ARSENIC FRACTIONATION, PLANT UPTAKE AND ITS CONCENTRATIONS IN RUNOFF FROM POULTRY LITTER-AMENDED PASTURES

by

SHADI ASHJAEI

(Under the Direction of William P. Miller)

ABSTRACT

In regions of concentrated poultry production, poultry litter (PL) is commonly surface-applied to pastures at high levels over multiple years. The effect of long-term applications of PL on soil concentrations of arsenic (As), copper (Cu), Zinc (Zn), and the uptake of these elements by bermudagrass showed that concentrations these trace elements in soils were significantly greater than unamended (control) soil at 0-2.5 and 2.5-7.5 cm depths. However, the levels were well below the USEPA loading limits. Concentrations of all As fractions were significantly greater in PL-amended soils compared to control at both depths. Residual fraction was the predominant form of As in all soils. In a rainfall simulation (RS) study, total concentrations of As in runoff were above USEPA drinking water limit immediately or few days after PL application and it remained elevated 4 weeks after PL application. However, concentrations of Cu and Zn even immediately after PL application were below USEPA drinking water limit. Roxarsone was the major As species in the fresh PL and runoff from third RS study.

INDEXWORD: Poultry litter, fractionation, USEPA, drinking water limit, rainfall simulation, Roxarsone, arsenic species.

ARSENIC FRACTIONATION, PLANT UPTAKE AND ITS CONCENTRATIONS IN RUNOFF FROM POULTRY-LITTER AMENDED PASTURES

by

SHADI ASHJAEI

B.S., University of Guilan, Iran, 2002

M.S., Georg-August University of Goettingen, Germany, 2005

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2010

ARSENIC FRACTIONATION, PLANT UPTAKE AND ITS CONCENTRATIONS IN RUNOFF FROM POULTRY-LITTER AMENDED PASTURES

by

SHADI ASHJAEI

Major Professor:	William P. Miller
Committee:	Miguel L. Cabrera Julia Gaskin

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2010

ACKNOWLEDGEMENTS

Special thanks are expressed to Dr. William Miller for his invaluable guidance and support in completion of this research. Great thanks to Dr. Miguel Cabrera, Dr. Sayed Hassan and Mrs. Julia Gaskin for their help and advice.

I would like to thank many people who helped me with field work and lab analyses including Gene Weeks, John Rema, and Frank Newsome. I also need to thank Dr. Dory Franklin and all the staff at Georgia Research and Education Center in Eatonton, GA, and the staff at the USDA-ARS in Watkinsville, GA.

I wish to thank my parents for their love and support and always being the most important motivation in my life. I also would like to thank my husband, Ramin Djaved, and my brother, Ali Ashjaei, for believing in me to achieve my goals.

TABLE OF CONTENTS

Page

CHAPTER

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Over 21 billion kg of broiler chicken was produced in the United States in 2006 (USDA-NASS, 2007). Georgia is one of the top states producing broiler chickens in the United States with 1.46 billion broilers produced in 2006 (Georgia Agricultural Statistic Services, 2006). The poultry industry is an important component of agricultural production in Georgia. In the areas with intensive poultry production, large amounts of litter produced are applied as organic soil amendment to local agricultural lands (Robinson and Sharpley, 1995). Poultry litter (PL) is a mixture of manure and bedding materials and is considered a valuable source of essential plant nutrients including phosphorus (P), potassium (K), nitrogen (N), and calcium (Ca). It also contains significant concentrations of trace elements such as arsenic (As), copper (Cu), and zinc (Zn) as a result of their use as growth promoters in poultry feed (Gupta and Charles, 1999; Menjoulet et al., 2009). Repeated applications of PL can result in elevation of trace elements in soil and pose potential environmental problems such as contamination of surface water. Land application of PL is regulated based on applying N and/or P for crop needs. There are no regulatory standards for trace elements concentrations or loading. In the United States, only municipal biosolids have regulatory limits on trace metals. The USEPA 503 regulation loading limits for As, Cu, and Zn in soil amended with biosolids are 20, 750, and 1400 mg kg^{-1}, respectively (Miller and Miller, 2000; USEPA, 1993). These standards are often used as a reference for other organic wastes.

Arsenic occurs in PL as a result of the use of organoarsenic compounds such as roxarsone (ROX, 3-nitro-4-hydroxyphenyl-arsenic acid) as a feed additive at concentrations of 25 to 50 mg kg^{-1} for prevention of fungal disease and for weight gain improvement (Jones, 2007). It has been reported that most of the ROX added to poultry diet is excreted unchanged and is introduced to the environment when litter is applied to agricultural land (Garbarino et al., 2003; Jackson et al., 2006). However, ROX is degraded and As mineralized as arsenate (As (V)) over time in the environment (Jackson et al., 2003). In addition, Cu and Zn are added to poultry feed as a sulfate salt or oxide to increase weight gain and feed efficiency (Adriano, 2001; Jackson et al., 2003). Several studies have shown that long-term PL application can elevate trace elements (e.g., As, Cu, and Zn) especially at the soil surface when surface-applied on pastures (Gupta and Charles, 1999; Kingery et al., 1994; Moore et al., 1998; Toor and Haggard, 2007; van der Watt et al., 1994).

Arsenic in soils occurs in various forms which are associated with different soil constituents. The bioavailability of arsenic to plants and its mobility and release to runoff from PL- amended soils is controlled by its distribution among various solid-phase components. In general, the water-soluble form is considered as readily bioavailable and is presumably susceptible to runoff losses compared to more tightly bound forms. To assess the distribution of As fractions in the PL-amended soils, the As fractionation associated with different soil solid phases can be examined by selective sequential extraction with reagents of increasing dissolution strength. This type of fractionation is used to predict what percentage of total As in the soil contributes to readily available As (water-soluble, weakly adsorbed As), Fe/Al –associated As, and stable As fractions (residual). Immediately after PL application there would be a high risk of mobilization of As from top soil but over time the water- soluble form of As converts to more stable fractions with less susceptibility to be lost by runoff (Han et al., 2004).

The environmental fate of trace elements in the PL-amended soils can be influenced by their solubility and transformation in PL, as well as soil texture and soil adsorption capacity.

Concentrations of As, Cu, and Zn in runoff from coarse-textured soils with low Fe/Al oxide content with long-term PL application may cause surface water contamination.

It is important to understand the chemical forms, speciation, and transformation of As in PL in order to be able to quantify the toxicity of different species to various organisms. ROX itself has been generally considered to be harmless. However, ROX is rapidly degraded to more toxic inorganic species such as As (V) and As (III) as well as variety of organic species such as dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) during composting of PL or after applying to soil. Therefore, PL management practices play an important role in ROX mineralization to other As species in PL and PL-amended soils.

The objectives of this research are to estimate the potential environmental concern associated with As, Cu, and Zn in long-term PL-amended pastures by; (1) assessing the distribution of different forms of As fractions in PL- amended pastures; (2) determining forage uptake of As, Cu, Zn, and P in PL-amended pastures; (3) assessing concentrations of As in runoff from natural precipitation from field-scale PL-amended plots containing tall fescue/bermudagrass; and (4) examining the effect of rainfall timing on total concentrations of As in runoff from rainfall simulation and As speciation in PL and runoff from rainfall simulation study.

Reference

Adriano, D. C. 2001. Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risks of metals, p. 219-261. New York Springer.

Garbarino, J. R., A. J. Bednar, D. W. Rutherford, R. S. Beyer, and R. L. Wershaw. 2003. Environmental fate of roxarsone in poultry litter. I. Degradation of roxarsone during composting. Environ. Sci. Technol. 37: 1509-1514.

Georgia Agricultural Statistic Services. 2006. Georgia poultry facts. Available at www.nass.usda.gov/QuickStats/PullData_US.jsp (verified 23 Feb. 2008).

Gupta, G., and S. Charles. 1999. Trace elements in soils fertilized with poultry litter. Poult Sci. 78: 1695-1698.

Han, F. X., W. L. Kingery, H. M. Selim, P. D. Gerard, M. S. Cox, and J. L. Oldham. 2004. Arsenic solubility and distribution in poultry waste and long-term amended soil. Sci Total Environ. 320:51-61.

Jackson, B. P., P. M. Bertsch, M. L. Cabrera, J. J. Camberato, J. C. Seaman, and C. W. Wood. 2003. Trace element speciation in poultry litter. J. Environ. Qual. 32: 535-540.

Jackson, B. P., J. C. Seaman, and P. M. Bertsch. 2006. Fate of arsenic compounds in poultry litter upon land application. Chemosphere. 65: 2028-2034.

Jones, F. T. 2007. A broad view of arsenic. Poult Sci. 86: 2-14.

Kingery, W. L., C. W. Wood, D. P. Delaney, J.C. Williams, and G. L. Mullins. 1994. Impact of long-term land application of broiler litter on environmentally related soil properties. J. Environ. Qual. 23: 139-147.

Menjoulet, B. C., K. R. Brye, A. L. Pirani, B. E. Haggard, and E. E. Gbur. 2009. Runoff Water Quality from Broiler Litter-Amended Tall Fescue in Response to Natural Precipitation in the Ozark Highlands. J. Environ. Qual. 38: 1005-1017.

Miller, D. M., and W. P. Miller. 2000. Land application of wastes. p G217-241. *In* M. E. Sumner (ed.) Handbook of soil science. CRC Press, Boca Raton, FL.

Moore, P. A., Jr., T. C. Daniel, J.T. Gilmour, B. R. Shreve, D. R. Edwards, and B. H. Wood. 1998. Decreasing metal runoff from poultry litter with aluminum sulfate. J. Environ. Qual. 27: 92-99.

Robinson, J. S. and A. N. Sharpley.1995. Release of nitrogen and phosphorus from poultry litter. J Environ Qual. 24: 62-67.

Toor, G., and B. Haggard. 2007. Trace metals runoff waters from soil amended with granulated poultry litter. *In* Annual meetings abstracts. ASA-CSSA-SSSA, New Orleans, Luisiana, 4-8 Nov. 2007. Avaiable at http://crops.confex.com/crops/2007am/techprogram (verified 12 Aug. 09)

USDA-NASS. 2007. Broilers: Production and value of production by year,US. Available at http://www.nass.usda.gov/Charts_and_Maps/Poultry/brprvl.asp (verified on 7 Aug. 2009). USDA-NASS, Washington,DC.

USEPA. 1995. Method 3051: Microwave assisted acid digestion of sediments, sludges, soils and oils. Availabe at http://www. epa.gov/SW-846/pdfs/3051.pdf (verified 22 July 2008). USEPA , Washington, DC.

van der Watt, H. v. H., M. E. Sumner, and M. L. Cabrera.1994. Bioavailability of copper, manganese, and zinc in poultry litter. J. Environ. Qual. 23: 43-49.

CHAPTER 1

LITERATURE REVIEW

Arsenic in the Environment

Arsenic (As) is metalloid that exists in nature in the oxidation states +5 (As (V)), +3 (As (III)), 0 (arsenic), and -3 (arsine) of which arsenate (As (V)) and arsenite (As (III)) are the main inorganic forms of As in most soils and sediments. The reaction of As in soil and its mobility is governed by its oxidized state. Arsenate compounds predominate in aerobic soils whereas As (III) compounds predominated in reduced conditions (Goh and Lim, 2005). Arsenate is present as $H_2AsO_4^-$ species under most environmental condition, and As (III) as the $H_3AsO_3^0$ species is predominant in low pH and low redox environments (Kabata-Pendias and Pendias, 2001). In the environment, inorganic As is a constituent of more than 200 minerals and is mainly associated with sulfide minerals. The most important As bearing minerals are arsenopyrite (FeAsS), orpiment (As_2S_3), realgar (AsS), and mispickel (FeAsS) (Bissen and Frimmel, 2003; Matschullat, 2000). Apart from natural sources, As contamination in soil and water sources are a result of human activities due to disposal of waste chemicals, burning of fossil fuels and application of arsenical herbicide and fungicide in agriculture. Today, contamination of surface and ground water due to anthropogenic activities or natural sources of As in ground water used for drinking water purposes are a major problem. Long-term exposure to As can cause skin, liver, kidney, and lung cancers. The U.S Environmental Protection Agency (USEPA) specifies a maximumcontaminant level for As in drinking water of 10 µg L^{-1} (Bissen and Frimmel, 2003; USEPA, 2006).

Arsenic in Poultry Litter Amended Soils

Arsenic occurs in poultry litter (PL) as a result of the use of organo arsenical compounds such as roxarsone (3-nitro-4-hydroxyphenyl-arsenic acid) as a feed additive at concentrations of 22.7 to 45.5 g ton^{-1} for prevention of fungal disease and for weight gain improvement. The practice is common in the U.S for production of broiler chickens, but feeding arsenic to laying hens is prohibited (Jones, 2007). It has been reported that most roxarsone (ROX) added to poultry diet is excreted unchanged and is introduced to the environment when litter is applied to agricultural lands (Garbarino et al., 2003; Jackson et al., 2006). In one study, fields that had received PL containing 15-35 mg kg^{-1} of As over a 20 years period in Arkansas was compared to control field and the result showed that 36 to 88% of the total arsenic in PL samples was ROX, and concentrations of As in drainage water from PL- amended soil averaged 0.29 mg L^{-1} (Morrison, 1969). The estimated amount of excreted ROX is about 150 mg per chicken during a 7-week growth period (Garbarino et al., 2003). If most of the 8 billion broiler chickens raised annually in the U.S are fed on a ROX supplemented diet, about 1,200 metric tons of ROX, corresponding to over 300 metric tons of arsenic, are released into the environment annually (O'Connor et al., 2005). However, this is an over-estimate because some broilers may not be fed ROX or fed ROX at a reduced rate (Chapman and Johnson, 2002). Repeated surface applications of PL could result in accumulation of heavy elements at shallow soil depth due to the immobility of these elements. Several studies have shown that long-term PL application results in elevation of trace elements (e.g., As, Cu, and Zn) at the soil surface (Gupta and Charles, 1999; Moore et al., 1998; Toor et al., 2007).

Factors Effecting Mobility and Bioavailability of Arsenic

The mobility of arsenic compounds in soil depends on the type and amount of adsorbing soil constituents, pH value, Fe and Al oxides, redox potentials, organic matter, and other anions competing with arsenic for adsorption sites on soil constituents. Inorganic arsenic compounds can be methylated by bacteria, fungi, and yeast to organic compounds such as monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and gaseous derivatives of arsine (Bissen and Frimmel, 2003). It has been reported that (As (V) and As (III)) are more toxic than methylated arsenic compounds, which are still approved for use as herbicides and defoliants in agriculture (Jackson et al. 2006). Among all As compounds, As (III) is the most soluble, mobile, and toxic species (Adriano, 2001; Jain and Ali, 2000) due to its weaker association with soil solids. Arsenic covalently bonds with many metals and nonmetals and forms As compounds both in its trivalent and pentavalent states. The most important compounds are white As (As_2O_3), calcium arsenate, aluminum arsenate, and lead arsenate. Although As minerals and compounds are readily soluble, As mobility is greatly limited due to the strong sorption by clays, hydrous oxides, and organic matter (Kabata-Pendias and Pendias, 2001). Chemical and biological process including adsorption/desorption and oxidation/reduction control the fate and transformation of As in the environment. The study of effects of acidification and natural organic materials on the mobility of As in the environment showed that sorption affinity of As depends on its chemical species. The adsorption of arsenic on alumina decreased at pH below 6 in the order As(V) > MMAA = DMAA > As(III) whereas at pH above 6 , the order was As(V) > As(III) > MMAA = DMAA (Xu et al. 1991). Clay minerals exhibited less As (III) adsorption than As (V) adsorption, and it was pH-dependent (Lin and Puls, 2000).

pH

The effect of pH on arsenic adsorption depends on many factors including Fe and Al oxide content of soil. In soils with low metal oxide content, increasing the pH has little effect on arsenic adsorption, while in highly oxidic soil, the adsorption of arsenic increases with decreasing pH. This increase of arsenic adsorption at low pH is attributed to two interacting factors: the decreasing of negative surface charge of Fe and Al oxides due to protonation of functional groups, and decreasing of negatively charged arsenic species present in the soil solution. Table 1 shows the dissociation constants of As compounds that occurs at different pH. As (V) appears as H_3AsO_4 at pH below 2 and dissociates to $H_2AsO_4^{-2}$ and $HAsO_4^{-1}$ at pH between 2 to 11, while As (III) as H_2AsO_3 does not dissociate up to pH 9. In general, mobility of arsenic species increases by increasing pH and decreases with decreasing pH. However, the maximum adsorption of As (V) occurs at lower pH compared to As (III). Figure 1.1 shows the percentage of adsorbed As on hydrous ferric oxide (HFO) as a function of pH using the chemical equilibrium program Visual MINTEQ (total As= 0.004 M, ionic strength= 0.001, HFO= 3g L^{-1} with diffuse double layer model, active site= 6.7 mM L^{-1}, and site density= 2.31 site nm^{-1}).

Oxides of Iron and Aluminum

The adsorption properties of the metal oxides are due to the presence of surface functional groups such as OH_2^+, OH, and O^-. The adsorption of solutes by solid surface can occur via outer-sphere or inner-sphere (bidenate or mondenate) surface complexation. Outer-sphere adsorption involves weak, electrostatic forces between the charged surface and charged As ions. However, inner-sphere adsorption is considered a stronger bond (covalent), and it is formed when As adsorbs by ligand exchange with OH and OH_2^+ surface functional groups

(Stollenwerk, 2003). The Fe (III) oxide surface has a high affinity for As(V) capable of forming inner-sphere bidentate, As(V)-Fe(III) complexes (Manning et al., 1998; O'Reilly et al., 2001).

One of the most important factors controlling the adsorption of arsenic on metal oxides is pH, as pH affects protonation and deprotonation of mineral surfaces. The points of zero charge (pzc) of iron oxides are in the range of 7 to 8.5. At pH above the pzc, oxyanions such as arsenate weakly adsorb due to the negatively charged surface of the solid. The pH_{pzc} for hydrated Fe (III) oxides is 8.6 which means in most aquatic environments hydrated Fe (III) has a positive surface charge, so arsenic compounds can be effectively adsorbed (Bissen and Frimmel, 2003). The Mn oxides generally carry a net negative surface charge in soils due to their low pzc (pH=6), and they are expected to have a limited role in adsorption of arsenic in soil with pH above 6. A study of competitive adsorption of arsenate and arsenite on oxides and clay minerals showed arsenate adsorption on oxides and clays was maximal at low pH and decreased with increasing pH, above pH 9 for Al oxide, pH 7 for Fe oxide, and pH 5 for clays. The maximum arsenite adsorption was around pH 8.5 for all materials (Goldberg, 2002).

Redox Potential

Redox potential (Eh) has an influence on mobility and speciation of arsenic in soil. Generally, inorganic arsenic is mainly present as As (V) in aerobic conditions (high Eh potential) and as As (III) in reducing environment (low Eh potential). Under reducing conditions (<200 mV) arsenic compounds bound to Fe and Mn oxide/hydroxide are solubilized because Fe (III) is reduced to Fe (II) and Mn (III/V) to Mn (II). An increase in As (III) under flooded conditions has been attributed to the release of As during dissolution of metal oxides that have high affinity for As (V) in aerobic condition. Since As (V) is less mobile and toxic compare to As (III), in order to prevent the mobilization of arsenic, the Eh potential of soil should be high and the pH should be in the alkaline range (Bissen and Frimmel, 2003; Fitz and Wenzel, 2002).The mobility of arsenic decreases at Eh potential of less than -250 mV due to precipitation of arsenic with iron sulfides as arsenopyrite (FeAsS) or As_2S_3 , while As sulfides easily oxidize under aerobic conditions (Carbonell-Barrachina et al., 2000). Iron oxyhydroxides are of main importance in regulating the concentration of As available for reduction, while Mn plays a lesser role (McGeehan and Naylor, 1994; Oscarson et al., 1983).

In flooded conditions, Mn solid phase dissolve before Fe (III) begin to be reduced and any desorbed As will be followed by readsorption by Fe (III) oxyhydroxides (Cullen and Reimer, 2002). A study of the sorption and Eh transformation of As (III) and As (V) in two flooded soil with different sorption capacity showed that the decrease in pH and Eh in flooded soils favors dissolution of Fe oxyhydroxide and desorption of $H_3AsO_3^0$ and $H_2AsO_4^-$ which regulates the soluble available As for Eh transformation. It was observed that Fe dissolution, As adsorption and desorption is slower in soil with higher sorption capacity (McGeehan and Naylor, 1994).

Competing Ions

Phosphate (P) and As (V) compete for similar sorption sites due to similar physicochemical properties, and this may affect As bioavailability. Furthermore, arsenate is thought to be taken up by plants via the P uptake system, and consequently plants metabolize it as phosphorus. As (V) competes with P in cell reactions and can uncouple oxidative phosphorylation so the high energy bonds of ATP are not conserved (Bissen and Frimmel, 2003). Goh and Lim (2005) assessed the influence of different anions (PO_4^{-3}, CO_3^{-2}, SO_4^{-2}, and Cl^-) on As mobility in As contaminated soil. It was observed that PO_4^{-3} was more efficient in mobilizing As which was believed to be due to a ligand exchange mechanism. It has been reported that texture and mineral content of soil affect on As-P interaction (Fitz and Wenzel, 2002). A study of the effect of P amendments on As mobility and uptake by hyperaccumulator *Petris Vittata L.* in both As-spiked contaminated and chromated-copper-arsenate soils showed that P amendment significantly enhanced plant As uptake in both soils due to replacement of As by P from sorption sites and increased the mobility of As (Cao et al., 2003). In sandy soils, P applications stimulate plants to take up additional As. In contrast, in silt and clay soils, P addition mobilizes As but decreases its plant uptake due to As adsorption by Fe/Al oxides and fine-textured particles (high surface area) (Bellows, 2005).

The coarse textured soils are likely associated with higher amounts of readily mobile As, while As in fine textured soils is mainly immobile due to higher content of minerals and organic constituents which are capable of binding anionic As species. However, As in fine-textured soils can be released with changes in environmental conditions such as acidification or changes in redox potential (Goh and Lim, 2005).

Forms and Chemical Fractionation of Arsenic in Soil

Arsenic in soils is distributed among various soil components which are associated with different soil constituents. Arsenic fractionation in different soil solid phases can be examined by selective sequential extraction with reagents of increasing dissolution strength. Based on the chemical similarity of P and As, modified versions of P fractionation have been adopted for As (Wenzel et al., 2001). Several schemes of selective sequential extraction procedures have been studied by researchers to fractionate solid phase As (Lombi et al., 2000; Onken and Adriano, 1997; Sarkar et al., 2007; Shiowatana et al., 2001; Wenzel et al., 2001). These schemes differ in their sequential extraction steps, reagents, and extraction conditions. A common feature among most of As extraction methods is extracting of the soluble (or exchangeable) As pool using NH_4Cl or H_2O, adsorbed using $NaHCO_3$, bound to Fe oxide with NaOH, Al oxide with

NH_4F, and the residual phase. The results of As fractionation aid in understanding the distribution of As among different soil constituents and their adsorption process in As retention, mobility and bioavailability.

The bioavailability of arsenic to plants and its mobility and possible release to runoff from PL- amended soil is governed by its distribution among various solid-phase components. In general, the water-soluble form is considered as readily bioavailable and is more susceptible to runoff losses compare to Fe/Al- , complexes of Ca/Mg-, and organic matter- associated As. A study of As distribution in PL and long-term PL amended soil (upper coastal plain, MS) that had received PL at rate of 10 Mg ha^{-1} per application showed that 47% of As in PL was accounted for in the water-soluble fraction and 7% in residual. However, 72% of As in PL-amended soil was in residual fraction and is considered as the least susceptible to runoff losses or downward movement. It was concluded that immediately after PL application there would be a high risk of mobilization of As from top soil and over time the water soluble form of As may transfer to more stable fractions (residual) with less susceptibility to be lost by runoff (Han et al., 2004). In another study, it was observed that Fe-bound As was the largest fraction in As contaminated soil (averaged 165 mg As kg^{-1}). However, As accumulated in soils primarily as Al- or Ca-As when the reactive Al or exchangeable Ca was high. It was also concluded that phytotoxicity of As was related more to its chemical form than its total amount. Soils with higher water-soluble As content were more phytotoxic than those with no detectable water-soluble As and soils with extremely high reactive Al levels were less phytotoxic than soils with low reactive Al contents (Woolson et al., 1971). An incubation study was conducted to estimate bioavailability of arsenic as a function of soil properties. It was observed that the amount of water soluble As decreased from 88% to 70% with an increase in Fe/Al bound fraction from 3% to 20% after 4 months of soil incubation with different rates of dimethylarsinic acid. However, the retention of As in the Fe/Al fraction was more pronounced in soil with greater content of Fe and Al-oxide content. Hence, there is probably a greater risk of contamination of surface water in sandy soils with low Fe and Al-oxide contents (Sarkar et al., 2005). A study of the effect of As uptake by Chinese Brake Fern (*P teris vittata L)* on As distribution from As contaminated soil showed that after 8 weeks of plant growth, Al-bound As and Fe-bound As fractions were significantly greater in As-spiked soil than in the control. Arsenic was taken up by the plants from all fractions, although Ca-bound As contributed the most (51-71% reduction) since As was present primarily in from of Ca-As in soil. In addition, it was observed that only water-extractable (soluble As) significantly correlated with As removed by the plant after 8 weeks of growth (Fayiga et al., 2007).

Bioavailability and Phytotoxicity of Arsenic

High concentrations of As in soil may interfere with plant metabolism, impair nutrient uptake, and compete for essential plant nutrients causing inhibition of plant growth, disruption of root functions, and sometimes leading to death (Carbonell et al., 1998). Plants vary in their resistance to As. Plant resistance is generally achieved through suppression of the high affinity phosphate/arsenate uptake system which reduces the As influx to a level that it can be detoxified by plant (Meharg and Macnair, 1992; Singh and Ma, 2006). The accumulation of As in plants occurs primarily through the root system (Azizur Rahman et al., 2008; Marin et al., 1993). It can be expected that tuber crops have higher As content compared to other crops in contaminated soils. Elevated concentration of soluble As in soils cause considerable concern with respect to plant uptake and subsequent movement through animal and human food chains. The bioavailability to As to plants depends on several physical and chemical factors in soil. The texture and chemical composition of the soils are important factors that govern the availability of arsenic to plants. Iron and Al oxides adsorb As species more in acidic soil than in alkaline soils. In general, soluble As is a more reliable indicator of As phytotoxicity than total concentration of

As in soil. The significant correlation between water- extractable As fraction and arsenic uptake by plants is a more accurate estimate of phytotoxicity than the relationship between total As in soil and plant growth. Moreover, not only the concentration of As available in soil solution but also the speciation of soluble As may affect As uptake (Quaghebeur and Rengel, 2005). In fact, most crop plants do not accumulate As to levels toxic to humans or animals health because the plant dies before it can be consumed (Adriano, 2001; Azizur Rahman et al., 2008). The use of As contaminated groundwater for irrigation of crops caused elevation of As concentration in agricultural soils in Bangladesh. A greenhouse and field study was conducted to examine the effect of different concentrations of As in irrigation water on growth of paddy rice (*Oryza sativa L.*). It was observed that increasing the concentration of As (V) in irrigation water significantly decreased plant growth while As concentrations increased in root, straw and rice husk. However, As concentration in grain were not significantly affected by the soil As concentrations and did not exceed food hygiene concentration limit (1.0 mg of As kg^{-1} dry weight) (Abedin et al., 2002; Azizur Rahman et al., 2008).

Environmental Fate of Roxarsone in Poultry Litter Amended Soil

It is important to understand the chemical forms, speciation, and transformation of As in PL in order to be able to quantify the toxicity of different species to various organisms. ROX itself has been generally considered to be harmless. However, ROX is rapidly degraded to other species including inorganic species such as As (V) and As (III) as well as a variety of organic species such as DMA and MMA during composting of PL or after applying to soil through chemical reactions such as reduction of nitro groups, oxidative aromatic ring fission, and rupture of C-As bonds (Cortinas et al., 2006). Arsenate was the major species in 50% of leachates from PL-amended soils (Jackson et al., 2003). Laboratory experiments on the effect of composting on degradation of ROX showed that the primary As species extracted with water from dried PL was ROX. However, when water was added to litter at about 50% by volume and the mixture was allowed to compost at 40°C for about 30 days, the speciation of As shifted to As (V) (Garbarino et al., 2003). Microbial activity was responsible for degradation of ROX in litter to inorganic As since increasing the amount of added water elevated the degradation rate, while addition of sodium azide and heat sterilization eliminated microbial activity and hence the rate of degradation. Arsenic in poultry waste or PL- amended soil may be present in organic forms and it can potentially degrade to more toxic inorganic species. As a result, PL management practices play an important role in degradation of organic As to other As species. During litter storage or even after PL application to soil, exposure to sunlight, elevated air temperature and precipitation most likely enhance photodegradation and microbial degradation (Garbarino et al., 2003).

Poultry litter applications tend to increase water soluble As levels in top soils, especially when the PL is surface applied but not incorporated. Surface runoff from intensive animal production areas potentially causes deterioration of water quality. A Maryland test plot that had received PL application for 2 years showed a measurable increase of water-extractable As in amended soil after two application at 3 and 6 tons per acre and one application at 6 tons per acre compared to control plots (Rutherford et al., 2003). Long-term application of PL can potentially led to environmental contamination of water sources via runoff or leaching. In a rainfall simulation study, PL- amended soil showed elevated concentrations of water-soluble As in runoff as the rate of PL application increased, both immediately and 7 days after application. When PL was applied at 8.9 Mg ha^{-1}, concentrations of As in runoff water from control field averaged 0.028 mg L^{-1} compared to 0.091 mg L^{-1} for PL-amended soil (Moore et al., 1998). In another study, rainfall simulations were conducted over three weeks and showed that concentrations of As in runoff from PL- amended soil ranged between 0.29 and 1.73 mg L^{-1}, compared to 0.12 mg L^{-1} from control plots. In subsequent rainfall events, concentrations of As in runoff declined to below the detection limit (Toor and Haggard, 2007). Concentrations of As in runoff from the two first rainfall events were greater than the U.S Environmental Protection Agency (USEPA) regulatory limit of 0.01 mg L^{-1} in drinking water (USEPA, 2006).

Environmental Effects of Arsenic on Human Health

Human may be exposed to As from variety of environmental sources including soil ingestion, food/ drinking water (food chain), inhalation, and dermal exposure. However, soil ingestion, food and water consumptions are the largest source of As intake with smaller contributions from As inhalation. Humans may drink water from wells contaminated by industrial, As-rich minerals, and agricultural activities. Arsenic compounds have a high affinity to sulfhydryl groups of proteins and cause disruption of metabolic processes including cellular glucose uptake and fatty acid oxidation (Bissen and Frimmel, 2003). Many aquatic organisms are capable accumulating As and methylate As species as a detoxifying process (Braman and Foreback, 1973; Kumaresan and Riyazuddin, 2001). It has been reported in some areas in the world such as Taiwan, China, Chile, and Argentina that drinking water supplies have been contaminated, leading to As poisoning and skin cancer. In was estimated that in Bangladesh where the source of As is geological in nature, over 20 million of country's 120 million populations are at the risk from As contaminated drinking water or irrigation of crops (Adriano, 2001). Groundwater in the As-contaminated areas in Bangladesh contain As concentration up to 2 mg kg^{-1} (Abedin et al., 2002; Tondel et al., 1999). In many countries including Bangladesh, rice straw is used as cattle feed. High concentration of As in rice straw that had been grown in areas with As contaminated irrigation water may pose adverse health effect on human and animals via the plant-animal-human pathway (Abedin et al., 2002; Azizur Rahman et al., 2008). Azizur Rahman et al. (2008) reported As concentration of 0.5 mg kg^{-1} in rice grain when soil As concentration was 40 mg kg^{-1}. The average daily rice consumption by an adult is between 400 to 650 g raw rice grains in As affected area of Bangladesh (Duxbury et al., 2003). Schoof et al. (1999) reported that between 30% to 85% of As in rice was present in inorganic species. The same result was observed by Azizur Rahman et al. (2008). These reports suggest that intake of As via rice grown in As rich soil irrigated with As contaminated water may cause adverse heath effects on humans.

Copper and Zinc in PL and PL- amended Soils

Copper (Cu) and Zinc (Zn) are added to poultry feed as a sulfate salt or oxide and are used to increase weight gain and feed efficiency (Adriano, 2001; Jackson et al., 2003). Dietary Cu levels of 125 to 250 mg kg^{-1} are adequate to promote growth of swine and poultry. It was reported that 80 to 90% of dietary Cu is excreted in manure (Adriano, 2001; Kpomblekou-A et al., 2002). Zinc is added to animal diets at a level of about 80 mg kg^{-1}. Poultry litter, on average, contains high amounts of Cu and Zn, at levels of approximately 380 mg kg^{-1} (Gupta and Charles, 1999).

Application of animal manure to meet the nitrogen (N) requirements can lead to accumulation of micronutrients in soil (Brock et al., 2006). Han et al. (2000) reported concentrations of Zn and Cu in soil in a pasture on a Mississippi poultry farm that had received PL applications over 25 years were significantly greater than unamended soil. Copper in the amended soil was present mostly in the organic matter fraction (47%), whereas Zn was found in the easily reducible oxide fraction (47%). The results suggested Cu and Zn in this soil are potentially bioavailable and mobile and could be potential environmental risks to plants and surface and groundwater (Han et al., 2000). The study of effects of broiler litter management practices on concentrations of Cu and Zn in a Maryland coastal plain soil that received broiler litter for 40 years showed higher concentrations of Cu and Zn in PL- amended soil compared to a wooded area (Codling et al., 2008). Copper and Zn are not particulary mobile in soil due to their high affinity for organic matter.

Land application of PL is regulated based on applying N and/or P for crop needs. There are no regulatory standards for trace elements concentrations or loading. In the United States, only municipal biosolids have regulatory limits on trace metals. The USEPA 503 regulation loading limits for As, Cu, and Zn in soil amended with biosolids are 20, 750, and 1400 mg kg^{-1}, respectively (Miller and Miller, 2000; USEPA, 1993). These standards are often used as a reference for other organic wastes.

Several studies reported high accumulation of Cu and Zn on soil surface due to long-term PL application (Kingery et al., 1994; van der Watt et al., 1994). These findings suggested an environmental concern related to losses of these metals via runoff from PL- amended soils to water sources.

A rainfall simulator study on small plots of PL- amended soil showed that concentrations of water-soluble Cu and Zn in runoff increased as the rate of PL application increased from two events, immediately and 7 days after application. Copper concentrations in runoff from PL amended soil were 93 times higher than control (0.93 mg L^{-1}). Concentrations of Zn in runoff from control fields were 0.047 mg L^{-1} compared to 0.44 mg L^{-1} for PL-amended soil (Moore et al., 1998). Concentrations of Cu and Zn in lakes and streams can have detrimental effects on aquatic plants. Moderate concentrations of Cu are extremely toxic to algae, and maximum concentrations of 0.02 mg L^{-1} are suggested to protect freshwater fish (Edwards et al., 1997). Although not directly applicable to surface waters, the regulated USEPA limit for Cu and Zn concentrations in drinking water is 1.3 and 5 mg L^{-1}, respectively (USEPA, 2006)

Factors Effecting Mobility and Bioavailability of Cu and Zn

Solubility and bioavailability of Cu and Zn to plants are governed largely by soil pH. At soil pH above 7, bioavailability of Cu and Zn dramatically decreases. The movement of Cu and Zn in sandy, acidic soil with low organic matter contents may occur if subjected to heavy rainfall or irrigation (He et al., 2006). The phytotoxicity of Cu and Zn decreases when soil pH increases due to binding to Fe/Al oxides and organic matter. The effect of liming on raising pH is the most efficient method in reducing plant uptake. In a study of the effect of liming on distribution of Mn, Cu, Fe, and Zn among soil fractions, increasing lime rates decreased exchangeable Zn and increased the organic fraction Zn and Mn (Shuman, 1986). Copper and Zn bioavailability is low in soil with high organic matter content. Besides other soil constituents such as Fe and Al oxides, organic matter plays an important role in controlling sorption of metals. Among divalent cations, Cu forms the most stable complex with organic matter compared to Pb, Fe, and Zn (Adriano, 2001). Generally, Cu and Zn movement through the soil profile is limited due to their high affinity to organic matter. The inner-sphere complex of Cu and Zn with organic matter lowers their solubility and potential leachability in soils (Brock et al., 2006; Codling et al., 2008).

Organic matter decomposition produces soluble organic ligands such as dissolved organic carbon that influence metal mobility and toxicity (Martinez and McBride, 1999). Soil texture, Fe and Al content of soil also play an important role in adsorption and bioavailability of Cu and Zn. Soil texture plays a key role in retention of Cu and Zn in soil. The study of Cu sorption and desorption on soil clay components showed that the fine clay fraction had higher Cu retention than did the coarse and medium clay (Wu et al. 1999). The Cu deficiency may occur in soils with high organic matter content (Histosol) and on coarse-texture mineral soils (Adriano, 2001). Several studies demonstrated the important role of metal oxides on adsorption and retention of heavy metals (Gadde and Laitinen, 2002; Mclaren et al., 1981).

References

Abedin, M. J., M. S. Cresser, A. A. Meharg, J. Feldmann, and J. Cotter-Howells. 2002. Arsenic accumulation and metabolism in rice (Oryza sativa L.). Environ. Sci. Technol. 36: 962-968.

Adriano, D. C. 2001. Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risks of metals, p. 219-261. New York Springer.

Azizur Rahman, M., H. Hasegawa, M. Mahfuzur Rahman, M. A. Mazid Miah, and A. Tasmin. 2008. Arsenic accumulation in rice (Oryza sativa L.): Human exposure through food chain. Ecotox Environ Safe. 69: 317-324.

Bellows, B. C. 2005. Arsenic in poultry litter: organic regulations. ATTRA the National Sustainable Agriculture Information Service. Available at http://attra.org/attra-pub/PDF/arsenic_poultry_litter.pdf (Verified 24 Aug. 2008).

Bissen, M., and F. H. Frimmel. 2003. Arsenic - a Review. Part I: occurrence, toxicity, speciation, mobility. Acta Hydrochimica et Hydrobiologica. 31: 9-18.

Braman, R. S., and C. C. Foreback. 1973. Methylated forms of arsenic in the environment. Science. 182: 1247-1249.

Brock, E. H., Q. M. Ketterings, and M. McBride. 2006. Copper and zinc accumulation in poultry and dairy manure-amended fields. Soil Sci. 171: 388-399.

Cao, X., L. Q. Ma, and A. Shiralipour. 2003. Effects of compost and phosphate amendments on arsenic mobility in soils and arsenic uptake by the hyperaccumulator, Pteris vittata L. Environ Pollut. 126: 157-167.

Carbonell-Barrachina, A. A., A. Jugsujinda, F. Burlo, R. D. Delaune, and W. H. Patrick. 2000. Arsenic chemistry in municipal sewage sludge as affected by redox potential and pH. Water Res. 34: 216-224.

Carbonell, A. A., M. A. Aarabi, R. D. DeLaune, R. P. Gambrell, and W. H. Patrick Jr. 1998. Arsenic in wetland vegetation: Availability, phytotoxicity, uptake and effects on plant growth and nutrition. Sci. Total Environ. 217: 189-199.

Chapman, H., and Johnson, Z. 2002. Use of antibiotics and roxarsone in broiler chickens in the USA: analysis for the years 1995 to 2000: Poult Sci, v. 81, p. 356-364.

Codling, E. E., R. L. Chaney, and C. L. Mulchi. 2008. Effects of broiler litter management practices on phosphorus, copper, zinc, manganese, and arsenic concentrations in Maryland coastal plain soils. Commun. Soil Sci. Plant Anal. 39:1193-1205.

Cortinas, I., J. A. Field, M. Kopplin, J. R. Garbarino, A. J. Gandolfi, and R. Sierra-Alvarez. 2006. Anaerobic biotransformation of roxarsone and related N-substituted phenylarsonic acids: Environ. Sci. Technol. 40: 2951-2957.

Cullen, W. R., and K. J. Reimer. 2002. Arsenic speciation in the environment. Chem Rev. 89: 713-764.

Duxbury, J. M., A. B. Mayer, J. G. Lauren, and N. Hasssan. 2003. Food chain aspects of arsenic contamination in Bangladesh: effects on quality and productivity of rice. J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng. 38: 61-69.

Edwards, D. R., P. A. Moore Jr, T.C. Daniel, P. Srivastava, and D.J. Nichols. 1997. Vegetative filter strip removal of metals in runoff from poultry litter-amended fescuegrass plots. Trans. ASAE. 40: 121-127.

Fayiga, A. O., L. Q. Ma, and Q. Zhou. 2007. Effects of plant arsenic uptake and heavy metals on arsenic distribution in an arsenic-contaminated soil. Environ. Pollut. 147:737-742.

Fitz, W. J., and Wenzel, W. W. 2002. Arsenic transformations in the soil-rhizosphere-plant system: fundamentals and potential application to phytoremediation: J. Biotechnol. 99: 259-278.

Gadde, R. R., and H. A. Laitinen. 2002. Heavy metal adsorption by hydrous iron and manganese oxides. Anal Chem. 46: 2022-2026.

Garbarino, J. R., A. J. Bednar, D. W. Rutherford, R. S. Beyer, and R. L. Wershaw. 2003. Environmental fate of roxarsone in poultry litter. I. Degradation of roxarsone during composting. Environ. Sci. Technol. 37: 1509-1514.

Goh, K. H., and T. T. Lim. 2005. Arsenic fractionation in a fine soil fraction and influence of various anions on its mobility in the subsurface environment. Appl Geochem. 20:229-239.

Goldberg, S. 2002. Competitive adsorption of arsenate and arsenite on oxides and clay minerals. Soil Sci. Soc. Am J. 66: 413-421.

Gupta, G., and S. Charles. 1999. Trace elements in soils fertilized with poultry litter. Poult Sci. 78:1695-1698.

Han, F. X., W.L Kingery, H. M. Selim, and P.D. Gerard. 2000. Accumulation of heavy metals in a long-term poultry waste-amended soil. Soil Sci. 65: 260-268.

Han, F. X., W. L. Kingery, H. M. Selim, P. D. Gerard, M. S. Cox, and J. L. Oldham. 2004. Arsenic solubility and distribution in poultry waste and long-term amended soil. Sci Total Environ. 320:51-61.

He, Z. L., M. Zhang, X. E. Yang, and P. J. Stoffella. 2006. Release behavior of copper and zinc from sandy soils. Soil Sci. Soc. Am J. 70: 1699-1707.

Jackson, B. P., P. M. Bertsch, M. L. Cabrera, J. J. Camberato, J. C. Seaman, and C. W. Wood. 2003. Trace element speciation in poultry litter. J. Environ. Qual. 32: 535-540.

Jackson, B. P., J. C. Seaman, and P. M. Bertsch. 2006. Fate of arsenic compounds in poultry litter upon land application. Chemosphere. 65: 2028-2034.

Jain, C. K., and I. Ali. 2000. Arsenic: occurrence, toxicity and speciation techniques: Water Res. 34: 4304-4312.

Jones, F. T. 2007. A broad view of arsenic. Poult Sci. 86: 2-14.

Kabata-Pendias, A., and H. Pendias. 2001. Trace elements in soils and plants. 3rd (ed). CRC Press, Boca Raton, FL.

Kingery, W. L., C. W. Wood, D. P. Delaney, J.C. Williams, and G. L. Mullins. 1994. Impact of long-term land application of broiler litter on environmentally related soil properties. J. Environ. Qual. 23: 139-147.

Kpomblekou-A, K., R. O. Ankumah, and H. A. Ajwa. 2002. Trace and nontrace element contents of broiler litter. Commun. Soil Sci. Plant Anal. 33: 1799-1811.

Kumaresan, M., and P. Riyazuddin. 2001. Overview of speciation chemistry of arsenic. Curr Sci. 80: 837-846.

Lin, Z., and R. W. Puls. 2000. Adsorption, desorption and oxidation of arsenic affected by clay minerals and aging process. Environ Geol. 39: 753-759.

Lombi, E., R. S. Sletten, and W.W. Wenzel. 2000. Sequentially extracted arsenic from different size fractions of contaminated soils. Water Air Soil Pollut. 124:319-332.

Manning, B. A., S. E. Fendorf, and S. Goldberg. 1998. Surface structures and stability of srsenic(III) on goethite: spectroscopic evidence for inner-Sphere complexes. Environ Sci Technol. 32: 2383-2388.

Marin, A. R., P. H. Masscheleyn, and W. H. Patrick. 1993. Soil redox-pH stability of arsenic species and its influence on arsenic uptake by rice. Plant and Soil. 152: 245-253.

Martinez, C. E., and M. B. McBride. 1999. Dissolved and labile concentrations of Cd, Cu, Pb, and Zn in aged ferrihydrite -organic matter systems. Environ. Sci. Technol. 33: 745-750.

Matschullat, J. 2000. Arsenic in the geosphere -- a review. Scio total Environ. 249: 297-312.

McGeehan, S. L., and D. V. Naylor. 1994. Sorption and redox transformation of arsenite and arsenate in two flooded soils. Soil Sci. Soc. Am J. 58: 337-342.

Mclaren, R. G., R. S. Swift, and J. G. Williams. 1981. The adsorption of copper by soil materials at low equilibrium solution concentrations. Eur J Soil Sci. 32: 247-256.

Meharg, A. A., and M. R. Macnair. 1992. Suppression of the high affinity phosphate uptake system: A mechanism of arsenate tolerance in holcus lanatus L. J. Exp. Bot. 43: 519-524.

Miller, D. M., and Miller, W. P. 2000. Land application of wastes. In *Handbook of Soil Science*, eds. Sumner, Malcolm. E. p. G-217-241. CRC Press, Boca Raton, FL.

Moore, P. A., Jr., T. C. Daniel, J.T. Gilmour, B. R. Shreve, D. R. Edwards, and B. H. Wood. 1998. Decreasing metal runoff from poultry litter with aluminum sulfate. J Environ Qual. 27: 92-99.

Morrison, J. L. 2002. Distribution of arsenic from poultry litter in broiler chickens, soil, and crops. J. Agric. Food Chem. 17: 1288-1290.

O'Connor, R., M. O'Connor, K. Irgolic, J. Sabrsula, H. Guerleyuek, R. Brunette, C. Howard, J. Garcia, J. Brien, J. Brien, and J. Brien. 2005. Transformations, air transport, and human impact of arsenic from poultry litter. Environ Forensics. 6: 83-89.

Onken, B. M., and D. C. Adriano.1997. Arsenic availability in soil with time under saturated and subsaturated conditions. Soil Sci. Soc. Am J. 61:746-752.

O'Reilly, S. E., D. G. Strawn, and D. L. Sparks. 2001. Residence time effects on arsenate adsorption/desorption mechanisms on goethite. Soil Sci. Soc. Am J. 65: 67-77.

Oscarson, D. W., P. M. Huang, W. K. Liaw, and U. T. Hammer. 1983. Kinetics of oxidation of arsenite by various manganese dioxides. Soil Sci. Soc. Am J. 47: 644-648.

Quaghebeur, M., and Z. Rengel. 2005. Arsenic speciation governs arsenic uptake and transport in terrestrial plants. Microchimica Acta. 151: 141-152.

Rutherford, D. W., A. J. Bednar, J. R. Garbarino, R. Needham, K. W. Staver, and R. L. Wershaw. 2003. Environmental fate of roxarsone in poultry litter. Part II. Mobility of arsenic in soils amended with poultry litter. J. Environ. Sci. Technol. 37: 1515-1520.

Sarkar, D., R. Datta, and S. Sharma. 2005. Fate and bioavailability of arsenic in organo-arsenical pesticide-applied soils. Part-I: incubation study. Chemosphere. 60:188-195.

Sarkar, D., K. C. Makris, M. T. Parra-Noonan, and R. Datta. 2007. Effect of soil properties on arsenic fractionation and bioaccessibility in cattle and sheep dipping vat sites. Environ Int. 33:164-169.

Schoof, R. A., L. J. Yost, J. Eickhoff, E. A.Crecelius, D. W. Cragin, D. M. Meacher, and D. B. MenzeL. 1999. A market basket survey of inorganic arsenic in food. Food Chem Toxicol. 37: 839-846.

Shiowatana, J., R. G. McLaren, N. Chanmekha, and A. Samphao. 2001. Fractionation of arsenic in soil by a continuous-flow sequential extraction method. J. Environ. Qual.30: 1940-1949.

Shuman, L. M. 1986. Effect of liming on the distribution of manganese, copper, iron, and zinc among soil fractions. Soil Sci. Soc. Am J. 50: 1236-1240.

Singh, N., and L. Q. Ma. 2006. Arsenic speciation, and arsenic and phosphate distribution in arsenic hyperaccumulator Pteris vittata L. and non-hyperaccumulator Pteris ensiformis L. Environ Pollut. 141: 238-246.

Stollenwerk, K. 2003. Geochemical processes controlling transport of arsenic in groundwater: a review of adsorption. p. 67–100. In A.H. Welch and K.G. Stollenwerk (ed). Arsenic in Groundwater, Geochemistry and Occurrence, Kluwer Academic Publishers, Boston.

Tondel, M., M. Rahman, A. Magnuson, I. A. Chowdhury, M. H. Faruquee, and S. A. Ahmad. 1999. The relationship of arsenic levels in drinking water and the prevalence rate of skin lesions in Bangladesh. Environ. Health Perspec. 107: 727-729.

Toor, G., and B. Haggard. 2007. Trace metals runoff waters from soil amended with granulated poultry litter. *In* Annual meetings abstracts. ASA-CSSA-SSSA, New Orleans, Luisiana, 4-8 Nov. 2007. Avaiable at http://crops.confex.com/crops/2007am/techprogram (verified 12 Aug. 09)

Toor, G. S., B. E. Haggard, and A. M. Donoghue. 2007. Water extractable trace elements in poultry litters and granulated products. J. Appl. Poult. Res. 16:351-360.

USEPA. 1993. Standards for the use or disposal of sewage sludge, Final Rules. 40 CFR Part 503. Fed. Regist. 58: 9387-9404.

USEPA. 2006. Drinking water Contaminants; List of Contaminants and their MCLs. http://www.epa.gov/safewater/contaminants/index.html#mcls (verified on 08.17.09).

van der Watt, H. v. H., M. E. Sumner, and M. L. Cabrera.1994. Bioavailability of copper, manganese, and zinc in poultry litter. J. Environ. Qual. 23: 43-49.

Wenzel, W. W., N. Kirchbaumer, T. Prohaska, G. Stingeder, E. Lombi, and D. C. Adriano. 2001. Arsenic fractionation in soils using an improved sequential extraction procedure. Anal. Chim. Acta. 436:309-323.

Woolson, E. A., J. H. Axley, and P.C. Kearney. 1971. The Chemistry and phytotoxicity of arsenic in soils: I. contaminated field soils. Soil Sci. Soc. Am. J. 35:938-943.

Wu, J., D. A. Laird, and M. L. Thompson. 1999. Sorption and desorption of copper on soil clay components. J. Environ. Qual. 28: 334-388.

Xu, H., B. Allard, and A. Grimvall. 1991. Effects of acidification and natural organic materials on the mobility of arsenic in the environment. Water, Air, & Soil Pollut. 57-58: 269-278.

Table and Figures

Table 1.1. Dissociation constant of H_3AsO_3, H_3AsO_4, monomethylarsonic acid (MMA), dimethylarsinic acid (DMA)

	pK_1	pK_2	pK_3
H_3AsO_3	9.2	12.1	13.4
H_3AsO_4	2.2	7.0	11.5
MMA	3.6	8.2	-
DMA	6.3	-	-

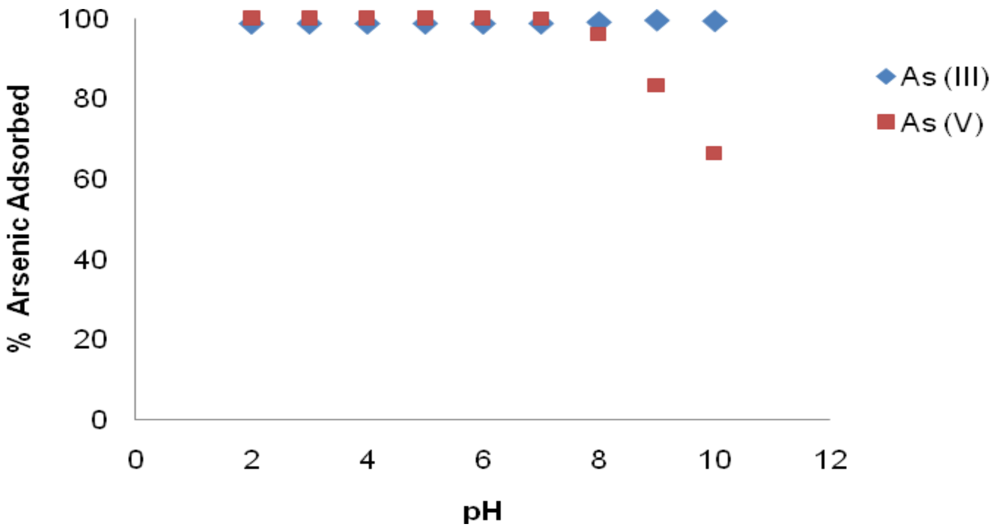


Figure 1.1. Effect of pH on arsenic adsorption by hydrous ferric oxide

CHAPTER 2

ARSENIC FRACTIONATION IN SOIL AND FORAGE UPTAKE FROM POULTRY LITTER –AMENDED PASTURES[1]

---

[1] Ashjaei, S., W. P. Miller, M. L. Cabrera, S. Hassan. To be submitted to J. Environ. Qual

Abstract

In regions of concentrated poultry production, poultry litter (PL) is commonly surface- applied to pastures at high levels over multiple years. This study examined the effect of long-term applications of PL on soil concentrations of arsenic (As), copper (Cu), Zinc (Zn), and the uptake of these elements by bermudagrass grown on Cecil (well-drained) and Sedgefield (somewhat poorly-drained) soils. The results showed that concentrations of As, Cu, and Zn in soils that had received surface-applied PL over a 14-year period were significantly greater than unamended soil at 0-2.5 and 2.5-7.5 cm depths. However, the levels were well below the USEPA loading limits for municipal biosolids. Arsenic fractionation conducted to assess the distribution of various forms of As in PL-amended soils showed that concentrations of all As fractions were significantly greater in PL-amended soils compared to control at 0-2.5 and 2.5-7.5 cm depths. There were no significant differences in concentrations of As fractions between two PL-amended soils at either depth except for $NaHCO_3$-associated As at 0-2.5 cm depth. The residual fraction was the predominant form of As in all soils. The water-soluble and $NaHCO_3$-associated As were only 1.9% and 1.8% of the total As, respectively. Significant differences were found in concentrations of these trace elements and phosphorus (P) in forage from PL-amended soils compared to that in control plot. The concentrations of Cu, Zn, As, and P were significantly greater in forage from Sedgefield amended soil compared to Cecil soil, but were in all cases below levels of environmental concern.

Introduction

The poultry industry is an important component of agricultural production in Georgia. Poultry litter (PL) is commonly surface-applied to pastures and is considered a valuable source of N, P, and K. It also contains high concentrations of trace elements such as arsenic (As), copper (Cu), and zinc (Zn) as a result of their use as growth promoters in poultry feed. Arsenic occurs in PL as a result of the use of organo arsenical compounds such as roxarsone (ROX, 3-nitro-4-hydroxyphenyl-arsenic acid) as a feed additive at concentrations of 25 to 50 mg kg^{-1} for prevention of fungal disease and for weight gain improvement (Jones, 2007). Georgia is one of the top broiler chicken producing regions in the United States. Every broiler produces between 1.46 and 2.67 kg of waste in its life span (Nachman et al., 2005; Sharpe et al., 2004). A broiler chicken fed the recommended dosage of 50 mg kg^{-1} ROX for 42 days life cycle will excrete 150 mg ROX in total (Rutherford et al., 2003). If 50% of total 1.46 billion produced broilers in Georgia in 2006 (Georgia Agricultural Statistic Services, 2006) were fed the recommended dosage of ROX, approximately 100 Mg of ROX or 28 Mg As was introduced to the environment. Repeated surface applications of PL could result in accumulation of heavy elements at shallow soil depth due to the immobility of these elements and result in environmental problems.

It has been reported that most of the ROX added to poultry diet is excreted unchanged and is introduced to the environment when litter is applied to agricultural land (Garbarino et al., 2003; Jackson et al., 2006). In one study, fields that had received PL containing 15-35 mg As kg^{-1} over 20 years in Arkansas showed that 36 to 88% of the total arsenic in PL samples was ROX (Morrison, 1969). Guptal and Charles (1999) reported greater concentrations of As, Cd, Cu, and Mn in fields that had received long-term PL applications compared to unamended soil.

Several studies have shown that long-term PL application has an effect on elevation of trace elements (e.g., As, Cu, and Zn) especially at the soil surface (Gupta and Charles, 1999; Kingery et al., 1994; Moore et al., 1998; Toor et al., 2007; van der Watt et al., 1994). A study of the long-term effect of broiler litter applications on Cu and Zn in Maryland Coastal Plain soil showed higher concentrations of these trace elements in PL-amended soil compared to a wooded area (Codling et al., 2008). Land application of PL is regulated based on applying N and/or P for crop needs. There are no regulatory standards for trace elements concentrations or loading. In the United States, only municipal biosolids have regulatory limits on trace metals. The USEPA 503 regulation loading limits for As, Cu, and Zn in soil amended with biosolids are 20, 750, and 1400 mg kg^{-1}, respectively (Miller and Miller, 2000; USEPA, 1993). These standards are often used as a reference for other organic wastes.

Arsenic in soils is distributed among various forms which are associated with different soil constituents. Arsenic fractionation in different soil solid phases can be examined by selective sequential extraction with reagents of increasing dissolution strength. Based on the chemical similarity of P and As, modified versions of P fractionation methods have been adapted for As (Wenzel et al., 2001; Lombi et al., 2000; Onken and Adriano, 1997; Sarkar et al., 2007; Shiowatana et al., 2001; Wenzel et al., 2001). These schemes differ in their sequential extraction steps, reagents, and extraction conditions. A common feature among most methods is extracting with NH_4Cl or H_2O (water-soluble As), $NaHCO_3$ (surface-adsorbed As), NaOH (bound to Fe oxide), NH_4F (bound to Al oxide) and the residual phase (acid digest). The results of As fractionation aid in understanding the distribution of As among different soil constituents and help in predicting its mobility and bioavailability.

The bioavailability of arsenic to plants and its mobility and possible release to runoff from PL-amended soils is governed by its distribution among various solid-phase components. In general, the water-soluble form is considered as readily bioavailable and is presumably susceptible to runoff losses compare to more tightly bound forms. A study of As distribution in PL and long-term PL amended soil (upper coastal plain, MS) that had received PL at a rate of 10 Mg ha^{-1} for 25 years showed that 47% of As in PL was water-soluble fraction. However, 72% of As in PL-amended soils was in the residual fraction and was considered as the least susceptible to runoff losses or downward movement. It was concluded that immediately after PL application there would be a high risk of mobilization of As from top soil but over time the water-soluble form of As converts to more stable fractions with less susceptibility to be lost by runoff (Han et al., 2004). An incubation study conducted to estimate bioavailability of arsenic as a function of soil properties found that the amount of water-soluble As decreased from 88% to 70% with an increase in Fe/Al bound fraction from 3% to 20% after 4 months of soil incubation with different rates of dimethylarsinic acid. The retention of As in the Fe/Al fraction was more pronounced in soils with greater content of Fe/Al-oxide content. Hence, there is probably a greater risk of contamination of surface water in coarse-textured soils with low Fe/Al-oxide contents (Sarkar et al., 2005). A study of the effect of As uptake by Chinese brake fern (*Pteris vittata L)* on As distribution from As contaminated soil showed that after 8 weeks of plant growth, Fe/Al-associated As fractions were significantly greater in As-spiked soil than in the control. Arsenic was taken up by plants from all fractions, although Ca-associated As contributed the most (51-71% reduction) since As was present primarily in the form of Ca-associated As in the soil. In addition, it was observed that only water-soluble As significantly correlated with As removed by the plant after 8 weeks of growth (Fayiga et al., 2007).

The objectives of this study are (1) to estimate the potential environmental concern associated with As, Cu, and Zn in long-term PL-amended pastures (2) to assess the distribution of different forms of As fractions in PL- amended pastures and (3) to determine forage uptake of

As, Cu, Zn, and P in PL-amended pastures. Two southern piedmont soils (well-drained Cecil series and somewhat poorly-drained Sedgefield) were compared in the experiments.

Materials and Methods

Soil Sampling and Characterization

Part of this research was conducted on six 0.8 ha PL-amended and two control plots with tall fescue (*Festuca arundinacea Scherb*) and bermudagrass (*Cynodon dactylon L.),* located at the College of Agricultural and Environmental Sciences Central Georgia Research and Education Center near Eatonton GA (39°24’ N, 83°29’ W, elevation 150 m). The soil series comprising the plots include Cecil (fine, kaolinitic, thermic Typic Kanhapludults), and Sedgefield (fine, mixed, active, thermic Aquic Hapludults). Poultry litter had been applied to each amended plot since 1995 at 5 Mg ha^{-1} annually and was applied on April 2008, November 2008, and March 2009 during this experiment. There were four PL-amended plots containing Cecil soil, two PL-amended plots containing Sedgefield soil and two unamended (control) plots containing Cecil soil. Composite soil sampling was conducted on June 2008 two months after PL application on April 2008 with six replications from PL-amended plots containing Cecil and Sedgefield soils and 3 replications from control plots (each replicate was a composite of 12 soil subsamples) at 0-2.5 and 2.5-7.5 cm depths. The samples were air-dried and passed through 2-mm sieve, mixed, and stored at room temperature before analysis. Soil particle-size distribution was determined by the micropipette method (Miller and Miller, 1987). Sodium dithionite was used to remove total free iron oxide and Tamm’s reagent was used to remove amorphous iron oxide in both PL-amended soils and they were determined by atomic absorption spectrometer (Loeppert and Inskeep, 1996). A 1:1 soil-to-water mixture was used to determine soil pH.

Forage Sampling

Forage was sampled twice from these experimental plots in October 2008, five months after PL application in April 2008 and in spring 2009, three months after PL application on March 2009. The pastures were predominantely bermudagrass (*Cynodon dactylon L.)* and tall fescue (*Festuca arundinacea Scherb*). Forage samples were taken from two PL-amended plots containing Cecil soil, two PL-amended plots containing Sedgefield soil, and two control plots (containing Cecil soil) with 3 replications per each plot. Each replicate was a composite sample of 10-12 randomly selected areas of bermudagrass which was clipped manually. The grass was in its vegetative stage approximately 20-30 cm tall, of which the top 10-15 cm was sampled. The samples were oven-dried at 65°C for 48 hr and ground prior to analysis.

Arsenic Fractionation

Soils were extracted sequentially to determine distinct fractions of soil arsenic using a method described by Shiowatana et al. (2001). Soil As pools extracted consist of (1) water-soluble As (0.01 *M* $Ca(NO_3)_2$); (2) surface- adsorbed As (0.5 *M* $NaHCO_3$); (3) Fe- and Al-associated As (0.1 *M* NaOH) (Shiowatana et al., 2001). In the As fractionation, 1 g of air-dried soil was extracted with 30 ml of 0.01 *M* $Ca(NO_3)_2$ in 30 ml polypropylene high speed centrifuge tubes and shaken for 16 hr and centrifuged at 3000 rpm for 15 min. The supernatant solution was filtered with Whatman No. 40 filter paper. The soil residue was resuspended with 30 ml $NaHCO_3$ and shaken for 16 hr and centrifuged as in step 1 and filtered with Whatman No.40 filter paper. The solution was diluted 4-fold prior to analysis in order to run the samples by ICP-MS (Inductively Coupled Plasma Mass Spectrometry, Perkin Elmer, Model Elan 9000). In the last step, the soil reside from step 2 was resuspended with 30 ml (0.1 *M*) NaOH for 16 hr, centrifuged as in previous steps and filtered with Whatman No.40 filter paper and diluted 10-fold prior to analysis by ICP-MS. The residual arsenic was calculated by subtracting the sum of all As fractions from total As in soil (described below). The water soluble As concentrations in PL were determined by extraction of 1 g PL with 10 ml distilled water in 50 ml polypropylene centrifuge tubes. After shaking for 2 hr, the extracts were centrifuged at 15000 rpm for 15 minutes, filtered (0.45 μm), and diluted 20-fold with distilled water prior to analysis by ICP-MS (Jackson et al., 2003).

Total Trace Elements Analysis

Total trace elements in PL–amended soil were determined using USEPA SW-846 hot plate acid digestion method 3050A (USEPA, 1995a). An air-dried soil sample (0.5 g) was transferred to a 250 ml glass Erlenmeyer flask and was covered with a small glass funnel to reflux vapors and placed on a hot plate under a fume hood. Ten ml of HNO_3 was added to the flask and heated to boiling point for about 3 hrs. In order to complete the digestion, 0.5 ml hydrogen peroxide (H_2O_2) was added slowly to the flasks after cooling down by removing the flask from the hot plate. This addition was repeated until a relatively clear solution was obtained. After cooling, flasks were taken to 100 ml final volume gravimetrically using deionized water and left overnight for settling of particulates. The decanted solutions were stored in polyethylene plastic vials and diluted at a 1:10 ratio prior to analysis by ICP-MS for As, Cu, and Zn. Replicates, blanks, calibration verification, and standard reference material (SRM-2709) were analyzed to ascertain data quality.

Poultry litter samples were freeze-dried (LabConco Lyph. Lock 6, Kansas, MO) and total As, Cu, and Zn in PL was analyzed using a microwave acid digest method USEPA 3051 (USEPA, 1995b). All microwave vessels were soaked in 5 % HNO_3 overnight and cleaned by microwaving with 2 ml HNO_3 and 10 ml distilled water (CEM corporation, Model 81D) for 20 minutes prior to use for sample digestion. The 0.12 g freeze-dried PL samples were transferred to microwave vessels and 5 ml 70% HNO_3 was added. Vessels were weighted before and after digestion to assure negligible solution loss during digestion. After removing from the microwave, vessels were cooled to room temperature and 25 ml distilled water was added to all vessels. The final samples were stored in polyethylene vials prior to analysis by ICP-MS. Trace elements (As, Cu, and Zn) and phosphorus concentrations in forage were determined by the same method using 0.1 g oven-dried forage samples.

Mehlich-I (Double Acid) extractable Cu and Zn

Extractable Cu and Zn in soils at two depths (0-2.5, 2.5-7.5 cm) were measured by the Mehlich-I method. The Mehlich-I extraction solution consists of 0.05 *M* HCl and 0.0125 *M* H_2SO_4 which was prepared by adding 4.17 ml of concentrated HCl (12 *M*) and 0.70 ml of concentrated H_2SO_4 (17.8 *M*) and brought to volume in a 1 litter volumetric flask. Five g of air-dried soil along with 20 ml of Mehlich-I extraction solution was transferred to 50 ml polypropylene centrifuge tubes and shaken for 5 min (Reed, 1996). The solution was filtered through Whatman no.42 filter paper. The filtered samples were diluted 1:100 prior to analysis by ICP-MS.

Statistical Analysis

Arsenic fractionation in control and PL-amended soils at 0-2.5 and 2.5-7.5 cm depths were examined using the PROC GLM procedure (SAS Institute, 2008). The main effect of soil type on concentrations of trace elements in forage tissue (soil treatments: control (Cecil) and PL-amended Cecil and Sedgefield soils) was significant at $p<0.0001$ and means were separated using LSMEANS procedure with PDIFF option. Mean concentration of trace elements in forage tissue were examined using the PROC MIXED procedure and means were separated using LSMEANS procedure with PDIFF option.

Result and Discussion

Total concentrations of As, Cu, and Zn in PL applied to the pasture on April 2008, November 2008, and March 2009 varied with sampling time (Table 2.1). These variations are due to differences in composition of poultry feeds and PL management practices. Trace element concentrations of As, Cu and Zn were low in all PL samples compared to pollutant limit metal concentrations for land application of biosolids (41 mg As kg^{-1}, 1500 mg Cu kg^{-1}, and 2800 mg Zn kg^{-1}) (Miller and Miller, 2000).

Table 2.2 shows the physical and chemical properties of both PL-amended soils. The clay content and total amounts of Fe oxide of amended Cecil soil were greater at both depths compared to those of Sedgefield soil. Hence, it may be presumed that higher Fe content and CEC of Cecil soil will increase the soil As retention capacity. Sarkar et al. (2005) reported that soils with higher concentrations of amorphous Fe/Al oxides retain more As and reduce its bioavailability. Rutherford et al. (2003) found a strong correlation between acid-extractable Fe and As content of PL-amended soils, suggesting adsorption of As with Fe oxides.

There were differences in total concentrations of As, Cu, and Zn in PL-amended soils compared to those in control plot at 0-2.5 cm and 2.5-7.5 cm depths (Table 2.3). Total concentration of As was greater in PL-amended Cecil and Sedgefield soils (3.67 mg kg^{-1} and 3.91 mg kg^{-1}) compared to those in control plots (1.46 mg kg^{-1}) at 0-2.5 cm depth. Similar results were found for total As concentrations in both PL-amended soils compared to those in control plots at 2.5-7.5 cm depth. Han et al. (2004) also found significant amount of As in PL-amended soil with a long-term history of PL application (25 years) compared to unamended soil and total As concentration in the amended soil was four times greater than that in the unamended soils (8.4 vs. 2.68 mg kg^{-1}).

Total concentrations of Cu and Zn were greater in both PL-amended soils compared to those in control plots. There was no difference in concentration of Cu in Cecil soil and concentration of Zn in Cecil and Sedgefield soils compared to control plots at the 2.5-7.5 cm depth. Han et al. (2000) reported concentrations of Cu and Zn in unamended soil (0-10 cm) were significantly lower (2.2 and 9.8 mg kg^{-1}) compare to PL-amended soil (74.5 and 88.8 mg kg^{-1}). Brock et al. (2006) also showed accumulation of Cu and Zn in soils with a history of 40 years of PL application in the plow layer (0-17.5 cm). The total Cu concentrations ranged from 5.9 to 30.1 mg Cu kg^{-1} soil and total concentrations of Zn were up to 112 mg Zn kg^{-1} soil. Other studies reported elevation of Cu and Zn concentration in the top 5-10 cm depth in short and long-term application of PL (Pederson et al., 2002; Han et al., 2000). Litter applications for 15 to 25 yr resulted in accumulation of Cu and Zn to a depth of 40 cm compared with unamended soil (Kingery et al., 1994).

The concentrations of As in Cecil and Sedgefield soils were not different compared with each other at both depths. Copper concentrations at 2.5-7.5 cm depth and Zn concentrations at 0-2.5 cm depth were different between Cecil and Sedgefield soils. Concentration of Cu was greater in PL-amended Cecil soil (0-2.5 cm) compared to that in 2.5-7.5 cm depth at $p<0.01$ (95.6 vs. 44.1 mg kg^{-1}). The same result was found for Zn with greater concentration in PL-amended Cecil soil at 0-2.5 cm soil compared to 2.5-7.5 cm depth (121 vs. 51.6 mg kg^{-1}). Concentration of Zn in PL-amended Sedgefield soil was greater at 0-2.5 cm depth compared to 2.5-7.5 cm depth at $p<0.01$ (88 vs. 56 mg kg^{-1}). Higher concentrations of Cu and Zn in both PL-amended soils at 2.5-7.5 than those in soil surface may reflect downward movement of Cu and Zn in both PL-amended soils following long-term PL additions. For the control plot, concentrations of Cu and Zn were not different in the surface soil compared to that at 2.5-7.5 cm depth. Han et al. (2000) have shown lower total concentrations of Cu and Zn in surface of unamended soil than those in subsoil which was related to plant uptake of these metals without replacement from surface zone.

The distribution of trace elements is clearly due to the surface application of the PL and lack of incorporation into the A horizon. However, concentrations of trace elements were well below USEPA loading limits at both depths. If we assume application rate of 5 Mg ha^{-1} per year over the 14 years with an average As concentration of 20 mg kg^{-1} in PL applied on the soil surface (0–7.5 cm), the average As input rate was approximately 1.4 kg As ha^{-1} yr^{-1}, which is equivalent to 1.4 mg As kg^{-1} yr^{-1} on the top soil (0-7.5 cm). This suggests that annual As loading at current application rate is below the annual ceiling rate (2.0 kg As ha^{-1} yr^{-1}) for safe land application of biosolids (Miller and Miller, 2000). The same estimation can be done for Cu and Zn with average concentrations of 540 mg Cu kg^{-1} and 443 mg Zn kg^{-1} in PL applied on top soil (0-7.5 cm) at a rate of 5 Mg ha^{-1} per year over the 14 years. The Cu and Zn input rates were approximately 38 mg Cu kg^{-1} yr^{-1} and 31 mg Zn kg^{-1} yr^{-1} which are below the annual celing rates (75 kg Cu ha^{-1} yr^{-1} and 140 kg Zn ha^{-1} yr^{-1}) for safe land application of biosolids.

There was a difference in main effect of soil type (treatments: control and PL-amended Cecil and Sedgefield soils) for all fractions. However, only water-soluble and Fe/Al-associated As showed a significant interaction effect between soil and depth at p<0.05 (Table 2.4). There were significant differences in concentrations of water-soluble As in PL-amended soils compared to control at both depths (Table 2.5). Only 1.9% and 1.8% of As was water- soluble two months after PL application in both soils at 0-2.5 cm depth. The same result was found for surface adsorbed As ($NaHCO_3$) at 0-2.5 cm depth. In addition, the amount of $NaHCO_3$- associated As was significantly greater in Sedgefield soil compared to Cecil soil only at depth of 0-2.5 cm. Since soil samples were taken two months after PL application, it could be expected to have less water-soluble As especially in soil with higher sorption capacity (Cecil soil). Solubility of As in PL-amended soils, indicated here by water-soluble and $NaHCO_3$-associated As, is linked to its mobility and plant uptake. The fact that long-term PL-amended Sedgefield soil has more $NaHCO_3$- associated As suggests the greater potential loss of As via runoff into surface water systems or uptake by plants compare to that in PL-amended Cecil soil. A similar result was found in an incubation study of two soils amended with dimethylarsinic acid with varying Fe/Al content (Sarkar et al., 2005). Immediately after application, dimethylarsenic acid was more mobile in soils with low amorphous Fe/Al content and it became less soluble after 4 months of incubation. Iron and Al-associated As (NaOH) in two PL-amended soils were significantly greater than control at both depths. There was no significant difference in concentration of Fe/Al-associated As between Cecil and Sedgefield soils. The residual fraction was the major form of As with approximately 50% of total As in both amended soils. Han et al. (2004) also found that As in the long-term PL-amended soil was mainly in the residual fraction (72% of total). Overall, there were no significant differences in concentrations of As fractions between PL-amended soils at 0-2.5 and 2.5-7.5 cm except for $NaHCO_3$ associated As at 0-2.5 cm depth. Apparently, As transforms to more stable forms, resulting in decreased As bioavailability and mobility months after PL application. This indicates possible loss of water-soluble As via runoff shortly after PL is applied to field.

The results of statistical analysis of trace elements concentrations in forage tissue from PL-amended soil and control showed that there was a significant main soil type effect of P, Cu, Zn, and As concentrations in forage tissues from control and PL-amended soils at $P> 0.028$, 0.0012, 0.034, and 0.0039, respectively (Table 2.6). However, no significant interaction was found between soil and date effect for all trace elements and P (October and June sampling). There were differences in concentrations of all trace elements in forage tissue from PL-amended soils compared to those in control plot except for amount of Zn in forage tissue from Cecil soil (Table 2.7). Another study of metal uptake by tall fescue as affected by PL application at low (5.6 t ha^{-1}) and high (11.3 t ha^{-1}) rates showed that Zn and Cu uptake were significantly greater in PL-amended soil compared to the control (Brye and Pirani, 2006). However, Kingery et al. (1994) showed that tall fescue grown in pastures with a history of long-term litter application had similar Cu and Zn concentrations as fescue grown in pastures with no history of litter application. The concentration of As in forage from Sedgefield soil was significantly greater than Cecil soil. The coarse-textured soils are likely associated with higher amounts of readily mobile As, while As in fine textured soils are mainly immobile due to lower content of minerals and organic constituents which are capable of binding anionic As species. There were no differences in concentrations on Cu and Zn in forage tissues from Cecil soil compared to those from Sedgefield soil. The concentrations of Cu and Zn in the forage samples did not exceed critical phytotoxic levels reported by Macnicol and Beckett (1985) (21-40, and 210- 560 mg kg^{-1} for Cu, and Zn, respectively). Long-term PL application did not produce tissue Cu and Zn levels above the NRC (National Research Council) maximum tolerance level of Cu for cattle at 40 mg Cu kg^{-1} feed (except for sheep, which is set at 15 mg Cu kg^{-1} feed) and Zn for cattle and sheep at 500 and 300 mg Zn kg^{-1} feed, respectively (NRC, 2005). The forage samples from both PL-amended soils were Cu deficient for animal feed based on the minimum recommended Cu concentration for cattle rations of 9 mg Cu kg^{-1} (NRC, 1980). Although the NRC tolerance limit for forage tissue Cu was not exceeded, continual PL applications to the pasture for 32 years might increase the Cu content beyond the 15 mg Cu kg^{-1} tolerance limit for sheep. Soon et al. (1980) reported small increases in the Cu content of corn grain and bermudagrass grown in soil amended with sewage sludge applications (31 kg Cu ha^{-1}) for five consecutive years. The minimum recommended Zn content in cattle rations average 35 mg kg^{-1}; forage samples from both PL-amended soils were close to that amount and considered Zn sufficient for animal feed. Warman and Termeer (2005) found that Zn content of grass forage and corn tissues grown in soil amended with sewage wastes for two consecutive years (applied twice a year) were below the 35 mg kg^{-1} minimum Zn requirement for cattle. The maximum tolerance levels of As for cattle and sheep are 30 mg As kg^{-1} feed (NRC, 2005). The data shown in Table 2.7 indicate that the As content of bermudagrass tissues grown in both PL-amended soils are below that amount.

The result of Mehlich-I extraction showed greater concentrations of Cu and Zn in PL-amended Cecil and Sedgefield soils compared to those in control plot at both depths (Table 2.8). Concentrations of extractable Zn were greater in PL-amended Sedgefield and Cecil soils (47.4 and 47.5 mg kg^{-1}) at 0-2.5 cm compared to those in 2.5-7.5 cm depth (10.45 and 11.29 mg kg^{-1}). Only Cu concentrations were greater in PL-amended Sedgefield soil at 0-2.5 cm (6.60 mg kg^{-1}) compared to that in 2.5-7.5 cm depth (4.60 mg kg^{-1}) at $p<0.01$. The fact that concentrations of extractable Cu and Zn were greater in PL-amended soils compared to those in control plot appear to be linked with the greater amounts of Cu and Zn in bermudagrass tissue from PL-amended soils and compared to that from control plots. Zhou et al. (2005) reported significant linear correlation between extractable soil Zn (and Cu) concentrations extracted by 1.0 *M* NH_4NO_3 and the Zn (and Cu) concentrations in radish tissues. Warman and Termeer (2005) found that only Mehlich-I Cu was highly correlated with forage tissue Cu ($r = 0.98$), and the correlation coefficients for extractable Zn and Zn uptake were not significant in soil with biosolids application.

Conclusions

Total concentrations of As, Cu, and Zn in soils that had received long-term surface-applied PL were significantly greater than unamended soil. If the USEPA annual loading limits are used as a frame of reference for serious environmental hazard, the concentrations were below those limits. The As fractionation study showed concentrations of all As fractions were significantly greater in PL-amended soils compared to controls at 0-2.5 and 2.5-7.5 cm depth. However, there were no significant differences in concentrations of any As fractions between two PL-amended soils at either depth except for $NaHCO_3$-associated As at 0-2.5 cm depth. The greater amounts of $NaHCO_3$-associated As seen in the somewhat poorly-drained Sedgefield soils amended with PL suggests greater potential loss of As via runoff or uptake by plants for these soils compared to PL-amended Cecil soils. Arsenic in the PL-amended soil was dominantly found in the residual fraction which is the least susceptible fraction to runoff losses as soluble As or downward movement. There were greater concentrations of As, Cu, Zn, and P in forage from PL-amended soils compared to those from control. Only As was significantly greater in forage grown in Sedgefield soil compared to Cecil soil. Although PL application resulted in elevated concentrations of As, Cu, Z, and P in forage tissues, it did not cause tissue As, Cu, and Zn levels to increase above the NRC domestic animal mineral tolerance levels. The forage samples from both PL-amended soils were Zn sufficient and Cu deficient for animal feed.

References

Brock, E. H., Q. M. Ketterings, and M, McBride. 2006. Copper and Zinc accumulation in poultry and dairy manure-amended fields. Soil Sci 171: 388-399.

Brye, K. R., and A. L. Pirani. 2006. Metal uptake by tall fescue (Festuca arundinacea) as affected by poultry litter application. Grass and Forage Sci. 61:192-199.

Codling, E. E., R. L. Chaney, and C. L. Mulchi. 2008. Effects of broiler litter management practices on phosphorus, copper, zinc, manganese, and arsenic concentrations in Maryland coastal plain soils. Commun. Soil Sci. Plant Anal. 39:1193-1205.

Fayiga, A. O., L. Q. Ma, and Q. Zhou. 2007. Effects of plant arsenic uptake and heavy metals on arsenic distribution in an arsenic-contaminated soil. Environ. Pollut. 147:737-742.

Georgia Agricultural Statistic Services. 2006. Georgia poultry facts. Available at www.nass.usda.gov/QuickStats/PullData_US.jsp (verified 23 Feb. 2008).

Goh, K. H., and T. T. Lim. 2005. Arsenic fractionation in a fine soil fraction and influence of various anions on its mobility in the subsurface environment. Appl Geochem. 20:229-239.

Gupta, G., and S. Charles. 1999. Trace elements in soils fertilized with poultry litter. Poult Sci. 78:1695-1698.

Han, F. X., W. L. Kingery, H. M. Selim, P. D. Gerard, M. S. Cox, and J. L. Oldham. 2004. Arsenic solubility and distribution in poultry waste and long-term amended soil. Sci Total Environ. 320:51-61.

Jackson, B. P., P. M. Bertsch, M. L. Cabrera, J. J. Camberato, J. C. Seaman, and C. W. Wood. 2003. Trace element speciation in poultry litter. J. Environ. Qual. 32:535-540.

Jones, F. T. 2007. A broad view of arsenic. Poult Sci. 86: 2-14.

Kingery, W. L., C. W. Wood, D. P. Delaney, J. C. Williams, and G. L. Mullins. 1994. Impact of long-term land application of broiler litter on environmentally related soil properties. J. Environ. Qual. 23:139-147.

Loeppert, R. H. and W.P. Inskeep. 1996. Iron. p. 645–650. *In* D.L. Sparks (ed.) Methods of soil analysis. Part 3. SSSA Book Series No 5. SSSA and ASA. Madison, WI.

Lombi, E., R. S. Sletten, and W.W. Wenzel. 2000. Sequentially extracted arsenic from different size fractions of contaminated soils. Water Air Soil Pollut. 124:319-332.

Macnicol, R. and P. Beckett. 1985. Critical tissue concentrations of potentially toxic elements. Plant and Soil. 85: 107-129.

Miller, D. M., and W. P. Miller. 2000. Land application of wastes. p. G217-241. *In* M. E. Sumner (ed.) Handbook of soil science. CRC Press, Boca Raton, FL.

Miller, D. M., and W. P. Miller. 1987. A micro-pipette method for soil mechanical analysis. Commun. Soil Sci. Plant Anal. 18:1-15.

Moore, P. A., Jr., T.C. Daniel, J.T. Gilmour, B. R. Shreve, D. R. Edwards, and B. H. Wood. 1998. Decreasing metal runoff from poultry litter with aluminum sulfate. J Environ Qual. 27: 92-99.

Nachman, K. E., J. P. Graham, L. B. Price, and E. K. Silbergeld.2005. Arsenic: A roadblock to potential animal waste management solutions. Environ. Health Perspec. 113: 1123-1124.

NRC. 2005. National Research Council. 2nd rev.(ed). Mineral tolerance of domestic animals, National Academy of Sciences, Washington, DC.

NRC. 1980. National Research Council. Mineral tolerance of domestic animals. National Academy of Sciences, Washington, DC.

Onken, B. M., and D. C. Adriano.1997. Arsenic availability in soil with time under saturated and subsaturated conditions. Soil Sci. Soc. Am J. 61:746-752.

Pederson, G. A., G. E. Brink and T. E. Fairbrother. 2002. Nutrient uptake in plant parts of sixteen forages fertilized with poultry litter: Nitrogen, Phosphorus, Potassium, Copper, and Zinc. Agron J. 94: 895-904.

Reed, S. T., and D.C. Martens. 1996. Copper and zinc. p. 703–722. *In* D.L. Sparks (ed.) Methods of soil analysis. Part 3. SSSA Book Series No 5. SSSA and ASA. Madison, WI.

Rutherford, D. W., A. J. Bednar, J. R. Garbarino, R. Needham, K. W. Staver, and R. L. Wershaw. 2003. Environmental fate of roxarsone in poultry litter. Part II. Mobility of arsenic in soils amended with poultry litter. J. Environ. Sci. Technol. 37: 1515-1520.

Sarkar, D., R. Datta, and S. Sharma. 2005. Fate and bioavailability of arsenic in organo-arsenical pesticide-applied soils. Part-I: incubation study. Chemosphere. 60:188-195.

Sarkar, D., K. C. Makris, M. T. Parra-Noonan, and R. Datta. 2007. Effect of soil properties on arsenic fractionation and bioaccessibility in cattle and sheep dipping vat sites. Environ Int. 33:164-169.

SAS Institute. 2008. SAS/STAT User's guide, Version 9.2. SAS, Cary, NC.

Sharpe, R. R., H. H. Schomberg, L. A. Harper, D. M. Endale, M. B. Jenkins, and A. J. Franzluebbers. 2004. Ammonia volatilization from surface-applied poultry litter under conservation tillage management practices. J. Environ. Qual. 33: 1183-1188.

Shiowatana, J., R. G. McLaren, N. Chanmekha, and A. Samphao. 2001. Fractionation of arsenic in soil by a continuous-flow sequential extraction method. J. Environ. Qual.30: 1940-1949.

Soon, Y. K., Bates, T. E.and Moyer, J. R. 1980. Land Application of Chemically Treated Sewage Sludge: III. Effects on Soil and Plant Heavy Metal Content: J Environ Qual. 9: 497-504.

Toor, G. S., B. E. Haggard, and A. M. Donoghue. 2007. Water extractable trace elements in poultry litters and granulated products. J. Appl. Poult. Res. 16:351-360.

USEPA. 1993. Standards for the use or disposal of sewage sludge, Final Rules. 40 CFR Part 503. Fed. Regist. 58: 9387-9404.

USEPA. 1995a. Method 3050A: Acid digestion of sediments, sludges, and soils. *In* Test methods for evaluating solid waste. 3rd (ed.). SW-846. USEPA, Washington, DC.

USEPA. 1995b. Method 3051: Microwave assisted acid digestion of sediments, sludges, soils and oils. Availabe at http://www. epa.gov/SW-846/pdfs/3051.pdf (verified 22 July 2008). USEPA , Washington, DC.

van der Watt, H. v. H., M. E. Sumner, and M. L. Cabrera.1994. Bioavailability of copper, manganese, and zinc in poultry litter. J. Environ. Qual. 23: 43-49.

Warman, P. R. and W. C. Termeer. 2005. Evaluation of sewage sludge, septic waste and sludge compost applications to corn and forage: Ca, Mg, S, Fe, Mn, Cu, Zn and B content of crops and soils. Biores Technol. 96: 1029-1038.

Wenzel, W. W., N. Kirchbaumer, T. Prohaska, G. Stingeder, E. Lombi, and D. C. Adriano. 2001. Arsenic fractionation in soils using an improved sequential extraction procedure. Anal. Chim. Acta. 436:309-323.

Woolson, E. A., J. H. Axley, and P.C. Kearney. 1971. The Chemistry and phytotoxicity of arsenic in soils: I. contaminated field soils. Soil Sci. Soc. Am. J. 35:938-943.

Zhou, D. M., X. Z. Hao, Y. J. Wang, Y. H. Dong, and L. Cang. 2005. Copper and Zn uptake by radish and pakchoi as affected by application of livestock and poultry manures. Chemosphere. 59: 167-175.

Tables

Table 2.1.Total concentrations of trace elements in poultry litter applied on April 2008, November 2009, and March 2009

	As	Cu	Zn
		mg kg^{-1}	
April 2008	23.6	207	229
November 2008	14.9	743	431
March 2009	26.7	672	671

Table 2.2. General chemical and physical properties of Cecil and Sedgefield soils at 0-2.5 and 2.5-7.5 cm depths.

					Particle size distribution		
Soil	Depth (cm)	pH (1:1 water)	Total Fe Oxide	Amorphous Fe Oxide	Sand	Silt	Clay
			mg kg^{-1}		%		
Cecil	0-2.5	6.00	7173	3397	58	34	8
Cecil	2.5-7.5	5.87	7511	3356	49	36	15
Sedgefield	0-2.5	5.99	4505	3259	72	23	2
Sedgefield	2.5-7.5	5.85	5281	3936	72	25	3

Table 2.3. Total concentrations of As, Cu, and Zn in poultry litter-amended pastures and control plot

	As	Cu	Zn
Depth (0-2.5cm)	mg kg^{-1}		
Control	1.46 a‡‡	13.6 a	32.0 a
Cecil (PL-amended)	3.67 b	95.6 b	121 b
Sedgefield (PL-amended)	3.91 b	88.2 b	88.0 c
Depth (2.5-7.5 cm)	mg kg^{-1}		
Control	1.57 a‡‡	29.3 a	34.0 a
Cecil (PL-amended)	3.04 b	44.1 ab	51.6a
Sedgefield (PL-amended)	3.46 b	66.5 c	56.0 a
USEPA loading Limit	20	750	1400

‡‡ Means (n=3) within a column at a given depth followed by the same letter are not different significantly according to LSD ($p<0.05$).

Table 2.4. Analysis of variance of arsenic concentrations in extracted fractions in poultry litter-amended soils

Source	WE	NaHCO3	NaOH	Residual	Total
			P>F		
Soil#	<0.0001	0.0005	<0.0001	<0.0001	<0.0001
Depth	<0.0001	<0.0001	0.0003	0.745	0.0198
Soil * Depth¶	0.028	0.183	0.0005	0.646	0.268

Main soil type effect (treatments: control (Cecil) and PL-amended Cecil and Sedgefield soils) significant at $p<0.05$

¶ Soil by depth interaction (significant at $p<0.05$)

Table 2.5. Arsenic fractionation in poultry litter-amended soils at 0-2.5 and 2.5-7.5 cm depths

	WE‡	NaHCO$_3$	NaOH	Residual	Total
Depth (0-2.5 cm)			mg kg^{-1}		
Control	0.03 a‡‡	0.17 a	0.55 a	0.55 a	1.29 a
Cecil (PL-amended)	0.07 b	0.36 b	1.43 b	1.81 b	3.67 b
Sedgefield (PL-amended)	0.07 b	0.53 c	1.52 b	1.79 b	3.91 b
Depth (2.5 -7.5 cm)			mg kg^{-1}		
Control	0.003 a‡‡	0.07 a	0.81 a	0.50 a	1.39 a
Cecil (PL-amended)	0.017 b	0.21 ab	1.09 b	1.68 b	3.03 b
Sedgefield (PL-amended)	0.014 b	0.24 b	1.19 b	2.00 b	3.46 b

‡: WE, NaHCO3, and NaOH stand for water-soluble-, adsorbed-, and Fe/Al-associated arsenic, respectively.

‡‡ Means (n=3) within column followed by the same letter are not different significantly according to LSD ($p<0.05$)

Table 2.6. Analysis of variance of P, Cu, Zn, and As concentrations in bermudagrass samples from poultry litter-amended soils

Source	P	Cu	Zn	As
		P>F		
Soil#	0.028	0.0012	0.034	0.003
Date	0.26	0.0003	0.045	0.75
Soil*Date¶	0.95	0.25	0.76	0.79

Main soil type effect (forage samples grown on control (Cecil) and PL-amended Cecil and Sedgefield soils) significant at $p<0.05$

¶ Soil by depth interaction (significant at $p<0.05$)

Table 2.7. Concentrations of elements in bermudagrass tissue from control and poultry litter-amended soils

	P	Cu[a]	Zn[b]	As[c]
	mg kg $^{-1}$			
Control	2189 a[‡‡]	5.10 a	25.7 a	0.16 a
Cecil (PL-amended)	2746 b	7.05 b	34.2ab	0.23 b
Sedgefield (PL-amended)	2927 b	7.23 b	41.3 b	0.31 c

‡‡ Means (n=3) within a column followed by the same letter are not different significantly according to LSD ($p<0.05$).

[a] The maximum tolerance levels of Cu in the feed of cattle and sheep are 40 and 15 mg kg $^{-1}$ respectively with assumption of normal concentration of molybdenum (1-2 mg kg $^{-1}$ diet) and sulfur (0.15-0.25%).

[b] The maximum tolerance levels of Zn in the feed of cattle and sheep are 500 and 300 mg kg $^{-1}$, respectively.

[c] The maximum tolerance levels of As in the feed of cattle and sheep are 30 mg kg $^{-1}$

Table 2.8. Mehlich-I extractable Cu and Zn in control and poultry litter-amended soils

	Cu	Zn
Depth (0-2.5 cm)	mg kg $^{-1}$	
Control	0.54 a[‡‡]	6.56 a
Cecil (PL-amended)	3.71 b	47.4 b
Sedgefield (PL-amended)	6.60 c	47.5 b
Depth (2.5-7.5 cm)	mg kg $^{-1}$	
Control	0.98 a	3.14 a
Cecil (PL-amended)	3.70 b	11.29 b
Sedgefield (PL-amended)	4.60 b	10.45 b

‡‡ Means (n=3) within a column in a given depth followed by the same letter are not different significantly according to LSD ($p<0.05$).

CHAPTER 3

ARSENIC IN RUNOFF FROM POULTRY LITTER-AMENDED PASTURES[1]

---

[1] Ashjaei, S., W. P. Miller, M. L. Cabrera, S. Hassan, D. H. Franklin, D. M. Endale. To be submitted to J. Environ. Qual.

Abstract

The organo arsenic compound 3-nitro-4-hydroxyphenyl-arsenic acid (Roxarsone, ROX), zinc (Zn), and copper (Cu) are used as feed additives in the poultry industry for prevention of fungal disease and enhance feed efficiency. Since most of the added trace elements in the feed are excreted in the poultry litter (PL), surface runoff from areas receiving long-term PL applications may have detrimental effects on water quality. Concentrations of arsenic (As) in runoff from PL-amended field-scale plots immediately after PL application were above USEPA drinking water limits (10 µg L^{-1}) but decreased over time. Three rainfall simulation (RS) events were conducted over a four-week period on PL-amended and control plots with tall fescue (*Festuca arundinacea Scherb*) and bermudagrass (*Cynodon dactylon L.*) with no history of PL application in randomized complete block design. Concentrations of As immediately and even 4 weeks after PL application were above USEPA drinking water limits in all RS events. However, concentrations of Cu and Zn were below USEPA drinking water limits (Cu: 1000 µg L^{-1} and Zn: 5000 µg L^{-1}). ROX was a major As species in fresh PL whereas As (V) was a predominant As species in PL that was stored for 4 months prior to application in RS study. The speciation of As in runoff from the third RS event showed that ROX was the major As species comprising 40% of total As compared to As (V) with 17% of total As in runoff immediately after litter application. The fact that ROX was the major As species in the PL an runoff from third RS event suggests that PL management practices play a key role in degradation of ROX in PL and As species distribution in runoff.

Introduction

The poultry industry is an important component of agricultural production in Georgia. Poultry litter (PL) is surface- applied to pastures and it is considered as a valuable source of N, P, and K content. It contains high concentration of trace elements such as arsenic (As), copper (Cu), and zinc (Zn) as a result of their use as growth promoters in poultry feed.

Arsenic occurs in PL as a result of the use of aromatic organo-arsenic compounds such as 3-nitro-4-hydroxyphenyl-arsenic acid (Roxarsone, ROX) or 4-aminophenylarsonic acid (*p*-arsanilic) as a feed additive at concentrations of 25 to 50 mg kg^{-1} for prevention of fungal disease and for weight gain improvement (Bellows, 2005; Bissen and Frimmel, 2003; Makris et al., 2008a). The practice is common in the U.S for production of broiler chickens, although feeding arsenic to laying hens is prohibited (Jones, 2007). Based on U.S. FDA regulation, in order to maintain the muscle tissue As concentration below 0.5 mg kg^{-1} and in edible organs below 2 mg kg^{-1} (21 CFR 556.60, Code of federal Regulations), arsenical additives are removed from poultry diet 5 days prior to slaughter (Anderson and Chamblee, 2001; Jackson and Bertsch, 2001). In addition, copper (Cu) and zinc (Zn) are also added to poultry feed as a sulfate salt or oxide to increase weight gain and feed efficiency (Adriano, 2001; Jackson et al., 2003). Dietary Cu levels of 125 to 250 mg kg^{-1} are adequate to promote growth of swine and poultry. It was reported that 80 to 90% of dietary Cu is excreted in manure (Adriano, 2001; Kpomblekou-A et al., 2002). Zinc is added to animal diets at a level of about 80 mg kg^{-1}. Poultry litter, on average, contains high amounts of Cu and Zn, at levels of approximately 380 mg kg^{-1} (Gupta and Charles. 1999).

It has been reported that most ROX added to poultry diets is excreted unchanged and is introduced to the environment when litter is applied to agricultural lands (Garbarino et al., 2003; Jackson et al., 2006). In a study of distribution of As in broiler chickens, it was reported that 36 to 88% of total As in the PL was ROX (Morrison, 2002). Georgia is one of the top broiler chicken producing regions in the United States. Every broiler produces between 1.46 and 2.67 kg of waste in its life span (Nachman et al., 2005; Sharpe et al., 2004). A broiler chicken fed the recommended dosage of 50 mg kg^{-1} ROX for 42 days life cycle will excrete 150 mg ROX (Rutherford et al, 2003). If 50% of total 1.46 billion produced broilers in Georgia in 2006 (Georgia Agricultural Statistic Services, 2006) were fed with recommended dosage of ROX for the 42 days of life cycle, approximately 100 Mg of ROX or 28 Mg As is introduced to the environment annually. Repeated surface applications of PL could result in accumulation of heavy elements at shallow soil depth due to the immobility of these elements and pose environmental problems.

Land application of PL like other organic wastes is not regulated based on trace elements concentrations but on the basis of nitrogen (N) and phosphorus (P) soil loading. Total As concentrations in PL are reported to be in range of <1 to 40 mg kg^{-1} (Garbarino et al., 2003; Jackson and Bertsch, 2001; Jackson et al., 2003; Makris et al., 2008b; Rutherford et al., 2003). Repeated surface applications of PL could result in accumulation of heavy elements at shallow soil depth due to the immobility of these elements and pose environmental concerns such as As runoff to surface water sources (Moore et al., 1998) and leaching to groundwater (Brown et al., 2005; Makris et al., 2008a). Guptal and Charles (1999) reported greater concentrations of As, Cd, Cu, and Mn in fields that had received long-term PL applications compared to unamended soils. Several studies have shown that long-term PL applications can cause elevation of trace elements (e.g., As, Cu, and Zn) especially at the soil surface (Gupta and Charles, 1999; Kingery et al., 1994; van der Watt et al., 1994). These findings indicate the potential for environmental concern related to losses of these metals via runoff from PL amended soils to water sources.

Arsenic (As) occurs in the environment in different soluble species. Inorganic arsenic compounds such as arsenate (As (V)) and arsenite As (III) (in reducing environments) can be methylated by bacteria, fungi, and yeast to organic compounds such as monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and gaseous derivatives of arsine (Bissen and Frimmel, 2003). It has been reported that As (V) and As (III) are more toxic than methylated arsenic compounds, which are still approved for use as herbicides and defoliants in agriculture (Jackson et al., 2006). It is important to understand the chemical forms, speciation, and transformations of As in PL in order to be able to quantify the toxicity of different species to various organisms. ROX itself has been generally considered to be harmless. However, ROX can be rapidly degraded to other species including inorganic species such as As (V) and As (III) as well as variety of organic species such as DMA and MMA during composting of PL or after applying to soil through chemical reactions such as reduction of nitro groups, oxidative aromatic ring fission, and rupture of C-As bonds (Cortinas et al., 2006). Jackson et al. (2003) studied the solubility of different trace elements in PL and speciation of As in the PL leachate. It was found that As was highly water soluble comprising 71% of total As in PL. The high extractability of ROX from PL suggests that ROX can easily be mobilized to the environment. The major species determined in the PL samples were either ROX or As (V) with minor amounts of MMA, DMA, and As (III). As (V) was the major species in 50% of PL leachates which suggests the mineralization of ROX (Jackson et al., 2003). Laboratory experiments on the effects of composting on degradation of ROX showed that the primary As species extracted with water from dried PL was ROX. However, when water was added to litter at about 50% and the mixture was allowed to compost at 40^{o}C for about 30 days, the speciation of As shifted to As (V) (Garbarino et al., 2003). Microbial activity was responsible for degradation of ROX to inorganic As since increasing the amount of added water elevated the degradation rate, while addition of sodium azide and heat sterilization eliminated microbial activity and hence the rate of degradation. Arsenic in poultry waste or PL amended soil may be present in organic forms and it can potentially degrade to more toxic inorganic species. It should be noted that PL management practices play an important role in degradation of organic As to other As species. During litter storage or even after PL application to soil, exposure to sunlight, elevated air temperature and precipitation most likely enhance photodegradation and microbial degradation (Garbarino et al., 2003).

Poultry litter applications tend to increase water soluble As level in top soils, especially when the PL is surface-applied but not incorporated. A Maryland test plot that had received PL application for 2 years showed a measurable increase of water-extractable As in amended soil after two application at 3 and 6 tons per acre and one application at 6 tons per acre compared to control plots (Rutherford et al., 2003). In a rainfall simulation study, PL-amended soil showed elevated concentrations of As in runoff as the rate of PL application increased, both immediately and 7 days after application. When PL was applied at rate of 8.9 Mg ha^{-1}, concentration of As in runoff water from control fields averaged 0.028 mg L^{-1} compared to 0.224 mg L^{-1} for PL-amended soil (Moore et al., 1998). In another study, rainfall simulation was conducted over a three week period and showed that concentrations of As in runoff from PL-amended soil ranged between 0.29 and 1.73 mg L^{-1}, compared to 0.12 mg L^{-1} from control plot. In subsequent rainfall events, concentrations of As in runoff declined to below detection limits (Toor and Haggard, 2007).

The effect of delay in rainfall after a PL application may play a key role in the magnitude of As cocnentration in runoff. Since As and P have similar physicochemical properties, the effect of rainfall timing on concentration of As in runoff from PL-amended soils can be comparable to P losses in runoff. Several researches have shown that concentration of P in runoff from PL-amended soils were generally greater immediately after PL application and declined with an increase in time to runoff-producing rainfall (Edwards and Daniel, 1993; Sauer et al., 1999;

Schroeder et al., 2004; Sharpley, 1997). The surface layer of thatch prevents the direct contact of manure with soil and increases the possibility of P adsorption by soil (Schroeder et al., 2004).

Moore et al. (1998) reported elevated concentrations of Cu and Zn in runoff as the rate of PL application increased from two rain events, immediately and 7 days after application. Copper concentration in runoff from PL amended soil was 93 times higher than control (0.93 mg L^{-1}) and concentration of Zn in runoff from control field was 0.047 mg L^{-1} compared to 0.44 mg L^{-1} for PL-amended soil. However, concentrations of Cu and Zn were below than the USEPA regulatory limits for Cu and Zn in drinking water which are 1,000 and 5,000 µg L^{-1}, respectively (USEPA, 2006). Concentrations of Cu and Zn in lake and stream water can have detrimental effect on aquatic plants. Moderate concentrations of Cu are extremely toxic to algae and a maximum concentration of 0.02 mg L^{-1} is suggested to protect freshwater fish (Edwards et al., 1997).

There are few studies investigating concentrations of As in runoff from PL-amended field-scale plots. The objectives of this study were (1) to assess concentrations of As in runoff from field-scale PL-amended plots planted to tall fescue/bermudagrass (received natural rainfall), (2) to investigate effect of rainfall-timing on concentrations of As in runoff from rainfall simulation study, and (3) to determine As speciation in the PL and runoff from rainfall simulation events on grassed plots over time after PL application.

Materials and Methods

Runoff Analysis

This research was conducted on six field-scale PL-amended plots (0.8 ha, 6-8% slope) and two control plots (unamended) with tall fescue (*Festuca arundinacea Scherb*) and bermudagrass (*Cynodon dactylon L.),* located at the College of Agricultural and Environmental Sciences Central Georgia Research and Education Center, near Eatonton, Georgia (39°24’ N, 83°29’ W, elevation 150 m). The soil series in the plot area include Cecil (fine, kaolinitic, thermic Typic Kanhapludults) and Sedgefield (fine, mixed, active, thermic Aquic Hapludults). Figure 3.1 shows the distribution of the soils in each plot. Plot 1 is mainly comprised of well-drained Cecil while plot 3 and 6 are occupied with somewhat poorly-drained Sedgefield. Plot 7 is well-drained Cecil with no history of PL application (not shown in Fig 3.1). Poultry litter has been applied to each amended plot since 1995 at 5 Mg ha^{-1} annually and it was applied in November 2008 and March 2009 during this experiment. Earthen berms (0.6 m high, 1.5 m wide) were constructed down slope of each plot to direct runoff to 0.45 m H-flumes equipped with SENIX (Burlington, VA) ultrasonic sensors to measure the depth of rainfall flow. The surface runoff was stored in refrigerated samplers (ISCO 3700FR, Lincoln, NE) until after collection. Surface runoff was collected from field 1 and 6 after natural rainfall events from November 2008 to May 2009. The runoff samples then transported to the laboratory, kept at $4^{o}C$, and filtered with 0.45 µm syringe filters. Concentrations of As, Cu, and Zn in runoff samples were analyzed by ICP-MS (Inductively Coupled Plasma Mass Spectrometry, Perkin Elmer, Model Elan 9000).

Rainfall Simulation Study

The rainfall simulation (RS) study used a portable rainfall simulator for plot-scale runoff studies (Humphry et al., 2002; Schroeder et al., 2004) at the Eatonton, Georgia location. Rainfall was applied three times during this experiment (September 2008 (RS-1), February 2009 (RS-2) and August 2009 (RS-3)) on 1.5 m^2 PL-amended and control plots in split plot experimental design (Fig 3. 2). The PL’s used in this study were sampled from different poultry houses in GA. The PL used for RS-1 was stored outside for 4 months covered with the plastic sheet before application. The history of PL applied in RS-2 was unknown. In RS-3, fresh PL was obtained from a poultry house. The PL was surface-applied by hand at a rate of 7 Mg ha^{-1}. All plots had tall fescue (*Festuca arundinacea Scherb*) and bermudagrass (*Cynodon dactylon L.*)

cover with Altavista soil (fine-loamy, mixed, semiactive, thermic Aquic Hapludults) with no history of PL application. All plots were surrounded with metal sheet in approximately 15 cm tall that was pressed into the ground. Aluminum flumes were placed at the down slope edge of each plot to direct surface runoff to collection containers. Plots received rainfall either immediately after PL application (week 0), or one week, two weeks, or 4 weeks after application. All plots were covered with transparent polymer covers prior to simulated rainfall in order to exclude natural rainfall from the plots. The rates of simulated rainfall were approximately 70 mm h^{-1}. Runoff samples were collected after initiation of steady runoff and continued for 30 min for each plot and immediately placed on ice. Time to runoff, collection time, and volume of runoff for were recorded for each plot. Runoff samples were transported to the laboratory, filtered with 0.45 µm syringe filters and kept at 4^{o}C. Concentrations of Cu, Zn and As in runoff were analyzed by ICP-MS (Perkin Elmer, Model Elan 9000).

Arsenic Speciation

Arsenic Standard Solutions: Sodium arsenite (Fisher scientific, Fairlawn, NJ), sodium arsenate (Sigma, St. Louis, MI), 4-hydroxy-3-nitrophenylarsonic acid (TCI America, Portland, OR), monosodium acid methane arsonate (Chem Service, West Chester, PA), and dimethylarsinic acid (Alfa Aesar, Heysham, Lancashire, England) were used to prepare As stock solutions of each species at 250 mg L^{-1} as As in deionized water on a daily basis. The concentrations of total As in stock solutions were verified by ICP-MS.

Ion Chromatography (High Performance Liquid Chromatography-Reversed Phase): The Ion chromatograph used was a Dionex Corporation system (Sunnyvale, CA) consisting of a gradient pump model GP40 in conjunction with AS3500 autosampler. Separation of As species was performed using a Dionex IonPac AS16 analytical column (4 x 250 mm, with ultralow hydrophobicity, 55% cross-linked) and IonPac AG16 guard column (4-50 mm). The eluants used were NaOH (50 mM) in 1% methanol in water and deionized water containing 2% methanol. Table 3.1 shows the gradient elution program for the AS16 column. The analytical column was connected by PEEK (polyetheretherketone) tubing to the nebulizer of the ICP-MS (Perkin Elmer, Model Elan 9000). The ICP-MS conditions were optimized by tuning and performing a daily check (e.g., static lens voltage, nebulizer gas flow, and RF power (1200 W)) each time prior to ion chromatography analysis. The calibration curves of mixed As standard solutions were produced with a three point calibration in the range of 1- 20 µg L^{-1} as As (all calibration curves were liner with R^2= 0.999). The calibration curves were used to identify the order of As species separation and their retention time. The pulse intensity for each As species in runoff samples were converted to µg L^{-1} concentration by using the calibration curve equation. Detection limits were calculated based on 3σ of the base line noise (blanks) and the values were converted to µg L^{-1} concentration based on calibration standard curves. Detection limits for As (V), As (III), MMA, DMA, and ROX were on average 0.01, 0.03, 0.03, 0.03, and 0.07 µg L^{-1}, respectively.

Arsenic Speciation in Poultry Litter and Runoff Samples: PL subsamples from all RS events were freeze-dried and total concentrations of As determined using microwave acid digest method USEPA 3051 (USEPA, 1995) by ICP-MS. The water-soluble As concentrations were determined by extraction of 1 g PL with 10 ml deionized water in 50 ml polypropylene centrifuge tubes. After shaking for 2 hr, the extracts were centrifuged at 15000 rpm for 15 minutes, filtered (0.45 μm), and diluted 20-fold with distilled water prior to analysis by ICP-MS (Jackson and Bertsch, 2001; Jackson et al., 2003). The diluted PL extract used for determination of As species in PL samples and sum of concentrations of all As species were compared with total As concentration in water-soluble PL samples analyzed with ICP-MS.

Runoff samples from RS-3 were used for determination of As species. Runoff samples collected immediately after PL application were analyzed within one day of collection, runoff samples collected one and four weeks after PL application were analyzed a week after collection, and runoff samples collected two weeks after PL application were analyzed two weeks after collection.

Statistical Analysis

The experimental design was a split-plot in which main plot treatments were time of rain (0, 1, 2 or 4 weeks after application) and subplot treatments were amendments (control (zero) or poultry litter). The main treatments were arranged in a completely randomized block design with four replications. The subplot treatments were randomized within each main plot. PROC GLM in SAS was used to carry out an analysis of variance, and mean separation was conducted with the PDIFF option in LSMEANS (SAS Institute, 2008).

Result and Discussion

Field-Scale Runoff Study

In the previous study (chapter 2, this thesis), significant elevation of concentrations of trace elements in the surface of PL-amended soils was found compared to control plots. Twelve runoff events occurred during the period of Nov 2008 through March 2009 on the 0.8 ha field-scale plots. The first rainfall on Nov 15 2008 was 3 days after PL application, and resulted in runoff that contained an average of 23 µg As L^{-1} for plot 6 with the somewhat poorly-drained Sedgefield soil, and about 5 µg As L^{-1} for the plot 1 containing well-drained Cecil soil (Fig 3.3). The unamended (control) plots (Cecil soil) had an average As concentration of 0.21 µg As L^{-1} for these runoff events. Over the following winter months subsequent runoff As concentrations decreased regularly for both PL-amended soils to levels of about 1-2 µg As L^{-1}, still higher than that from control plots. However, the As concentrations decreased tp below USEPA drinking water limits (10 µg As L^{-1}). Litter was applied for the second time on March 24 2009, and a rainfall event 5 days after PL application resulted in runoff As concentrations for both soil series of 16 µg As L^{-1}. The following rainfall events again showed a regular decline in runoff As over the subsequent 3 runoff events in April and May. The PL applied in November 2008 had a total As content of 15 mg kg^{-1}, and that applied in March 2009 was 27 mg As kg^{-1}. The runoff As concentrations shown in Fig 3.3 were occasionally greater than the USEPA drinking water limit of 10 µg As L^{-1}. Concentrations greater than drinking water limit only occurred in the one or two rainfall events directly after PL application and showed a decreasing trend to lower values thereafter. A similar study using rainfall simulation showed that concentrations of As in runoff from PL-amended soil ranged between 0.29 and 1.73 mg kg^{-1} and it decreased to below detection limit in subsequent rainfall events (Toor and Haggard, 2007).

Since not all the fields containing control, Cecil and Sedgefield soils produced sufficient amounts of runoff from November 2008 through May 2009, statistical analysis of concentrations of As and Cu in runoff from control and PL-amended soils was done only for two dates (December 01, 2008 and March 02, 2009) that contained sufficient replicates (Cecil soil: field 1 and 5; Sedgefield soil: field 3 and 6; Control: field 7 and 8). It was found that concentrations of As in runoff from Sedgefield amended soils were significantly greater compared to control plots on these dates (Fig 3.3). Concentrations of As in runoff from Sedgefield soil were significantly greater compared to that from Cecil soil in December 2008. However, there were no significant differences in concentrations of As in runoff from Sedgefield and Cecil soils four months after PL application in March 2009. Concentrations of As in runoff from Sedgefield soil significantly decreased from December 2008 to March 2009 ($P<0.01$) (data not shown). On the other hand, there were no significant differences in concentrations of As in runoff from Cecil soil between December 2008 and March 2009.

Concentrations of Cu in runoff from both PL-amended soils were greater compared to that of control plots in December 2008 and March 2009 (Fig 3.4). Total concentrations of Cu in runoff from both PL-amended Cecil and Sedgefield soils were below the USEPA drinking water limit of 1000 µg L^{-1} even when runoff occurred a few days after PL applications on November 15 2008 (334 µg Cu L^{-1}) and March 29 2009 (270 µg Cu L^{-1}). The control plots (Cecil soil) had an average Cu concentration of 9 µg Cu L^{-1} for these runoff events. Over the winter months following PL application, runoff Cu concentrations decreased regularly for both PL-amended soils. Similar results were observed for Zn concentrations in runoff (data not shown). Apparently, concentrations of Cu and Zn in runoff even a few days after PL application have little potential to contribute to surface water contamination.

As in Runoff from Rainfall Simulation Study

Total and water-soluble concentrations of As, Cu, and Zn in dried PL are given in Table 3.2. Since PL used in RS events were obtained from different PL houses, there is some variation in concentrations of trace elements in PL. The effect of rainfall delay on concentrations of As, Cu, and Zn in runoff from PL-amended soils immediately (week 0) or 1, 2, or 4 weeks after PL application was studied to determine how long after PL application the concentrations of these trace elements in runoff still remain elevated above the USEPA drinking water limits.

Concentrations of As, Cu, and Zn in runoff (mg ha^{-1}) from control and PL-amended plots in first RS event (RS-1) are shown in Table 3.3. Concentrations of As, Cu, and Zn in runoff from PL-amended soils were significantly greater than control plots immediately (week 0) or 4 weeks after PL application. Concentrations of all trace elements in runoff significantly decreased 4 weeks after PL-application. However, concentrations of As and Zn in runoff were not significantly different 1, 2, or 4 weeks after litter application. The percentage of As lost via runoff immediately after PL application (worse-case scenario) was only 5% of total applied As to soil by PL application, indicating the great majority of applied As remained on the soil surface.

The result of RS-2 showed that concentrations of trace elements in runoff from amended soils were significantly greater compared to that from control plot immediately or 1, 2, or 4 weeks after PL application (Table 3.4). Concentrations of As in runoff from amended plots decreased 4 weeks after PL application compared to those from week 0. Concentrations of Cu in runoff from PL-amended soil did not change significantly from week 0 compared to that 1 or 2 weeks after PL application. There were no significant differences in concentrations of Zn in runoff immediately after PL application compared to those 4 weeks after application. The percent amount of As lost by runoff in RS-2 was greater compared to RS-1. This is may be due to either higher As content of PL applied in RS-2 or because it was conducted in winter when the soil had a higher moisture content and thus the volume of collected runoff was greater in RS-2 compare to that in RS-1.

The As concentrations in runoff from amended plots compared to control in RS-3 were statistically similar to the results in RS-1 (Table 3.5). Total concentrations of soluble As, Cu, and Zn in runoff from plots that received PL application were significantly greater compared to those from control plots. It was found that concentrations of As and Cu in runoff immediately after PL application were significantly greater than 1, 2, or 4 weeks after PL application. There were no significant differences in concentrations of As and Cu in runoff from PL amended soil 1, 2, or 4 weeks after PL application. Although the As content in PL applied in RS-3 was greater compared to that in RS-1 and -2, the percent amounts of As lost via runoff were lower compared to RS-1 and -2. The soil was drier and the total volumes of collected runoff were less compared to that in RS-1 and -2. It should be noted that there were no differences in total runoff volumes from control plots compared to those from PL-amended soil in RS -1, -2, and -3. In addition, total volumes of collected runoff from PL-amended plots were not significantly different immediately, 1 week, 2 weeks, or 4 weeks after PL application in RS-1, -2, and -3.

Total concentrations of As in runoff (µg L^{-1}) from control and PL-amended plots over the 4 week period of all RS events are shown in Fig. 3. 5. Concentrations of As in runoff from plots that received PL application were significantly greater ($p<0.01$) compared to those from control plots rained on immediately, or 1 week, 2 weeks, or 4 weeks after PL application. It was found that concentrations of As in runoff 4 weeks after PL application significantly decreased compared to that in week 0 in all RS events. However, there were no significant differences in concentrations of As in runoff 1 week or 4 weeks after PL application. Concentrations of As in runoff were greater immediately after PL application in RS-1, -2, and -3 (37, 83, and 58 µg L^{-1}, respectively) compared to those 4 weeks after litter application (22, 48, and 30 µg L^{-1}, respectively). It is possible that either transformation of As via microbial activity resulted in depletion of soluble As species, or As adsorption occurred at the soil surface during 4 weeks of incubation after PL application. However, even 4 weeks after PL application, the concentrations of As in runoff remained elevated above the USEPA drinking water limits. Moore et al. (1998) found that concentrations of As in runoff from PL-amended plots were significantly greater than those from control plots, and As concentrations were much lower in runoff from PL-amended soil that received PL application at 9 Mg ha^{-1}, 7 days after PL application (0.091 mg L^{-1}) compared to that immediately after application (0.22 mg L^{-1}). It should be noted that As tends to have similar behavior in soil as P, which is considered immobile and tends to accumulate in the soil surface. Schroeder et al. (2004) studied the effect of rainfall timing on loss of P in runoff immediately or 30 days after PL application. It was found that the mass of P lost in runoff immediately after PL application was more pronounced compared to that 30 days after PL application. The same result was found by Sharpley (1997), who noted that increasing the time between PL application and rainfall from 1 to 35 days reduced dissolved P concentrations from 0.74 to 0.45 mg L^{-1} due to sorption of P.

It was found that total concentrations of Cu and Zn in runoff from PL- amended plots were significantly greater ($p<0.01$) compared to those from control plots immediately or 1, 2, or 4 weeks after PL application in all RS events (Fig 3.6 and 3.7). Total concentrations of Cu in runoff from amended soils in RS-1 and RS-3 were significantly ($p<0.01$) greater immediately after PL application compared to those 4 weeks after litter application. It should be noted that Cu has a high affinity for organic matter and it is possible that adsorption of Cu to organic matter occurred 4 weeks after PL application. There were significant differences in Zn concentrations in runoff from amended soils immediately after litter application and 4 weeks after application only in RS-1. However, concentrations of Cu in runoff even immediately after PL application in RS-1, -2, and -3 (506, 49, and 363 µg L^{-1}, respectively) and concentrations of Zn (431, 224, and 114 µg L^{-1}, respectively) were all below USEPA drinking water limits for Cu and Zn (1000 and 5000 µg L^{1}, respectively) and this suggests little potential threat to water contamination. Moore et al. (1998) found that concentrations of Zn in runoff immediately after PL application (9 Mg ha^{-1}) were below USEPA drinking water limits, but concentrations of Cu were close to drinking water limits.

As Speciation in PL and Runoff from Rainfall Simulation Study

The result of Ion chromatography of PL extract used in RS-1 that was stored for 4 months before application showed that As (V) is the major As species with detectable amount of DMA, As (III), and ROX at low concentrations .The same trend was found for the PL used in RS-2, and this similarity suggests that PL was also stored for some time before PL application (Fig. 3.8). Figure 3.9 shows that in fresh PL used in RS-3 ROX were the major As species with an almost 10-fold greater concentration than As (V) in the water extract (14 µg L^{-1} vs. 1.4 µg L^{-1}). It was found that ROX and As (V) comprised 26% and 2.6% of the total As in the PL, respectively. The sum of the As species in PL used in RS-1, -2, and -3 totaled about 40%, 43%, and 64% of the total water soluble As determined by ICP-MS analysis. It indicates that there are other unidentified As species, potentially metabolites of ROX, in PL that were not eluted from the column either due to high affinity to column or their size (Garbarino et al., 2003; Jackson and Bertsch, 2001). The fact that ROX was the predominant As species in fresh PL (RS-3) implies that degradation of ROX to inorganic and other organic As species is controlled by poultry litter management practices. Similar results were obtained by Garbarino et al. (2003) when the composting of PL degraded ROX mainly to As (V). During storage or after PL application to fields, moisture and temperature have an influential effect in mineralization of ROX to other, potentially more toxic inorganic As compounds.

The speciation of As in runoff from RS-3 showed that ROX is the major As species with 40% of total As compared to As (V) with 17% of total As in runoff immediately after litter application (Fig. 3.10). Since ROX was the predominant As species in fresh PL applied in RS-3, it was expected to find ROX as a major As species in runoff from PL-amended plots. The percentage of ROX and As (V) decreased to 17% and 9% of total As in runoff with 4 weeks delay after PL application. Concentrations of ROX and As (V) were significantly greater in runoff from PL-amended plots compared to those from control plots immediately or 1, 2 or 4 weeks after PL application, and concentrations of ROX and As (V) were significantly greater in runoff immediately after PL application compared to those 4 weeks after litter application (ROX: 24 µg L^{-1} vs. 9 µg L^{-1} and As (V): 10 µg L^{-1} vs. 3 µg L^{-1}).

Conclusion

Concentrations of As in runoff immediately after PL application on field-scale plots and in a RS study were above USEPA drinking water limits and showed a decreasing trend over time.

Concentrations of As in runoff in the RS study remained elevated even 4 weeks after PL application and were above USEPA drinking water limits. Apparently, a delay of precipitation for a month after PL application did not effectively reduce the concentration of As in runoff below regulatory limits. Although runoff water is not directly used for drinking water purposes, in small watersheds with intensive PL application, large amounts of As lost via runoff can pose potential environmental concerns if it reaches drinking water supplies. Concentrations of Cu and Zn in runoff immediately or few days after litter application were below USEPA drinking water limits in both RS and field-scale runoff studies. The effect of rainfall-timing on As loss via runoff were not as prominent as the effect of natural rainfall events over time on As loss in runoff from field-scale plots. Concentrations of As in runoff were greater immediately after PL application in RS study compared to those few days after litter application in field-scale runoff study. This is either due to greater amounts of applied PL with higher As contents or larger volume of collected runoff in RS compared to that in field-scale study. Poultry litter management practices have a significant effect on degradation of ROX to other organic and toxic inorganic As compounds in the PL which consequently affects As species distribution in runoff from PL-amended soils. The losses of As via runoff from PL-amended soils can be minimized by appropriate PL management strategies such as weather forecasts to avoid PL application prior to intensive precipitation.

References

Adriano, D. C. 2001. Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risks of metals, p. 219-261. New York Springer.

Anderson, B. K., and T. N. Chamblee. 2001. The effect of dietary 3-nitro-4-hydroxyphenylarsonic acid (roxarsone) on the total arsenic level in broiler excreta and broiler litter. J. Appl. Poult. Res. 10: 323-328.

Bellows, B. C. 2005. Arsenic in poultry litter: organic regulations. ATTRA the National Sustainable Agriculture Information Service. Available at http://attra.org/attra-pub/PDF/arsenic_poultry_litter.pdf (Verified 24 Aug. 2008).

Bissen, M., and F. H. Frimmel. 2003. Arsenic - a Review. Part I: occurrence, toxicity, speciation, mobility. Acta Hydrochimica et Hydrobiologica. 31: 9-18.

Brown, B. L., A. D., Slaughter, and M. E. Schreiber. 2005. Controls on roxarsone transport in agricultural watersheds. Appl Geochem. 20: 123-133.

Cortinas, I., J. Field, J. A , M. Kopplin, J. R. Garbarino, A. J. Gandolfi, and R. Sierra-Alvarez. 2006. Anaerobic biotransformation of roxarsone and related N-substituted phenylarsonic acids. Environ. Sci. Technol. 40: 2951-2957.

Edwards, D. R., P. A. Moore Jr, T.C. Daniel, P. Srivastava, and D.J. Nichols. 1997. Vegetative filter strip removal of metals in runoff from poultry litter-amended fescuegrass plots. Trans. ASAE. 40: 121-127.

Edwards, D. R. and T. C. Daniel. 1993. Effects of poultry litter application rate and rainfall intensity on quality of runoff from Fescuegrass plots. J. Environ Qual. 22: 361-365.

Garbarino, J. R., A. J. Bednar, D. W. Rutherford, R. S. Beyer, and R. L. Wershaw. 2003. Environmental fate of roxarsone in poultry litter. I. Degradation of roxarsone during composting. Environ. Sci. Technol. 37: 1509-1514.

Georgia Agricultural Statistic Services. 2006. Georgia poultry facts. Available at www.nass.usda.gov/QuickStats/PullData_US.jsp (verified 23 Feb. 2008).

Gupta, G., and S. Charles. 1999. Trace elements in soils fertilized with poultry litter. Poult. Sci. 78: 1695-1698.

Humphry, J. B., T. C. Daniel, D.R. Edwards, and A. N. Sharpley. 2002. A portable rainfall simulator for plot-scale runoff studies. Appl. Eng. Agric. 18: 199-204.

Jackson, B. P., and P.M. Bertsch. 2001. Determination of arsenic speciation in poultry wastes by IC-ICP-MS. Environ. Sci.Technol. 35: 4868-4873.

Jackson, B. P., P. M. Bertsch, M. L. Cabrera, J. J. Camberato, J. C. Seaman, and C. W. Wood. 2003. Trace element speciation in poultry litter. J. Environ. Qual. 32: 535-540.

Jackson, B. P., J. C. Seaman, and P. M. Bertsch. 2006. Fate of arsenic compounds in poultry litter upon land application. Chemosphere. 65: 2028-2034.

Jones, F. T. 2007. A broad view of arsenic. Poult Sci. 86: 2-14.

Kingery, W. L., C. W. Wood, D. P. Delaney, J.C. Williams, and G. L. Mullins. 1994. Impact of long-term land application of broiler litter on environmentally related soil properties. J. Environ. Qual. 23: 139-147.

Kpomblekou-A, K., R. O. Ankumah, and H. A. Ajwa. 2002. Trace and nontrace element contents of broiler litter. Commun. Soil Sci. Plant Anal. 33: 1799-1811.

Makris, K. C., S. Quazi, P. Punamiya, D. Sarkar, and R. Datta. 2008a. Fate of arsenic in swine waste from concentrated animal feeding operations. J. Environ. Qual. 37: 1626-1633.

Makris, K. C., J. Salazar, S. Quazi, S. S. Andra, D. Sarkar, S. B.H. Bach, and R. Datta. 2008b. Controlling the fate of roxarsone and inorganic arsenic in poultry litter. J. Environ. Qual. 37: 963-971.

Moore, P. A., Jr., T.C. Daniel, J.T. Gilmour, B. R. Shreve, D. R. Edwards, and B. H. Wood. 1998. Decreasing metal runoff from poultry litter with aluminum sulfate. J Environ Qual. 27: 92-99.

Morrison, J. L. 2002. Distribution of arsenic from poultry litter in broiler chickens, soil, and crops. J. Agric. Food Chem. 17: 1288-1290.

Nachman, K. E., J. P. Graham, L. B. Price, and E. K. Silbergeld.2005. Arsenic: A Roadblock to potential animal waste management solutions. Environ. Health Perspec. 113: 1123-1124.

Rutherford, D. W., A.J. Bednar, J. R. Garbarino, R. Needham, K. W. Staver, and R. L. Wershaw. 2003. Environmental fate of roxarsone in poultry litter. Part II. Mobility of arsenic in soils amended with poultry litter. J. Environ. Sci. Technol. 37: 1515-1520.

SAS Institute. 2008. SAS/STAT User's guide, Version 9.2. SAS, Cary, NC.

Sauer, T. J., T. C. Daniel, P. A. Moore, Jr., K. P. Coffey, D. J. Nichols, and C. P. West. 1999. Poultry litter and grazing animal waste effects on runoff water quality. J. Environ. Qual. 28: 860-865.

Schroeder, P. D., D. E. Radcliffe, and M. L. Cabrera. 2004. Rainfall timing and poultry litter application rate effects on phosphorus loss in surface runoff. J. Environ. Qual. 33: 2201-2209.

Sharpe, R. R., H. H. Schomberg, L. A. Harper, D. M. Endale, M. B. Jenkins, and A. J. Franzluebbers. 2004. Ammonia volatilization from surface-applied poultry litter under conservation tillage management practices. J. Environ. Qual. 33: 1183-1188.

Sharpley, A. N. 1997. Rainfall frequency and nitrogen and phosphorus runoff from soil amended with poultry litter. J. Environ. Qual. 26: 1127-1132.

Toor, G., and B. Haggard. 2007. Trace metals runoff waters from soil amended with granulated poultry litter. *In* Annual meetings abstracts. ASA-CSSA-SSSA, New Orleans, Luisiana, 4-8 Nov. 2007. Avaiable at http://crops.confex.com/crops/2007am/techprogram (verified 12 Aug. 09)

USEPA. 1995. Method 3051: Microwave assisted acid digestion of sediments, sludges, soils and oils. Availabe at http://www. epa.gov/SW-846/pdfs/3051.pdf (verified 22 July 2008). USEPA , Washington, DC.

USEPA. 2006. Drinking water contaminants; list of contaminants and their MCLs. Avaiable at http://www.epa.gov/safewater/contaminants/index.html (verified on 17 Aug. 09).

van der Watt, H. v. H., M. E. Sumner, and M. L. Cabrera.1994. Bioavailability of copper, manganese, and zinc in poultry litter. J. Environ. Qual. 23: 43-49.

Tables and Figures

Table 3.1. Gradient elution program of AS16 column

Time	Eluent A¶ (D.I.H_2O)	Eluent B¶ (NaOH 50 mM)	Flow Rate
0	40%	60%	1.0 ml min^{-1}
3	100%	0.0%	1.5 ml min^{-1}
10	40%	60%	1.5 ml min^{-1}

¶ Eluent A and B contain 2% and 1% methanol, respectively

Table 3.2. Total and water-soluble concentrations of trace elements in poultry litter applied in rainfall simulation 1 (RS-1), 2 (RS-2), and 3 (RS-3)

	Cu		Zn		As	
	Total	WE[¶]	Total	WE	Total	WE
	mg kg^{-1}					
Poultry Litter (RS-1)	369	179	503	135	21.8	10.2
Poultry Litter (RS-2)	123	36.2	620	90.8	38.4	16.8
Poultry Litter (RS-3)	675	268	711	55.6	53.4	25.6

¶ Concentrations of water-soluble Cu, Zn, and As in poultry litters used in rainfall simulation events

Table 3.3. Total concentrations of trace elements in runoff from rainfall simulation-1 (summer 2008)

	As		Cu		Zn	
	mg ha^{-1}					
	Control	PL-amended	Control	PL-amended	Control	PL-amended
Week 0[¶]	181 a[‡] A[‡‡]	5750 b A	1580 a A	79300 b A	6410 a A	68000 b A
Week 1	55.0 a A	2310 b B	1740 a A	36800 b BC	2650 a A	25500 b B
Week 2	45.0 a A	2150 b B	940 a A	30300 b C	1990 a A	19000 b B
Week 4	57.0 a A	3020 b B	1500 a A	46400 b B	2450 a A	26300 b B

¶ Weeks after poultry litter application; week 0 (immediately), 1 week, or 2, or 4 weeks after poultry litter application

‡ Means in the same row followed by different lower case letter are significantly different at $p<0.01$

‡‡ Means within each column followed by different upper case letter are significantly different at $p<0.01$

Table 3.4. Total concentrations of trace elements in runoff from rainfall simulation-2 (Winter 2009)

	As		Cu		Zn	
	mg ha^{-1}					
	Control	PL-amended	Control	PL-amended	Control	PL-amended
Week 0[¶]	142 a[‡] A[‡‡]	20000 b A	519 a A	11500 b B	3120 a A	52200 b A
Week 1	164 a A	9190 b C	444 a A	9990 b B	2530 a A	28600 b B
Week 2	101 a A	14800 b AB	335 a A	11400 b B	2500 a A	28900 b B
Week 4	137 a A	11300 b BC	689 a A	15900 b A	3920 a A	46200 b A

¶ Weeks after poultry litter application; week 0 (immediately), 1 week, or 2, or 4 weeks after poultry litter application

‡ Means in the same row followed by different lower case letter are significantly different at $p<0.01$

‡‡ Means within each column followed by different upper case letter are significantly different at $p<0.05$

Table 3.5. Total concentrations of trace elements in runoff from rainfall simulation-3 (Summer 2009)

	As		Cu		Zn	
	mg ha^{-1}					
	Control	PL-amended	Control	PL-amended	Control	PL-amended
Week 0[¶]	116 a[‡] A[‡‡]	3990 b A	612 a A	24800 b A	2580 a A	8010 b A
Week 1	63.0 a A	1810 b B	472 a A	12000 b B	1580 a A	6100 b AB
Week 2	47.0 a A	1330 b B	426 a A	9080 b B	1590 a A	3400 a B
Week 4	29.0 a A	1960 b B	585 a A	1310 b B	4650 a A	7100 a A

¶Weeks after poultry litter application: weeks 0 (immediately), 1 week, or 2, or 4 weeks after litter application

‡ Means in the same row followed by different lower case letter are significantly different at $p<0.05$ except in week 2, As, Cu, and Zn are significantly different at $p<0.1$

‡‡ Means within each column followed by different upper case letter are significantly different at $p<0.05$

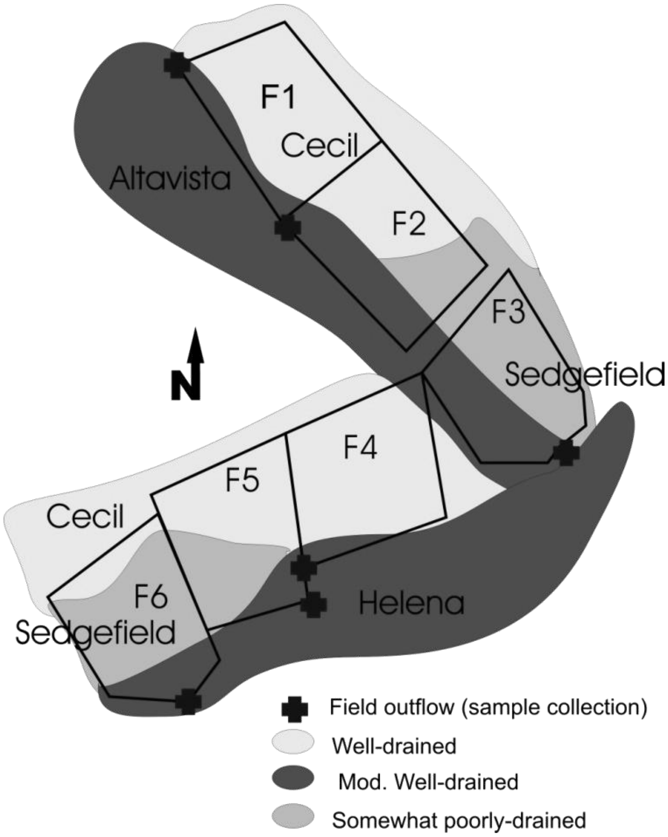
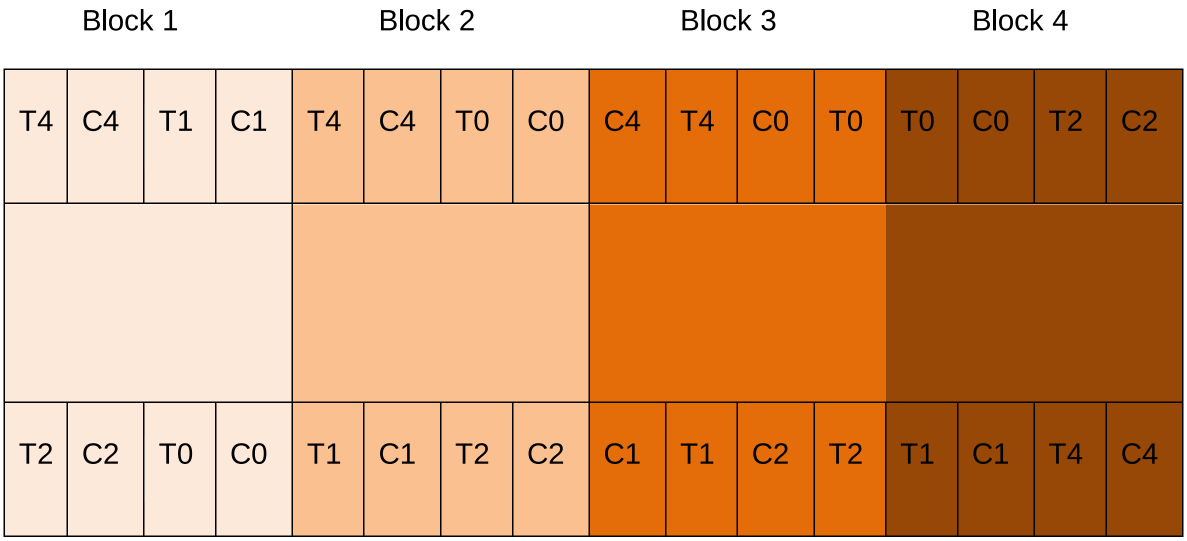


Fig 3.1. Soil series in field 1 and field 6; Cecil (fine,kaolinitic, thermic Typic Kanhapludults) and Sedgefield (fine, mixed, active, thermic Aquic Hapludults, respectively. The soil drainage classes are represented by shades of gray color



Block 1				Block 2				Block 3				Block 4			
T4	C4	T1	C1	T4	C4	T0	C0	C4	T4	C0	T0	T0	C0	T2	C2
T2	C2	T0	C0	T1	C1	T2	C2	C1	T1	C2	T2	T1	C1	T4	C4

Fig 3.2. Rainfall Simulation study: split plot experimental design; T and C stand for poultry litter amended and control plots, respectively. Number 0 to 4 stand for time of rainfall simulation event with four replications; runoff collected from plots immediately after poultry litter application (T0, C0), or 1 week (T1, C1), 2 weeks (T2, C2), or 4 weeks (T4, C4) after application

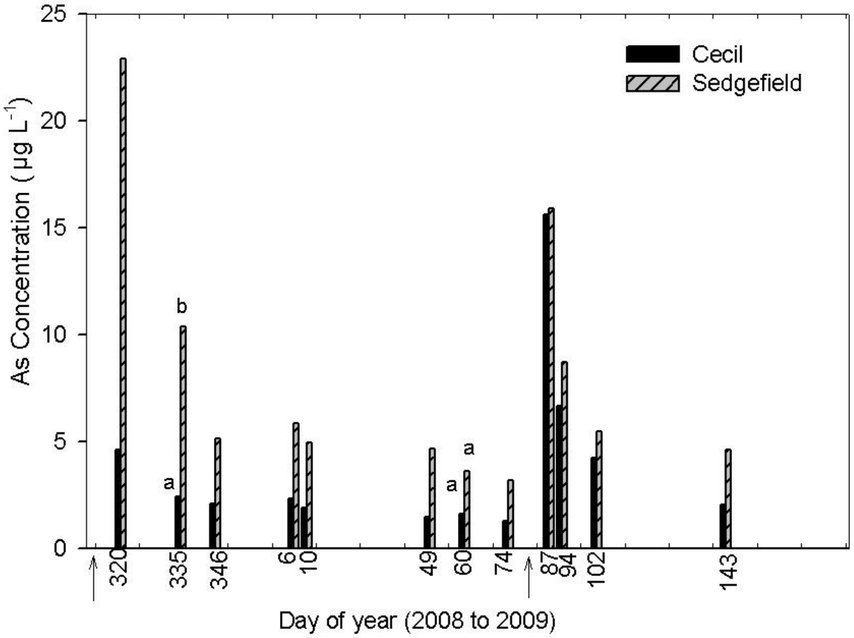


Fig 3.3. Total concentrations of arsenic (As) in runoff from field-scale plots from PL-amended Sedgefield (somewhat poorly-drained) and Cecil (well-drained) soils. Arrows indicate the approximate date when poultry litter was applied

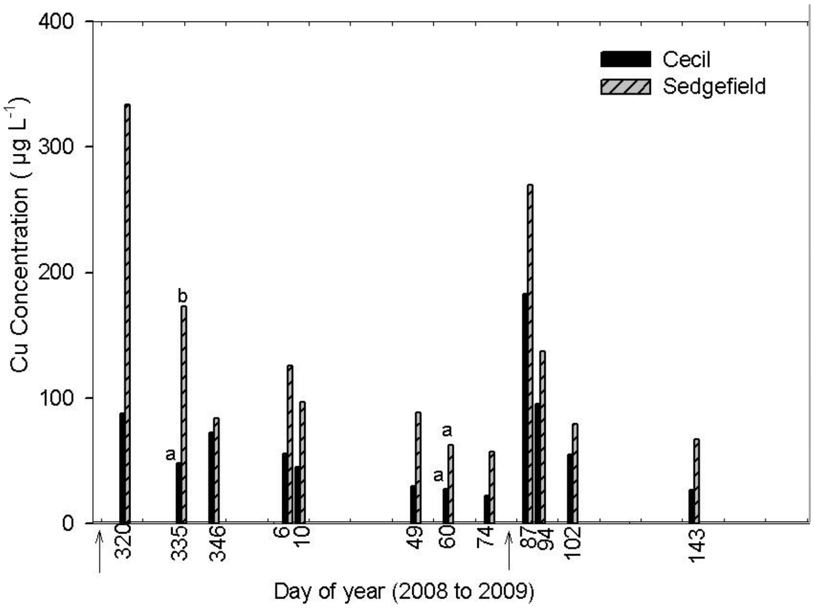


Fig 3.4. Total concentrations of copper (Cu) in runoff from field-scale plots from PL-amended Sedgefield (somewhat poorly-drained) and Cecil (well-drained) soils. Arrows indicate the approximate date when poultry litter was applied

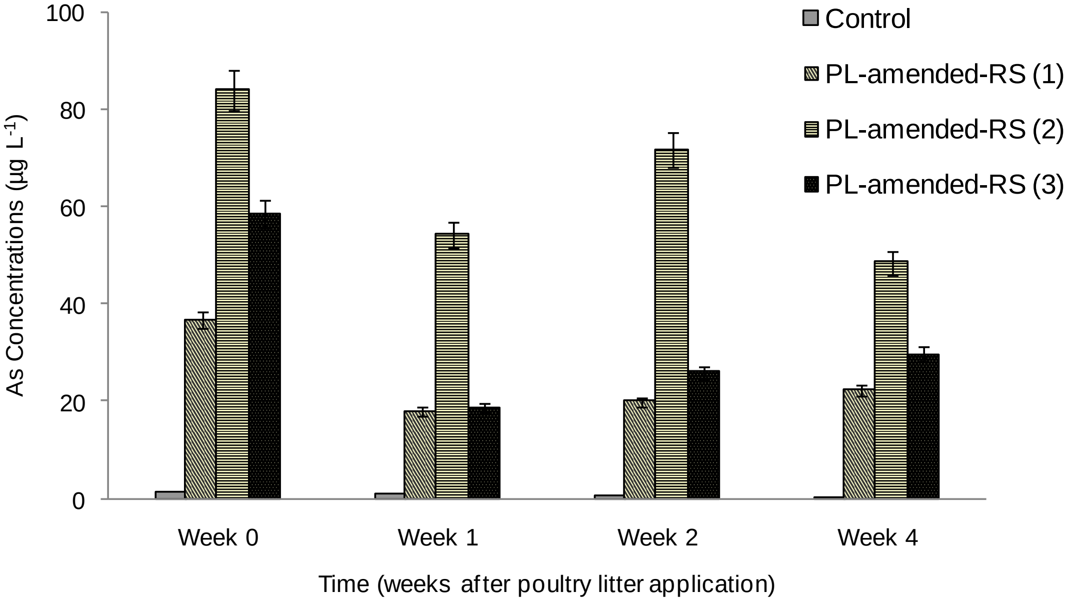


Fig 3.5. Total concentrations of arsenic (As) in runoff over a 4-week period, immediately (week 0) or with 1 week, or 2, or 4 weeks delay in rainfall after poultry litter application from rainfall simulation-1 (Summer 08), rainfall simulation-2 (Winter 09), and rainfall simulation-3 (Summer 09)

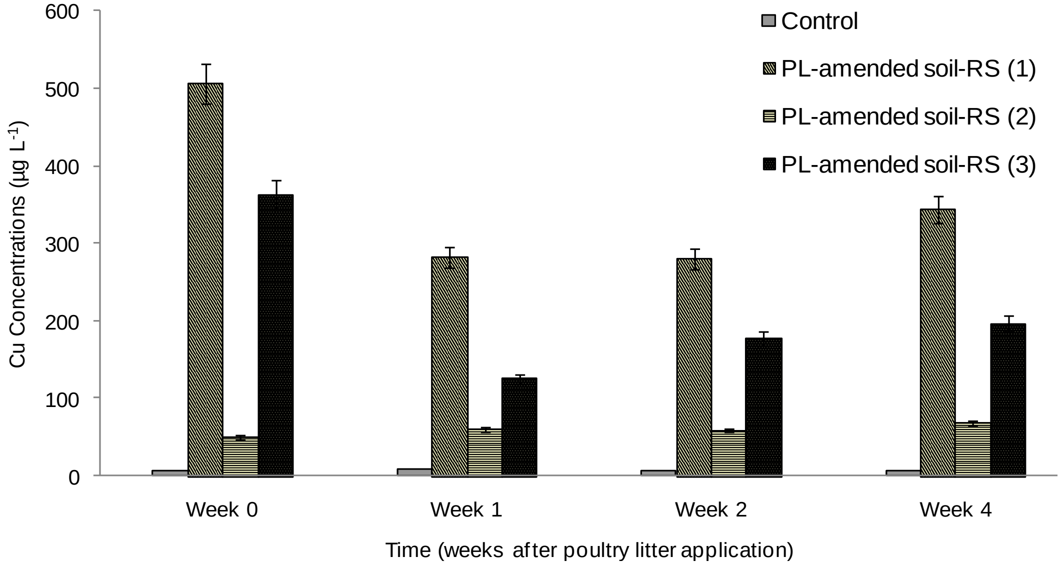


Fig 3.6. Total concentrations of Copper (Cu) in runoff over a 4-weeks period, immediately (week 0) or with 1 week, or 2, or 4 weeks delay in rainfall after poultry litter application from rainfall simulation-1 (Summer 08), rainfall simulation-2 (Winter 09), and rainfall simulation-3 (Summer 09)

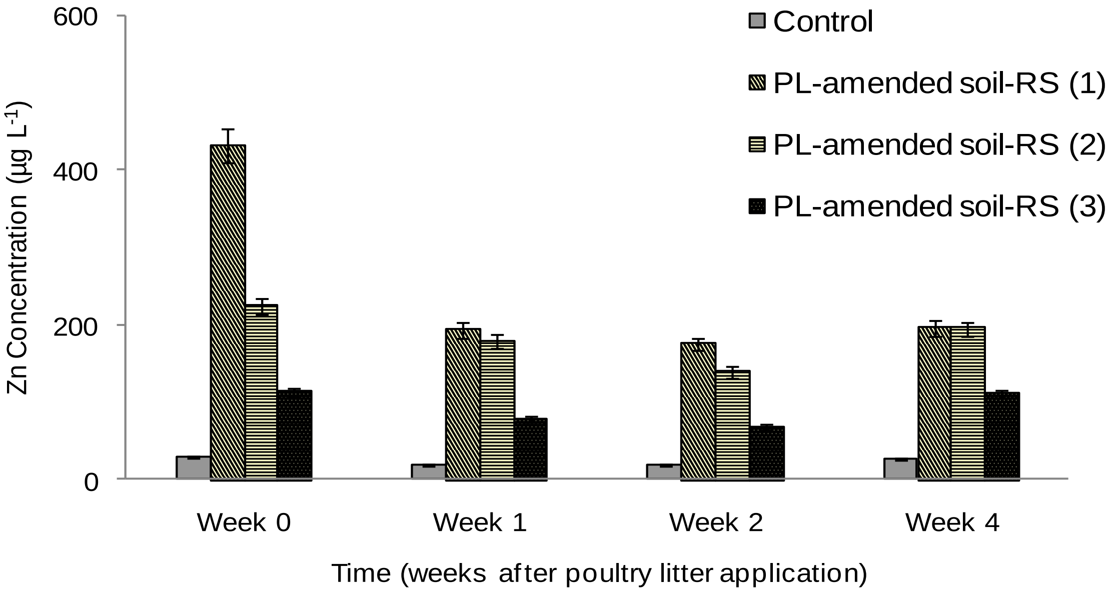


Fig 3. 7. Total concentrations of Zinc (Zn) in runoff over a 4-weeks period, immediately (week 0) or with 1 week, or 2, or 4 weeks delay in rainfall after poultry litter application from rainfall simulation-1 (Summer 08), rainfall simulation-2 (Winter 09), and rainfall simulation-3 (Summer 09)

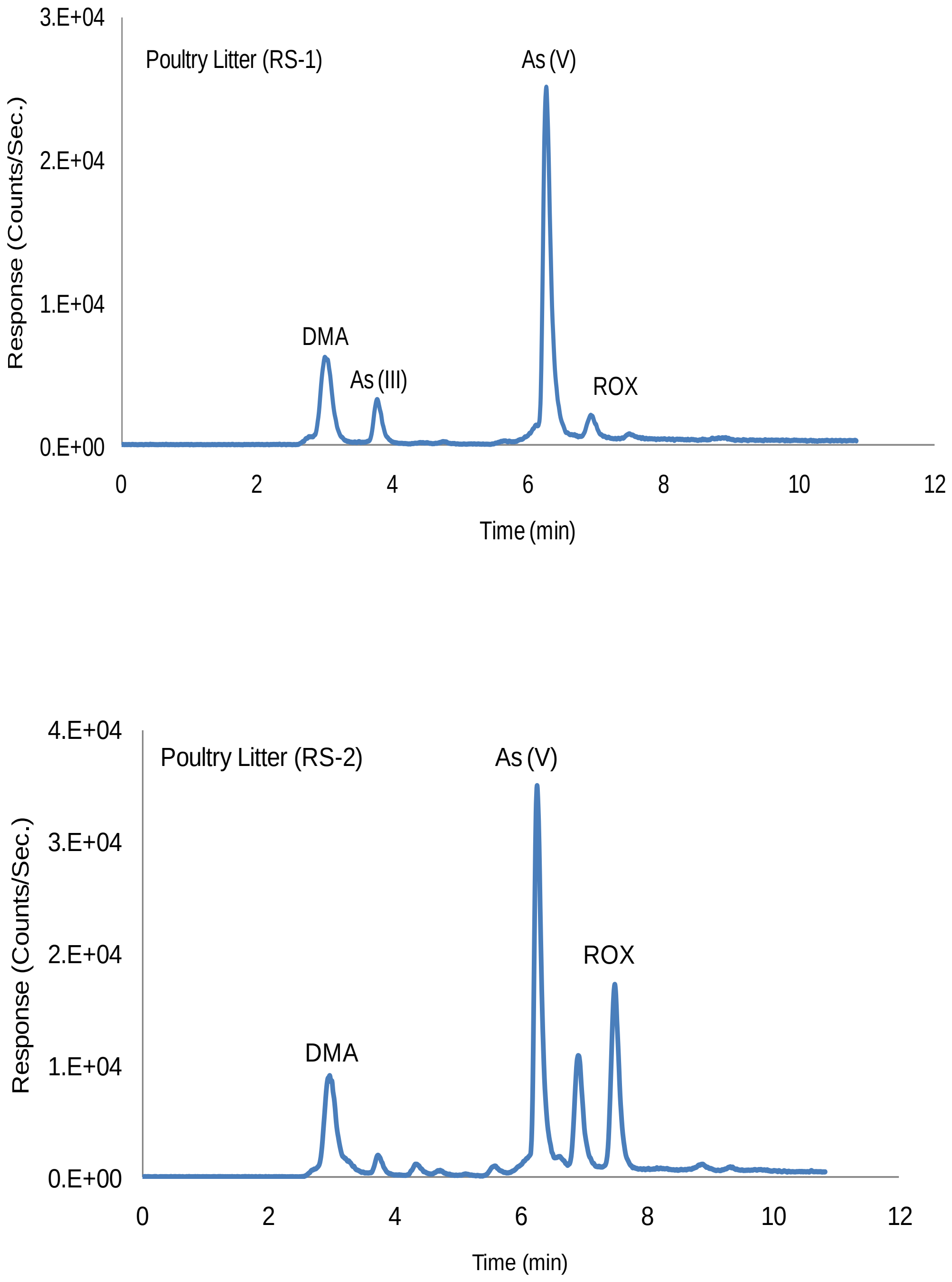


Fig 3.8. As speciation in water-extract of poultry litters used in rainfall simulation events (rainfall simulation-1 (RS-1), -2 (RS-2)

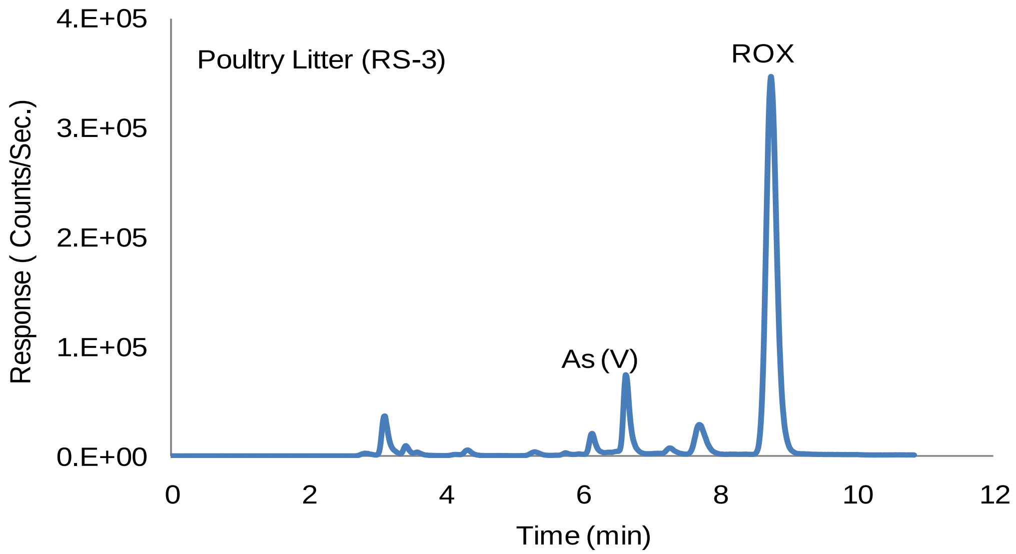


Fig 3.9. As speciation in water-extract of poultry litters used in rainfall simulation events (rainfall simulation-3 (RS-3)

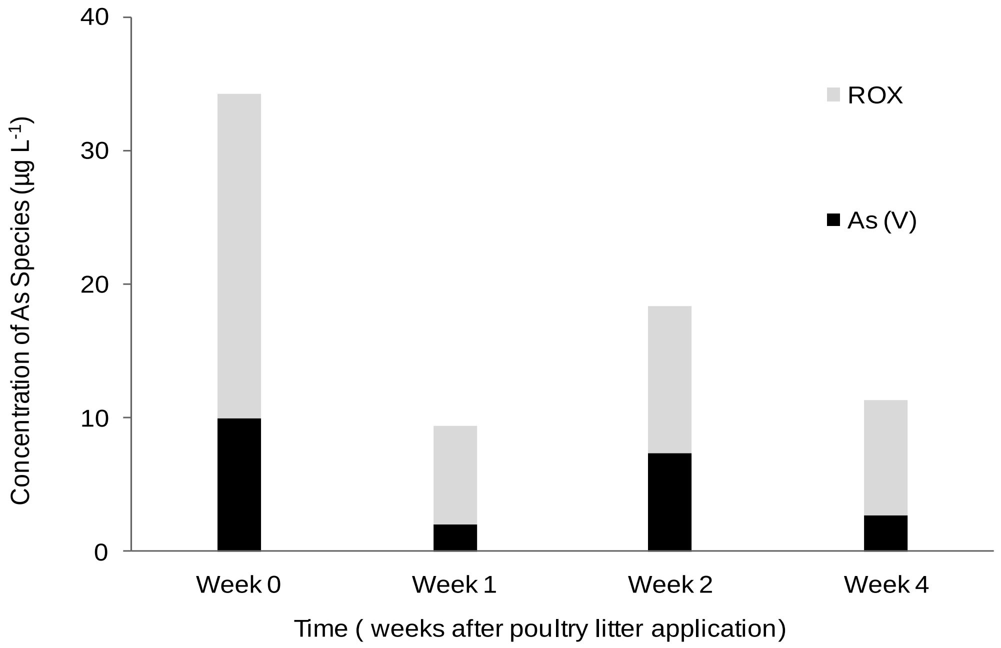


Fig 3.10. Concentrations of major As species (Roxarsone, ROX and arsenate, As (V)) in runoff from poultry litter-amended plots immediately (0), or 1, 2, or 4 weeks after poultry litter application from third rainfall simulation event

CHAPTER 4

CONCLUSION

This thesis explored the potential for soil and water (runoff) contamination due to the practice of adding arsenic (As) to chicken feed, resulting in elevated As levels in land-applied poultry litter (PL). While this practice apparently allows chicken producers to grow broiler chickens more rapidly in confined feed operations, the resultant As levels in manures (10-20 ppm As) were shown to have a measureable environmental impact. Specific findings of this research are:

1) Total concentrations of As, Cu, and Zn in field soils that had received long-term surface-applied PL were significantly greater than unamended (control) soil. However, the concentrations were below USEPA annual loading limits used for sewage sludge. The As fractionation study showed concentrations of all As fractions were significantly greater in PL-amended soils compared to control at 0-2.5 and 2.5-7.5 cm depth. However, there were no differences in concentrations of any As fraction between two PL-amended soils (a well-drained and somewhat poorly drained soil) at either depth except for $NaHCO_3$-associated As at 0-2.5 cm depth. Residual was the predominant As fraction from total in all soils.

2) The analysis of forage samples showed that there were differences in concentrations of As, Cu, Zn, and P in forage from PL-amended soils compared to control plots, and As was significantly greater in forage from coarse-textured, well-drained soil compared to fine-textured, wetter soil. However, concentrations of As, Cu, Z, and P in all forage tissues were below NRC (National Research Council) domestic animal mineral tolerance levels.

Concentrations of As in forage from both PL-amended soils were below the maximum tolerance level of 30 mg As kg^{-1} for domestic animals. Although the NRC tolerance limit for forage tissue Cu was not exceeded, continual PL applications might increase the Cu content beyond the 15 mg Cu kg^{-1} tolerance limit for sheep. On the other hand, forage samples from all plots were close to the minimum recommended levels of 35 mg Zn kg^{-1} and 9 mg Cu kg^{-1} for cattle feed, and thus additions of these micronutrients may be advantageous on these soils.

3) Concentrations of As in runoff immediately after PL application in large field-scale plots and smaller rainfall simulation (RS) runoff plots were above USEPA drinking water limits (10 µg L^{-1}); however, As levels decreased over time after application. Concentrations of As in runoff were greater immediately after PL application in the RS study compared to As losses via runoff a few days after litter application in the field-scale runoff study. This is either due to greater amounts of applied PL with higher As contents or larger volume of collected runoff in RS compared to that in field-scale study. In RS study, the As concentrations in runoff from PL-amended soils 4 weeks after PL-application were significantly lower compared to that in runoff immediately after PL application. It is possible that either transformation of As via microbial activity resulted in depletion of soluble As species or As adsorption in soil occurred during the 4 weeks after PL application. However, total As concentrations in runoff from PL-amended soil remained elevated even 4 weeks after PL application and were above USEPA limit drinking water limits. Apparently, a delay of precipitation for a month after PL application did not effectively reduce the concentration of As in runoff below the USEPA limit. Concentrations of Cu and Zn in runoff immediately after litter application were well below USEPA limits.

4) Roxarsone (RX) was the major soluble As species in water extracts of PL, and also in runoff during the rainfall simulation experiments. Poultry litter management practices have a

significant effect on degradation of ROX to other organic and toxic inorganic As compounds in PL and it consequently affects the As species distribution in runoff from PL-amended soils.

Thus, these results indicate the tendency for increased soil concentrations and runoff losses of As, Cu, and Zn after 15 years of repeated PL applications on two soils of the Georgia Piedmont. Given that significant acreages of the Piedmont likely have received similar or higher amounts of As-contaminated PL over time, there is a clear potential for long-term elevation of soil As levels as well as sporadic increases in streamflow As concentrations draining from these areas. The threat of water contamination is clearly higher directly following PL application, as As levels in runoff decrease over time after application (due to removal of soluble As species, soil adsorption, and/or microbial transformation).

Risk assessment is beyond the scope of this study, but may warrant further examination as to the size of populations that are exposed to levels of As resulting from runoff from PL-amended pastures. Surface water is extensively used domestically in the Piedmont, largely from impoundments in watersheds that have large poultry production just to the north and east of the Atlanta-Athens corridor. However, no reports of elevated As levels have been reported from major reservoirs in this region, which may suggest that the land area generating As-containing runoff is too small to have a significant effect, or that As transported off field areas is attenuated in riparian or streambed processes. Too little is known of transport pathways or attenuation mechanisms to be able to predict the later processes; in addition, there is no spatial data on areas or rates of PL application that might be used to estimate land areas that contribute As-bearing runoff to larger reservoirs.

Overall, it is clear that the practice of using As in animal feeds is not sustainable, for the simple reason that As will continue to accumulate in soils over time. Other potential contaminants originating from manures (hormones, antibiotics) are degradable and unlikely to accumulate to any degree (although may still constitute a short-term water quality hazard); inorganics, however, not readily lost from the system. It is clear from this study that As export via runoff is significant, but obviously not a desirable process. Similarly, plant uptake of As is higher on PL-amended soils; while the magnitude of this increase is small compared to levels deleterious to animal health, it will only become increasingly prevalent as soil As levels increase over time. Thus, this study would suggest a critical look be taken at practices such as As additions to animal feeds to ensure that long-term soil and water contamination is not the ultimate price we pay for cheaper chicken at the grocery.