the trend MTM > ILL > KAO for LAS, and MTM>KAO>ILL for MON. Thus, Hypotheses 1 and 2 were shown to be incorrect. Our traditional views on clay sorptive capacity does not hold true for these compounds.
3.1.1 Adsorption Kinetics

A kinetic study was used to evaluate adsorption rates to the sorbents. The amount (%) adsorbed at each time interval was calculated using Equation 3 and used to establish a time of equilibrium for future experimentation. Results of adsorption kinetics for all sorbents are presented in Appendix 1 and summarized in Table 3.1, and for clay sorbents in Figure 3-1.

Table 3.1 Adsorption Kinetics by Percent Adsorption

<table>
<thead>
<tr>
<th>Matrix</th>
<th>% Adsorbed (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>GLRI</td>
<td></td>
</tr>
<tr>
<td>Time (h)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>34.8(17)</td>
</tr>
<tr>
<td>OTS</td>
<td>32.6 (21)</td>
</tr>
<tr>
<td>MTM</td>
<td>81.6 (4)</td>
</tr>
<tr>
<td>ILL</td>
<td>93.4 (4)</td>
</tr>
<tr>
<td>KAO</td>
<td>67.8 (10)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Matrix</th>
<th>% Adsorbed (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>GLRI</td>
<td></td>
</tr>
<tr>
<td>Time (h)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>74.9 (6)</td>
</tr>
<tr>
<td>OTS</td>
<td>16.9 (8)</td>
</tr>
<tr>
<td>MTM</td>
<td>37.8 (5)</td>
</tr>
<tr>
<td>ILL</td>
<td>97.3 (1)</td>
</tr>
<tr>
<td>KAO</td>
<td>56.6 (17)</td>
</tr>
</tbody>
</table>

GLRI- soil; OTS- Ottawa sand; MTM- montmorillonite; ILL-illite; KAO- kaolinite; *(Standard Deviation, n=3).
Adsorption was very rapid to all sorbents and occurred in ≤ 1 hour; this was expected and is common for hydrophobic compounds (Voice and Weber, 1983). Equilibrium (defined as < 5% change) was reached within ≤ 4 hours. ILL adsorbed the greatest percentage of both compounds compared to the other sorbents, 94.6% for LAS and 99% for MON, with little variance between samples. This suggests that sorption of both compounds to ILL is strong. Both compounds adsorbed similarly to each other with respect to all matrices with the exception of MTM. LAS had high affinity for MTM and KAO. No significant difference in LAS adsorption was noticed between MTM and KAO at equilibrium. MTM adsorbed nearly two times the percentage of LAS than MON. At equilibrium time of 8h, LAS adsorption to MTM was 74.8% and for MON 37.8%. LAS adsorption onto GLRI at 4h was 8.7%. LAS lowered significantly at 12h and spiked again at 24h while MON gradually increased over the 24h. OTS had the least adsorption percentages of all sorbents as expected, for MON ~19% and LAS ~32%. Quartz is the least reactive sorbent, has lower specific surface area and has no sites to facilitate ionic adsorption processes, however, sorption was as high as 47% for LAS at 2h. This was one indication that sorption mechanisms for LAS and MON are not typical chemical mechanisms observed for other compounds. Eight hours was established as the equilibrium time for all samples.
Figure 3-1 Percent adsorption with time for Lasalocid and Monensin. Error bars represent standard deviation n=3.
3.1.2 Adsorption Coefficients

Batch equilibrium experiments were used to evaluate partitioning of chemicals between solid and liquid phases of soil without distinction of processes. This information can be used to predict and model the behavior of chemicals in soil, such as the availability of a chemical for leaching, run-off, transformation, uptake, or degradation. Adsorption data include linear adsorption distribution coefficients ($K_d$), $R^2$ values, Freundlich distribution coefficients ($K_f$), and linearity constants (n) for both non-competitive and competitive studies calculated using Equations 4 and 5. Results are presented in Appendix 2 and Tables 3.2 and 3.3, respectively.

Adsorption was generally non-linear within the range of concentrations tested in this study and isotherms were not well fitted using either the Freundlich or linear equations. The coefficient of determination ($R^2$) quantifies the fit of the data to the assumed model. $R^2$ values averaged 0.82 for all treatments. Particularly low $R^2$ values were associated with non-competitive LAS adsorption to ILL (mean 0.59), and MAN (mean 0.47). During the competitive study, low $R^2$ values were found for ILL (mean 0.57), MAN (mean 0.41), and MON for MAN (mean 0.55). Transformation to the Freundlich equation increased $R^2$ from the linear $K_d$ value in only some cases. For example, in the case of ILL non-competitive adsorption for LAS, the $R^2$ was improved from an average 0.59 to 0.65 and for MON decreased from an average 0.91 to 0.81.
Linearity constants \( (n) \) ranged from 0.6 to 2.8 with no apparent trend. EPA guidelines suggest that \( n \) values usually range from 0.7-1.0 (USEPA, 2008a). The lowest \( n \) values in this study were from the soil and illite. Sassman and Lee (2007) reported the lowest \( n \) value associated with a soil with the highest clay percentage.

### Table 3.2 Non-Competitive Adsorption Isotherm Parameters

<table>
<thead>
<tr>
<th>Adsorption</th>
<th>( K_d ) (L kg(^{-1}))</th>
<th>( R^2 )</th>
<th>( n )</th>
<th>( K_f ) (µg(^{-1}) - n L kg(^{-1}))</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LAS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTM</td>
<td>30.8 (0.6)</td>
<td>0.85 (0.1)</td>
<td>1.4 (0.4)</td>
<td>20.5 (11)</td>
<td>0.93 (0.0)</td>
</tr>
<tr>
<td>KAO</td>
<td>267.3 (52)</td>
<td>0.80 (0.2)</td>
<td>1.5 (0.3)</td>
<td>127.6 (55)</td>
<td>0.85 (0.1)</td>
</tr>
<tr>
<td>ILL</td>
<td>426.7 (51)</td>
<td>0.59 (0.2)</td>
<td>0.8 (0.1)</td>
<td>1417.9 (863)</td>
<td>0.65 (0.1)</td>
</tr>
<tr>
<td>OTS</td>
<td>3.2 (0.4)</td>
<td>0.62 (0.4)</td>
<td>1.8 (0.3)</td>
<td>1.5 (1)</td>
<td>0.80 (0.2)</td>
</tr>
<tr>
<td>MAN</td>
<td>4.1 (2)</td>
<td>0.47 (0.3)</td>
<td>1.4 (0.5)</td>
<td>3.0 (2)</td>
<td>0.55 (0.2)</td>
</tr>
<tr>
<td>GLRI</td>
<td>1470.2 (246)</td>
<td>0.87 (0.1)</td>
<td>1.1 (0.2)</td>
<td>1345.3 (625)</td>
<td>0.87 (0.0)</td>
</tr>
<tr>
<td><strong>MON</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTM</td>
<td>221.9 (37)</td>
<td>0.83 (0.4)</td>
<td>1.5 (0.2)</td>
<td>84.5 (12)</td>
<td>0.83 (0.1)</td>
</tr>
<tr>
<td>KAO</td>
<td>429.3 (54)</td>
<td>0.82 (0.2)</td>
<td>1.3 (0.1)</td>
<td>219.8 (38)</td>
<td>0.94 (0.0)</td>
</tr>
<tr>
<td>ILL</td>
<td>1374.7 (265)</td>
<td>0.91 (0.1)</td>
<td>0.9 (0.1)</td>
<td>2387.8 (906)</td>
<td>0.81 (0.1)</td>
</tr>
<tr>
<td>OTS</td>
<td>3.7 (0.3)</td>
<td>0.81 (0.1)</td>
<td>0.9 (0.3)</td>
<td>10.8 (8)</td>
<td>0.90 (0.1)</td>
</tr>
<tr>
<td>MAN</td>
<td>26.8 (2)</td>
<td>0.83 (0.0)</td>
<td>1.0 (0.3)</td>
<td>34.5 (16)</td>
<td>0.76 (0.1)</td>
</tr>
<tr>
<td>GLRI</td>
<td>1247.5 (239)</td>
<td>0.92 (0.1)</td>
<td>0.8 (0.1)</td>
<td>2248.4 (854)</td>
<td>0.81 (0.1)</td>
</tr>
</tbody>
</table>

GLRI- soil; OTS- Ottawa sand; MTM- montmorillonite; ILL-illite; KAO- kaolinite; MAN- manure; *(Standard deviation n=3)*
3.1.3. Adsorption Isotherms

Linear adsorption isotherms and Freundlich isotherms for both the non-competitive and competitive studies are presented in Appendix 1. $K_d$ values for both the non-competitive and competitive studies indicate that sorption was strongest to GLRI and ILL for both compounds, followed by moderate sorption to KAO > MTM. These values agree with the kinetic study.

In the non-competitive study, $K_d$ values for MON were 2-4 times greater than those of LAS, suggesting that MON should have out competed LAS in the competitive study. Adsorption of both compounds was weakest to OTS which was predicted from preliminary kinetic study results. Adsorption to MAN was also very low (MON showed slightly more affinity than LAS) and not significantly different from OTS. This result was not expected since manure is rich in organic material, often considered a strong adsorbent for other organic contaminants. Low adsorption of MON and LAS to MAN suggests that this particular organic matter plays a small role in enhancing partitioning to soil and its presence may not inhibit leaching of these compounds in a agricultural setting. Adsorption of MON was greater than that of LAS with all sorbent materials with the exception of GLRI (non-competitive) and OTS (competitive). Both compounds bonded strongly with GLRI. Substantial compositional differences exist between a pure clay and a natural soil thus, strong adsorption of LAS and MON to GLRI may be attributed to numerous factors. However, some factors include the presence of other ions in the soil and the age and composition
of soil organic matter. Another contributing factor could be the glacial origin of the GLRI parent material and the possibility of high ILL content common to glacial sediments, although this alone would not raise \( K_d \) values as high as were shown here.

Table 3.3 Competitive Adsorption Isotherm Parameters

<table>
<thead>
<tr>
<th>Competitive Adsorption (*)</th>
<th>( K_d ) (L kg(^{-1}))</th>
<th>( R^2 )</th>
<th>( n )</th>
<th>( K_f ) (( \mu )g(^{1-n})* mL g(^{-1}))</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTM</td>
<td>109.0 (3)</td>
<td>0.60 (0.1)</td>
<td>2.8 (0.1)</td>
<td>28.3 (2)</td>
<td>0.91 (0.0)</td>
</tr>
<tr>
<td>KAO</td>
<td>245.1 (34)</td>
<td>0.83 (0.1)</td>
<td>1.5 (0.6)</td>
<td>160.1 (172)</td>
<td>0.86 (0.1)</td>
</tr>
<tr>
<td>ILL</td>
<td>1154.1 (280)</td>
<td>0.57 (0.2)</td>
<td>2.5 (0.2)</td>
<td>55.3 (20)</td>
<td>0.61 (0.3)</td>
</tr>
<tr>
<td>OTS</td>
<td>11.4 (1)</td>
<td>0.81 (0.1)</td>
<td>1.1 (0.2)</td>
<td>13.8 (8)</td>
<td>0.72 (0.2)</td>
</tr>
<tr>
<td>MAN</td>
<td>14.0 (1)</td>
<td>0.41 (0.3)</td>
<td>1.1 (0.1)</td>
<td>12.0 (2)</td>
<td>0.70 (0.1)</td>
</tr>
<tr>
<td>GLRI</td>
<td>116.6 (12)</td>
<td>0.59 (0.3)</td>
<td>1.7 (0.4)</td>
<td>48.5 (21)</td>
<td>0.86 (0.1)</td>
</tr>
<tr>
<td>MON</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTM</td>
<td>169.7 (42)</td>
<td>0.70 (0.4)</td>
<td>1.6 (0.4)</td>
<td>73.8 (30)</td>
<td>0.93 (0.1)</td>
</tr>
<tr>
<td>KAO</td>
<td>369.4 (62)</td>
<td>0.78 (0.2)</td>
<td>0.7 (0.0)</td>
<td>886.3 (715)</td>
<td>0.90 (0.2)</td>
</tr>
<tr>
<td>ILL</td>
<td>1606.8 (411)</td>
<td>0.57 (0.2)</td>
<td>1.8 (0.2)</td>
<td>163.1 (54)</td>
<td>0.78 (0.1)</td>
</tr>
<tr>
<td>OTS</td>
<td>9.2 (4)</td>
<td>0.65 (0.3)</td>
<td>1.1 (0.4)</td>
<td>12.9 (14)</td>
<td>0.65 (0.2)</td>
</tr>
<tr>
<td>MAN</td>
<td>19.5 (1)</td>
<td>0.70 (0.1)</td>
<td>0.6 (0.2)</td>
<td>58.3 (4)</td>
<td>0.84 (0.1)</td>
</tr>
<tr>
<td>GLRI</td>
<td>250.9 (21)</td>
<td>0.75 (0.1)</td>
<td>1.7 (0.2)</td>
<td>80.9 (24)</td>
<td>0.90 (0.0)</td>
</tr>
</tbody>
</table>

GLRI- soil; OTS- Ottawa sand; MTM- montmorillonite; ILL-illite; KAO- kaolinite; MAN- manure; *(Standard deviation n=3)

In the competitive study, concentrations of each compound were the same as in the non-competitive study, which means that total concentration of available compound was doubled. \( K_d \) values for MON remained higher than those for LAS. However, LAS adsorption strength increased for several sorbents, particularly ILL and MTM, indicating enhanced adsorption when the compounds are used in tandem. Enhanced adsorption in this case could be the
result of multi-layering preferential attraction of MON over LAS upon the sorbent surface. KAO adsorption showed no significant differences from the non-competitive study, which is likely caused by the 1:1 structure of KAO with lack of isomorphous substitution and limitation of pH-dependent sites to basal planes and broken edges on the crystal structure. K_d values were one order of magnitude lower for MON (from ~1248 to 251 L kg⁻¹) and LAS (from ~1470 to 117 L kg⁻¹) with GLRI where organics (1.8%) are present, suggesting competition between MON and LAS for sorption and/or possible suppression of adsorption when organic matter is present. OTS and MAN also showed no significant differences from the non-competitive study.
Figure 3-2 Linear Adsorption Isotherm ($K_d$) and Freundlich Isotherm ($K_f$) for ILL. Series number indicates one unit of the triplicate set.
3.1.4 Desorption Kinetics

According to USEPA Test Guidelines (USEPA, 2008a), a compound must desorb >75% from a sorbent in at least 2x the time of adsorption equilibrium to be considered reversibly adsorbed. Desorption is relative to the adsorption amount which is accommodated for in calculations. Hysteresis is the inability of desorption rates and processes to mirror adsorption rates and process. Considerable hysteresis resulted during both competitive and non-competitive studies, with more occurring during the non-competitive experiment. Equilibrium time was sorbent dependent for both compounds. Results of desorption further support the interpretation of pH-dependent adsorption. Percent desorption was calculated according to Eq. 6 and results are presented in Appendix 3, and Tables 3.4 and 3.5.

All combinations of adsorption observed from the experimental treatments can be considered irreversible. In the non-competitive study MON desorption was generally greater than that of LAS. Although OTS and MTM reached equilibrium in ≤ 1 hour MTM only desorbed up to ~55% in replicate. The highest desorption percentage occurred with was MON from OTS. The lowest percentage desorbed was MON from ILL and LAS from GLRI, as low as ~0.7% and ~1.5%, respectively. Equilibrium times for GLRI and KAO were ≤ 8h, ILL and MAN ≤ 24h.
<p>| | | | | |</p>
<table>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time (h)</td>
<td>% Desorbed (*)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>8</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>KAO</td>
<td>9.10 (2.6)</td>
<td>6.1 (0.7)</td>
<td>8.4 (1.8)</td>
<td>6.9 (0.9)</td>
</tr>
<tr>
<td>ILL</td>
<td>10.1 (3.7)</td>
<td>12.4 (3.6)</td>
<td>18.2 (4.4)</td>
<td>13.2 (3.7)</td>
</tr>
<tr>
<td>MTM</td>
<td>29.4 (3.0)</td>
<td>34.6 (0.7)</td>
<td>41.8 (7.5)</td>
<td>41.9 (7.0)</td>
</tr>
<tr>
<td>GLRI</td>
<td>1.9 (0.4)</td>
<td>4.5 (0.2)</td>
<td>4.8 (0.2)</td>
<td>3.27 (1.0)</td>
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<tr>
<td>MAN</td>
<td>14.5 (4.6)</td>
<td>12.3 (3.5)</td>
<td>12.3 (3.5)</td>
<td>5.9 (7.1)</td>
</tr>
<tr>
<td>OTS</td>
<td>19.7 (4.5)</td>
<td>16.8 (5.0)</td>
<td>21.8 (4.3)</td>
<td>20.4 (1.6)</td>
</tr>
</tbody>
</table>

GLRI- soil; OTS- Ottawa sand; MTM- montmorillonite; ILL- illite; KAO- kaolinite; MAN- manure; *(Standard Deviation, n=3)
<table>
<thead>
<tr>
<th></th>
<th>% Desorbed (*)</th>
<th>Time (h)</th>
<th>0</th>
<th>8</th>
<th>24</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAO</td>
<td>29.4 (18.1)</td>
<td>25.5 (11.7)</td>
<td>53.9 (35.0)</td>
<td>44.9 (32.8)</td>
<td>41.5 (19.9)</td>
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<td></td>
</tr>
<tr>
<td>ILL</td>
<td>19.8 (19.7)</td>
<td>15.9 (16.5)</td>
<td>34.2 (10.7)</td>
<td>55.2 (43.8)</td>
<td>21.9 (11.6)</td>
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</tr>
<tr>
<td>MTM</td>
<td>7.6 (0.4)</td>
<td>4.2 (0.9)</td>
<td>9.6 (2.8)</td>
<td>6.4 (1.2)</td>
<td>6.1 (0.9)</td>
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</tr>
<tr>
<td>GLRI</td>
<td>11.0 (6.2)</td>
<td>11.2 (6.0)</td>
<td>15.5 (2.6)</td>
<td>22.3 (1.7)</td>
<td>16.0 (1.3)</td>
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<tr>
<td>MAN</td>
<td>0.8 (0.1)</td>
<td>0.8 (0.2)</td>
<td>0.8 (0.2)</td>
<td>1.4 (0.3)</td>
<td>0.9 (0.1)</td>
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</tr>
<tr>
<td>OTS</td>
<td>3.5 (1.8)</td>
<td>8.8 (3.4)</td>
<td>22.5 (5.4)</td>
<td>45.6 (11.3)</td>
<td>58.8 (53.8)</td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>% Desorbed (*)</th>
<th>Time (h)</th>
<th>0</th>
<th>8</th>
<th>24</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAO</td>
<td>16.9 (5.9)</td>
<td>10.7 (4.2)</td>
<td>33.7 (5.9)</td>
<td>46.7 (8.8)</td>
<td>55.5 (4.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ILL</td>
<td>23.6 (3.3)</td>
<td>21.7 (7.3)</td>
<td>68.7 (26.0)</td>
<td>84.7 (14.3)</td>
<td>76.5 (25.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTM</td>
<td>19.1 (0.2)</td>
<td>11.9 (3.1)</td>
<td>29.8 (4.5)</td>
<td>29.7 (3.2)</td>
<td>25.9 (1.1)</td>
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<td></td>
</tr>
<tr>
<td>GLRI</td>
<td>8.5 (1.5)</td>
<td>10.4 (3.3)</td>
<td>17.5 (2.7)</td>
<td>18.8 (2.1)</td>
<td>20.3 (2.7)</td>
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</tr>
<tr>
<td>MAN</td>
<td>7.4 (1.3)</td>
<td>8.0 (0.6)</td>
<td>10.4 (1.4)</td>
<td>16.3 (2.0)</td>
<td>14.2 (1.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OTS</td>
<td>8.8 (4.1)</td>
<td>9.6 (3.7)</td>
<td>6.2 (1.5)</td>
<td>12.9 (3.4)</td>
<td>18.0 (14.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GLRI- soil; OTS- Ottawa sand; MTM- montmorillonite; ILL- illite; KAO- kaolinite; MAN- manure; *(Standard deviation, n=3)
Desorption kinetics changed in the competitive study, although all adsorption was irreversible as in the non-competitive study, desorption percentages were higher in the competitive study and equilibrium times were greater. Overall, LAS was retained by each sorbent preferentially to MON. This may be a reflection of the type of sites MON occupies on the crystal structure of the sorbent. The greatest percentage of compound desorbed was MON from ILL up to ~77%, but this only occurred after 48h contact time. The greatest hysteresis occurred with MAN, which desorbed MON ~6% and LAS ~0.6%. MAN adsorbed very little compound but retained most of what was adsorbed. The amount of both compounds desorbed from OTS and KAO increased with time and did not reach equilibrium within 72 h. GLRI, MTM and MAN reached equilibrium within 24h, and ILL equilibrium was reached in ≤ 1 h. There was a higher degree of variance in competitive desorption which seemed to increase proportionally with length of the experiment particularly with KAO, OTS and ILL.

3.1.5 Isotherm Shapes

The shape of plotted data can be compared to that of other chemicals and used to predict mechanisms of adsorption (Giles et al., 1974b). Calvet (1989) applied the parameters (slope in relation to the partitioned phases at equilibrium) with regard to organic compounds (Figure 3-3).
Figure 3-3 Shapes of Adsorption Isotherms
Isotherms in this study conform to the S and L shape categories. S-shaped isotherms are associated with solute compounds that have hydrophobic parts; facilitating sorption with increased solute concentration and strong competition for sites on sorbents by other molecules in solution (Giles et al., 1974a; 1974b). Interaction between first-layer adsorbed molecules with those still in solution facilitates adsorption of the next molecules (Giles et al., 1974b). Giles et al. (1974a) defined S-shaped curves as indicative of “cooperative sorption” of clustered, clumped molecules. Calvet (1989) found S isotherms commonly associated with organic molecules adsorbing to clay sorbents, particularly montmorillonite, but only with polar compounds. However, this review was likely prior to attention focused upon adsorption of ionophores. Sposito (1984) explains the S-curve as representative of solution ions in competition to coordinate at surface sites until surface sites are filled and any remaining adsorption occurring through other interactions.

L-shaped isotherms correspond to neutral molecules with low solubility, decreased sorption strength with solution concentration increase, and layered positioning upon the sorbent without competition from other molecules in solution (Calvet, 1989). Giles et al. (1974b) stated that close packing of sites and specific hydrophobicity of a sorbent is one determinant difference between the L and S curves. S curves occur with close packing and lower hydrophobicity, with L curves relating to limited and/or sparse sites and a more hydrophobic surface. Multi-layered curves in both L and S categories represent completion of a first
layer and a change in adsorption mechanism to fill successive layers (Giles et al., 1974b).

LAS and MON produced multi-layered L-shaped isotherms with sand in the non competitive study. Both compounds were adsorbed to capacity. Both isotherms would appear as flat in comparison with the other sorbents. Giles et al. (1974b) associate long plateaus with low affinity for the molecule by the sorbent, which would be expected with the sand. However, in the competitive study, both isotherms adjusted to multi-layered S shape. Adsorption capacity was reached with a low preference for both compounds. The competitive LAS isotherm also illustrated desorption which indicates that forces are not sufficient to bind additional molecules (Figure 3-4). Isotherms for manure were also multi-layered and S-shaped. These responses reflect low $K_d$ values for both sorbents. KAO adsorption was also S-shaped. Multi-layered S-shaped trends may indicate repulsion by the sorbent until certain conditions in the system are met, such as changes in available sites on the sorbent surface.

In the case of sand, there are few available sites, thus primary adsorption mechanisms would be Van der Waals forces amplified by hydrophobic exclusion and some desorption could be expected. However, an opposite trend was observed with ILL, which was the strongest sorbent. In the non-competitive study the isotherms were multi-layered S-shaped for LAS and L-shaped for MON with very short plateaus, which is an indication of very strong adsorption of both ionized (negatively charged) and neutral molecules (Figure 3-5). In the
competitive study, the isotherms are L-shaped and sharply multi-layered, possibly the result of rapid change in sorption mechanism with concentration increase.

Figure 3-4 Isotherm Shapes for Ottawa Sand
X axis is constant to illustrate isotherm shape. (A) Non-competitive study. (B) Competitive study. † Max adsorption. M- Multi-layer; S- S-shaped; L- L-shaped
All adsorption isotherms for montmorillonite are multi-layered L shape (Figure 3-6). These isotherms are very similar in shape which indicates that processes are similar for adsorption. Enhanced adsorption was well illustrated by the Y-axis of the competitive and non-competitive isotherms. The exception, MON in the competitive study, illustrates a leveling off of sorption with the higher concentrations possibly caused by the competition with LAS.

![Figure 3-5 Isotherm Shapes for Illite](image)

**Figure 3-5 Isotherm Shapes for Illite**
X axis is constant to illustrate isotherm shape. (A) Non-competitive study. (B) Competitive study. † Max adsorption. M- Multi-layer; S- S-shaped; L- L-shaped
Isotherms of GLRI soil are similar to those for ILL (Figure 3-7). In the non-competitive study, isotherms are S-shaped and multi-layered. For the competitive study they are transformed to L-shaped with no multi-layer. The lack of multi-layering for the GLRI indicates that adsorption strength levels off after a saturation point of available sites. This effect is shown in the competitive study, in which the LAS isotherm indicates achievement of adsorptive capacity, whereas MON appears to be able to absorb more.

MAN isotherms have a shape that was not comparable with the other matrices in the study. MAN showed a very long plateau for the first layer adsorption (Figure 3-8). This phenomenon indicates a reluctance to adsorb, also evidenced by low $K_d$ value for this sorbent. Reasons for poor adsorption to manure may be humic acid complexation with available metal ions that consume available sites and/or elimination of MON and LAS by the hydrophilic layer of the humic acid complex on the surface of the sorbent (Avena and Koopal, 1999), or the absence of sites with appropriate charge.

In the competitive experiments, adsorption of LAS to ILL and OTS reached maximum capacity. For MON, maximum capacity was only reached with GLRI. As in the non-competitive experiment, LAS did not reach maximum capacity with GLRI
Figure 3-6 Isotherm Shapes for Montmorillonite
X axis is constant to illustrate isotherm shape. (A) Non-competitive study. (B) Competitive study. † Max adsorption. M- Multi-layer; S- S-shaped; L- L-shaped
Figure 3-7 Isotherm Shapes for GLRI
X axis is constant to illustrate isotherm shape. (A) Non-competitive study. (B) Competitive study. † Max adsorption. M- Multi-layer; S- S-shaped; L- L-shaped
Figure 3-8 Isotherms Shapes for Manure
X axis is constant to illustrate isotherm shape. (A) Non-competitive study. (B) Competitive study. † Max adsorption. M- Multi-layer; S- S-shaped; L- L-shaped

3.2 Adsorption Mechanisms

Many aspects of these results show a departure from more commonly studied pollutants. Metals that are commonly positively charged are attracted to negatively charged colloidal sites. Neutral compounds, such as hydrocarbons, are repulsed from solution and adsorbed to organic matter and other neutral sites. MON and LAS are negatively charged when ionized and neutral when
complexed. Species of the compound available for different sorption mechanisms are dependent upon environmental conditions.

Acid dissociation constants (pKa) are used to determine the strength of an acid in solution. Lower values, those < -2, are associated with strong acids which dissociate or ionize readily under environmental conditions. There is some conflict in the literature regarding values for MON and LAS which directly impact interpretation of sorption mechanisms. Russell and Houlihan (2003) report pKa values of 7.95 and 5.8 for MON and LAS, respectively. Sassman and Lee (2007) report calculated pKa values for MON and LAS of 4.1-4.2 and 2.6-2.74 respectively, which are more widely accepted (Hansen et al., 2009b). Generally, organic acids become ionized when pH is greater than pKa. If so, under the parameters of this study 90-100% of the compounds would be ionized (negatively charged) and available for chemical reactions either with other solutes or with solids. In this case, little or no adsorption would occur to negatively charged surfaces of adsorbents. Hansen et al. (2009b) reports that ionophores complex with monovalent and divalent (LAS) metals when pH is greater than pKa. In that case there would be no adsorption to KAO, little adsorption to ILL, and more yet to MTM.

Sassman and Lee (2007) tested adsorption of MON and LAS two soils with differing pH and similar clay and organic matter content. The pH adjustment made for both soils caused significant changes in $K_d$ values. They also found that the highest $K_d$ values were associated with soils with the lowest soil pH, which
was a trend also found in this study, except in the case of GLRI. At lower pH the compound would be less dissociated and the neutral species would be more abundant. This would cause more adsorption onto organic neutral constituents.

Thus, a relationship between soil pH and adsorption of these compounds exists and some degree of ionization and cation exchange occurs with clay fractions of soil. However, if cation exchange is the primary driver of adsorption, CEC would be a strong predictor of $K_d$, which is not the case for the results of Sassman and Lee (2007) or this study. The characteristics of the sorbent surface may also have to be considered.

Calvet (1989) referred to adsorption of organic compounds onto soil constituents as usually being the result of ion exchanges between an organic cation and the negatively charged sorbent; or in the case of organic anions, but to a much lesser degree, ion exchanges with oxide and hydroxide constituents. Sassman and Lee (2007) concluded that sorption of MON and LAS must be the result of exchanges with oxide and hydroxide constituents. MON and LAS are organic anions. For chemical adsorption of MON and LAS to occur, there must be ionized molecules and a positively charged sorbent surface. Clay surfaces are positively charged when solution pH drops below the point at which surface positive and negative charges are equal, referred to as the zero point of charge (Figure 3-9). Cation exchange, pH, and zero point of charge (ZPC) for each sorbent is presented in Table 3.6. Quartz sand, KAO, and MTM are all at pH ranges where surface charge would be negative during the experimentation.
phase of this study, thus chemical sorption would be unlikely. ILL is within a pH range to have a positively charged surface.

Figure 3-9 Example of Zero Point of Charge
Table 3.6 Adsorbent ZPC, CEC and test solution pH

<table>
<thead>
<tr>
<th>Sorbent</th>
<th>Montmorillonite</th>
<th>Illite (Mica)</th>
<th>Kaolinite</th>
<th>Ottawa Sand</th>
<th>GLRI Soil</th>
<th>Manure</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH TS (SD)</td>
<td>6.85 (0.06)</td>
<td>5.01 (0.01)</td>
<td>6.18 (0.05)</td>
<td>6.03 (0.02)</td>
<td>7.03 (0.05)</td>
<td>7.87 (0.04)</td>
</tr>
<tr>
<td>CEC</td>
<td>105</td>
<td>70</td>
<td>11</td>
<td>0.06</td>
<td>22.81/24.24</td>
<td>U</td>
</tr>
<tr>
<td>ZPC</td>
<td>1-3</td>
<td>9.6</td>
<td>2-5</td>
<td>1-3</td>
<td>U</td>
<td>U</td>
</tr>
</tbody>
</table>

ZPC- Zero Point of Charge; CEC- cation exchange capacity (cmol kg\(^{-1}\)); () indicate Standard deviation n=3; U- unknown. Alternate CEC for soil is GLRI + Manure at common application rates as determine by outside laboratory.

Results of this study suggest that some portion of the compound may be ionized, a larger portion is complexed in solution. Therefore, a working hypothesis is that if MON and LAS are complexed, chemical adsorption processes occur but are limited to and dependent upon pH of the soil or test solution. The ionized portion (anions) may be adsorbed via interactions with inorganic cations at the sorbent surface. Calvet (1989) referred to this mechanism as coordination bonds with 1A, 2A and transition metals. This would explain why sorption to ILL is much greater than to the other sorbents. For ILL, the surface is positively charged and the greatest portion of compound is ionized. This explains why adsorption for one 2:1 clay (ILL) is much stronger than the other (MTM). Strong adsorption to GLRI may be due to difference in the state of the organic matter, and/or the presence of aluminum and iron oxides in the soil.

The ranges of pH used in this experiment, not unlike those of the natural environment, produced differing ranges of sorption that could not be entirely
attributed to anion exchange. Some adsorption of compounds also adsorbed to a non-reactive, low surface area comparison sorbent (OTS), as well as to a non-swelling clay (KAO) with equal or greater affinity than a swelling clay (MTM). This suggests that adsorption of the complexed species of the compound are physical. Although, desorption for OTS and KAO was irreversible by definition of the guidelines (USEPA, 2008). These sorbents would also have negatively charged surfaces at the pH ranges of these experiments. Calvet (1989) describes the sorption of organic compounds in soil as being mainly driven by attraction to organic matter, particularly in the case of hydrophobic compounds. Although MON and LAS complexes are hydrophobic and considered insoluble according to USEPA Epiweb calculations, attraction to organic matter (MAN) was very low and limiting in the case of the GLRI soil. Calvet (1989) described a physical adsorption mechanism that may apply to the complexed species of MON and LAS as the result of Van der Waals attraction/London dispersion forces amplified by hydrophobic interactions (i.e. hydrophobic exclusion) at the water/sorbent interface (Ebril, 2006). In this process, water molecules surround and isolate (exclude) hydrophobic molecules at the surface of the sorbate resulting in enhanced adsorption strength. Hydrophobic exclusion and the insolubility of complexed MON and LAS may also explain “loss” experienced as matrix effects in the test solution as opposed to degradation, which is most commonly associated with loss and variance between sample sets illustrated by low coefficients of determination ($R^2$) (Pinkerton and Steinrauf, 1970).
Chapter 4

Conclusions and Future Research

4.1 Objectives and Hypotheses

The objectives of this research have been met. Objective 1, the detection and quantification of Monensin and Lasalocid was achieved through multiple trial and error experiments. Properties unique to these types of antibiotics required method development beyond that required for organic cations commonly researched. The second and third objectives were completed using the developed methods for batch equilibrium experiments for the three clay constituents of soil and subsequent analysis using LC/MS-MS. The results were compared to those of pure sand, a natural soil, and liquid manure. Proposed sorption mechanisms were based upon the shape of resultant isotherms and properties of the target compounds and sorbents with relation to cation exchange capacity, anion exchange capacity, zero point of charge and pH. The following summary highlights significant results from the various experimental treatments.
- Illite shows a strong affinity for both LAS and MON and has optimal properties for sorption of these compounds. At the pH (5.01) of these experiments, illite had an abundance of positively charged surface sites and negative ions were available for adsorption. Thus, this type of clay, particularly when abundant in soils may be a strong driver of adsorption. Desorption was very high in the competitive study.

- Kaolinite showed unexpected moderate adsorption. Test solution pH was 6.18, which suggests that some positively charged surface sites were still available and some negatively charged molecules were still present in the solution. Desorption was nearly twice as high when the compounds were paired. Kaolinite did not perform as a strong adsorbent and did not retain MON and LAS effectively.

- Montmorillonite had very few positively charged sites available at a solution pH of 6.85 because the zero point of charge is considerably low at a pH of 1-3. Even though some portion of the compound was still negatively charged, sorption sites were limited. While desorption was higher in the non-competitive study, a preference for retention of LAS was apparent in the competitive one. Low adsorption and retention may occur in soils with high clay fractions of montmorillonite clay.
- Manure used in this study did not show strong affinity for MON or LAS complexes that were initially expected. Although, hydrocarbons are usually adsorbed to organic constituents by hydrophobic expulsion, and these two compounds are often described as having a “hydrocarbon-like” exterior when complexed they did not adsorb readily. Desorption was very low, particularly for LAS in the competitive study.

- Ottawa Sand, which is 99% pure quartz, has an un-reactive surface due to its chemical composition and structure. Little adsorption was expected and little was found. Desorption was generally moderate (~15-30%), but higher in the non-competitive study for MON 56% and for LAS 59%.

- GLRI adsorption was generally high was one order of magnitude higher for the non-competitive study. Retention of compounds was high in both studies.

Results of batch equilibrium adsorption experiments indicate that although some portion of the compound may be ionized, a larger portion is complexed in solution. The ionized portion becomes involved in chemical adsorption by interactions with inorganic cations such as, coordination complexes with metals at the sorbent surface when positively charged as a function of pH. Adsorption however, is not entirely chemical. The differing ranges of pH for test treatments of this experiment enable complexation of MON
and LAS. Complexation is proportionally greater with higher pH, resulting in a neutral hydrophobic molecule which would be excluded from anion exchange. Adsorption mechanisms for complexed species of the molecules are physical. Adsorption of the complexed portion is the result of Van der Waals attractions amplified by hydrophobic interactions (i.e. hydrophobic expulsion) at the water/sorbent interface. More study is necessary concerning pH-dependent activity of the compounds with one soil of a known mineralogy.

The first proposed hypothesis was that both compounds would exhibit rapid and strong adsorption in the order montmorillonite > illite > kaolinite for both compounds. This was based on cation exchange capacity and previously researched adsorption behavior of organic compounds. Adsorption was rapid and strong, but proceeded in the order illite > kaolinite > montmorillonite. Competition between Monensin and Lasalocid occurred, but also enhanced adsorption of Lasalocid by Monensin. This is possibly attributed to multilayering of the compounds upon the surface of the sorbents after sites have been filled. Thus, under saturated conditions Monensin would be more readily available for transport than Lasalocid if concentrations of these compounds were introduced to the environment in tandem such that equilibrium conditions were obtained. No hypothesis was made for the adsorption onto OTS. It was assumed that no adsorption would occur. Adsorption to OTS was minimal and what was adsorbed was readily desorbed, particularly after 24h of contact with the test solution.
Hypothesis 2 predicted slower desorption rates in the order kaolinite > illite > montmorillonite. Desorption was slower, in reverse order to what was hypothesized and irreversible. The parameters of this experiment would mimic those of a continuously saturated CAFO holding pond in the natural environment. Sediments containing a high percentage of sand may facilitate MON and LAS transport through leaching and/or runoff, and leave high percentages of these compounds available for transformation and plant uptake. Although adsorption occurred with OTS, only small concentrations of these compounds were adsorbed and retained during desorption. Thus, OTS would not be recommended for remediation of these compounds as pollutants.

Hypothesis 3 considered transfer of Monensin and Lasalocid to soil via manure application and the possible implications of enhanced adsorption. Although background concentrations (ng L\(^{-1}\)) were found in the manure used for this research, treatment with additional concentrations yielded very little adsorption. Manure samples may have been saturated with compound prior to experimentation, or the particular composition of the manure was resistant to adsorption. Retention of the compounds was high during desorption; most of what was adsorbed remained. Thus, the addition of this manure to soils may enhance leaching through the soil.

GLRI soil comparatively had the highest adsorption strength and lowest desorption rates of all the sorbents. With a natural soil, additional competition for adsorption occurs because of other inherent ions and compounds. This high
adsorption and retention indicates somewhat limited availability of the compounds for leaching, bioaccumulation, plant uptake, and transformation of the compounds in the natural environment. However, these results do not indicate reactions in other soils. It is also unknown what higher concentrations, whether due to experimental spike or additional loading in the natural environment (beyond the saturation limits of the soil) may have on adsorption. Percentages not adsorbed and/or those desorbed prior to degradation may pose an environmental concern.

4.2 Future Research

Because of their widespread use, toxicity, and association with resistant bacteria, further research is suggested for the adsorptive and desorptive behavior of these antibiotic compounds.

- Understanding the effect of changing pH on the adsorptive and desorptive behavior with Monensin and Lasalocid in soils of known mineralogy by adjusting pH to straddle pKa could isolate sorption mechanisms. Illite and the natural soil both had strong affinity for these compounds; different properties or some common property shared by these two sorbents may drive adsorption of MON and LAS.
- Understanding availability of Monensin and Lasalocid for uptake by plants can aid in development of better practices for handling and containing these potential contaminants.
• Comparison of competitive adsorption kinetics may improve interpretation of desorption processes.

• Desorption isotherms may increase understanding of the effects of concentration on desorption.

• Adsorption and desorption at higher concentrations could help to determine capacities of various soils and the clays used for this study.

• Batch equilibrium experiments need to be conducted to obtain more data points to better the shape of isotherms for interpretations.
References


Appendix A

Adsorption Kinetics

LASALOCID

Percent Adsorbed

Time (h)

0 20 40 60 80 100

LASALOCID

GLRI
OTS
KAO
ILL
MTM

Percent Adsorption of LAS to selected sorbents. Error bars represent standard deviation, n=3
Percent Adsorption of MON to selected sorbents. Error bars represent standard deviation, n=3.
Appendix B

Adsorption Isotherms Raw Data

Adsorption Isotherms for clay sorbents (non-competitive).

**K_d ILL-LAS**

- Series 1
  - $y = 483.16x$
  - $R^2 = 0.4509$
- Series 2
  - $y = 412.03x$
  - $R^2 = 0.5563$
- Series 3
  - $y = 384.82x$
  - $R^2 = 0.7697$

**K_f ILL-LAS**

- Series 1
  - $y = 1.2264x + 3.0912$
  - $R^2 = 0.5724$
- Series 2
  - $y = 1.1125x + 2.8204$
  - $R^2 = 0.6340$
- Series 3
  - $y = 1.3706x + 3.3727$
  - $R^2 = 0.7575$

$C_s$ (µg g$^{-1}$) vs. $C_{aq}$ (µg L$^{-1}$)
Adsorption Isotherms for clay sorbents (non-competitive).

**K_d ILL-MON**

- **Series 1**
  - $y = 1657.5x$
  - $R^2 = 0.9564$

- **Series 2**
  - $y = 1333.9x$
  - $R^2 = 0.9151$

- **Series 3**
  - $y = 1132.7x$
  - $R^2 = 0.8657$

**K_f ILL-MON**

- **Series 1**
  - $y = 1.06x + 3.3296$
  - $R^2 = 0.9497$

- **Series 2**
  - $y = 1.0532x + 3.2133$
  - $R^2 = 0.7845$

- **Series 3**
  - $y = 1.3675x + 3.5306$
  - $R^2 = 0.6953$
Adsorption Isotherms for clay sorbents (non-competitive).

**K_d KAO-LAS**

- **Series 1**
  - $y = 222.84x$
  - $R^2 = 0.6056$
- **Series 2**
  - $y = 254.57x$
  - $R^2 = 0.9441$
- **Series 3**
  - $y = 324.34x$
  - $R^2 = 0.8499$

**K_f KAO-LAS**

- **Series 1**
  - $y = 0.5451x + 1.8175$
  - $R^2 = 0.771$
- **Series 2**
  - $y = 0.8091x + 2.1644$
  - $R^2 = 0.9695$
- **Series 3**
  - $y = 0.7666x + 2.2333$
  - $R^2 = 0.8164$
Adsorption Isotherms for clay sorbents (non-competitive).

**$K_d$ KAO-MON**

- **Series 1**
  \[ y = 474.69x \]
  \[ R^2 = 0.648 \]

- **Series 2**
  \[ y = 369.19x \]
  \[ R^2 = 0.8648 \]

- **Series 3**
  \[ y = 443.87x \]
  \[ R^2 = 0.9371 \]

**$K_f$ KAO-MON**

- **Series 1**
  \[ y = 0.7579x + 2.3629 \]
  \[ R^2 = 0.9263 \]

- **Series 2**
  \[ y = 0.7774x + 2.2483 \]
  \[ R^2 = 0.9136 \]

- **Series 3**
  \[ y = 0.8336x + 2.4009 \]
  \[ R^2 = 0.9838 \]
Adsorption Isotherms for clay sorbents (non-competitive).

**K_d MTM-LAS**

- Series 1: $y = 30.219x$, $R^2 = 0.9002$
- Series 2: $y = 31.161x$, $R^2 = 0.8654$
- Series 3: $y = 30.964x$, $R^2 = 0.7833$

**K_f MTM-LAS**

- Series 1: $y = 0.7379x + 1.1919$, $R^2 = 0.9739$
- Series 2: $y = 0.9658x + 1.526$, $R^2 = 0.9201$
- Series 3: $y = 0.5754x + 1.0915$, $R^2 = 0.9058$
Adsorption Isotherms for clay sorbents (non-competitive).

**$K_d$ MTM-MON**

- **Series 1**
  - $y = 261.54x$
  - $R^2 = 0.8783$
  - Symbols: ♦

- **Series 2**
  - $y = 189.63x$
  - $R^2 = 0.7913$
  - Symbols: ○

- **Series 3**
  - $y = 214.69x$
  - $R^2 = 0.8318$
  - Symbols: △

**$K_f$ MTM-MON**

- **Series 1**
  - $y = 0.6741x + 1.9572$
  - $R^2 = 0.7013$
  - Symbols: ♦

- **Series 2**
  - $y = 0.8029x + 1.9661$
  - $R^2 = 0.8056$
  - Symbols: ○

- **Series 3**
  - $y = 0.6065x + 1.8478$
  - $R^2 = 0.985$
  - Symbols: △
Adsorption Isotherms for comparison sorbents (non-competitive).

**K_d OTS-LAS**

- **Series 1**
  - $y = 6.9066x$
  - $R^2 = 0.8843$

- **Series 2**
  - $y = 5.5034x$
  - $R^2 = 0.0467$

- **Series 3**
  - $y = 7.0034x$
  - $R^2 = 0.5207$

**K_f OTS-LAS**

- **Series 1**
  - $y = 0.5345x + 0.3943$
  - $R^2 = 0.6035$

- **Series 2**
  - $y = 0.478x + 0.3543$
  - $R^2 = 0.8589$

- **Series 3**
  - $y = 0.6656x + 0.6205$
  - $R^2 = 0.9478$
Adsorption Isotherms for comparison sorbents (non-competitive).

**K\textsubscript{d} OTS-MON**

![Graph showing adsorption isotherms for K\textsubscript{d} OTS-MON with regression lines and R\textsuperscript{2} values for Series 1, Series 2, and Series 3.]

**K\textsubscript{f} OTS-MON**

![Graph showing adsorption isotherms for K\textsubscript{f} OTS-MON with regression lines and R\textsuperscript{2} values for Series 1, Series 2, and Series 3.]

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Series 1:
- \( y = 3.2799x \)
- \( R^2 = 0.9616 \)

Series 2:
- \( y = 3.7399x \)
- \( R^2 = 0.7616 \)

Series 3:
- \( y = 3.9534x \)
- \( R^2 = 0.7168 \)
Adsorption Isotherms for comparison sorbents (non-competitive).

**K_d MAN-LAS**

- Series 1
  - \( y = 13.923x \)
  - \( R^2 = 0.3686 \)
- Series 2
  - \( y = 6.0426x \)
  - \( R^2 = 0.2036 \)
- Series 3
  - \( y = 4.492x \)
  - \( R^2 = 0.837 \)

**K_f MAN-LAS**

- Series 1
  - \( y = 1.0019x + 1.0613 \)
  - \( R^2 = 0.5801 \)
- Series 2
  - \( y = 0.7344x + 0.5953 \)
  - \( R^2 = 0.6841 \)
- Series 3
  - \( y = 0.5121x + 0.1601 \)
  - \( R^2 = 0.3733 \)
Adsorption Isotherms for comparison sorbents (non-competitive).

**K_d MAN-MON**

- Series 1
  \[ y = 24.239x \]
  \[ R^2 = 0.81 \]
- Series 2
  \[ y = 28.308x \]
  \[ R^2 = 0.7838 \]
- Series 3
  \[ y = 28.105x \]
  \[ R^2 = 0.9042 \]

**K_f MAN-MON**

- Series 1
  \[ y = 0.7659x + 1.1955 \]
  \[ R^2 = 0.7156 \]
- Series 2
  \[ y = 1.2681x + 1.6532 \]
  \[ R^2 = 0.6491 \]
- Series 3
  \[ y = 1.2017x + 1.6317 \]
  \[ R^2 = 0.912 \]
Adsorption Isotherms for comparison sorbents (non-competitive).

**K\textsubscript{d} GLRI-LAS**

- **Series 1**
  - \(y = 1662.7x\)
  - \(R^2 = 0.9819\)
- **Series 2**
  - \(y = 1554.2x\)
  - \(R^2 = 0.7718\)
- **Series 3**
  - \(y = 1193.8x\)
  - \(R^2 = 0.8489\)

**K\textsubscript{f} GLRI-LAS**

- **Series 1**
  - \(y = 1.0697x + 3.3141\)
  - \(R^2 = 0.9141\)
- **Series 2**
  - \(y = 0.7874x + 2.9594\)
  - \(R^2 = 0.8388\)
- **Series 3**
  - \(y = 0.9266x + 3.0269\)
  - \(R^2 = 0.8455\)
Adsorption Isotherms for comparison sorbents (non-competitive).

**K_d GLRI-MON**

- **Series 1**  
  \( y = 1504.1x \)  
  \( R^2 = 0.9601 \)

- **Series 2**  
  \( y = 1207.3x \)  
  \( R^2 = 0.9164 \)

- **Series 3**  
  \( y = 1031.1x \)  
  \( R^2 = 0.8679 \)

**K_f GLRI-MON**

- **Series 1**  
  \( y = 1.0751x + 3.3042 \)  
  \( R^2 = 0.9502 \)

- **Series 2**  
  \( y = 1.0678x + 3.1862 \)  
  \( R^2 = 0.7835 \)

- **Series 3**  
  \( y = 1.391x + 3.5045 \)  
  \( R^2 = 0.6944 \)
Adsorption Isotherms for clay sorbents (competitive).

**$K_d$ ILL-LAS**

- **Series 1**
  
  \[ y = 868.69x \]
  \[ R^2 = 0.3979 \]

- **Series 2**
  
  \[ y = 1428.8x \]
  \[ R^2 = 0.7634 \]

- **Series 3**
  
  \[ y = 1164.8x \]
  \[ R^2 = 0.5526 \]

**$K_f$ ILL-LAS**

- **Series 1**
  
  \[ y = 0.367x + 1.5974 \]
  \[ R^2 = 0.8982 \]

- **Series 2**
  
  \[ y = 0.3917x + 1.6909 \]
  \[ R^2 = 0.3671 \]

- **Series 3**
  
  \[ y = 0.4443x + 1.8885 \]
  \[ R^2 = 0.5583 \]
Adsorption Isotherms for clay sorbents (competitive).

\[ y = 1174.5x \quad R^2 = 0.8498 \]

\[ y = 1653.7x \quad R^2 = 0.3935 \]

\[ y = 1992.1x \quad R^2 = 0.5518 \]

\[ y = 0.229x + 1.0963 \quad R^2 = 0.5519 \]

\[ y = 0.6323x + 2.3377 \quad R^2 = 0.5275 \]

\[ y = 0.5007x + 2.0369 \quad R^2 = 0.4256 \]
Adsorption Isotherms for clay sorbents (competitive).

$K_d$ KAO-LAS

- **Series 1**
  - $y = 206.67x$
  - $R^2 = 0.8162$

- **Series 2**
  - $y = 255.6x$
  - $R^2 = 0.7202$

- **Series 3**
  - $y = 272.93x$
  - $R^2 = 0.954$

$K_f$ KAO-LAS

- **Series 1**
  - $y = 0.4717x + 1.5629$
  - $R^2 = 0.7634$

- **Series 2**
  - $y = 0.6335x + 1.9273$
  - $R^2 = 0.9304$

- **Series 3**
  - $y = 1.0729x + 2.5526$
  - $R^2 = 0.9813$
Adsorption Isotherms for clay sorbents (competitive).

**K_d KAO-MON**

- Series 1
  - $y = 235.67x$
  - $R^2 = 0.4709$
- Series 2
  - $y = 584.18x$
  - $R^2 = 0.9172$
- Series 3
  - $y = 343.23x$
  - $R^2 = 0.9629$

**K_f KAO-MON**

- Series 1
  - $y = 0.6358x + 1.9142$
  - $R^2 = 0.7349$
- Series 2
  - $y = 1.6094x + 3.5862$
  - $R^2 = 0.9914$
- Series 3
  - $y = 1.4824x + 3.1146$
  - $R^2 = 0.8963$
Adsorption Isotherms for clay sorbents (competitive).

**K_{d} MTM-LAS**

- Series 1:
  \[ y = 106.74x \]
  \[ R^2 = 0.708 \]

- Series 2:
  \[ y = 111.65x \]
  \[ R^2 = 0.6947 \]

- Series 3:
  \[ y = 108.65x \]
  \[ R^2 = 0.5414 \]

**K_{f} MTM-LAS**

- Series 1:
  \[ y = 0.3532x + 1.4079 \]
  \[ R^2 = 0.9014 \]

- Series 2:
  \[ y = 0.3773x + 1.4774 \]
  \[ R^2 = 0.9413 \]

- Series 3:
  \[ y = 0.3598x + 1.4667 \]
  \[ R^2 = 0.8743 \]
Adsorption Isotherms for clay sorbents (competitive).

$y = 194.31x$
$R^2 = 0.8632$

$y = 194.74x$
$R^2 = 0.9694$

$y = 120.16x$
$R^2 = 0.2724$

$K_d$ MTM-MON

$y = 0.7148x + 1.9289$
$R^2 = 0.8759$

$y = 0.7296x + 1.9855$
$R^2 = 0.9932$

$y = 0.4743x + 1.5999$
$R^2 = 0.9009$
Adsorption Isotherms for comparison sorbents (competitive).

\[ y = 12.573x \quad R^2 = 0.8701 \]
\[ y = 11.019x \quad R^2 = 0.8807 \]
\[ y = 10.694x \quad R^2 = 0.668 \]

\[ K_d \text{ OTS-LAS} \]
\[ \diamond \text{ Series 1} \]
\[ y = 12.573x \]
\[ R^2 = 0.8701 \]
\[ \circ \text{ Series 2} \]
\[ y = 11.019x \]
\[ R^2 = 0.8807 \]
\[ \triangle \text{ Series 3} \]
\[ y = 10.694x \]
\[ R^2 = 0.668 \]

\[ K_f \text{ OTS-LAS} \]
\[ \diamond \text{ Series 1} \]
\[ y = 1.3522x + 1.3616 \]
\[ R^2 = 0.5907 \]
\[ \circ \text{ Series 2} \]
\[ y = 1.0587x + 1.0736 \]
\[ R^2 = 0.8852 \]
\[ \triangle \text{ Series 3} \]
\[ y = 0.8034x + 0.8218 \]
\[ R^2 = 0.6841 \]
Adsorption Isotherms for comparison sorbents (competitive).

**$K_d$ OTS-MON**

- **Series 1**
  - $y = 13.47x$
  - $R^2 = 0.9156$

- **Series 2**
  - $y = 6.0184x$
  - $R^2 = 0.3895$

- **Series 3**
  - $y = 7.9975x$
  - $R^2 = 0.6471$

**$K_f$ OTS-MON**

- **Series 1**
  - $y = 1.4315x + 1.4631$
  - $R^2 = 0.7343$

- **Series 2**
  - $y = 0.9592x + 0.8221$
  - $R^2 = 0.7564$

- **Series 3**
  - $y = 0.6672x + 0.4689$
  - $R^2 = 0.4547$
Adsorption Isotherms for comparison sorbents (competitive).

$$y = 14.034x$$  \(R^2 = 0.6812\)

$$y = 13.32x$$  \(R^2 = 0.4443\)

$$y = 13.177x$$  \(R^2 = 0.7068\)

For $K_d$ MAN-LAS:

- Series 1: \(y = 14.034x\)  \(R^2 = 0.6812\)
- Series 2: \(y = 13.32x\)  \(R^2 = 0.4443\)
- Series 3: \(y = 13.177x\)  \(R^2 = 0.7068\)

For $K_f$ MAN-LAS:

- Series 1: \(y = 0.8319x + 1.0246\)  \(R^2 = 0.6465\)
- Series 2: \(y = 0.8717x + 1.045\)  \(R^2 = 0.7091\)
- Series 3: \(y = 1.0853x + 1.1587\)  \(R^2 = 0.8526\)
Adsorption Isotherms for comparison sorbents (competitive).

$K_d$ MAN-MON

- Series 1: $y = 19.735x$, $R^2 = 0.7292$
- Series 2: $y = 17.975x$, $R^2 = 0.7327$
- Series 3: $y = 20.801x$, $R^2 = 0.6395$

$K_f$ MAN-MON

- Series 1: $y = 1.4931x + 1.5874$, $R^2 = 0.8466$
- Series 2: $y = 2.1898x + 1.998$, $R^2 = 0.9525$
- Series 3: $y = 1.4769x + 1.5637$, $R^2 = 0.7279$
Adsorption Isotherms for comparison sorbents (competitive).

\[ y = 114.17x \quad R^2 = 0.6764 \]

\[ y = 105.55x \quad R^2 = 0.2241 \]

\[ y = 129.91x \quad R^2 = 0.8908 \]

\[ y = 0.5263x + 1.589 \quad R^2 = 0.9732 \]

\[ y = 0.5038x + 1.5379 \quad R^2 = 0.702 \]

\[ y = 0.7666x + 1.8575 \quad R^2 = 0.9096 \]
Adsorption Isotherms for comparison sorbents (competitive).

\[
y = 231.01x \\
R^2 = 0.7044
\]

\[
y = 248.12x \\
R^2 = 0.6775
\]

\[
y = 273.44x \\
R^2 = 0.87
\]

\[
K_f \text{ GLRI-MON}
\]

\[
\begin{align*}
\text{Series 1:} & \\
y &= 0.5152x + 1.7751 \\
R^2 &= 0.9089
\end{align*}
\]

\[
\begin{align*}
\text{Series 2:} & \\
y &= 0.5839x + 1.8864 \\
R^2 &= 0.8823
\end{align*}
\]

\[
\begin{align*}
\text{Series 3:} & \\
y &= 0.6732x + 2.026 \\
R^2 &= 0.9076
\end{align*}
\]
Appendix C

Desorption Kinetic Percent over Time

Non-competitive Desorption

**ILL-LAS**

**ILL-MON**
Non-competitive Desorption

**KAO-LAS**

- % Desorbed vs Time (h)
- Data points showing desorption percentages over time for KAO-LAS.

**KAO-MON**

- % Desorbed vs Time (h)
- Data points showing desorption percentages over time for KAO-MON.

**MTM-LAS**

- % Desorbed vs Time (h)
- Data points showing desorption percentages over time for MTM-LAS.

**MTM-MON**

- % Desorbed vs Time (h)
- Data points showing desorption percentages over time for MTM-MON.
Non-competitive Desorption

**OTS-LAS**

% Desorbed

Time (h)

**OTS-MON**

% Desorbed

Time (h)

\*note Y axes

Non-competitive Desorption

**MAN-LAS**

% Desorbed

Time (h)

**MAN-MON**

% Desorbed

Time (h)
Non-competitive Desorption

**GLRI-LAS**

% Desorbed

Time (h)

**GLRI-MON**

% Desorbed

Time (h)
Competitive Desorption

**ILL-LAS**

![Graph showing desorption over time for ILL-LAS]

**ILL-MON**

![Graph showing desorption over time for ILL-MON]

**KAO-LAS**

![Graph showing desorption over time for KAO-LAS]

**KAO-MON**

![Graph showing desorption over time for KAO-MON]
Competitive Desorption

MTM-LAS

MTM-MON

OTS-LAS

OTS-MON
Competitive Desorption

**MAN-LAS**

% Desorbed vs Time (h)

**MAN-MON**

% Desorbed vs Time (h)

*note Y axes

**GLRI-LAS**

% Desorbed vs Time (h)

**GLRI-MON**

% Desorbed vs Time (h)