ARSENIC UPTAKE BY BEETS (*BETA VULGARIS*) CULTIVATED IN A ROXARSONE-CONTAMINATED MEDIUM

By

Joel Andrew Houser

Approved:

Sean M. Richards
Professor of Biological and Environmental Sciences
(Director of Thesis)

Jennifer N. Boyd
Assistant Professor of Biological and Environmental Sciences
(Committee Member)

J. Hill Craddock
Professor of Biological and Environmental Sciences
(Committee Member)

Jeffery S. Elwell
Dean of the College of Arts and Sciences

A. Jerald Ainsworth
Dean of the Graduate School

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ABSTRACT

Arsenic (As) is a global toxicant that negatively impacts human health. Roxarsone (ROX) is an organoarsenical administered to poultry to control internal parasites. ROX is excreted from poultry unchanged and the waste may be used for vegetable fertilizer. This experiment was conducted with beets (*Beta vulgaris*) by adding 0, 1, 10, and 100 mg/kg As (T_1, T_2, T_3, and T_4 respectively, with ROX, presented as As concentrations) to a growing medium prepared with topsoil and other ingredients in a greenhouse pot experiment. The study aimed to determine effects of As-contaminated soils on biomass production, uptake of As by beets, and allocation of As to tissues. Results showed that biomass production of beets was negatively correlated with As concentrations in the growing medium ($r = -0.3286$, $p < 0.0001$). As uptake by beets was positively correlated with As concentrations in the growing medium (roots, $r_s = 0.7577$, $p < 0.0001$; shoots, $r_s = 0.8406$, $p < 0.0001$). As uptake by beets was observed with median values in the roots of 0.267 ± 0.004 mg/kg, 0.271 ± 0.001 mg/kg, 0.271 ± 0.289 mg/kg, and 3.76 ± 1.92 mg/kg for T_1, T_2, T_3, and T_4 respectively; the shoots took up 0.259 ± 0.006 mg/kg, 0.263 ± 0.313 mg/kg, 0.271 ± 0.373 mg/kg, and 3.94 ± 0.72 mg/kg for the respective treatments. Beets took up 4.3 ± 2.3% of available As and distributed it equally into tissues. The results suggest that As could be transferred to humans through the food chain via beet consumption.

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LIST OF ABBREVIATIONS

µg, Microgram

Al, Aluminum

ANOVA, Analysis of Variance

As (III), Arsenite

As (V), Arsenate

As, Arsenic

BCF, Bioaccumulation Factor

Broilers, Poultry Raised for Meat Consumption

Ca, Calcium

CAFO, Animal Feeding Operation

Cd, Cadmium

Cu, Copper

CV, Coefficient of variation

DMAA, Dimethylarsonic Acid

EFSA, European Food Safety Authority

EPA, United States Environmental Protection Agency

FDA, United States Food and Drug Administration

Fe, Iron

g, Gram

H, Hydrogen

IARC, International Agency for Research on Cancer

ICP-AES, Inductively Coupled Plasma – Atomic Emission Spectrometry

ICP-MS, Inductively Coupled Plasma – Mass Spectrometry

IQR, Interquartile Range

K, Potassium

kg, Kilogram

L, liter

LOC, Level of Concern

LOQ, Limit of Quantification

mg, Milligram

Mg, Magnesium

mm, Millimeter

MMA, Monomethylarsonic acid

N, Nitrogen

OAs, Organoarsenical

OH-, Hydroxide

P, Phosphorus

Pb, Lead

pH, $-\log[H^+]$

PL, Poultry Litter

PO_4, Phosphate

ROX, Roxarsone

T_x, Treatment (x denotes Treatment #)

TF, Translocation Factor

U.S., United States of America

Zn, Zinc

CHAPTER 1

INTRODUCTION

As in the Human Environment

Arsenic (As) is ubiquitous in nature and exists in all compartments of the environment (Mandal & Suzuki 2002). Concentrations of As in the environment vary widely depending on natural and anthropogenic factors (Schulman 2000). Average As concentrations in the Earth's crust range from 1.5 mg/kg to 5.0 mg/kg, but soil concentrations may be much higher (Cullen & Reimer 1989). Background soil As concentrations in Tennessee, U.S., range from 0.1 to 120 mg/kg, averaging 10 mg/kg (Kopp 2001). Though metallic As does exist naturally, As is usually associated with mineral complexes containing metals and other elements (Schulman 2000).

Anthropogenically, As is released from agricultural and industrial processes, as well as from the weathering of lumber treated with chromated copper arsenate (CCA); CCA-treated lumber was phased out of production in the U.S. in 2003, but continues to leach As (Schulman 2000, Wormell 2011). How As compounds behave in the environment depend on the oxidation state of the As, oxidation-reduction potential of the system, pH, and presence of metals and sulfides (Schulman 2000).

Roxarsone

Roxarsone (ROX; $C_6H_6AsNO_6$) is an organoarsenical (OAs) pesticide used to control internal parasites in poultry production and to a lesser extent in swine production (Schulman

2000). In the U.S., the maximum allowable dosage of ROX in poultry feed is 50 mg/kg (Moody & Williams 1964, Chapman & Johnson 2002). ROX is 28.5% As by weight (Acros 2009). It is estimated that as of 2000, 70% of broiler chickens raised in the U.S. were treated with ROX or the closely related *p-arsanilic* acid (Chapman & Johnson 2002).

Though ROX has been used in the U.S. since the 1940s, its popularity in the U.S. is currently waning under increased scrutiny and societal pressure (Kerr et al. 1969, Morrison 1969, Hileman 2007). In June 2011, Pfizer (the primary manufacturer of ROX) announced a voluntary cessation of the production of ROX to be sold in the U.S. in response to relatively high As concentrations found by U.S. Food and Drug Administration (FDA) researchers in the livers of treated broiler chickens (Conklin et al. 2012). However, the use of ROX in the U.S. is not explicitly prohibited; moreover, it continues to be used in most other countries with few exceptions (Chapman & Johnson 2002, Rutherford et al. 2003).

Potential Pathway of ROX human exposure

The As in ROX eventually may enter the human food chain through plant uptake by vegetable crops (Figure 1) (Wang et al. 2006). Plants tend to accumulate inorganic species of As preferentially to OAs (Abedin et al. 2002b). Many plant species grown in As-contaminated soils consistently exhibit higher concentrations of As their tissues (Wang et al. 2006, Yao et al. 2009a). But vegetable food crops vary in their ability to uptake As from contaminated soils; some, such as water spinach (*Ipomea aquatica*), preferentially uptake As, while others, such as amaranth (*Amaranthus tricolor*), do not (Yao et al. 2009a). Some vegetable species have a hormetic response in the form of increased biomass production when exposed to low levels of

As; however, in the majority of species, As retards plant growth and reduces vegetable crop yields (Walsh & Keeney 1975, Marin 1993, Wang et al. 2006).

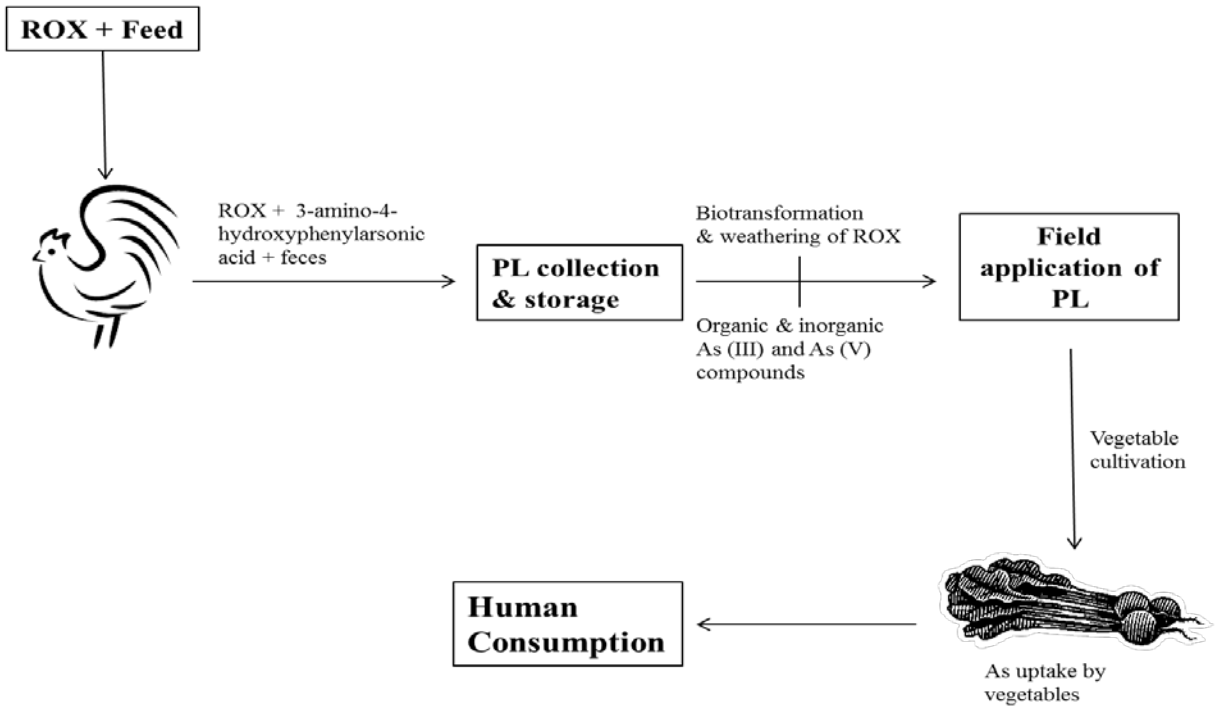

Figure 1 Potential pathway from ROX administration to poultry, deposition into poultry litter (PL) (Moody & Williams 1964), biotransformation of ROX into more mobile As species, application to agricultural fields (Kpomblekou et al. 2002, Garbarino et al. 2003), uptake of As by food crops (Wang et al. 2006), and subsequent human exposure.

No published studies have measured ROX-sourced As uptake in beets (*Beta vulgaris*). Beet pectin has been shown to readily sorb to Pb, Zn, Cu, and Cd, indicating its potential to accumulate metals (Ali 2010). Research of As uptake by beets is warranted because in many plant species, As tends to partition in roots and leaves; both beet tissues are commonly consumed by humans (Abedin et al. 2002a, Abedin et al. 2002b, Wang et al. 2006, Schmidt et al. 2008, Yao

et al. 2010). Root tissues in some plants tend to accumulate more As than other tissues (Wang et al. 2006). Though beets are in the minority of plants that are considered to be fairly tolerant to high concentrations of soil As, As does decrease biomass production in many plants (Walsh & Keeney 1975).

To estimate the relative potential for humans to ingest As from plants grown in ROX-laden soil, beets were grown in soils spiked with environmentally relevant concentrations of ROX. Concentrations of As were then quantified in the soil and beet tissues (subterranean and above ground). The data collected were used to test the following hypotheses: 1) plant biomass production of beets is indirectly proportional to soil As concentrations, 2) As concentrations in beet tissues are directly proportional to concentrations of As in ROX-contaminated soils, and 3) beets assimilates more As into the subterranean tissues than those above ground.

CHAPTER 2

LITERATURE REVIEW

Toxicological Consequences of Human As Exposure

Chronically, humans are exposed to low doses of As through food and water (Mandal & Suzuki 2002). That exposure greatly increases the incidence of preventable diseases (Yumei et al. 2012). The greatest toxicological concern of chronic exposure to As is its carcinogenic effects (Mandal & Suzuki 2002). Chronic As exposure has been linked to a variety of cancers including bladder, kidney, colon, and pancreatic cancer (Mandal & Suzuki 2002). As is also associated with increased risks of non-cancer endpoints such as type II diabetes, hypertension, and neuropathy (Navas-Acien & Guallar 2008, Silbergeld & Nachman 2008, Abhyankar et al. 2012). However, some organic phenylarsenic compounds, including salvarsan and melarsoprol, are used medicinally for treatment of syphilis and sleeping sickness (Schmidt et al. 2008).

While much research has been conducted concerning direct inorganic As exposure to humans, the lower exposure concentrations and lower toxicity of organic arsenicals (OAs) has produced less research (Marin 1993). The most absorbable forms of As in the human body are soluble anionic species, such as inorganic arsenite (As (III)) and inorganic arsenate (As (V)) compounds. Most OA compounds, such as ROX, *p*-arsanilic acid, and dimethylarsonic acid (DMAA) are relatively insoluble (Mandal & Suzuki 2002). As (III) is the most toxic form of As to humans (Mandal & Suzuki 2002).

As in the Human Diet

As contamination in food is relatively common and found throughout many food groups including grains, fruits, vegetables, meats (aquatic and terrestrial), and drinking water (EFSA 2009). Regulations for acceptable levels of As in foods differ not only by country, but also by food type (EFSA 2009). The U.S. does not have standard As limits in foods. Rather, the Level of Concern (LOC) varies by food (e.g. pear juice LOCs range from 50 to 262 µg/L depending on a person's age) (DHHS 2008). The statutory limit for inorganic As in all foods in China is 0.15 mg/kg (Liu et al. 2009).

Fish and fish products are the most likely foods to contain As concentrations sufficient to cause concern (EFSA 2009). As associated with seafood is typically organic; however, some species such as the blue mussel (*Mytilus edulis*) may have concentrations of inorganic As greater than 30 mg/kg (EFSA 2009). Shiber (2011) tested sardines and found that all contained As concentrations that ranged from 0.49 mg/kg to 1.87 mg/kg (Shiber 2011). The highest mean As concentrations in sardines were from Thailand, all of which contained concentrations higher than 1.33 mg/kg (Shiber 2011). Mean fish levels reported to the European Food Safety Authority (EFSA) ranged from 1.453 mg/kg As to 5.011 mg/kg As (EFSA 2009).

Recent attention has focused on As accumulation in cereal grains and fruits (Wang et al. 2006, Wilson et al. 2012). Rice (*Oryza sativa*) is known to accumulate As and is a primary contributor to total As body burden (Wang et al. 2006). Boiled brown rice has greater As concentrations than boiled white rice, mostly in the inorganic form (Heitkemper et al. 2001, EFSA 2009). Abedin et al. (2002) and Wang et al. (2006) observed accumulation of As in roots of rice to concentrations similar to adjacent soils (Abedin et al. 2002a, Wang et al. 2006). Wilson et al. (2012) tested many commercially available juices and found that all contained As, most in

the µg/L range (Wilson et al. 2012). Although the As concentrations in juices were low compared to those found in foods, many were greater than the 10 µg/L U.S. limit for drinking water (Wilson et al. 2012).

ROX Chemical Properties

ROX is a crystalline powder at room temperature that is relatively insoluble (Acros 2009). ROX is 28.5% total As by weight, the remainder is C, H, N, or O. All of the As contained in pure ROX is in the form of organic As (V). ROX is not acutely toxic when used as directed (Moody & Williams 1964). The toxicological properties of ROX have not been fully studied, though it is known that it targets the kidneys, liver, lungs, nervous system, pancreas, and skin in humans and the International Agency for Research on Cancer (IARC) classifies it as a Group 1, known human, carcinogen (IARC 2006, Acros 2009).

Use of ROX in Poultry Production

Based on a survey of 90% of U.S. poultry production units, from 1995 to 2000, ROX was used in 69.8% of the poultry starter and 73.9% of the grower feed (Chapman & Johnson 2002). Chapman and Johnson (2002) found that most poultry production units used ROX-supplemented poultry feed at a rate of 50 mg/kg, the maximum permissible concentration by the USDA (Chapman & Johnson 2002).

Though the exact quantity of ROX introduced into the environment is not known, estimates range from 9×10^5 kg to 10^6 kg annually from U.S. poultry production (pre-2011 levels) (Christen 2001, Garbarino et al. 2003). Such data are sparse, but Brown et al. (2005)

estimated that 9.9×10^3 kg of As had been intentionally applied annually in the Shenandoah Valley, Virginia, U.S., in the form of poultry litter (PL) (Brown et al. 2005).

ROX is a useful prophylaxis in poultry production because it provides effective prevention of coccidiosis, improving feed utilization (Anderson & Chamblee 2001, Bray et al. 2009). ROX is typically administered as a feed additive at a rate of 50 mg/kg (Anderson & Chamblee 2001). A feeding rate of 50 mg/kg ROX in poultry feed results in 14.25 mg/kg As in poultry feed. In U.S. poultry, medication with ROX is not permitted for egg layers because of likely As deposition into eggs (Bellows 2005).

The primary method of ROX excretion by poultry is through urine (Aschbacher & Feil 1991). Early research indicated that after 14 days of depuration, poultry was effectively void of all As associated with ROX medication (Kerr et al. 1969). Another study found that ROX-treated roosters expelled 70% of the As within 6 hours (Aschbacher & Feil 1991). Of the tissues (leg, breast, liver, and heart) tested by Kazi et al. (2013) the liver contained the highest concentrations of As (7.17 ± 1.1 µg/kg); the period of depuration for tissues tested in this study were not defined (Kazi et al. 2013).

Up to 88% of ROX dosages administered to poultry are excreted as the parent compound (Moody & Williams 1964). Of the remaining As compounds in the excrement, the majority is the ROX metabolite 3-amino-4-hydroxyphenylarsonic acid (Moody & Williams 1964). The ROX-associated As is deposited into PL where it may accumulate (Anderson & Chamblee 2001).

ROX in Poultry Litter

It is estimated that every chicken produces 1.5 kg of manure (dry weight) per year (Kpomblekou et al. 2002). At a typical poultry farm, the manure combines with bedding

material, feathers, and unconsumed feed – collectively known as poultry litter (PL) (Nicholson et al. 1999, Kpomblekou et al. 2002). The amount of As in PL, as a result, is variable and inconsistent depending on husbandry practices, bedding (type and volume), and amount of ventilation (Marin 1993, Anderson & Chamblee 2001, Makris et al. 2008b). Kpomblekou et al. (2002) studied 33 PL samples from 31 different growers in Alabama (U.S.) and found As concentrations ranging from < 2.0 mg/kg to 70.4 mg/kg (Kpomblekou et al. 2002), which is substantially higher than concentrations observed by Anderson and Chamblee (2001) of 31.50 mg/kg (Anderson & Chamblee 2001).

Typically, PL is disposed of by field application, stored in waste lagoons, or composted for later field application as fertilizer (Garbarino et al. 2003). Because of centralized production, disposal of litter is a continual problem and PL often is applied to lands adjacent to the concentrated animal feeding operations (CAFOs) (Kpomblekou et al. 2002). When applied to agricultural lands, PL improves field drainage and fortifies the soil with Ca, Mg, K, N, and P (Kpomblekou et al. 2002). However, over-application of litter can result in degraded soil, unhealthy plants, and groundwater and/or surface water contamination (Kpomblekou et al. 2002). The typical application rate of PL to agricultural fields for fertilization is 11,000 kg/acre (Garbarino et al. 2003).

ROX Degradation in Poultry Litter and Soil

Anderson and Chamblee (2001) concluded that As concentrations in PL were inconsistent and unpredictable, largely due to biotransformation of ROX within PL (Anderson & Chamblee 2001). Degradation of ROX typically occurs within stored PL, but may also happen after field application.

Once OAs species enter the environment, both biotic and abiotic degradation occurs resulting in more mobile inorganic arsenical compounds (Bednar et al. 2003). In most natural soil systems, As (V) is the dominant oxidation state of As (Walsh & Keeney 1975). As (III) will not persist for more than a few days in well aerated soils. Regardless of treatment, all As in PL is typically oxidized to As (V) after a short time (Garbarino et al. 2003).

ROX and 3-amino-4-hydroxyphenylarsonic acid ultimately are degraded into variable and unpredictable inorganic As species such as inorganic As (III) and As (V) compounds within the PL (Bednar et al. 2003, Garbarino et al. 2003). ROX metabolites found in PL include inorganic As: As (III), As (V), and OAs: monomethylarsonic acid (MMA), dimethylarsonic acid (DMAA), and phenylarsenic compounds among other minor varieties of As compounds (Bednar et al. 2003, Makris et al. 2008b, Yumei et al. 2012).

Though the dominant As species in PL is As (V) (Makris et al. 2008a), Abedin et al. (2002) hypothesized that some ROX metabolites in PL, such as arsine (AsH_3), readily volatilize, reducing the concentrations of As accumulation in PL (Abedin et al. 2002a). In one greenhouse pot experiment, 23% of As present volatilized (Abedin et al. 2002b). Anderson and Chamblee (2003) hypothesized that this mode of transport is responsible for lower As concentrations in some PL than expected (Anderson & Chamblee 2001).

ROX tends to degrade to inorganic species via microbial degradation when composted, (Brown et al. 2005, Jackson et al. 2006). As availability and ROX stability are dependent on litter management practices such as maintenance of moisture content and temperature (Kpomblekou et al. 2002, Garbarino et al. 2003). Wetting litter during storage (naturally or artificially) affects stability of ROX by increasing its solubility (Garbarino et al. 2003). Because of this transformation, As concentration of leachates can exceed greatly that of the source PL

(Bednar et al. 2003). A large amount (92%) of As in PL may be water soluble (Jackson & Bertsch 2001). Makris et al. (2008) found water soluble As in aged PL at concentrations of approximately 2000 µg/L (Makris et al. 2008b). Leaching and runoff occur when ROX-laden wastes are applied to fields, resulting in elevated As concentrations in soils and surface waters (Schmidt et al. 2008).

Transport and Fate of ROX-source As

Research in the Shenandoah Valley, Virginia, U.S. indicates that there is a downward movement of inorganic As through soils as a result of the application of ROX-contaminated PL (Brown et al. 2005). Increased sorption of As to soil negatively impacts the bioavailability and mobility of As, thus reducing a downward movement of As through the soil (Yao et al. 2009b). As sorption is dependent on soil parameters such as mineral concentrations and pH (Jackson & Miller 2000). As (V) exhibits competitive sorption with PO_4 on Fe- and Al-oxides (Jackson & Miller 2000). Consequently, higher Fe- and Al-oxide concentrations in soil cause As to become less mobile in soils (Jackson & Miller 2000, Jackson et al. 2006). Inorganic As is extracted most easily from geolithe at a low pH (< 3) and most stable at more neutral pH values (Jackson & Miller 2000). Due to lower soil sorption potential (from a lack of Fe- and Al-oxides), As is considered more mobile and bioavailable when present in sandy soils rather than in soils with higher clay content (Walsh & Keeney 1975).

As Uptake by Plants

Plant species show varying tendencies to accumulate As (Yumei et al. 2012). Research indicates that As accumulated by rice, amaranth, and water spinach is directly proportional to

soil As concentrations (Wang et al. 2006, Yao et al. 2009a). Some plants show the ability to hyperaccumulate arsenic. The brake fern (*Pteris vittata*) was the first known As hyperaccumulator and has been shown to accumulate As to concentrations up to 22,630 mg/kg (Ma et al. 2001). The growth of brake ferns is also stimulated by the presence of As (Ma et al. 2001).

Water-soluble species of As, inorganic As (III) and As (V), are taken up at a higher rate than OAs in rice (Abedin et al. 2002b, Schmidt et al. 2008). Rice exhibits preferential uptake for As (III) over the less toxic As (V) when subjected to high concentrations of both species (Abedin et al. 2002b). However, at low concentrations, rice shows comparable uptake between As (III) and As (V) (Abedin et al. 2002b). In turnips (*Brassica rapa*), Yao et al. (2009) found that As (V) is taken up and is then biotransformed into As (III) (Yao et al. 2009b). Unlike uptake of As (III), uptake of As (V) is affected by the presence of PO_4 (Kpomblekou et al. 2002).

As tends to partition at different concentrations in different plant tissues (Wang et al. 2006). In nasturtium (*Tropaeolum majus*), As is more likely to concentrate in leaves compared with flowers and stalks (Schmidt et al. 2008). Turnips accumulate more As in the shoots than roots (Abedin et al. 2002a, Abedin et al. 2002b, Wang et al. 2006, Yao et al. 2010). Some research indicates that slower growing tissues, such as roots, tend to accumulate higher concentrations of As than faster growing tissues such as spikelets and grains (Abedin et al. 2002a, Wang et al. 2006). Rice has been shown to accumulate root and straw As concentrations of 192 mg/kg and 201 mg/kg respectively, while grain As concentrations did not exceed 0.74 mg/kg (Abedin et al. 2002a).

Root As concentration often is similar to ambient soil concentrations (Abedin et al. 2002a, Wang et al. 2006). However, Schmidt et al. (2008) and Walsh and Keeny (1975)

hypothesize that As more likely is sorbed to soil attached to the roots rather than accumulated within the roots resulting in an overestimation of As accumulation by plants (Walsh & Keeney 1975, Schmidt et al. 2008). Walsh and Keeny (1975) discount the concern of As in food crops; their research indicated that As killed the plant before levels of As could accumulate to levels considered hazardous to humans (Walsh & Keeney 1975).

Extremely high As concentrations in growing media negatively impact biomass production in most observed plants (Walsh & Keeney 1975, Wang et al. 2006). As concentrations negatively correlate with plant height ($r = -0.9866$) and grain yield in both number ($r = -0.9915$) and weight ($r = -0.9916$) in rice (Wang et al. 2006, Liu et al. 2009). Though it is generally accepted that As has a tendency to negatively affect plant growth and vigor, hormetic responses have been observed with low applications of As to potatoes, corn, rye, and wheat (Walsh & Keeney 1975).

CHAPTER 3

MATERIALS AND METHODS

Medium Materials and Preparation

The growing medium in the present study was a combination of supplemented potting mix and topsoil. The components for the growing medium were topsoil collected from Crabtree Farms, Chattanooga, TN, U.S., potting mix (MetroMix®, Marysville, Ohio, U.S.), perlite, worm castings, and pelleted organic fertilizer (NatureSafe® 8-5-5, Griffin Industries, Inc., Cold Springs, Kentucky, U.S.) (Prasanna Kumar & Raheman 2008, Migliaccio et al. 2010). A premade soilless potting mix was used for the base of the soil mixture used in this study. The potting mix was composed of 75-85% composted bark, Canadian sphagnum peat moss, dolomite lime, and gypsum. The growing medium was augmented with standard horticultural perlite and worm castings (Morton's Horticultural Products in McMinnville, TN, U.S.). A slow-release fertilizer (Nature Safe®) was used to provide essential macronutrients to the test plants via a proprietary blend of meat, bone, and blood meals (N-P-K ratings of 8-5-5). Topsoil used in the growing medium was an Etowah silt loam from Crabtree Farms (Chattanooga, TN, U.S.). The soil has been managed organically (i.e., without the use of synthetic fertilizers or pesticides) since 1998 and has been used for diversified vegetable crop cultivation since 2004. Soils were collected from multiple, randomly selected, locations (N = 3) and placed in an open shed to dry to ambient atmospheric humidity for a period of several weeks. Soils were mixed repeatedly by hand to ensure heterogeneity. After allowing topsoil to dry to ambient relative humidity, it was

sifted through galvanized hardware cloth with holes ranging from 5.5 to 7.0 mm^2 (Liu et al. 2009, Yao et al. 2009a, Yao et al. 2009b). Worm castings and the potting mix were dried in the same fashion, with the exception that forced ventilation was used in order to facilitate drying (Liu et al. 2009, Yao et al. 2009b).

After air-drying the bulk materials, they were mixed at consistent ratios (Table 1). The proportions were chosen after several growing trials with varying ratios of topsoil and other materials. The highest rate of topsoil that would support beet cultivation in pots was used to better simulate field soil conditions with respect to sorption and microbial communities (Yao et al. 2009a). To mix the bulk materials, a new concrete mixer was used (to avoid potential contamination from previously-mixed concrete) (Bhunia et al. 2007). Fourteen batches, approximately 32 kg each, were mixed for the study. After mixing, each batch was stored in a plastic bag until experiment initiation.

Table 1 Growing medium base formula. The growing medium was mixed using weight percentages to ensure consistency. The final ingredient ratios were chosen based on pilot experiments and available research (Alexander 2009, Migliaccio et al. 2010).

Component	**Weight %**	**Purpose**
Topsoil	71.4	Base soil
Potting mix	20.7	Drainage and organic matter
Worm castings	3.2	Micronutrients and microflora
Perlite	2.5	Drainage
Fertilizer	2.2	Macro- and micro-nutrients

To ensure accurate dosage with ROX, the moisture of each soil batch was measured. To do this, three samples from each batch were collected and weighed. Samples were dried in drying ovens for approximately 45 hr. before the final sample weight was taken. The soils

averaged 14.21% moisture ([(Wet Soil (g)-Dry Soil (g))÷Wet Soil (g)]×100=% Moisture). Previous studies concerning the uptake of As from ROX sources have neglected this step and mixed soil based on weight of air-dried materials alone (Wang et al. 2006, Yao et al. 2008, Yao et al. 2009a).

A sample of the growing medium was sent to A&L Analytical Laboratories in Memphis, TN, U.S. for quantification of medium components and determination of texture (Table 2). The texture, classified as a loam, was optimum for the purposes of the present study. The reported Ca-to-Mg ratio was lower than desired (2.97), indicating that the medium used was slightly deficient of Ca (Table 2).

Table 2 Physiochemical properties of base growing medium (without ROX) determined by A&L Analytical Laboratories, Memphis, TN, U.S.

Property	**Value**
As (mg/kg)	5.94
pH	5.2
Buffer pH	7.59
Ca (meq/100g)	12.91
Mg (meq/100g)	4.34
K (meq/100g)	3.02
Cation Exchange Capacity (meq/100g)	23.6
Organic Matter (%)	13
Sand (%)	34
Silt (%)	42
Clay (%)	23
Texture Classification	Loam

ROX Dosage

ROX (+98% purity, solid powder, 28.5% As) was obtained from Acros Organics (Geel, Belgium). ROX was added to and mixed with the prepared growing medium base on a dry weight basis to create the growing media for the four experimental treatments: 0 mg/kg additional As (Treatment 1; control), 1 mg/kg additional As (Treatment 2), 10 mg/kg additional As (Treatment 3), and 100 mg/kg additional As (Treatment 4). Per kg of soil mix, 0 mg ROX, 3.5088 mg ROX, 35.088 mg ROX, and 350.88 mg ROX were added to Treatments 1 (T_1), 2 (T_2), 3 (T_3), and 4 (T_4), respectively, to attain desired As concentrations. Each treatment was mixed separately in small batches and wetted to approximately 20% moisture by weight with tap water. After all batches for each treatment were mixed, the batches were combined and homogenized manually. At this point, three grab samples from each of the four treatments were collected and shipped to A&L Laboratories, Inc. in Memphis, TN for total As concentration verification. The samples were shipped in Whirl-Pak® bags at ambient conditions.

A&L Laboratories, Inc. quantified total As present in the growing medium using USEPA method #3050B for digestion and #6010B for total As quantification. Method #3050B requires a vigorous digestion of materials prior to concentration quantification using nitric acid and hydrogen peroxide followed by dilution either with nitric acid or hydrochloric acid (EPA 1996). Method #6010B is used for determination of metal concentrations in environmental samples. This method utilizes inductively coupled plasma-atomic emission spectrometry (ICP-AES) for elemental quantification (EPA 1996). Atomic emissions of nebulized samples at a wavelength of 193.696 nm are observed for As determination with an estimated detection limit of 35 µg/L (EPA 1996). ICP-AES is susceptible to spectral interferences and the detection limit may also be skewed by complex matrices (EPA 1996).

Cultivation

Beets were cultivated in a greenhouse in Chattanooga, TN, U.S. from fall 2011 through spring 2012. Three-quart pots (N=75) were filled with 1.30 kg - 1.43 kg of the growing medium for each treatment; amounting to 300 pots total. At least three beet seeds, cultivar 'Detroit Dark Red' obtained from Fedco Seeds (Waterville, ME), were placed in each pot at a depth of 1 - 2 cm and wetted with tap water. Pots (of all treatments) were randomly placed on three greenhouse benches. The greenhouse temperature was maintained between 11^{o}C and 21^{o}C for the winter and between 13^{o}C and 29^{o}C in the spring; the greenhouse was continually covered with a 50% reflective shade cloth.

During cultivation, pots were amended with tap water as needed. Intervals between watering ranged from 14 days to 2 days depending on the weather and stage of the plants. As the seeds germinated, beets were thinned to one individual per pot (the most vigorous individual was kept) and weeded as needed. Predominant weeds were multiple unknown species of Poaceae, chickweed (*Stellaria media*), and henbit (*Lamium amplexicaule*). Weeds continued to germinate throughout the experiment and were removed as needed.

At 22 days after seeding, an unknown fungal infection of the seedlings was observed. The cause of this is not known, but treatment of pots with Banrot® 40WP (Scotts®, Marysville, Ohio, U.S.) controlled the outbreak. Banrot® was applied at a rate of 1/3 tsp./gallon as directed by product label.

At 42 and 107 days after seeding, an outbreak of fungus gnats (*Bradysia* sp.) was observed. To treat the outbreak, a soil drench of ACE Malathion 50 Insect Spray was used on all treatments. The drench was mixed at a nominal concentration of 1 TBSP/gallon. This treatment provided sufficient control of the fungus gnat population.

At 83 and 118 days after seeding, all pots were amended with Neptune's Harvest® Fish Emulsion + Kelp Extract (Oceans Crest Seafoods Inc., Gloucester, Massachusetts, U.S.) with use of a Siphonject® (Dramm, Manitowoc, Wisconsin, U.S.) passive vacuum feeder. The Siphonject® dosed tap water at a nominal rate of 1:16. Approximately two liters of the fish emulsion + kelp extract were used to treat all four treatments in both applications.

Sample Preparation

At 163 days after seeding, beets were harvested and processed. Beets were divided by treatment (N = 75 per treatment). From the 300 available beets, 20 from each treatment were randomly selected for biomass determination and total As quantification without bias for size or growing location. Samples were processed in order of increasing treatment soil As concentration. Each individual to be sampled was removed from its pot and tagged with its identification number.

Samples were washed with tap water and scrubbed with nylon brushes to remove soil from the roots (Wang et al. 2006). Samples were then rinsed with deionized water to remove potential As impurities from tap water (Yao et al. 2008). Beet stems and leaves were separated from the roots with a stainless steel knife (Wang et al. 2006). The fine roots were removed and discarded so as to quantify only As in the edible portions of the plant by minimizing contamination by sorbed soil particles associated with fine roots (Schmidt et al. 2008). Leaves, stems, and napiform roots were minced separately, placed in Whirl-pak® bags, and frozen at -52^{o}C until biomass determination (Yao et al. 2008). Between sample preparations, knives and cutting surfaces were washed with tap water and rinsed with deionized water.

Biomass Determination

Samples were dried in plastic weigh boats in ovens at approximately 60^{o}C for 2-5 days and their masses recorded (Liu et al. 2009). Treatments were dried separately in ascending order of As concentration in the growing medium. Stems and leaves were dried separately from the napiform root and the combined masses for each individual represented the total biomass produced (Yao et al. 2008). Dried samples were transferred to new Whirl-pak® bags and returned to the freezer for storage (Yao et al. 2008).

Quantification of Total As in Beets

Twenty complete samples, consisting of the combined dried shoots and dried napiform roots, for each treatment were shipped to A&L Analytical Laboratories in Memphis, TN, U.S. for total As quantification utilizing USEPA standard #6010B. Determination of metal concentrations using USEPA standard #6010B requires an acid digestion of dry materials followed by elemental quantification utilizing inductively coupled plasma-atomic emission spectrometry (ICP-AES) (EPA 1996). This method observes atomic emissions of nebulized samples at a wavelength of 193.696 nm and has an estimated detection limit of 35 µg/L (EPA 1996). ICP-AES is susceptible to spectral interferences and the detection limit may also be skewed by complex matrices (EPA 1996).

Biomass Statistical Analyses

All biomass statistics were produced with the SAS® System for Windows 9.0, 2002 (Cary, NC, USA). For biomass production, proc univariate tests were performed as well as a one-way fixed effects analysis of variance (ANOVA) with the Tukey-Kramer test for means

comparisons ($\alpha = 0.05$). Data considered for biomass production were the means of total biomass production from each sampled individual. Biomass production was analyzed as a function of additional As to the growing medium. Correlation between biomass production and growing medium As concentration was calculated using the Pearson product-moment correlation coefficient (r). The closer that the r value is to 1, the stronger the correlation between two factors (negative correlations are closer to -1). The slope of biomass production over additional As was calculated by the following formula: $[\frac{\Delta\ Biomass\ (g)}{\Delta\ Additional\ As\ \left(\frac{mg}{kg}\right)}]$.

As Concentration Statistical Analyses

As concentration statistics were produced with the SAS® System for Windows 9.0, 2002 (Cary, NC, U.S.). Data considered were the results of As quantification by A&L Laboratories (Memphis, TN, U.S.). In the event that reported data were below the limit of quantification (LOQ), the value that was half of the LOQ was used for analyses (Breckenridge & Crockett 1995, OPP 2000). For the shoot portions quantified, the following values were reported below the LOQ by treatment: T_1 – 17 of 20 samples, T_2 – 13 of 20 samples, T_3 – 12 of 20 samples, and T_4 – 0 of 20 samples. For the root portion quantified, the following values were reported below the LOQ by treatment: T_1 – 16 of 20 samples, T_2 – 20 of 20 samples, T_3 – 14 of 20 samples, and T_4 – 0 of 20 samples.

The reported concentrations of As were not normally distributed; therefore, As concentrations were analyzed using the one-way Wilcoxon rank sum test in order to generate a Kruskal-Wallis output. Differences in rank scores were analyzed with Fisher's least significant difference (LSD) test ($\alpha = 0.05$). Correlations between As concentrations in tissues and the

growing medium were calculated using Spearman's rank correlation coefficient (r_s). The closer that r_s is to 1, the stronger the correlation (negative correlations are closer to -1).

The Bioaccumulation Factor (BCF) was calculated for each treatment and values were reported as the mean for each treatment. BCF is a measure of the ability of an organism to assimilate contaminants from its environment, whether that is from absorption or consumption (Neely et al. 1974, Branson et al. 1975). The BCF was calculated using the following formula: $\left[(\text{Beet As}\frac{\text{mg}}{\text{kg}})\div(\text{Growing Medium As}\frac{\text{mg}}{\text{kg}})\right]$ (Branson et al. 1975). The average of the four treatments was considered the BCF of As in the present study.

The Translocation Factor (TF) was calculated for the individuals of each treatment TF is a measure of the ability of a plant to translocate metals from their roots to the above-ground portions of the plants (Singh & Agrawal 2007, Yilmaz & Temizgül 2012). For this study, the TF was the ratio of As located in above ground portions of the plant to the As concentration of the below ground portions (Singh & Agrawal 2007, Yilmaz & Temizgül 2012). TF for As in beets was calculated by the following equation: $\left[(\text{Shoot As}\frac{\text{mg}}{\text{kg}})\div(\text{Root As}\frac{\text{mg}}{\text{kg}})\right]$

(Singh & Agrawal 2007, Yilmaz & Temizgül 2012).

CHAPTER 4

RESULTS

Effects of ROX on Biomass Production

As contamination of the growing media influenced biomass production of beets. There was a significant negative correlation between growing medium As concentrations and total beet (shoot biomass + root biomass) biomass production ($r = -0.3286$, $p = 0.0029$), though not between all treatments (Table 3, Figure 2). Mean biomass production in T_1 and T_2 were similar ($p > 0.05$), as were T_3 and T_4 ($p > 0.05$) (Figure 2). Recorded mean biomass production for each treatment was 15.48 g, 14.01 g, 11.64 g, and 11.35 g for T_1, T_2, T_3, and T_4 respectively.

Based on calculated slope, the largest rate of decline in mean biomass production (g) as a function of additional As (mg/kg) was between T_1 and T_2 [-1.775 g biomass / (mg/kg As in growing medium)] (Figure 2, Table 3). The smallest decrease in biomass production with respect to As concentrations occurred between T_3 and T_4 [0.0003 g biomass / (mg/kg As in growing medium)] (Figure 2, Table 3). The largest decrease in biomass between treatments was between T_2 and T_3 (Table 3).

Table 3 As concentration of growing medium by treatment, median As concentration by tissue and treatment, range of As concentrations in tissues by treatment, biomass by tissue and treatment, bioaccumulation factor (BCF), and translocation factor (TF) for the total plant. Treatment medians or means within a given treatment denoted by the same letter are not significantly different ($\alpha = 0.05$).

Sample	Trmt	Growing Medium [As] (mg/kg)	Median [As] (mg/kg) ± IQR	[As] Range (mg/kg)	Biomass (g) ± st. dev	BCF	TF
Root	T_1	5.937	0.267 ± 0.004^a	0.177 – 0.573	7.46 ± 2.39	0.055	-
	T_2	6.757	0.271 ± 0.001^a	0.263 – 0.281	7.14 ± 2.43	0.040	-
	T_3	15.62	0.271 ± 0.289^a	0.263 – 0.828	5.09 ± 1.86	0.024	-
	T_4	98.32	3.76 ± 1.92^b	2.06 – 7.50	5.73 ± 1.84	0.043	-
Shoots	T_1	5.937	0.259 ± 0.006^a	0.219 – 0.664	8.25 ± 1.63	0.053	-
	T_2	6.757	0.263 ± 0.313^a	0.258 – 0.717	7.05 ± 1.39	0.071	-
	T_3	15.62	0.271 ± 0.373^a	0.262 – 0.908	6.39 ± 1.56	0.029	-
	T_4	98.32	3.94 ± 0.72^b	2.55 – 6.22	5.85 ± 1.10	0.043	-
Total Plant	T_1	5.937	0.262 ± 0.005^a	0.215 – 0.460	15.71 ± 3.39^a	0.053^a	1.055^a
	T_2	6.757	0.266 ± 0.157^a	0.262 – 0.541	14.19 ± 3.26^a	0.055^a	1.759^b
	T_3	15.62	0.444 ± 0.307^a	0.263 – 0.743	11.48 ± 2.68^b	0.027^b	$1.354^{a,b}$
	T_4	98.32	3.97 ± 1.00^b	2.39 – 6.74	11.58 ± 2.14^b	$0.042^{a,b}$	1.052^a

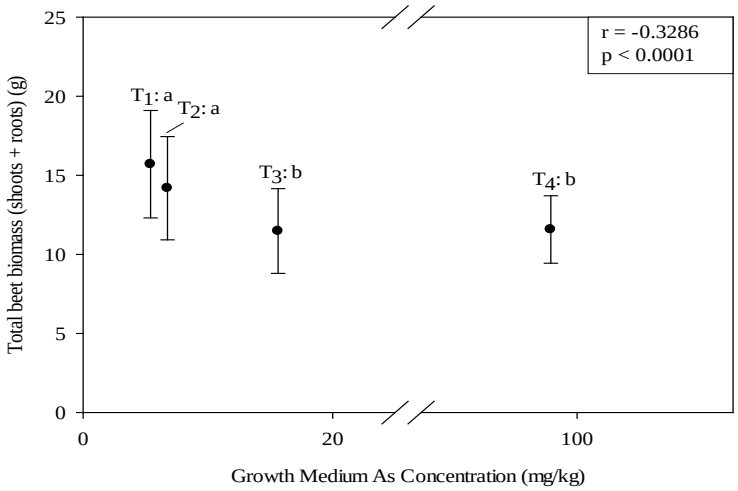

Figure 2 Mean biomass production (g) of sampled beets as a function of growing medium As concentration (mg/kg). Means denoted by the same letter indicate no significance (Tukey-Kramer, $\alpha = 0.05$). A marked decrease in biomass production between T_2 and T_3 indicate a potential threshold of phytotoxicity. See Table 3 for concentrations of As in each treatment.

ROX Effect on As Concentrations in Beet Roots

Presence of increased ROX in the growing medium positively correlated with increased concentrations of As within beet roots (r_s = 0.7577, $p < 0.0001$). T_4 accumulated significantly higher concentrations of As in root samples than all other treatments ($p < 0.0001$) (Table 3, Figure 3). All root samples exhibited high coefficient of variation (CV) of As values indicating high variation within each treatment compared to treatment means.

The median (± IQR) reported As concentrations taken up into the root portion of sampled beets were 0.267 ± 0.004 mg/kg, 0.271 ± 0.001 mg/kg, 0.271 ± 0.289 mg/kg, and 3.76 ± 1.92 mg/kg for T_1, T_2, T_3, and T_4 respectively (Table 3). The only treatment that was significantly different was T_4 ($p < 0.0001$) (Table 3, Figure 3). The total range of reported values was 0.177 mg/kg As to 7.50 mg/kg As in T_1 and T_4 respectively (Table 3).

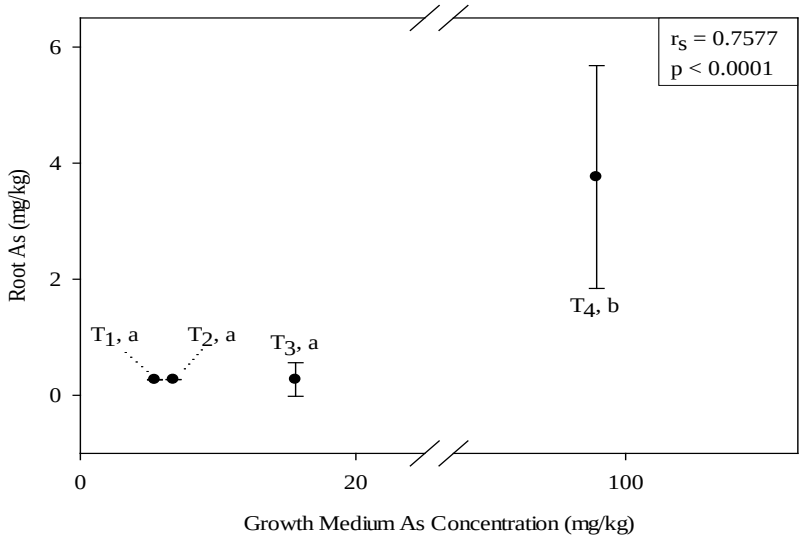

Figure 3 Median (± IQR) As concentrations of the root portion of sampled beets as a function of As concentrations in growth medium. Medians denoted by the same letter indicate no significance (Fischer's LSD, $\alpha = 0.05$). See Table 3 for concentrations of As in each treatment.

ROX Correlation with As Concentrations in Beet Shoots

Presence of increased ROX in the growing medium positively correlated with increased concentrations of As in beet shoots (r_s = 0.8406, $p < 0.0001$). All treatments exhibited high CV values indicating high variation within treatments compared to treatment means.

The median As concentrations in the shoot portion of the plant was 0.259 ± 0.006 mg/kg, 0.263 ± 0.313 mg/kg, 0.271 ± 0.373 mg/kg, and 3.94 ± 0.72 mg/kg for T_1, T_2, T_3, and T_4 respectively (Table 3). T_4 accumulated significantly higher concentrations of As in shoot samples ($p < 0.0001$) (Table 3, Figure 4). The total range of reported values for the sampled population of shoots was 0.219 mg/kg As to 6.22 mg/kg As in T_1 and T_4 respectively (Table 3).

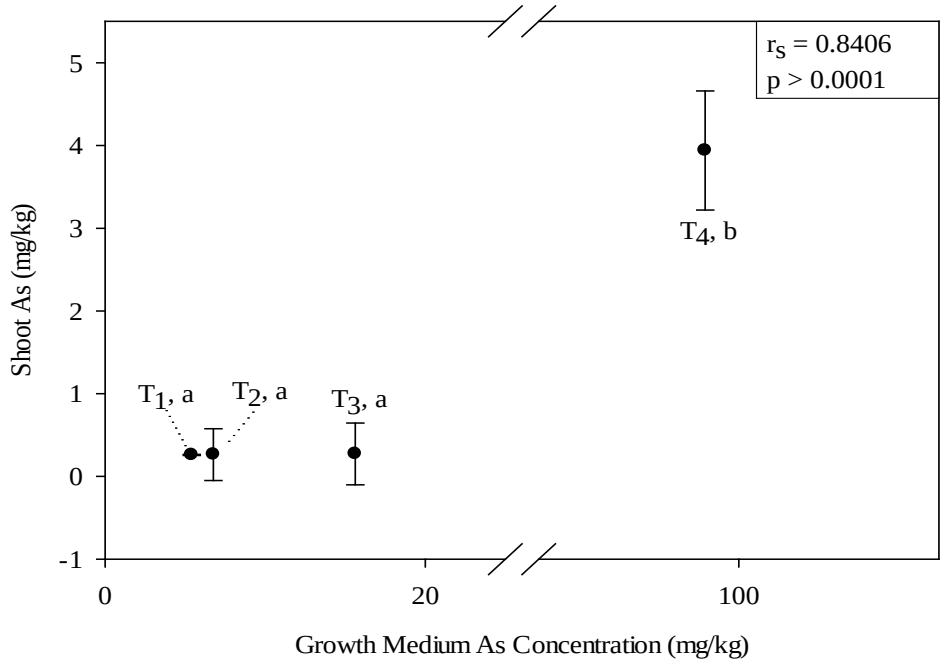

Figure 4 Median (± IQR) As concentrations of the shoot portion of sampled beets as a function of As concentrations in growing medium. Medians denoted by the same letter indicate no significance (Fischer's LSD, $\alpha = 0.05$). See Table 3 for specific values.

Bioaccumulation Potential of Beets as Determined by Bioaccumulation Factor

The mean calculated BCF for beets from all treatments observed in this study was 0.043 ± 0.023 for combined tissues. T_2 exhibited the highest BCF, with a value of 0.055. T_3 exhibited the lowest BCF of 0.027. T_1, T_2, and T_4 were not significantly different from one another ($p > 0.05$) nor were T_3 and T_4 ($p > 0.05$). T_1 and T_2 exhibited BCF values that were significantly different from T_4 ($p < 0.05$) and T_3 exhibited a similar BCF to all other treatments ($p > 0.05$) (Table 3, Figure 5). On average for this study, beets accumulated 4.3 ± 2.3% of As available in the growing medium. The mean total As that could be ingested by consuming one average-sized beet grown in soil containing 98.32 mg/kg As (T_4) would be 47.619 µg. For T_1, T_2, and T_3, based on calculated BCF, this amount would be 4.924 µg, 5.270 µg, and 4.773 µg, respectively.

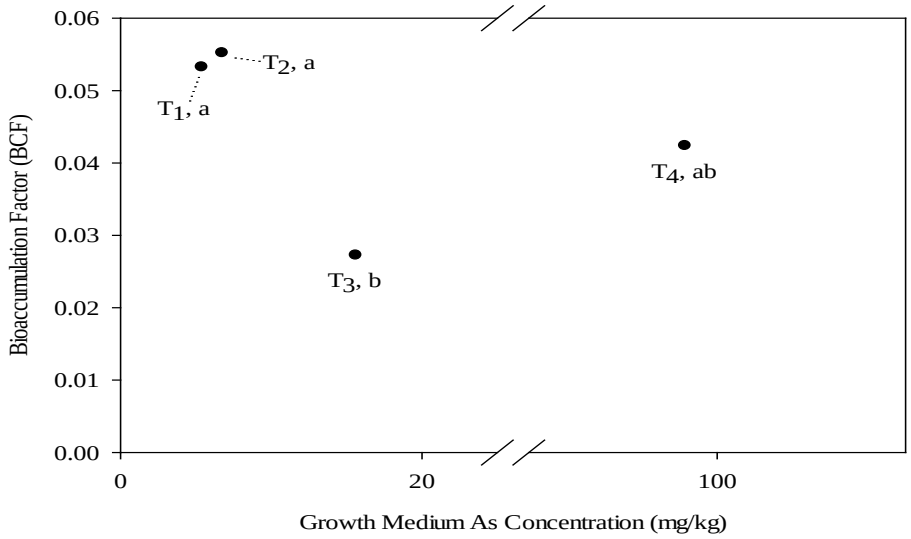

Figure 5 Bioaccumulation Factor (BCF). BCF is a standardized dimensionless value that represents the potential for an organism to accumulate toxins from its surroundings (Branson et al. 1975). Higher BCF values indicate that a higher proportion of available As was accumulated into the tissues of the beets for that treatment. BCF values denoted by the same letter are not significantly different ($\alpha = 0.05$). See Table 3 for specific values.

Distribution of As in Beet Tissues as Determined by the Translocation Factor (TF)

The difference between accumulated concentrations of As in the shoots and roots were not significant ($p = 0.0902$). The TF were not significant for T_1, T_3, and T_4 ($p > 0.05$), and the TF for T_2 and T_3 were not significant ($p > 0.05$). The TF for T_1 and T_4 were significantly different from T_2 ($p < 0.05$); the TF for T3 was similar to those of all other treatments ($p > 0.05$) (Figure 6). The range of TF values for T_1, T_3, and T_4 is 0.96 to 1.19 indicating that similar concentrations of As accumulated in the above- and below-ground portions of the tested plants. T_2 (TF = 1.76) exhibited a tendency to translocate the available As to the above ground portions of the plant (Table 3). Though apparent differences between As accumulation in the shoots and the roots may be seen in Table 3, no values for T_1, T_3, or T_4 are significant ($p = 0.0956$).

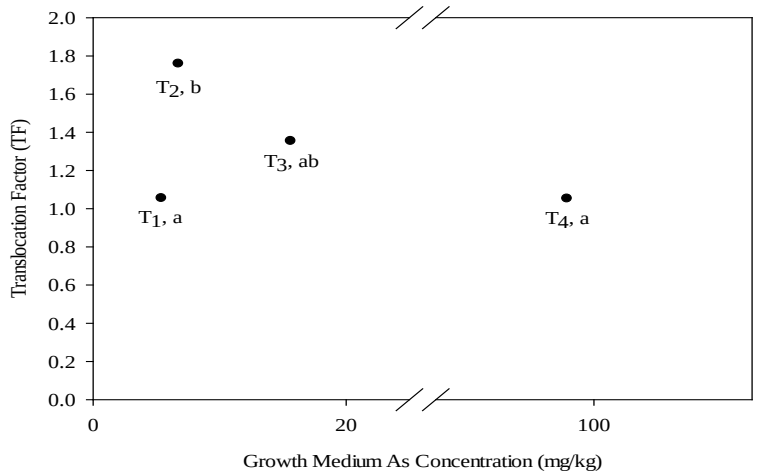

Figure 6 Translocation Factor (TF) as a function of growing medium As concentration. TF is a dimensionless value that is a measure of the tendency for a plant to translocate a toxicant from the subterranean portion to the above ground portion of the plant. A value of < 1 indicates that more of the toxicant (in this case As) remains in the subterranean tissues. A value that is > 1 indicates that the plant tends to translocate the toxicant to the above ground portion, and a value of 1 indicates that there is no preference by the plant (Singh & Agrawal 2007). TF values denoted with the same letter are not significant ($\alpha = 0.05$). See Table 3 for specific values.

CHAPTER 5

DISCUSSION

This study was designed to determine both the potential hazards of cultivating beets in soils contaminated with ROX as well as the potential negative impacts to biomass production of beets from the introduction of ROX to the growing medium. To our knowledge, no previous studies have researched: 1) the relationship between soil concentrations of ROX and the uptake of As by beets, or 2) the relationship between soil As concentrations and biomass production of beets. Studies that have attempted to correlate ROX-sourced As uptake by plants often used a much lower sample size than the present study (Wang et al. 2006, Schmidt et al. 2008, Yao et al. 2008, Yao et al. 2010, Yilmaz & Temizgül 2012). The following hypotheses were supported: 1) As concentrations in beet tissues *are* directly proportional to concentrations of As in ROX-contaminated soils, 2) plant biomass production of beet individuals *is* indirectly proportional to soil As concentrations. However, beets do *not* assimilate more As into the subterranean tissues than those above ground.

Significant Findings of this Study

As content in beets was directly proportional to the concentration of the growing medium ($r_s = 0.7574$). All treatments exhibited this trend, though the only treatment that was significantly different was T_4 ($p < 0.0001$). The relationship between growing medium As concentration and beet As concentration existed in the shoot portion as well as the root portion.

Results of this study indicate that As is toxic to the 'Detroit Dark Red' cultivar of beets at relatively low concentrations. Beet biomass was negatively impacted as As concentrations increased in the growing medium ($p < 0.0001$). A threshold of decreased biomass production exists between 6.76 mg/kg and 15.62 mg/kg As in the growing medium. This indicates that the mechanism by which As negatively impacts biomass production is overwhelmed somewhere between these two values. Further studies are needed to determine if this threshold is closer to 6.76 mg/kg or 15.62 mg/kg As.

Concerning As uptake, two likely causes for the lack of significance in the lower treatments in this study may be the high CV observed in most treatments and the number of samples reported below the LOQ. This may be typical of studies of this type as the majority of similar studies have not reported CV values (Wang et al. 2006, Singh & Agrawal 2007, Yao et al. 2009a). The nonparametric Wilcoxon sign rank test gives more robust results because the sample populations were not normally distributed, however the large variance within treatments still may have interfered with significant findings. As values reported below the LOQ are marginalized due to the method of data analysis. Values that are well below the LOQ (e.g. T_1 and T_2 As concentrations) are skewed positively and values that are just below the LOQ (e.g. T_3 As concentrations) are skewed negatively.

Other studies have found that subterranean portions of plants tend to accumulate As more readily than their surface counterparts (Wang et al. 2006, Yao et al. 2008). The present study, however, found that the tissues of beets accumulated As uniformly into all tissues. These results are supported by findings of Walsh and Keeny (1975) who hypothesized that As associated with roots was more likely sorbed to the outside of the roots than incorporated into tissues (Walsh & Keeney 1975).

Impact of Physiochemical Soil Characteristics on As Uptake

The growing medium for this study was selected based on similar research (Wang et al. 2006, Yao et al. 2009a). The intention of the selection was to provide sufficient cation exchange while providing abundant organic matter and aeration to ensure that the plants would grow unimpeded. High amounts of organic matter are associated with the use of PL fertilizers by the nature of the composition of PL (Kpomblekou et al. 2002). In addition to offering high amounts of organic matter, animal manures and manure byproducts contain a wide range of plant-essential metals and micronutrients (Bolan et al. 2004). To simulate the effects of manure in this pot experiment, a complete fertilizer, in addition to worm castings and well-balanced topsoil were used.

Although the nutrient content of the growing medium was well-balanced for cultivation of beets, the pH of 5.2 was lower than desired for this study. In acidic (reducing) conditions, plants are more likely to uptake inorganic As species, particularly inorganic As (III) species (Yao et al. 2008). Although research shows that heavy metals are more available for accumulation by plants at lower pH's, Yao, Li et al. (2008) found that at a pH of 6.5, OAs compounds are more likely to be assimilated into plant tissues than in more acidic conditions (Jackson & Miller 2000, Yao et al. 2008). If the proper conditions were not present for ROX to be either biotically or abiotically degraded into inorganic metabolites, the low pH of the soil may have contributed to the relatively low rate of As assimilation by the beets compared to prior research.

Comparative Uptake of As by Beets in Different Tissues

The As TF values found in this research indicate that the As concentrations in the below- and above-ground portions of sampled beets were similar. Yilmaz and Temizgül (2012) observed

As TF values in beets grown in varying levels of sewage sludge to range from 0.001 to 0.98 (Yilmaz & Temizgül 2012). A value of 0.001 indicates that concentrations of As within the subterranean portion of the beets was 1000× that of the above-ground portion. Our research found the As TF in beets to range from 0.96 to 1.76, suggesting that this was not the case.

One reason for this discrepancy may be that our research concentrated on the edible portion of the plants instead of the total plant. For the present study, the inedible fine roots of the beets were removed prior to biomass determination and As quantification. The intention of this step was to minimize the possibility of skewed As quantification results identified by Schmidt et al. (2008) that were due to contamination by As sorbed to the surface of fine roots (Schmidt et al. 2008). Yilmaz and Temizgül (2012) did not identify whether fine roots were removed from their samples prior to analysis (Yilmaz & Temizgül 2012).

Other factors differentiating the current study from Yilmaz and Temizgül (2012) include timing of quantification, population size, and method of quantification (Yilmaz & Temizgül 2012). In the current study, beets were allowed to reach a harvestable stage (with a developed napiform root), which took 163 days. Yilmaz and Temizgül (2012) harvested and quantified plants after 30 days (Yilmaz & Temizgül 2012). At this stage, the napiform root has not developed and reported As concentrations do not present an accurate reflection of actual risk of human exposure. Additionally, Yilmaz and Temizgül (2012) only used five replications per treatment (Yilmaz & Temizgül 2012); the current study quantified 20 replications per treatment (randomly selected from the larger population of 75 per treatment). Concerning As quantification, the current study quantified As from individuals by means of an ICP-AES in singlicate and Yilmaz and Temizgül (2012) quantified As using and ICP-MS (which is more sensitive) in triplicate (Yilmaz & Temizgül 2012).

Data Gaps and Uncertainties

Complex interactions between plants, inputs, and soils are well researched, but not always predictable. Research focusing on As uptake by plants varies widely both by species and by soil type (Jackson & Miller 2000, Liu et al. 2009, Yao et al. 2009b, Yumei et al. 2012). Other factors may influence the phytoavailability of As as well. Research by Anderson and Chamblee (2001) indicates that As concentration in PL is highly variable and dissipates over time, presumably through volatilization (Anderson & Chamblee 2001). This variable was not considered in our experimental design as soil As concentrations were not monitored throughout the experiment. Due to the slow growth rate of beets, volatilization into the atmosphere is certainly a possibility, leaving less available As in the growing medium. Also, leachate was not collected and analyzed; it is possible that As leached from the growing pots during the course of the experiment despite efforts made to minimize potential leaching such as avoiding over-watering.

Limitations of the ICP-AES could have impacted the results of this study. The size of plant material used during processing can impact As recovery through digestion for quantification by up to seven-fold (Schmidt et al. 2008). It is highly unlikely that the present study suffered from such variation; Schmidt et al. (2008) compared the extractability of As from plant materials that were very coarsely processed to those very fine (Schmidt et al. 2008). However, a matrix interaction could exist; the root portion was not processed as finely as shoots due to the nature of the material. This is also a possible explanation for the higher TF found during this research compared to other beet research (Yilmaz & Temizgül 2012).

The contracted laboratory, A&L Analytical Laboratories, Memphis, TN, utilized an EPA-approved method (#6010B) for determining As in plant tissues that included an acid digestion

and quantification with an ICP-AES. This method has been shown to have higher LOQs than utilization of Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) (Schmidt et al. 2008). The EFSA has determined that the use of an ICP-AES is not suitable for the detection of As in food samples due to the relatively low sensitivity of the machine as compared to other available technologies (e.g. ICP-MS) (EFSA 2009).

For As concentration values reported to be less than the LOQ, the value that was one half of the LOQ was used for statistical analysis (Breckenridge & Crockett 1995). The limitations of the ICP-AES may have skewed positively As reporting for T_1 and T_2 as most of the samples had reported As concentrations below the LOQ. Likewise, reported As concentrations in T_3 were likely negatively skewed for the same reason. This is standard procedure for handling environmental data found below the LOQ (Breckenridge & Crockett 1995, OPP 2000). Handling of data in this manner may have reduced the significance of the experimental treatments by lessening the reported As gap between treatments. This could have been a cause for the lack of significance between T_1, T_2, and T_3.

Risk of Human As Exposure with Consumption of Beets

Beets will accumulate increased concentrations of As when grown in soils that have increased concentrations of the heavy metal. The present study found that the BCF of beets range from 0.026 to 0.054 BCF when soils are supplemented with ROX; this is in contrast with Yilmaz and Temizgül (2012) who reported values as high as 19.3 BCF (Yilmaz & Temizgül 2012). The BCF values found in the current study are relatively low, indicating that beets do not preferentially take up As, although they do accumulate it into their tissues.

The beets cultivated in the highest concentration of the present study may pose a chronic human health threat if consumed. The highest concentration of As found in a root was 7.50 mg/kg. This is much higher than the levels that are regarded as safe for human consumption (EFSA 2009). The concentrations of As found in the beets of T_1, T_2, and T_3 are within the range considered safe in most countries of the world (EFSA 2009). For perspective, mean fish levels reported to the European Food Safety Authority ranged from 1.4526 mg/kg As to 5.011 mg/kg As, all of which are higher than the reported means of accumulated As in T_1, T_2, and T_3 (EFSA 2009).

CHAPTER 6

CONCLUSION

ROX is a global source of anthropogenic As. Often, it is applied directly to croplands in the form of ROX-contaminated PL. Because metals do not degrade in the environment, plant accumulation and subsequent human exposure to As from ROX application is a real possibility.

The present study indicates that beets are impacted by As in the growing medium. Significant effects were observed in both biomass production and As concentrations in beets with increasing concentrations of As in the growing medium. Beet biomass production is negatively correlated with increased As concentrations of the growing medium and As concentrations within the plants are positively correlated with increased As concentrations of the growing medium.

The correlation between soil As and that taken up by beets was not as pronounced in the present study as in prior research. This could be due to differences in research methodology, but it may also be to the fact that ROX was used as the As amendment and not weathered As-laden manure. ROX metabolites are often more soluble and readily taken up by plants. Moreover, the topsoil used for this study had no known prior exposure to ROX; the microbes necessary for ROX mineralization may not have been present in the study soils. It is likely that in practice, more As is taken up by beets grown in contaminated soils than observed in the present study.

The results of this study indicate that care should be taken when applying ROX-laden PL to croplands. It is likely that one-time application of the average PL to agricultural fields would

not result in the contamination of vegetable crops. However, repeated applications should be avoided to ensure that As does not accumulate in the soil, which could result in As-contaminated food crops. If PL is to be used as a fertilizer, the PL and receiving soils should be tested for As; As concentrations in PL are variable and without batch-testing, the As load into an agricultural field would be unknown.

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VITA

Joel Houser, the youngest of three boys, was born in Nashville, TN to Tom and Sarah Houser. Joel graduated from Hillwood Comprehensive High School (Nashville, TN) in 1999 and moved to Knoxville, TN to begin his undergraduate career at the University of Tennessee. After a brief stint in Knoxville, he moved to Chattanooga, TN to earn his B.S. in Environmental Science from the University of Tennessee at Chattanooga, which was completed in 2004. Meanwhile, he courted and married his wonderful wife, Shannon Houser, in 2002. Joel worked as the Farm Manager of Crabtree Farms of Chattanooga after earning his B.S. until he returned to UTC to earn his M.S. in Environmental Science in 2010. In 2010, he was named Executive Director of Crabtree Farms and held the position while earning his M.S. At UTC. Joel earned the Outstanding Graduate Student Award before graduating in May, 2013. He is considering either continuing his graduate education by pursuing a Ph.D., or continuing to work in the nonprofit or governmental sector.