University of Alberta

Life cycle assessment of arsenic from poultry feed to agricultural crops

by

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Abstract

A series of experiments were conducted to study the fate and distribution of arsenic (As) when it passes through various components of biosphere. Roxarsone, an As containing compound is added to poultry feed for enhanced feed utilization. Mass balance calculations revealed that chickens excreted ~80% of ingested As into poultry manure. A high dose of As-containing poultry litter was applied to silt-loam and loam soils to study As distribution. Sequential extraction analyses revealed that ~60% of applied As remained in highly labile water soluble fraction. Mobility of As was found more in loam soil. To study its translocation from soil to plants, barley and canola plants were grown in litter-amended soils in a growth chamber. More As was taken up from loam soil, and by canola than barley. This study suggested that As moved from poultry feed to crop plants, accumulated in roots and shoots but rarely concentrated in grains.

Preface

This dissertation is a part of a multi-disciplinary research project initiated at the University of Alberta to study the fate of arsenic during its transport from poultry feed to arable crops. In 2009, a team under the leadership of Dr. Martin J. Zuidhof from the Department of Agricultural, Food and Nutritional Science laid out a poultry feed study. Dr. Chris Le in the Department of Laboratory Medicine and Pathology was involved in studying arsenic in poultry meat of roxarsone-fed chickens. Dr. Gary Kachanoski in the Department of Renewable Resources supervised the research on investigating arsenic release into poultry litter and its subsequent fate in poultry litter-amended soil. Xiaomei Sun, a Master's student with Dr. Kachanoski, collected poultry litter samples from this joint study and stored them, and started a field experiment but unfortunately could not continue her study program. In May 2011, the undersigned continued this research under the supervision of Dr. Tariq Siddique in the Department of Renewable Resources who joined Dr. Kachanoski in his research. Subsequently, a series of experiments were conducted in the laboratory, field and growth chambers to assess the fate of arsenic during its transport from poultry feed, poultry litter, poultry litter-amended soil to agricultural crops.

This dissertation is an original intellectual product of the undersigned. The Table 2.1 in Chapter 2 in this dissertation was used for mass balance calculations with the permission of Dr. Martin (Permission Letter attached) (Appendix H). Rest of the data (tables and figures) and photographs in this dissertation have been generated by the undersigned as a Master's student under the supervision of Dr. Tariq Siddique.

Following oral presentations have been made using the data from this dissertation:

1. Sanjay Gupta, Martin Zuidhof, Gary Kachanosky and Tariq Siddique. 2013. Mass balance of arsenic from poultry feed to poultry litter. Joint Annual Meeting of the Canadian Society of Soil Science, Manitoba Soil Science Society and Canadian Society of Agricultural and Forest Meteorology, Winnipeg, Manitoba, July 16-21, 2013.
2. Sanjay Gupta, Gary Kachanosky and Tariq Siddique. 2013. Assessment of arsenic-rich poultry litter as manure in barley (*Hordeum vulgare* L.) in an Orthic Black Chernozemic soil of Canadian Prairies. Alberta Soil Science Workshop, Lethbridge, Alberta, February 19-21, 2013.

(Sanjay Kumar Gupta)

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Chapter 1: Introduction

1.1 Arsenic toxicity and spread

Historically, owing to its use by the ruling class for killing one another and because of its incredible potency and discreetness, arsenic (As) was called the 'Poison of Kings and the King of Poisons' (Vahidnia, 2007). More recently, As has become an element of public concern because of its widespread presence in the environment and its toxic and carcinogenic effects on humans.

A single acute exposure to a high dose of As may lead to severe reactions such as diarrhea, vomiting, pain, dehydration, coma and even death due to heart failure. While acute exposures nowadays are rare occurrences, chronic poisoning is much more insidious in nature and is occurring at an alarming rate. Numerous studies have revealed that continual exposure to As can result in peripheral neuropathy and various forms of cancers (Brouwer et al., 1992; WHO, 1993). The International Agency for Research on Cancer (IARC) and the Canadian Environmental Protection Act of 1993, list As and its compounds in Group 1 of the Priority Substances List because they are harmful to the environment and dangerous to human health.

Chronic As poisoning represents a global health concern because of chronic environmental exposure resulting from contaminated drinking water, polluted air from mining and other industrial processes, or ingestion of food of low As concentrations (Kelynack and Lond, 1900; Centeno et al., 2007). Rahman et al. (2006) reported that 20 countries in different parts of the world were affected by elevated levels of As in groundwater. Ravenscroft (2007) predicted that As in drinking water was affecting 137 million people in more than 70 countries. While the number of affected countries and

people did not increase because of sudden rise in As contamination, it does signify the worldwide attention As received as a contaminator of soil and groundwater, and the extensive work conducted in many countries of the world (Naidu et al., 2006). Bangladesh is the worst affected country in the world where 35-77 million people were exposed to As through drinking water which was the major cause of death especially among children (Mukherjee et al., 2006).

1.2 Sources of arsenic in environment

Arsenic is ubiquitous in nature. It is the 20th most common element in the earth's crust. The average As concentration in earth's crust is about 2-5 mg kg^{-1} (USDHHS, 2000). The most common sources of As in the natural environment are volcanic rocks specifically their weathering products and ash, marine sedimentary rocks, hydrothermal ore deposits and associated geothermal waters, and fossil fuels including coal and petroleum (Smedley and Kinniburgh, 2002). Arsenic occurs naturally in a wide range of minerals. The most common As-containing minerals are: arseno-pyrite (FeAsS), realgar (AsS) and orpiment (As_2S_3). These minerals are usually associated with sulfide or other metal ores and work as a major starting point for the introduction of As into the environment (Wang and Mulligan, 2006).

The global average concentration of As in uncontaminated soil is 5-6 mg kg^{-1} (Peterson et al., 1981). However, it may vary among geographic regions. The As levels in soil derived from As-enriched sedimentary rocks may attain a value of 20-30 mg kg^{-1} (Zou, 1986). Bennett and Dudas (2003) reported an acid sulfate soil that contained up to 37.9 mg As kg^{-1} soil.

Local geology, hydrogeology and geochemical characteristics of the aquifer are responsible for the presence of As in natural waters. Several geochemical processes such as oxidation of As-bearing sulfides; desorption from (hydro)oxides of iron, aluminum and manganese; reductive dissolution of As-bearing iron (hydro)oxides as well as leaching from sulfides have been attributed as the source of As in natural waters (Kim et al., 2000; Bennett and Dudas, 2003). Worldwide reported As concentrations in natural waters vary from 0.00002 mg L^{-1} to >5 mg L^{-1} (Smith et al., 2002). Global natural As emissions have been estimated to be around 7900 tons $year^{-1}$ (Nriagu and Pacyna, 1988) which originate from wind erosion, volcanic emissions and low-temperature volatilization from soil and water surfaces.

While As enters the environment from natural sources, its concentration is further enriched by anthropogenic activities. Arsenic and As-containing compounds have been produced and used commercially in pharmaceuticals, wood preservatives, agricultural chemicals, metallurgy, glass-making and semiconductor industries (IARC, 2012). These anthropogenic activities along with processing and combustion of fossil fuel, and disposal and incineration of industrial wastes release As into the environment (Popovic et al., 2001). Furthermore, in agriculture, As has historically been used in pesticides, herbicides and defoliants, and as a feed additive for poultry (Bishop and Chisholm, 1962; Chapman and Johnson, 2002). With the increasing use of As, its release into the environment from anthropogenic activities far exceeds those from natural sources. Most anthropogenic releases of As are to land or soil, primarily in the form of solid wastes, however, substantial amounts are also released to air and water (USDHHS, 2000). Soluble forms of As are known to leach into the groundwater and may enter surface waters from runoff.

Slimak and Charles (1984) estimated that 19% of As follows soil-related pathways via runoff and leaching.

1.3 Arsenic contamination in Canada and Alberta

In Canada, high levels of As have been reported in the air (up to 6.5 µg m^{-3}), surface and groundwater (up to 1570 mg L^{-1}), as well as soils and sediments (up to 25,000 mg kg^{-1}) in several regions (Newhook et al., 2003). The release of As from weathering and erosion of As-bearing rocks and minerals is the main natural As source in the environment (Wang and Mulligan, 2006). The principal anthropogenic sources of As release into the environment are metallurgical applications, manufacturing and use of wood preservatives, processing of base-metal and gold, use of arsenical pesticides, coal-fired power generation, and disposal of domestic and industrial wastes (CCME, 1997).

Elevated levels of dissolved As in well waters have been reported in the Cold Lake Region, three Regional Health Authorities in Northern Alberta, and Athabasca River Delta and Western Lake Athabasca in Fort Chipewyan (Stein et al., 2000; Alberta Health and Wellness, 2000; Timoney, 2007) indicating towards As-bearing geological formations as the source of As. Very high concentrations of As have also been reported in the acid sulfate soils developed on pyrite-rich shale in the Peace River Region (Dudas, 1987), and bedrock geologic units in the Cold Lake Area (Andriashek, 2000).

1.4 Arsenic in poultry industry

The Canadian poultry industry has been growing steadily over the years. The contribution of poultry industry to Canada's Gross Domestic Product was $ 6.8 billion

(CFC, 2011). In 2009, Canada produced 1.01 billion kilogram chicken (AAFC, 2013). Considering 1.91 as the average feed conversion efficiency of a chicken (NCC, 2012), it can be estimated that millions of tons of poultry feed is consumed annually in Canada. In this industry, organo-arsenical drug roxarsone (3-nitro-4-hydroxyphenylarsonic acid) is used extensively. This compound contains As, and its use is often contentious though it is used legally in poultry feed under Canada Feeds Act.

Roxarsone is fed to chickens to increase feed consumption, improve weight gain, pigmentation and to save chickens from the coccidial parasite of intestine (Chapman and Johnson, 2002). It has been reported that roxarsone is not completely metabolized by poultry; the bulk of it is excreted in feces unaltered (Moody and Williams, 1964) which becomes part of poultry litter after mixing with bedding material (O'Connor et al., 2005). Fresh poultry litter contains predominately organic roxarsone (Fisher et al., 2011) which may be converted to inorganic arsenate (As^{V}) under aerobic conditions (Jackson et al., 2006) or arsenite (As^{III}) under anaerobic conditions in the poultry litter (Cortinas et al., 2006). This conversion is both microbially mediated and through photolysis (Fisher et al., 2011). Total As concentrations in poultry litter usually range from <1 to 40 mg kg^{-1} (Jackson et al., 2003). Since poultry litter is rich in nutrients, more than 90 per cent of it is land-applied as fertilizer (Jackson and Bertsch, 2001). A growing body of data suggests that inorganic As present in poultry litter could contaminate soil and end up in crops, food and water bodies (Christen, 2001; Williams et al., 2007) as shown in Fig. 1.1. However, the ultimate fate of As derived from roxarsone is largely unknown because the chemistry of As in the environment is complicated and often difficult to predict. Several environmental processes such as ion exchange, adsorption/desorption, biological activity,

and intrinsic factors such as pH, Eh and competition for adsorption sites add to this complexity (Robertson, 1989; Jain and Ali, 2000).

1.5 Research rationale and objectives

The poultry industry in Canada is faced with an imminent ban of roxarsone, a routine preventive antimicrobial drug and growth promoter, because it contains As. However, there is little data available in the public domain regarding degradation/transformation of roxarsone after addition in the poultry feed. The maximum acceptable concentrations of As are: 0.01 mg L^{-1} in drinking water (Health Canada, 2006), 1-2 mg kg^{-1} in food (FSANZ, 2013) and 12 mg kg^{-1} in agricultural soils (CCME, 1997). With such low thresholds, even a little increase in As concentration can become hazardous to public health. Hence, there is an ongoing debate about the danger of using As containing poultry litter in soil because the fate of As in soil can be extremely complex as it can be retained by the solid phase of the soil, taken up by the plants, volatilized into the atmosphere, leached down to groundwater or move with the runoff water. Therefore, in order to minimize the potential risks to human health and environment from As in poultry litter, it is critical to understand the retention/release mechanisms of As in agricultural soils. A complete life cycle assessment of As starting from poultry feed to poultry litter and then to agricultural crop plants will help take an informed decision about roxarsone and its use in the poultry industry as well as the disposal of As-rich poultry litter on agricultural land.

A multi-disciplinary project (Fig. 1.2) was initiated at the University of Alberta in 2009 to understand the fate of As after addition of roxarsone in the poultry feed. A team

from the Department of Agricultural, Food and Nutritional Science laid out a feed study to gain practical insights into the effects of feeding sub-therapeutic levels of Roxarsone on chicken performance and production economics. A second team from the Department of Laboratory Medicine and Pathology collected samples from different body parts of chicken to study the accumulation of As in the chicken body. A third team, which I am a part of, in the Department of Renewable Resources collected poultry litter samples and assessed the transference of As from poultry feed to poultry manure and then its fate in soil and its uptake by agricultural crops.

In this thesis, three major aspects of As transference were studied in detail. First, transfer of As from poultry feed to poultry manure was evaluated using mass balance analysis to know how much As is coming into the poultry manure from poultry feed. Then, As fractionation in soils amended with poultry litter was performed to understand the phase distribution, potential mobility and bioavailability of As within the soils. Finally, As uptake by crop plants from poultry litter amended soils was investigated to determine As translocation from soil to plants. The findings are presented and discussed in the next chapters.

Tables and Figures

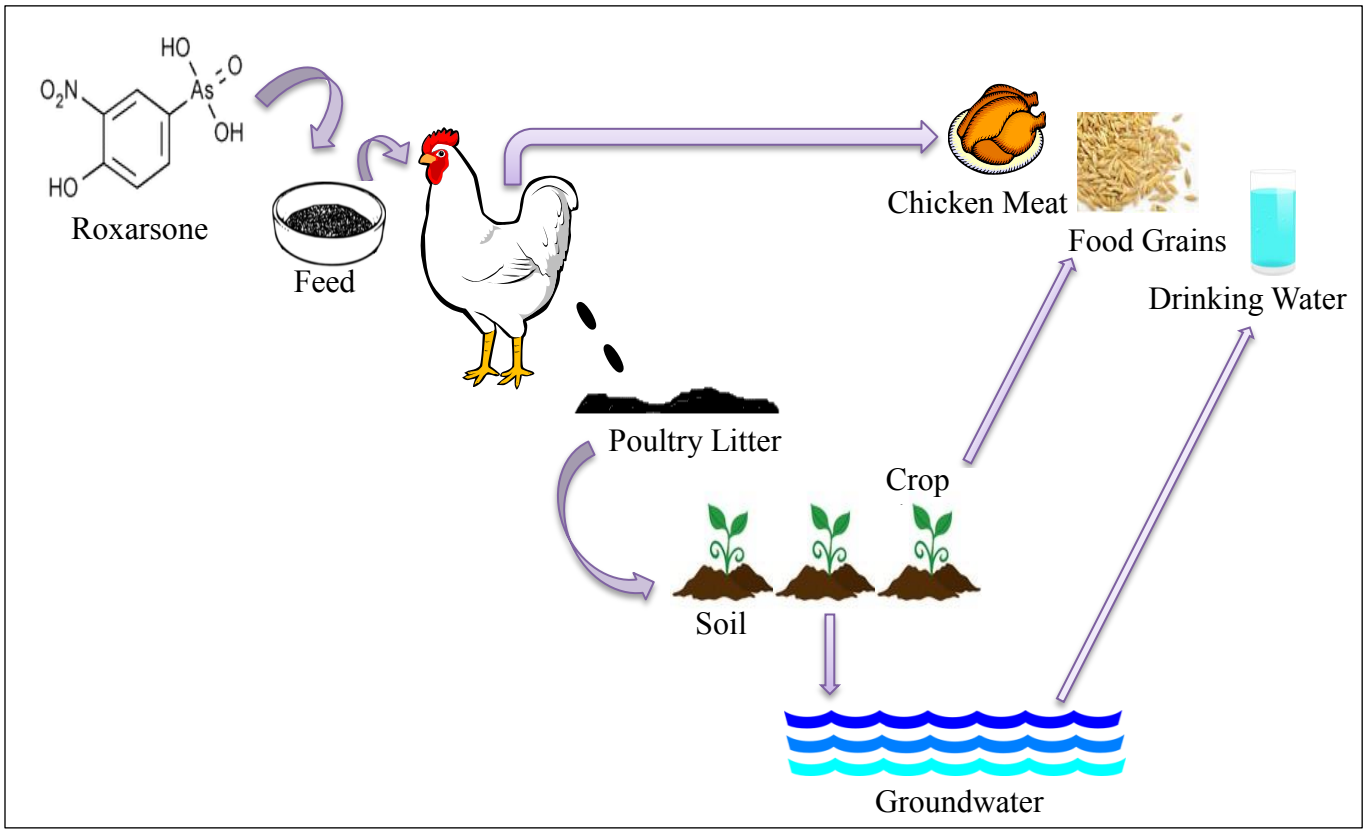

Fig. 1.1 Schematic diagram showing the possible transference of As from roxarsone in poultry feed to poultry litter, soil, crop, water and food (inspired and partly-adopted from Christen, 2001).

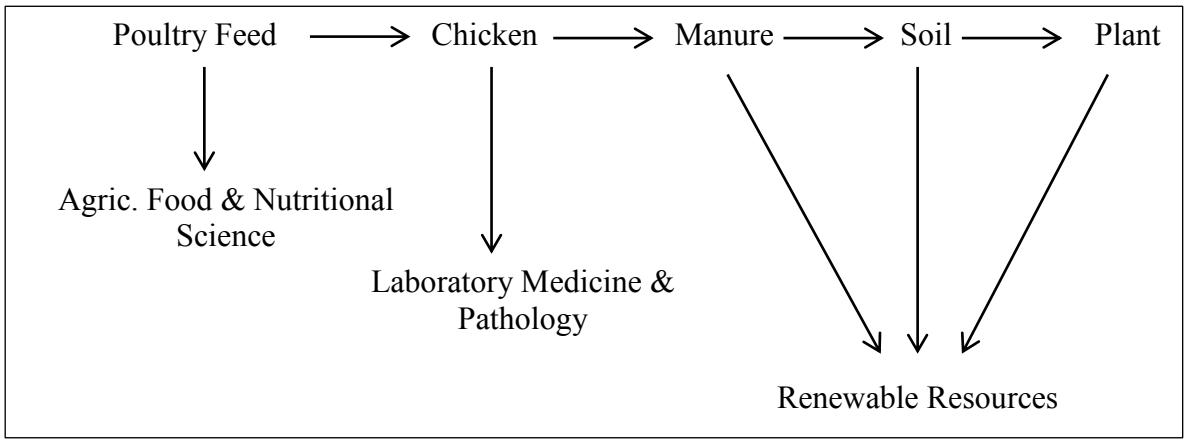

Fig.1.2 Schematic diagram showing multi-disciplinary approach adopted for the project.

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Chapter 2: Transference of arsenic from poultry feed to poultry manure

2.1 Introduction

In Canada, the poultry industry has been growing steadily over the years. The contribution of poultry industry to Canada's Gross Domestic Product was $ 6.8 billion (CFC, 2011). In 2009, Canada produced 1.01 billion kilogram chicken (AAFC, 2013). Based on the average feed conversion efficiency of a chicken (1.91) reported by NCC (2012), it can be estimated that millions of tons of poultry feed is consumed annually in Canada. Roxarsone, an organic compound containing As (3-nitro-4-hydroxyphenylarsonic acid), is added routinely to the poultry feed individually or in combination with other drugs for disease prevention, growth promotion, enhanced feed utilization and improved meat pigmentation (USFDA, 2011).

At the time Roxarsone was approved, scientists believed that the non-toxic organic As present in roxarsone would not change into toxic inorganic As inside the chicken body, rather organic As would be excreted unchanged and chicken meat would be safe for human consumption (Schmidt, 2012). However, in a recent study, Kawalek et al. (2011) found higher incidence of inorganic As in the livers of roxarsone-treated chickens compared to untreated ones. This indicates that chickens can metabolize organic As to inorganic forms and can result in As exposure to humans through consumption of such meats. Following the release of this study by the US Food and Drug Administration (USFDA), Pfizer's Alpharma Unit, manufacturer of Roxarsone, voluntarily suspended the sale of roxarsone in the United States. Earlier in 1999, the European Union discontinued the use of arsenicals as feed additives due to environmental and human health issues (European Commission, 1999), and that ban is still in effect. In Canada, however,

roxarsone is still being used as a feed additive under the jurisdiction of Canada Feeds Act.

In addition to increasing the level of inorganic As in chicken meat, roxarsone can also be related to other potential environmental concerns that can adversely impact humans. In poultry farms, the chicken excreta (or manure) get mixed with the sorbent bedding material (wood shavings, sawdust, etc.) producing poultry litter. This poultry litter is considered one of the best organic fertilizers and is applied to the agricultural fields (Wilkinson, 1979). Roxarsone in feed is not completely metabolized by the chickens, bulk of it is excreted in feces which during storage and after land application undergoes photo (Bednar et al., 2003) and microbial (Garbarino et al., 2003) degradation thus enriching the poultry litter with inorganic As. There is large volume of scientific literature which shows a wide range (0-77 mg kg^{-1}) of inorganic As in poultry litter (Sims and Wolf, 1994; Moore et al., 1998; Arai et al., 2003; Toor et al., 2007). A growing bulk of data suggests that inorganic As present in poultry litter could contaminate soil, crops, plants, water bodies, and air in the nearby homes. In light of such risks, the ban in the European Union and a suspension of sale in the United States, poultry industry in Canada is under pressure to ban this compound. However, the industry is concerned about the effects of this ban on the production, performance and chicken welfare in Canada.

Roxarsone in poultry feed contributes As to poultry litter (Fig. 2.1). This As-rich poultry litter is then used as an organic fertilizer in the agricultural fields, and can potentially contribute As to the crops. In this study, mass balance calculations were performed on the basis of poultry feed consumption and poultry litter analysis to find the amount of As taken in by chickens with their feed, the amount of As excreted into the

manure and the amount of As possibly retained in the chicken body. Though the samples of poultry litter (excreta mixed wooden chips) were analyzed but the results have been presented on poultry manure (excreta only) basis to avoid any discrepancy due to addition of different amounts of bedding material (wooden chips) in different pens at the beginning of the experiment. The results will help in accurate assessment of health risks associated with the consumption of poultry meat if As is retained in the chicken body, and environmental impacts associated with high concentration of As in poultry litter if As-rich poultry litter is applied as fertilizer for crop growth. Furthermore, these results will facilitate in designing of regulations for land application of As-rich poultry litter in Canada. Fractionation of As in soil and uptake of As by crops from soils amended with As-rich litter are explored in next chapters.

2.2 Materials and Methods

A chicken (or broiler) feed study was conducted at Poultry Research Centre, University of Alberta, in May 2009 to gain practical insights into the effects of feeding sub-therapeutic levels of antibiotics on broiler performance and production economics. Complete details of the study have been described by Wenger et al. (2013). From that study, poultry litter samples were collected and analyzed for As concentration, and then mass-balance calculations were performed using some of the data from that study and the results of poultry litter analysis.

Poultry feed study: In this study, effect of two treatments of roxarsone was tested on two strains of poultry. In Control treatment, chickens were fed with the feed prepared without any roxarsone but in Rox treatment, roxarsone was mixed with the feed to feed

the chickens. The drug, 3-NITRO®20% (trade name) from Alpharma, was the source of roxarsone which was added to the feed at the rate of 250 g ton^{-1}. This drug contains 20% roxarsone (3-nitro-4-hydroxyphenylarsonic acid) which means the roxarsone content of the Rox feed used in the experiment was 50 g ton^{-1}. Roxarsone contains 28.48% As, hence As content of the Rox feed was 14.24 mg kg^{-1} of feed. The Control treatment had all the feed ingredients except roxarsone. Standard Poultry Research Centre diet composition (Appendix A) was used at levels approved for poultry feed in Canada. The energy levels as well as all essential amino acids in the two feeds were equal. All chickens received a pelleted standard Starter Diet (3068 kcal kg^{-1}; 23.0% crude protein) up to two weeks, a Grower Diet (3152 kcal kg^{-1}; 20.2% crude protein) from two to four weeks, and a Finisher Diet (3196 kcal kg^{-1}; 19.0% crude protein) from four to five weeks of poultry age. Roxarsone was added to the Starter and Grower Diets only. Amprolium (coccidiostat) was added to all starter rations to prevent coccidiosis. Chickens had *ad libitum* access to both feed and water.

In total, there were 16 pens with a treatment combination of two commercial poultry strains: Cobb500 and Ross308, two types of feed: Control and Rox, and four replications. To each pen, 102 chicks were assigned who were one-day old and mixed-sex. All the chicks were weighed before putting them in the pen. The study was conducted in an environmentally controlled barn, and the age related temperature decrease was followed as per the strain Management Guides of Cobb (Cobb-ventress, 2012) and Ross (Aviagen, 2009). The lighting program followed a photoperiod of 24 hours of light for the first three days, and then 20 hour light and 4 hour dark from fourth day to the end of the experiment. Ventilation requirements of a commercial breeder were

maintained. Cumulative Feed Consumption was recorded on pen basis at 14, 28 and 35 days of age.

Specification of the pen and sampling of poultry litter: The size of the individual pen was 169cm x 420cm. New softwood shavings of known weight were used as the bedding material in each pen, the depth of which was 7.5 cm above the pen floor. The samples of poultry litter (excreta mixed bedding material) were collected from each pen on 14, 24, 28, 31, 35 and 37th day. For 14, 28 and 35th day sampling, a steel core of 10 cm diameter was used to collect 15 fresh samples up to the full depth of the litter randomly from each pen and then the total weight of litter in each pen was estimated by extrapolation using pen area and sample weights. These 15 samples, on each respective sampling day, were pooled together and a representative sample (a quarter of the composite sample, approximately half kg) was put into a double Ziplock@ polyethylene bag, sealed and frozen at -20 ^{0}C until chemical analysis. The remaining sample was put back into the pen and mixed well with the litter. The total litter weight was also recorded on 37th day (when pens were cleaned out) by physically weighing the litter. Afterwards, litter of the whole pen was mixed together and a composite sample was drawn. The data pertaining to number of chickens per pen, cumulative feed consumption and weight of poultry litter per pen were received from this feed study. The weight of wooden chips recorded on 1st day was subtracted from the weights of poultry litter of 14, 28, 35 and 37th days to get the weight of poultry manure on respective days. The loss of manure weight due to periodical sampling over the study period was accounted for (as loss) during the final calculations.

Microwave digestion of poultry litter: The moist samples of poultry litter, equivalent to 1g oven-dried sample, were used for digestion. To decide whether to use dry or moist

litter samples, a preliminary study was performed. It was observed that litter samples lost some of As during air-drying process, hence only moist samples were used for analysis. However, the results were reported on dry-weight basis after using moisture correction factor. The samples were digested with concentrated HNO_3 (nitric acid) (A509P212, trace metal grade, Fisher Scientific) in a microwave oven (Ethos Sel, Milestone) using Method 3052 (USEPA, 1996) with some modifications where only HNO_3 acid was used in the digestion. Hydrochloric acid (HCl) was not used with HNO_3 to avoid formation of polyatomic ions ($^{40}Ar\ ^{35}Cl$) which interfere with ^{75}As determination on ICP-MS due to same mass to charge ratio. Similarly, Hydrofluoric acid (HF) was avoided as it can react with glass components of ICP-MS and disturb its functioning. Hydrogen peroxide (H_2O_2) was not used because it reacts with the sample violently and may create a very high pressure inside the digestion vial which may cause venting of the vessel with potential loss of sample. However, with the adjustment of temperature and time, total sample decomposition was achieved using HNO_3 only. Prior to acid digestion, the samples were homogenized in an all-plastic blender. After digestion, the digestion vials were allowed to cool, the solution was diluted to 100 ml using nanopure water (Barnstead nanopure, Thermo Scientific) and the diluted solution was filtered through 0.45 µm PTFE syringe filter (033911C, Fisher Scientific). One milliliter of filtered solution was taken in a 50 ml centrifuge tube and diluted to a final volume of 50 ml using 1% nitric acid solution. This acidification of samples was done to prevent any precipitation before ICP-MS analysis.

Analysis of poultry litter for arsenic using ICP-MS: Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to determine As in the poultry litter. Approximately 10 ml of the prepared solution of digested poultry litter (process described in the preceding

section) was transferred to a sampling tube and placed on the auto-sampler. After every ten samples, a rinse, two external standards of 2 ppb and 20 ppb followed by a rinse again, were run for quality control. Analysis was performed on (ELAN 9000, ICP-MS, PerkinElmer) housed in Geotech lab, Department of Civil and Environmental Engineering, University of Alberta, wherein the samples were spiked with 250 µL of multi-element internal standard (CLMS-1, SPEX CertiPrep Inc.). Later on, the same samples were analyzed on (iCAP Q, ICP-MS, Thermo Scientific), housed in the Department of Renewable Resources. The results obtained from iCAP Q were more consistent as this instrument is equipped with collision cell technology which helps remove interferences from polyatomic ions by breaking these ions into individual atoms.

Statistical Analysis: The experimental design was a 2x2 factorial with two strains (Cobb500 and Ross308) and two treatments (Control and Rox). Initially the experiment was designed with four replications but the loss of some samples during storage allowed us to use only three replications during poultry litter analysis. Though the whole study was performed using poultry litter but the results were presented on poultry manure basis to avoid any discrepancy due to presence of different amounts of wood chips in the different pens at the start of the experiment. Statistical analysis was performed on IBM SPSS Statistics version 20 (IBM, 2011) using Linear Mixed Model. Differences between the means were tested at significance level of 0.05 using Least Significant Difference test.

Mass Balance Calculations: To quantify how much As retained in the chicken body and how much As was excreted in the manure, a mass balance approach was used using the feed consumption and manure production data. Though all the data were recorded on per

pen-basis, the calculations were done on per chicken-basis because the number of chickens varied on different days of observations (some died and a few others were removed for other studies). The following equations were used to calculate the mass balance of As:

$$I = Wf * Cf \quad (1)$$

Where, I = Intake of As (total weight of As taken in by the chickens from feed, mg As $chicken^{-1}$)

Wf = Weight of feed consumed by the chickens, kg $chicken^{-1}$

Cf = Concentration of As in poultry feed, mg kg^{-1} (14.24 mg As kg^{-1} feed)

$$E = (Wm * Cm) + L - Cmc \quad (2)$$

Where, E = Excretion of As (weight of As excreted by the chickens in manure, mg As $chicken^{-1}$)

Wm = Total weight of manure, kg $chicken^{-1}$ (weight of wood chips recorded on 1^{st} day was subtracted from weight of poultry litter on all the days of observations to get the weight of poultry manure)

Cm = Concentration of total As in manure, mg kg^{-1} (concentration of As in poultry litter was converted mathematically to concentration in manure)

L = Cumulative loss of As from manure due to sampling of poultry litter, mg As $chicken^{-1}$. (For example, on day 14^{th}, one sampling was done, which meant 500 g of litter was removed from the pen. Suppose, As concentration of litter on that day was 17.40 mg kg^{-1}, then it was assumed that 8.70 mg As was lost due to sampling from

that pen. From 14th to 28th day, 2 more samplings were done. If arsenic concentration was 32.12 mg kg^{-1}, it was assumed that 32.12 mg As got lost from 14th to 28th day. Cumulatively, 8.70 + 32.12 = 40.82 mg As was lost from that pen till 28th day because of 3 samplings. This loss in weight of As was also converted on manure-basis. It was then divided by the number of chickens on that day to convert the loss value on per chicken basis)

Cmc = Amount of As present in the manure of Control chickens, mg As chicken^{-1} (This term is included in the equation because the source of this As was not Rox in the feed but this source was unidentified)

Mass Balance = I - E (3)

Recovery of As (%) = $\frac{E}{I} * 100$ (4)

2.3 Results

2.3.1 Data received from poultry feed study

The data pertaining to the number of chickens, cumulative feed consumption and weight of poultry litter in each pen on different days of observation were received from poultry feed study. The data were recorded on per pen basis but then converted to per chicken basis (Table 2.1) because the number of chickens in each pen varied on different days of observation as some died and a few others were removed for other studies. The experiment was started with 102 chickens in each pen. On 35th day, chickens of Cobb500

were less in number (69 and 67 in Control and Rox treatment, respectively) as compared to Ross308 (72 each in Control and Rox).

Cumulative feed consumption: Cumulative feed consumption is the amount of feed consumed by a chicken cumulatively over a period of time. It increased significantly with age for both the strains (Cobb500 and Ross308) under both the treatments (Control and Rox) (Table 2.1). Except on 28^{th} day under Control treatment wherein average Cobb chicken consumed significantly more feed (2.38 kg $chicken^{-1}$) than Ross chicken (2.24 kg $chicken^{-1}$) ($P = 0.01$), feed consumption of two strains under two treatments was statistically no different on any other day of observation. However, in 35 days, a chicken belonging to Cobb strain consumed 4.29 kg feed and a chicken belonging to Ross strain consumed 4.17 kg feed under Control treatment. Under Rox treatment, the cumulative feed consumption by a Cobb chicken was 4.19 kg and by a Ross chicken was 4.08 kg, respectively.

Weight of poultry manure: On 0 day, a known weight of wood chips was spread on each pen floor as bedding material. As the chickens grew in age and size, they consumed more feed and water, and excreted more urine and feces on to the bedding material. Consequently, weight of poultry litter (mixture of wood chips and excreta) increased significantly with time during the study period of 35 days (Table 2.1). The chickens were sent to the slaughter house thereafter. It would be worth noting that on 14^{th}, 28^{th} and 35^{th} days, weight of poultry litter in each pen was estimated based on sample weight and sampled area which was then extrapolated to the full pen area, while on 37^{th} day, litter in each pen was weighed physically. That is why a little discrepancy in litter weight between 35^{th} and 37^{th} days (1.49 to 2.01%) was observed. However, some evaporation

loss is also expected in the pens after removal of the chickens. Weight of poultry manure was calculated by subtracting initial weight of wood chips from the weight of poultry litter on all the days of observation. However, there was no significant effect of either the type of strain (Cobb or Ross) or type of treatment (Control or Rox) on the manure weight on any day of observation. Under Control treatment, 0.15 kg manure was produced by the Cobb chicken and 0.14 kg by the Ross chicken till 14^{th} day which increased to 0.96 kg and 0.88 kg in the respective strains on 35^{th} day. Similarly, under Rox treatment, the manure production was 0.16 kg in Cobb strain and 0.14 kg in Ross strain on 14^{th} day which increased to 1.02 kg and 1.01 kg on 35^{th} day in the two strains, respectively.

2.3.2 Total arsenic intake by chicken from poultry feed

In Control feed, roxarsone was not added (Appendix A), hence it was presumed that no As was taken up by the chickens from Control treatment. In Rox treatment, roxarsone was added at the rate of 50 g ton^{-1} of feed (0.005%) which meant As content of the feed was 14.24 mg kg^{-1} (roxarsone has 28.48% As content). As the chickens matured in age, they consumed more feed for their growth, consequently, significantly more As was taken in from roxarsone treated Starter Diet (0-14 days) and Grower Diet (14-28 days). Supply of As was stopped after 28^{th} day as roxarsone containing Grower Diet was replaced by Finisher Diet which did not contain roxarsone. As the two strains were not different in their feed consumption (Table 2.1), As intake too was not significantly different between them (Fig. 2.2). On 14^{th} day, As intake was 7.49 mg $chicken^{-1}$ in Cobb500 and 7.67 mg $chicken^{-1}$ in Ross308 which increased to 33.39 mg $chicken^{-1}$ in Cobb500 and 32.63 mg $chicken^{-1}$ in Ross308 on 28^{th} day indicating significant increases (Appendix B - statistical analysis) of 346 and 325 per cent, respectively.

2.3.3 Arsenic concentration in poultry manure

Under Control treatment, small quantities of As were detected in the manures from both the strains (Fig. 2.3) even though the Control feed was free from roxarsone. The As concentration in Control treatment ranged from 0.59 mg kg^{-1} to 2.10 mg kg^{-1} in Cobb and 1.40 mg kg^{-1} to 2.15 mg kg^{-1} in Ross on different days of observation. In manures of Rox fed chickens, As concentration was significantly more than the Control fed chickens on all days of observation in both the strains. If we make comparisons between the strains in Rox fed treatment, the As concentrations in the manures from two strains were statistically same except day 35^{th} when As concentration was significantly lower in Cobb (25.89 mg kg^{-1}) than Ross (30.10 mg kg^{-1}) (Appendix C - statistical analysis). Age-wise, concentration of As increased significantly in the poultry manures from both the strains from 14^{th} to 28^{th} day, and then decreased significantly on 35^{th} and 37^{th} days. On 14^{th} day, mean As concentration was 37.29 mg kg^{-1} in Cobb500 and 36.61 mg kg^{-1} in Ross308 which increased to 53.11 mg kg^{-1} and 50.08 mg kg^{-1} respectively on 28^{th} day, and went down to 27.49 mg kg^{-1} and 26.75 mg kg^{-1} respectively on 37^{th} day (the clean-out day).

2.3.4 Arsenic excretion by chicken in poultry manure

It is important for the farmer or manager to know the concentration of As in poultry litter as it tells the level of As contamination in this organic fertilizer. But, As concentration in poultry litter does not give the actual amount of As retained in the chicken body or released into the excreta. Presence of bedding material may dilute the actual amount of As released by the chicken. Moreover, the type and amount of bedding

material used by the chicken farmers may be different throughout Canada which may further provide different data from different locations. But from consumer safety point of view, data pertaining to As excretion in poultry manure is very important which may help quantify the actual amount of As retained by the chicken in its body. That is why an attempt was made to calculate As excretion in poultry manure.

Excretion of As in poultry manure increased significantly from 14^{th} to 28^{th} day in both the poultry strains (Fig. 2.4). Arsenic excretion was 5.6 mg $chicken^{-1}$ in Cobb and 4.9 mg $chicken^{-1}$ in Ross on 14^{th} day which increased to 26.5 mg $chicken^{-1}$ in Cobb and 23.5 mg $chicken^{-1}$ in Ross on 28^{th} day. In Cobb500, there was no significant change in As excretion after 28^{th} day, however in Ross308, As excretion increased significantly from 28^{th} to 35^{th} day (Appendix D - statistical analysis).

The two strains also excreted significantly different As amounts in poultry manure on 35^{th} day but on 37^{th} day, the two values were very close being 26.66 mg $chicken^{-1}$ in Cobb and 25.95 mg $chicken^{-1}$ in Ross. The discrepancy in excretion data on 35th day might be because of the different sampling methodology followed to collect the poultry litter samples as discussed in the previous section.

2.3.5 Mass balance calculations and recovery of arsenic in poultry manure

Mass balance calculations for As intake through poultry feed and As excretion in poultry manure indicated that an average chicken belonging to Cobb500 ingested 7.5 mg As in 14 days and excreted out 5.6 mg in poultry manure showing a recovery of 75% (Fig. 2.5). In case of Ross308, an average chicken took in 7.7 mg As and excreted out 4.9 mg showing a recovery of 64% on the 14^{th} day. On 28^{th} day, 79% of the ingested As was recovered in Cobb chicken as compared to 72% in Ross. From recovery perspective, the

two strains were not significantly different on 14, 28 and 37 days. Age-wise, recovery of As in Cobb was steady throughout the experiment ranging from 75% to 80%. In Ross, however, recovery differed significantly from 63% to 90%, particularly on 35th day, the recovery was exceptionally high. Considering that 37th day data for recovery was more realistic because the whole litter in every pen was mixed before sampling, the recovery figures for both the strains come very close and match at 80%. The unaccounted As, in that case, was 20% (6.7 mg chicken^{-1}) for both the strains.

2.4 Discussion

2.4.1 Arsenic intake

In Canada, roxarsone containing 3-NITRO®20% has been promoted for stimulating growth and increasing feed efficiency in chickens (ACC, 2000). The recommended dose of 3-NITRO®20% for mixing in feed is 0.025%. Roxarsone content in 3-NITRO®20% is 20%, hence the recommended dose of roxarsone in the feed is 0.005%. The same concentration of 3-NITRO®20% or roxarsone was used in this experiment. Feed consumption increased with age significantly but remained statistically similar in Control and Rox treated chickens (Cobb500 and Ross308) on all days of observation. Since 1940's, researchers have been examining the effects of feeding roxarsone to chickens singularly or in combination with antibiotics, anti-coccidial compounds or antibiotics and anti-coccidial compounds together, and demonstrated that it improved growth and feed utilization (Morehouse, 1948; West, 1956). But, Damron et al. (1975) and Anderson and Chamblee (2001) found no difference in feed efficiency or feed consumption between Control and Rox treatments. A broad spectrum of factors such

as flock size, stocking rate, temperature, lighting, noise, physical form of feed, feed flavor and water supply affect feed intake of chickens (World Poultry, 2013). In this study conducted in the Department of Agricultural, Food and Nutritional Science (Wenger et al., 2013), most of the management, environment and physical factors were optimized, still no significant difference in feed consumption was observed between the two treatments.

However, As intake of the chickens kept increasing with time in Rox treatment as Starter and Grower Diets contained recommended dose of roxarsone. Though supply of As was stopped after 28th day as the roxarsone containing Grower Diet was replaced by Finisher Diet which was free from roxarsone, but by that time 33.4 mg As chicken^{-1} in Cobb500 and 32.6 mg As chicken^{-1} inRoss308 had already been taken in.

2.4.2 Arsenic excretion

Excretion of As was calculated using equation (2) wherein weight of poultry manure, concentration of As in poultry manure, loss of As due to sampling and amount of As present in manure of Control chickens were considered as contributing parameters. Various estimates of amount of poultry litter production are available in literature. On the basis of dry matter digestibility of diet (87.5%), it has been estimated that 0.34 kg dry manure is excreted by a 35 day old bird (FSA, 2007). Bolan et al. (2010) reported that poultry litter production ranges from 0.7 to 2.0 tons/1000 broilers/flock on the dry weight basis. In this experiment, however, the amount of poultry litter generated by a 35 day old chicken was in the range of 1.07 to 1.20 kg chicken^{-1} and the amount of manure generated was in the range of 0.88 to 1.02 kg chicken^{-1} on dry weight basis. A number of factors such as feed composition, efficiency of feed utilization, type of bedding material,

final live weight of poultry, and handling and storage factors may affect the actual quantity and quality of litter generated from various types of poultry units (Maguire et al., 2006).

A small concentration of As ranging from 0.59 mg kg^{-1} to 2.15 mg kg^{-1} was detected in the manures of Control chickens of both the strains on all observation days. This implied that at least one more source of As was present in the feed other than the roxarsone. Garbarino et al. (2003) also reported a concentration of 0.6 mg kg^{-1} As in poultry litter from a poultry house that did not use roxarsone in feed indicating the presence of one more source of As in feed. The finger of suspicion pointed towards fish meal which was added in the feed at the rate of 3.002, 5.003 and 3.509 % in Starter, Grower and Finisher Diets, respectively. Presuming that as the As concentration built up inside the chicken through consumption of this feed over a period of time, chicken started excreting it out and As appeared in the litter from the Control chickens. Edmonds and Francesconi (2003) reported that marine organisms are capable of bioaccumulation of As directly from both ambient water and from food organisms. Their earlier reports that total As concentrations ranging from 1 to 100 mg kg^{-1} (w/w) are typically found in marine animals and algae including fish, shellfish and seaweed, support the above presumption.

Regarding As in the litter of roxarsone-fed chickens, there is a general consensus in the available scientific literature that chickens fed on roxarsone-mixed feed produce a litter that contains As. Theoretically, very little As should remain in the chicken body, with the majority of As being excreted into the litter (Fisher et al., 2011). This suggests that whatever amount of As is taken up by the chickens during the first four weeks should be excreted out shortly after. The left over amount inside the body should also come out

during the last week because the intake of As is stopped at the end of 4th week by replacing the Grower Diet (roxarsone-mixed) with Finisher Diet (roxarsone-free). In literature, the reported concentration of As in poultry litter varies significantly from 0 to 77 mg kg^{-1} (Morrison, 1969; Sims and Wolf, 1994; Adeli et al., 2007; Toor et al., 2007) but there is no mention of recovery or mass balance anywhere. In this experiment, the maximum concentrations of As were detected on 28^{th} day in poultry manures of roxarsone fed chickens being 53.1 mg kg^{-1} in Cobb500 and 50.1 mg kg^{-1} in Ross308. This concentration was found decreased on 35^{th} day because of two-step dilution (Table 2.1). First, as the chickens grew in age and size after 28^{th} day, they consumed ~81% more of roxarsone-free (Finisher) diet between 28^{th} and 35^{th} day, and second, they produced ~98% more excreta between 28^{th} and 35^{th} day which had comparatively less As than 28^{th} day. Further reduction in As concentration on 37^{th} day can be ascribed to some volatilization loss of As from manure.

After considering weight of poultry manure, concentration of As in poultry manure, loss of As due to sampling and amount of As present in manure of Control chickens, As excretion was calculated for the roxarsone-fed chickens of the two strains. Excretion values of 35^{th} day appeared erratic and out of sync in both the strains. This could have been because of auger sampling approach adopted for drawing the representative sample of poultry litter and estimation approach adopted for measurement of weight of poultry litter in a pen. On 37^{th} day, however, both these approaches were right because the poultry litter of the whole pen was mixed before drawing the representative sample and the weight of poultry litter of the whole pen was measured physically. Hence, an excretion of 26.66 mg $chicken^{-1}$ out of 33.39 mg $chicken^{-1}$ intake of

As in Cobb500 and 25.95 mg chicken^{-1} excretion out of 32.63 mg chicken^{-1} intake of As in Ross308 was considered realistic. These results indicated a recovery of 80% in both the strains on 37th day which also meant a shortfall of 20% of ingested As. This shortfall was 6.73 mg chicken^{-1} in Cobb500 and 6.68 mg chicken^{-1} in Ross308. Possible fate of this un-accounted As could be: a) retention in chicken body tissues, and/or b) volatilization loss to the atmosphere. However, researchers are still trying to determine the volatile biological/environmental transformation products of roxarsone (arsine and methylated derivatives such as mono-, di- and tri-methylarsines) in poultry litter (Momplaisir, 2013).

If the factors such as volatilization loss of As from poultry litter, and some sampling or analytical errors are assumed to be insignificant, then accumulation of As in chicken body parts at 6.73 or 6.68 mg As chicken^{-1} can be a human health concern. With an average body weight of chicken being 2.2 kg on 35th day as observed in the feed study, As concentration in chicken body would be 3.06 mg kg^{-1} in Cobb500 and 3.04 mg kg^{-1} in Ross308 . These concentrations are alarming as they fall within the range of As carcinogenicity (FSANZ, 2013). Very recently, Kawalek et al. (2011) reported that chickens can metabolize organic As to the more toxic inorganic form, and they found a concentration of 2.8 $\pm$ 1.4 mg kg^{-1} total As in the livers of roxarsone-treated chickens. Based on these results, Pfizer Inc. suspended the sale of roxarsone in the United States (Pfizer, 2011).

2.5 Conclusions

This study was designed to calculate the amount of As chickens take in with feed, and the amount of As they excrete into the manure. The maximum recovery of As in poultry manure was found to be 80% in the two strains of poultry (Cobb500 and

Ross308). These results raise two concerns. First, about 6.7 mg chicken^{-1} unaccounted As in Cobb500 and Ross308 was either retained in chicken body or lost into the environment. If this unaccounted amount of As was retained in the chicken body contrary to the popular scientific belief, it would pose a direct human health issue. With an average body weight of a 35-day old chicken being 2.2 kg in this study, As concentration in chicken body would be 3.06 mg kg^{-1} in Cobb500 and 3.04 mg kg^{-1} in Ross308, which is within carcinogenicity range of As. However, if this loss was due to volatilization then chicken meat would not have an As concentration in the carcinogenicity range. The report from Kawalek et al. (2011) confirmed that As is being accumulated in the chicken body defeating the speculation of volatilization loss of As.

Secondly, this study also confirmed that the poultry litter from chickens fed with roxarsone was indeed richer in As than that from those chickens who were not fed roxarsone. Since this litter is commonly used as organic fertilizer for crops, this can pose further health issues because As in poultry litter/manure can find its way into our food chain via soil and agricultural products. Therefore, in order to assess this concern, further insight is needed to know how As behaves in different soil environments and how much As is actually taken up by the crops. The fate of As in soil is explored in the next chapter.

Tables and Figures

Table 2.1 Data pertaining to number of chickens, cumulative feed consumption and weight of poultry manure received from poultry feed study (Wenger et al., 2013)

Measured characteristics	Strain	Treatment	Age (Days)				
			0	14	28	35	37
Number of chickens pen^{-1}	Cobb	Control	102±0.6*	88±1.5	81±5.3	69±5.5	
	Cobb	Rox	102±0.6	88±0.6	80±5.3	67±4.7	
	Ross	Control	102±0.0	88±1.0	83±1.2	72±2.1	
	Ross	Rox	102±0.6	89±0.6	84±0.6	72±1.5	
**Cumulative feed consumption, kg chicken^{-1}	Cobb	Control		0.534±0.006	2.384±0.104	4.285±0.187	
	Cobb	Rox		0.526±0.011	2.345±0.046	4.190±0.098	
	Ross	Control		0.529±0.023	2.236±0.062	4.171±0.450	
	Ross	Rox		0.538±0.010	2.292±0.068	4.080±0.096	
***Weight of poultry manure, kg chicken^{-1}	Cobb	Control		0.152±0.031	0.509±0.080	0.959±0.163	0.942±0.162
	Cobb	Rox		0.155±0.034	0.514±0.049	1.017±0.034	0.993±0.034
	Ross	Control		0.137±0.057	0.458±0.104	0.880±0.199	0.860±0.196
	Ross	Rox		0.138±0.041	0.476±0.045	1.012±0.050	0.991±0.046

* Values are mean of three with associated standard deviation.

** Cumulative feed consumption measured on per pen basis was converted into per chicken basis.

*** On 0 day, a known weight of wood chips was spread on each pen floor. On 14th, 28th and 35th days, the weight of poultry litter was estimated based on sample weight and pen area, while on 37th day, litter in each pen was weighed physically. Weight of poultry manure was calculated by subtracting weight of wood chips from the weight of poultry litter on all the days of observation.

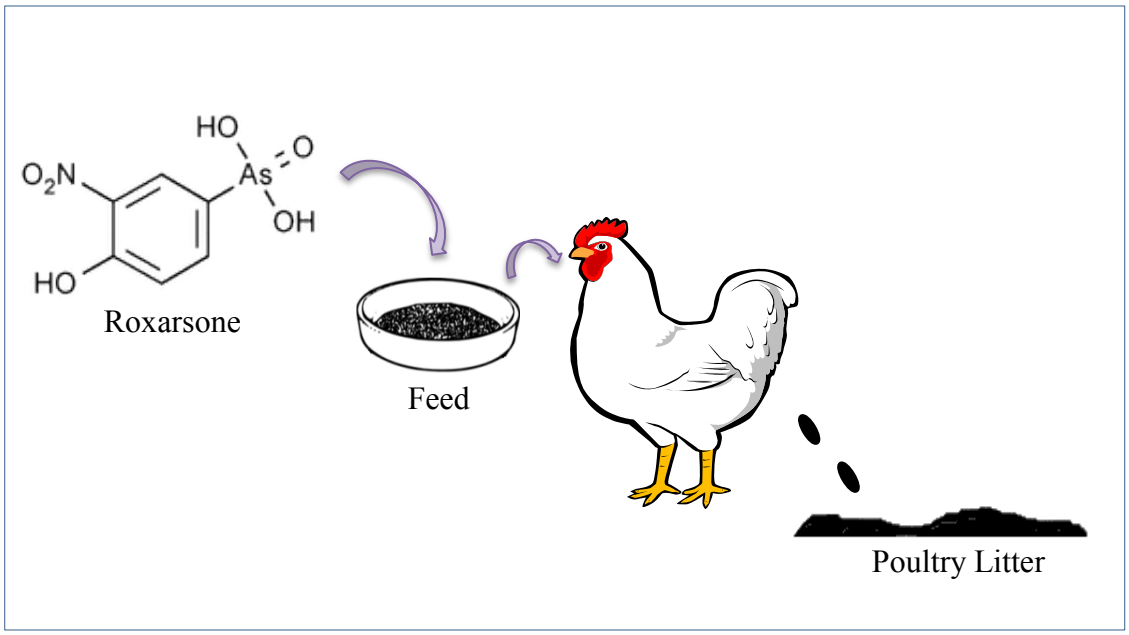

Fig. 2.1 Schematic diagram showing transfer of As from roxarsone in poultry feed to chicken body, and from there to poultry manure/litter.

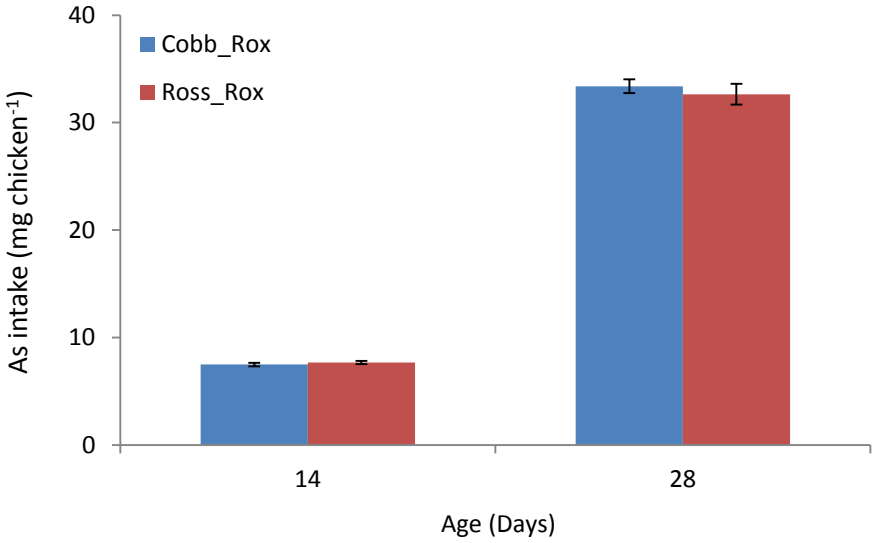

Fig. 2.2 Total As intake by two strains of poultry (Cobb500 and Ross308) consuming Rox feed. Error bars represent ± standard deviation around the mean values.

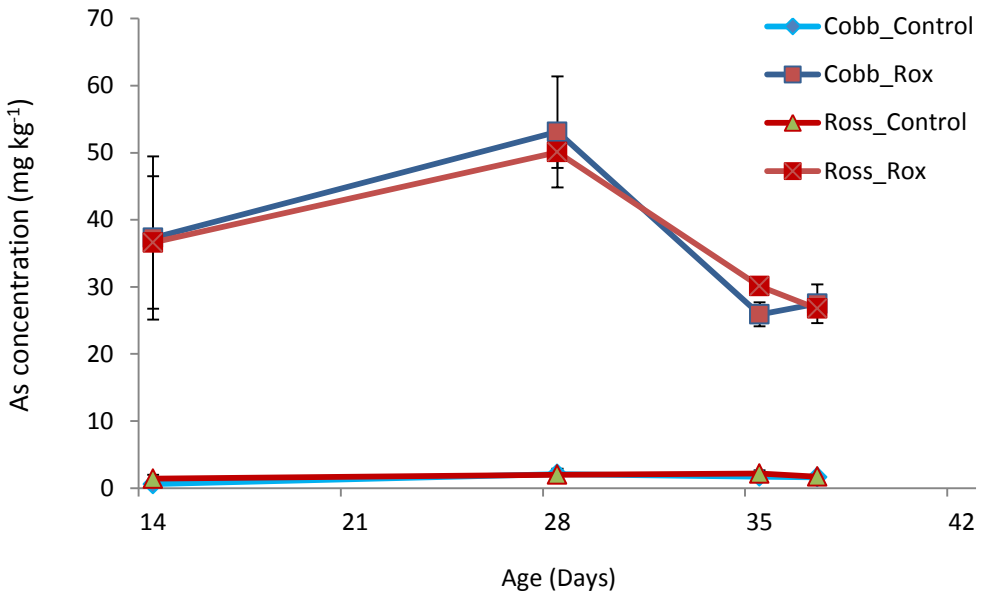

Fig. 2.3 Arsenic concentration in poultry manure produced by the chickens of two strains (Cobb500 and Ross308) consuming two types of feed (Control and Rox). Error bars represent ± standard deviation around the mean values. On 14th, 28th and 35th day, poultry litter samples were drawn from 15 different locations in each pen, pooled together and then one quarter of the mixed sample was saved for analysis. On 37th day, poultry litter of the whole pen was mixed together and a composite sample was drawn. The As concentration in poultry litter was converted to concentration in manure on weight proportion basis.

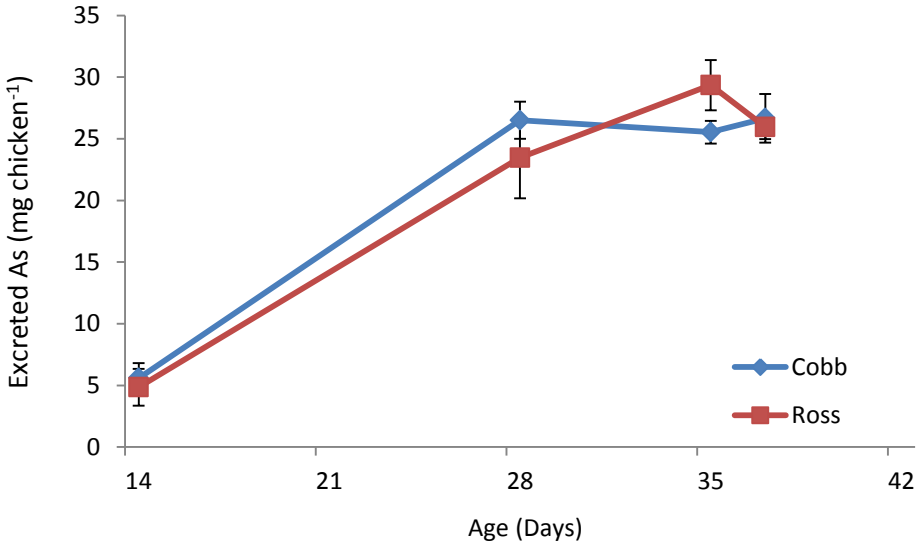

Fig. 2.4 Weight of excreted As in manure of two strains of poultry (Cobb500 and Ross308) consuming Rox feed. Error bars represent ± standard deviation around the mean values. On 35th and 37th days, approaches for sample collection and weight measurement of poultry litter were different as described in Materials and Methods.

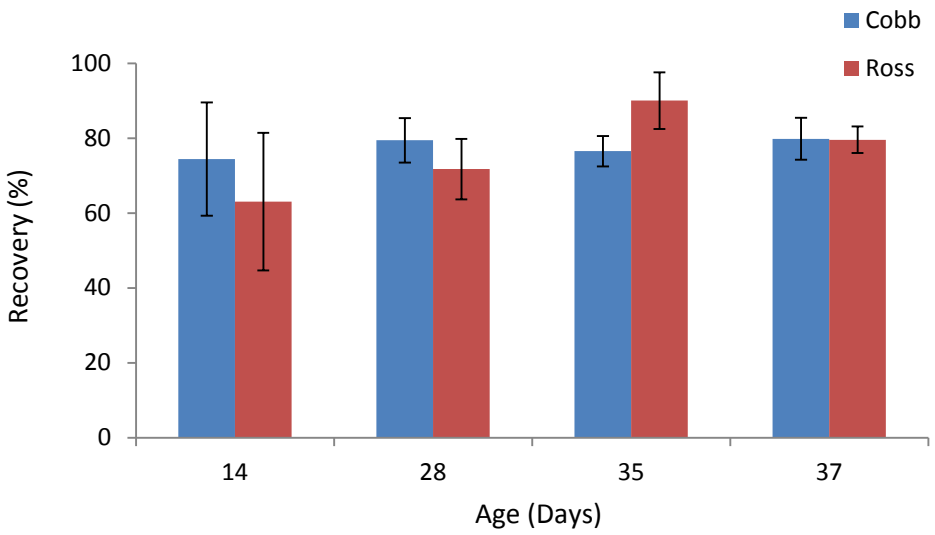

Fig. 2.5 Recovery of As from the manure of two strains of poultry (Cobb500 and Ross308) consuming Rox feed. Error bars represent ± standard deviation around the mean values.

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Chapter 3: Fate of arsenic in soils amended with poultry litter

3.1 Introduction

Worldwide, there is increasing concern regarding the contamination of soils with arsenic (As) and the potential risk to human and environmental health arising from such contamination (McLaren et al., 2006). Contamination of soils with As can occur as a result of both natural and anthropogenic activities. While rock minerals are the prominent natural source of As in soil, it also accumulates in soil as a result of atmospheric deposition (Scudlark and Church, 1988). Anthropogenic contamination is associated with the use of arsenical pesticides and herbicides, mining and smelting activity, and land disposal of sewage sludge and tannery wastes (Code of Federal Regulations, 2011). Spills, leaks and run-off from wood treatment facilities, and use of As containing groundwater for irrigation also contribute to this contamination (McLaren and Smith, 1996; Burgess and Ahmed, 2006).

Roxarsone-medicated feed used in poultry production might be another source of As in the soil. As we explored in Chapter 2 that chickens fed on roxarsone-mixed feed produced a litter that contained As. Since poultry litter is considered as one of the best organic fertilizers, it is applied to agricultural fields for crop production and serves as another medium for As to find its way into soil (Gupta and Charles, 1999). With the steady growth of the Canadian poultry industry, high As concentration in ever-growing volume of poultry litter can be a big concern. In areas where litter is applied to enrich the soil with nutrients, As concentration can exceed the background As level. The maximum acceptable concentration of As in agricultural soils is 12 mg kg^{-1} (CCME, 1997). Repeated application of As-rich poultry litter on the same parcel of agricultural land year

after year can exceed even this threshold level, and can either leach down to groundwater or taken up by the crop plants, thus becoming hazardous to public health.

The fate of As in a soil environment is extremely complex. Several soil processes such as ion exchange, precipitation, adsorption/desorption and biological activity contribute to this complexity (Jain and Ali, 2000). Other intrinsic factors such as pH, Eh and competition for adsorption sites further add to the complexity (Robertson, 1989). However, presence of microbes in the soil, and changing soil environmental conditions due to moisture, air, temperature or agricultural operations can alter the thermodynamic equilibrium of As in soil. Consequently, As can change its forms and association with soil particles depending upon the changed soil conditions and interaction with the biota, making it difficult to predict its mobility and bioavailability in soil.

Inorganic As in poultry litter is highly water soluble having potential for downward movement, but the movement is limited in most soils due to their high capacity to bind As to clay minerals and oxides of iron (Fe), aluminum (Al) and manganese (Mn) (Fisher et al., 2011). Co-precipitation of As with Fe oxyhydroxides has also been defined as the major mechanism that strongly retains As in sediments (Larios et al., 2012) but As-bearing Fe oxyhydroxides may also act as a source of As during reductive dissolution of both amorphous and crystalline oxyhydroxides (Sadiq, 1997). Arsenic associated with hydrous Mn oxides is relatively more labile than Fe oxyhydroxides (Tessier et al., 1979). Arsenic adsorption on the edges of clay minerals and on the surface of calcite has also been observed (Larios et al., 2012). Some other stable forms of As are: naturally occurring As-bearing sulfide ores and silicate minerals, but weathering of these minerals can release As into the soil environment (Sadiq, 1997).

However, even in relatively low concentrations, As can pose high risk to soil pore water and biota (Edwards et al., 2004). It is, therefore, important to quantify the concentration of As in soil which tells about the status of the problem and thus helps in proper characterization of the site. But, total concentrations do not give any information about the solid-phase partitioning (Cottenie et al., 1980), biological availability or potential mobility of As within the soils (Anawar et al., 2006). Hence, it is of paramount importance to identify and quantify As in different fractions associated with solid constituents in soil because some of these fractions are considered to be the most available to biota and most easily leached to groundwater (Brandstetter et al., 1999).

Arsenic distribution in different soil constituents can be studied by sequential extraction procedure (SEP). Several SEP schemes have been developed by researchers to fractionate As (Tessier et al., 1979; Keon et al., 2001; Wenzel et al., 2001). These schemes vary in terms of number of fractions, extraction solutions, conditions and the sequence of extraction steps. In this study, a modified SEP developed by Javed et al. (2013) has been used to quantify As associated with different solid constituents in soil after application of poultry litter in soil and incubation. This SEP had an edge over other procedures as ~95% of the applied As could be traced back in ten different meaningful fractions. The results of this study will help quantify the mobility and bio-availability of As in soils which will ultimately help assess the risk associated with movement of As to groundwater or uptake of toxic levels of As by plant parts, and later help design proper management techniques.

3.2 Materials and Methods

Poultry Litter: After completion of the feed study (described in detail in the previous chapter), poultry litter from Rox pens and Control pens was mixed separately, and two bulk samples of Rox poultry litter and Control poultry litter were collected and stored in a freezer at -20 ^{0}C until further use. Selected characteristics of these samples are provided in Table 3.1. However, in this study, only Rox poultry litter was applied in the soils.

Soil sample collection: Two sites were selected for soil sample collection based on soil texture and organic matter content. Soil1 was collected from Ellerslie Research Station (53^0 25' 27" N and 113^0 32' 47" W) and Soil2 from Breton Plots (53^0 5' 17.85" N and 114^0 26' 37.11" W), University of Alberta, Canada. The soil from Ellerslie Research Station was Eluviated Black Chernozem while the soil from Breton Plots was Orthic Gray Luvisol. Bulk soil samples were collected manually from the surface (0-20 cm), air-dried, ground with mortar and pestle of agate, and passed through 2 mm sieve. Background As concentrations in Soil1 and Soil2 were 8.06 and 7.87 mg kg^{-1}, respectively. There was no earlier record of use of As in any form on these soils. Some selected characteristics of these two soils are also provided in Table 3.1.

Two treatments of poultry litter were planned for this study. In Control treatment, 4 kg soil alone (without any addition of poultry litter) was poured in a PVC pot lined with polyethylene bag. Height of this soil rose up to ~20 cm in the pot. In Rox treatment, first, two kg soil was poured in the pot which went up to ~10 cm in the pot, and then on top of this soil, a mixture of poultry litter and soil (500 g poultry litter and 1400 g soil) was poured. It meant that in the top layer, poultry litter was applied at the rate of 714 tons ha^{-1} with As concentration of 9.75 mg kg^{-1}. The reason for adding this high dose of poultry

litter was to mimic the build-up of As in soil due to repeated applications of poultry litter over years on the same parcel of land. Schematic representation of the PVC pots used in this study is shown in Fig. 3.1. Pots were irrigated with nanopure water (Barnstead nanopure, Thermo Scientific). Moisture content was maintained at 80% of the field capacity by periodically weighing the pots and adding water to compensate for any loss in weight. All pots were placed in an environmentally controlled chamber (23±1/ 20±1 ^{0}C day/night temperature, 50±5% humidity, 16/8 h light/dark photoperiod, 325±10 µ mol m^{-2} s^{-1} photo-synthetically active radiation). Pots were randomized on the growth chamber bench and their positions were changed every week to minimize variations in the micro environments. After 6 weeks, polyethylene bags were taken out of the pots without disturbing the profile and soil samples were collected from Depth1 (10 cm) and Depth2 (18 cm) using a plastic knife. Collected soil samples were immediately transferred to a freezer (-20 ^{0}C) to avoid any volatilization loss.

Soil analysis for total As concentration: To determine total As concentration in soil samples, moist soil equivalent to1 g dry soil was digested in conc. HNO_3 (Trace Metal Grade, 67-70% concentration, Fisher Scientific) in a microwave (Ethos Sel, Milestone) using Method 3052 (USEPA, 1996) where except HNO_3 no other acid or reagent was used in digestion (reasons explained in the previous chapter). However, total sample decomposition was achieved with the adjustment of temperature and time. After cooling of the digestion vials, the solution was diluted to 100 ml using nanopure water, filtered through 0.45 µm PTFE syringe filter (033911C, Fisher Scientific) and analyzed using Inductively Coupled Plasma Mass Spectrometry (iCAP Q, ICP-MS, Thermo Scientific).

Total As concentration was quantified using external and internal standards and dilution factor.

Soil analysis for different As fractions: For As fractionation, soil samples were extracted using ten different extractants sequentially. Sequential extraction procedure (Javed et al., 2013) has been summarized in Table 3.2. All the extractants were prepared using trace metal grade acids, reagent grade chemicals and nanopure water. For analysis, 0.4 g soil was taken in 50 mL centrifuge tube of polypropylene (430829, Corning) and 40 mL of first extractant was added to it to maintain a soil to extractant ratio of 1:100. For each step, the same ratio was maintained to ensure that the extractant did not exhaust. Soil-extractant solution was shaken on a mechanical shaker for specified duration and then centrifuged for 30 minutes at 7000 *g*. The supernatant was decanted in a clean beaker. Samples were washed with nanopure water (shaking for 30 minutes and centrifugation for 30 minutes at 7000 *g*). Water-wash was pooled with the first decanted solution. The same procedure was followed for the next nine steps. All extracts were filtered through 0.45 µm PTFE syringe filters and stored at 4 ^{0}C prior to analysis on ICP-MS. Reagent blanks of all the extractants were also analyzed in parallel.

Particle size analysis (sand, silt and clay) was performed according to ASTM Method D422-63 (ASTM, 2007) and the soils were classified as per the Canadian System of Soil Classification (AAFC, 1998). Soil pH and EC were determined using 1:2 ratio of soil to de-ionized water using pH/Conductivity Meter (AR20, Accumet). For total organic carbon and nitrogen analyses, samples were digested using dry combustion Method 972.43 (AOAC, 2000) and analyzed using Costech (EA 4010).

Statistical Analysis: The experiment design was a 2x2x2 factorial with two soils (Soil1 and Soil2), two treatments (Control - no addition of poultry litter, and Rox - addition of As-rich poultry litter in soil at 714 tons ha^{-1} contributing 9.75 mg As kg^{-1}soil) and two sampling depths (Depth1 = 10 cm and Depth2 = 18 cm) with two replications, and the data were analyzed using IBM SPSS Statistics version 20 (IBM, 2011). Differences between the means were tested at significance level of 0.05 using Least Significant Difference test.

Distribution of arsenic in soil: To examine the fate (recovery, volatilization and mobility/leaching) of As in two soils after application of As-rich poultry litter and 6 weeks of incubation, mass balance calculations were performed as under:

$$R = [\{(A - B) + (C - D)\}/ E]* 100 \quad (1)$$

where R = Total recovery of As after 6 weeks of incubation, %

A = Total As at Depth1 in Rox treatment, mg kg^{-1}

B = Total As at Depth1 in Control treatment, mg kg^{-1}

C = Total As at Depth2 in Rox treatment, mg kg^{-1}

D = Total As at Depth2 in Control treatment, mg kg^{-1}

E = Total As added through poultry litter, mg kg^{-1}

$$EL = 100 - R \quad (2)$$

where EL = Environmental/volatilization loss of As, %

$$LL = \{(C - D)/E\}*100 \quad (3)$$

where LL = Leaching loss of As, %

C, D and E - same as above

3.3 Results

3.3.1 Total arsenic concentration in soils

Total As concentration in soil samples was due to both geogenic and anthropogenic sources. In Control treatment where poultry litter was not added, the source of As was geogenic only while in the Rox treatment where As-rich poultry litter was added to the soils, the source included both geogenic and anthropogenic sources. The background arsenic concentrations in Soil1 and Soil2 were 8.1 and 7.9 mg kg^{-1}, respectively. After 42 days of incubation, As concentration was significantly different in Control and Rox treatments at both the depths in the two soils (Fig. 3.2, ANOVA table in Appendix E). Total As concentration in Soil1 at Depth1 increased from 8.12 mg kg^{-1} in Control to 15.29 mg kg^{-1} in Rox, and at Depth2, concentration increased from 8.21 mg kg^{-1} in Control to 10.47 mg kg^{-1}in Rox. In Soil2 at Depth1, total As concentration increased from 7.96 mg kg^{-1} in Control to 14.65 mg kg^{-1} in Rox, and at Depth2, the significant increase was from 8.00 mg kg^{-1} in Control to 10.85 mg kg^{-1} in Rox, respectively. With Rox treatment, As concentration increased at Depth1 in both the soils, and the two soils had slightly (but significant) different As concentrations whereas at Depth2, the two soils had almost similar concentrations of As.

3.3.2 Arsenic fractionation in Soil1

First, this surface soil (Ah/Ahe horizon) of Eluviated Black Chernozem soil without any Rox poultry litter addition (Control soil before incubation) was sequentially extracted to observe what fractions of As were present in the soil. Geogenic As was identified and quantified in eight fractions including the residual one (Fig. 3.3a). Water

soluble fraction (F1) and loosely adsorbed - ionically bound fraction (F2) were absent from this soil. Carbonate bound (F4), organic matter and secondary sulfide bound (F9), and residual As (F10) fractions were present but in very small amounts (<4.0% each). However, strongly adsorbed As (F3), As co-precipitated with amorphous Fe, Al and Mn oxyhydroxides (F5), As co-precipitated with crystalline Fe, Al and Mn oxyhydroxides (F6), As associated with As oxides and silicate clays (F7) and As co-precipitated with pyrite and amorphous orpiment (F8) were the dominant fractions representing 16.3%, 23.9%, 15.7%, 14.6% and 21.8% of the total As in this soil, respectively. F5 was the largest fraction (23.9%) with a concentration of 2.0 mg As kg^{-1}soil.

After addition of As-rich poultry litter as Rox treatment to the upper layer (Depth1) of Soil1, As concentration at Depth1 increased significantly in F1, F2, F3 and F4 fractions after 42 days of incubation as compared to Control treatment (Fig. 3.3b). Concentration increased from 0.02 to 4.68 mg kg^{-1} in F1, 0.03 to 0.59 mg kg^{-1} in F2, 1.50 to 3.73 mg kg^{-1} in F3 and 0.11 to 0.34 mg kg^{-1} in F4 fractions. Fractions F5, F7, F8, F9 and F10 contained almost same amount of As in Control as well as Rox treatments. However, in fraction F6, As was absent from Rox treatment but 0.99 mg As kg^{-1} soil was present in Control treatment which was still lower than the native soil (1.29 mg As kg^{-1} soil).

At Depth2 (lower layer) where As-rich poultry litter was not mixed with soil, As concentrations were still significantly higher in Rox treatment than Control treatment in F1, F2 and F3 fractions (Fig. 3.3c) wherein 1.42 mg kg^{-1}, 0.21 mg kg^{-1} and 2.92 mg kg^{-1} As was present in the three fractions, respectively. In F6 fraction, significantly more As was present in Control (1.06 mg kg^{-1}) than Rox (0.28 mg kg^{-1}) treatment indicating that

something different is happening with this fraction in soil. In all other fractions, As concentration was statistically unchanged.

3.3.3 Arsenic fractionation in Soil2

Surface soil (Ah+Ae horizons) of Orthic Gray Luvisol (Soil2) without any Rox poultry litter treatment (Control soil before incubation) was also sequentially extracted. In this soil, geogenic As was absent from F1 and F2 fractions just like Soil1 (Fig. 3.4a). Fraction F4, F9 and F10 contained <5.0% As. Fraction F3 (14.4%), F6 (18.1%), F7 (12.1%) and F8 (19.3%) were the significant phases while F5 with 2.24 mg kg^{-1} (28.2%) was the largest pool of As in this soil.

After mixing of Rox poultry litter with Depth1 soil of Soil2 and 42 days of incubation, As concentrations of Rox treated Depth1 soil increased significantly from nil to 4.06 mg kg^{-1} in F1, nil to 0.53 mg kg^{-1} in F2, 1.32 to 3.54 mg kg^{-1} in F3 and 2.38 to 2.93 mg kg^{-1} in F5 fractions which were significantly higher than Control treated F1, F2, F3 and F5 fractions (Fig. 3.4b). Pool of As in F4, F7, F8, F9 and F10 fractions remained unchanged while in F6, As concentration was significantly less in Rox treatment (0.43 mg kg^{-1}) than Control treatment (1.24 mg kg^{-1}).

At lower depth (Depth2), As concentration in Rox treated soil was significantly more than background concentration (Control treatment) in F1 (1.61 verses 0.05 mg kg^{-1}), F2 (0.29 verses 0.03 mg kg^{-1}) and F3 (3.00 verses 1.44 mg kg^{-1}) fractions while in F6, As concentration was significantly more in Control treatment than Rox treatment (1.24 verses 0.49 mg kg^{-1}) (Fig. 3.4c). All other fractions contained almost the same concentration of As in both the treatments.

Further statistical calculations revealed that in untreated soils (Control treatment), As concentrations in all the fractions were not significantly different between Depth1 and Depth2 showing no significant mobilization and downward movement of As. However in Rox treatment, As moved downwards significantly in F1, F2 and F3 fractions of both the soils, F4 and F6 fractions of Soil1 only, and F5 fraction of Soil2 only. Concentration of As in Soil1 differed significantly from Soil2 at Depth1 in Rox treatment in F1, F4, F5, F6 and F9 fractions only. Similarly, As concentration differed significantly between Rox and Control treatment in the two soils at both the depths in F1, F2, F3 and F6 fractions only.

3.4 Discussion

3.4.1 Total arsenic concentration in soils

The two soils selected for this study had quite a high concentrations of As (8.06 mg kg^{-1} in Soil1 and 7.87 mg kg^{-1} in Soil2) though these are within the range of uncontaminated soils (Pendias and Pendias, 2001). Across Canada, As can be found naturally in soils at concentrations ranging from 4.8 to 13.6 mg kg^{-1} (CCME, 1999/2002). Horseshoe Canyon Formations on which Soil1 developed and Paskapoo Formations on which Soil2 developed may have some As-containing minerals, or marine shale because of underlying Bearpaw Formations and Lea Park Formations which are rich in marine shale (Barker et al., 2011). Shale of marine origin often contains elevated levels of As (Dudas, 1987) which might have contributed As to these soils.

After 42 days of incubation, As concentration increased a little bit at Depth2 in both the un-amended soils (Control treatment): from 8.06 mg kg^{-1} to 8.21 mg kg^{-1} in Soil1 and from 7.87 mg kg^{-1} to 8.00 mg kg^{-1} in Soil2. This slight increase might be due to

oxidative weathering (Muloin and Dudas, 2013) and/or reductive dissolution of As-containing minerals present in these soils, and then with the downward movement of irrigation water, As moved to the lower depth. Though we did not determine these processes but these were the possibilities.

In poultry litter amended soils (Rox treatment), there was a loss of As from upper layer (Depth1) and gain at lower layer (Depth2). The loss from the upper layer suggested either leaching loss to the lower layer with water, or volatilization loss to the environment (Sparks et al., 2007). Soil fungal species play a dominant role in As volatilization aerobically in agricultural soils (Peterson et al., 1981), and have been demonstrated to be efficient arsine producers (Edvantoro et al., 2004). Various bio-transformations of As in soil, in particular oxidation (Ehrlich, 1996), reduction (Langner and Inskeep, 2000), methylation (Gao and Burau, 1997) or photo-degradation (Bednar et al., 2003) play an important role in As cycles under certain conditions and influence its solubility and transport. Increase in As concentration at the lower depth represented mobility and leaching of As. Increased concentration of total As was also observed at lower depths (up to 60 cm) of agricultural soils (Gupta and Charles,1999) and pasture soils (Rutherford et al., 2003) to which poultry litter had been applied for 15 to 30 years. Soil2 lost more As from Depth1 than Soil1. This might be because Soil2 had less clay, organic matter, P, Ca and Fe but more Mn and pH (Table 3.1) which indicated that Soil2 was short of As binding materials, and the soil conditions were not favorable to hold As in this soil. That is why, Soil2 lost more As from upper layer than Soil1. The reported effect of pH (Anderson et al., 1975), P (Roy et al., 1986), Ca (Smith et al., 2002), Fe (Elkhatib et al., 1984), clay content (Livesay and Huang, 1981) and Mn (Oscarson et al., 1983) on

retention/release of As in soils corroborated with our observations.

Mass balance calculations for total As concentrations in the two soils clearly informed that 97% and 98% of the applied As was recovered back, 3% and 2% was lost to the environment, 74% and 69% was retained in the upper layer (Depth1), and 23% and 29% moved to the lower layer (Depth2) in Soil1 and Soil2, respectively.

However, if we consider a different approach with an experimental set-up wherein only poultry litter-amended soils (Rox treatment) and pots are present (Control soils, pots and data being absent), some of the discrepancy arising due to unequal heights (or unit area) of soils in Control and Rox treated pots may be taken care of because in this new set-up, there will be no reference of Control and As movement will be considered on whole area basis. In that case, total As concentration in Rox treated soils will remain the same being 15.29 mg kg^{-1} at Depth1 and 10.47 mg kg^{-1} at Depth2 in Soil1, and 14.65 mg kg^{-1} at Depth1 and 10.85 mg kg^{-1} at Depth2 in Soil2, respectively, but total recovery figures will change from 97% to 99% in Soil1 and from 98% to 100% in Soil2; loss to environment figures will change from 3% to 1% in Soil1 and from 2% to 0% in Soil2; As retention in upper layer will change from 69% to 70% in Soil2; and leaching to the lower layer will change from 23% to 25% in Soil1 and from 29% to 31% in Soil2, respectively.

3.4.2 Arsenic fractionation in the soils

In un-amended Control soils before and after incubation, water soluble (F1) and loosely adsorbed (F2) fractions of As were absent. That was understandable because marine shale and all other minerals in soils, after passing through the cycles of weathering, precipitation-dissolution and immobilization reactions, must be in their geochemical equilibrium. That is why, the soils may not loose the tightly bound, strongly

adsorbed or co-precipitated As to the soft extractants of F1 and F2 fractions. Elkhatib et al. (1984) also mentioned that much less As desorption occurred in the presence of water alone. Extraction of a small quantity of As in F4, F9 and F10 fractions pointed out that these fractions occupied a small proportion of total As present in these two soils. However, big pools of As were present in F3, F5, F6, F7 and F8 fractions indicating that in these soils, As was present mostly as strongly adsorbed As, co-precipitated with amorphous and crystalline Fe, Al and Mn-oxyhydroxides, associated with As oxides and silicate clays, or co-precipitated with pyrite and amorphous orpiment. Previously, it had been examined and reported that As in soils and sediments was tightly bound mostly with amorphous and crystalline iron hydr(oxides) (Fendorf et al., 1997), phyllo-silicate clay minerals (Goldberg and Glaubig, 1988), and pyrite and orpiment (Keon et al., 2001).

In poultry litter amended soils (Rox treatment) at Depth1, a big change in As pool at various soil phases was noticed. The major pools of As shifted to water soluble (F1), loosely adsorbed (F2), strongly adsorbed (F3), carbonate bound (F4) and co-precipitated As with amorphous Fe, Al and Mn oxyhydroxides (F5) fractions whereas F7, F8, F9 and F10 remained unchanged. This indicated that As coming from poultry litter enriched F1, F2, F3, F4 and F5 fractions only, and the rest of the fractions neither lost nor gained As from the poultry litter. However, F6 was the only exception in this case where As either disappeared or got reduced to low amounts. At Depth2, pools of As got bigger at F1, F2 and F3 fractions. The biggest gainer was F1 fraction at both the depths.

Loss of As from F6 (crystalline oxyhydroxides of Fe, Al and Mn) fraction indicated that addition of poultry litter might have helped mobilize As from this pool by facilitating aqueous complexation (Redman et al., 2002) between As and organic acids

generated by the decomposition of poultry litter. Generation of a strong competition (Redman et al., 2002) between organic acids and As for sorption on crystalline oxyhydroxides of Fe, Al and Mn might be the another process responsible for As mobilization from F6 fraction. Organic matter has been reported to increase As mobility in mine tailings from Fe fraction by providing organic substrate to the indigenous bacteria who facilitated As release through ligand- and proton-promoted dissolution or reductive dissolution under both aerobic and anaerobic conditions (Lee et al., 2009). It is speculated that presence of arsenite-oxidizing microbes (Ehrlich, 1996) at the upper depth and arsenate-reducing (Jones et al., 2000) microbes at the lower depth might have mediated these processes. However, in poultry litter amended soils at both the depths, the maximum concentration of As was present in water soluble fraction (F1) followed by strongly adsorbed (inner-sphere complexation) fraction (F3). Rutherford et al. (2003) and Arai (2010) also reported similar results for water soluble and soil bound As in poultry litter amended soils.

Arsenic-rich poultry litter contributed 58-62% As to the water soluble fraction (F1) in the two soils. In light (coarse) textured soil (Soil2), concentration of As at the lower depth was more than the heavy textured soil in this fraction showing more mobility and leaching in light textured soil. This observation is in agreement with Yang (1983) who reported that coarse textured soils low in Fe oxides are likely to yield the higher amounts of readily available As. In fine textured soils, As is typically immobilized by Fe oxides that are homogeneously disseminated in the clay fraction (Lombi et al., 2000).

3.5 Conclusions

It is known that As adsorbs strongly to metal oxides and clays in soil, and that the adsorption is highly pH dependent. But changing soil environmental conditions and the microbial population can alter this equilibrium and, in turn, make immobile As mobile, or vice versa. Mobile As, even in relatively low concentrations, can pose high risk to soil pore waters and biota. This study was designed to understand the distribution of As in poultry litter amended soils.

Application of poultry litter increased the concentration of labile pool of As (water soluble + exchangeable) in soil, and mobility and leaching of As in loam soil was more than silt-loam soil. Silt-loam soil retained more As in upper layer (74% of the applied As through poultry litter) as it had higher contents of clay, organic matter, Fe, P and Ca. Plant uptake of As may be less in such soil in the short term but As will continue to accumulate with further application of poultry litter. In the long term, if this As becomes mobile due to changed environmental conditions, it can be a big concern of As toxicity through food and groundwater. Loamy soil, on the other hand, contributed more As to the lower depth (29% of the applied As through poultry litter). Higher mobility of As in this soil may initiate more uptake by plants and more leaching to groundwater thus becoming an immediate concern in the short term. It is, therefore, suggested that before application of As-rich poultry litter to any soil, the possible contamination of plant products and groundwater should be carefully weighed over on the basis of inherent characteristics of the soil. The uptake of As by plants is investigated in the next chapter.

Tables and Figures

Table 3.1 Selected characteristics of soils and poultry litters

Characteristic	Soil		Poultry litter	
	Soil 1	Soil 2	Rox	Control
pH (1:2)	6.02	6.68	8.66	8.44
EC (1:2), dS m^{-1}	0.12	0.04	8.75	9.70
Total C, g kg^{-1}	63.70	10.30	595.80	480.90
Total N, g kg^{-1}	5.60	0.90	68.40	58.40
Total P, g kg^{-1}	1.04	0.52	14.07	12.89
Total Ca, g kg^{-1}	1.25	0.57	23.69	22.71
Total Fe, g kg^{-1}	29.53	24.18	0.96	0.97
Total Mn, mg kg^{-1}	534.48	765.55	525.11	474.89
Total Zn, mg kg^{-1}	88.56	54.16	639.84	598.49
Total Cu, mg kg^{-1}	19.70	8.84	64.28	69.71
Total Ni, mg kg^{-1}	26.78	21.45	5.75	5.34
Total As, mg kg^{-1}	8.06	7.87	27.29	1.61
Moisture (0.33 bar)	43.91	24.24		
Clay, %	25.06	21.26		
Texture	SiL	L		
Soil Classification	Eluviated Black Chernozem	Orthic Gray Luvisol		

Table 3.2 Sequential extraction procedure (adopted from Javed et al., 2013)

Fraction	Target phase	Extractant	Extraction conditions
F1	Soluble As	De-ionized Water	0.5h shaking, 25 ^{0}C
F2	Loosely adsorbed As	1M NaOAc, pH 8.2	2h shaking, 25 ^{0}C, one water wash
F3	Strongly adsorbed As	1M NaH_2PO_4, pH 5.0	16, 24h shaking, 25 ^{0}C, one repetition of each time duration + one water wash
F4	Carbonate bound As	1M NaOAc, pH 5.0	5h shaking, 25 ^{0}C, one water wash
F5	As co-precipitated with amorphous Fe, Al and Mn oxyhydroxide	Tamm's reagent (ammonium oxalate/oxalic acid, pH 3.0)	2h shaking, 25 ^{0}C, one water wash
F6	As co-precipitated with crystalline Fe, Al and Mn oxyhydroxide	Ti-citrate-EDTA-bicarbonate, pH 7.0	2h shaking, 25 ^{0}C, one repetition + one water wash
F7	As associated with As oxides and silicate clays	10M HF	1 and 24 h* shaking, 25 ^{0}C, one repetition of each time duration + one boiling water wash
F8	As co-precipitated with pyrite and amorphous orpiment	16N HNO_3	2h shaking, 25 ^{0}C, one repetition + one water wash
F9	Organic matter and secondary sulfides bound As	30% H_2O_2 + 1M NH_4OAc (1:2), pH 2.0	16h shaking, 25 ^{0}C, one water wash
F10	Residual As	Concentrated HNO_3	Microwave digestion

* Add 5g boric acid at 16th hour of 24 hour extraction

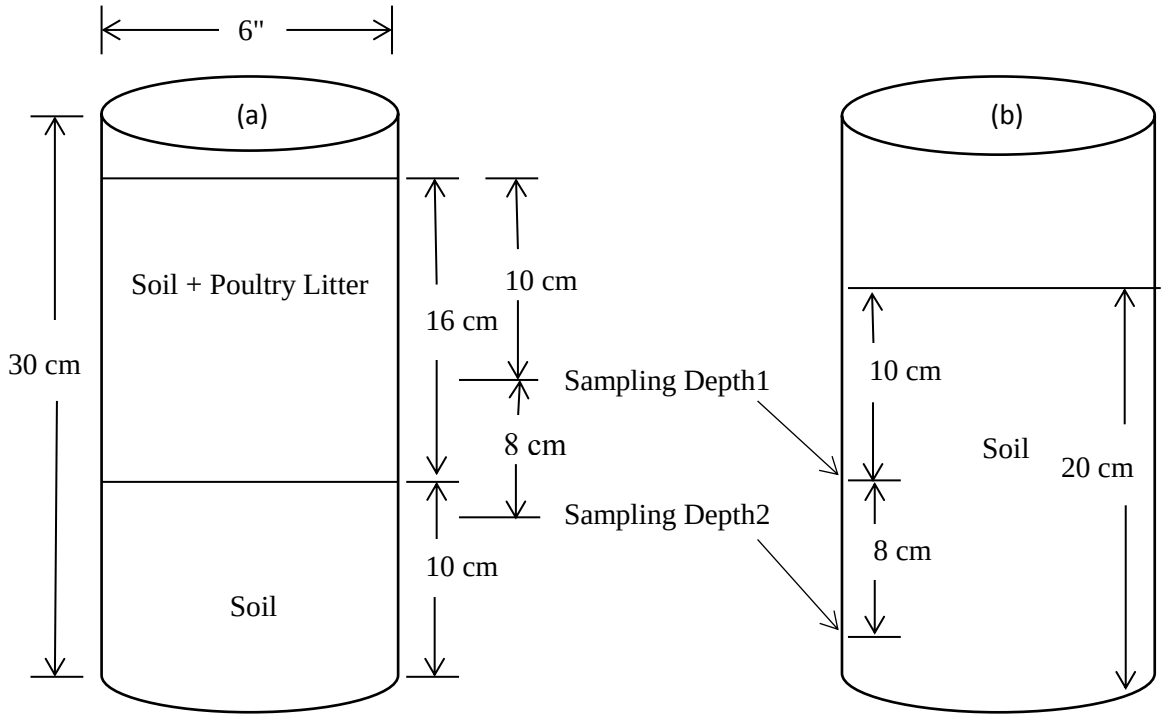

Fig. 3.1 Schematic representation of PVC pots used for a) Poultry litter-amended, and b) Control soil treatments.

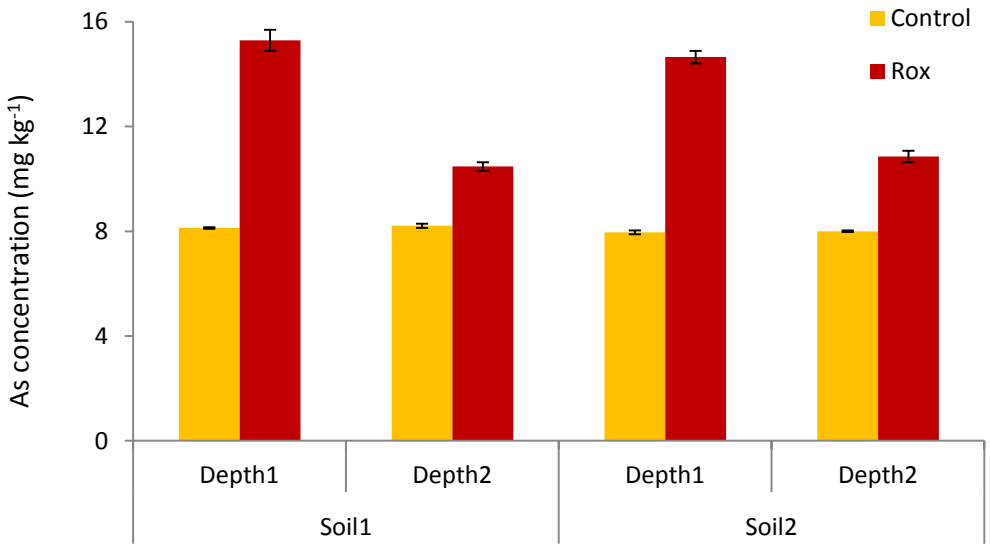

Fig. 3.2 Total As concentration in two soils at two depths after 42 days of incubation with Control and Rox treatments of poultry litter. Error bars represent ± standard deviations around the mean values.

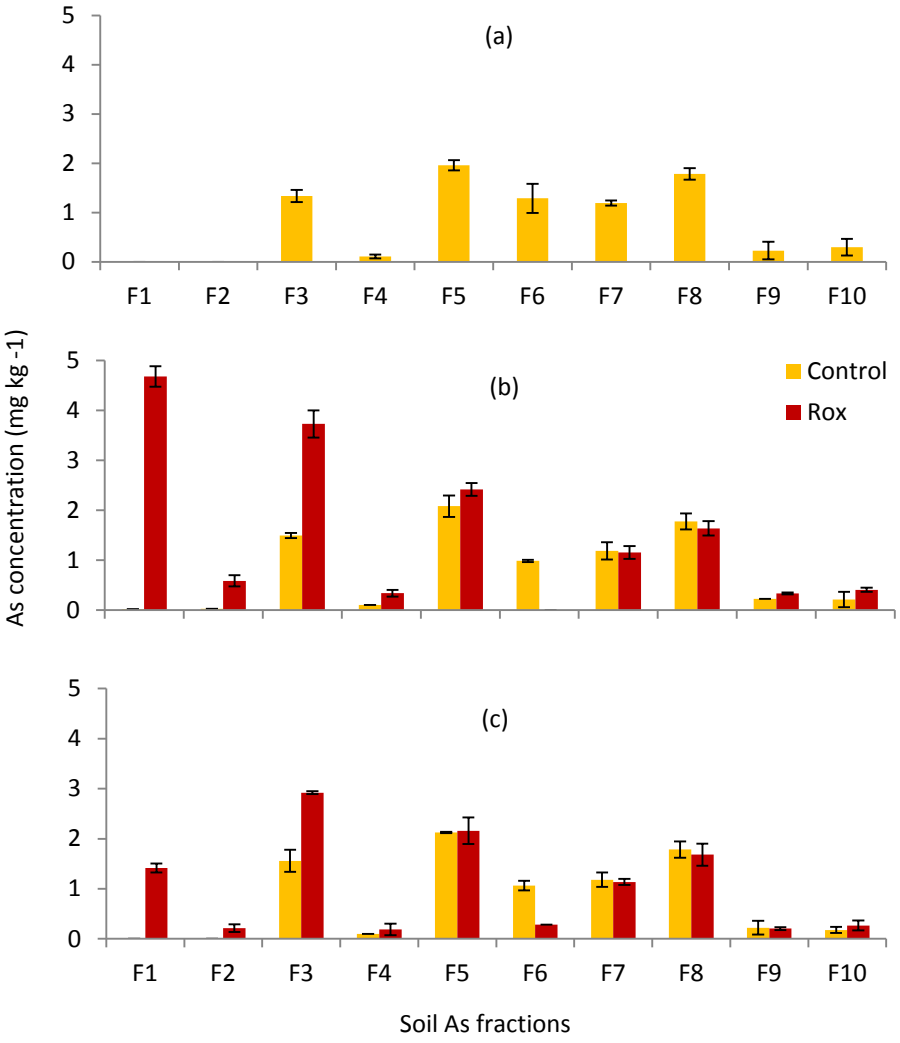

Fig. 3.3 Fractional distribution of As in Soil1: a) As fractions in soil before incubation, b) As fractions at Depth1 after 42 days of incubation (P values for F1, F2, F3, F4 and F6 fractions are <0.05 in each case), c) As fractions at Depth2 (P values for respective F1, F2, F3 and F6 fractions are: <0.05, 0.02, <0.05 and <0.05). Error bars represent ± standard deviation around the mean values.

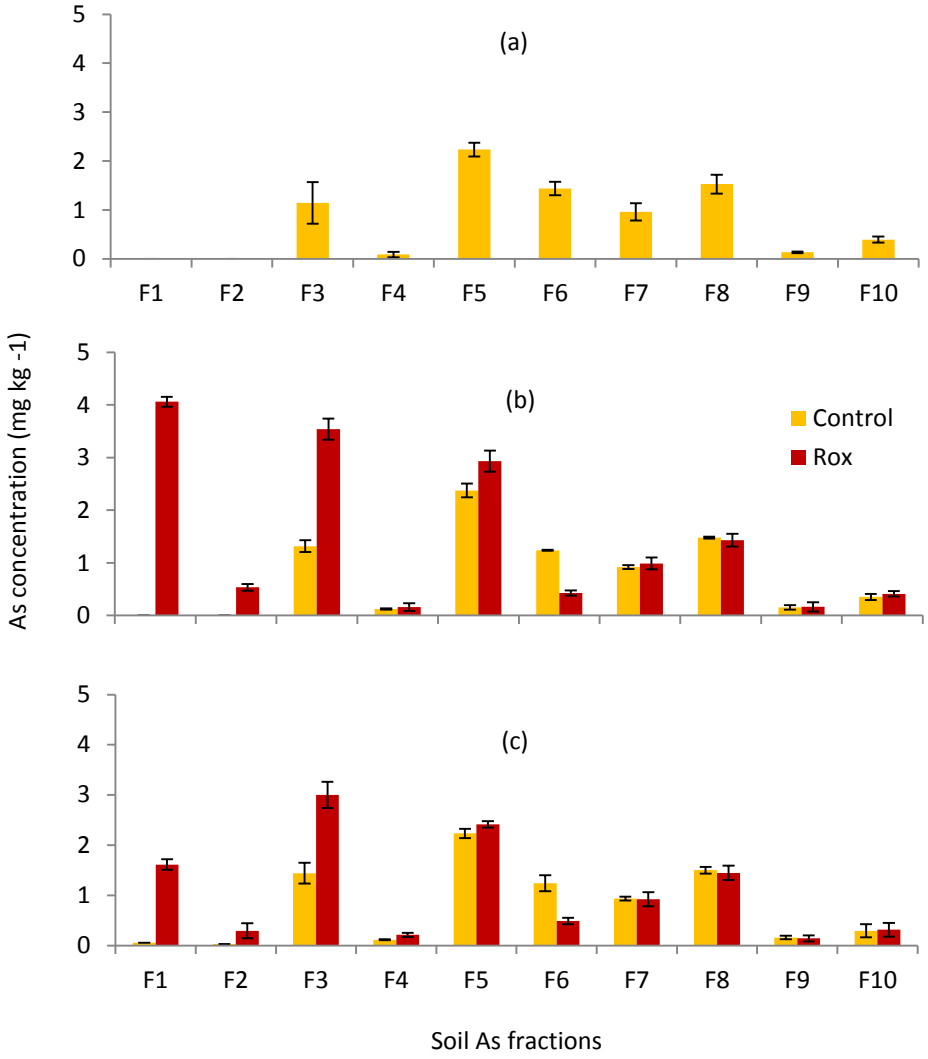

Fig. 3.4 Fractional distribution of As in Soil2: a) As fractions in soil before incubation, b) As fractions at Depth1 after 42 days of incubation (P values for F1, F2, F3, F5 and F6 fractions are <0.05 in each case), c) As fractions at Depth2 (P values in F1, F2, F3 and F6 fractions are <0.05 in each case). Error bars represent ± standard deviation around the mean values.

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Chapter 4: Arsenic uptake by crop plants from soils amended with poultry litter

4.1 Introduction

The poultry industry is one of the largest and fastest growing agro-based industries in the world due to the acceptance of poultry meat by most societies and its relatively low cholesterol content (Bolan et al., 2010). In Canada (AAFC, 2006) as well as Alberta (ACP, 2012), this industry is expanding steadily over the years because it is a profitable sector of production. However, a huge amount of waste is also generated by this industry in the form of poultry litter - a mixture of bedding material and excreta of the chickens. Poultry litter is one of the best organic fertilizers available (Wilkinson, 1979), hence most of it is applied to nearby agricultural land (Simpson, 1991). However, along with many major, secondary and micro nutrients, poultry litter may also contain toxic elements, such as arsenic (As) (Bolan et al., 1992) which is a known carcinogen. A growing body of data suggests that inorganic As present in poultry litter could contaminate soil, crops, food and water bodies (Williams et al., 2007).

Natural levels of As in vegetation rarely exceed 1-2 mg kg^{-1} on a dry weight basis (NRCC, 1978). Research indicates that on un-contaminated soils, concentrations of As in edible plant portions (fruit and grain) are low and do not exceed recognized health-based food standards (1-2 mg kg^{-1}) (Carbonell-Barrachina et al., 1995). However, elevated levels of As in the shoots of *Brassica juncea* grown on historically contaminated sites (cattle dip) in South Australia have been reported by Niazi et al. (2011). Ashjaei et al. (2011) also found significantly higher As concentrations (0.23-0.31 mg kg^{-1}) in bermuda grass (*Cynodon dactylon* L.) grown in the long term poultry litter amended pasture fields. In Bangladesh, the farmlands irrigated with As-contaminated water showed a 10-fold

higher As concentration than the normal level in rice grain (Meharg and Rahman, 2003). This may pose a health risk to people consuming large amounts of rice in their diet (Zhu et al., 2008).

Though the poultry litter will continue to be applied to croplands because of its nutritional and economic values, it is important to understand the release of As from poultry litter and its movement from soil to roots, shoots and other edible parts of the major crops to predict its entry into the food chain. In Canada, barley and canola are grown extensively because of the profitability, resilience of crops and less potential risk to the environment. Canola contributes $15.4 billion to the Canadian economy each year (CCC, 2011). Barley is the second most widely grown cereal crop in Canada after wheat. Barley grows successfully on almost any topography or land conformation in Alberta that can be cultivated, provided other conditions do not limit growth (AARD, 2011a). Barley is used to make beverages like beer and whisky, sweetener for a variety of foods, noodles, breakfast cereals and instant baby formulas, and canola products include oil for human consumption and meal for livestock feed. Canola oil is further processed into a wide range of consumer and commercial food products (CCC, 2011). Uptake of As by these plants can be a cause of concern in near future because these beverages and food items may become the potential source of inorganic As intake for the Canadian population.

In the previous chapter, we studied the fate and distribution of As in soils supplied by the poultry litter. It was clearly highlighted that 58 to 62% of As coming from poultry litter was present in water soluble fraction (F1) which is mobile and easily available to the plants. Considering that soil-crop-food transfer can become a major source of As

exposure pathways (Correll et al., 2006), this study was designed to understand the fate of As if plants are grown in such soils. The questions of particular interest were: how much As do these plants take up from poultry litter amended soils; in which plant parts As gets accumulated and how much; and which soil As fractions contribute to plant uptake of As?

4.2 Materials and Methods

In order to find the answers to the above mentioned questions, two experiments were conducted - one in the field with agronomic rate of poultry litter application, and another in a growth chamber with enhanced rate of poultry litter application (to mimic the high soil As concentration due to the repeated use of poultry litter on the same parcel of land).

4.2.1 Field study

In 2009, bulk samples of poultry litter from the poultry feed study mentioned in Chapter 2 were collected and stored in a freezer (-20 ^{0}C). In 2010, a 1600 m^2 plot was selected at Ellerslie Research Station (53^0, 25', 25.32" N and 113^0, 32', 47.85" W), University of Alberta, having Eluviated Black Chernozemic soil (Soil1, Table 3.1) where no earlier record of use of As in any form existed. The plot was divided into 12 equal sub-plots of 80 m^2 each. Field layout and dimensions are shown in Fig. 4.1. Three treatments (Check - no application of poultry litter, Control - application of poultry litter without roxarsone in poultry feed, and Rox - application of poultry litter with roxarsone in the feed) were replicated four times. Poultry litter was applied at 10 tons ha^{-1} (on dry weight basis). In Control treatment, concentration of As in poultry litter was 1.61 mg kg^{-1}

whereas in Rox treatment, As concentration in poultry litter was 27.29 mg kg^{-1}. Barley seeds (*Hordeum vulgare* L.) var. Trochu were sown at the rate of 90 kg ha^{-1} on 1st June, 2010 and harvested on 31st August, 2010. After air-drying and thrashing, grain and straw weights were recorded on sub-plot basis and samples were stored in polyethylene bags at room temperature for further analysis. Plots remained under snow in the winter. In June 2011, barley var. Trochu was sown again in the same plots to study the residual effect of poultry litter. This time, no poultry litter was added into the field. Grain and straw weights were recorded, and samples were stored at room temperature for further analysis. Root samples were also collected from the field. Roots were washed following the best procedure (Azcue, 1996), wiped dry and stored in a freezer at -20 ^{0}C.

Unfortunately, straw samples from 2010 crop were found missing, and root samples were not collected in the first year because translocation of As in plant parts was not thought of during the first year. Hence, grain samples from 2010 crop, and grain, straw and root samples from 2011 crop were analyzed for total As concentration using ICP-MS.

Grain and straw samples were ground in an all plastic grinder. A sample of 0.5 g weight (dry weight basis) of grain, straw or root samples was digested in conc. HNO_3 (Trace Metal Grade, 67-70% purity, Fisher Scientific) in a microwave (Milestone, Ethos Sel) using Method 3052 (USEPA, 1996) with some modifications as mentioned in Chapter 2. After digestion, the solution was diluted to 100 ml using nanopure water, filtered through 0.45 µm PTFE syringe filter (033911C, Fisher Scientific) and analyzed using Inductively Coupled Plasma Mass Spectrometry (iCAP Q, ICP-MS, Thermo Scientific). Total As concentration was quantified using external and internal standards,

and the dilution factor.

Soil samples were collected from the field during both the crop years at the time of sowing, flowering and immediately after harvesting from a depth of 5-10 cm (root zone where root-soil interaction is maximum) and immediately transferred to a freezer (-20 ^{0}C) to avoid volatilization loss. To determine total As concentration in soil, 1 g soil (dry weight basis) was digested in conc. HNO_3 (Trace Metal Grade, 67-70% purity, Fisher Scientific) in a microwave (Milestone, Ethos Sel) using method 3052 (USEPA, 1996) with some modifications as mentioned in Chapter 2. After digestion, the solution was diluted, filtered, analyzed using Inductively Coupled Plasma Mass Spectrometry (iCAP Q, ICP-MS, Thermo Scientific), and As concentration was calculated.

The experiment was laid out as Randomized Complete Block Design (RCBD) with three treatments (Check, Control and Rox) and four replications. The results have been presented as average values from four replications with standard deviations for the yield data, and as average values from three replications with standard deviations for soil and plant analyses data. Analyses of variance were performed using Linear Mixed Model algorithm on IBM SPSS Statistics 20 (IBM SPSS, 2011). Differences between the means were tested at significance level of 0.05 using Least Significant Difference test procedure.

4.2.2 Growth chamber study

With one agronomic application of poultry litter that fulfills the N or P requirements of the crop, not much As goes into the soil (Oyewumi and Schreiber, 2012), but with continuous application of poultry litter year after year on the same parcel of land, As gets accumulated in the soil (Adeli et al., 2007). Since we did not observe much As compartmentalization in soil and plants in the field study because very little As was

added to soil using the recommended application rate of poultry litter, this growth chamber experiment was conducted to understand the uptake mechanism of As by the crop plants at elevated soil As level and the fractionation of As in poultry litter-amended cultivated soils.

Two different types of surface soil were collected for this experiment (characteristics are shown in Table 3.1). One kg soil was filled in the plastic pot of 2 kg capacity lined with polyethylene bag. For Control treatment, only 1 kg soil was used without any addition of poultry litter while for Rox treatment, 45 g poultry litter (dry weight basis) was mixed with 1 kg soil which was equivalent to 90 tons ha^{-1} of poultry litter. It also meant that 1.23 mg As kg^{-1} soil was added through poultry litter in the Rox treatment which was over and above the As concentration naturally present in the native soil. Four 1-day old soaked seeds of barley (*Hordeum vulgare* L.) var. Trochu and canola (*Brassica napus* L.) var. HiQ were planted in the pots which were thinned to two after one week. Pots were irrigated with Nano-pure water. Moisture content was maintained at 80% of the field capacity by periodically weighing the pots and adding water to compensate for any loss in weight. All pots were placed in an environmentally controlled chamber ($23\pm1\ ^0C$/ $20\pm1\ ^0C$ day/night temperature, $50\pm5\%$ humidity, 16/8 h light/dark photoperiod, 325 ± 10 µ mol $m^{-2}\ s^{-1}$ photo-synthetically active radiation). Pots were randomized on the growth chamber bench and their positions were changed every week to minimize variations in the micro environments. After 30 days, polyethylene bags were taken out of the pots, leaves and roots were harvested, and soil samples were collected. Leaves were wiped, and roots were washed and wiped dry as per the best procedure of Azcue (1996). Fresh weights of leaves and roots were measured. After measurement,

leaves, roots and soil samples were transferred to a freezer (-20 ^{0}C) to avoid any volatilization loss. These samples were digested in concentrated HNO_3 in a microwave following Method 3052 (USEPA, 1996), and analyzed for total As concentration using ICP-MS (iCAP Q, ICP-MS, Thermo Scientific) as mentioned in the previous section. Sequential extraction procedure (Javed et al., 2013) as described in Chapter 3 was used to fractionate As in the soil samples.

In 2011, while collecting root samples, root weight per unit area was not measured. The concentration of As in roots was determined but for the lack of root weight per unit area, it was impossible to calculate As uptake by roots in the field. However, during the growth chamber study, two plants of same barley variety were sown in three pots each and raised up to maturity. Their root weight and total plant weight (shoot and grain) were recorded, and root to total above ground plant weight ratio was calculated. This ratio was very close to the reported one (Gregory et al., 1992), and hence used to calculate the root uptake of As by barley plants in the field.

With two soils, two treatments and two crops, the experiment was laid out in 2x2x2 factorial design but failure of canola crop in one of the soils allowed to present the results as 2x2 factorial design. Canola plants could not grow in Soil2 may be because of low buffering capacity of this soil to high concentration of uric acid produced from decomposition of higher application of poultry litter. Average values from three replications with standard deviations have been shown. IBM SPSS Statistics 20 (IBM, 2011) was used for statistical analyses. Differences between the means were tested at significance level of 0.05 using Least Significant Difference test procedure.

4.3 Results

4.3.1 Field study

Soil arsenic concentration: Arsenic concentration was measured in soil samples collected from the field during both the crop years at the time of sowing, flowering and immediately after harvesting. Initial As concentration in this soil was 8.06 mg kg^{-1}. There was no significant change in this concentration either due to treatments (Check, Control and Rox), crop growth stages (Sowing, Flowering and Harvesting), years (2010 and 2011) or any of their interactions (Fig. 4.2).

Barley crop yield: In 2010, grain, straw and total dry matter (grain + straw) yields of barley increased with the application of poultry litter. The three types of yield in case of Control and Rox treatments were significantly more than the Check, but almost equal amongst themselves. In grain yield, the increase from Check to Control and Check to Rox was 80% and 82% while in straw yield, the increase was 103% and 112%, respectively. During the subsequent year, the residual effect of poultry litter was still very prominent in grain and total dry matter yields where these two yields were significantly more in Control and Rox over Check (Fig. 4.3). Except the straw yield under Check, all other yields were significantly higher in all the three treatments in 2010 (the year of poultry litter application) than one year after (2011). However, there was no visible or statistical effect of As toxicity on barley yield with the application of poultry litter in this Elluviated Black Chernozemic soil.

Arsenic concentration in barley plant parts: During the year of poultry litter application (2010), As concentration in barley grains under Check treatment was below

detection limit (BDL). Under Control and Rox treatments, the As concentrations in grains were 0.01 mg kg^{-1} and 0.06 mg kg^{-1}, respectively (Fig. 4.4) which were significantly different ($P < 0.05\%$). Unfortunately, straw samples were lost, and root samples were not collected that year.

In the year 2011, As concentration was below detection limit in grains in all the three treatments. In straw, As was present under both Control and Rox treatments at a concentration of 0.01 mg kg^{-1}. However, the roots accumulated a noticeable quantity of As under all the treatments. As concentration was 0.33 mg kg^{-1} under Check treatment, 0.53 mg kg^{-1} under Control treatment and 0.82 mg kg^{-1} under Rox treatment, indicating significant increases from Check to Rox and Control to Rox ($P < 0.05$), respectively (Fig. 4.4).

Arsenic uptake by barley plant parts: Uptake of As by barley grains in 2010 under Control treatment was 78 mg ha^{-1} which increased significantly to 359 mg ha^{-1} under Rox treatment. In 2011, barley grains did not show any uptake of As. Barley straw extracted 35 mg ha^{-1} under Control and 46 mg ha^{-1} under Rox treatment. Roots having the maximum concentration, accumulated 596 mg ha^{-1} under Check which increased significantly to 1292 mg ha^{-1} under Control and 1990 mg ha^{-1} under Rox treatment, indicating a significant increase between Control and Rox treatment as well ($P < 0.05$, Fig. 4.5).

4.3.2 Growth chamber study

In the field study, we could not observe the proper compartmentalization of As in soil and plants as the quantity of As added through one agronomic dose of poultry litter

was very low. Hence, this growth chamber experiment was conducted to understand the uptake mechanism of As by the crop plants at elevated soil As level and to see which soil fractions are contributing As to plant uptake in poultry litter-amended cultivated soils. In this experiment, poultry litter was applied to two different soils at the rate of 90 tons ha^{-1}. With that addition of poultry litter, As was enriched by 1.23 mg kg^{-1} soil on top of the native concentrations of As in the two soils. Thereafter, barley and canola plants were grown for 30 days.

Total As concentration in soils: After 30 days of plant growth, soil samples were collected and analyzed for total As concentration. Arsenic concentration was significantly more under Rox treatment than Control both in barley (9.26 mg kg^{-1} verses 8.12 mg kg^{-1}) and canola (9.23 mg kg^{-1} verses 8.11 mg kg^{-1}) in Soil1, and barley crop (8.96 mg kg^{-1} verses 7.99 mg kg^{-1}) in Soil2 (Fig. 4.6). However, total As concentration in soil did not change significantly due to different crops (barley and canola) in Soil1, or different soils (Soil1 and Soil2) under barley.

Arsenic fractionation in soils: With the use of modified sequential extraction procedure of Javed et al. (2013), ten different fractions of As were identified and quantified in the two soils. In Soil1 under barley, As concentration differed significantly between Control and Rox treatments in water soluble fraction (F1), loosely adsorbed - ionically bound fraction (F2), strongly adsorbed fraction (F3), carbonate bound fraction (F4) and crystalline Fe, Al and Mn oxyhydroxides bound fraction (F6) (Fig. 4.7a). The maximum As concentration under Control treatment was observed in F5 (2.36 mg kg^{-1}) followed by F8 (1.73 mg kg^{-1}) and F3 (1.45 mg kg^{-1}) fractions. Under Rox treatment, however, the

maximum concentration of As was found in F5 (2.48 mg kg^{-1}) followed by F3 (2.23 mg kg^{-1}) and F8 (1.68 mg kg^{-1}), respectively.

Under canola in Soil1, As concentration again differed significantly between Control and Rox treatments in F1, F2, F3, F4 and F6 fractions, and only a little bit in all other fractions (Fig. 4.7b). The maximum As concentration under Control treatment was observed in F5 (2.36 mg kg^{-1}) fraction followed by F8 (1.70 mg kg^{-1}) and F3 (1.46 mg kg^{-1}) fractions whereas under Rox treatment, the maximum As concentration was found in F5 (2.47 mg kg^{-1}) followed by F3 (2.15 mg kg^{-1}) and F8 (1.68 mg kg^{-1}) fractions, respectively.

In Soil2 under Barley crop, As concentration followed almost the same trend as in Soil1 being significantly different between Control and Rox treatments in F1, F3, F4 and F6 fractions, and remained unchanged in F5, F7, F8, F9 and F10 fractions (Fig. 4.8). The only exception was fraction F2 wherein As concentration was not significantly different in this soil though it was significant in Soil1 under Barley and Canola. The maximum concentration of As under Control treatment was 2.29 mg kg^{-1} in F5 followed by 1.63 mg kg^{-1} in F3 and 1.45 mg kg^{-1} in F8 whereas under Rox treatment, the maximum As concentration was found in F5 (2.37 mg kg^{-1}) followed by F3 (2.23 mg kg^{-1}) and F8 (1.43 mg kg^{-1}) fractions, respectively.

Shoot and root dry weights: In Soil1, shoot weight of barley and canola, and in Soil2, shoot weight of barley increased significantly with the application of As-rich poultry litter (Rox treatment) as compared to Control treatment (Fig. 4.9a). Unfortunately, canola crop failed in Soil2. Shoot weight of barley was significantly more in Soil1 than Soil2 both in Control and Rox treatments. However, in Soil1, shoot weight of barley and canola

under Control treatment was significantly different (1.64 g pot^{-1} and 2.89 g pot^{-1}) but under Rox, both the crop plants had almost equal shoot weight (5.85 g pot^{-1} and 5.69 g pot^{-1}).

Root weight of barley and canola in Soil1 was significantly more under Rox treatment (2.93 g pot^{-1} and 1.87 g pot^{-1}, respectively) than under control treatment (0.71 g pot^{-1} and 0.92 g pot^{-1}, respectively) (Fig. 4.9b). However, in Soil2, root weight of barley in Control treatment was not significantly different from Rox treatment ($P = 0.16$). In Soil1 under Rox treatment, root weight of canola was significantly more than barley ($P < 0.05$) but under Control treatment, the difference was not significant ($P = 0.48$). Barley root weight in Soil1 under Rox treatment (2.93 g pot^{-1}) was significantly more than the barely root weight (0.73 g pot^{-1}) grown in Soil2 ($P = <0.05$).

Arsenic concentration in shoots and roots: Concentration of As was higher in roots as compared to shoots by one order of magnitude (Fig. 4.10). The shoot As concentration in barley and canola in Soil1, and in barley in Soil2 was significantly more in Rox treatment than Control treatment (Fig. 4.10a). In canola shoots, As concentration was significantly higher than barley shoots in Soil1 both under Control and Rox treatments. However, As concentrations in shoots of barley in Soil1 and Soil2 under Control and Rox treatments were no different being 0.07 and 0.08 mg kg^{-1} in Control and 0.09 and 0.10 mg kg^{-1} in Rox, respectively.

Concentrations of As in roots of barley and canola in Soil1, and barley in Soil2 were significantly more in Rox treatment than Control treatment (Fig. 4.10b). Under Control treatment in Soil1, As concentration in barley and canola roots were almost same being 0.70 mg kg^{-1} and 0.75 mg kg^{-1} but under Rox treatment, canola roots had

significantly more (3.79 mg kg^{-1}) As concentration than barley roots (2.07 mg kg^{-1}). Though the concentration of As increased significantly in the roots of barley grown on Rox treatment as compared to Control treatment, there was no significant effect of soil type on root As concentration being 0.70 mg kg^{-1} in Soil1 and 0.73 mg kg^{-1} in Soil2 in Control treatment, and 2.07 mg kg^{-1} in Soil1 and 2.18 mg kg^{-1} in Soil2 in Rox treatment, respectively.

Arsenic uptake by shoots and roots: Like As concentration, As uptake by roots was more than shoots by one order of magnitude (Fig. 4.11). Uptake of As by shoots of barley in Soil1 and Soil2, and canola in Soil1 was significantly higher under Rox treatment than Control treatment (Fig. 4.11a). In Soil1 under Control treatment, As uptake was significantly more in canola ($27x10^{-5}$ mg pot^{-1}) than barley ($11x10^{-5}$ mg pot^{-1}). Similarly in this soil under Rox treatment, As uptake was significantly more in canola ($67x10^{-5}$ mg pot^{-1}) than barley ($51x10^{-5}$ mg pot^{-1}). For As uptake, barley shoots in Soil1 and Soil2 were no different under Control treatment ($P = 0.07$) but under Rox treatment, barley shoots in Soil1 showed significantly more uptake ($11x10^{-5}$ mg pot^{-1}) than Soil2 ($6x10^{-5}$ mg pot^{-1}).

In roots, uptake of As was much more than shoots. Under Rox treatment, root uptake of As by barley in Soil1 and Soil2 was significantly higher than Control treatment (Fig. 4.11b). Comparing barley and canola in Soil1, uptake of As by barley and canola roots was no different in Control ($5x10^{-4}$ mg pot^{-1} and $7x10^{-4}$ mg pot^{-1}, $P = 0.90$) as well as Rox treatment ($6x10^{-3}$ mg pot^{-1} and $7x10^{-3}$ mg pot^{-1}, $P = 0.54$). However, barley roots under Rox treatment showed significantly more As uptake in Soil1 ($6x10^{-3}$ mg pot^{-1}) than Soil2 ($2x10^{-3}$ mg pot^{-1}).

Phosphorus (P) uptake by shoots and roots: To examine the role of P in As uptake, total P uptake by the shoots and roots was also determined in this study. Rox treatment increased P uptake by shoots significantly over Control treatment in Soil1 under barley and canola, and Soil2 under barley (Fig. 4.12a). Comparing barley and canola in Soil1, P uptake by barley and canola shoots was not significantly different under Control (P = 0.40) as well as under Rox (P = 0.32) treatments. However, under Rox treatment, P uptake by barley was significantly more in Soil1 (31.1 mg pot^{-1}) than Soil2 (13.1 mg pot^{-1}).

In roots of barley and canola, P uptake was significantly more in Rox treatment than Control treatment in Soil1, but in Soil2, Rox treatment could not increase P uptake by barley over Control significantly (P = 0.54). However, P uptake by barley roots under Rox treatment in Soil1 (7.35 mg pot^{-1}) was significantly higher than Soil2 (1.30 mg pot^{-1}).

4.4 Discussion

4.4.1 Field study

Soil As concentration: There was no significant change in soil As concentration either due to treatments (Check, Control and Rox), crop growth stages (Sowing, Flowering and Harvesting), years (2010 and 2011) or any of their interactions (Fig. 4.2). This was because of one agronomic dose of poultry litter application which added only 0.008 mg As kg^{-1} soil in Control treatment (As concentration in Control poultry litter was 1.61 mg kg^{-1}) and 0.136 mg As kg^{-1} soil in Rox treatment (As concentration in Rox poultry litter was 27.29 mg kg^{-1}), which were very small quantities of As added to the soil. That is

why, these quantities could not produce any significant change in As concentration in the soils and crops over time.

Barley crop yield: Barley grain, straw and total dry matter yields increased with the application of poultry litter (Control as well as Rox), and there was no visible or statistical effect of As toxicity on barley yield during the year of application or a year after. Poultry waste contains all essential nutrients including micronutrients (Williams et al., 1999; Chan et al., 2008), and its addition improves the fertility of cultivated soil by increasing the organic matter content, water holding capacity, oxygen diffusion rate and the aggregate stability (Adeli et al., 2009). In Alberta (home to some of the very fertile Chernozemic soils), fertilizers are recommended not only to optimize plant and grain yields but also to improve grain quality (AARD, 2011b). Secondly, in Alberta, growing season is very short (3-4 months), and the land remains under snow for much of the year, therefore, nutrients supplied through poultry litter could not be exhausted completely by barley crop in the year of application. Consequently, some residual effect was also seen in the yield during the subsequent year.

Arsenic uptake by barley: During 2010, in un-amended soil condition (Check treatment), barley plants could not transfer any As to the grains. With application of plain poultry litter (Control treatment) wherein only 0.008 mg As kg^{-1} soil was applied, some As could be mobilized from native soil, that is why, 0.01mg As kg^{-1} grain was present in grains. However, with application of As-rich poultry litter (Rox treatment) wherein 0.136 mg As kg^{-1} soil was applied, plants could take up some As. Though the root and straw data is not available but 0.06 mg As kg^{-1} grain reached the grain. Wiersma et al. (1986) from the Netherlands and Williams et al. (2007) from Scotland reported barley grain As

levels as 0.08 µg g^{-1} (0.08 mg kg^{-1}) and 0.04 µg g^{-1} (0.04 mg kg^{-1}), respectively, which are comparable to the concentration observed in this experiment. As concentration of 0.06 mg kg^{-1} grain is well below the permissible limit for crop plants (1-2 mg kg^{-1}) but whether this concentration will increase or decrease during processing of barley grains for beer or whisky making is not yet known, and needs further investigation because the maximum tolerance for As in fruit juices, fruit nectar and ready-to-serve beverages is 0.1 ppm (mg L^{-1}) in Canada (CFIA, 2013). In 2011, high As concentration in roots as compared to grains or straw indicated that As moved from soil to barley roots but got accumulated there, and only a very small quantity could move up into shoots and grains.

In this field experiment, the maximum amount of applied As (Rox treatment) stayed in soil, a very small amount accumulated in the barley roots, and even smaller quantity reached the grains (359 mg As from 1 ha field soil). Though elevated levels of As in plants grown on contaminated sites have been reported by Mitchell and Barr (1995), Pitten et al. (1999) and Niazi et al. (2011), and on poultry litter amended fields by Ashjaei et al. (2011), the amount that reached the grains in this study was negligible. Moreover, root accumulation of As and very less transfer to the grains indicated towards a defense mechanism operating in barley plant to save itself from As toxicity (Pickering et al., 2000; Raab et al., 2007; Shaibur et al., 2008).

4.4.2 Growth chamber study

Total As concentration in soils: Initial As concentrations in Soil1 and Soil2 were 8.06 mg kg^{-1} and 7.87 mg kg^{-1}, respectively. With the application of Rox poultry litter, 1.23 mg As kg^{-1} soil was added to Rox treated soils. After 30 days of plant growth when soil was analyzed, As concentration was found increased in Rox treated soils (9.26 mg kg^{-1}

under barley and 9.23 mg kg^{-1} under canola in Soil1, and 8.96 mg kg^{-1} under barley in Soil2). However, these concentrations were a little bit lower than the total concentrations (9.29 mg kg^{-1} in Soil1 and 9.10 mg kg^{-1} in Soil2) indicating that some As was taken up by the plants even though it is non-essential for and toxic to plants (Zhao et al., 2009).

Arsenic fractionation in soils: From the data in Fig. 4.7 and Fig. 4.8, some inferences can be drawn. First, upon application of As-rich poultry litter in the two soils, As concentration in F1, F2, F3 and F4 fractions increased significantly over Control. Second, F6 fraction lost its As in Control as well as Rox treated soils, and more so in Rox than Control treatment. Third, As concentration in F5, F7, F8, F9 and F10 fractions was unaffected even after application of As-rich poultry litter and 30 days of plant growth. The overall trend of all these three observations match with our data from Chapter 3 wherein the two soils were incubated in growth chamber without plant growth though the rate of poultry litter application (or As addition) in soils was different in the two experiments. However, two significant differences were also observed in case of F6 and F1 fractions.

In this study with plants, F6 fraction (As co-precipitated with crystalline Fe, Al and Mn oxyhydroxides) constituted 8-12% of the total As present in Control treatment whereas in the previous study (Chapter 3, without plants), F6 fraction constituted 12-16% of the total As present in Control treatment. This observation implied that in the presence of plants, less As was present in F6 fraction. Romheld and Marschner (1986), Fan et al. (2001) and Kumpiene et al. (2012) reported that in case of Fe deficiency in soil, barley root exudates (phytosiderophores) solubilize inorganic Fe^{III} from crystalline Fe oxyhydroxides and take it up for its growth and reproduction. Both the soils used in this

experiment were deficient in available Fe (Appendix F: photographs of Fe deficiency symptoms in barley in the two soils) and a noticeable amount of Fe was taken up by the barley plants from these soils in Control treatment (Appendix G: Fe uptake by the plants) supporting the above observation. It is speculated that As which was also bound to F6 was left behind in soil and might got re-distributed in other fractions (F1, F2, F3 and F4) leaving behind less As in F6 fraction. Romheld and Marschner (1986) reported that non-graminaceous plant species (canola in this case), use different mechanism for acquisition of Fe under Fe deficiency in soil which is not as efficient as phytosiderophores, and hence less As is released from Fe-oxyhydroxides leaving more As in F6 fraction than barley. In this study, however, As concentration in F6 fraction under barley growth in Soil1 was minimum as the demand of Fe was more (maximum dry matter production), followed by canola inSoil1 and barley in Soil2 (minimum dry matter production).

In case of Rox treatment, F6 fraction constituted 5-9% of total As in this study whereas only 0-5% in the previous study (Chapter 3). Production of phytosiderophores by the plants (Romheld and Marschner, 1986), microbial siderophores by the microbial community (Neilands, 1981; Woodridge and Williams, 1993) in the poultry litter, and organic acids (Lee et al., 2009) from the poultry litter, might have increased the solubilization of crystalline Fe-oxyhydroxides leaving behind more As which ultimately got transferred to some other fractions. And, because the rate of poultry litter application in soil in the previous study was very high, the microbial demand of Fe would be much higher. In this way, more As will be left behind in soil in other fractions.

Concentration of As in F1 fraction was 28 - 31% in poultry litter-amended uncultivated soil (previous study) but with the plants in it, As concentration in F1 fraction

got reduced to 2- 3% only. The reason for this finding can be explained on the basis of a report by Zhao et al. (2009) who mentioned that in aerobic conditions, As is present in soil as arsenate (As^{V}) which is taken up by the plants with phosphate transporters. In root cells, arsenate is rapidly reduced to arsenite (As^{III}). Some of this arsenite is translocated to shoots but more of it is complexed by thiol peptides and sequestered in the root vacuole itself. But most of the arsenite is effluxed to the external medium (soil in this case). In our study, all these results indicated that the maximum contribution of As for the plant uptake was done by F1 fraction, consequently As concentration in F1 got reduced, and effluxing of As from plant roots increased As concentration in F2, F3 or F4 fractions.

Shoot and root dry weights: In this study, a higher dose of poultry litter was applied to the soil to mimic the build-up of As in soil due to repeated application of poultry litter on the same parcel of land year after year. This time, As-rich poultry litter was added at 90 ton ha^{-1} rather than normal 10 ton ha^{-1}. In this way, N and many other essential nutrients were supplied in much more quantity than desired for normal crop production. Fresh and dry weights of shoots and roots of barley and canola in Soil1 and barley in Soil2 increased with application of poultry litter. However, response of canola to high dose of nutrients was less than barley in Soil1, and in Soil2 it did not grow even. It is speculated that excessive dose of poultry litter in soil produced large amounts of uric acid on decomposition which got converted to ammonia gas (Ritz et al., 2004) that killed the canola plants in Soil2. However in Soil1, canola plants survived and grew well may be because of the favorable inherent characteristics of this soil than Soil2 (Table 3.1).

P uptake by shoots and roots: Chicken feeds are high energy diets (Appendix A) formulated to help them attain optimum genetic potential in growth and feed efficiency as

well as skeletal development (Applegate and Angel, 2008). Poultry litter becomes rich in P (Mullins et al., 2002) as some of P is excreted back by chickens during their stay in the barn. Phosphorus is an essential nutrient for plant growth, hence application of poultry litter (Rox treatment) increased P uptake by the roots and shoots of the plants. Phosphorus accumulation was much more in shoots than roots because the roots transfer P to young plant parts (Shen et al., 2011) where it is needed for the development of new cells and for the transfer of genetic code from one cell to another (IPNI, 1999). Uptake of P by both roots and shoots was the highest in canola in Soil1, followed by barley in Soil1 and Soil2. This might be because the P requirement of oilseeds is higher than that of cereals as P is involved in the synthesis of energy rich oils and proteins (Sahrawat and Islam, 1989).

Arsenic uptake by shoots and roots: In poultry litter-amended soil (Rox treatment), much of As was present in water extractable form (inferred from As fractionation study), which was easily available for plant uptake (Ashjaei et al., 2011). Proliferating tap roots of canola extracted comparatively more As from soil than fibrous roots of barley. Comparing the effect of soil, barley roots extracted more As from Soil1 than Soil2. This was so because the root weight of barley in Soil1 was more than Soil2 even though the concentration of As in barley roots of Soil2 was more than Soil1.

In shoots, uptake of As was much lower than roots indicating limited translocation of As from roots to shoots. The probable explanation of limited translocation of As from roots to shoots is that in root cells, arsenate is rapidly reduced to arsenite which gets complexed with thiols and possibly gets sequestered in the root vacuoles (Zhao et al., 2009). Furthermore, a very strong correlation was observed

between P uptake and As uptake in roots (R^2 = 0.90) and shoots (R^2 = 0.89) (Fig. 4.13) indicating that it is P which facilitates As uptake from soil to roots and from roots to shoots. This observation is in agreement with Meharg et al. (1994) who reported on the basis of physiological and electrophysiological studies that arsenate and phosphate share the same transport pathway even though the transporters have a higher affinity for phosphate than for arsenate.

Root uptake of As was very low in all the three cases which varied from $2x10^{-3}$ mg pot^{-1} to $7x10^{-3}$ mg pot^{-1}. Shoot uptake of As was there but that was almost negligible (varied from $6x10^{-5}$ mg pot^{-1} to $67x10^{-5}$ mg pot^{-1}). Chances of As accumulation by edible plant parts seemed to be even lower. Almost all the As supplied through poultry litter was found in soil being present in water soluble (F1), loosely adsorbed (F2), strongly adsorbed (F3) and carbonate bound (F4) fractions.

4.5 Conclusions

With one application of agronomic dose of As-rich poultry litter in the field, yield of barley grain, straw and total dry matter increased in Elluviated Black Chernozemic soil. Concentration of As in the grains increased but it was well below the food standards during the first year of poultry litter application, and in the subsequent year, As concentration in barley grains was below detection limit. In the growth chamber study wherein a high dose of As-rich poultry litter was applied in the soils (to mimic the As accumulation in soil due to repeated application of As-rich poultry litter) and the plants were raised for 30 days, the yield of barley and canola, and uptake of As increased in their roots and shoots. Most of the applied As remained in soil even though a large quantity of As was present in soil in water soluble (easily available) fraction. However, as

the plants do not stop growing at this stage, they will continue extracting nutrients, particularly P along with As from the soil which means that As concentration may definitely go up with the crop age. As barley grains are processed further for beer and whisky making, and canola grains for oil extraction, even low concentration of As in the grains may help cross the limit of 0.1 ppm set for ready-to-serve beverages. This aspect needs to be explored further before taking final decision regarding the use of As-rich poultry litter on agricultural fields. It is also recommended that before applying poultry litter to any soil, proper consideration should be given to the As content of the poultry litter, type of crop to be raised and type of soil to be used because in light textured soil, availability of As to plants and its downward mobility was found to be more. Some plants (with high P requirements) may take up more As than others, and accumulate it in their roots, stems, leaves or the edible parts thus facilitating its entry in our food chain. And, high mobility of water soluble As in light textured soil may contaminate our waters over the years.

Tables and Figures

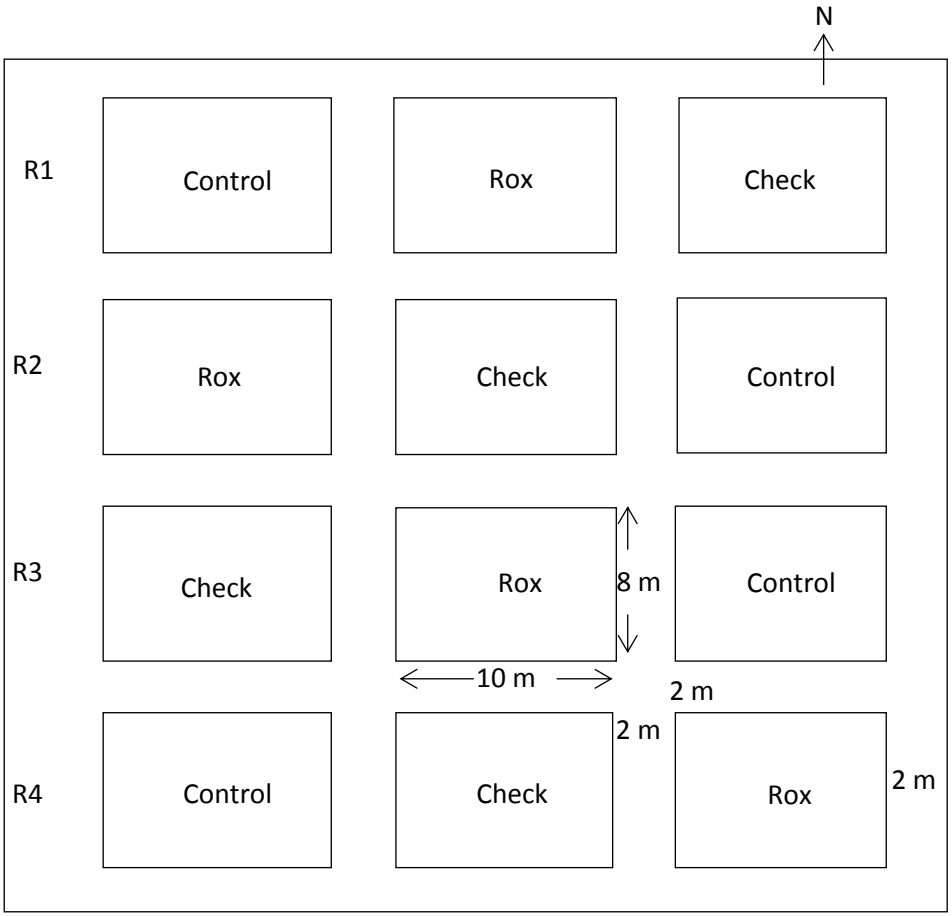


Fig. 4.1 Schematic diagram showing field layout for the Field Study. Twelve equal plots of 80 m^2 each accommodating four replications of three treatments (Check - no poultry litter addition, Control - poultry litter of chickens not fed with roxarsone in the feed, Rox - poultry litter of chickens fed with roxarsone in the feed).

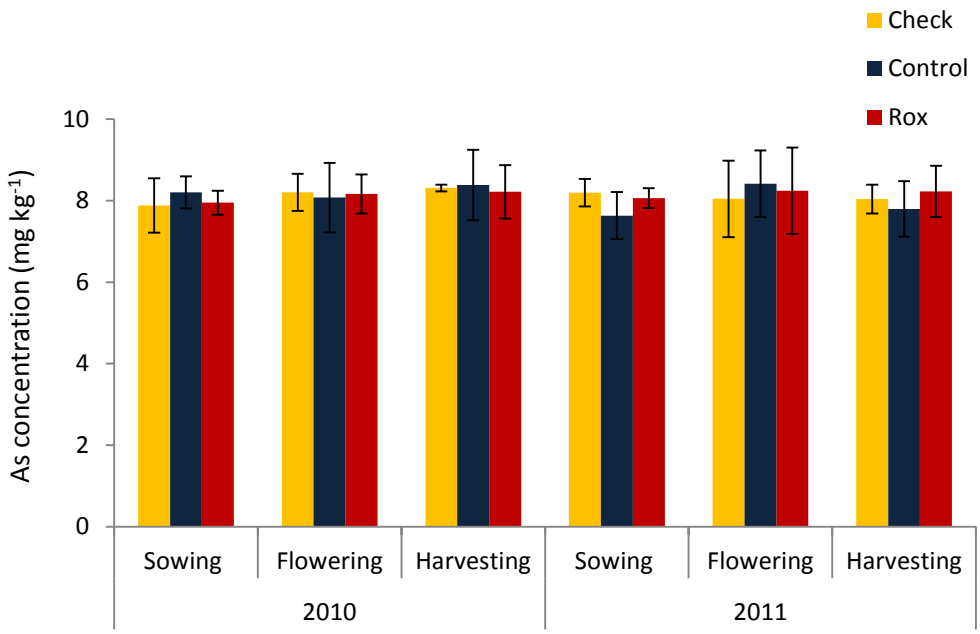


Fig. 4.2 Soil As concentration after three critical crop growth stages (sowing, flowering and harvesting) determined in two crop years (2010 and 2011) for Field Study. Error bars represent ± standard deviations around the mean values.

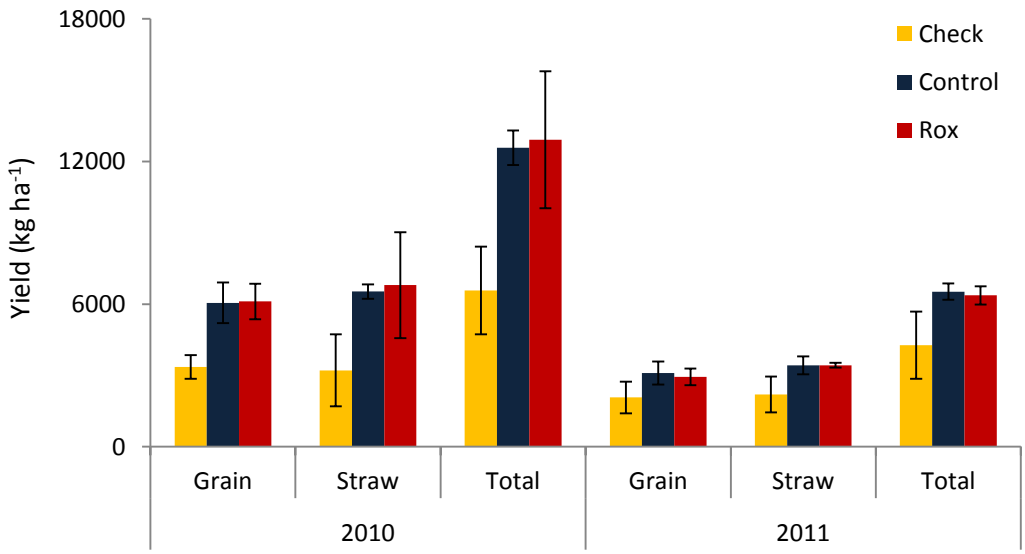
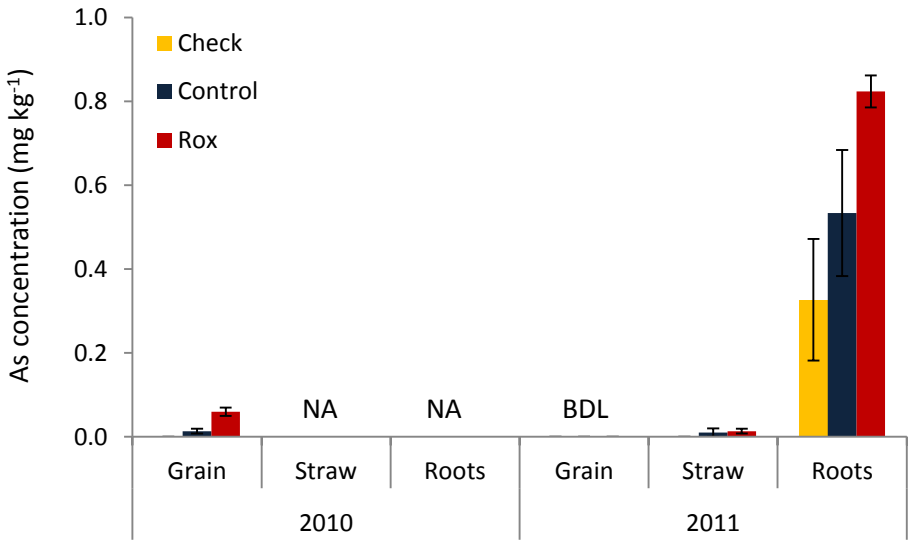
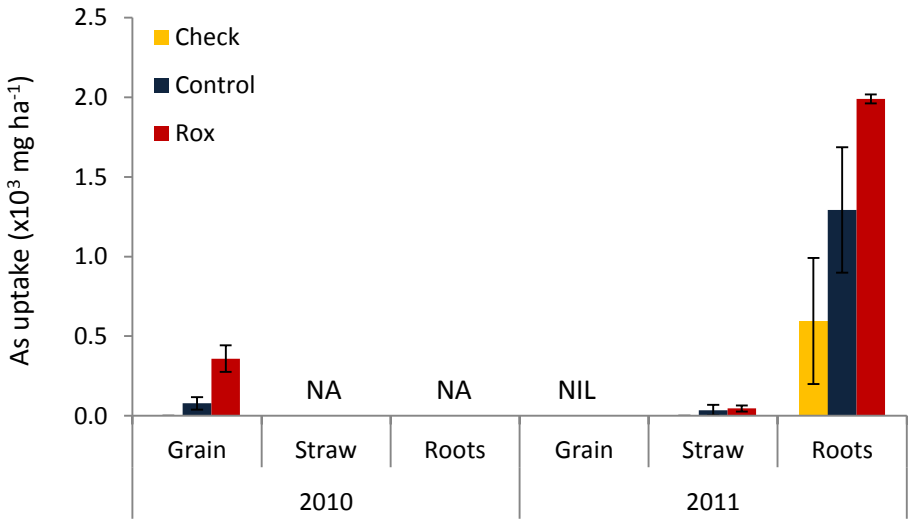


Fig. 4.3 Yields of barley - Grain, Straw and Total dry matter (Grain + Straw) as affected by the three treatments (Check, Control and Rox) measured in 2010 and 2011 for Field Study. Error bars represent ± standard deviations around the mean values.



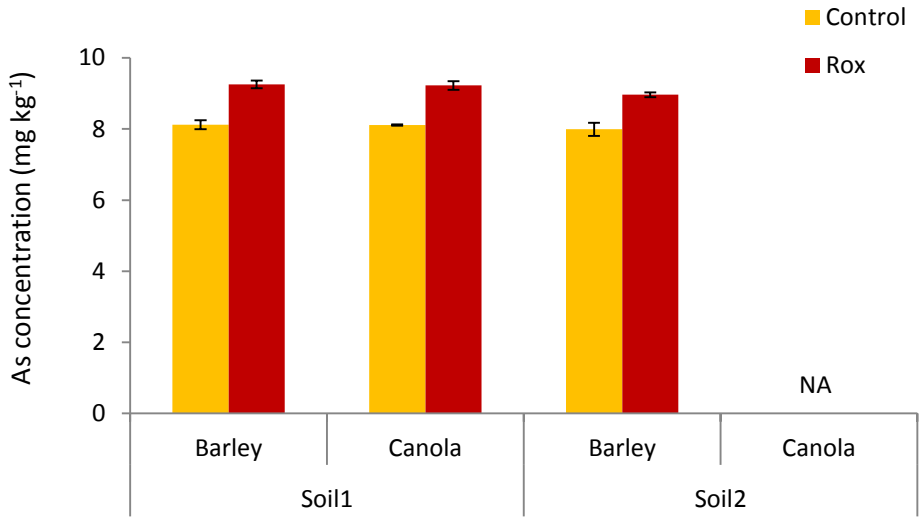
NA - Not Applicable (Straw samples lost; Root samples not collected in 2010)
BDL - Below Detection Limit

Fig. 4.4 Arsenic concentration in barley plant parts (Grain, Straw and Roots) as affected by the three treatments (Check, Control and Rox) determined in 2010 and 2011 crops for Field Study. Error bars represent ± standard deviations around the mean values.



NA - Not Applicable (Straw samples lost; Root samples not collected in 2010)
NIL - No data value because of BDL concentration

Fig. 4.5 Arsenic uptake by barley plant parts (Grain, Straw and Roots) as affected by the three treatments (Check, Control and Rox) calculated for 2010 and 2011 crops for Field Study. Error bars represent ± standard deviations around the mean values.



NA - Not Applicable (Canola plants could not grow in Soil2 with Rox treatment)

Fig. 4.6 Total As concentration in two soils determined after 30 days of barley and canola plant growth with Control and Rox treatments of poultry litter in the Growth Chamber. Error bars represent ± standard deviations around the mean values.

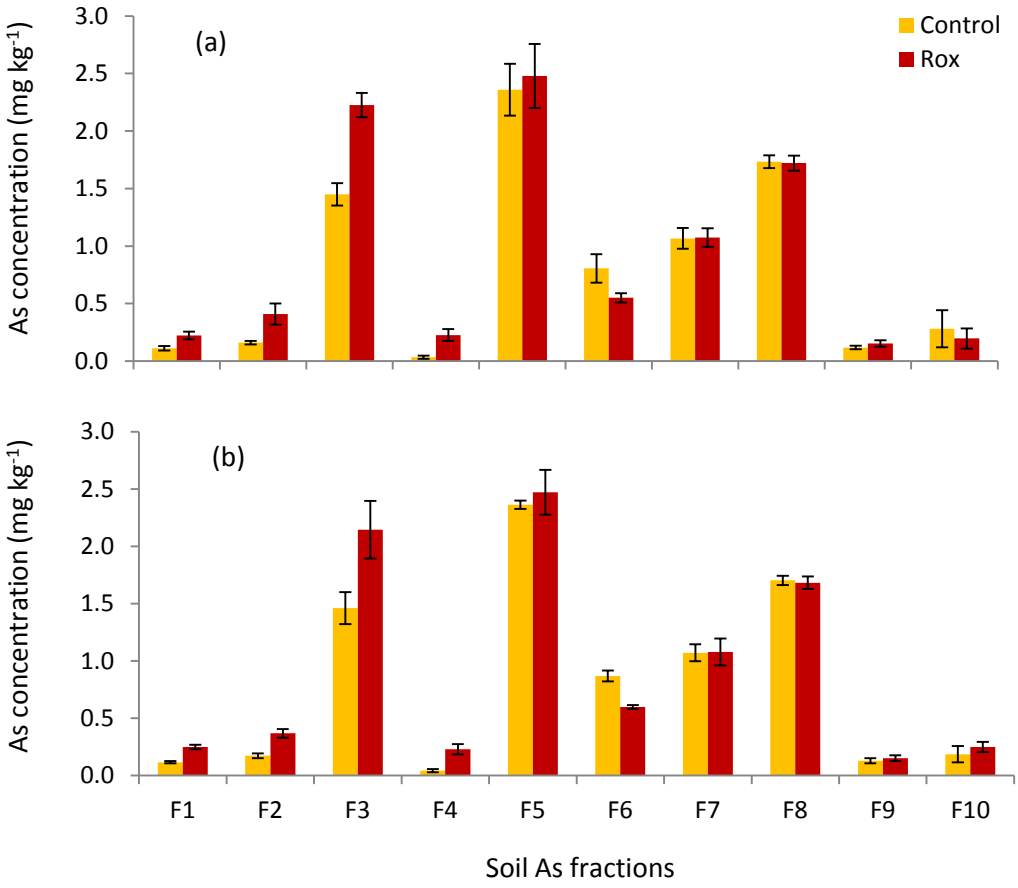


Fig. 4.7 Concentration of As in different soil As factions in Soil1 determined after 30 days of a) barley and b) canola plant growth under Control and Rox treatments of poultry litter in the Growth Chamber. Error bars represent ± standard deviations around the mean values.

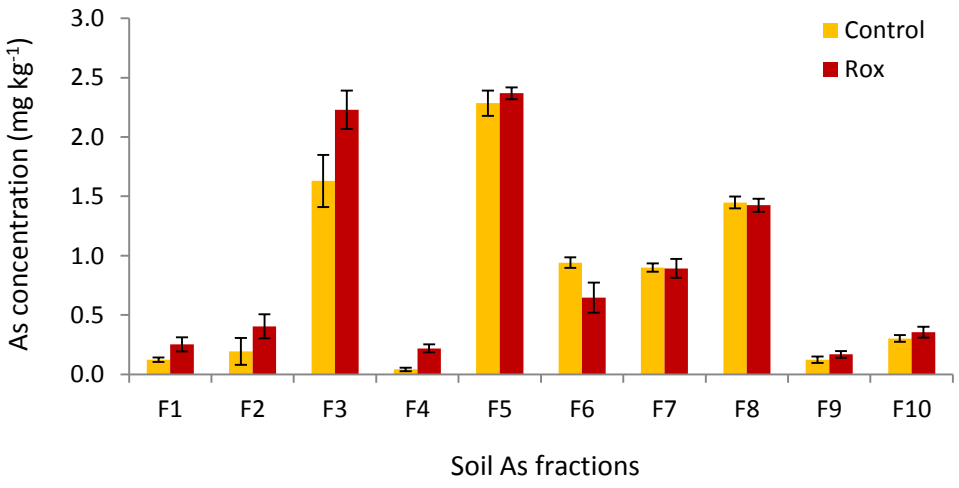
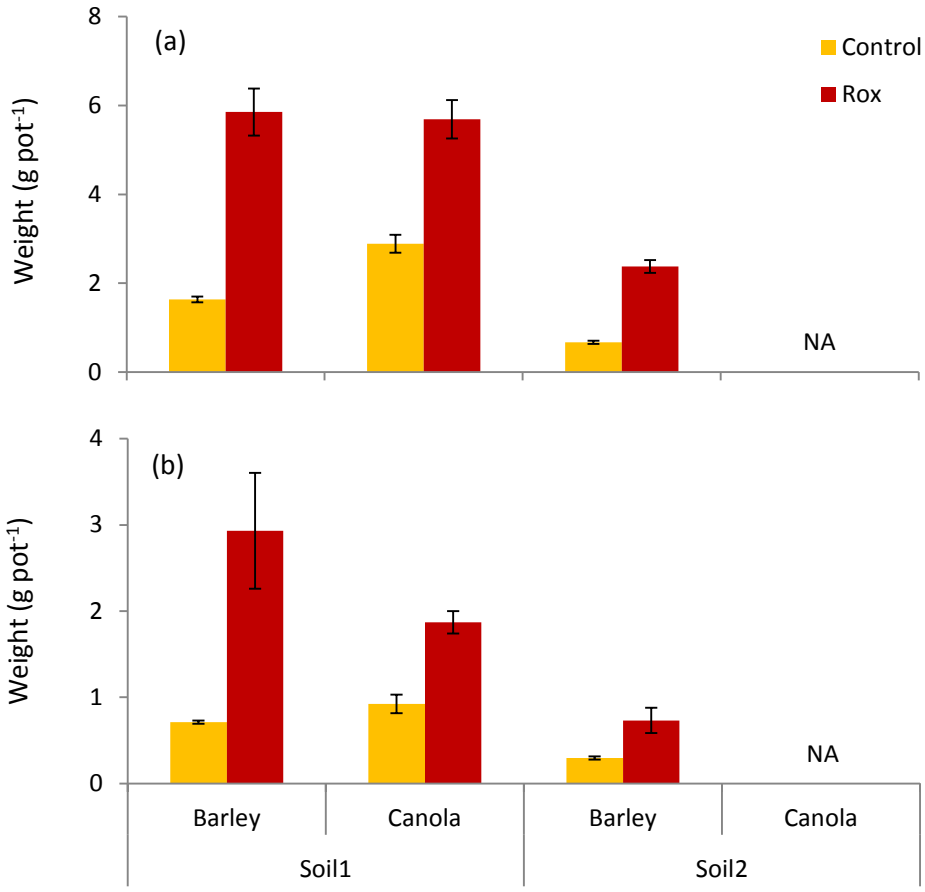
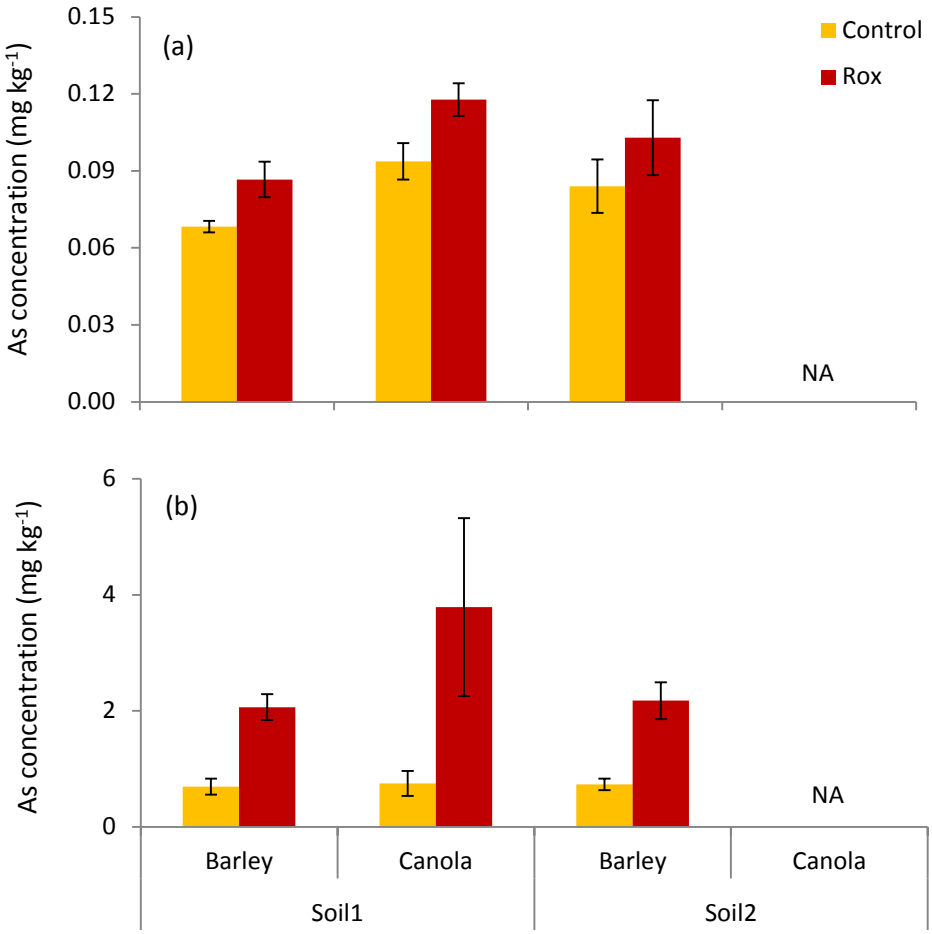


Fig. 4.8 Concentration of As in different soil As fractions in Soil2 determined after 30 days of barley plant growth under Control and Rox treatments of poultry litter in the Growth Chamber. Error bars represent ± standard deviations around the mean values.



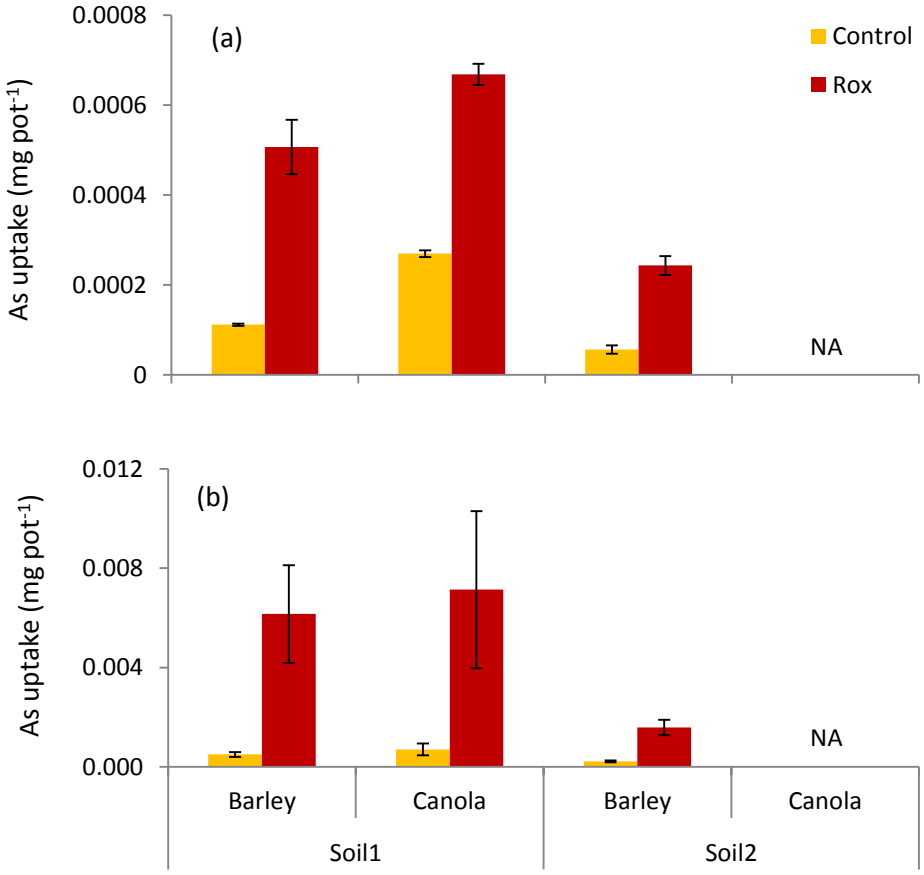
NA - Not Applicable (Canola plants could not grow in Soil2 with Rox treatment)

Fig. 4.9 Dry weights of a) Shoots and b) Roots of barley and canola plants as affected by Control and Rox treatments of poultry litter in two soils measured after 30 days of plant growth in Growth Chamber. Error bars represent ± standard deviations around the mean values.



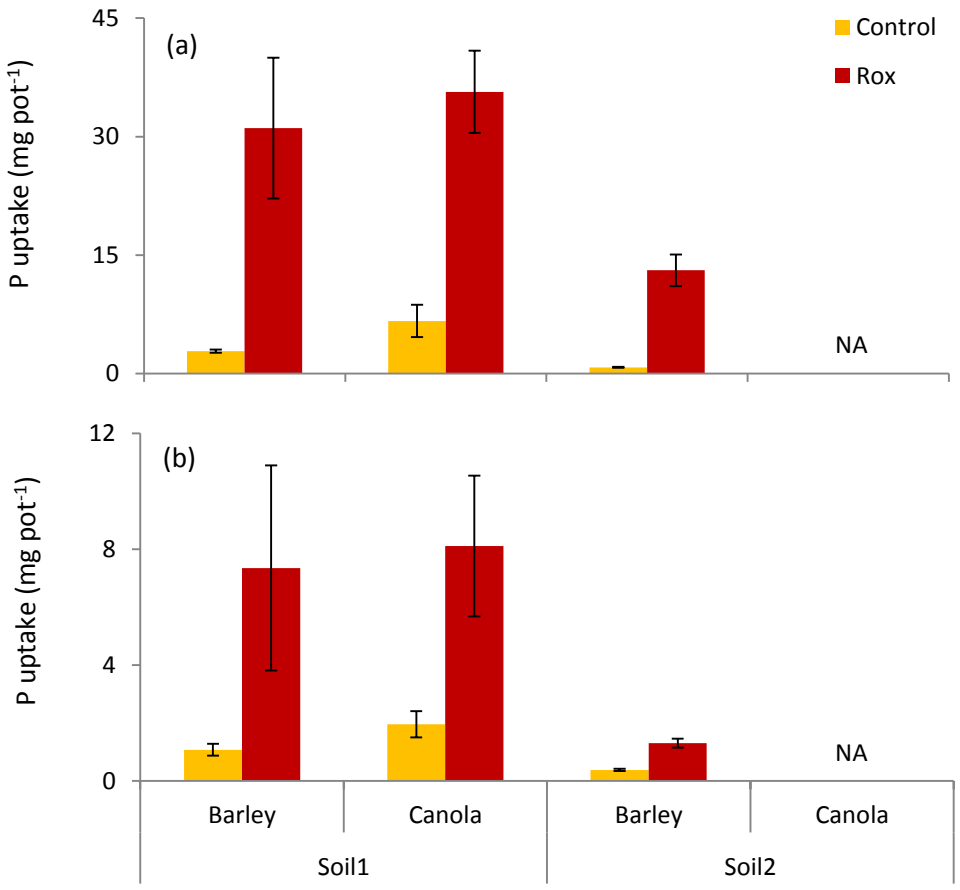
NA - Not Applicable (Canola plants could not grow in Soil2 with Rox treatment)

Fig. 4.10 Arsenic concentration in a) Shoots and b) Roots of barley and canola plants as affected by Control and Rox treatments of poultry litter in two soils determined after 30 days of plant growth in Growth Chamber. Error bars represent ± standard deviations around the mean values.



NA - Not Applicable (Canola plants could not grow in Soil2 with Rox treatment)

Fig. 4.11 Arsenic uptake by a) Shoots and b) Roots of barley and canola plants as affected by Control and Rox treatments of poultry litter in two soils calculated after 30 days of plant growth in Growth Chamber. Error bars represent ± standard deviations around the mean values.



NA - Not Applicable (Canola plants could not grow in Soil2 with Rox treatment)

Fig. 4.12 Phosphorus uptake by a) Shoots and b) Roots of barley and canola plants as affected by Control and Rox treatments of poultry litter in two soils calculated after 30 days of plant growth in Growth Chamber. Error bars represent ± standard deviations around the mean values.

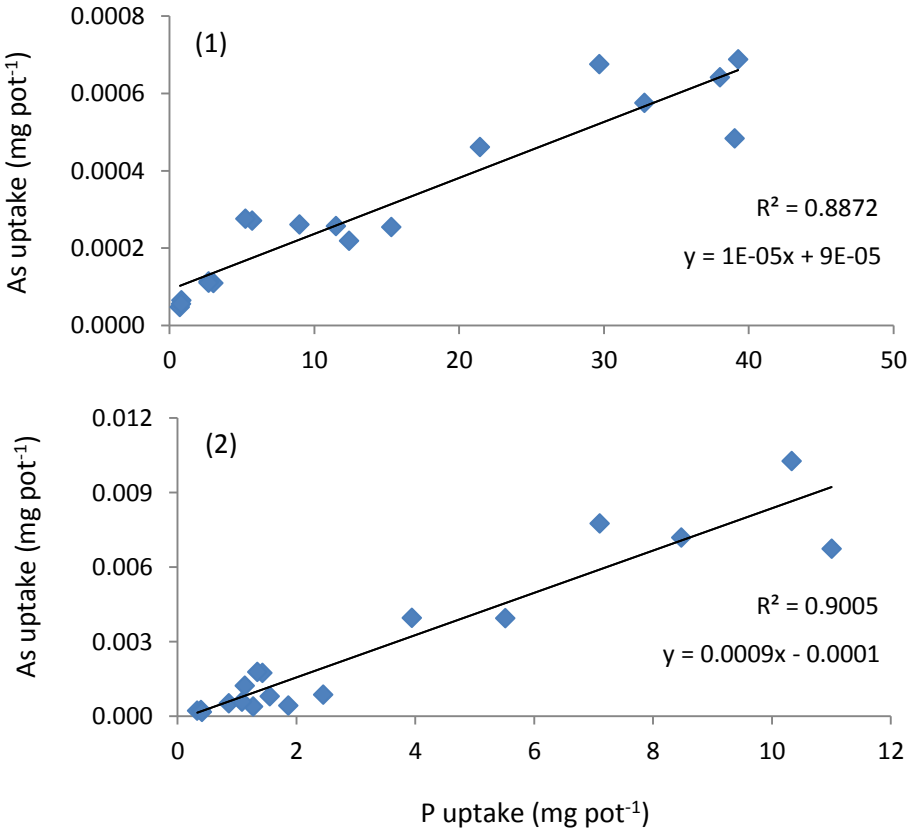


Fig. 4.13 Correlation between As and P uptake in a) Shoots and b) Roots of plants determined for the Growth Chamber study.

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Chapter 5: Conclusions

Arsenic (As) is a toxic trace element and it is carcinogenic in humans. While As enters the environment from natural sources, its concentration is further enriched by anthropogenic activities. Therefore, for efficient and cost effective management of As, it is imperative that the sources of As contamination be traced, its extent and severity be defined, and finally remedial action be implemented (Naidu et al., 2006).

Poultry litter is a waste from poultry industry but it is one of the best organic fertilizers available. Poultry litter is enriched with As because of the use of roxarsone as a poultry feed additive. This poultry litter is applied to agricultural fields as fertilizer which could contaminate soil with As; and the As may end up in crops, food and water bodies. However, with little data available in public domain regarding degradation or transformation of roxarsone after addition in the poultry feed, after excretion of roxarsone in the poultry litter or after addition of As-rich poultry litter in agricultural soil it became imperative to understand the retention/release mechanisms of As in chicken body, poultry litter/manure and agricultural soils so that an informed decision about roxarsone and its use in the poultry industry as well as the disposal of As-rich poultry litter on agricultural land could be taken.

This dissertation is a part of a multi-disciplinary research project. In here, my specific goal was to assess the fate of As from poultry feed to agricultural crops. The research objectives were to: 1) evaluate transference of As from poultry feed to poultry manure and to calculate indirectly how much As is being retained by the chicken body; 2) study fate of As in soils amended with poultry litter; and 3) investigate As uptake by crop plants from poultry litter amended soils.

5.1. Research findings

The first experiment described in Chapter 2, was designed to calculate the amount of As chickens take in with feed, and the amount of As they excrete into the manure. It was confirmed that the poultry manure from chickens fed with roxarsone was indeed richer in As (27-28 mg kg^{-1}) than that from those chickens who were not fed roxarsone (<2 mg kg^{-1}). Mass balance calculations revealed that 80% of the ingested As was excreted in to the poultry manure leaving 20% un-accounted. Three possible destinations were thought of for this un-accounted As: a) accumulation in chicken body (as reported by Kawalek et al., 2011), b) volatilization loss (as was observed during our lab experiment), or c) experimental error (because of sub-sampling and dilutions during analysis in the laboratory).

In the second experiment described in Chapter 3, fate of As in poultry litter amended soils was investigated. Two different soils having loam and silt-loam textures were treated with litter and incubated in a growth chamber. After six weeks, soil samples were collected from two depths, and analyzed for total As concentration. All these samples were also analyzed using sequential extraction procedure to quantify As associated with different solid constituents in soil. Results informed that application of As-rich poultry litter increased the concentration of labile pool of As (water soluble + exchangeable) in soil; and mobility and leaching of As in loam soil was more than silt-loam soil. Silt-loam soil retained more As in upper layer (74% of the applied As through poultry litter). Plant uptake of As may be less in such soil in the short term but As will continue to accumulate with further application of poultry litter. In the long term, if this As becomes mobile due to changed environmental conditions, it can be a big concern of

As toxicity through food and groundwater. Loamy soil, on the other hand, contributed more As to the lower depth (29% of the applied As through poultry litter). Higher mobility of As in such soil may initiate more uptake by plants and more leaching to groundwater thus becoming an immediate concern.

In the first part of the third experiment described in Chapter 4, barley was grown in the field in Elluviated Black Chernozem soil till maturity with one application of agronomic dose of As-rich poultry litter (10 tons ha^{-1}). Concentration of As in grains increased to 0.06 mg kg^{-1} which was 17 times less than the critical limit of food items (1 mg kg^{-1}). Secondly, As accumulation was more in roots, less in shoots and the least in grains indicating As sequestration in roots and limited translocation to shoots and grains.

In growth chamber study, a high dose of As-rich poultry litter was applied in the soils to mimic As accumulation in soil due to repeated application of As-rich poultry litter, and the plants were raised for 30 days. Higher uptake of As was observed in canola than barley (may be because more phosphorus uptake of an oil-seed plant facilitated more As uptake). Secondly, higher uptake of As was recorded in loam soil than silt-loam soil.

Overall, it can be summarized that out of the total As fed to the chickens, 80% was excreted in to the poultry manure; after application of As-rich poultry litter in soil, major portion of As was present in water soluble fraction; and very minute quantity (0.06 mg kg^{-1}) of As was taken up by the barley grains after one application of agronomic dose of As-rich poultry litter in the agricultural field.

5.2 Recommendations

As far as the responsible use of As-rich poultry litter in agricultural fields is concerned, it is recommended that before application, proper consideration should be

given to the As content of the poultry litter, the type of soil to be used and the type of crop to be raised. In light textured soil, availability of As to plants and its downward mobility was found higher. Some plants with high P requirements may take up more As, and accumulate in their edible parts thus facilitating its entry in to our food chain.

5.3 Future research and the big question

It would be interesting to quantify the different As species in the poultry litter, litter-amended soils and plants to estimate mobility and toxicity of As. Secondly, different crop plants could be grown till maturity on agricultural fields where As-rich poultry litter is being applied continuously for years to better understand the As uptake.

This suggested future work along with the findings of the team from the Department of Laboratory Medicine and Pathology (who is studying the accumulation of As in different body parts of the chicken) will help decide whether to continue the use of roxarsone in poultry feed or not. The approach used in this thesis to determine As accumulation in chicken body was an indirect one. However, the data generated in this thesis will prove to be a very good reference material for any such decision making process.

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Appendix A

Composition of poultry feed

Ingredient (%)	Control			Roxarsone		
	Starter*	Grower**	Finisher***	Starter	Grower	Finisher
Corn, Yellow Grain	18.009	18.009	15.008	18.005	18.005	15.004
Fat, Vegetable	3.775	3.365	4.131	3.774	3.364	4.130
Fish Meal, Menhaden	3.002	5.003	3.509	3.001	5.001	3.508
Soybean Meal, Deh-Plant 1	26.880	16.221	15.105	26.873	16.217	15.102
Wheat, Hard Grain	42.952	53.263	58.074	42.941	53.250	58.059
Calcium Carbonate	1.501	1.048	1.066	1.500	1.048	1.066
Dicalcium Phosphate	1.546	1.005	1.081	1.546	1.005	1.081
Sodium Chloride	0.426	0.337	0.358	0.426	0.337	0.358
L - Lysine	0.232	0.151	0.154	0.232	0.151	0.154
DL - Methionine	0.229	0.096	0.089	0.229	0.096	0.089
L - Threonine	0.048	0.101	0.025	0.048	0.101	0.025
Broiler Vitamin Premix (0.5% inclusion)	0.500	0.500	0.500	0.500	0.500	0.500
Choline Chloride Premix (0.5% inclusion)	0.500	0.500	0.500	0.500	0.500	0.500
Vitamin E 5000 IU kg^{-1}	0.300	0.300	0.300	0.300	0.300	0.300
Generic Enzyme (0.5% inclusion)	0.050	0.050	0.050	0.050	0.050	0.050
Coccidiostat (Amprol)	0.050	0.050	0.050	0.050	0.050	0.050
Growth Promoter (Roxarsone)	0.000	0.000	0.000	**0.005**	**0.005**	0.000

Starter*: 0-14 days, Grower**: 15-28 days, Finisher***: 29-35 days

Appendix B

Mixed Model Analysis for Total Arsenic Intake under Rox Treatment

Model Dimension

	Parameter
Fixed effects	Strain
	Time
	Strain x Time
Random effects	Pan
Repeated effects	Time

Tests of Fixed effects

Source	df	F	Sig.	
Strain	1	1.521	0.243	NS
Time	1	13707.492	0.000	*
Strain x Time	1	4.638	0.061	NS

Pairwise comparisons

Time	Strain	df	F	Sig.	
14 Day	Cobb:Ross	1	3.539	1.000	NS
28 Day	Cobb:Ross	1	2.944	0.121	NS

Pairwise comparisons

Strain	Time	df	F	Sig.	
Cobb	14 Day:28 Day	1	7108.212	0.000	*
Ross	14 Day:28 Day	1	6603.919	0.000	*

NS - Non significant

* - The mean difference is significant at 0.05 level

Dependent variable = Total Arsenic Intake (mg chicken^{-1})

Adjustment of multiple comparisons = LSD (Least Significant Difference)

Appendix C

Mixed Model Analysis for arsenic concentration in poultry manure

Model Dimension

	Parameter
Fixed effects	Strain
	Treatment
	Time
	Strain x Treatment x Time
Random effects	Pan
Repeated effects	Time

Tests of Fixed Effects

Source	df	F	Sig.	
Strain	1	0.014	0.908	NS
Treatment	1	1013.178	0.000	*
Time	3	46.785	0.000	*
Strain x Treatment x Time	10	16.323	0.000	*

Pairwise comparisons

Treatment	Time	Strain	df	F	Sig.	
Control	14 Day	Cobb:Ross	1	0.024	0.879	NS
	28 Day	Cobb:Ross	1	0.001	0.974	NS
	35 Day	Cobb:Ross	1	0.408	0.535	NS
	37 Day	Cobb:Ross	1	0.006	0.939	NS
Rox	14 Day	Cobb:Ross	1	0.017	0.898	NS
	28 Day	Cobb:Ross	1	1.103	0.314	NS
	35 Day	Cobb:Ross	1	35.101	0.000	*
	37 Day	Cobb:Ross	1	0.576	0.462	NS

Pairwise comparisons

Strain	Time	Treatment	df	F	Sig.	
Cobb	14 Day	Control:Rox	1	49.35	0.000	*
	28 Day	Control:Rox	1	312.824	0.000	*
	35 Day	Control:Rox	1	1159.501	0.000	*
	37 Day	Control:Rox	1	708.886	0.000	*
Ross	14 Day	Control:Rox	1	45.404	0.000	*
	28 Day	Control:Rox	1	277.886	0.000	*
	35 Day	Control:Rox	1	1547.425	0.000	*
	37 Day	Control:Rox	1	664.967	0.000	*

Pairwise comparisons

Strain	Treatment	Time	Sig.	
Cobb	Control	14 day:28 day	0.725	NS
		14 day:35 day	0.771	NS
		14 day:37 day	0.787	NS
		28 day:35 day	0.853	NS
		28 day:37 day	0.830	NS
		35 day:37 day	9.333	NS
	Rox	14 day:28 day	0.001	*
		14 day:35 day	0.010	*
		14 day:37 day	0.022	*
		28 day:35 day	0.000	*
		28 day:37 day	0.000	*
		35 day:37 day	0.075	NS
Ross	Control	14 day:28 day	0.889	NS
		14 day:35 day	0.844	NS
		14 day:37 day	0.937	NS
		28 day:35 day	0.943	NS
		28 day:37 day	0.892	NS
		35 day:37 day	0.603	NS
	Rox	14 day:28 day	0.005	*
		14 day:35 day	0.106	NS
		14 day:37 day	0.021	*
		28 day:35 day	0.000	*
		28 day:37 day	0.000	*
		35 day:37 day	0.001	*

NS - Non significant

* - The mean difference is significant at 0.05 level

Dependent variable = Arsenic concentration (mg kg^{-1})

Adjustment of multiple comparisons = LSD (Least Significant Difference)

Appendix D

Mixed Model Analysis for arsenic excretion under Rox Treatment

Model Dimension

	Parameter
Fixed effects	Strain
	Time
	Strain x Time
Random effects	Pan
Repeated effects	Time

Tests of Fixed Effects

Source	df	F	Sig.	
Strain	1	0.035	0.857	NS
Time	3	1248.875	0.000	*
Strain x Time	3	6.766	0.011	*

Pairwise comparisons

Time	Strain	df	F	Sig.	
14 day	Cobb:Ross	1	0.681	0.437	NS
28 day	Cobb:Ross	1	3.351	0.106	NS
35 day	Cobb:Ross	1	8.821	0.023	*
37 day	Cobb:Ross	1	0.526	0.489	NS

Pairwise comparisons

Strain	Time	Sig.	
Cobb	14 day:28 day	0.000	*
	14 day:35 day	0.000	*
	14 day:37 day	0.000	*
	28 day:35 day	0.470	NS
	28 day:37 day	0.892	NS
	35 day:37 day	0.234	NS
Ross	14 day:28 day	0.000	*
	14 day:35 day	0.000	*
	14 day:37 day	0.000	*
	28 day:35 day	0.001	*
	28 day:37 day	0.062	NS
	35 day:37 day	0.006	*

NS - Non significant

* - The mean difference is significant at 0.05 level

Dependent variable = Arsenic excretion (mg chicken^{-1})

Adjustment of multiple comparisons = LSD (Least Significant Difference)

Appendix E

ANOVA table for total As concentrations in soils

Tests of Between-Subjects Effects

Source	df	SS	MS	F	Sig.	
Soil	1	0.101	0.101	2.618	0.144	NS
Depth	1	17.988	17.988	466.504	0.000	*
Treat	1	89.932	89.932	2332.289	0.000	*
Soil x Depth x Treatment	4	19.637	4.909	127.313	0.000	*
Error	8	0.308	0.039			

Pairwise comparisons

Soil	Treatment	Depth	SS	df	MS	F	Sig.	
Soil1	Control	D1:D2	0.008	1	0.008	0.203	0.664	NS
Soil1	Rox	D1:D2	23.232	1	23.232	602.507	0.000	*
Soil2	Control	D1:D2	0.002	1	0.002	0.044	0.840	NS
Soil2	Rox	D1:D2	14.379	1	14.379	372.91	0.000	*

Pairwise comparisons

Depth	Treatment	Soil	SS	df	MS	F	Sig.	
D1	Control	Soil1:Soil2	0.027	1	0.027	0.706	0.425	NS
D1	Rox	Soil1:Soil2	0.413	1	0.413	10.722	0.011	*
D2	Control	Soil1:Soil2	0.045	1	0.045	1.171	0.311	NS
D2	Rox	Soil1:Soil2	0.148	1	0.148	3.844	0.086	NS

Pairwise comparisons

Depth	Soil	Treatment	SS	df	MS	F	Sig.	
D1	Soil1	Control: Rox	51.352	1	51.352	1331.746	0.000	*
D1	Soil2	Control: Rox	44.729	1	44.729	1160.007	0.000	*
D2	Soil1	Control: Rox	5.096	1	5.096	132.167	0.000	*
D2	Soil2	Control: Rox	8.151	1	8.151	211.388	0.000	*

NS - Non significant

* - The mean difference is significant at 0.05 level

Dependent variable= As concentration (mg kg^{-1})

Adjustment of multiple comparisons= LSD (Least Significant Difference)

Appendix F

Iron **(**Fe) deficiency symptoms in barley in Soil1 (a) and (b), and Soil2 (c)

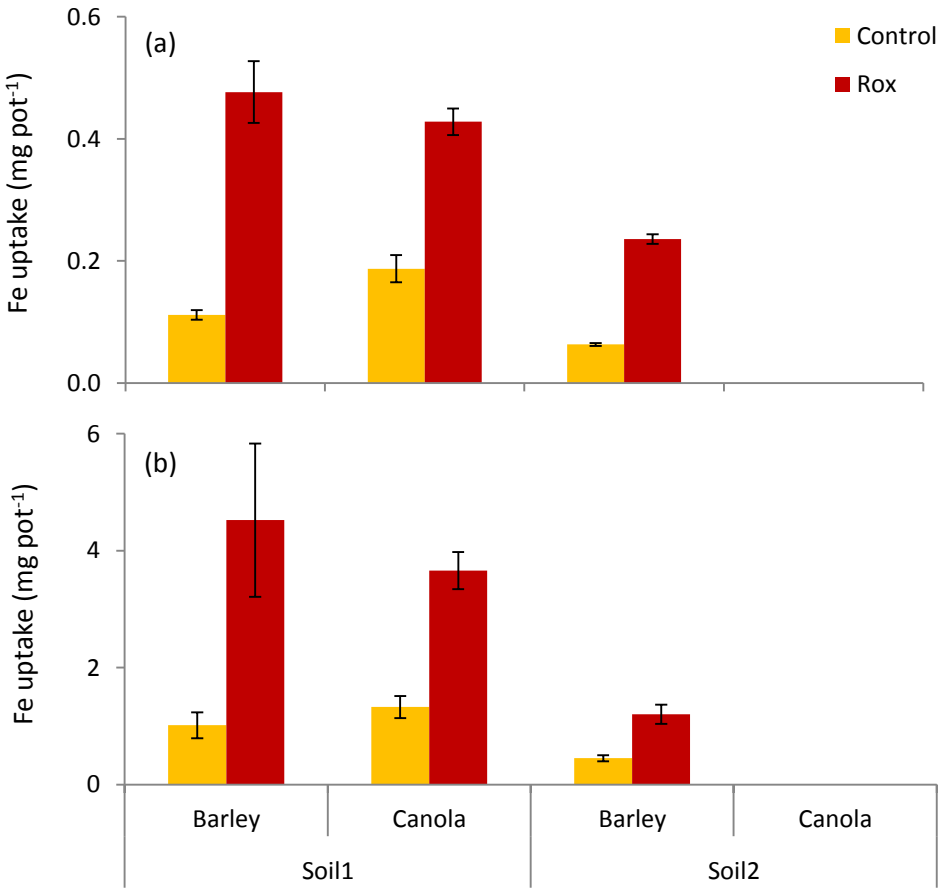
(a)

(b)

(c)

Appendix G

Iron (Fe) uptake by plant a) Shoots and b) Roots



Appendix H

Permission Letter

UNIVERSITY OF ALBERTA

DEPARTMENT OF AGRICULTURAL, FOOD AND NUTRITIONAL SCIENCE
FACULTY OF AGRICULTURAL, LIFE & ENVIRONMENTAL SCIENCES

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I, Martin J. Zuidhof, team leader of the research project "Nutritional mitigation strategies for antibiotic free broiler production: Performance and economics" consent to the use of my data (number of chickens, cumulative feed consumption and weight of poultry litter on different days of observation) by Sanjay Gupta, a Master's student with Dr. Tariq Siddique in the Department of Renewable Resources in his thesis entitled "Life cycle assessment of arsenic from poultry feed to agricultural crops" because both these projects are part of a multi-disciplinary project.
I understand that the data will be used for his Master's thesis and subsequent research papers and/or presentations only, and that copyright of the data will be retained by the University of Alberta.
I also declare that every ethical issue related with chicken health and welfare was duly taken care of in my research project.
I require that my name be retained in association with this data.

JAN 6, 2014

Dated

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