THE FATE AND SPECIATION OF ARSENIC IN SOILS AND POULTRY PRODUCTION SYSTEMS

by

Jennifer M. Seiter

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant and Soil Sciences

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The path that brought me to the University of Delaware was relatively straight forward. While I was an undergraduate at the State University of New York at Brockport, I discovered a love for soil science. A resource soil scientist from the USDA in New York State inspired me to pursue an advanced degree in soil science, particularly soil chemistry. I was astounded and amazed at how complex it was, and I had to know more. It turns out that my advisor at SUNY Brockport, Dr. Mark Noll, graduated from the University of Delaware. It wasn't until after I applied to the Plant and Soil Sciences Department that I realized he had actually graduated from Dr. Sparks' group. This is truly a small world.

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~ Jennifer Seiter

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ABSTRACT

Arsenic (As) is a toxic metalloid found in soil and water environments. One source of As in Delaware soils is the incorporation of poultry litter into agricultural fields. The most common source of As in poultry litter is an organic arsenical called roxarsone.

Poultry litter amended and wooded (background) soils were sampled to assess the arsenic status of Delaware soils. The litter amended soils were not elevated with respect to the wooded soils. Arsenic retention in the presence of P was investigated, and the results indicate that regardless of the phosphate concentration, P is preferred over As.

Arsenic speciation in poultry litter was investigated. A flock of birds was grown on a roxarsone diet. Litter was collected from the poultry house, and at the conclusion of the experiment, the litter was stored and sampled over the course of one year. Arsenic content and speciation were determined for all litter samples using X-ray absorption (XAS) and X-ray fluorescence (XRF) spectroscopy and Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometry (IC-ICP-MS). The results indicate that roxarsone degrades into a mix of As species. Both reduced (arsenite) and oxidized (arsenate) were found in all litter samples. In general, the litter samples collected in the poultry house contained more roxarsone than the stored litter samples. Roxarsone was identified in litter stored up to one year, demonstrating that organic As can remain for long periods of time.

Arsenic content and speciation of As treated broiler excreta and tissues were also determined. Excreta, liver, breast muscle, digestive tract (ileum) contents, skin, and feathers were collected. As content in all samples were determined. As speciation and distribution using XAS and XRF was determined for excreta, ileal contents, breast and liver tissues. Ileal and excreta samples contained a mix of As species, including reduced, oxidized, and organic As. Speciation of liver and breast revealed that both were dominated by reduced arsenic species. The results of these studies indicate that roxarsone degradation is occurring within the bird, meaning that land application of poultry litter may introduce a mix of As compounds into soil and water environments.

Chapter 1

LITERATURE REVIEW:
ARSENIC AND ITS ROLE IN THE ENVIRONMENT

1.0. Research Rationale and Objectives:

1.0.0. Rationale and Focus:

There are many long-standing environmental concerns about surface and ground water quality in the Mid-Atlantic U.S. Historically, the primary pollutants of interest in Delaware have been nutrients, such as N and P, but growing concerns have shifted the interest to trace metal(loid)s introduced to the soil and water due to excessive land application of manures/fertilizers and industrial activities. Arsenic (As) is commonly known for its lethal and carcinogenic characteristics, therefore within the past few years, environmental arsenic regulations have become more stringent, including lowering the drinking level standard to 10 ppb. Although some regulations are being made to protect drinking water from As contamination, researchers are still trying to determine the fate of As in Delaware soils and waters.

One major source of As in Delaware soils is the incorporation of poultry litter (a mixture of bedding materials such as wood shavings or sawdust and manure) into the

agricultural fields. Poultry litter contains high concentrations of certain trace elements such as Cu, Zn, and As. The sources of As in the poultry litter are 4-amino-phenylarsonic acid (p-ASA/ars), 4-nitrophenylarsonic acid (nitarsone), or 3-nitro-4-hydroxyphenly-arsonic acid (roxarsone). Of these arsenicals roxarsone is most common; it is used as a feed additive to prevent coccidiosis, increase weight gain, improve feed efficiency and pigmentation. In 2000, 620 million broilers were produced, which resulted in manure and poultry litter containing approximately 26,000 kg of As (Delmarva Poultry Industry, 2000; Garbarino et al., 2003). Poultry litter is generally applied at the rate of 9-20 Mg ha^{-1} on agricultural lands, and its total annual As inputs on the Delmarva Peninsula are estimated between 20 and 50 metric tons of total As (Christen, 2001a). Land application of poultry litter is not currently regulated with regards to trace metals at either the federal or state level at this time. Therefore, a better understanding of arsenic fate and transport in Delaware soils is required.

1.0.1. Objectives:

Understanding the fate and speciation of As in Delaware soils resulting from poultry litter amendments, and the factors involved in the transport of As is critical in order to protect ground water quality, and human and ecological health. Many of Delaware's agricultural soils are highly susceptible to As leaching to ground waters due to their sandy texture, low organic matter, clay, and metal oxide contents.

The objectives of this study are fourfold: 1) Determine the arsenic status of agricultural soils in Delaware, in particular the fields that have received litter

amendments for decades. 2) Determine what fate arsenic will have after it is incorporated into Delaware soils via litter amendments, and determine what environmental factors will govern its fate in the soil and water environment. 3) Determine what form of As is found in poultry litter and excreta both before and after storage in order to determine exactly what forms of As are being introduced into the natural environments. Arsenic speciation is the key to determining the fate of As in soil and water systems. 4) Determine the effect that arsenic speciation and concentration have on the birds and what forms of arsenic, if any, are accumulated within the muscle and organ tissues of poultry.

1.1. Arsenic and Its Origin

1.1.0. What is Arsenic?

Arsenic (As) is a ubiquitous contaminant in soil and water environments due to natural geologic processes and anthropogenic inputs. It ranks 20th in abundance in the Earth's crust, 14th in seawater and 12th in the human body (Mandal and Suzuki, 2002; Woolson, 1975). As has been used in medicine, agriculture, electronics, wood processing, livestock, industry and metallurgy (Mandal and Suzuki, 2002; Nriagu, 1990). Over the past few decades, the health of humans, farm animals, wildlife, microorganisms, and some plants in the U.S. have been jeopardized by As contaminated soil and water. It is estimated that the "average" human in the U.S., Canada, and the UK consumes between 53 and 63 micrograms of As per day (Abernathy, 2001). Due to its high carcinogenic, phytotoxic and biotoxic characteristics, As exposure is a serious concern. Long-term human exposure to As in drinking water can result in bladder, lung, skin,

kidney, dietary, immunological, neurological, and endocrine effects. The USEPA announced that it was lowering the maximum contaminant level (MCL) for As in drinking water from 50 μg L^{-1} (ppb) to 10 μg L^{-1}, and all water systems must comply (Christen, 2001b). Studies have shown that the consumption of only 3 μg L^{-1} of As creates risk of bladder and lung cancer in 4 to 10 people per 10,000 people, which indicates the new MCL may still be too high (Christen, 2001b). This exceeds EPA's maximum acceptable level of risk of 1 in 1,000,000 people by 1000-fold.

1.1.1. Where Does As Come from?

Arsenic in the environment comes from both natural and anthropogenic sources. The average terrestrial abundance of arsenic varies from 1.5-3.0 mg kg^{-1}(Mandal and Suzuki, 2002). A portion of As contamination in soil and aqueous environments is due to natural sources, with the weathering of As-containing rocks being the main source. This process releases 45,000 metric tons of As per year (Tamaki and Frankenberger, 1992). Arsenic is a major constituent in more than 200 minerals including arsenates (60%); sulfides and sulfosalts (20%); and oxides, arsenites, arsenides, silicates, and elemental As (20%) (Gao et al., 1994; Onishi, 1969; Yan-Chu, 1994). The most common of these minerals are: arsenopyrite, galena, iron pyrite, chalcopyrite, realgar, orpiment, and sphalerite (Mahimairaja et al., 2005; Mandal and Suzuki, 2002). Mineral distribution depends on the parent rock composition and the extent of weathering that has occurred. The concentration of As in sedimentary and igneous rocks ranges from 0.1 to 2,000 mg kg^{-1} (ppm). Sedimentary rocks have a mean As concentration of 13 mg kg^{-1} in shales and

25 mg kg^{-1} in coal. In general igneous rocks have a lower mean concentration of 1.5 mg kg^{-1} and metamorphic rocks contribute the least to As release, having a range of 0.4 to 18 mg kg^{-1} As (Mandal and Suzuki, 2002).

Arsenic can co-precipitate with a variety of elements (Zn, Cu), iron oxides, and sulfides in sedimentary rock (Grafe and Sparks, 2005; McLean and Beveridge, 2001; McLean et al., 2000). It is commonly found in correlation with iron deposits, sedimentary iron ores, and manganese nodules (Mandal and Suzuki, 2002). While As is mainly released into soil and aqueous environments through the weathering of rocks, it is also deposited into the atmosphere by volcanic and geyser activities (Smith et al., 1998). Volcanic activity and other natural sources of As contamination account for over half of arsenic's atmospheric flow.

Arsenic concentrations in water range from 0.1 to 1 mg L^{-1} with a mean of 3 μg L^{-1} in sea water, 1.7 μg L^{-1} in river water, 1 μg L^{-1} in precipitation, and 280 μg L^{-1} in saline lakes (Gao et al., 1994). Long-term anthropogenic inputs (e.g., inorganic and organic arsenical pesticides, defoliants, wood preservatives, manures, and biosolids) to agricultural fields have increased total As levels up to as high as 165 mg kg^{-1}in soil. The average concentration of As varies considerably from region to region with the average As concentration in uncontaminated soils 5-6 mg kg^{-1}(Mandal and Suzuki, 2002).

1.2. Arsenic Speciation in Natural Environments

Arsenic can be found in both inorganic and organic forms in soil. It is found predominantly in the inorganic form (+5, +3, 0, and -3), but can be found as the organic

mono- and dimethyl-arsenates/aresenites (Pongratz, 1998). Speciation is critical because it predicts the fate, transport, toxicity, and bioavailability of the metalloid in the soil and water system. Many factors affect speciation including oxidation-reduction, pH, time, type and quantity of inorganic and organic sorbent phases, other ions, and organisms. These factors will determine whether a given contaminant will adsorb, precipitate, chelate, leach through the soil profile, or be absorbed by plants.

Arsenate (As(V)) is found in oxidizing conditions and aerobic environments. It is very stable and will readily adhere to soil constituents such as: clays, metal oxides/hydroxides, and sometimes organic matter. Under reducing conditions, arsenite (AsIII) will be prevalent. This form of As is more mobile and more toxic than As(V) (Bhumbla and Keefer, 1994; Pongratz, 1998; Sadiq, 1997; Scott and Morgan, 1995). Although these are the common trends, some studies have found exceptions. A recent X-ray absorption study found both As(V) and As(III) species in reduced soils (Mitsunobu et al., 2006).

Methyl-arsenic species are produced by microorganisms from inorganic As under oxidizing conditions. These species include: monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and trimethylarsine oxide (TMAsO) (Mandal and Suzuki, 2002). Biomethylation is an enzymatic process in which one methyl group is transferred from one atom to another (also referred to as ligand replacement) (Thayer, 2002). Enzymatic transmethylation occurs in all organisms, however not all organisms can methylate every element (Thayer, 2002). During this process, As(III) is oxidized to As(V); CH_3^+ is reduced to CH_3^-; then stable As species are formed when these methyl

groups are added to the As. See Figure 1.1 from Jiang et al. (2003) that depicts the methylation process. The theoretical oxidation-reduction potential of the system is 0.557 V at 20°C (Mandal and Suzuki, 2002). When methylating heavier elements, the bonding of methyl groups increases their ability to volatilize. Arsenic volatilization can occur in the form of $(CH_3)_2AsH$ (Thayer,2002).

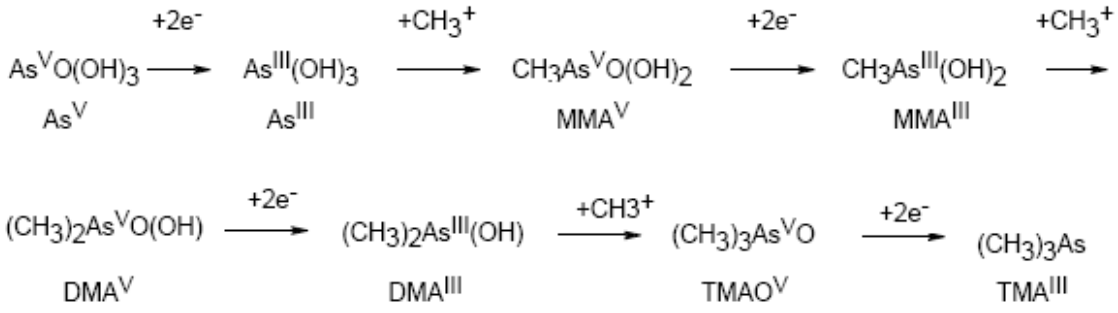

Figure 1.1. The stepwise methylation process which involves a 2 electron transfer from As(V) to As(III) followed by oxidative addition of methyl groups (adapted from Jiang et al., 2003).

Arsenic speciation in ocean and fresh water is not usually as complex as that in the soil environment. As^o and As^{3-} and large organic As compounds are not commonly found in aquatic environments. (Mandal and Suzuki, 2002). Arsenate is thermodynamically more stable in water than arsenite, though some arsenite is found in ocean and fresh water. This As(III) contribution is mostly likely due to biological reduction of seawater. The form of As(V) found in water are: H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$, AsO_4^{3-}. When reducing conditions or lower redox potentials are present in the soil/water environment, the trivalent H_3AsO_3, $H2AsO_3^-$, $HAsO_3^{2-}$, AsO_3^{3-} As species will predominate. Thermodynamic calculations predict that the As(V) species $HAsO_4^{2-}$

>H_2AsO^{4-} at pH 7, and As(III) is expected to be $HAsO_2^0 = H_3AsO_3^0 > AsO_2^- = H_2AsO_3^-$ at pH 7 as is likely to be found in natural waters (Sadiq, 1997). The soil and water pH will have a dramatic impact on the As(V) species, this is due to the pKa at which the various anions will form. Since the first pKa of As(III) is so high, the neutral As(III) species are the most prevalent forms found. Table 1.1 depicts pKa values for As species commonly found naturally in aquatic and soil systems.

Table 1.1: pKa values for relevant arsenic compounds found in nature.

Species			Dissociation Constant pK_1	pK_2	pK_3	Reference
Arsenate	As(V)	H_3AsO_4	2.2	6.97	11.53	Bowell (1994)
Arsenite	As(III)	H_3AsO_3	9.22	12.13	13.4	Bowell (1994)
Monomethylarsonic acid	MMA	$CH_3AsO(OH)_2$	3.6	8.2		Bowell (1994)
Dimethylarsinic acid	DMA	$(CH_3)_2AsO(OH)$	6.2			Bowell (1994)
p-arsanilic acid	p-ASA	$C_6H_3(NH_2)AsO(OH)_2$	2	4.02	8.92	Bowell (1994)
Roxarsone	ROX	$C_6H_3(NO_2)AsO(OH)_3$	3.5	6.5	9.6	Baert et al., (2006)
Phenylarsonic Acid	PAA*	$C_6H_5AsO(OH)_2$	3.6	8.8		Jaafar et al., (2007)
Phosphate		H_3PO_4	2.15	7.1	12.4	Cotton et al., (1972)
S-bearing Amino Acid Species			**α-carboxylic acid**	**α-amino**	**Side chain**	**Reference**
Cysteine	Cyst	$C_2H_7NO_2S$	1.8	10.8	8.6	Parril (1997)
Methionine	Meth.	$C_5H_{11}NO_2S$	2.3	9.2		Parril (1997)

1.3. Arsenic in Agriculture

1.3.0. General Agricultural Uses of Arsenic in Agriculture

Arsenic has been used in agricultural and horticultural settings for decades. In the 1950s, As production was a source of revenue for China, USSR, France, Mexico, Germany, Peru, Namibia, Sweden and the U.S. During the 1970s, about 80% of the

consumption of arsenic was for agricultural purposes (Nelson, 1977). These uses include: pesticides, herbicides, desiccants, and feed additives. For decades, As was readily used as a pesticide or insecticide on orchards and agricultural fields. In 1955, it was estimated that 18,000 tons of arsenic were used for agricultural purposes and that most of it was used as pesticides in the form of lead arsenate, Ca_3AsO_4, copper acetoarsenite, Paris-Green, H_3AsO_4, and others (Mandal and Suzuki, 2002; Peryea, 1991; Peryea and Kammereck, 1997). Both organic and inorganic As species were used as herbicides, and arsenic acid was used extensively as a cotton desiccant for many years.

National surveys and research provide evidence that poultry production practices introduce As into the environment at higher rates than first estimated (Christen, 2001). More than 11.4 million Mg of poultry litter (PL) were produced in the U.S. in 1996 and about 90% of this litter was land applied (Cabrera and Sims, 2000). Poultry litter contains high concentrations of certain trace elements such as Ni, Cu, Zn, Mn, Fe, and As, the sources of which are growth promoters and agristats added to poultry feed. The total As concentrations in PL vary depending mostly on the concentration of As fed to the birds. For example, Sims and Wolf (1994) found levels ranging from 0 to 77 mg kg^{-1}. Others have shown As concentrations in PL within this same range, for example, 30-37 mg kg^{-1} (Van der Watt et al., 1994), 43 mg kg^{-1} (Moore et al., 1998), 35 mg kg^{-1} (Jackson et al., 1999), 45 mg kg^{-1} (Sims and Luka-McCafferty, 2002), and 1-39 mg kg^{-1} (Jackson et al., 2003). In comparison, the As concentrations set by USEPA as As ceiling concentration limits and As pollutant concentration limits for land application of sewage

sludge are 75 and 41 mg kg^{-1}, respectively (Christen, 2001), it would appear that these limits need be applied to manure amendments.

1.3.1. The Use of As in Poultry Industry

The sources of As in PL are 4-amino-phenylarsonic acid (p-ASA), 4-nitrophenylarsonic acid (Nitarsone), or 3-nitro-4-hydroxyphenly-arsonic acid (*Roxarsone*, abbreviated ROX), used as feed additives to prevent coccidiosis, increase weight gain and improve feed efficiency (See Figure 1.2 for the roxarsone structure). ROX was used in about 70% of broiler industry operations from 1999-2000 (Chapman and Johnson, 2002). The estimated 8.7 billion broilers produced each year generate 26 to 55 billion pounds of PL (Nachman et al., 2005). The poultry litter that is disposed on nearby agricultural lands may add half a million to 2.6 million pounds of roxarsone a year (O'Connor et al., 2005).

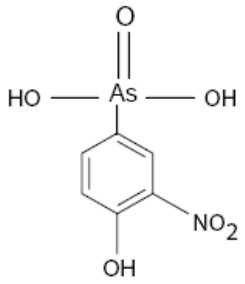

Figure 1.2. Roxarsone skeletal structure.

The organo-As compounds added to the feed are primarily excreted in the organo-As forms. For example, Morrison (1969) found that ROX constituted 36-88% of the total As in 10 PL samples. Jackson et al. (2003) found that the major As species in 40 PL

extracts were either ROX or As(V). For 20 of the 40 PL samples, As(V) was the major As species in the water extract, showing that mineralization of the initial organo-As had occurred. The quantity of ROX excreted by a single broiler when fed the typical 45.4 g As ton^{-1} formulation is about 150 mg over the normal growth period of 42 days (equal to 43 mg of As) (Garbarino et al., 2003). Feed spillage and undigested materials can increase mean total As concentration in PL to 14-76 mg kg^{-1} (Moore et al., 1998). Thus, assuming PL is applied at ~7 Mg ha^{-1} (~ 3 tons ac^{-1}) it is estimated that about 100-530 g of As ha^{-1} could be added with each land application. Addition of As to agricultural lands via PL is not specifically regulated at either the federal or state levels nor is nonpoint source pollution of soils by As considered under current nutrient management laws in Delaware. It is unclear whether total maximum daily load agreements (TMDLs) established between Delaware and USEPA to protect surface water quality will affect the application of As in PL to cropland.

Roxarsone has been used to promote growth in the poultry and swine industries for the past 50 years. The toxic effects of this compound have been reported in both field (Rice et al., 1980) and experimental (Kerr et al., 1963; Sullivan and Al-Timimi, 1972; Wise and Hartley, 1974) environments and studies. The fate of roxarsone inside of the chicken is not well understood. Research has provided information indicating that As preferentially sorbs to S bearing compounds upon entering a biological entity (Duncan et al., 2006; Jaing et al., 2003). There are a variety of trace metals and sulfur amino acid supplements used as macro and micro-nutrients in the poultry feed, therefore these must be taken into account when determining As transport processes.

1.3.2. Government Regulation on Arsenic in Poultry Feeds

The Food & Drug Administration (FDA) demands that animal drugs only be used in accordance with their respective FDA approval guidelines in order to protect animal and human health. It is required that human risk drugs be premixed at a licensed feed mill. All animal drugs are divided into one of two catergories: Category I or Category II (Howie, 2004). Category I drugs are those for which no withdrawal period is necessary at the lowest use level in each species for which they are approved. Category II drugs are those: (1) for which a withdrawal period is required at the lowest use level for at least one species for which they are approved; or (2) that are regulated on a "no-residue" basis or with a "zero" tolerance because of a carcinogenic concern, regardless of whether a withdrawal period is required (Howie, 2004). Since roxarsone and all other arsenical drugs have a withdrawal period, they are classified as Category II drugs.

Roxarsone is mixed with a maximum value of 2.275 g/lb (0.5%) in basal feeds. Arsanillic acid and Nitarsone are mixed with a maximum value of 4.5 g/lb (1.0%) and 8.5 g/lb (1.87%), respectively. Roxarsone is commonly used in combination with a series of other drugs to control disease, weight gain, and pigmentation. Amprolium, Salinomycin, and Ethopabate are added for development of active immunity to coccidiosis and for treatment of the disease in poultry. Bacitracin Methylene Disalicylate, Penicillin, and Bambermycins are added for increased rate of weight gain and improved feed efficiency. The FDA has limited the amount of As in muscle tissue to 0.5 ppm (Vandiver, 2004).

The industry is strictly regulated by a series of provisions provided by the FDA to sustain "Current Good Manufacturing Practices (CGMPs)"(Howie, 2004). These provisions cover every aspect of the manufacturing practice from maintenance of facilities and equipment to labeling and laboratory assays. Common production errors are intercepted using specifically designed tests to monitor drug levels in processed feeds. Proper mixing is a major concern when adding small amounts of additives to feeds, often at less than 5 lb. per ton. Feed manufacturers are forced to mix ingredients that vary in composition, particle size and shape, bulk density and electrostatic charge making it difficult to evenly distribute the additives within each batch. This means that roxarsone levels in feeds may vary from batch to batch, and from mill to mill (Howie, 2004). Poultry nutritional needs must be met, therefore inadequately mixed feeds can also cause dietary health problems (Howie, 2004). Studies conducted to evaluate mixer performance indicate that mixers do not always produce satisfactory mixes often due to poor maintenance or improper operation of the equipment (Wicker and Poole, 1991; Wilcox and Unruh, 1986).

The use of arsenic in the poultry industry is not necessary to prevent disease and sustain growth. Europe has condemned and outlawed the use of As in poultry production, and organic poultry producers do not add As to their feeds. Both are examples of successful poultry production free of As contamination. The European Medicines Agency determined in 1994 that they could not assure consumers that the product would be safe to consume, so they banned the use of ROX.

1.3.3. Poultry Industry on the Delmarva Peninsula

The United States produces more chickens annually than any other country in the world (Christen, 2001a). The Delmarva Peninsula is one of the most concentrated poultry production areas in the US. Sussex county, Delaware produces more broilers annually than any other county in the country; it has been number one in the amount of chicken meat produced since 1944 (Delmarva Poultry Industry, 2000). There are approximately 1,900 growers, and 5,100 houses on the Delmarva Peninsula (Delmarva Poultry Industry, 2004(a)). The poultry industry is reported to have a wholesale value of $1.7 billion in 2004 alone (Delmarva Poultry Industry, 2005(a)). Approximately 71% of Delaware's farm income was from broilers in 2003 (Delmarva Poultry Industry, 2005(b)). The poultry industry also supports other agriculture in the region; most of the grain grown in this area is used in poultry feed and most of the feed mills are found in the region.

In 2000, 620 million broilers were produced, which resulted in manure and poultry litter containing approximately 26,000 kg of As (Christen, 2001a; Garbarino et al., 2003). Poultry litter is generally applied at the rate of 9-20 Mg ha^{-1} on agricultural lands, and total annual As inputs on the Delmarva Peninsula are estimated between 20 and 50 metric tons of total As (Christen, 2001). It is unclear whether total maximum daily load agreements (TMDLs) established between Delaware and USEPA to protect surface water quality will affect the application of As in PL to cropland. Groundwater studies implemented by both the Delaware and Maryland state agencies have failed to detect As concentrations in surface or groundwater in levels above the EPA's health

standard, which means the water supply on these coastal plains should be safe (Water Resources, 2007).

1.4. Poultry Digestion and Internal Environment

1.4.0. Avian Digestive and Excretory Systems

The chemical environment of the intestinal system is important in understanding nutrition and the health of the birds. The feed will enter the mouth then travel to: the esophagus, crop, lower esophagus, proventriculus (the glandular stomach), gizzard (muscular stomach), small intestine, ceca, large intestine (rectum), and the cloaca (Ensminger, 1992; Moreng and Avens, 1985).

The digestive system of the bird is very different from most non-ruminants. The absence of teeth means that reduction in size of the food particles will take place along the digestive system. Birds that consume dry feeds have well formed salivary glands. Saliva is important in digestion because it used as a lubricant, buffers ingested materials, protects membranes and enhances taste (Ensminger, 1992). The crop is a storage pouch where the food is moistened to aid in the rest of the digestion process. The food is then moved to the proventriculus where digestion officially begins. Digestive secretions are released containing the enzyme, pepsin, and hydrochloric acid, where each of these components begins the digestion process. Since the proventriculus has limited space, the moistened food and digestive secretions pass on to the gizzard. Rhythmic movement of the muscles and the presence of grit or stones cause physical breakdown of the feed. The physical breakdown of food is important because it increases the surface area of the food

particles allowing a more complete enzymatic breakdown and absorption of nutrients (Moreng and Avens, 1985). Digestive enzymes are the primary means by which digestion takes place. They are organic catalysts which speed biochemical reactions (Ensminger, 1992). The food leaving the gizzard then enters the small intestine.

The small intestine is comprised of three sections: duodenum, jejunum and the ileum. The jejunum is the site where most absorption takes place (North, 1984). It is often difficult to differentiate the jejunum from the ileum, so these portions are often considered the lower small intestine (Ensminger, 1992). The food from the gizzard enters the duodenal loop, it is where the food is mixed with bile produced in the liver. The bile is an alkaline substance that breaks down fatty products in the feed. The bile also serves a neutralizing agent for the digestive juices. The duodenal loop and upper small intestine is where the breakdown of most compounds occurs. In the remaining portion of the small intestine the digestive process is completed and absorption of nutrients, minerals and vitamins begin. The digested nutrients travel from the small intestine to the liver where nutrient metabolism and storage takes place (Ensminger, 1992). The ceca are located at the point where the small and large intestines come together. Not all ingested food will proceed to the ceca, these organs are primarly for the breakdown of dietary fiber (Moreng and Avens, 1985). The main function of the large intestine is the storage of undigested waste and the absorption of water (Moreng and Avens, 1985).

The urinary system or excretory system is closely linked with the digestive process. This system is responsible for the elimination of waste products created during

metabolism. The urine produced by birds is not liquid but is a white pasty material that is deposited with the feces (Moreng and Avens, 1985).

The liver and kidneys can be considered the filtration systems in the bird. The liver is an important organ in the digestive tract of any animal. It has numerous functions that serve in digestion and absorption. Some physiological functions include: secretion of bile; detoxification of harmful/foreign compounds; metabolism of proteins, carbohydrates, and lipids; storage of vitamins and carbohydrates; and other important processes. The production of bile is one of the most important roles of the liver. The kidneys are considered an important part of the excretory system. Their primary functions are: to filter the blood so as to remove waste products and water; and to reabsorb any nutrients. They filter and recycle water in the body, and therefore control osmotic balance in the body (Ensminger, 1992).

1.4.1. Chemical Environment of the Digestive Tract

The pH is important in maintaining some substances in solution; influencing absorption of nutrients and metallic ions; and maintaining enzyme activitiy (Ensminger, 1992; Ford, 1974). The activity of the digestive enzymes is regulated by pH, it is often responsible for activation of enzymes. Ford (1974) found that microflora will alter the pH of the intestine, which in turn may alter nutrient availability. Studies have found that the bird has the ability to maintain the pH of the intestinal system regardless of the feed (Hurwitz and Bar, 1968; Mussehl et al., 1933). A slightly acid crop was followed by the acid proventriculus and gizzard. At the exit of the gizzard the pH increased within the

first few centimeters of the duodenum, then the rest of the intestine was circum-neutral (±1) until the end. Table 1.2 is adapted from Ford (1974) illustrating the pHs of the digestive tract of the bird. This study showed that the presence of microflora actually decreased the pH of the bird gut, indicating that the microbes may secrete acidic metabolites which alter the chemistry of the internal environment, decreasing anywhere from 0 to 0.6 of a pH unit.

Table 1.2. pH of the chicken gut, adapted from Ford (1974) and Hewitt et al. (1955).

	Ford (1974)	Hewitt (1955)
Position in the tract	**pH**	**pH**
Crop	5.1	4.67
Proventriculus	2.1	4.48
Gizzard	2.3	2.94
Duodenum	6.9	6.13
Jejunum	7.0	6.29
Ileum (at yolk sac)	6.8	6.58
Ileum (at cecal junction)	7.4	
Cecum	6.8	6.14
Rectum	6.5	6.82

Understanding the process by which digestion takes place in the bird is essential when formulating a diet. Most components of the diet are fully digested and the end-products are then absorbed into the bird. However, some vitamins and most minerals cannot undergo digestion and are directly absorbed from the intestinal tract in the same form that they are fed (North, 1984). Trace minerals commonly added to feed (eg., Cu, Zn, Fe, Mn, Se) are sometimes incorporated as a part of a protein or enzyme (North,

1984). So these trace metals are essential to metabolic process, but an excess of some leads to problems (e.g. toxicity, bioaccumulation, etc…), therefore it is important to properly regulate the amounts of these trace metals added to the feed. Drugs and antibiotics enter the bloodstream in their original form, and in most cases are not affected by the digestive processes (North, 1984).

1.4.2. Microbial Communities and Their Functions in the Digestive System.

Microbes found within the bird can be classified based on their functions: there are microbes that assist in the various processes occurring inside the bird; and there are pathogens that produce detrimental reactions (*Salmonella pullorum*) (North, 1984). Bacteria can also be classified by the types of environments in which they are found: anaerobic and aerobic. The anaerobic bacteria found in the intestine of the bird are mostly located in the ceca, 10^8 to 10^9 anerobes/g (Barnes, 1977; Barnes et al., 1972; Shapiro and Sarles, 1949), ceca located at the junction of the small and large intestine. It has been found that most birds empty the cecal material once every 24 to 48 hours, while there are usually about 6 fecal droppings a day (Barnes, 1977). The typical adult intestinal flora is established by about 2 weeks, but the cecal flora takes a lot longer to develop. The general trend in the ceca is that there is a dominance of aerobic bacteria in the first few days, but as time goes on anaerobic bacteria take over and the population increases in complexity. Some of the microbes found in the beginning stages of intestinal development include: streptococci, coliform bacteria, clostridia, and bacilli (Barnes, 1977; Shapiro and Sarles, 1949). At 2 weeks the anaerobic cocci (peptostreptococci)

outnumbered all the other groups of bacteria, but then the numbers decreased as the birds got older. During 2 to 6 weeks the gram negative bacteria (fusiforms) increased in number, typical bacteroids and bifidobacteria became major components at 4 and 6 ½ weeks (Barnes, 1977). Shapiro and Sarles (1949) found that coliform, particularly *Escherichia coli* were the most dominant of the aerobic bacteria found in the cecum. This study examined the microflora species in the digestive tract of birds with time. The cecum, ileum, duodenum and colon contents were sampled and extracts were plated on various growth supplements to determine the different forms of microbes found inside the bird at various stages. Shapiro and Sarles (1949) found that *Lactobacilli* were the most numerous group of bacteria in the cecum, ileum, and duodenum. They found that the lactic acid, or *Lactobacilli* bacteria were the dominant forms in the ileum. The duodenum contained fewer microbes than the other parts of the digestive tract tested. The study determined that the anaerobic population consisted almost solely of *Clostridium perfringens*. There was a strong presence of *Streptococcus faecalis* in all parts of the tract. The results indicated that there was a different population of microbes throughout the digestive tract (Shapiro and Sarles, 1949). More recent studies (Garrido et al., 2004) confirmed the results from this earlier study by finding coliforms, *Lactrobacillus* spp, *Clostridium perfringens*, and *Enterococcus* spp in the ceca and ileum.

Bacteria vary in both As resistance and their ability to take up As. Some bacteria were found to convert organic arsenicals into inorganic species (Anke, 1986). Forsberg (1978) found that rumen microflora are sensitive to As. It was found that As(III) inhibits

the fermentative activity and growth of some rumen bacteria more than the oxidized form. Isolated *Escherichia coli* strains were found to be resistant to As(V) (Anke, 1986).

1.5. Bioaccumulation of Arsenic

1.5.0. Arsenic Accumulation in Plants and Animals

Arsenic has been found to accumulate in living tissue. As is transported very slowly throughout all living organisms if at all. Therefore, the amount of As accumulation that occurs is dependent on the amount of As the organism was exposed to. Some plants have the ability to take As out of the soil and transport it to their above ground shoots and leaves. Plants accumulate between 0.01 to 5 μg (dry weight) As. At these levels, most animals could consume plants that were grown on contaminated soils, and not be concerned about As poisoning. Usually the plant will die or suffer greatly before accumulating potentially dangerous levels of As (1975).

Arsenic also accumulates in animal and human tissues. Marine animals contain more As than any other animal on Earth. Arsenic is found to be accumulative to levels from 0.005 to 0.3 mg kg^{-1} in some mollusks, crustaceans, and coelenterates (Bowen, 1966). The average As concentration in freshwater fish is about 0.54 μg g^{-1}, based on total wet weight (Whitacre and Pearse, 1972). In mammals it was found that As accumulates in certain areas of the ectodermic tissues, primarily hair and nails. The total As that the human body contains, varies between 3 and 4 mg and tends to increase with age. The absorption of As in the human body is high for anionic and soluble species, but does not easily react with insoluble As species. Inorganic As has a special affinity for

keratin rich tissues, teeth, and hair (Anke, 1986; Matsui et al., 1999; Smith, 1964). Examination of these tissues is part of the process of diagnosing As poisoning (Anke, 1986). One study used arsenic-76 to track As in poultry, accumulation in feathers 12 hrs after the oral dose (Anke, 1986). Arsenic accumulates in the liver and kidneys, since these organs are part of the excretory cycle, and it is believed that the reduction of As(V) to As(III) occurs in these organs (Armstrong et al., 1984). In general it is found that organic arsenicals are excreted more rapidly than inorganic As, where pentavalent species are found to clear more quickly than trivalent As species (Anke, 1986; Council and Selenium, 1971; Lauwerys et al., 1979). Human exposure to a number of different As species complicates the study of As contamination and accumulation in tissues. Older studies have studied the rate at which As passes through animals and the effect of As speciation on As retention in the body. Two older studies examined the rate at which As passed through the human body. One study showed that humans excreted 74 percent of the 25 mg of As ingested from lobster within 48hr (Chapman, 1926). One study found that the As found in shrimp passed through the body within 4 days (Coulson et al., 1935). Another study found that renal excretion rate of arsenic in humans depends on the form (Buchet et al., 1981). The study indicates that 46, 78 and 75% of the single 500 μg dose of arsenic in the forms of sodium arsenite, monomethylarsonate (MMA), or cacodylate (DMA), respectively, were recovered. The cacodylate was excreted unchanged, while approximately 75 percent of the As excreted after As(III) ingestion was methylated, and 13 percent of the MMA was slightly methylated. Tam et al. (1979) found that 51% of

ingested As(III) was transformed into DMA, 21% was MMA, and about 27% inorganic As was found in the urine.

Early studies investigated the rate at which arsenicals commonly added to feeds are eliminated from the body. Overby and Frost found that arsanilic acid is well absorbed but rapidly disappeared from the tissues into the feces (1960). Frost et al. (1955) found that these organic As compounds did not accumulate in tissues to "excessive concentrations". Ferslew and Edds found that by discontinuing the addition of arsenicals to feed, As concentrations were reduced to <0.5 and 2.0 mg kg^{-1} in muscle and liver/kidneys, reaching the upper limit of acceptable As concentrations (Ferslew and Edds, 1979).

The bioavailability of ingested arsenic is dependent on many factors including: the matrix in which it was ingested, As speciation, the presence of other nutrients in the digestive tract, and the concentration of the As consumed. Arsenic distribution in tissue is also dependent on a number of factors: tissue volumes, diffusion coefficients, membrane characteristics, and tissue affinities (Mandal and Suzuki, 2002). Absorption, incorporation, and excretion are also affected by the interposition of rumen and rumen flora (Anke, 1986). Consecutive methylation reactions and oxidation and reduction reactions between As(III) and As(V) are the two major reactions that will govern the fate of ingested As.

1.5.1. Arsenic Toxicity.

As accumulation occurs in both plant and animal tissue and can occur by a series of mechanisms. Arsenate (As(V)) can replace phosphate in biochemical reactions. One such example is phosphate replacement in ATP. Replacing the phosphate anion with arsenate can cause rapid hydrolysis of high energy bonds and effectively uncoupling oxidative phosphorylation. Arsenate can replace phosphate because they are similar in charge and size, not because they are isoelectronic. Elemental As has an electron configuration of $[Ar]4s^2 3d^{10} 4p^3$ and P has an electron configuration of $[Ne]3s^2 3p^3$. Both elements have 3 electrons in their p orbitals, the difference being As has a complete d orbital with 10 electrons. Arsenate may also replace the phosphorus in DNA, thus inhibiting the DNA repair mechanism and causing mutanegentic effects that can be passed on for generations (Dixon, 1997; Goyer, 1991).

The most common toxic mode of arsenic is the inactivation of enzymes, is known that As(III) will inhibit more than 200 different enzymes (Abernathy et al., 1999). Arsenite (As(III)) forms strong bonds with sulfhydryl and disulfide groups disrupting sulfur bearing enzymes and amino acids, such as cysteine and methionine. As(III) inhibits pyruvate and succinate oxidation pathways and the tricarboxylic cycle, and can greatly impair gluconeogenesis which with extended exposure can eventually lead to diabetes (Tseng, 2004). It is this strong bond with S that may be the reason that As accumulates in keratin tissues (Mandal and Suzuki, 2002).

Arsenite is a weak acid and exists primarily in its non-ionic form H_3AsO_3 in most environments due to its high pKa_1 of 9.2 (see Table 1.1). Theoretically As should be in

this non-ionic form in the chicken breast since the pH is 5.7-5.9, which is well under the pKa of As(III) (McMeekin, 1975). The electron configuration for As(III) has a full set of 3d-orbitals and a full 4s-orbital with empty 4p-orbitals ($[Ar]4s^2 3d^{10}$). The highest occupied molecular orbital (HOMO) is the 3d orbital and the lowest unoccupied molecular orbital (LUMO) is the 4p orbital. This configuration leaves As(III) with complete outer orbitals, making it a poor electron donor.

1.5.2. Arsenic and Amino Acid Binding Processes

Cysteine and methionine are sulfur bearing amino acids that studies have shown to readily bind with As(III). Methionine contains a single S that is incorporated into the chain structure making it less accessible to binding. Cysteine contains a sulfhydryl (thiol) side chain (SH); it is this group that will bind the As(III) (See Figure 1.3 for skeletal diagrams of the amino acids). Metabolic conversions make it possible for methionine to convert into cysteine, but the reverse process is not possible (Lewis, 2003). The cysteine amino acid is unstable in solution, and it readily oxidized to the dimer form, cystine. Therefore, it is likely that this dimer is found in solution, not cysteine. The two cysteine molecules will bind together at the two thiol groups creating a disulfide bond (Lewis, 2003). The thiol group can display nucleophility, which means it has the ability to form a chemical bond by donating both bonding electrons. Sulfur is nucleophilic due to its large size, which makes it easily polarizabled and in some cases it has a lone pair of electrons available for donation (McNaught and Wilksin, 1997). Elemental sulfur has an electron

configuration of [Ne]$3s^2 3p^4$, therefore S^{2-} and S^{1-} would have an electron configuration of [Ne] $3s^2 3p^6$ and [Ne] $3s^2 3p^5$.

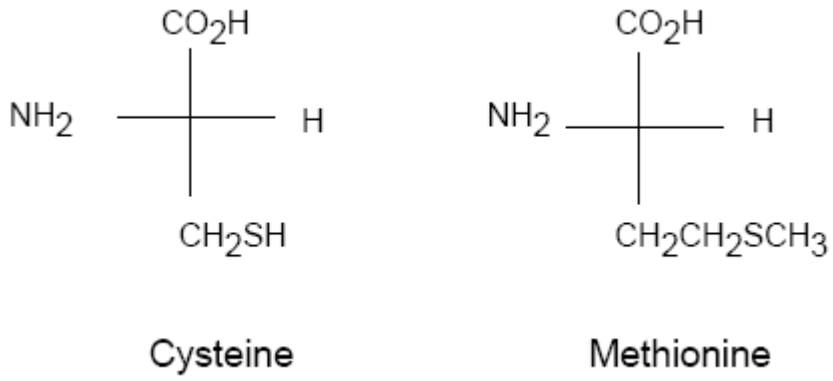

Figure 1.3. The skeletal diagrams for amino acids cysteine and methionine.

Metallothionine is a metallated protein that has stimulated many areas of active research including metal binding properties. It is known to bind with many trace metals with Zn, Cu, and As just being a few (Rigby-Duncan et al., 2006). Metallothionein is composed of 20 cysteine residues, which makes this protein active in As(III) binding (Rigby-Duncan et al., 2006). This protein has two domains (α and β), and it is known that six As(III) will bind to each protein (Jiang et al., 2003). It is through metallothionein research that researchers determined that the amino acid, cysteine, is the active As(III) binding reagent within the protein structure.

Arsenite-thiolate complexes occur frequently in natural systems and many studies have been conducted in order to better understand how As and S react. As(III)-thiolate complexes show a predominantly distorted trigonal pyramidal geometry. It is believed

that one As(III) will bind to 3 S atoms (3 cysteine molecules/residues) (Rigby-Duncan et al., 2006). Figure 1.4 shows As(III) binding to 3 cysteine molecules. It was noted that in As metabolism a major step is biomethylation of arsenic. This step in humans usually occurs in the liver, where arsenic methyltransferase enzymes mediate the methylation process with sulfur bearing compounds as the electron donor. During this process a series of electron shuffling occurs, and as methyl species are added to the As, the amount of cysteine compounds bound decreases (Jiang et al., 2003).

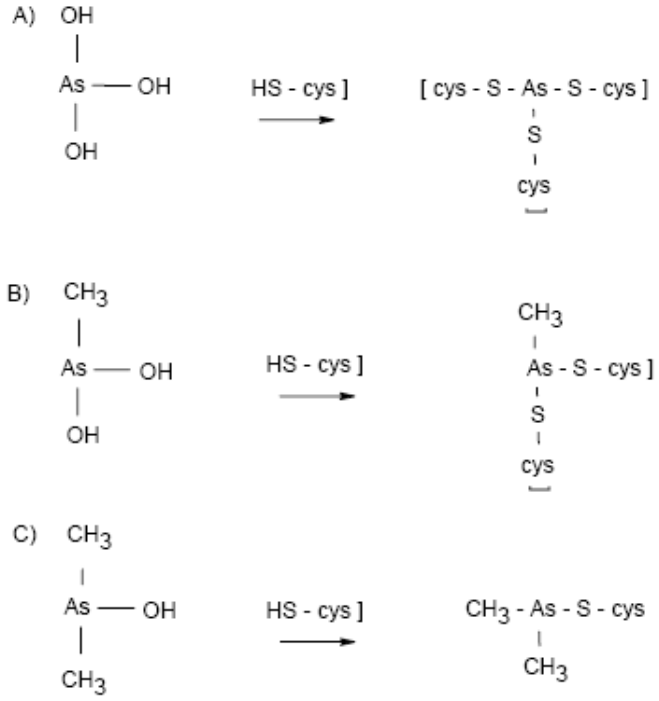

Figure 1.4. Schematic Representation of the binding stoichiometry between metallothionine and (a) As(III), (b) MMA^{III}, and (c) DMA^{III} (as suggested by and adapted from Jiang et al., 2003).

A number of animal studies have shown that dimethylarsenate (DMA) is the main metabolite, while in human urine excretion about 20% is inorganic As, 20% is MMA, and 60% is DMA. This plethora of species is found due to the rigorous environments and cycles that As will follow on its path through the body, see Figure 1.4 (Buchet et al., 1981; Vahter et al., 1984). More recent studies have found that the MMA and DMA are reduced to their trivalent species MMAIII and DMAIII (Le et al., 2000a; Le et al., 2000b; Mandal et al., 2001)

Studies noted that pH has an effect on how readily As will bind with cysteine. Toyama et al. (2002) studied As(III) and metallothionein at pH 2 and 7.4. The study found that the 1 As: 3 S ratio was maintained at 7.4, but not at 2. It would appear that this reaction is inhibited at lower pHs. This pH dependence indicates that at 5.7-5.9 (the pH of chicken breast), the cysteine-As(III) binding reaction should be favorable. Although this pH (7.4) is lower than the pKa at which the S side chain will deprotonate, the reaction still proceeds in the presence of cysteine. This is most likely due to the nucleophility of S. These reactions are more likely to occur in the later portion of the digestive system due to very acidic levels in the upper part of the system.

There are a variety of trace metals and sulfur amino acids used as macro and micro-nutrients in the poultry feed, therefore these must be taken into account when determining As transport processes. Robbins and Baker (1980) determined that copper toxicity is decreased in the presence of cysteine. Early studies indicate that a copper-arsonic acid interaction was present in turkeys, wherein copper sulfate was shown to decrease the efficacy of three arsenic acid compounds (Bowen et al., 1971). Studies by

Czarnecki et al. (1982, 1985) examined the interactions between roxarsone, cysteine and copper. The most weight gain in chicks was seen when the chicks were fed low levels of ROX (0-50 mg kg^{-1}), high levels of L-cysteine·HCl·H_2O, and high concentrations of Cu. The As levels in the kidneys indicated that As and cysteine concentration determined the amount of As found in the tissue. Increased concentrations of both compounds resulted in increased As accumulation in the kidneys. The study also found that the mortality rate of chicks fed high levels of ROX was increased by supplemental cysteine. Cysteine increased ROX toxicity in these studies. Cysteine may preferentially bind to copper, therefore when supplemental copper is present in the diet, cysteine may not exert as strong an effect on ROX (Czarnecki, 1982). Czarnecki and Baker (1985) found that Cu concentrations in tissues were reduced in the presence of ROX, but not in the presence of other arsenicals (arsanilic acid, As_2O_3, or As_2O_5) indicating that arsenic may not have an effect on Cu accumulation in liver tissue, but that a chelate was formed between Cu and the nitroso and hydroxyl groups of the ring structure of the ROX (Czarnecki and Baker, 1985). (A nitroso group is the reduction product of the nitro group found on the ROX structure.) ROX did not cause redistribution of Cu from one tissue to another, therefore ROX may have been acting by decreasing Cu absorption from the gut or by enhancing Cu removal from the body (Czarnecki and Baker, 1985). It could also be possible that ROX and Cu may form a complex that is keeping the Cu from being taken up by the animal tissues, this complex can then be excreted from the body. The copper binding by the ring structure may be occurring by means of the Baudisch reaction. It is a reaction in which a chelate is formed between Cu and adjacent nitroso and hydroxyl groups (See

Figure 1.5) Two moles of o-nitrosophenol chelate 1 mol of Cu (Baudisch, 1940; Czarnecki and Baker, 1985). Co was included in these experiments because it is known to chelate with adjacent nitroso and hydroxyl groups (Smith and Garst, 1973; Windholz et al., 1976). A final experiment conducted by Czarnecki and Baker (1985) indicated that the presence of As is indeed important to the Cu uptake reaction, it appears that the acidic functional group aids in the binding of Cu. It is possible that this reaction is not specific to Cu, other divalent trace metals may experience a similar behavior in the presence of ROX.

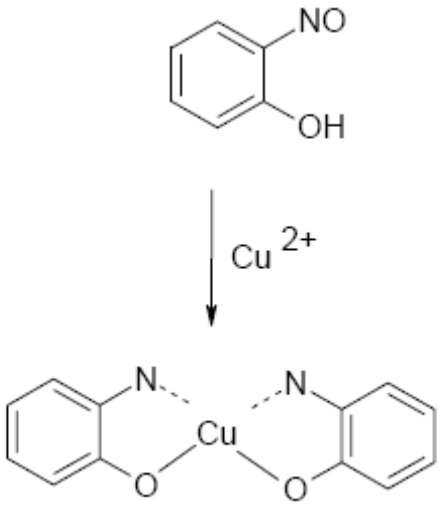

Figure 1.5. Baudisch reaction, how trace metals could be chelated by ROX, adapted from Czarnecki and Baker, 1985.

Roxarsone and cysteine reactions are believed to occur between the sulfhydryl group of cysteine and the arsenic acid portion of the ROX structure. Due to sulfur's strong affinity for As, an intense As-S attraction may weaken and break the arsenic acid-

nitrophenol bond releasing an arsenic-cysteine complex and orthonitrophenol. The resulting compounds may be more toxic than ROX, this would explain the increased mortality seen in Czarnecki et al. (1982). Additionally, the thiol group on the cysteine may act as an electron donor causing the recently cleaved As(V) to be reduced to the more toxic (and labile) As(III). Doull et al. (1980) found that As(III) is more toxic and results in greater tissue accumulation than As(V).

1.5.3. Arsenic Accumulation in Poultry Tissues.

The poultry industry is a large business in the United States. A total of 8.7 billion broilers was produced in 1994 and about 70% of these birds are fed an arsenic diet (Wallinga, 2006). Annual chicken consumption has risen 253 percent from 1966 to 2000, from 32.1 to 81.2 pounds per person (Taylor, 2004). A combination of antibiotics to promote growth, organic arsenic, and an anti-parasite drug called a coccidiostat are typically added to poultry feed. It is estimated that 1.7 to 2.2 million pounds of ROX are fed to poultry each year. Each bird is fed about 3.5 mg of As a day throughout their 6 week life (Momplaisir et al., 2001).

Arsenic accumulation in animals has been investigated. One recent study by Xie et al., (2004) investigated As accumulation in the liver of mice, and found that regardless of As speciation (organic vs. inorganic) As concentration in the liver increased when As was introduced to the diet. Lasky et al. (2004) found an average 0.39 ppm As in chicken livers and some muscle tissues collected from the USDA's data sources. The data showed that As accumulation in livers could be 2 to 11 times higher in the liver than in

the muscle tissue, depending on how long the As was removed from the feed before slaughter. Silbergeld (2004) suggests that repeated As exposure could eventually lead to As accumulation in muscle tissue, meaning that eventually the concentration of As in the muscle tissues could surpass that of the livers. Wallinga et al.(2006) tested and found arsenic in 55% of the chicken products that they purchased in the supermarket. The arsenic levels found in the chicken meat ranged from 1.5 to 22 ppb As.

Arsenic can cycle between inorganic and organic phases in the environment and inside the body. As was already discussed, the process of methylation can take inorganic As and transform it into organic As. The opposite process is true. Upon examining the pKa values for arsenic in the environment, it is seen that As(III) in the environment should be found in the non-ionic form (pKa1 ~9.0). Theoretically As should be in this non-ionic form in the chicken breast being that the pH is 5.7-5.9, which is well under the pKa of As(III) (McMeekin, 1975)

1.6. Poultry Litter Management Practices

More than 26-55 billion pounds of poultry litter is produced in the U.S. each year (Wallinga, 2006). Therefore, manure disposal becomes an important issue, and trying to dispose of this waste in an environmentally friendly manner complicates the problem. There are a number of different practices used by the poultry industry to dispose of PL. Spreading manure is a common practice used to dispose of poultry wastes. In some areas it is reasonable to dispose of the waste in this manner, however for lands that have had years of PL amendments this becomes an issue. In many instances there is not enough

available land to spread the manure, and due to the difficulty and expense of transporting it the lands surrounding the houses usually experience excessive amounts of litter applications. Regulations state that about 3-4 tons of manure may be spread on an acre of land (devoted to the production of corn) (North, 1984). Following best management practices (BMPs) are essential when spreading manure. These BMPs limit not only the amount of litter being spread, but also the time of year that it can be applied and the components found in the litter. These regulations imply that manure must be stored until the appropriate time of year. Phosphorus is considered as part of most BMPs; however most trace metal(loid)s are not considered at this time.

Poultry litter storage is a problem that most poultry producers encounter. There are a number of practices used, including: dehydration, composting, litter accumulation on floor operations, lagoons and oxidation ditches. Dehydration is used to reduce the volume of PL being stored and land applied. The litter is dried at temperatures ranging from 700^{o} to 1800^{o}F; at these temperatures bacterial action is prevented therefore reducing the amount of odor released from the PL. Composting is a more common practice for managing PL. During the composting process, there are essentially three separate layers: the aerobic surface, the composting layer, and the anaerobic layer at the bottom. The litter can accumulate for years and could pile up to 2 to 4 feet in depth (Miner et al., 2000; North, 1984). However, at the end of the process, the litter still needs to be dealt with. Litter on floor operations consist of litter being placed on the floor of the poultry house, then rearing a flock of birds. At the end of this cycle, the litter can be mixed, the top cake-layer can be removed, and/or additional litter is applied on top and

then new birds are introduced (Miner et al., 2000). All of these practices are ways to manage the massive amounts of litter produced, yet it is expensive to upkeep these practices (North, 1984). Liquid land application equipment; building lagoons, troughs, and composting structures cost money that the farmers may not have to spend on PL management.

Researchers and the poultry industry have created a variety of products to reduce the negative effects associated with the massive amounts of PL generated. One problem commonly associated with PL is a large influx of P and N into surface and ground waters. PL is often amended with metal salts e.g., aluminum sulfate (alum) to reduce ammonia emissions and stabilize P such that it may be less mobile in soil and water environments. Alum amendments do lower water soluble P levels (Moore et al., 2000; Sims and Luka-McCafferty, 2002). Moore et al. (2000) also reported trace metals were less mobile via runoff after PL was amended with alum. Sims and Luka-McCafferty (2002) conducted a large scale study on 200 farms on Delmarva to determine effects of alum on PL properties, elemental composition, and the solubility of inorganic elements such as P and As. With respect to As, total levels in untreated PL samples averaged 45 mg kg^{-1} and soluble As averaged 19 mg kg^{-1}, in contrast alum-treated samples water soluble As content was lower, averaging 7 mg kg^{-1}.

1.7. Arsenic Speciation of Poultry Litter

1.7.0. Arsenic Speciation.

The main form of arsenic used in the poultry industry is an organic arsenic called 3-nitro-4-hydroxyphenly-arsonic acid, more commonly known as roxarsone (ROX). There is evidence that the organic As transforms into organic and inorganic As, primarily As(V) (Garbarino et al., 2003; Jackson et al., 2003; Rosal et al., 2005; Stolz et al., 2007). Garbarino et al. (2003) mixed PL samples with water (50 wt %) and the mixture was allowed to compost at 40C, the organo-As converted to As(V) in about 30 days. These studies suggest that after litter storage and land application, and subsequent exposure to sunlight, elevated temperatures, and precipitation, As could undergo transformations to inorganic As species such as As(V). It has been suggested that this conversion is biologically mediated (Cortinas et al., 2006; Garbarino et al., (2003); Stolz et al., 2007). However arsenate is not the only degradation product found in PL, a variety of both organic and inorganic arsenic compounds have been found. Aerobic and anaerobic conditions will have an impact on the speciation of As found in the litter. Aerobic studies have found more As(V) and some organic molecules (Arai et al., 2003; Garbarino et al., 2003; Jackson et al., 2003; Rosal et al., 2005; Stolz et al., 2007), while anaerobic studies have shown more As(III) and organic breakdown products (Arai et al., 2003; Cortinas et al., 2006). Common organic degradation products are 4-hydroxy-3-aminophenylarsonic acid (HAPA) and 4-aminophenylarsonic acid (4-APA/p-ars). These organic structures are depicted in Figure 1.6.

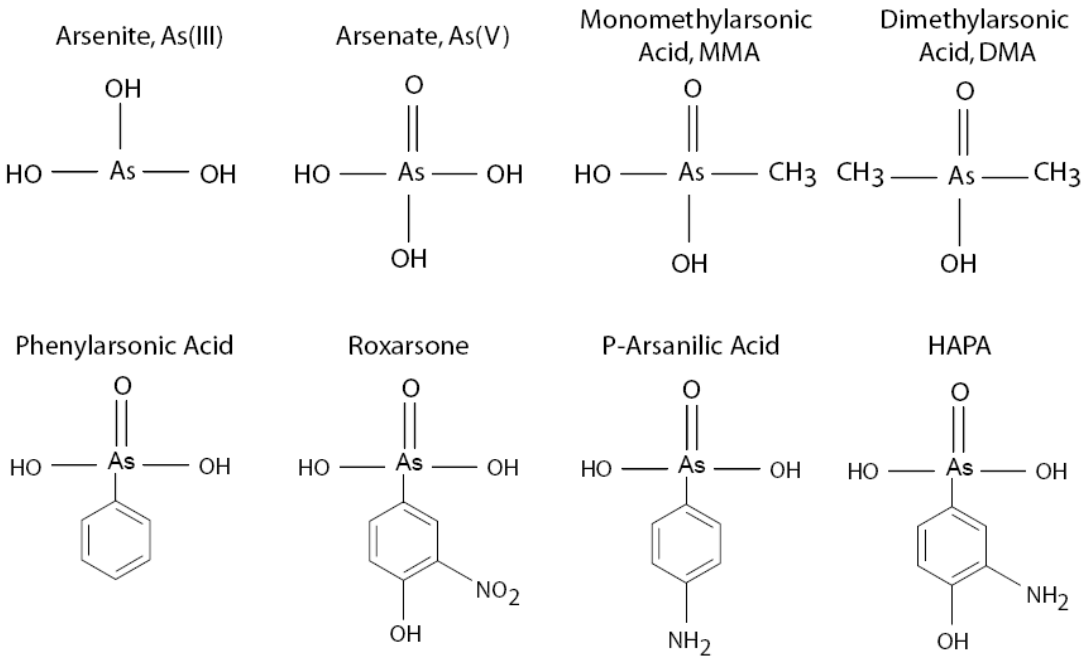

Figure 1.6. Roxarsone and its degradation products: arsenite (As(III), arsenate (As(V)), Monomethylarsonic Acid (MMA), Dimethylarsonic Acid (DMA), p-arsanillic acid (p-ASA/ars), HAPA, and phenylarsonic acid (PAA).

The inorganic species As(V) and As(III) are much more soluble and toxic than ROX and could be readily mobile in soils and potentially contaminate shallow ground waters, most of which are inter-connected with fresh and estuarine surface waters (Bednar et al,; 2004; Brown et al,; 2005). Brown et al. (2005) found As (V) to be readily mobile in soil systems, and found ROX to be more mobile in the subsurface soil horizons. Arai et al. (2003) employed micro-focused x-ray absorption fine structure (XAFS) and x-ray fluorescence (XRF) spectroscopies to directly speciate As in PL samples and long-term PL amended soils. The predominant inorganic species in the PL was As(V). The As(V), which is more toxic than the organo-As species could sorb on soil components such as metal oxides or leach into waters. Jackson and Miller (1999) found

that 72% of the total As in PL samples was water soluble, Jackson and Bertsch (1999) reported 71% of the As in PL samples was water soluble, and Garbarino et al (2003), 70-90% of As in dried PL samples from Kansas was water soluble.

The organo-As compounds added to the feed are primarily excreted in the organo-As forms. For example, Morrison (1969) found that ROX constituted 36-88% of the total As in 10 PL samples. Jackson et al. (2001) found that the major As species in 40 PL extracts were either ROX or As(V). For 20 of the 40 PL samples, As(V) was the major As species in the water extract, showing that mineralization of the initial organo-As had occurred. Jackson and Bertsch (2001) used IC-ICP-MS to differentiate between the different As species in PL. They found ROX to be the dominant species with DMA and As(V) being detected in solution.

1.7.1. Roxarsone Degradation.

Roxarsone degradation will occur in strictly anoxic conditions. This is an important finding because there are many places where anaerobic conditions exist within the poultry management regime. Cortinas et al. (2006) found that under anaerobic conditions, nitrogen-substituted phenylarsonic compounds degrade into As(III). Stolz et al. (2007) found pure ROX to degrade rapidly under strictly anaerobic conditions. Their inoculated studies did not find ROX to degrade under aerobic conditions. The microbes that Stolz et al. (2007) found in the PL were most closely related to the *Clostridium celerecrescens* which is also found in the gastrointestinal tract of the bird. It is speculated that ROX degradation to 3-amino-4-hydroxybenzene arsenic acid and inorganic As may

be part of a respiratory process. Stolz et al. (2007) used DFT methodology to determine which portions of the ROX compound are more prone to oxidation and reduction. They found the benzene ring will be oxidized first, which would ultimately release inorganic As. The research suggests that ROX may serve as an electron acceptor for anaerobic respiration. These results are not out of line with established research. Prokaryotes are known to use As oxyanions for energy generation, either by oxidizing arsenite or by respiring arsenate. Both aerobic and anaerobic microbial activity has been documented (Oremland and Stolz, 2003).

1.7.2. Arsenstruvite.

Chemical compounds found in animal waste and their impacts on the environment have been studied for years. One such phosphorus compound, called Struvite ($NH_4MgPO_4 \cdot 6H_2O$), is a crystalline ammonia magnesium phosphate mineral that has been found in diary, sheep, and poultry manures (Fettman et al., 1992; Gungor and Karthikeyan, 2005a; Gungor and Karthikeyan, 2005b; Gungor and Karthikeyan, 2008; Gungor et al., 2007; Qureshi et al., 2006; Shand et al., 2005; Uludag-Demirer et al., 2005; Uludag-Demirer et al., 2008) It is considered to be a slow release compound and in some cases manure management practices have been instated to encourage the formation of this compound to limit soluble P (Gungor and Karthikeyan, 2008; Uludag-Demirer et al., 2005; Uludag-Demirer et al., 2008). Recently Hunger et al. (2008) documented its formation in stored poultry litter (Hunger, 2008). Struvite has an analogue called arsenstruvite ($NH_4MgAsO_4 \cdot 6H_2O$). It has been poorly documented in literature

(FERRARIS, 1973; Stefov, 2008), and its environmental impact is unknown. It is expected to behave similarly to struvite under natural conditions.

1.8. Arsenic Sorption onto Soils

1.8.0. Arsenic Characterization in Soils.

Pollution assessment and remediation efforts represent a significant financial burden for agriculture, industry, and government. Frequently, remedial actions are undertaken based on the total metal(loid) content because precise speciation information on the contaminants is not known. Speciation of metals can determine what fate the contaminant might have in soil/water environments. Although total content of metals can give insight into the degree of pollution, these analyses provide limited information on the fate and transport of the metal(loid)s through the soil/water environment or bioavailability (de Groot, 1995). A series of more effective, widely-used and accepted approaches to characterize the potential for As to move through soils to ground waters include: (i) chemical extraction; (ii) sorption-desorption studies; and (iii) chemical and spectroscopic studies.

Chemical extraction approaches use solutions of dilute acids, bases, chelates, or even water to rapidly and inexpensively characterize the solubility and bioavailability of metals in soils, also known as a sequential extraction. Sequential extraction techniques use a series of progressively stronger and selective chemical extractants to partition As into soluble, Al-bound, Fe-bound, organic, and very recalcitrant residual phases in soils and sediments. A variety of chemical extractants used alone, or in sequence, have been

evaluated with As. Examples include the method more recently used by Rodriguez et al., (2003) and the (Tessier et al., 1979) method designed for trace metals in 1979. The Rodriguez method entails: deionized water, phosphate solutions, agronomic soil tests, and solutions specifically designed to extract As from Al and Fe oxides in soils. The Tessier method includes: magnesium chloride, sodium acetate adjusted to pH 5.0, sodium citrate-dithionite, dilute nitric acid, followed by a complete digestion using HF-$HClO_4$. These tests have been shown to be reasonably well correlated with the potential for As to leach through soils, to be bioavailable, and to become soluble under anaerobic conditions that can occur in aquifers and sediments from fresh and estuarine water bodies. These methods are very useful to conduct large-scale screening studies of a variety of soils and sediments. These procedures help determine the distribution of As, describe As retention, and speciation of As in soils.

Sorption-desorption studies are widely used to obtain quantitative information about an environmentally relevant component and its ability to adhere to various sorbents. This information is often used to set parameters needed for environmental fate and transport models that characterize the potential for metals to leach into ground waters or be transported via overland flow processes to surface waters. Sorption refers to the binding of a metal(loid) to soil constituents (e.g., clays, Al or Fe oxides, organic matter). Desorption refers to the release of a metal(loid) from these constituents into the soil solution (Sparks, 2003). Understanding sorption-desorption processes in soils and how they are affected by soil physical and chemical properties is critical to quantifying the concentration of As that will be in the soil solution and thus will directly interact with

leaching or runoff waters. These studies provide numerical parameters, such as partition coefficients, sorption maxima, and binding strengths that are useful in accessing and modeling the potential for As to move through different soil profiles into the ground water system. Sorption-desorption studies are more process-oriented than chemical extractions and can quantify the relationship between As in solid and aqueous phases, such as soils, metal oxides, leachate, ground water, or surface water.

Speciation is critical to predicting the fate, transport, toxicity, and bioavailability of metals and metalloids. Many factors affect speciation including oxidation-reduction, pH, time, type and quantity of inorganic and organic sorbent phases, other ions, and organisms. These factors will determine whether a given contaminant will adsorb, precipitate, chelate, leach through the soil profile, or be absorbed by plants. Traditionally, speciation of metals/metalloids such as As in soils has been assessed by indirect approaches such as sequential extraction and/or modeling based on equilibrium data from laboratory studies (Manning and Goldberg, 1997; Manning and Suarez, 2002). These approaches are limited in their ability to assess the precise speciation of metals/metalloids in soils, primarily because of the heterogeneous nature within and among soils, and the assumptions used in the processes. Sequential extraction techniques, while useful for characterization purposes, may introduce artifacts by transforming chemical forms and may overlook minor but important phases. Extraction of sorbed As with subsequent chromatographic identification is another technique that has been employed to identify previously bound arsenic phases, yet it fails to describe the actual sorption complex (Garcia-Manyes et al., 2002; Pongratz, 1998). To alleviate such shortcomings, more

sophisticated techniques that provide direct identification of species (i.e., X-ray diffraction (XRD), thermal gravimetric analysis (TGA), X-ray photoelectron spectroscopy (XPS)) have been adopted to characterize metals in contaminated soils (Ding et al., 2000; Farquahr et al., 1996; Nesbitt et al., 1998). Despite their accurate descriptions of metal speciation relative to extraction approaches, these techniques may also introduce artifacts from sample alterations, and detection limits are often far above background concentrations of the target metal.

1.8.1. Fate and Transport of Arsenic in Poultry Litter Amended Soils.

The effects of continuous poultry litter amendments on As contamination in Mid-Atlantic soil and water environments are not known. Limited data have shown ground water from agricultural fields of the Pocomoke River Basin in Maryland and Delaware having total dissolved As concentrations as high as 23 μg L^{-1} (Hancock et al., 2003). Moore et al. (1998) reported initial soluble As concentrations of > 200 μg L^{-1} were found in runoff water from a field that had been amended with 9 Mg PL ha^{-1} and after 7 days, the As concentrations were still > 50 μg L^{-1}. Many of the PL degradation products are water soluble which could potentially contaminate shallow ground waters, most of which are inter-connected with fresh and estuarine surface waters (Bednar et al., 2004; Brown et al., 2005).

The amount of arsenic sorbed onto the soil is dependent on many different soil physiochemical characteristics including: pH, redox potential, metal and mineral content, and soil texture. A sandy soil and a clayey soil will react very differently under the same

pH and Eh. Metal (oxy)hydroxides are the As stabilizing components in the soil, it is the organic fraction that allows As solubility to occur (Rutherford et al., 2003; Sadiq, 1997). Unlike most metals, As is not known to adhere to the organic fraction of soils. This is most likely due to the negative charge associated with both the As oxyanion and the negative charge of organic materials (Sadiq, 1997). Results by Pantsar-Kallio and Manninen (1997) suggest that pH changes in the soil system would have to be very drastic in order to cause serious contamination and health risks because As is well retained at natural soil pHs.

Poultry litter is generally applied at the rate of 9-20 Mg ha^{-1} on agricultural lands, and its total annual As inputs on the Delmarva Peninsula are estimated between 20 and 50 metric tons of total As (Christen, 2001a). The soils in this region are mostly sandy, prone to leaching, and overlie shallow ground waters commonly used as sources of drinking water. Sussex county is entirely within the Atlantic Coastal Plain (Ireland and Matthews, 1974). The elevation change is about 78ft from the coast to the highest point in the county. Because this area originated as a coastal plain, it does not have dominant bedrock that influences the composition of the soils, it contains loosely consolidated sediment that dates back to the Jurassic (Survey, 2007).

Rutherford et al. (2003) conducted sequential water extractions on soil samples taken from the Delmarva and Oklahoma areas that had been amended for an extended period of time with PL, and compared these to the same soils from forested areas that had no history of PL applications. Water-extractable As was 6.4 times higher for the PL-amended Delmarva soil than the unamended soil. They found that water-extractable As

decreased with depth in the soils. Acid-extractable As and Fe increased with depth in the amended soils with concentrations of As being twice as high in the amended soil at all depths compared to the unamended field. The correlation between As and Fe suggests that Fe-oxides in the soils could be retaining the As, however, this was not established by Rutherford et al. (2003). Many studies have investigated As sorption to Fe oxides and are reported in a later section. Gupta and Charles (1999) also found increased levels of arsenic in manure amended fields (15.72 mg kg^{-1}) when compared to control fields (9.26 mg kg^{-1}). Yet other studies did not find significant As accumulation in long-term amended PL agricultural soils (Arai et al., 2003). These results indicate that As retention in soils is not occurring in all areas, and that there many be certain factors such as soil composition, the presence of competitive anions and general chemistry influencing As sorption.

There are very limited data on the speciation and distribution of As in long-term PL-amended soils. The fate and transport of As in Delaware soils is not well understood. The extent of which competing ions, such as phosphate, affect As retention and release to groundwater has not thoroughly been assessed. This information has the potential to aid in the development of management practices that will mitigate As transport

1.8.2. Quantifying Retention and Speciation of Arsenic in Soils.

A number of researchers have studied As adsorption on soils and minerals using macroscopic techniques and surface complexation models. As(V) sorption studies on metal oxides (eg., Fe, Al, Mn) have shown that As(V) is strongly adsorbed on amorphous

$Al(OH)_3$, $\alpha - Al_2O_3$, hydrous ferric oxides (HFO), hematite at acidic pH (Arai and Sparks, 2002; Arai et al., 2001; Darland and Inskeep, 1997; Deschamps et al.; Fendorf et al., 1997; Ford, 2002; Garcia-Manyes et al., 2002; Grafe et al., 2001; Grafe et al., 2002; Halter and Pfeifer, 2001; Hansel et al., 2002; Jain and Loeppert, 2000; Jain et al., 1999; Lafferty and Loeppert, 2005; O'Reilly et al., 2001; Smith et al., 1998; Smith et al., 1999; Su and Puls, 2001a; Su and Puls, 2001b; Sun and Doner, 1996; Yang et al., 2005). Conversely, As(III) adsorption has been found to increase with increasing pH when sorbed on goethite, ferrihydrite, kaolinite, illite, montmorillonite, and amorphous aluminum oxides (Arai and Sparks, 2001; Arai and Sparks, 2002; Arai et al., 2001; Barrachina et al., 1996a; Barrachina et al., 1996b; Elkhatib et al., 1984a; Elkhatib et al., 1984b; Goldberg, 2002; Grafe et al., 2001; Grafe et al., 2002; Jain and Loeppert, 2000; Jain et al., 1999; Lafferty and Loeppert, 2005; Pierce and Moore, 1982; Raven et al., 1998; Su and Puls, 2001a; Su and Puls, 2001b; Sun and Doner, 1996; Sun et al., 1999; Yang et al., 2005). Hsia et al. (1994) used the triple layer model to describe As(V) adsorption on amorphous iron oxides from pH 4-10, the data suggested the formation of an inner-sphere monodentate mononuclear species. As(V) sorption to coprecipitated Al:Fe hydroxides was studied, in hopes of using this hydroxide in remediation processes. The hopes were to reduce the amount of As(V) reduction occurring by the iron oxides (which are commonly used in remediation strategies). The Al oxides did reduce the amount of As(III) produced, yet it may not be the best option since Al oxides do not readily retain As(III) (Masue et al., 2007).

However, the coupling of macro and nano(micro)-scaled techniques potentially provide more information about how a trace metal(liod) is bound to soil constituents. X-ray absorption fine structure (XAFS) spectroscopic studies were used to describe As sorption to soil components. As(V) form both inner-sphere bidentate binuclear and monodentate complexes on ferrihydrite, goethite, hematite, and lepidocrocite at pH 6-8 (Deschamps et al., 2003; Farquhar et al., 2002; Fendorf et al., 1997; O'Reilly et al., 2001; Waychunas et al., 1993,1995}. Suarez (1999) using attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopic studies, electrophoretic mobility (EM) measurements, and titration studies also suggested inner-sphere adsorption mechanisms of As(V) and As(III) on ferrihydrite. An in situ XAFS study focusing on As(III) adsorption to goethite reported a bidentate binuclear bridging configuration (Manning et al., 1998). Using a combination of macroscopic and XAFS, Arai et al.(2002) and Arai and Sparks (2001) observed inner-sphere complexes with bidentate binuclear configurations for both As(III) and As(V) sorbed on γ- Al_2O_3 as a function of pH and ionic strength. In some instances a combination of both inner and outer-sphere complexes were seen.

Other soil constituents need to be considered when discussing arsenic adsorption in soils. Manganese oxides are important sorbent phases in soils, while their sorption capacity may be less due to smaller specific surface areas and their low point of zero charge of 2. However they do posses the ability to oxidize As(III) to As(V) (Arai et al., 2003; Sadiq, 1997). Many recent studies have examined and confirmed that As oxidation can naturally occur in soils due to Mn oxides (Chen et al.; Deschamps et al., 2003; Jessen

et al.; Nesbitt et al., 1998; Ouvrard et al., 2002; Tournassat et al., 2002). The studies found that it is a two step process, where an intermediate Mn(III) oxy-hydroxide phase followed by subsequent oxidation of As(V) and reduction to Mn(II). Both of these products are released into solution, and over time an As(V)-Mn precipitate is formed (Manning et al., 2002; Tournassat et al., 2002). The As(V)-MnO_2 complex is a bidentate binuclear corner sharing complex occurring at interlayer sites and crystallite edges (Manning et al., 2002). A natural Mn sand was examined for Mn speciation, then the sand was used for an As(V) sorption experiment. When using X-ray photoelectron spectroscopy (XPS) to analyze As content, they discovered As(III) bound to the surface. Ouvrard et al. (2005) state that the As(V) was reduced by the presence of iron particles. A recent study states that the amount and rate that Mn oxides will oxidize As(III) is dependent on the pH and the presence of competing ions. It was found that the presence of competing ions will decrease the amount of As(V) produced (Power et al., 2005). Arsenic retention on ferrihydrite coated quartz formed a bidentate-binuclear attachment, which is a strong binding mechanism (Manceau et al., 2007)

Clay minerals are typically strong sorbent phases in soils when dealing with metals. However when considering oxyanions like As(III) and As(V), a great deal of sorption is not typically seen. Following a similar trend as organic materials, the negatively charged clays do not readily sorb the As oxyanions. Secondary clay minerals such as kaolinite, montmorillonite, and vermiculite have low sorption capacities for inorganic As species. Although these reactions are not as prevalent as Fe or Al oxide sorption, it can occur by chemisorption or ligand exchange processes (Sadiq, 1997).

Goldberg (2002) found the sorption maximum for As(V) and As(III) to be near their respective pKa1 values. However, when the clay minerals are coated with organic materials or iron nanoparticles As(V) sorption is seen (Dousova et al., 2006; Grafe et al., 2001; Li and Bowman, 2001). This is an unusual observation because organic acids and As(V) are believed to compete for sites on variably charged clay surfaces. Lin and Puls (2000) found 1:1 clays will retain some levels of As(V) and very small amounts of As(III). Kaolinites sorbed 0.9-1.3 μg/g As(V) while halloysites adsorbed 40-70 μg/g As(V). As(III) was about 0.4 to 0.6 μg/g on both clay minerals. FTIR was employed to investigate As(V) binding mechanisms to clay minerals. It is suggested that OH groups on the clay mineral surface may influence As(V) binding, and a hydroxy-arsenate interlayer in halloysite may contribute to the greater binding characteristics of this clay mineral (Lin and Puls, 2000). Another study suggested that 2:1:1 clays also have a greater ability than most secondary clay minerals to sorb As(V) (Lin and Puls, 2000).

Another interesting phenomenon incorporates both oxidation of As(III) to As(V) and sorption by clay minerals. A series of studies have detected As(III) oxidation by clay mineral surfaces (Lin and Puls, 2000; Manning and Goldberg, 1997). It was found that As(III) sorption increases with aging time, and its subsequent desorption decreases with time. Oxidation of As(III) was seen, but reduction of As(V) was not detected on the clay surfaces. It is postulated that the oxidation of As(III) is enhanced by impurities in the clay or the presence of FeO (Manning and Goldberg, 1997). Oxidation of As(III) to As(V) would lead to greater As retention in soils.

1.8.3. Quantifying Desorption of Arsenic in Soils.

Arsenic desorption from soils is another critical process that predicts the extent to which As will remain bound to soil components, and therefore important in assessing its bioavailability and mobility. There have been numerous studies examining As sorption to soils and various soil constituents, however there are fewer studies on its release. Zhang and Selim (2005) determined that desorption of As(V) from soils was hysteric in nature and that irreversible sorption processes may be present in some soils.

Arsenic desorption is directly affected by environmental conditions and the presence of competing anions. Arsenate and arsenite speciation are both directly influenced by the pH of the soil solution. Arsenate retention is greater at lower pH and decreases as pH increases, while the opposite is true of As(III) (Fendorf et al., 1997; Lafferty and Loeppert, 2005; O'Reilly et al., 2001). However, competing oxyanions commonly found in poultry litter such as phosphate, selenate, nitrate, and sulfate need to be considered when discussing As sorption to soil components. These oxyanions are all similar in size and charge and will therefore compete for the same soil constituents.

As was previously described, As is strongly bound to soil by inner-sphere complexes. Researchers have found that other anions, particularly phosphate, can effectively compete with As(V) for sorption sites, hence releasing As into the soil solution and possibly contaminating groundwater (Darland and Inskeep, 1997; Manning and Goldberg, 1996a; Manning and Goldberg, 1996b; O'Reilly et al., 2001; Peryea; Peryea and Kammereck, 1997; Woolson et al., 1973). However, some oxyanions, like sulfate, compete poorly with As(V), and pose little threat to As desorption (Lafferty and

Loeppert, 2005; O'Reilly et al., 2001). In both studies, phosphate was significantly more competitive than arsenic. However, a rather detailed study examined As(V) and phosphate sorption on a series of soils and soil components. In about half of the components examined As(V) would out compete PO_4^{3-} for sorption sites (eg., birnessite, pyrolusite, goethite, nontronite, and iron bearing smectites), while PO_4^{3-} was preferred on amorphous Al precipitation products, allophane, gibbsite, goehmite, and kaolinite. They found that in all soils and components decreasing the pH increased the amount of As(V) sorbed on the sample and increasing residence time decreased the amount of PO_4^{3-} sorbed (Violante and Pigna, 2002). Manning and Goldberg (1996) found that PO_4^{3-} and As(V) were comparable in their ability to be retained on Fe and Al oxide minerals. These studies suggests that the amount of As(V) sorbed and released is limited by a number of factors: soil components, pH, residence time, and competing anions.

Agricultural practices introduce high amounts of phosphate and arsenate together, and then later exposure of phosphates added by fertilizers can potentially mobilize the As. Davenport and Peryea (1991) recorded high rates of monoammonium phosphate or monocalcium phosphate fertilizers significantly increased the amount of As leached from the soil. Mixing these fertilizers with orchard soils that had experienced lead arsenate pesticide contamination induced As release and it was positively correlated to the level of P input. In these systems As solubility is regulated by $H_2PO_4^-$ - AsO_4 exchange (Peryea, 1991). The trends seen in these studies indicate that extensive use of P fertilizers on As contaminated soils has the potential to stimulate downward movement of As (Peryea and Kammereck, 1997). Poultry litter amendments can introduce 200 times more P than As

in a single application, thus it would seem that excessive P could greatly impact As retention in litter amended soils.

Ligand displacement experiments conducted by Jackson and Miller (1999) demonstrate PO_4^{3-} and OH^- abilities to desorb a wide array of As compounds from both crystalline and amorphous iron oxides. In this study they monitored the desorption of As(III), As(V), DMA, MMA, p-arsanilic acid, ROX, Se(IV), and Se(VI). Hydroxide was the most efficient extractant, however it was inefficient in desorbing As(V) from goethite, and As(III) from amorphous iron oxide. Phosphate was most effective at low pHs (3) and high concentrations 0.5 M phosphate. ROX and p-arsanilic acid are both readily removed from the Fe oxide surface, apparently the arrangement of the side chains do not have an effect on sorption. DMA was readily desorbed from both minerals, while MMA was bound more tightly and behaved similarly to As(III) or As(V).

A major factor when considering desorption is residence time. The longer the contaminant (As) is in the soil, often the more difficult it becomes to remove (Ainsworth et al., 1994; Ford and Sparks, 2000; Ford et al., 1999; Lin and Puls, 2000; O'Reilly et al., 2001; Pignatello, 2000; Strawn and Sparks, 2000; Woolson et al., 1971). Residence time effects on As(V) desorption from γ-Al_2O_3 and goethite found biphasic As(V) desorption reactions were present at both pH 4.5 and 7.8 (Arai and Sparks, 2002). At both pHs the amount of As(V) desorbed decreased with increasing aging time (3d to 1yr), these results indicate that residence time does affect As(V) retention on mineral surfaces.

As residence time increases, arsenic can become incorporated into inorganic mineral structures (eg., interparticle diffusion and precipitation) (Sadiq, 1997). As(V)-Ca

coprecipitates were found in soils contaminated lead arsenate years prior to sampling (Arai et al., 2006). Arsenic and zinc precipitates formed on goethite after 6 months of time (Grafe and Sparks, 2005). Many times metal contamination occurs with a complex mixture of metals, therefore it is important to evaluate how these will react with time. Arsenate and phosphate posses isomorphic tendencies for one another, this process can be seen in natural minerals. Apatite is a phosphate mineral that varies in chemical, physical and crystallographic properties. Studies have reported that a variety of metals can be immobilized by apatite. A recent study found As incorporated into both processed and mined apatite, indicating that there is a substitution process actively occurring in nature (Knox et al., 2006).

1.9. The use of Synchrotron Spectroscopy in As Speciation

1.9.0. X-ray Absorption Spectroscopy Described

X-ray absorption spectroscopy (XAS) is a technique that can provide detailed chemical and structural information about a specific element. This element can be a major component of a solid phase (crystalline and amorphous), a trace component of the bulk phase, or a surface-associated component {Bertsch and Hunter, 1998; Bertsch and Sayers, 1998). Molecular scale information can be invaluable when dealing with environmental samples. Extended X-ray absorption fine structure (EXAFS) spectroscopy and X-ray absorption near edge structure (XANES) spectroscopy are two techniques commonly used in synchrotron work. EXAFS spectroscopy is used to elucidate chemical information such as coordination number, bonding distances, and nearest neighbors

{Sparks, 2003}. XANES spectroscopy is used primarily as a fingerprinting technique to elucidate oxidation states of the element of interest (Arai et al., 2003).

An XAS experiment consists of exposing a sample to an incident monochromatic beam of synchrotron X-rays, which is scanned over a wide range of energies both above and below the adsorption edge of the element of interest, resulting in a spectrum of data (Arai et al., 2003). Figure 1.7 depicts an As spectrum and points out the various regions of concern when discussing XAS techniques. When X-rays interact with elements a number of processes can occur: X-ray scattering of optical photons, production of photoelectrons, production of fluorescence X-ray photons, and positron-electron pair production (Arai et al., 2003). Within the X-ray range of As XAS experiments, photoelectron production dominates over the other processes. The spectrum in Figure 1.7 is produced based on the energy of the incident X-ray beam vs. the binding energy of the core electron of the element of interest. When the energy of the incident X-ray beam is below that of the excitation energy of the core electron, absorption is minimal (pre-edge region). When the incident X-ray beam energy is equal to that of the binding energy, excitation of the core electron occurs and the electron transitions to unoccupied bound energy levels, therefore contributing to the appearance of the main absorption edge. As the energy increases (about 50eV above the edge) the electrons stay within the vicinity of the absorber atom (in our case As) and are multiply scattered among its nearest neighbors (XANES region) (Arai et al., 2003). At energies above the XANES region, electrons are ejected from the central atom (absorber) and singly or multiply scattered from first or second nearest neighboring atoms and back to the central absorber, and then leave the

vicinity of the absorber (Arai et al., 2003; Brown et al., 2005). This portion of the spectrum (EXAFS region), is caused by interference between outgoing and backscattered photoelectrons.

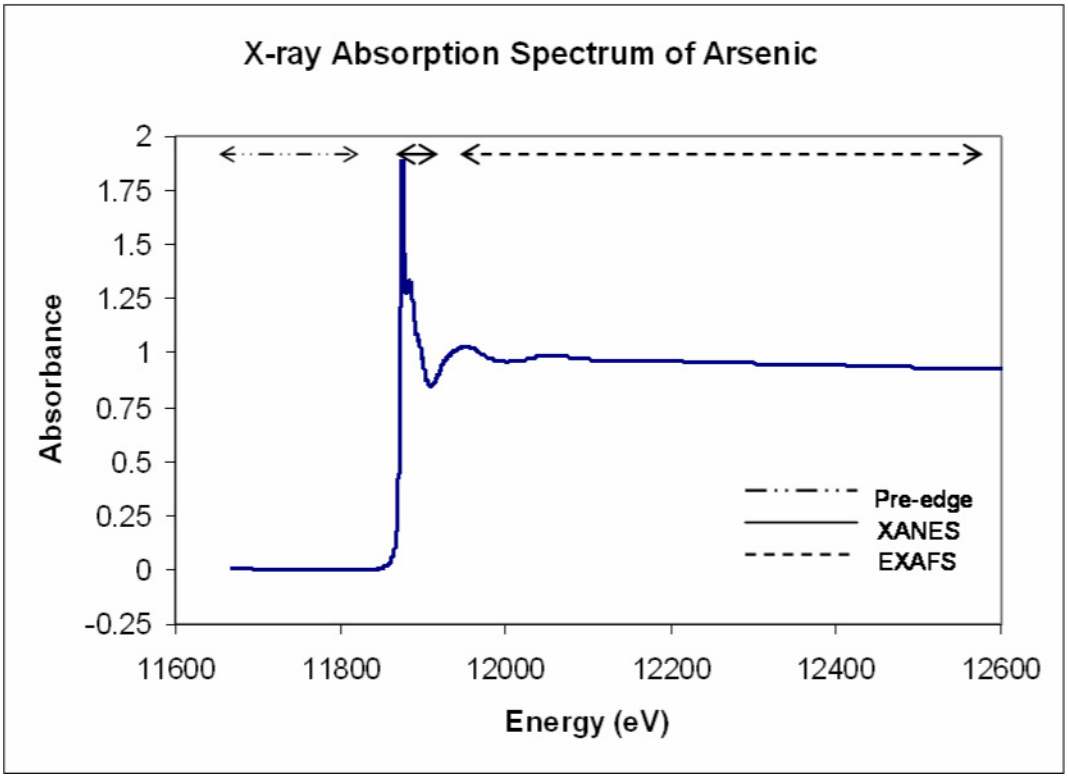

Figure 1.7. X-ray spectrum of As (DMA) collected at beamline X11A at the NSLS. It depicts the different X-ray regions within a single scan.

These techniques have been used on all media from geodermic samples to biological samples. Even poultry litter has been examined using this technique (Arai et al., 2005; Arai et al., 2003; Shober et al., 2006). Several research studies have demonstrated the utility of XAFS to elucidate sorption mechanisms of metal ions on single-component metal oxides and clay mineral systems (Bargar et al., 1995; Charlet

and Manceau, 1992; Fendorf et al., 1997; Scheidegger et al., 1998; Schlegel et al., 1999; Strawn and Sparks, 1999). Subsequently, XAFS studies have been performed using mixtures of oxides and clay minerals to better simulate metal sorption behavior in natural systems (Elzinga and Sparks, 1999; Scheckel and Sparks, 2000; Scheckel et al., 2000b). These studies have enabled researchers to extend this technique one step further, allowing one to use XAFS to successfully characterize metal-contaminated environmental samples (Hesterberg et al., 1997; Manceau et al., 1996; Morin et al., 1999; Morris et al., 1996; O'Day et al., 1998; Ostergren et al., 1999). Arai et al. (2006) found a complex mixture of As species present in lead arsenate contaminated soils. This study shows the importance of residence time and environmental conditions on As speciation in soils.

While these studies have been critical in improving the understanding of metal sorption mechanisms in all media, one must realize that standard (bulk) XAFS or XANES probes an area of several millimeters in a sample, providing only an average speciation of the metal(loid) of interest in a sample. This may pose a problem when analyzing XAS data collected on heterogeneous samples (eg. soils, poultry litter, plants), since the spectrum may represent several species. A proper database of reference samples are crucial in deciphering any data collected (Hunter and Bertsch, 1998; Manceau et al., 2000). Moreover, in samples where the metal/ metalloid may be present in numerous phases, the detection limit for minor species is indefinite and all species may not be represented upon spectral analyses since high Z elements in coordination to the central absorbing atom are preferentially represented over low Z elements (Manceau et al., 2000).

1.9.1. Advantages of Using a Synchrotron Microprobe

Other techniques that are capable of probing an element in an environmental sample at a scale more indicative of the most reactive sites in soils (micron level) may give insight into spatial distribution of a contaminant. Electron microscopy and electron microprobe analysis can attain both quantitative (elemental composition) and qualitative (contaminant distribution) with good spatial resolution (< 1 μm) (Webb et al., 2000). However, the information gleaned from these techniques only provides elemental concentrations, making it difficult to distinguish between individual species. One of the most promising techniques to examine heterogeneous environmental samples is spatially resolved, micro-focused XAFS (μ-XAFS), whereby discrete regions within a complex mixture can be investigated on a micron scale (Manceau et al., 2000; Roberts, 2002). Advantages of using synchrotron-based radiation relative to standard electron probe microprobe techniques are the increased sensitivity to metal/metalloid concentrations and its ability to distinguish between phases. Some microprobes are equipped with μ-X-ray diffraction (μ-XRD) abilities in which an XRD pattern can be taken *in-situ* and of different particles within a sample. These data provide structural information about crystalline materials.

1.9.2. Using X-ray Absorption Spectroscopy with Redox Sensitive Elements.

As with all analytical techniques there are issues that need to be taken into consideration when analyzing samples and interpreting data. Redox sensitive elements, such as manganese (Mn), Copper (Cu), Gold (Au), Chromium (Cr), and arsenic, can experience chemical alteration when handled (Ross et al., 2001(b))or analyzed improperly (Brown and Sturchio, 2002; Farges et al., 2002; Jayanetti et al., 2001; Manceau et al., 2002; Ross et al., 2001a; Ross et al., 2001b; Zachara et al., 2004). One such issue seen in redox sensitive elements is beam-induced reduction. Beam-induced reduction can cause chemical alteration of the element of interest and sample damage leading to misinterpretation of samples. This issue occurs most commonly at third generation sources (APS), but still occur at sources with less intense X-ray beams (Manceau et al., 2002). Ross et al. (2001) found that when using a synchrotron microprobe, when leaving the beam on a single spot for an extended period of time (about 150 minutes) a shift in energy up to 1.7eV. The researchers found that by moving the sample in the x-ray beam, beam-reduction did not occur. This indicates that beam-induced reduction occurs in the x-ray beam path only. This effect has been seen in both wet and dried samples. Another source of potential sample alteration/damage are secondary electrons emitted from the sample itself (Brown and Sturchio, 2002). Certain materials naturally exude more electrons under intense energy than others based on the solid type (insulator vs. metal), composition, crystal perfection, etc.(Brown and Sturchio, 2002; Cazaux, 2001). A number of methods have been suggested to limit the effect of beam-induced sample alteration, these include: cooling of redox-sensitive samples,

reducing additional potential sources of electrons when mounting samples (Manceau et al., 2002), and reducing the amount of time spent on a single location, particularly when using a microprobe.

1.9.3. Synchrotron Light Sources

Synchrotron light sources in the U.S. include: the Advanced Photon Source (APS) at Argonne National Laboratory, the Advanced Light Source (ALS) at Lawrence Berkeley National Laboratory, the National Synchrotron Light Souce (NSLS) at Brookhaven National Laboratory, and the Stanford Synchrotron Radiation Laboratory (SSRL). Each of these facilities has a broad array of XAS techniques available to users. Micro-XAS analyses of materials can be conducted at these facilities. At beamline 13-ID/GeoSoilEnviroCARS (GSECARS) at the APS and beamline 10.3.2 at the ALS state-of-art- μ-EXAFS (micro-extended X-ray absorption fine structure) spectroscopic analyses can be determined (Sutton et al., 2002). Micro-XANES analyses can be performed at beamline X-26A at the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory (Sutton et al., 2002). Thus, the ability to determine analyses of the entire micro-XAFS (micro-XANES and micro-EXAFS) spectra will provide not only the molecular symmetry and the oxidation state but also binding mechanisms within the samples. Such bright light sources enable one to employ a beam size of several hundred square micrometers and increased detection limits. Since the micrometer scale of most environmental samples (eg., mineral, litter particles) contain diverse reactive sites (e.g., oxide and organic matter coatings), μ-XANES is extremely

useful for gaining detailed data on contaminant speciation. In addition, μ-synchrotron X-ray fluorescence (μ-SXRF) spectroscopic analyses can also be conducted which provides significant information on elemental association with a targeted element.

1.10. Introduction to Subsequent Chapters

The following chapters will thoroughly discuss various aspects of arsenic chemistry in poultry litter amended soil, poultry litter, excreta and tissues. The main objective is to fully understand the impact of the agricultural practice of feeding arsenicals to poultry and the subsequent disposal of the As enriched-excreted materials.

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Chapter 2

ARSENIC STATUS IN DELAWARE SOILS

2.0. Abstract:

The poultry industry is highly concentrated on the Delmarva Peninsula. Millions of birds are produced each year, resulting in mass amounts of poultry litter which is later land applied to the surrounding soils. The litter contains high amounts of trace metal(loids), including arsenic (As). As is incorporated into the feed in the form of an organic arsenical (roxarsone, ROX). Studies have shown that ROX will degrade into organic and inorganic As species, with As(V) or arsenate being among one of the dominant species.

This study was conducted to examine As content in litter amended soils, and to determine the soils' ability to retain As under a series of environmental conditions. The results indicate that although the soils of this region have the ability to retain high amounts of As, As accumulation due to poultry litter amendments is not seen. Phosphate is another oxyanion found in poultry litter. As(V) and phosphate are chemical analogues of one another, meaning they will compete for the same sorption sites in the soil. Phosphate and As(V) competitive studies indicate that phosphate will greatly inhibit As accumulation at any concentration. Since phosphate can be up to a 1000 times more

abundant than As in litter, the As may not be able to adhere to soil components in the presence of phosphate.

2.1. Introduction:

2.1.0. Background Information.

Arsenic is a toxic metalloid found in soil/water environments due to natural and anthropogenic inputs. Poultry litter contains high concentrations of trace elements such as Ni, Cu, Zn, Mn, Fe, and As, the sources of which are growth promoters and basic mineral mixes added to poultry feed. The total As concentrations in poultry litter (PL, a mixture of bedding such as wood shavings or sawdust and manure) vary depending on the concentration of As in the feed. More than 11.4 million Mg of PL were produced in the U.S. in 1996 and about 90% of this litter was land applied (Cabrera and Sims, 2000).

Arsenic supplements commonly added to poultry litter are 4-amino-phenylarsonic acid (p-ASA), 4-nitrophenylarsonic acid (Nitarsone), or 3-nitro-4-hydroxyphenly-arsonic acid (*Roxarsone*, abbreviated ROX). These arsenicals are used to prevent coccidiosis, increase weight gain, improve feed efficiency and pigmentation. Roxarsone has been used to promote growth in the poultry and swine industries for the past 50 years. ROX was used in about 70% of broiler industry operations in the U.S. from 1999-2000 (Chapman and Johnson, 2002). Studies report that the organo-As compounds added to the feed are primary end products found in the excreta. Morrison (1969) found that

ROX constituted 36-88% of the total As in 10 PL samples, and Jackson et al. (2003) found that the major As species in 40 PL extracts were either ROX or inorganic arsenate (As(V)). Feed spillage and digested materials can increase the mean total As concentration in PL to 14-76 mg kg^{-1} (Moore et al., 1998). Research suggests that during litter storage, land application and then subsequent exposure to sunlight, elevated temperatures, and precipitation; As could undergo transformations to organic degradation products (phenylarsonic acid) and inorganic As species such as As(V) (Garbarino et al., 2003; Jackson et al., 2003; Rosal et al., 2005; Stolz et al., 2007). Studies suggest that this conversion is at least partially biologically mediated (Bednar et al.; Cortinas et al., 2006; Stolz et al., 2007).

The Delmarva Peninsula (an area encompassing parts of Delaware, Maryland and Virginia) is one of the most concentrated poultry production areas in the US. Sussex county, Delaware produces more broilers than any other county in the U.S.; it has produced more chicken meat than any other county in the country since 1944 (Delmarva Poultry Industry, 2000). In 2000, 620 million broilers were produced, which resulted in poultry litter containing approximately 26,000 kg of As (Christen, 2001; Garbarino et al., 2003). National surveys and research provide evidence that poultry production practices introduce As into the environment at higher rates than first estimated, and with the total As inputs on the Delmarva Peninsula are estimated between 20 and 50 metric tons of total As (Christen, 2001).

The effects of continuous poultry litter amendments on As contamination in Mid-Atlantic soil and water environments are not known. Limited data have shown ground

water from agricultural fields of the Pocomoke River Basin in Maryland and Delaware having total dissolved As concentrations as high as 23 μg L^{-1} (Hancock et al., 2001). Since the PL degradation products are water soluble, they could potentially contaminate shallow ground waters and therefore contaminate the inter-connected fresh and estuarine surface waters (Bednar et al., 2004; Brown et al., 2005).

The amount of arsenic (As(V)) sorbed onto the soil is dependent on many different soil physiochemical characteristics including: pH, redox potential, metal and mineral content, and soil texture. A sandy soil and a clayey soil will react very differently under the same pH and Eh. Metal (oxy)hydroxides are the As stabilizing components in the soil, it is the organic fraction that allows As solubility to occur because unlike most metals, As is not known to adhere to the organic materials (Rutherford et al., 2003; Sadiq, 1997). This is most likely due to the negative charge associated with both the As oxyanion and the negative charge of organic materials (Sadiq, 1997). Arsenate is tightly bound to soil components at natural soil pHs, therefore Pantsar-Kallio and Manninen (1997) suggest that pH changes in the soil system would have to be very drastic in order to cause serious contamination and health risks.

The soils in the Delmarva region are mostly sandy, prone to leaching, and overlie shallow ground waters commonly used as sources of drinking water. Sussex county is entirely within the Atlantic Coastal Plain (Ireland and Matthews, 1974). The elevation change is about 78ft from the coast to the highest point in the county. Because this area originated as a coastal plain, it does not have dominant bedrock that influences the composition of the soils, it contains loosely consolidated sediment that dates back to the

Jurassic (Survey, 2007). The upper part of the state, New Castle County, has a completely different geologic history. This part of the state has metamorphic bedrock, mostly gneiss and gabbro dating back to the Pre-Cambrian period (2007).

Rutherford et al. (2003) conducted sequential water extractions on soil samples taken from the Delmarva and Oklahoma areas that had been amended for long times with PL, and compared these to the same soils from forested areas that had no history of PL applications. Water-extractable As was 6.4 times higher for the PL-amended Delmarva soil than the unamended soil and found that water-extractable As decreased with depth in the soils. Gupta and Charles (1999) also found increased levels of arsenic in manure amended fields (15.72 mg kg^{-1}) when compared to control fields (9.26 mg kg^{-1}). Yet other studies did not find significant As accumulation in long-term amended PL agricultural soils (Arai et al., 2003). These results indicate that As retention in soils is not occurring in all areas, and that there many be certain factors such as soil composition, the presence of competitive anions and general chemistry that may be influencing As sorption.

A number of researchers have studied As adsorption on soils and minerals using macroscopic techniques and surface complexation models. As(V) sorption studies on metal oxides (eg., Fe, Al, Mn) have shown that As(V) is strongly adsorbed on amorphous $Al(OH)_3$, $\alpha - Al_2O_3$, hydrous ferric oxides (HFO), hematite at acidic pH and Mn oxides (Arai and Sparks, 2001; Arai and Sparks, 2002; Arai et al., 2001; Darland and Inskeep, 1997; Fendorf et al., 1997; Ford, 2002; Garcia-Manyes et al., 2002; Grafe et al., 2001; Grafe et al., 2002; Halter and Pfeifer, 2001; Hansel et al., 2002; Jain and Loeppert, 2000;

Jain et al., 1999; Lafferty and Loeppert, 2005; O'Reilly et al., 2001; Smith et al., 1998; Smith et al., 1999; Su and Puls, 2001a; Su and Puls, 2001b; Sun and Doner, 1996; Yang et al., 2005). Clay minerals are typically strong sorbent phases in soils when dealing with metals, but when considering oxyanions like As(V), a great deal of sorption is not typically seen. Secondary clay minerals such as kaolinite, montmorillonite, and vermiculite have low sorption capacities for inorganic As species. Although these reactions are not as prevalent as Fe or Al oxide sorption, they do occur by chemisorption or ligand exchange processes (Sadiq, 1997).

Arsenic desorption from soils is another critical process that predicts the extent to which As will remain bound to soil components, and therefore impact its bioavailability and mobility. Arsenic desorption is directly affected by environmental conditions and the presence of competing anions. Researchers have found that other anions, particularly phosphate, can effectively compete with As(V) for sorption sites, hence releasing As into the soil solution and possibly contaminating groundwater (Darland and Inskeep, 1997; Manning and Goldberg, 1996a; Manning and Goldberg, 1996b; O'Reilly et al., 2001; Peryea; Peryea and Kammereck, 1997; Woolson et al., 1973). However, some oxyanions, like sulfate, compete poorly with As(V), and pose little threat to As desorption (Lafferty and Loeppert, 2005; O'Reilly et al., 2001). A rather detailed study examined As(V) and phosphate sorption on a series of soils and soil components. In about half of the components examined As(V) would out compete PO_4^{3-} for sorption sites (eg., birnessite, pyrolusite, goethite, nontronite, and iron bearing smectites), while PO_4^{3-} was preferred on amorphous Al precipitation products, allophane, gibbsite, goehmite, and

kaolinite. They found that in all soils and components decreasing the pH increased the amount of As(V) sorbed on the sample and increasing residence time decreased the amount of PO_4^{3-} sorbed (Violante and Pigna, 2002). Manning and Goldberg (1996a,b, and 1997) found that PO_4^{3-} and As(V) were comparable in their ability to be retained on Fe and Al oxide minerals. These studies suggests that the amount of As(V) sorbed and released is limited by a number of factors: soil components, pH, residence time, and competing anions. Poultry litter amendments can introduce 200 times more P than As in a single application, based on these studies it would seem that excessive P could greatly impact As retention in litter amended soils.

2.1.1. Objectives and Focus.

Addition of As to agricultural lands via poultry litter is not specifically regulated at either the federal or state levels, nor is it considered a nonpoint source pollution of soils, under current nutrient management laws in Delaware. Therefore, the objectives of the study were two-fold i) to determine how decades of poultry litter amendments have affected Delaware soils and ii) to better understand the processes of As sorption and desorption in the heterogeneous Delaware soils As and the impact of anions on As competitive sorption/desorption.

2.2. Methods and Materials:

2.2.0. Soil Sample Collection.

Soil samples from 13 soil profiles representing 10 of Delaware's benchmark soil series were collected (Tables 2.1 and 2.2; and 2.A.1-6). Five of the 10 soil series were located on two farms in New Castle County, Delaware with no history of animal manure application and provided a range in topography, drainage class, and soil properties typical to this geographic region: Elkton, Woodstown, Sassafras, Nassawango, and Reybold. The other five soil series were located on farms in Sussex County either on i) crop land (Rumford, Sassafras, Downer, Corsica, Greenwich) or ii) forests adjacent to the sampled crop land (Sassafras, Ingleside, Greenwich). Soil profiles sampled in Sussex County also represented the typical range in topography, drainage, and soil properties characteristic of this area. Manure application history ranged from infrequent (Rumford, Sassafras soils) to a regular part of the crop fertilization program (Corsica, Downer, Greenwich soils).

Soils were collected by horizon to a depth of about 90-100 cm, depending upon the characteristics of the soil. The soil was collected on an S based sampling regime across the amended fields. Soil description and classification information at each site was provided by USDA-NRCS soil scientists who assisted in collection of the soil samples. A total of 42 distinct soil horizons were obtained for analysis.

2.2.1. Soil Characterization and Analysis.

After collection, all soil samples were air-dried, ground and sieved to pass a 2 mm screen. All soils were analyzed for the following physical and chemical properties: total

elemental composition (Al, As, B, Ca, Co, Cu, Fe, K, Mg, Mn, Ni, P, S, Zn) by two acid digestion methods - USEPA 3051 (microwave-assisted) and USEPA 3050B (digestion block); routine soil test analysis (pH, organic matter, soil test (Mehlich 3 (M3): 0.2 *M* CH_3COOH + 0.25 *M* NH_4NO_3 + 0.015 *M* NH_4F + 0.13 *M* HNO_3 + 0.001 *M* EDTA) extractable elements (P, K, Ca, Mg, Al, B, Cu, Fe, Mn, S, Zn); textural class (percentage of sand, silt, and clay); effective cation exchange capacity (ECEC) by summation of exchangeable Ca, K, and Mg and 1*M* KCl-exchangeable acidity; plant-available (M3) soil As; amorphous and crystalline iron and aluminum oxide content using a sodium carbonate-dithionite extraction and an oxalate reaction, respectively; water soluble As and P were conducted using 1 g of soil in 10 mL of distilled dionized water. Concentrations of As in all soil extracts were determined by inductively coupled optical emission spectroscopy (ICP-OES). As standard solutions were made as an internal calibration to analyze samples by ICP-OES. Trace metal grade acids were used in the digestion of the soils.

2.2.2. Arsenic Sorption by Selected Soil Horizons.

Arsenate (As(V)) was chosen as the sorptive, based on research indicating that As(V) is the primary degradation product of roxarsone (Arai et al., 2003; Garbarino et al., 2003). The As(V) concentration (as $Na_2HA_SO_4.7H_2O$) reacted with the soils was based on typical application rates of As(V) in PL-amended soils (20 pmm or 266 μmol As). Two horizons from each of the four agricultural or background soils were chosen in the isotherm and pH studies (Table 2.2).

Preliminary kinetic studies determined that after 48 h there was minimal increase in As sorption onto the soils, therefore all sorption studies were conducted for this period of time. All studies were conducted using a 5g/L suspension and a 0.01 $NaNO_3$ electrolyte solution and the pH of all reactions was monitored using a pH stat with trace metal grade 0.01 M HNO_3 or 0.01M NaOH.

The pH studies were conducted over a pH range of 3 to 10. Initial pH studies purely examined the effect of pH on the soils' ability to retain As under varying environmental conditions. The studies were conducted as batch reactions with each pH and soil conducted as its own batch study. Each soil was allowed to react with As at a specific pH with all acid or base additions recorded. The results of these studies are depicted in Figure 2.1. Subsequent pH studies were conducted to directly evaluate the effect of changing the natural pH of the soil. Each soil was allowed to equilibrate for 24 hours at pH 5.0 (about the average the pH of soils) before addition of As. After 48 hours, a sample was taken and the pH of the soil solution was adjusted to pH 4 or 6. This progression continued until the solutions were allowed to react at either pH 3 or 10. All solution removal and additions were recorded. Results of these reactions are depicted in Figure 2.2.

Arsenate sorption isotherm studies were conducted as individual batch reactions similar to the first set of pH edge studies except that the pH was maintained at 5.5 and temperature and pressure were held constant. The concentrations used to determine the As sorption maximum were 5, 10, 25, 50, 75, 100, 250, 500, 750, 1000 μmol L^{-1} of As(V) (Figure 2.3). The Langmuir sorption equation was then used to determine the sorption

maxima for the soils, (Figures 2.4-5 and Table 2.2).

2.2.3. Arsenate Competitive Studies.

The interaction of As(V)-PO_4 binary systems with selected soils was studied at typical soil pH values and at different As:PO_4 ratios. A series of As and P competitive studies were conducted to determine which oxyanion was preferred in these soils and to suggest possible sorption mechanisms. The first of the experiments was a simple As and P competition study. We used 1:1, 1:50 and 50:1 ratios of As:P based on past published studies that have investigated the mechanisms of competitive oxyanion sorption on soils. The concentrations used were 4 ppm As to 4 ppm P, 4 ppm As to 200 ppm P, and 200 ppm As to 4 ppm P. The oxyanions were introduced at the same time and measurements were made at 24 and 48 h. The pH of the solutions was maintained at 5.5. A 5 g/L soil solution and a 0.01M $NaNO_3$ background electrolyte were used in all competitive sorption reactions. Phosphate concentrations (as $Na_2HPO_4 \cdot H_2O$) used in sorption studies were based on those found in the surface and subsurface horizons of Delaware soils. Figures 2.6-9 depict the relationships between As and P sorption.

Arsenic kinetic sorption studies were performed upon three (Corsica 0-30cm, Corsica 40-81cm and Sassafras 20-40cm) of the eight soils. These soils were chosen based upon the results of the isotherm and pH edge studies. The first step was to assess how quickly As would sorb to soils without the presence of PO_4^{3-}, see Figure 2.10. Then 1:1, 1:50 and 50:1 As:P and P:As ratio studies were conducted over time to assess As's ability to adhere to the soil in the presence of P. All experiments were kept at a pH of 5.5

with a background electrolyte of 0.01*M* NaNO3, a 5g L^{-1} soil suspension, and they were monitored for 48 hours, (Figure 2.11-13 and Table 2.3). The concentrations used were 4 ppm As to 4 ppm P, 4 ppm As to 200 ppm P, and 200 ppm As to 4 ppm P.

2.2.4. Arsenic Desorption from Soil Horizons.

The purpose of the desorption studies was to assess the extent and rate of release of native and freshly sorbed As from selected soil horizons. The first competitive experiment assessed P's ability to remove As from the soil (Figure 2.14-16). The first step was to sorb As(V) onto the soil using 4 ppm As(V) and allow the solution to equilibrate for 24 h. The soils were then subjected to washing with 0.01*M* NaCl to mimic reactions with the soil solution, and the solution was analyzed for As. The soil was washed again with 4 ppm with phosphate solutions (Na_2HPO_4) for 24 hours, then the resulting solutions were analyzed for As and P. The conditions where then reversed, where the release of freshly sorbed PO_4^{3-} by As(V) was monitored, see Figures 2.17-19. This experiment monitored how tightly bound As was and assessed if P could displace As from the soil surface.

2.3. Results and Discussion

2.3.0. Soil Classification.

Five of the 10 soil series (Elkton silt loam, Reybold silt loam, Woodstown loam, Nassawango silt loam, and Sassafras sandy loam) were located on two farms in New Castle County, Delaware with no record of animal manure application and provided a

wide range in topography, drainage class, and soil properties typical to this geographic region (Appendix Table 2.A.1). The other soil series were located on farms in Sussex County either on i) crop land - Rumford sandy loam, Sassafras sandy loam, Corsica loamy sand, Downer sandy loam, Greenwich sandy loam; or in ii) forests adjacent to the sampled crop land – Sassafras sandy loam, Ingleside sandy loam, Greenwich loam. The taxonomic class and properties are found in Table 2.1. The physical properties of these soils can be found in the appendix at the end of the chapter (Table 2.A.2).

Table 2.1. Taxonomic characteristics of the soil samples.

Soil Series	Drainage Class	Taxonomic Class
Elkton	Poorly drained	(fine-silty, mixed, active, mesic Typic Endoaquults)
Reybold	Well drained	(fine-loamy, mixed, semiactive, mesic Typic Hapludults)
Woodstown	Mod. well drained	(fine-loamy, mixed, active, mesic Aquic Hapludults)
Nassawango	Well drained	(fine-silty, mixed, semiactive, mesic Typic Hapludults)
Sassafras	Well drained	(fine-loamy, siliceous, semiactive, mesic Typic Hapludults)
Rumford	Well drained	(coarse-loamy, siliceous, subactive, thermic Typic Hapludults)
Corsica	Very poorly drained	(fine-loamy, mixed, active, mesic Typic Umbraquults)
Downer	Well drained	(coarse-loamy, siliceous, semiactive, mesic Typic Hapludults)
Greenwich	Well drained	(coarse-loamy, mixed, semiactive, mesic Typic Hapludults)
Ingleside	Well drained	(coarse-loamy, siliceous, semiactive, mesic Typic Hapludults)

2.3.1. Physiochemical Characteristics of Delaware Agricultural and Forested Soils.

The soil profiles on crop land and forests had physical and chemical properties that were representative of the Coastal Plain (Sussex County) and Piedmont soils (New Castle County) that are typical of agricultural land in Delaware. All soils had pH values less than 7.0, and most of the agricultural field topsoils were in or near the pH range recommended for crop production in Delaware (pH 5.6 - 6.0) (Sims and Gartley, 1996).

The forested soils were more acidic ranging from 4.1 to 5.3, these soils are not routinely limed and there is a constant input of organic materials which can increase soil acidity. The soil organic content in the forested topsoils was higher (4.8%) than the agricultural soils(<3%). However, the Corsica soil, whose lower horizons are classified as a clay loam, is poorly drained with a soil organic matter content of 7.4%. On average, the soil organic matter content of the New Castle County soils (1.4%) were higher than Sussex County (1.0%). Subsoils had lower OM contents (mean = 0.6 ±0.4%) than topsoils (mean = 2.9 ±2.1%), which can be explained by the direct input of organic material to the soil surface.

The Coastal Plain soils of Sussex County were coarser textured (sandy loams and loamy sands), than the finer-textured Upper Coastal Plain and Piedmont soils from New Castle County. Clay content increased in the subsoils in most soils, which is indicative of long-term weathering of soils. The weathering process causes illuviation of fine particles into the lower subsurface horizons (typically the B horizon, argillic horizons). Effective cation exchange capacity (ECEC) values tended to be lower in topsoils (1.48 to 6.60 meq 100 g^{-1}) than subsoils (1.10 to 12.4 meq 100 g^{-1}) and followed the expected trend of increasing in finer-textured subsoils where clay, silt, Al and Fe had accumulated. Clays and (oxy)hydroxides are the predominant sorption surfaces in soils, and therefore the CEC should increase with increasing concentrations of these components. As was previously mentioned, although clays do not readily sorb As they do play a role in As retention and limit water percolation allowing As residence time in subsurface soils to increase.

The elemental composition of all soils on these farms was dominated by aluminum (Al: mean = 11797 ± 5153 mg kg^{-1}) and iron (Fe: mean = 9979 ± 6375 mg kg^{-1}); all other elements measured had concentrations < 2000 mg kg^{-1} (Tables A-3 and A-4). Total Al and Fe were also highly correlated with clay content (Al, r=0.77***; Fe, r=0.75***), reflecting both the composition of clay minerals (alumino-silicates) typical to Delaware soils and the association of amorphous Al and Fe (hydr)oxides with soil clays. Iron and aluminum oxide analyses indicate that crystalline Fe oxides were predominant in subsurface soils (81.2% ± 7.4), while Al oxides are predominant in subsurface soils (72.8% ± 8.15). For most soils, amorphous Al content was higher in the topsoils than in the subsurface soils. The litter amended topsoils from Sussex county had slightly higher levels of Cu (9.36 mg kg^{-1} ± 2.8) and Zn (21.4 mg kg^{-1} ±4.8) than forested soils (4.6 mg kg^{-1} ± 2.5; 17 mg kg^{-1} ± 3.6). Trace metals are added to poultry diets to aid in growth, feed conversion and serve as macro and micro nutrients. It is possible that these trace metals may serve as an indicator of past poultry litter amendments.

The distribution of soil test P (PO_4^{-3}, HPO_4^{-2} or $H_2PO_4^{-1}$) and S (sulfate, SO_4^{-2}) in these soil profiles illustrates the typical behavioral patterns of anion retention by Delaware soils. The trend consistently observed for P, as shown in past research (Mozaffari and Sims, 1994) is for accumulation in topsoils with increased leaching of P into subsoils gradually occurring as topsoils become more P-saturated (Sims and Luka-McCafferty, 2002). This is supported by the significant correlation (r=0.80) determined for these soils between topsoil M3-PSR and the M3-P content in underlying subsoils. In contrast, for sulfate, known to be more leachable in soils than phosphate (Kline et al.,

1989; O'Reilly et al., 2001), accumulation often occurs in high clay/Al/Fe subsoils underlying sandy surface horizons. The Downer soil series, which has experienced decades of poultry litter amendments had an accessive amount of M3-P in its surface horizon with more than 100 mg P kg^{-1}. Chemical and elemental composition of soils (Tables 2.A.3 and 2.A.4.) can be found in the appendix at the end of the chapter.

(Results from this section were used for the Delaware Department of Natural Resources and Environmental Control (DNREC) report that was compiled by D.L. Sparks, J.T. Sims, J.M. Seiter, and S.M. Gardner. Therefore, these authors have contributed to these calculations and data interpretation).

2.3.2. Arsenic Status of Agricultural and Forested Soils.

Total As concentrations in the 42 soil horizons collected varied from below detection to 9.2 mg kg^{-1} (mean = 3.0 ± 1.9 mg kg^{-1}) by the EPA 3051 method and from 0.4 to 7.8 mg kg^{-1} (mean = 3.1 ± 1.7 mg kg^{-1}) by the EPA 3050B method (Table 2.A.5., 2.A.6.). Arsenic values for some selected soils can be seen in Table 2.1. Unlike the other oxyanions commonly found in poultry litter (as was previously mentioned), As accumulation did not follow any distinct patterns with regards to physiochemical characteristics of the soils (e.g. texture, metal oxide content, organic matter). However, there was one trend related to geographic location seen within the soil profiles. The soils collected in New Castle county contained slightly higher total As concentrations in agricultural soil profiles (mean = 4.1 ± 1.9 mg kg^{-1}) than those found in Sussex county (mean = 2.6 ± 1.8 mg kg^{-1}). These results are unexpected because Sussex county soils

receive regular inputs of As introduced from poultry litter amendments. However, total As concentrations were not statistically different for the three farms where soil profiles were sampled on crop land and in adjacent forests (mean = 2.8 ± 1.6 and 2.6 ± 1.9 mg kg^{-1}, respectively). The increase in As content of the piedmont soils could be from previous agricultural activity (pesticides, defoliants, and possible dietary supplements) or naturally introduced from the metamorphic bedrock found in the northern part of the state.

2.3.3. Arsenic Sorption by Soil Horizons.

Due to the large number of soil samples, eight soils were chosen to perform sorption experiments upon. The soils were chosen based on their texture, Fe and Al oxide content, P and As levels, and organic matter content. Table 2.1. provides a brief description of the physical and chemical properties and As status of the soils used in the sorption studies.

The pH edge/envelope studies provide important information about the soils' ability to retain As under varying environmental conditions. Agricultural soils can experience a number of applications which can cause pH to change; such practices include liming and applying fertilizer. Therefore, it is important to assess how these practices can affect As retention on these soils. A number of pH studies were conducted to determine how a change in pH can alter As sorption. The reaction conditions were chosen based on environmentally relevant conditions, particularly at the interface between the poultry litter and the soil.

The first pH studies were done in the traditional manner, where each soil was equilibrated separately at a different pH ranging from pH 3 to pH 10. In all cases subsurface soils sorbed more As than surface soils. Figure 2.1 demonstrates the importance of pH to As retention. As(V) is an oxyanion, which means that it has a negative net charge. Oxyanions traditionally sorb more at lower pHs than at higher pHs and sorption results in what is known as an adsorption envelope, as seen in Figure 2.1. The soils retained more As(V) at the lowest pH values and then experienced another sorption maximum around pH 5.5 to 7. The target pH range for Delaware agricultural soils is from pH 5.5-6.5, similar to the pH values where greatest As sorption occurred.

A second pH sorption study was conducted in order to more directly assess As retention as the pH of the soil varies. These pH studies show that As sorption is most dominant in the pH range of 5.5 to 7; outside of this range, As retention decreases (Figure 2.2). Therefore, when land applying PL onto agricultural soils maintaining a pH over this range will maximize As retention by soils. In both studies, subsurface soils retained an average of 30-50% more As than surface soil horizons (Figs. 2.1 and 2.2). This is probably due to overall higher Fe and Al oxides and clay content in the subsoils (Table 2.1).

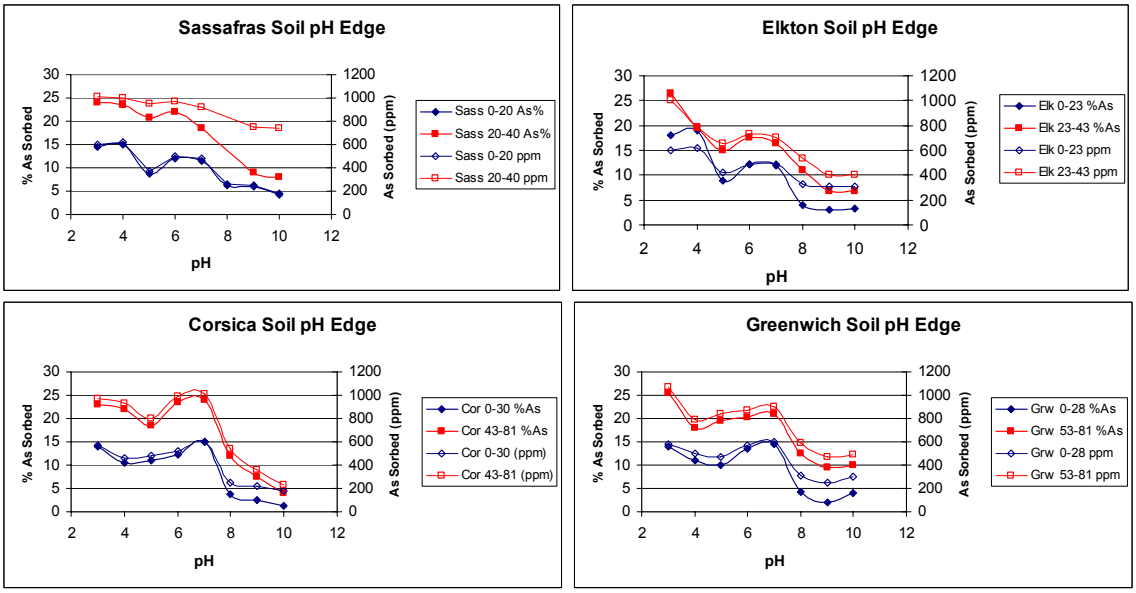

Figure 2.1. Arsenic sorption pH edge studies conducted on selected agricultural soils. Sorbed As is reported in ppm (mg kg^{-1}).

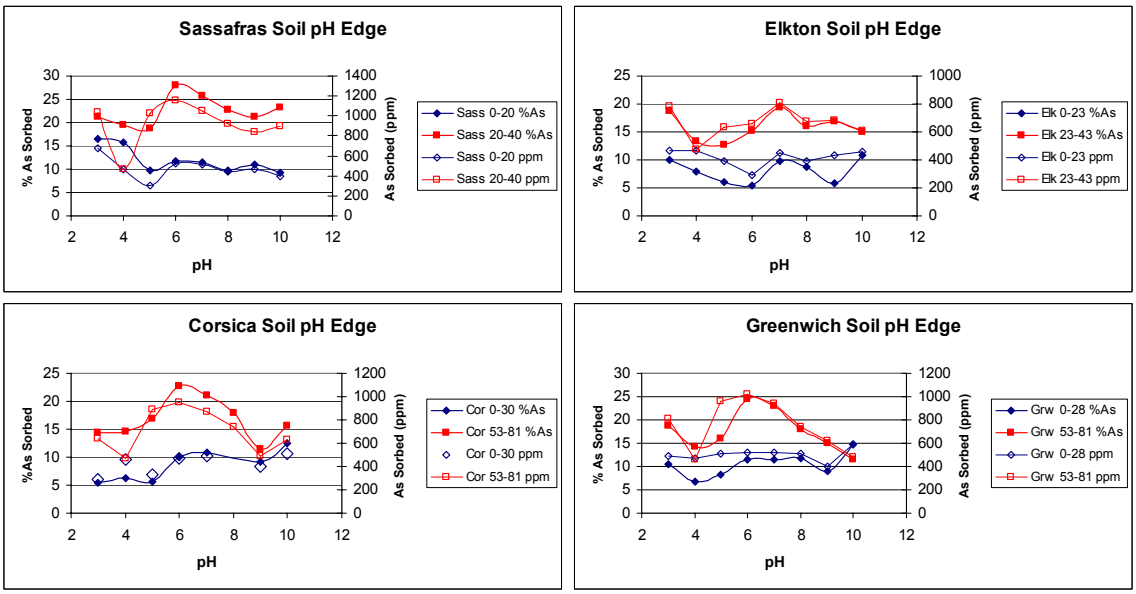

Figure 2.2. Monitoring As retention as pH increases or decreases from a pH of 5.5. Sorbed As is reported in ppm (mg kg^{-1}).

Sorption isotherms are used to describe the relationship between equilibrium concentrations of the sorptive and the quantity of sorbate on the soil surface (Sparks, 2003). Sorption isotherms were conducted to determine the maximum amount of As these soils could retain. Figure 2.3 depicts the relationship between the final (equilibrium) As concentration and As sorption by the soils. The final (equilibrium) concentration of As in solution is plotted against q, which is the amount of As per unit mass of soil. The following is the equation used to calculate q:

$$\frac{(C_o V_o) - (C_f V_f)}{m} = q$$

Where: C_o and C_f are the initial and final adsorptive (As) concentrations in mol L^{-1}, V_o and V_f are the initial and final sorptive volumes in liters and m is the mass of the sorbent (soil) in kg.

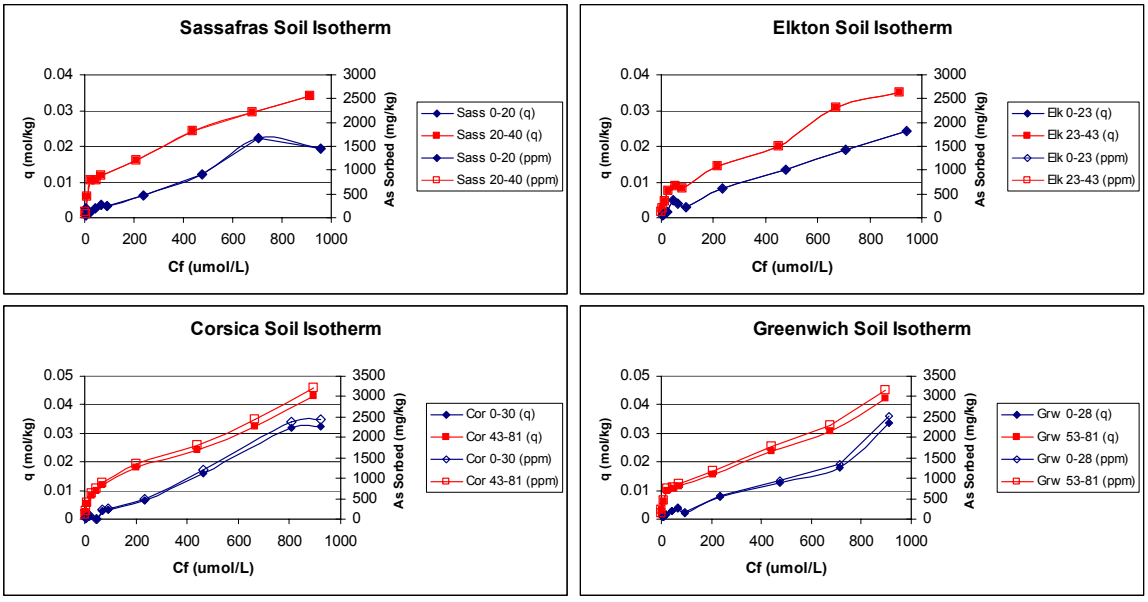

Figure 2.3. Sorption isotherms for the eight selected soils. Data are plotted C_f, final sorptive concentration vs. q, the amount of adsorption (sorbate per unit mass of sorbent).

The isotherms (Figure 2.3) show sorption behavior similar to what was observed in the pH sorption envelopes (Figures. 2.1-2). The subsurface soil horizons sorbed more As than the surface horizons. By applying the Langmuir equation the theoretical, maximum amount of As(V) that can be sorbed on the Delaware agricultural soils can be determined (Figure 2.4). The following linearized form of the Langmuir equation was used to determine As sorption maxima for the soils, where C is the final As concentration in solution, q is the amount of As sorbed, k is a constant related to binding strength and b is the sorption maximum.

$$C/q = 1/kb + C/b$$

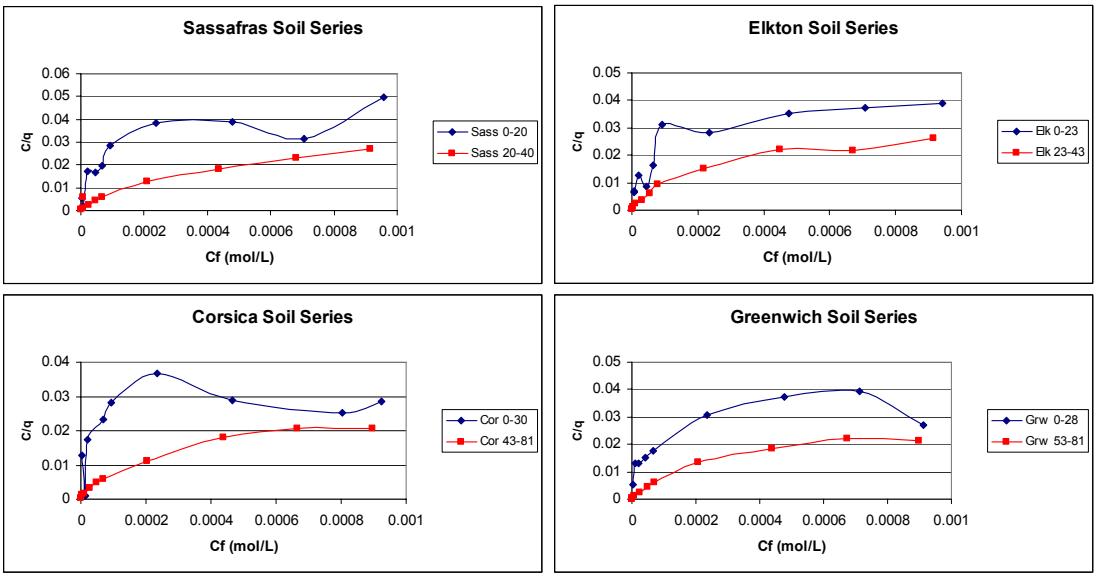

Figure 2.4. The Langmuir plots for the Delaware agricultural soils. C_f is the final As concentration in solution plotted against C/q (final As concentration/As sorbed). The slope of the lines will provide the sorption maximum.

Table 2.2. Soils chosen for sorption experiments based on their variability in physiochemical characteristics. Elkton is a New Castle county agricultural crop soil while Sassafras, Corsica and Greenwich are poultry litter amended soils from Sussex county.

Soil Series	Depth (cm)	Sand (%)	Silt (%)	Clay (%)	P 3050B (mg/kg)	As 3050B (mg/kg)	Fe 3050B (mg/kg)	Crystalline Fe (mg/kg)	Amorphous Fe (mg/kg)	Al 3050B (mg/kg)	Crystalline Al (mg/kg)	Amorphous AL (mg/kg)	pH	OM %	As (mg/kg) WS	P (mg/kg) WS	PSR	P Melich-3 (mg/kg)	As Melich-3 (mg/kg)
Elkton	0-23	23	57	20	442.5	0.8	6900	4926	2775	9014	321	2661	5.7	2.15	0.09	4.97	0.065	52	0.08
Elkton	23-43	15	57	28	83.2	1.2	15687	16601	4694	9495	544	2875	4.85	0.9	0.13	0.25	0.002	2	0
Sassafras	0-20	68	22	10	294.1	2.8	6288	5741	2797	6941	675	2950	5.1	1.2	0.15	2.01	0.072	47	0.19
Sassafras	20-40	52	23	25	130.9	4.9	13809	15878	2837	12950	1225	3708	5.9	0.8	0.05	0.39	0.003	2	0.28
Corsica	0-30	79	14	7	486.9	0.4	1882	325	709	14368	772	2987	4.65	7.4	0.13	4.65	0.092	86	0.16
Corsica	43-81	30	35	35	40.6	2.3	17785	4061	2284	23840	653	2047	4.35	0.65	0.02	0.04	0.001	2	0.26
Greenwich	0-28	55	30	15	384.5	3.6	7148	2185	2381	11173	1484	3958	5.2	1.25	0.09	2.11	0.089	87	0.26
Greenwich	53-81	49	30	21	164.9	4	14008	5711	1807	15281	1398	2088	5.7	0.8	0.02	0.29	0.003	3	0.18

The initial linear portion of the Langmuir plot is depicted in Figure 2.5 for each of the soils. The sorption maxima were calculated from the slopes of these lines.

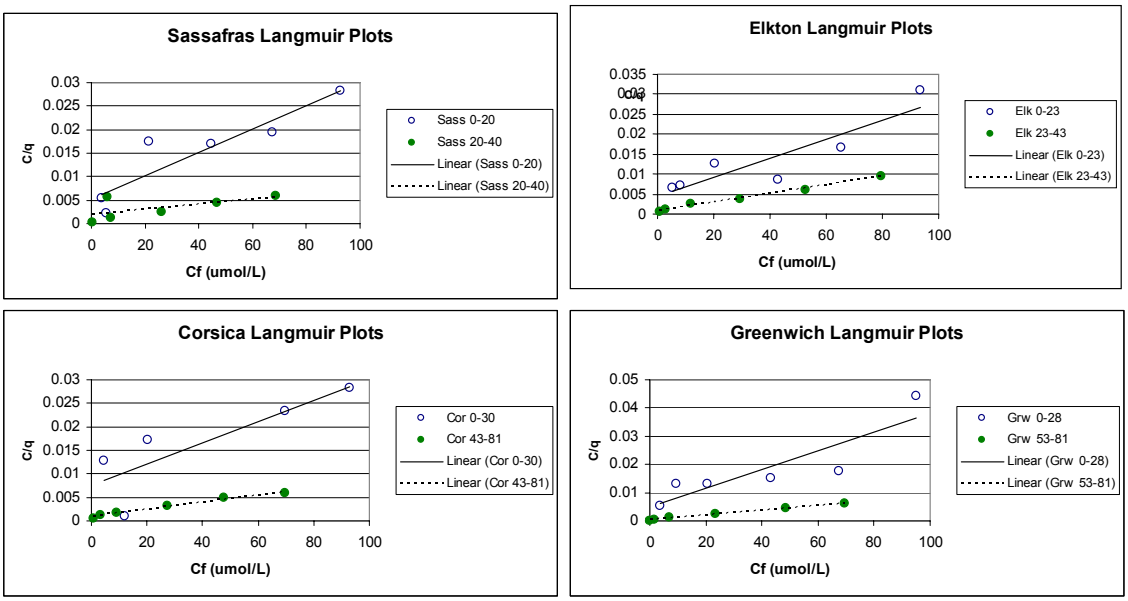

Figure 2.5. Initial linear portion of Langmuir plots that are used to calculate the sorption maxima.

The sorption maxima, for a particular soil depth, for each of these soils were similar (Table 2.3). According to the calculations, the subsurface soils had the ability to retain more As than surface soils. The Sassafras and Corsica soils displayed the greatest ability to sorb As, 18.2 µmol g^{-1} (1367 mg kg^{-1}) and 13.0 µmol g^{-1} (980.7 mg kg^{-1}) respectively. The pH edge sorption values were estimated at pH 5.5 and at the field soil pHs from the pH edge plots. When comparing Langmuir sorption maxima values (Table 2.3) with the quantity of As(V) that is present in the soils, one can see that on average, the soil is now retaining a small percentage of the total As it can sorb.

Table 2.3. Calculated Langmuir sorption maxima and sorption maxima derived from pH edges at pH 5.5 and the pH of the soils, where values are in ppm or mg kg^{-1}.

Soil Series (cm depth)	Langmuir Calculated pH 5.5 (ppm)	pH edge Estimated at pH 5.5 (ppm)	pH edge Estimated pH of the soil (ppm)
Sassafras 0-20	302.4	449	473
Sassafras 20-40	1367.0	715	850
Elkton 0-23	312.7	464	425
Elkton 23-43	709.7	675	716
Corsica 0-30	334.1	493	455
Corsica 43-81	980.7	849	870
Greenwich 0-28	224.8	503	465
Greenwich 53-81	902.0	812	815

The Langmuir sorption maximum calculations and the actual observed amount of As sorption, based on the pH studies, are in correlated. Although the values are not identical, the trends predicted by the Langmuir model and the quantity measured. The Elkton series was predicted (on average) to sorb less As than most of the others, and in general it sorbed the least amount of As. This soil is from New Castle county and contains relatively high concentrations of fine particles (silt and clay), yet it inefficient to sorb As. The model also predicted that the subsurface soils of the Sassafras and the Corsica series would be able to retain the most As, and the pH edge results were consistent with this predictions.

The Langmuir sorption maximum calculations provide important information about As sorption on the soils. The agricultural soils commonly found in Sussex County, Delaware appear to be able to retain a significant amount of As. If the surface soils are not able to retain the As, it would appear that the subsurface soils

should have the ability to sorb the remaining As that leaches through. The presence of phosphate however, may inhibit As retention on the soils.

2.3.4. Competitive Studies.

Oxyanions are dominant in poultry litter and the litter applied soils. When trying to determine arsenate sorption by soils, it is important to note the role that other oxyanions such as phosphate play in the retention of As. Phosphate and As(V) behave similar chemically, they are of similar size and charge, and previous studies have found that P will out compete As for sorption sites (Peryea, 1991; Peryea and Kammereck, 1997). There is at least 100 times more P than As in Delaware agricultural soils (Table 2.1), and at times up to 1000 times more P than As in PL. Therefore, it is worthwhile to study the impact that P has on As sorption.

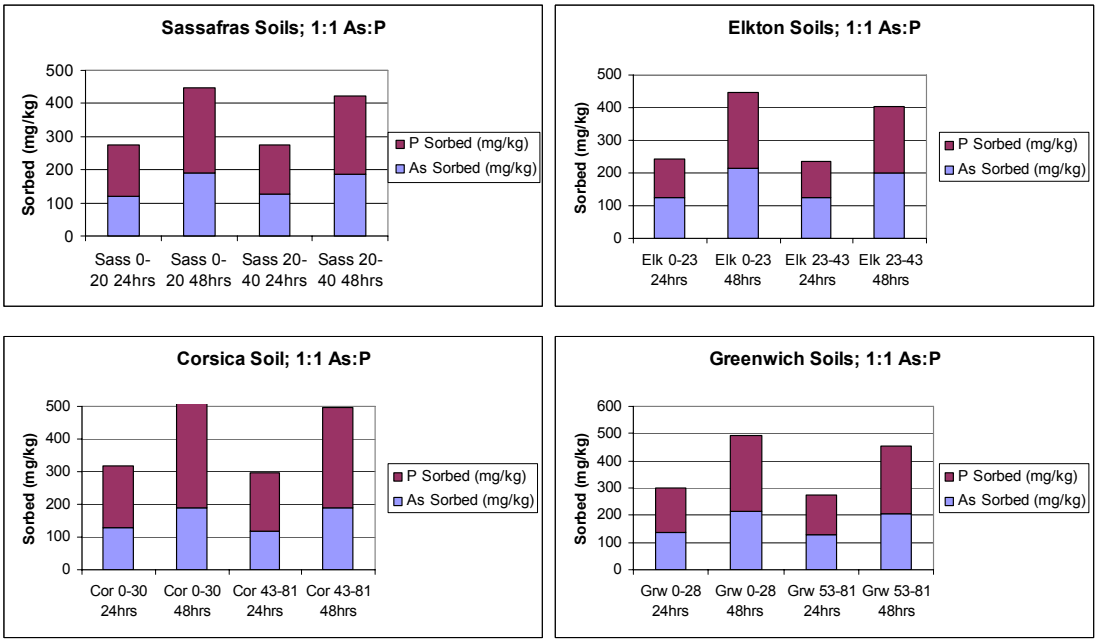

Figure 2.6. Amount of As and P mg kg^{-1} sorbed by soils after 24 and 48 h at a 1:1 As:P ratio.

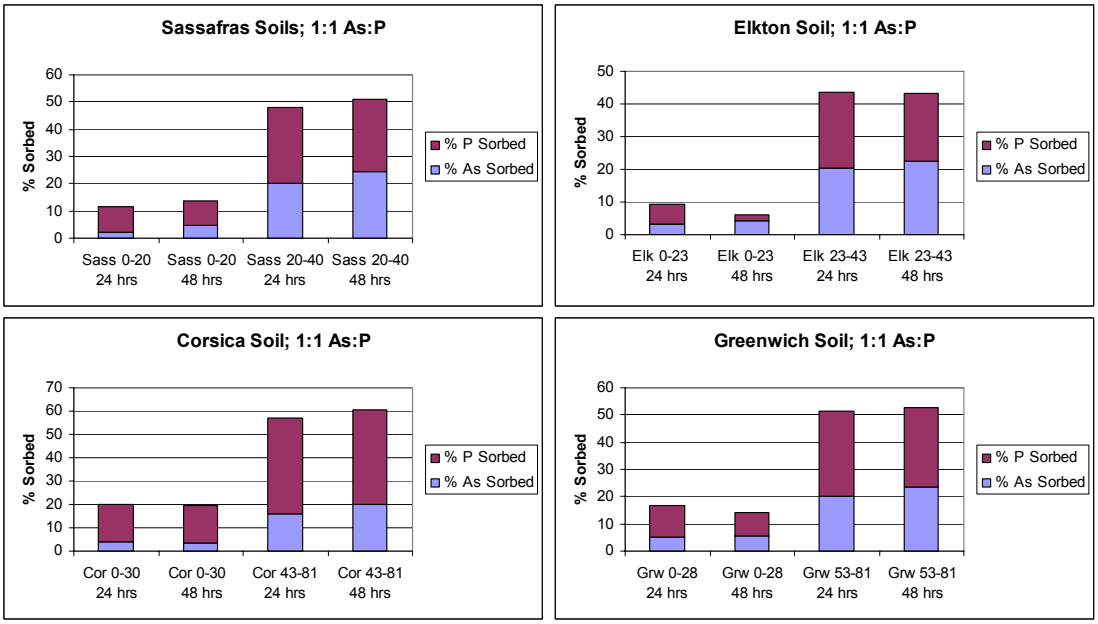

Figure 2.7. Amount of As and P (%) sorbed by soils after 24 and 48 h at a 1:1 As:P ratio.

The 1:1 competitive study demonstrated that the soils prefer P over As when introduced together at the same concentration, 4 mg L^{-1} (Figures 2.6 and 2.7). In most cases the amount of P and As sorbed increased with time. In all cases the subsurface soils sorbed more of the oxyanions than the surface soils. This would indicate that when P and As are introduced together, P may out compete As for sorption sites.

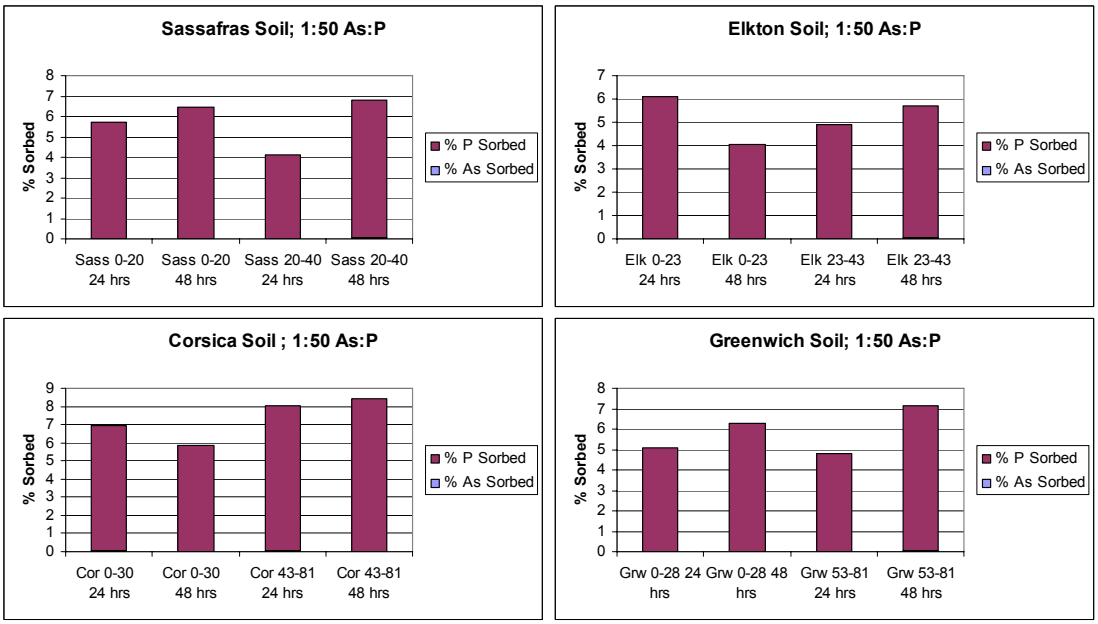

Figure 2.8. The percent of As and P (%) sorbed onto the soils after 24 and 48 hours at a 1:50 As:P ratio.

When As and P were introduced together at a 1:50 ratio, there was minimal As sorption (<1 μmol g^{-1}) (Figure 2.8). These results indicate that when P is in excess, it is preferred over As(V).

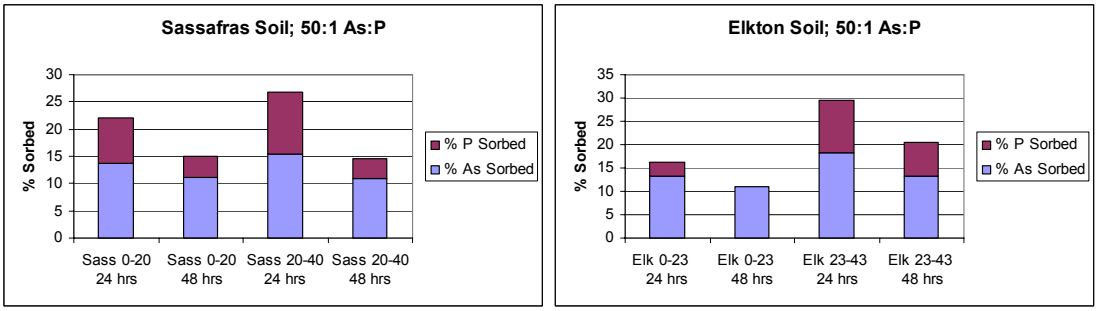

Figure 2.9. Amount of As and P (%) sorbed by soils after 24 and 48 h at a 50:1 As:P ratio.

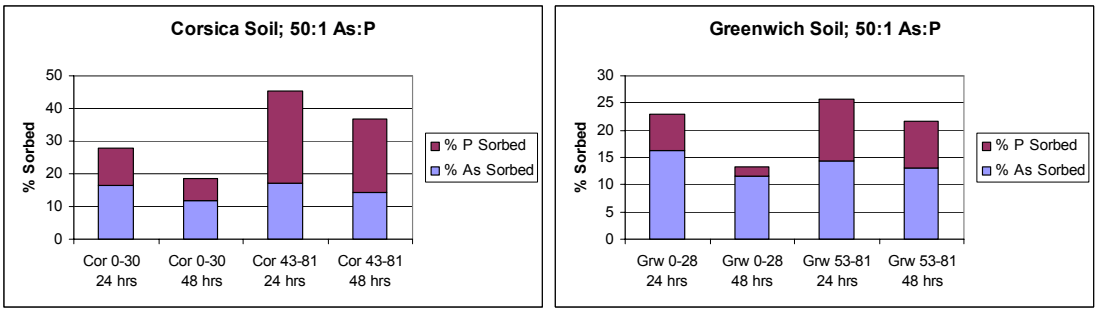

Figure 2.9. (cont.)

When As and P were introduced at a 50:1 As:P ratio more As was sorbed than P (Figure 2.9). However, in contrast with the 1:50 As:P study, a noticeable amount of P was bound to the soil. Even though As was in excess in solution, P was still taken up by the soil. In general, more P was sorbed by the surface soils, than by the subsurface soils. Higher P concentrations were seen in the surface soils, therefore the increase in P sorption to these soils is expected.

Three soils were chosen to perform a series of kinetic sorption studies. These studies helped determine how quickly As sorption occurred in the soils. The Corsica surface and subsurface soils, and the Sassafras subsurface soil were chosen based on their ability to sorb As.

The first of the studies examined As sorption kinetics by the soils to determine how quickly the As sorbs onto the soil surface without the presence of an inhibiting compound. Arsenic sorption exhibited typical kinetic behavior, a rapid increase in sorption followed by a slow uptake over time. Most of the As sorption occurs within the first 60 minutes (Figure 2.10). The maximum amount of As sorbed was similar to what was seen in the pH edge and isotherm experiments.

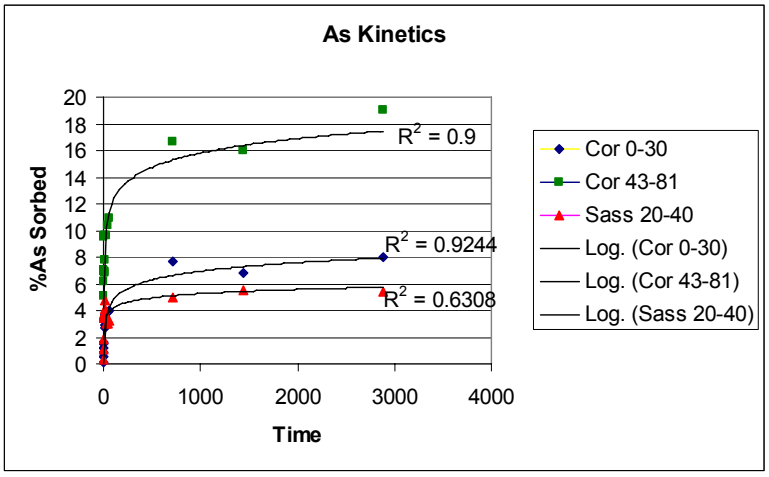

Figure 2.10. As sorption with time conducted on Corsica 0-30 cm, 43-81 cm, and Sassafras 20-40 cm depth soils.

The 1:1, 1:50 and 50:1 As:P ratio studies were conducted on the soils over time to assess As's ability to adhere to the soil in the presence of P (Figs. 2.11-13). All experiments were kept at a pH of 5.5, had a background electrolyte of 0.01*M* $NaNO_3$, 5g L^{-1} soil suspension, and were monitored for 48 hours. The concentrations were 4 ppm As to 4 ppm P, 4 ppm As to 200 ppm P, and 200 ppm As to 4 ppm P.

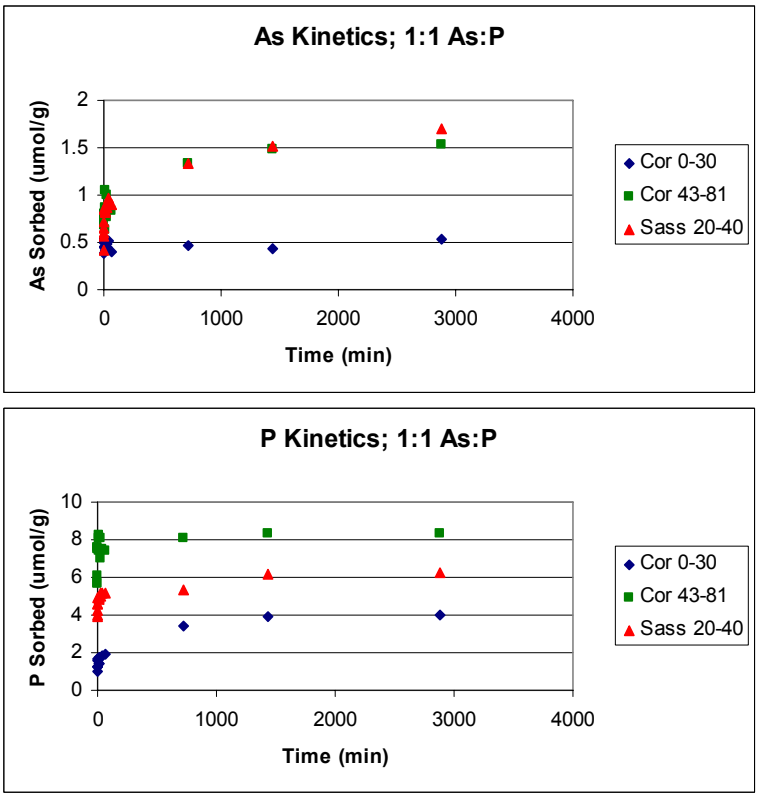

Figure 2.11. 1:1 As:P kinetic studies conducted on Corsica 0-30 cm, 43-81 cm, and Sassafras 20-40 cm depth soils.

The 1:1 As:P kinetic study demonstrates that when introduced at the same time at the same concentration, the soils prefer P over As. In all cases the soils immediately sorbed more P than As. The final percent sorbed for all experiments is noted in Table 2.4. The Corsica soils sorbed significantly more P than As, while the Sassafras soil sorbed an equal amount of both oxyanions. Once again the subsurface soils sorbed more of both As and P.

Table 2.4. Percent As and P sorbed at the end of 48 h for each of the three soils and treatments in Figures 2.11-13.

Soil	Treatment	% As Sorbed	% P Sorbed
Cor 0-30	1As:1P	5.70	16.91
Cor 43-81	1As:1P	21.45	41.68
Sass 20-40	1As:1P	23.16	28.65
Cor 0-30	1As:50P	2.32	7.37
Cor 43-81	1As:50P	5.17	5.28
Sass 20-40	1As:50P	4.00	6.12
Cor 0-30	50As:1P	17.23	11.63
Cor 43-81	50As:1P	6.26	21.12
Sass 20-40	50As:1P	15.55	11.97

Table 2.4 illustrates the effect that As and P concentrations have on the soils' ability to retain these compounds. When one oxyanion is in excess, sorption of the other was inhibited. One trend that should be noted is that when P is in excess, As sorption is reduced. However, when As was in excess, there was still a significant amount of P sorption.

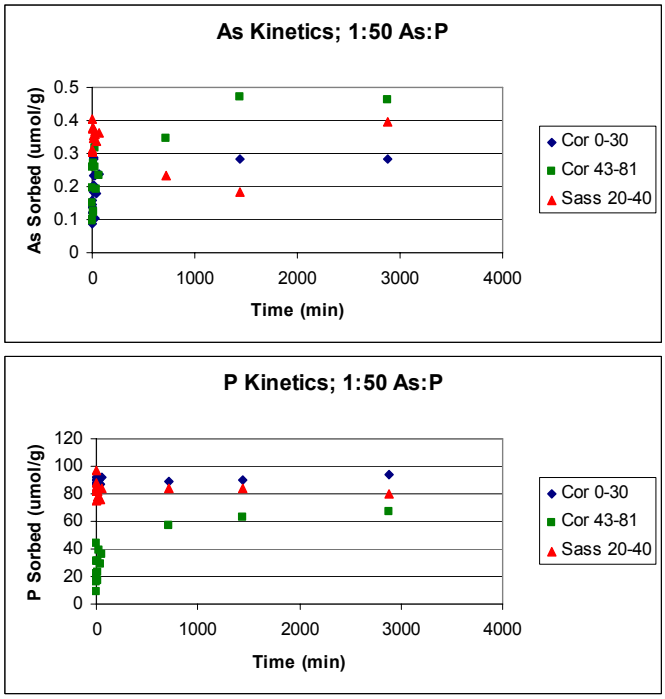

Figure 2.12. 1:50 As:P kinetic studies conducted on Corsica 0-30 cm, Corsica 43-81 cm, and Sassafras 20-40 cm depth soils.

Figure 2.12 depicts the uptake of As and P when in a 1:50 As:P ratio. Table 2.4 demonstrates that the amount of As sorbed was greatly reduced when the P concentration increased.

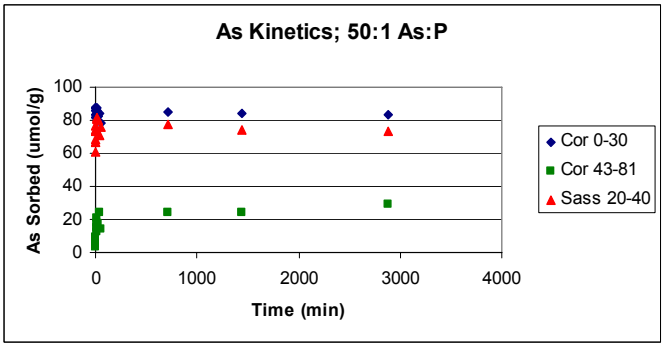

Figure 2.13. 50:1 As:P kinetic studies conducted on Corsica 0-30 cm, Corsica 43-81 cm, and Sassafras 20-40 cm depth soils.

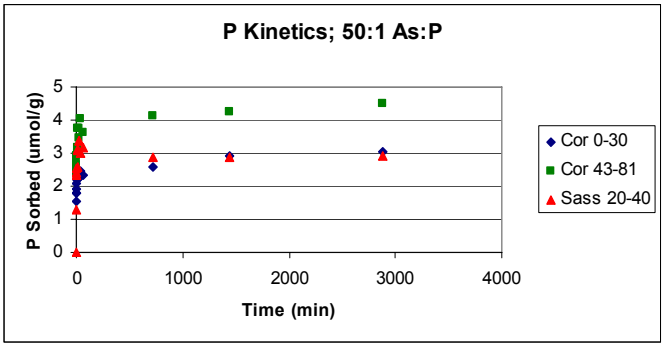

Figure 2.13. (cont.)

Figure 2.13 illustrates the uptake of As and P when in a 50:1 As:P ratio. Most of the sorption was completed within the first 60 minutes. Table 2.4 demonstrates that the amount of P sorbed was reduced when the P concentration increased. This study implies that even when As was in excess, P can still be retained in significant amounts by the soil.

2.3.5. Desorption Studies.

The purpose of the desorption studies was to assess the extent and rate of release of native and freshly sorbed As from selected soil horizons. Two separate experiments were completed. The first involved sorbing As(V) onto the selected soils, and then later introducing P into the system to determine how readily P can displace As. Then the inverse of this experiment was conducted to determine how readily As can displace P.

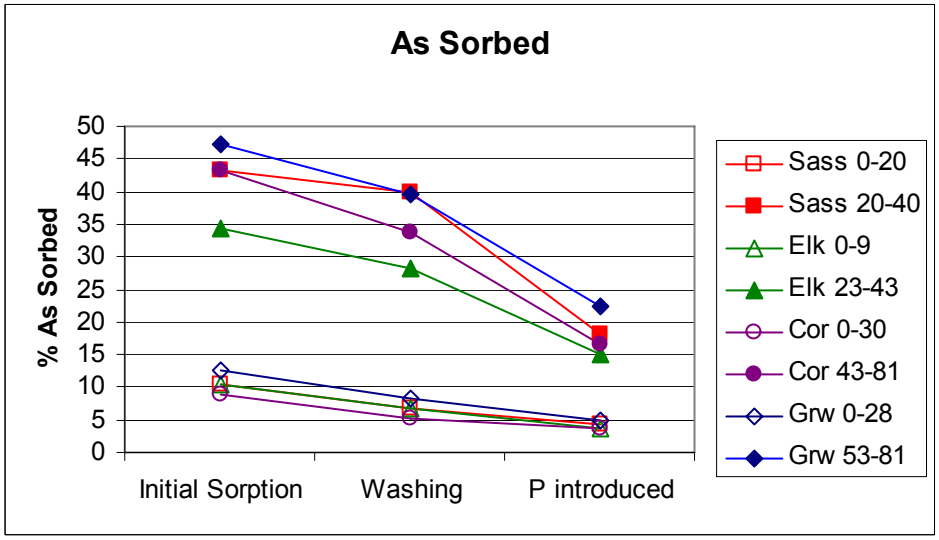

Figure 2.14. As removal from the soils after washing and P introduction.

Arsenic removal from the soil surface is shown in Figure 2.14. The greatest impact was seen in the subsurface soils, where at least 20% of the initially sorbed As was removed by the end of the experiment. Figure 2.14 depicts the % As sorbed in the beginning, after washing and after P was introduced for each of the eight soils. It appears that P had the ability to displace a significant amount of As from the soil surface. More As was displaced with P than with the NaCl electrolyte.

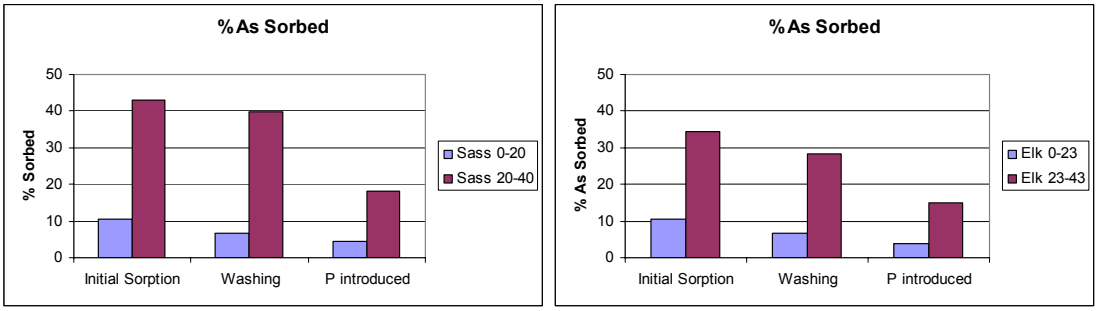

Figure 2.15. Percent As sorption before and after 0.01*M* NaCl addition and P sorption.

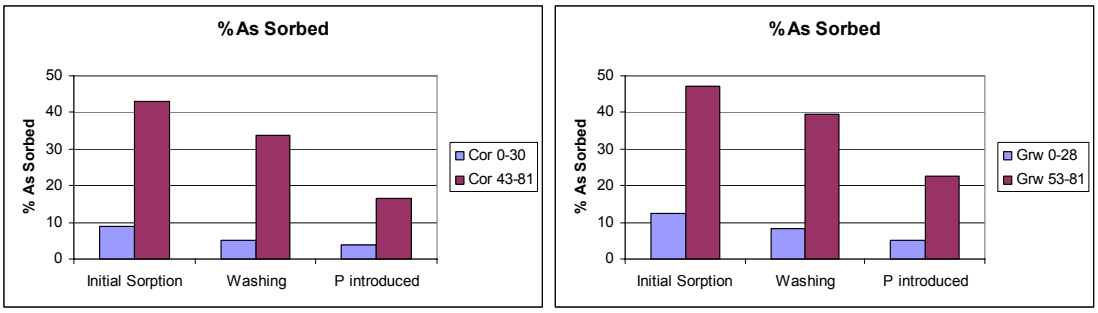

Figure 2.15. (cont.)

Figure 2.15 demonstrates what percentage of the initial As added remained sorbed to the soil surface after the washing and the introduction of P to the system. Figure 2.16 shows the soils' ability to sorb P when As is already present in the soil system.

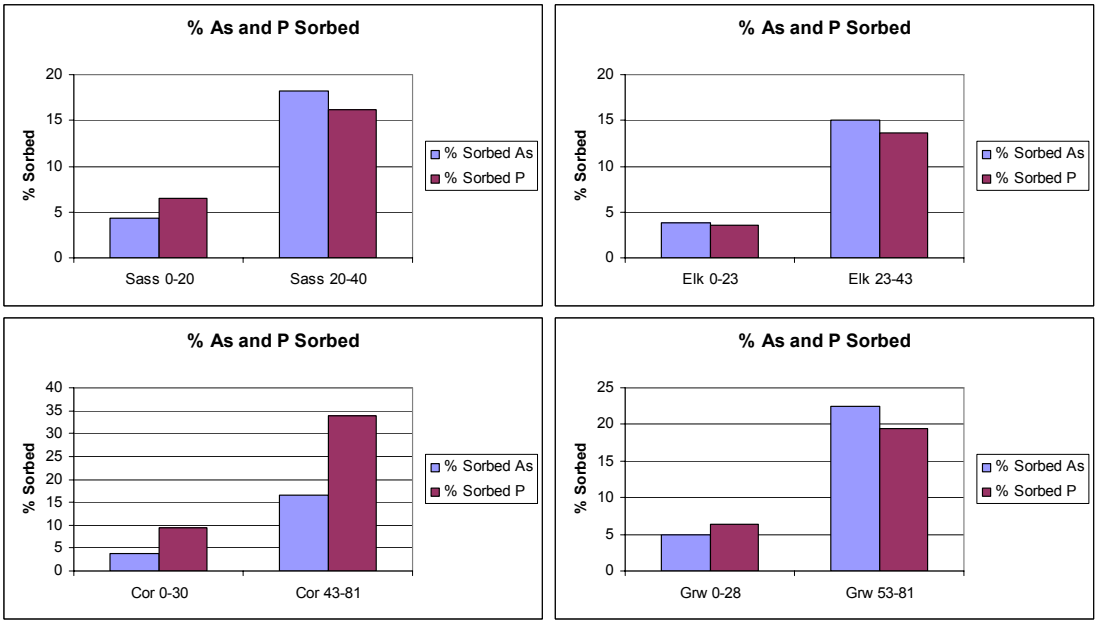

Figure 2.16. Percent As and P concentrations sorbed at the completion of the As then P sorption study after washing and P introduction.

In most cases more As remained sorbed than P, although the presence of P could displace a significant portion of As. This indicates that As is tightly bound to soils and may not easily be desorbed. The Corsica subsoil (43-81cm) sorbed significantly more As and P than other soils, indicating that texture and the presence of Fe and Al oxides play a role in As sorption.

The purpose of the second experiment was to assess As's ability to remove previously bound P from soils (Figure 2.17). The first step was to sorb PO_4 onto the soil and take a sample after 24 hours. The soil was then washed with 0.01M NaCl to simulate soil solution, and then the solution was analyzed for P. As(V) was then added to the solution and allowed to react for 24 h.

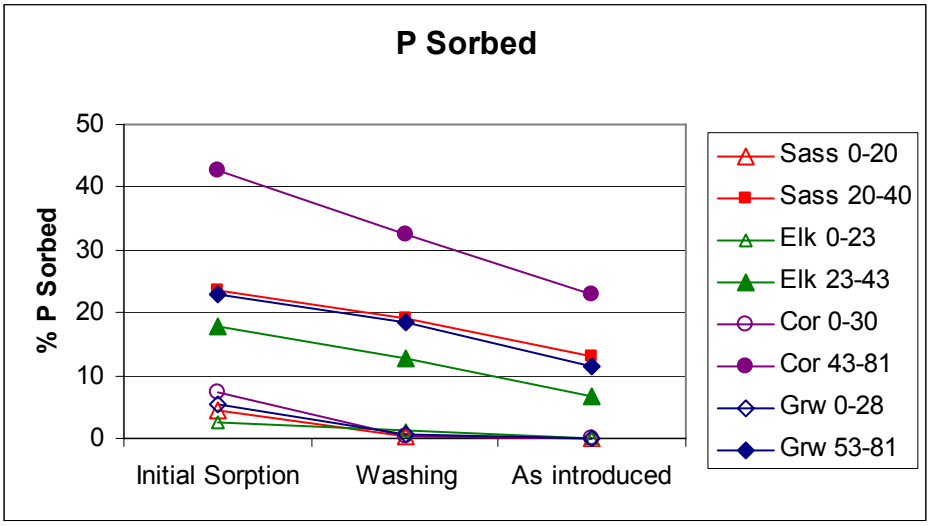

Figure 2.17. P removal from the soils after washing and As introduction.

Figure 2.17 shows a significant amount of P was displaced in all soils. All soils showed the impact of the washing, which indicates that P can be removed.

Comparing this graph to the initial As sorbed experiment, it seems that As is more tightly bound than P in Delaware soils. This would indicate that once As is sorbed onto the soils it is less likely to be removed.

Figure 2.18 shows the percentage of P sorbed in the beginning, after washing and after As was introduced. It appears that As has the ability to desorb a significant amount of P from the soil surface.

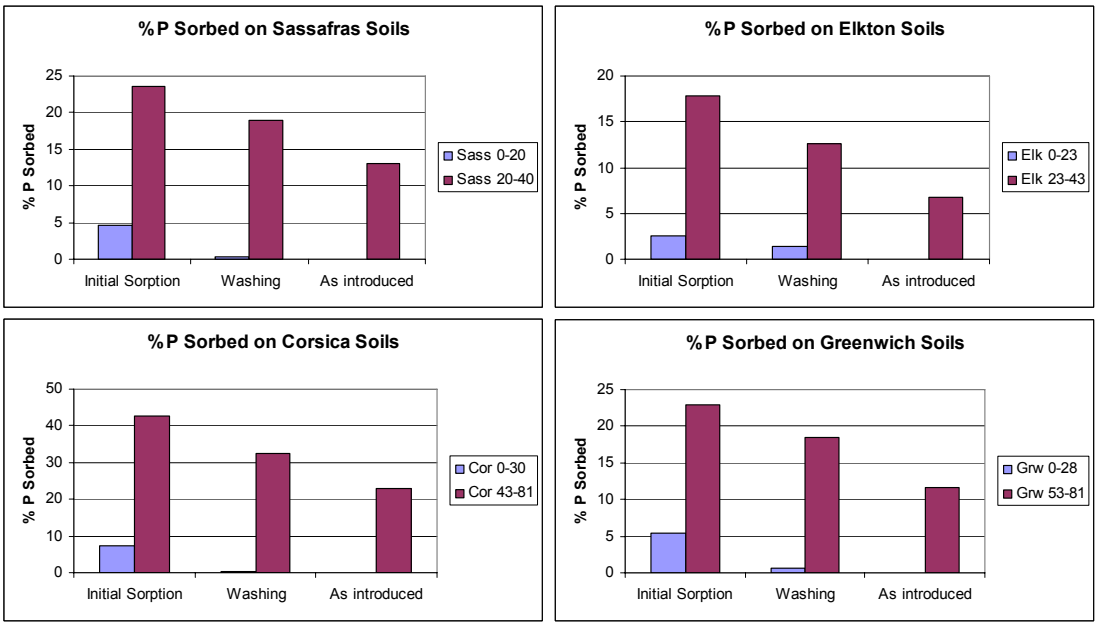

Figure 2.18. Percent P sorption before and after 0.01M NaCl addition and As sorption.

It is evident that the topsoils are not able to retain as much P as the subsurface soils (Figure 2.18). This could be due to the fact that there already is a significant amount of P bound to these soils. Once again the Corsica subsurface soil sorbed

more P than any other soil. In all cases As had the ability to completely remove P from the surface soils (Figure 2.19).

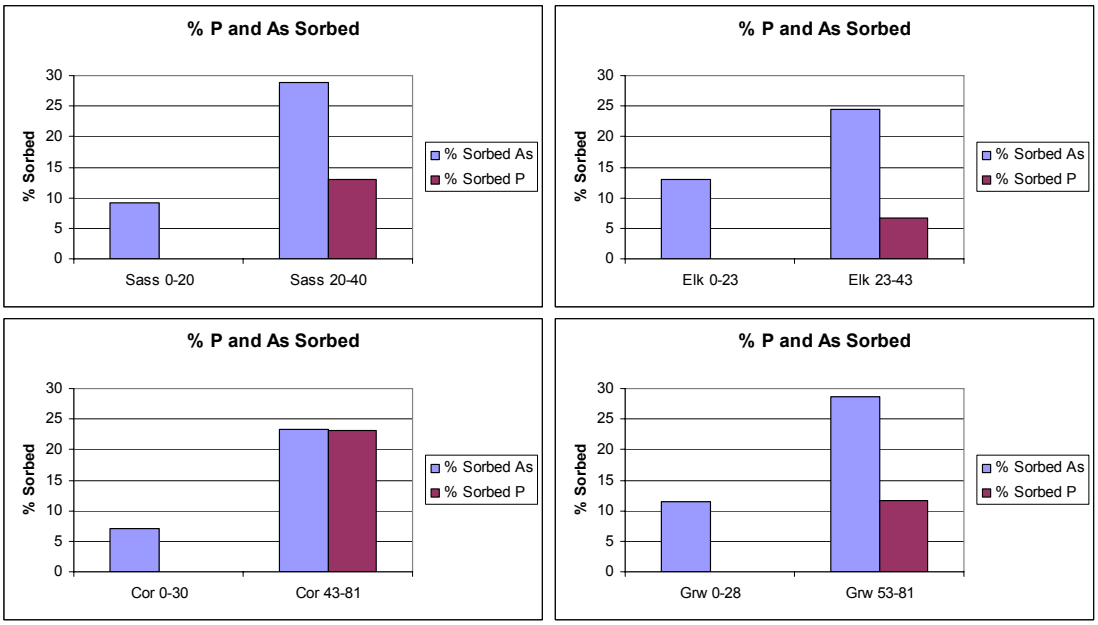

Figure 2.19. Percent As and P concentrations sorbed at the completion of the P then As sorption study, after washing and As introduction.

These studies indicate that As has the ability to displace sorbed P from the soil surface. As stated above, it would appear that As may adhere more tightly to the soil surface than P.

2.4 Conclusions

Soils from farms where poultry litter was land applied did not have elevated concentrations of total arsenic concentrations. Studies indicate that Delaware soils have the capability to retain dissolved As that is released from litters, manures,

fertilizers, and other soil amendments. In all cases, subsoils had greater capacities to retain As than the topsoils, primarily due to higher concentrations of iron and aluminum oxides. Arsenic sorption was greatest at low pH and at natural pH values. These values fall within the soil pH range recommended for crop production (pH 5.5 to 7.0). Kinetic studies demonstrate that As sorption by soils occurs rapidly, with a majority of adsorption occurring within the first 60 minutes, followed by a slow phase that continued to remove As from solution for days. Oxyanions, such as phosphate and sulfate, are present in poultry litter and therefore are present at high concentrations in many agricultural soils in Delaware due to long-term applications of manures and fertilizers. These oxyanions are similar in size and charge to arsenate, and therefore could potentially have an impact on As sorption. The studies showed that phosphate was preferentially sorbed by soils, relative to As, and therefore has the potential to inhibit As sorption through competition on soil constituents. Desorption studies showed that solutions with high phosphate concentrations could displace previously sorbed As from soils, particularly from subsoils. As does have the ability to remove a small portion of previously sorbed phosphate, which indicates that As sorption onto elevated P soils may still occur.

Although As accumulation is not being seen in Delaware soils amended with poultry litter, As should still be incorporated into best management practices (BMPs). These studies indicate that elevated levels of phosphorus may inhibit As sorption and enhance As release from soil. Phosphorus input is highly regulated in land application of biosolids, however the impact of the input of other oxyanions, such as

As(V), is not considered. New BMPs should be formulated considering both phosphate and arsenic additions to Delaware soils.

2.5. Appendix for Chapter 2:

2.5.0. Soil characteristics for Agricultural Soils

Table 2.A.1. Selected properties for soil profiles of benchmark Delaware soil series located in forests adjacent to crop land at three poultry farms in Sussex County

Soil Series	Depth	pH	OM	Sand	Silt	Clay	Textural Class
	--cm--		----------%----------				
	Sussex County Poultry Farms (Forests)						
Farm #1 -	0-10	5.0	4.6	65	27	8	Sandy loam
Sassafras	10-40	4.8	0.7	72	11	17	Sandy loam
	40-61	4.7	0.3	70	14	16	Sandy loam
	61-81	4.7	0.1	85	5	10	Loamy sand
Farm #2 -	0-15	4.2	5.3	70	24	6	Sandy loam
Ingleside	15-40	4.6	0.6	68	22	10	Sandy loam
	40-61	4.5	0.3	62	24	14	Sandy loam
	60-91	4.3	0.4	61	19	20	Sandy loam
Farm #5 -	0-8	5.3	4.6	49	36	15	Loam
Greenwich	8-46	4.2	1.0	47	34	19	Loam
	46+	4.1	1.1	51	26	23	Sandy clay loam

Table 2.A.2. Properties of soil profiles for ten Delaware benchmark soil series on agricultural crop land at five poultry farms in Sussex County and two farms in New Castle County

Soil Series	Depth	pH	OM	Sand	Silt	Clay	Textural Class
	--cm--		----------%----------				
	Sussex County Poultry Farms (Crop land)						
Farm #1 -	0-20	5.8	0.7	80	14	6	Sandy loam
Rumford	20-40	5.7	0.1	81	9	10	Loamy
	40-61	5.4	0.2	72	9	19	Sandy loam
	61-81	5.3	0.2	74	10	16	Sandy loam
Farm #2 -	0-20	5.1	1.2	68	22	10	Sandy loam
Sassafras	20-40	5.9	0.8	52	23	25	Sandy clay loam
	40-61	6.1	0.4	72	10	18	Sandy loam
	61-81	6.5	0.2	80	4	16	Sandy loam
Farm #3 -	0-30	4.7	7.4	79	14	7	Loamy sand
Corsica	30-43	4.7	1.6	45	26	29	Clay loam
	43-81	4.4	0.7	30	35	35	Clay loam
Farm #4 -	0-28	5.3	1.5	75	16	9	Sandy loam
Downer	28-45	5.8	0.7	69	18	13	Sandy loam
	45-73	5.7	0.7	76	7	17	Sandy loam
	73-101	5.7	0.2	67	16	17	Sandy loam
Farm #5 -	0-28	5.2	1.3	55	30	15	Sandy loam
Greenwich	28-53	5.6	0.5	55	28	17	Sandy loam
	53-81	5.7	0.8	49	30	21	Loam
	81-109	5.9	0.2	73	13	14	Sandy loam
	New Castle County Cash Grain Farms						
Farm #6 -	0-23	5.7	2.2	23	57	20	Silt loam
Elkton	23-43	4.9	0.9	15	57	28	Silt loam
Farm #6 -	0-23	6.5	2.1	31	48	21	Loam
Reybold	23-30	6.5	1.1	33	46	21	Loam
	30-56	6.5	1	23	50	27	Silt loam
Farm #6 -	0-23	5.4	1	47	39	14	Loam
Woodstown	23-46	5.6	0.4	39	42	19	Loam
Farm #7 -	0-18	6.2	1.6	33	49	18	Loam
Nassawango	18-76	6.7	0.9	19	48	33	Silty clay loam
Farm #7 -	0-30	5.4	4.8	53	30	17	Sandy loam
Sassafras	30-56	5.1	0.6	53	28	19	Sandy loam
	56-91	5.6	0.6	53	24	23	Sandy clay loam

Table 2.A.3. Elemental composition of New Castle County Soils.

	Depth	P	Mn	Zn	Cu	Fe	Al	S	As	pH	OM	ECEC
	--- cm ---	----------	----------	----------	---mg kg^{-1}	----------	----------	----------	----------		--- % ---	meq 100g^{-1}
New Castle County Cash Grain Farms												
Farm #6	0-23	392	107	34	4.7	8226	8370	214	3.4	5.7	2.2	5.31
Elkton	23-43	89	21	15	2.2	18293	8367	123	2.5	4.9	0.9	3.28
Farm #6	0-23	364	182	32	5.6	12062	11265	198	3.3	6.5	2.1	6.6
Reybold	23-30	313	191	30	5	11499	10623	134	3.7	6.5	1.1	4.41
	30-56	286	161	33	6.9	17119	14865	121	4.8	6.5	1	5.07
Farm #6	0-23	208	76	23	3.5	6387	8475	112	2.2	5.4	1	2.73
Woodstown	23-46	123	57	21	4.9	9053	11484	68	2.1	5.6	0.4	2.79
Farm #7	0-18	380	306	35	5.8	11535	11533	173	4.4	6.2	1.6	4.48
Nasswango	18-76	397	143	37	8.9	24243	17428	125	4.8	6.7	0.9	4.48
Farm #8	0-30	652	814	43	6	15480	12854	200	5.4	5.4	4.8	2.44
Sassafras	30-56	432	324	28	3.9	16241	10060	127	3.7	5.1	0.6	1.94
	56-91	464	111	36	7.7	28393	15143	166	9.2	5.6	0.6	5.04

Table 2.A.4. Elemental Composition of Sussex County Soils.

	Depth	P	Mn	Zn	Cu	Fe	Al	S	As	pH	OM	ECEC
	--- cm ---	---mg kg^{-1}---									--- % ---	meq 100g^{-1}
Sussex County Poultry Farms (Crop Land)												
Farm #1	0-20	302	39	18	9	2785	3432	71	2.8	5.8	0.7	1.48
Rumsford	20-40	184	30	11	5.9	3967	4293	25	2.8	5.7	0.1	1.13
	40-61	147	48	16	5.5	8517	14851	44	3.4	5.4	0.2	2.89
	61-91	132	45	16	5.8	9539	12458	68	1.5	5.3	0.2	2.99
Farm #2	0-20	269	121	28	7.2	6499	7714	105	5.4	5.1	1.2	2.12
Sassafras	20-40	144	100	22	3.5	14704	14458	68	5.4	5.9	0.8	4.89
	40-61	116	64	15	1.5	11910	10279	49	0.8	6.1	0.4	4.05
	61-91	73	41	9	2.2	7396	8231	39	3.4	6.5	0.2	2.30
Farm #3	0-30	395	22	16	7	1480	10163	226	2.1	4.7	7.4	5.88
Corsica	30-43	39	22	12	9.8	10546	13802	98	0.5	4.7	1.6	10.20
	43+	33	17	17	15.2	16080	15182	119	0	4.4	0.7	12.36
Farm #4	0-28	442	64	24	14	3446	5399	93	2.6	5.3	1.5	4.40
Downer	28-46	176	55	14	5.8	5107	6368	23	1.1	5.8	0.7	3.21
	46-69	139	68	16	8.8	9069	8712	23	6.3	5.7	0.7	6.32
	69-100	40	26	8	2.9	3236	5191	13	3.2	5.7	0.2	1.38
Farm #5	0-28	322	136	21	9.6	5951	7348	96	2.6	5.2	1.3	6.45
Greenwich	28-54	163	110	20	8.6	8574	9902	45	0.5	5.6	0.5	12.31
	54-43	143	47	19	11.4	13319	11212	38	3.2	5.7	0.8	5.50
	43-108	84	33	11	6.7	7804	6980	29	1.6	5.9	0.2	4.83
Sussex County Poultry Farms (Forested Soil)												
Farm #1	0-10	209	59	18	2.1	4812	6209	6209	1.5	5.0	4.6	4.49
Sassafras	20-40	110	49	22	3.1	9745	13016	13016	5.4	4.8	0.7	2.42
	40-61	90	40	15	3.6	9307	11804	11804	1.5	4.7	0.3	3.05
	61-91	66	26	10	2.4	6535	7492	7492	0.8	4.7	0.1	1.92
Farm #2	0-10	159	35	13	4.6	3914	4791	4791	0.2	4.2	5.3	3.75
Ingleside	20-40	73	32	10	0	3985	5674	5674	2.1	4.6	0.6	1.21
	40-61	85	43	13	0	6087	7294	7294	1.5	4.5	0.3	1.10
	61-91	138	50	16	1.8	12259	10469	10469	6.1	4.3	0.4	3.48
Farm #5	0-8	324	144	20	7	6563	10039	10039	4.2	5.3	4.6	13.31
Greenwich	8-46	292	127	21	7.2	8679	13420	13420	2.1	4.2	1.0	8.29
	46+	424	92	21	12.5	14176	12223	12223	3.3	4.05	1.1	7.11

Table 2.A.5. Total, and plant-available (Mehlich 3) arsenic (As) in soil profiles for two cash-grain farms in New Castle County.

Soil Series	Depth	Total As EPA 3050B	Total As EPA 3051	Mehlich 3 As
	--cm--	---------- mg kg^{-1} ----------		
	New Castle County Cash Grain Farms			
Farm #6 - Elkton	0-23	0.8	3.4	0.1
	23-43	1.2	2.5	0
Farm #6 - Reybold	0-23	5	3.3	0.1
	23-30	4.3	3.7	0.1
	30-56	5.8	4.8	0
Farm #6 - Woodstown	0-23	2.5	2.2	0.1
	23-46	1.2	2.1	0.1
Farm #7 - Nassawango	0-18	2.4	4.4	0.1
	18-76	2.4	4.8	0
Farm #7 - Sassafras	0-30	7.8	5.4	0.2
	30-56	5.4	3.7	0.1
	56-91	5.8	9.2	0.1

†bd = below detection limit

Table 2.A.6. Total and plant-available (Mehlich 3) arsenic (As) in soil profiles for Delaware benchmark soil series on agricultural cropland and forested soils from five poultry farms in Sussex County.

Soil Series	Depth	Total As EPA 3050B	Total As EPA 3051	Mehlich 3 As
	--cm--	----------mg kg^{-1} ----------		
	Sussex County Poultry Farms (Crop Land)			
Farm #1 - Rumford	0-20	1.2	2.8	0.2
	20-40	1	2.8	0.3
	40-61	2.9	3.4	0.2
	61-81	3.3	1.5	0.2
Farm #2 - Sassafras	0-20	2.8	5.4	0.2
	20-40	4.9	5.4	0.3
	40-61	4.7	0.8	0.1
	61-81	3	3.4	0.2
Farm #3 - Corsica	0-30	0.4	2.1	0.2
	30-43	0.9	0.5	0.2
	43-81	2.3	bd	0.3
Farm #4 - Downer	0-28	4.9	2.6	0.4
	28-45	3.5	1.1	0.3
	45-73	3.9	6.3	0.2
	73-101	1	3.2	0.2
Farm #5 - Greenwich	0-28	3.6	2.6	0.3
	28-53	3.3	0.3	0.2
	53-81	4	3.2	0.2
	81-109	1.9	1.6	0.1
	Sussex County Poultry Farms (Forested Soil)			
Farm #1 - Sassafras	0-10	2.9	1.5	0.1
	Oct-40	2.2	5.4	0.1
	40-61	2	1.5	0.1
	61-81	1.6	0.8	0.3
Farm #2 - Ingleside	0-15	5.2	0.2	0.3
	15-40	2	2.1	0
	40-61	0.9	1.5	0.2
	60-91	4.3	6.1	0.1
Farm #5 - Greenwich	0-8	5.1	4.2	0.3
	Aug-46	2.8	2.1	0.2
	46+	4.9	3.3	0.1

†bd = below detection limit

Additional Information:

Due to the lack of arsenic accumulation in litter amended soils, this research indicates that arsenic may not be the best indicator of poultry litter amendments in Delaware soils. However, there are a variety of trace metals found in poultry litter that are not naturally found at elevated levels in Delaware soils. Such trace metals include: Cu, Co, Zn, and Ni. Data that we do have on trace metals in poultry litter amended soils can be found in Table 2.A.4. As was already reported, Cu concentrations in the litter amended topsoils from Sussex county had slightly higher levels of Cu (9.36 ± 2.8) and Zn (21.4±4.8) than forested soils (4.6 ± 2.5; 17 ± 3.6). I feel that examining poultry litter amended soils for these trace metals and possibly incorporating them into BMPs might be an area worth investigating. The trace metal concentrations for soils are found in Table 2.5. Both average metal concentrations and top soil metal concentrations are listed in order to describe metal status in the soils. When examining the top soils, in almost all cases the metal concentration is elevated in the litter amended soils, indicating that these trace metals may indeed be a better indicator than As of past litter applications.

Table 2.A.7. Trace metal concentrations in litter amended and background soils.

	Soil Series	Treatment	Co (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Zn (mg/kg)	Co (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Zn (mg/kg)
			average				top soil			
Site #1	Sassafras	Amended	3.11	9.26	9.72	25.13	8.94	8.94	8.22	29.28
	Ingleside	Forested	1.02	3.97	4.63	11.01	0.69	4.44	3.63	10.79
Site #2	Greenwich	Amended	1.93	6.99	6.76	18.12	2.12	7.41	7.26	21.68
	Greenwich	Forested	2.52	6.12	9.13	20.76	2.04	5.68	9.45	22.19
Site #3	Corsica	Amended	0.66	7.43	5.53	14.35	0.63	5.61	4.83	12.65
Site #4	Elkton	Agricultural	1.79	7.36	5.03	20.11	2.25	6.37	5.44	16.26

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Chapter 3

MONITORING THE EFFECTS OF STORAGE ON ARSENIC SPECIATION AND DISTRIBUTION IN POULTRY LITTER USING A MULTI-SCALE APPROACH

3.0. Abstract:

Speciation determines the toxicity, mobility, and bioavailability of As in soil environments. Therefore, a study was conducted to determine the As speciation of As in poultry litter taken from a poultry house, and litter that was stored for up to one year.

A series of techniques, including: X-ray Absorption Near Edge Structure (XANES) spectroscopy, X-ray fluorescence (XRF), X-ray diffraction (XRD), and liquid chromatography – inductively coupled plasma- mass spectrometry (LC-ICP-MS) were used to determine As distribution and speciation in poultry litter samples. The results indicate that roxarsone (ROX) and various organic degradation products (monmomethylarsenate, MMA; dimethlyarsenate, DMA; and 4-hydroxy-3-aminophenylarsonic acid, HAPA) are found in most of the litter samples, but tend to be more prevalent in litter samples taken from within the poultry house. Reduced As(III) (arsenite) and oxidized As(V) (arsenate) are found in all samples with a higher percentage found in stored litter. X-ray diffraction identified many environmentally relevant compounds, including: ROX, phosphates, carbonates, and ammonia-bearing

minerals. One mineral of interest that was found after one year in storage is arsenstruvite ($NH_4MgAsO_4 \cdot 6H_2O$). Its phosphate analogue is considered a slow release compound. Therefore, formation of this mineral may present one way to limit As solubility in poultry litter, thus reducing As release into the soil and water environment.

3.1. Introduction:

3.1.0. Background

Arsenic (As) is a toxic metalloid used in a number of commercial and industrial practices. As has been used in agricultural practices for decades, including: pesticides, herbicides, desiccants, and feed additives. The Delmarva Peninsula, an area encompassing parts of Delaware, Maryland, and Virginia, is one of the most concentrated areas of poultry production in the United States. Poultry litter (PL), a mix of excreta and wooden bedding materials, contains high concentrations of trace elements such as Cu, Zn, Ni, Mn, Fe, and As, the sources of which are mineral and vitamin mixes, and dietary supplements added to the poultry feed. Agricultural input of As from poultry litter amendments is one of the main sources of As inputs in soil and water systems in this region. In 2000, 620 million broilers were produced on the Delmarva Peninsula, which resulted in manure and poultry litter containing approximately 26,000 kg of As (Delmarva Poultry Industry, 2000; Garbarino et al., 2003). Poultry litter is generally applied at the rate of 9-20 Mg ha^{-1} on agricultural lands, therefore the total annual As

inputs on the Delmarva Peninsula are estimated between 20 and 50 metric tons of total As (Christen, 2001a; Christen, 2001b).

The total As concentrations in PL vary depending mostly on the concentration of As added to the diets. Sims and Wolf (1994) found levels ranging from 0 to 77 mg kg^{-1}. Other research has shown PL As concentrations to fall within this range, for example, 30-37 mg kg^{-1} (Van der Watt et al., 1994), 43 mg kg^{-1} (Moore et al., 1998), 35 mg kg^{-1} (Jackson and Miller, 1999; Jackson et al., 1999), 45 mg kg^{-1} (Sims and Luka-McCafferty, 2002), and 1-39 mg kg^{-1} (Jackson et al., 2003). Of the total As in the litter, a significant portion is readily water soluble, indicating a potential for ground water contamination. Jackson and Miller (1999) found that 72% of the total As in PL samples was water soluble, Jackson and Bertsch (2001) reported 71% of the As in PL samples was water soluble, and Garbarino et al. (2003), reported that 70-90% of As in dried PL samples was water soluble.

The common sources of arsenic in PL are 4-amino-phenylarsonic acid (p-ars), 4-nitrophenylarsonic acid (Nitarsone), or 3-nitro-4-hydroxyphenly-arsonic acid (Roxarsone, abbreviated ROX). These compounds are used as feed additives to prevent coccidiosis, increase weight gain, and improve feed efficiency and pigmentation. ROX was used in about 70% of broiler industry operations from 1999-2000 (Chapman and Johnson, 2002), however its use is on the decline. Studies have found that the organo-As compounds added to the feed are primarily excreted in the organo-As forms. For example, Morrison (1969) found that ROX constitutes 36-88% of the total As in 10 PL samples. Jackson et al. (2001) found that the major As species in 40 PL extracts were either ROX or As(V).

For 20 of the 40 PL samples, As(V) was the major As species in the water extract, showing that mineralization of the initial organo-As had occurred. The quantity of ROX excreted by a single broiler when fed the typical 45.4 g As ton^{-1} formulation is about 150 mg over the normal growth period of 42 days (equal to 43 mg of As) (Garbarino et al., 2003). Feed spillage, and excreted materials can increase the total As concentration in PL to levels between 14-76 mg kg^{-1} (Moore et al., 1998). Thus, assuming PL is applied at ~7 Mg ha^{-1} (~ 3 tons/ac^{-1}) about 100-530 g of As ha^{-1} could be added to the soil with each application.

Although the main form of arsenic used in the poultry industry is the organic arsenical ROX, there is evidence that the organic As transforms to other organic and inorganic As. (Garbarino et al., 2003; Jackson et al., 2003; Rosal et al., 2005; Stolz et al., 2007). Garbarino et al. (2003) mixed PL samples with water (50 wt %) and the mixture was allowed to compost at 40C. The speciation of As converted from organo-As to As(V) in about 30 days. These studies suggest that litter storage; land application; and then exposure to sunlight, elevated temperatures, and precipitation will cause changes in As speciation. The ROX could undergo transformations to organic degradation products and inorganic As species such as As(V) or As(III). It has been suggested that this conversion is biologically mediated (Cortinas et al., 2006; Garbarino et al., 2003; Stolz et al., 2007). These studies indicate that arsenate is not the only degradation product found in PL, a variety of both organic and inorganic arsenic compounds also have been identified.

The inorganic species As(V) and As(III) are much more soluble and toxic than ROX and could be readily mobile in soils and potentially contaminate shallow ground waters, most of which are inter-connected with fresh and estuarine surface waters (Bednar et al., 2004; Brown et al., 2005). Brown et al. (2005) found As (V) to be readily mobile in soil systems, and found ROX to be more mobile in the subsurface soil horizons. Arai et al. (2003) employed μ-focused X-ray absorption fine structure (XAFS) and X-ray fluorescence (XRF) spectroscopies to directly speciate As in PL samples and long-term PL amended soils. The predominant inorganic species in the PL was As(V). The As(V), which is more toxic than the organo-As species could sorb on soil components such as metal oxides or leach into waters.

Aerobic and anaerobic conditions will have an impact on the speciation of As found in the litter. Aerobic conditions result in As(V) and some organic molecules (Arai et al., 2003; Garbarino et al., 2003; Jackson et al., 2003; Rosal et al., 2005; Stolz et al., 2007), while anaerobic conditions result in more As(III) and organic breakdown products (Arai et al., 2003; Cortinas et al., 2006). Common organic degradation products are 4-hydroxy-3-aminophenylarsonic acid (HAPA) and 4-aminophenylarsonic acid (4-APA or p-ars).

Chemical compounds found in animal waste and their impacts on the environment have been studied for years. One such phosphorus compound, called struvite ($NH_4MgPO_4 \cdot 6H_2O$), is a crystalline ammonia magnesium phosphate mineral that has been found in diary, sheep, and poultry manures (Fettman et al., 1992; Gungor and Karthikeyan, 2005a; Gungor and Karthikeyan, 2005b; Gungor and Karthikeyan, 2008;

Gungor et al., 2007; Qureshi et al., 2006; Shand et al., 2005; Uludag-Demirer et al., 2005; Uludag-Demirer et al., 2008). It is considered to be a slow release compound. In some cases, manure management practices have been altered in order to encourage the formation of this type of compound to limit soluble P (Gungor and Karthikeyan, 2008; Uludag-Demirer et al., 2005; Uludag-Demirer et al., 2008). Recently Hunger et al. (2008) documented its formation in stored poultry litter (Hunger, 2008). Struvite has an analogue called arsenstruvite ($NH_4MgAsO_4 \cdot 6H_2O$). It has been poorly documented in literature (Ferraris, 1973; Stefov, 2008), and its environmental impact is unknown. It is expected to behave similarly to struvite under natural conditions.

Determining speciation of As in poultry litter could predict the fate of As after incorporation into the soil and water environments. A previous study conducted by the University of Delaware and the Delaware Department of Natural Resources and Environmental Control (DNREC), reported that accumulation of As was not observed in Delaware soils having received decades of poultry litter amendments. Therefore, it is crucial to fully understand what form of As is potentially being introduced into the soils. Few studies have examined poultry litter speciation as a function of storage. Poultry litter can accumulate in either the poultry house or be stored outside the house until land application is allowed.

This study consists of a series of PL samples collected from a research poultry house at the University of Delaware Research and Education Center in Georgetown, DE. At the end of the experiment PL was collected and stored up to one year. The poultry litter was periodically sampled and analyzed for As status and speciation.

3.1.1. Objectives

The objectives of this study are to determine As speciation of poultry litter in both poultry house settings and after storage up to two years. X-ray absorption spectroscopy, X-ray Diffraction, and Liquid Chromatography coupled with Mass Spectrometry were used to determine arsenic speciation. Understanding the impacts of these practices will help determine if addition of As to agricultural lands via PL amendments should be regulated and/or incorporated into best management plans (BMPs).

3.2. Methods and Materials:

3.2.0. Poultry House Study.

A large scale poultry house study was conducted at the University of Delaware Research and Education Center in Georgetown, DE. A flock of birds was grown on a roxarsone (ROX) diet, with As concentrations similar to those used in industry settings (2.275 g/lb). The broilers were fed a series of diets throughout their 44 day life cycle. The first was a starter diet with no arsenicals added to the feed (days 0-17). The second and third diets contained varying concentrations of ROX (days 17-33, 33-39). The final diet was a basal diet from which the As was removed, in keeping with the industry standard which requires As to be removed from the feed in order to allow time for As to be removed from the bird (days 39-44). Litter samples were collected from five pens each containing 45 birds fed the same diets. The pens were five by eight feet, creating a bird per square foot ratio of 0.82 (birds/sq ft).

3.2.1. Poultry Litter Collection.

Poultry litter was collected from five identical pens at the end of each diet feeding period (days 17, 33, 39 and 44) in order to allow maximum accumulation of poultry excreta in the litter. Subsamples were taken from multiple locations within each pen and homogenized. The sub sample was removed, placed in a bag and stored on ice until refrigeration (-20^{o}C) in cold storage was available. A portion of each sample was air dried, at 65^{o}C for 48 hours shortly after sampling for X-ray analysis.

Samples collected within the house will be referred to by number and by the days they were in the house they represent. For example, the second litter sample collected within the poultry house (the concentrated ROX diet) is called Litter 2 (days 17-31) and the final sample taken within the poultry house is called Litter 4 (days 38-44).

3.2.2. Litter Storage.

At the conclusion of the experiment, the pens were cleaned out and the litter was placed into 25 gallon garbage bins. The bins were stored in a garage where temperature was not regulated in an attempt to reproduce realistic, seasonal variations in temperature. Temperature in the litter was monitored and recorded at the time of sampling at both the center and edge of the bin. This experimental set up is similar to what was used during a P storage study conducted by McGrath et al. (2005). The purpose of temperature monitoring was to determine the extent of microbial activity in the litter pile. The litter was not turned over, in order to replicate what may be occurring at the bottom of the pen and in the stockpiles of poultry litter during storage.

Litter was sampled biweekly for the first three months and monthly up to one year. Samples were taken from areas on the edge of the bin and in the center of the pile in order to assess the differences in As status. At each sampling, multiple subsamples were collected and homogenized. pH and Eh measurements were monitored directly after litter collection. The pH and Eh measurements were collected on a 1:10 litter to water ratio. After collection the samples were monitored for moisture content and were kept frozen (-20^{o}C) until analysis. A subsample was air-dried for X-ray analysis.

The naming scheme applied to the stored litters applies to the length of time since the litters were removed from the pens and placed into the storage bins. Therefore, time zero is considered the day the PL was removed from the house and placed into storage. For example, month two litter means the litter has been stored two months since it was removed from the house.

3.2.3. Chemical Analysis.

Total As and trace metal concentrations in the poultry litter and feeds were determined using the EPA 3050B digestion method. This method involves the complete dissolution of 0.5g of the dried sample by a hot nitric acid digestion followed by a peroxide digestion heated to 95^{o}C for 2 hours. After digestion, the products were diluted to a final volume of 50mL. The samples were digested using a DigiPrep digestion block in order to maintain temperature throughout the digestion process. The eluent was then filtered using a 0.22μm filter, and samples were kept refrigerated until analyzed. The samples were analyzed using ICP-OES. As standard solutions were also analyzed and

used as an internal calibration. Trace metal grade acids were used in the digestion of litters. The reliability of this method was examined by digesting samples with known As concentrations and comparing the results.

Water soluble As was determined on all litter samples in order to assess what percentage of the litter is readily labile, and how storage affects the water soluble As content. One gram of litter in 10mL of distilled dionized water was shaken for 24 hours. The eluent was then filtered using a 0.22μm filter, and samples were kept refrigerated until analyzed.

Arsenic speciation in the poultry litter samples was determined using liquid chromatography coupled with inductively coupled plasma and mass spectrometry (LC-ICP-MS). The poultry litter samples were extracted with water in a 1 gram per 10 mL ratio. The samples were then diluted 20:1 (DI H_2O: extract). The column is a Phenomemex 150 x 4.6mm polar phenyl-ethyl 5 μm column made by Prodigy. It is stable from pH 2 to 9. The flow rate was 1mL min^{-1} with a 10 μL injection. Liquid As standards were also run alongside the samples, so that As speciation and quantification could be determined.

3.2.4. Arsenstruvite Synthesis

Arsenstruvite is an ammonia magnesium arsenate mineral ($NH_4MgAsO_4 \cdot 6H_2O$). The literature provides very little information about this mineral and how it behaves in the environment. It may be possible to extrapolate some information from struvite ($NH_4MgPO_4 \cdot 6H_2O$). Since phosphate and arsenate are analogues of one another, it is

assumed that arsenstruvite should exhibit similar characteristics to struvite. Therefore, arsenstruvite was synthesized using an adapted struvite method used by Hunger et al. (2008). All reagents were analytical grade and DI water was used. Arsenstruvite was synthesized by titrating an equimolar solution of $MgCl_2.6H_2O$ and KH_2AsO_4 to pH 9 with 1.0 M NH_4OH. The precipitate was stirred over night, filtered, washed with DI water and acetone, and allowed to dry at room temperature in air. The solid was confirmed to be struvite by synchrotron XRD.

3.2.5. X-ray Absorption Spectroscopy Analysis.

Known arsenic standards were analyzed to aid in the identification of unknown As species within experimental samples. These standards were analyzed at beamlines X11A, X11B, and X26A at the National Synchrotron Light Source (NSLS) at the Brookhaven National laboratory in Upton, New York. The standards were calibrated to 11874 eV using an inline As(V) standard, $Ca_3(AsO4)_2$. The normalized derivative of these standards can be seen in Figure 3.5.

Experimental X-ray absorption near edge structure (XANES) spectroscopy was conducted at beamline X26A at the NSLS, and initial assessment studies were conducted at beamline 10.3.2 at the Advanced Light Source (ALS) at Lawrence Berkeley National Lab in Berkeley, CA. The assessment studies determined the samples were susceptible to beam reduction, therefore these samples require a different beamline to conduct these studies. Beamline X26A is a microprobe capable of microspectroscopy, microdiffraction, fluorescence microtomography, and fluorescence mapping. When the

monochromatic beam is focused, the spot size is approximately 10 μm^2 with a flux at 18 keV being $1x10^9$ photons/sec. Canberra 9-element Ge array and Radiant Vortex-EX silicon drift detectors were used in the collection of fluorescence data. A channel-cut, silicon crystal monochromator with a (111) lattice cut was used in the collection of experimental data. A Bruker SMART 1500 CCD detector was used to collect μ-X-ray diffraction data. Additional bulk synchrotron based X-ray diffraction was collected at 10 KeV at beamline 11-3 at the Stanford Synchrotron Radiation Lightsource (SSRL) in Menlo Park, California.

Litter samples were air dried (65^oC) and ground in order to establish a homogeneous sample. Samples were mounted on mylar film using petroleum jelly to adhere the samples to the film. The samples were applied in a single layer on the film. Both the mylar film and jelly were analyzed for As content, in order to minimize contamination of the As signal. Samples were prepared directly before arrival at the national lab.

The mylar film and petroleum jelly were used in order to minimize the effects of beam-induced As reduction. Preliminary studies indicate that mounting poultry litter samples on tapes with adhesives increase the rate at which As reduction occurs (Figure 3.1).

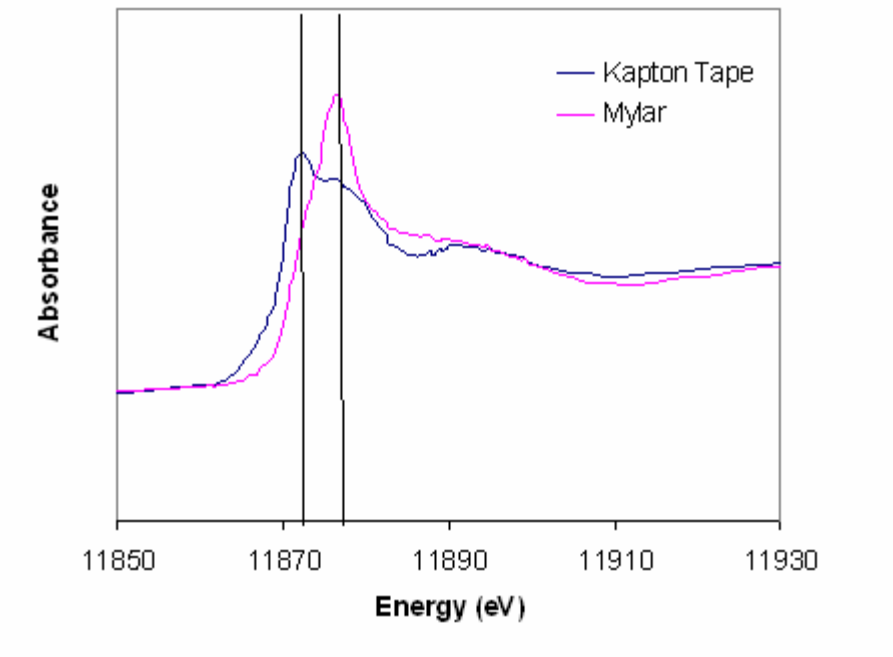

Figure 3.1. The importance of sample preparation in data analysis. A leftward shift in the data indicates reduction of As (As(V) or ROX reduced to As(III)).

The beamline was calibrated to 11874 eV using $Ca_3(AsO4)_2$. Reducing beam-induced sample damage and As reduction was a major priority when collecting XANES data. The amount of time spent in the pre-edge region of the scan was reduced to minimize the amount of time the beam was in one spot before the whiteline, since this is the fingerprinting region (See Figure 3.3 for explanation of these terms). Scans were not taken at the same spot within a single particle, the beam was shifted a few micrometers in between scans in order to collect reasonable and representative data. Figure 3.2 depicts the effect of taking multiple scans at a single point taken at beamline 10.3.2. Figure 3.4 is a test run at X26A on poultry litter in order to asses the rate at which As reduction occurs, in order to be certain that the data did not have artifacts introduced by the beam. Each scan was less than two minutes. Figure 3.4 shows that obvious beam reduction

occurs around the fourth or fifth scan, and is dominant by the 11th scan (purple line). The scan parameters created for the poultry litter are well within this time frame, and therefore the samples should exhibit minimal beam-induced As reduction.

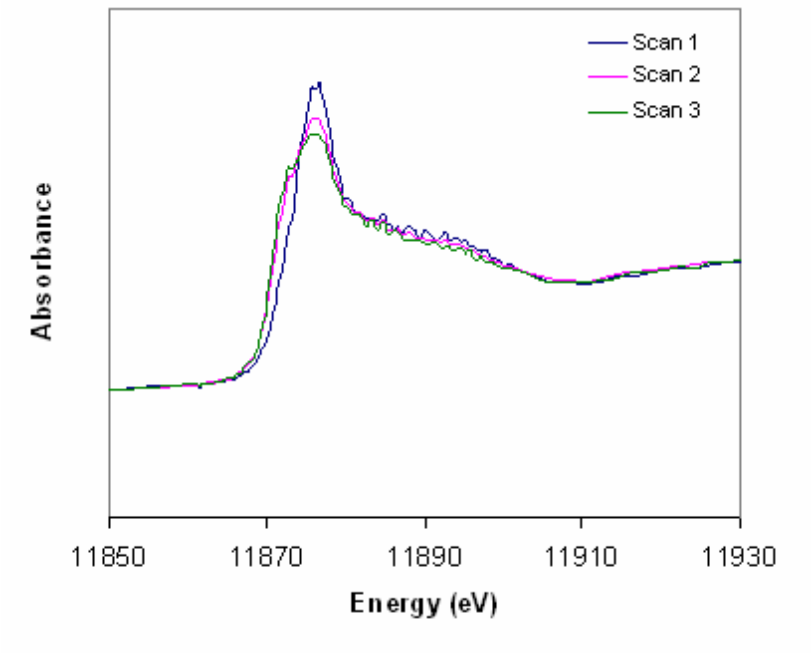

Figure 3.2. Beam induced beam reduction with three scans taken at the same spot. Scans taken at the ALS.

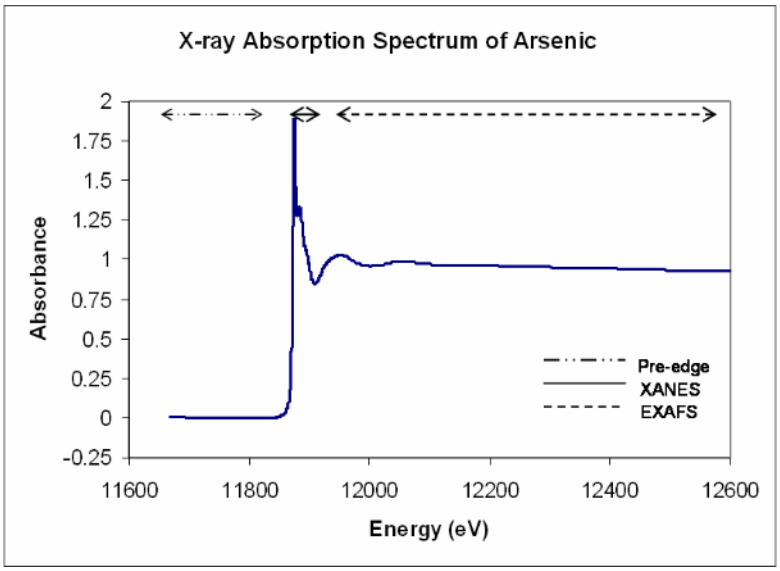

Figure 3.3. X-ray absorption spectroscopy scan of As taken at X11B. The figure depicts the various regions of a scan and which parts are considered in the data analysis. A majority of the performed research in this chapter focuses on the XANES region of the data and therefore the data was only collected out to about 12000 eV.

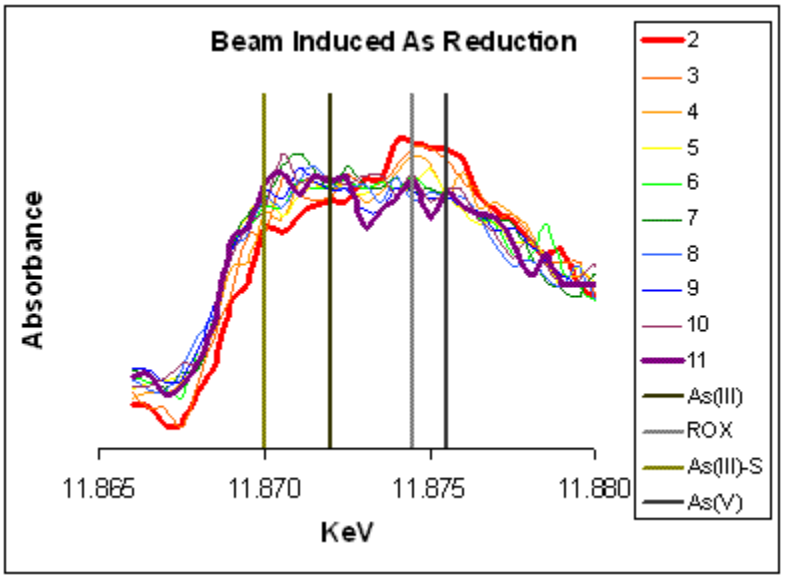

Figure 3.4. A study where multiple scans were taken at a single spot on poultry litter in order to assess the rate at which As reduction takes place, and to ensure the data collected does not contain artifacts.

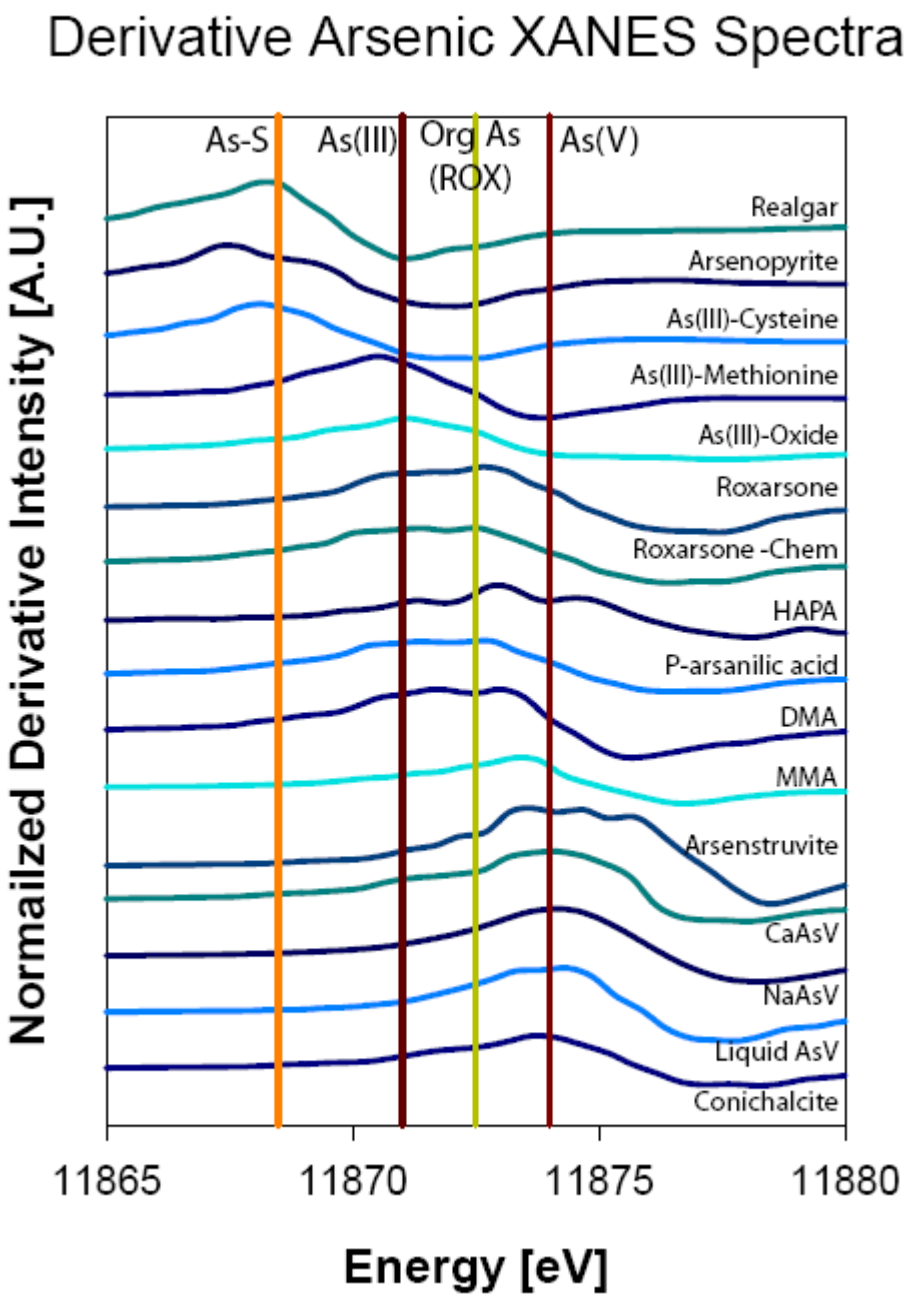

Figure 3.5. XANES spectra of the As standards used in data analysis. The vertical lines represent the energies at which the As species should align with. For instance: the highest point of the liquid As(V) scan should be aligned with the As(V) vertical line.

X-ray fluorescence mapping was collected at 13 KeV and X-ray Diffraction data was collected at 17 KeV. The average map was 0.5mm by 0.5mm with each pixel being 0.01mm by 0.01mm.

After collection, the XANES data and XRF maps were analyzed using the X26A Plot program that consolidates detector channels of XANES scans, and then outputs XANES scans into binary files that can be read by other programs. The X26A Plot was also used in the formation of X-ray fluorescence maps and correlation plots. XANES analysis and As speciation was determined using WinXAS 3.1 and Athena 0.8.051 (Newville, 2001; Ressler, 1998). The determination of As speciation was accomplished by comparing the whiteline and derivative values of the experimental and standard spectra. Principal Component analysis was completed on all experimental scans and it was determined that four components were required to fit the spectra. Linear combination fitting (LCF) was performed using Athena 0.8.051 (Newville, 2001) and Six Pack's Linear Least Squares Fitting (Webb, 2005). An error of about 5-10% is associated with LCF results (Manceau et al., 2002). To see the standards used in the LCF fitting see Table 3.A.3 in the appendix. The XRD data were analyzed using Fit2D and Match! software programs (Davies, 2006).

The total metal concentrations present in the XRF maps were quantified using a series of IDL (interface definition language) programs. The MCA (multi-channel analyzer) program and the NRLXRF IDL programs were used for conventional XRF analysis where a standard material with known elemental concentration is used to quantify elemental abundance in experimental materials. It is more accurate to quantify

elemental abundance in homogeneous samples. Poultry litter samples are heterogeneous in nature and can change in thickness throughout a sample. This can present problems when trying to accurately describe elemental abundance.

3.2.6. Scanning Electron Microscopy

A Field Emission Scanning Electron microscope (FE-SEM), the Hitachi S-4700, was used to collected micrographs of the poultry litter and ROX samples. This system is coupled with an Oxford INCA Energy (EDS) system that allows for elemental distribution analysis of the samples. This research was performed at the Delaware Biotechnology Institute at the University of Delaware.

3.3. Results and Discussion:

3.3.0. Arsenic in the Diets

The study was engineered to represent the practices used in the poultry industry. The arsenic concentrations used in the study fall under the industry maximum value of 2.275 g/lb (0.5%) in basal feeds. The starter (first) diet fed to the broilers did not have ROX added to the feed, yet it has the second highest As concentration out of all of the feeds (Table 3.1). The As could be the result of improper cleaning of the mixing machinery, natural substitution of As into mineral structures, or contamination of individual feed components.

Table 3.1. As status of the litters collected from the poultry house inlcuding As concentrations in the feed and the total As, and water soluble As concentrations.

Litter #	Age (days)	Diet	Total As in Feed (mg kg^{-1})	Total As in Litter (mg kg^{-1})	Water Soluble As in Litter (mg kg^{-1})	pH Of Litter	Moisture Content of Litter %
1	0-16	No As	2.7	2.6 (0.6)	0.42 (0.2)	8.4	21.4
2	17-31	ROX	13.3	12.0 (5.2)	12.8 (1.5)	8.6	24.2
3	32-37	ROX	0.6	15.2 (1.2)	7.8 (1.5)	8.8	25.7
4	38-44	No As	0.3	13.0 (1.1)	7.5 (1.5)	8.8	30.5

* indicates concentration values with standard deviations using n=3.

The total As concentrations were high in the poultry litter (2.6 mg kg^{-1}) and the feed (2.7 mg kg^{-1}) that did not have arsenic supplements, therefore the source of the As in the feed was investigated. The individual components used to compile a basal diet are listed in Table 3.2.a. Samples of basic feed components were digested and the As concentrations are reported in Table 3.2.b. Both inorganic and organic trace metal mineral supplements can be used as part of the feed. When comparing the trace metal constituents, arsenic is most highly associated with the manganese minerals. The general trend in As association (concentration) is Fe ~ Zn < Cu < Mn. The individual component with the highest As concentration is the inorganic Mn compound (10.87 mg kg^{-1} ± 0.73) and the dicalcium phosphate (5.56 mg kg^{-1} ± 0.51). Although these concentrations are higher than perhaps first expected, it is not likely that these concentrations alone are contributing to the increased As values in the feeds. The total percentage of the mineral and/or vitamin mixes are only 0.075% each, therefore even with an As concentration of ~11ppm this contribution alone is not enough to achieve bulk As concentrations of ~2.6

ppm. Dicalcium phosphate composes a larger percentage of the total feed, however at ~6ppm As at 1.75% of the total weight of the feed, it does not appear that the total As concentration can be explained by these values alone. Trace concentrations of As were found in most of the mineral components, but were negligible in the basic compounds (corn, soy beans, and limestone). Some limestone and dicalcium phosphate components are natural minerals, while other times they are synthesized. The nature of these compounds in these samples is not known. Arsenate (As(V)) substitution for phosphate in mineral structures is possible in natural environments. As residence time increases, As can become incorporated into inorganic mineral structures (eg., interparticle diffusion and precipitation) (Sadiq, 1997). Arsenic coprecipitation with other metals has been observed in a number of studies, which could explain the As concentrations in the mineral mix compounds. The source of limestone and dicalcium phosphate for these feeds is not known.

Table 3.2 a,b. The first chart (a) shows an example of the composition of a common basal diet. The second table (b) depicts arsenic concentrations of these basic mineral mix ingredients and feed components.

a)

Basal Diet: Ingredient	% Composition of the feed
Corn	53.7
Soybean meal	37.8
Soybean Oil	4.5
Limestone	1.33
Dicalcium Phosphate	1.75
Salt	0.4
L-Met	0.19
Vitamin mix	0.075
Mineral mix	0.075
Choline Chloride	0.1

b)

Ingredient	As (mg/kg)	As St.Dev. ±		Ingredient	As (mg/kg)	As St.Dev. ±
Org Mn	**4.28**	0.11		$MnSO_4$	**10.87**	0.73
Org Fe	**0.69**	0.47		$FeSO_4$	**0.20**	0.28
Org Zn	**1.33**	1.08		$ZnSO_4$	**0.55**	0.71
Org Cu	**0.92**	0.08		$CuSO_4$	**1.18**	0.82

Organic Mineral Mix Constituents

Inorganic Mineral Mix Constituents

Ingredient	As (mg/kg)	As St.Dev. ±
Corn	**0.44**	0.42
Soy B	**1.52**	1.22
Limestone	**0.44**	0.58
Di-Cal	**5.65**	0.51

Basic Feed Components

3.3.1. Physicochemical Properties and As Status of the Poultry House Litter

The poultry litter sampled from the house ranged from 2.6 to 15.1 mg kg^{-1} As (Table 3.1). Three of the four samples had water soluble concentrations that were at least 50% of the total As values, indicating that a large portion of the As in the poultry litter is readily soluble and could easily be transported in soil and water systems. The first litter sample had a water soluble concentration of 0.5 ppm. This indicates less than 20% of the As in the litter is water soluble which indicates the source of As in the litter is more insoluble and may be incorporated into a mineral structure or bound to one of the basal components.

Moisture content and pH of the poultry litter sampled from the house did not vary widely through the 44 day cycle. Moisture content is indicative of different environments within the litter. A drier litter will have decreased microbial activity and may not as efficiently promote changes in speciation (McGrath et al., 2005). The pH of the litter is slightly basic (8.4-8.8), which is higher than most soils, and will influence the As speciation in the litter. At this pH, arsenite should be in its zero valent form, while As(V) should have an over all 2- charge.

3.3.2. Physicochemical Properties and As Status of the Stored Poultry Litter

At the conclusion of the study, PL from the five pens was collected and placed in bins and stored up to one year (see Table 3.3 for As status). The total As concentration did not fluctuate greatly over the one year period with a slight increase in total As concentration, see Figure 3.6 and Table 3.3. A similar trend in total P concentration of

litters stored dry was noted in the study conducted by McGrath et al. (2005), and no information was provided to explain this increase. This variation in poultry litter concentration could simply be due to the heterogeneity of the litter samples. The most likely reason for the increase in As content is a decrease in biomass due to microbial degradation of the litter. Another subject that is up for debate in the literature is the ability of As to volatilize. If this process is occurring in the litter, then this could explain the increase on the total As content of the litter.

The water soluble arsenic is a huge concern when dealing with poultry waste disposal. The data in Tables 3.1 and 3.3 illustrate that about 50% of the arsenic in the litter is water soluble. As time increases the amount of water soluble As remains constant, which means that after 2 years of storage, the litter still possesses a considerable amount of labile As. The concentrations for water extractable trace metals (Table 3.A.1) does not appear to follow any specific trends.

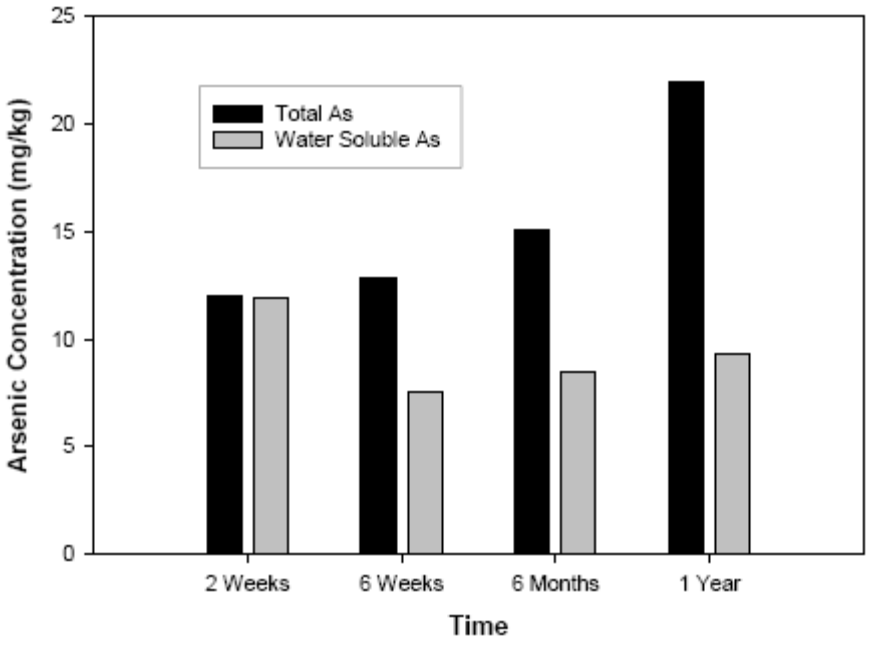

Figure 3.6. Arsenic Water soluble and total As concentrations for selected litters in mg kg^{-1}.

Table 3.3. Arsenic content and other physicochemical characteristics of stored litters.

Time in Storage	Total As* mg kg^{-1}	Water Soluble As* mg L^{-1}	pH	Eh (mV)	Moisture Content %
1 Month	19.8 (4.1)	8.3 (2.8)	8.33	-74.65	33.82
2 Months	22.4 (4.7)	7.3 (1.9)	8.29	-77.00	31.95
4 Months	21.2 (1.0)	7.3 (1.8)	7.86	-50.17	35.38
5 Months	19.0 (4.1)	6.4 (1.7)	7.89	-43.67	30.62
6 Months	15.0 (3.1)	7.0 (2.0)	8.17	-65.93	28.23
1 Year	22.0 (8.8)	6.2 (2.4)	6.63	22.55	25.77

* indicates concentration values with standard deviations using n=3.

Environmental conditions within the litter can play an important role in As speciation. The litter pH and Eh go through a series of fluctuations as storage time increases. These conditions seem to coincide with one another. During the earlier

months, the pH is higher, fluctuating between 6.4 and 8.3, then after a year in storage the pH decreased down to 6.6. During the first few months, the redox potential is lower indicating the presence of a reducing environment, as time increases the system becomes slightly more oxidized. An Eh value of about -100 mV is considered to represent reducing conditions. Although this point is not reached, reducing conditions are approached in the stored litter samples. The conversion to an oxidizing environment after 1 year, also coincides with a decrease in moisture content and pH (Table 3.3). The water content may have an effect on the physicochemical properties of the litter. There was a period where there was a noticeable change in redox potential of the litter at month 4 (Figure 3.7).

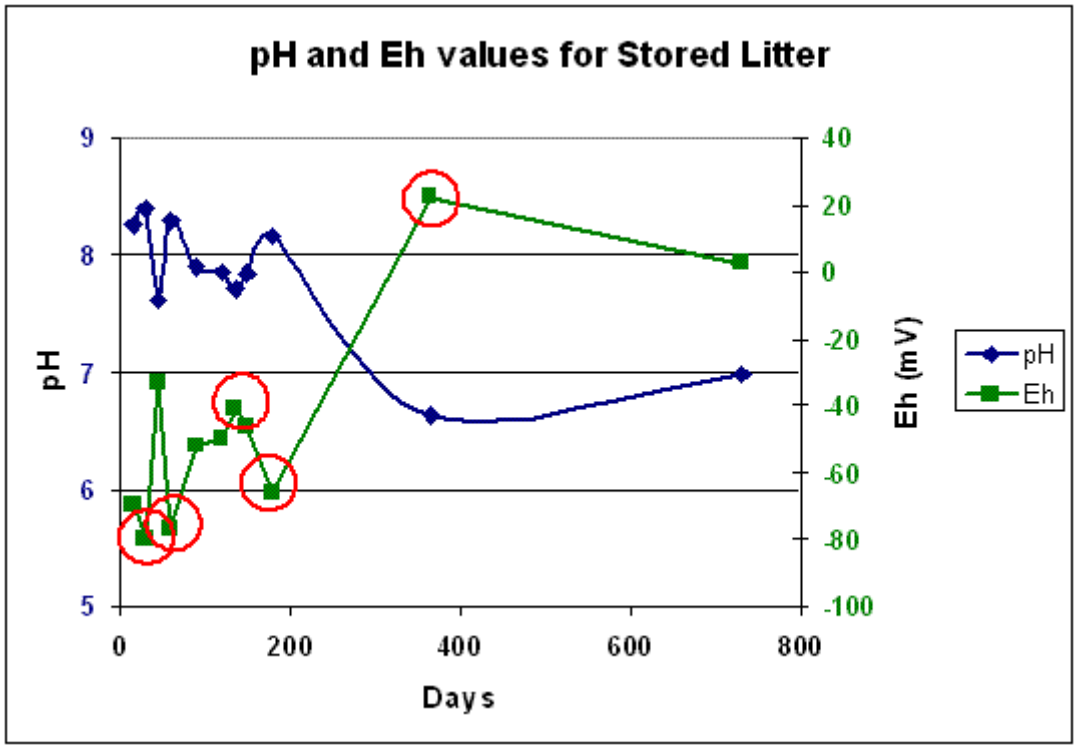

Figure 3.7. pH and Eh values for the stored litter, up to 2 years. Circles indicate time periods where As speciation was conducted.

Throughout the 1 year storage period, the moisture content of the litter decreased with time. However, the change in temperature between the outer and inner pile did not significantly change, a 0-4 ^{o}C change in temperature was noted with the greatest differences being during the winter months. The average temperature increased during the warmer months, though never reaching 40^{o}C, a value set by Zibilske (1997) indicating the presence and activity of thermophilic microorganisms. This indicates that complete composting should not have occurred in these litter samples (McGrath et al., 2005). Figure 3.8 depicts average litter temperature against the average high and low temperature for the year. During the colder months, the average temperature of the stored litter was higher than the air temperature outdoors.

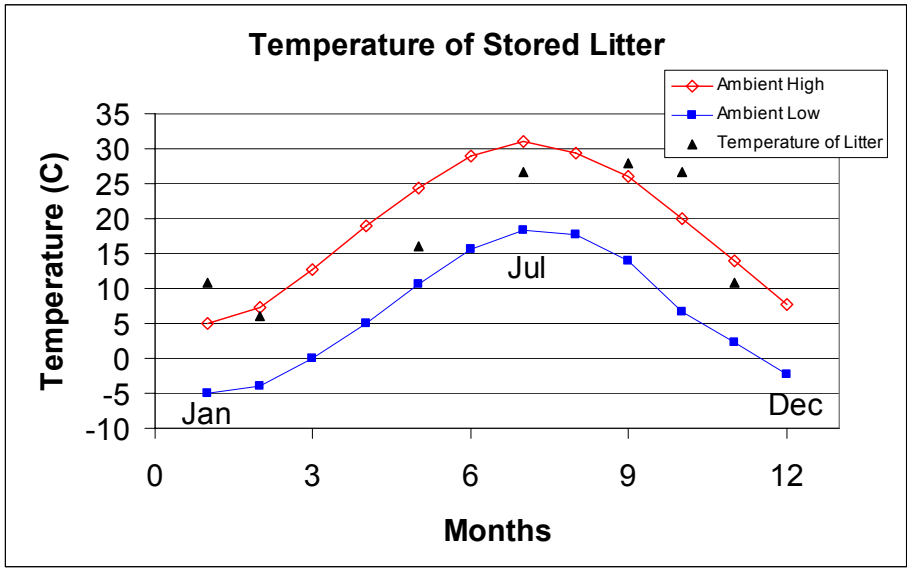

Figure 3.8. Seasonal average temperature of the stored litter. The chart shows that the temperature of the stored litter does not reach 40^{o}C or fluctuate much above the average seasonal temperatures.

In addition to using synchrotron based X-ray fluorescence to determine elemental distribution, Scanning Electron Microscopy (SEM) was conducted at the University of Delaware to determine As distribution in poultry samples. Below are some micrographs of the commercial ROX used in the study. The picture on the left, resembles a corn-like structure. However the scale bar on the bottom left is of 1mm, a corn kernel is larger than what is depicted here.

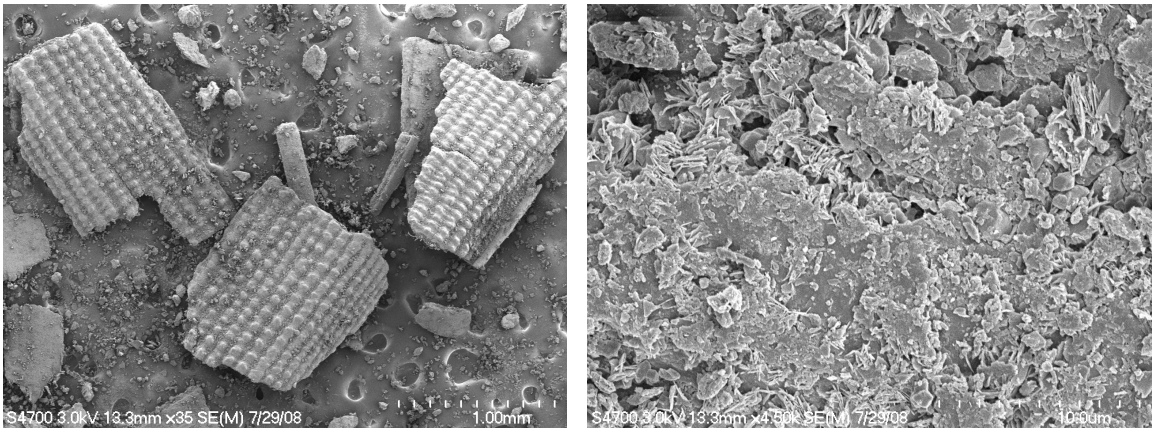

Figure 3.9. SEM image of the commercial Roxarsone used in the study.

3.3.3. As Speciation of Poultry Litter Samples Using X-ray Absorption Spectroscopy of Poultry Litter House Samples

The arsenic speciation of poultry litter will determine the fate of arsenic in the soil once the litter is land applied. Results from Chapter 2 indicate that As accumulation is not seen in poultry litter amended Delaware soils, even though the soils have the ability to retain large quantities of As. This could be due to competition from other oxyanions or to the As speciation. As was discussed in the literature review, some As species will

behave differently in the environment. Therefore, determining As speciation in the source material (litter) is very important.

Two poultry litter samples taken at two separate time periods, days 17-31 (Litter 2) and days 38-44 (Litter 4), from within the poultry house were examined for As speciation, trace metal association and elemental distribution. X-ray fluorescence mapping indicates that As and other trace metals are not evenly distributed throughout the litter and are often localized into concentrated spots or "hotspots", as indicated by the bright warmer colors (yellows and white). Figure 3.10 depicts As distribution in poultry litter 2, and four points of interest where XANES analyses were conducted (Figure 3.11 A and B). The litter particles are found in both needle shaped and small spherical shaped particles. Ca and K analysis indicate that more of the small spherical shaped particles are excreta, while the longer needlelike pieces are the bedding material (the excreta has higher concentrations of Ca and K). The XRF maps provide a liberal quantitative display of elemental distribution throughout the litter sample. All of the XRF maps, like Figure 3.10, are presented in concentration of the specified element (in this case As in mg kg^{-1}).

The X-ray absorption near edge structure spectroscopy (XANES) results indicate that there is a mixture of As species in the poultry litter. These findings are in agreement with previous studies which have shown that poultry waste is primarily excreted in the form of ROX, and is then degraded into inorganic and organic compounds. A larger percentage of ROX and organic As compounds are usually found in the areas of highest As concentration.

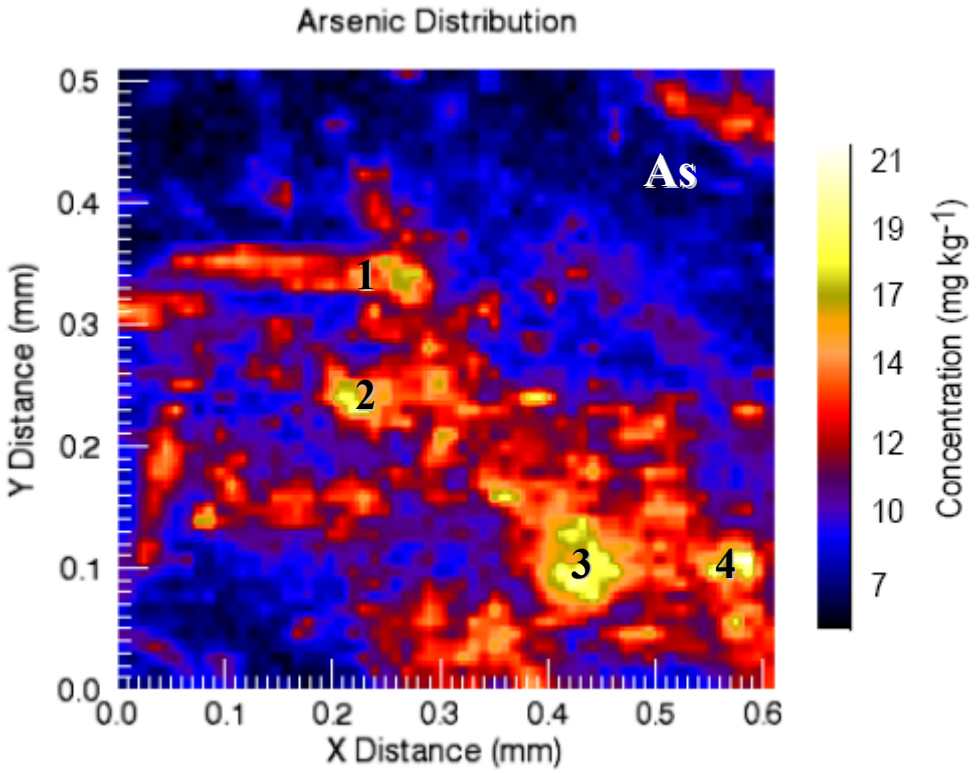

Figure 3.10. Arsenic distribution in poultry litter 2 indicates that As is not evenly distributed, and that it is found in isolated "hotspots".

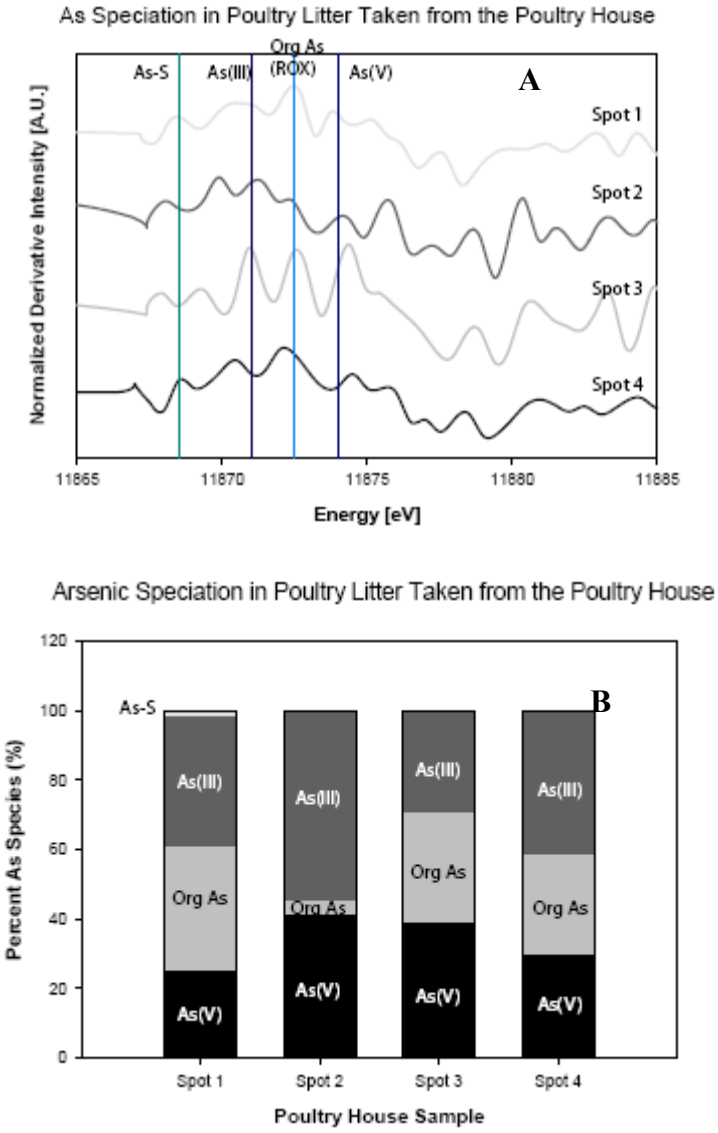

Figure 3.11. Figure A) XANES analyses of poultry litter 2 (days 17-31). Figure B) depicts Linear Combination Fitting (LCF) for this sample. Data indicates a mix of As species in these samples.

XANES scans can be interpreted a number of ways. One method is to examine the position of the whiteline, or the form of the scan that is collected at the beamline. Examples of this are depicted in Figures 3.1-3. This can be an efficient way of looking at differences in arsenic speciation. For this study examining the first derivative of the

spectrum was a more efficient way to display and analyze the data. Therefore, the data presented here will be in normalized derivative intensity. Different arsenic species are correlated with a specific energy. For instance, arsenate's derivative spectrum is associated with energy 11874, while ROX is 11872.5, and arsenite is 11871 eV. The vertical lines depicted on the XANES spectrum slides are the individual As species. The organic arsenic species are grouped together around 11872.5 to keep interpretation simple. ROX was the dominant organic As species found using XANES analysis. Although some of the organic As compounds do not have energies at exactly 11872.5 they are very close. The XANES scans are then analyzed using Linear Combination Fitting (LCF). This data analysis technique assigns a percentage value to the As species that are present in the sample. You can then in turn approximate how much of each of the individual species are present in the samples.

Arsenic speciation in the litter 2 (days 17-31) consisted of ROX, arsenite (As(III)), arsenate (As(V)), and monomethylarsonic acid (MMA). The "Org As" label on the LCF charts stands for organic As. The organic As species included in this list are: roxarsone, HAPA, p-ars, DMA, and MMA. The presence of As(III) may be indicative of anaerobic conditions in the litter. Considering that most of the digestive tract of the bird is anaerobic, it may not be surprising to find As(III) in the poultry litter. In fact, one scan from Spot 2 on the map is mostly comprised of As(III). This area is highly correlated with Cu (see the Cu map depicted in Figure 3.12). Also, it is important to note in areas where As concentration is the highest (Spots 3 and 4) there is a larger percentage of organic arsenic or ROX present. Bulk scans taken of these samples indicate that the

majority of the As in the litter is ROX, with trace amounts of As(V) and As(III). A bulk XAS sample was run at beamline 11.2 at the Stanford Synchrotron Radiation Laboratory, SSRL. Bulk XAS data is useful because it provides an overall average As speciation for the sample. The beamline at the SSRL, has 30 channels, which creates a more sensitive detection limit, even on samples containing only 20ppm of As. See Figures 3.29 and 3.30 for bulk XANES scans and LCF analysis. Also, as a side note: the bulk XANES were collected before the micro-focused work was done, so the differences in As speciation may be an effect of the amount of time the samples were stored after collection. Freezing samples at -20^{o}C may not be cold enough to limit microbial degradation.

Arsenic is associated with a number of trace elements commonly found and added to poultry feeds. These associations can be seen using X-ray fluorescence mapping (XRF). Copper (Cu), manganese (Mn), nickel (Ni), iron (Fe), zinc (Zn) and in some cases calcium (Ca) are associated with As (compare Figures 3.10 and 12). Arai et al. (2003) found that As was commonly found with Ca in poultry litter samples, however this relationship does not appear to be as common in these poultry litter samples. The strongest associations are seen between Cu, Mn, Zn and As.

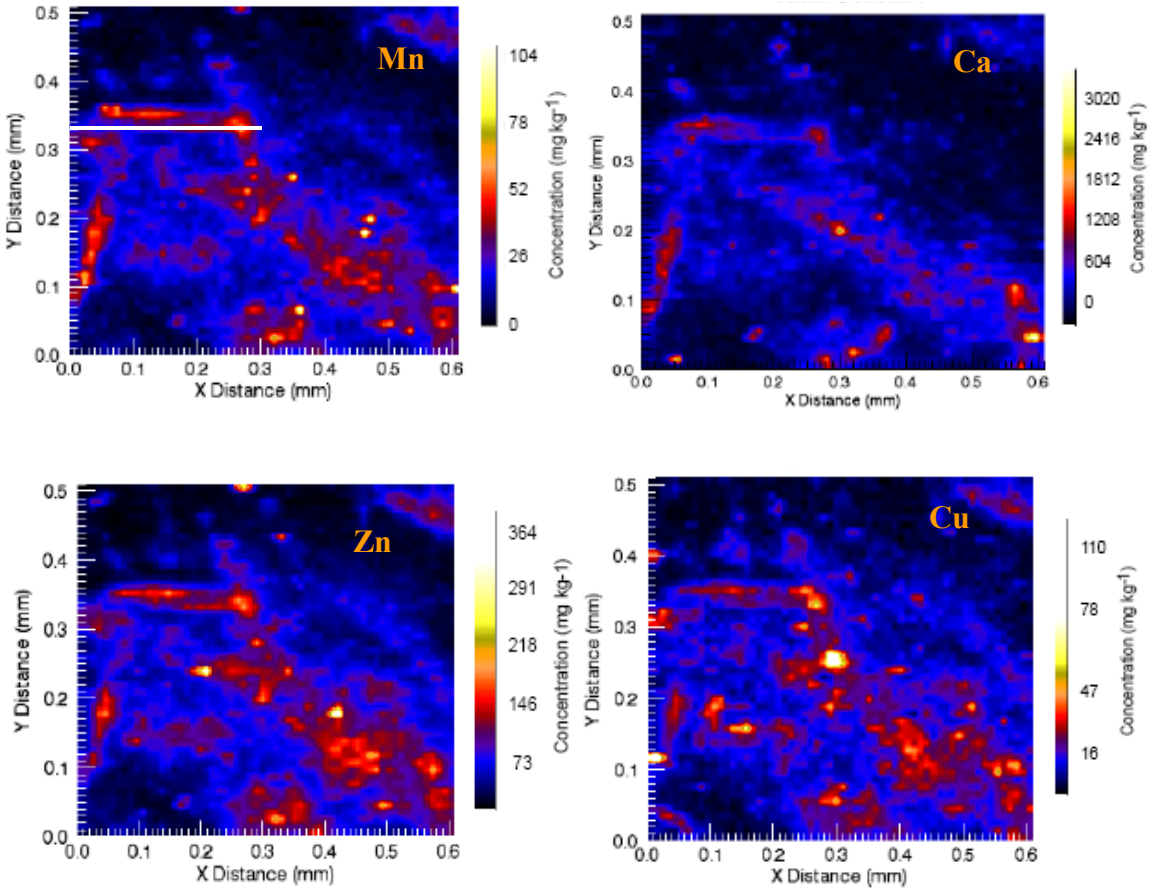

Figure 3.12 XRF maps of trace metal (Mn, Ni, Zn, Cu) distribution in poultry litter 2 (days 17-31) is depicted in this figure. Compare Figures 3.10 to 3.11 to see trace metal distribution vs. arsenic distribution.

Notice there is a line drawn across the Mn map depicted in Figure 3.12. Figure 3.13 depicts the changes in As, Mn, Cu, and Zn concentration (mg kg^{-1}) moving from left to right along this line. Arsenic and all the trace metals exhibit a similar pattern indicating that they are located at relatively the same place, indicating that they are correlated in the PL samples. Total metal concentrations can be found in Table 3.A.1 of the appendix. When comparing the maps above and the total values, one will notice that As concentrations are much lower (almost tenfold lower) than that of the other trace

metals. Another way to look at elemental correlation is to plot maps on top of one another. A bicolor or tricolor map can be used to examine such trends. Often times, true correlations can be more easily seen by looking at bicolor maps, and assigning an R^2 value to the correlation plots, see Figure 3.14. So, although it appears that Mn may not be as highly correlated, the correlation plots reveal R^2 values of 0.6746 and 0.6818 for Cu and Mn respectively.

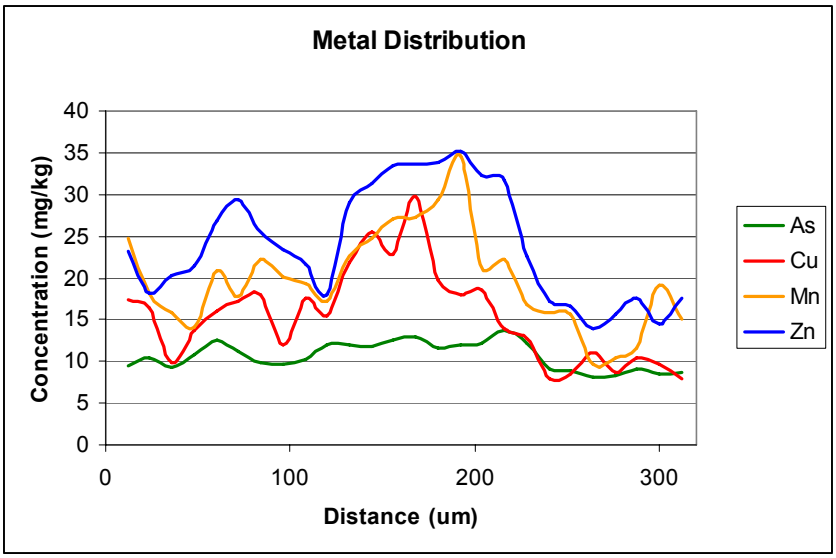

Figure 3.13 Metal distribution(As, Mn, Zn, Cu) in poultry litter 2 (days 17-31) across the line in Figure 3.11.

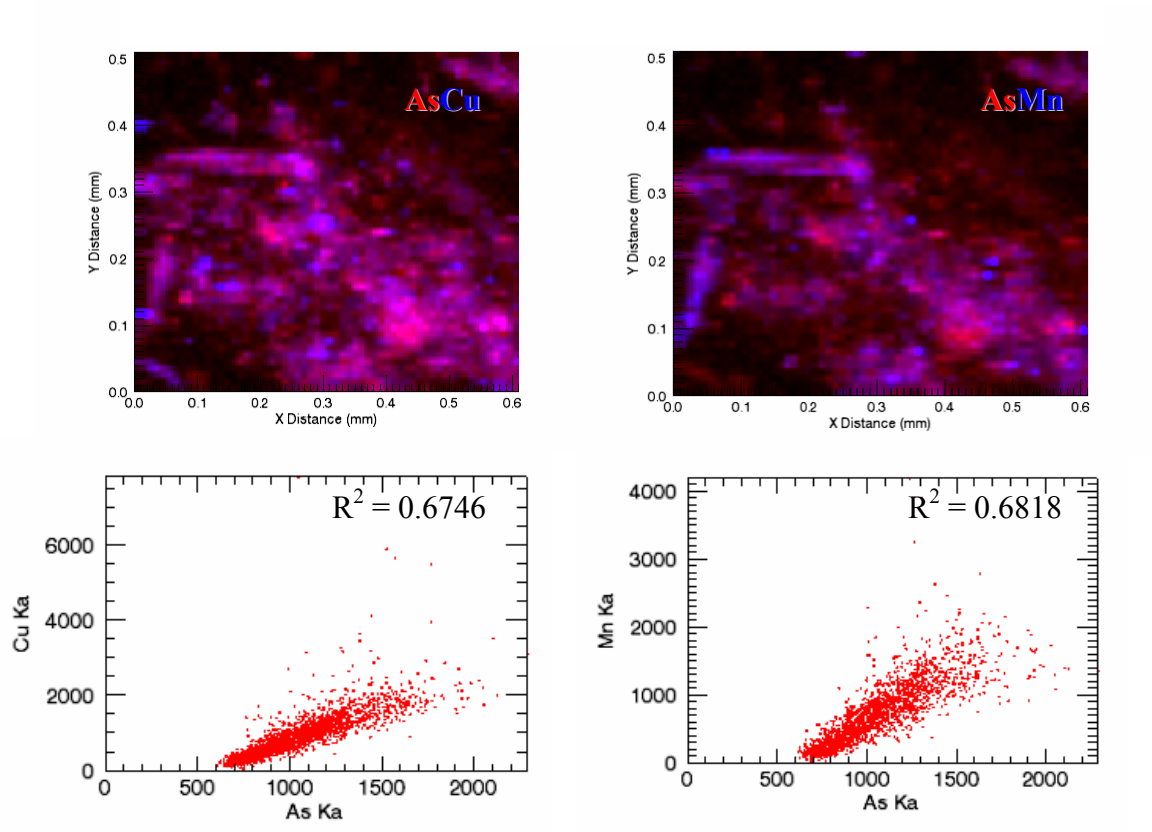

Figure 3.14 XRF maps and correlation plots of poultry litter 2 (days 17-31) depicting trends between As (red), Cu (blue), and Mn (blue). The thin straight lines on the correlation plots indicate that there is a trend between these metals and As.

One feature at beamline X26A is that a MCA (Multiple Channel Analyzer) scan is taken at each pixel on the map. An MCA display is beneficial because it shows relative elemental concentrations at a given point. An MCA plot for spot 1 is shown in Figure 3.A.1 in the appendix. It shows that at this spot there are many trace metals that greatly outweigh As in relative concentration. These plots can be used to help determine actual concentration gradients throughout a map. The MCA plots are valuable because they can help determine what is present at any given point. They can also provide information about elements that you may have not been considering.

SEM coupled with EDS/X can also be used to determine elemental distribution in samples. However, the SEM does not reach the high energy or have the resolution of the synchrotron techniques and therefore did not produce sufficient As data to provide additional information about As distribution. Nonetheless, it was able to provide elemental distribution of the other trace metals and elements present in the samples. Some elements like P and Si that are not mapped using the synchrotron based techniques are possible using SEM. Figure 3.15 shows a few SEM micrographs coupled with EDX results displaying elemental distribution of P, Ca, and Mg. We find similar trends in trace metal distribution using the SEM. Figure 3.16 shows some photographs of litter particles. One can see the difference between the excreta and wood shavings. It is also important to note that most of these particles are amorphous in nature; we do not see many crystalline or well structured compounds/particles.

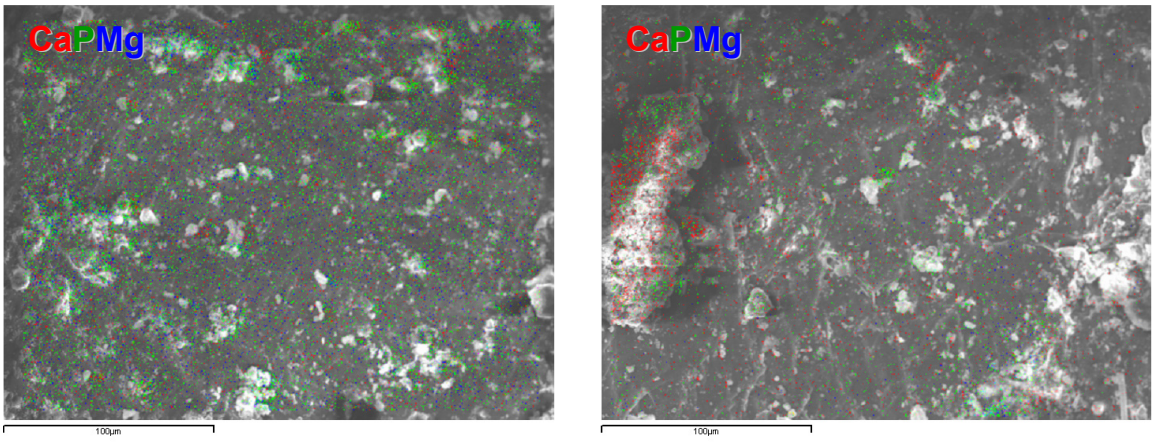

Figure 3.15. SEM image and EDX of Poultry litter from the house. Calcium is red, Phosphorus is green, and Magnesium is blue.

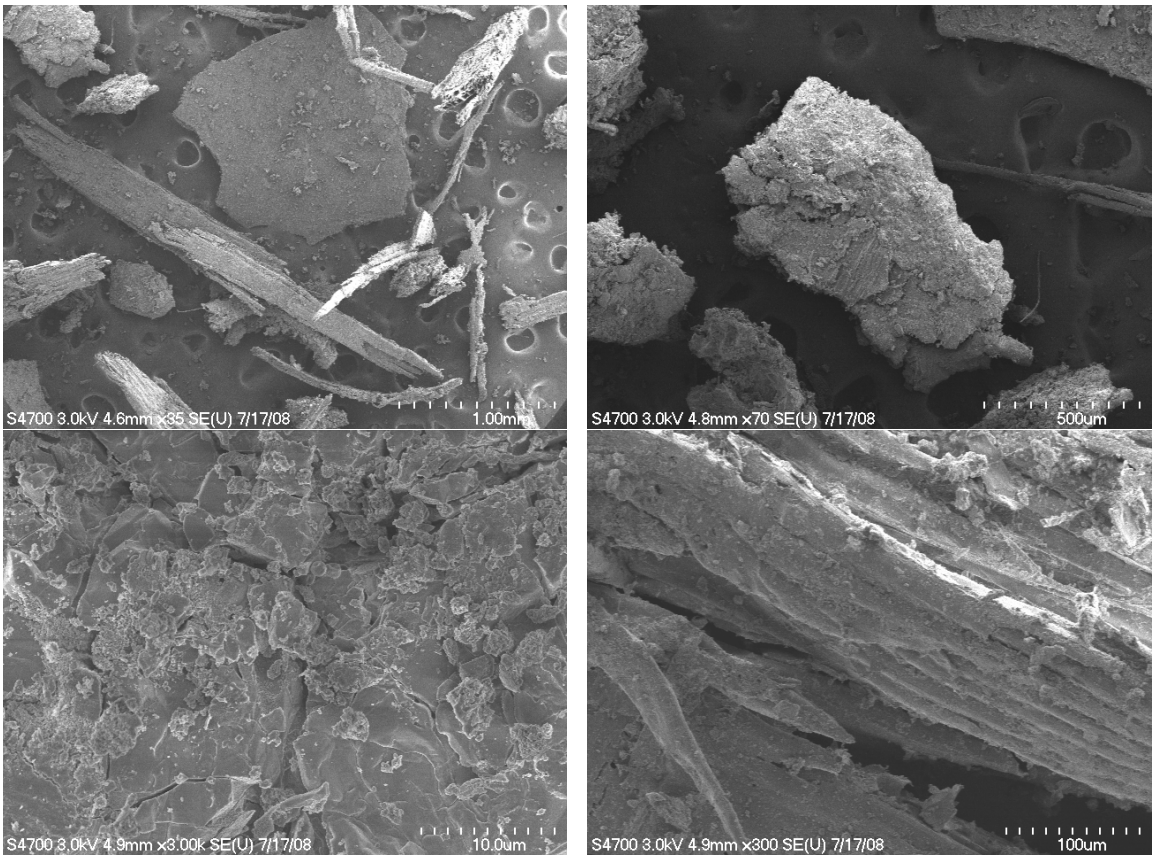

Figure 3.16. SEM images of poultry litter from the house (poultry litter 4).

A couple of older studies examined the relationship between As (ROX) and Cu supplements on Cu and As accumulation and toxicity in birds (Czarnecki and Baker, 1985). They observed a trend between ROX and Cu toxicity and concentration levels in broiler livers. It is believed that ROX and Cu may be forming a complex which minimizes Cu toxicity in birds. The findings in the litter X-ray absorption spectroscopy (XAS) studies indicate that there may be an As-Cu complex forming in some samples.

Arsenic distribution and speciation in the fourth and final sample taken from the poultry house (days 38-44) is similar to poultry litter 2 (days 17-31) (See Figures 3.29 and 3.30). However, the XANES analysis revealed some differences between the two

house litters. There was an increase in the amount of As(III) seen, which may indicate the presence of anaerobic conditions forming and/or an increase in microbial activity. Also it is important to note that as the birds get older, there is a change in gastrointestinal microflora. In the beginning there are aerobic conditions in the gut, followed by a progression towards an anaerobic environment as demonstrated by the overwhelming majority of anaerobic bacterial species found in the GI tract. Therefore, it would make sense that more As(III) would be excreted than As(V).

The data suggest that As speciation is altered with respect to spatial distribution and surface areas of the As in the litter. The largest areas of concentrated As are predominantly ROX, which indicates that the organic arsenical will persist especially in condensed particles. If this process is microbially or environmentally mediated, the particles with the greatest surface area would be transformed more quickly than concentrated particles.

If the poultry litter was removed from the house and land applied after this flock of birds, a portion of the As in the litter is As(III), which is more soluble than the more oxidized As species and the organic As species. Maps, scans and MCA plots of poultry litter 4 can be found in the appendix of this chapter (Figures 3.A.2-5).

3.3.4. As Speciation using X-ray Absorption Spectroscopy of Stored Poultry Litter.

Arsenic content and speciation were investigated in a number of stored poultry litter samples. Arsenic distribution and metal associations are not greatly altered with length of storage time. The strong associations between As and the trace metals are still

observed, however there is a noticeable change in As speciation with time. Each sample spectrum contained multiple As species. Arsenic speciation in litters stored for six months and one year is discussed in the following paragraphs. Data and a brief discussion of two month samples are located in the appendix for this chapter (Figures 3.A.6-8).

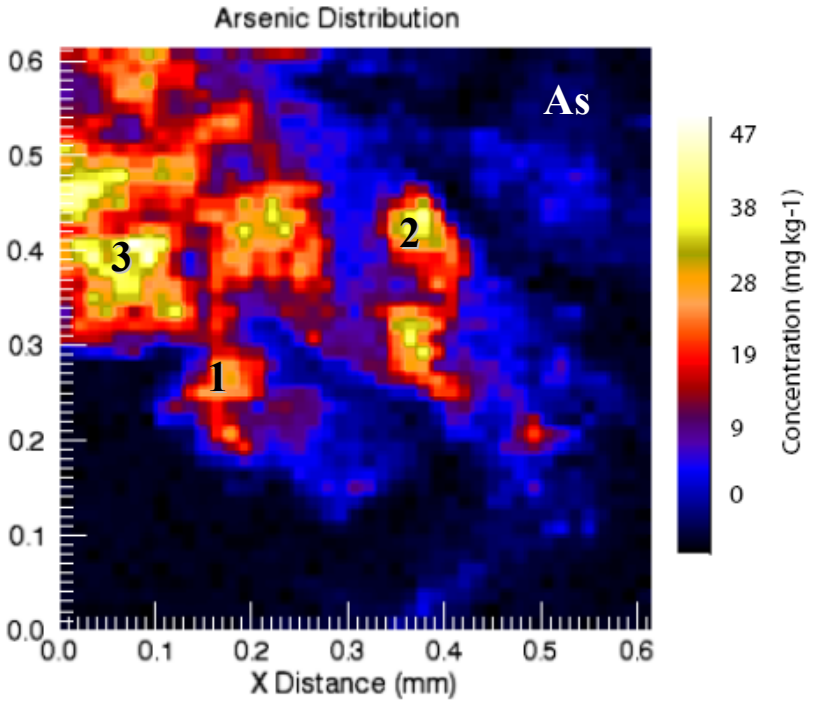

Figure 3.17. Arsenic distribution in poultry litter stored for 6 months. Arsenic is not evenly distributed in the litter materials. Arsenic concentration is higher in lighter colored areas.

Figure 3.17 shows As distribution in poultry litter stored for six months. Similar to what was seen in the poultry house samples, the As is not evenly distributed. It is found in localized hot spots. XANES scans were collected at each of the numbered spots in Figure 3.17 and the scans and LCF results can be seen in Figures 3.18 A and B.

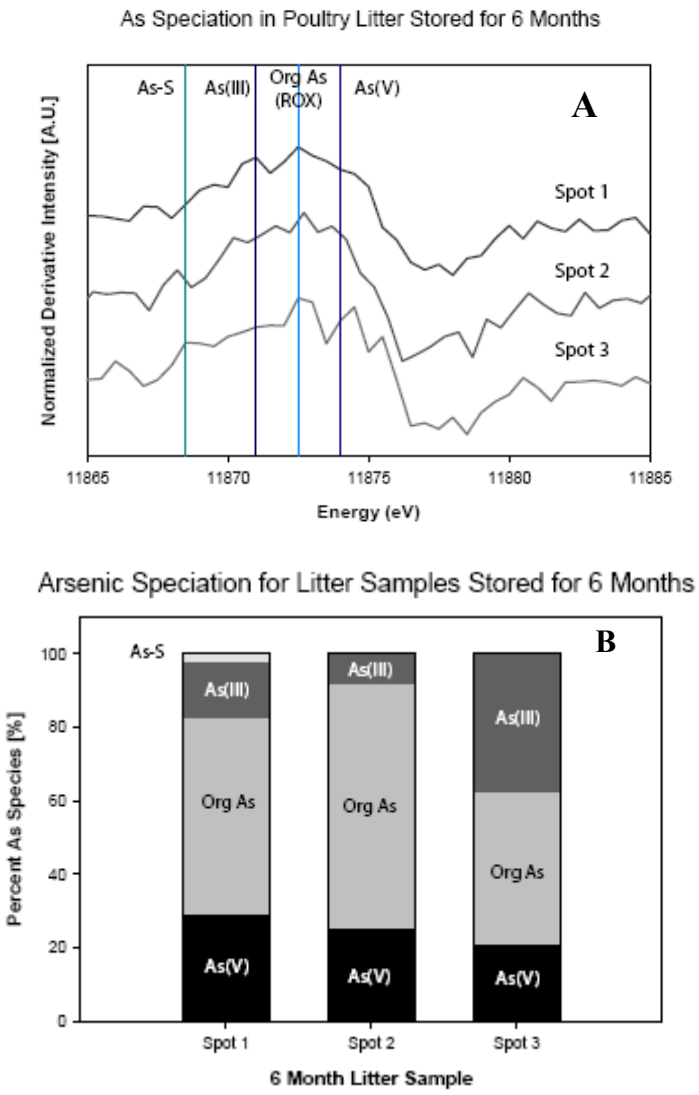

Figure 3.18 A and B. Figure A) As XANES analyses of poultry litter stored for 6 months. Figure B) the Linear Combination Fit of the XANES spectra in Figure A. XANES analysis indicates that there are a number of different As species, making this litter heterogeneous in nature. The presence of As(III) and As(V) species are becoming more dominant than the house samples.

Storage of poultry litter plays a role in As speciation. The scans in Figure 3.18 A and B demonstrate the variability that is seen within one sample. Roxarsone was the dominant organic species found in these samples. However, MMA and HAPA were found in the LCF fitting as well which indicates a change in environmental conditions.

HAPA is mostly found in litter samples under anaerobic conditions. In fact, there is a large amount of organic arsenic at these locations. Spot 1 consists of a number of As species, including reduced As, organic As, oxidized As and a very small amount of As-S, while spot 3 is composed of more inorganic As species. The micro-focused X-ray analyses provides information about the speciation on a smaller scale, but definite changes in overall As speciation can be seen on the bulk level (Figure 3.29 and 3.30). An unidentified As species came out with whiteline (and derivative) values between DMA and As(III). When considering land application of poultry litter, it is imperative to consider how all of these species will react once introduced into the soil and water environments. Land application of PL will not only introduce ROX, but also reduced and oxidized As species. Oxidized and reduced As will behave very differently from the organic As forms. Reduced As is more likely to leach into ground water than the oxidized forms. Reduced As(III) is neutrally charged in most natural systems, meaning that it is not likely adhere to soil particles.

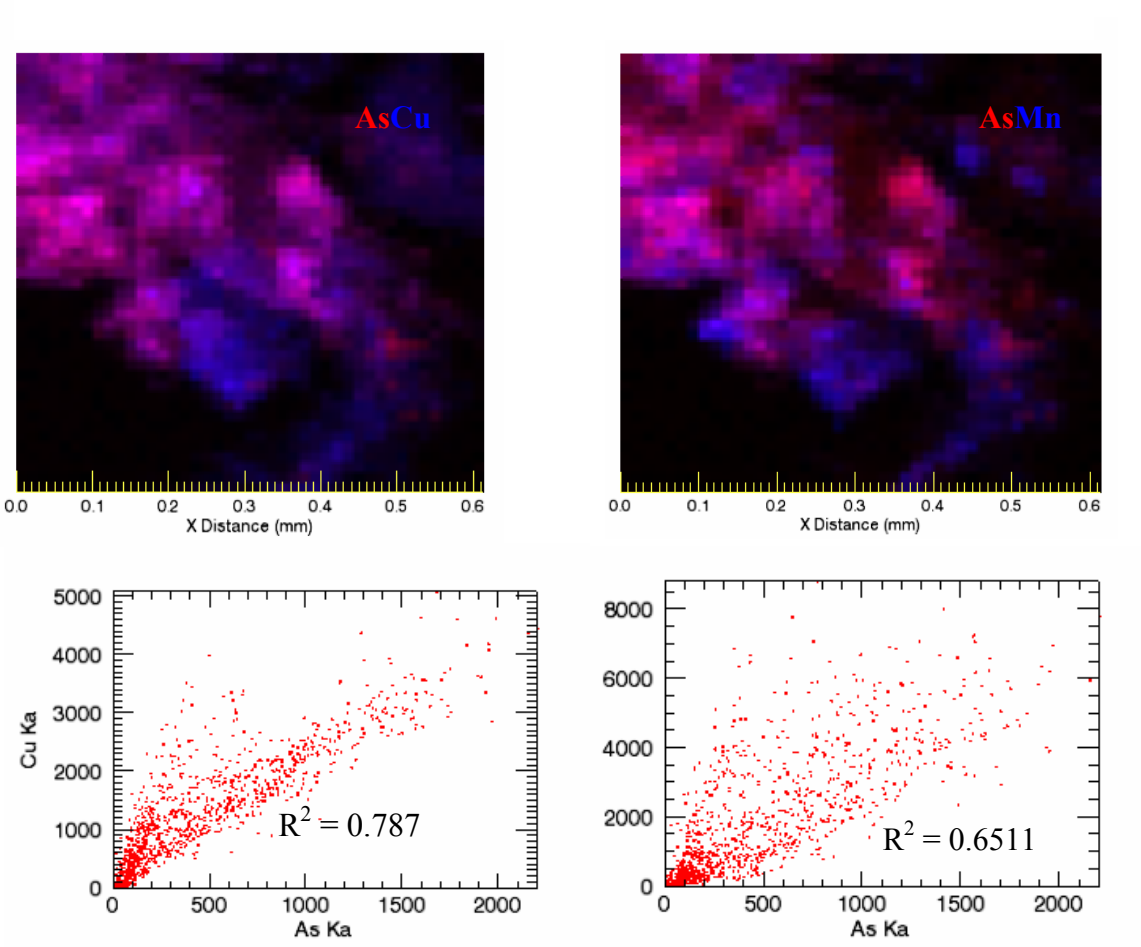

Figure 3.19. As distribution with Cu and Mn are depicted in XRF maps and correlation plots of poultry litter stored for 6 months.

Bicolor maps depicted in Figure 3.19 show the strong As and trace metal correlations seen in the earlier samples. However, the As-Cu (R^2 = 0.787) trend has become stronger than the As-Mn (R^2 0.6511) relationship in these poultry litters. The As-Cu trend has also become stronger in comparison to the litters taken from the samples (litter 2) taken from poultry house (R^2 0.6746). It is hard to definitively say if this is sample heterogeneity or if this is a true trend. Since µ-XRF is taken across a small area of the PL sample, it may not be an excellent representation of the PL sample as a whole.

Individual XRF maps for various elements are shown in Figure 3.20. Comparing Figure 3.20 to 3.17 allows the As-trace metal correlations to be seen. A line was drawn across one of the XRF maps in Figure 3.20 and the elemental concentrations across this line are plotted in Figure 3.21. As, Cu, Zn all exhibit similar trends in concentration when moving across the particle (left to right). Mn shows similar trends, but is not as highly correlated.

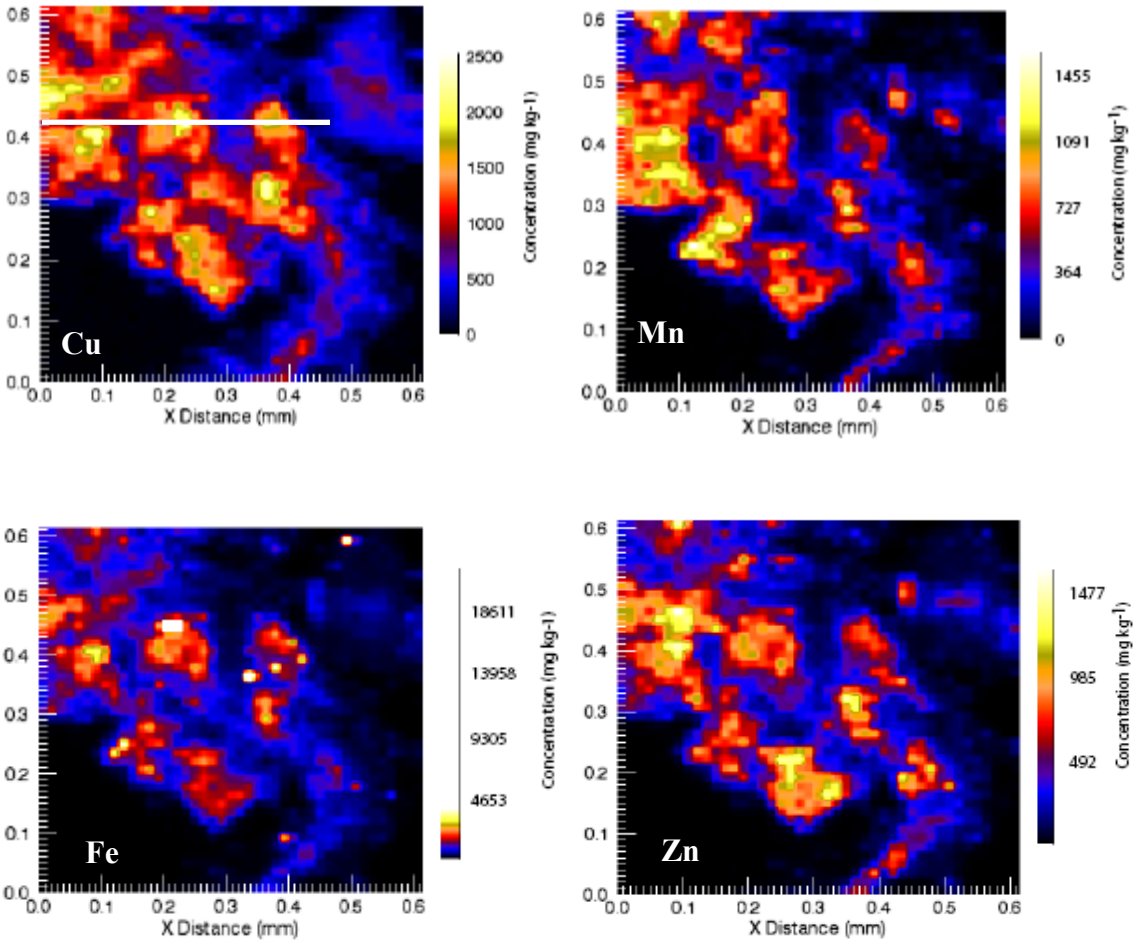

Figure 3.20. Trace metal distribution in poultry litter stored for 6 months.

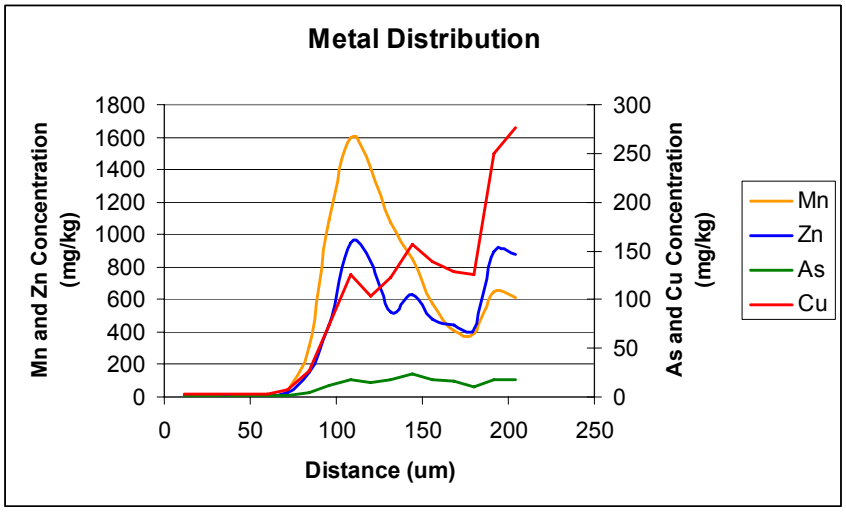

Figure 3.21. Trace metal distribution along the line drawn across in Figure 3.20 in poultry litter stored for 6 months (left to right).

Arsenic speciation data for the six month storage litter sample exhibited some differences when compared to the poultry house litter. The bulk XANES data shows a breakdown of organic As into inorganic As. This relationship is also seen at the micro-level, but not as prevalently. The As-trace metal correlations continue and may even be more highly associated as the litter ages.

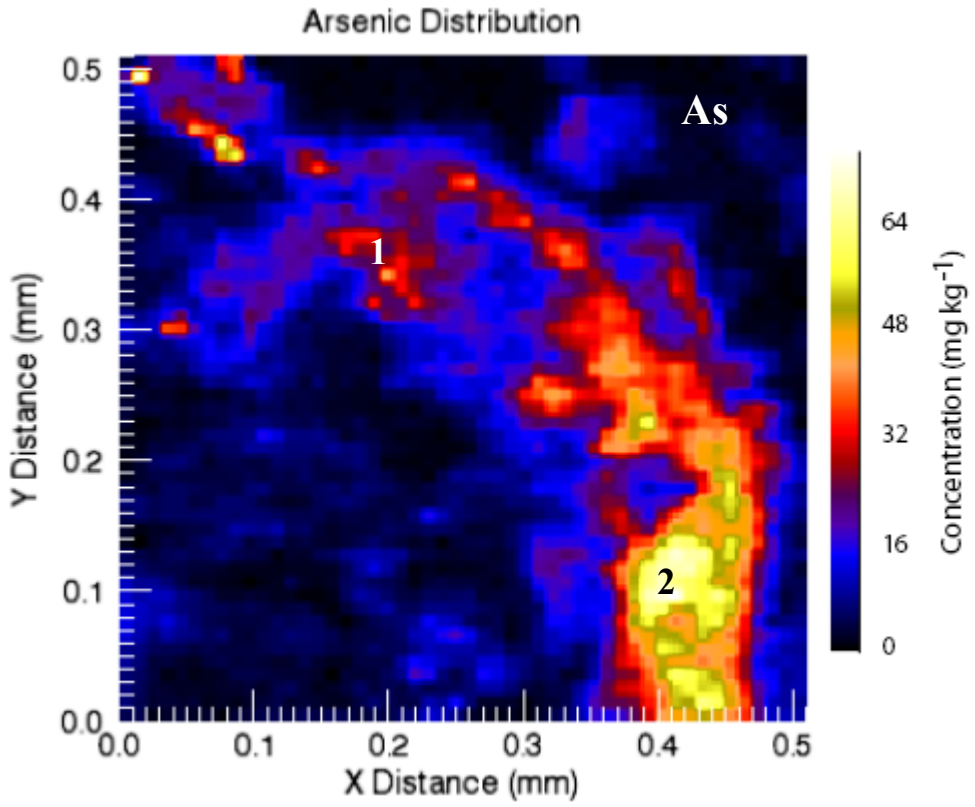

Figure 3.22. Arsenic distribution in poultry litter stored for 1 year.

Arsenic distribution in poultry litter stored for one year is depicted in Figure 3.22. Once again, a similar pattern is clear. Arsenic is still localized into hotspots. The XANES and LCF results are seen in Figure 3.23 A and B.

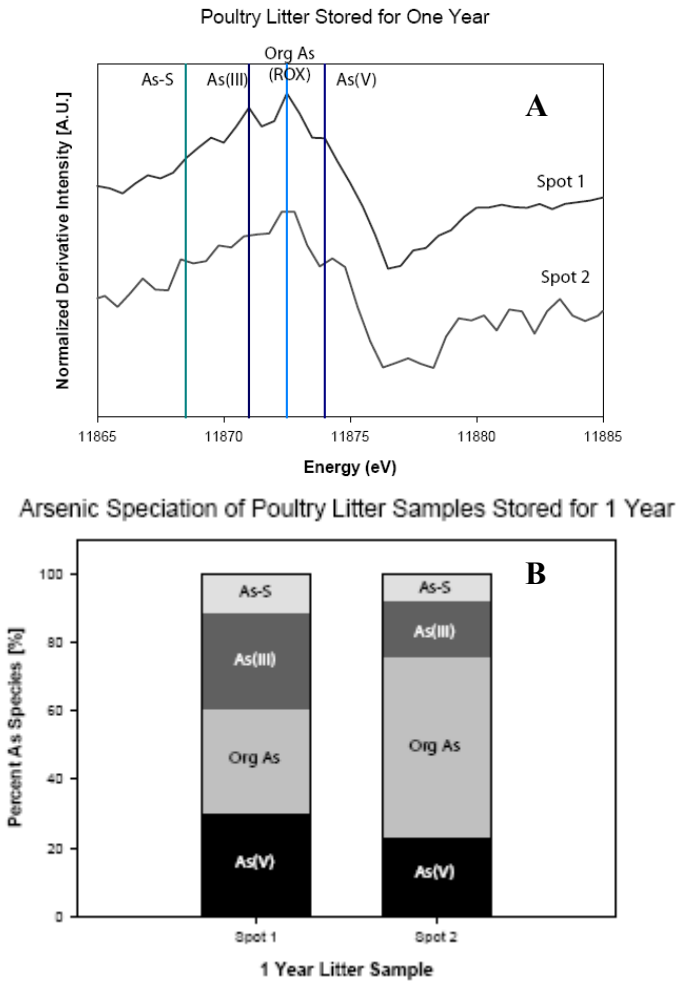

Figure 3.23 A and B. Figure A) As XANES analyses of poultry litter stored one year. Figure B) the Linear Combination Fit of the XANES spectra in Figure A. XANES analysis indicates that there are a number of different As species, making this litter heterogeneous in nature. The presence of As(III) and As(V) species are continuing to become more dominant than the house samples.

After one year of storage, inorganic As species are also present in this litter. Roxarsone is found in Spot 2, which has a high concentration of As (figure 3.22). The increased concentration of As(III) and As(III)-S found, and organic degradation products in these samples is indicative of a change in environmental conditions. The temperature of the pile is still warmer in the center of the pile, which indicates that microbial activity is most likely still taking place. Also, perhaps more importantly, the Eh values have been

low (-66 mV at six months) and after one year have increased drastically (+23 mV) indicating a change to more oxidizing environmental conditions in the litter piles.

The elemental distribution in the one year litter is similar to the rest of the samples. When examining the maps from the one year sample and the MCA plot (Figures 3.24-27), elemental distribution is well understood. Comparing Figures 3.22 and 3.24 shows trace metal distribution relative to As distribution. This sample shows that As andCu are once again very highly correlated with an R^2 value of 0.8090. This value is both higher than Mn and higher than any other R^2 value we have seen for As-Cu. It is possible that as storage time increases, As and Cu may be forming some type of precipitate or forming some type of complex.

Elemental distribution across the line drawn in Figure 3.24 (Figures 3.24 and 25) show the relative elemental concentration. As, Cu, Mn, and Zn all appear to be following similar trends.

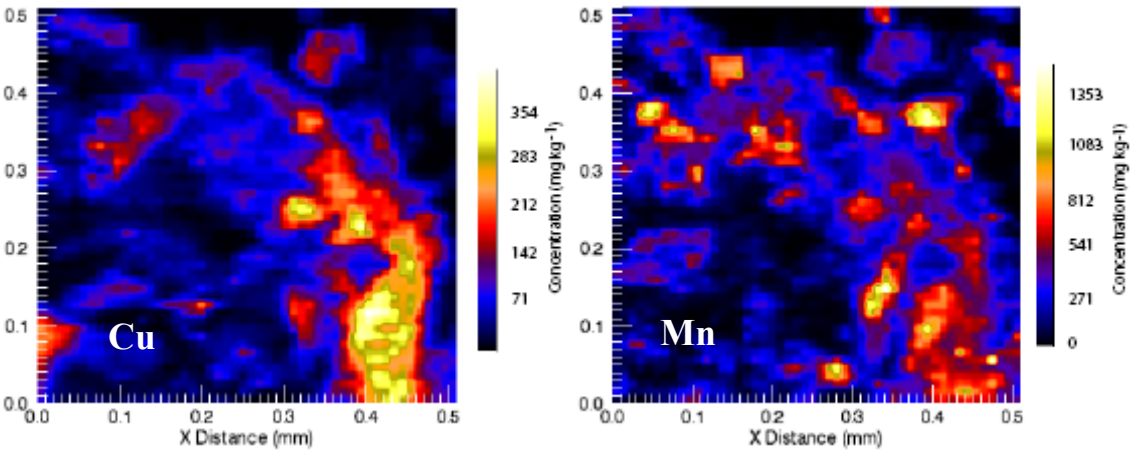

Figure 3.24. Trace metal (Cu, Mn, Zn, Ca) distribution in poultry litter samples stored for 1 year.

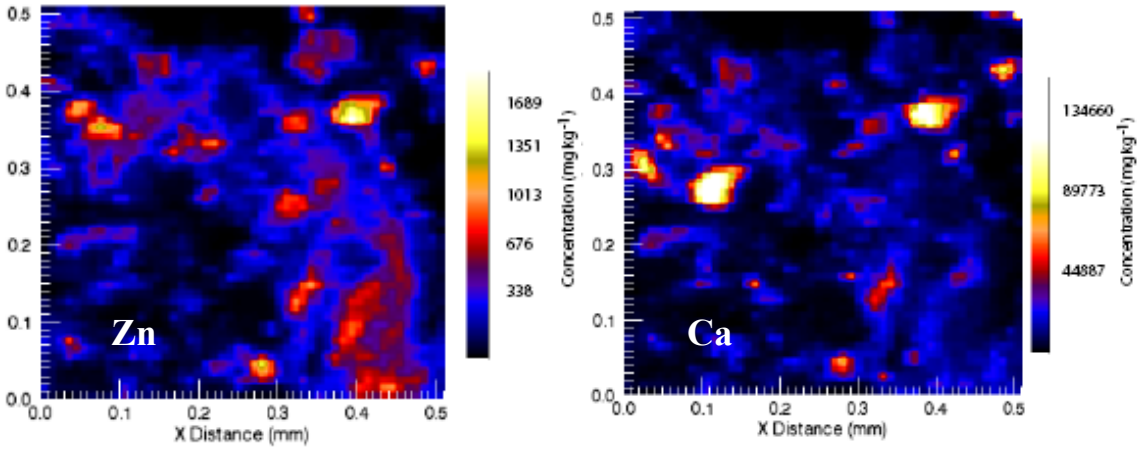

Figure 3.24.. (cont.)

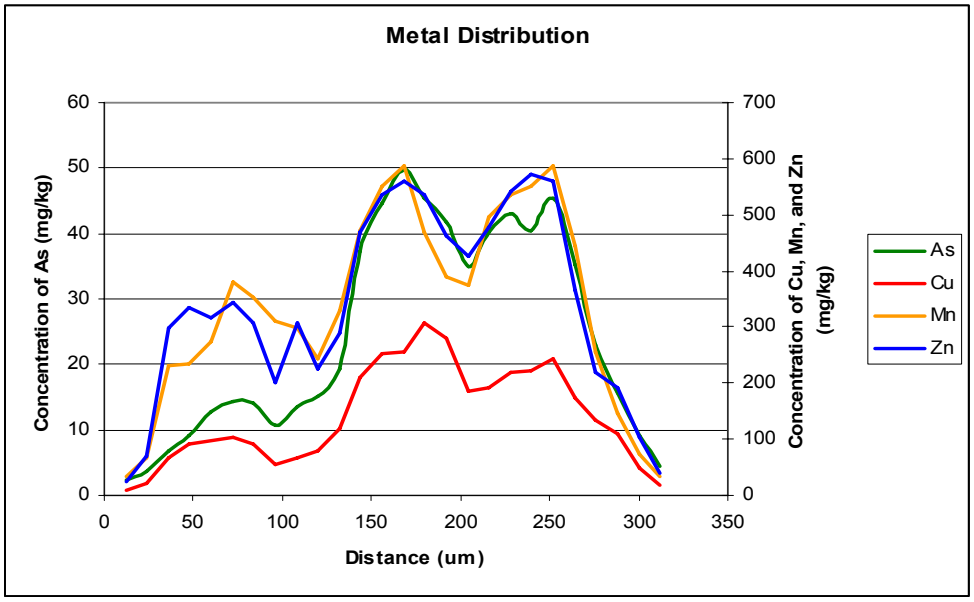

Figure 3.25. Trace metal distribution across the line drawn across in Figure 3.24 in poultry litter stored for one year.

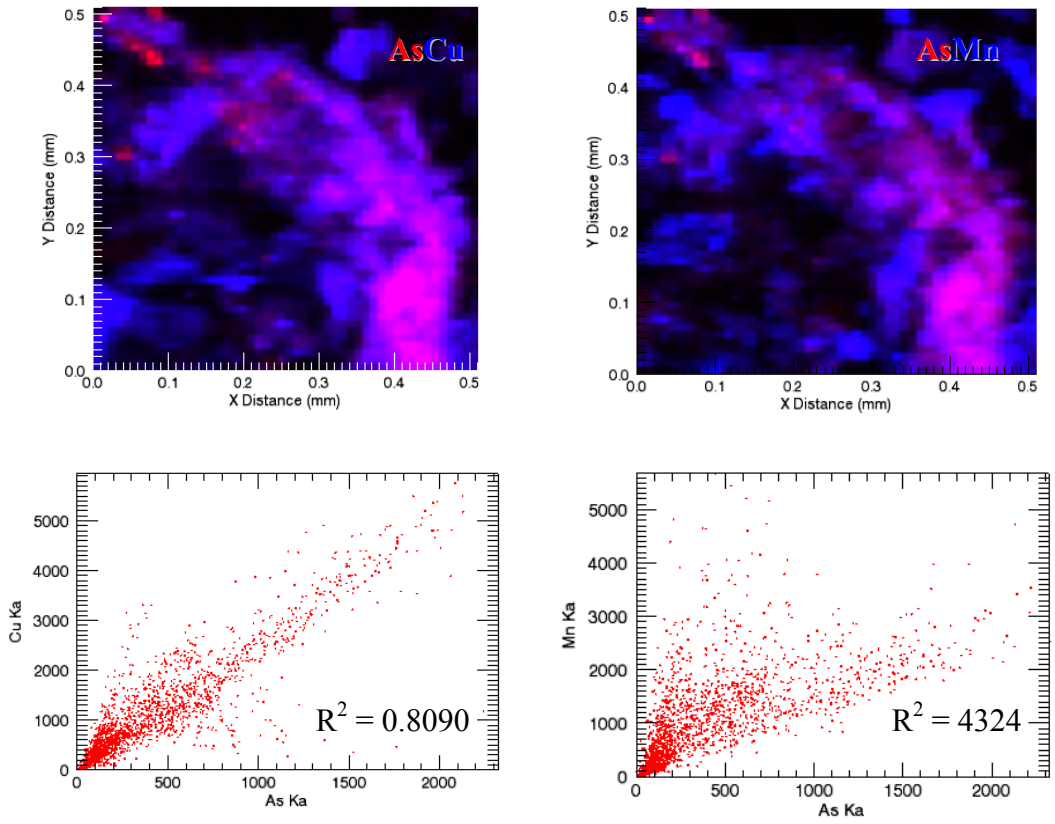

Figure 3.26. XRF maps and correlation plots demonstrating the relationships between As and Cu and Mn in poultry litter stored for one year.

The MCA plot depicted in Figure 3.27 shows MCA plots for both Spot1 (the lighter line) and Spot 2 (the darker line). Both spots exhibit similar elemental trends with Spot 2 having more of all of the elements. However, Spot 2 has significantly more Fe than Spot 1.

SEM micrographs are depicted in Figure 3.28. The litter appears to be slightly more crystalline or ordered in the micrograph on the right. This may prove that the litter is becoming more structured as it ages and dries.

There are additional maps and XANES scans of poultry litter stored for one year in the appendix of this chapter (3.A.11-14). Those scans shown in 3.A.11 and 12 show even more inorganic degradation products than the map shown here. This demonstrates the sample heterogeneity found within the litter samples.

Storing poultry litter for one year causes breakdown of ROX into inorganic and organic degradation products. Similar to other As speciation studies, the XANES studies suggests that ROX does degrade into inorganic and organic degradation products. There is a large amount of both oxidized and reduced As species present in these samples. The XANES analysis illustrates that simply moving a few microns within a litter particle can show differences in As speciation.

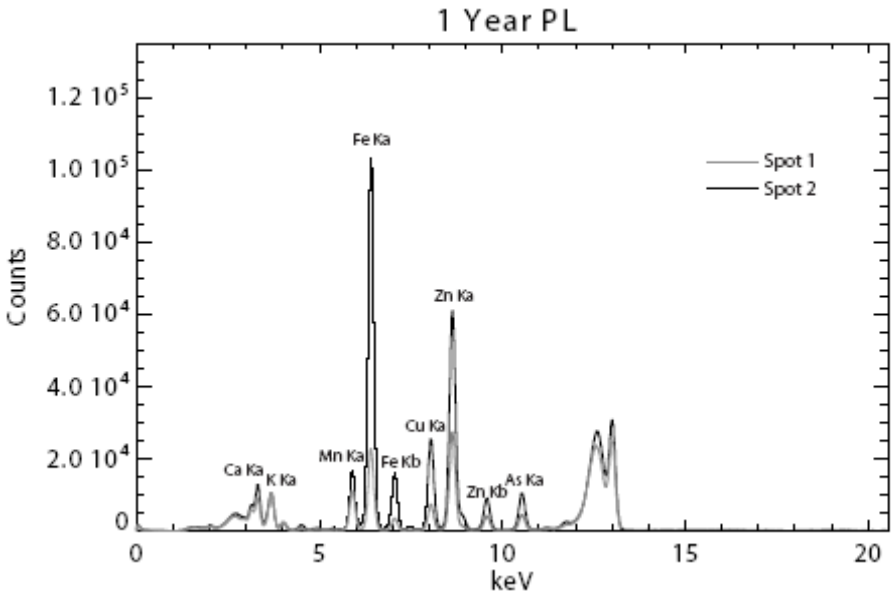

Figure 3.27. MCA plot of elemental distribution of spot 1 and 2 (Figure 3.22) for poultry litter stored for one year.

Figure 3.28. SEM micrographs of 1 year poultry litter.

3.3.5. A Summary of As Speciation of Poultry Litter using X-ray Absorption Spectroscopy.

In summary, As speciation in poultry litter may be time dependent. As speciation changed over the 44 days the litter remained in the poultry house. Once the litter was removed from the poultry house and stored for up to one year, more changes in As speciation were observed. Figures 3.29 and 30 depict the overall changes in As speciation with time. As speciation for the litter samples collected from the poultry house are predominantly oxidized and organic As species with some reduced As. However, after six months of storage the general trend shifts to more reduced As species with some organic and oxidized species remaining in the sample. As(III) constitutes almost as much if not more than the organic components. After the poultry litter is stored for one year, a portion of the As in the samples is reduced As(III) and As bound to S. One of the more notable and noticeable trends is the increase in the amount of As(V) in the litter samples. There is more oxidized As present at the end of the storage period than

there was in the beginning (Figure 3.30). As the litter dried, it was subjected to more oxidizing conditions. Therefore, these results indicate that there are trends in As speciation as length of storage time increases, indicating that this may need to be considered when managing poultry litter in agricultural settings.

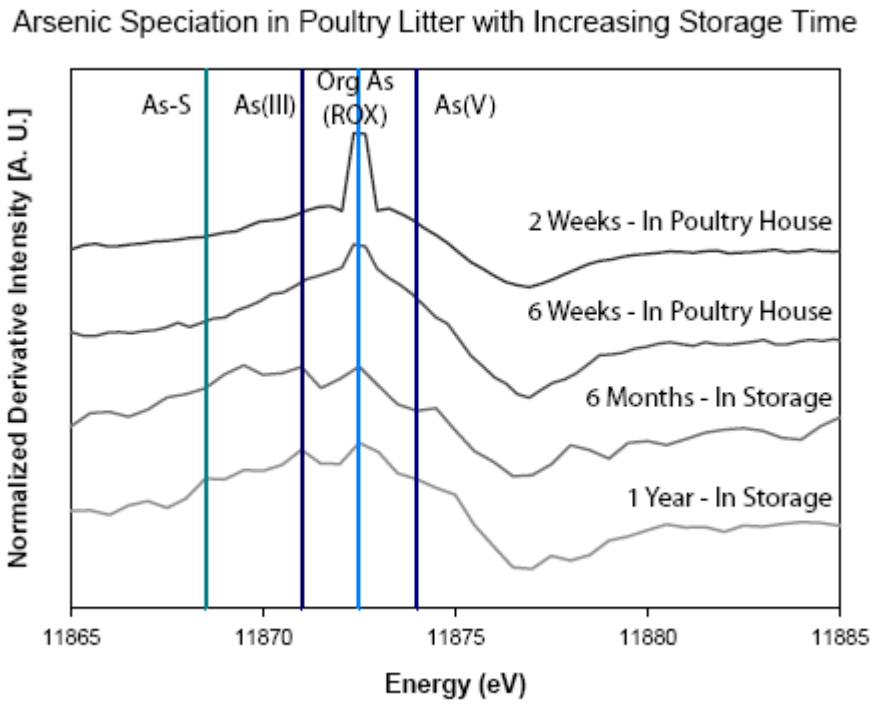

Figure 3.29. As Speciation of poultry litter with increasing storage time. One notices a broadening of the peaks indicating a mix of arsenic species as time increases. The two and six week poultry house litters contain primarily organic As species, while those stored for six months and one year in storage have multiple As species. There is a mix of both organic As, oxidized As(V), and reduced As species.

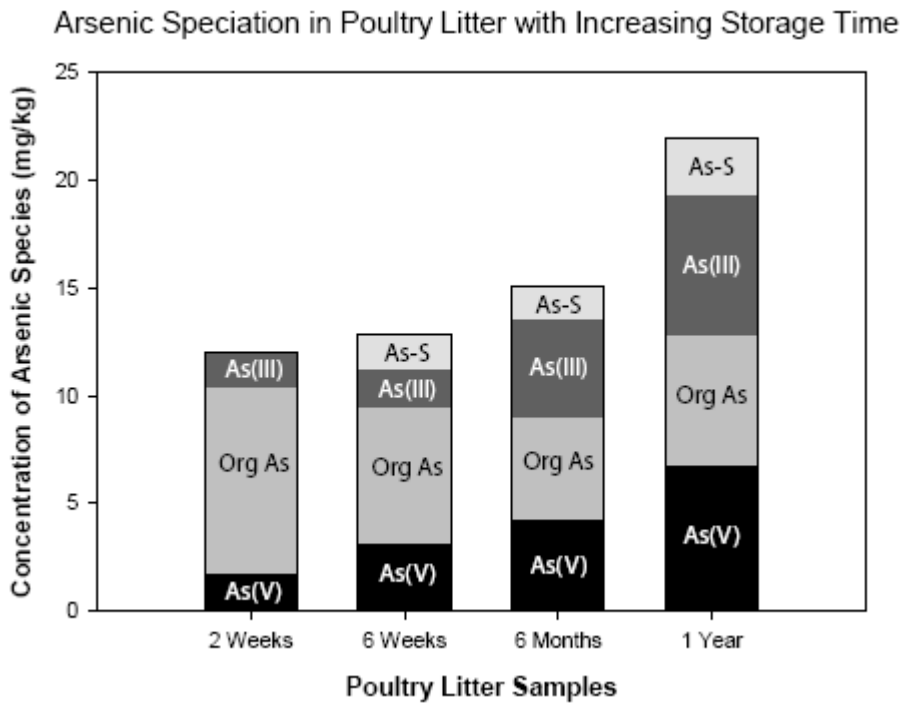

Figure 3.30. Linear Combination Fitting of As spectra from Figure 3.29 depicting As speciation of poultry litter with increasing storage time. One notices a progression away from roxarsone (early time points) towards reduced and oxidized As species. This indicates a change in oxidation state.

3.3.6. Investigation of Crystalline Compounds Using Synchrotron Based X-ray Diffraction.

X-ray diffraction has been used for years to determine the identity of crystalline compounds in a variety of materials. It is commonly used in soils in order to identify clay mineral composition. Similar to the XANES analysis conducted in the earlier sections, two different forms of synchrotron based X-ray diffraction were used to identify crystalline materials in the litter samples. Bulk X-ray diffraction was collected at beamline 11-3 at the Stanford Synchrotron Radiation Light Source in Menlo Park, CA. The bulk X-ray data provide an overall picture of the crystalline compounds found in the

litter samples. Microfocused XRD was collected at beamline X26A at the National Synchrotron Light Source in Upton, NY.

Similar to XANES analysis, the data analysis is only as good as the database or standards used as a reference tool to identify crystalline compounds present in the sample. Since ROX is not a mineral commonly found in XRD databases, a ROX standard had to be collected. Many crystalline compounds have already been described in the literature, and in many cases crystallographic information files (CIF) are available. These CIF files contain peak information for the compound. The CIF files can then be used in addition to the already established database to identify compounds. A CIF file for ROX did exist in the literature (Hunter, 1995). Figure 3.31 shows the XRD pattern (blue line) and CIF (vertical red lines) for ROX. The CIF file peaks should line up with the peaks taken from the sample or in this case ROX. Since, the sample and the CIF match we can now use this information to identify ROX in the unknown samples.

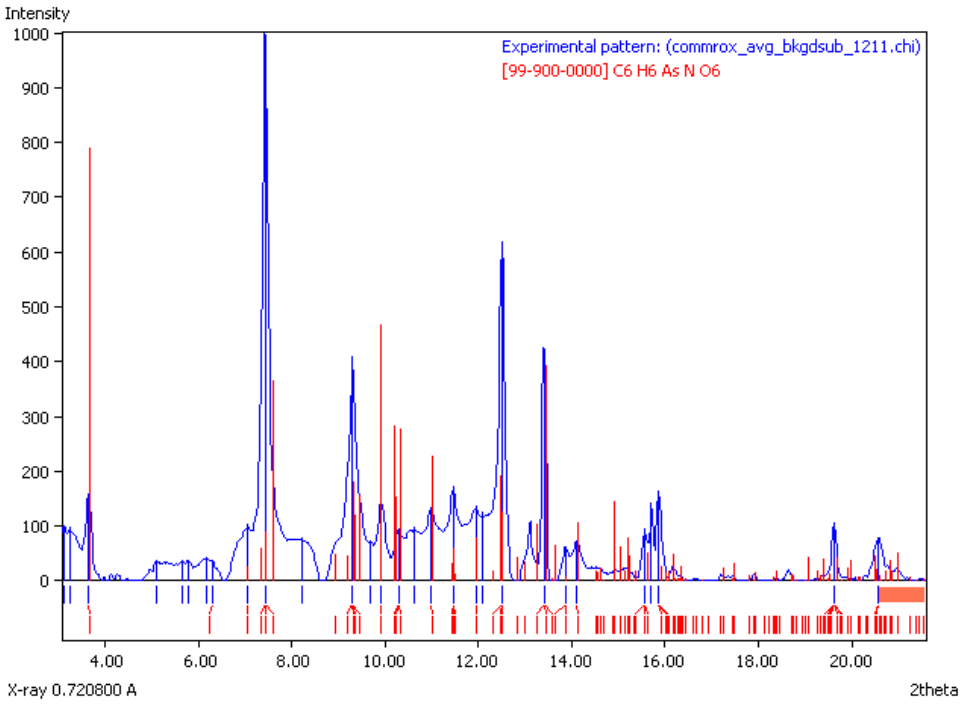

Figure 3.31. X-ray diffraction pattern for the roxarsone fed to the birds (blue line), and the crystallographic information file (red lines) taken from Hunter et al., 1995.

Figure 3.32 shows both the collected two dimensional CCD pattern collected at the beamline and Figure 3.37 shows the integrated 1 dimensional line plot. Looking first at the CCD patterns in Figure 3.32, one sees that sample A has a bright center with a few bright flecks within the red circle, while Sample B has a bright center with many flecks of bright light. These flecks or spots are part of a broken ring structure. A highly regular and crystalline compound would produce a pattern with complete concentric circles. The more rings or flecks of light, the more crystalline the material is. Sample A is a litter sample taken from within the poultry house, while sample B was poultry litter stored for one year. One sees that as the poultry litter ages it becomes more crystalline. This most

likely means that as the litter ages, the compounds in the sample may become less soluble or bioavailable.

The identity of the compounds in the litter samples are determined by examining the 1D line plots (Figure 3.33). The litter sample taken from within the poultry house shows two pieces of information. First, it has many peaks that are indicative of quartz. At first glance, these results seem odd since these birds were grown in a cement floor, but after some investigation it turns out that silicates are often fed, in the form of bentionite, as part of a bird's dietary regime in order to serve as a lubricant {Quisenberry, 1968}. Bentonite is a 2:1 clay mineral composed mostly of montmorillonite. Silicates continue to turn up in most litter XRD samples. The other piece of information gathered from the first sample was that ammonia bearing minerals are beginning to form while in the poultry house. There were not enough peaks present to determine the exact identity of the mineral. However, the peaks that were present were indicative of either struvite ($NH_4MgPO_4 \cdot 6H_2O$) or arsenstruvite ($NH_4MgAsO_4 \cdot 6H_2O$). In comparison, the majority of the peaks for the stored litter indicates an overwhelming presence of arsenstruvite. This is an interesting development. Struvite, the phosphate bearing analog of this mineral, has been found in dairy, sheep, and recently poultry manures. This compound is considered to be a slow release P compound. In many cases, land owners are trying to promote the formation of this compound in the manure in order to reduce runoff of P during land application of manure. If the formation of this ammonia bearing mineral causes phosphate to become less soluble, then it should have the same affect on the arsenate

mineral. A recent study by Hunger et al (2008) showed that struvite formed during storage of poultry litter materials.

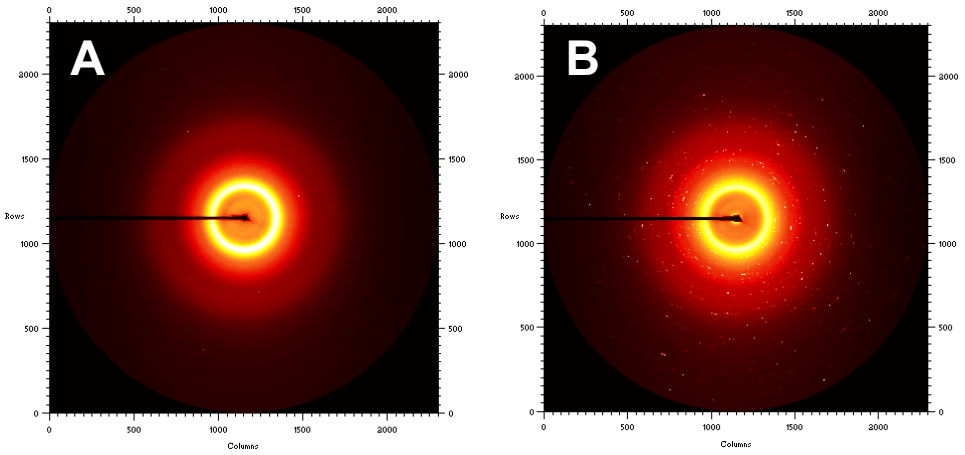

Figure 3.32. Bulk XRD CCD pattern collected of poultry litter taken from within a poultry house (A) and after one year in storage (B). The bright spots in Figure B indicate that stored litter contains more crystalline materials/compounds.

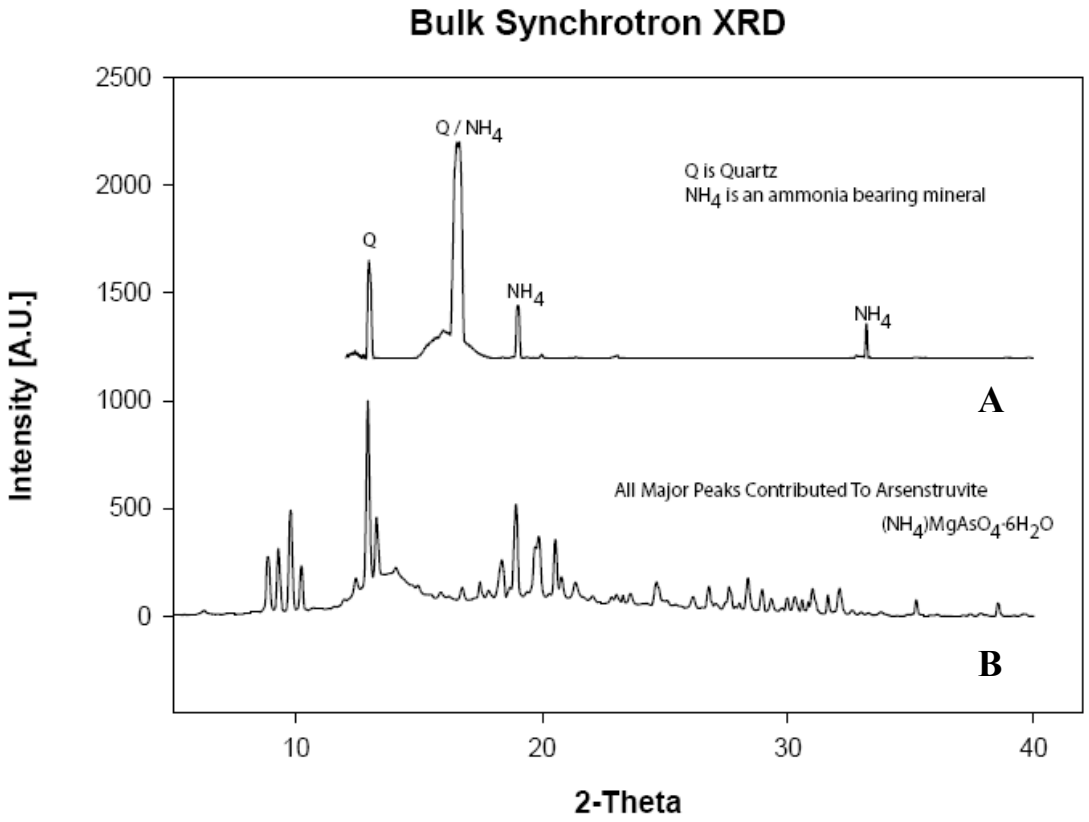

Figure 3.33. Bulk XRD spectra of poultry litter taken from the house and after one year in storage. Line A indicates the presence of silica and the initial formation of ammonia bearing minerals, while line B indicates a more crystalline ammonia bearing mineral. This shows that the litter becomes more crystalline as it is stored.

X-ray diffraction can also be collected at the micro-scale. X26A at the NSLS also has the ability to collected X-ray diffraction at a given point on an XRF map (similar to how the XANES scans are collected). XRD patterns were collected at most places where XANES scans were collected. The μ-XRD data provided even more information about the As and other crystalline compounds present in the litter samples. Figure 3.34 shows a few representative scans from the poultry litter samples. The first 1D pattern, labeled "poultry house sample" was collected on a litter sample taken at the end of the poultry

house study (6 weeks). Unlike the bulk XRD data, one sees that ROX is present in this sample. The peaks are labeled with letters, the letters correspond with a given mineral explained in the legend. The letter 'R' indicates all peaks that are representative of ROX. Oxalate is also found in the poultry house sample, along with a silicate mineral. Poultry litter stored for 6 months also shows struvite formation. There is also some Zinc Nitrate minerals in the sample. The poultry litter stored for 1 year also shows evidence of struvite, and for the first time we see arsenstruvite formation. These results indicate that although struvite may form at an earlier stage, the arsenstruvite may require a longer period of time or perhaps different environmental conditions to form. There are also many peaks indicative of potassium nitrate present in the one year poultry litter sample.

It is possible to use X-ray diffraction to determine As speciation in environmental samples. The data provided by the XRD supports the XANES data. None of the μ-XRD patterns for the six month or one year stored samples indicated the presence of ROX. Therefore, although the XANES data demonstrates that it can persist, it may not be as crystalline or as dominant as it was in the poultry house. The presence of phosphate and nitrate minerals is not surprising considering the large amounts of P and N found in litter materials.

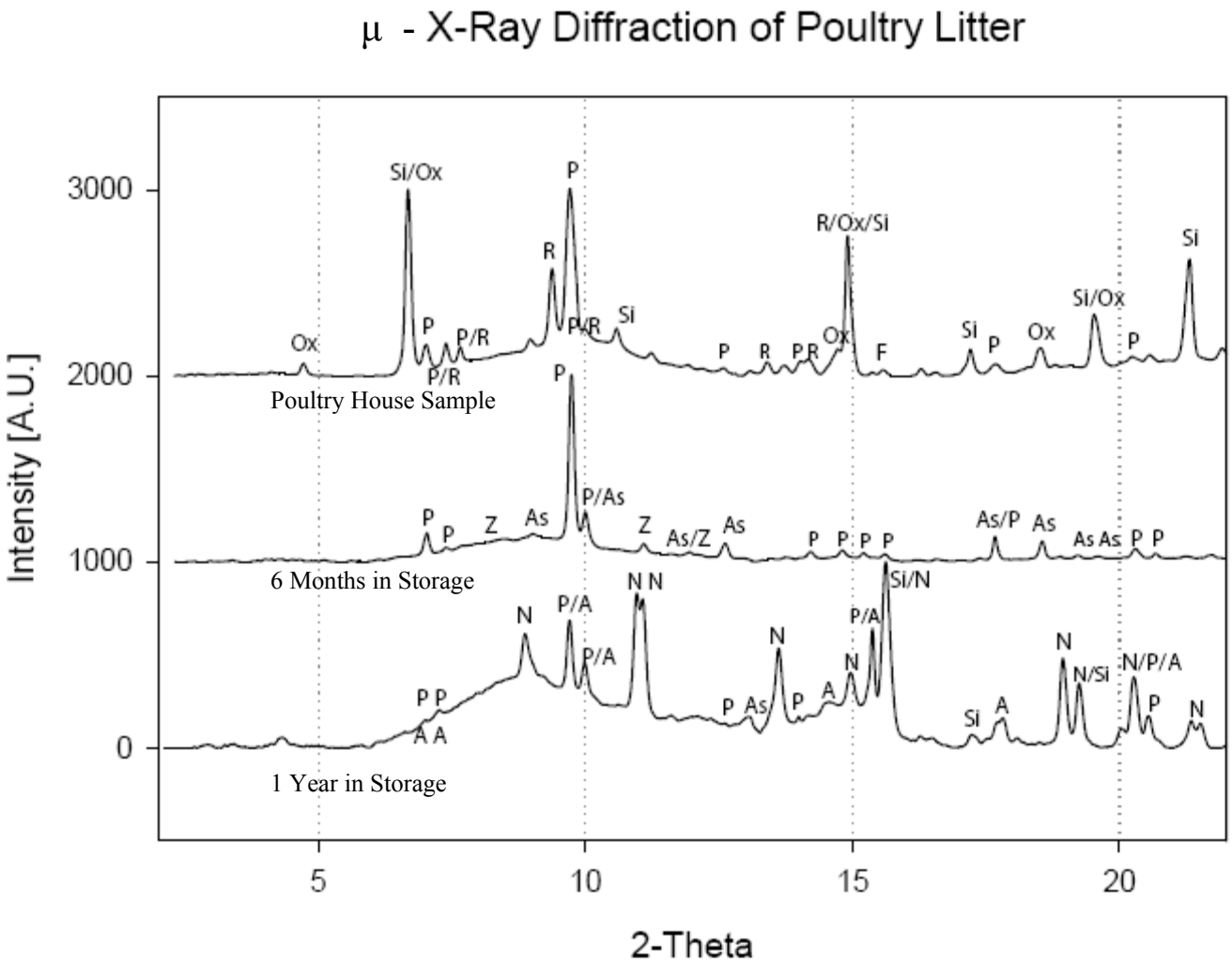

Figure 3.34. μ-X-ray diffraction of poultry litter with increasing time. One notices a progression away from roxarsone and towards reduced As species (leftward migration) indicating a change in oxidation state.

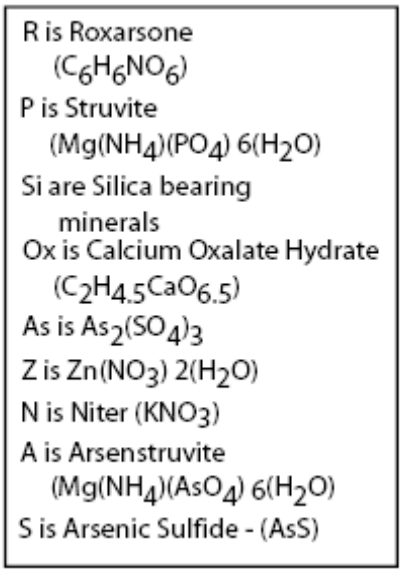

Figure 3.34 (cont.)

3.3.7. Arsenic Speciation of Poultry Litter using Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometry.

Liquid chromatography coupled with ICP mass spectrometry can be used to separate out different arsenic species in a liquid sample or extract by mass. X-ray Absorption Near Edge Structure (XANES) spectroscopy and X-ray Diffraction (XRD) are both used to examine arsenic speciation or chemical composition of a solid material, while LC-ICP-MS provides speciation in a liquid sample. The first step was to run the individual As species: As(III), As(V), DMA, MMA, ROX, HAPA, and p-arsanilic acid (p-ars). This was done in order to determine at what time these species would separate out on the column. It was found that As(III) would come out first at a time of 1.81 minutes, followed by DMA, MMA, ROX/P-ars, HAPA and As(V) at 2.66, 3.61, 4.29,

4.88, and 5.68 minutes, respectively. Figure 3.35 is a chromatogram of the six As species analyzed. ROX and P-ars acid came out at the same time, therefore making it impossible to determine one from the other. Changing the pH of the eluent solution, or flow times may allow us to separate these species. Once this information was determined, a series of mixed As standards were run for calibration purposes.

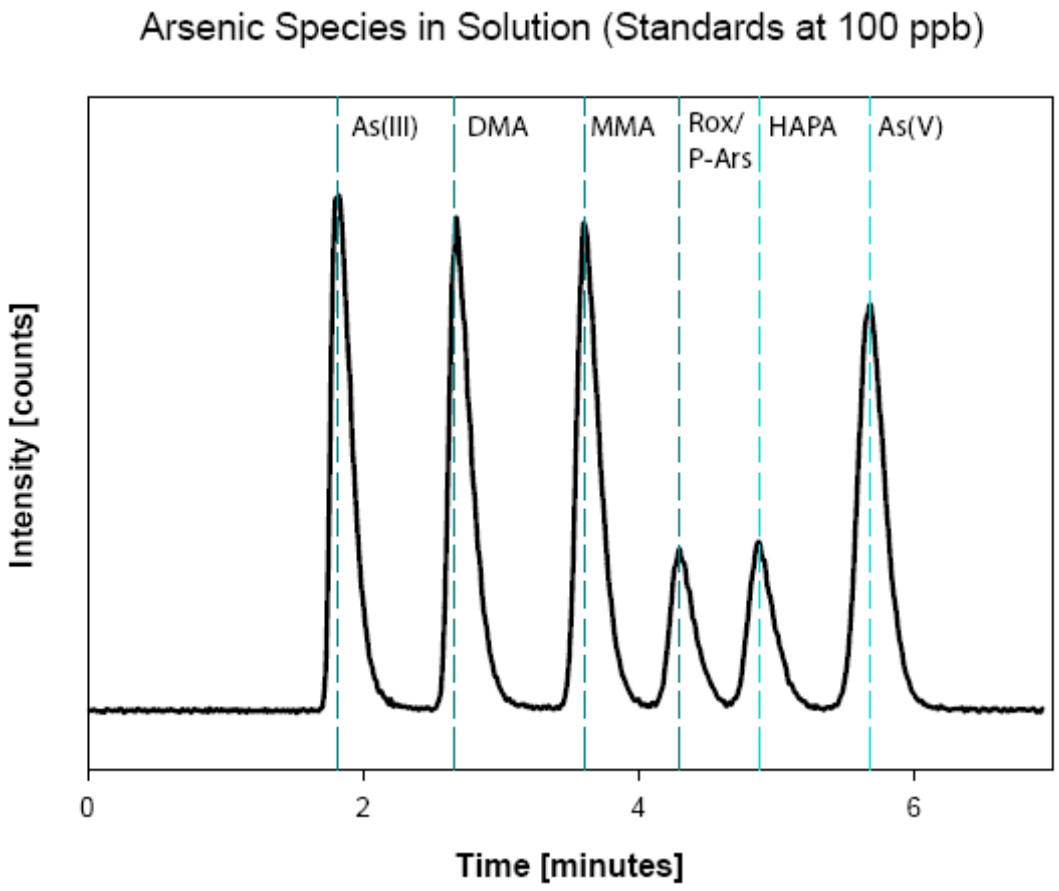

Figure 3.35. Chromatogram of arsenic species: Arsenite (As(III)), Dimethylarsenate (DMA), monomethylarsenate (MMA), Roxarsone/P-arsanilic acid (Rox/P-ars), 4-hydroxy 3-amine arsenic acid (HAPA), and Arsenate (As(V)).

The poultry litter samples were extracted with DI H_2O, in order to represent the water soluble fraction of the poultry litter. This extract was 20-fold diluted and then injected into the LC-ICP-MS. The results are depicted in Figure 3.36. The liquid extract data is similar to the XANES data. Discrepancies can be attributed to the difference between examining a solid sample and a liquid extract. Oxidized arsenic in the form of As(V) and DMA are found in all poultry litter samples. The percent and amount of each form of arsenic can be found in Tables 3.4A and B. A considerable amount of reduced arsenic is found in the six month sample and some in the one month sample. These samples exhibited the most As(III) in the bulk XANES analysis as well. The litter samples from one-six months also have the lowest Eh values. However, after one year in storage there is not extractable As(III) present, but almost 90% of the extractable As is in the As(V) form. As speciation does not change much between the two poultry litter samples taken from in the poultry house. It is interesting to note that we do not find extractable ROX in most of the samples. The most predominant organic As form is DMA.

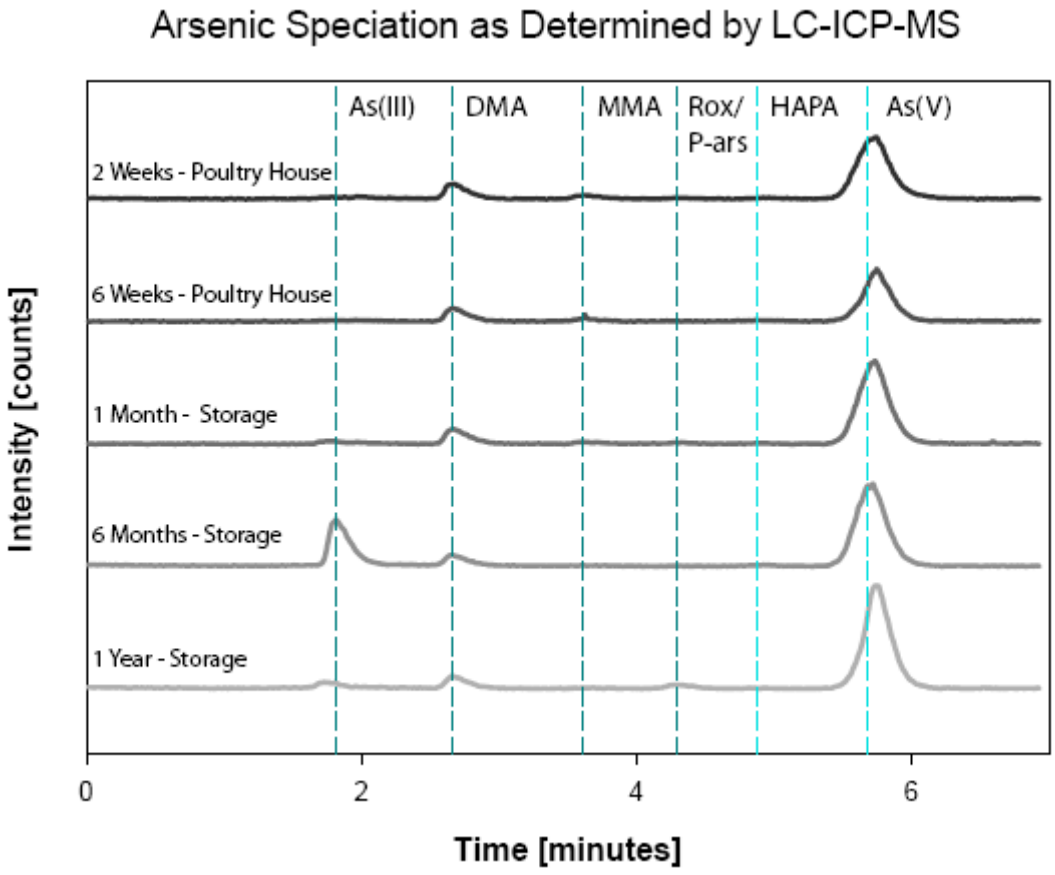

Figure 3.36. Chromatograms of water extractable As species from poultry litter. As(V) is the most dominant water extractable As species in all litter samples.

There are differences between the XANES and water extractable As depicted in Figure 3.36. These differences can be attributed to examining a solid and liquid sample. The liquid sample is only a fraction of the total available As. The liquid extractable As may also be an artifact of the extraction method. These results are similar to other liquid PL As speciation studies. However, the XANES are similar to the findings of Arai et al. (2003) who investigated As speciation in PL using XANES.

Tables 3.4.A and B. Table 3.A shows the percent that each As species makes up the total water extractable As. Table 3.B. shows the concentration in parts per billion of each of the water extractable As species.

A

Percent Arsenic Species in Poultry Litter as Determined by LC-ICP-MS

Sample	As(III)	DMA	MMA	Rox/P-ars	HAPA	As(V)
2 Week	--	12.2	4.4	--	0.4	82.9
4 Week	--	16.7	3.4	--	0.4	79.5
1 Month	1.5	11.9	--	--	--	86.6
6 Month	25.6	5.9	--	--	0.2	68.4
1 Year	--	7.9	--	2.4	0.0	89.6

B

Concentration (ppb) Arsenic Species in Poultry Litter as Determined by LC-ICP-MS

Sample	As(III)	DMA	MMA	Rox/P-ars	HAPA	As(V)
2 Week	--	1855.2	674.3	--	59.6	12647.0
4 Week	--	1727.4	355.3	--	40.9	8222.8
1 Month	259.1	2051.5	--	--	--	14895.9
6 Month	5727.7	1311.3	--	--	46.1	15305.8
1 Year	--	1444.9	--	445.9	16.2	16490.5

* indicate samples taken from in the poultry house, all others were samples taken during storage of litter.

3.4 Conclusions.

The addition of arsenic to poultry feeds is controversial in many countries throughout the world. Europe has completely banned the practice to minimize public concerns about ingesting As. Although diminishing in use, the practice is still common in the United States. The impacts of this dietary supplement and its subsequent effects on the environment are important in evaluating effects on human and ecological health.

The results from this study indicate that ROX and a series of both organic and inorganic degradation products are found in poultry litter. It is difficult to say from these studies if the bird is solely excreting ROX, since the litter collected from the poultry house would remain in the house for a period of time. For litter 2 (days 17-31),

essentially the excreta was in the poultry house for a week and half and microbial activity may have caused ROX degradation. The results do indicate that poultry litter taken from the house after 44 days includes a variety of As species that if land applied at this time could possibly take a number of routes in water and soil environments.

The storage portion of this study provides data that shows a progression towards the formation of inorganic As species taking place through the course of one year. The XANES, XRD, and liquid extractions show a number of As species are present at any given time. More reduced As(III) is found during XANES analysis than with the other two techniques. If land application of poultry litter occurred after 1 year of storage, the majority of the As being applied would be in the inorganic form (As(III) or As(V)). As(III) is more mobile then As(V) and ROX. Also, after a few months to a year in storage, the litter becomes more crystalline indicating that some of the more soluble elements like As, P, and N may become less water soluble. The percentage of water soluble As did decrease with storage time. The formation of crystalline compounds like struvite, arsenstruvite, and zinc and potassium nitrates are examples of less soluble forms of environmentally relevant elements that are formed as litter is stored. This is the first study to document the formation of arsenstruvite in poultry litter materials. This compound may have a significant impact on the amount of soluble As found in the poultry litter, and therefore can impact the amount of As released into the surrounding ecosystems. If soluble As is a concern for landowners, then perhaps growers should store the litter for at least one year before land application of the poultry litter materials.

The storage study did not include turning over the litter to allow aeration; however there are definitely places in the house and composting piles that are not regularly turned over or exposed until land application. If aeration or turning of the litter had occurred, different As species would most likely be present in the litter. Turning the pile over would create a more oxidizing environment in the litter, which could result in more As(V) species present in the litter.

Trace metals such as Cu, Mn, Zn, Fe and Ni are widely distributed in the litter samples. A number of these metals, if found at high enough concentrations can be toxic to both humans and plants, and therefore may need to be incorporated into best management plans. The strong correlations found between As and the divalent trace metals (in particular Cu) are worth examining, since these relationships are consistent throughout the data. In many of the six month and one year litter samples areas containing high concentrations of As and Cu, ROX was identified.

As was discussed in Chapter 2, the presence of oxyanions like phosphate can greatly reduce the soils' ability to retain even the more resilient forms of As. Since the concentration of phosphate is often 1000 times that of arsenic, it may limit the amount of As sorption occurring in the Delaware soils. However, elevated concentrations of As have not been found in the limited groundwater and surface water data that are available. The Delmarva Peninsula would greatly benefit from more extensive ground and surface water data.

3.5 Appendix for Chapter 3:

Tables 3.5.0. Total and Water Soluble Values in Poultry Litter

Table 3.A.1. Total trace metal concentration in poultry litter.

Litter Sample	Sample Location	As (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Ca (mg kg^{-1})
2 Week	Poultry House	12.0 (5.2)	66.6 (39.2)	127.4 (97.2)	91.3 (54.8)	362.2 (238.6)	6002.5 (1707.1)
6 Week	Poultry House	13.0 (1.1)	92.6 (23.9)	338.7 (20.5)	235.5 (41.2)	404.8 (108.1)	11302.2 (616.5)
1 Months	In Storage	19.8 (4.1)	97.2 (17.1)	458.8 (55.8)	1314.3 (671.2)	385.0 (46.2)	13598.9 (1618.0)
2 Months	In Storage	22.4 (4.7)	133.1 (9.8)	568.2 (71.4)	1225.1 (222.3)	490.5 (52.0)	19213.4 (1893.1)
4 Months	In Storage	21.2 (1.0)	116.9 (10.4)	523.4 (14.1)	1450.0 (407.7)	441.2 (97.7)	14802.0 (1364.2)
5 Months	In Storage	19.0 (4.1)	104.2 (33.1)	474.5 (99.6)	930.4 (238.9)	406.6 (16.2)	14836.0 (4999.2)
6 Months	In Storage	15.0 (3.1)	118.0 (3.8)	452.4 (18.4)	833.7 (45.3)	453.2 (14.7)	14964.7 (902.6)
1 Year	In Storage	22.0 (8.8)	112.7 (4.9)	395.8 (30.8)	739.3 (72.1)	405.5 (23.9)	13053.3 (1090.2)

Numbers in parentheses indicate standard deviations using n=3.

Table 3.A.2. Water Soluble trace metal concentrations in poultry litter

Litter Sample	Sample Location	As (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Ca (mg kg^{-1})
2 Week	Poultry House	12.8 (1.5)	23.5 (3.1)	9.3 (2.3)	32.0 (5.0)	21.9 (2.2)	291.1 (28.5)
6 Week	Poultry House	7.5 (1.5)	26.6 (1.6)	14.3 (1.2)	35.2 (2.2)	42.6 (1.2)	282.6 (17.8)
1 Months	In Storage	8.3 (2.8)	14.2 (6.3)	13.2 (8.2)	16.1 (11.1)	7.4 (4.4)	438.0 (294.7)
2 Months	In Storage	7.3 (1.9)	15.2 (5.6)	5.1 (2.3)	15.0 (12.3)	13.0 (14.6)	266.1 (100.0)
4 Months	In Storage	7.3 (1.8)	10.4 (2.0)	6.6 (4.0)	10.0 (5.1)	4.5 (2.0)	298.2 (105.7)
5 Months	In Storage	6.4 (1.7)	10.7 (3.2)	6.5 (4.0)	10.8 (6.7)	4.4 (2.0)	270.1 (113.7)
6 Months	In Storage	7.0 (2.0)	18.4 (11.8)	8.4 (5.6)	22.8 (20.6)	9.5 (8.2)	272.6 (146.5)
1 Year	In Storage	6.2 (2.4)	9.9 (2.7)	10.5 (8.0)	10.8 (6.5)	5.1 (2.5)	347.6 (166.8)

Numbers in parentheses indicate standard deviations using n=3.

Table 3.5.1. Arsenic Standards Used in XAS Analyses

Table 3.A.3. Arsenic standards used in linear combination fitting (LCF). The first column depicts compounds that were commonly found to be fits of the experimental litter scans. The category indicates which As species group the standard belongs to. Note: the As(III) methionine is under the As(III) category because there was not an As-S bond established in solution.

LCF Fit	Standard/ As Compound	Category	Formula
x	Liquid Arsenate	As(V) - Arsenate	$Na_2HAsO_4{\cdot}7H_2O$
	Arsenstruvite	As(V) - Arsenate	$NH_4MgAsO_4{\cdot}6H_2O$
	Conichalcite	As(V) - Arsenate	$CaCuAsO_4(OH)$
x	Calcium Arsenate	As(V) - Arsenate	$Ca_3(AsO_4)_2$
x	Sodium Arsenate	As(V) - Arsenate	$Na_2HAsO_4{\cdot}7H_2O$
x	Roxarsone (Commercial) (3-nitro-4-hydroxyphenly-arsonic acid)	Organic Arsenic	$C_6H_3(NO_2)AsO(OH)_3$
	Roxarsone (Chemical) (3-nitro-4-hydroxyphenly-arsonic acid)	Organic Arsenic	$C_6H_3(NO_2)AsO(OH)_3$
x	HAPA (4-hydroxy-3-aminophenylarsonic acid)	Organic Arsenic	$C_6H_3(NH_2)AsO(OH)_3$
	P-arsanilic Acid (4-amino-phenylarsonic acid)	Organic Arsenic	$C_6H_3(NH_2)AsO(OH)_2$
x	Monomethylarsenate (MMA)	Organic Arsenic	$CH_3AsO(OH)_2$
x	Dimethylarsenate (DMA)	Organic Arsenic	$(CH_3)_2AsO(OH)$
	Arsenic Oxide	As(III) - Arsenite	As_2O_3
x	Liquid Arsenite	As(III) - Arsenite	$NaAsO_2$
	Sodium Arsenite	As(III) - Arsenite	$NaAsO_2$
x	As(III) - Methionine	As(III) - Arsenite	$C_5H_{11}NO_2S$
x	As(III) - Cysteine	AsS-Sulfur	$C_3H_7NO_2S$
x	Realgar	AsS-Sulfur	As_4S_4
	Pararealgar	AsS-Sulfur	As_4S_4
	Orpiment	AsS-Sulfur	As_2S_3

Figure 3.5.2. Additional Poultry Litter Figures

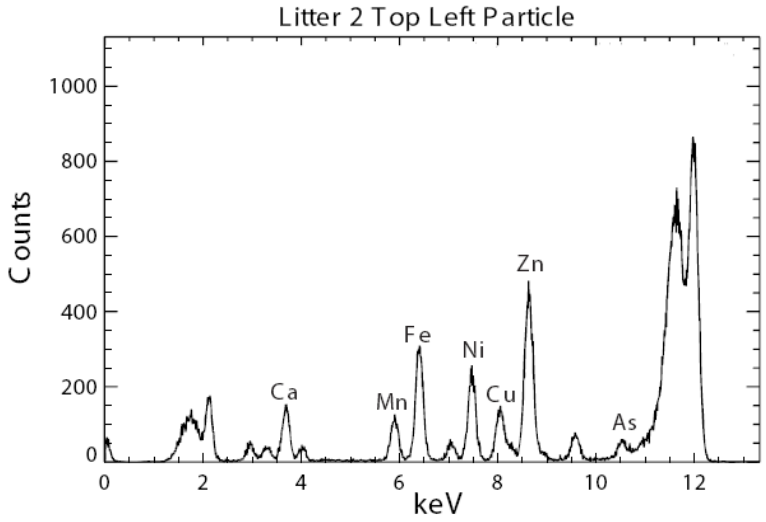

Figure 3.A.1. MCA display of spot 1 from the XRF map in Figure 3.8 of poultry litter 2 (days 17-31). Zn, Cu, Fe and Mn are the elements with the highest concentration at this place on the map. Most As "hot spots" were similar in composition.

The following are data from my proposal defense. The XANES data are presented in a different manner, but they are still relevant and useful.

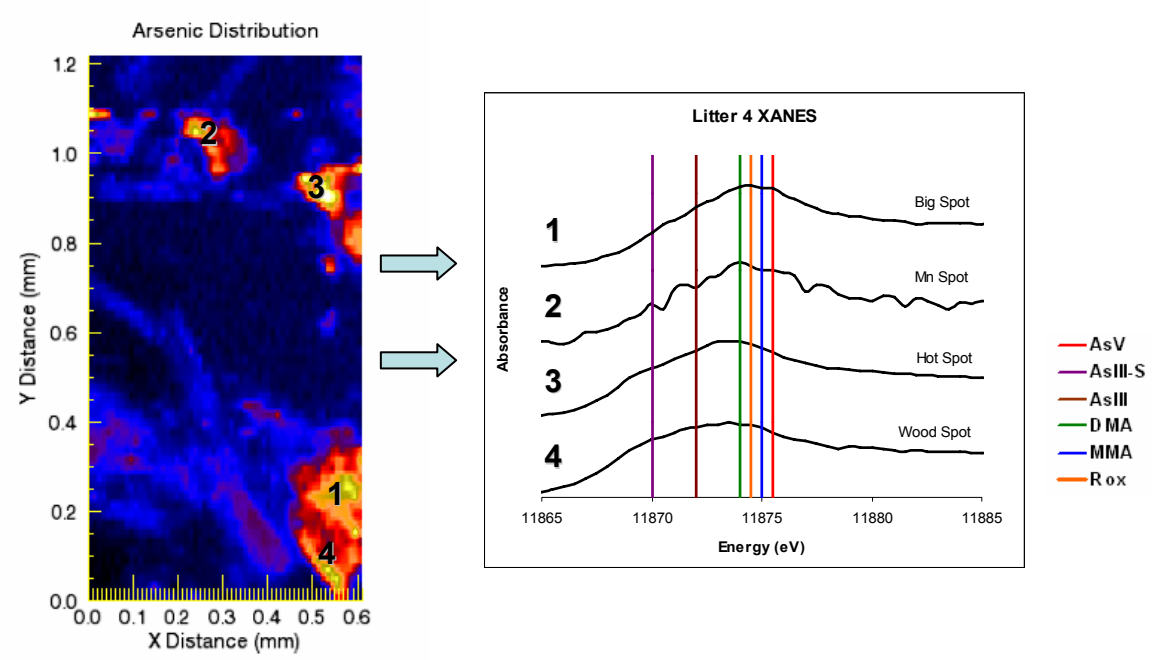

Figure 3.A.2 XRF map of As distribution in poultry litter 4 (days 38-44). XANES scans of four particles within litter 4. Arsenic distribution changes depending on elemental association. Warmer colors (yellow and white) indicate regions of concentrated As, while cooler colors (blues and black) indicate ares of low or minimal As concentration.

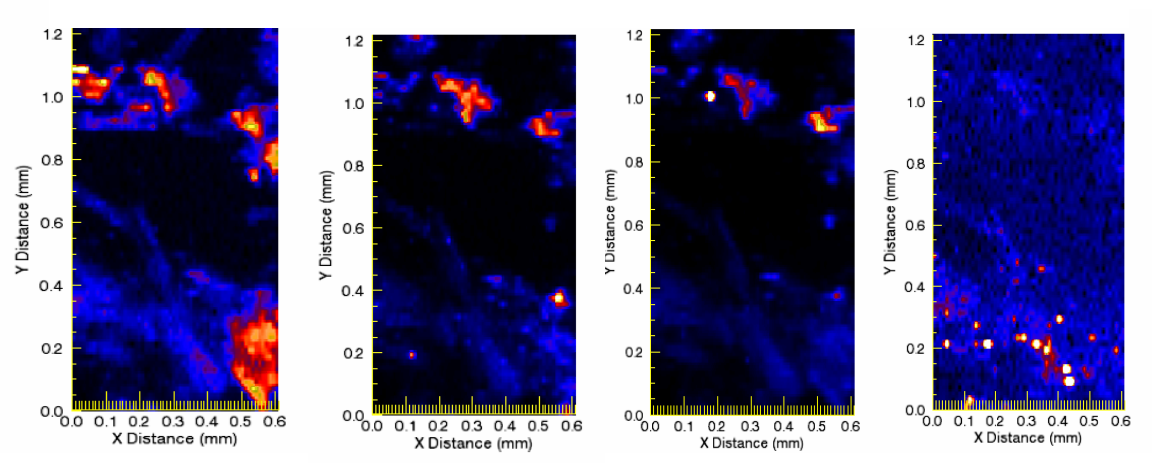

Figure 3.A.3 XRF maps of copper, manganese, zinc, and nickel distribution in poultry litter 4 (days 38-44). When comparing As distribution to the other trace metals, it appears that Cu may be more highly associated with As than with the other metals.

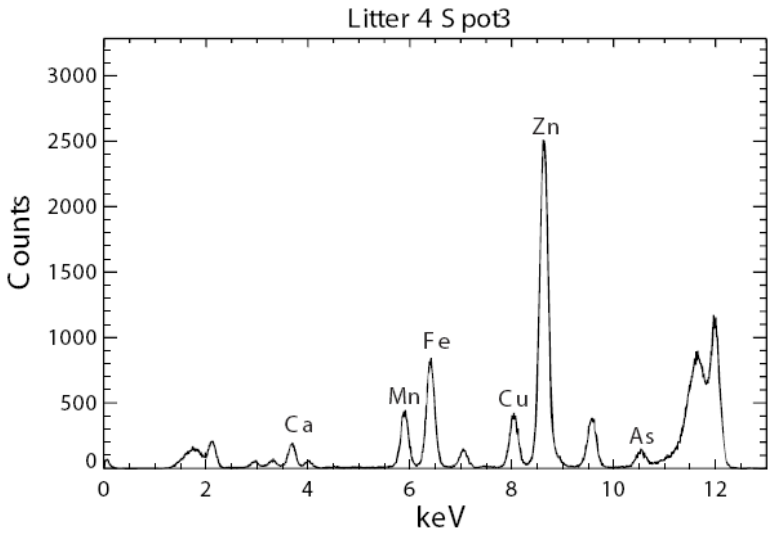

Figure 3.A.4. MCA (Multi-Channel) plot for Litter 4 (days 38-44) spot 3. This shows the elemental distribution at the spot. Allows one to see more elemental concentration associations. As concentration is relatively low in relation to Zn, Cu, or Mn.

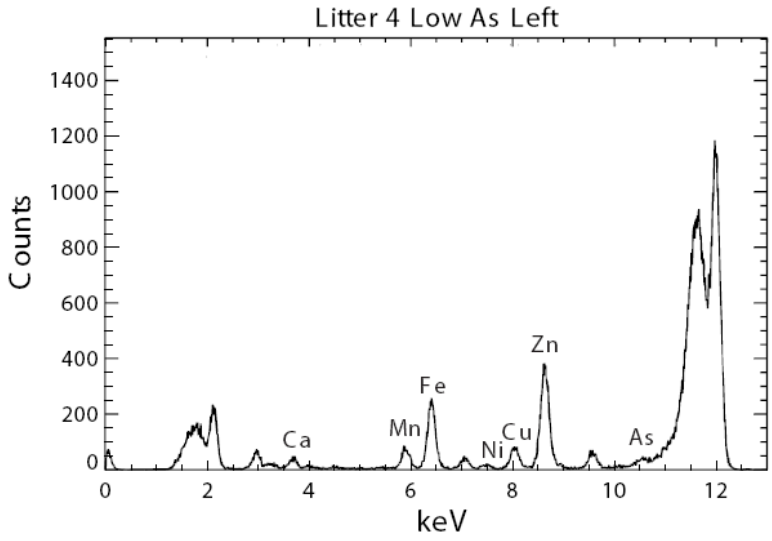

Figure 3.A.5. MCA plot for an area outside of an As concentrated area to give an idea of the other elemental distribution for Litter 4 (days 38-44) in the house. The dominance of the trace metals is seen here. The litter exhibits high Zn, Fe, Mn and Cu values.

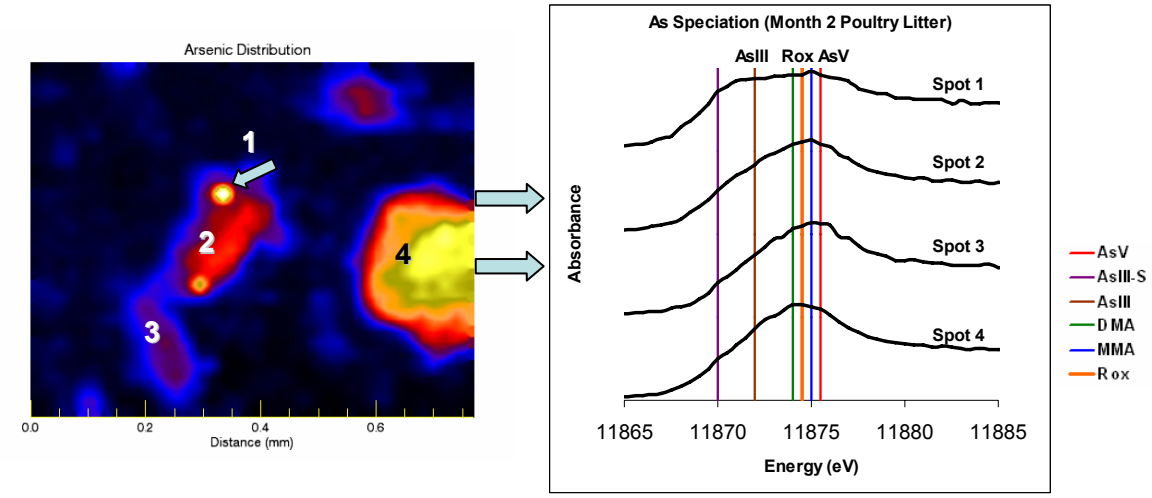

Figure 3.A.6. The XRF map above illustrates the As distribution in poultry litter stored for 2 months. XANES analysis indicates a large variability in As speciation in this sample.

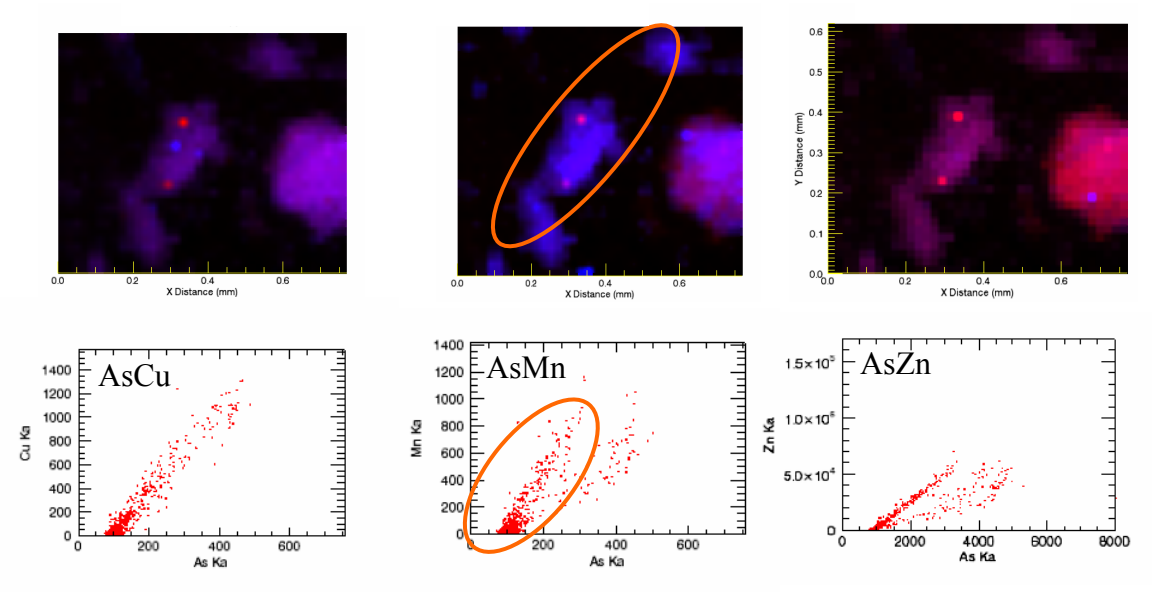

Figure 3.A.7. XRF and correlation plots of As for poultry litter stored for 2 months and three trace metals (Cu, Mn, and Zn).

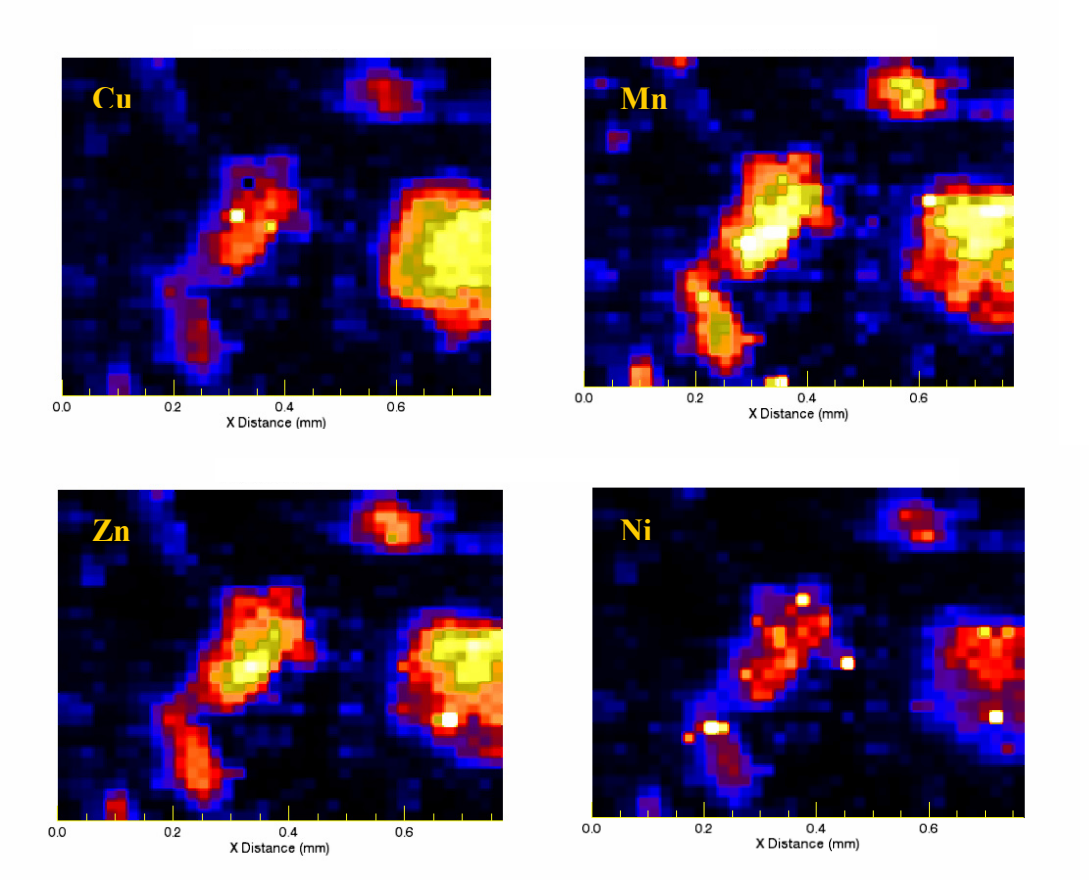

Figure 3.A.8. Trace metal distribution in poultry litter after 2 months of storage. (note Ni map decreased the max value multiplier in order to see Ni distribution)

Arsenic speciation in poultry litter that was aged for 4 months is very similar to that of litter that was stored for 2 months. The major difference being that oxidized As species are found in the litter. More MMA and As(V) are found in these litter samples than the Month 2 samples. This change to oxidizing As species coincides with the decrease in pH and a movement towards less reducing conditions in the poultry litter. The organic As species found in the Month four samples are roxarsone, MMA, DMA, p-arsanilic acid, and again the unidentified "organic reduced" As component. The metal distribution found within these samples is very similar to that found in the previous

samples (Litter 2(days 17-31) and Litter 4 (days 38-44)). The same strong relationships between As and Mn, Cu, and Zn are found. The XANES scans and XRF maps of these litter samples can be found in the appendix at the end of the chapter.

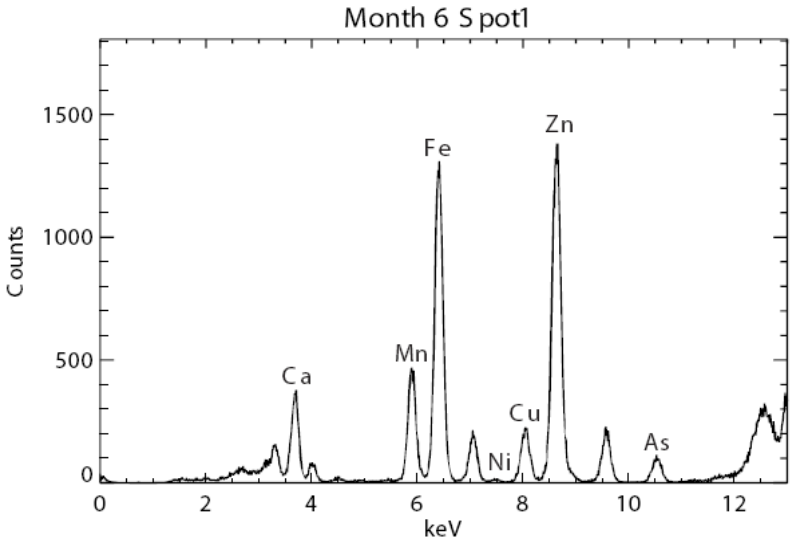

Figure 3.A.9. MCA plot from XRF map from Figure 3.19. spot 1 (6 Months). As and trace metals are found at this spot. Notice that Ni is missing from this spot.

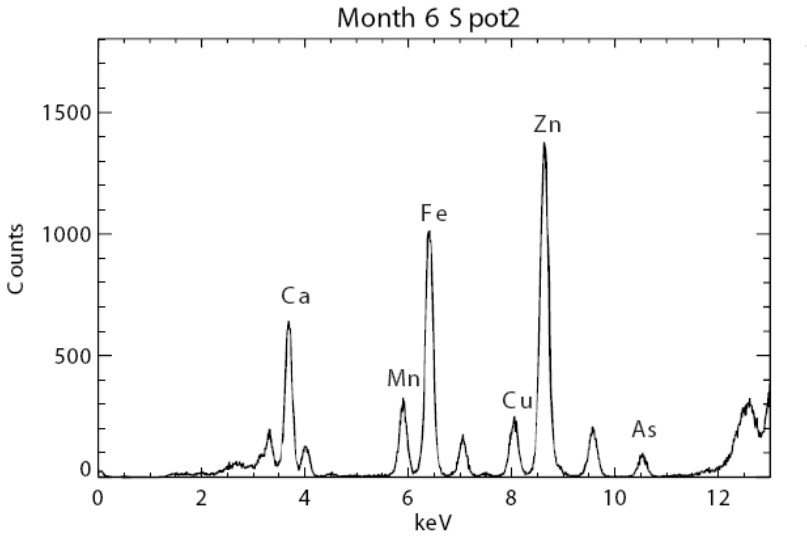

Figure 3.A.10. MCA plot from XRF map 3.19. spot 2. A similar trend is seen between these two spots in the 6 month sample.

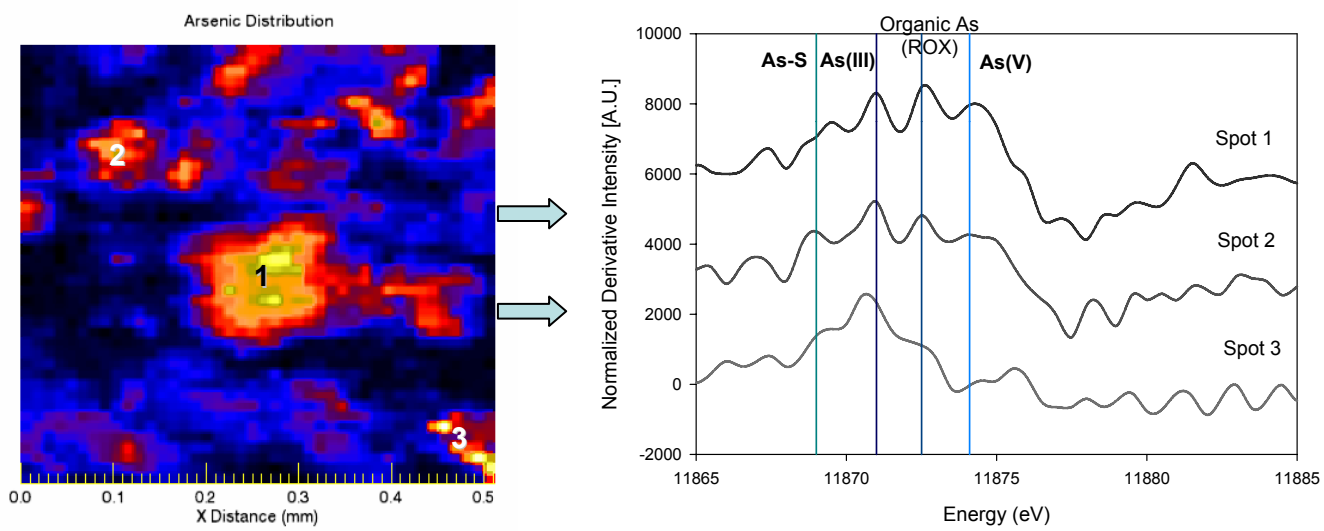

Figure 3.A.11. Arsenic distribution and speciation in poultry litter stored for one year. XRF map located on the left, and XANES scans taken at those numbered locations are on the right. Reduced As species are prevalent in most XANES scans. The map shows warmer colors (yellow and white) which indicate areas of increased As concentration, while blues and black indicate areas of very low or no As concentration.

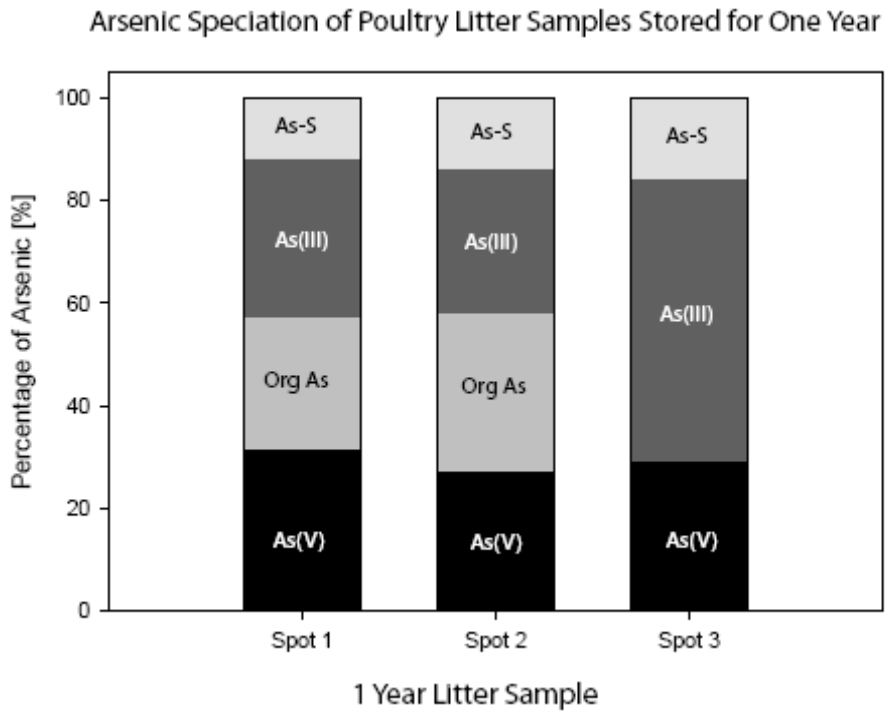

Figure 3.A.12. Linear combination fitting results for the XANES scans in Figure 3.A.11. These results show that arsenic speciation does change from point to point.

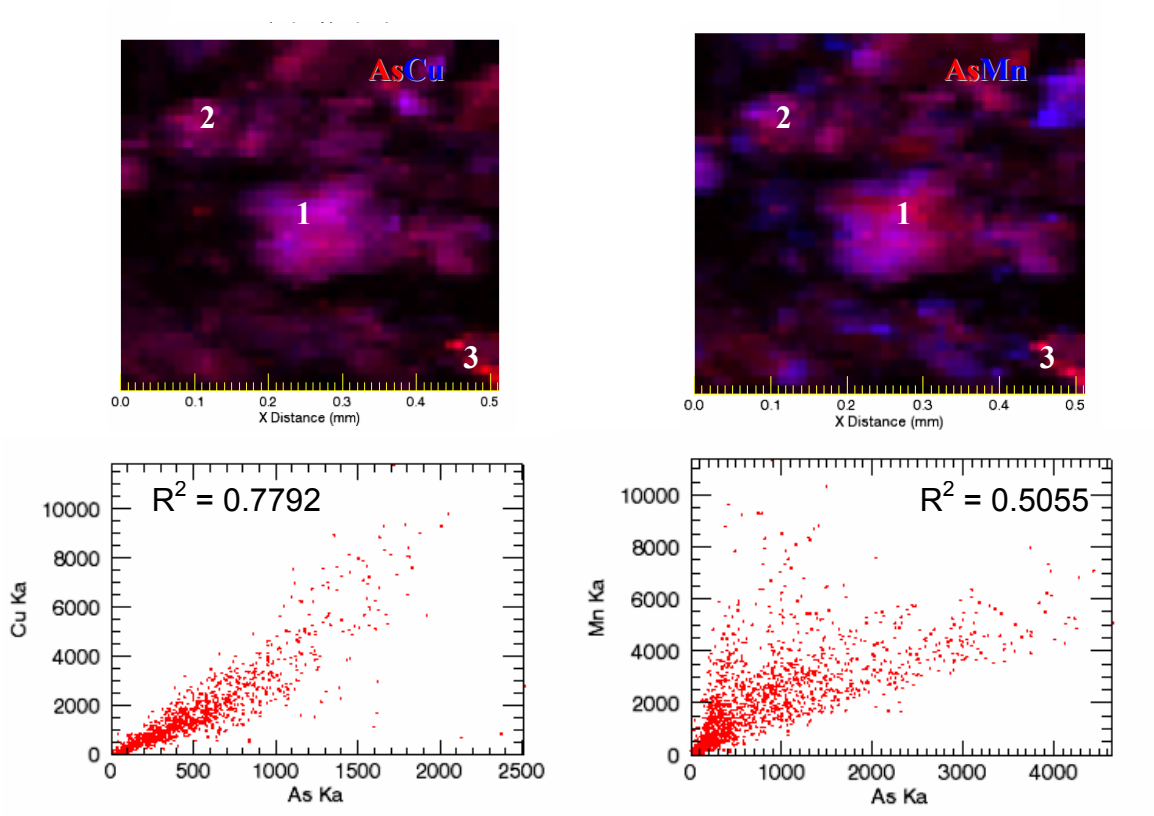

Figure 3.A.13. XRF maps and correlation plots demonstrating the relationships between As and Cu and Mn in poultry litter stored for one year. There is a stronger relationship between Cu and As, than As and Mn.

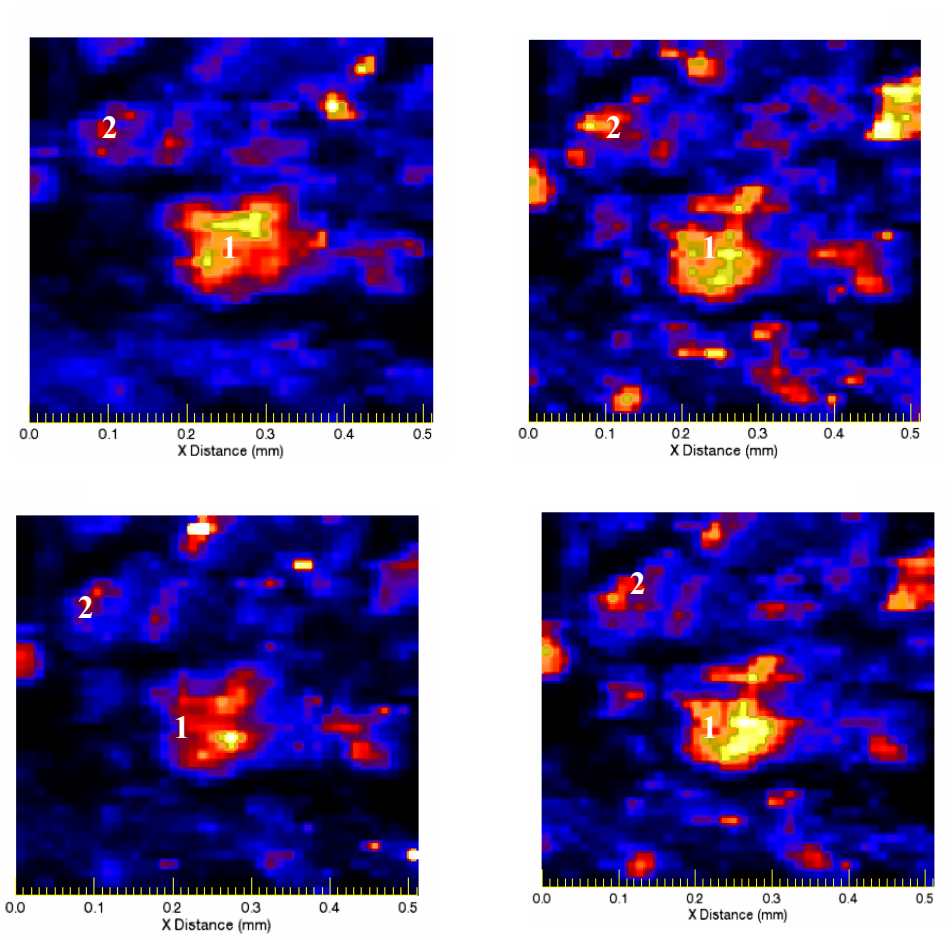

Figure 3.A.14. Trace metal (Cu, Mn, Fe, Zn) distribution in poultry litter samples stored for 1 year. (Note Fe max value multiplier was lowered in order to display Fe distribution)

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Chapter 4

DIRECT SPECIATION AND DIDSTRIBUTION OF DIETARY ARSENIC IN POULTRY EXCRETA AND TISSUES

4.0. Abstract:

Arsenic distribution and speciation in poultry excreta, ileal contents, and poultry tissues were examined using a number of techniques including: X-ray Absorption Near Edge Structure (XANES) spectroscopy, X-ray fluorescence (XRF), X-ray diffraction (XRD), and liquid chromatography – inductively coupled plasma- mass spectrometry (LC-ICP-MS).

Results indicate that As does accumulate in some poultry tissues no matter what form of As is fed to the birds. More total As was found in liver and breast muscle than in feathers and skin. As analyses of breast and liver indicate that all trace metal(loid)s, with the exception of Ca, are evenly distributied throughout the tissues. XANES spectra indicate that reduced As(III) (arsenite) is found in tissue. XANES and XRD analyses show that a mix of As species are found in the poultry excreta. Roxarsone was identified in both ileal and excreta samples by XANES and XRD.

4.1. Introduction:

4.1.0. Background

Arsenic has been used in agricultural and horticultural settings for decades. Poultry litter (a mixture of excreta and bedding material) contains trace elements such as Ni, Cu, Zn, Mn, Fe, and As, the sources of which are growth promoters and dietary supplements added to poultry feed. The total As concentrations in PL vary depending mostly on the concentration of As fed to the birds. Reported As levels range from 0 to 77 mg kg^{-1} (Jackson et al., 1999; Jackson et al., 2003; Moore et al., 1998; Sims and Wolf, 1994; Sims and Luka-McCafferty, 2002; Van der Watt et al., 1994). The most common source of As in poultry litter is 3-nitro-4-hydroxyphenly-arsonic acid (*Roxarsone*, abbreviated ROX), used as feed additives to prevent coccidiosis, increase weight gain and improve feed efficiency (Figure 4.1). The organo-As compounds added to the feed are primarily excreted in the organo-As or As(V) forms (Garbarino et al., 2003; Jackson et al., 2003; Morrison, 1969).

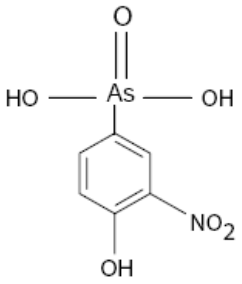

Figure 4.1. Roxarsone skeletal diagram.

The Food & Drug Administration (FDA) demands that animal drugs only be used in accordance with their respective FDA approval guidelines in order to protect animal

and human health. Roxarsone is mixed with a maximum possible value being 2.275 g/lb (0.5%) in basal feeds. The FDA has limited the amount of As in muscle tissue to 0.5 ppm (Vandiver, 2004).

The fate of roxarsone inside of the chicken is not well understood. The chemical environment of the digestive system is important in understanding nutrition and the health of the birds. Digestive enzymes are the primary means by which digestion takes place. They are organic catalysts which speed biochemical reactions (Ensminger, 1992).

The small intestine is comprised of three sections: duodenum, jejunum and the ileum. The jejunum is the site where most absorption takes place (North, 1984). The food from the gizzard enters the duodenal loop, where the food is mixed with bile produced in the liver. The bile is an alkaline substance that breaks down fatty products in the feed, and it serves a neutralizing agent for the digestive juices. The duodenal loop and upper small intestine is where the breakdown of most compounds occurs. In the remaining portion of the small intestine the digestive process is completed and absorption of nutrients, minerals and vitamins begin. The digested nutrients travel from the small intestine to the liver where nutrient metabolism and storage takes place (Ensminger, 1992). The liver has numerous functions that serve in digestion and absorption. Some physiological functions include: secretion of bile; detoxification of harmful/foreign compounds; metabolism of proteins, carbohydrates, and lipids; and storage of vitamins and carbohydrates. The ceca are located at the point where the small and large intestines come together. Not all ingested food will proceed to the ceca, these organs are primarily for the breakdown of dietary fiber (Moreng and Avens, 1985). The main function of the

large intestine is the storage of undigested waste and the absorption of water (Moreng and Avens, 1985).

The pH of the digestive tract is important in maintaining some substances in solution; influencing absorption of nutrients and metallic ions; and maintaining enzyme activitiy (Ensminger, 1992; Ford, 1974). Digestive enzymes are regulated by pH, which is often responsible for activation of enzymes. Ford (1974) found that microflora will alter the pH of the intestine, which in turn may alter nutrient availability. Studies have found that the bird has the ability to maintain the pH of the intestinal system to aid in digestion (Hurwitz and Bar, 1968; Mussehl et al., 1933). Table 4.1 is adapted from Ford (1974) and Hewitt and Schelkopf (1955) illustrating the pH of the digestive tract of the bird.

Table 4.1. pH of the digestive tract of the chicken.

	Ford (1974)	Hewitt (1955)
Position in the tract	**pH**	**pH**
Crop	5.1	4.67
Proventriculus	2.1	4.48
Gizzard	2.3	2.94
Duodenum	6.9	6.13
Jejunum	7.0	6.29
Ileum (at yolk sac)	6.8	6.58
Ileum (at cecal junction)	7.4	
Cecum	6.8	6.14
Rectum	6.5	6.82

Understanding the process by which digestion takes place in the bird is essential when formulating a diet. Some vitamins and most minerals (trace metals) undergo digestion and are directly absorbed from the intestinal tract in the same form that they are fed; are incorporated as a part of a protein or enzyme; or are undigestable and are excreted (North, 1984). Trace metals are essential to the metabolic process, but an excess of some can lead to environmental issues from groundwater contamination to plant toxicity, therefore it is important to properly regulate the amounts of these trace metals added to the feed.

A number of microbial species are found in the digestive tract of the bird. The general trend in the cecum is that there is a dominance of aerobic bacteria in the first few days, but with time, anaerobic bacteria take over and the population increases in complexity. Shapiro and Sarles (1949) found that coliform, particularly *Escherichia coli,* were the most dominant of the aerobic bacteria found, while the anaerobic population consisted almost solely of *Clostridium perfringens* (Barnes, 1977). Bacteria vary in both As resistance and their ability to take up As. Some were found to convert organic arsenicals into inorganic species (Anke, 1986). It was found that As(III) inhibits the fermentative activity and growth of some rumen bacteria more than the oxidized form.

Arsenic accumulation in biological tissues is noted in many organisms. The amount of As accumulation is dependent on the amount of As the organism was exposed to. The absorption of arsenic in the human body is high for anionic and soluble species, but does not easily react with insoluble As species. Inorganic As has a special affinity for keratin rich tissues, teeth, and hair (Anke, 1986; Matsui et al., 1999; Smith, 1964).

Arsenic accumulation in feathers 12 hrs after the oral dose was seen when using arsenic-76 (Anke, 1986). Arsenic accumulates in the liver and kidneys, since these organs are part of the excretory cycle, and it is believed that the reduction of As(V) to As(III) occurs in these organs (Armstrong et al., 1984). In general it is found that organic arsenicals are excreted more rapidly than inorganic As, where pentavalent species are found to clear more quickly than trivalent As species (Anke, 1986; Lauwerys et al., 1979). A number of animal studies have shown that DMA is the main metabolite. There are similar results for human urine excretion with about 20% inorganic As, 20% MMA, and 60% DMA.

Early studies investigated the rate at which arsenicals commonly added to feeds are eliminated from the body. Overby and Frost (1960) found that arsanilic acid is well absorbed but rapidly disappeared from the tissues into the feces. Frost et al. (Frost et al.) found that these organic As compounds did not accumulate in tissues to "excessive concentrations". Ferslew and Edds (1979) found that by discontinuing the addition of arsenicals to feed, the As concentrations were reduced to <0.5 and 2.0 mg kg^{-1} in muscle and liver/kidneys, reaching the upper limit of acceptable As concentrations (Ferslew and Edds, 1979).

As can accumulate in both plant and animal tissue by a series of mechanisms. Arsenate (As(V)) can replace phosphate in biochemical reactions (ATP). Replacing the phosphate anion with arsenate can cause rapid hydrolysis of high energy bonds and effectively uncouple oxidative phosphorylation. Arsenate may also replace the phosphorus in DNA, thus inhibiting the DNA repair mechanism and causing mutanegentic effects that can be passed on for generations (Dixon, 1997; Goyer, 1991).

The most common toxic mode of arsenic is the inactivation of enzymes. It is known that As(III) will inhibit more than 200 different enzymes (Abernathy et al., 1999). Arsenite (As(III)) forms strong bonds with sulfhydryl and disulfide groups disrupting sulfur bearing enzymes and amino acids, such as cysteine and methionine. As(III) inhibits pyruvate and succinate oxidation pathways and the tricarboxylic cycle, and can greatly impair gluceonogenesis, which with extended exposure can eventually lead to diabetes (Tseng, 2004). It is this strong bond with S that may be the reason that As accumulates in keratin tissues (Mandal and Suzuki, 2002). As(III) is a weak acid and exists primarily in its non-ionic form H_3AsO_3 in most environments due to its high pKa_1 of 9.2. Theoretically As should be in this non-ionic form in the chicken breast since the pH is 5.7-5.9, which is well under the pKa of As(III) (McMeekin, 1975).

There is a variety of trace metals and sulfur amino acids used as macro and micro-nutrients in the poultry feed, therefore these must be taken into account when determining As transport processes. Robbins and Baker (1980) determined that copper toxicity is decreased in the presence of cysteine. Early studies indicate that a copper-arsonic acid interaction was present in turkeys, wherein copper sulfate was shown to decrease the efficacy of three arsenic acid compounds (Bowen et al., 1971). Studies by Czarnecki et al. (1982, 1985) examined the interactions between roxarsone, cysteine and copper. Increased concentrations of both compounds resulted in increased As accumulation in the kidneys.

Arsenic accumulation in animals has been investigated for years, yet our understanding is still lacking. One recent study by Smith et al. (2008) used X-ray

absorption spectroscopy and found As(III) accumulation in rodent fur and bird feathers. Xie et al. (2004) investigated As accumulation in the liver of mice, and found that regardless of As speciation (organic vs. inorganic) As concentration of the liver increased when As was introduced to the diet. Lasky et al. (2004) found an average 0.39 ppm As in chicken livers and some muscle tissues collected from the USDA's data sources. The data showed that As accumulation in livers could be 2 to 11 times higher than in the muscle tissue, depending on how long the As was removed from the feed before slaughter. Wallinga et al. (2006) tested and found arsenic in 55% of the chicken products that they purchased in the supermarket with the average levels found ranging from 1.5 to 22 ppb As.

4.1.1. Objectives.

The objectives of this study are threefold: 1) to assess the effect of As speciation on As bioaccumulation 2) to determine As and trace metal distribution, association, and As speciation of the excreta from broilers fed roxarsone and 3) to determine As and trace metal distribution, association and As speciation in excreta and broiler tissues. Arsenic speciation of poultry litter was described in the previous study, however we could not definitively determine the As speciation of the excreta leaving the bird because the samples were collected days after removal from the body. This study aims at determining the As speciation both after excretion and while in the bird.

4.2. Materials and Methods

4.2.0. Experimental Design.

A broiler experiment utilizing five different As feeding regimes was conducted. Each feeding regime had 6 pens with 6 birds per pen. In the first treatment, the control, the birds were fed a basal diet containing no As supplements. The second was a roxarsone diet, consisting of values commonly found in commercial settings. The third treatment consisted of an inorganic As treatment with As(III) oxide as the source of inorganic As. The fourth treatment was also a roxarsone treatment with the difference being that roxarsone was fed for one week, and the control diet was fed for the remaining week. The fifth treatment was similar to the fourth, except that As(III) was fed the first week instead of ROX. The excreta were depositeded on plastic covered trays and collected every three days. At the termination of the experiment, liver, breast muscle tissue, ileum contents, skin, and feathers were collected. Liver and ileum contents were collected from all birds from all replicates. However, breast, skin, and feathers were collected from three birds from each replicate chosen at random. All samples were kept frozen at -20^{o}C, with excreta and ileum samples dried for X-ray analysis. Arsenic concentrations of all samples can be seen in Tables 4.4-8 and Tables 4.A.3-7.

Table 4.2. Ingredient composition of basal diet.

Basal Diet: Ingredient	% Composition of the feed
Corn	53.7
Soybean meal	37.8
Soybean Oil	4.5
Limestone	1.33
Dicalcium Phosphate	1.75
Salt	0.4
L-Met	0.19
Vitamin mix	0.075
Mineral mix	0.075
Choline Chloride	0.1

4.2.1. Chemical Analysis

All samples were digested using the EPA 3050B method, which involved dissolving 0.5g of an air-dried sample in nitric acid at 95^{o}C for 2 hours followed by digestion of the sample with peroxide at 95^{o}C for another 2 hours. For samples containing lower concentrations of As, 1 g of air-dried sample was digested in order to obtain reliable values. The samples were diluted to a final volume of 50 mL, and filtered using a 0.22 μm filter. All samples were analyzed using ICP-AES, and As standards were run as an internal calibration standard. Trace element grade acid was used in all of the digestions. Total metal concentration of feather, skin, breast, liver, and ileum samples were also determined using ICP-mass spectrometry (MS). This technique allows for much lower detection limits in environmental samples. However, the precision of the samples with concentrations less than 500 ppb is suspect, since this is approaching the detection limit for these samples.

Statistical analysis conducted using SAS version 8 found that based on Shapiro-Wilks test results, all data were log-normally distributed. Log transformed data were analyzed using the proc mixed procedure. Means were compared using the Tukey Kramer adjusts means separation with alpha value of 0.05. Means presented are the inverse of log transformed calculated means.

Water soluble metal and As concentrations were conducted on excreta and these data are presented in the appendix for this chapter (Table 4.A.2), in order to assess what fraction of the excreta is most labile. One gram of dried excreta in 10 mL of distilled dionized water was shaken for 24 hrs and was vacuum filtered using a 0.45 μm filter. All samples were kept refrigerated until analysis to minimize evaporation and sample alteration.

Arsenic speciation was determined by liquid chromatography coupled with inductively coupled plasma and mass spectrometry (LC-ICP-MS). The poultry excreta and ileal samples were extracted with water in a 1 gram per 10 mL ratio. The samples were then diluted 20:1 (DI H_2O: extract) and As speciation was determined using LC-ICP-MS. The column is a Phenomemex 150 x 4.6mm polar phenyl-ethyl 5 μm column made by Prodigy. It is stable from pH 2 to 9. The flow rate was 1mL min^{-1} with a 10 μL injection. Liquid As standards were also run alongside the samples, so that As speciation and quantification could be determined.

4.2.2. X-ray Absorption Spectroscopy Analysis.

Known arsenic species were run as standards to aid in the identification of unknown As species within experimental samples. These standards were run at beamlines X11A and X11B at the National Synchrotron Light Source (NSLS) at the Brookhaven National laboratory in Upton, New York. The standards were calibrated to 11874 eV using an inline As(V) standard, $Ca_3As(O_4)_2$.

Experimental X-ray absorption near edge structure (XANES) spectroscopy was conducted at beamline X26A at the NSLS. Beamline X26A is a microprobe capable of microspectroscopy, microdiffraction, fluorescence microtomagraphy, and fluorescence mapping. When the monochromatic beam is focused, the spot size is approximately 10 μm^2 with flux at 18 KeV being $1x10^9$ photons/sec. Canberra 9-element Ge array and Radiant Vortex-EX silicon drift detectors were used in the collection of fluorescence data. A channel-cut, silicon crystal monochromator with a (111) lattice cut was used in the collection of experimental data. A Bruker SMART 1500 CCD detector was used to collect μ-diffraction data.

Excreta, ileum, liver and breast samples were air dried (65^oC) and ground in order to establish a homogeneous sample. Breast samples were also mounted as whole sections in order to preserve muscle tissue structure. Samples were mounted on mylar film using petroleum jelly to adhere the samples to the film. The samples were applied in a single layer on the film. Both the mylar film and jelly were analyzed for As content, in order to minimize contamination of the As signal. Samples were prepared directly before arrival at the national lab. The mylar film and petroleum jelly were used in order to minimize

the effects of beam-induced As reduction (see Chapter 3 for details).

The beamline was calibrated to 11874 eV using $Ca_3As(O_4)_2$. Reducing beam-induced sample damage and As reduction was a major priority when collecting XANES data. The amount of time spent in the pre-edge region of the scan was reduced to minimize the amount of time the beam was in one spot before the whiteline, since this is the fingerprinting region. Scans were not taken at the same spot within a single particle and the beam was shifted a few micrometers in between scans in order to collect reasonable and representative data. Similar reduction studies to those described in Chapter 3 were also conducted on these samples, and the same scan parameters are used for all of these samples. X-ray fluorescence mapping was collected at 13 KeV and XRD data was collected at 17 KeV. The average map was 0.5mm by 0.5mm with each pixel being 0.01mm by 0.01mm.

After collection, the XANES data and XRF maps were analyzed using the X26A Plot program for consolidating detector channels of XANES scans and output XANES scans into binary files that can be read by other programs. The X26A Plot was also used in the formation of X-ray fluorescence maps and correlation plots. XANES analysis and As speciation was determined using WinXAS 3.1 and Athena 0.8.051 (Newville, 2001; Ressler, 1998). The determination of As speciation was accomplished by comparing the whiteline and derivative values of the experimental and standard spectra. Principal Component analysis was completed on all experimental scans and it was determined that four components were required to fit the spectra. Linear combination fitting (LCF)was performed using Athena 0.8.051 (Newville, 2001) and Six Pack's Linear Least Squares

Fitting (Webb, 2005). An error of about 5-10% is associated with LCF results (Manceau et al., 2000). To see the standards used in the LCF fitting see Table 3.A.3 in the appendix. The XRD data were analyzed using Fit2D and Match! software programs (Davies, 2006).

4.2.3. Scanning Electron Microscopy

Scanning electron microscopy (SEM) was used to examine poultry breast samples. A Field Emission Scanning Electron Microscopy (FE-SEM), the Hitachi S-4700, was used to collect micrographs of the poultry litter and roxarsone samples. This system is coupled with an Oxford INCA Energy (EDS) system that allows for elemental distribution analysis of the samples. This research was performed at the Delaware Biotechnology Institute at the University of Delaware. SEM/EDX is similar in nature to XRF in that both are based on an energy scale. Therefore, the higher ranges of the SEM had to be used in order to potentially see the heavier As atoms (15 to 20 KeV). The samples were carbon coated and placed on carbon coated aluminum plates for analysis.

4.3. Results and Discussion

4.3.0. Total Arsenic and Trace Metal Contents in Excreta and Poultry Tissues

Determining the effect of As speciation on As bioaccumulation in poultry is prudent because broilers are potentially exposed to both organic and inorganic As in the feed. As was described in the previous chapter, the basal feed often contains a number of possible sources of As contamination and exposure. Therefore, the birds are potentially

exposed to inorganic As, and these As species may be taking different routes within the birds. In this study, both organic and inorganic As supplements were used to assess how As is consumed and distributed within the bird.

The study was designed to address issues of As bioaccumulation in broilers, particularly the effect of As species on As uptake. The As and trace metal concentrations in the feed, excreta, ileal contents, and poultry tissues are depicted in Tables 4.3-13, Tables 4.A.2-7, and Figures 4.2-4. The As concentration in the basal feed is detectable (2.2 mg kg^{-1}). The diet used for the roxarsone treatment contains an As concentration within a range of standard industrial practices (19.6 mg kg^{-1}). The As content in the inorganic As diet for the bird study was 36.61 mg kg^{-1}. The As content in the inorganic feed (36.6 mg kg^{-1}) was higher than the roxarsone diet (19.6 mg kg^{-1}). The values were supposed to be within a similar range of one another.

The poultry tissue samples collected in this study were chosen based upon knowledge of As bioaccumulation in living organisms. The liver is the natural filtration system for the body, and therefore should be exposed to higher concentrations of As than most of the other organs and tissues of the body. Skin and feathers were investigated because they are high in keratin, which has been found to accumulate As in humans (hair and nails). Breast tissues were collected for As analysis because these tissues are among the most commonly consumed poultry tissues.

Statistical analysis was conducted upon the excreta samples and then on all day 14 samples (excreta days 10-14, ileal content, feathers, skin, breast, and liver). The statistics provide information about As content in these samples, and how As speciation

in the feed will affect As bioaccumulation in the bird. Values with the same letter ("a" for example) indicate that there is not a significant difference between the values. However, values with an "ab" distinction indicate that these values are not significantly different from "a" or "b", but "a" is significantly different from "b". The most noteable trends and distinctions will be discussed in the following paragraphs. It is important to note that the statistical data for Cu, Mn, Fe, Ca, and Zn in poultry tissues are not shown here because trace metal content did not vary greatly with changes in feeding regimes.

Arsenic distribution, as an effect of arsenic speciation in broiler feeds, is examined in this experiment. The inorganic As species used in this study is the reduced As(III), introduced as arsenic oxide. Since the speciation of the As in the basal feed is unknown, it is useful to examine the effects of many As species. Since organic and inorganic As species behave differently within the body, these patterns are critical in assessing As accumulation in broiler tissues.

A practice associated with the use of arsenicals is the removal of As from the feed at least five days before slaughter. This practice allows As time to be evacuated from the body before human consumption. The feasibility and reliability of this practice was assessed using both types of As diets.

The arsenic and trace metal concentrations from the control treatment are described in Tables 4.3 and 4.4. The As concentration in the excreta are highest in the first two excreta sampling periods (Days 1-4 and 4-7) and decrease to 1.0 mg kg^{-1} in the last two days of excreta collection, although these values are not significantly different from one another. SAS analysis conducted on these excreta samples indicate that there is

not a significant difference in the amount of As, or any of the other trace metals, excreted throughout the course of the control study. Time does not have an effect on the amount of metals or metalloids excreted in the control study. Statistical analysis indicates that the trace metal and As concentrations for the ileum and last excreta contents were similar for all evaluated elements (elements depicted in these tables). The As concentrations for ileum and last excreta samples are depicted in Table 4.4 the control treatment. The statistical analysis for all tissue samples for all As treatments can be found in Table 4.13. The As trend with respect to the roxarsone and inorganic As is plotted in Figures 4.2 and 4.3. The results will be discussed in a few paragraphs.

Table 4.3. Arsenic and total metal mean concentrations in the control excreta. Means followed by the same letter are not significantly different at an alpha value of 0.05.

Excreta Sample	As	Ca	Cu	Mn	Fe	Zn
	----------	----------	(mg kg^{-1})	----------	----------	----------
Days 1-4	2.0 a	10557.1 a	15.1 a	54.7 a	208.3 a	177.2 a
Days 4-7	4.1 a	15206.2 a	19.1 a	73.2 a	293.8 a	200.6 a
Days 7-10	1.0 a	10436.3 a	21.6 a	61.1 a	180.1 a	211.3 a
Days 10-14	1.0 a	14157.2 a	26.8 a	81.1 a	460.2 a	266.6 a

Arsenic accumulation in poultry tissues is seen to a small degree when the broilers are fed the basal diet. The As concentration in liver, skin and feathers is lower than the FDA's maximum limit of 0.5 ppm. However, the breast concentrations are higher with an average value of 1.2 mg kg^{-1}. Arsenic accumulation in feather and skin

was minimal. Values lower than 0.5 ppm are approaching the detectable limit for these samples and techniques.

Table 4.4. Arsenic and trace metal mean concentrations for the excreta (Days 10-14), ileal samples, and poultry tissues for the control diet. These birds were fed the basal diet for the full 14 days. (SAS analysis of these samples was conducted and significant results are discussed in the text of this section.)

Treatment	Sample	As	Ca	Cu	Mn	Fe	Zn
		----------	----------	(mg kg^{-1})	----------	----------	----------
	Excreta	1.0	14157.2	26.8	81.1	460.2	266.6
	Ileum	2.5	17265.6	46.4	125.7	1130.6	313.0
Control	Feather	0.2	280.3	4.8	5.7	8.6	57.7
	Skin	0.4	197.9	1.9	0.7	15.1	25.9
	Breast	1.2	845.2	0.9	1.2	50.7	36.0
	Liver	0.4	1038.3	10.0	8.4	366.8	114.9

Arsenic concentrations in feed, excreta, ileal samples and poultry tissues for the roxarsone diet are depicted in Tables 4.5-6. When fed roxarsone for the full 14 days, As concentration in the excreta fluctuates across the 14 day period with the maximum As excretion taking place during the 4-7 day period. However, statistical analysis indicates that although there is a fluctuation there is not a significant difference between these excreta samples for any of the metals examined here. As content in the excreta (45.1-84.6 mg kg^{-1}) is higher than in the feed (19.6 mg kg^{-1}), this is likely due to the removal of soluble feed components. The trace metal and As concentrations of the ileal samples and the last excreta sample were similar in value(Table 4.6). When As is fed for the full 14 days, the birds excrete As and trace metals on a consistent time scale.

All broiler tissue samples collected from the roxarsone study contain As concentrations higher than the FDA's maximum allowable As concentration (0.5 ppm). The liver contained more As (10.4 mg kg^{-1}) than any of the other tissues sampled with the skin and feathers containing less As than the breast and liver. In fact, the only tissue that is significantly different from the others is the liver. The As concentration in the liver is higher than that seen in the control liver sample (0.4 mg kg^{-1}). Table 4.13 indicates that the As content in the liver is significantly different from the control liver. This indicates that feeling organic As to the birds will increase the amount of As found in the liver. The As concentration in the breast sample is not significantly higher than that of the control breast. However, copper content in the breast was statistically significant in the roxarsone breast when compared to the control breast tissue. This is something that was noted in a study by Czarnecki and Baker (1985) where they noted As feed content affected Cu accumulation in mice. This appears to be occurring in these poultry liver samples as well. This could be significant because consuming extraneous amounts of Cu could pose a health threat. This effect is not seen in the liver, skin or feather samples.

Table 4.5. Arsenic and total metal mean concentrations in the roxarsone excreta. Means followed by the same letter are not significantly different at an alpha value of 0.05. These birds were fed the roxarsone diet for the full 14 days.

Excreta Sample	As	Ca	Cu	Mn	Fe	Zn
	----------	----------	(mg kg^{-1})	----------	----------	----------
Days 1-4	45.1 a	13044.8 a	19.6 a	52.0 a	252.8 a	159.4 a
Days 4-7	84.6 a	17651.8 a	27.2 a	85.1 a	272.2 a	229.4 a
Days 7-10	61.8 a	11563.6 a	18.0 a	59.3 a	84.5 a	212.0 a
Days 10-14	75.6 a	14230.8 a	21.0 a	71.4 a	402.3 a	227.8 a

Table 4.6. Arsenic and trace metal mean concentrations for the excreta (Days 10-14), ileal samples, and poultry tissues for the roxarsone diet. These birds were fed the roxarsone diet for the full 14 days. SAS analysis of these samples was conducted and significant results are discussed in the text of this section.

Treatment	Sample	As	Ca	Cu	Mn	Fe	Zn
		----------	----------	(mg kg^{-1})	----------	----------	----------
	Excreta	75.6	14230.7	22.6	77.0	438.2	245.2
	Ileum	75.1	22745.8	34.4	132.4	1079.0	283.3
Roxarsone	Feather	0.7	284.9	5.2	5.6	8.7	61.2
	Skin	0.9	205.2	1.3	1.1	12.8	22.3
	Breast	2.6	834.4	3.6	0.5	21.7	29.2
	Liver	10.4	913.5	10.4	9.7	453.4	120.3

The form of arsenic fed to the broilers has an effect on some tissues. The inorganic As(III) oxide treatment for the full 14 days illustrates some different trends from that of the full roxarsone study. The As concentrations in the excreta do not follow a similar trend to that seen in the roxarsone treatment. The rate of As excretion is not consistent throughout the growth period. There is a dip in the As content of the excreta during the day 7-10 period. This result is unexpected, and the excreta was redigested a number of times. The highest amount of As is seen at the end of the excreta study (days 10-14). Trace metal excretion is not significantly different with time, with the exception of Cu, which increases with time. The ileal contents did not contain significantly more As than the last excreta sample. The other trace metals found in the excreta (Ca, Cu, Mn, Fe and Zn) did not vary significantly with time when fed the full roxarsone diet. It does not appear that feeding organic As for the full 14 days has any effect on trace metal excretion.

Table 4.7. Arsenic and total metal mean concentrations in the full As(III) excreta trial. Means followed by the same letter are not significantly different at an alpha value of 0.05. These birds were fed the inorganic As(III) diet for the full 14 days.

Excreta Sample	As	Ca	Cu	Mn	Fe	Zn
	----------	----------	(mg kg^{-1})	----------	----------	----------
Days 1-4	87.8 ab	13317.8 a	19.0 a	66.7 a	236.0 a	214.2 a
Days 4-7	248.8 ab	13888.0 a	16.9 a	67.9 a	352.6 a	170.7 a
Days 7-10	135.5 a	14043.3 a	31.8 b	79.3 a	238.3 b	282.4 a
Days 10-14	326.3 b	21535.2 a	37.0 c	115.1 a	606.6 a	376.2 a

Table 4.8. Arsenic and trace metal mean concentrations for the excreta (Days 10-14), ileal samples, and poultry tissues for the As(III) diet. These birds were fed the inorganic As(III) diet for the full 14 days. SAS analysis of these samples was conducted on these samples and significant results are discussed in the text of this section.

Treatment	Sample	As	Ca	Cu	Mn	Fe	Zn
		----------	----------	(mg kg^{-1})	----------	----------	----------
	Excreta	326.3	21535.2	37.0	115.1	606.6	376.2
	Ileum	914.3	24408.9	33.6	163.6	1266.7	287.2
Inorganic As(III)	Feather	2.1	209.6	4.1	4.6	8.4	53.6
	Skin	0.8	193.4	2.6	1.0	15.1	26.9
	Breast	4.4	646.6	3.3	0.7	30.4	31.4
	Liver	4.5	668.7	15.0	11.3	367.9	117.7

All tissue values, except the skin, contained values that are relatively above the FDA's maximum As content. The As content in the feathers (2.1 ppm) is significantly higher than that of the control feathers (0.2 ppm), indicating that introducing As in the inorganic form may cause an increase in the amount of As accumulation in keratin materials. This trend was not seen in the organic As study. The control (1.2 mg kg^{-1}) and the full inorganic As(III) breasts (4.4 mg kg^{-1}) were significantly different from one

another. A similar trend was also seen in the inorganic As(III) treated liver. The inorganic liver (4.5 mg kg^{-1}) was higher than the control (0.4 mg kg^{-1}), but not significantly different from the roxarsone study (10.4 mg kg^{-1}). Statistics indicate that As accumulation in the liver is not impacted by the type of As introduced into the system. The breast muscle may be more affected by As speciation than the liver. Copper accumulation was only significant in the breast muscle. The liver did not significantly accumulate Cu like the breast did.

The second roxarsone treatment consisted of one week of the roxarsone diet followed by consumption of the basal feed. These results can be found in Tables 4.9 and 10. This experiment was designed to mimic the common practice of arsenic withdrawal from the feed at least five days before slaughter. This resulted in decreased As concentrations in all excreta and tissue samples. There was a significant drop in As concentration from days 4-7 to 7-10, which is indicative of the removal of As from the feed. The SAS statistical analysis indicates that three of the excreta samples are similar, while the second sampling period (Days 4-7) is different from the rest. All arsenic diets see a slight (but not always significant) increase in the amount of As excreted between the first two excreta sampling periods. The ileum contents and final excreta values were not similar, and the ileal values were significantly lower (0.8 mg kg^{-1}) than the ileal contents of the full roxarsone study (75.1 mg kg^{-1}). Liver contents decreased from 10.4 to 2.3 mg kg^{-1} and breast concentrations decreased from 2.6 to 1.5 mg kg^{-1} (when comparing the full roxarsone diet to the As-removal study), however these were not deemed to be significantly different. Although removing As from the feed decreased the

total As content in the tissues, it did not drop the values below the FDA's maximum As concentration limit.

Figure 4.2 depicts the As concentration trends in the excreta of both the roxarone treatments when compared to the control excreta values. SAS analysis shows three different trends in As content when comparing the three treatments. During days 4-7, the full and half roxarsone treatments are not different from one another, but they are both significantly different from the control. This result is not a surprise considering roxarsone is being fed to both roxarsone trials at this time. The days 7-10 excreta all have different values from one another. The As has been removed from the industry-like treatment. Therefore, the amount of As present in the samples is lower than the full roxarsone treatment and greater than the control. The final excreta sample (days 10-14) shows that the control and final excreta sample in the roxarsone removal study are similar with both samples being significantly different from the full roxarsone study. The second roxarsone study had an initial increase in As content followed by decreasing As values. Although, removing As from the diets did decrease total As content in all samples, it may not decrease As concentration enough to satisfy all regulations. The trace metal content (Ca, Cu, Mn, and Zn) in the organic As withdrawal treated excreta did not vary significantly with time. However, the iron content in the excreta did vary with time, which may indicate the removal of organic As from the diet may have an effect on Fe excretion. These results also indicate that after 3 days, not all of the As is flushed from the digestive tract of the bird.

Table 4.9. Arsenic and total metal mean concentrations in the ROX withdrawal excreta. Means followed by the same letter are not significantly different at an alpha value of 0.05. These birds were fed the roxarsone diet days 1-7, and the control diet days 7-14.

Excreta Sample	As	Ca	Cu	Mn	Fe	Zn
	----------	----------	(mg kg^{-1})	----------	----------	----------
Days 1-4	44.0 a	10968.1 a	17.8 a	60.4 a	225.7 c	190.9 a
Days 4-7	76.7 b	12064.7 a	16.9 a	61.7 a	186.1 a	181.9 a
Days 7-10	8.5 a	11211.6 a	17.6 a	57.5 a	129.5 b	202.1 a
Days 10-14	1.7 a	14878.0 a	22.1 a	80.5 a	401.1 c	256.7 a

Table 4.10. Arsenic and trace metal mean concentrations for the excreta (Days 10-14), ileal samples, and poultry tissues for the second ROX diet. These birds were fed the ROX diet days 1-7, and the control diet days 7-14. SAS analysis of these samples was conducted and significant results are discussed in the text of this section.

Treatment	Sample	As	Ca	Cu	Mn	Fe	Zn
		----------	----------	(mg kg^{-1})	----------	----------	----------
	Excreta	1.7	14878.0	22.1	80.5	401.1	256.7
	Ileum	0.8	17498.0	46.7	123.6	1266.6	306.1
Roxarsone	Feather	0.5	250.7	6.0	4.2	9.0	57.4
Withdrawal	Skin	0.7	212.2	4.0	1.9	14.9	28.2
	Breast	1.5	752.9	1.1	0.5	20.3	25.1
	Liver	2.3	1005.2	14.4	11.0	347.1	105.8

The second inorganic As treatment exhibited As excretion trends similar to that of the previous As(III) oxide treatment during the first two time periods. However once As was removed from the feed, As content dropped drastically (from 201.0 to 2.9 mg kg^{-1}) in the final excreta sample. The statistical analysis depicted in Table 4.11 and Figure 4.3 illustrate these trends. The first two excreta samples are similar to one another, and both are significantly different from the last two excreta samples. The ileal contents were drastically lower after the removal of As compared to the full As(III) treatment (from

914.3 to 1.1 mg kg^{-1}). It appears that the inorganic As flushes from the body at a greater rate than the roxarsone removal. The third and fourth excreta samples were significantly different from one another. There was not a significant difference in the amount of As retained in the liver, breast, feather, or skin when comparing the full and half As(III) treatments (Table 4.13). The arsenic that was accumulated in the full As(III) study is no longer an issue. There was not a difference in the total amount of As accumulated in the tissues when comparing the organic and inorganic removal studies (Table 4.13). There was also no significant difference between the tissues of inorganic As(III) withdrawal treated birds when compared to the tissues of the control birds. These results indicate that although the inorganic As(III) may accumulate in certain tissues, after a withdrawal period the As can be flushed from the body. Bioaccumulation of As(III) across these time periods is reversible.

The trace metal concentrations remained consistent in the excreta samples. The copper content was significantly higher in the skin when the As(III) was removed from the diet. The Cu that accumulated in the breast for both the full organic and full inorganic treatments has dropped in the As removal breast tissues.

The trends in As excretion exhibited by both inorganic excreta treatments can be seen in Figure 4.3. The days 4-7 and 7-10 excreta sampling periods exhibit similar trends, due to the strange anomaly in the days 7-10 excreta As content in the full inorganic study. The two As treatments are similar to one another and both are different from the control study. However, the days 10-14 sampling period exhibits a different As content pattern. The final excreta sampling period (days 10-14) is similar to the control

and both are different from the full As study. This indicates that As content in the excreta has dropped down to background (basal feed) levels. Arsenic content was not lower than the FDA's maximum As contaminant level, except for the skin. The trace metal content in the inorganic As(III) withdrawal treatment did not vary significantly with time in the excreta with the exception of Fe.

Table 4.11. Arsenic and total metal mean concentration in the As(III) excreta. Means followed by the same letter are not significantly different at an alpha value of 0.05. These birds were fed the As(III) diet days 1-7, and the control diet days 7-14. The effects of removing As from the diet before slaughter were evaluated in this study.

Excreta Sample	As	Ca	Cu	Mn	Fe	Zn
	----------	----------	(mg kg^{-1})	----------	----------	----------
Days 1-4	91.1 c	12953.2 a	20.7 a	70.6 a	179.5 a	218.6 a
Days 4-7	201.0 c	15283.1 a	24.4 a	77.9 a	194.4 a	216.0 a
Days 7-10	20.9 b	10176.1 a	25.9 a	73.3 a	115.8 a	177.6 a
Days 10-14	2.9 a	18244.2 a	26.6 a	101.9 a	574.9 b	317.7 a

Table 4.12. Arsenic and trace metal mean concentrations for the excreta (Days 10-14), ileal samples, and poultry tissues for the second As(III) study. The birds were fed the As(III) diet days 1-7, and the control diet days 7-14. SAS analysis of these samples was conducted on these samples and significant results are discussed in the text of this section.

Treatment	Sample	As	Ca	Cu	Mn	Fe	Zn
		----------	----------	(mg kg^{-1})	----------	----------	----------
	Excreta	3.4	18000.7	26.4	100.3	586.6	310.5
	Ileum	1.1	13830.6	30.4	101.3	613.5	365.7
Inorganic As(III) Withdrawal	Feather	1.0	342.7	4.9	1.5	16.0	68.1
	Skin	0.2	197.6	4.1	0.2	13.0	25.8
	Breast	1.6	5688.3	13.5	35.8	266.1	150.2
	Liver	1.7	1022.5	13.8	11.1	358.7	95.2

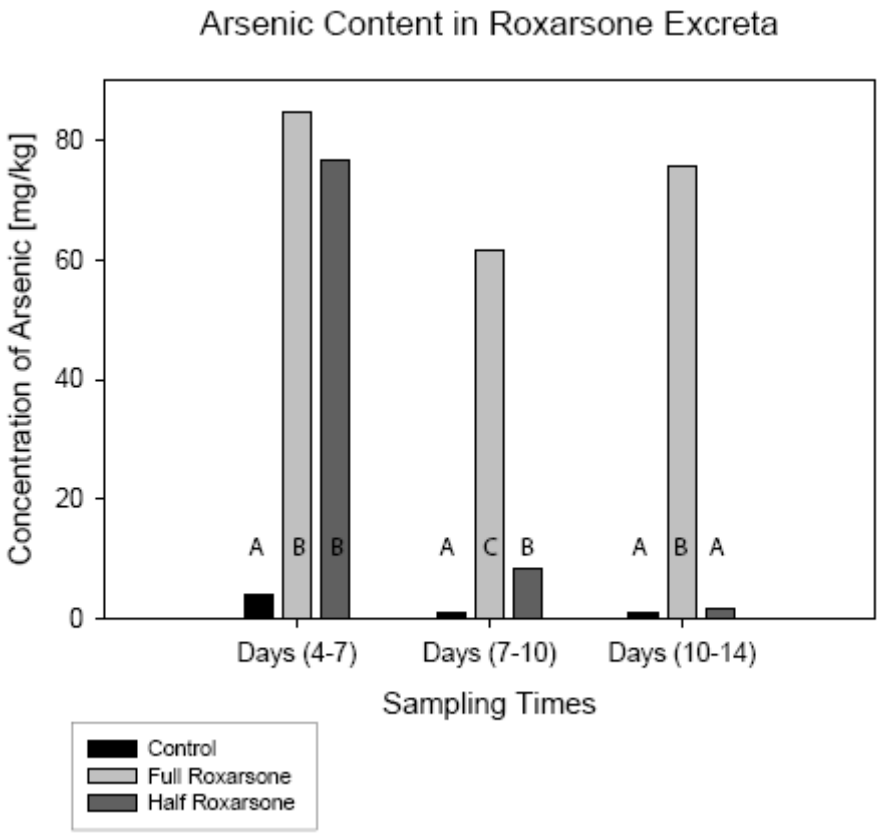

Figure 4.2. Mean arsenic concentration with increasing time for the roxarsone studies. Means labeled with the same letter are not significantly different at an alpha value of 0.05.

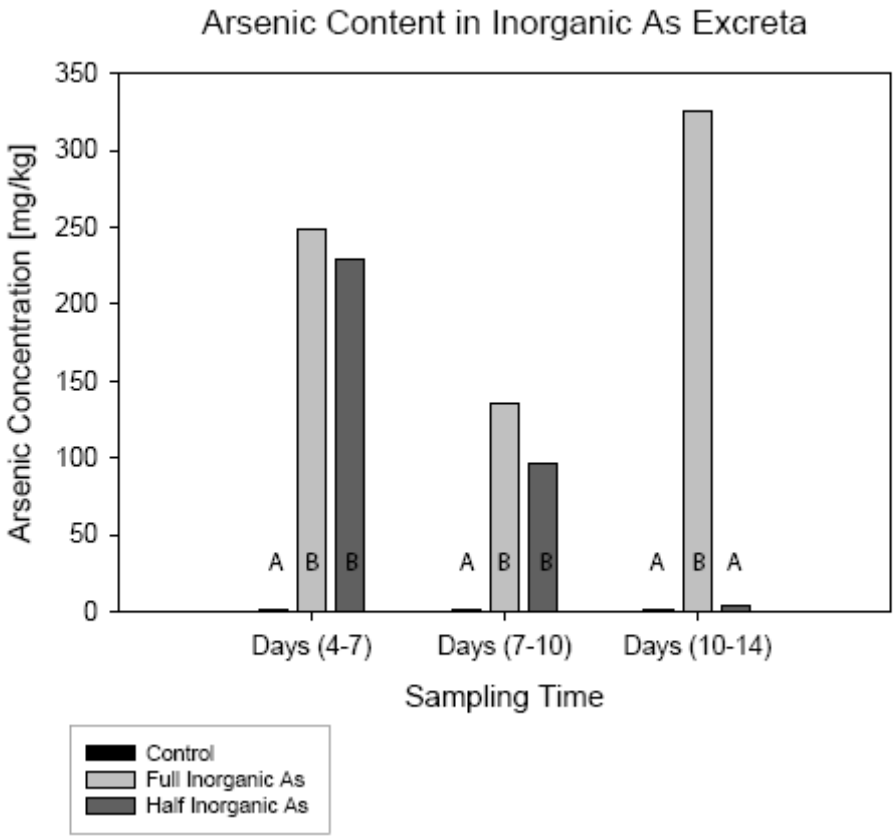

Figure 4.3. Mean arsenic concentration with increasing time for the inorganic As(III) studies. Means labeled with the same letter are not significantly different at an alpha value of 0.05.

Total arsenic concentrations in the poultry tissues for all treatments can be seen in Figure 4.4 and Table 4.13. In summary, there is relatively more As accumulation in the liver and breast tissues, than the feather and skin for most treatments. Some arsenic accumulation was seen in feathers and liver for As treatments when compared to the control. In most cases, the As was removed from the tissues when the As was no longer added to the feed.

Table 4.13. Arsenic mean concentration in poultry tissues. Statistics should be considered within a tissue type, and not compared across tissue samples. Means followed by the same letter are not significantly different at an alpha value of 0.05. These birds were fed the different As feeding regimes. The effects of removing As from the diet before slaughter were evaluated in this study. ROX stands for roxarsone, and INORG stands for the inorganic As(III) diet.

Treatment	Feather	Skin	Breast	Liver	Ileal
	----------	----------	(mg kg^{-1})	----------	----------
Control	0.2 a	0.4 a	1.2 a	0.4 a	2.5 a
ROX Full	0.7 ab	0.9 a	2.6 ab	10.4 b	75.1 b
INORG Full	2.1 b	0.8 a	4.4 b	4.5 b	914.3 c
ROX Withdrawal	0.5 ab	0.7 a	1.5 ab	2.3 b	0.8 a
INORG Withdrawal	1.0 ab	0.2 a	1.0 ab	1.7 ab	1.1 a

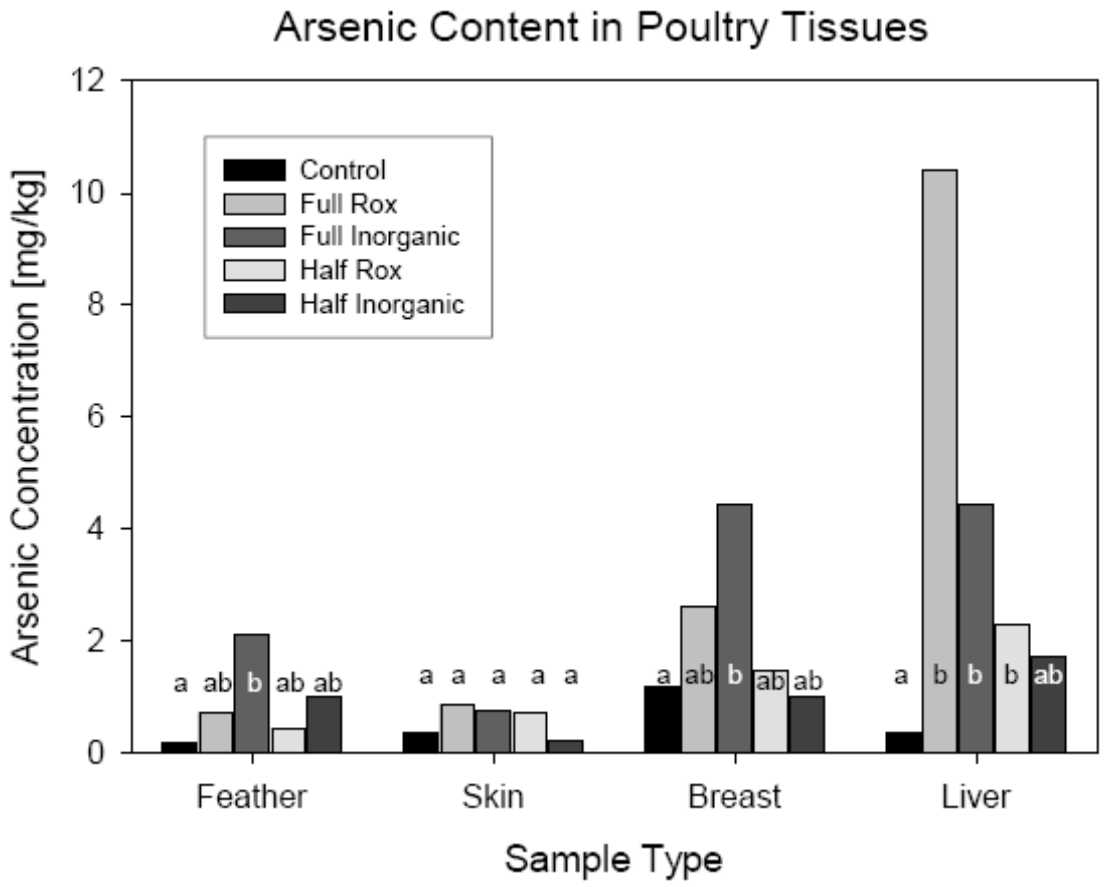

Figure 4.4. Graph depicting total As concentration in feather, skin, breast and liver samples from birds fed a series of As diets.

An attempt to study elemental distribution in poultry breast was made on the FE-SEM at the Delaware Biotechnology Institute at the University of Delaware (Figure 4.5). The experiments were conducted using an energy dispersive X-ray in hopes of getting As distribution in the breast tissue sample. Since the SEM can collect lighter elements like S and P, it would be a nice complement to the synchrotron X-ray work discussed in the next section. However, the As counts were too low. Below are two micrographs of breast tissue.

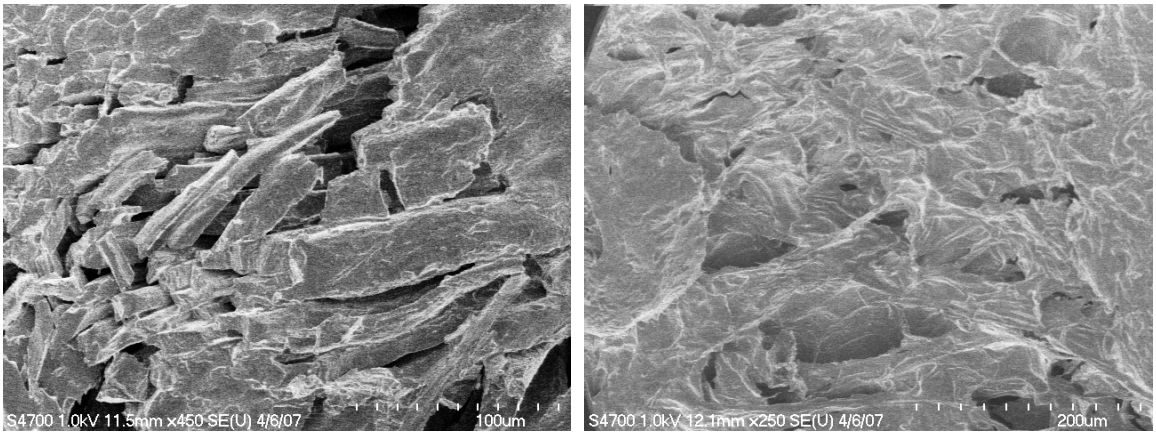

Figure 4.5. SEM micrographs of poultry breast tissue from the full roxarsone treatment.

4.3.1. Arsenic Speciation, Distribution, and Elemental Associations in Excreta

Determining As and trace metal distribution, association, and As speciation of the excreta from broilers fed roxarsone and inorganic As is essential to determine the fate of these contaminants once incorporated into soil and water systems. The form of As present in the excreta may not be the only factor affecting the fate of As in natural systems, co-precipitation and complexation may also change As availability in the excreta.

Excreta samples from the bird experiment were brought to beamline X26A for X-ray analysis. X-ray fluorescence (XRF) mapping provided insight into the spatial distribution, elemental association and As speciation in both roxarsone and inorganic excreta and ileal samples. X-ray analysis was conducted on excreta and ileal samples that were treated with As for the full 14 days. The last excreta sample from the roxarsone removal study was mapped in order to look for As hot spots in the excreta material, but none were found. Therefore, the first (days 1-4) and last excreta (days 10-14), and ileal samples were analyzed from the roxarsone treatment, and the last excreta (days 10-14) and ileal samples were analyzed from the inorganic As(III) treatment.

Arsenic speciation in poultry litter (a mix of excreta and bedding materials) was conducted, and the results indicate that there are a variety of As species found in the excreted materials (roxarsone (ROX), organic degradation products, As(III), and As(V)). However, few studies have used fresh excreta samples or samples taken from within the bird itself.

Arsenic distribution in the first excreta sample from the roxarsone diet (days 1-4) demonstrated that As is not evenly distributed in the sample, and that it is localized in "hotspots" (Figure 4.6). The concentration of these "hotspots" is higher than the total As value for the excreta sample as demonstrated by the quantified values on the XRF map. It is these "hotspots" that add to the total-overall As value of the sample. The X-ray absorption near edge structure (XANES) spectroscopy provided detailed information about As speciation in the excreta during this time period (Figure 4.6-7). This sample had a range of As species with As(III) and roxarsone being the dominant species found in

the excreta. Oxidized As species were found but in limited amounts. The changing internal environment within the bird during the beginning developmental stages, may play a role in As speciation. Roxarsone and other organic As species were found in these samples. Roxarsone was found in areas of concentrated As. After the XRF maps and XANES scans were collected, a hotspot located out of the range of the map depicted in Figure 4.6 was found and XANES scans were collected. These scans were averaged and the results are shown in Figures 4.7A and B. This scan contains more organic As than any of the other scans. However, even this area that contained high levels of As still contains multiple As species.

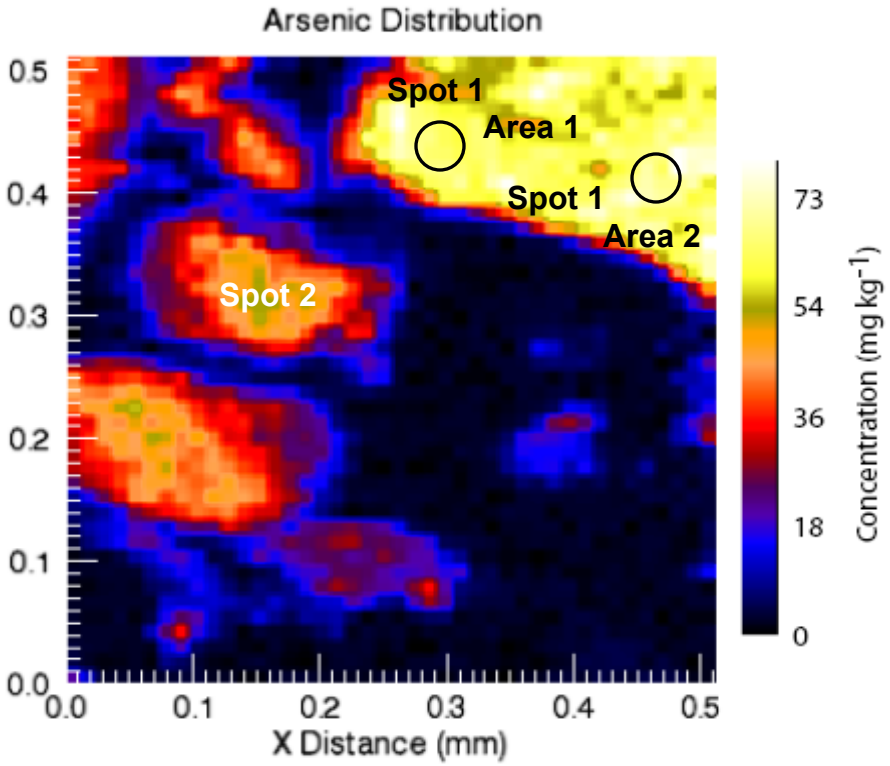

Figure 4.6. Arsenic distribution in the first roxarsone excreta sample (days 1-4) using XRF represented in mg kg^{-1}. XANES scans were taken at the areas located on the map.

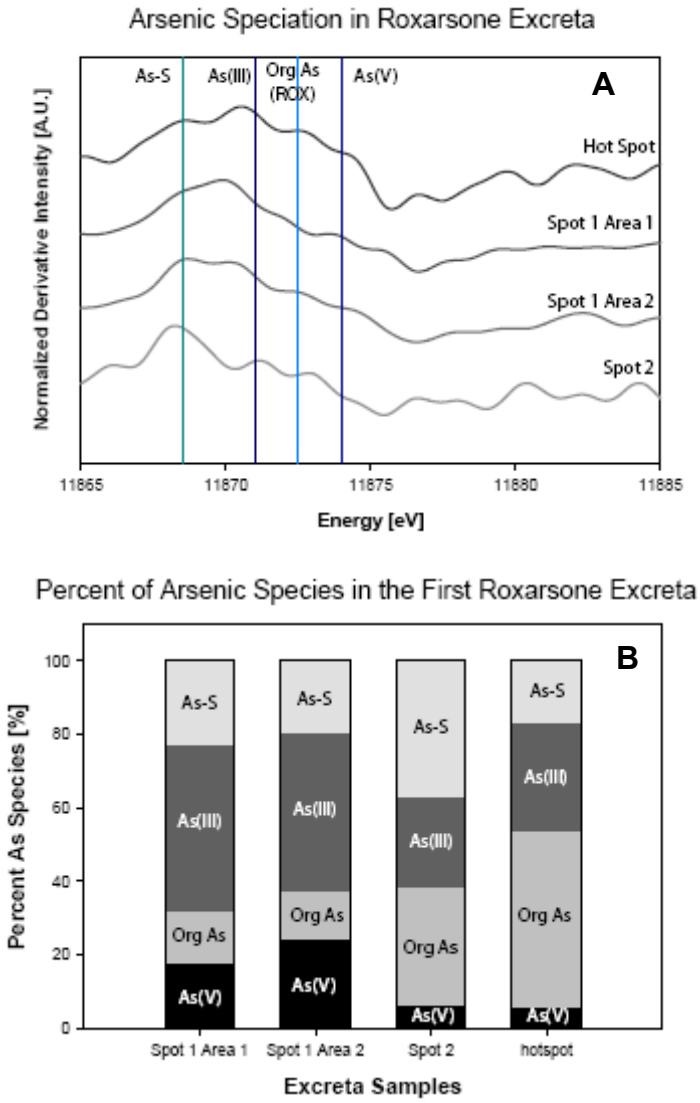

Figure 4.7. Normalized derivative XANES scans in Figure 4.7 A and the linear combination fitting (B) of those scans presented in mg kg^{-1} are depicted in Figure 4.7B. An additional "hotspot" scan was taken at an area on this sample that was not located on the map.

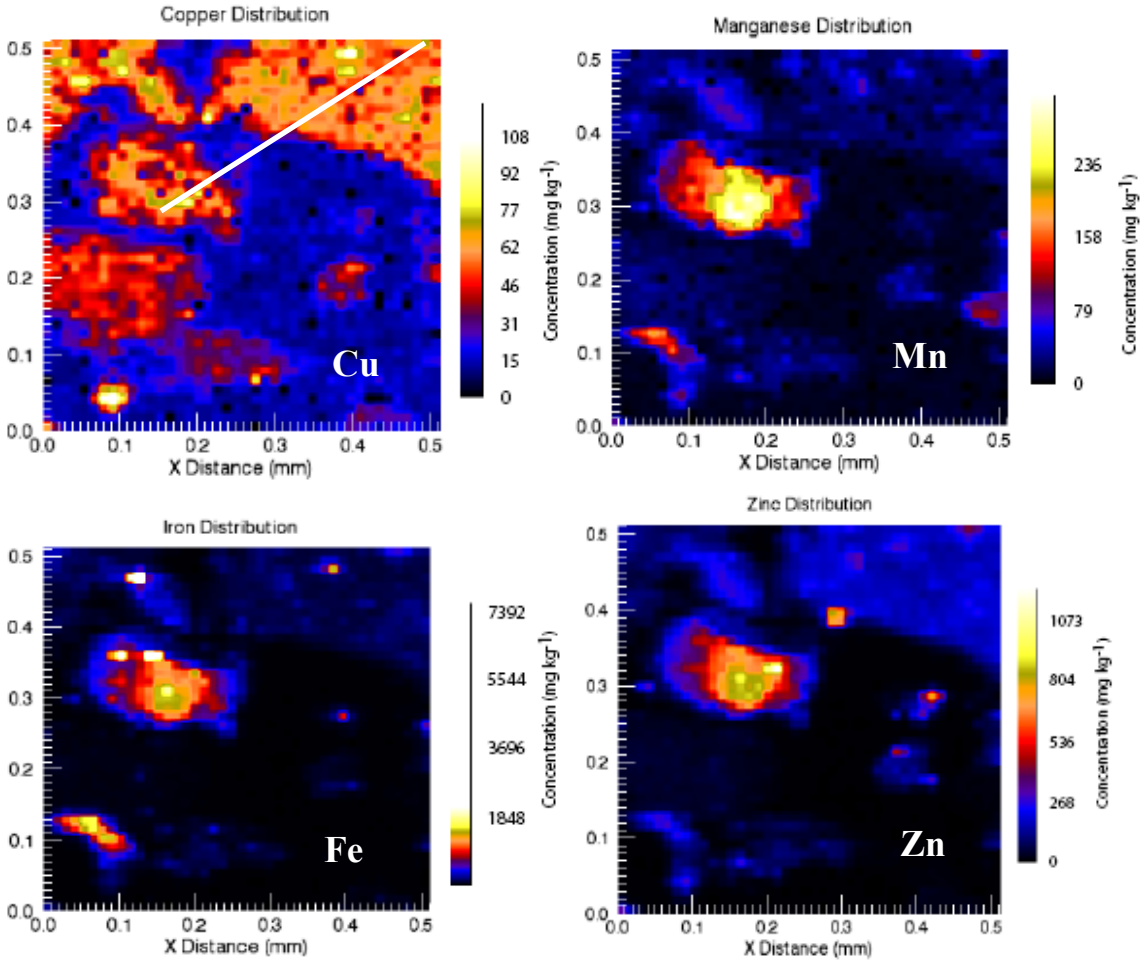

Figure 4.8. XRF maps of trace metal distribution in the first roxarsone excreta sample represented in mg kg^{-1} .

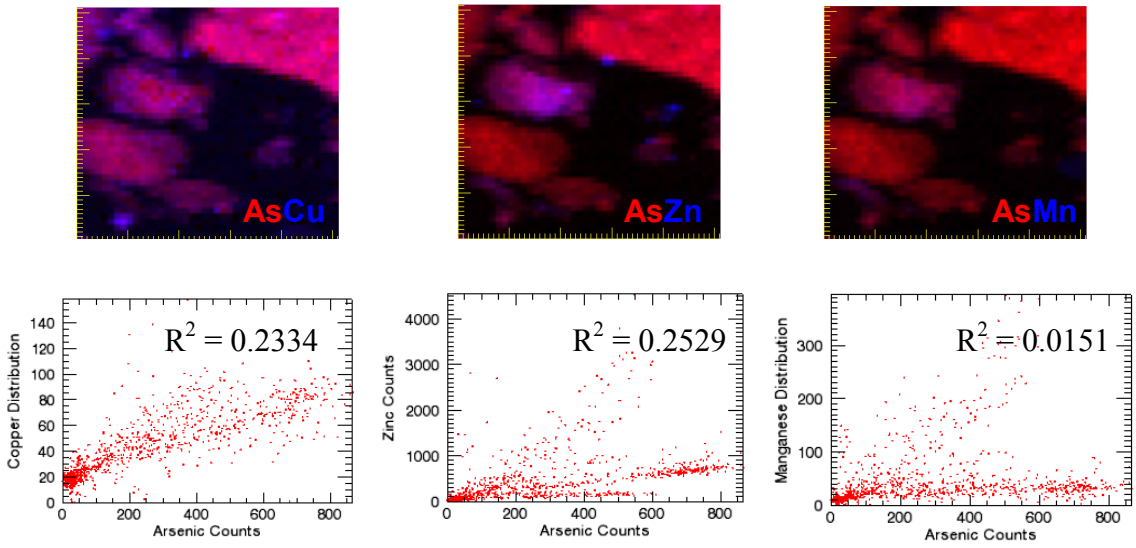

Figure 4.9. XRF maps of arsenic and trace metal associations and correlations in the first roxarsone excreta sample (days 1-4).

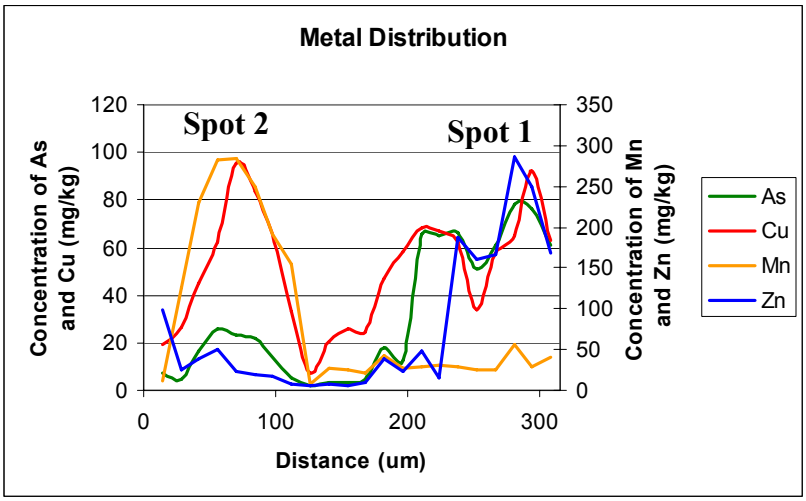

Figure 4.10. Metal Distribution in mg kg $^{-1}$ across the line drawn on the Cu map in Figure 4.8.

Arsenic and trace metal association and distribution in excreta illustrate a series of interesting relationships that may have an effect on nutrient availability both within the bird and in the soil once the excreta (poultry litter) is land applied. In Chapter 3, all of the poultry litter samples contained strong divalent trace metal-As associations. This also

happens in the poultry excreta, just not to the same degree. The trace metals found in the excreta samples appear to be more localized. This localization of both As and trace metals causes the associations to not be as prevalent as they were in the poultry litter samples. An example of this can be seen when comparing Figures 4.6 and Figures 4.7-10. As is not evenly distributed in this excreta sample. There is a large particle in the upper right hand corner of the map. This particle does not contain high amounts of other trace metals. This is best illustrated when comparing the individual XRF maps in Figure 4.8. Cu is located in this large particle, but Mn, Fe and Zn are not. This is also seen in the bicolor maps depicted in Figure 4.9. In the bicolor maps, if this large particle was evenly occupied by As and the trace metal, then the particle would appear purple. However, in each of these maps this area is largely dominated with the reddish-pink color indicating a predominance of As. Cu appears to be slightly more correlated than the other trace metals. However the Cu correlation factor ($R^2 = 0.2334$) is not higher than Zn (0.2529) or Mn (0.0151).

Another way to look at metal distribution in a sample is to look at metal distribution across an area of the sample. Figure 4.10 shows elemental distribution across the line drawn on the Cu map in Figure 4.9 (from left to right). The green line is As. The first set of peaks is correlated with the area labeled spot 2 on the As distribution map (Figure 4.6), while the second set of peaks is representative of the larger particle (Spot 1). From this information you can see that Spot 2 contains less As and Zn than Spot 1. Whereas, Spot 1 contains more As, Cu, and Zn. This information is also depicted in the

Multiple Channel Analyzer (MCA) plots in Figure 4.11-12 to see relative elemental abundance for these two locations.

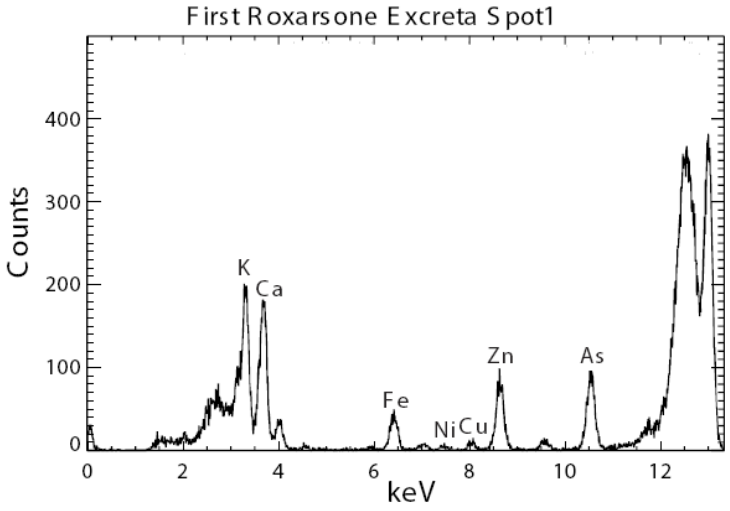

Figure 4.11. MCA plot of relative elemental distribution in the 1st roxarsone excreta sample spot 1.

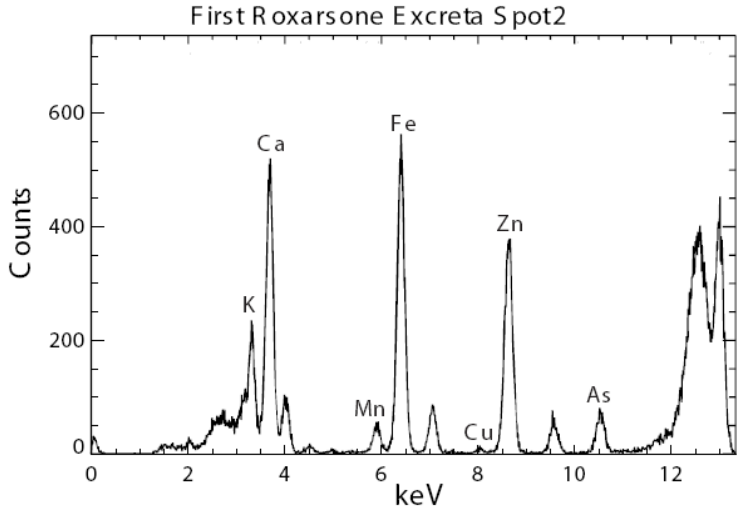

Figure 4.12. MCA display depicting relative elemental distribution in the first roxarsone excreta spot 2 (days 1-4)

It has been suggested that microorganisms may play a role in roxarsone degradation. These microbes have the ability to reduce As(V) into As(III), and oxidize As(III) into As(V). These processes are mitigated by the presence of suitable electron donors, electron acceptors and potential food sources. Under reducing environments, oxygen is limited, therefore the electron shuttling experienced during microbial respiration must come from another source. The oxidized As(V) serves as a suitable electron acceptor, and will therefore be reduced to As(III). This helps explain why As(III) is more commonly found in reducing environments than As(V). As(V) alone in a reducing environment will generally not transform into As(III), this process needs to be catalyzed by an outside source such as microbes. Respiration is not the only microbial process that can dictate As speciation. Metabolism causes electron shuffling that can cause As(III) to oxidize into As(V). When sufficient electron donors, or food sources, are not present, some microbes can use As(III) as an energy source and therefore convert As(III) into As(V). These processes have been noted in natural environments, but have not been reported in the digestive system of the bird.

The changes in inorganic As speciation have been extensively studied, however the exact process of roxarsone degradation is unknown. Studies have shown that the first step is the rearrangement of the nitrogen group on the ring. The NO_2 group converts into a NH_2 group, this reduction is commonly seen under reducing conditions. This new As-bearing compound is what is referred to as HAPA. Next to roxarsone, HAPA is the most common organic As compound found when computing the linear combination fitting. I would hypothesize that the first step involved when cleaving the As group from the ring

structure would most likely involve breaking the ring to form a more linear C chain. The phenyl ring is very strong and the pi-bonds can keep this ring structure very stable and resistant to microbial degradation. Once the ring is broken, the C can readily affect microbial processes and the individual side chains (including the As group) will be easier to cleave from the C structure. Once this As group is detached from the roxarsone structure it has the potential to form As(III), As(V), and organic C-AsO_3 like structures. Therefore, predicting roxarsone degradation is very difficult especially in a complex environment such as the digestive tract of the bird.

Arsenic speciation changes are not purely limited to biological input. Various minerals can cause As oxidation in natural systems. Both iron and manganese minerals have been found to readily oxidize As in natural settings. Although these minerals may not be directly added to the feed, conditions within the bird or litter may allow metal mineral formation and subsequent As speciation changes to occur. A simple study conducted when preparing As standards for an NSLS trip involved forming mixed metal precipitates (AsMn, AsCu, AsMnCu). The studies showed As will precipitate at pHs higher than 6.5, indicating that the formation of Mn mineral and/or precipitates are possible in the litter environments (pH 8) and the digestive tract of the bird at pHs around 6-7. Therefore, highly redox sensitive surfaces may be forming in these environments, causing As speciation alteration.

The last roxarsone excreta sample (days 10-14) exhibited both similar and unique patterns to that of the first excreta sample (days 1-4). The As distribution in the last ROX excreta collected was again uneven with localized “hotspots”. The As speciation was

more diverse in the last excreta sample than in the first sample (Figure 4.A.2). There were more organic degradation products and reduced As species present in the samples. The As species found in the sample include: roxarsone, As(V), DMA, and As(III). The change from mostly aerobic microorganisms to anaerobic microorganisms suggest that the internal environment of the bird undergoes a change in redox environment, thus a change in its redox potential. This change is noted to happen within the first few days of the bird's life, therefore this change may be occurring or has occurred between the time of sampling the first and last excreta samples. The presence of an increased amount of reduced As species in the last excreta sample, therefore may be expected. The variety of As species detected in this sample may be indicative of the complex internal environment of the digestive tract discussed in the previous paragraphs. Arsenic association with trace metals is a continuing trend throughout all of the samples. The maps for the last roxarsone excreta sample can be found in the appendix (Figures 4.A.2-5). Strong trace metal correlations were not limited to As and Cu in these samples, other metals such as Zn and Mn also displayed strong relationships with As. As time increases, the internal environment of the birds may be changing, thus resulting in changes in As speciation and its behavior in the digestive system. These changes in behavior may result in changes in its reactivity with divalent trace metals. As time goes on, more As-trace metal correlations are seen.

The digestive contents collected from the upper section of the small intestine of the birds from the roxarsone study were analyzed for As distribution, elemental associations and As speciation. This provides direct evidence for the As speciation inside

the bird and may aid in the understanding of the transformation processes occurring within the digestive tract. Arsenic is not evenly distributed, and is localized into "hotspots". XANES analysis of this sample indicates that there is a very complex distribution of As species found within the digestive contents of the upper small intestine (Figure 4.14). The As species detected in this sample includes: ROX, As(V), As(III), with trace amounts of DMA, MMA and As bound to S. Areas with concentrated amounts of As, like the hot spot on Figure 4.13, were mainly roxarsone and oxidized As species, while the more dispersed areas were organic degradation products and reduced As species. This trend is similar to what was seen in the previous chapter with the poultry litters. It would make sense that the ileal samples would be similar in nature to the last excreta samples since they are collected within a similar time frame and internal digestive environment.

Elemental distribution is interesting in these samples. Figures 4.13 and Figures 4.15-18 depict the various relationships and trends seen within this sample. The elemental distribution between spots 1 and 2 differs greatly. This differentiation impacts the quality of the R^2 values for As Vs. divalent trace metals. The correlation values are higher for the ileal sample than they were for the excreta sample, but some are still not as high as the As- metal values in the poultry litter. Cu and As once again had the strongest correlation with a R^2 value of 0.8888. This trace metal analysis indicates that As and various trace metals commonly added to poultry feed are strongly correlated within the digestive tract of the bird. The trends seen in excreta and the poultry litter samples from Chapter 3 apply even at this early stage in excreta formation.

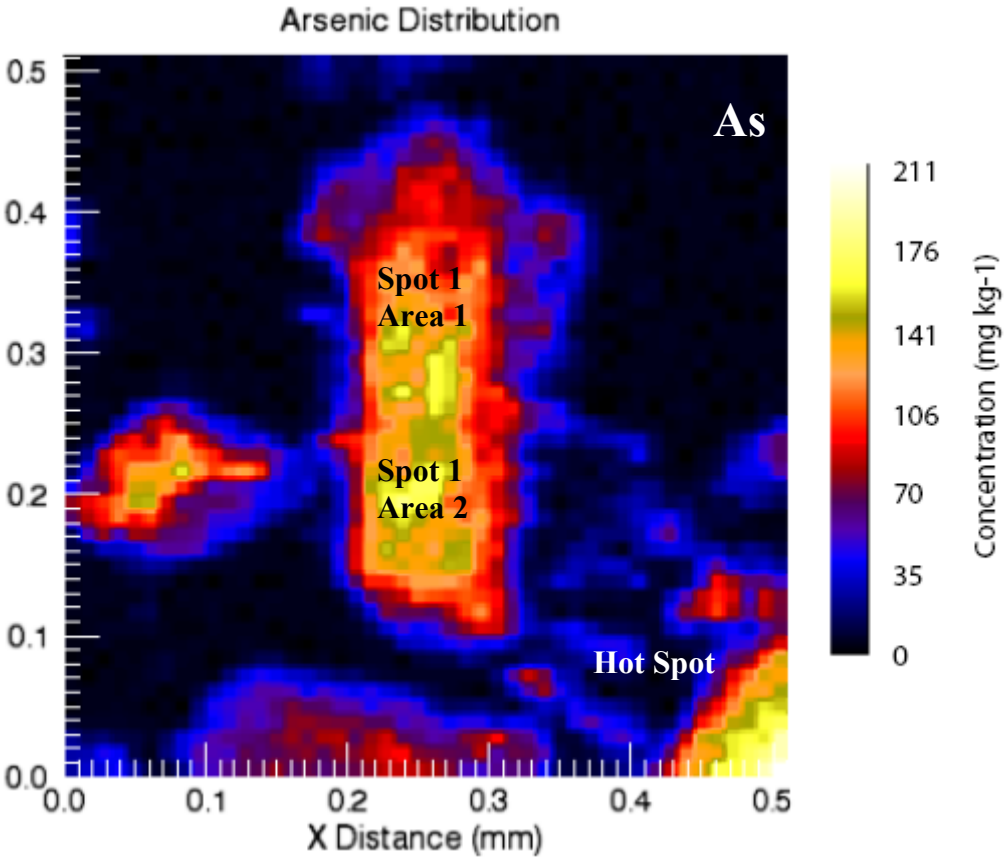

Figure 4.13. Arsenic distribution in roxarsone ileal samples represented in mg kg^{-1} .

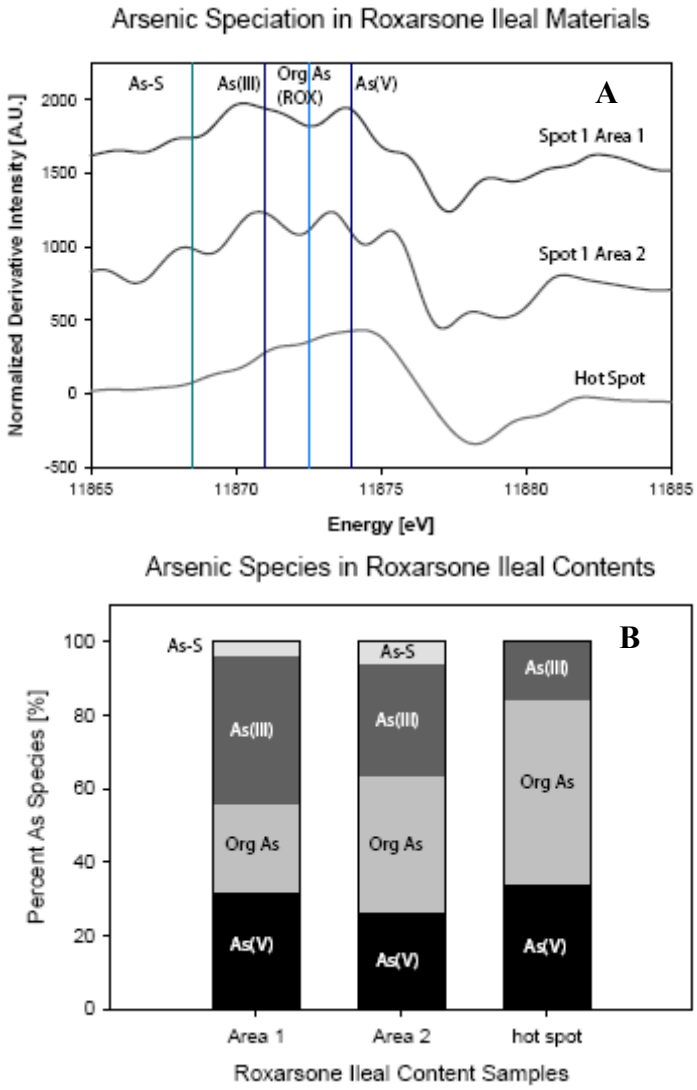

Figure 4.14A and B. Figure A depicts normalized derivative intensity of the XANES scans collected at the locations on the As XRF map in Figure 4.13. Figure B depicts the As compounds found by LCF in the scans in Figure 4.A. in mg kg^{-1}.

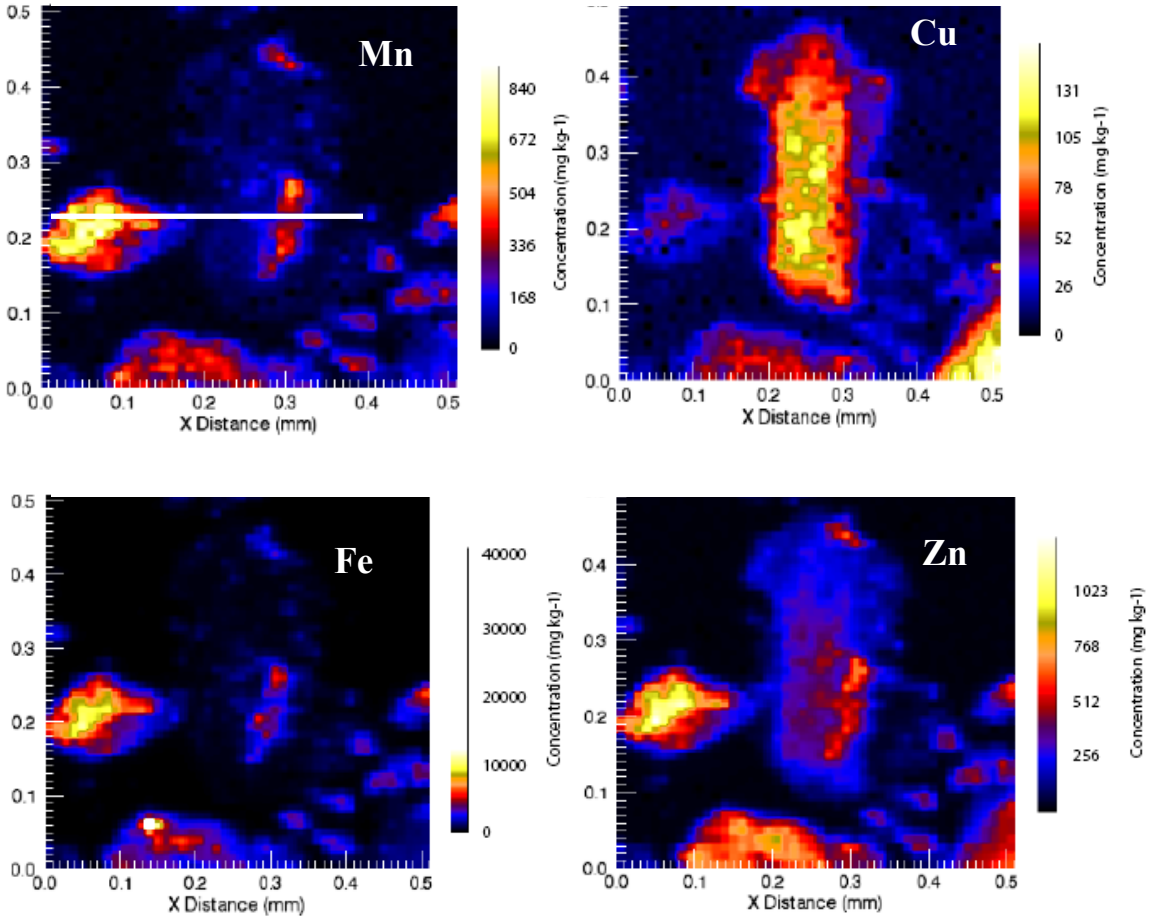

Figure 4.15. Metal distribution in the ileum contents from the roxarsone treatment represented in mg kg^{-1}.

The trace metal distribution can be examined by looking at the data in Figures 4.13 and Figures 4.15-4.18. As and Cu are more highly correlated than the other elements. However, Zn is slightly more correlated than Mn. The correlation values back up these observations, As-Mn (0.2645) and As-Zn (0.5862). The correlation plots for both Mn and Zn are forked meaning that there are two or more separate As-metal trends happening within the map. One fork is most likely the center particle, while the other represents the other smaller particles.

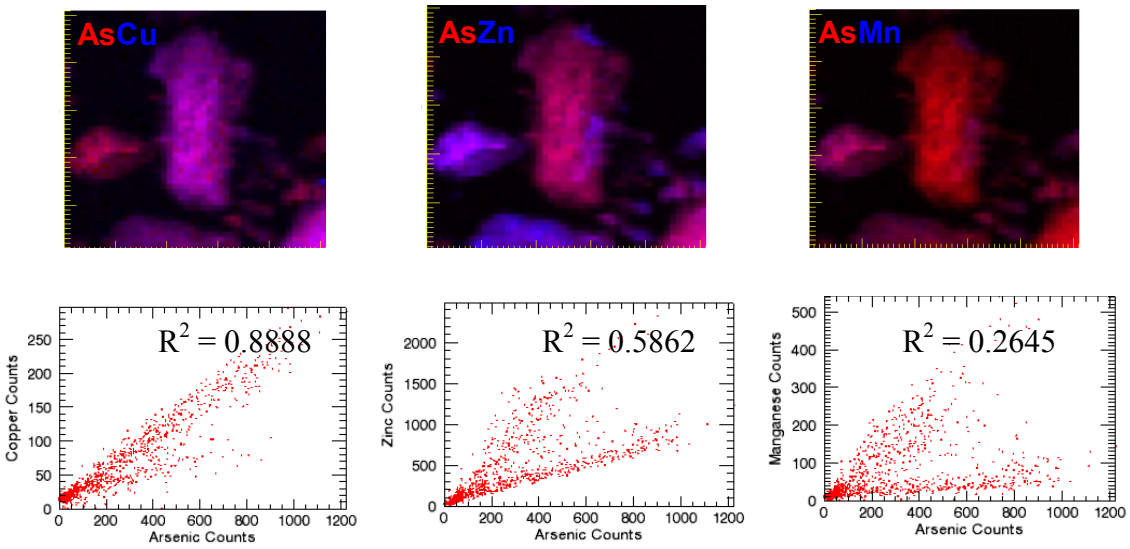

Figure 4.16. Arsenic and trace metal associations and correlations within the ileal contents of a broiler fed a roxarsone diet.

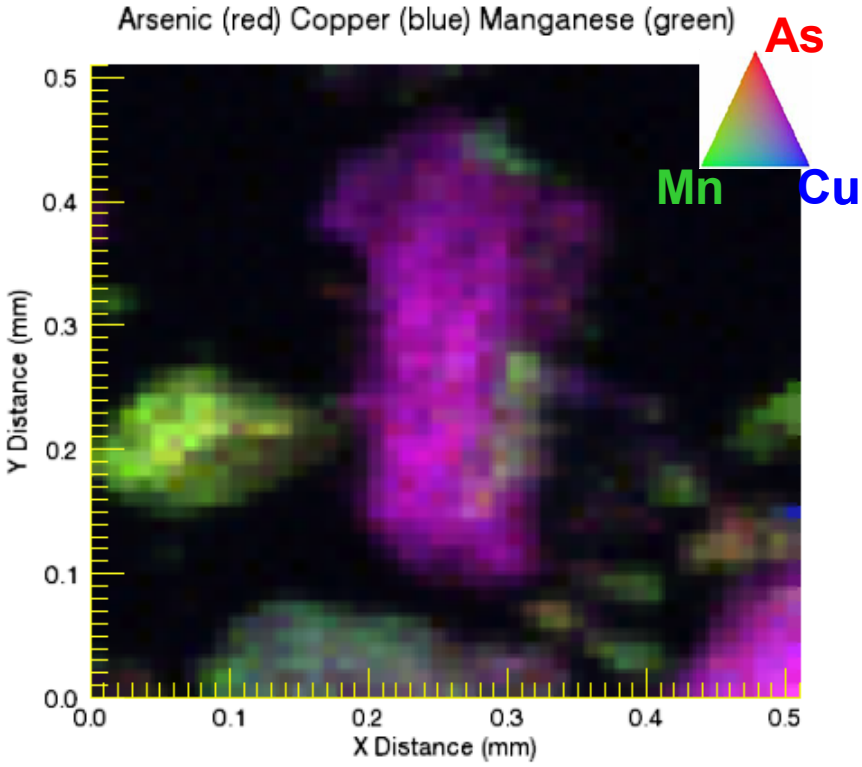

Figure 4.17. Arsenic (red), copper (blue), and manganese (green) associations in the ileal contents of a broiler fed a roxarsone diet. The central particle has a strong As-Cu relationship while the particle on the left has a stronger As-Mn relationship with Mn dominating.

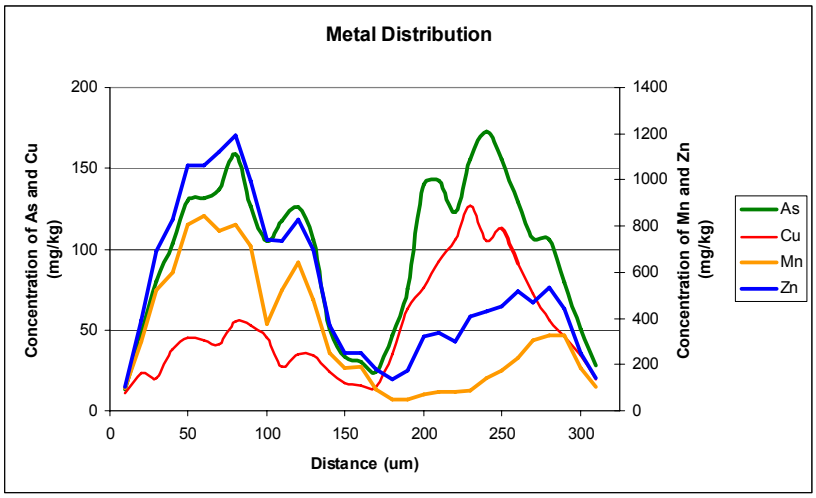

Figure 4.18. Metal distribution across the line drawn in Figure 4.15 (left to right). The central particle has a strong As-Cu relationship while the particle on the left has a stronger As-Mn relationship with Mn dominating. The XRF maps show high Mn on the left and not as much in the middle. This relationship is depicted here.

Inorganic arsenic distribution and speciation in excreta and in the digestive tract of the bird was investigated at beamline X26A at the NSLS. Figure 4.19 depicts the XANES and LCF data for two inorganic ileal scans and one excreta (days 10-14) scan. The XRF maps for these samples are located in the appendix for this chapter (4.A.6-8). As is not evenly distributed throughout these samples. The As speciation results from these samples are opposite of what is seen in the roxarsone samples. The areas of concentrated As are mostly reduced As species (As(III) and As bound to S), while the more dispersed areas are a combination of reduced As and oxidized As species. Since the As was fed in the reduced As(III) state, oxidation of the As(III) must have taken place either within the digestive tract of the bird or once the sample was exposed to the air prior to sample collection. The As species found in both ileal and excreta samples are similar. There is a small fraction of As that is bound to carbon. The organic As compound that

the LCF used for the fitting was DMA. The organic As(III) may be forming organic complexes. The inorganic As(III) treatment behaves oppositely to that of the organic As treatment, indicating that As behavior and As speciation is dependent on the type of As being fed to the birds.

Arsenic speciation in the excreta is highly dependent on the source of As introduced to the system. Organic As has the potential to introduce a wide variety of As species into the environment, while potential inorganic As sources are more limited in terms of the amount of different As species present. Although inorganic As is not intentionally added to the poultry feeds, it may be found in the feeds as a result of natural substitution into mineral structures or the formation of co-precipitates or sorption complexes with minerals commonly added to the feed. Understanding the forms of As being applied to the soils can aid in the understanding of As transport in soil and water environments.

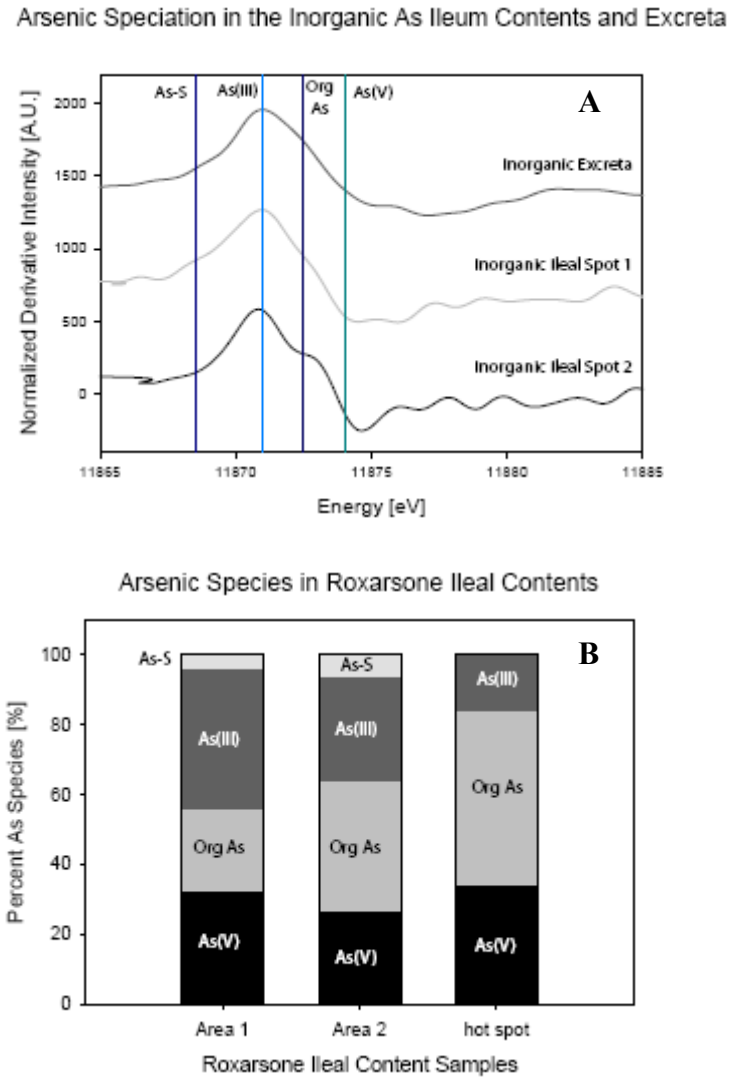

Figure 4.19. XANES (A) and LCF (B) results of inorganic excreta and ileal content. The LCF is presented in mg kg^{-1}.

4.3.2. Arsenic Distribution, Association and Speciation in Broiler Tissues.

A major concern when feeding As to broilers, is the amount of As accumulation in the muscles and tissues of the birds, particularly the portions sold to consumers. The digest results presented in section 4.2.0. suggest that As is accumulating to some degree in the tissues of the birds. Breast and liver samples taken from the roxarsone treatment

study (As for 0-14 days) were analyzed for As and trace metal distribution and association and As speciation.

In both treatments, the liver contains relatively high concentrations of As and trace metals. In order to get a true understanding of elemental distribution in tissue samples, breast and liver samples were cut to 100 μm thicknesses and mounted on mylar film. The tissues were cut using a cryosectioning tool, Leica CM3050 S Cryostat at the Delaware Biotechnolgy Institute at the University of Delaware. This thickness allows for the beam to pass through the sample with minimum sample thickness issues.

The XRF analysis indicates that As is more evenly distributed in the tissue samples than the excreta samples. The trace metals are also more evenly distributed throughout the sample (Figures 4.20-22). It appears that the liver is selective about the types and amounts of trace metals introduced into the organ due to its relatively low amount of Mn, and higher concentrations of Cu and Zn. Bicolor and tricolor maps were not created because the trends were not clear. The maps were always dominated by one stronger element making the trends impossible to see. One noteable trend that is seen in these liver samples is the Ca localization. The intensities on the Ca map have been altered in order to see Ca distribution beyond the hotspots, see the colorbar in Figure 4.21. This indicates that these are very high concentration areas, probably at least 10,000 ppm. Calcium does not follow the same trends throughout the tissue like As, Zn, Fe, and Cu. The MCA plot in Figure 4.22 shows overall elemental distribution in the sample. It is clear that Mn is very low (almost undetectable) and As is very low in comparison to Fe and Zn. Arsenic speciation in liver will be discussed later.

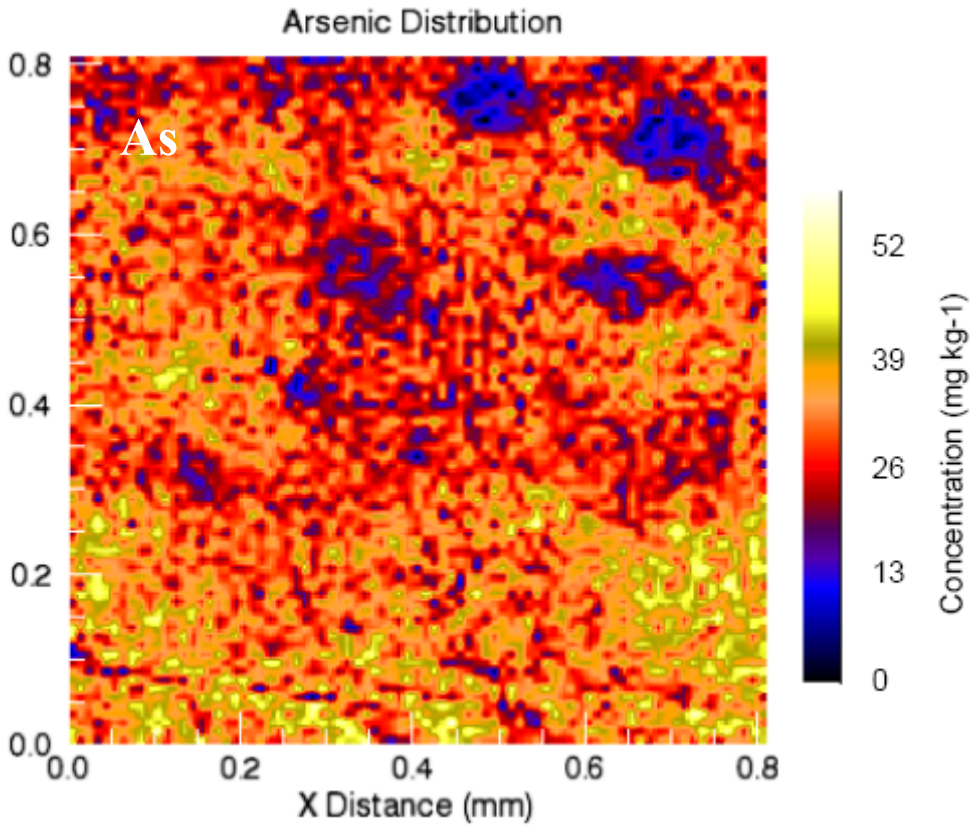

Figure 4.20. XRF and XANES analysis of the liver sample from a broiler fed 14 days of roxarsone represented in mg kg^{-1} .

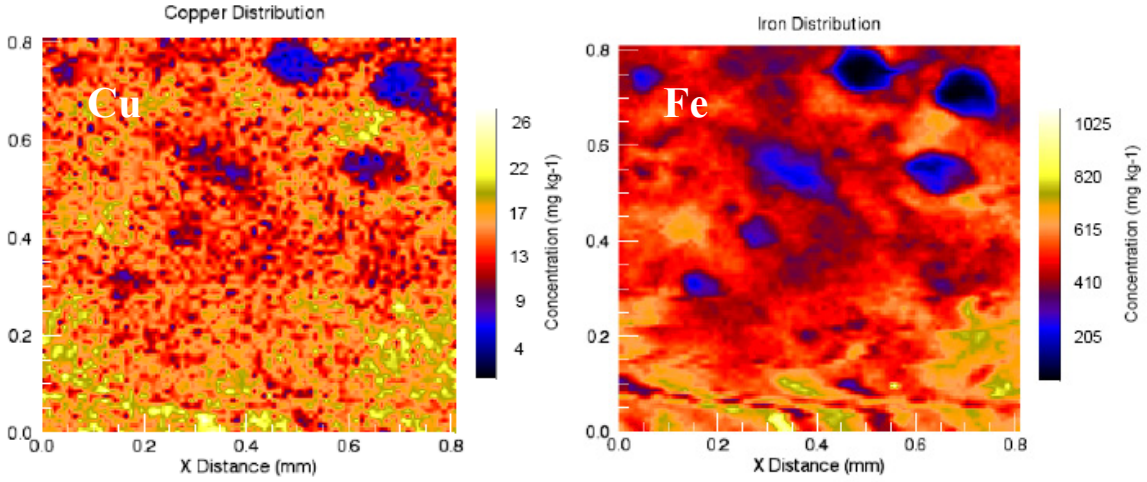

Figure 4.21. XRF maps of trace metal distribution (represented in mg kg^{-1}) in liver samples from broilers fed a roxarsone diet

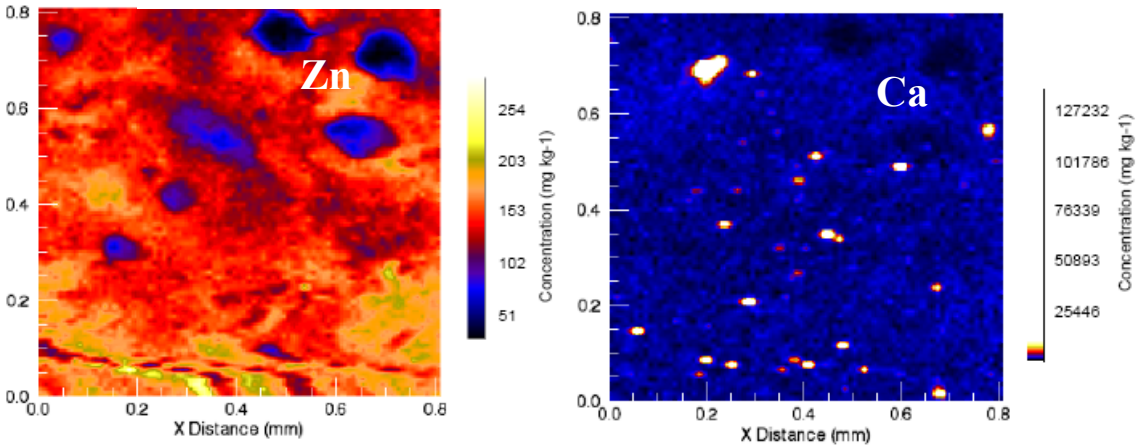

Figure 2.21. (cont.)

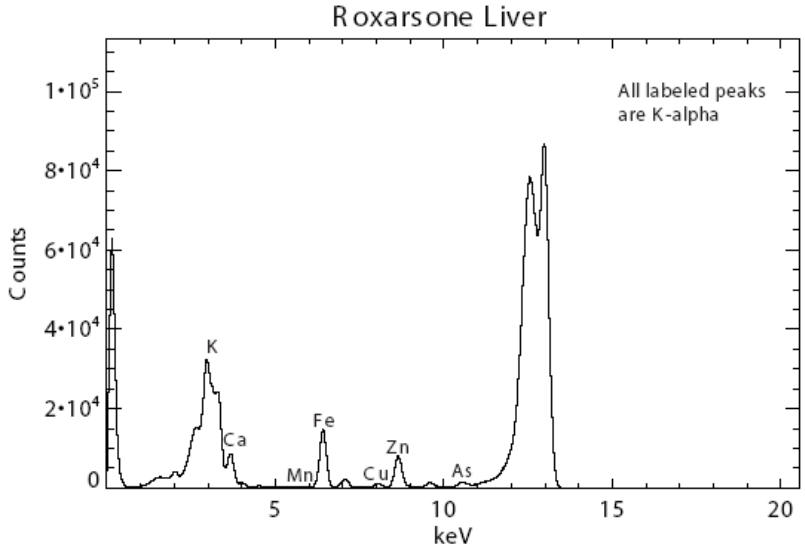

Figure 4.22. MCA summed for the whole liver map. This provides information about the relative total distribution of metals in the roxarsone liver sample.

Arsenic distribution in poultry breast tissues was investigated using XRF mapping and XANES spectroscopy. Figure 4.23 depicts the relative As distribution in a 100 µm cryosectioned poultry breast tissue. The map indicates that As is evenly distributed

throughout the tissue sample and may not have a preferential pattern. Similar to the liver, the trace metals found in the breast muscle also follow a similar pattern. Mn maps were left out of this analysis because they do not provide much valuable information.

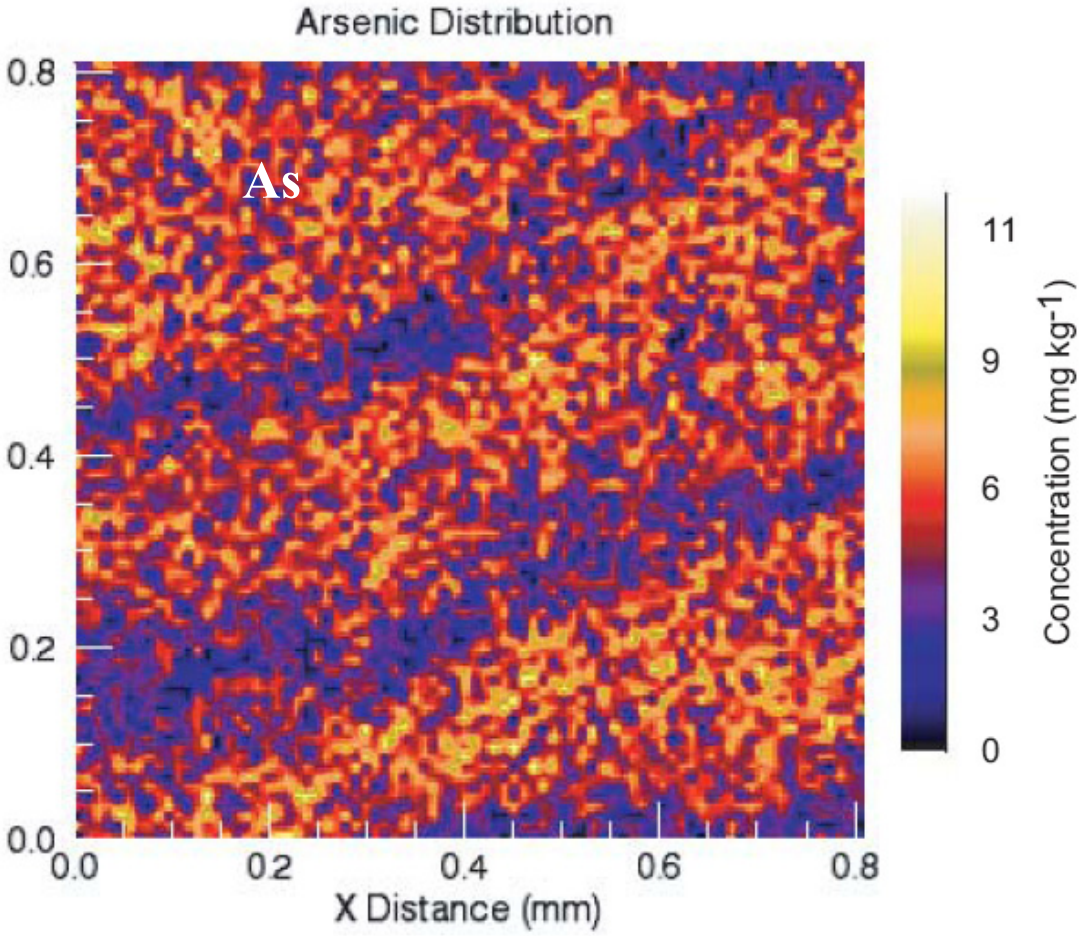

Figure 4.23. XRF map of As distribution (mg kg^{-1}) of breast muscle from a broiler fed a roxarsone diet.

The trace metal distribution data gathered from the XRF maps are limited due to low concentrations of trace metals that accumulated in the breast tissue. Copper, iron, zinc, and calcium distributions are depicted in Figure 2.24. In general, the trace metals are following a similar trend to As. It is interesting to note here that Ca is not found in

localized hotspots like it was in the liver, and levels depicted on the maps are much lower in the breast tissue. The MCA depicted in Figure 2.25 shows an overall metal distribution across most of the map. The As content of these samples is low in comparison to the Zn and Fe. Again, the results indicate that As and the divalent trace metals do not preferentially sorb to muscle components.

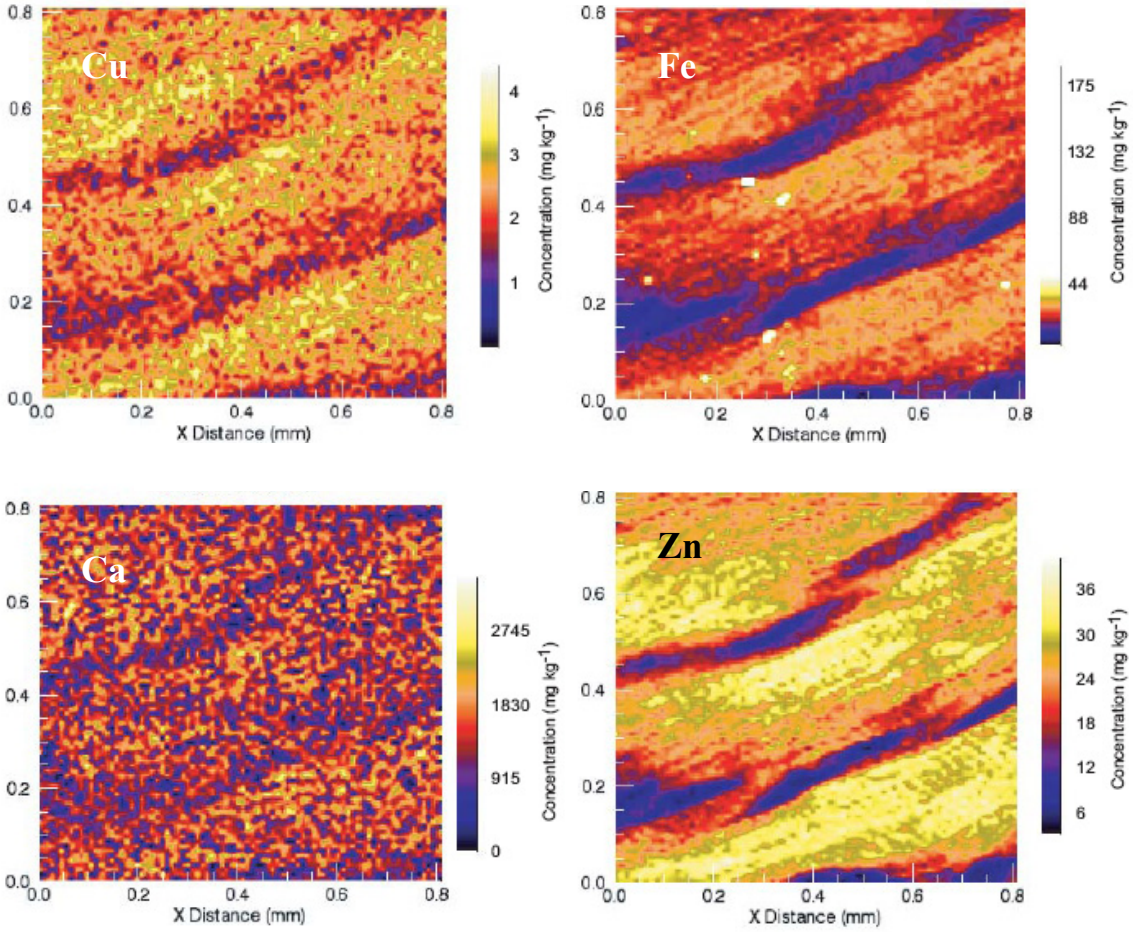

Figure 4.24. XRF maps of trace metals (mg kg^{-1}) found in the breast tissue of a broiler fed roxarsone.

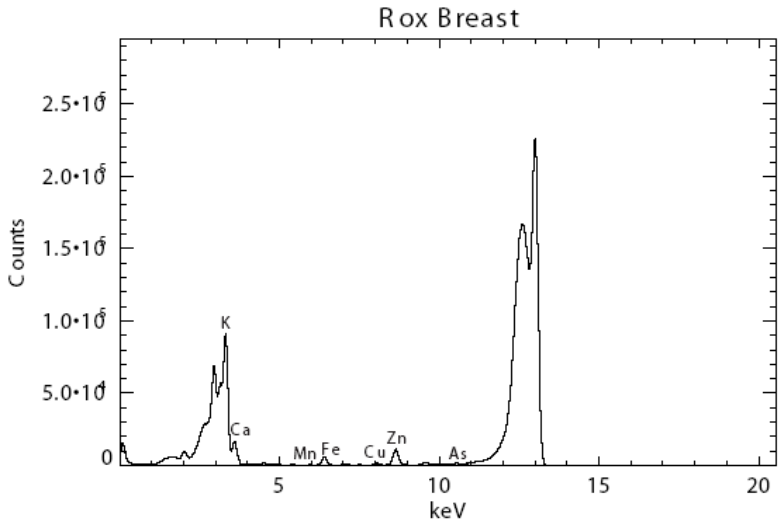

Figure 4.25. MCA plot depicting relative elemental distribution across the breast map.

XANES scans of the roxarsone breast and liver samples were collected on random maps and samples not depicted here. The large maps shown previously did not contain high enough As values to collect As speciation information. XANES scans were collected on hotspots found in the tissue samples. Figure 4.26A and B show the XANES scans and the LCF results for liver and breast. The As speciation is presented in percent.

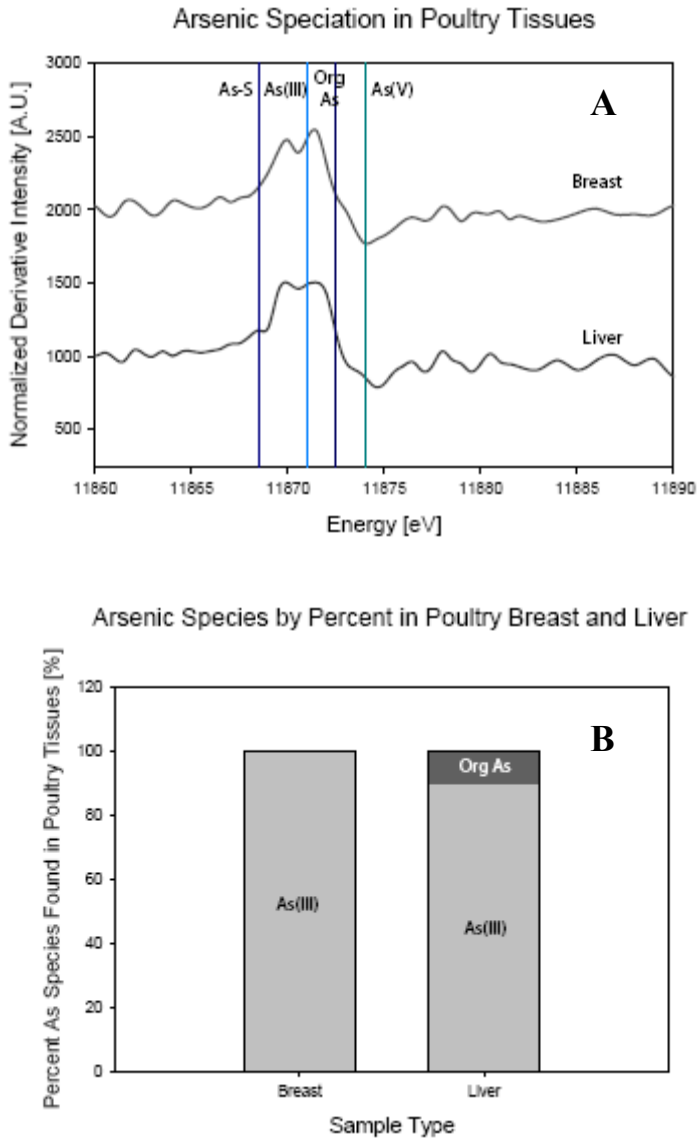

Figure 4.26 A and B. Normalized derivative XANES scans for breast and liver. The LCF results indicate that the majority of the As found in the breast and liver is reduced As(III).

The XANES results indicate that the majority of the As found in the liver and breast is reduced inorganic As(III). As(III) accumulation in tissues is commonly associated with S bearing compounds, commonly S-bearing amino acids. As(III) can also bind with other S compounds and can bind to over 200 enzymes. However, As

bound to S and As bound to O have significantly different whitelines and derivative values, and based on the XANES data, the As(III) in this sample is not bound to sulfur. The concentrations of As in cryosectioned tissues were not concentrated enough to collect XANES scans. Finding As(III) in the poultry tissues indicates that roxarsone degradation had to occur in order for As(III) accumulation to happen. No matter what form As is present in the feed, the majority of the As found in the body is inorganic As(III). It is important to note that these scans are preliminary and possibilities of beam reduction need to be properly ruled out.

Breast and liver taken from birds fed the inorganic As(III) were examined using XRF to determine As distribution in these samples. The maps of the inorganic As(III) treated samples were identical to the roxarsone treated samples. As and the trace metals all followed a similar trend. Even the Ca was localized into hot spots in the liver. As XRF maps for the breast and liver are found in Figure 4.27. These results indicate that the form of As fed to the bird does not change the way As is distributed in the breast and liver.

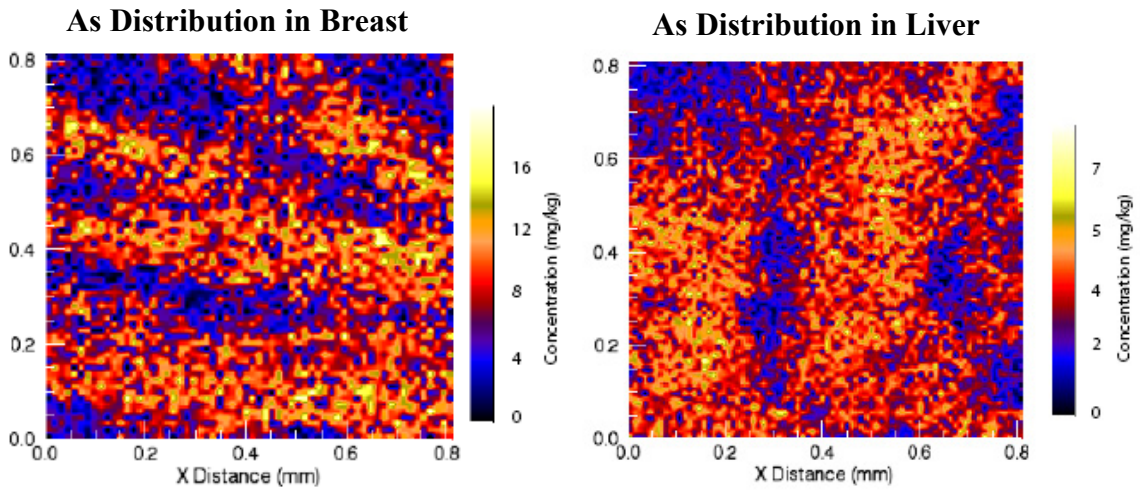

Figure 4.27. XRF maps of As in Breast and Liver from the full As(III) treatment.

4.3.3. Investigating Crystalline Compounds Using Synchrotron Based X-ray Diffraction.

X-ray diffraction has been used for years to determine the identity of crystalline compounds in a variety of materials. It is commonly used in soils in order to identify clay mineral suites. Similar to the XANES analysis conducted in the earlier sections, two different forms of synchrotron based X-ray diffraction were used to identify crystalline materials in the litter samples. Bulk X-ray diffraction was collected at beamline 11-3 at the Stanford Synchrotron Radiation Lightsource in Menlo Park, CA. The bulk X-ray data provides an overall picture of the crystalline compounds found in the litter samples. Microfocused XRD was collected at beamline X26A at the National Synchrotron Light Source in Upton, NY.

The identity of the compounds in the roxarsone excreta and ileal samples are shown by the 1D line plots, also called XRD patterns (Figure 4.28). The XRD patterns

depicted in this diagram are representative μ-XRD taken at beamline X26A. The excreta and ileal samples did not exhibit much variability and in many cases were very amorphous. There were a few compounds that were identified in all of these scans. Roxarsone was found in almost all of the XRD patterns that were collected in As-bearing areas. There are two main forms of P found in these samples: struvite and divalent metal forms of phosphate (Zn and Mn). Silicates also continue to turn up in excreta and ileal XRD patterns, similar to the poultry litter samples. Due to the high levels of trace metals and phosphate in poultry excreta, it should not be surprising to find divalent phosphate minerals in the excreta.

The first XRD pattern in Figure 4.28 is from the roxarsone ileal map that was presented earlier in this chapter. It was taken in the center spot of the map. It is dominated by roxarsone and a few other compounds. The other two patterns are of excreta samples. Note the large mound in the middle of these scans. At first glance it appears to be improper background subtraction. However, the poultry litter scans from the previous chapter and the bottom scan in Figure 4.28 background subtract out nicely. There appears to be some unidentified amorphous compound present in these samples. The same amorphous compound is found in all of the bulk XRD samples collected at the SSRL. These patterns are located in the appendix for this chapter (Figure 4.A.9). The compounds are not labeled on the scans, but the results were very similar to the μ-XRD data with roxarsone, silicates, and phosphate minerals being the most dominant crystalline species. Crystalline roxarsone was found in both the full roxarsone excreta samples and the full roxarsone ileal sample. However, it was not found in the samples

where As was removed. It appears that the As is readily flushed from the digestive tract after As removal from the feed. Although the arsenstruvite (NH_4MgAsO_4) compound that was found in the aged poultry litter was not found in these samples, a number of other ammonia bearing minerals were found. Ammonium copper sulfate was identified in the full roxarsone ileal sample, the first roxarsone excreta sample (days 1-4), and the last excreta sample in the roxarsone removal study (days 10-14). The trace metals added to the poultry feeds are usually added as sulfate minerals. Therefore, finding divalent-sulfate minerals is not surprising. However, this means that not all of the minerals added to the poultry feeds are thoroughly dissolved in the digestive tract. The high amount of ammonia in the excreta appears to form less soluble compounds like ammonia copper sulfate and ammonia magnesium arsenate/phosphate. Again, these types of compounds should reduce the amount of water soluble trace metals in excreta and soil environments.

Coupling XRD along with XANES analysis can provide additional information about As speciation. XRD also provides important information about environmentally relevant compounds that are not discovered using element specific techniques.

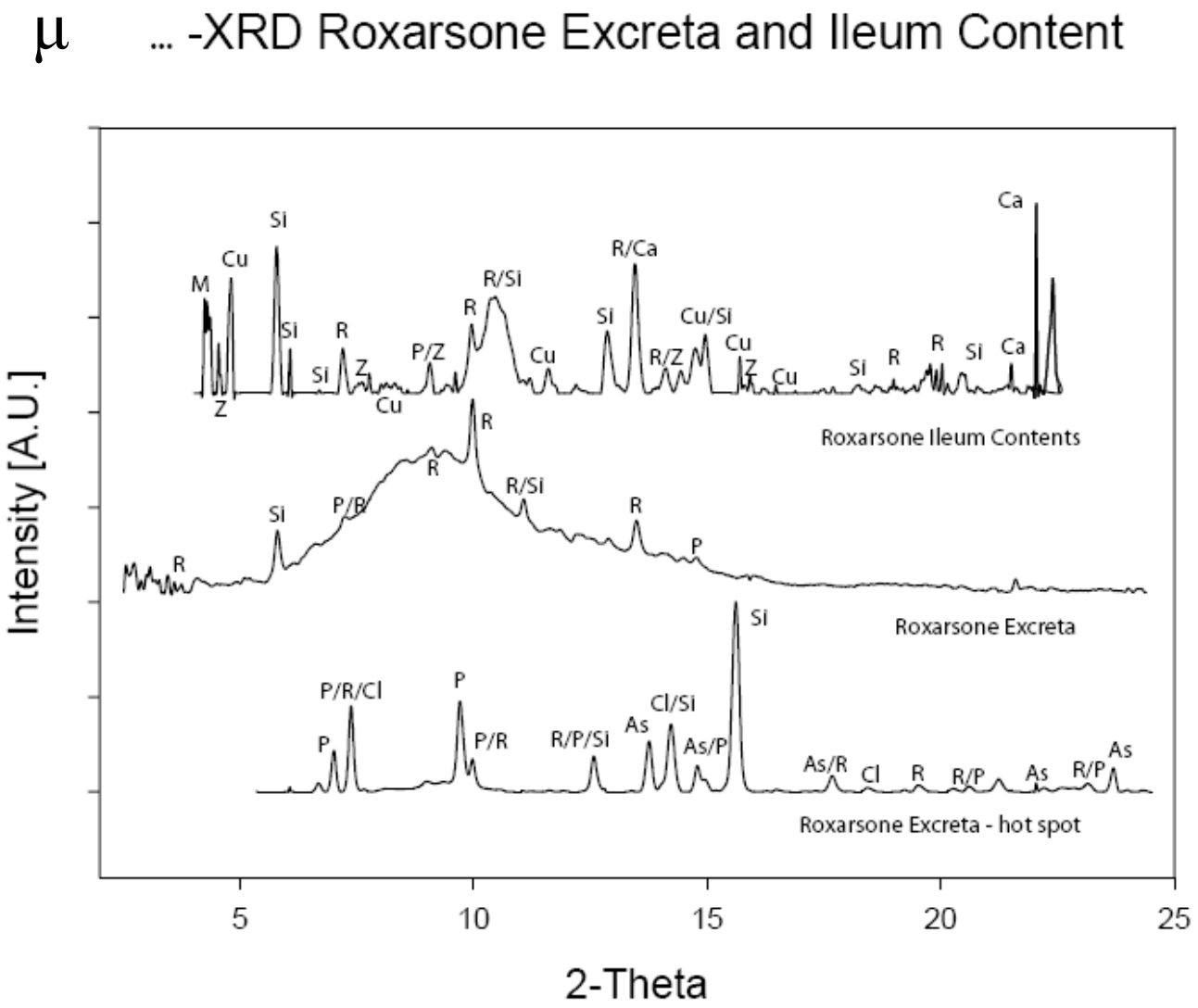

Figure 4.28 Micro-XRD patterns for ROX excreta and ileal samples. The samples contain a combination of P, Si, carbonate, and ROX.

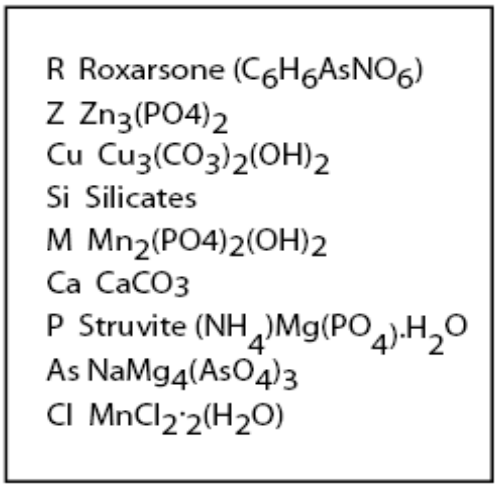

Figure 4.28. (cont.)

4.3.4. Arsenic Speciation of Poultry Excreta and Ileal Contents Using Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometry.

Liquid chromatography coupled with ICP mass spectrometry can be used to separate out different arsenic species in a liquid sample or extract by mass. X-ray Absorption Near Edge Structure (XANES) spectroscopy and X-ray Diffraction (XRD) are both ways to examine arsenic speciation or chemical composition of a solid material, while LC-ICP-MS is speciation in a liquid sample. The first step was to run the individual As species: As(III), As(V), DMA, MMA, Roxarsone, HAPA, and p-arsanilic acid. This was done in order to determine at what time these species would separate out on the column. It was found that As(III) would come out first at a time of 1.81 minutes, followed by DMA (2.66), MMA (3.61), Roxarsone/P-arsanilic acid (4.29), HAPA (4.88) and As(V) at 5.68 minutes. Figure 4.29 is a chromatogram of the six As species analyzed. Roxarsone and P-arsanilic acid came out at the same time, therefore making it

impossible to determine one from the other. Once this information was determined, a series of mixed As standards were run for calibration purposes.

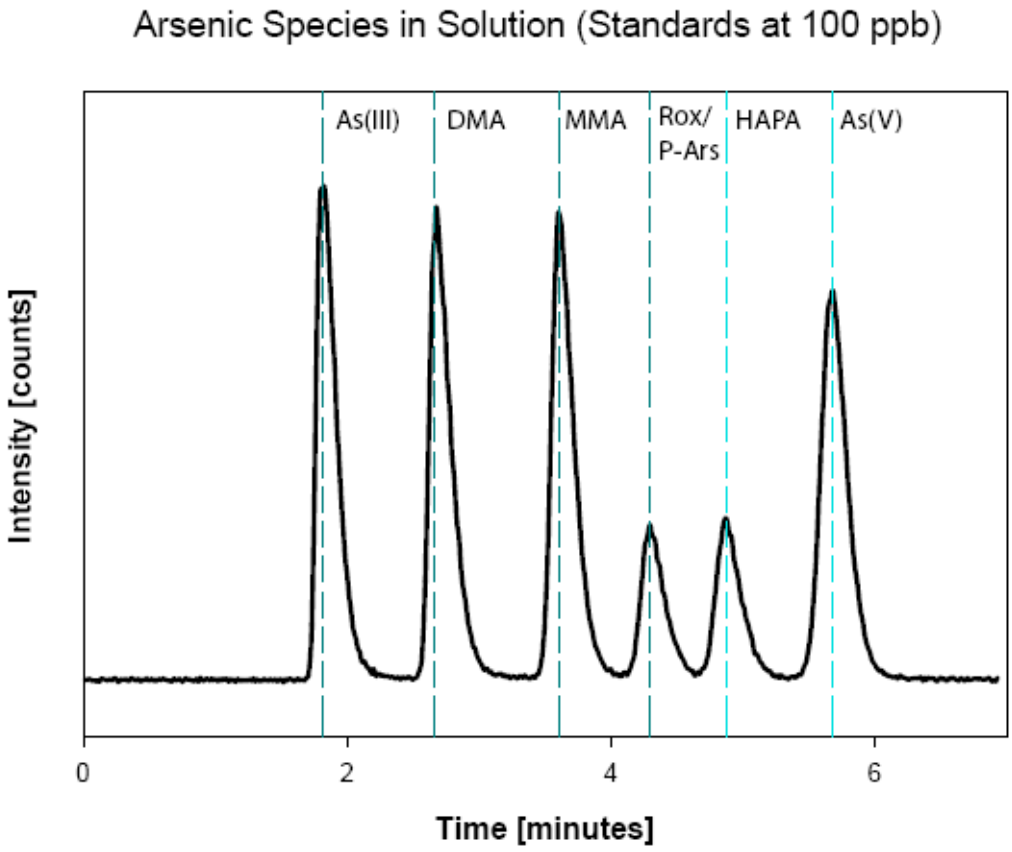

Figure 4.29. Chromatogram of arsenic species: Arsenite (As(III)), Dimethylarsenate (DMA), monomethylarsenate (MMA), Roxarsone/P-arsanilic acid (Rox/P-ars), 4-hydroxy 3-amine arsenic acid (HAPA), and Arsenate (As(V)).

The roxarsone excreta and ileal samples were extracted with DI H_2O, in order to represent the water soluble fraction of the poultry excreta materials. This extract was 20-fold diluted and then injected into the LC-ICP-MS. The results are depicted in Figure 4.30A and B. Figure 4.30B is a smaller portion of Figure A (from 1 to 4 minutes). The liquid extract data are similar to the XANES data. Discrepancies can be attributed to the

difference between examining a solid sample and a liquid extract. Oxidized arsenic in the form of As(V) is found in all poultry excreta and ileal samples. Some amount of As(III), DMA, MMA, and HAPA are found in all the samples. Roxarsone is the one As species that was not present or was very limited in these extracts. Extractable roxarsone was not found in the poultry litter samples either. The percent and amount of each form of arsenic can be found in Tables 4.14 A and B. The total As concentrations were much lower than the water soluble values presented in Table 4.A.2. This could be attributed to the As retention exhibited in the IC column.

A considerable amount of reduced arsenic is found in the two excreta samples (about 3-12%). These samples contain As(III) in the XANES analysis as well. The ileal samples have the most As(III) out of all of the samples. The XANES analysis also showed a high amount of reduced As in the scans. These results indicate that roxarsone degradation is occurring within the bird, and continues after exiting the body. Similar to current research, a larger portion of the extractable As is As(V). It is interesting to note that HAPA and DMA were found in the samples and not roxarsone. As a side note, when a blank sample (water) was run after the excreta samples a series of unidentified As species came out about 7-10 minutes after these samples were run through the column. It is possible that some As species are being retained in the column. It seems likely that organic species would be attracted to the charges in the column. However, it is impossible to make any conclusions about the identity of these compounds without the proper standards.

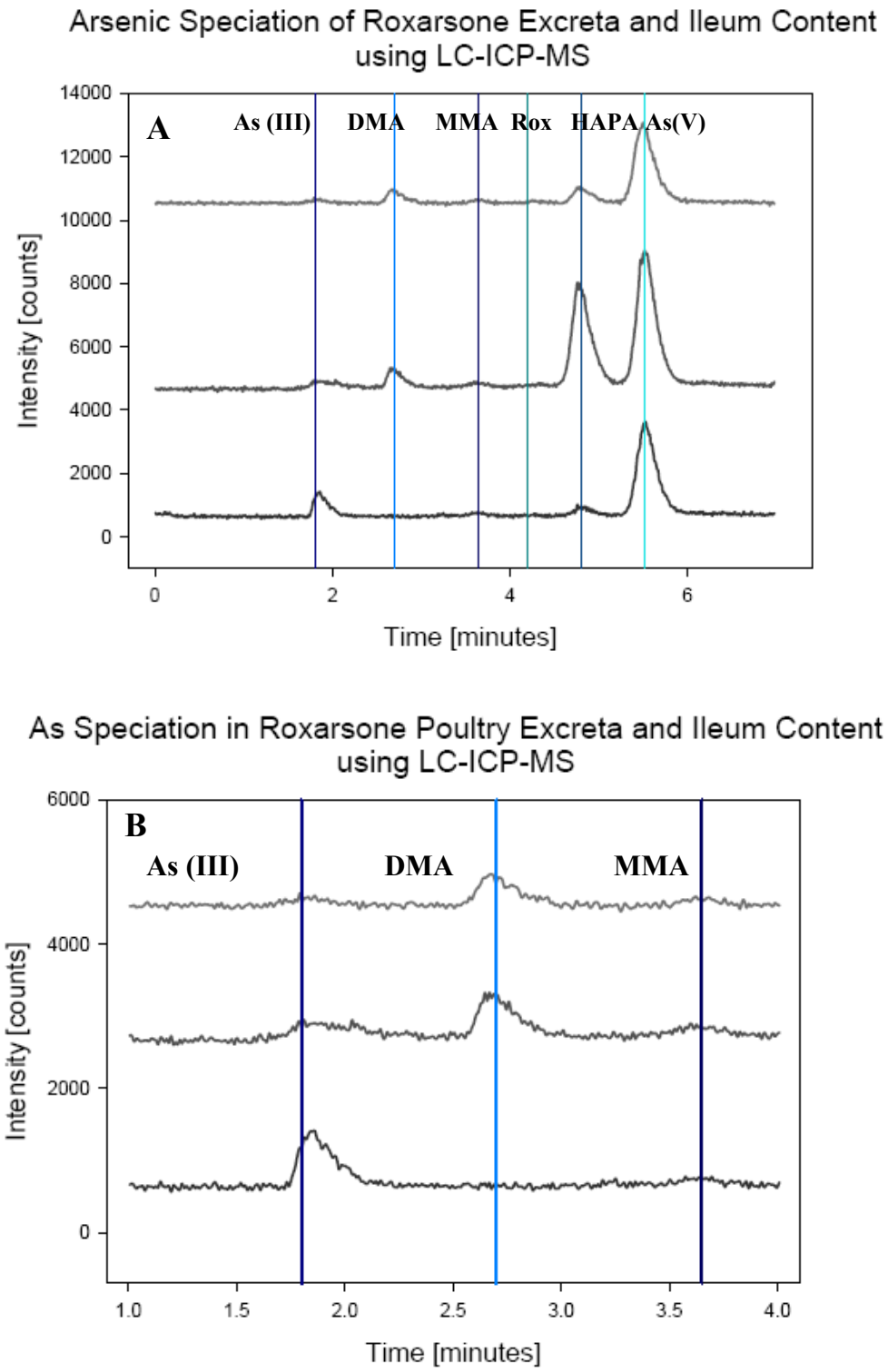

Figure 4.30 A and B. Water extractable As species in excreta and ileal samples. Figure A is the whole chromatogram, while Figure B shows a section of the chromatogram.

Table 4.14 A and B. Percent and total amount of As species in water exctracts of roxarsone excreta and ileal contents.

A

Percent Arsenic Species in Poultry Litter as Determined by LC-ICP-MS

Sample	As(III)	DMA	MMA	Rox/P-ars	HAPA	As(V)
Rox Exc. (Days 1-4)	2.7	10.7	2.4	--	17.5	66.3
Rox Exc. (Days 10-14)	12.2	12.6	8.4	2.8	33.5	30.5
Rox Ileum Contents	21.5	4.8	12.4	2.0	7.1	52.3

B

Concentration (ppb) Arsenic Species in Poultry Litter as Determined by LC-ICP-MS

Sample	As(III)	DMA	MMA	Rox/P-ars	HAPA	As(V)
Rox Exc. (Days 1-4)	243.1	951.4	216.3	--	1566.6	5917.5
Rox Exc. (Days 10-14)	536.0	555.2	371.8	122.7	1475.4	1343.4
Rox Ileum Contents	620.9	138.4	357.7	56.5	205.4	1511.0

4.4. Conclusions.

4.4.0. Impacts of As Supplements on As Content and Speciation in Excreta.

The consequences of adding arsenic to poultry feeds was examined in this study. Arsenic speciation played a role in As distribution in the bird, rate of excretion, elemental association, and As speciation in excreta. Studies have documented that organic As will be removed from the body before inorganic As. This study does indicate that organic As and the inorganic As(III) were excreted along different time frames. The difference between the control and the excreta (when As was withdrawn) is different in the 7-10 days excreta, yet they are not significantly different in the 10-14 days excreta. This indicates that after a few days the As is flushed from the body, and the As content in the final excreta sample (for both As regimes) is similar to the As content in the basal feed. In most cases, except for the roxarsone As removal study, the ileal concentrations were

not different from the last excreta. There is a complete set of metal values for the excreta samples in the appendix of this chapter.

Changes within the digestive tract of the bird may play a role in As speciation. A mix of As species is found in all of the excreta and ileal samples. The XANES data show a large amount of inorganic As(III) and As(V) in the excreta samples. Both roxarsone and a number of other organic compounds are found and fit the data well. The LC-ICP-MS data also showed that there are many As species found in these materials. As(III) was found to some degree in all samples with the most found in the ileal samples. The XANES data show a trend towards more reduced As species in the last excreta sample and the ileal samples indicating there are conditions conducive to roxarsone degradation within the bird. These results indicate that roxarsone degradation does begin inside the bird itself. The XAS data analysis of the inorganic As(III) treatment indicates that As(III) remains the dominant As species in the system. Some traces of oxidized As species and some As bound to C were found in the inorganic As(III) treated last excreta sample. Changes in As speciation are more noteworthy in the roxarsone treatments than in the inorganic treatments.

The results from this study both agree and contradict with studies found in the literature. Many studies, most of which are liquid analyses, find As(V) to be the dominant form of As in the excreta. The LC-ICP-MS data indicates that this is the case. However, the XANES data show a higher percentage of both organic and reduced As species. This could be attributed to differences between solid and liquid speciation techniques. The current understanding of roxarsone bioaccumulation and degradation

indicates that roxarsone is excreted as roxarsone and that limited breakdown occurs within the body. However, both the liquid and solid analysis of the roxarsone ileum contents show a mix of As species present in these samples. This study also indicates that there may be even more different forms of As in the excreta than initially imagined.

Similar to the trends seen in Chapter 3, As is highly associated with the trace metals added to poultry feeds (Cu, Mn, Zn, and Fe). As and Cu appear to have a strong relationship in these materials. If roxarsone is combining with the divalent trace metals, then it may have an impact on roxarsone and Cu reactivity in the digestive tract of the bird. If roxarsone does form a complex with Cu, this may lead to As stability in the excreta and potentially in soil and water environments after land application of the litter. Further investigation of the reactivity of roxarsone and trace metals should be investigated in order to determine if ROX is limiting nutrient availability.

The data suggest that land application of poultry excreta introduces a number of As species into soil and water systems. Each of these species will behave differently in these complex environments. The presence of reduced As(III) indicates that there is a considerable amount of soluble and potentially very mobile As in these samples. Therefore, this information should be taken into consideration during BMP development, but also when determining if roxarsone should be used at all.

4.4.1. The Impacts of As Supplements on As Content and Speciation in Broiler Tissues.

The form of As being ingested by the birds may play a role in As and Cu bioaccumulation. The other trace metals that were analyzed in this data set were not impacted by the form of As or the change in As dietary patterns. As and Cu values in the skin were not affected by treatment. More As and Cu accumulation were seen in the feathers when the birds were fed inorganic As(III), but not when fed organic As. This metal(loid) accumulation was not seen in the feathers of the As(III) removal study indicating that removing As a week before slaughter did reduce the amount of As and Cu in the feathers. Arsenic accumulation in the breast also followed a similar pattern. A significant amount of Cu accumulation in the breast was seen when both As species were fed for the full 14 days. In both cases, the Cu content dropped after As removal from the diet. The liver contained more As than any other tissue. There was a significant difference between the control liver and almost all of the As treatments, except for the As(III) removal study. This indicates that As is not fully removed from the liver when the bird is fed roxarsone. Significant Cu accumulation was not seen in the liver of the birds.

The As found in the roxarsone and inorganic As(III) fed broiler tissue is evenly distributed. As accumulation in the breast does not appear to follow any specific trends. It was first thought that As may be more prone to accumulate in specific locations within the muscle, but these results do not support this theory. Other detectable trace metals in the poultry tissues follow a similar trend. As and most of the trace metals are evenly

distributed in the liver, and do not appear to follow a strict trend. However, Ca is located in localized hotspots in both As treatments. As speciation of the roxarsone liver and breast samples indicate that As(III) is predominant in these samples. As(III) accumulation is commonly found in animal tissues and is commonly associated with S bearing compounds and enzymes.

Removing As from the diet seven days before slaughter does remove As from the organs and tissues of the bird. However, all of these values are above the FDA's level of 500 ppb in poultry tissue. So, although this does appear to work, As levels found in this study does not currently meet the standards. It appears that either less As needs to be used or roxarsone should not be used in US poultry operations.

4.5 Appendix for Chapter 4

4.5.0. Arsenic Standards Used for Linear Combination Fitting

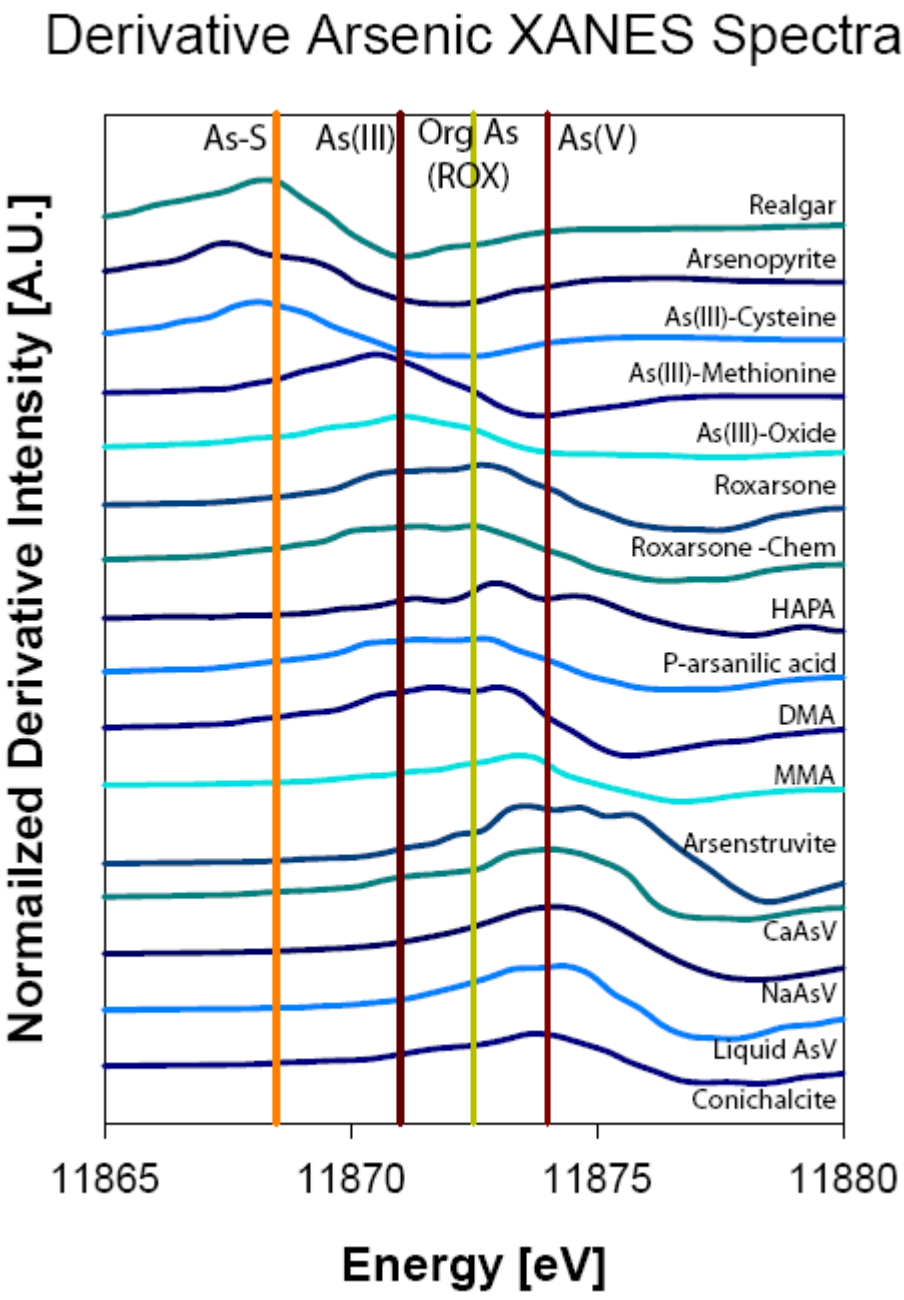

Figure 4.A.1. XANES spectra of As standards used in As speciation.

Table 4.A.1. A table of arsenic standards used in linear combination fitting (LCF). The first column depicts compounds that were commonly found to be fits for the litter experimental scans. The category indicates which As species group the standard belongs to. Note: the As(III) methionine is under the As(III) category because there was not an As-S bond established in solution.

LCF Fit	Standard/ As Compound	Category	Formula
x	Liquid Arsenate	As(V) - Arsenate	$Na_2HAsO_4{\cdot}7H_2O$
	Arsenstruvite	As(V) - Arsenate	$NH_4MgAsO_4{\cdot}6H_2O$
	Conichalcite	As(V) - Arsenate	$CaCuAsO_4(OH)$
x	Calcium Arsenate	As(V) - Arsenate	$Ca_3(AsO_4)_2$
x	Sodium Arsenate	As(V) - Arsenate	$Na_2HAsO_4{\cdot}7H_2O$
x	Roxarsone (Commercial) (3-nitro-4-hydroxyphenly-arsonic acid)	Organic Arsenic	$C_6H_3(NO_2)AsO(OH)_3$
	Roxarsone (Chemical) (3-nitro-4-hydroxyphenly-arsonic acid)	Organic Arsenic	$C_6H_3(NO_2)AsO(OH)_3$
x	HAPA (4-hydroxy-3-aminophenylarsonic acid)	Organic Arsenic	$C_6H_3(NH_2)AsO(OH)_3$
	P-arsanilic Acid (4-amino-phenylarsonic acid)	Organic Arsenic	$C_6H_3(NH_2)AsO(OH)_2$
x	Monomethylarsenate (MMA)	Organic Arsenic	$CH_3AsO(OH)_2$
x	Dimethylarsenate (DMA)	Organic Arsenic	$(CH_3)_2AsO(OH)$
	Arsenic Oxide	As(III) - Arsenite	As_2O_3
x	Liquid Arsenite	As(III) - Arsenite	$NaAsO_2$
	Sodium Arsenite	As(III) - Arsenite	$NaAsO_2$
x	As(III) - Methionine	As(III) - Arsenite	$C_5H_{11}NO_2S$
x	As(III) - Cysteine	AsS-Sulfur	$C_3H_7NO_2S$
x	Realgar	AsS-Sulfur	As_4S_4
	Pararealgar	AsS-Sulfur	As_4S_4
	Orpiment	AsS-Sulfur	As_2S_3

Tables 4.5.1.Total Water Soluble Metals in Poultry Excreta

Table 4.A.2. Water soluble metal concentrations of poultry excreta for all treatments.

Treatment	Excreta Sample	As	Ca	Cu	Mn	Fe	Zn
		----------	----------	($mg\ kg^{-1}$)	----------	----------	----------
1. Control	Days 1-4	2.7	1679.8	1.4	2.7	12.8	27.4
	Days 4-7	8.4	920.1	1.6	8.4	14.6	38.5
	Days 7-10	2.2	1067.3	1.8	2.2	16.9	9.4
	Days 10-14	1.0	1531.8	1.7	1.0	4.7	20.0

Treatment	Excreta Sample	As	Ca	Cu	Mn	Fe	Zn
		----------	----------	($mg\ kg^{-1}$)	----------	----------	----------
2. Roxarsone	Days 1-4	57.4	1667.6	1.4	57.4	10.3	31.8
	Days 4-7	80.8	1938.7	1.5	80.8	15.5	27.3
	Days 7-10						
	Days 10-14	91.3	2239.4	1.7	91.3	11.9	35.1

Table 4.A.2. (Cont.)

Treatment	Excreta Sample	As	Ca	Cu	Mn	Fe	Zn
		----------	----------	(mg kg^{-1})	----------	----------	----------
	Days 1-4	12.4	1652.2	0.9	12.4	-5.3	23.4
3. Inorganic	Days 4-7	45.7	1013.1	1.5	45.7	7.7	31.9
As(III)-oxide	Days 7-10	40.8	462.6	1.7	40.8	10.5	39.6
	Days 10-14	52.8	816.2	2.0	52.8	6.5	34.2

Treatment	Excreta Sample	As	Ca	Cu	Mn	Fe	Zn
		----------	----------	(mg kg^{-1})	----------	----------	----------
4. Roxarsone	Days 1-4	67.0	1132.8	1.2	67.0	4.6	42.1
(days 1-7)	Days 4-7	90.9	891.6	1.6	90.9	17.0	35.9
No Roxarsone	Days 7-10	13.4	365.4	1.6	13.4	25.8	52.2
(days 7-14)	Days 10-14	4.3	1511.7	1.6	4.3	11.8	19.0

Treatment	Excreta Sample	As	Ca	Cu	Mn	Fe	Zn
		----------	----------	(mg kg^{-1})	----------	----------	----------
5. Inorganic As	Days 1-4	36.1	755.3	1.9	36.1	6.0	41.5
(days 1-7)	Days 4-7	37.9	1567.1	1.6	37.9	12.9	13.5
No Arsenic	Days 7-10	33.5	354.6	4.7	33.5	13.0	8.6
(days 7-14)	Days 10-14	378.2	1552.7	1.4	378.2	6.5	26.2

Tables 4.5.2. Total Metal Content in All Poultry Excreta, Ileal, and Tissue Samples

Table 4.A.3. Total Arsenic and mean metals concentrations in all poultry samples for the control treatment.

Treatment	Sample	As	Ca	Cu	Mn	Fe	Zn
		----------	----------	(mg kg^{-1})	----------	----------	----------
	Feed (days 1-14)	2.2		8.4	20.3		84.1
Control	Days 1-4	2.0	10557.1	15.1	54.7	208.3	177.2
	Days 4-7	4.1	15206.1	19.1	73.2	293.8	200.6
	Days 7-10	1.0	10436.3	21.6	61.1	180.1	211.3
	Days 10-14	1.0	14157.2	26.8	81.1	460.2	266.6
	Ileum	2.5	17265.6	46.4	125.7	1130.6	313.0
	Feather	0.2	280.3	4.8	5.7	8.6	57.7
	Skin	0.4	197.9	1.9	0.7	15.1	25.9
	Breast	1.2	845.2	0.9	1.2	50.7	36.0
	Liver	0.4	1038.3	10.0	8.4	366.8	114.9

Table 4.A.4. Total Arsenic and mean metals concentrations in all poultry samples for the roxarsone treatment.

Treatment	Sample	As	Ca	Cu	Mn	Fe	Zn
		----------		(mg kg^{-1})	----------		
	Feed (days 1-14)	19.6		8.3	17.1		77.0
Roxarsone	Days 1-4	45.1	13044.84	22.4	55.2	296.7	168.9
	Days 4-7	84.6	17651.77	31.6	89.3	286.7	239.4
	Days 7-10	61.8	11563.63	19.1	63.3	97.1	223.7
	Days 10-14	75.6	14230.75	22.6	77.0	438.2	245.2
	Ileum	75.1	22745.8	34.4	132.4	1079.0	283.3
	Feather	0.7	284.9	5.2	5.6	8.7	61.2
	Skin	0.9	205.2	1.3	1.1	12.8	22.3
	Breast	2.6	834.4	3.6	0.5	21.7	29.2
	Liver	10.4	913.5	10.4	9.7	453.4	120.3

Table 4.A.5. Total Arsenic and mean metals concentrations in all poultry samples for the inorganic As(III) treatment.

Treatment	Sample	As	Ca	Cu	Mn	Fe	Zn
		----------	----------	(mg kg^{-1})	----------	----------	----------
	Feed (days 1-14)	36.6		9.3	23.6		80.1
Inorganic	Days 1-4	87.8	13317.8	19.0	66.7	236.0	214.2
As(III)-oxide	Days 4-7	248.8	13888.0	16.9	67.9	352.6	170.7
	Days 7-10	135.5	14043.3	31.8	79.3	238.3	282.4
	Days 10-14	326.3	21535.2	37.0	115.1	606.6	376.2
	Ileum	914.3	24408.9	33.6	163.6	1266.7	287.2
	Feather	2.1	209.6	4.1	4.6	8.4	53.6
	Skin	0.8	193.4	2.6	1.0	15.1	26.9
	Breast	4.4	646.6	3.3	0.7	30.4	31.4
	Liver	4.5	668.7	15.0	11.3	367.9	117.7

Table 4.A.6. Total Arsenic and mean metals concentrations in all poultry samples for the roxarsone removal treatment.

Treatment	Sample	As	Ca	Cu	Mn	Fe	Zn
		----------	----------	(mg kg^{-1})	----------	----------	----------
	Feed (days 1-7)	19.6		8.3	17.1		77.0
	Feed (days 7-14)	2.2		8.4	20.3		84.1
Roxarsone	Days 1-4	44.0	10968.1	17.8	60.4	225.7	190.9
(days 1-7)	Days 4-7	76.7	12064.7	16.9	61.7	186.1	181.9
No Roxarsone	Days 7-10	8.5	11211.6	17.6	57.5	129.5	202.1
(days 7-14)	Days 10-14	1.7	14878.0	22.1	80.5	401.1	256.7
	Ileum	0.8	17498.0	46.7	123.6	1266.6	306.1
	Feather	0.5	250.7	6.0	4.2	9.0	57.4
	Skin	0.7	212.2	4.0	1.9	14.9	28.2
	Breast	1.5	752.9	1.1	0.5	20.3	25.1
	Liver	2.3	1005.2	14.4	11.0	347.1	105.8

Table 4.A.7. Total Arsenic and mean metals concentrations in all poultry samples for the inorganic As(III) removal treatment.

Treatment	Sample	As	Ca	Cu	Mn	Fe	Zn
		----------		(mg kg^{-1})	----------		
	Feed (days 1-7)	36.6		9.3	23.6		80.1
Inorganic As (days 1-7)	Feed (days 7-14)	2.2		8.4	20.3		84.1
No Arsenic (days 7-14)	Days 1-4	78.0	13540.3	22.3	71.4	191.2	227.0
	Days 4-7	229.2	15059.8	25.1	76.8	246.6	213.5
	Days 7-10	96.7	16459.4	29.9	84.2	232.9	300.4
	Days 10-14	3.4	18000.7	26.4	100.3	586.6	310.5
	Ileum	1.1	13830.6	30.4	101.3	613.5	365.7
	Feather	1.0	342.7	4.9	1.5	16.0	68.1
	Skin	0.2	197.6	4.1	0.2	13.0	25.8
	Breast	1.0	4261.2	13.9	30.5	287.6	122.8
	Liver	1.7	1022.5	13.8	11.1	358.7	95.2

Figure 4.5.3. Additional XAS Analysis for Chapter 4

The data presented in the next section are data taken from my proposal defense. They are presented, concentrating on the whiteline, not the derivative, but these data are still useful and provides additional information on the subject.

XANES analysis of the Last Roxarsone Excreta Sample (Days 10-14).

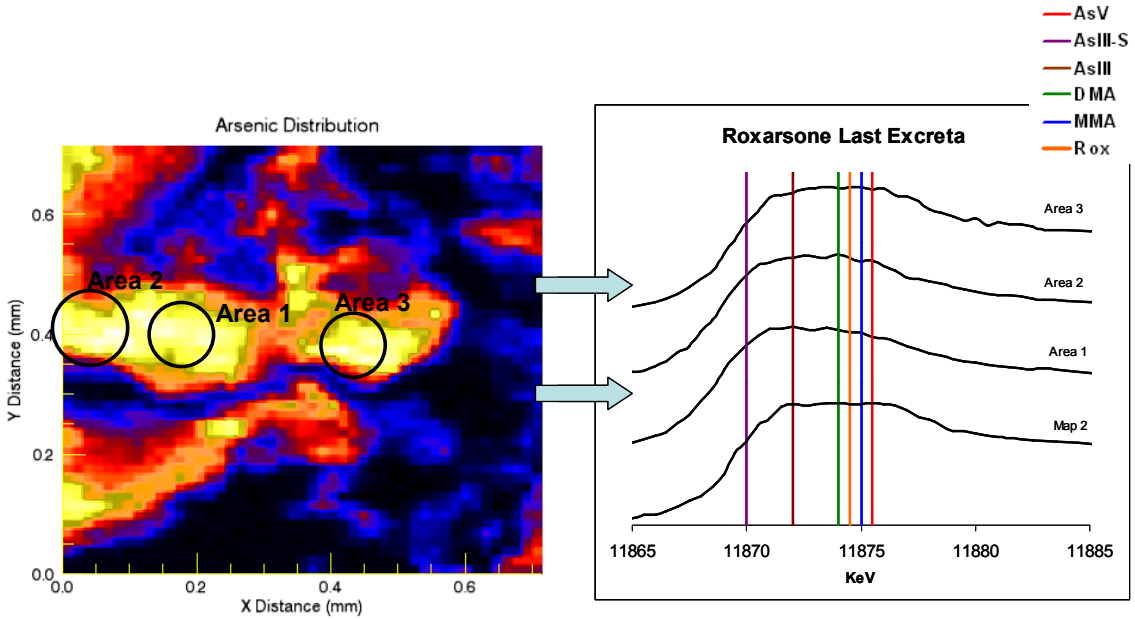

Figure 4.A.2. XRF map depicting trace metal distribution in the last roxarsone excreta sample (days 10-14).

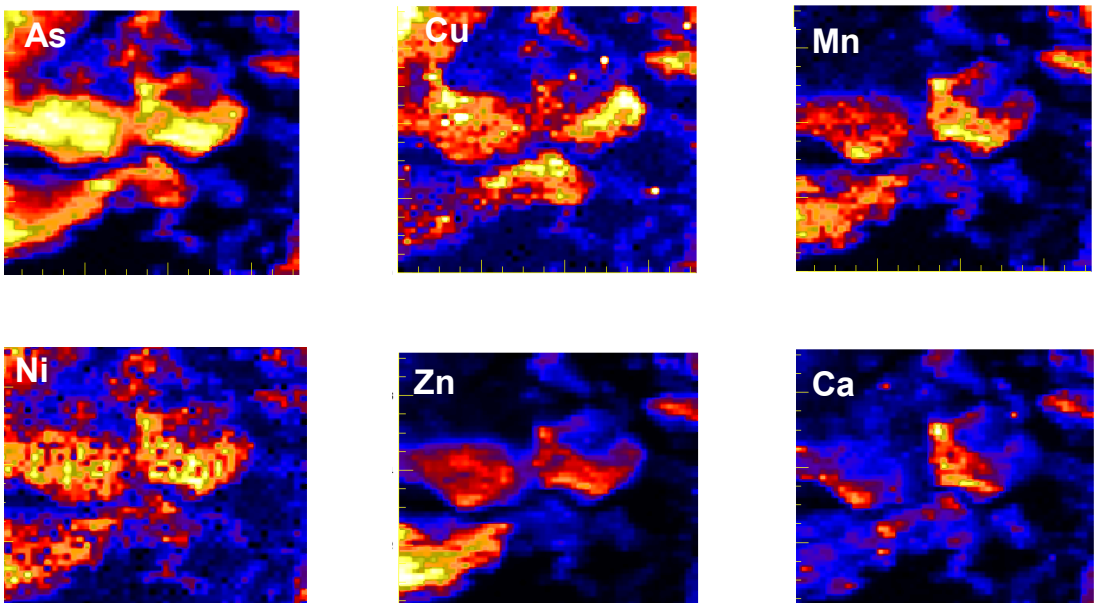

Figure 4.A.3. XRF map depicting trace metal distribution in the last roxarsone excreta sample (days 10-14).

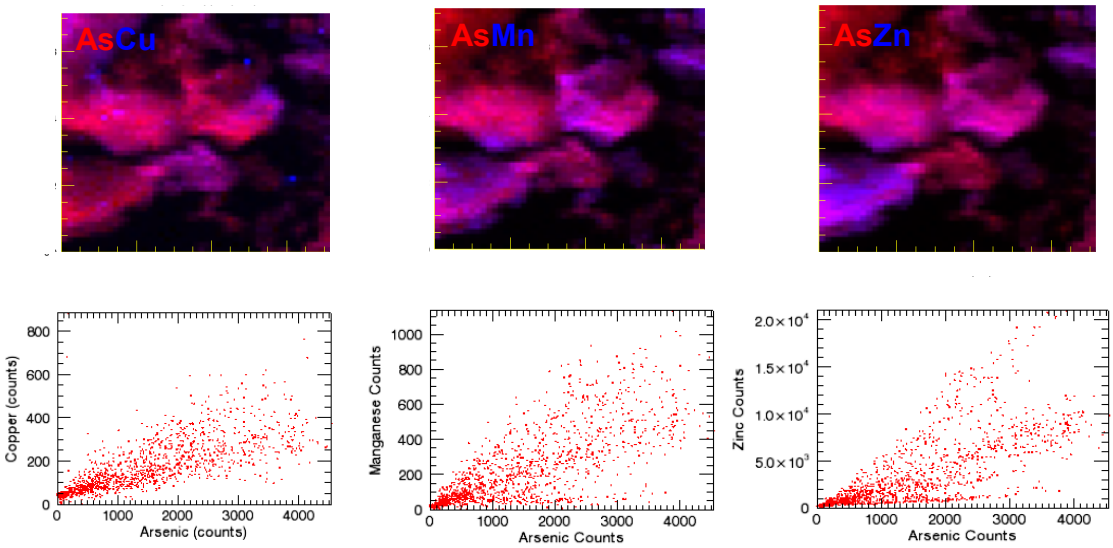

Figure 4.A.4. XRF maps of arsenic and trace metal associations and correlations seen in the last roxarsone excreta sample.

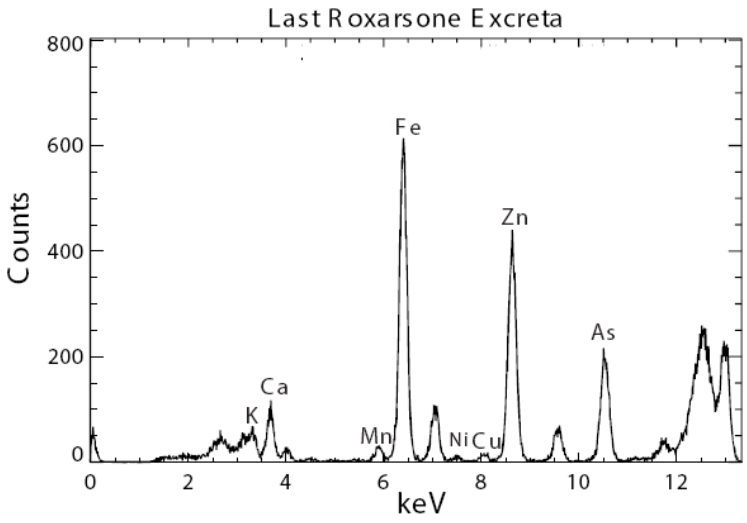

Figure 4.A.5. MCA plot of elemental distribution in the last roxarsone excreta sample.

XANES analysis of the Inorganic Ileal and Excreta Samples:

Inorganic arsenic distribution and speciation in excreta and in the digestive tract of the bird were investigated at beamline X26A at the NSLS. Once again, As is not evenly distributed throughout the sample. The As speciation results from these samples are opposite of what is seen in the roxarsone samples (Figure 4.18). The areas of concentrated As are mostly reduced As species (As(III) and As bound to S), while the more dispersed areas are a combination of reduced As and oxidized As species. Since the As was fed in the reduced As(III) state, oxidation of the As(III) must have taken place either within the digestive tract of the bird or once the sample was exposed to the air during sample collection. It is interesting to note that Area 2 in the inorganic excreta is dominated by Mn, while Area 1 has relatively low concentrations of Mn (Figure 4.18). It is possible that a Mn complex may be causing As oxidation

Trace metal analyses of the inorganic excreta are depicted in Figures 4.18-21 and Figures 4.A.6-8. The strong relationships noted in the organic As excreta and ileum content samples are not as definitive in these samples. There is definitely a relationship between As and Cu, Mn and to some extent Zn and possibly Ni, but they are not as closely associated as was seen in previous samples.

The ileal contents from the birds fed the inorganic As diet were analyzed for As and metal content, distribution, association and As speciation. The results indicate that a majority of the species found in the digestive contents from the ileum are reduced As species. These results suggest that As oxidation is primarily occurring outside of the body of the bird. XANES scans and an XRF map of As distribution are illustrated in Figure 4.21. Trace metal and As associations and distribution are similar to the inorganic excreta samples, except that stronger correlations are seen between As and the commonly occurring trace metals. The trace metal analysis figures are located in the appendix of this chapter (Figures 4.A.9-11).

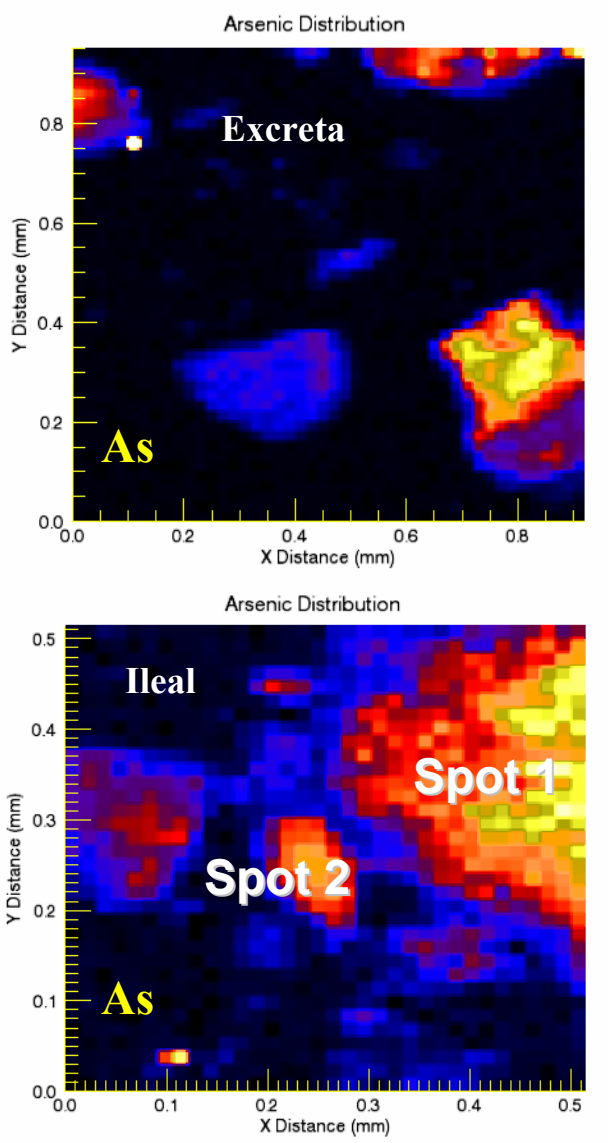

Figure 4.A.6. As XRF maps of ileum contents and excreta of a bird fed As(III) oxide,

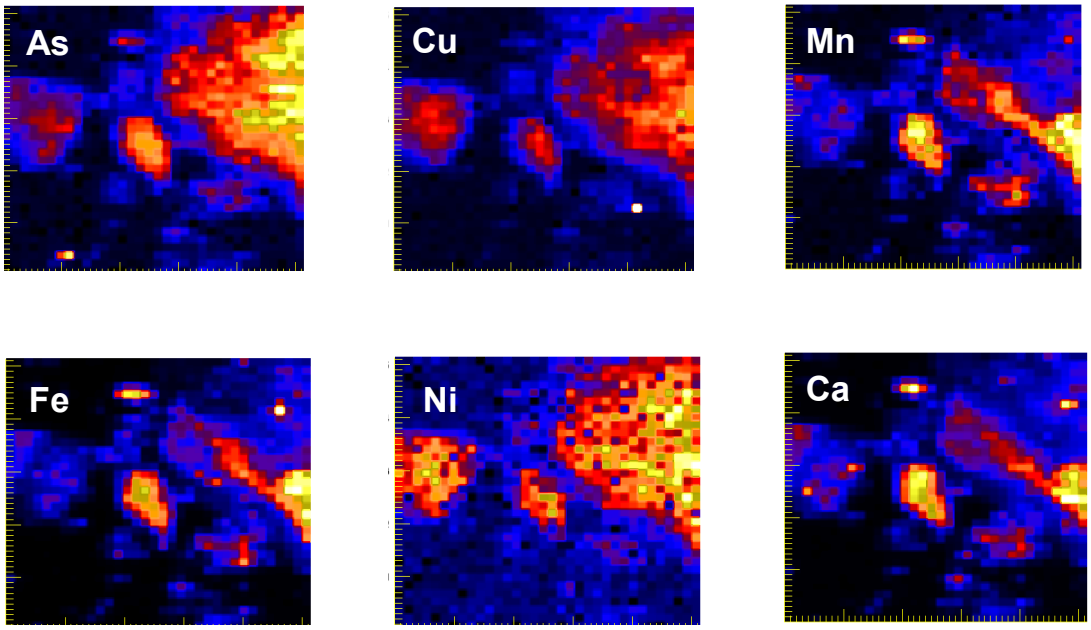

Figure 4.A.7. XRF maps of trace metals commonly found in poultry feeds and litters for the inorganic ileal sample. *Note the max value multiplier for Ni was lowered in order to display Ni distribution.

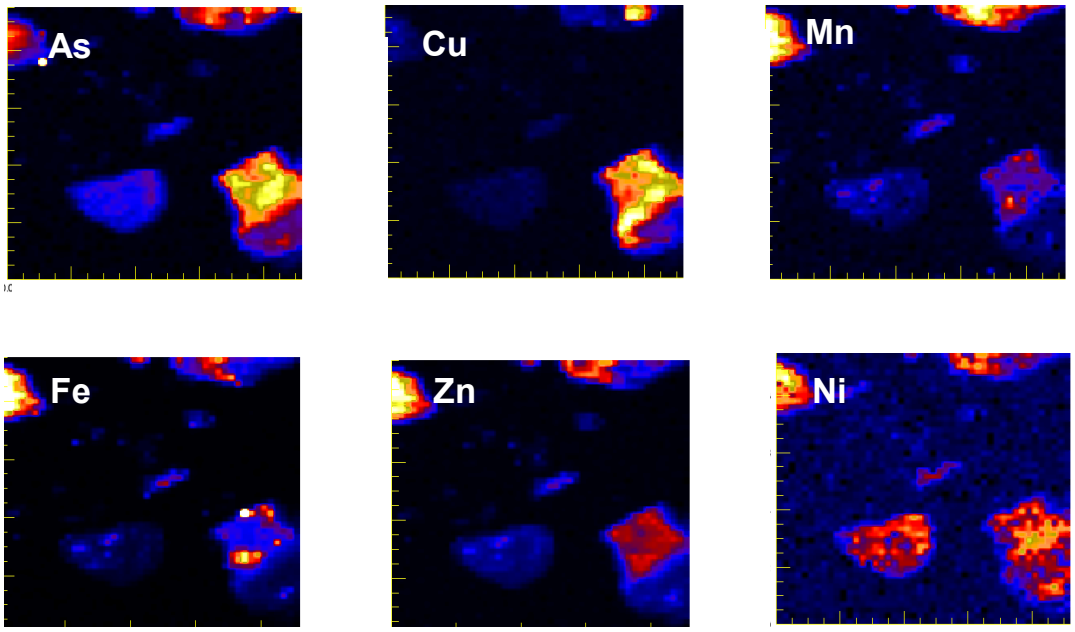

Figure 4.A.8. XRF maps of trace metals commonly found in poultry feeds and litters for the inorganic ileal sample. *Note the max value multiplier for Ni was lowered in order to display Ni distribution.

Bulk XRD of Roxarsone Excreta and Ileal Samples:

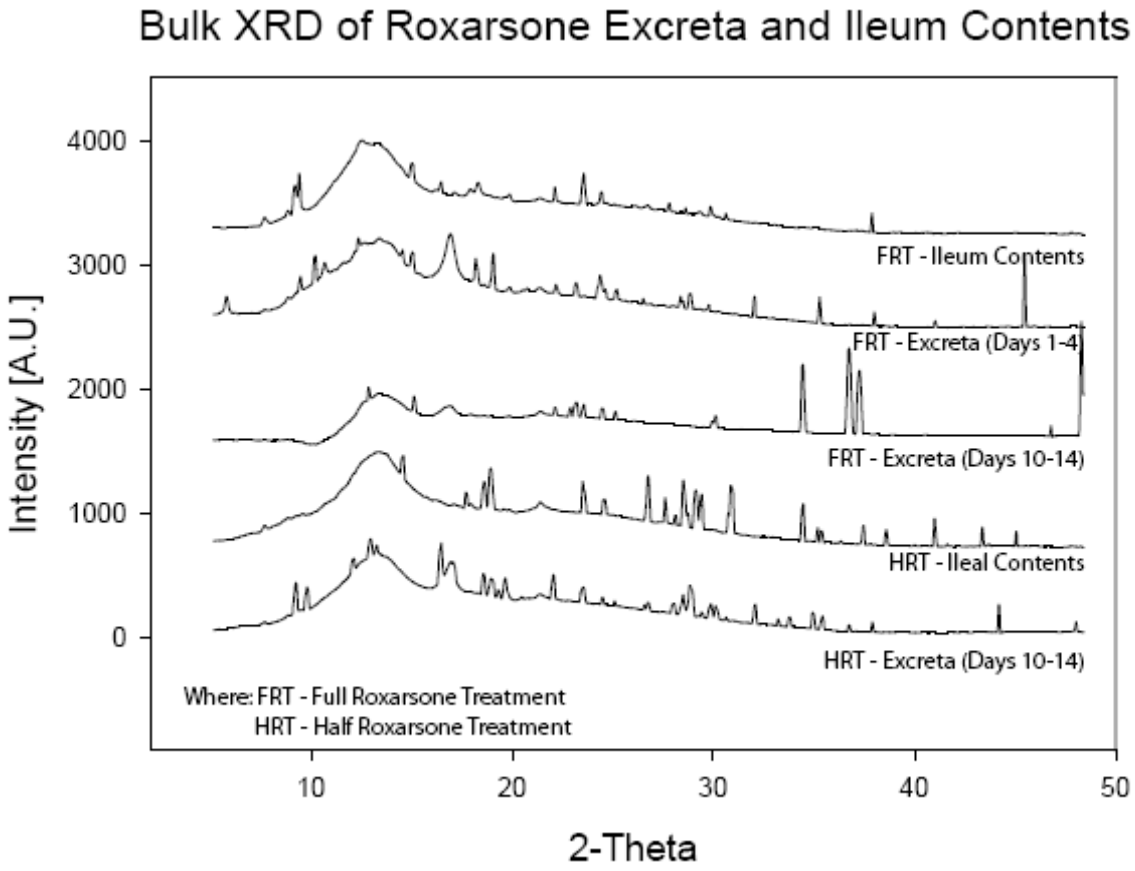

Figure 4.A.9. Bulk XRD of roxarsone samples taken at the SSRL.

Table 4.A.1. A table of arsenic standards used in linear combination fitting (LCF). The first column depicts compounds that were commonly found to be fits for the litter experimental scans. The category indicates which As species group the standard belongs to. Note: the As(III) methionine is under the As(III) category because there was not an As-S bond established in solution.

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